

**A HISTOPATHOLOGICAL
AND
IMMUNOHISTOCHEMICAL EVALUATION
OF
SCAR BASAL CELL CARCINOMAS**

**by
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DEDICATION

I dedicate this thesis to my sons Kowin and Cole Sydney, and my wife Karen Sydney, for their patience, tolerance and understanding, and for being at my side every step of the way.

PREFACE**DECLARATION**

This study represents original work by the author and has not been submitted in any form to another university. Where use was made of the work of others it has been duly acknowledged in the text. The biostatistical analysis was performed by Annapurna Ghosh. School of Statistical and Actuarial Sciences, University of KwaZulu Natal, Howard College Campus, Durban.

The research described in this thesis was conducted in the Department of Pathology, Nelson R Mandela School of Medicine, College of Health Sciences, University of KwaZulu Natal, under the supervision of Professor PK Ramdial.

ABSTRACT

Infiltrative morphological mimicry at sites of biopsy-proven nodular basal cell carcinoma has been described. The immunoprofile of scar BCCs (scar BCCs, SBCCs) has not been documented. The aim of this study was to assess the histopathological spectrum, stromal (fibronectin, laminin, actin, desmin and vimentin) response and proliferation (bcl-2, MIB1 and p53) status of SBCCs. Twenty nine BCCs occurring in scars, unrelated to previous malignancy (*de novo* scar BCCs, DN-SBCCs), 27 BCCs that were incompletely excised and regrew at the same site (regrowth scar BCCs, RG-SBCCs) and 25 BCCs that were completely excised with tumour free margins, but recurred at the same site (recurrent scar BCCs, R-SBCCs) were accessed from the files of the Department of Pathology and Plastic and Reconstructive Surgery of the Faculty of Medicine, University of KwaZulu Natal, and formed the basis of this study.

The morphological features of DN-SBCCs was pure (3%), predominantly nodular (79%), micronodular (7%) and infiltrative (11%). RG-SBCCs were predominantly nodular (82%), micronodular (7%) and infiltrative (11%). R-SBCCs were predominantly nodular (80%), micronodular (4%) and infiltrative (16%).

The majority of DN-SBCCs, RG-SBCCs and R-SBCCs showed intact basement membrane laminin staining, while two (7%) DN-SBCCs showed 1+ and 2+ loss of basement membrane laminin staining. Three (11%) and two

(8%) RG-SBCCs and R-SBCCs, respectively, showed 2+ or 3+ basement membrane laminin discontinuity. The majority of DN-SBCCs (83%), RG-SBCCs (75%) and R-SBCCs (88%) were actin negative. No desmin immunopositivity was demonstrated in the epithelial or stromal components of DN-SBCCs, RG-SBCCs and R-SBCCs. All BCC groups showed high 3+ or 4+ vimentin immunopositivity. The majority (>50%) of the SBCCs showed low ($\leq 2+$) fibronectin immunopositivity. DN-SBCCs and R-SBCCs showed low ($\leq 2+$) fibronectin immunopositivity in 76%, while 52% of RG-SBCCs showed low ($\leq 2+$) fibronectin immunopositivity. MIB1 immunostaining revealed a range, but no significant trend of proliferative activity in all cell groups. The majority (77%) of the SBCCs showed high (>2+) bcl-2 immunopositivity. There was no significant difference in p53 immunopositivity in all SBCCs.

SBCCs demonstrate phenotypic and immunophenotypic heterogeneity. That DN-SBCCs with the infiltrative and micronodular patterns have not recurred implies that the histomorphology is a pseudo-aggressive pattern. A similar view could pertain to RG-SBCCs, but because the scar did not cicatrise the incompletely excised BCC implies that the histomorphology of RG-BCC may be a potentially more aggressive phenotype. The recurrence of a completely excised basal cell carcinoma may be viewed as a feature of an aggressive tumour, especially when the recurrent BCC contains micronodular and infiltrative components. However, as most R-SBCCs occurred at head and neck sites that are exposed to ultraviolet light, it is also possible that these are simply new BCCs occurring within scars in head and neck sites prone to BCCs. Furthermore, these R-SBCCs were not destructive tumours.

CONCLUSION:

None of the infiltrative foci of DN-SBCCs demonstrated laminin loss. Three of 5 with intra-epithelial actin immunopositivity also demonstrated low bcl-2 and high p53 staining, immunoprofiling these with an aggressive infiltrative component.

Of 11 RG-SBCCs with high p53 staining, 4 had high p53 staining in the infiltrative component, but only one had a low bcl-2 composite score and low bcl-2 score in the infiltrative focus. In addition, these infiltrative foci demonstrated intraepithelial MSA positivity and a "VA" immunophenotype of the stromal cells, indicating one RG-SBCC with an established, aggressive immunophenotype. Those positive with one or more, but not all, aggressive immunostains, are hypothesised to be RG-SBCCs evolving/developing an aggressive immunophenotype.

Only one R-SBCC, with a predominantly infiltrative pattern, had a "full-house" of aggressive immunostaining in the infiltrative foci: low bcl-2, high p53, 2+ laminin discontinuity and intra-epithelial and stromal MSA positivity. Of significance is that 7 with a predominant nodular pattern had a high p53 score. Of these, 5 had high bcl-2 scores. Hence, while high p53 may be a feature of aggressive growth, it is important that this staining be complemented with that of bcl-2, laminin and MSA.

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GLOSSARY

BCC	Basal Cell Carcinoma
High immunopositivity	> 2+
Low immunopositivity	≤ 2+
Predominantly Nodular	≥ 60% of the growth patterns are nodular
Predominantly Micronodular	≥ 60% of the growth patterns are micronodular
Predominantly Infiltrative	≥ 60% of the growth patterns are infiltrative
URD	Papillary dermis and upper half of reticular dermis
DRD	Lower half of reticular dermis
DN-SBCC	BCCs that arise in areas of scarring from various causes, which include burns, trauma, vaccination, tattooing, chicken pox and leg ulcers
RG-SBCC	BCCs that have been incompletely excised and have regrown at the same site
R-SBCC	BCCs that have been completely excised with tumour free margins, but recurred at the same site
MSA	Muscle specific actin

CHAPTER 1 – INTRODUCTION	PAGE
1.1 Introduction	2
1.2 Objectives	4
CHAPTER 2 – BACKGROUND AND LITERATURE REVIEW	
2.1 Clinical and histopathological aspects	6
2.1.1 BCCs: General	6
2.1.2 Recurrent BCC	9
2.1.3 Scar BCC	13
2.1.3.1 Extracutaneous malignancies and scars	13
2.1.3.1.1 The late 19 th century up to the 1940s	13
2.1.3.2 Cutaneous malignancies and scars	17
2.1.3.3 BCCs and scars	19
2.1.4 Treatment options	21
2.2 BCC variants	24
2.3 The Extracellular matrix	26
2.3.1 General features of the basement membrane	26
2.3.2 Ultrastructure of basement membrane	28
2.3.3 Basement membrane in tumours	28
2.3.4 Basement membrane function	29
2.3.5 Normal basement membrane of the skin	29
2.4 Immunohistochemistry	29
2.4.1 Laminin	30
2.4.1.1 Functions	31
2.4.1.2 Laminin expression in BCCs	31
2.4.2 Fibronectin	32

2.4.2.1	Structure	33
2.4.2.2	Functions	33
2.4.2.3	Expression in normal tissue	34
2.4.2.4	Fibronectin in disease states	34
2.4.3	Ki-67	35
2.4.3.1	MIB1 antibody	35
2.4.3.2	Ki-67 antigen	36
2.4.3.3	Antigen distribution	36
2.4.3.4	Function: Ki-67 antigen	37
2.4.3.5	Expression in skin	38
2.4.3.6	Expression in BCCs	38
2.4.4	<i>bcl-2</i>	39
2.4.4.1	Function	39
2.4.4.2	Structure	39
2.4.4.3	<i>bcl-2</i> family of genes	40
2.4.4.4	Expression of <i>bcl-2</i> in normal tissue	40
2.4.4.5	<i>bcl-2</i> expression in malignancies of the skin	41
2.4.5	<i>p53</i> -gene	41
2.4.5.1	What are tumour suppressor genes?	41
2.4.5.2	<i>p53</i> structure	43
2.4.5.3	Expression in normal skin	43
2.4.5.4	Mutant <i>p53</i>	44
2.4.5.5	<i>p53</i> and the cell cycle	44
2.4.5.6	<i>p53</i> expression in malignancies of the skin	44
2.4.6	The stromal cellular response	45

2.4.6.1	The myofibroblast	45
2.4.6.1.1	Actin	47
2.4.6.1.2	Desmin	49
2.4.6.1.3	Vimentin	50

CHAPTER 3 – PATIENTS AND METHODS

3.1	Introduction	52
3.2	Definitions and review parameters	52
3.2.1	Clinical parameters	52
3.2.1.1	Scar BCC	52
3.2.1.2	Regrowth BCC	53
3.2.1.3	Recurrent BCC	53
3.2.2	Histological parameters	53
3.2.2.1	Histologic growth pattern	53
3.2.2.1.1	Superficial BCC	53
3.2.2.1.2	Nodular BCC	53
3.2.2.1.3	Micronodular BCC	54
3.2.2.1.4	Infiltrative BCC	54
3.2.2.1.5	Nodulocystic BCC	54
3.2.2.1.6	Morphoeaform BCC	54
3.2.2.1.7	Pigmented BCC	54
3.2.2.1.8	Fibroepithelioma	54
3.2.2.1.9	Solid BCC	55
3.2.2.1.10	Metatypical BCC	55

3.2.2.1.11	Basosebaceous BCC	55
3.3	Specimen Collection	55
3.4	Clinical Data	56
3.5	Histological Data	56
3.5.1	Growth pattern	56
3.5.2	Palisade	56
3.5.3	Cell group shape	56
3.5.3.1	Cell group shape: round	56
3.5.3.2	Cell group shape: spiky	56
3.5.4	Peritumoural lacunae formation	57
3.5.5	Invasion	57
3.5.6	Perineural lymphocytes	57
3.5.7	Differentiation	57
3.5.8	Mitoses	57
3.5.9	Apoptosis	57
3.5.10	Cell shape	57
3.5.11	Inflammatory host response	58
3.6	Immunohistochemical studies	59
3.6.1	Primary antibodies used	59
3.6.2	Universal Kit	60
3.6.3	Sample preparation for immunohistochemistry	60
3.6.4	Procedure following antigen retrieval	61
3.6.4.1	Following antigen retrieval using the microwave technique	61

3.6.4.2	Procedure following antigen retrieval using the enzyme pre-treatment technique	62
3.6.4.2.1	Following protease pre-treatment: Laminin immunostaining	62
3.6.4.2.2	Following pronase pre-treatment: Fibronectin immunostaining	63
3.6.5	Procedure following DAB colour development	64
3.6.6	Scoring of immunohistochemical staining	65
3.6.6.1	Epithelial component	65
3.6.6.2	Stromal component	66
3.6.6.2.1	Laminin immunostaining	66
3.6.6.2.2	Fibronectin	66

CHAPTER 4 – RESULT

4.1	Sample	68
4.2	Clinical details	68
4.3	Histological details	69
4.3.1	Growth patterns	69
4.3.1.1	DN-SBCCs, RG-SBCCs and R-SBCCs	69
4.3.1.2	RG-SBCCs: initial and second tumour	70
4.3.1.3	R-SBCCs: initial and second tumour	71
4.3.2	Depth of invasion of DN-SBCCs, RG-SBCCs and R-SBCCs	71
4.3.3	Architectural contour, palisade formation and peritumoural lacunae	72
4.3.4	Differentiation	72

4.3.5	Cellular details	73
4.3.6	Stromal features	73
4.3.7	Hyalinisation, stromal spindle cells and haemosiderin	74
4.3.8	Nerve involvement	75
4.4	Immunohistochemical studies: Stromal markers	76
4.4.1	Laminin	76
4.4.1.1	Laminin discontinuity in DN-SBCCs, RG-SBCCs and R-SBCCs	76
4.4.1.2	Initial versus second RG-SBCCs	77
4.4.1.3	Initial versus second R-SBCCs	77
4.4.2	Fibronectin	78
4.4.2.1	Peritumoural fibronectin immunopositivity in DN- SBCCs, RG-SBCCs and R-SBCCs	78
4.4.2.2	Initial versus second RG-SBCCs	79
4.4.2.3	Initial versus second R-SBCCs	80
4.4.3	Actin	80
4.4.3.1	Actin immunopositivity in DN-SBCCs, RG-SBCCs and R-SBCCs	81
4.4.3.2	Initial versus second RG-SBCCs	81
4.4.3.3	Initial versus second R-SBCCs	82
4.4.4	Desmin	82
4.4.4.1	Desmin immunoreactivity	82
4.4.5	Vimentin	83
4.4.5.1	Vimentin immunoreactivity	83
4.5	Immunohistochemical studies: Proliferation marker	84

4.5.1	MIB1 Immunoreactivity	84
4.5.1.1	MIB1 immunopositivity in DN-SBCCs, RG-SBCCs and R-SBCCs	84
4.5.1.2	Initial versus second RG-SBCCs	85
4.5.1.3	Initial versus second R-SBCCs	85
4.5.2	Cell cycle proteins	86
4.5.2.1	p53 protein	86
4.5.2.2	p53 immunopositivity in DN-SBCCs, RG-SBCCs and R-SBCCs	86
4.5.2.3	Initial versus second RG-SBCCs	87
4.5.2.4	Initial versus second R-SBCCs	87
4.5.3	bcl-2 protein	88
4.5.3.1	bcl-2 immunopositivity in DN-SBCCs, RG-SBCCs and R-SBCCs	88
4.5.3.2	Initial versus second RG-SBCCs	89
4.5.3.3	Initial versus second R-SBCCs	89

CHAPTER 5 – DISCUSSION

5.1	Histological features of S-BCCs	92
5.2	Immunohistochemical features of S-BCCs	98
5.2.1	bcl-2	98
5.2.2	MIB1	102
5.2.3	p53	103
5.2.4	Fibronectin	109
5.2.5	Laminin	112

5.2.6	Actin, Desmin and Vimentin	115
-------	----------------------------	-----

CHAPTER 6 – CONCLUSION

6.0	Conclusion	121
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LIST OF TABLES	PAGE
1. Clinical details of SBCCs	68
2. Growth pattern: DN-SBCCs, RG-SBCCs and R-SBCCs	69
3. Summary of growth patterns: DN-SBCCs, RG-SBCCs and R-SBCCs	69
4. RG-SBCCs: growth pattern of initial and second tumour	70
5. R-SBCCs: growth pattern of initial and second tumour	71
6. Extent of invasion: DN-SBCCs, RG-SBCCs and R-SBCCs	71
7. Hyalinisation, stromal spindle cells and haemosiderin	75
8. Laminin discontinuity: DN-SBCCs, RG-SBCCs and R-SBCCs	76
9. Laminin discontinuity: initial and second RG-SBCCs	77
10. Laminin discontinuity: initial and second R-SBCCs	78
11. Fibronectin immunopositivity: DN-SBCCs, RG-SBCCs and R-SBCCs	78
12. Fibronectin immunopositivity: initial and second RG-SBCCs	79
13. Fibronectin immunopositivity: initial and second R-SBCCs	80
14. Actin immunopositivity: DN-SBCCs, RG-SBCCs and R-SBCCs	81
15. Actin immunopositivity: initial and second RG-SBCCs	82
16. Actin immunopositivity: initial and second R-SBCCs	82
17. MIB1 immunopositivity: DN-SBCCs, RG-SBCCs and R-SBCCs	84
18. MIB1 immunopositivity: initial and second RG-SBCCs	85
19. MIB1 immunopositivity: initial and second R-SBCCs	86
20. p53 immunopositivity: DN-SBCCs, RG-SBCCs and R-SBCCs	86
21. p53 immunopositivity: initial and second RG-SBCCs	87
22. p53 immunopositivity: initial and second R-SBCCs	88
23. bcl-2 immunopositivity: DN-SBCCs, RG-SBCCs and R-SBCCs	88
24. bcl-2 immunopositivity: initial and second RG-SBCCs	89
25. bcl-2 immunopositivity: initial and second R-SBCCs	90

LIST OF PLATES	PAGE
1. Growth Pattern: DN-SBCC	155
2. Growth Pattern: DN-SBCC	155
3. Growth Pattern: DN-SBCC	156
4. Growth Pattern: DN-SBCC	156
5. Growth Pattern: DN-SBCC	157
6. Growth Pattern: RG-SBCC	157
7. Growth Pattern: RG-SBCC	158
8. Growth Pattern: RG-SBCC	158
9. Growth Pattern: RG-SBCC	159
10. Growth Pattern: R-SBCC	159
11. Growth Pattern: R-SBCC	160
12. Growth Pattern: R-SBCC	160
13. Growth Pattern: R-SBCC	161
14. Growth Pattern: R-SBCC	161
15. Laminin Immunostaining: DN-SBCC	162
16. Laminin Immunostaining: DN-SBCC	162
17. Laminin Immunostaining: DN-SBCC	163
18. Laminin Immunostaining: DN-SBCC	163
19. Laminin Immunostaining: DN-SBCC	164
20. Laminin Immunostaining: DN-SBCC	164
21. Laminin Immunostaining: RG-SBCC	165
22. Laminin Immunostaining: RG-SBCC	165
23. Laminin Immunostaining: R-SBCC	166
24. Fibronectin Immunostaining: DN-SBCC	167
25. Fibronectin Immunostaining: DN-SBCC	167
26. Fibronectin Immunostaining: DN-SBCC	168
27. Fibronectin Immunostaining: RG-SBCC	168
28. Fibronectin Immunostaining: RG-SBCC	169
29. Fibronectin Immunostaining: RG-SBCC	169
30. Fibronectin Immunostaining: R-SBCC	170
31. Fibronectin Immunostaining: R-SBCC	170
32. Fibronectin Immunostaining: R-SBCC	171

33. Actin Immunostaining: DN-SBCC	172
34. Actin Immunostaining: DN-SBCC	172
35. Actin Immunostaining: DN-SBCC	173
36. Actin Immunostaining: RG-SBCC	173
37. Actin Immunostaining: RG-SBCC	174
38. Actin Immunostaining: RG-SBCC	174
39. Actin Immunostaining: RG-SBCC	175
40. Actin Immunostaining: RG-SBCC	175
41. Actin Immunostaining: R-SBCC	176
42. Actin Immunostaining: R-SBCC	176
43. Actin Immunostaining: R-SBCC	177
44. Actin Immunostaining: RG-SBCC	177
45. Actin Immunostaining: R-SBCC	178
46. Desmin Immunostaining: DN-SBCC	179
47. Desmin Immunostaining: RG-SBCC	179
48. Desmin Immunostaining: R-SBCC	180
49. Vimentin Immunostaining: DN-SBCC	181
50. Vimentin Immunostaining: DN-SBCC	181
51. Vimentin Immunostaining: R-SBCC	182
52. Vimentin Immunostaining: RG-SBCC	182
53. Vimentin Immunostaining: RG-SBCC	183
54. Vimentin Immunostaining: R-SBCC	183
55. Vimentin Immunostaining: RG-SBCC	184
56. Vimentin Immunostaining: R-SBCC	184
57. Vimentin Immunostaining: R-SBCC	185
58. Vimentin Immunostaining: R-SBCC	185
59. MIB1 Immunostaining: DN-SBCC	186
60. MIB1 Immunostaining: DN-SBCC	186
61. MIB1 Immunostaining: DN-SBCC	187
62. MIB1 Immunostaining: DN-SBCC	187
63. MIB1 Immunostaining: DN-SBCC	188
64. MIB1 Immunostaining: R-SBCC	188
65. MIB1 Immunostaining: R-SBCC	189
66. MIB1 Immunostaining: R-SBCC	189

67. MIB1 Immunostaining: R-SBCC	190
68. MIB1 Immunostaining: R-SBCC	190
69. MIB1 Immunostaining: RG-SBCC	191
70. MIB1 Immunostaining: RG-SBCC	191
71. MIB1 Immunostaining: RG-SBCC	192
72. MIB1 Immunostaining: RG-SBCC	192
73. MIB1 Immunostaining: RG-SBCC	193
74. MIB1 Immunostaining: RG-SBCC	193
75. MIB1 Immunostaining: RG-SBCC	194
76. MIB1 Immunostaining: DN-SBCC	194
77. MIB1 Immunostaining: R-SBCC	195
78. MIB1 Immunostaining: Nodal	195
79. p53 Immunostaining: DN-SBCC	196
80. p53 Immunostaining: DN-SBCC	196
81. p53 Immunostaining: DN-SBCC	197
82. p53 Immunostaining: DN-SBCC	197
83. p53 Immunostaining: DN-SBCC	198
84. p53 Immunostaining: DN-SBCC	198
85. p53 Immunostaining: DN-SBCC	199
86. p53 Immunostaining: R-SBCC	199
87. p53 Immunostaining: R-SBCC	200
88. p53 Immunostaining: R-SBCC	200
89. p53 Immunostaining: R-SBCC	201
90. p53 Immunostaining: R-SBCC	201
91. p53 Immunostaining: R-SBCC	202
92. p53 Immunostaining: R-SBCC	202
93. p53 Immunostaining: RG-SBCC	203
94. p53 Immunostaining: RG-SBCC	203
95. p53 Immunostaining: RG-SBCC	204
96. p53 Immunostaining: RG-SBCC	204
97. p53 Immunostaining: RG-SBCC	205
98. p53 Immunostaining: RG-SBCC	205
99. p53 Immunostaining: Adjacent solar keratotic epidermis	206

100. bcl-2 Immunostaining: DN-SBCC	207
101. bcl-2 Immunostaining: DN-SBCC	207
102. bcl-2 Immunostaining: DN-SBCC	208
103. bcl-2 Immunostaining: DN-SBCC	208
104. bcl-2 Immunostaining: DN-SBCC	209
105. bcl-2 Immunostaining: DN-SBCC	209
106. bcl-2 Immunostaining: DN-SBCC	210
107. bcl-2 Immunostaining: RG-SBCC	210
108. bcl-2 Immunostaining: RG-SBCC	211
109. bcl-2 Immunostaining: RG-SBCC	211
110. bcl-2 Immunostaining: RG-SBCC	212
111. bcl-2 Immunostaining: RG-SBCC	212
112. bcl-2 Immunostaining: R-SBCC	213
113. bcl-2 Immunostaining: R-SBCC	213
114. bcl-2 Immunostaining: R-SBCC	214
115. bcl-2 Immunostaining: R-SBCC	214
116. bcl-2 Immunostaining: Epidermal downgrowth	215
117. bcl-2 Immunostaining: DN-SBCC	215

CHAPTER 1

1.1 INTRODUCTION

BCC is a “low grade malignant epithelial neoplasm composed of cells simulating the basal cell layer of the epidermis” (Heenan *et al*, 1996). It is the most common type of skin cancer in the world. They are seen commonly on hair bearing skin, especially on the face and neck. Most BCCs are characterized by indolent clinical growth and a recurrence rate of 10-40%, depending on the mode of therapy employed (Horlock *et al*, 1998). A minority of BCCs, however, display aberrant, destructive behaviour and have been labeled aggressive, mutilating, giant or horrifying BCCs. BCCs can be subtyped according to their clinical appearance and histological features. Identification of these features may give the clinician an idea of the prognosis and growth pattern of the tumour. A range of non-aggressive and aggressive histological subtypes of BCCs have been described (Lever, 1990; Lang and Maize, 1986; Sloane, 1977). The former includes superficial and nodular subtypes while the latter encompasses morphoeic, sclerosing, desmoplastic, micronodular and infiltrative subtypes. Aggressive and non-aggressive BCC subtypes differ, not only in terms of histopathological profile, but also by the immunohistochemical stromal and proliferation profile. A group of workers documented aggressive infiltrative histopathological mimicry at sites of biopsy-proven nodular BCC, a histologically non-aggressive subtype (Swetter *et al*, 1998). The immunoprofile of this cohort of tumours was not assessed. Apart from this report, there is no documentation on the proliferation status or stromal (basement membrane and stromal cellular and matrix) features of BCCs occurring in scars (scar BCCs).

Scar BCCs arise in scars induced by burns, trauma, vaccination, tattoos, chickenpox, leg ulcers or following cardiac surgery (Koga and Sawada, 1997; Kulwin, 1975; Hendricks, 1980; Dolan, 1998). BCCs may also regrow at scar sites of previously incompletely excised BCCs (Richmond and Davie, 1987). Scarring can cause changes in the connective tissue stroma that may play a role in subsequent BCC growth. The Veterans Affairs Palo Alto Health Care System [VAPAHCS] has reported that a focal infiltrative pattern developed in the region of biopsy scars in re-excision specimens of a number of their cases which were examined histopathologically over a 2 year period (Swetter *et al*, 1998). The initial biopsies were diagnosed clinically and histologically as nodular BCC. The VAPAHCS also stated that they could not exclude the possibility that focal infiltrative changes were present in the original nodular BCCs [ie. mixed pattern tumours], but the presence of fibrosis and a foreign body reaction adjacent to the infiltrative changes in the re-excision specimens supported the concept that focally infiltrative BCC was induced by the biopsy procedure and scar formation.

This study therefore seeks to evaluate the histopathological spectrum of all scar BCCs. Because infiltrative aggressive BCCs are characterized by alterations in the proliferation marker status, a need exists to determine whether the infiltrative BCCs in scar tissue that mimic aggressive infiltrative BCCs histologically, also share the aggressive proliferation status of the aggressive infiltrative tumours. Because BCCs have a well-known interdependence with the adjacent mesenchyme, it is undocumented whether the basement membrane and stromal cellular and matrix characteristics of the

surrounding scar tissue, at an immunohistochemical level, mimic that of the non-aggressive or aggressive BCC subtypes. This study therefore aims to assess whether the scar BCCs mimic aggressive infiltrative BCCs at an immunohistochemical level as well, or, whether immunohistochemical markers can differentiate between scar-induced infiltrative “pseudo-aggressive” tumours and those that are *de novo* infiltrative and aggressive.

1.2 OBJECTIVES

- 1.1.1 To assess the histopathological spectrum of scar BCCs.
- 1.1.2 To assess the stromal response in scar BCCs.
- 1.1.3 To assess the proliferation status of scar BCCs.

CHAPTER 2

2.0 BACKGROUND AND LITERATURE REVIEW

2.1 CLINICAL AND HISTOPATHOLOGICAL ASPECTS

2.1.1 BCCs: GENERAL

BCCs are the commonest of all malignant skin tumours that can be treated in several different ways, including surgical excision, curettage and electrodesiccation, radiotherapy, cryotherapy and Moh's surgery (Lauritzen *et al*, 1965). Arthur Jacob, an Irish ophthalmologist, was the first to give a description of "extensive rodent ulcers" in the face of 3 patients (Jacob, 1827). Cecil Beadles described the first "metastatic rodent ulcer". He reported metastases from a primary lesion on the face to a submandibular lymph node (Beadles, 1894). In his description, the histology of metastatic BCC was described for the first time. In 1897, BCC was described in Hyde and Montgomery's textbook on diseases of the skin as "superficial epithelioma" (Ramdial, MMed Thesis, University of KwaZulu-Natal, 1999). In their text, the BCC was described clinically as "rodent ulcer" and histologically as "tubular epithelioma". Krompecher, for the first time in 1903, described in detail, the histological appearance of BCCs (Lever and Schaumburg-Lever, 1990). He also recognized that the BCCs originated in the basal layer of the epidermis and epidermal appendages as none of the other cell types were seen in a typical rodent ulcer.

In 1910 and 1912, MacCormac and Korbi, in independent studies, recognised tumours that were intermediate between that of a BCC and squamous cell carcinoma (MacCormac, 1910; Borel, 1973).

In 1928, Montgomery felt that these tumours were morphologically similar to BCCs but behaviourally identical to squamous cell carcinoma and referred to them as "basal squamous cell epithelioma". Foot (1947) classified these basal cell tumours into pilar, sudorific and primordial, based on resemblances to the hair follicle, the sweat gland, and the "primordium", a common stage through which both of these structures pass in development.

In 1951, Lennox and Wells discussed differentiation within the "rodent ulcer group of tumours". They collected a series of rodent ulcers, classified them according to Foot's (1947) criteria, and then applied to them all available clinical behaviour in the hope of separating them, but unfortunately this was not possible. Their forms of differentiation after analysis were: presence or absence of melanin pigment, palisade formation, fluid formation and whorl formation. Thackray (1951) attempted to classify rodent ulcers histologically and found that the only method of dividing the tumours which seemed to bear any relationship to prognosis, was that based on the growth pattern, into circumscribed or infiltrative. The fact that an intermediate group was necessary indicates the difficulty in making this distinction. Similar views were expressed by Lever (1948), who stated that the appearances usually interpreted as baso-squamous cell carcinoma were simply areas of keratinisation in basal-cell carcinomas and were of no prognostic significance. Burston and Clay (1959) stated that tumours with separate basal cell and squamous components should be detailed as such. The tumours with benign keratinisation were in fact keratotic BCCs, and that basosquamous carcinomas were simply areas of keratinisation in BCCs and were of no

prognostic significance. At the turn of this decade, a form of BCC with the clinical behaviour and histology different to that of a rodent ulcer was still a controversial issue, and, although debate on the cell of origin continued, very little new information was added to the world literature on the subject.

A large number of reports on metastatic BCCs were documented in the sixties. In 1961, Cotran, in his study of 9050 cases of BCCs, reported metastatic lesions in 0.1% of cases, with the tumour cells in the metastatic foci having the histological appearance of metatypical carcinoma. In the 1960s the best treatment option for BCCs to prevent recurrences, were analysed. Knox *et al* (1960) stated that curettage followed by electrodesiccation is a widely used method. Van Scott and Reinertson (1961) demonstrated the stromal dependency of the epithelial components of BCCs. In their studies, BCCs only grew when the epithelial component was transplanted with the stromal component. The issue of sub-typing with respect to recurrence emerged with the study of Hayes in 1962. He concluded that the multifocal type of lesion was more prone to recur.



In the 1970s, the importance of the typing of BCCs in predicting recurrence of tumour emerged (Rintala, 1971; Freeman and Duncan, 1973; Jackson and Adams, 1973). In addition, this decade expanded the histopathological spectrum of BCCs (Borel, 1973).

In 1977, Sloane examined 156 BCCs in his attempt to address and predict tumour recurrence. He divided the tumours into 4 main groups:

1. nodular

2. nodular with an infiltrative margin
3. infiltrative
4. multifocal

The infiltrative and multifocal types had a high rate of recurrence while recurrence was rare in the nodular type. In addition, some parameters which were not contributory in terms of prognostic value, included the various types of epithelial differentiation and stromal factors. Ten-Seldam and Helwig (1974) added the morphea-type of lesion to the group of lesions with a greater potential to recur or progress.

2.1.2 RECURRENT BASAL CELL CARCINOMAS

Taylor and Barisoni (1973) stated that many factors influenced their diagnosis of a recurrent BCC. Often they had great difficulty in deciding whether a lesion which reappeared in the same site was a true recurrence or a new lesion. They eventually designated lesions as recurrent when suspicion of recurrence was queried by the surgeon on the clinical notes or the pathology requisition. Thus the referring physician, the patient or the surgeon himself noted the presence of some abnormalities in or immediately adjacent to the scar of previous removal or repair (Taylor and Barisoni, 1973). Some have thought that a recurrent BCC was a different lesion from a primary BCC (Sakura and Calamel, 1979). Because it recurred, they thought it had been a more aggressive lesion from its onset (Sakura and Calamel, 1979).

In 1986, Lang and Maize chose to study recurrent basal cell carcinomas to determine whether these BCCs become more biologically active with each recurrence or whether these BCCs have aggressive histopathologic features

in the original lesions. Their study included 47 patients with 51 original tumours and all their recurrences. The patients that were included had to have at least one suitable slide from their original lesions and from each recurrent lesion. The treatment modality for the original tumours were also known. Forty patients were treated by electrodesiccation and curettage and 10 BCCs had been treated by excision with 4 having margins involved by tumour. Examination of the original tumours showed that 65% had a prominent micronodular and/or infiltrative element that usually showed poor palisading. Lang and Maize (1986) believed that it was important to note the infiltrative and micronodular elements, because they appeared to correlate with an increased tendency for recurrence. Lang and Maize (1986) concluded that most of the recurrent tumours are aggressive from the beginning. They also believed that this aggressiveness could often be predicted from pathologic examination of original lesions. These aggressive tumours may be very destructive if inappropriately or inadequately managed. The findings of Lang and Maize (1986) were similar to those of Sloane (1977), Jacobs *et al* (1982), Thackray (1951), Afzelius *et al* (1980) and Mehregan (1983). These authors observed BCCs that showed poor palisading and consisted of micronodular and infiltrating strands of tumour, concluding that these tumours were more likely to recur, invade deeply and show aggressive biologic behaviour (Lang and Maize, 1986). They also believed that blind procedures such as curettage and electrodesiccation, cryosurgery and radiation therapy, contraindicated in tumours with aggressive histologic pattern, should also be used in carefully selected nodular BCCs. Analysis of recurrent BCC lesions is difficult because initial treatment, as well as location, histology and size

appear to play a role in their development. Primary BCCs are commonly located on the head and neck; these areas are exposed to ultraviolet rays (Koplin and Zarem, 1980). The malar area and nose account for 41.5% of all head and neck BCCs with the nose comprising 25% of all facial lesions. Ninety seven percent of all recurrent lesions are located in the head and neck region with recurrences on the trunk and extremities being very rare (Dubin and Kopf, 1983). The combined nasal, malar and peri-orbital areas comprise over 75% of all recurrent BCCs (Koplin and Zarem, 1980). These areas have high recurrences. Due to fears of deformity and loss of function when these lesions are treated, many patients are also reluctant to undergo further treatment for a BCC when symptoms such as bleeding and crusting have cleared after the biopsy (Sakura and Calamel, 1979). In 1983, Dubin and Kopf included several factors that were correlated with recurrence rates, treatment modality, age and gender of patient, anatomic site, largest diameter of lesion, and previous treatment to determine which factors were associated with recurrence. One thousand four hundred and seventeen BCCs treated between 1955 and 1969 were analysed. The majority of lesions (758/1417) were treated with curettage-electrodessication, of which 197 (26%) recurred within 5 years. None of the lesions <2mm in diameter recurred within the 5 years. The recurrence rate increased with increasing diameter, such that half of all lesions over 30mm recurred. Lesions that received prior therapy (40%) and those that did not receive prior therapy (25%) recurred. Recurrent rates also increased substantially with the patient age at treatment: Seventeen percent for patients under 40 years of age and ranging up to 33% for patients over 70 years. Anatomic sites with increased risk of recurrence were eyes,

nose, face, ears and forehead, accounting for 94.5% of all recurrences (Taylor and Barisoni, 1973; Koplin and Zarem, 1980). Conservative modes of treatment in these crucial areas is thought to play a role in recurrence. Non-head location is generally associated with non-recurrence. Of four hundred and twelve lesions treated by X-ray therapy, 9.7% recurred within 5 years. When study factors were examined individually, only sex was significantly associated with the risk of recurrence. Men (12.9%) had double the recurrence rate of women (6.6%). A total of 247 lesions were treated with surgical excision, of which 23 (9.3%) recurred. The only significant factor was previous therapy. Patients whose lesions had received previous therapy had a significant higher recurrence rate of 19.4% than the patients whose lesions had not previously been treated (7.6%). Taylor and Barisoni (1973) stated that due to an increase in the risk of recurrence, BCCs in the following categories probably required observation over a period of 3 years:

- a) lesions in the nasal and periorbital regions
- b) lesions which had already recurred following any form of treatment
- c) lesions in which residual tumour was reported in the margin of resection.

Some authors have clearly shown that the type of treatment modality plays an important role in both primary and subsequent recurrence, with Moh's microsurgery demonstrating lower recurrence rates and electrodesiccation and curettage having highest recurrence rates (Sakura and Calamel, 1979). Some authors assert that the risk of BCC recurrence varies with its histopathologic type. Morphoeic and metatypical (basosquamous) subtypes

and BCCs with a prominent micronodular or infiltrative component are associated with recurrence (Lang and Maize, 1986; Siegle *et al*, 1986; Dixon *et al*, 1991). While some authors state that no one histologic growth pattern of BCC is more likely to recur than another, attributes such as spiky shape of cell groups, infiltrating invading edge, poorly developed peripheral palisading, moderate to marked degree of nuclear pleomorphism and increased mitotic figures are associated with recurrence (Dixon *et al*, 1989). Some authors have suggested that tumour size is a clinical factor predisposing to recurrence and increased subclinical extensions are seen with larger lesions (Roeningk *et al*, 1986; Dixon *et al*, 1989; Ko *et al*, 1992). The distance to the closest resection margins has also been regarded as a factor predisposing to recurrence.

2.1.3 SCAR BCCs

2.1.3.1 EXTRACUTANEOUS MALIGNANCIES AND SCARS

2.1.3.1.1 THE LATE 19TH CENTURY UP TO THE 1940s

In 1828, Jean-Nicolas Marjolin was the first to describe a malignant ulcer which occurred in areas of chronic scarring (Bostwick *et al*, 1976). However, the true description of this process was made by Robert Smith who named the process "Marjolin's ulcer" (Celikoz *et al*, 1997). Scar carcinomas have been known to arise in areas of scarring from various causes which include scars induced by burns, trauma, vaccination and chickenpox. The earliest observation of malignant degeneration in burn scars is credited to Celsus, in the first century (Giblin *et al*, 1965). In 1833, Hawkins had reported two instances of malignant degeneration in burn scars which had developed in the

scars of English soldiers who had been burned in India (Giblin *et al*, 1965). Broca (Giblin *et al*, 1965) observed malignant degeneration and ulceration on a 75 year old man who had been burned 51 years previously. Treves and Pack (1930) presented an excellent analysis of the development of cancer in 34 burned patients. Scar formation is a distinct process in malignant tumours. Several studies have argued that the scar might follow, rather than precede the carcinoma. According to Treves and Pack (1930) scar is less highly organized than the original skin and therefore more liable to ulceration and degeneration. Treves and Pack (1930) believed that repair and regeneration in marginal epithelium, which result from recurring ulceration in poorly vascularized scar tissue lead to neoplastic change. Roffo (Clements *et al*, 1995) suggested that misplaced epithelial cell group were responsible, while Virchow (Clements *et al*, 1995) believed that chronic irritation played the primary role and Treves and Pack (1930) attributed it to the release of toxins. Scar cancer of the lung was first described by Freidrich in 1939 as a peripheral carcinoma growing around a scar (Madri and Carter, 1984). Impressed by the mature acellular appearance of the central scar, Friedrich concluded that scarring preceded the development of neoplasia and acted as a carcinogenic focus. As a zone of necrosis separated the central scar from viable neoplastic tissue in many scar cancers, he argued that neither healing by scar nor accumulation of anthracotic pigment could occur in the center of a necrotic neoplasm, and considered this observation supportive of his interpretation (Kolin and Koutoulakis, 1988). MacKenzie and Rous (1941) proposed an elaborate two-stage mechanism. In the "initiating" phase, normal cells are changed to dormant cells secondary to exposure to irritants such as

sunlight. Subsequently a “promoting” phase induced by a burn encourages dormant cellular dedifferentiation into neoplastic cells. Schrek (1941) has stated that 18% of all malignant tumours of the scalp, trunk, legs and arms develop in pre-existing scars.

Neuman (Arons *et al*, 1966) summarized the four possible aetiological mechanisms relating to the formation of cancers in scars. They are:

- 1) Local environmental changes in both epidermis and dermis.
- 2) Action of cancer as a co-carcinogen.
- 3) Carcinogenic agents, which may accompany the trauma.
- 4) Implantation of living epithelial elements into the dermis.

Warren (1943) provided a list of minimal criteria that are critical to implicate mechanical trauma as the aetiopathogenesis of tumour:

- 1) The integrity of the tumour site prior to injury must be established
- 2) The injury must be sufficiently severe to disrupt the continuity of the tissue at the site, and so initiate reparative proliferation of cells
- 3) The tumour must follow the injury by a reasonable length of time
- 4) The tumour must be of a type, which might reasonably develop as a result of the regeneration and repair of those tissues which had received the injury.

Other workers have emphasized the importance of the alteration in connective tissue, probably hyalinization, and the presence of exogenous collagen with fibrinoid change, in burn scars (Iregbulem, 1987). These changes are said to

precede post burn malignancy, induced by initiating abnormal metabolic activity. Mouly (Arons *et al*, 1966) observed that lesions such as cigarette burns of the lower lip produce a special type of "acute" cancer. Bondi (1960) reported cases of carcinoma developing in amputation stump scars. Giblin *et al* (1965) estimated that 2% of burn scars undergo malignant change, and the changes usually occurred on the extremities and scalp. The behaviour pattern of scar tissue carcinoma has often been minimized and the problems of prevention, recognition, pathologic diagnosis and surgical therapy have often been overlooked (Arons *et al*, 1966). Castillo *et al* (1968) have proposed that a depressed immunological state surrounding a burned area was involved in the initiation and development of burn scar cancer. Crawley *et al* (1978) stated that scar carcinoma is a notoriously aggressive tumour with a high potential for metastases, due to reports suggesting that this is due to the origin of the carcinoma from an "immunologically privileged site", or it is due to the anaplastic nature of the tumour itself.

Various observers have reported that scar carcinoma accounts for approximately 7-15% of primary lung malignancies (Auerbach *et al*, 1979; Carroll, 1962; Limas *et al*, 1971). Oscar *et al* (1979) defined scar carcinoma of the lung as a peripherally located tumour having no evidence of bronchial origin and occurring in intimate relation to scar tissue. In their review of 1186 cases of lung cancer over a 21 year period, Oscar *et al* (1979) found a total of 7% peripheral cancers that were related to scars. Most authors suggest that the scar is the result of a focal fibrosing process such as tuberculosis, organized pneumonias and pulmonary infarction, or exposure to certain

substances, such as asbestos and silica. They stated that while most of the scars were of tuberculous origin, some were caused by infarcts. In addition to these reports of tuberculosis or infarcts as the cause of scar, there have been few reports in which scar resulting from trauma (foreign body such as bullet, knife-blade or grenade splinters, or a ruptured abscess) have been related to the carcinoma (Cagle *et al*, 1985).

Myofibroblasts, described in wound healing and conditions of scarring, are also a notable feature of desmoplasia in carcinoma of the breast (Lipper *et al*, 1980; Seemayer *et al*, 1980). Battersby and Anderson (1985) were the first to report the presence of myofibroblasts in radial scars of the breast. Cagle *et al* (1985), in an autopsy study, suggested that the prognosis of pulmonary scar cancers tends to be poor due to the peripheral location of these tumours and because of a tendency to metastasize, while clinically undetectable. Although there is no universally accepted theory for the development of burn scar carcinoma, it would appear that chronic inflammation, the presence of infection and poor initial wound management are implicated in the pathophysiology of this condition (Celikoz *et al*, 1997).

2.1.3.2 CUTANEOUS MALIGNANCIES AND SCARS

Cutaneous scars are considered poorly organized and vascularized areas and therefore, are more liable to ulceration, degeneration and eventually malignant transformation. Malignant change in smallpox vaccination scars has been infrequently described in the world medical literature. According to Kulwin (1975), a total of 54 cases have been reported, of which 31 were

BCCs arising in small pox vaccination scars, nine were squamous cell carcinomas, and a surprising 13 (24%) were malignant melanomas. The patient age ranged from 40-60 years, and in most cases vaccinations had been performed more than 30 years before the appearance of the lesions. Malignant changes in burn scars have long been reported in the literature (Nishimoto *et al*, 1996). These tumours in most cases are squamous cell carcinomas, although other types of malignancies, such as malignant melanomas and sarcomas, can be seen rarely (Yucel *et al*, 2000). The second most common histological type of malignancy originating from chronic wounds is BCCs (Yucel *et al*, 2000). Squamous cell carcinomas on burn scars occur predominantly on the extremities, while most BCCs occurring in burn scars are located in the head and neck area, with a few exceptions occurring on the extremities and chest (Martin *et al*, 2005). In 1996, Chowdri and Darzi studied 72 post burn carcinomas in the Kashmir valley. Flame burns, of which 32 were due to Kangri burns, accounted for 56 cases, scalds occurred in 11 patients and electric burns in the remaining 5 patients. All the post-burn scar cancers were well-differentiated squamous cell carcinomas. In 1930, Treves and Pack treated 1091 patients' with squamous skin carcinomas and 1374 patients with BCCs. Of these 21/1091 (2%) squamous and 7/1374 (0.3%) BCCs arose in burn scars. In 1983, Nancarrow assessed malignancies arising in scars, labelling them cicatricial cancers'. BCCs were excluded from the study, since BCC histories were much longer than the history of the scar, making "retrospective substantiation of a pre-existing benign lesion less easy" (Nancarrow, 1983). Of 18 cicatricial cancers, 5 arose in burn scars, 5 in radiotherapy induced, 4 in varicose ulcers, 3 in post-

traumatic scars and one in a scar induced by lupus vulgaris. All of 18 cicatricial cancers were squamous cell carcinomas. The age of onset of cicatricial squamous is lower than in spontaneous onset squamous cell carcinomas (Nancarrow, 1983).

2.1.3.3 BCCs AND SCARS

Carcinomatous changes have been reported as occurring in scars from a multiplicity of causes; such cutaneous cancers have been referred to as cicatricial or scar cancers (Nancarrow, 1983). Since the turn of the 19th century, reports have confirmed that cancer can arise in old burn scars, chronic ulcers, sinus tracts, fistulas and long-existing wounds where a prolonged process of repair had taken place (Giblin *et al*, 1965). Previous reports have described BCCs developing with heat, involving small areas of the skin, such as splattering of hot oil, metal chips, or molten metal.

Histologically, all have been of the nodulo-ulcerative type (Treves and Pack, 1930). It was experimentally proven that within the scarred area, tumour cells that are normally rejected, are allowed to grow (Freund *et al*, 1976). The scar acts as an immunologically privileged site that delays the recognition of tumour antigens, enabling tumour growth to a size where the immune mechanism is no longer able to prevent continued tumour progression and spread (Futrell and Myers, 1972). It is also hypothesised that scarring can cause changes to the connective tissue stroma that may play a role in BCC growth (Pollack *et al*, 1982). BCCs have also been described in scars induced by burns, trauma, vaccination and chickenpox (Pollack *et al*, 1982). Carcinomas arising in burn scars have been described in the medical

literature for about 150 years. Burn carcinomas are more common in Japan (Kairo burns) and in Kashmir (Kangri burns) than in the United States. They occur more frequently in male patients although burns are more frequent in females (Stone and Monteil, 1970). Margolis (1970) reported the first case of a superficial multicentric basal cell epithelioma arising on a thermal burn scar. Malignant change in smallpox vaccination scars has been infrequently described in the world medical literature. Zelickson (1968) reported a case of a basal cell epithelioma developing at a site of a vaccination scar after a one-year duration, and proposed that the vaccination site did not heal but led directly to a basal cell epithelioma. Sunlight has been implicated in the pathogenesis of BCCs arising in smallpox vaccination scars (Hendriks, 1980). What role the scarring process itself plays in the pathogenesis of these skin cancers is not well understood. Connolly (1960) proposed that scar tissue may be more sensitive to the effects of sunlight because of its decreased vascularity and atrophic adnexal and epidermal structures. Several other explanations have been proposed regarding the pathogenesis of BCCs developing in burn scars (Martin *et al*, 2005). Ultraviolet light has been implicated as a causative factor because the most common location of the scar BCCs is the head and neck, a site exposed to sunlight constantly (Martin *et al*, 2005). A relationship between embryological closure lines and BCCs on burn scars has also been suggested, as well as BCCs occurring in lesions where the sweat glands and hair follicles do not appear to have been destroyed (Martin *et al*, 2005). Malignant change may also be a function of the nature of the scar; scars that are prominently depigmented and thickened are more likely to undergo neoplastic change (Nancarrow, 1983). As the

scarred areas are less elastic and respond poorly to tension, the stress in scarred skin over anatomically mobile areas, such as joints, is another hypothesised factor for neoplastic change (Nancarrow, 1983).

2.1.4 TREATMENT OPTIONS

Most physicians are confident to diagnose and recommend treatment of BCCs yet recurrences of these lesions occur with a certain degree of frequency (Shanoff *et al*, 1967). Small tumours with a benign histologic pattern can recur and be destructive if inadequately managed especially if the tumour occurs in an area noted for its high recurrence rate, such as the perinasal area (Lang and Maize, 1986). The average BCC, however, can be effectively controlled without danger to life, but a significant number of cases are seen in which the tumour becomes difficult to eradicate with all reasonable attempts and eventually becomes fatal, usually because of an intracranial extension (Shanoff *et al*, 1967). In other cases, slow but relentless progression of this lesion creates widespread tissue destruction and severe functional or cosmetic deformity (Shanoff *et al*, 1967). Treatment of BCCs vary, according to size, type, and site of the lesion as well as the age and sex of the patient (Hacker *et al*, 1993). It is generally agreed that no single type of treatment is better than any other method at all times (Knox *et al*, 1960). The goal of therapy is permanent cure of the patient with the best cosmetic results (Hacker *et al*, 1993). Therapeutic options include electrodesiccation and curettage, cryosurgery, excisional surgery, Moh's surgery and ionising radiation. Knox *et al* (1960) stated that curettage followed by electrodesiccation is a widely used method for the treatment of

skin cancers, but these methods were criticized by other observers that preferred surgical excision or irradiation. Knox *et al* (1960) decided that in order for this method to receive proper respect the basic principles involved in its use, indications, contraindications and data presenting a cure rate has to be published. Knox *et al* (1960) studied patients who had a histopathological diagnosis of BCC or squamous cell carcinoma, had been treated by curettage and electrodesiccation during the period between 1 January 1939 and 1 January 1959 and followed for a 5 year period. The method was used whenever lesions were well circumscribed and not large or destructive. The results of this study, which included 67 patients with 90 lesions, was a 5 year cure rate of 96,67% with only 3 recurrences. Knox *et al* (1960) agreed that lesions treated by this method should be ≤ 2 cm in diameter and should be non-destructive (no invasion of deeper structures).

CURETTAGE AND ELECTRODESSICATION

In curettage and electrodesiccation, the sharp cutting blade of the curette is used to remove the tumour mass of either inward or outward growing lesions, making it a valuable tool in determining the depth and extent of the epithelioma (Knox *et al*, 1960). Clarke in 1910 defined "desiccation" as the method by which benign or malignant growths may be destroyed by the utilization of heat of just sufficient intensity to dehydrate the tissue (Knox *et al*, 1960). After curettage, electrodesiccation is utilized to destroy any abnormal cells that may remain at the base or sides of the operative site and also at the same time provide hemostasis (Knox *et al*, 1960). The cells become shrunken and shriveled, and nuclei are condensed and elongated (Knox *et al*,

1960). This procedure can be performed quickly and requires a minimum of office visits, the cosmetic results are usually excellent and the cure rate is very high in properly selected areas. The disadvantages are that this method is not appropriate for large and invasive lesions, an electrodesiccated surface will not heal as rapidly as an excision site and healing may be slow over areas of poor vascular supply.

Cryosurgery, that employs liquid nitrogen at a temperature of -195.8°C , is applied to the lesion (Hacker *et al*, 1993), and followed by 1-3 minutes of thawing time. The advantages are that it is easy, fast and cheap to perform and does not cause blood loss or require anesthesia. The disadvantages are patient discomfort with therapy, delayed healing time and occasionally hypopigmentation (Hacker *et al*, 1993).

Surgical excision is a very effective method, particularly for large lesions that are situated in high risk areas. Often a simple ellipse with suturing is feasible. However, in more difficult anatomic sites, the use of skin flaps and grafts may be necessary. A minimal margin of 4mm is required for tumours smaller than 2cm to eradicate the entire cancer (Hacker *et al*, 1993).

The cure rate with MOH'S Surgery is 99% for primary BCCs and 96% for recurrent lesions. This technique is used for the following tumours:

- tumours situated in areas where the risk of recurrence is high
- tumours with poorly defined clinical borders or surface changes
- recurrent tumours

- morphoeaform tumours
- incompletely excised tumours
- large tumours

By using Moh's surgery a tumour can be completely excised in a day without removing more than 1-2mm of normal skin (Hacker *et al*, 1993).

Radiation therapy may be used when surgery is not feasible or surgical destruction is not desirable. This method should not be used in the following cases:

- lesions in areas such as in the inner canthus, where recurrences might be catastrophic
- lesions in patients younger than 50 years, who would be subject to long-term sequelae such as carcinogenesis and chronic radiation dermatitis
- lesions in previously irradiated areas
- morphoeaform BCCs (Hacker *et al*, 1993)

2.2 BCC VARIANTS

The criteria for defining a giant BCC included a lesion that was more than 10cm in size or had an average area of greater than 80cm² (Randle *et al*, 1993; Sahl *et al*, 1994; McElroy *et al*, 1996). At present the acceptable criteria for a giant BCC is 5cm, although other factors, including age of the lesion, the site location, histologic subtype and possible previous therapeutic modalities also influence outcome. Granular cell BCCs, described in 1979 by Barr and Graham, contain typical neoplastic aggregates composed partially or

completely of granular cells. Reichel (1997) described a granular cell BCC in which granular cells arose from the base of a typical nodular BCC.

White *et al* (1991) reported a case of signet ring BCC, which had the presence of keratin within these signet ring cells as opposed to the presence of altered lysosomes and glycogen in signet ring clear cell BCC that was described by Cohen and Zaim (1988). Although pigmented BCCs were described in the 1920s, Maloney *et al* (1992) recognised deficiencies in the literature, and investigated the frequency, type of pigment and its location, the histologic growth pattern and the frequency of margin involvement in pigmented BCCs. In their findings pigment was present most commonly in the mixed nodular/micronodular subtype. The pigment was confirmed to be melanin in all lesions. In 1993, a new variant of BCC, in which monster cells were indentified, was described by Elston *et al*. Unlike the BCCs with giant cells, these monster cells were not multinucleate in type and they did not contain eosinophilic nuclear granules and spherical basophilic bodies that were described by Ochiai *et al* in 1987. Fibro-epithelioma of Pinkus, which was recognised as a distinct entity in 1953, received greater attention in the nineties (Pinkus, 1953). In 1994, Stern *et al* suggested that most, but not all, cases of fibroepithelioma of Pinkus, owed their characteristic histologic pattern to the growth and extension of BCC cells down eccrine ducts.

Requena *et al* (1996) described a new clinicopathological variant of BCC that they designated keloidal basal cell carcinoma. In their two cases, keloidal tissue was present in the stroma of primary BCCs. In 1997, a variant of BCC with thickened basement membrane was described (El-Shabrawi and LeBoit, 1997). The basement membranes stained positively with periodic-acid Schiff

and with antibodies to type IV collagen and laminin. While type IV collagen stained the entire basement membrane and the hyaline globules within the tumour, laminin showed continuous staining only at the inner edge of the membrane. According to the authors, the existing theory that thickened basement membrane around epithelial cell nests signified a benign cutaneous epithelial neoplasm, was flawed by the new BCC variant.

2.3 THE EXTRACELLULAR MATRIX

The extracellular matrix is a dense lattice work of collagen and elastin embedded in a viscoelastic ground substance composed of a mixture of collagenous and non-collagenous proteoglycans and glycoproteins (D'Ardenne, 1989). The composition and arrangement of basement membranes differ in different sites according to functional requirements (Damjanov, 1990). Basement membranes are a supporting scaffold which isolates tissue compartments, mediates cell attachment and influences tissue architecture. The matrix also acts as a selective macromolecular filter and plays a role in mitogenesis and differentiation. Interactions between the normal cells and the matrix may be altered in neoplasia and this may influence tumour proliferation and invasion. The vertebrate organism is separated into tissue compartments bordered by the basement membrane and interstitial stroma (Liotta, 1986).

2.3.1 GENERAL FEATURES OF THE BASEMENT MEMBRANE

Basement membranes are ubiquitous, thin, sheet-like structures located beneath epithelia and endothelia: They surround muscle, nerve and other tissues of mesenchymal origin (Zhou *et al*, 1994). Basement membranes are

extracellular matrices that are found in various regions of the skin (Weber *et al*, 1982). These membranes are complex structures composed of a mixture of collagenous glycoproteins, non-collagenous glycoproteins and proteoglycans (D'Ardenne, 1989). The collagenous glycoproteins is a meshwork of type IV collagen which is the structural backbone of the basement membrane (Liotta, 1986) and the high molecular weight non-collagenous protein is known as laminin (Timpl *et al*, 1979). Although the basement membrane is actively involved in preserving the integrity of organs, it also contributes to their specialised functions (Vracko, 1974; Gorstein, 1988). Fibronectin is another large glycoprotein that is present in plasma, on the cell surface, and in many basement membranes. Fibronectin mediates the attachment of a variety of cell types, whereas type IV collagen and laminin may be involved in the attachment of epithelial cells. Basement membranes separate the parenchymal cells from their underlying connective tissue stroma, forming a barrier to the passage of macromolecules from blood (Foidart, 1980). They also form the normal substrate for endothelial and epithelial cells and are involved in the morphologic differentiation of tissues (Foidart, 1980). Interactions between cells and basement membranes occur via specific cell surface receptors for different basement membrane constituents, and influence cell morphology and differentiation and promote cellular adhesion and movement. Several studies have indicated the importance of basement membranes in tissue regeneration and repair for example, an intact membrane is necessary for regeneration of renal tubular epithelium following acute tubular necrosis and for repair of the epidermis after skin damage (D'Ardenne, 1989). Basement membranes also act as a

filtration barrier and are important in morphogenesis, tissue differentiation and regeneration (Vracko, 1974). They play a critical role in tumour invasion (Albrechtsen *et al*, 1981).

2.3.2 ULTRASTRUCTURE OF BASEMENT MEMBRANE

Basement membranes are characterised by the presence of a basal lamina. This is further subdivided into the lamina densa, which is sometimes referred to as the basement membrane proper, and the lamina lucida. The lamina densa consists of tightly matted, randomly orientated fibrils, 3-4nm in diameter, which is embedded in a dense matrix. The basement membrane is composed of several proteins such as type IV collagen, laminin, proteoglycan, and entactin (Hudson *et al*, 1993).

2.3.3 BASEMENT MEMBRANE IN TUMOURS

Basement membranes are affected in a number of genetic and acquired diseases (Zhou *et al*, 1994). One of the first barriers to carcinoma invasion into surrounding tissues is transgression of basement membranes (Zhou *et al*, 1994). Benign epithelial neoplasms are always surrounded by an intact basement membrane even though they may "expand" into adjacent host tissues (Barsky *et al*, 1983). These basement membranes are structurally and biochemically indistinguishable from those in normal adult tissues (Willebrand *et al*, 1986). Malignant tumours on the other hand lose their extracellular basement membrane, during the process of tumour invasion. Immunohistochemical techniques using antibodies against different basement membrane constituents have been undertaken to:

- confirm tumour invasion
- distinguish malignant from benign “look-alikes”
- determine tumour prognosis
- determine tumour histogenesis (D’Ardenne, 1989).

2.3.4 BASEMENT MEMBRANE: FUNCTION

The basement membrane plays a role in cell adhesion and differentiation, as well as in tissue regeneration. They also have an important function in the size-selective sieving of macromolecules in renal glomeruli.

2.3.5 NORMAL BASEMENT MEMBRANE OF SKIN

The basement membrane of the epidermis is an interface between the dermis and epidermis. It supports the basal cells, moors the epidermis to the dermis and is a zonal barrier to cells and molecules that are localised to one or other compartment (Stenn and Bhawan, 1990). On light microscopy, the basement membrane is highlighted with glycoprotein stains, as a thin, well defined sheath beneath the epidermis.

2.4 IMMUNOHISTOCHEMISTRY

The rate at which a tumour proliferates has long been considered to bear a relationship to its clinical course. Histopathologists have therefore sought means of determining this as an adjunct to diagnosis. A variety of methods have been adapted, including mitotic counts, use of tritiated thymidine or bromodeoxyuridine incorporation and flow cytometry. Some of the above methods are not reliable in terms of accuracy, eg. the mitotic count of a BCC

may be high, but this is due to the prolonged M-phase of the cell cycle rather than a high proliferation rate in the tumour itself. Some of these methods are difficult, expensive and time consuming and are not within the domain of the general surgical pathologist. By contrast, immunohistochemistry is simple, familiar to most pathologists and is in general, diagnostic. In distinguishing benign from malignant tumours, a positive result is supported by the negative observation (lack of basal lamina immunoreactivity). It is critical, therefore, to ensure that the immunohistochemical stains have actually "worked" (D'Ardenne, 1989).

2.4.1 LAMININ

Laminin is a high molecular weight non-collagenous glycoprotein (Foidart, 1980). It consists of three chains: α , β and γ chains connected by an alpha-helical coiled domain that is synthesized by epithelial cells, endothelial cells or differentiated myotubes. Laminin was originally thought to be a third chain of type IV collagen, but resistance to collagenase indicated its non-collagenous nature. Ultrastructurally, laminin is localized in the lamina lucida of the human epidermal basement membrane. The laminin molecule has a cross-like structure with one long arm and three short arms (Kallunki, 1992). Laminin consists of one heavy α chain of 400KD and two light chains β 1 and β 2 of 200KD each. The α chains have three globular domains at their amino terminus which are separated by EGF-like repeats, a coiled-coil domain and a large globular domain at the carboxy terminus containing five globules (Malinda and Kleinman, 1996). The β and γ chains are shorter than the α chains. They contain two globular domains and two EGF repeats at the

amino terminus only. The long arm is formed by heptad repeats typical of α -helical coiled-coil proteins and the short arms are formed of EGF-like modules and domains.

2.4.1.1 FUNCTIONS

The functions of laminin include stimulation of cell growth and differentiation, and promotion of neurite outgrowth, cell adhesion and locomotion. Purified antibody against laminin was used to localize basement membranes by indirect immunofluorescence in various anatomical regions of normal and diseased human skin. Albrechtsen *et al* (1981) discovered from their studies that the presence of cytoplasmic staining for laminin in ductal epithelial cells in areas with normal breast morphology suggested that laminin is continuously synthesized by the normal epithelium. Albrechtsen *et al* (1981) stated that the biological significance of this production was not clear, but hypothesized that the synthesis was probably necessary to replenish laminin that had been broken down. Since malignant cells stained even more strongly for laminin than normal epithelial cells, it seems reasonable to conclude that carcinoma cells are able to synthesize laminin *in vivo*. The significance of this synthesis is not known, but recent results on the cell-adhesion promoting properties of laminin suggest that laminin could be important for the attachment and growth of such tumours.

2.4.1.2 LAMININ EXPRESSION IN BCCs

Laminin expression in BCCs is either continuous and distinct or discontinuous and indistinct basement membrane around tumour aggregates. Generally,

aggressive BCCs, including the morphoeic, infiltrative and basosquamous types tend to have discontinuous and indistinct basement membranes around the tumour aggregates (Barsky, 1983; De Rosa *et al*, 1994), while the converse is true of the non-aggressive BCCs (Markey *et al*, 1991). The presentation of single laminin chains on tumour cell surfaces, and the loss of structural basement membrane organisation, may lead to retention of cellular adhesion and support tumour cell invasion. A structurally altered basement membrane, in addition, is able to modulate the adhesive and invasive properties of tumour cells, possibly by virtue of its altered laminin isoforms (Albrechtsen *et al*, 1981). The laminin receptors of normal epithelium may be polarised at the basal surface and occupied with laminin in the basement membrane (Liotta *et al*, 1983). In contrast, laminin receptors on invading carcinoma cells may be distributed over the entire surface of the cell. They may be unoccupied because of the loss of formed basement membrane associated with the invading cells.

2.4.2 FIBRONECTIN

Fibronectin is a high molecular weight glycoprotein that is found in a soluble form in blood and extracellular tissue fluids, and in an insoluble form in connective tissues (Erickson *et al*, 1981). Fibronectin is also present widely on the cell surface of fibroblasts, monocytes, endothelial cells and several other types of cells (Nagata *et al*, 1985). Fibronectin is derived from the Latin language, "fibra" meaning "fibre" and "nectere" meaning to bind or tie.

2.4.2.1 STRUCTURE

Plasma fibronectin and the cell surface or extracellular matrix forms are dimers, comprising two subunits of 220,000 molecular weight, covalently linked by a single disulphide bond near their carboxyl termini. Cell surface fibronectin differs from plasma fibronectin in carbohydrate content and solubility and exists as oligomers of the basic dimeric molecule, but the two forms are very similar in amino acid and carbohydrate composition and are also immunologically indistinguishable. Plasma and cell surface fibronectins are elongated, rather than globular proteins.

2.4.2.2 FUNCTIONS

Fibronectin promotes interaction between cells and the extracellular matrix, and is present at the dermal-epidermal junction (DEJ) and throughout the dermis, but absent in the normal epidermis (Nelson *et al*, 1983). Fibronectin plays an important role in many biologic activities which include:

- cell-cell adhesion
- cell-substrate adhesion
- migration and differentiation of cells
- maintenance of cellular structure
- wound healing
- blood coagulation and opsonic function.

It is also well known that fibronectin has a special affinity for collagen, heparin, fibrin, fibrinogen and hyaluronic acid.

2.4.2.3 EXPRESSION IN NORMAL TISSUE

Fyrand (1979) had undertaken a study on fibronectin in normal skin. The group of individuals that made up the study consisted of women with a mean age of 40 years and men with a mean age of 41 years. Using indirect immunofluorescence Fyrand (1979) discovered that fibronectin was seen mainly in the basement membrane, in the papillary dermis and epidermal appendages (pilosebaceous units and eccrine sweat glands). According to Fyrand (1979) the reticular dermis showed the presence of fibronectin to a lesser degree with a different type of distribution. In areas of the skin with more ground substance, such as the papillary dermis, the immunofluorescence pattern of fibronectin shows a reticular character, whereas in the reticular dermis larger bundles of fibronectin give a fibrillar pattern. These reticular and fibrillar patterns may be based upon the binding of fibronectin to collagen fibres. Nagata *et al* (1985) demonstrated from their studies of normal skin, that fibronectin was found along the dermo-epidermal junction, reticulately in the papillary dermis, intercalated between arrector pili muscle cells, in the vascular walls of the papillary dermis, and along eccrine sweat glands and hair follicles. The epidermis and deep reticular dermis did not show any specific immunofluorescence.

2.4.2.4 FIBRONECTIN IN DISEASE STATES

Fibronectin plays an important role during tissue re-modelling in atherosclerosis, pulmonary fibrosis and glomerulosclerosis (Kosmehl *et al*, 1996). Terranova *et al* (1984) showed that cultured cancer cells from which fibronectin was excluded exhibited reduced invasive and metastatic abilities.

In 1985, Matsuura and Hakomori documented the existence of two types of fibronectin, which were subsequently named “normal fibronectin” and “oncofoetal fibronectin”. The latter is expressed in various tumours and foetal tissues. Fibronectin has diagnostic histopathologic relevance in embryogenetic, inflammatory and neoplastic conditions (Kosmehl *et al*, 1996).

2.4.3 **Ki-67**

Immunohistochemical detection of Ki-67 was originally limited to frozen material. However, with the development of monoclonal antibody MIB1, this has become possible on archival paraffin embedded tissue although a prior antigen retrieval step using microwave incubation is required (Brown and Gatter, 1990).

2.4.3.1 **MIB1 ANTIBODY**

MIB1 is a monoclonal antibody which can be used to assess the growth fraction, ie. the number of cells in the cell cycle, of normal, reactive and neoplastic tissues. MIB1 is an IgG class murine monoclonal antibody (Brown and Gatter, 1990), which was generated when mice were immunized with nuclear extract from the L428 cell line in the course of raising monoclonal antibodies to nuclear antigens specific for Hodgkin and Reed Sternberg cells. The antigen was named Ki-67 after its place of production in Kiel, West Germany and because the clone producing the antibody was grown in the 67th well of the tissue culture plate.

2.4.3.2 Ki-67 ANTIGEN

Antibody MIB1 recognizes an antigen which is associated with the cell nucleus. Ki-67 antigen is present in the nucleus of proliferating cells. Gerdes *et al* (1991) have shown that Ki-67 antigen is exposed in all stages of the cell cycle, except G₀. Some authors found that the amount of Ki-67 antigen varies with the phases of the cell cycle. Sasaki *et al* (1987) found that the Ki-67 antigen expression increases with cell cycle progression, rising during the latter half of the S-phase, and peaking in the G₂ and M-phase. Guillard *et al* (1989) also demonstrated an increase in Ki-67 expression through the cell cycle in normal and malignant cell lines. Verheijen *et al* (1989) used flow cytometry and showed the highest staining intensities in mitotic cells with similar results described by others. However, some workers reported uniform expression of Ki-67 antigen throughout the cell cycle using different cell lines. Verheijen *et al* (1989) revealed that there is a decrease or lack of antigenic expression of Ki-67 in all phases of the cell cycle in nutritionally deprived or slow growing cells. Brown and Gatter (1990) suggested that tissue from the central area of a large tumour might give an erroneously low value for the growth fraction.

2.4.3.3 ANTIGEN DISTRIBUTION

Investigation of the spatial and temporal patterns of expression of the antigen in a variety of normal and neoplastic human cell lines, including morphological analyses of the distribution of Ki-67 antigen expression during the cell cycle, has revealed that:

- the topographical distribution of the antigen appears to be cell cycle dependent.
- in the late G₁ phase, the Ki-67 antigen is present in the perinucleolar region.
- there is homogenous distribution of Ki-67 in the karyoplasm in the S-phase.
- in G₂, the perinucleolar staining within the karyoplasm is finely speckled or granular.
- during prophase, perichromosomal and karyoplasmic staining is present.
- during metaphase, perichromosomal and cytoplasmic staining is seen.
- with progression from S into G₂, the Ki-67 antigen is tightly associated with chromatin.
- during mitosis, all Ki-67 immunoreactivity is co-localised with chromatids.

2.4.3.4 FUNCTIONS: Ki-67 ANTIGEN

The exact function of the Ki-67 antigen is not known. However, Braun *et al* (1988) suggested that because of its increasing level and continuous spread throughout the cell cycle, it acts as a timer molecule. Falini *et al* (1989) have shown that the antigen is present in both neoplastic and normal tissues, thereby indicating that this antigen plays an important role in regulation of cell proliferation.

2.4.3.5 EXPRESSION IN SKIN

MIB1 immunoreactivity is present throughout the epidermis of the skin. In a study by Smith *et al* (1995), MIB1 positivity was detected in the basal layer and suprabasal layer keratinocytes. Although positivity was confined to the nucleus, it was heterogeneous in strength. Mitotic figures were strongly positive. MIB1 positivity was also noted in cells of hair follicles and sebaceous glands. Dermal fibroblasts, sweat glands and endothelial cells were non-reactive.

2.4.3.6 EXPRESSION IN BCCs

In 1993, Baum *et al* investigated MIB1 expression and the growth patterns of BCCs. In the nodular group of BCCs, 7-67% of cells were MIB1 immunoreactive, while 18-49% were MIB1 immunopositive in the superficial type and 4-33% were positive in the fibrosing group. The staining pattern in the tumours varied. In some tumours MIB1 positive nuclei were confined to the peripheral 3 to 5 rows, or, were scattered in the central and peripheral parts of the tumour. In 1995, Healy *et al* investigated the MIB1 proliferation characteristics of BCCs that recurred and compared results with non recurrent BCCs using MIB1 antibodies on 4 μ m paraffin embedded sections as described by Catorretti *et al* (1992). They found that MIB1 expression was significantly higher in primary BCCs which later recurred after complete surgical excision, as compared to non-recurrent primary BCCs. Although the recurrent BCCs also showed increased MIB1 expression in comparison with primary non-recurrent tumours, the increase was not statistically significant.

2.4.4 *bcl-2*

The 14;18 translocation was first described in association with follicular lymphoma in 1979. In this chromosomal abnormality the gene coding for the immunoglobulin heavy chain on chromosome 14 is brought into apposition with a previously undescribed gene on chromosome 18. This gene is now known as *bcl-2* since it was believed to be the second member of a potential family of genes involved in the deregulation of lymphoid cells when a particular chromosomal translocation has occurred (Pezzella and Gatter, 1995). The translocation results in the production of abnormally high levels of otherwise normal *bcl-2* protein and its immunohistochemical detection was reported to be a specific marker for tumours with the 14:18 translocation.

2.4.4.1 **FUNCTION**

The physiological function of *bcl-2* is different from other oncogenes in that its expression does not induce proliferation or transformation but allows cells to survive, even without essential growth factors, due to its ability to suppress cell death by apoptosis (programmed cell death) in which the cell and its nucleus condenses and fragments. This is believed to be the most important mechanism of cell death in humans. Vaux *et al* (1988) were the first to report that *bcl-2* can prolong cell survival.

2.4.4.2 **STRUCTURE**

The 25-26kD *bcl-2* protein from humans, mice, rats and chickens is a stretch of 19 hydrophobic amino acids near the COOH terminus with 2 charged residues that presumably serve to anchor the protein in membranes (Cazals-Hatem *et al*, 1992; Sato and Kennard, 1993). Mutagenesis studies have

confirmed that the hydrophobic COOH - terminal region allows post-translational insertion into membranes such that the majority of the *bcl-2* protein should be orientated towards the cytosol (Chen-Levy *et al*, 1989), demonstrating the importance of membrane insertion for *bcl-2* function as a blocker of apoptosis (Hockenberry *et al*, 1990). A second shorter form of the *bcl-2* protein that lacks a hydrophobic tail can potentially be produced through alternative splicing (Tsujimoto and Croce, 1986), but this protein (termed *bcl-2* beta) has never been seen *in vivo* in appreciable amounts.

2.4.4.3 ***bcl-2* FAMILY OF GENES**

cDNA has been cloned for several human genes. One of these, termed *bcl-X*, encodes a 241-amino acid protein with 74% homology to *bcl-2*. An additional *bcl-2* homologue, called *bax*, was discovered through analysis of *bcl-2* associated proteins. A 21-kD (192 amino acids) isoform of *bax* contains a transmembrane domain and has 21% homology (43% similarity) with *bcl-2*. Additional members of the *bcl-2* family are called *Mcl-1* and *A1-gene*. *Mcl-1* encodes a 37-kD protein that contains a stretch of 139 amino-acids with 35% homology (59% similarity). *A1* encodes a 20kD protein that has 40% homology (Reed, 1994).

2.4.4.4 **EXPRESSION OF *bcl-2* IN NORMAL TISSUES**

In adult epithelial tissues *bcl-2* expression is related to endocrine activity. Proliferative endometrial glands, which are under endocrine control show increased *bcl-2* expression allowing the cells of the endometrium to survive as the menstrual cycle progresses. In the thyroid gland, epithelial cells lining the follicles show cytoplasmic staining of *bcl-2*. Epithelia under hormonal control

exhibit variable expression and localisation of bcl-2 protein. bcl-2 expression has also been demonstrated in cells that are under endocrine control, such as stem cells or proliferating cells including basal cells of the skin (Yang and Korsmeyer, 1996; Verhaegh *et al*, 1995). It is also expressed in duct cells in endocrine glands as well as in salivary and sweat glands.

2.4.4.5 **bcl-2 EXPRESSION IN MALIGNANCIES OF THE SKIN**

BCCs tend to be more bcl-2 positive than squamous cell carcinomas.

Morales-Ducret *et al* (1995) demonstrated 100% positivity in BCCs in contrast to 10% in squamous cell carcinomas. Nakagawa *et al* (1994) demonstrated bcl-2 positive staining in 100% of squamous cell carcinomas in contrast to 67% of BCCs. Crowson *et al* (1996) compared bcl-2 expression in aggressive and non-aggressive BCCs and found that non-aggressive BCCs exhibited greater bcl-2 expression than their aggressive counterparts.

2.4.5 **p53 GENE**

The p53 tumour suppressor gene has acquired great importance in the understanding of the origins of neoplasia in human beings (Levine *et al*, 1994).

2.4.5.1 **WHAT ARE TUMOUR SUPPRESSOR GENES?**

Tumour suppressor genes encode specific proteins that function to prevent cancer development by blocking the division of cells that have sustained DNA damage, and in some cases triggering cell death by apoptosis (Lane, 1994).

Tumour suppressor genes have been divided into two distinct groups based

on their functions. This is useful when considering how they prevent cancer. The first group, called the “Gatekeeper” genes, are genes that have a direct effect on tumour growth by either inhibiting proliferation or promoting cell death. These are the classical tumour suppressor genes. The second group called the “Caretaker” genes, are genes that influence growth indirectly by encouraging cells to produce “genetic mistakes” which increase the likelihood that other genes, including “Gatekeeper genes”, are knocked out. Functional studies have shown that *p53* plays a major role in the arrest of cell growth and induction of apoptosis following DNA damage. Abnormalities of *p53* are one of the most frequent abnormalities identified in human skin cancers to date (Swale and Quinn, 2000).

p53 protein was first identified through its ability to form a tight, stable complex with the SV40 large T antigen (Lane and Crawford, 1979). The large T antigen is a multifunctional protein that has the ability to transform a wide range of vertebrate cells and also acts to initiate viral replication (Rigby and Lane, 1983; Fried and Prives, 1986). Large T antigen can block the action of *p53* as a DNA-binding protein and as a transcription factor (Mietz *et al*, 1992). It can also act to block the growth arrest and apoptotic functions of *p53* thereby permitting viral replication/tumour spread. One of the striking early findings in the field was of the radical changes in *p53* protein levels that were associated with transformation (DeLeo *et al*, 1979). Normal cells contain two copies of each tumour suppressor gene and cancer development normally requires that both copies of the genes be inactivated. There are several different ways in which tumour suppressor genes can be inactivated. In

numerous cancers, tumour suppressor genes are inactivated by a combination of gene deletion due to chromosome loss and point mutations (Swale and Quinn, 2000).

2.4.5.2 **p53 STRUCTURE**

The human *p53* gene is located on the short arm of chromosome 17 in the 17p13 position. Translation to a nuclear phosphoprotein occurs and consists of 393 amino acids contained in 11 exons (Levine *et al*, 1994). The first exon is non-coding. When the *p53* protein is produced, it turns on the gene for a 21kD protein that blocks cdk enzymes and thus cell division (Marx, 1993).

2.4.5.3 **EXPRESSION IN NORMAL SKIN**

The levels of *p53* in normal cells and tissues are always low, yet in transformed cells, the levels are much higher (Lane, 1994). In normal skin, the levels of wild-type *p53*, a potent suppressor gene, increase following exposure to ultraviolet (UV) radiation. This is thought to be an important protective response as demonstrated by studies in mice which lack *p53*, so called knockout mice, that show a marked increase in the rate of skin cancer development following exposure to UV irradiation. The loss of wild type *p53* expression in most solid tumours is usually the result of a large chromosomal deletion (Batsakis and El-Naggar, 1995). Wild type *p53* protein blocks tumourigenicity of cancer cells in nude mice and suppresses transformation of cells in culture (Donehower and Bradley, 1993). *p53* mutations have been identified in small clones of keratinocytes in sun-exposed human skin which suggests that inactivation of this protein is an early event in skin cancer

development.

2.4.5.4 **MUTANT *p53***

Mutant *p53* genes produce proteins that do not bind to DNA and do not promote transcription of a usually responsive promoter enhancer and gene.

Mutant *p53* may act in a dominant negative manner (like an oncogene) by entering into oligomeric complexes with wild-type *p53* and blocking its function thereby enhancing oncogenic potential (Deppert, 1994).

2.4.5.5 ***p53* AND THE CELL CYCLE**

p53 regulates the initiation of the S-phase and helps to ensure the replication of intact DNA alone (Hartwell, 1992; Weinert and Lyndall, 1993; Rotter *et al*, 1994). The G₁-S and the G₂-M transition phases are regulated in response to DNA damage, thus allowing for DNA repair before entry into the S or M phase. Wild-type *p53* influences cell growth or division negatively. It is able to prevent uncontrolled cellular proliferation by binding to specific DNA sequences and inhibiting adjacent gene transcription. Excessive wild-type *p53* in the late G₁ phase of the cycle blocks progression into the S-phase. Loss of wild-type activity results in a loss of control at the G₁-S cell cycle check point following DNA damage (Sturzbecher and Deppert, 1994). The end result is the production of genetically damaged cells that are vulnerable to further damage and capable of yielding neoplastic clones.

2.4.5.6 ***p53* EXPRESSION IN MALIGNANCIES OF THE SKIN**

McGregor *et al* (1992) used CM-1 anti-*p53* antibody to identify *p53* protein expression in solar keratoses, Bowen's disease, keratoacanthomas, squamous cell carcinomas, BCCs, viral warts and normal skin. *p53* immunoreactivity was not seen in normal skin or in viral warts but strong nuclear staining was seen throughout the thickness of 50% of squamous cell carcinomas, 46% of BCCs, 80% of Bowen's disease and 55% of solar keratoses. In 64% of keratoacanthomas, *p53* immunopositivity was seen mainly in basal keratinocytes. In squamous cell carcinomas, although there was no significant difference in *p53* immunopositivity in the well, moderately and poorly differentiated tumours, strong immunostaining was observed more frequently in poorly differentiated tumours.

2.4.6 THE STROMAL CELLULAR RESPONSE

2.4.6.1 THE MYOFIBROBLAST

The discovery of the myofibroblast occurred in electron micrographs from contracting (healing) experimental granulation tissue (Gabbiani *et al*, 1971).

The myofibroblast is defined by the essential morphologic elements, including the presence of bundles of actin filaments with interspersed dense bodies running parallel to the long axis of the cell, called stress fibres, as well as the presence of well developed cell-to-stroma attachment sites (fibronexus) and intercellular intermediate and gap junctions (Schürch *et al*, 1998).

Although the myofibroblast is best defined when visualised with an electron microscope, the myofibroblast has several histological features that allow their recognition in routine paraffin sections. The myofibroblast has features in common with smooth muscle cells and fibroblasts (Seemayer *et al*, 1980).

Histologically, myofibroblasts are usually large, spindle shaped, often stellate (spider-like) with long cytoplasmic extensions, and possess distinct acidophilic and fibrillar cytoplasm. The nuclei are indented or show strangulations of nuclear segments, a feature reflecting cellular contractions. Myofibroblasts contain finely granular chromatin and nucleoli are conspicuous.

Immunohistochemical staining reveals heterogenous cytoskeletal phenotypes with regard to their content of intermediate filaments, smooth muscle actin, and myosin expression. The myofibroblastic phenotype is determined by the expression of vimentin, actin, myosin and desmin (Schürch *et al*, 1997).

The various cytoskeletal phenotypes disclosed by the myofibroblasts are as follows:

- Phenotype V is represented by cells that only express vimentin
- Phenotype VAD is represented by cells that express vimentin, α -smooth muscle actin and desmin
- Phenotype VA is represented by cells that express vimentin and α -smooth muscle actin
- Phenotype VD is represented by cells that are positive for vimentin and desmin.

Myofibroblasts have been described in normal tissue of human and animals.

Stromal cells with myoid features were identified in rat and human lymph nodes, as well as in human spleen and expressed desmin, α -smooth muscle actin, smooth muscle myosin, and vimentin (Toccanier-Pelte *et al*, 1987).

Myofibroblasts have been described during analyses of many pathological conditions, during which three processes emerge:

- 1) Responses to injury and repair phenomena: Myofibroblasts have been demonstrated in granulation tissue where they are well developed and numerous in the exudatoproduktive layer (Ryan *et al*, 1974).
- 2) The quasineoplastic proliferative conditions: Include fibromatoses of soft tissue and bone and other soft tissue proliferations that mimic sarcomas (Schürch *et al*, 1998).
- 3) The stromal response to neoplasia: Many invasive and metastatic carcinomas are characterized by a desmoplastic stromal reaction that is rich in myofibroblasts. Although it was believed that the myofibroblastic response represented a host response to the presence of cancer in an attempt to limit the spread of tumour, it has been hypothesized that the myofibroblastic response promotes cancer invasion by secreting stromal degrading enzymes (Seemayer *et al*, 1980).

2.4.6.1.1 ACTIN

Actin is a ubiquitous cytoskeletal protein that is composed of microfilaments (Tsukada *et al*, 1987a; Enzinger and Weiss, 1995). Actin is present in large amounts in muscle as a thin 5-7 μ m diameter filament composed of globular subunits organised in a double stranded helix (Junqueira *et al*, 1992). It is also present in non-muscle cells. Structural and biochemical analyses have shown that actin may be an important component of the total protein of all cells. In skeletal muscle, actin assumes a paracrystalline array that is

integrated with thick (16 μ m) myosin filaments. In most cells, actin filaments are present as a thin sheath just beneath the plasmalemma. They may be intimately associated with cytoplasmic organelles, vesicles and granules or they may form a "purse-string" ring of microfilaments, whose constriction results in cleavage of mitotic cells. Different cell types contain immunochemically distinct actins that vary in amino acid sequence, and isoelectric point (Junqueira *et al*, 1992).

With regards to actin expression, there are six isoforms found in mammals which share more than 90% homology throughout the entire molecule, but each has a unique sequence in the first 18 residues (Collins and Elzinger, 1975; Vandekerckhore and Weber, 1978). The six actin isoforms are:

- 1) two non-muscle actins which are β and γ isoforms; these are called the cytoplasmic actins
- 2) two smooth muscle actins which are the α and γ isoforms
- 3) two sarcomeric actins which are the α -cardiac and α -skeletal isoforms.

Skeletal, cardiac and vascular smooth muscle each contain unique α -actins.

Actins of β and γ -motility are found to be in non-muscle cells. Chicken gizzard smooth muscle contains a γ -actin that differs from the non-muscle isozyme.

The presence of distinct actin isotypes in muscle cells suggests that some actin variants are specifically tailored for cell contraction (Rungger-Brandle and Giabbiani, 1983). The expression of actin isoforms in smooth muscle cells is modulated during physiological, pathological and *in vitro* conditions, but is always characterised by the presence of α -and- γ -smooth muscle and of β -and- γ -cytoplasmic actins in variable amounts (Tsukada *et al*, 1987b). The

distinction between α -smooth muscle actin and the sarcomeric actins is an important means by which to evaluate the smooth muscle or striated muscle derivation of neoplasms of mesenchymal origin (Tsukada *et al*, 1987b; Enzinger and Weiss, 1995). Actin has been hypothesised to play an important role in the modifications of cell motility during *in vivo* regenerative and tumoural conditions.

Monoclonal and polyclonal antibodies to actin isotypes have been described. A monoclonal antibody specific to smooth and skeletal muscle has been reported by Lessard *et al* (1983), while Lubit and Schwartz (1980) generated a polyclonal anti-actin antibody that was reactive with non-muscle (β) actin isotypes. Gown *et al* (1985) described a smooth muscle-cell-specific monoclonal antibody, CGA7, which reacted with smooth muscle alpha and gamma actin isotypes only. Tsukada *et al* (1987) reported the generation of HHF35, an actin antibody, which detected an epitope common to the actin isotypes of all muscle cell types, but not the actin isotypes of non-muscle cells. In pathologic tissues, HHF35 was present in myofibroblasts, intimal cells in atherosclerotic plaques, reactive mesothelial cells and sarcomas of muscle origin (Gown *et al*, 1985; Tsukada *et al*, 1987b).

2.4.6.1.2 **DESMIN**

Desmin is an intermediate filament which has a molecular weight of 53 000 kDa and can be found to be present in smooth and striated muscle (Enzinger and Weiss, 1995). With synemin and filamin, desmin forms the peripheral domain of Z-bands in adult striated muscle (Tokuyasu *et al*, 1983), as well as

an interconnecting network that ensures proper myofibril alignment during the contraction relaxation cycle. Desmin is associated with cytoplasmic dense bodies and subplasmalemmal dense plaques in smooth muscle. Apart from desmin expression in benign and malignant smooth and skeletal muscle tumours, myofibroblasts also express desmin positivity (Altmannsberger *et al*, 1985). Desmin immunoreactivity in various spindle cell lesions, not traditionally considered to be of smooth muscle origin, has been interpreted as evidence of focal myofibroblastic differentiation, which include fibromatoses, malignant fibrous histiocytoma and myofibroblastoma of the breast (Enzinger and Weiss, 1995; Taccagni *et al*, 1997).

2.4.6.1.3 VIMENTIN

Vimentin is one of the five major types of cytoplasmic intermediate filaments, with a molecular weight of 57kDa (Rosai, 1996). Vimentin is ubiquitously expressed in all cells during early embryogenesis and is gradually replaced in many cells by type-specific intermediate filaments (Enzinger and Weiss, 1983). In some mesenchymal tissues vimentin is co-expressed along with the type-specific intermediate filaments eg. desmin and vimentin co-expression in muscle cells (Enzinger and Weiss, 1983). Vimentin is so ubiquitous that some people use it as a control of the immunohistochemical reaction, in the sense of questioning its reliability if there is no staining for vimentin in the tissue.

CHAPTER 3

3.0 PATIENTS AND METHODS

3.1 INTRODUCTION

The study has a 19 year retrospective component. The patient records and computer files of the Departments of Pathology and Plastic and Reconstructive Surgery, College of Health Sciences, University of Kwazulu-Natal, Durban, were accessed to identify patients with Scar BCCs (SBCCs). The Department of Pathology has computerised surgical pathology records of all cases diagnosed in the Department from 1986. The scar BCCs included all BCCs arising in scars, with the scar being a significant finding, significant enough to have been included on the histopathology request form. Hence, the scar BCCs included BCCs arising in scars from a range of causes, but excluded scars occurring as a result of the previous excision. These scar BCCs are labelled "*de novo* scar BCCs" (DN-SBCCs). The second and third group of SBCCs encompass BCCs occurring in scar sites of previously excised BCCs. The scars had to be clinically and histologically prominent and the BCCs were non-destructive. Destructive, mutilating second BCCs occurring within scars were excluded from the study. The second group of SBCCs are referred to as "Regrowth scar BCCs" (RG-SBCCs) as they occurred in scars' occurring at sites of previously incompletely excised BCCs. The third group of SBCCs are referred to as "Recurrent scar BCCs" as they arose in scars occurring at sites of previously completely excised BCCs.

3.2 DEFINITIONS AND REVIEW PARAMETERS

3.2.1 CLINICAL PARAMETERS

3.2.1.1 *De novo* Scar BCCs

BCCs that arise in areas of scarring from various causes. These may include scars induced by burns, trauma, vaccination, tattooing, chickenpox, leg ulcers or even hair transplantation. BCCs arising in scars induced by previous excision of cutaneous BCCs were excluded from this group.

3.2.1.2 **Regrowth BCCs**

BCCs that have been incompletely excised and have regrown at the same site, within scar tissue.

3.2.1.3 **Recurrent BCCs**

BCCs that have been completely excised with tumour free margins, but recurred at the same site, within scar tissue.

3.2.2 **HISTOLOGICAL PARAMETERS**

3.2.2.1 **Histologic Growth Pattern**

3.2.2.1.1 **Superficial BCC**

A BCC growth pattern in which small buds of proliferating basal cells grow down from the epidermis or hair follicle into the superficial dermis, whilst maintaining their attachment to the base of the epidermis. They present clinically as a patch or plaque resembling Bowen's disease or eczema. These patches slowly increase in size by peripheral extension. Superficial BCC occurs predominantly on the trunk.

3.2.2.1.2 **Nodular BCC**

A BCC growth pattern in which tumour cells grow in rounded masses with a prominent peripheral palisade of nuclei and may have a surrounding retraction artefact, due to retraction of fibrous stroma from the tumour mass. Nuclei lie in parallel alignment at right angles to those at the centre of the lobules

(peripheral palisading). The BCC may be differentiated or undifferentiated (solid).

3.2.2.1.3. **Micronodular BCC**

A variant of BCC in which small cell groups containing less than 50 cells and a less prominent peripheral palisade than nodular BCC.

3.2.2.1.4 **Infiltrative BCC**

Infiltrative BCCs are "hybrids" of the nodulocystic and morphoeaform varieties, in that they show a combination of expansile solidly cellular branched, sharply angulated, and linear cell groupings. The tumour cells in these lesions have been described as "spiky" by Jacobs et al (1982).

Palisading is absent or poorly developed.

3.2.2.1.5 **Nodulocystic BCC**

A BCC presenting as a dome-shaped translucent papular nodule which may be variably fluid-filled. The nodule usually increases slowly in size and often undergoes central ulceration.

3.2.2.1.6 **Morphoeaform BCC**

The morphoeic subtype is a subgroup or variant of infiltrative BCC in which all the cell groups are small and irregular islands and cords of cells infiltrate into a dense, sclerotic, fibrous stroma. Appears as a sclerotic plaque or scar rather than a tumour, the borders are difficult to view clinically and the tumour may spread out beyond the obvious margins. This subtype of BCC is often not detected by the patient and physician at an early stage of development.

3.2.2.1.7 **Pigmented BCC**

The features and growth are similar to that of the nodulocystic subtype except for the presence of brown pigmentation.

3.2.2.1.8 **Fibroepithelioma**

Commonly located on the back of patients, this type of BCC usually consists of one but occasionally of several raised, moderately firm nodules covered by smooth, slightly reddened skin. Clinically they resemble fibromas.

3.2.2.1.9 **Solid BCC**

Seen as an umbilicated pearly papule or nodule with rolled telangiectatic borders, there is often central necrosis. Microscopically, nests of basaloid cells are seen extending into the dermis with a palisade of cells at the periphery. The tumour is undifferentiated.

3.2.2.1.10 **Metatypical BCC**

The clinical appearance is not characteristic, but occasionally lesions are very extensive, ulcerated and grow rapidly. Histologically the tumour has features of BCC and squamous cell carcinoma.

3.2.2.1.11 **Basosebaceous BCC**

Rare subtype of BCC that expresses another line of ectodermal differentiation: the presence of mature sebaceous cells.

3.3 **Specimen Collection**

Histology case numbers were obtained from the records of the Departments of Pathology and Plastic and Reconstructive Surgery, College of Health Sciences, University of KwaZulu-Natal. In the Department of Pathology, a computer search using SNOMED classification codes was performed. All the relevant slides and wax blocks were retrieved from the Archives of the Department of Pathology and Regional Laboratory Services. Scar BCCs, initial biopsies with subsequent regrowth and recurrent BCCs were included in

the study. Recurrent and regrowth BCCs in which the original biopsy could not be traced were also used provided that the original reports commented on the excision margins. Slides that were faded or damaged were re-cut from the paraffin wax embedded tissue blocks. 3µm sections were cut, stained with routine Mayer's haematoxylin and eosin and then viewed by light microscopy.

3.4 Clinical Data

The following clinical details, extracted from the files of Pathology and Plastic and Reconstructive Surgery were documented: patient name, age, gender, race, site of lesion, size of specimen, size of lesion, clinical diagnosis and histology number.

3.5 Histological Data

A range of histologic parameters were assessed and qualitatively evaluated as described by Dixon *et al* (1989) and Jacobs *et al* (1982).

3.5.1 Growth pattern

The growth pattern was classified into four groups, namely, included superficial, nodular, micronodular and infiltrative groups. A predominant subtype referred to a BCC with $\geq 60\%$ constitution of a particular pattern.

3.5.2 Palisade

Refers to the roughly parallel alignment of the peripheral nuclei at right angles to those in the center of the tumour nodules and to the basement membrane.

3.5.3 Cell group shape

The cell group shape was recorded as round or spiky.

3.5.3.1 Cell group shape: round

Tumours classified as round were composed entirely of cell nests with a round, smooth configuration.

3.5.3.2 **Cell group shape: spiky**

Tumours composed entirely of cell nests with a jagged, irregular outline.

3.5.4 **Peritumoural lacunae formation**

This refers to the separation of the tumour from its stroma, with the formation of lacunae (or clefts), which may contain necrotic cells and pyknotic nuclei.

3.5.5 **Invasion**

Tumour invasion was assessed in terms of the micro-anatomic extent of disease and was categorized as follows:

Extension into the upper reticular dermis.

Extension into the deep reticular dermis.

Extension into subcutaneous tissue.

Extension into muscle.

Perineurial invasion.

3.5.6 **Perineural lymphocytes**

Were noted as present or absent.

3.5.7 **Differentiation**

The tumour was classified as differentiated or undifferentiated. When differentiation was present, the type of differentiation was recorded.

3.5.8 **Mitoses**

Mitotic figure that has a tripolar or tetrapolar form. Mitoses with the characteristic morphology as seen during the mitotic cycle were regarded as typical, but tripolar forms were regarded as "atypical".

3.5.9 **Apoptosis**

Apoptosis was identified as cells with condensed, eosinophilic cytoplasm containing nuclear fragments.

3.5.10 **Cell shape**

The cell shape was classified as either polygonal, elongated or spindled shape.

3.5.11 **Inflammatory host response**

The presence of lymphocytes and/or plasma cells at the tumour stroma interface or within the tumour.

3.6 IMMUNOHISTOCHEMICAL STUDIES**3.6.1 PRIMARY ANTIBODIES USED****FROM: Signet Pathology Systems, Inc., Dedham, MA.****Desmin**

(prediluted antibody, Clone D33; 1:3, monoclonal mouse antibody).

Vimentin

prediluted antibody, Clone V9; 1:40, monoclonal mouse antibody).

FROM: Novocastra Laboratories Ltd, Benton Lane, U.K.**bcl-2**

(lyophilised, reconstituted with 1ml Phosphate buffered saline (PBS), Clone bcl-2100/D5, 1:40, monoclonal mouse antibody).

Laminin

(lyophilised, reconstituted with 0.1ml PBS, Clone Lam 89, 1:200, monoclonal mouse antibody).

FROM: DAKO A/S Glostrup, Denmark.**Anti-Human Ki-67 Antigen**

(prediluted antibody, Clone MIB1, 1:40, monoclonal mouse antibody).

Anti-Human p53 Protein

prediluted antibody, Clone DO-7, 1:100, monoclonal mouse antibody).

Anti-Human Fibronectin

prediluted antibody, Code No.A0245, 1:400, rabbit antibody).

FROM: Dako Corporation, Carpinteria, USA

Anti-Human muscle Actin

(prediluted antibody, Clone HHF35, 1:50, monoclonal mouse antibody).

3.6.2 UNIVERSAL KIT

FROM: Dako Corporation, Carpinteria, USA

Dako LSAB2 System, HRP Kit

The labeled streptavidin-biotin (LSAB) method was used for immunohistochemical studies, which was designed for use with primary antibodies from rabbit and mouse. The kit consists of Link (110ml), which is composed of biotinylated anti-rabbit and anti-mouse immunoglobulins in phosphate buffered saline (PBS) containing a carrier protein and 15mM sodium azide and streptavidin peroxidase in PBS, containing a carrier protein and anti-microbial agent.

3.6.3 SAMPLE PREPARATION FOR IMMUNOHISTOCHEMISTRY

1. Tissue sections were cut at 2 μ M thickness using sterile disposable blades on a rotary microtome.
2. They were floated onto a water bath and picked on poly-L-lysine coated slides, (see Appendix C).
3. The sections were baked on a hotplate at 60°C for 20mins before dewaxing by agitating in two changes of xylene (3minutes in each).

4. This was followed by passage through two changes of 100% alcohol for 3 minutes each and two changes of 95% alcohol for 3 minutes each.
5. The sections were re-hydrated in running tap water for 5 minutes.
6. Antigen retrieval using the microwave, or enzyme pretreatment (see Appendix A) was then performed.

ANTIGEN RETRIEVAL METHOD

ANTIBODY	ANTIGEN RETRIEVAL TECHNIQUE
Actin	Microwave
Desmin	Microwave
Vimentin	Microwave
MIB1	Microwave
p53	Microwave
bcl-2	Microwave
Laminin	Enzyme pretreatment (protease)
Fibronectin	Enzyme pretreatment (pronase)

3.6.4 PROCEDURE FOLLOWING ANTIGEN RETRIEVAL

3.6.4.1 Following antigen retrieval using the microwave technique.

1. Slides were allowed to cool at room temperature for 5 minutes.
2. Slides were then washed in tap water for 1 minute.
3. Slides were incubated in 3% hydrogen peroxide (see Appendix B) in a coplin jar at room temperature for 5 minutes.
4. Slides were washed in tap water.

5. A circle was drawn around tissue sections with a Dako pen (Dako A/S, Glostrup, Denmark) to form a well for reagents on the slide. Slides were then placed in PBS.
6. Excess PBS buffer was removed from slide.
7. Primary antibody (at the correct dilution) was added.
8. The slides were then incubated in a Sharp Carousel microwave at "LOW" temperature for 4 minutes and 30 seconds.
9. The slides were then rinsed three times with PBS.
10. Excess PBS was removed.
11. Link antibody was applied to the sections.
12. The slides were then incubated in a Sharp Carousel microwave at "LOW" temperature for 3 minutes and 30 seconds.
13. The slides were then rinsed three times with PBS.
14. Excess PBS was removed.
15. Label antibody was then applied to the tissue sections.
16. The slides were then incubated in a Sharp Carousel microwave at "LOW" temperature for 3 minutes and 30 seconds.
17. The slides were then rinsed three times with PBS and excess PBS was tapped off.
18. DAB-chromogen solution (see APPENDIX B) was applied to the slides. Colour development was observed microscopically.

3.6.4.2 Procedure following antigen retrieval using the enzyme pretreatment technique

3.6.4.2.1 Following Protease pretreatment: Laminin immunostaining

1. The slides were rinsed in water.

2. Slides were then quenched in methanol/hydrogen peroxide for 30 minutes.
3. The slides were then rinsed in PBS.
4. Normal horse serum (5% in PBS) was then applied to the sections for 20 minutes at room temperature.
5. Monoclonal antibody (1:200) was applied to the sections and then incubated in a Sharp Carousel microwave at "LOW" temperature for 4 minutes and 30 seconds, and then allowed to stand at room temperature for 50 minutes.
6. The slides were then rinsed three times with PBS.
7. Excess PBS was removed.
8. Link antibody was applied to the sections.
9. The slides were then incubated in a Sharp Carousel microwave at "LOW" temperature for 3 minutes and 30 seconds.
10. The slides were then rinsed three times with PBS.
11. Excess PBS was removed.
12. Label antibody was then applied to the tissue sections.
13. The slides were then incubated in a Sharp Carousel microwave at "LOW" temperature for 3 minutes and 30 seconds.
14. The slides were then rinsed three times with PBS and excess PBS was tapped off.
15. DAB-chromogen solution (APPENDIX B) was applied to the slides.
Colour development was observed microscopically.

3.6.4.2.2 Following Pronase pretreatment: Fibronectin immunostaining (use 3% BSA for all dilutions)

1. The slides were rinsed in water.
2. Normal horse serum (5% in PBS) was then applied to the sections for 20 minutes at room temperature.
3. Monoclonal antibody (1:400) was applied to the sections and then incubated in a Sharp Carousel microwave at "LOW" temperature for 4 minutes and 30 seconds, and then allowed to stand at room temperature for 50 minutes.
4. The slides were then rinsed in PBS.
5. Excess PBS was removed.
6. Link antibody was applied to the sections.
7. The slides were then incubated in a Sharp Carousel microwave at "LOW" temperature for 3 minutes and 30 seconds.
8. The slides were then rinsed three times with PBS.
9. Excess PBS was removed.
10. Label antibody was then applied to the tissue sections.
11. The slides were then incubated in a Sharp Carousel microwave at "LOW" temperature for 3 minutes and 30 seconds.
12. The slides were then rinsed three times with PBS and excess PBS was tapped off.
13. DAB-chromogen solution (APPENDIX B) was applied to the slides. Colour development was observed microscopically.

3.6.5 Procedure following DAB colour development

1. The slides were left in running tap water for 5 minutes.

2. The sections were then counterstained in Mayer's haematoxylin (see APPENDIX B) for one minute.
3. The slides were washed in running tap water for 2 minutes.
4. The slides were then immersed in ammoniated water (see APPENDIX B) to develop the counterstain.
5. The slides were rinsed in running tap water for 2 minutes.
6. The sections were then dehydrated in two changes of 90% alcohol (2 minutes each) and 2 changes of absolute ethanol (3 minutes each).
7. The sections were then cleared in two changes of xylene for 3 minutes each.
8. The sections were mounted using DPX and coverglass.

3.6.6 SCORING OF IMMUNOHISTOCHEMICAL STAINING

3.6.6.1 Epithelial component

Ki-67, p53 and bcl-2 immunostaining was scored as follows:

Negative: No staining of cells

0: $\leq 5\%$ of cells positive

1+: 6-25% of cells positive

2+: 26-50% of cells positive

3+: 51-75% of cells positive

4+: 76-100% of cells positive

These scores were obtained by counting the number of positive cells and expressing this as a percentage of the total number of cells present in a high power field (X40). A maximum of 10 high power fields was counted and an average was calculated.

3.6.6.2 Stromal component**3.6.6.2.1 Laminin immunostaining**

- 0: All cell groups with intact basement membrane
- 1+: 1-25% of cell groups with basement membrane discontinuity
- 2+: 26-50% of cell groups with basement membrane discontinuity
- 3+: 51-75% of cell groups with basement membrane discontinuity
- 4+: 76-100% of cell groups with basement membrane discontinuity

3.6.6.2.2 Fibronectin

- 0: Fibronectin present around $\leq 5\%$ of tumour cell groups
- 1+: Fibronectin present around 6-25% of tumour cell groups
- 2+: Fibronectin present around 26-50% of tumour cell groups
- 3+: Fibronectin present around 51-75% of tumour cell groups
- 4+: Fibronectin present around 76-100% of tumour cell groups

CHAPTER 4

4.0 RESULTS

4.1 SAMPLE

Seventy three patients with scar BCCs were accessed from the files of Pathology and Plastic and Reconstructive Surgery. A total of 81 completely excised BCCs formed the study cohort. There were 29 *de novo* scar BCCs (DN-SBCCs), 27 regrowth scar BCCs (RG-SBCCs) and 25 recurrent scar BCCs (R-SBCCs). The initial excision biopsies of 8 RG-SBCCs and 7 R-SBCCs were also analysed.

4.2 CLINICAL DETAILS

		DN-SBCCs	RG-SBCCs	R-SBCCs
Age(years)	Range	21-85	42-86	30-87
	Mean	67.4	66.5	69.2
Race	African (Albino)	2	2	0
	White	27	25	25
Gender	Male	21	14	17
	Female	8	13	8
Sites	Head/neck	17(59%)	25(93%)	19(76%)
	Extracranial	12(41%)	2(7%)	6(24%)
Size specimen:range (mm)		8-45	10-160	11-70
	Mean	27	38.6	29.9
Lesion size:range (mm)		1-15	3-45	5-30
	Mean	8.7	16.7	13

TABLE 1: Clinical details of Scar BCCs.

The nose was commonest head and neck site that was involved by DN-SBCCs and RG-SBCCs. The cheek and ear were the commonest sites of R-SBCC involvement. In the periorbital area, the left eye-lid was involved by DN-SBCCs in 2 (7%) patients and the lateral canthus in 1 (3%). R-SBCCs of the periorbital involved the right lower eyelid (1 patient) and left medial canthus (1 patient).

The smallest DN-SBCC and RG-SBCC measured 1 x 1mm and 3 x 4mm each, respectively. The largest DN-SBCC measured 15 x 15mm. Nineteen DN-SBCCs were ≤ 15 mm in largest diameter. The largest RG-SBCC and R-SBCC measured 45 x 60mm and 30 x 10mm each, respectively. Ten R-SBCCs measured < 20 mm.

4.3 HISTOLOGICAL DETAILS

4.3.1 Growth Patterns

4.3.1.1 DN-SBCCs, RG-SBCCs and R-SBCCs

	Pure N	Pred N	Pred M	Pred I	TOTAL
DN-SBCCs	1(3%)	23(79%)	2(7%)	3(11%)	29
RG-SBCCs	0	22(82%)	2(7%)	3(11%)	27
R-SBCCs	0	20(80%)	1(4%)	4(16%)	25

Table 2: Growth patterns of DN-SBCCs, RG-SBCCs and R-SBCCs

Key:

N = Nodular
M = Micronodular
S = Superficial
I = Infiltrative
Pred = Predominant

	Pure	Pred
DN-SBCCs	3%	97%
RG-SBCCs	0%	100%
R-SBCCs	0%	100%

Table 3: Summary of growth patterns of DN-SBCCs, RG-SBCCs and R-SBCCs

(Plates 1-14)

In 28 (97%) DN-SBCCs a predominant BCC subtype was identified while in 1 a pure nodular subtype was evident (tables 2-3). All RG-SBCCs and R-SBCCs demonstrated a predominance of one histological subtype (table 2).

4.3.1.2 RG-SBCCs: initial and second tumour

	Pure N	Pred N	Pred In
Initial <i>Minor</i>	1(12%)	6(76%) S,M,I: 2 M,I: 3 S,M: 1	1(12%) S,N,M: 1
second <i>Minor</i>	0	8(100%) S,M,I: 4 M,I: 2 S: 1 I: 1	0

Table 4: RG-SBCCs: Growth patterns of initial and second tumours

A pure nodular pattern was seen in 1 (12%) initial RG-SBCC. Of those RG-SBCCs with a predominantly nodular growth pattern, the majority had minor micronodular and infiltrative patterns. One initial RG-SBCC that had a predominantly infiltrative growth pattern also contained minor superficial, nodular and micronodular growth patterns. In all initial biopsies with a combination of architectural patterns, the superficial, micronodular and nodular components were situated higher in the dermis than the infiltrative components. Similarly, in the second biopsy of RG-SBCC, the majority of tumours had a predominantly nodular pattern and minor micronodular and infiltrative patterns.

4.3.1.3 R-SBCCs: initial and second tumours

	Pred N	Pred I
Initial <i>Minor</i>	6(86%) I: 1 M,I: 1 S,M,I: 1 M: 2 S: 1	1(14%) N:1
second <i>Minor</i>	7 (100%) M,I: 1 S,M, I: 3 M: 1 S: 1 S,M: 1	0

Table 5: R-SBCC: Growth patterns of initial and second tumours'

Of those R-SBCCs with a predominantly nodular growth pattern, the majority had minor micronodular and infiltrative patterns. One initial R-SBCC that had a predominantly infiltrative growth pattern also contained a minor nodular growth pattern. The majority of R-SBCCs with a predominantly nodular pattern had minor superficial, micronodular or infiltrative patterns.

4.3.2 Depth of invasion of DN-SBCCs, RG-SBCCs and R-SBCCs

	URD	DRD	Subcutaneous	Muscle	Lateral margin	Deep margin
DN-SBCCs	6(21%)	19(66%)	4(13%)	0	5	4
<u>RG-SBCCs</u>						
Initial	0	7(87%)	1 (13%)	0	8	4
RG-SBCCs	2(7%)	19(71%)	6(22%)	0	17	11
<u>R-SBCCs</u>						
Initial	0	7(100%)	0	0	0	0
R-SBCCs	5(20%)	8(32%)	11(44%)	1(4%)	8	4

Table 6: Extent of invasion in DN-SBCCs, RG-SBCCs and R-SBCCs

Only 1 (4%) BCC extended into muscle. Five (17%) DN-SBCCs had incompletely excised lateral margins and 4 (14%) incompletely excised deep margins. All initial RG-SBCCs had incompletely excised lateral margins. The lateral and deep margins of all initial R-SBCCs were clear of tumour.

4.3.3 Architectural contour, palisade formation and peritumoural lacunae.

The superficial, nodular and micronodular growth patterns of all DN-SBCCs, RG-SBCCs and R-SBCCs showed palisade formation. These patterns also demonstrated smooth, rounded contours, while the infiltrative component had a spiky configuration. One (3%) DN-SBCC lacked peritumoural lacunae; 3 (11%) had focal and 25 (86%) had prominent peritumoural lacunae. All initial biopsies of RG-SBCCs had prominent peritumoural lacunae. Six (22%) RG-SBCCs lacked peritumoural lacunae; 2 (7%) had focal and 19 (71%) had prominent peritumoural lacunae. One (14%) initial R-SBCC had focal and 6 (86%) had prominent peritumoural lacunae. Four (16%) R-SBCCs lacked peritumoural lacunae; 2 (8%) had focal and 19 (76%) had prominent peritumoural lacunae.

4.3.4 Differentiation

Sixteen (55%) DN-SBCCs showed adenoid and keratotic differentiation, 5 (17%) showed exclusive adenoid and 8 (28%) exclusive keratotic differentiation. Four (50%) initial biopsies of RG-SBCCs showed adenoid and keratotic differentiation. Thirteen (48%) RG-SBCCs showed adenoid and keratotic differentiation; while 5 (19%) and 2 (7%) showed exclusive adenoid

and keratotic differentiation, respectively. Seven (26%) RG-SBCCs were undifferentiated. Four (57%) initial R-SBCCs showed adenoid and keratotic differentiation; one (14%) showed adenoid differentiation only and two (29%) were undifferentiated. Eleven (44%) R-SBCCs showed adenoid and keratotic differentiation; 5 (20%) showed adenoid differentiation only and 6 (24%) showed keratotic differentiation only. Three (12%) R-SBCCs were undifferentiated.

4.3.5 Cellular details

The superficial, nodular and micronodular growth patterns had polygonal to elongated cells in all 3 BCC groups, while the infiltrative component had spindle shaped cells. Giant cells and pseudoinclusions were present in one (3%) DN-SBCC and one (4%) RG-SBCC. Twenty six (90%) DN-SBCCs demonstrated focal clear cell change, while 4 (50%) initial RG-SBCCs demonstrated the same. Nineteen (70%) RG-SBCCs had focal clear cells. Three (43%) initial and 19 (76%) R-SBCCs had focal clear cells. In 2 (8%) R-SBCCs clear cells were prominent. Vesicular nuclei were seen in 26 (90%) DN-SBCCs. Six (75%) initial and all RG-SBCCs contained vesicular nuclei. Vesicular nuclei were seen in all initial and R-SBCCs. Nucleoli were conspicuous in 26 (90%) DN-SBCCs. Nucleoli were conspicuous in 5 (63%) initial and 20 (74%) RG-SBCCs. Nucleoli were conspicuous in 6 (86%) initial and 21 (84%) R-SBCCs. Mitoses and apoptotic figures were present and prominent in all BCCs.

4.3.6 Stromal features

In DN-SBCCs, fibrosis was present in advancing front of the tumour in all cases. In 13/29 DN-SBCCs fibrosis was confined to the upper reticular dermis (URD). Sixteen of 29 DN-SBCCs demonstrated fibrosis in the advancing front of the tumour in the URD and deep reticular dermis (DRD). The majority of DN-SBCCs showed solar elastotic masses (SEM) in the DRD (21/29), while 7/29 DN-SBCCs showed SEM in the URD. Only 1 case did not show any SEM.

Nineteen of 27 RG-SBCCs showed fibrosis around (URD and DRD) the tumour. Five showed fibrosis in the DRD in the advancing front of the tumour. One each had fibrosis in the advancing front of the tumour, localized to the URD and DRD. One RG-SBCC demonstrated fibrosis within but not around the tumour. Twenty of 27 RG-SBCCs showed SEM around the tumour and 5 RG-SBCCs showed SEM in the DRD. Two RG-SBCCs did not show any SEM. All of 25 R-SBCCs demonstrated fibrosis in the advancing front, localized to the DRD in 24/25 and to the URD in 1/25 cases. SEM was present in the DRD in 24/25 biopsies and in the URD in 1/25 case.

4.3.7 Hyalinisation, stromal spindle cells and haemosiderin.

	Hyalinisation	Stromal spindle cells	Haemosiderin
DN-SBCCs	23(79%)	21(72%)	7(24%)
<u>RG-SBCCs</u>			
Initial	6(67%)	7(87%)	0
RG-SBCCs	22(81%)	24(89%)	5(17%)
<u>R-SBCCs</u>			
Initial	5(71%)	4(57%)	0
R-SBCCs	23(92%)	24(96%)	2(8%)

TABLE 7: Hyalinisation, stromal spindle cells and haemosiderin

Hyalinisation and stromal spindle cells were seen in the majority of the DN-SBCCs, while haemosiderin was demonstrated in 7 (24%) tumours. None of the initial biopsies of RG-SBCCs or R-SBCCs demonstrated haemosiderin. The majority of RG-SBCCs and R-SBCCs had hyalinisation and stromal spindle cells. Five (17%) and 2 (8%) RG-SBCCs and R-SBCCs demonstrated haemosiderin, respectively. Capillaries, lymphocytes and plasma cells were present at the tumour-stroma interface and within the fibrotic component of all BCCs.

4.3.8 Nerve involvement

Perineural lymphocytes were present in 23 (79%) DN-SBCCs and perineural tumour invasion was seen in 3 (10%) DN-SBCCs. Perineural lymphocytes were present in 2 (25%) and perineural invasion was present in 1 (13%) initial biopsies of RG-SBCC. Perineural lymphocytes were present in 16 (59%) and perineural tumour invasion was seen in 7 (26%) RG-SBCCs. Perineural lymphocytes were present in 2 (8%) initial biopsies of R-SBCCs, while no perineural invasion was seen. Perineural lymphocytes were present in 16 (64%) and perineural tumour invasion was seen in 6 (24%) R-SBCCs.

4.4 IMMUNOHISTOCHEMICAL STUDIES: STROMAL MARKERS.

4.4.1 LAMININ

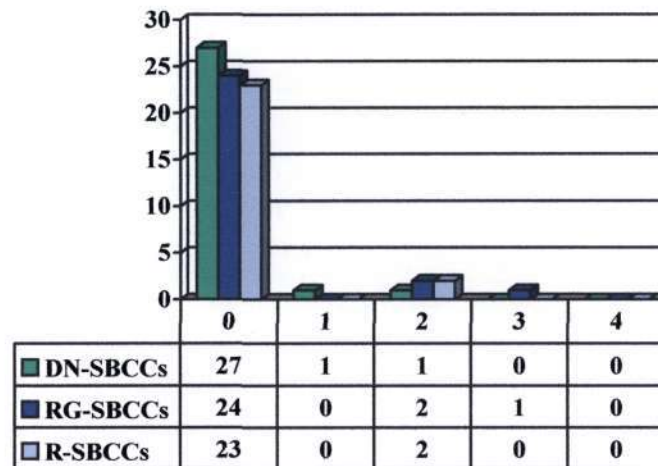


Table 8: Laminin discontinuity in DN-SBCCs, RG-SBCCs and R-SBCCs

4.4.1.1 Laminin discontinuity in DN-SBCCs, RG-SBCCs and R-SBCCs

(Plates 15-23)

Twenty seven (93%) of 29 DN-SBCCs showed intact basement membrane laminin staining around tumour cell nests, while 2/29 (7%) showed 1+ and 2+ loss of basement membrane laminin. This was present in areas containing lymphocytes at the tumour-stroma interface. Twenty four (89%) of 27 RG-SBCCs showed intact basement membrane laminin staining around tumour cell nests, while 3/27 (11%) RG-SBCCs showed 2+ or 3+ basement membrane laminin discontinuity in infiltrative foci. Twenty three (92%) of 25 R-SBCCs showed intact basement membrane laminin staining around tumour cell nests, while 2/25 (8%) showed 2+ basement membrane laminin discontinuity in infiltrative foci. Comparison of the basement membrane

laminin staining pattern in DN-SBCCs, RG-SBCCs and R-SBCCs did not reveal any statistically significant results ($p=1.0$).

4.4.1.2 Initial and second RG-SBCCs

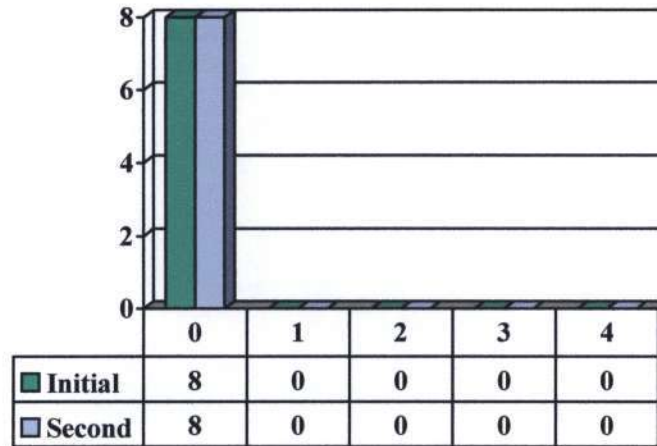


Table 9: Laminin discontinuity of initial and second RG-SBCCs

All of eight (100%) initial and RG-SBCCs showed intact basement membrane laminin staining around the tumour cell nests. Comparison of the basement membrane laminin staining pattern in the initial and RG-SBCCs did not reveal any statistically significant results ($p=1.0$).

4.4.1.3 Initial and second R-SBCCs

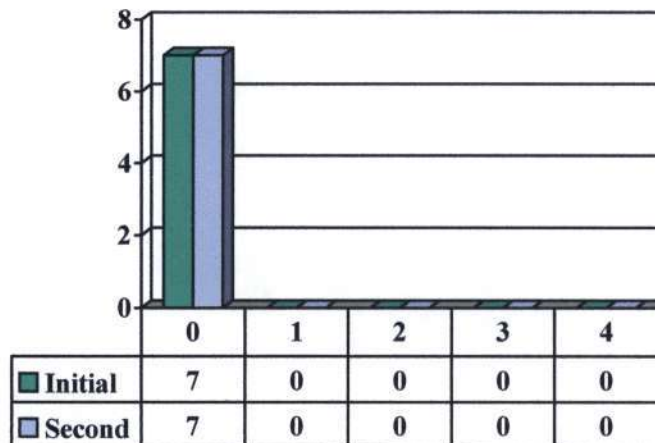
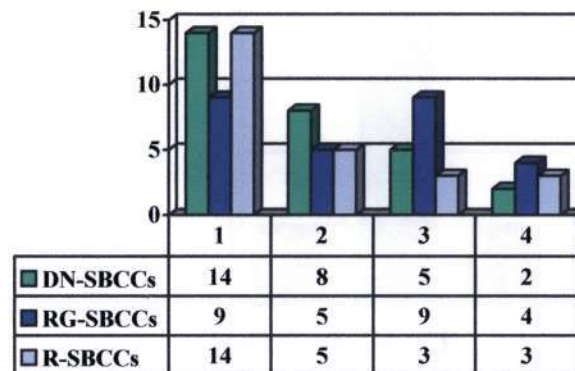


Table 10: Laminin discontinuity of initial and second R-SBCCs

All of seven (100%) initial and RG-SBCCs showed intact basement membrane laminin staining around the tumour cell nests. Comparison of the basement membrane laminin staining pattern in the initial and R-SBCCs did not reveal any statistically significant results ($p=1.0$).

4.4.2 FIBRONECTIN

**Table 11: Fibronectin immunopositivity in DN-SBCCs, RG-SBCCs and R-SBCCs**

4.4.2.1 Peritumoural fibronectin immunopositivity in DN-SBCCs, RG-SBCCs and R-SBCCs

(Plates 24-32)

Seven (24%) of 29 DN-SBCCs showed high (>2+) peritumoural fibronectin immunopositivity, while 22/29 (76%) showed low ($\leq 2+$) peritumoural fibronectin immunopositivity. Thirteen (48%) of 27 RG-SBCCs showed high (>2+) and 14/27 (52%) showed low ($\leq 2+$) peritumoural fibronectin immunopositivity. Six (24%) of 25 R-SBCCs showed high (>2+) peritumoural fibronectin immunopositivity, while 19/25 (76%) R-SBCCs showed low ($\leq 2+$) peritumoural fibronectin immunopositivity. Comparison of fibronectin staining

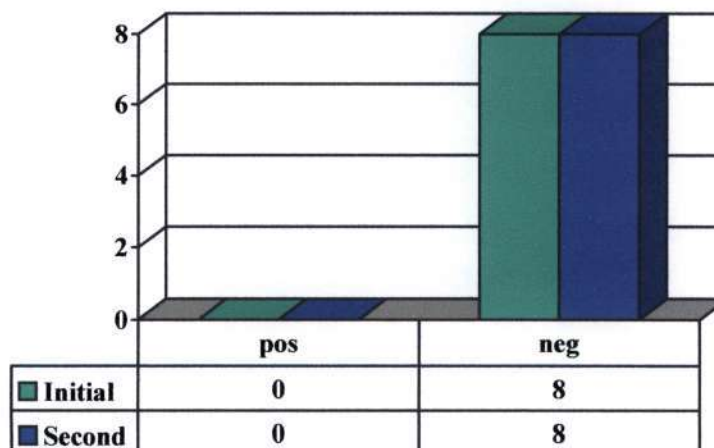
Table 14: Actin immunopositivity in DN-SBCCs, RG-SBCCs and R-SBCCs.

(Plates 33-45)

4.4.3.1 Actin immunopositivity in DN-SBCCs, RG-SBCCs and R-SBCCs.

Five (17%) of 29 DN-SBCCs showed focal epithelial actin immunopositivity. None demonstrated stromal actin immunopositivity. Three RG-SBCCs demonstrated focal epithelial actin immunopositivity in the infiltrative component only, 2 demonstrated exclusive stromal actin immunopositivity and 2 demonstrated epithelial and stromal actin immunopositivity. The stromal and epithelial components in 20/27 (75%) RG-SBCCs were actin immunonegative. Three of 25 (12%) R-SBCCs showed actin immunopositivity; two showed focal epithelial and stromal actin immunopositivity and one showed stromal actin immunopositivity exclusively. Twenty two (88%) of 25 R-SBCCs were actin negative. Comparison of stromal actin staining (low [$\leq 2+$] versus high [$> 2+$] staining) in DN-SBCCs and RG-SBCCs ($p=0.42$), DN-SBCCs and R-SBCCs ($p=0.71$), and, RG-SBCCs and R-SBCCs ($p=0.29$) revealed no statistical significance.

4.4.3.2 Initial versus second RG-SBCCs



(low [$\leq 2+$] versus high [$> 2+$] staining) in DN-SBCCs and RG-SBCCs ($p=0.06$), DN-SBCCs and R-SBCCs ($p=0.99$), and, RG-SBCCs and R-SBCCs ($p=0.07$) revealed no statistical significance. All demonstrated focal intratumoural fibronectin immunopositivity.

4.4.2.2 Initial versus second RG-SBCCs

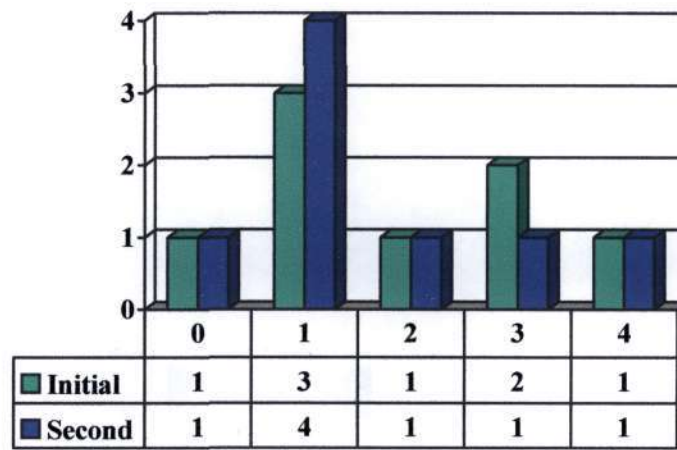


Table 12: Fibronectin immunopositivity of initial and second RG-SBCCs

Five (62%) of 8 initial RG-SBCCs showed low ($\leq 2+$) and 3/8 (38%) showed high ($> 2+$) peritumoural fibronectin immunopositivity. Six (75%) of 8 RG-SBCCs showed low ($\leq 2+$) peritumoural fibronectin immunopositivity and 2/8 (25%) showed high ($> 2+$) peritumoural fibronectin immunopositivity.

Comparison of the fibronectin staining pattern in the initial and RG-SBCCs did not reveal any statistically significant results ($p=0.7$).

4.4.2.3 Initial versus second R-SBCCs

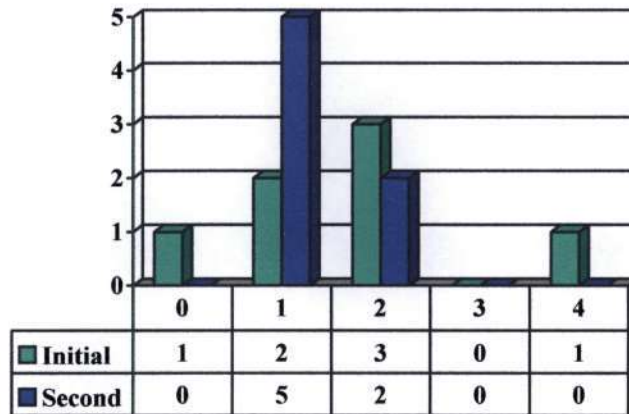
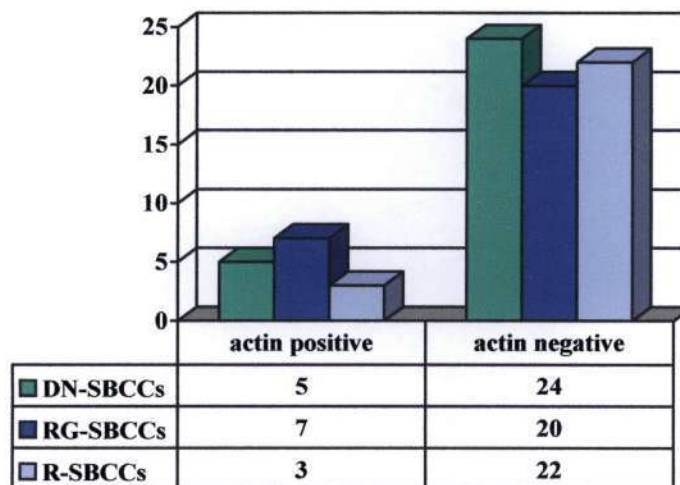


Table 13: Fibronectin immunopositivity of initial and second R-SBCCs

Six (86%) of 7 initial R-SBCCs showed low ($\leq 2+$) and 1/7 (14%) showed high ($> 2+$) peritumoural fibronectin immunopositivity. All of 7 (100%) R-SBCCs showed low ($\leq 2+$) peritumoural fibronectin immunopositivity. Comparison of the fibronectin staining pattern in the initial and R-SBCCs did not reveal any statistically significant results ($p=1.0$).



4.4.3 ACTIN (MSA)

Table 14: Actin immunopositivity in DN-SBCCs, RG-SBCCs and R-SBCCs.

(Plates 33-45)

4.4.3.1 Actin immunopositivity in DN-SBCCs, RG-SBCCs and R-SBCCs.

Five (17%) of 29 DN-SBCCs showed focal epithelial actin immunopositivity.

None demonstrated stromal actin immunopositivity. Three RG-SBCCs demonstrated focal epithelial actin immunopositivity in the infiltrative component only, 2 demonstrated exclusive stromal actin immunopositivity and 2 demonstrated epithelial and stromal actin immunopositivity. The stromal and epithelial components in 20/27 (75%) RG-SBCCs were actin immunonegative. Three of 25 (12%) R-SBCCs showed actin immunopositivity; two showed focal epithelial and stromal actin immunopositivity and one showed stromal actin immunopositivity exclusively. Twenty two (88%) of 25 R-SBCCs were actin negative. Comparison of stromal actin staining (low [$\leq 2+$] versus high [$> 2+$] staining) in DN-SBCCs and RG-SBCCs ($p=0.42$), DN-SBCCs and R-SBCCs ($p=0.71$), and, RG-SBCCs and R-SBCCs ($p=0.29$) revealed no statistical significance.

4.4.3.2 Initial versus second RG-SBCCs

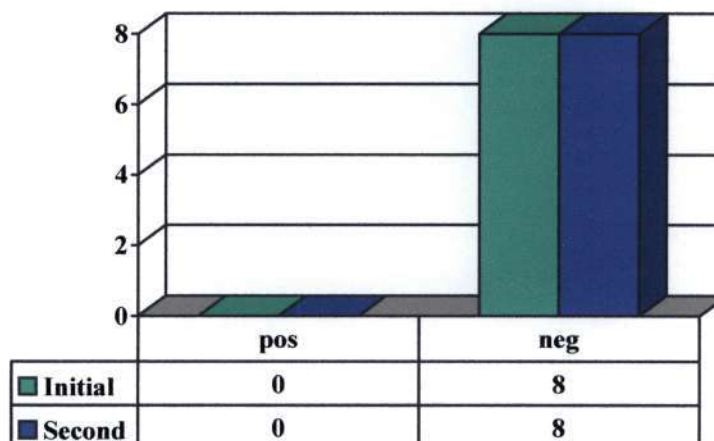


Table 15: Actin immunopositivity of initial and RG-SBCCs

All of 8 initial and RG-SBCCs were actin negative. Comparison of the actin staining pattern in the initial and RG-SBCCs did not reveal any statistically significant results ($p=1.0$).

4.4.3.3 Initial versus second R-SBCCs

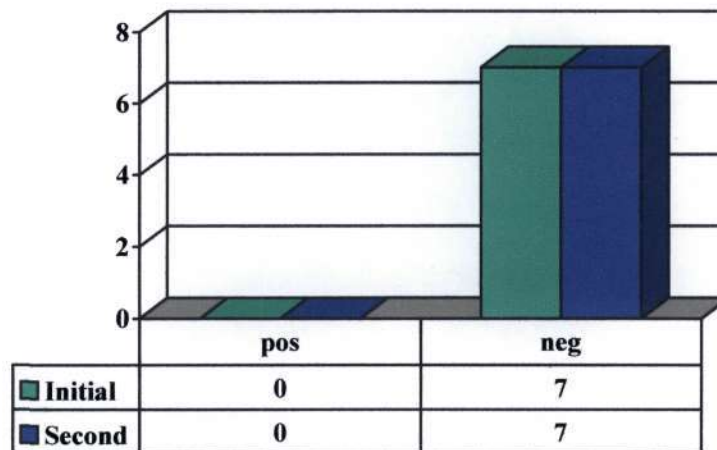


Table 16: Actin immunopositivity of initial and second R-SBCCs

All of 7 initial and R-SBCCs were actin negative. Comparison of the actin staining pattern in the initial and R-SBCCs did not reveal any statistically significant results ($p=1.16$).

4.4.4 DESMIN

4.4.4.1 Desmin immunoreactivity

(Plates 46-48)

No desmin immunoreactivity was demonstrated in the epithelial or stromal components of DN-SBCCs, RG-SBCCs and R-SBCCs. Desmin immunopositivity in vascular smooth muscle served as an inbuilt control.

4.4.5 **VIMENTIN**

4.4.5.1 **Vimentin immunoreactivity**

Plates (49-58)

All the BCC groups showed 3+ or 4+ stromal vimentin immunopositivity.

4.5 IMMUNOHISTOCHEMICAL STUDIES: PROLIFERATION MARKER

4.5.1 MIB1 Immunoreactivity

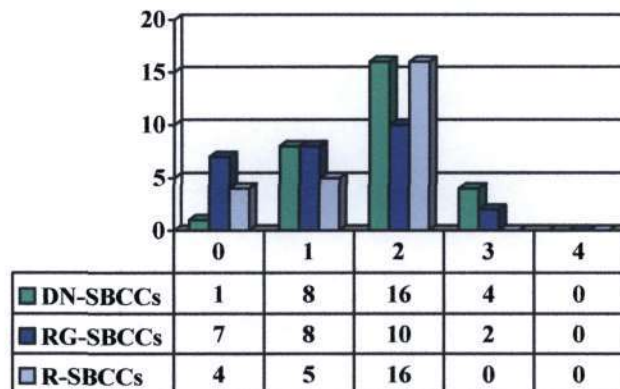


Table 17: MIB1 immunopositivity in DN-SBCCs, RG-SBCCs and R-SBCCs

(Plates 59-78)

4.5.1.1 MIB1 immunopositivity in DN-SBCCs, RG-SBCCs and R-SBCCs

The immunopositive signal varied from pale to bright in different parts of an individual tumour. In some tumours there was peripheral immunopositivity, in some, central, and in others, peripheral and central immunopositivity. Four (14%) of 29 DN-SBCCs showed high (>2+) immunostaining (Plate K1), while 25/29 (86%) showed low ($\leq 2+$) MIB1 immunopositivity (Plate K2). Two (7%) of 27 RG-SBCCs showed high (>2+) MIB1 immunopositivity (Plate K3), while 25/27 (93%) showed low ($\leq 2+$) (Plate K4) MIB1 immunopositivity. All of 25 R-SBCCs showed low ($\leq 2+$) MIB1 immunopositivity. Immunopositive cells were randomly distributed, in the peripheral palisade of cells as well as centrally within the cell nests, in all the DN-SBCCs, RG-SBCCs and R-SBCCs. Focal infiltrative foci demonstrated accentuated MIB1 staining at the tumour-stroma interface. Comparison of MIB1 staining (low [$\leq 2+$] versus high [$> 2+$] staining)

in DN-SBCCs and RG-SBCCs ($p=0.67$), DN-SBCCs and R-SBCCs ($p=0.11$), and, RG-SBCCs and R-SBCCs ($p=0.49$) revealed no statistical significance.

4.5.1.2 Initial versus second RG-SBCCs.

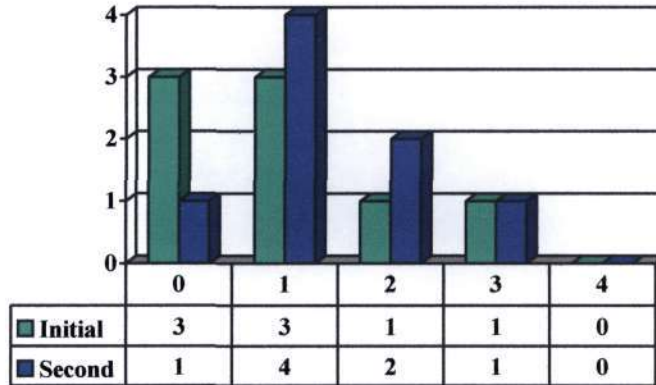


Table 18: MIB1 immunopositivity of initial and second RG-SBCCs

Seven (88%) of 8 initial RG-SBCCs showed low ($\leq 2+$) MIB1 immunopositivity, while 1/8 (12%) showed high ($> 2+$) MIB1 immunopositivity. Seven (88%) of 8 RG-SBCCs demonstrated low ($\leq 2+$) MIB1 immunopositivity, while 1/8 (12%) showed high ($> 2+$) MIB1 immunopositivity. Comparison of the MIB1 staining pattern in the initial and RG-SBCCs did not reveal any statistically significant results ($p=0.55$).

4.5.1.3 Initial versus second R-SBCCs.

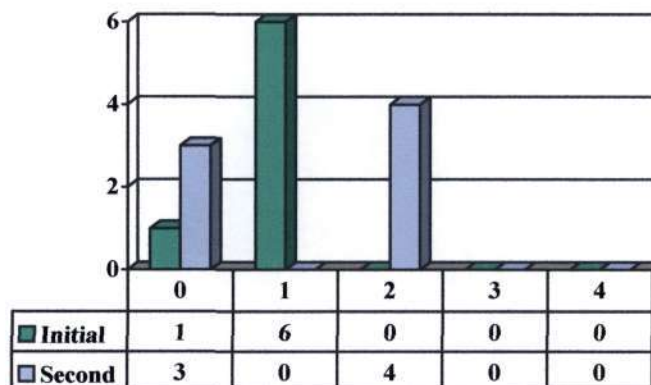


Table 19: MIB1 immunopositivity of initial and second R-SBCCs

All of the initial and R-SBCCs demonstrated low ($\leq 2+$) MIB1 immunopositivity. Comparison of the MIB1 staining pattern in the initial and R-SBCCs did not reveal any statistically significant results ($p=1.0$).

4.5.2 Cell cycle proteins

4.5.2.1 p53 protein

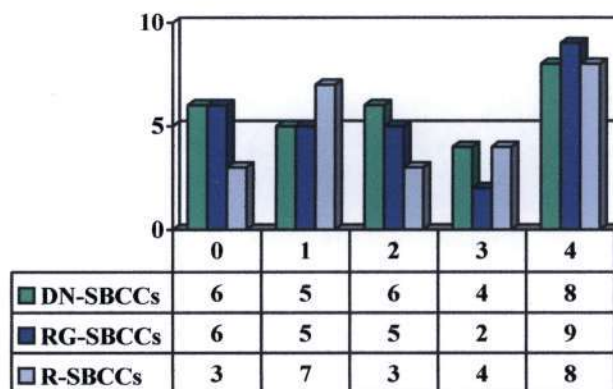


Table 20: p53 immunopositivity in DN-SBCCs, RG-SBCCs and R-SBCCs.

4.5.2.2 p53 immunopositivity in DN-SBCCs, RG-SBCCs and R-SBCCs.

(Plates 79-99)

p53 immunostaining was nuclear and randomly distributed in the tumour cell nests in all cases. The immunopositive signal varied from pale to bright.

Twelve (41%) of 29 DN-SBCCs showed high ($>2+$) p53 immunostaining, while 17/29 (59%) showed low ($\leq 2+$) p53 immunostaining. Sixteen (59%) of 27 RG-SBCCs showed low ($\leq 2+$) p53 immunostaining, while 11/27 (41%) showed high ($>2+$) p53 immunostaining. Thirteen (52%) R-SBCCs showed low ($\leq 2+$) p53 immunostaining and 12/25 (48%) R-SBCCs showed high ($>2+$) p53 immunostaining. Comparison of p53 staining (low [$\leq 2+$] versus high [$>2+$] staining) in DN-SBCCs and RG-SBCCs ($p=0.96$), DN-SBCCs and R-SBCCs

($p=0.62$), and, RG-SBCCs and R-SBCCs ($p=0.59$) revealed no statistical significance.

4.5.2.3 Initial versus second RG-SBCCs

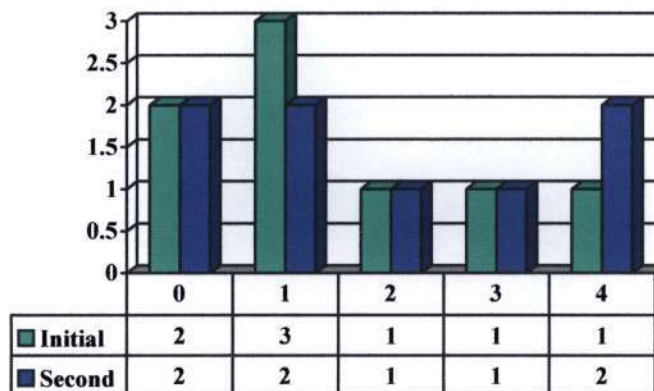


Table 21: p53 immunopositivity of initial and second RG-SBCCs

Two (25%) of 8 initial RG-SBCCs showed high ($>2+$) p53 immunopositivity, while 6/8 (75%) showed low ($\leq 2+$) p53 immunopositivity. Five (63%) of 8 RG-SBCCs showed low ($\leq 2+$) p53 immunostaining, while 3/8 (37%) showed high ($>2+$) p53 immunostaining. Comparison of p53 staining pattern in the initial and RG-SBCCs did not reveal any statistically significant results ($p=0.42$).

4.5.2.4 Initial versus second R-SBCCS

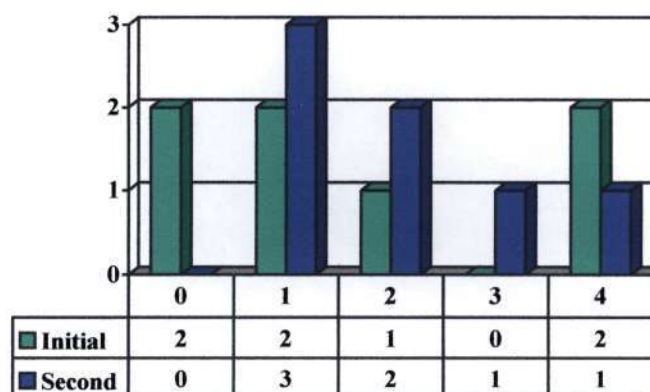
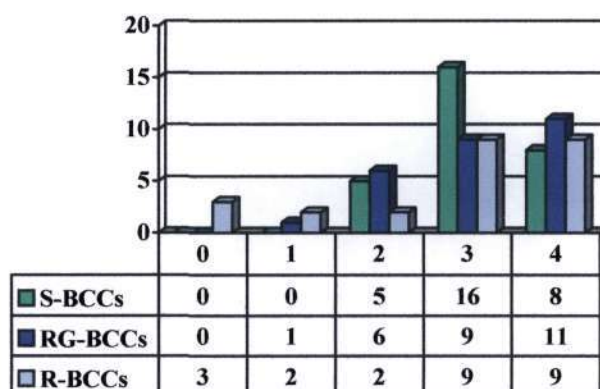


Table 22: p53 immunopositivity of initial and second R-SBCCs

Two (29%) of 7 initial R-SBCCs showed high (>2+) and 5/7 (71%) showed low (\leq 2+) p53 immunopositivity. Five of 7 (72%) R-SBCCs showed low (\leq 2+) and 2/7 (28%) showed high (>2+) p53 immunostaining. Comparison of the p53 staining pattern in the initial and R-SBCCs did not reveal any statistically significant results ($p=0.42$).

4.5.3 bcl-2 protein

**Table 23: bcl-2 immunopositivity in DN-SBCCs, RG-SBCCs and R-SBCCs.**

4.5.3.1 bcl-2 immunopositivity in DN-SBCCs, RG-SBCCs and R-SBCCs.

(Plate 100-117)

Twenty four (83%) of 29 DN-SBCCs showed high (>2+) bcl-2 immunopositivity, while 5/29 (17%) showed low (\leq 2+) bcl-2 immunopositivity. Twenty (74%) of 27 RG-SBCCs showed high (>2+) bcl-2 immunopositivity, while 7/27 (26%) showed low (\leq 2+) bcl-2 immunopositivity. Eighteen (72%) of 25 R-SBCCs showed high (>2+) bcl-2 immunopositivity, while 7/25 (28%) showed low (\leq 2+) bcl-2 immunopositivity. Comparison of bcl-2 staining (low

[$\leq 2+$] versus high [$> 2+$] staining) in DN-SBCCs and RG-SBCCs ($p=0.42$), DN-SBCCs and R-SBCCs ($p=0.34$), and, RG-SBCCs and R-SBCCs ($p=0.86$) revealed no statistical significance.

4.5.3.2 Initial versus second RG-SBCCs

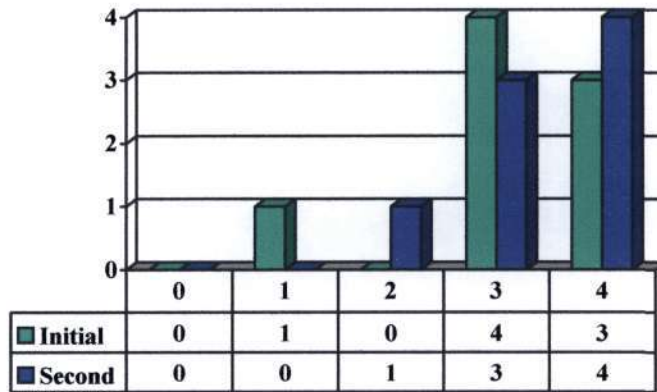


Table 24: bcl-2 immunopositivity of initial and second RG-SBCCs

Seven (88%) of 8 initial RG-SBCCs showed high ($> 2+$) bcl-2 immunopositivity, while one (12%) showed low ($\leq 2+$) bcl-2 immunopositivity. Seven (88%) of 8 RG-SBCCs showed high ($> 2+$) bcl-2 immunopositivity, while 1/8 (12%) showed low ($\leq 2+$) bcl-2 immunopositivity. Comparison of the bcl-2 staining pattern in the initial and RG-SBCCs did not reveal any statistically significant results ($p=0.64$).

4.5.3.3 Initial versus second R-SBCCS

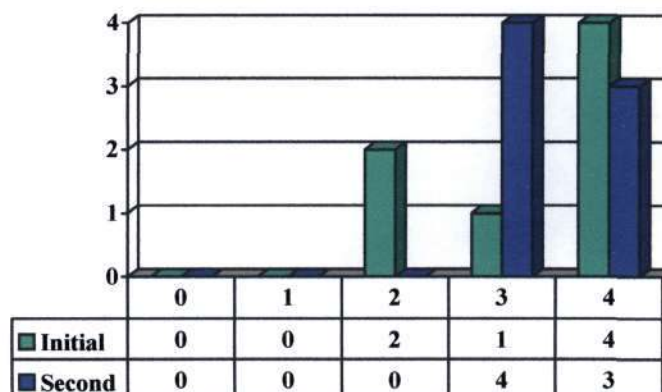


Table 25: bcl-2 immunopositivity of initial and second R-SBCCs

Five (72%) of 7 initial R-SBCCs showed high (>2+) bcl-2 immunopositivity, while 2 (28%) showed low (\leq 2+) bcl-2 immunopositivity. All of 7 (100%) R-SBCCs showed high (>2+) bcl-2 immunopositivity. Comparison of the bcl-2 staining pattern in the initial and R-SBCCs did not reveal any statistically significant results ($p=1.0$).

CHAPTER 5

5.0 DISCUSSION

5.1 Histological features of S-BCCs

In 1828, Jean-Nicolas Marjolin described malignant ulceration in chronic scars (Bostwick *et al*, 1976). Subsequently, a detailed description of this evolution was described, and the entity was labelled "Marjolin's ulcer" (Celikoz *et al*, 1997). The exact incidence of carcinogenesis in cutaneous scars is undetermined. However, while Broders (1921) estimated that 24% of all extremity cancers arose in scars, Schrek (1941) reported an 18% incidence of scar cancers in the scalp, trunk, legs and arms. Squamous cell carcinomas are the most common, and BCCs, the second most common malignancy in wounds (Kowal-Vern and Criswell, 2005). While scar formation is a distinct process in malignant tumours, it is controversial whether the scar predisposes to or follows carcinogenesis (Warren, 1943). In the present study, 66% of BCCs occurring within scars demonstrated a predominantly nodular growth pattern. However 69% of DN-SBCCs contained an infiltrative subtype as a predominant (13%) or minor (56%) component. A micronodular component was present in 58% of DN-SBCCs as a predominant (3%) or minor (55%) subtype. Aggressive infiltrative and micronodular histologic growth patterns were therefore identified in 92% of DN-SBCCs in the present study, confirming the findings of several workers that BCCs within scars may contain histologically aggressive subtypes. The exact pathogenetic mechanism underlying the aggressive infiltrative and micronodular morphology in DN-SBCCs is uncertain. Speculated hypotheses include aggressive and pseudoaggressive phenotypic features in an altered milieu (Swetter *et al*, 1998). The former is speculated to be induced by matrix metalloproteinases

at sites of wound healing, while the latter is hypothesized to be a function of “tracks” along collagen and fibrous tissue, formed within the healing scar (Swetter *et al*, 1998). While the pseudoaggressive phenotypic hypothesis is applicable to DN-SBCCs in the present study since none recurred after complete excision, assessment of the tumour biology, especially cellular proliferation and stromal characteristics, may play a pivotal role in confirming this theory. The role, significance and impact of the scar in R-SBCCs and RG-SBCCs, however, appear multifaceted and more complex. Scar cancers of the lung, that show little elastin, are usually associated with areas of proliferating spindle cells (fibroblasts). Such scarring is usually seen in carcinomas with invasive or destructive growth and occurs randomly throughout the tumour (Kolin and Koutoulakis, 1988). Elastin-rich type of scar was more restricted in distribution. In RG-SBCCs the elastic and scar tissue was seen mainly around tumour lobules, while in DN-SBCCs and R-SBCCs the elastic tissue was seen mainly at the advancing border of lobules. Therefore the elastic tissue stain may be of value in differentiating RG-SBCCs from R-SBCCs and DN-SBCCs. Fibrosis, hyalinization and stromal spindle cells were present in majority of the *denovo* scar, regrowth and recurrent BCCs. All RG-SBCCs and R-SBCCs showed fibrosis, while 86% of the DN-SBCCs showed the same. Jacobs *et al* (1982) stated that hyalinisation of the stroma and fibrosis were more common in aggressive tumours. In this present study hyalinisation and fibrosis was a prominent feature although the growth patterns were predominantly nodular.

Regardless of the mode of therapy, 0.5 to 14% of BCCs recur (Dixon *et al*, 1991). The biological behaviour and management of recurrent BCCs is a contentious topic. While some studies have demonstrated that an adequately treated recurrent BCC is no more likely to recur than an untreated primary tumor (Koplin and Zarem, 1980; Sakura and Calamel, 1979), other studies have found significantly higher recurrence rates for these tumors (Taylor and Barisoni, 1973; Monballiu, 1968; Menn *et al*, 1971). In the present study, while a nodular subtype predominated in 20/32 (63%) and micronodular and infiltrative patterns predominated in 6/32 (19%) recurrent SBCCs, only 3/32 (9%) lacked at least a minor aggressive or infiltrative component. Coupled with these findings, it is controversial whether a recurrent BCC is aggressive from the onset or whether it becomes more aggressive with each recurrence. Assessment of the initial biopsy of seven R-SBCCs confirmed the conversion to more aggressive and less aggressive phenotypes in one case each. In 5/7 cases, a micronodular or infiltrative pattern was present in initial and second tumours. One tumour phenotype changed from predominantly infiltrative in BCC1 to predominantly nodular in BCC2. Although the majority of the initial and second tumours had a predominant nodular component, it is possible that the biologically aggressive nature of the BCC is the function of the minor aggressive histologic components. However, it is difficult to ascribe the aggressive recurrent nature of the BCCs to the minor components when all the initial BCCs were completely excised with adequate margins. Initial and second tumors of R-SBCCs were characterized by minor aggressive histological subtypes in the majority of cases. The profile of the R-SBCCs was similar to that of DN-SBCCS, none of which recurred and similar to the

findings of some previous workers, where inadequately treated BCCs did not recur (Koplin and Zarem, 1980; Sakura and Calamel, 1979). Why some BCCs with aggressive histological subtypes recur and others do not, despite adequate excision, remains unexplained and enigmatic. It is tempting to speculate that the pathogenetic evolution of the aggressive histological subtypes in the different study cohorts differ, resulting in the biological heterogeneity despite the shared morphology. It is therefore critical that the cellular and stromal factors be investigated, to elucidate the biological profile of BCCs within scars, not only for an improved understanding of the pathogenesis of R-SBCCs, but also to ensure optimal, including preventative, management of all scar BCCs. At a practical level, despite histological confirmation of complete excision with adequate margins, based on the behaviour of these tumors, it is still not possible to guarantee complete cure.

There are no statistically valid guidelines for the management of patients with incompletely excised BCCs (Dellon *et al*, 1985). While the destructive potential of regrowth BCCs at sites of incomplete excision is well recorded (Sakura and Calamel, 1979; Jackson and Adams, 1973; Mikhail *et al*, 1977; Dvoretzky *et al*, 1978), re-excision of margin positive BCCs has been advocated in some reviews (Koplin and Zarem, 1980). The opposing view is that since one third of margin positive BCCs regrow and since regression of regrowth BCCs may occur, the BCC site could be observed at regular intervals for regrowth (Dellon *et al*, 1985). In 1985, Dellon *et al* assessed the histopathologic features promoting regrowth of incompletely excised BCCs. Their results demonstrated a 95% probability of regrowth if there was more

than 75% irregularity of the peripheral palisade of tumor cords or nests. The sites of irregularities were hypothesized to be foci of invasion. No degree of host lymphocyte response impacted on the regrowth potential. However, in tumors with 25-75% irregularities of the peripheral palisade and an absent lymphocytic host response, 88% of BCCs regrew. In the presence of a strong host response, 25% of BCCs regrew. In the present study, 89% RG-SBCCs contained infiltrative and/or micronodular subtypes. Comparison of initial and the second regrowth tumours demonstrated infiltrative and/or micronodular subtypes in 7/8 cases. Similar to R-SBCCs, the majority of RG-SBCCs had extended into the deep dermis and subcutis. The histopathological profile was no different from that of DN-SBCCs or R-SBCCs. While the importance of identifying an infiltrative component as a predictor of regrowth of incompletely excised BCCs was detailed most intensively by Thackray in 1951, coinage of the term "micronodular" as a distinct subtype, that was predictive of regrowth, was detailed about 30 years later by Lang and Maize (1986). Similar to the study of these workers and that of several other workers, the majority of RG-SBCCs in the present study had aggressive micronodular or infiltrative subtypes. However, this study also demonstrates that the nodular subtype was the predominant component of all BCCs occurring in scars of varied origin. In addition, morphological confirmation of the presence of infiltrative and micronodular components as markers of aggressive growth appears an oversimplification; Swetter *et al* (1998) demonstrated this in incompletely excised BCCs and the present study highlights DN-SBCCs with morphologically aggressive phenotypes that have not recurred. While the morphological features may serve as a tocsin for

possible regrowth, the gold standard for management guidelines must still be multifaceted, and individualized.

From a histopathological perspective, SBCCs are characterized by a range of histopathological subtypes. While aggressive micronodular and infiltrative subtypes occurred in 79/81 biopsies, the majority contained a nodular subtype. BCCs containing these aggressive morphologic, albeit minor, subtypes within iatrogenic and non-iatrogenic induced scars require further profiling of biological markers to determine their true biological potential. Epithelial proliferation markers and cell cycle status, and stromal basement membrane and cellular characteristics may hold clues to the true aggressive versus non-aggressive nature of SBCCs with aggressive histological subtypes.

In the present study *de novo* scar BCCs (DN-SBCCs) which were induced by burns, trauma (biopsy scar), vaccinations, as well as tattooing were re-appraised. BCCs that were incompletely excised and regrew (RG-SBCCs), as well as BCCs that were completely excised and recurred (R-SBCCs) formed cohorts that occurred in iatrogenic scars. In 1986, Lang and Maize chose to study recurrent basal cell carcinomas to determine whether these BCCs become biologically more active with each recurrence or whether these BCCs have aggressive histopathologic features in the original lesions. They concluded that most of the recurrent tumours are aggressive from the very beginning. It has also been stated that BCCs that regrow at sites of previously incomplete excision have an infiltrative pattern and therefore mimic aggressive BCCs. Scar formation appears to cause changes in connective

tissue stroma that may play a role in subsequent BCC growth (Swetter et al, 1998). The possibility is that BCCs track along collagen and fibrous tissue produced by scar formation and simulates an aggressive growth pattern.

The majority of the DN-SBCCs, RG-SBCCs and R-SBCCs showed a predominantly nodular growth pattern, with 11% of the DN-SBCCs and RG-SBCCs and 10% of R-SBCCs having predominantly infiltrative patterns. Although 63% of RG-SBCCS had a predominantly nodular growth pattern and 60% of R-SBCCs showed same, 33% RG-SBCCs showed minor infiltrative growth patterns and 40% R-SBCCs showed minor infiltrative patterns. The nodular BCCs showed palisade formation and demonstrated smooth rounded contours, therefore demonstrating a non-aggressive architectural appearance. The infiltrative BCCs had spindle shaped basaloid cells with no palisade formation. Jacobs et al (1982) hypothesised aggressive behaviour in BCCs with an infiltrating pattern.

Perineurial invasion was not significantly different in the 3 groups. Three (10%) DN-SBCCs, 7 (26%) RG-SBCCs and 6 (24%) R-SBCCs showed perineurial invasion. Perineurial invasion was seen in BCCs from the head and neck area, except for one BCC that was from the chest wall.

5.2 Immunohistochemical features of S-BCCs

5.2.1 *bcl-2*

bcl-2 was discovered adjacent to the breakpoints of a chromosome rearrangement which is commonly seen in B-cell lymphomas. *bcl-2* was

cloned more than a decade ago, but its unique characteristics have become evident only relatively recently (Tsujiimoto *et al*, 1986). The *bcl-2* proto-oncogene encodes for a 24-kd protein expressed in the nuclear envelope, endoplasmic reticulum and mitochondrial membranes. The exact mechanism of action of *bcl-2* is not known but high expression of *bcl-2* is associated with cell longevity through reduced apoptosis. *bcl-2* promotes cell survival even in the absence of a high rate of cell proliferation, thus providing a growth advantage that may eventually lead to neoplastic transformation (Cerroni and Kerl, 1994). The relationship between the prognosis of solid tumours and *bcl-2* expression has not been fully elucidated. Although *bcl-2* positive tumours may have a better prognosis than *bcl-2* negative tumours, exceptions have been described, wherein some carcinomas demonstrated a better prognosis despite showing low *bcl-2* positivity (Colombel *et al*, 1993; Pezzella *et al*, 1993; Chetty R *et al*, 1997). While Nakagawa *et al* (1994) reported *bcl-2* immunopositivity in 67% of BCCs, other studies have reported staining in 100% of BCCs (Mooney *et al*, 1995; Morales-Ducret *et al*, 1995; Verhaegh *et al*, 1995). High levels of *bcl-2* immunostaining in non-aggressive BCCs has also been described by several other workers (Morales-Ducret *et al*, 1995; Verhaegh *et al*, 1995; Crowson *et al*, 1996; Wikonkal *et al*, 1997). High *bcl-2* expression was a manifestation of all non-aggressive BCC subtypes, while low *bcl-2* expression, was a statistically significant marker of aggressiveness (Ramdial *et al*, 2000).

In 1996, Crowson *et al* investigated *bcl-2* immunoexpression in different histologic subtypes of BCCs. They found that aggressive histologic subtypes,

such as the infiltrative metatypical and morphoeic BCCs, showed less bcl-2 immunostaining, than did the superficial and circumscribed BCCs. Ramaili (MMed Sci Thesis, University of KwaZulu-Natal, 2000) revealed that there was reduced expression of bcl-2 in the infiltrative component of non-aggressive BCCs, implying that cells had less protection from apoptotic death. Therefore despite high MIB-1 labelled cells, cells with damaged DNA will not survive and will be eliminated through apoptosis in the presence of low bcl-2, resulting in the reduction of tumour volume.

Ramdial (MMed Thesis, University of KwaZulu-Natal, 1999) suggested that the proliferation of neoplastic cells in BCCs may be due to protection from apoptosis that is mediated by aberrant persistence of high levels of bcl-2 protein. However, similar to the findings of Cerroni and Kerl (1994) and Ramdial (MMed Thesis, University of Natal, 1999) the occurrence of high bcl-2 immunopositivity in association with a high apoptotic count in the BCCs studied, is unexplained. Factors such as apoptosis and mitotic activity, do not appear influential, since there was no statistically significant difference in the apoptotic and mitotic indices in the aggressive and non-aggressive BCCs.

The majority (>70%) of the DN-SBCCs in the present study that showed high (>2+) bcl-2 immunopositivity had a predominantly nodular growth pattern with minor superficial, micronodular and infiltrative patterns. The minority (<30%) of cases displayed low (\leq 2+) bcl-2 immunostaining. Only 1 initial biopsy of both RG-SBCC and R-SBCC showed low (\leq 2+) bcl-2 immunopositivity and the predominant growth pattern in these biopsies were of an infiltrative nature.

Of the 17% of DN-SBCCs that showed low ($\leq 2+$) bcl-2 immunopositivity, 10% had a predominantly nodular growth pattern with minor superficial, micronodular and infiltrative patterns, while 7% had a predominant infiltrative growth pattern. Twenty six percent of the RG-SBCCs and 28% of the R-SBCCs showed low ($\leq 2+$) bcl-2 immunopositivity. Of the 26% of RG-SBCCs that showed low ($\leq 2+$) bcl-2 immunopositivity, 11% had a predominantly infiltrative or micronodular pattern, while the other 15% had a predominantly nodular growth pattern with minor superficial, micronodular and infiltrative patterns. Twelve percent of R-SBCCs that showed low ($\leq 2+$) bcl-2 staining had a predominantly infiltrative growth pattern with minor superficial, nodular and micronodular growth patterns, while the other 16% had predominantly nodular growth patterns with minor superficial, micronodular and infiltrative patterns. Although bcl-2 staining was noted in all DN-SBCCs in the above study, it was similar to that reported by Ramdial *et al* (2000), wherein all non-aggressive BCCs showed high ($> 2+$) bcl-2 immunostaining, while the majority of the aggressive BCCs showed low ($\leq 2+$) bcl-2 immunostaining. High bcl-2 immunostaining in non-aggressive BCCs has also been described by several other workers (Morales-Ducret *et al*, 1995; Verhaegh *et al*, 1995; Crowson *et al*, 1996; Wikonkal *et al*, 1997). Although DN-SBCCs were characterized by infiltrative histological features, the high bcl-2 immunopositivity of DN-SBCCs in the present study is indicative of a non-aggressive phenotype. These BCCs mimic aggressive BCCs by showing infiltrative growth patterns, possibly due to the BCCs tracking along collagen and fibrous tissue produced by scar formation, simulating an aggressive growth pattern.

5.2.2 MIB1

Cell proliferation characteristics are a fundamental biological determinant of the behaviour of many tumour types (Tubiana and Courdi, 1987; Kanataki *et al*, 1997). Tumour growth is determined by a combination of cell cycle time for each tumour cell (the growth fraction) and balance between cell production and cell loss. A range of MIB1 expression has been described in the different histologic subtypes of BCC (Baum *et al* 1993; Healy *et al* 1995; Horlock *et al* 1998). Healy *et al* (1995), found a higher growth fraction in BCC which recurred after complete excision. In the present study on scar BCCs, the initial and recurrent biopsies were similar in that both showed low MIB1 immunopositivity. The initial and regrowth biopsies in the present study which had minor micronodular growth patterns also showed low MIB1 immunopositivity. This is similar to the study by Horlock *et al* (1998), wherein micronodular BCCs, also prone to incomplete excision and subsequent recurrence, were characterized by a relatively low growth fraction. Ramiali (MMed Sci Thesis, University of KwaZulu-Natal 2000) demonstrated high MIB-1 staining in micronodular BCCs. One of the reasons postulated for the high MIB-1 staining in micronodular BCCs was that micronodular aggregates are usually present at the tumour/stromal interface, where adequate oxygenation is present, rather than centrally, where the vascular supply is reduced. Horlock *et al* (1998) found a lower MIB1 growth fraction in micronodular and nodular BCCs in comparison to tumours with a morphoeic or infiltrative pattern of growth. Horlock *et al* (1998), in their study of horrifying and non-horrifying BCCs found no difference in growth fraction in the tumours of similar histological growth patterns. They suggested that infiltrative or

nodular tumours of horrifying and non-horrifying tumours are biologically similar. Similar to the findings of Horlock *et al* (1998), the study on aggressive and non-aggressive BCCs subtypes (Ramdial MMed Thesis, University of KwaZulu-Natal, 1999) demonstrated no significant differences. The majority (>80%) of the scar BCCs in the present study showed low ($\leq 2+$) MIB1 immunopositivity in a variety of BCC subtypes. There was no definite MIB1 qualitative or quantitative staining patterns noted in different scar BCCs. MIB1 staining was random central and peripheral in all tumours; there was no defined spatial distribution. Similar staining in all subtypes implies that cell proliferation alone does not account for growth patterns of DN-SBCCs. Baum *et al* (1993), have claimed that proliferating cells are scattered randomly, whereas other investigators have found proliferative activity predominantly in the peripheral cells of BCC strands, especially in the palisaded array (Baum *et al*, 1993). In the study by Baum *et al* (1993) a peripheral and a random distribution of MIB1 positive cells in BCCs of the nodular and superficial type was described.

5.2.3 p53

Damage to the genome, occurring during the G₁ phase of the cell cycle, causes rapid cell arrest which is driven by wild type p53 protein, the levels of which rise rapidly within 30 minutes of DNA damage (Kastan *et al*, 1991). The high levels of p53 protein, induced in response to DNA damage, can be detected immunohistochemically. The p53 block occurs in the G₁ and G₂/M phases of the cell cycle; this delays progression into the cell cycle, until damage is repaired or cells are mobilized into the apoptotic pathway. In

addition, a p21-induced G₁ block can either hold cells in G₁, thereby delaying the cell cycle or reverts cells back into the G₀ quiescent stage. The end result of both pathways is the reduction of cell numbers and tumour size. The study by De Rosa *et al* (1993) of p53 protein in aggressive and non-aggressive BCCs revealed that aggressive BCCs have a higher p53 immunolabeling than non-aggressive BCCs, findings similar to that of Ramaili (MMed Sci Thesis, University of KwaZulu-Natal, 2000). Shea *et al* (1992) documented p53 staining in 83% of the BCCs of the head and neck region. Therefore, high levels of p53 might be an indicator of increased aggressiveness of tumour cells and an indicator of dedifferentiation. At the point of infiltration, p53 staining was lower than the dominant nodular component in 23 and the same in 27 infiltrative non-aggressive BCCs Ramaili (MMed Sci Thesis, University of KwaZulu-Natal, 2000). In the infiltrative front, at the tumour/stromal interface, there was high staining. In the deeper free-lying, aggregates in the dermis, p53 staining was decreased. Although a range of p53 immunostaining was noted in all subtypes of non-aggressive BCCs (Ramdial MMed Thesis, University of KwaZulu-Natal, 1999), 80% revealed >2+ p53 immunopositivity. All superficial BCCs showed p53 immunopositivity. The p53 immunoprofile of micronodular BCCs was similar to that of the other non-aggressive subtypes indicating that p53 mutation is not an important driving force behind the deeply invasive characteristics of the BCC subtype. Low (<2+) p53 immunostaining emerged as a statistically significant predictor of aggressiveness (Ramdial MMed Thesis, University of KwaZulu-Natal, 1999). In this study, 23/25 aggressive BCCs revealed low ($\leq 2+$) p53 immunopositivity, unlike the non-aggressive BCCs, in which high (>2+)

immunostaining was prominent. Although the percentage of p53 immunostaining in the aggressive and non-aggressive BCCs ranged from $\leq 5\%$ to 100%, the reason why the aggressive cohort of BCCs had low ($\leq 2+$) immunoexpression is uncertain (Ramdial, MMed Thesis, University of KwaZulu-Natal, 1999). Other factors that require assessment, in view of the present study, include suboptimal p53 staining methodology, or that the mutational event in the aggressive cohort was not identified by the antibody used. p53 staining difficulties in the tissue sections had necessitated heat-assisted pressure cooker antigen retrieval; hence other factors might have contributed to the results. In the study of Barrett *et al* (1997) an equal number of superficial BCCs displayed low (5-20%) or no p53 immunostaining. An intriguing aspect of the study by Barrett *et al* (1997) is that the histologically aggressive subtypes of BCC (sclerosing, infiltrative and metatypical) revealed high ($>2+$) p53 immunopositivity. The study of De Rosa *et al*, (1993) of p53 protein in aggressive and non-aggressive BCCs revealed that aggressive BCCs have a higher p53 immunolabeling than non-aggressive BCCs.

In the present study, there was no significant difference in p53 immunopositivity in scar BCCs. This is similar to the findings of Healy *et al* (1995) who reported an absence of significant differences in p53 immunopositivity in non-recurrent BCCs, in primary BCCs which later recurred and in recurrent BCCs. p53 immunopositive nuclei were randomly distributed in the different growth patterns. The immunopositive signal varied from pale to bright. The brightest signals were consistently found in the superficial and nodular growth patterns. Low and pale staining was mainly seen in the infiltrative components. In the present study, 17/29 (59%), 16/27 (59%) and

13/25 (52%) of DN-SBCCs, RG-SBCCs and R-SBCCs, respectively, demonstrated low (<2+) p53 staining. According to that described by several workers (Shea *et al*, 1992; De Rosa *et al*, 1993; Barrett *et al*, 1997) these would signify BCCs with a non-aggressive immunophenotype.

Seventeen of 29 DN-SBCCs had low p53 immunostaining. Of 12 with a high p53 score, 11 had a predominant nodular histological subtype and one a predominant micronodular. All but one had a minor infiltrative subtype. If the high p53 score is hypothesized to be a marker of aggressiveness (Shea *et al*, 1992; De Rosa *et al*, 1993; Barrett *et al*, 1997), the high score is incongruent with the non-aggressive clinical outcome and the predominant nodular subtype which is associated with a good outcome. Three of these 11 DN-SBCCs with a high overall p53 score demonstrated a low bcl-2 score in the infiltrative component. Eight of these 11 DN-SBCCs demonstrated high bcl-2 scores in the predominant nodular and minor infiltrative components, indicating a non-aggressive phenotype, from a bcl-2 perspective.

Furthermore, only 4 of the 11 DN-SBCCs with a minor infiltrative component, had high p53 staining in the nodular and infiltrative components. Another interesting comparison is that, of five with intra-epithelial actin immunopositivity, three had high p53 immunostaining, this was accentuated in some of the infiltrative foci. These three BCCs also had low bcl-2 scores.

This combination of staining may also be a signal of heightened potential and tumour "preparation" for stromal invasion. The intra-epithelial actin immunopositivity in the infiltrative foci may be indicative of an altered immunophenotype in preparation for the tumour invasiveness, prior to BM disruption, as the laminin was still intact.

Eleven of 27 RG SBCCs had a high p53 score, of which 9 had a dominant nodular phenotype and two had a predominant infiltrative phenotype. All of nine predominant nodular growth patterns had minor infiltrative patterns, while two that had a predominant infiltrative pattern had minor nodular histological patterns. One with a predominant nodular phenotype had a low bcl-2 score, while one with a predominant infiltrative component was accompanied by a low bcl-2 score and intra-epithelial and stromal actin immunopositivity. While this combination of p53, actin and bcl-2 staining is indicative of an aggressive BCC, three RG-SBCCs with a low p53 score of "0" had a high bcl-2 score and focal epithelial actin immunopositivity. The difference in this setting probably lies in the specific staining within the infiltrative foci. Of the eleven RG-SBCCs with high p53 staining, 4 had high p53 staining in the infiltrative component. The bcl-2 score within the infiltrative component was low in one case and high in the rest. The case with the high p53 and low bcl-2 scores, also had intraepithelial and stromal actin immunopositivity indicating that one of the 11 RG-SBCCs with high p53 staining is a truly aggressive immunophenotype, while the others were developing/evolving an aggressive immunophenotype.

Thirteen of 25 R-SBCCs had low p53 immunostaining. Twelve of 25 R-SBCCs had a high p53 score, of which seven had a predominant nodular histological subtype, with minor infiltrative and micronodular patterns in five and a superficial pattern in two. Four had a predominant infiltrative with minor nodular and micronodular patterns and one a predominant micronodular with minor nodular histological subtypes. Two R-SBCCs with a high p53 score had a low bcl-2 score. While both had predominant infiltrative growth patterns, one of these R-SBCCs had 2+ laminin discontinuity. One R-SBCC

with intra-epithelial actin immunopositivity, had increased p53 immunopositivity staining with a predominant infiltrative growth pattern, but a high bcl-2 score.

The p53 findings in the present study cannot be used as a predictor of aggressive potential. However, the literature in general, has a range of p53 results, including reports in which no p53 staining was present. As differing methodologies were used in the local departmental studies, and may be important in the outcome of the local, so too, the use of different antibodies, frozen versus formalin-fixed tissue and staining techniques may be responsible for the disparate results.

- Although a range of p53 immunostaining was noted in all subtypes of non-aggressive BCCs, 80% revealed >2+ p53 immunopositivity (Ramdial, MMed Thesis, University of KwaZulu-Natal, 1999).
- In the study of Barret *et al* (1997), an equal number of superficial BCCs displayed low (5-20%) or no p53 immunostaining.
- It could be speculated that from Ramaili (MMed Sci Thesis, University of KwaZulu-Natal, 2000), high expression of p53 correlates with aggressiveness of micronodular BCCs. Results of this study showed that micronodular BCCs not only demonstrated high p53 but also low bcl-2.
- In one study of Moles *et al* (1993), p53 immunopositive was found in 48% of BCCs, suggesting that p53 mutations had occurred.

What does emerge, however, is that the infiltrative foci of scar BCCs are characterised by immuno-phenotypic heterogeneity. The majority have a non-aggressive immunophenotype. The use of laminin, muscle specific actin and bcl-2 are important prognostic markers. Laminin discontinuity, intra-epithelial and/or stromal actin, and low bcl-2 immunopositivity potentially indicate an aggressive immunophenotype. RG-SBCCs with any of the above marker profiles should be subjected to adequate complete re-excision, rather than adoption of a wait-and-see policy. p53 does not appear to play a reproducible prognostic role, but high staining may be an alarm bell.

5.2.4 Fibronectin

Fibronectin plays a major role in cell adhesion and migration, maintenance of normal cell morphology, cytoskeletal organisation, haemostasis, cell differentiation and oncogenic transformation. In the present study, the majority of the scar BCCs showed low ($\leq 2+$) fibronectin immunopositivity. DN-SBCCs and R-SBCCs showed low ($\leq 2+$) fibronectin immunopositivity in 76%, while 52% of RG-SBCCs showed low ($\leq 2+$). In a previous study (Ramdial, MMed Thesis, University of KwaZulu-Natal, 1999) on aggressive and non-aggressive BCCs, 60% of non-aggressive BCCs did not reveal peritumoural fibronectin immunopositivity and 40% revealed 1+ peritumoural fibronectin immunopositivity. De Rosa *et al* (1994), in their study of aggressive and non-aggressive BCCs, found that 18/19 aggressive BCCs revealed diffuse granular or fibrillar peritumoural fibronectin positivity, which was similar to Ramdial (MMed Thesis, University of KwaZulu-Natal, 1999), wherein peritumoural fibronectin immunopositivity was present in 88% of the

aggressive BCCs, but was not a prominent feature of any non-aggressive BCCs. DeRosa *et al* (1994) did not suggest a reason for the negative staining in one aggressive BCC. In their study, De Rosa *et al* (1994) demonstrated absence of peritumoural fibronectin immunopositivity in 16/19 non-aggressive BCCs. The present study showed low ($\leq 2+$) fibronectin immunopositivity in 68% of the scar BCCs, indicating alignment to a non-aggressive BCC growth profile. Twenty four percent of DN-SBCCs and R-SBCCs that showed high ($>2+$) fibronectin immunopositivity had a predominantly nodular growth pattern. Nelson *et al* (1983) also reported intense peritumoural fibronectin immunopositivity in 7/7 non-aggressive BCCs. The presence of fibronectin in all cases is hypothesised to be a function of the peritumoural scarring process. Being a prominent matrix component, fibronectin plays important roles in various stages of wound healing (Brown *et al*, 1993) and during tissue re-modelling in atherosclerosis, pulmonary fibrosis and glomerulosclerosis (Kosmehl *et al*, 1996). Furthermore, it is a marker of angiogenesis in neoplastic and non-neoplastic conditions (Kosmehl *et al*, 1996). The exact significance of 4+ fibronectin staining in two DN-SBCCs is intriguing. Comparison of other markers reveals low (2+) bcl-2 staining, intact laminin, 2+ MIB1 scoring and 3+ or 4+ p53 staining. While the MIB1 had a 2+ score in the tumours, there was higher staining in the infiltrative foci. It is therefore interpreted that the high fibronectin is a function of scarring plus a more aggressive biology, especially in view of the low bcl-2 staining and high p53 staining. Four RG-SBCCs and 3 R-SBCCs had 4+ fibronectin staining. One RG-SBCC had a predominantly infiltrative morphology, a bcl-2 score of 1+, p53 score of 4+, MIB1 of 1+ and intact BM laminin. Two of the RG-SBCCs

had a bcl-2 score of 2+, one of which had a predominant infiltrative and the other, predominant micronodular pattern. The MIB1 scores were 2+ and p53 was 4+ and 1+. One demonstrated 2+ laminin discontinuity, while the other demonstrated intact laminin staining. The fourth RG-SBCC with high fibronectin staining had a predominant nodular and minor micronodular, superficial, infiltrative patterns and demonstrated 2+ laminin loss in the infiltrative component, p53 of 3+, and a bcl-2 score of 2+. All of the RG-SBCCs with 4+ fibronectin staining had an aggressive immunoprofile in the proliferation and other stromal markers.

Three R-SBCCs had a fibronectin score of 4+. One had a predominant infiltrative pattern, a p53 of 3+, bcl-2 score of 1+ and 2+ laminin loss. Two had a predominant nodular morphology, minor micronodular or a combination of superficial, micronodular and infiltrative patterns. While both R-SBCCs had a bcl-2 score of 1+, the laminin profile varied but p53 scores were high. The R-SBCCs with a nodular/micronodular subtype demonstrated intact laminin and a p53 score of 3+, while that with a nodular/micronodular/superficial/infiltrative combination demonstrated 2+ laminin discontinuity and p53 score of 3+.

Hence, whilst the presence of fibronectin in the stroma of the scar BCCs is hypothesised to be a function of scarring process, 4+ staining is accompanied by low bcl-2 and high p53 staining is hypothesised to portend an aggressive immunophenotype. That the BM laminin was intact in 4 cases suggests that laminin disruption is a later event in the evolution of an aggressive infiltrative component in BCCs.

5.2.5 Laminin

Basement membranes (BMs) are ubiquitous structural components of the extracellular matrix of normal and neoplastic tissues (Damjanov, 1990). Ultrastructurally basement membranes are characterised by the presence of basal laminae, which are further subdivided into a lamina densa and lamina lucida (Vracko, 1974). The former consists of tightly matted randomly orientated fibrils 3-4nm in diameter, embedded in a dense matrix. The principal constituents of basal lamina are type IV collagen, the high molecular weight glycoprotein, laminin, and proteoglycans (Timpl *et al*, 1979; Timpl *et al*, 1981; Poole, 1986). Although originally considered to be an inert framework for cells or for delineating parenchymal and stromal tissue compartments (Stenback *et al*, 1985; Vracko, 1974), the basement membrane is now known to be involved in the maintenance of organ integrity and to participate in their specialised functions (Gorstein, 1988). BMs form a barrier to the passage of macromolecules from the blood, serve as substratum for attachment of endothelial and epithelial cells and contribute to the differentiation of tissue (Stenback *et al*, 1985). Similar to resident cells of an organ, the neoplastic cells, stromal cells or both may produce various BM components and even assemble complete BM (Liotta, 1986; Martinez-Hernandez and Amenta, 1983). Although benign tumors form BMs that are structurally and functionally indistinguishable from those in normal adult tissues, in malignant tumours, the BM is defective. In such tumors, it is debatable whether the tumor produces defective or destroys basement membrane. Invasion and metastasis are hallmarks of malignant neoplasia and frequently determine the course of the disease (Havenith *et al*, 1987). In carcinomas, a dynamic interaction occurs

at the interface between tumor cells and the surrounding mesenchymal stroma.

In the present study, the majority of scar BCCs (91%) showed intact basement membrane laminin staining around the tumour aggregates and the rest displayed 1+, 2+ or 3+ basement membrane discontinuity. The initial biopsies and regrowth and recurrent SBCCs demonstrated intact basement membrane laminin staining. The scar BCCs that showed 2+ (6%) laminin discontinuity had a predominant nodular growth pattern with minor superficial and micronodular patterns, as well as the presence of a lymphocytic infiltrate at the tumour stroma interface. Micronodular BCCs has been described as a histologically aggressive subtype (Lang and Maize, 1986). However, in the present study, although the invasive nature of the micronodular subtype was confirmed, the laminin staining was continuous. One RG-SBCC that showed 3+ laminin discontinuity had a predominant nodular growth pattern, while the other two RG-SBCCs that showed 2+ basement membrane laminin discontinuity had a predominant nodular growth pattern in one and an infiltrative growth pattern in the other with minor superficial, nodular and micronodular patterns. These did not contain a lymphocytic host response at the tumour stroma. Instead they were intimately apposed to scar tissue and a myofibroblastic response. Although DN-SBCCs have shown infiltrative growth patterns with the presence of spindle shaped cells, resembling those of the aggressive BCC subtype, the basement membrane laminin staining pattern is continuous. Nine percent of scar BCCs that demonstrated discontinuous laminin staining contained a host lymphocytic infiltrate at the tumour-stroma interface. These findings were similar to the findings in the study of non-

aggressive BCCs (Ramdial, MMed Thesis, University of KwaZulu-Natal, 1999) wherein 56% of tumours that demonstrated laminin discontinuity contained a host tumour-stroma lymphocytic infiltrate. Some workers have reported continuous basement membrane around BCC islands in non-aggressive BCCs (Nelson *et al*, 1983; Barsky *et al*, 1987; De Rosa *et al*, 1994), while others have found modifications in the distribution of basement membrane in the different histological subtypes of BCCs. Kallionen *et al* (1984) reported continuous basement membrane around tumour aggregates of non-sclerosing BCCs. Van Cauwenberge (1983) made a similar observation, but their studies were unrelated to clinical behaviour, recurrence rates or metastases.

Why some non-aggressive BCCs manifested laminin discontinuity while others did not, is not known. In the study by De Rosa *et al* (1994), the cause of the laminin discontinuity in 2/19 non-aggressive BCCs was not addressed. Barsky *et al* (1983), in their study of invasive tumours, stated that loss of basement membrane components is associated with invasion. They also stated that while many epithelial lesions may mimic the appearance of invasion, histologically, these lesions demonstrate intact basement membrane components. D'Ardenne (1989) stated that breaks in basement membrane at sites of inflammation are small and sharply defined in contrast to the irregular discontinuities found in *in-situ* carcinoma. Others have suggested that the gaps in basement seen in dysplasia may be due to enzymes produced by inflammatory cells and this might promote invasion (D'Ardenne, 1989). High levels of type1 collagenase have been described in BCCs. Such high levels of type1 collagenases, derived from stromal fibroblasts that surround epithelial

islands of basal cells, are purported to be a characteristic of stromal modelling by activated fibroblasts. Type1 collagenases are known to degrade basement membranes. It is therefore tempting to speculate that stromal factors are responsible for basement membrane degradation, and BCCs with an infiltrative phenotype and basement membrane laminin loss in the absence of a host lymphocytic response at the tumour-stroma interface, represent biologically aggressive infiltrative foci in scar BCCs.

5.2.6 Actin, Desmin and Vimentin

Actin is a highly conserved ubiquitous, microfilamentous, cytoskeletal protein. Although it normally exists in large amounts in muscle, it is known to exist in non-muscle cells. Different cell types contain immunochemically distinct actins that vary in sequence and iso-electric point. While skeletal, cardiac and vascular smooth muscle each contain unique α -actins, non-muscle cells contain actins of β and γ motility. The presence of distinct actin isotypes in muscle cells suggest that some actin variants are specifically tailored for cell contraction. An increase in contractile proteins and a relationship with cell movement in epithelial cells during reactive and neoplastic processes has been described (Low *et al*, 1981; Pollard and Weihing, 1974). The presence of these contractile proteins in human cancer cells has been studied by means of immunofluorescence staining using anti-actin antibodies, and, by ultrastructural demonstration of cytoplasmic microfilaments. The presence of these microfilaments was related to enhanced motility of invasive carcinoma cells. Strong peripheral actin immunofluorescence has been described in the invasive component of malignant cells that ultrastructurally reveal

pseudopodlike protrusions and contain a well developed microfilamentous network. In addition, actin immunopositivity has been described in myofibroblasts, which are an integral component of the desmoplastic stroma of malignant tumours. Since 1980, several monoclonal and polyclonal antibodies to actin isotypes have been described. Tsukada *et al* (1987) generated an actin antibody, HHF35, which detects an epitope common to the α and γ actin of isotypes of all muscle cells. In addition, HHF35 antibody also localizes to myoepithelium, myofibroblasts, reactive mesothelial and submesothelial cells and in cells of adenomas and carcinomas of salivary gland origin. In malignant tumours, actin expression has paralleled that described in normal tissue. In mesenchymal tumours, actin immunopositivity is reflective of tumour lineage. In carcinomas, however, actin immunopositivity has been described, not only in the desmoplastic stromal response but also in the malignant epithelial component (Bertheim *et al*, 2004). In carcinomas, ultrastructural demonstration of pseudopodlike cytoplasmic protrusions with a well developed microfilamentous network, in the periphery and advancing edge of the tumour have been described. The periphery of these cells have also displayed strong positivity after immunofluorescence staining with actin antibodies. In cutaneous BCCs, immunofluorescent intracytoplasmic actin immunopositivity has been described, in addition to ultrastructural localization of actin as a microfilamentous network. The role of actin immunohistochemical labelling as a marker of aggressiveness in basal cell carcinomas has been described in terms of peritumoural stromal myofibroblastic actin immunopositivity.

However, the value of intracytoplasmic actin immunopositivity, as a predictor of aggressiveness has not been described before.

While it is accepted that tumours are dependent on their surrounding stroma, the exact relationship has been insufficiently characterized (Bertheim *et al*, 2004). The stroma, or matrix, is made up of a ground substance that is composed of glycosaminoglycans containing a complex meshwork of collagen, fibronectin and elastin (Moy *et al*, 2000). It has been hypothesised that tumour pathogenesis, progression and metastases may be caused by tumour cell interaction with its extracellular stroma (Parrott *et al*, 2001).

Myofibroblasts, which have been investigated extensively in the stroma of carcinomas of the breast, are also a common finding in the stroma of primary invasive carcinomas in various extra-mammary sites and in metastatic carcinomas. Myofibroblasts are not a normal constituent of the skin, but their presence in skin tumours are indicative of a stromal response to neoplasia. In BCCs, an intimate association between tumour parenchyma and the stroma exists. Experimental and autotransplant studies of BCCs have shown a striking growth-related interdependence between the neoplastic cells and stroma (Kirkham, 1997). Seemayer *et al* (1980) have hypothesised that the myofibroblastic reaction is an expression of a non-immune stromal response to neoplasia and serves to retard or contain the growth of the malignant cellular population. The density and contractile state of such tissue is hypothesised to limit the mobility and accessibility of tumour cells to vascular channels or perineural spaces. Schurch *et al* (1998) have, however, suggested that the myofibroblasts within the desmoplastic response secrete

enzymes that degrade the extracellular matrix, facilitating tumour invasion. They concluded that carcinomas were not uniform with regard to stromal reactions and that even within the same neoplasm, mechanisms co-exist that either facilitate or prevent/delay tumour invasion. However, no description exists on the actin immunohistochemistry in paraffin wax BCC sections. The peritumoural stromal cells in DN-SBCCs in the present study were actin and desmin negative, but vimentin immunopositive. The myofibroblasts were therefore of "V" immunophenotype. The findings in the present study were similar to that of De Rosa *et al* (1994). In their study of aggressive and non-aggressive BCCs, all non-aggressive BCCs were actin negative and the majority were desmin negative, but all aggressive BCCs showed actin, focal desmin and vimentin immunopositivity. In a similar study to De Rosa *et al* (1994), of aggressive and non-aggressive BCCs, all non-aggressive BCCs showed no actin or desmin immunopositivity, while all aggressive BCCs were positive for stromal actin (Ramdial, MMed Thesis, University of KwaZulu-Natal, 1999). Ramdial (MMed Thesis, University of KwaZulu-Natal, 1999) concluded that actin immunopositivity in BCCs represents a feature of aggressiveness and although the desmoplastic response was also a feature of aggressiveness, its exact significance as a promoter of, or barrier to, invasion remains unknown. The presence of epithelial actin immunopositivity, however, similar to previous studies, may be indicative of the initiation step of aggressive behaviour. While the majority of RG-SBCCs and R-SBCCs did not demonstrate epithelial or stromal actin immunopositivity, 25% of BCCs in both groups demonstrated actin positivity. This was noted either in stromal myofibroblasts and/or the focally infiltrative spindle cell epithelial components.

Hence, it is hypothesised from this study that the actin immunostaining in DN-SBCCs highlights the tumours with a biologically aggressive profile or with the potential for aggressive growth. Not all DN-SBCCs with a focally infiltrative pattern demonstrated actin positivity. In five DN-SBCCs with intact laminin and exclusive epithelial actin immunopositivity, it is hypothesised that this is the initial event where the epithelial cells are being remodelled for infiltration. In RG-SBCCs and R-SBCCs, all those with exclusive epithelial or mixed epithelial and stromal actin immunopositivity also demonstrated laminin immunopositivity. In these cases, the actin and laminin stains are critical markers predictive of aggressive growth in DN-SBCCs with an infiltrative pattern. It also serves to highlight the fact that not all RG-BCCs and R-BCCs with an infiltrative morphology have an aggressive biological immunophenotype. The infiltrative morphology in these tumours is therefore hypothesised to be a product of cellular scaffolding along the connective tissue planes of the scar. The repeated growth of these purported non-aggressive BCCs is hypothesised to be a function of tumour occurrence at a site that is prone to BCC occurrence.

CHAPTER 6

6.0 CONCLUSION

Historically, scar cancers have been recognised since 100AD. Originally described in burn scars, the term cicatricial or scar cancer of the skin was coined in the 1980s to describe a spectrum of cancers occurring in scars and chronic ulcerative conditions. The majority of scar cancers are squamous cell carcinomas. Basal cell carcinomas are poorly recognised as a form of scar cancer. Furthermore, although BCCs are characterised by a spectrum of histological subtypes; these are poorly characterised in scar cancers. In the late nineties, Swetter *et al* reported the presence of an infiltrative, pseudo-aggressive phenotype in incompletely excised nodular BCCs (Swetter *et al*, 1998).

As all the present tumours occurred within scar tissue, tumour growth occurred in an altered milieu. As there are recognised aggressive histological patterns and stromal features, it is critical that truly aggressive BCC attributes be delineated from pseudo-aggressive features. While all but 2 cases contained infiltrative and micronodular patterns, the exact significance of the histomorphology is multifaceted:

1. That DN-SBCCs with the infiltrative and micronodular patterns have not recurred implies that the histomorphology is a pseudo-aggressive pattern.
2. A similar view could pertain to RG-SBCCs. However, the fact that the scar did not cicatrise the incompletely excised BCC implies that the histomorphology of RG-BCC may be a potentially more aggressive phenotype.

3. The recurrence of a completely excised basal cell carcinoma may be viewed as a feature of an aggressive tumour, especially when the recurrent BCC contains micronodular and infiltrative components. However, as most R-SBCCs occurred at head and neck sites that are exposed to ultraviolet light, it is also possible that these are simply new BCCs occurring within scars in head and neck sites prone to BCCs. Furthermore, these R-SBCCs were not destructive tumours.

At a light microscopic H&E morphological level, none of the above possibilities can be proven or disproven and remain mainly rhetorical. However, as immunohistochemistry has played a vital role in revealing the biological characteristics of many tumours, including BCCs, the profile of stromal and proliferation/cell cycle markers are also critical in evaluating the morphological characteristics of the S-BCCs:

1) DN-SBCCs

While the DN-SBCCs contained infiltrative foci, none of the infiltrative foci demonstrated laminin loss; this is a non-aggressive feature of BCCs. However, some of the infiltrative foci demonstrated intra-epithelial MSA positivity, indicating a reorganisation of the intra-epithelial actin network. This is hypothesised to be one of the earliest cellular alterations for true invasion in BCCs. It is important to note that not all infiltrative foci within a single tumour demonstrated intra-epithelial actin immunopositivity. Some of these infiltrative foci also demonstrated accentuated MIB1 and p53 immunostaining. However, while some of the infiltrative foci demonstrated high bcl-2 staining, albeit of

variable intensity, indicating a non-aggressive bcl-2 profile, 3/5 DN-SBCCs with intraepithelial actin immunopositivity, also demonstrated low bcl-2 staining and high p53 staining, despite an intact laminin pattern. This would immunoprofile 3 DN-SBCCs with an aggressive profile of the infiltrative components.

2) RG-SBCCs

Of 11 RG-SBCCs with high p53 staining, 4 had high p53 staining in the infiltrative component, but only one had a low bcl-2 composite score and low bcl-2 score in the infiltrative focus. In addition, these infiltrative foci demonstrated intraepithelial MSA positivity and a “VA” immunophenotype of the stromal cells, indicating one RG-SBCC with an established, truly aggressive immunophenotype. Those positive with one or more, but not all, aggressive immunostains, are hypothesised to be RG-SBCCs evolving/developing an aggressive immunophenotype.

3) R-SBCCs

Only one R-SBCC, with a predominantly infiltrative pattern, had a “full-house” of aggressive immunostaining in the infiltrative foci: low bcl-2, high p53, 2+ laminin discontinuity and intra-epithelial and stromal MSA positivity. Of significance is that 7 with a predominant nodular pattern had a high p53 score of which 5 had high bcl-2 scores. Hence, while high p53 may be a feature of aggressive growth, it is important that this staining be complemented with that of bcl-2, laminin and MSA.

Hence, SBCCs demonstrate phenotypic and immunophenotypic heterogeneity. In relation to aggressive, destructive BCCs, SBCCs with laminin discontinuity, intra-epithelial and stromal MSA immunopositivity, low bcl-2 and high p53 staining are aggressive; complete excision is critical to prevent destructive growth and recurrence. However, tumours expressing lower combinations of aggressive immunomarkers are hypothesized to be evolving aggressive BCCs. While high fibronectin staining had been demonstrated as a marker of aggressive BCCs in previous studies, high fibronectin staining in SBCCs is not a reliable indicator, as the scar tissue contains stromal fibronectin.

New Protocol for Histopathologists:

All SBCCs with infiltrative foci should be subjected to immunostaining with laminin, bcl-2, MSA and p53, and guidelines to surgeons should be modelled on the above findings.

Guidance to Surgeons:

Excision with adequate margins should be the minimum undertaking in SBCCs with this immunoprofile, rather than a wait-and-watch stance.

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APPENDIX A: ANTIGEN RETRIEVAL**1. Microwave Technique**

- Slides were placed in a thermoresistant plastic coplin jar (50ml) filled with 0,01M buffered sodium citrate solution at pH 6.0 (appendix).
- They were then incubated at 85°C for 10 minutes in a H2500 Microwave Processor (Energy Beam Sciences, Inc., Agawam, Massachusetts).
- The sections were allowed to cool in the coplin jar for 5 minutes.

2. Enzyme Pretreatment Technique**a) Laminin: Protease pretreatment**

- The sections were pretreated with protease (0.05% in PBS) for 5 minutes at room temperature.

b) Fibronectin: Pronase pretreatment

- The sections were pretreated with pronase (0.05% in PBS) for 5 minutes at room temperature.

APPENDIX B: PREPARATIONS OF SOLUTIONS FOR IMMUNOHISTOCHEMISTRY**a) 0.01M Sodium Citrate antigen retrieval buffer.**

- 2,94g sodium citrate was dissolved in 950ml of distilled water.
- 400µl of 1N HCl was added and using distilled water, the volume was raised to 1000ml.

- The solution was kept at room temperature.

b) Phosphate buffered saline, pH 7.4 (PBS)

- 10 tablets of PBS were dissolved in 1000ml of distilled water in a 2 litre conical flask.
- The solutions were stored at room temperature.

c) 3 % Hydrogen peroxide

- 3ml of hydrogen peroxide was added to 97ml of distilled water
- The solution was stored at 4°C.

d) DAB chromogen solution

- 20µl of the DAB chromogen was added to 200µl of substrate buffer.
- This solution was stored at 4°C.

e) 37mM Ammonium hydroxide (ammoniated water)

- 2.5ml of 15M ammonium hydroxide solution was added to 950ml of distilled water
- Using distilled water, the volume was raised to 1000ml.
- The solution was kept at room temperature.

f) Mayer's haematoxylin

- Using heat, 1g of haematoxylin was dissolved in 950ml of distilled water in a 2 litre flask.

- While heating, alum was added and was followed by 0.2g sodium iodate.
- 1g citric acid and 50g chloral hydrate were added. Heating continued to allow solution of the mixture.
- The mixture was cooled and using distilled water, the volume was raised to 1000ml.
- The solution was filtered and stored at room temperature.

APPENDIX C: Preparation of Poly-L-Lysine slides (SIGMA DIAGNOSTICS)

- Poly-L-Lysine coating solution (50ml) was diluted in 450ml of distilled water in a trough.
- New slides were arranged in a staining rack and submerged in the Poly-L-Lysine solution for 5 minutes
- The rack containing the slides was removed from the solution and placed on thick absorbent paper to facilitate drainage of excess solution.
- The subsequent emersions were increased by 30 seconds.
- The solutions was used to coat a maximum of 12 packets of poly-L-Lysine slides.
- The coated slides were allowed to dry at room temperature overnight.
- The coated slides were packed in boxes, in the order of coating.
- The coated slides were employed in picking sections for immunohistochemical staining.

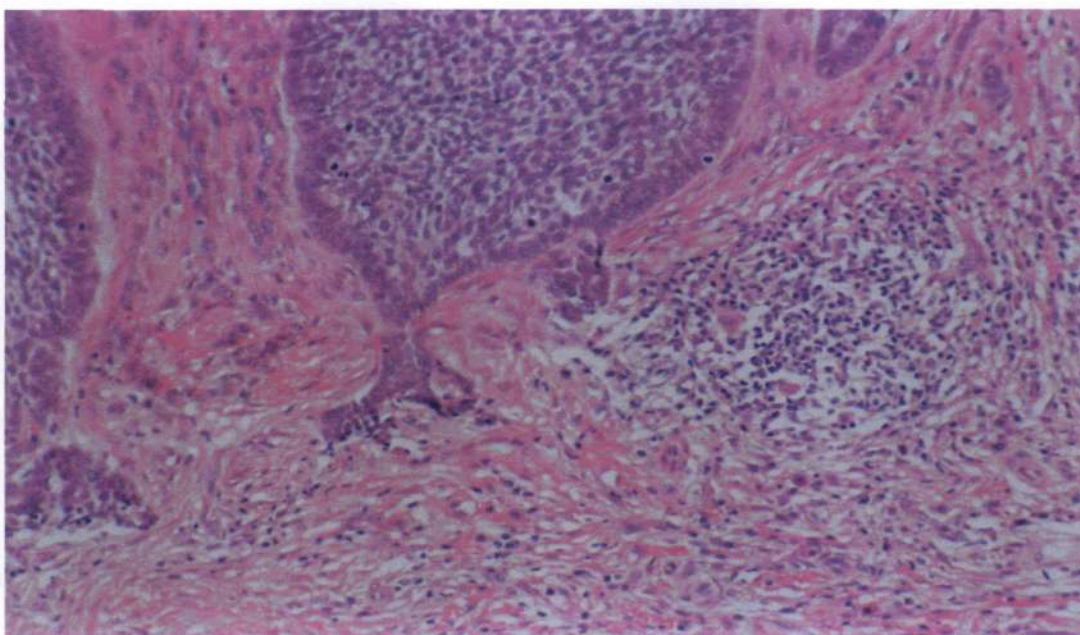


PLATE 1: GROWTH PATTERN

DN-SBCC: Nodular and infiltrative patterns in fibrotic stroma containing a lymphocytic infiltrate.

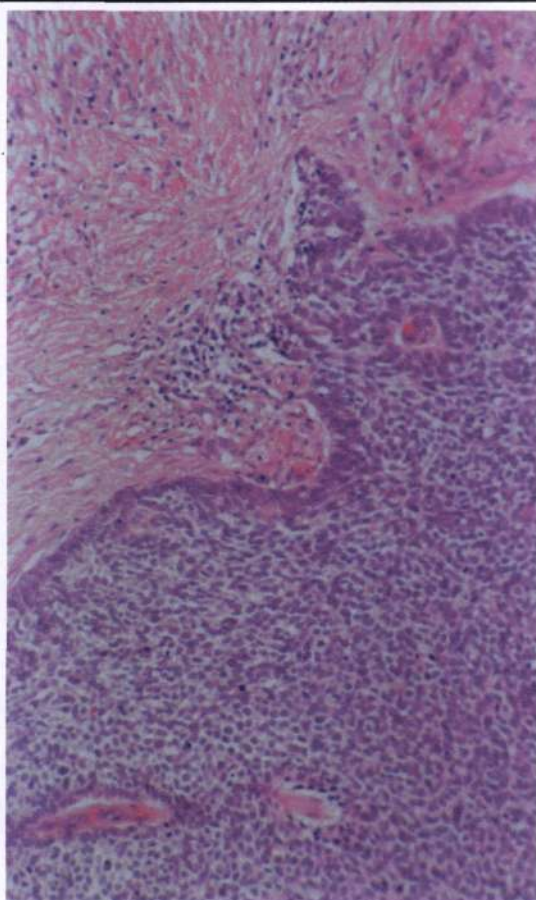


PLATE 2: GROWTH PATTERN

DN-SBCC: Nodular and infiltrative patterns in fibrotic stroma containing a lymphocytic infiltrate.

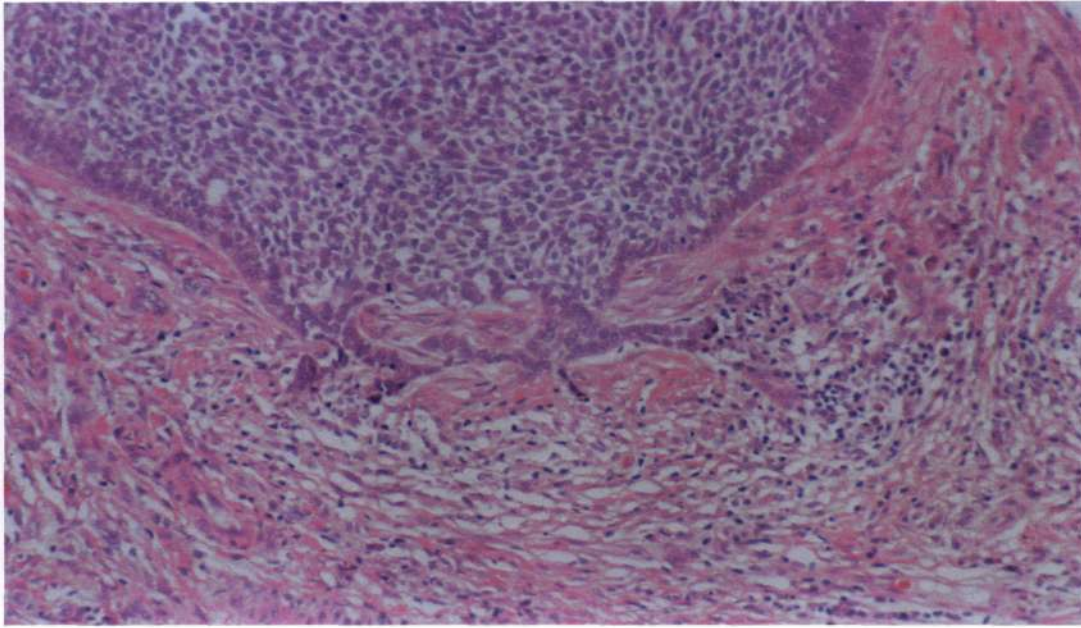


PLATE 3: GROWTH PATTERN

DN-SBCC: Nodular and infiltrative patterns in fibrotic stroma containing a lymphocytic infiltrate.

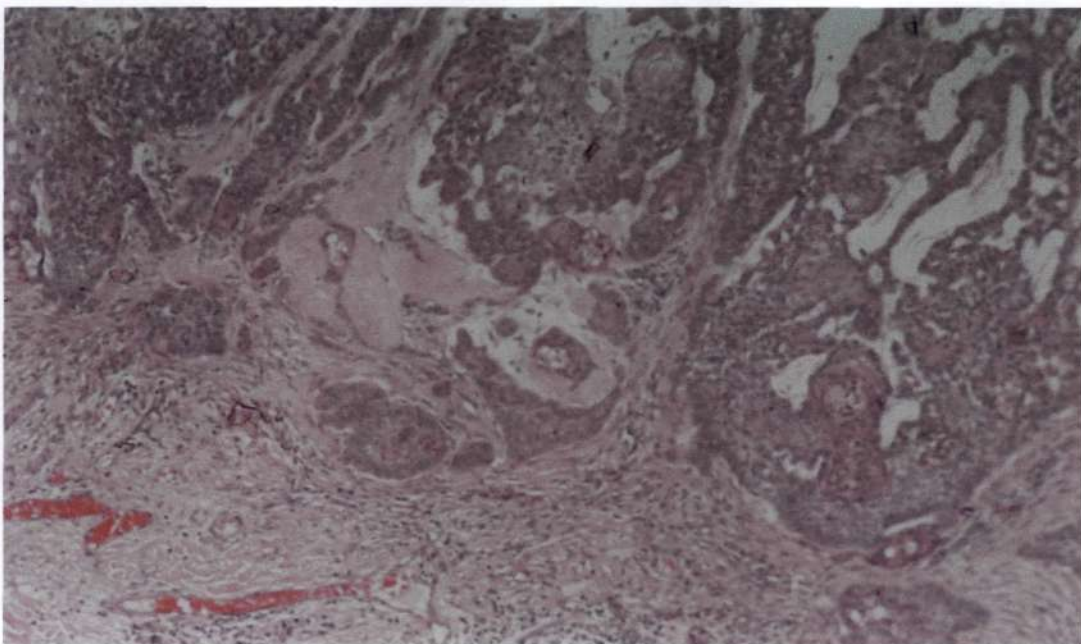


PLATE 4: GROWTH PATTERN

DN-SBCC: Nodular and infiltrative patterns in fibrotic stroma containing a lymphocytic infiltrate.

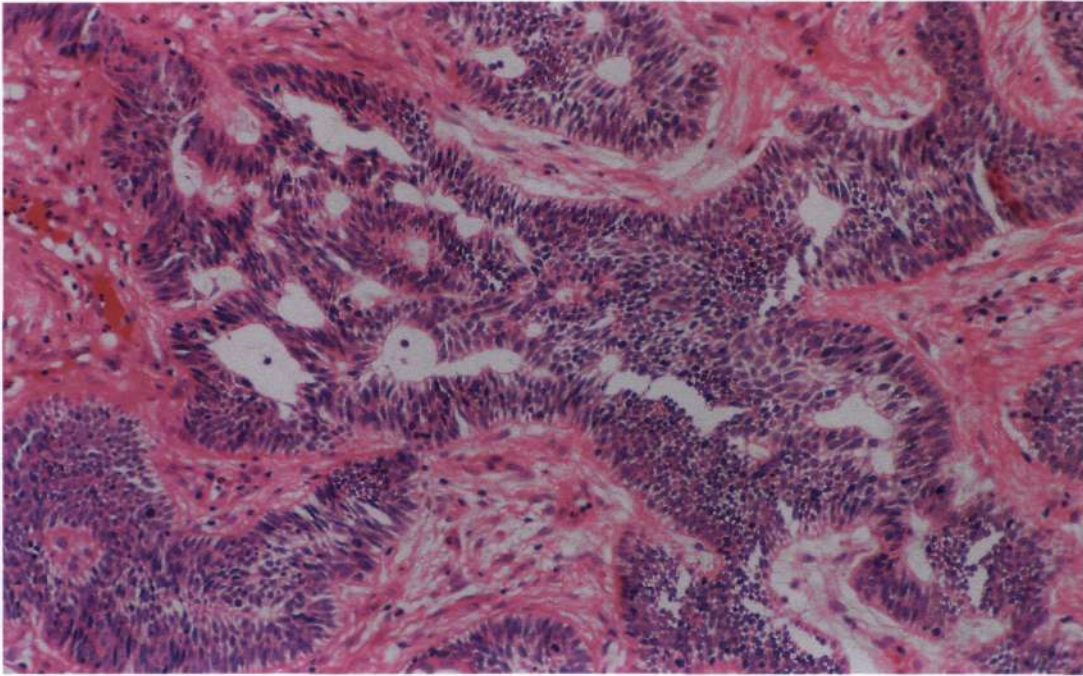


PLATE 5: GROWTH PATTERN

DN-SBCC: Nodular pattern with adenoid differentiation in scar tissue.

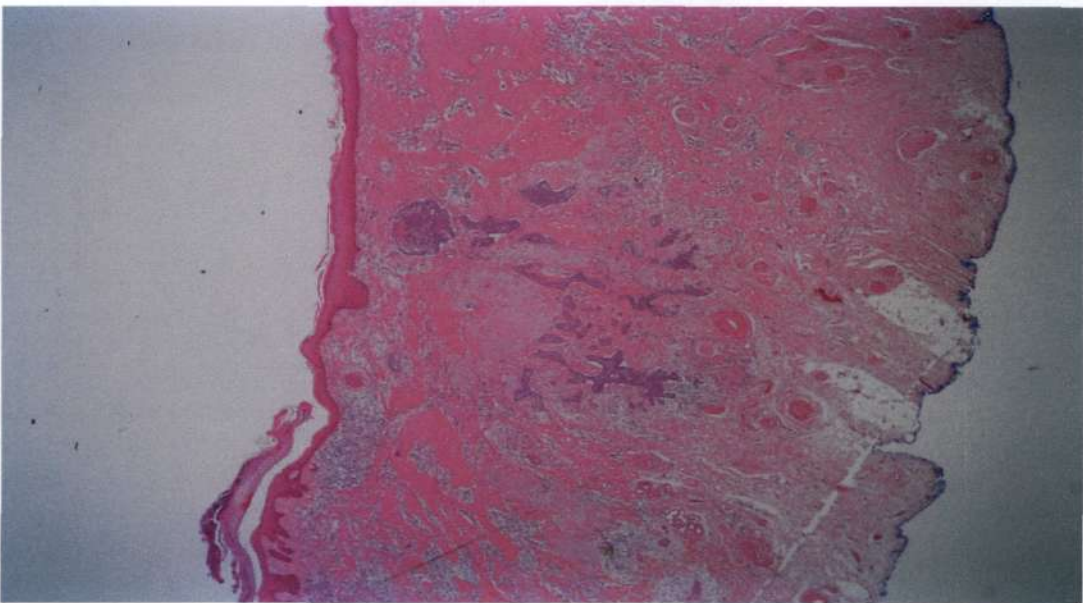


PLATE 6: GROWTH PATTERN

RG-SBCC: Intradermal regrowth with nodular, micronodular and infiltrative patterns in scar tissue containing elastotic material and chronic inflammatory cells.

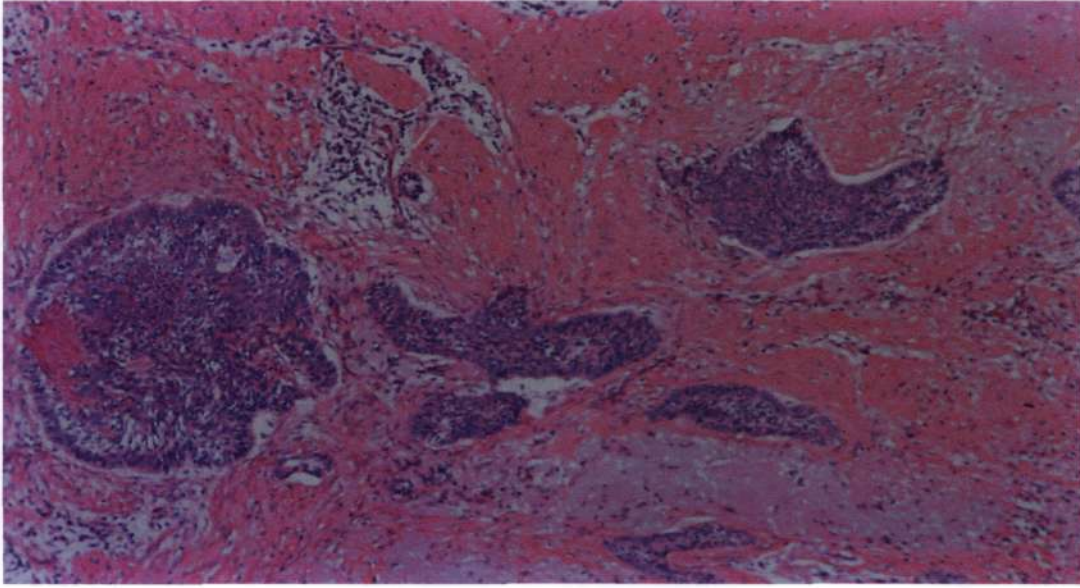


PLATE 7: GROWTH PATTERN

RG-SBCC: Intradermal regrowth with nodular, micronodular and infiltrative patterns in scar tissue containing elastotic material and chronic inflammatory cells.

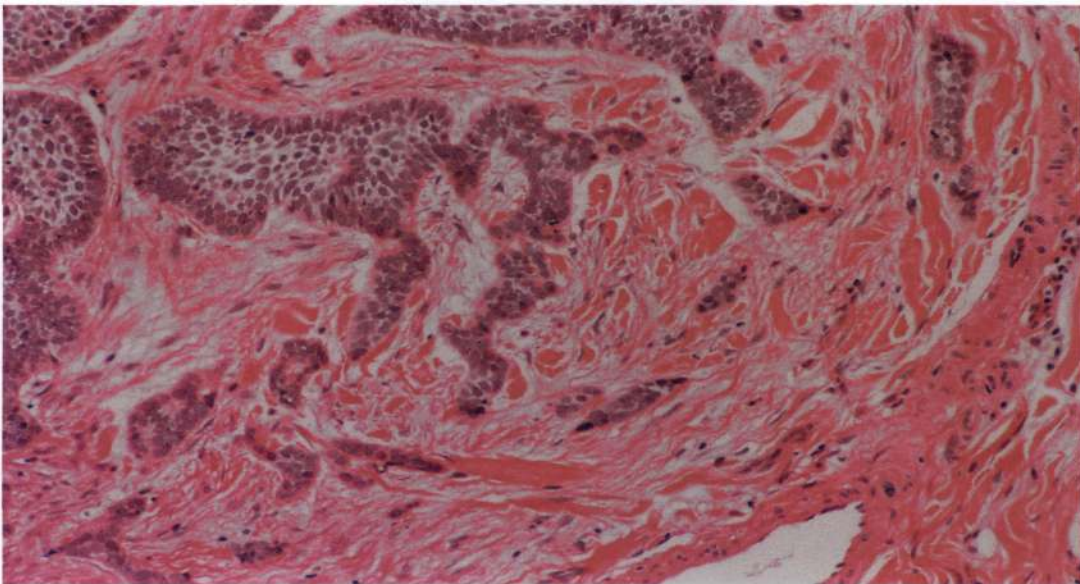


PLATE 8: GROWTH PATTERN

RG-SBCC: Intradermal regrowth with nodular, micronodular and infiltrative patterns in scar tissue containing elastotic material and chronic inflammatory cells.

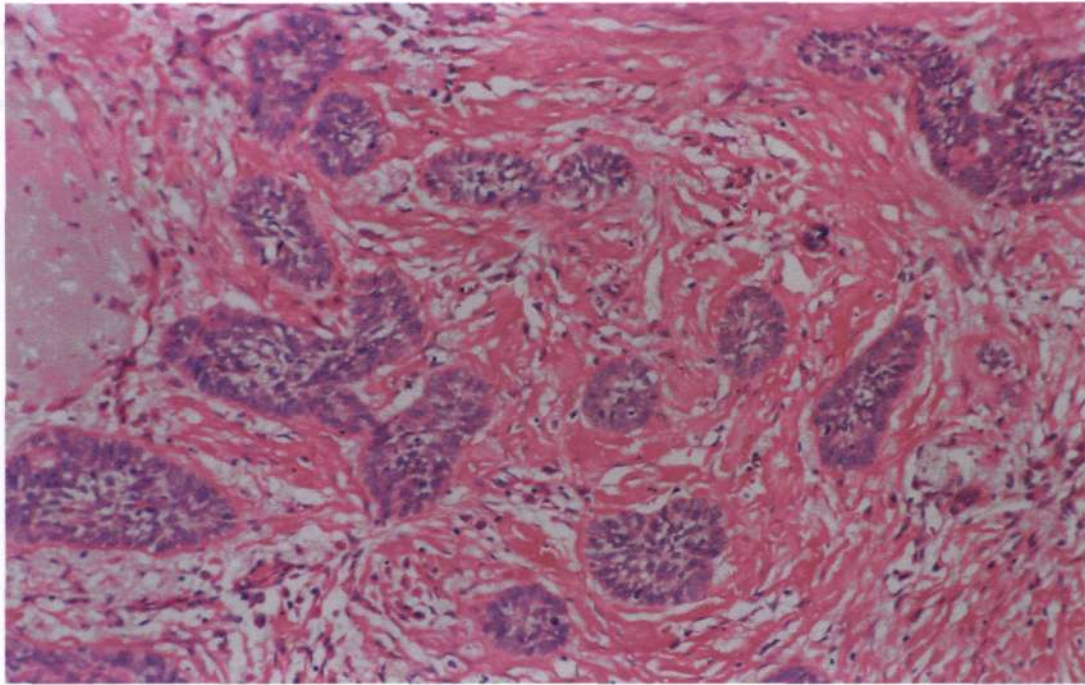


PLATE 9: GROWTH PATTERN
RG-SBCC from epidermis into scar tissue.

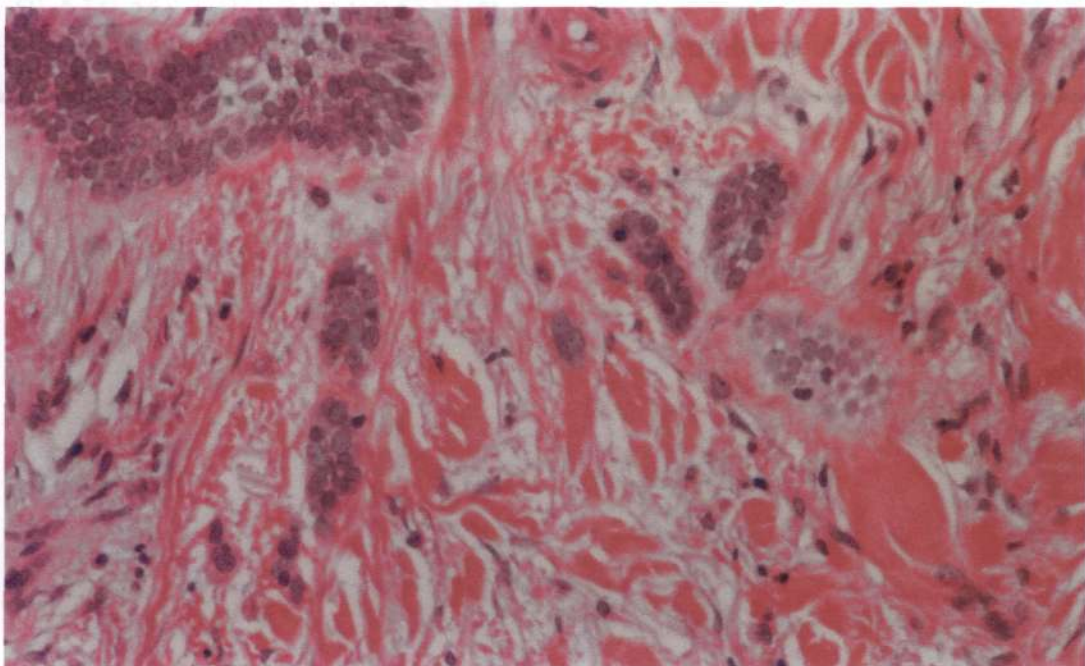


PLATE 10: GROWTH PATTERN
R-SBCC: Nodular, micronodular and infiltrative patterns in scar tissue.

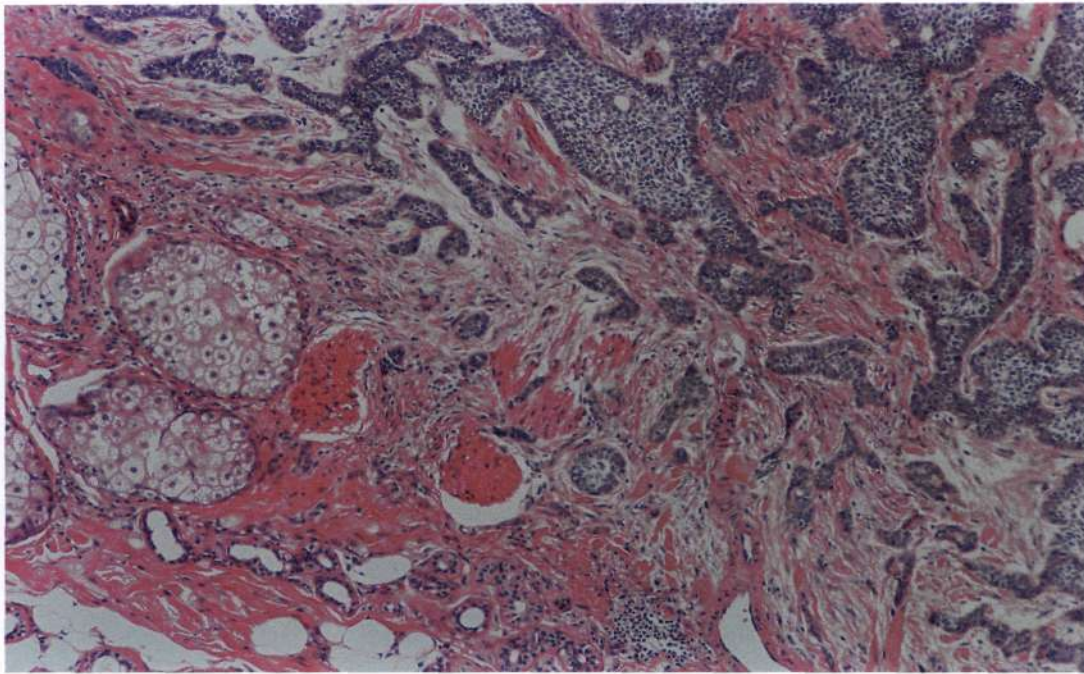


PLATE 11: GROWTH PATTERN

R-SBCC: Nodular, micronodular and infiltrative patterns in scar tissue.

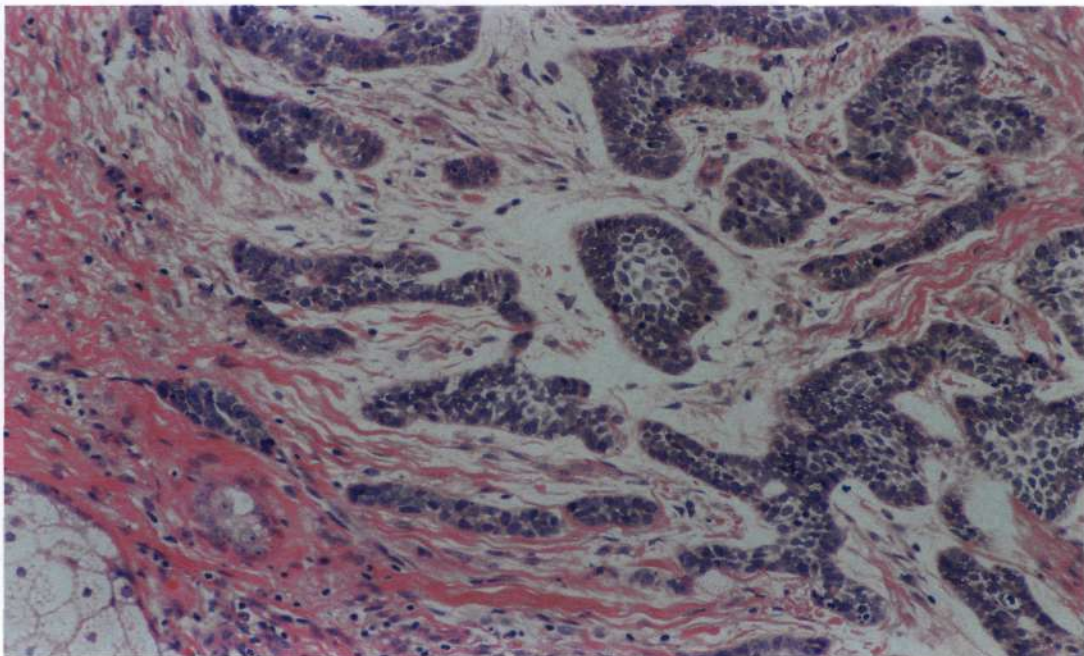


PLATE 12: GROWTH PATTERN

R-SBCC: Nodular, micronodular and infiltrative patterns in scar tissue.

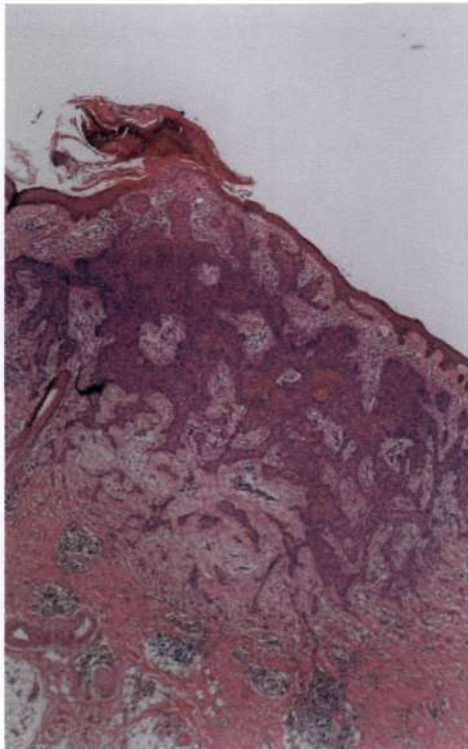


PLATE 13:
GROWTH PATTERN
R-SBCC: Nodular,
micronodular and
infiltrative patterns in
scar tissue.

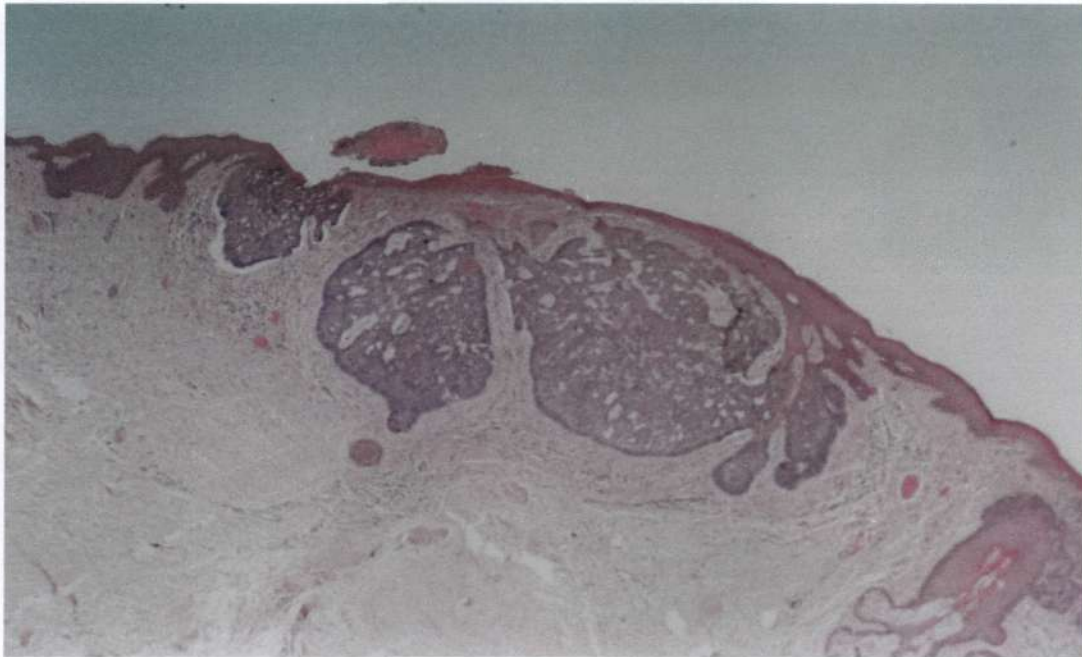


PLATE 14: GROWTH PATTERN
R-SBCC: Nodular, micronodular and infiltrative patterns in scar tissue.

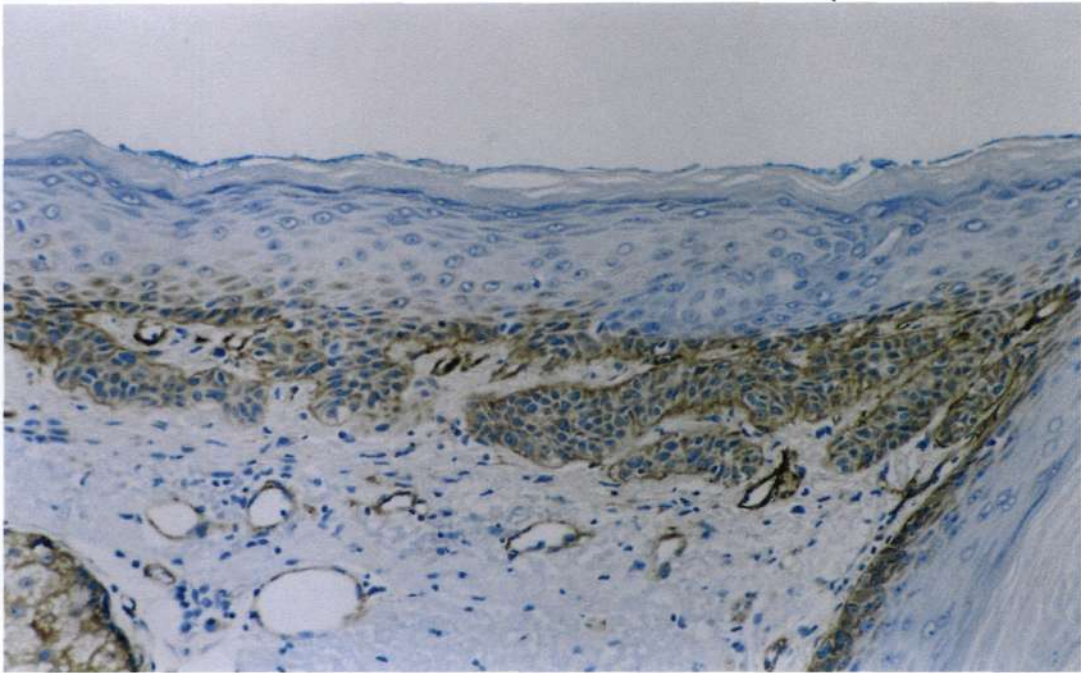


PLATE 15: LAMININ IMMUNOSTAINING

DN-SBCC: Superficial pattern with intact laminin staining.

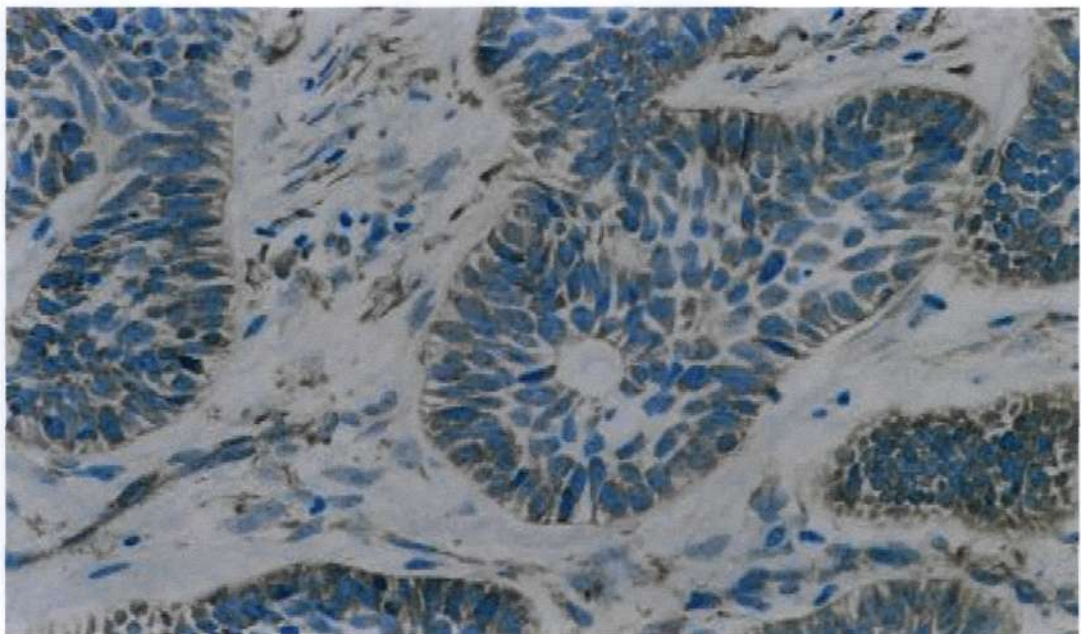


PLATE 16: LAMININ IMMUNOSTAINING

DN-SBCC: Nodular pattern with intact basement membrane laminin staining.

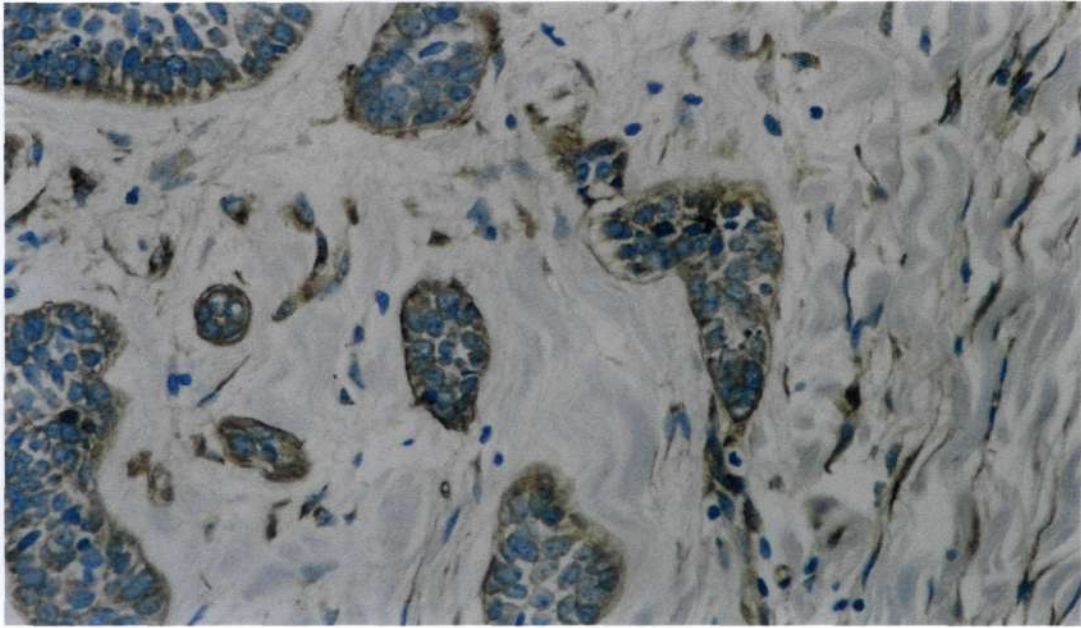


PLATE 17: LAMININ IMMUNOSTAINING

DN-SBCC: Micronodular pattern with intact basement membrane laminin staining.

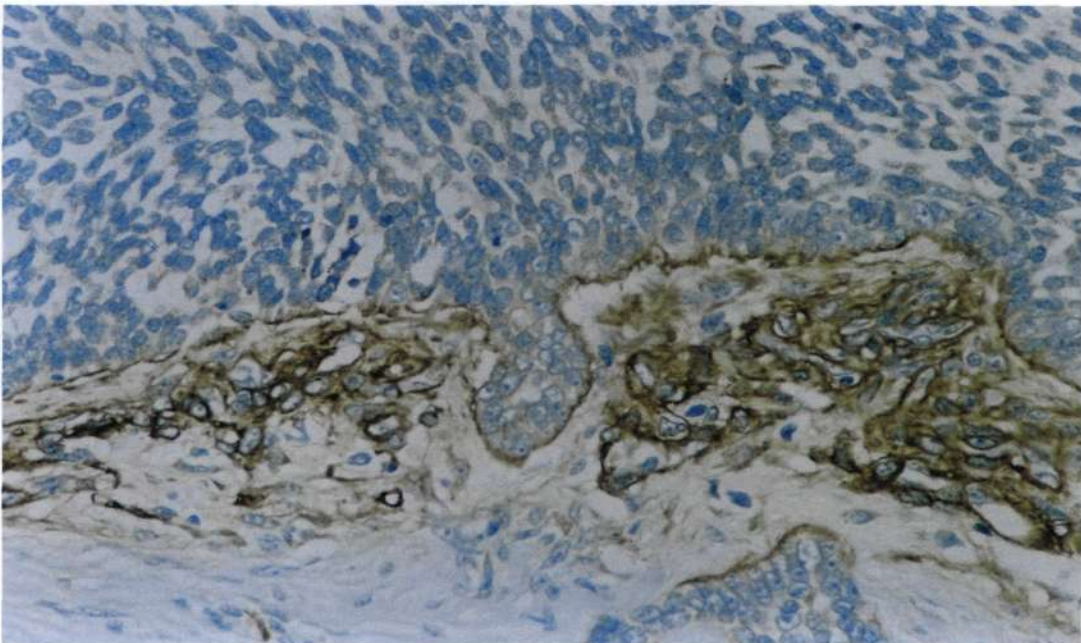


PLATE 18: LAMININ IMMUNOSTAINING

DN-SBCC: Focally infiltrative pattern with intact basement membrane laminin.

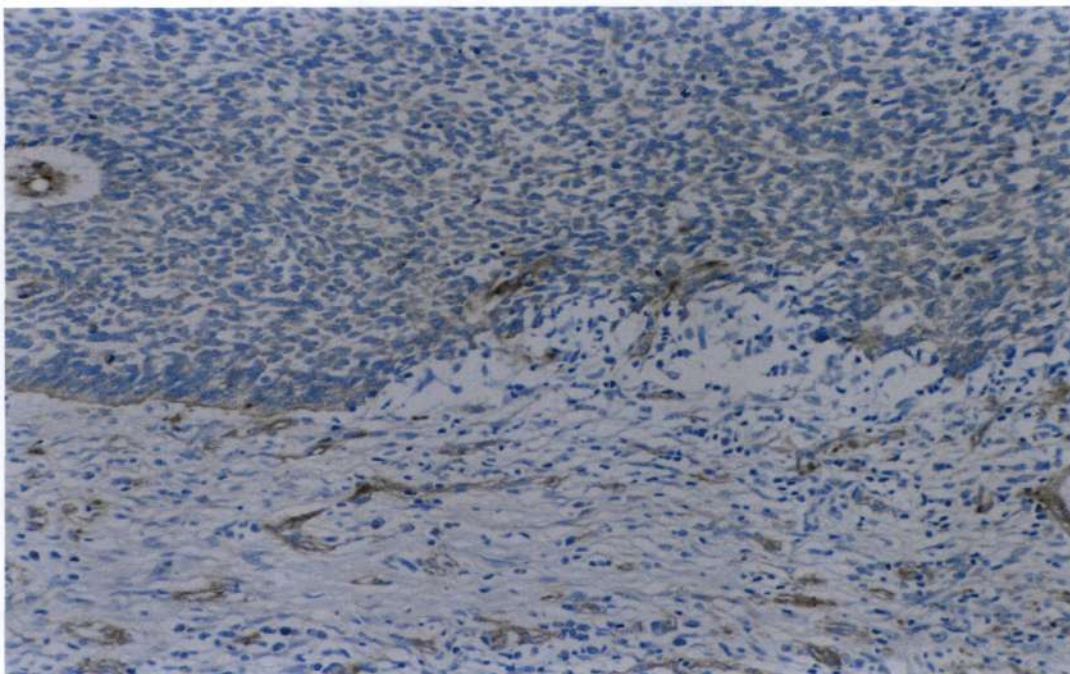


PLATE 19: LAMININ IMMUNOSTAINING
DN-SBCC with loss of laminin staining in area of lymphocytic infiltration.

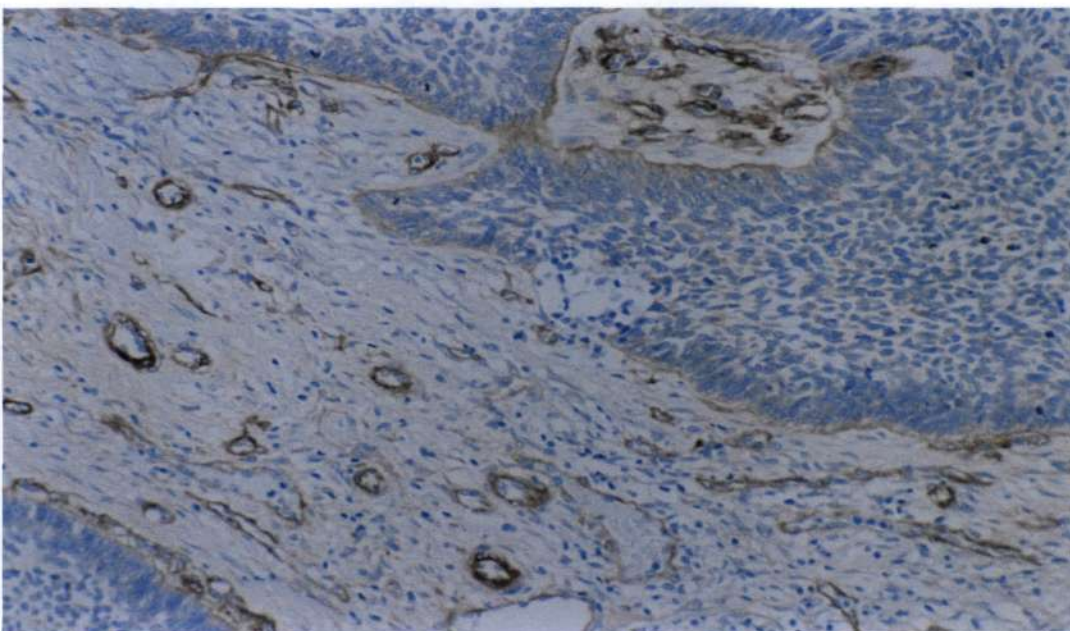


PLATE 20: LAMININ IMMUNOSTAINING
DN-SBCC with loss of laminin staining in focus of lymphocytic infiltration and adjacent infiltrative focus with intact laminin.

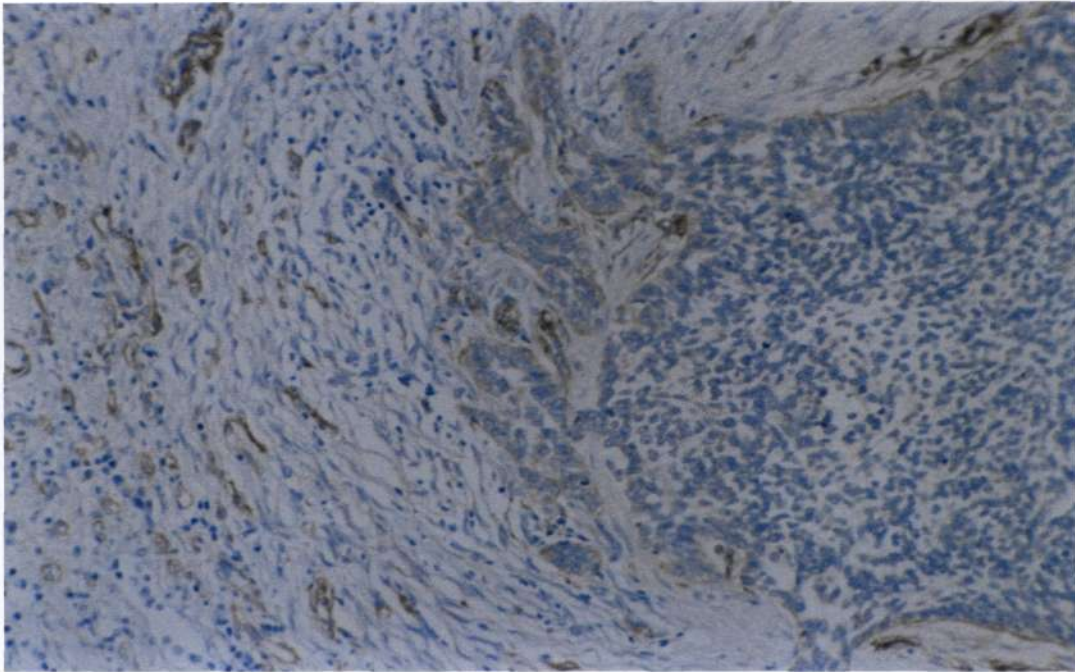


PLATE 21: LAMININ IMMUNOSTAINING

RG-SBCC: Nodular and infiltrative patterns with intact laminin staining.

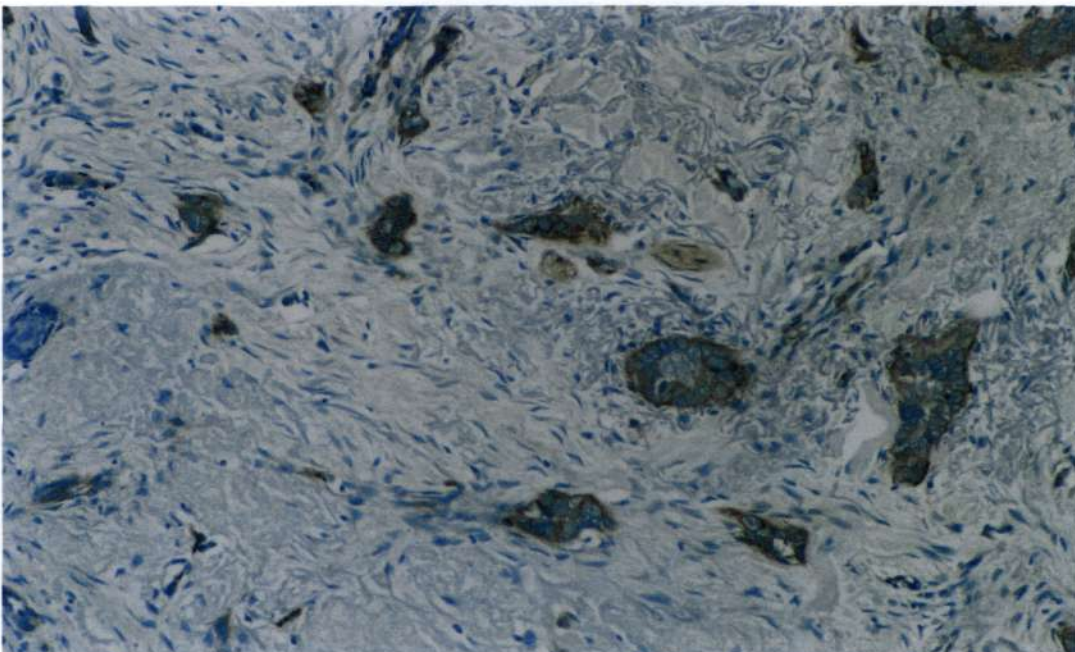


PLATE 22: LAMININ IMMUNOSTAINING

RG-SBCC: Micronodular and infiltrative patterns with intact laminin staining.

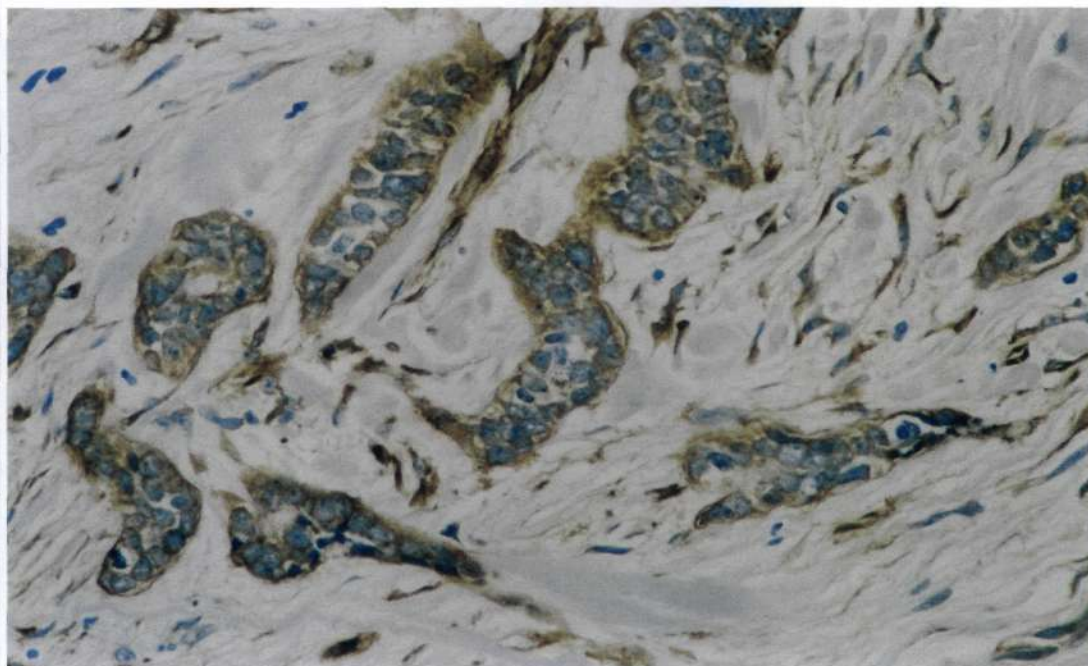


PLATE 23: LAMININ IMMUNOSTAINING

R-SBCC: Intact laminin staining in micronodular and infiltrative components

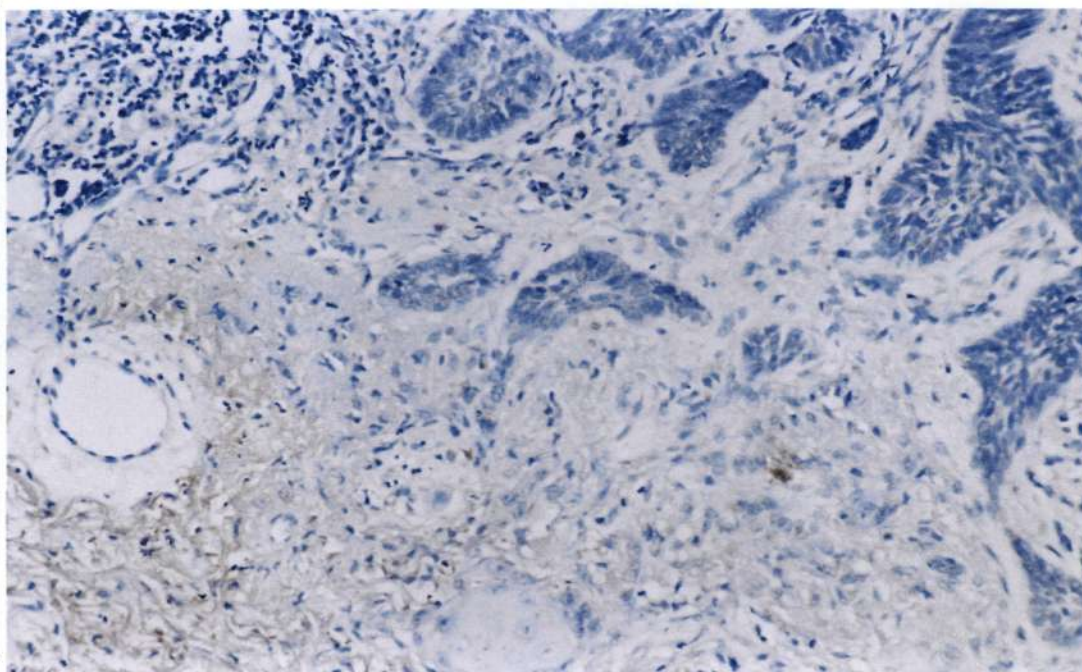


PLATE 24: FIBRONECTIN IMMUNOSTAINING
DN-SBCC: Pale, low fibronectin staining.

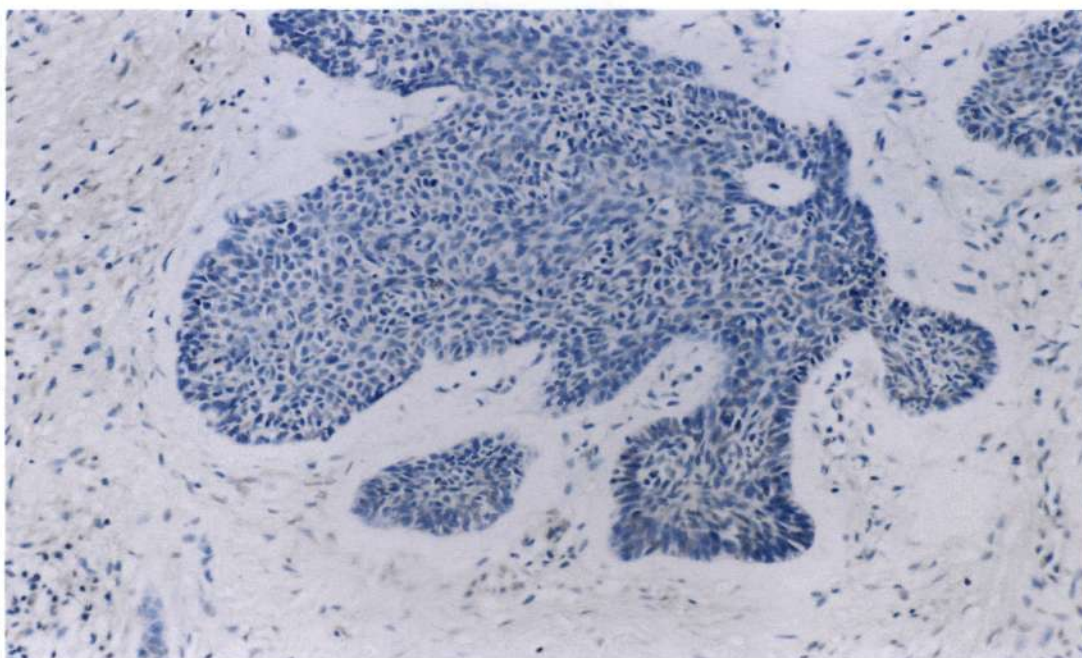


PLATE 25: FIBRONECTIN IMMUNOSTAINING
DN-SBCC: Low fibronectin staining.

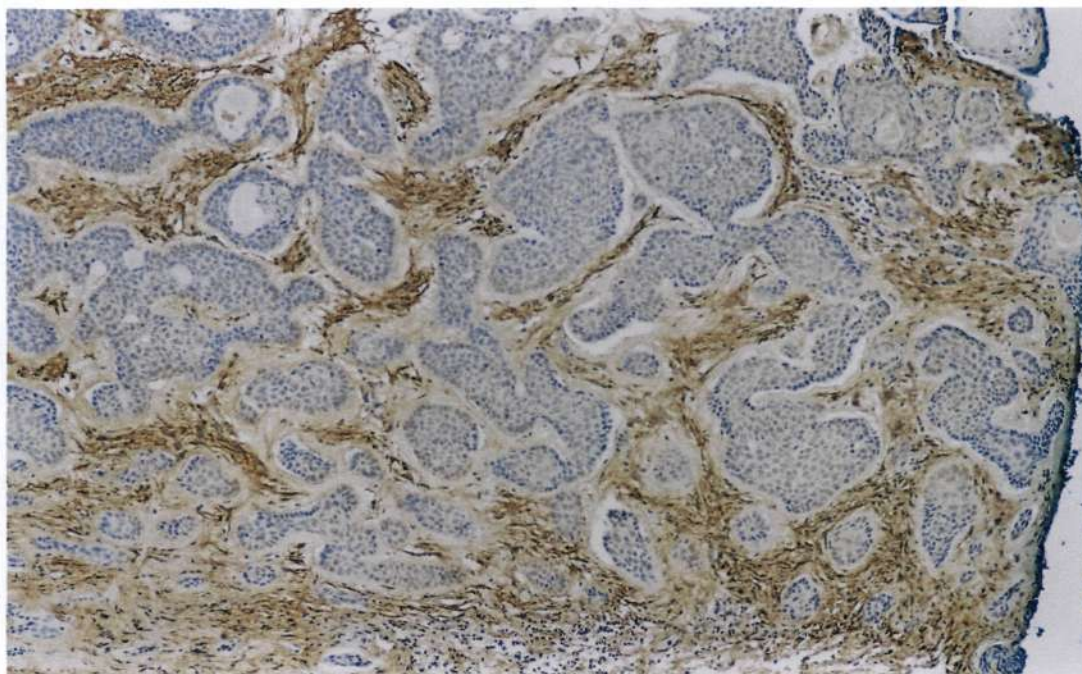


PLATE 26: FIBRONECTIN IMMUNOSTAINING
DN-SBCC: High, bright fibronectin staining.

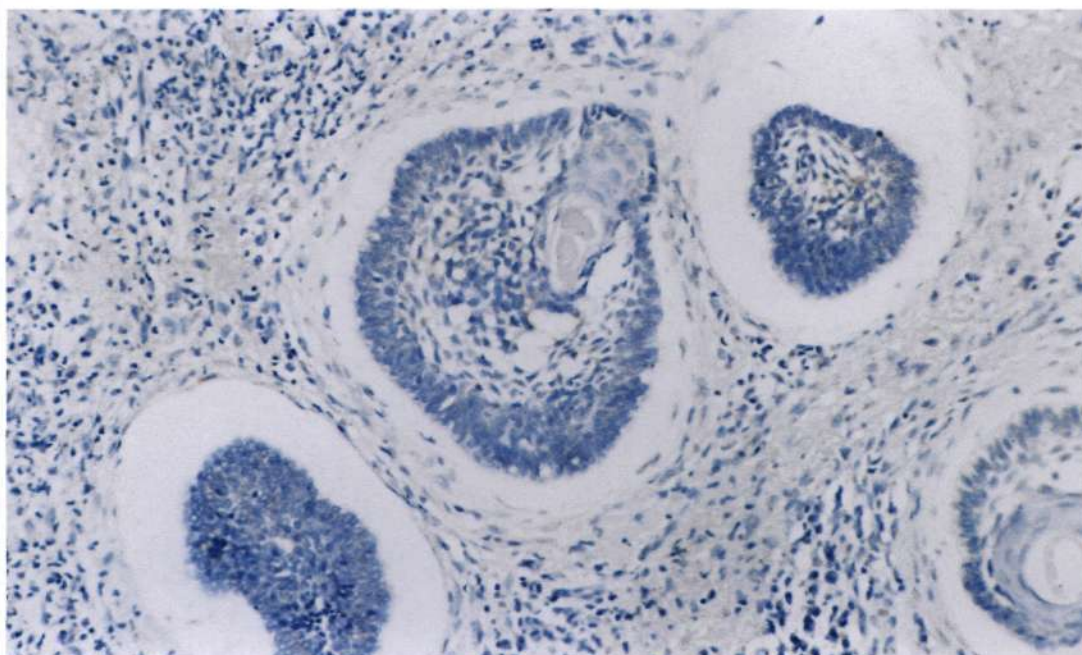


PLATE 27: FIBRONECTIN IMMUNOSTAINING
RG-SBCC: Low fibronectin staining.

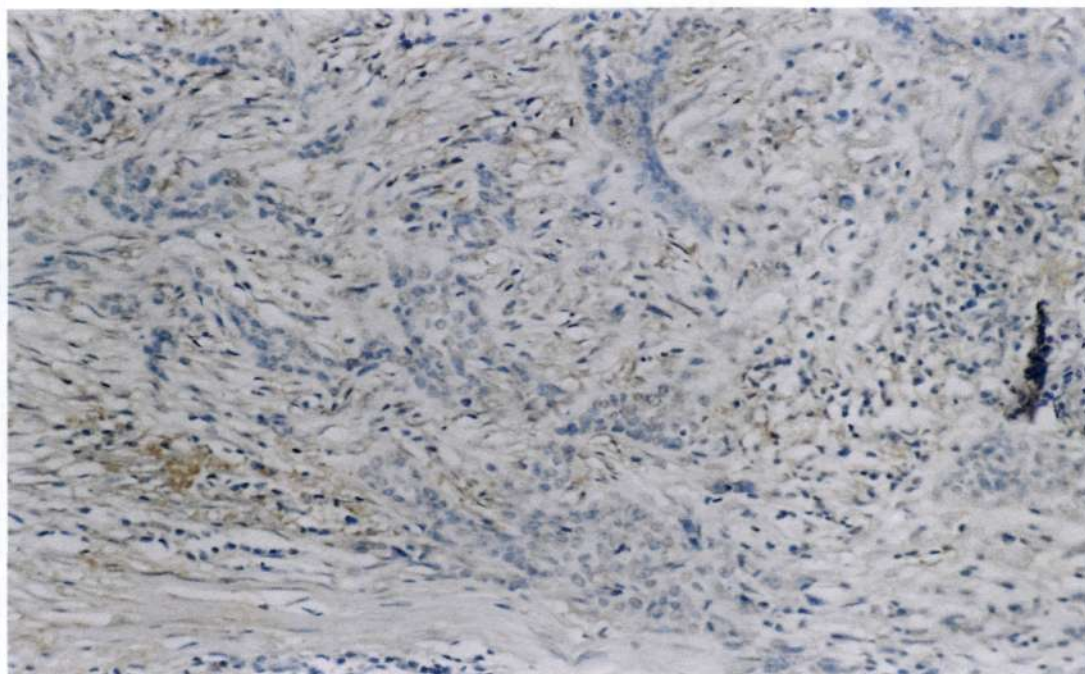


PLATE 28: FIBRONECTIN IMMUNOSTAINING
RG-SBCC: Pale, high fibronectin staining.

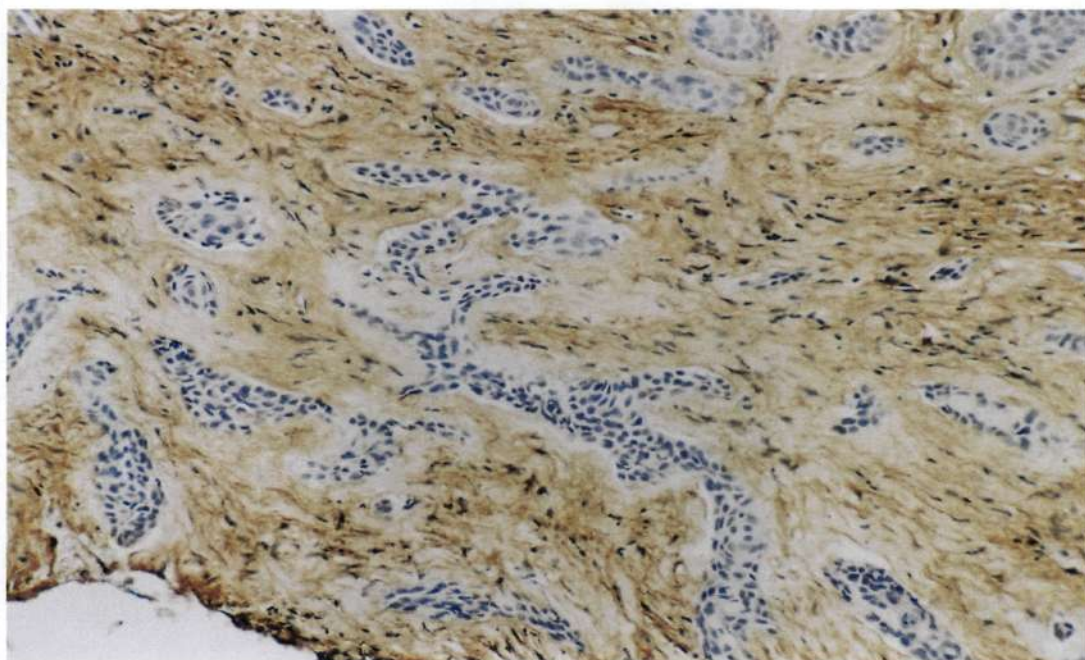


PLATE 29: FIBRONECTIN IMMUNOSTAINING
RG-SBCC: High, bright fibronectin staining.

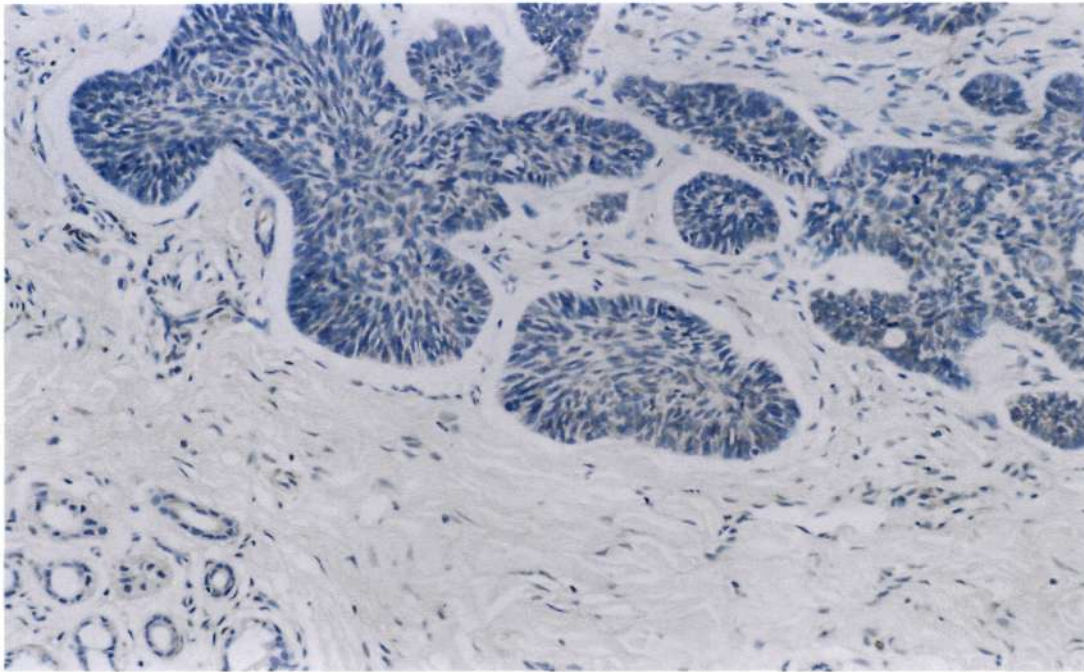


PLATE 30: FIBRONECTIN IMMUNOSTAINING
R-SBCC: Low fibronectin staining.

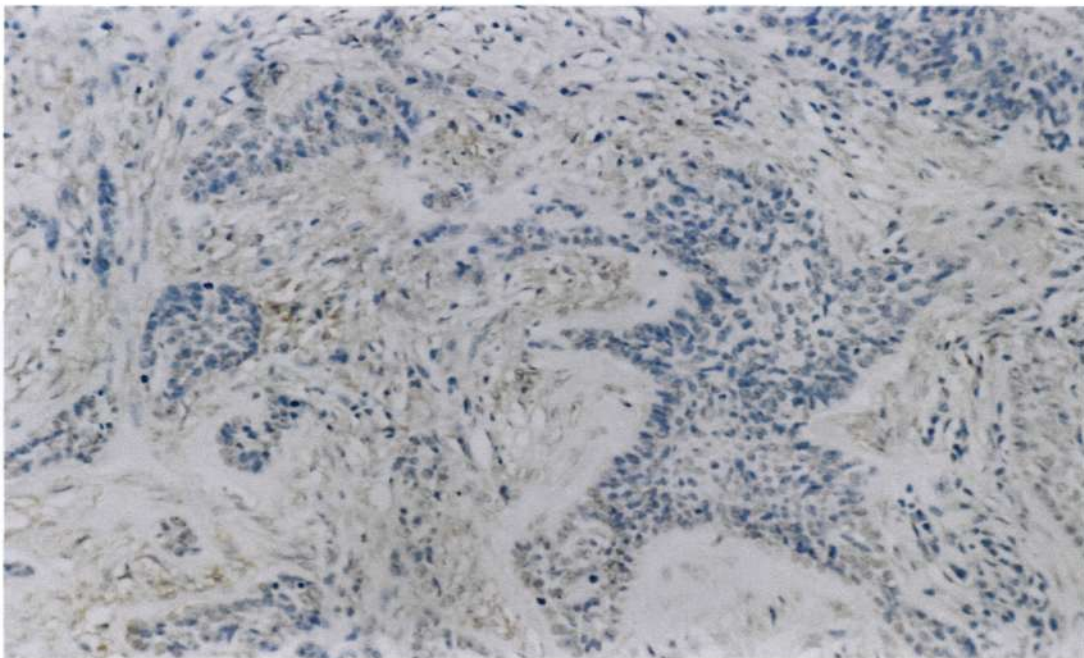


PLATE 31: FIBRONECTIN IMMUNOSTAINING
R-SBCC: Pale, high fibronectin staining.

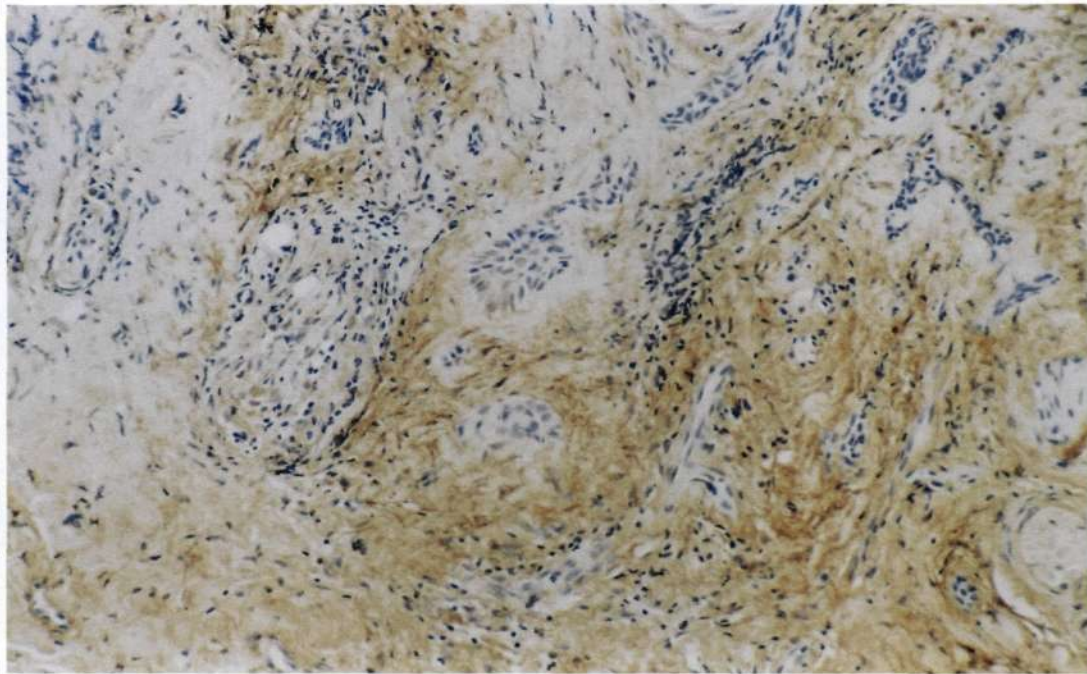


PLATE 32: FIBRONECTIN IMMUNOSTAINING

R-SBCC: High, bright fibronectin staining.

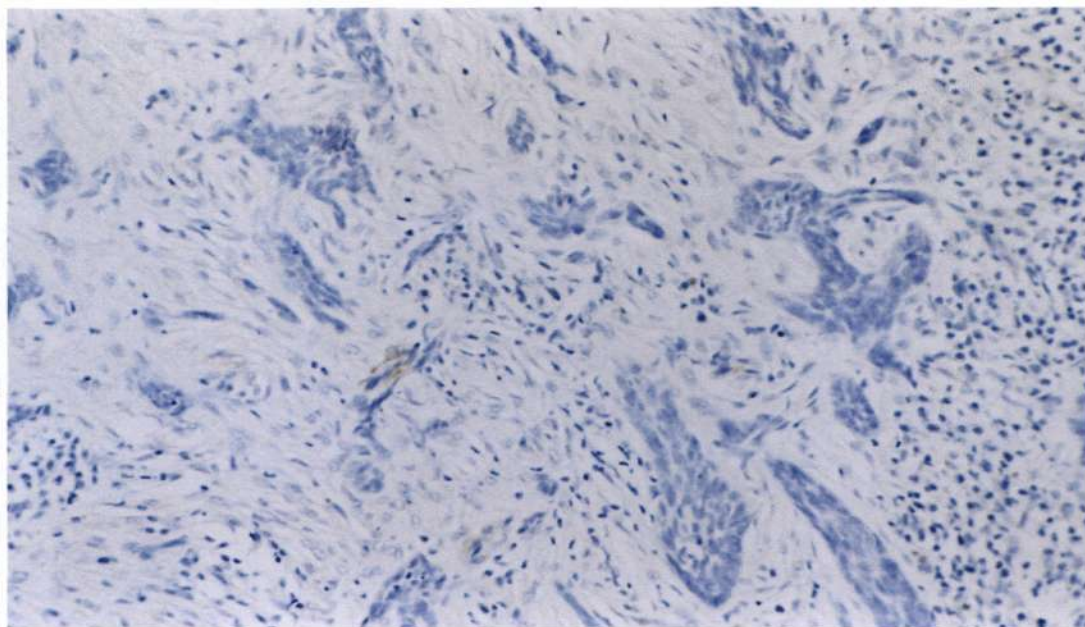


PLATE 33: ACTIN IMMUNOSTAINING

DN-SBCC: MSA negativity in peritumoral stromal cells. Inbuilt control positive.

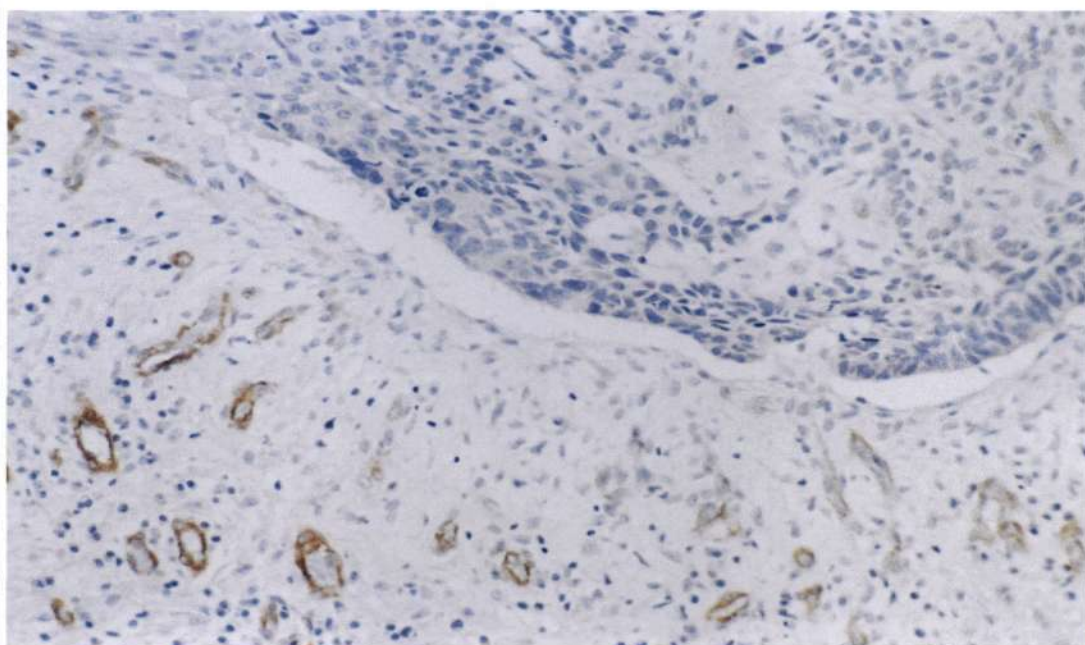


PLATE 34: ACTIN IMMUNOSTAINING

DN-SBCC: MSA negativity in peritumoral stromal cells. Inbuilt control positive.

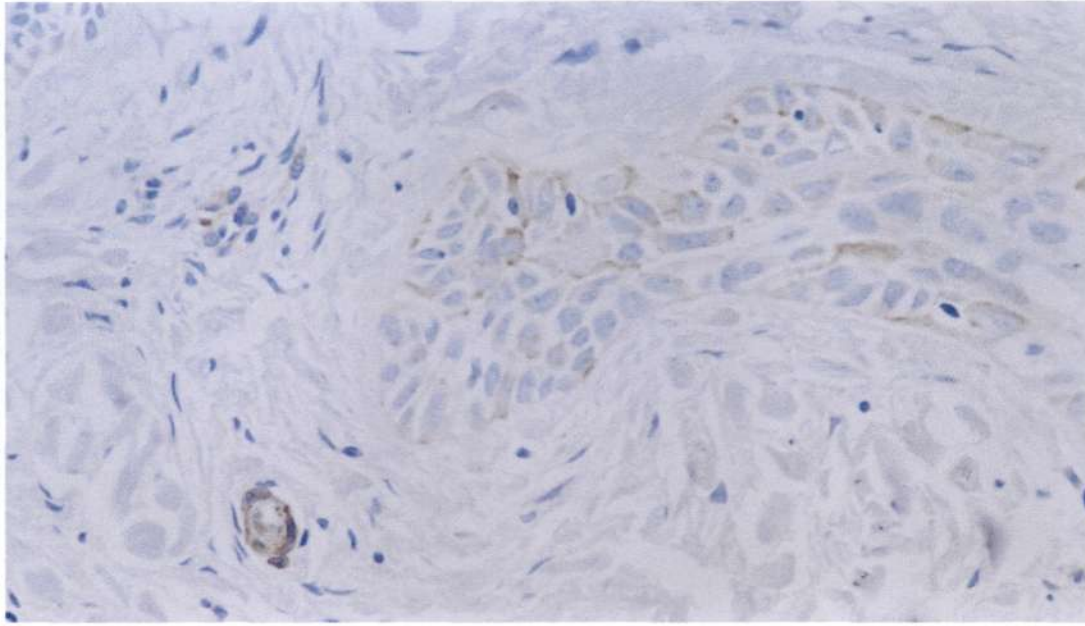


PLATE 35: ACTIN IMMUNOSTAINING

DN-SBCC: Exclusive epithelial MSA positivity. In-built control (blood vessels) positive.

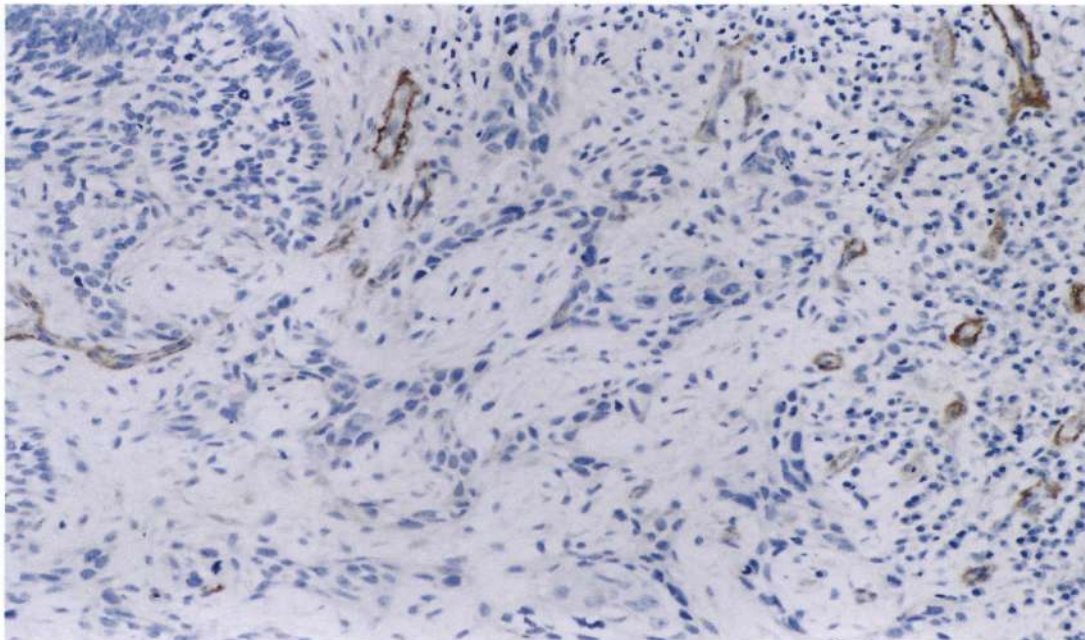


PLATE 36: ACTIN IMMUNOSTAINING

RG-SBCC: MSA negativity in peritumoral stromal cells. Inbuilt control positive.

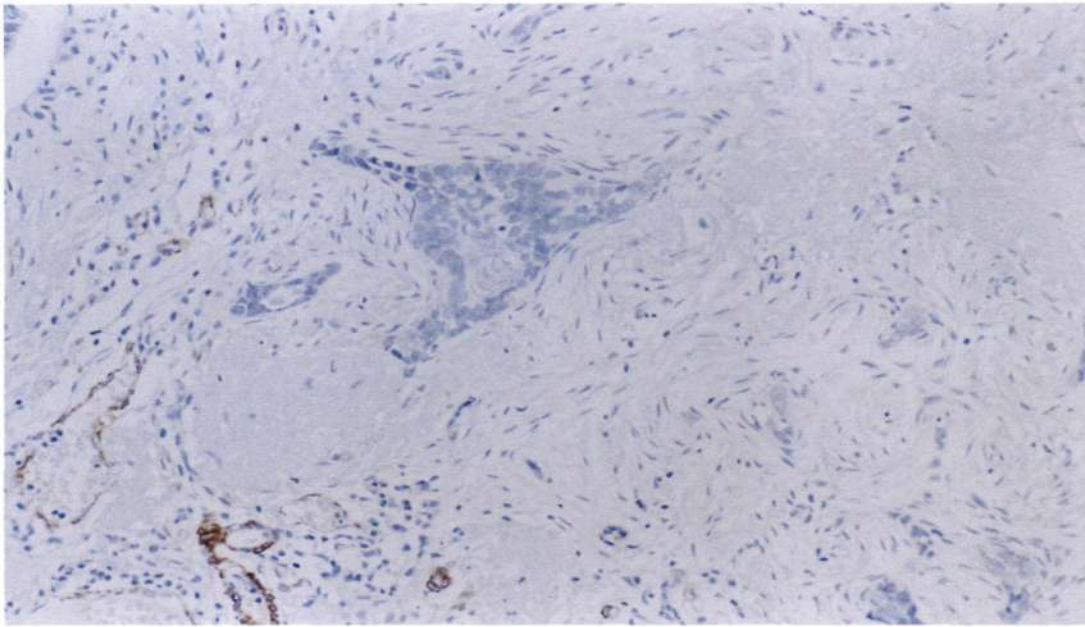


PLATE 37: ACTIN IMMUNOSTAINING

RG-SBCC: Negative MSA stromal response. In-built control (blood vessel) positive.

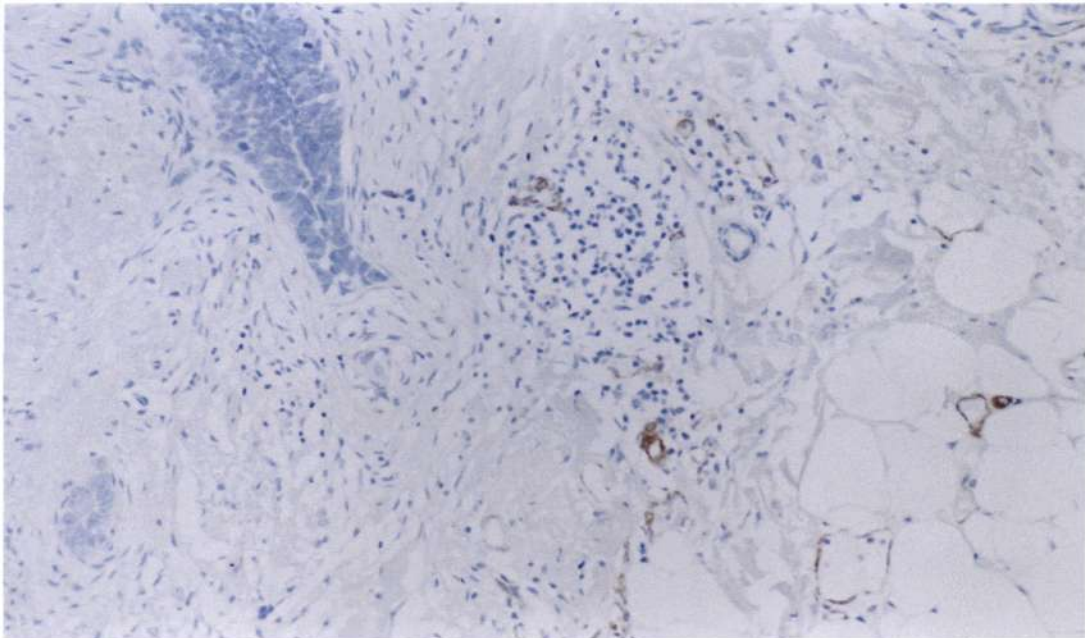


PLATE 38: ACTIN IMMUNOSTAINING

RG-SBCC: Negative MSA stromal response. In-built control (blood vessel) positive.

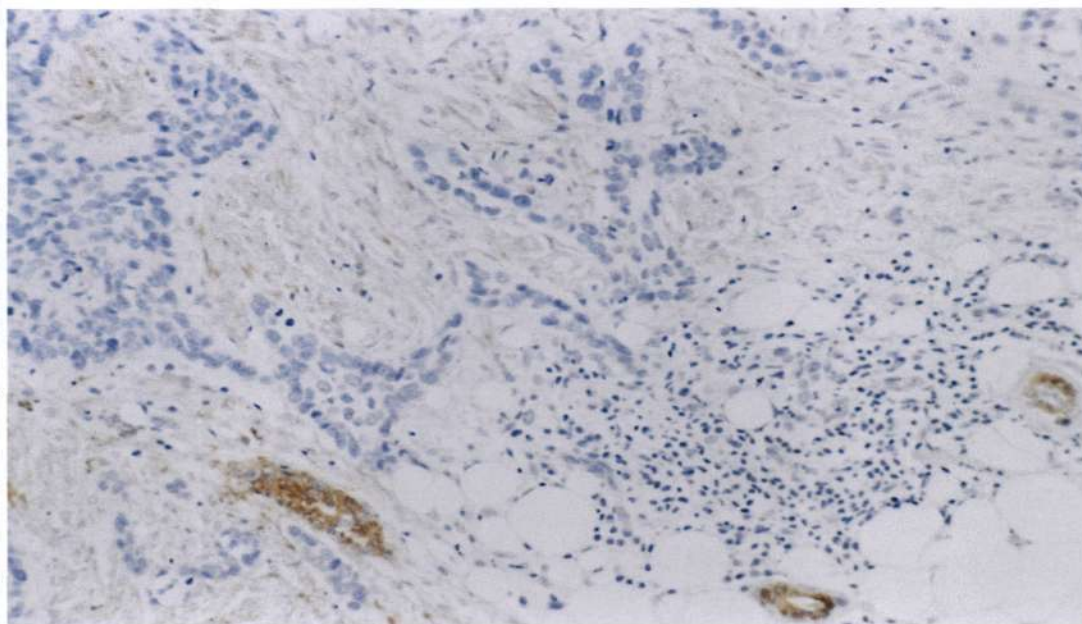


PLATE 39: ACTIN IMMUNOSTAINING

RG-SBCC: Exclusive peritumoral MSA immunopositivity. In-built control (arterioles) positive.

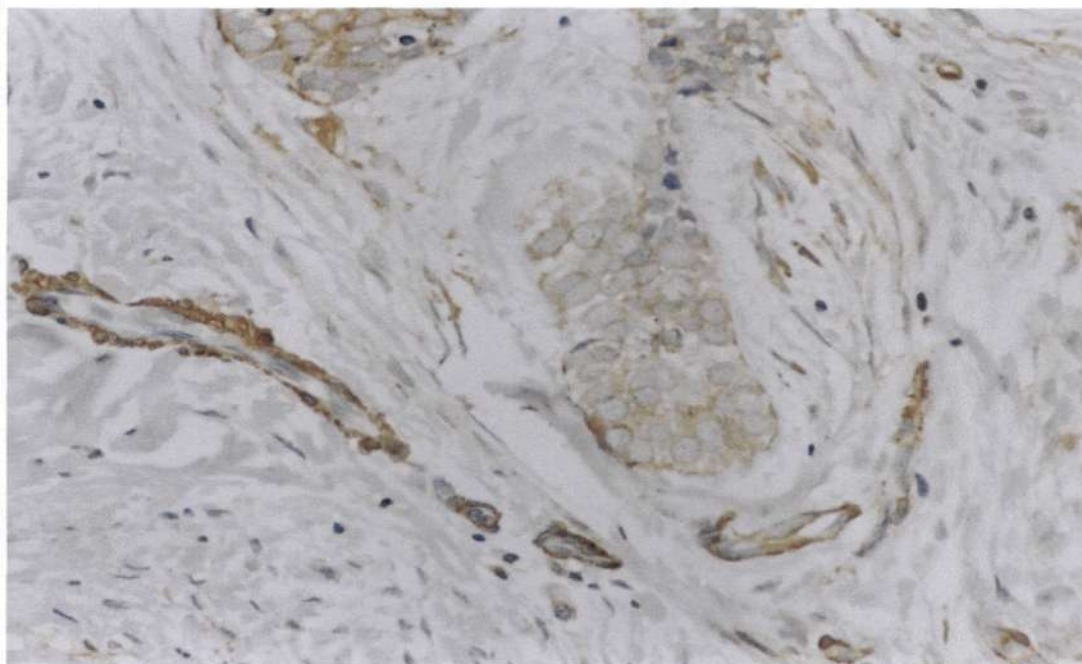
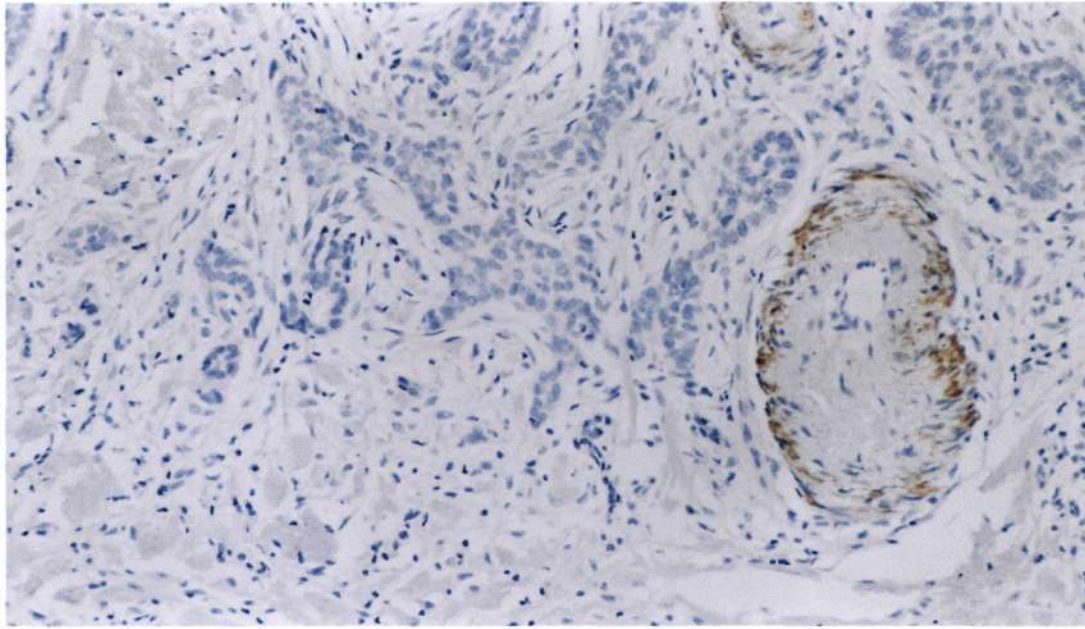
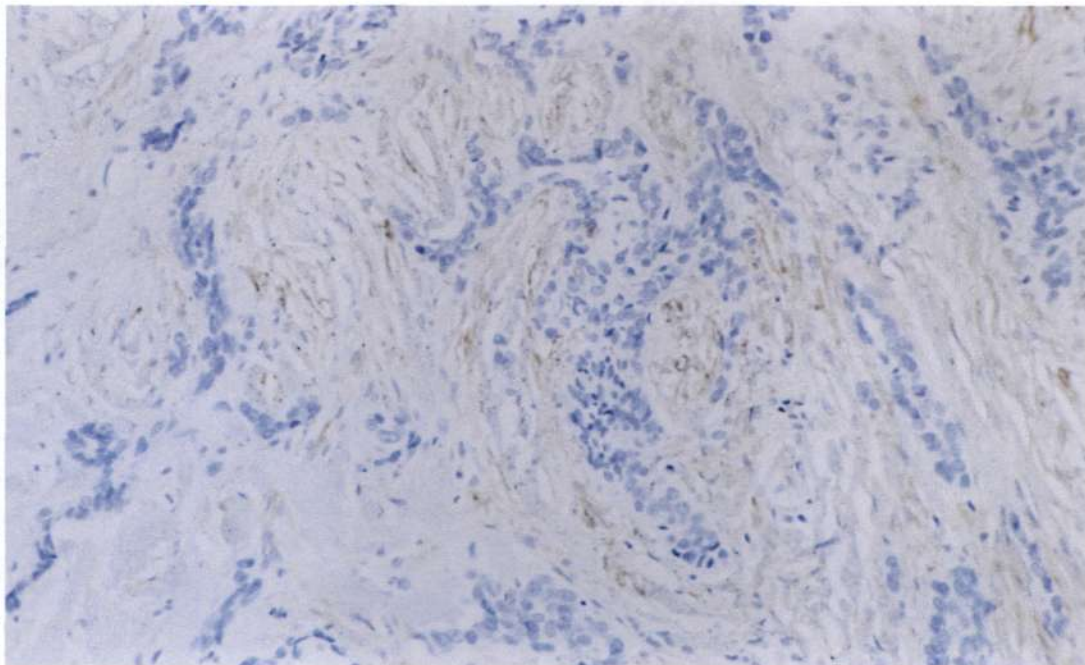


PLATE 40: ACTIN IMMUNOSTAINING

RG-SBCC: MSA immunopositivity in epithelial and stromal components.

**PLATE 41: ACTIN IMMUNOSTAINING**

R-SBCC: Negative epithelial and stromal response to MSA. In-built control (blood vessel) positive.

**PLATE 42: ACTIN IMMUNOSTAINING**

R-SBCC: Exclusive stromal MSA positivity.

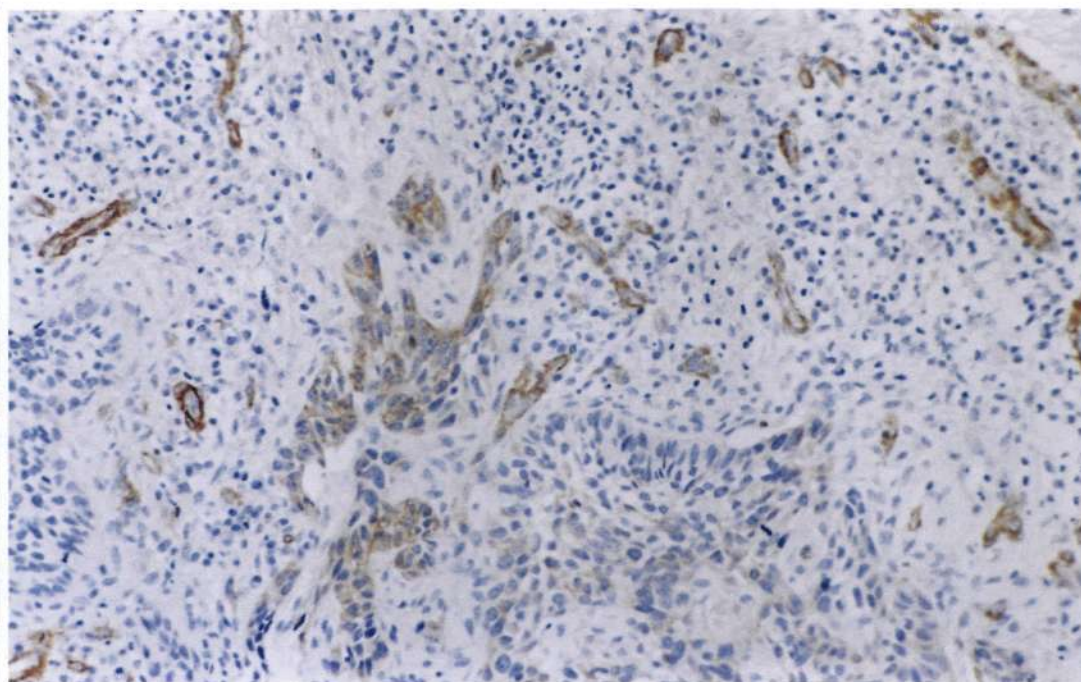


PLATE 43: ACTIN IMMUNOSTAINING
R-SBCC: Exclusive epithelial MSA positivity.

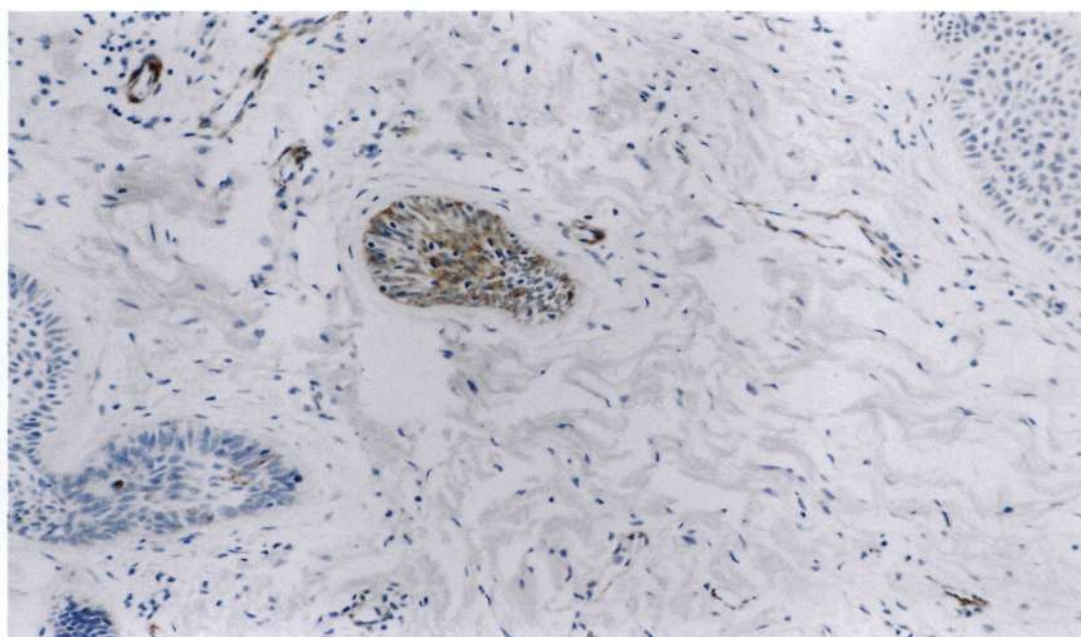


PLATE 44: ACTIN IMMUNOSTAINING
RG-SBCC: Exclusive epithelial MSA positivity. In-built control (blood vessels) positive.

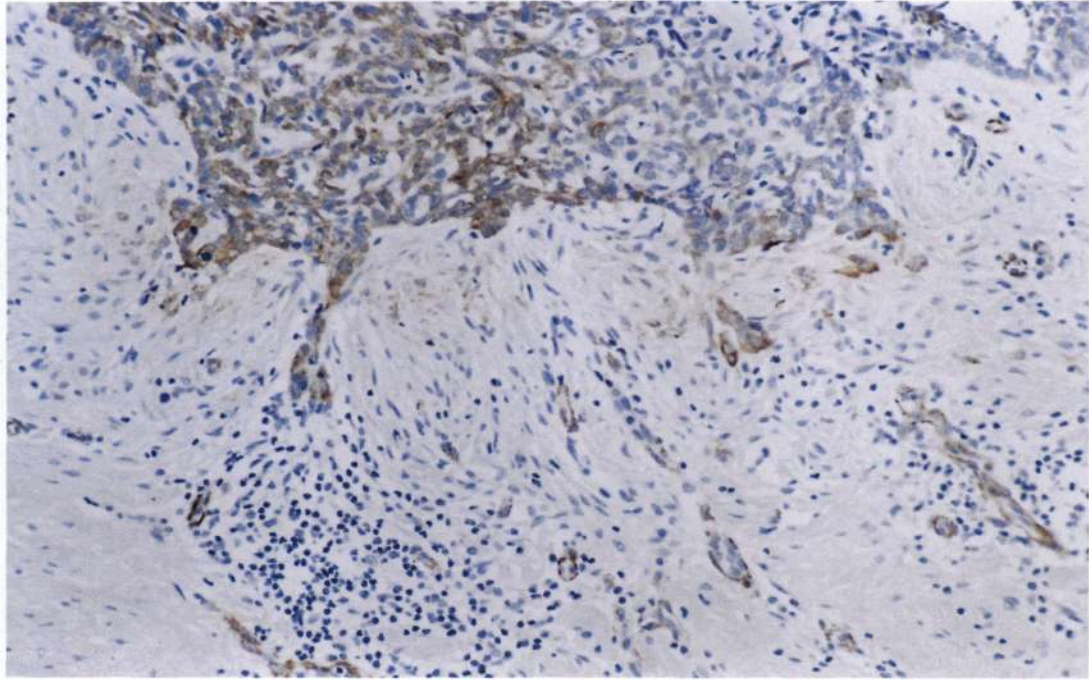


PLATE 45: ACTIN IMMUNOSTAINING

R-SBCC: MSA immunopositivity in epithelial and stromal components.

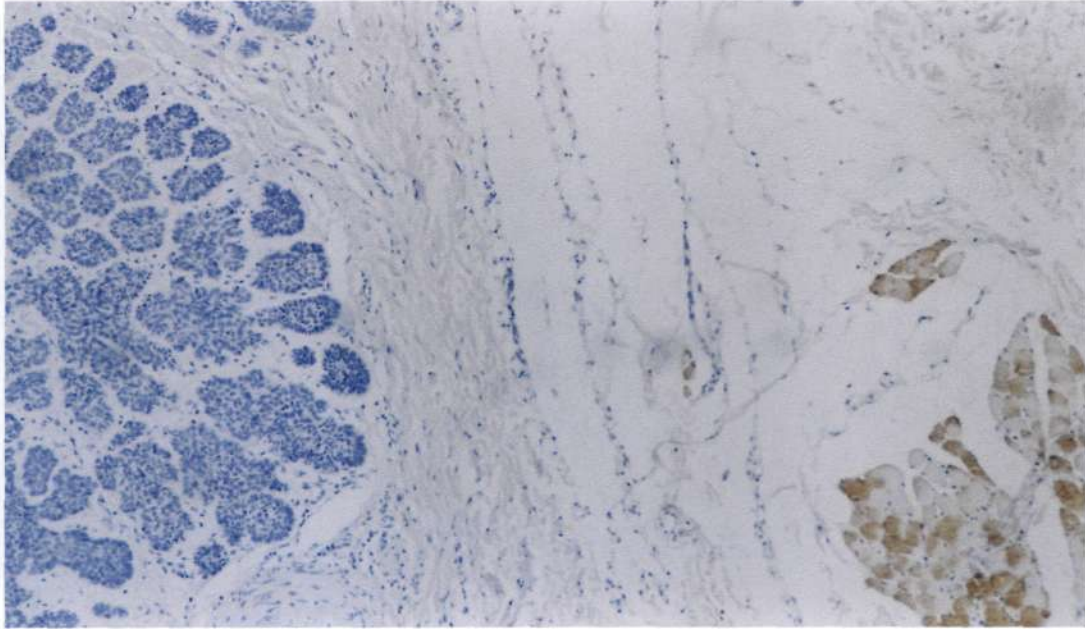


PLATE 46: DESMIN IMMUNOSTAINING

DN-SBCC: Negative peritumoral stromal cells. In-built control (skeletal muscle) positive.

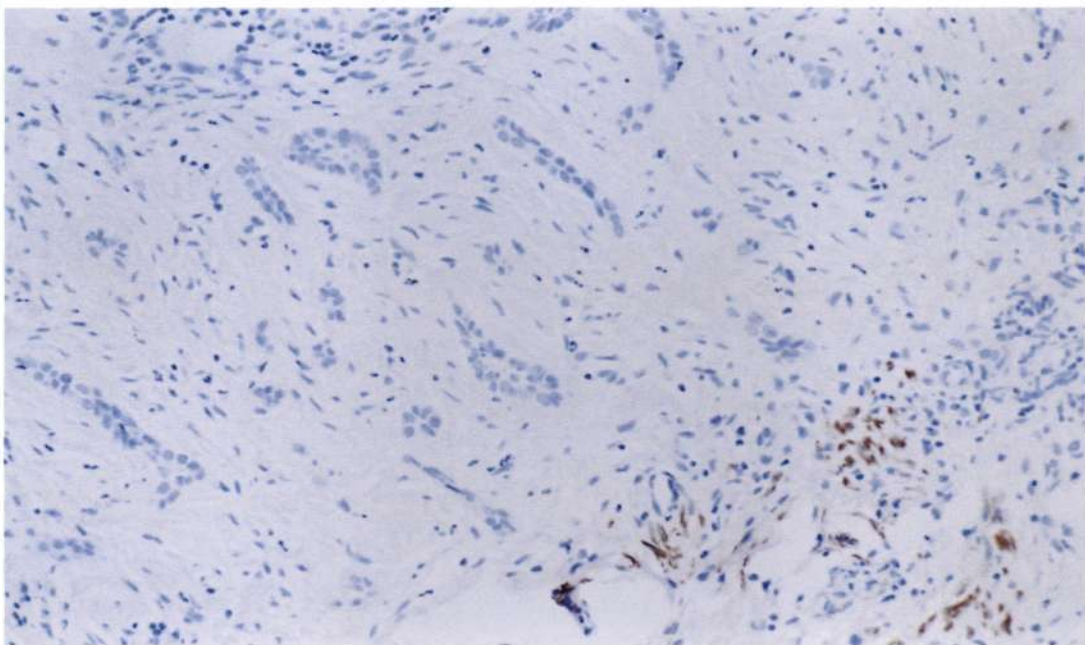


PLATE 47: DESMIN IMMUNOSTAINING

RG-SBCC: Negative peritumoral stromal cells. In-built control (muscular artery) positive.

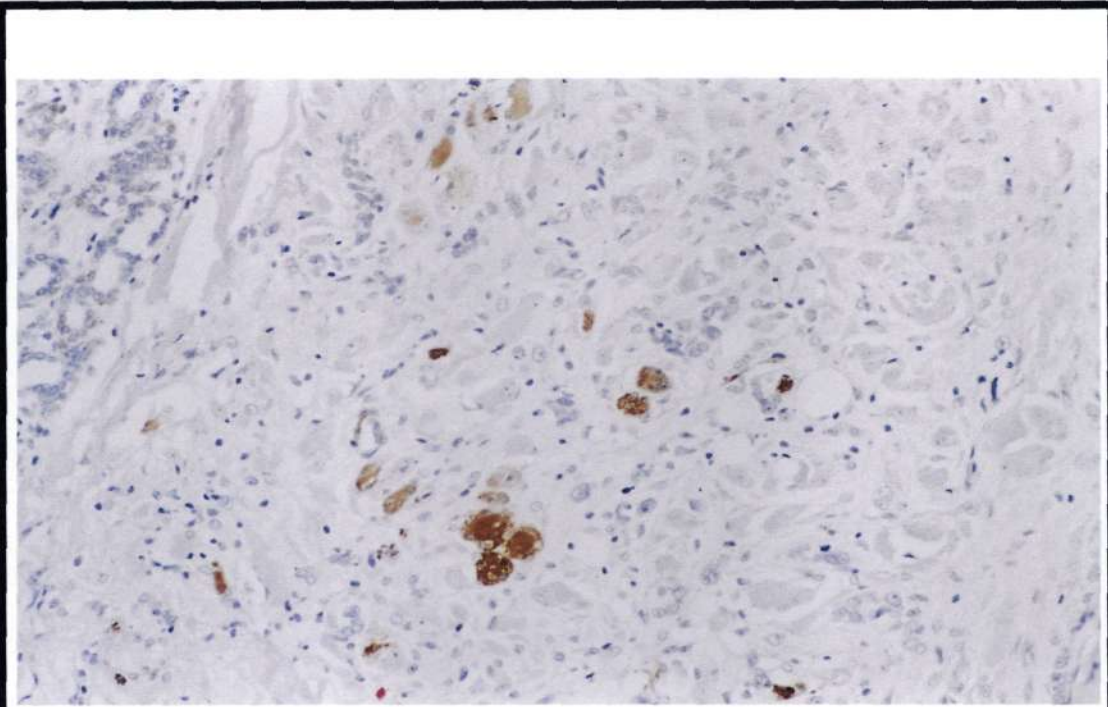


PLATE 48: DESMIN IMMUNOSTAINING

R-SBCC: Desmin negativity in scar tissue. In-built control (skeletal muscle) positive.

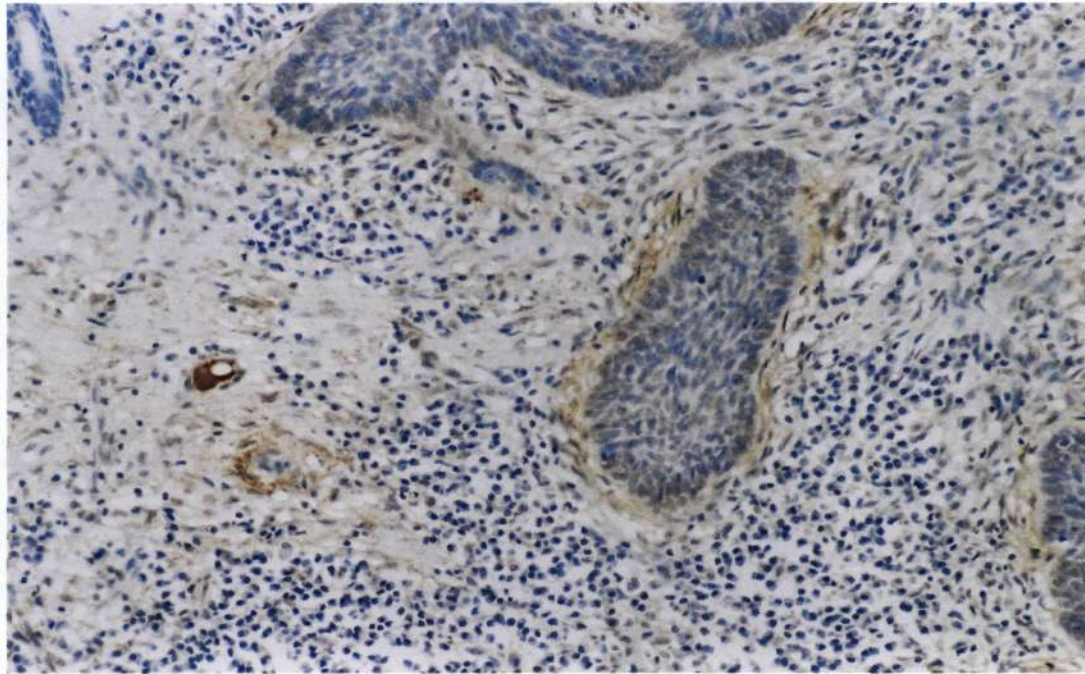


PLATE 49: VIMENTIN IMMUNOSTAINING
DN-SBCC: Vimentin staining of peritumoral spindle cells.

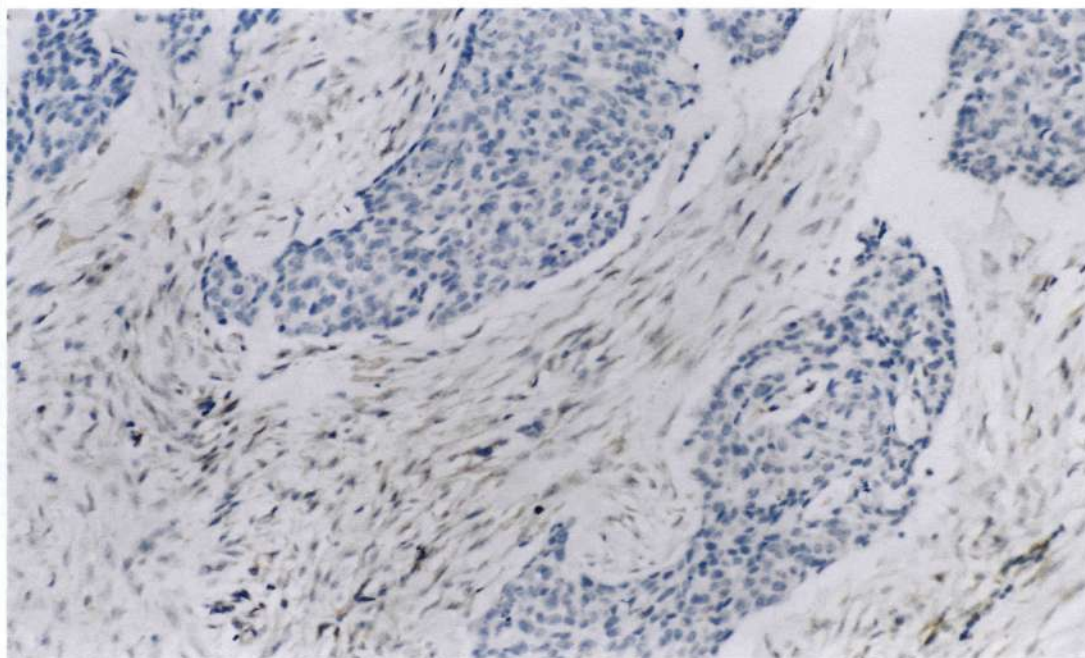


PLATE 50: VIMENTIN IMMUNOSTAINING
DN-SBCC: Vimentin-positive peritumoral stromal cells.

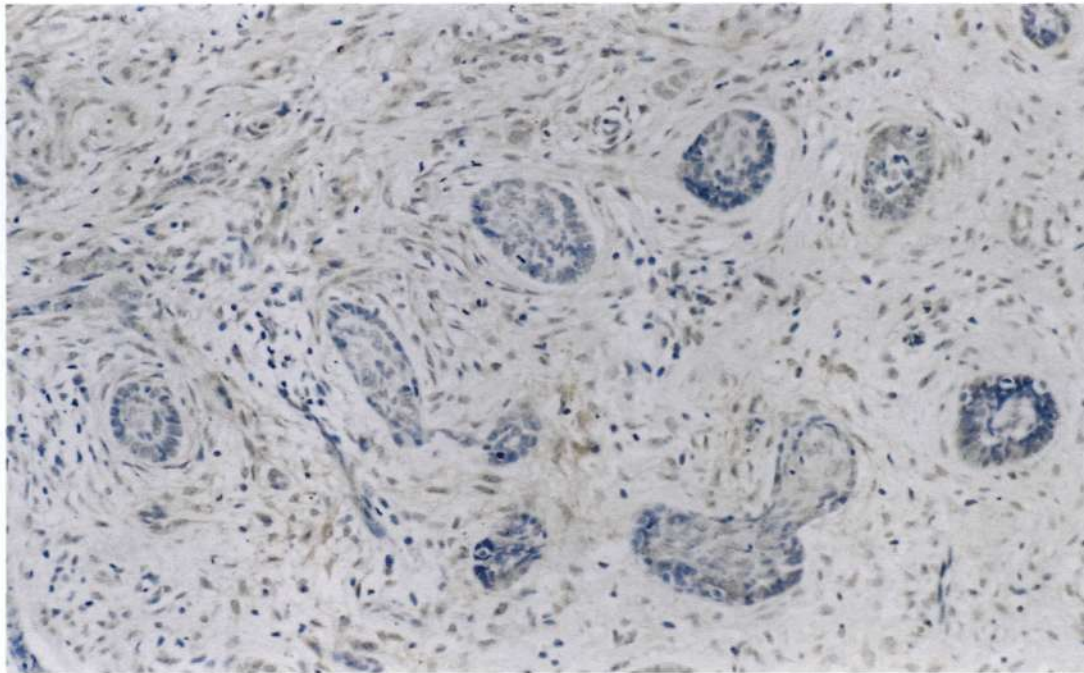


PLATE 51: VIMENTIN IMMUNOSTAINING

R-SBCC: Vimentin positive peritumoral stromal cells.

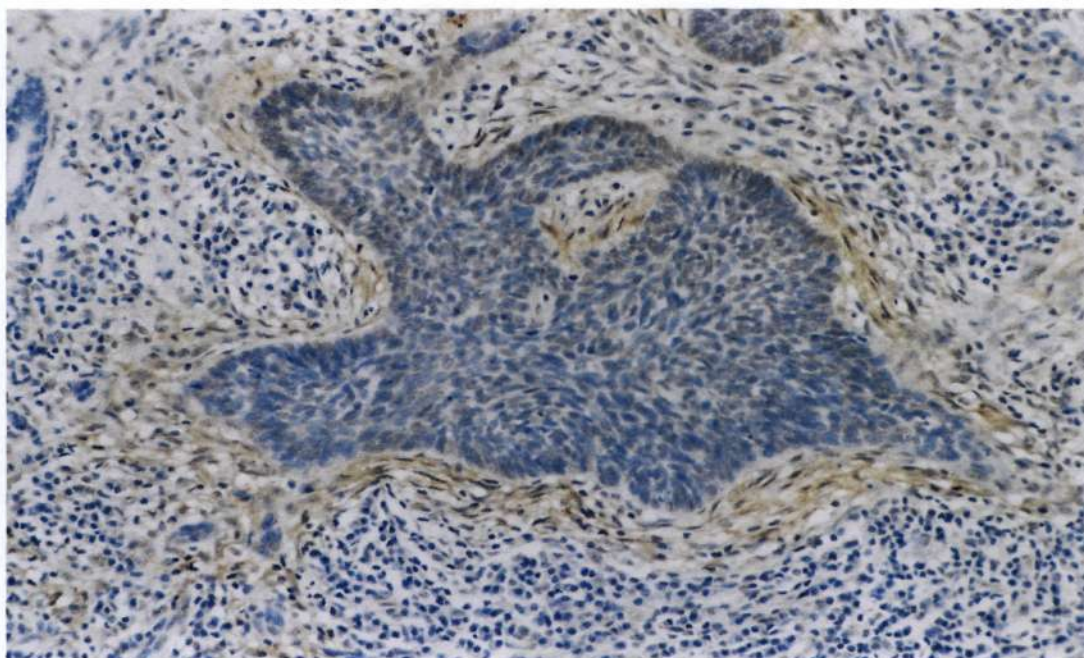


PLATE 52: VIMENTIN IMMUNOSTAINING

RG-SBCC: Vimentin staining of peritumoral stromal cellular response.

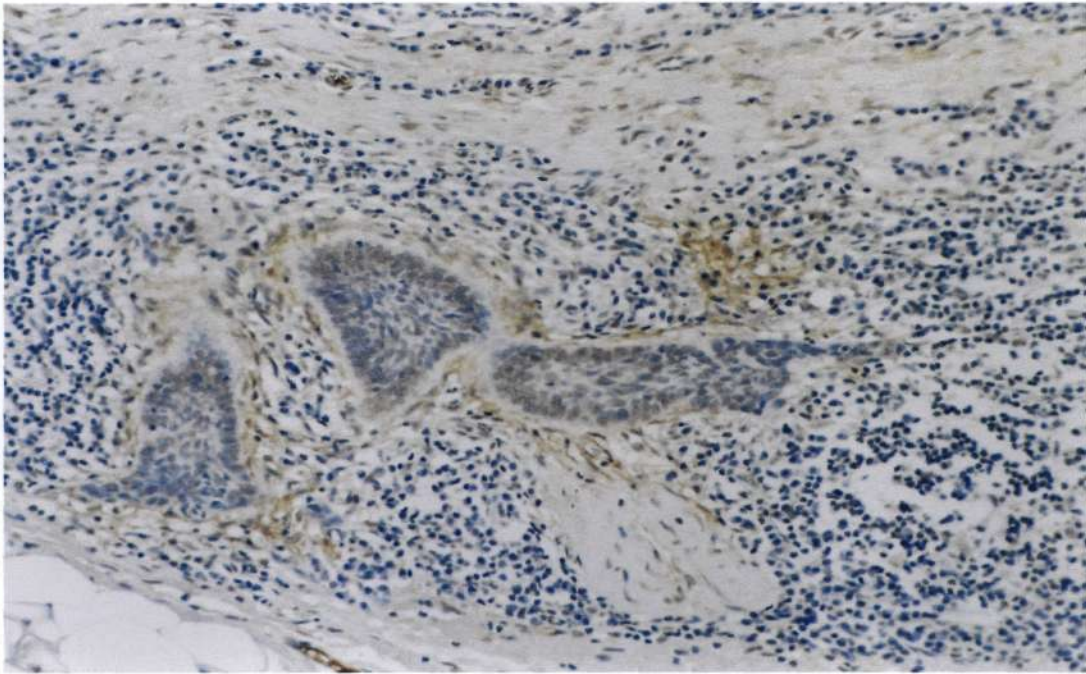


PLATE 53: VIMENTIN IMMUNOSTAINING

RG-SBCC: Vimentin positivity of peritumoral stromal cells.

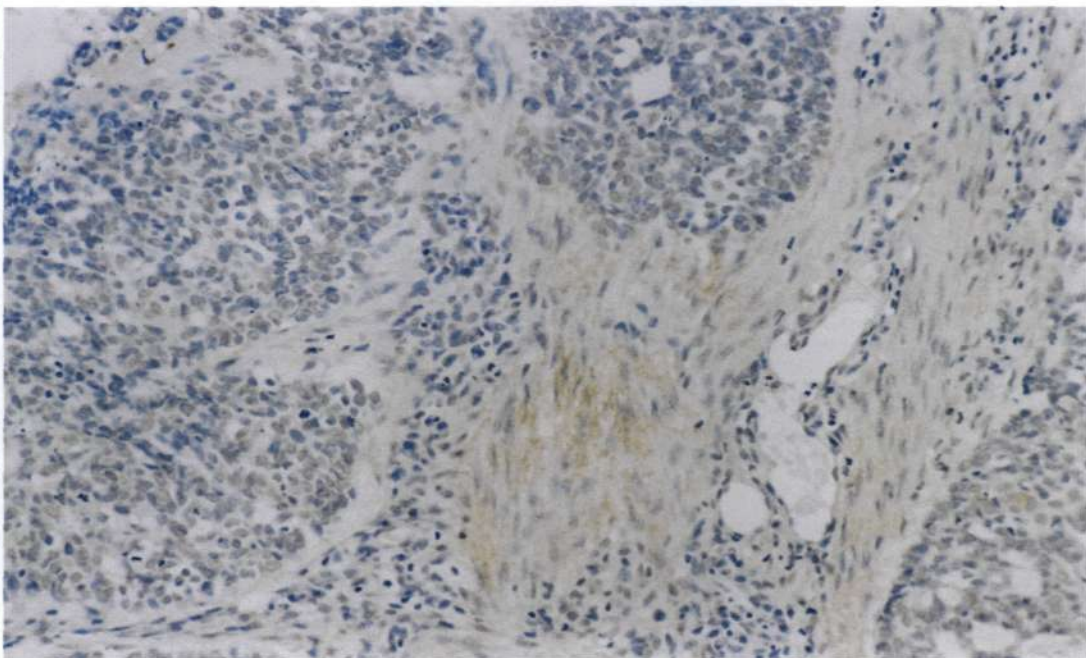


PLATE 54: VIMENTIN IMMUNOSTAINING

R-SBCC: Vimentin positive stromal cellular response.

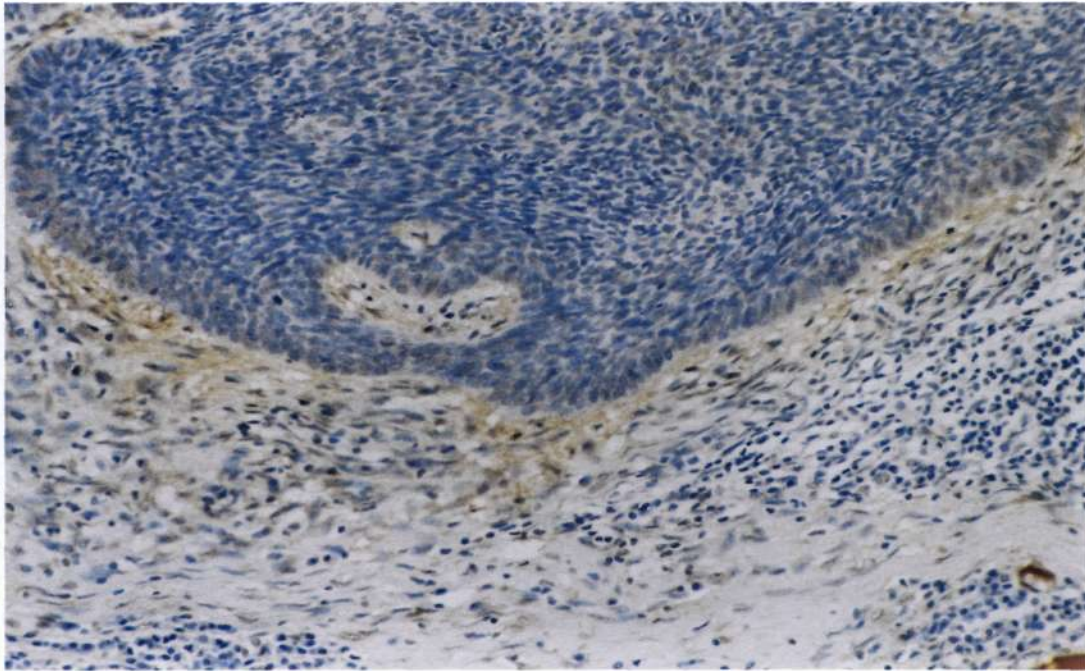


PLATE 55: VIMENTIN IMMUNOSTAINING

RG-SBCC: Peritumoral vimentin positive cells around nodular component.

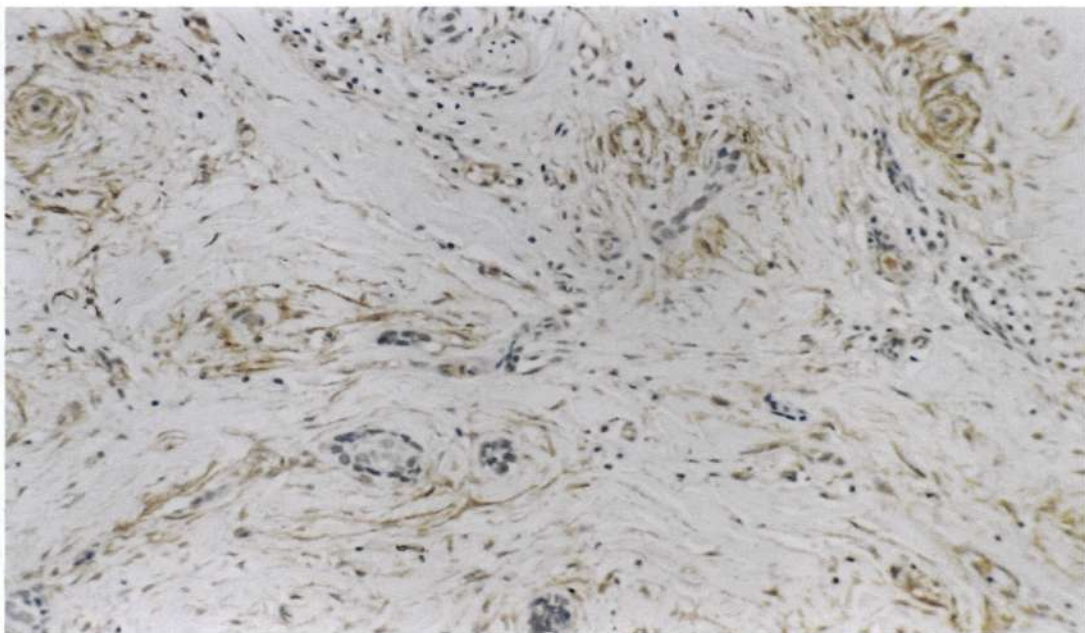


PLATE 56: VIMENTIN IMMUNOSTAINING

R-SBCC: Vimentin-positive stroma around micronodular and infiltrative tumour patterns.

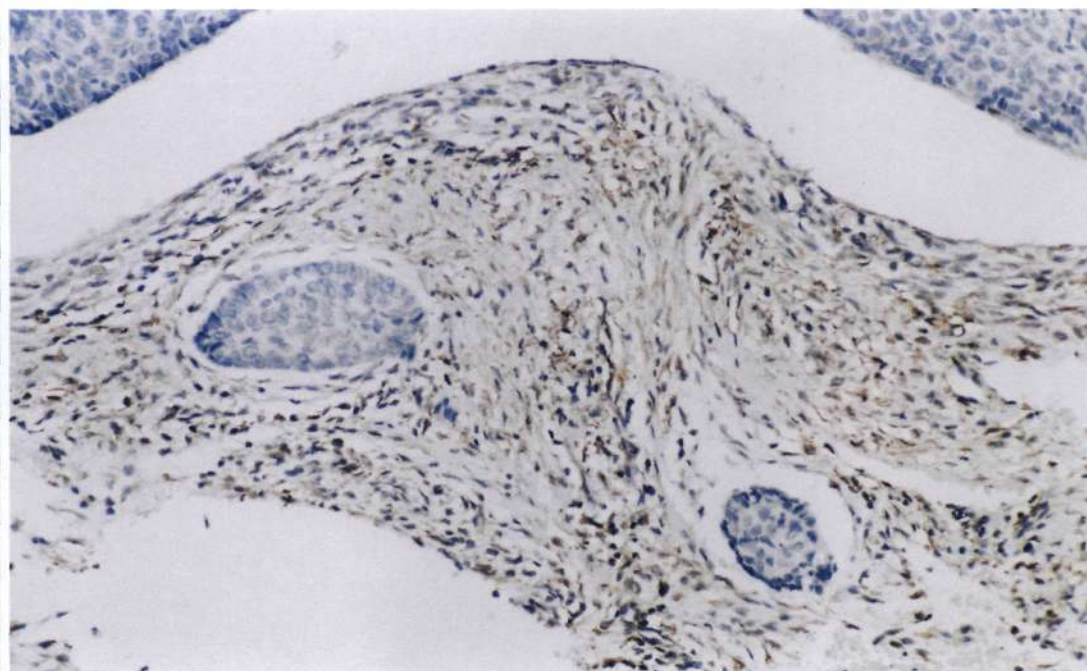


PLATE 57: VIMENTIN IMMUNOSTAINING

R-SBCC: Vimentin-positive peritumoral stromal cells.

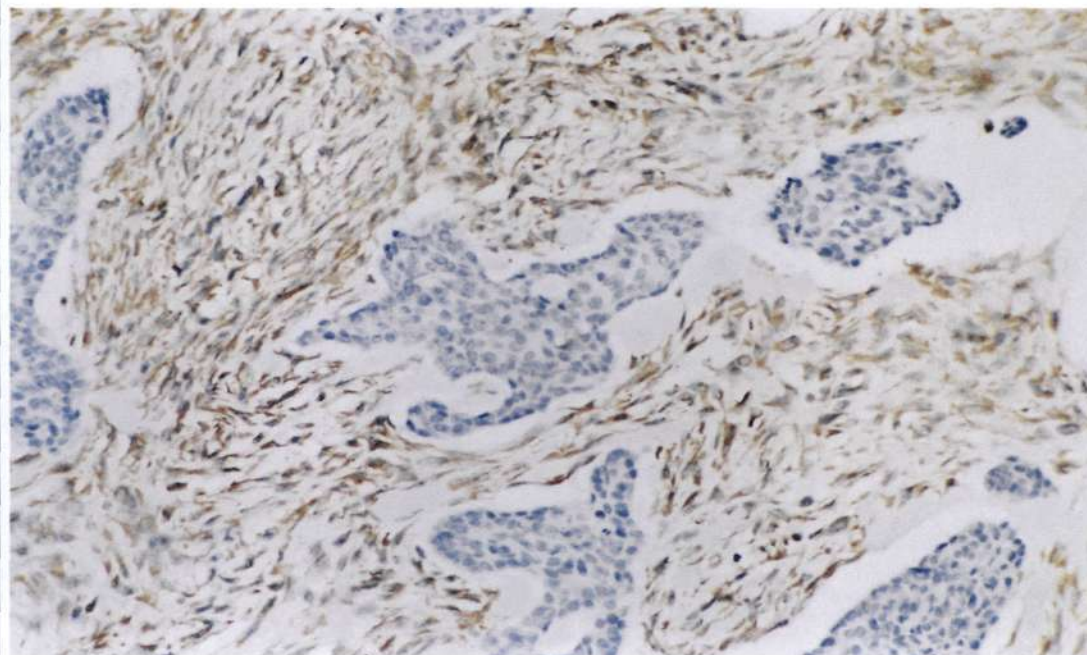


PLATE 58: VIMENTIN IMMUNOSTAINING

R-SBCC: Vimentin-positive peritumoral stromal cells.

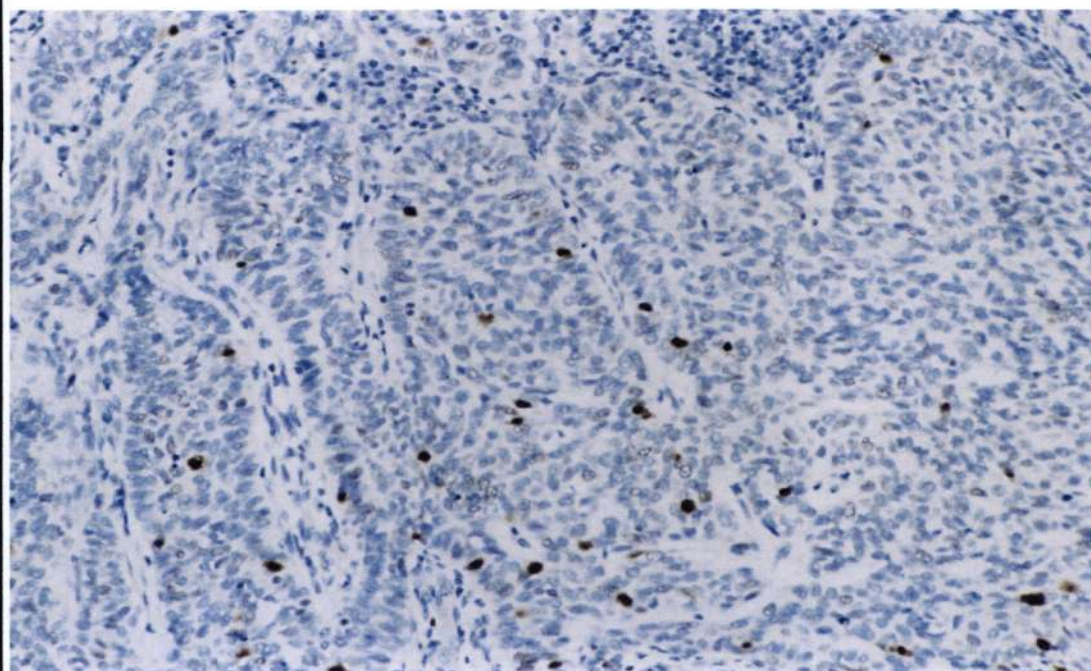


PLATE 59: MIB1 IMMUNOSTAINING

DN-SBCC: Low peripheral and central MIB1 staining.

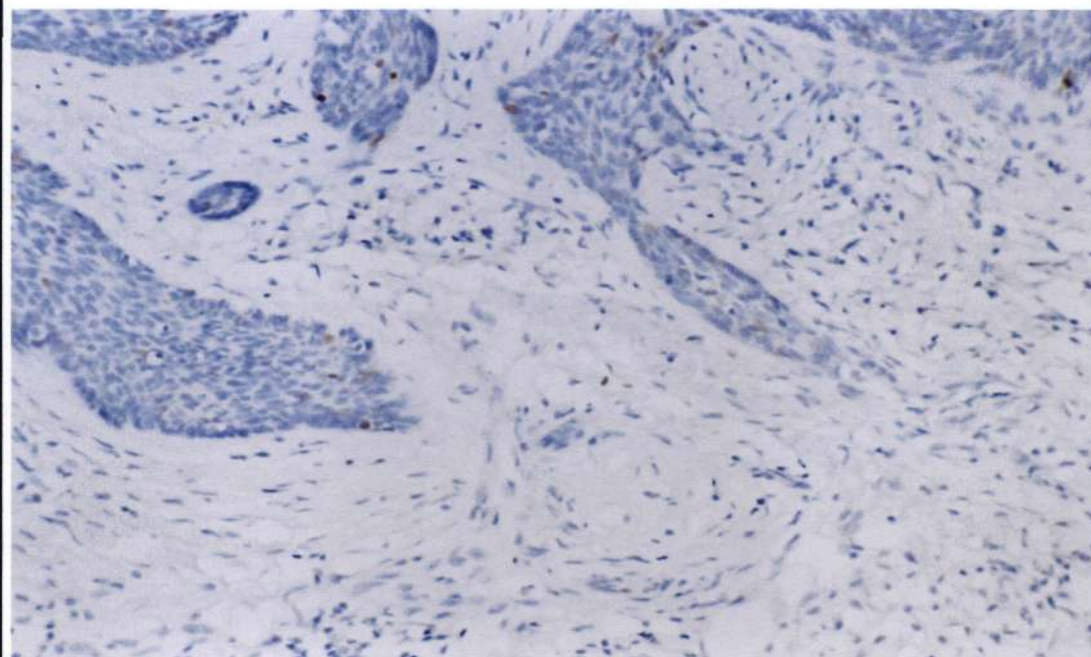


PLATE 60: MIB1 IMMUNOSTAINING

DN-SBCC: Low (1+) MIB1 staining in infiltrative component.

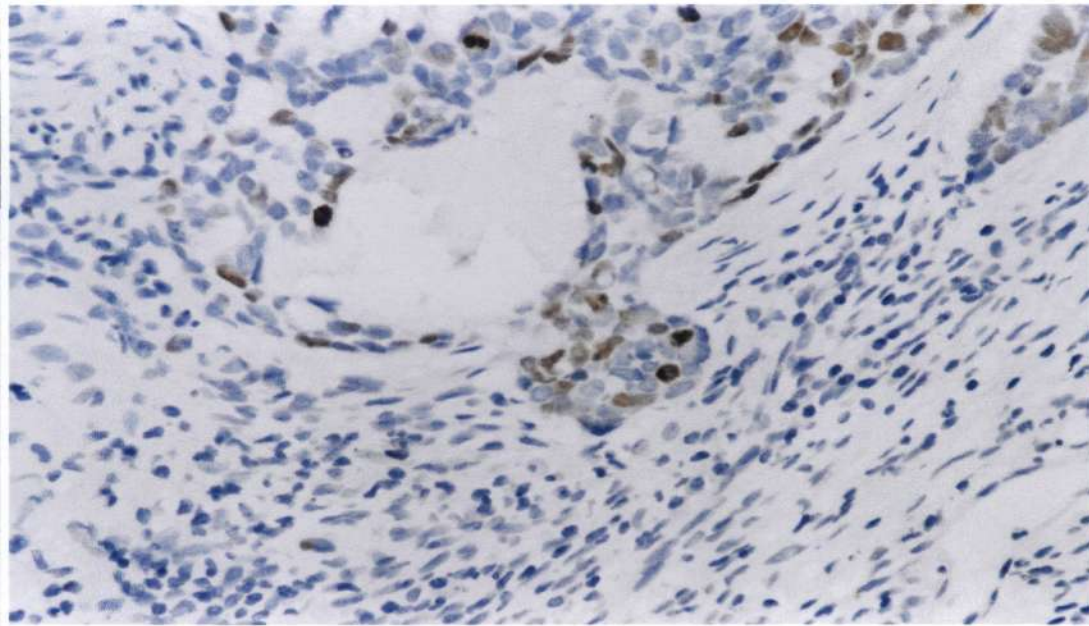


PLATE 61: MIB1 IMMUNOSTAINING

DN-SBCC: Accentuated staining in infiltrative focus at tumor-stroma interface.

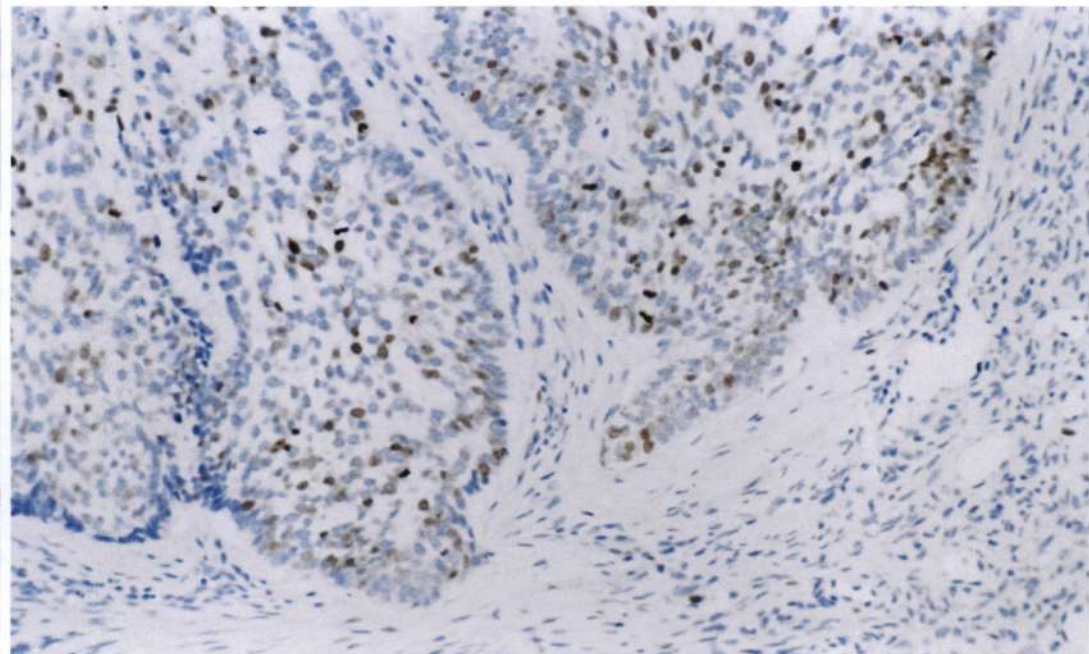


PLATE 62: MIB1 IMMUNOSTAINING

DN-SBCC: High (3+) peripheral and central MIB1 staining.

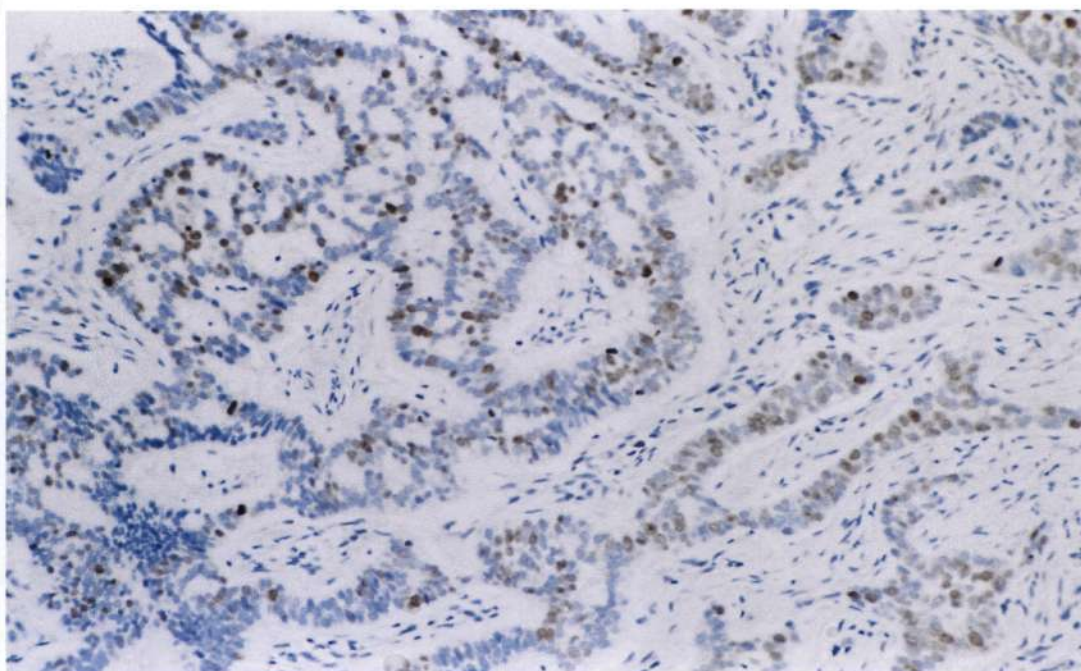


PLATE 63: MIB1 IMMUNOSTAINING

DN-SBCC: High (3+) MIB1 staining in infiltrative nests and trabeculae.

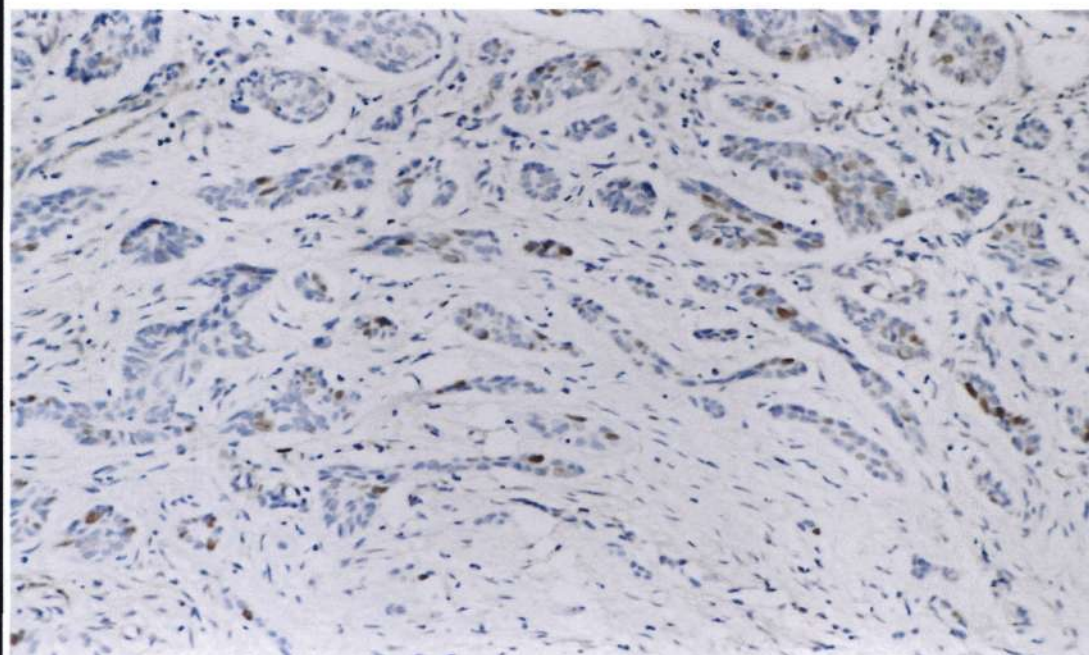


PLATE 64: MIB1 IMMUNOSTAINING

R-SBCC: Low MIB1 staining in infiltrative component.

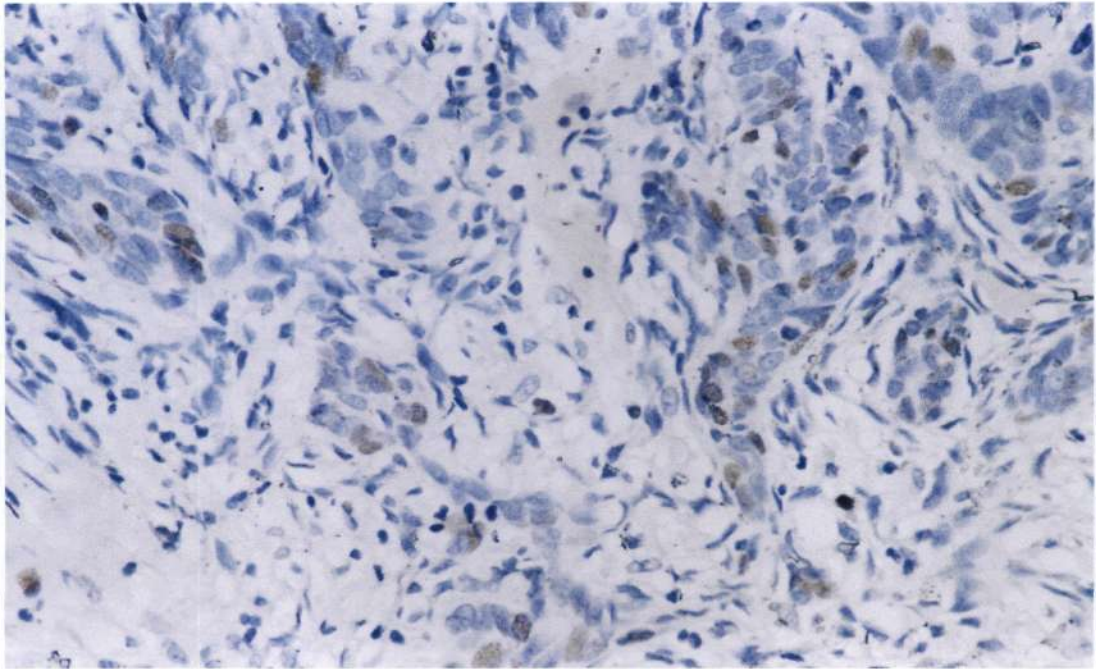


PLATE 65: MIB1 IMMUNOSTAINING
R-SBCC: Low MIB1 staining in infiltrative focus.

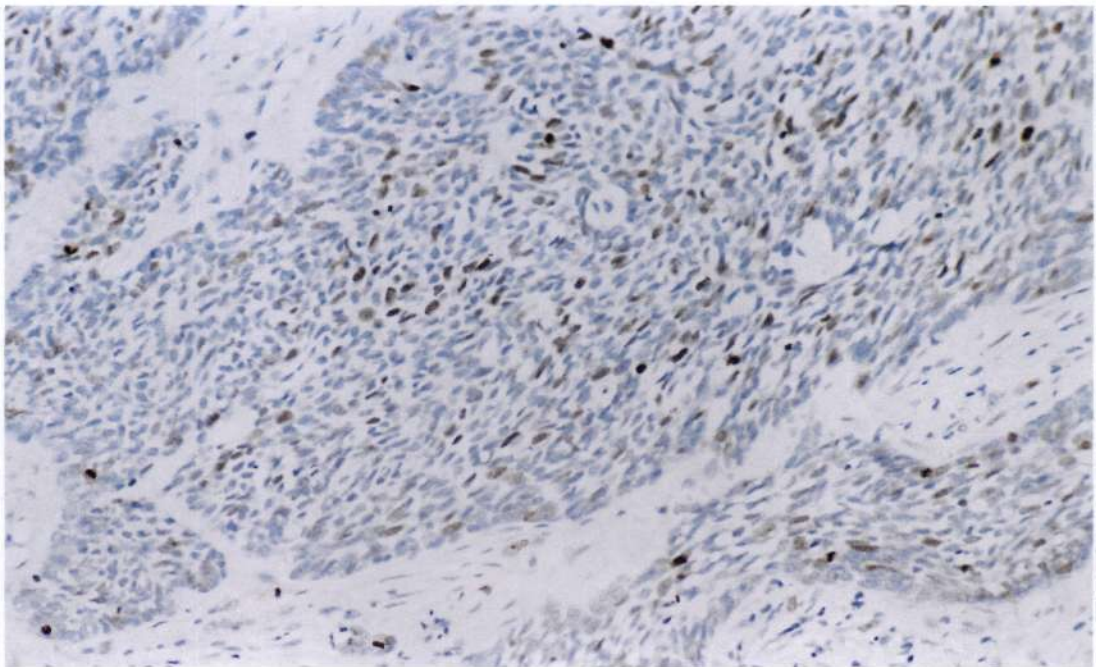


PLATE 66: MIB1 IMMUNOSTAINING
R-SBCC: High MIB1 staining in infiltrative component.

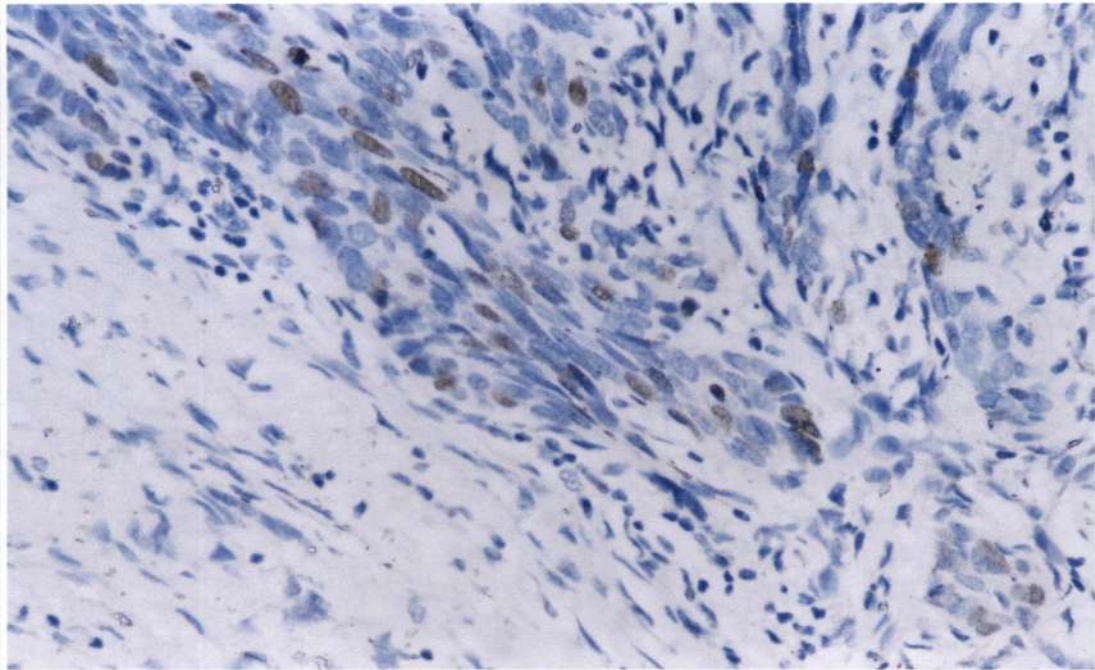


PLATE 67: MIB1 IMMUNOSTAINING
R-SBCC: High MIB1 staining in infiltrative focus.

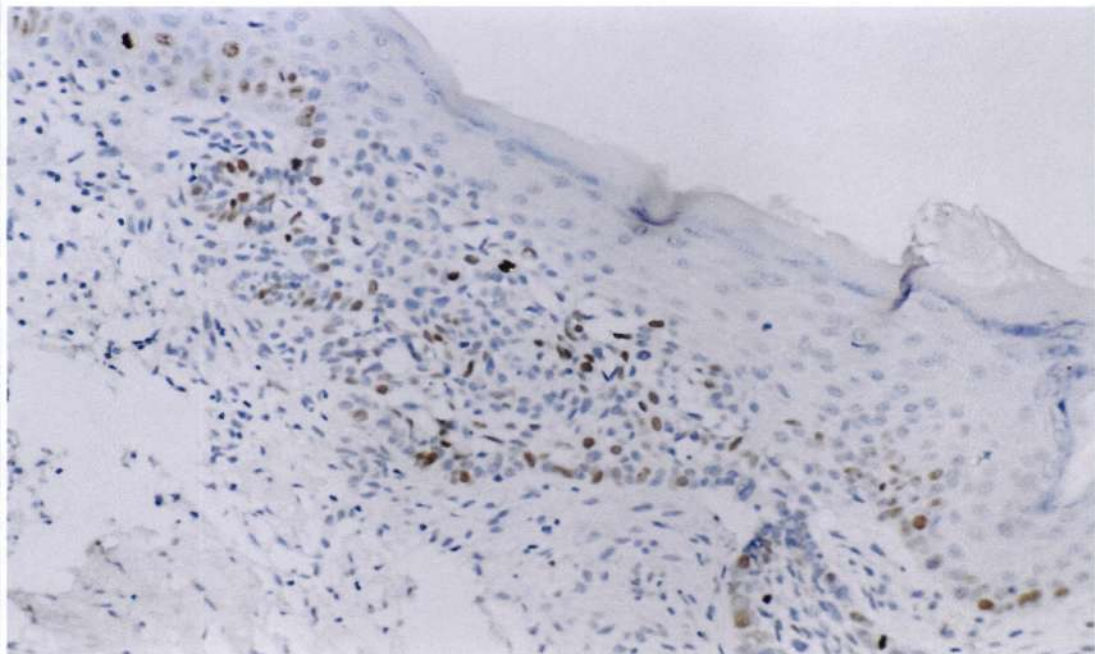


PLATE 68: MIB1 IMMUNOSTAINING
R-SBCC: High MIB1 staining in superficial pattern.

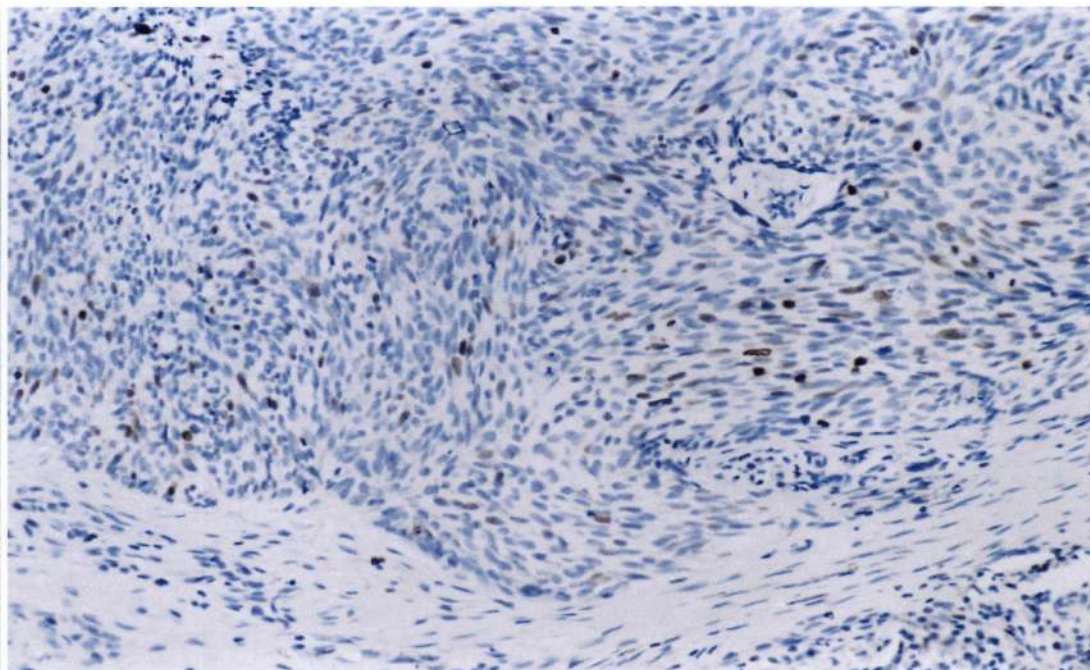


PLATE 69: MIB1 IMMUNOSTAINING

RG-SBCC: Low (2+) MIB1 staining in nodular and infiltrative focus.

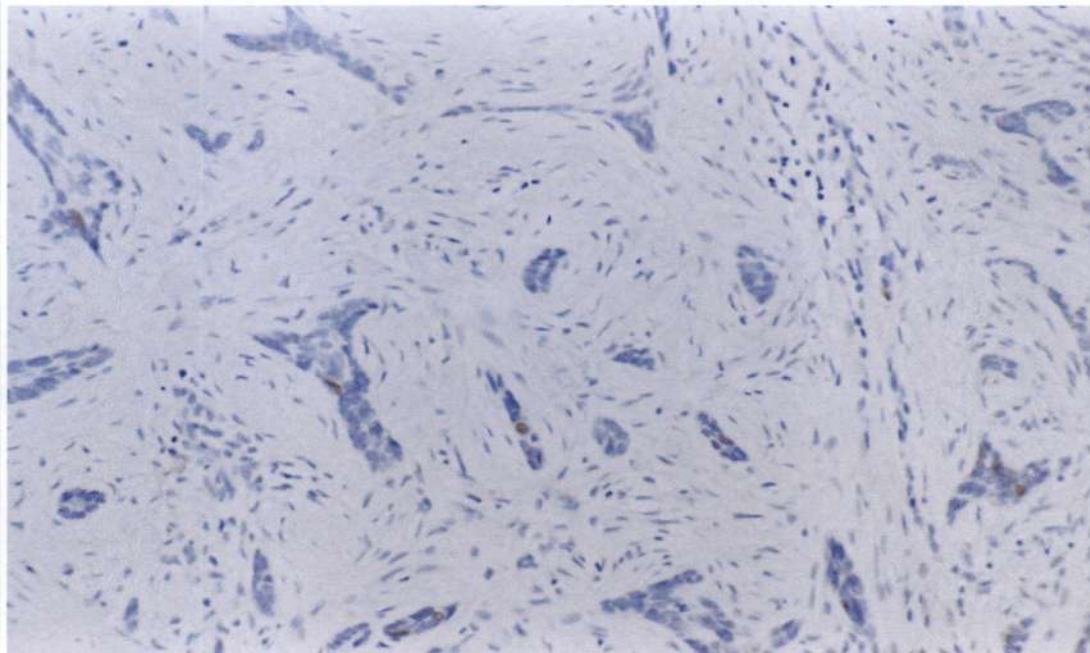


PLATE 70: MIB1 IMMUNOSTAINING

RG-SBCC: Low MIB1 staining in infiltrative component.

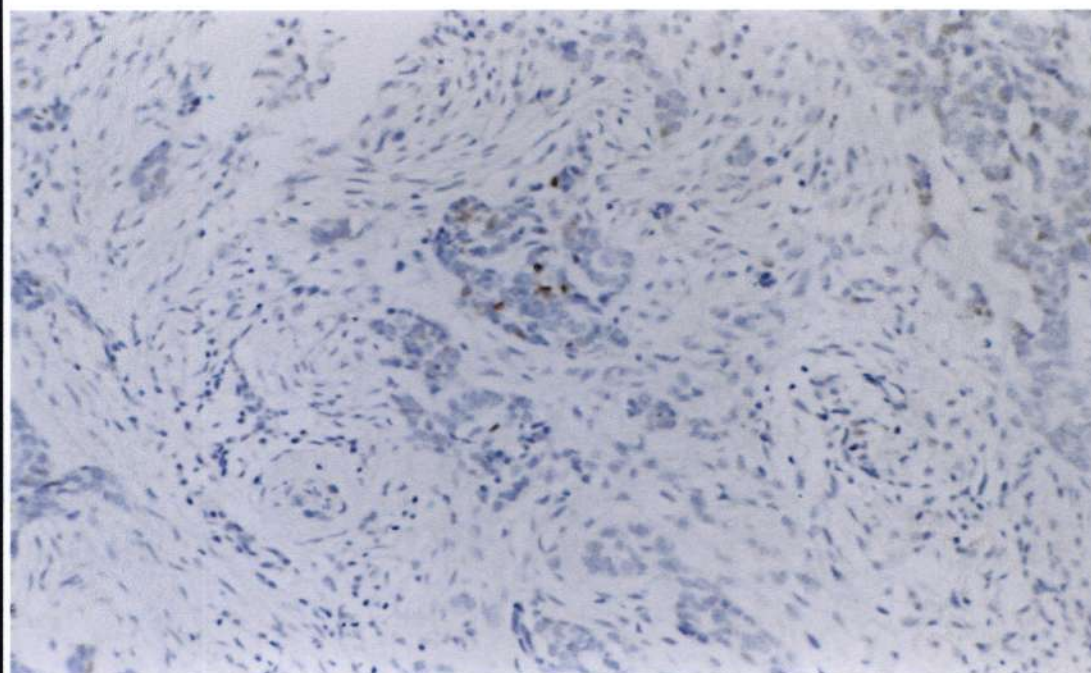


PLATE 71: MIB1 IMMUNOSTAINING

RG-SBCC: Low MIB1 staining in infiltrative focus.

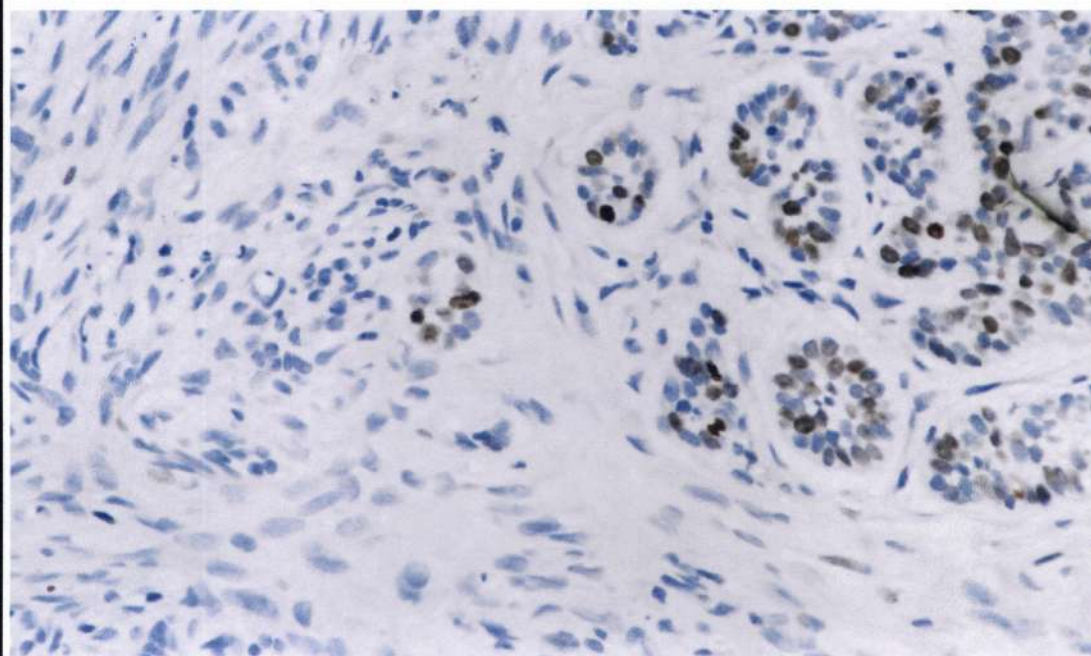


PLATE 72: MIB1 IMMUNOSTAINING

RG-SBCC: High MIB1 staining in micronodular aggregates.

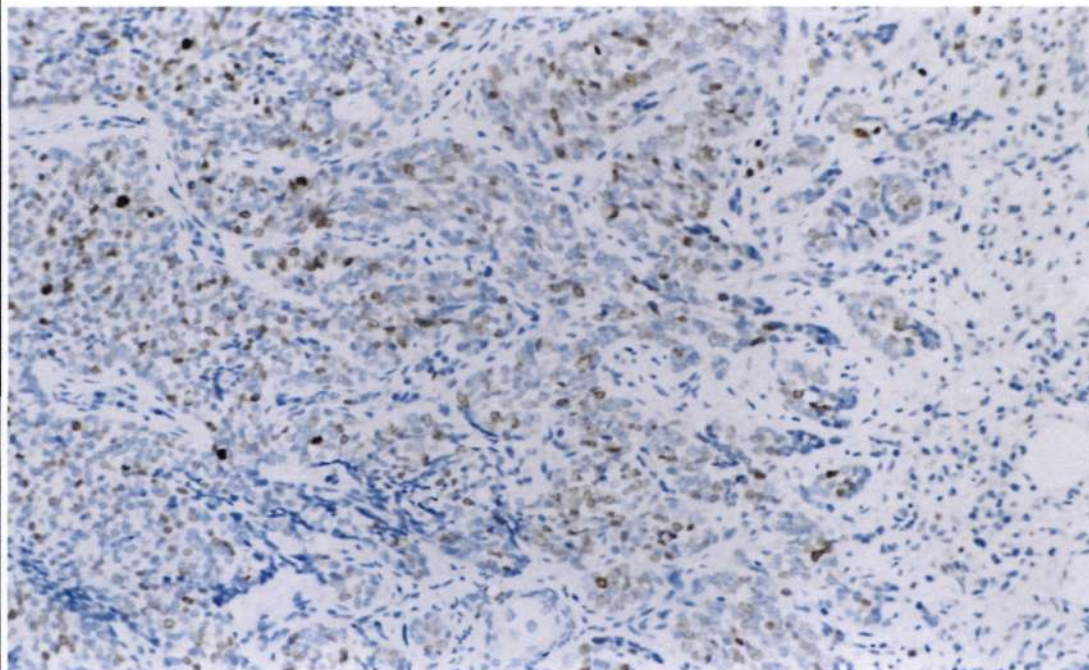


PLATE 73: MIB1 IMMUNOSTAINING

RG-SBCC: High MIB1 staining in infiltrative component.

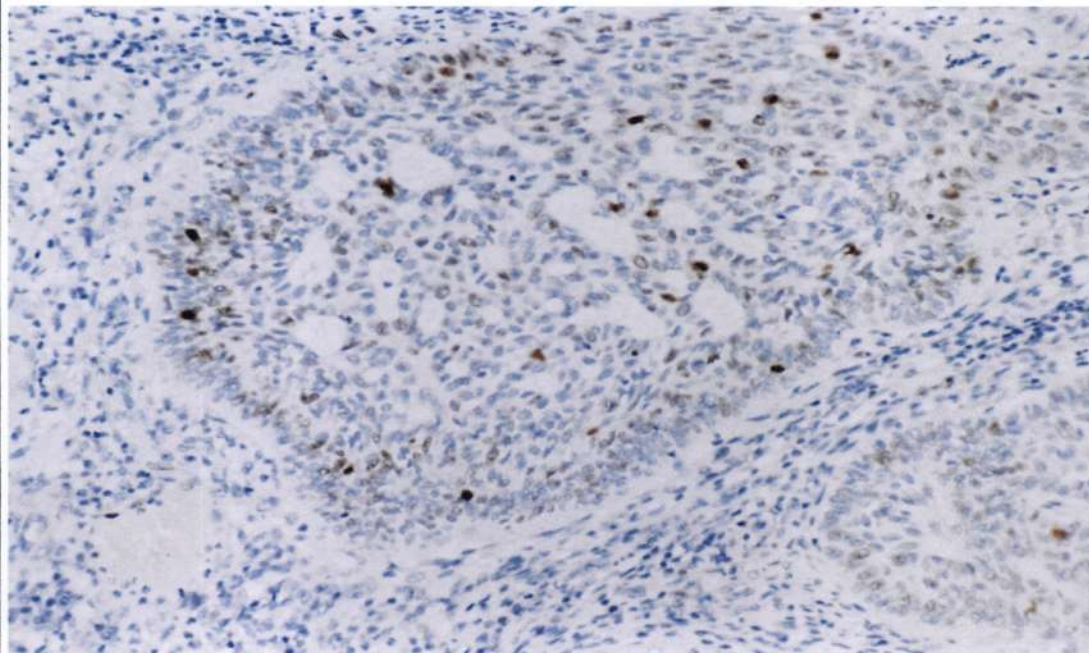


PLATE 74: MIB1 IMMUNOSTAINING

RG-SBCC: High MIB1 staining in nodular component.

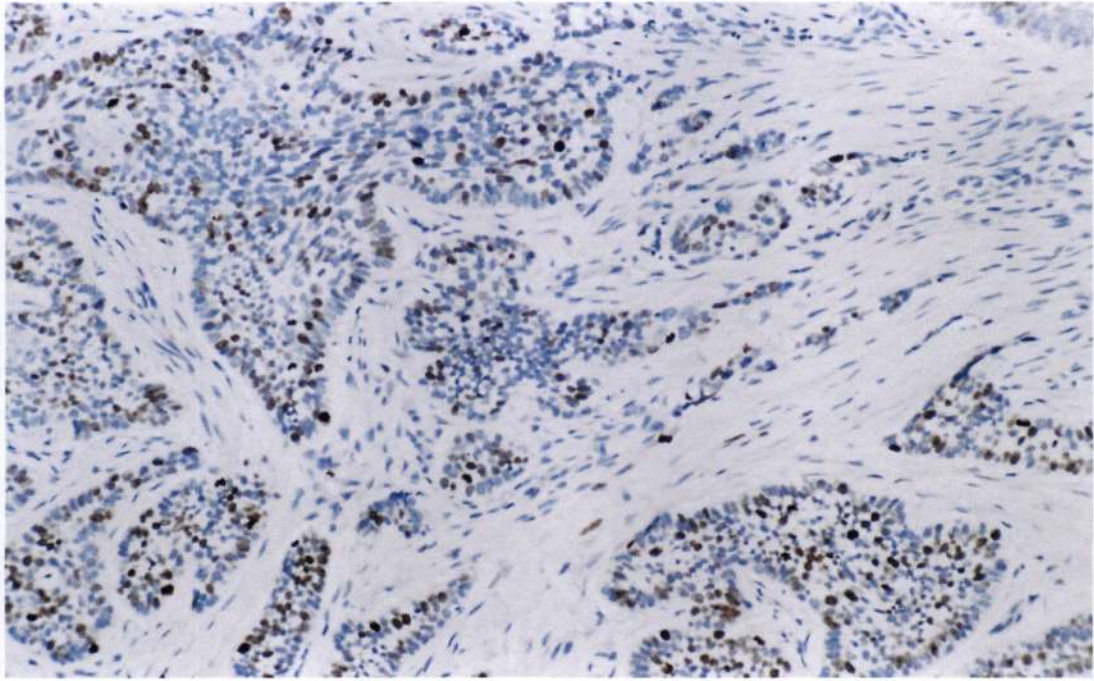


PLATE 75: MIB1 IMMUNOSTAINING

RG-SBCC: High MIB1 staining in nodular and infiltrative component

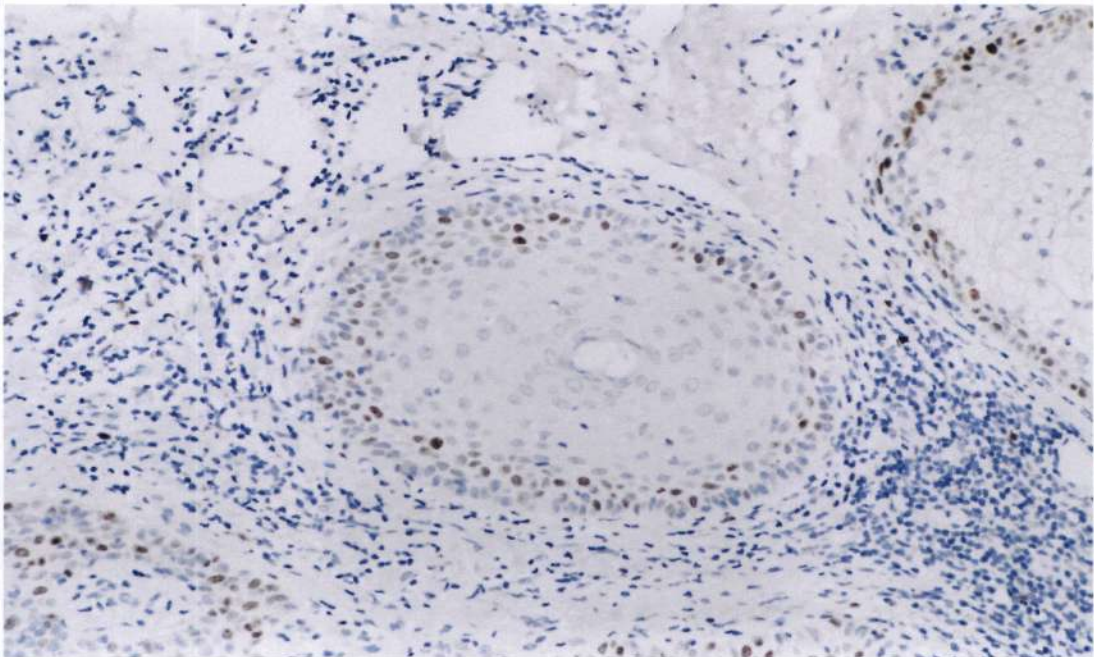


PLATE 76: MIB1 IMMUNOSTAINING

DN-SBCC: Sebaceous glands and hair follicle epithelium: in-built control.

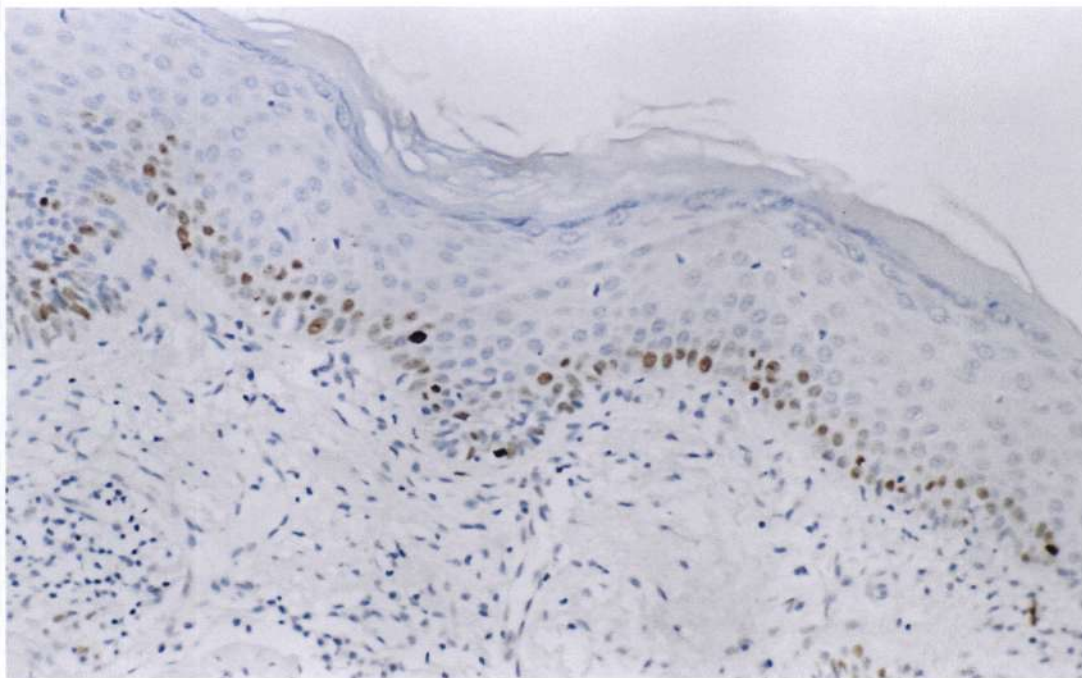


PLATE 77: MIB1 IMMUNOSTAINING

R-SBCC: Basal layer MIB1 positivity in epidermis: in-built control.

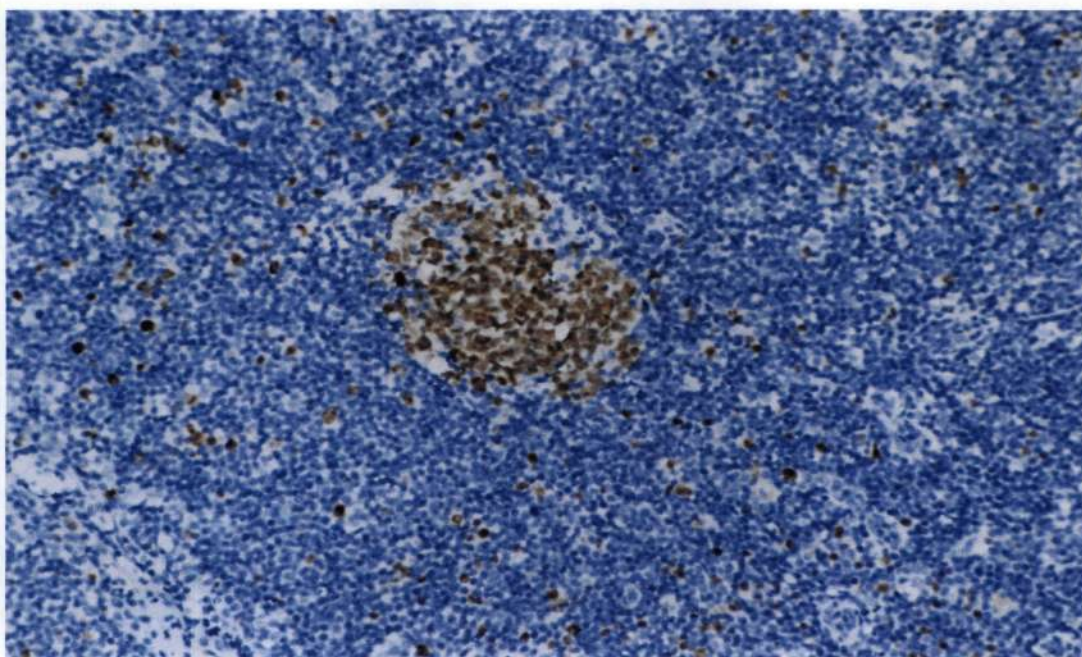


PLATE 78: MIB1 IMMUNOSTAINING

Nodal + MIB1 positivity: External positive control.

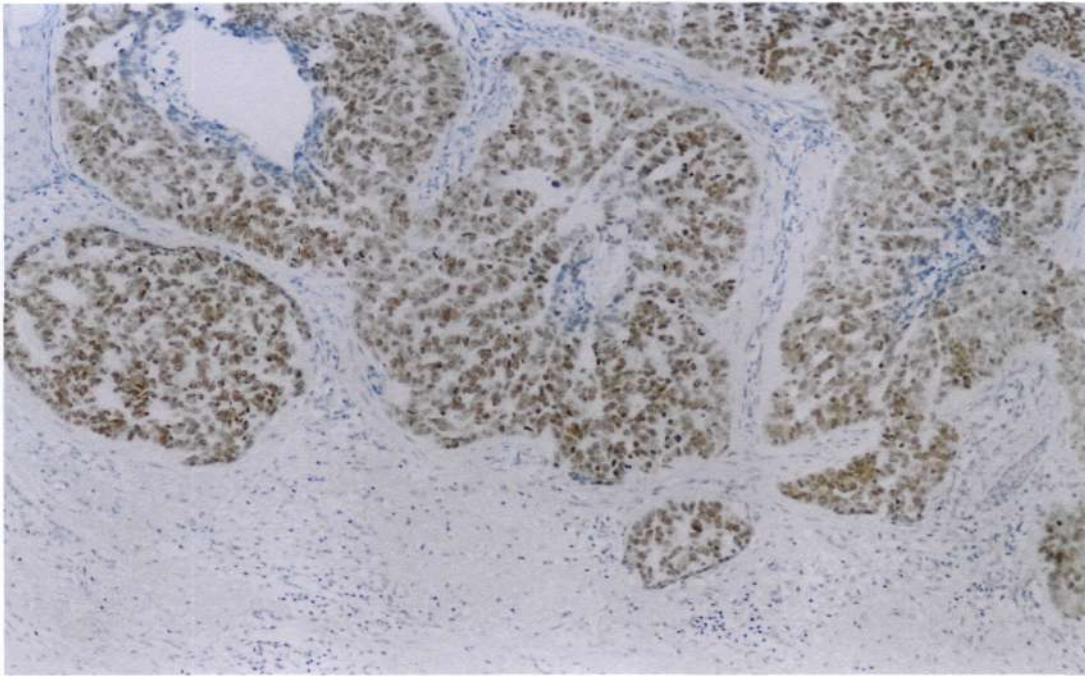


PLATE 79: p53 IMMUNOSTAINING
DN-SBCC: High p53 immunopositivity.

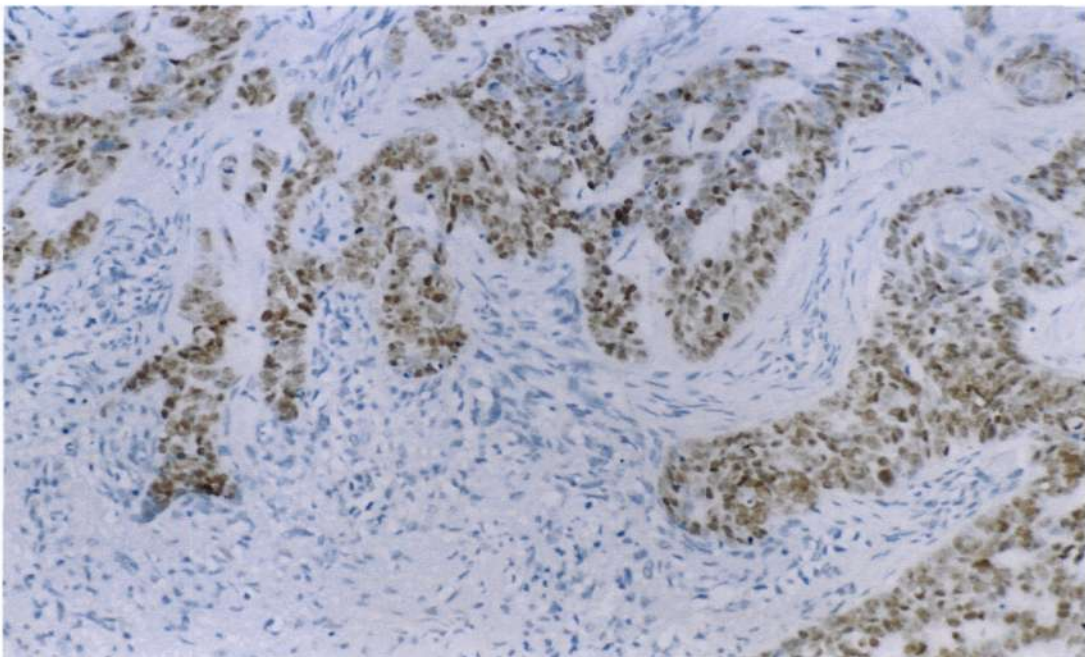


PLATE 80: p53 IMMUNOSTAINING
DN-SBCC: High p53 immunopositivity.

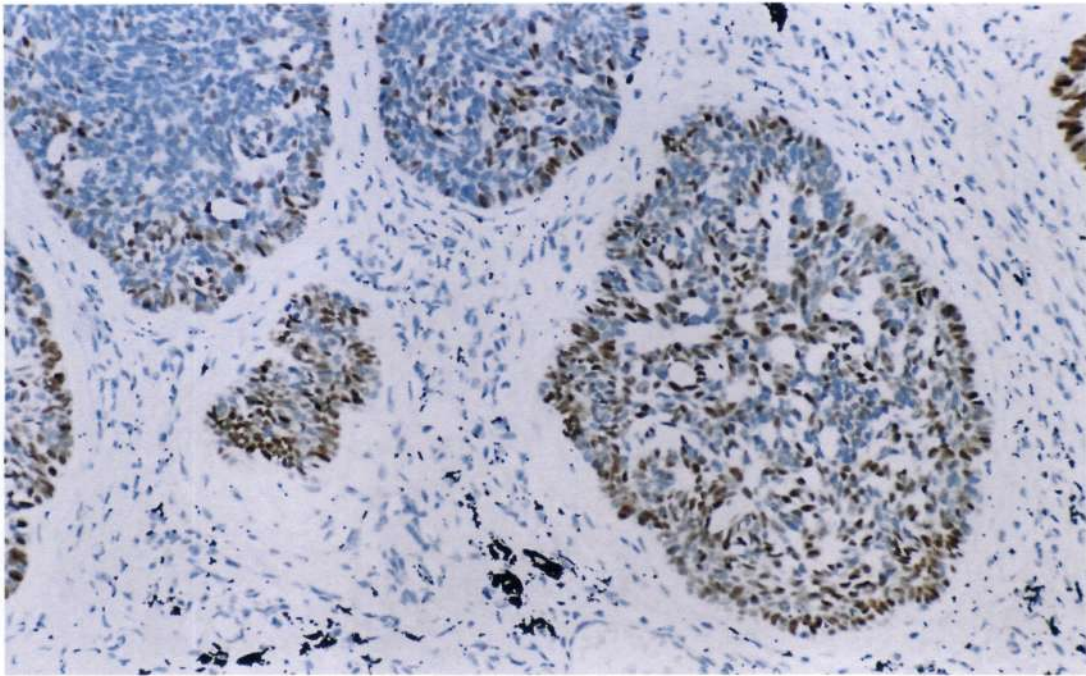


PLATE 81: p53 IMMUNOSTAINING
DN-SBCC: High p53 immunopositivity.

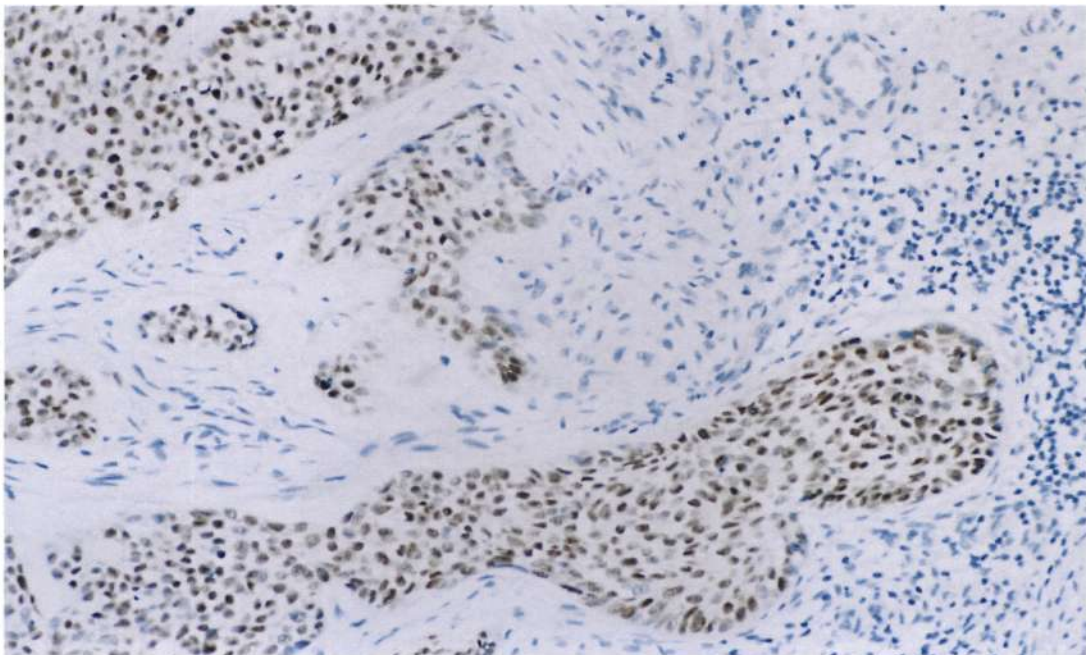


PLATE 82: p53 IMMUNOSTAINING
DN-SBCC: High p53 immunopositivity.

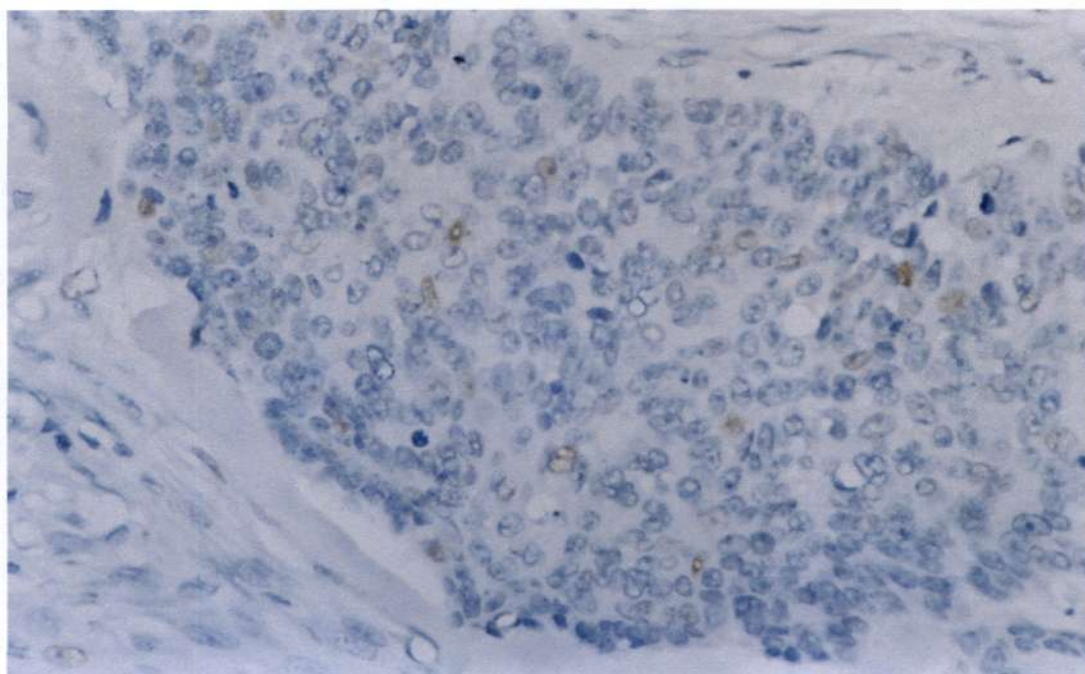


PLATE 83: p53 IMMUNOSTAINING
DN-SBCC: Low p53 immunopositivity.

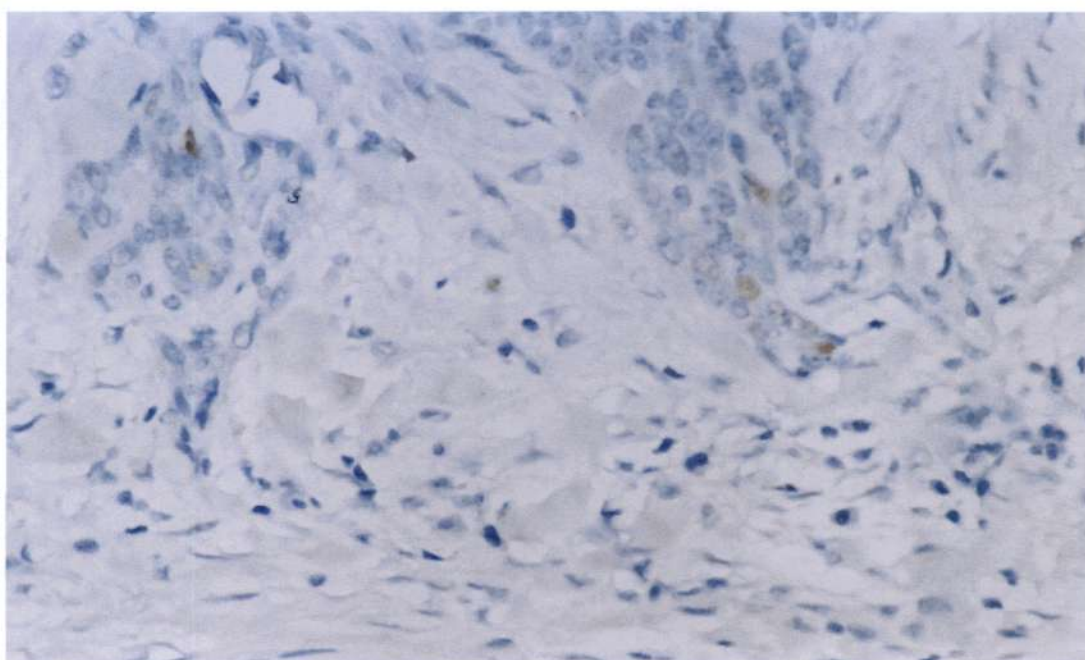


PLATE 84: p53 IMMUNOSTAINING
DN-SBCC: Low p53 immunopositivity.

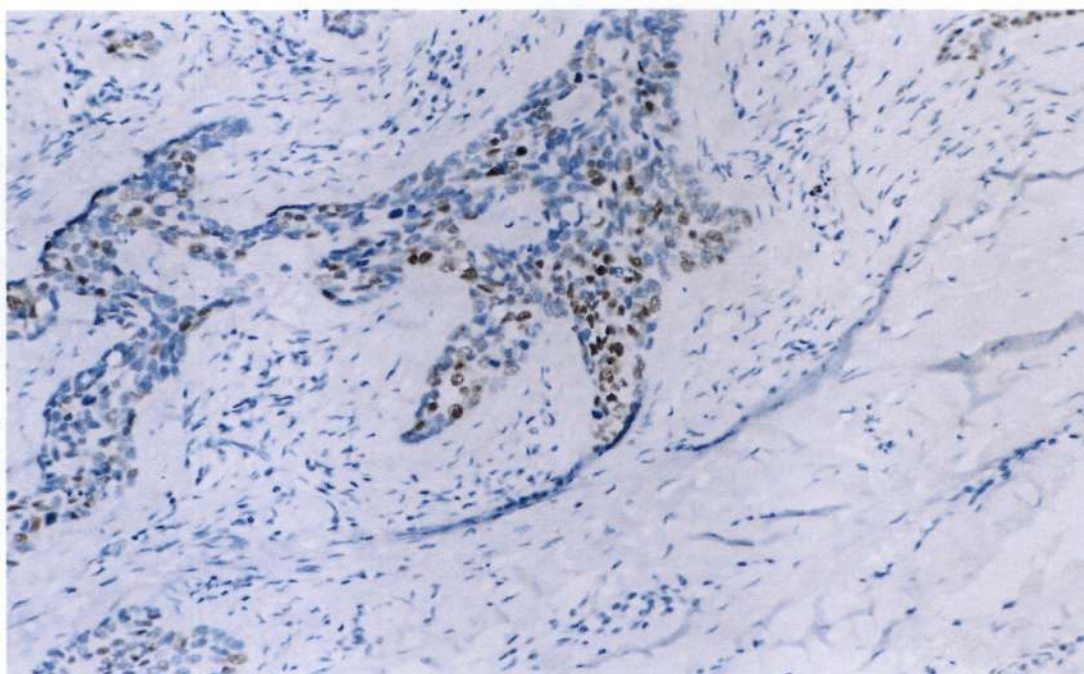


PLATE 85: p53 IMMUNOSTAINING

DN-SBCC: Accentuated p53 immunostaining in infiltrative tongue.

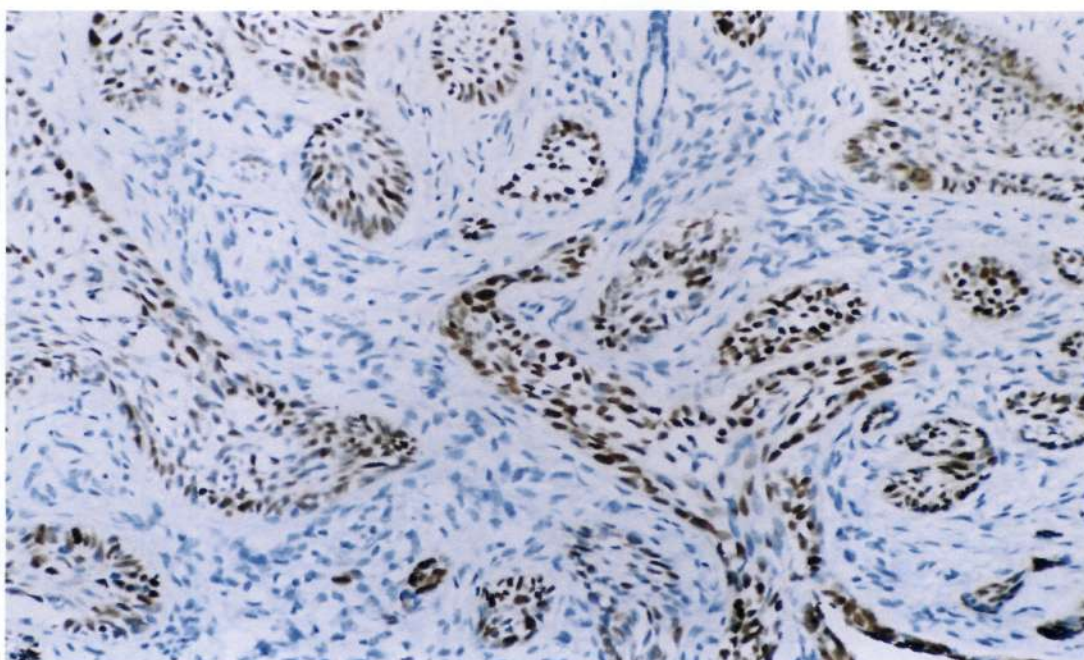


PLATE 86: p53 IMMUNOSTAINING

R-SBCC: High p53 immunopositivity.

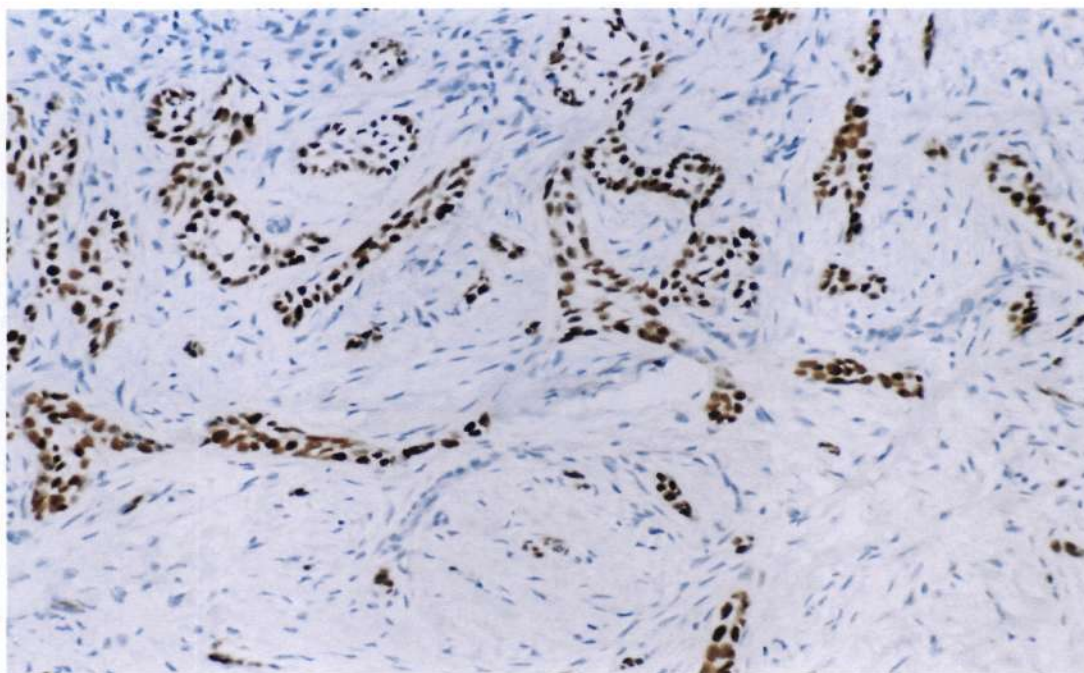


PLATE 87: p53 IMMUNOSTAINING
R-SBCC: High p53 immunopositivity.

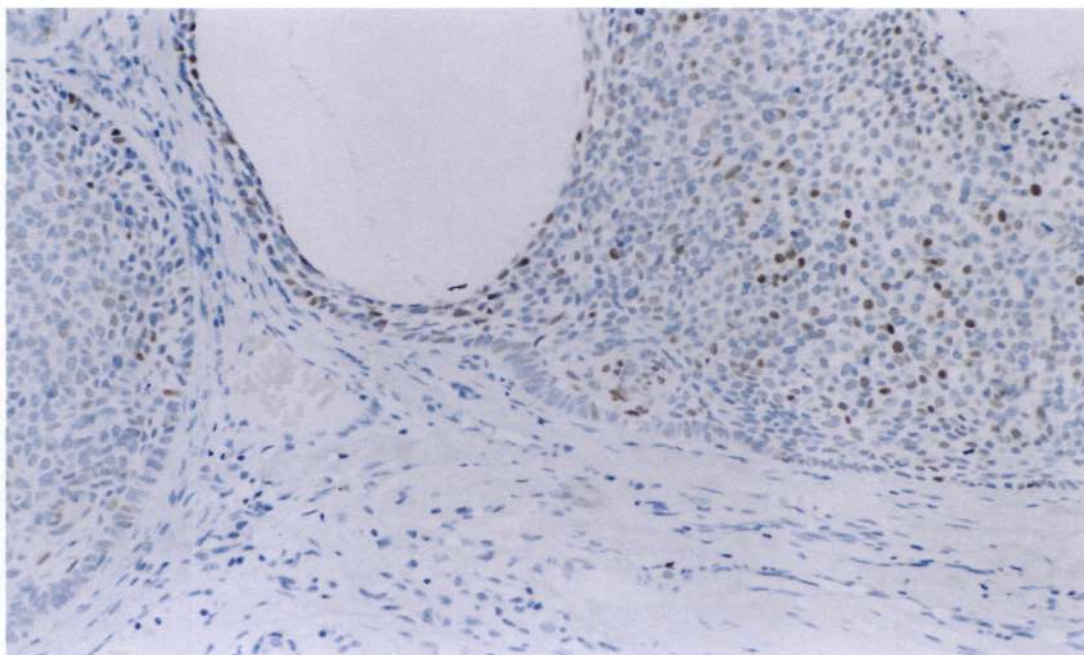


PLATE 88: p53 IMMUNOSTAINING
R-SBCC: High p53 immunopositivity.

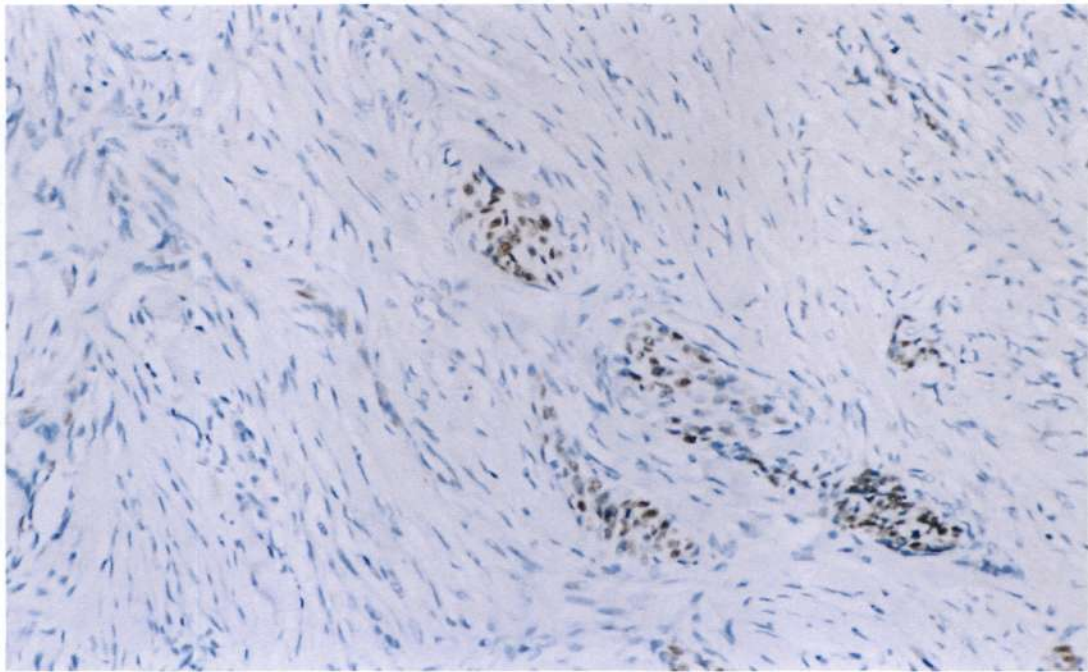


PLATE 89: p53 IMMUNOSTAINING
R-SBCC: High p53 immunopositivity.

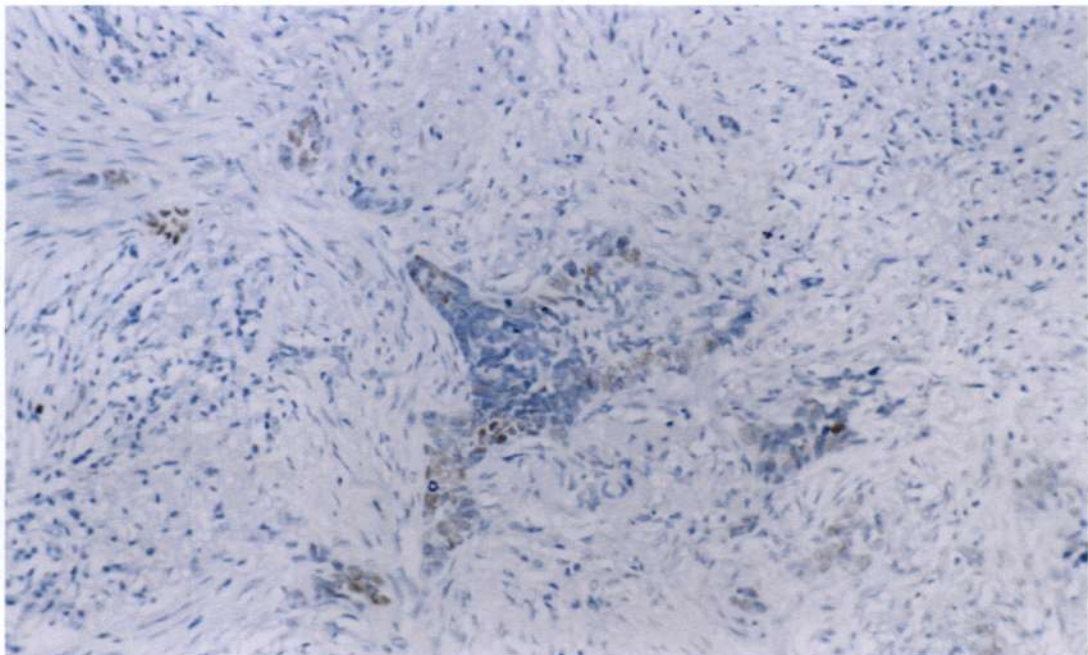


PLATE 90: p53 IMMUNOSTAINING
R-SBCC: Low p53 immunopositivity.

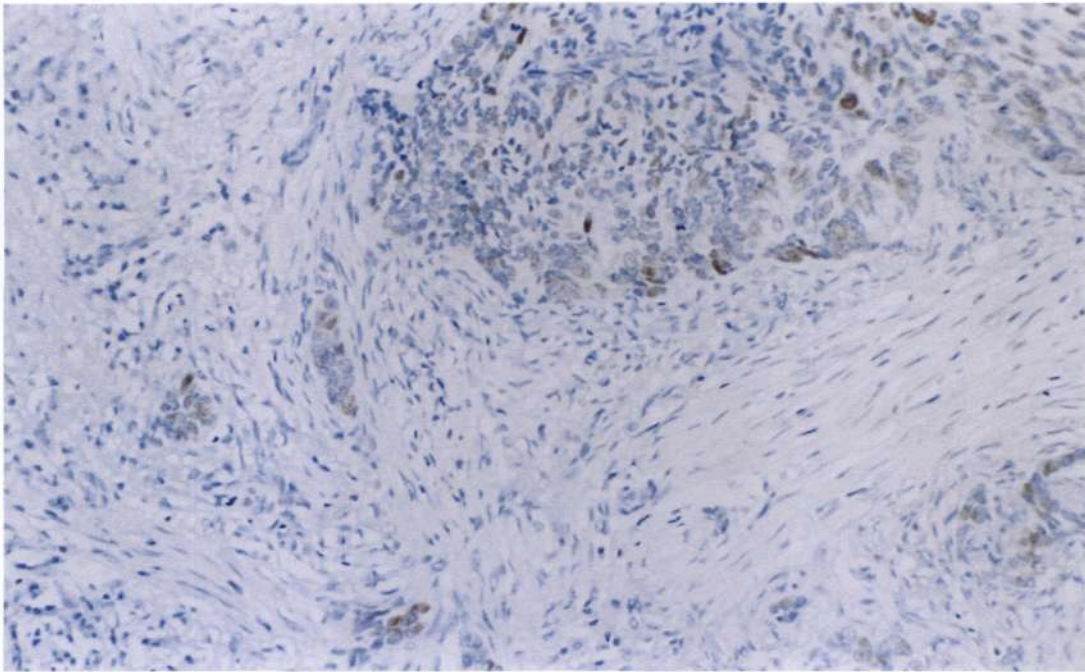


PLATE 91: p53 IMMUNOSTAINING
R-SBCC: Low p53 immunopositivity.

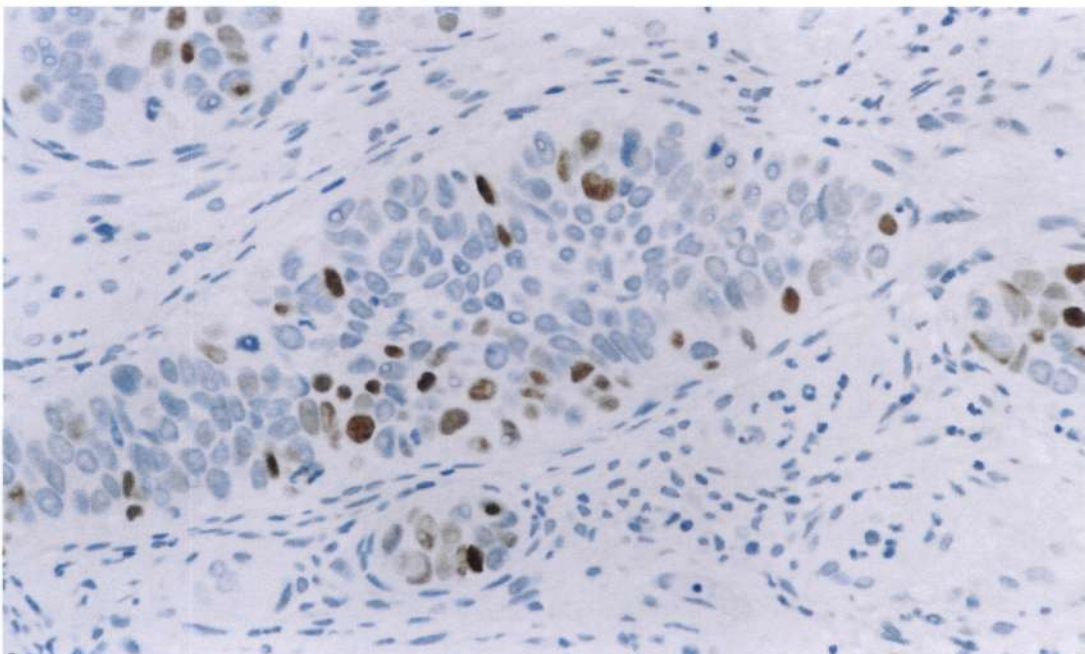


PLATE 92: p53 IMMUNOSTAINING
R-SBCC: Low p53 immunopositivity.

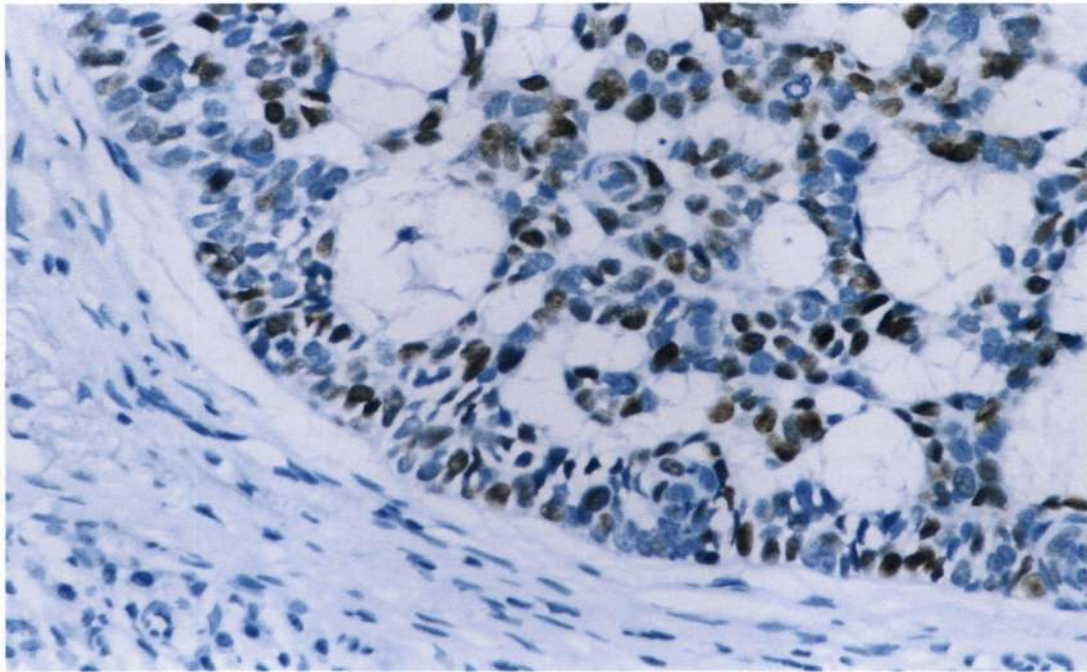


PLATE 93: p53 IMMUNOSTAINING
RG-SBCC: High p53 immunopositivity.

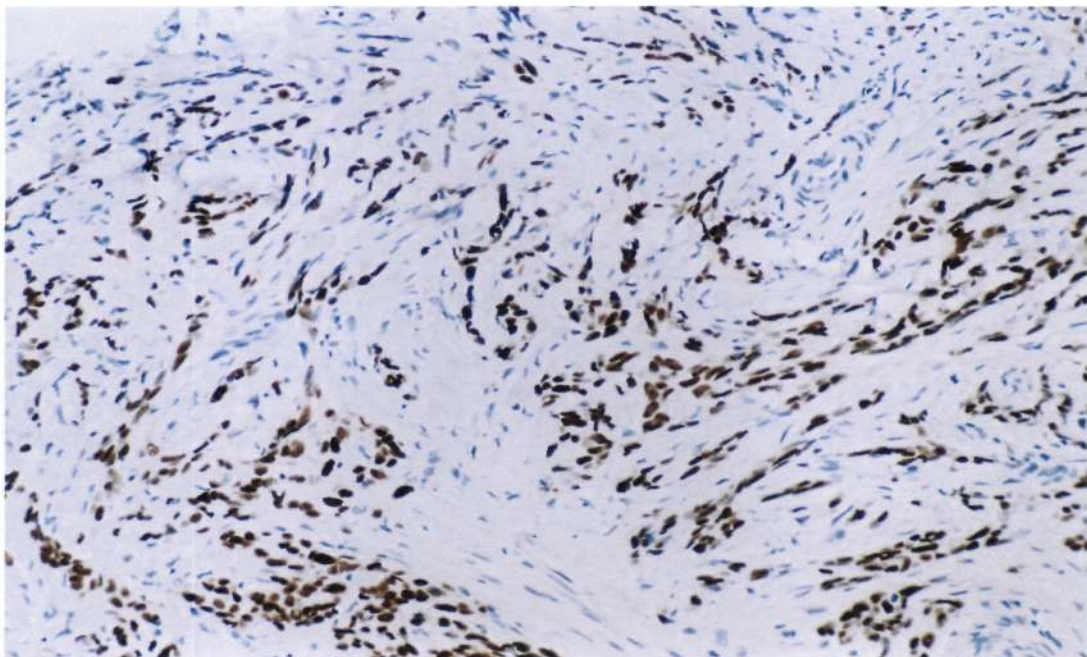


PLATE 94: p53 IMMUNOSTAINING
RG-SBCC: High p53 immunopositivity.

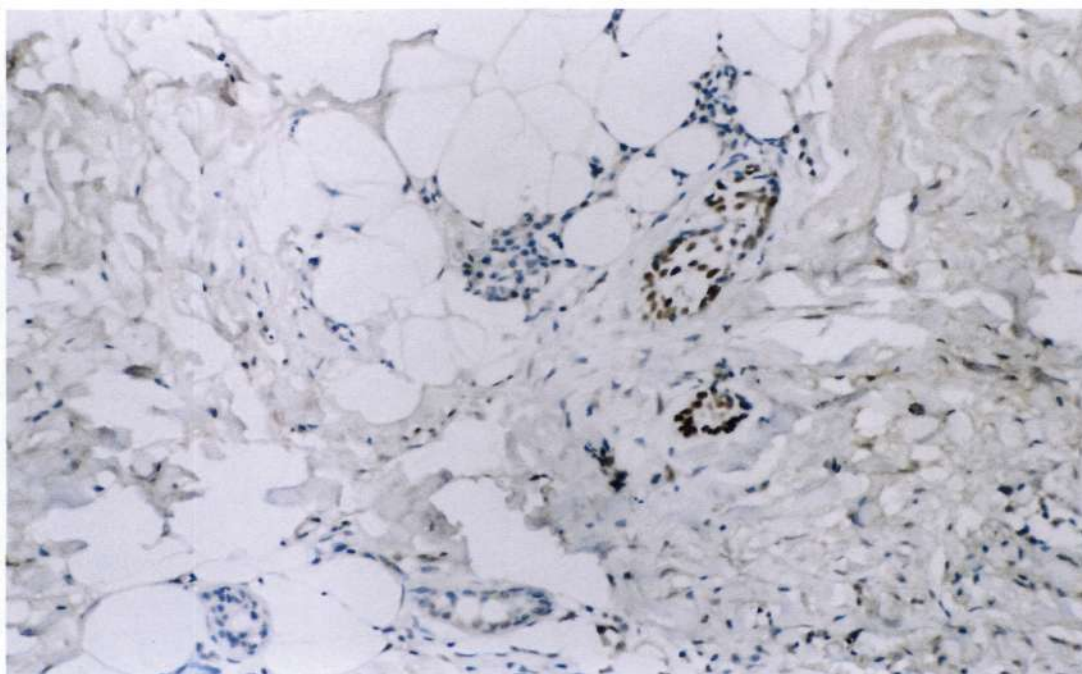


PLATE 95: p53 IMMUNOSTAINING
RG-SBCC: High p53 immunopositivity.

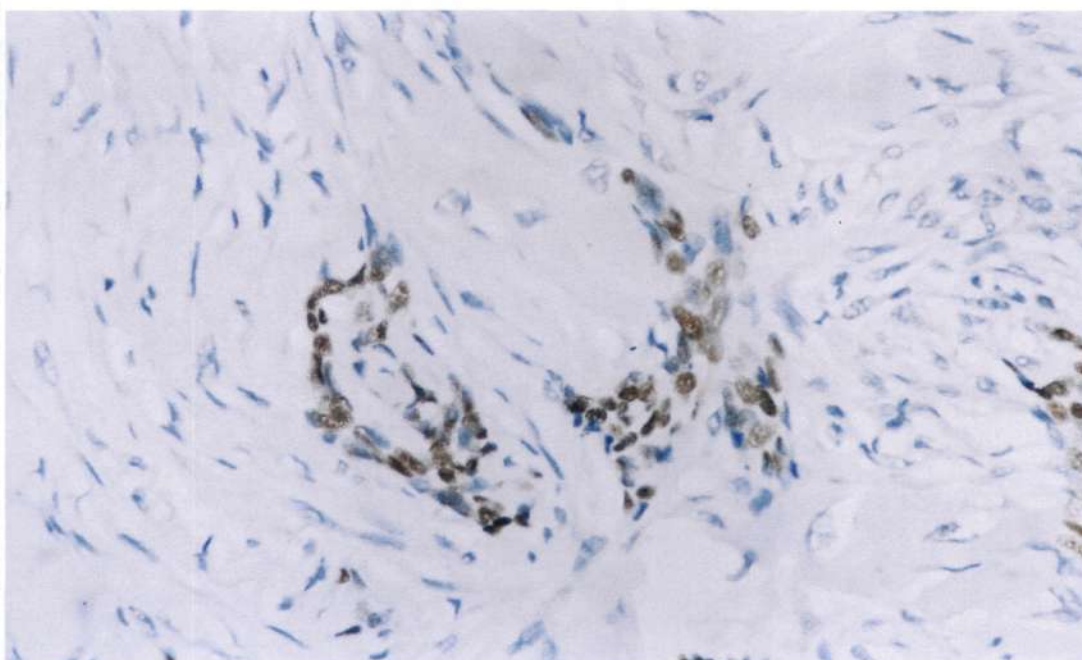


PLATE 96: p53 IMMUNOSTAINING
RG-SBCC: High p53 immunopositivity.

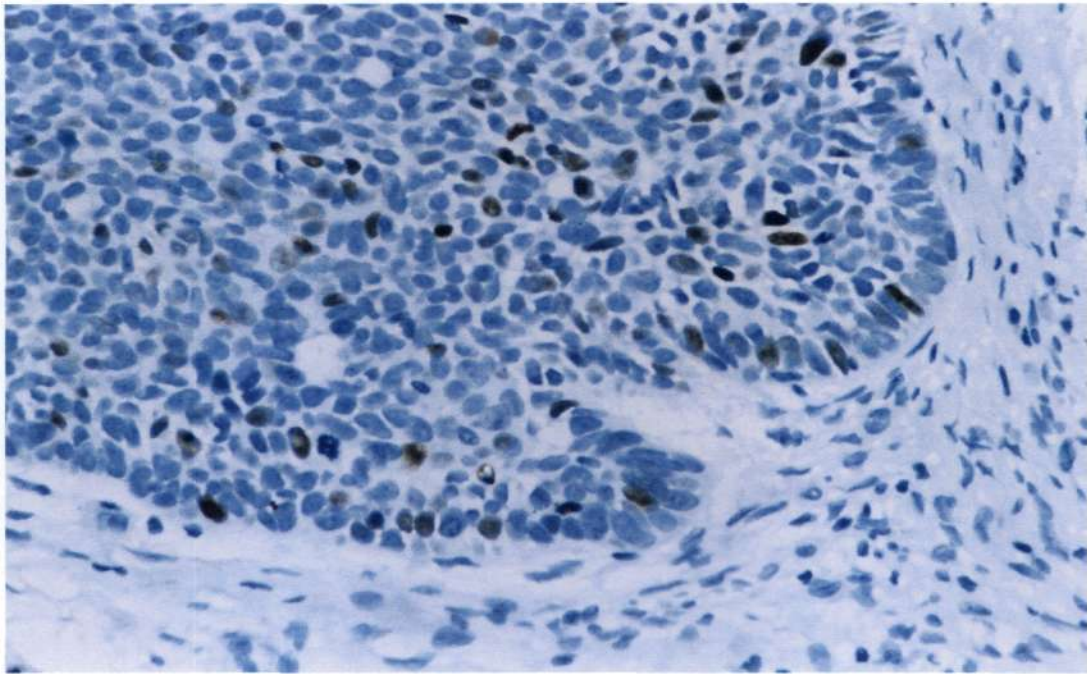


PLATE 97: p53 IMMUNOSTAINING
RG-SBCC: Low p53 immunopositivity.

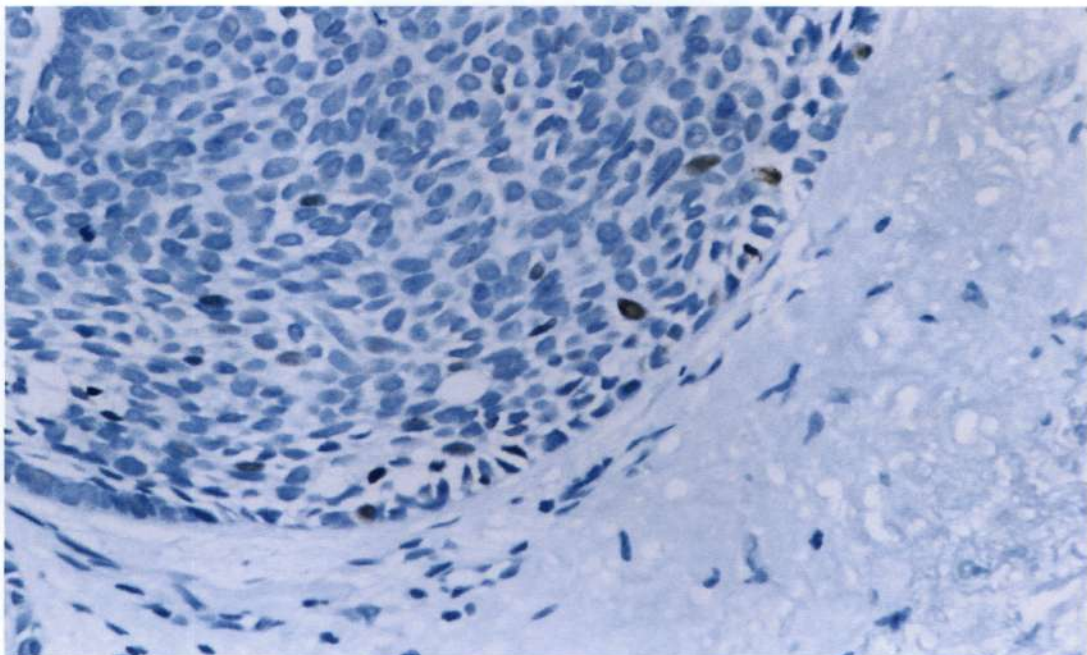


PLATE 98: p53 IMMUNOSTAINING
RG-SBCC: Low p53 immunopositivity.

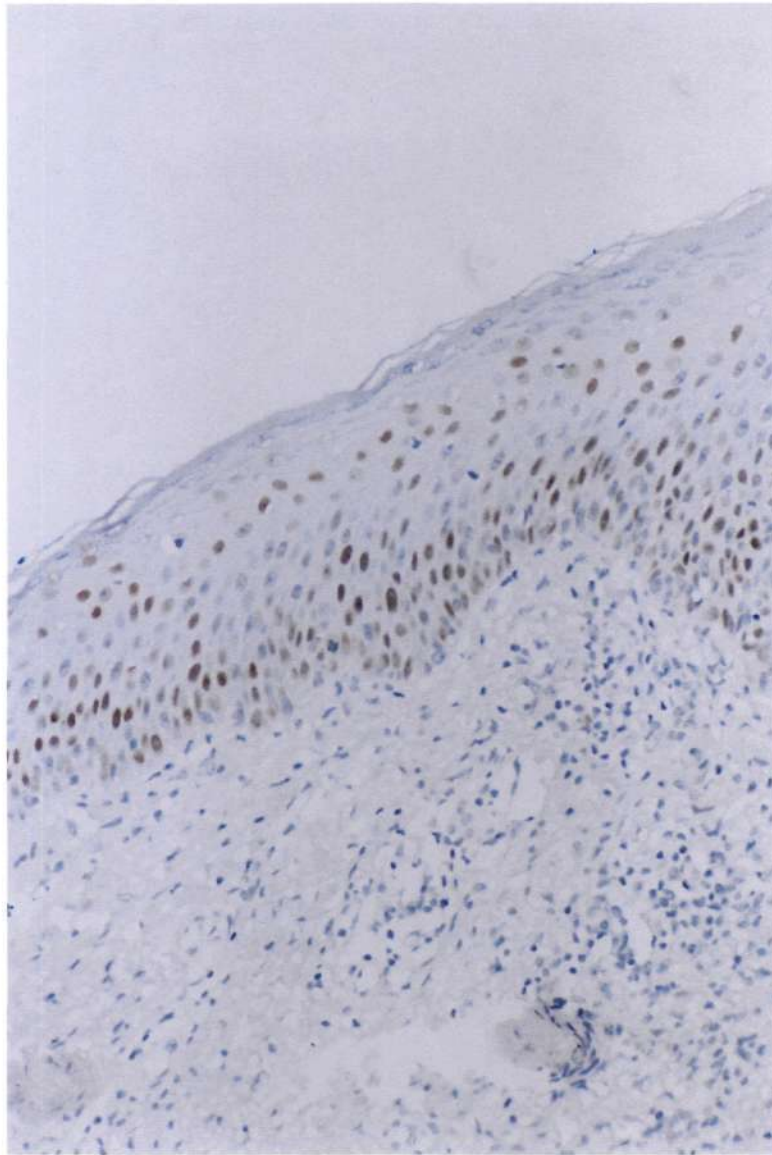


PLATE 99: p53 IMMUNOSTAINING

Adjacent solar keratotic epidermis: In-built positive control.

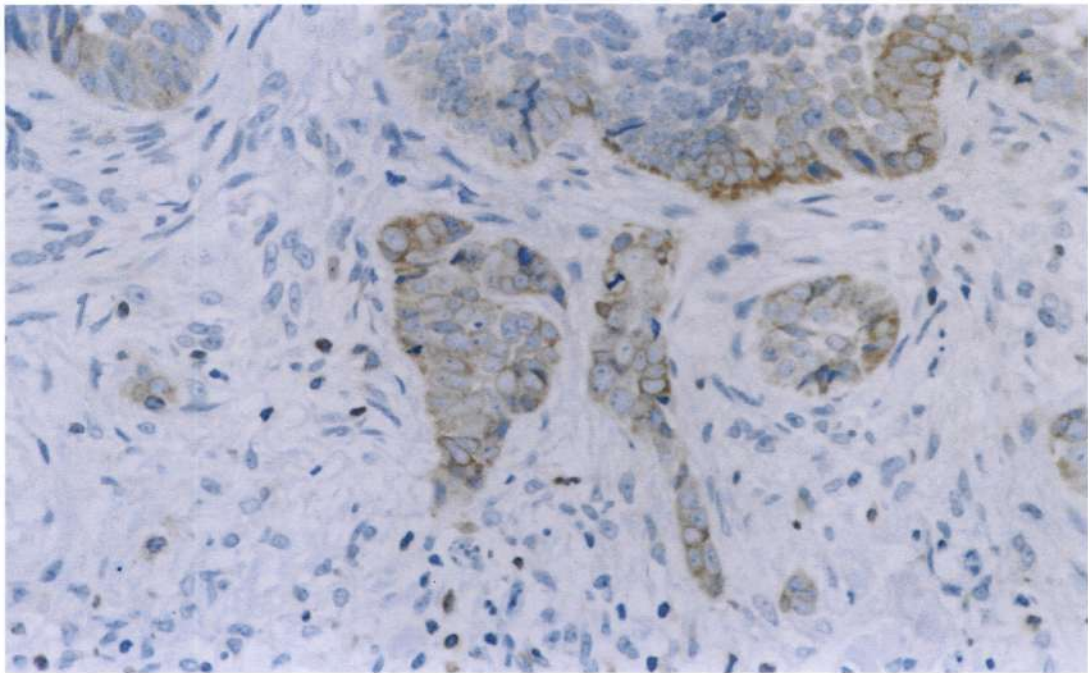


PLATE 100: bcl-2 IMMUNOSTAINING
DN-SBCC: High, bright bcl-2 immunopositivity.

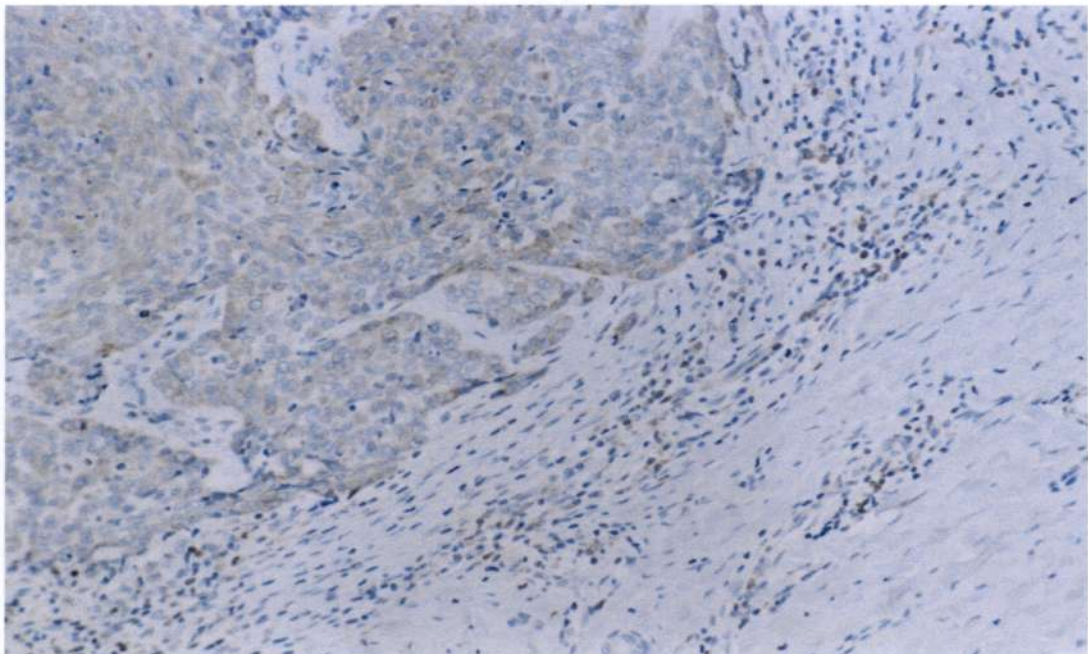


PLATE 101: bcl-2 IMMUNOSTAINING
DN-SBCC: High, bright bcl-2 immunopositivity.

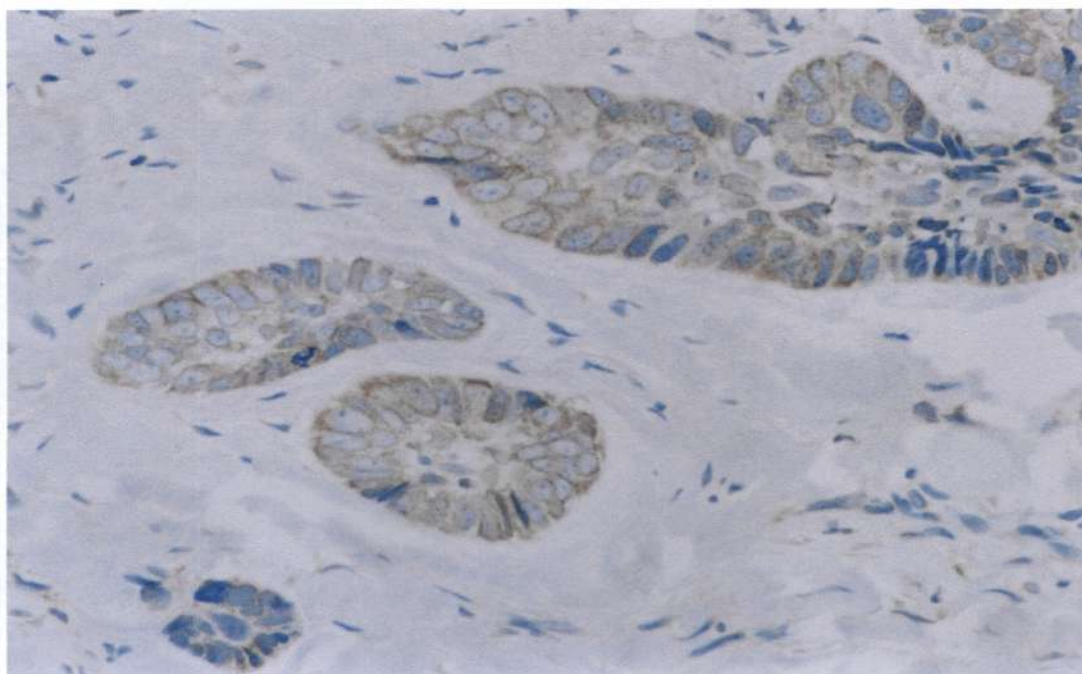


PLATE 102: bcl-2 IMMUNOSTAINING

DN-SBCC: High, bright bcl-2 immunopositivity.

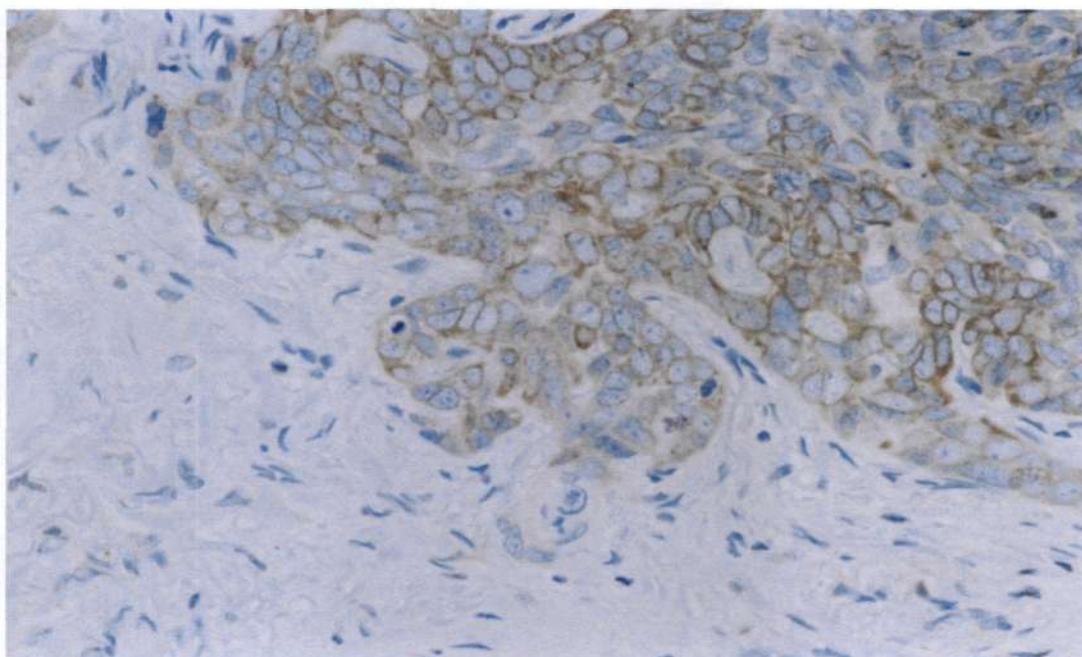


PLATE 103: bcl-2 IMMUNOSTAINING

DN-SBCC: High, bright bcl-2 immunopositivity.

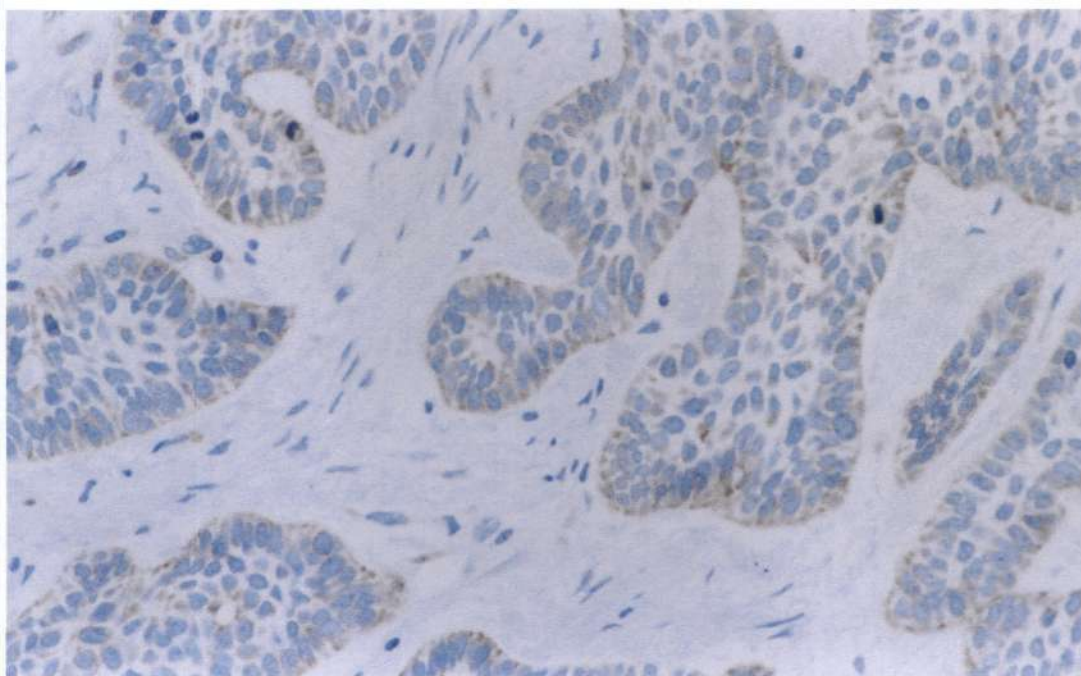


PLATE 104: bcl-2 IMMUNOSTAINING
DN-SBCC: High, pale bcl-2 positivity.

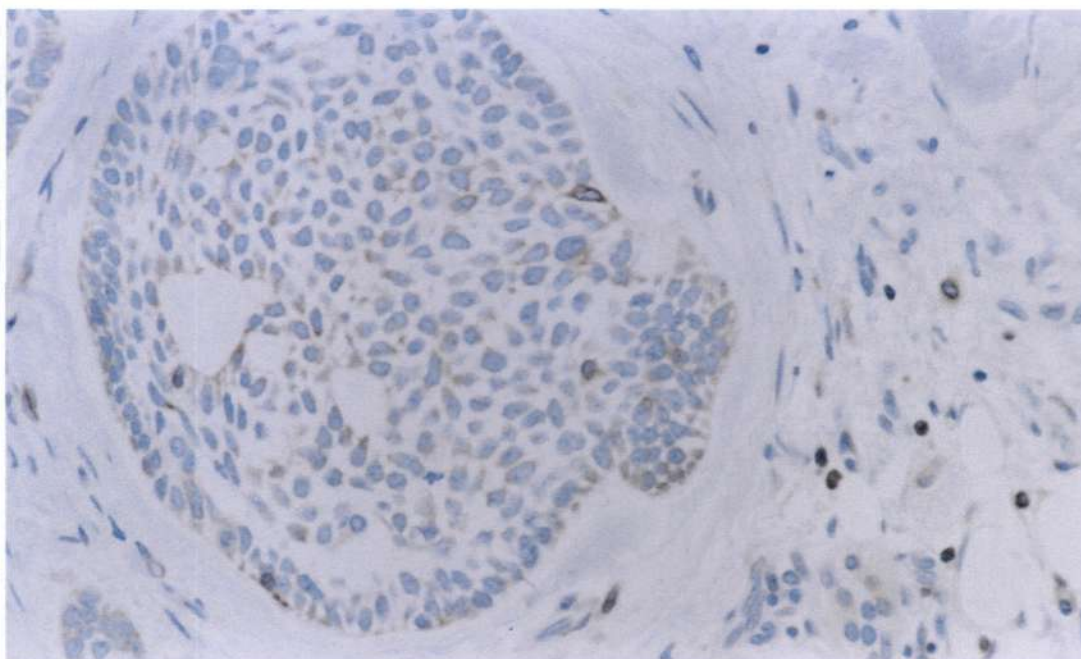


PLATE 105: bcl-2 IMMUNOSTAINING
DN-SBCC: High, pale bcl-2 positivity.

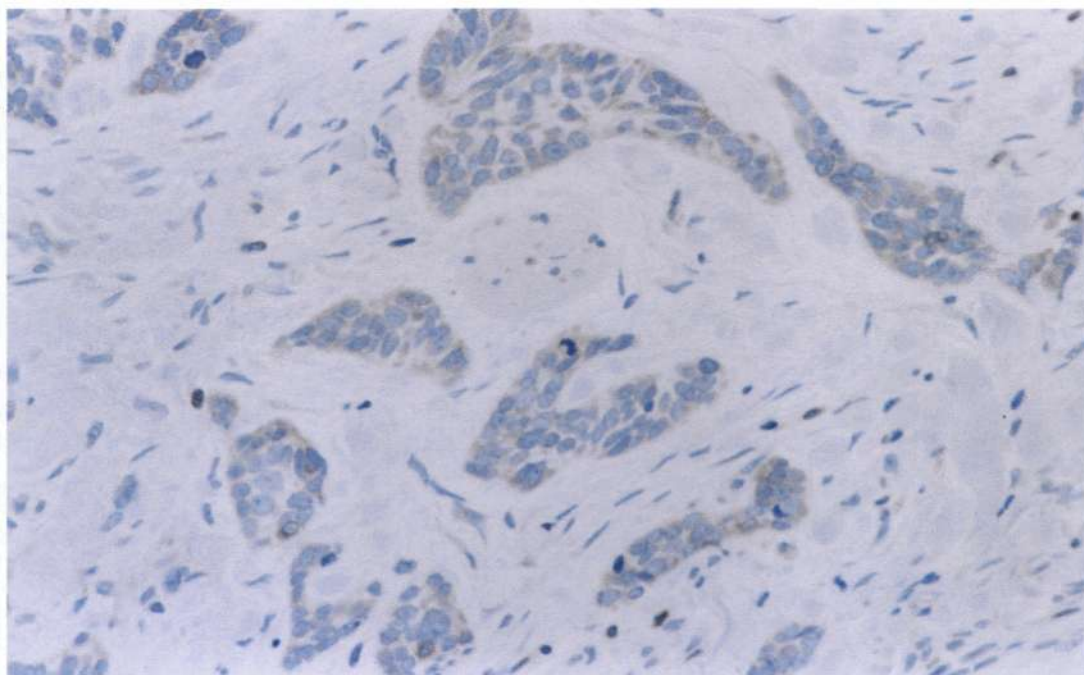


PLATE 106: bcl-2 IMMUNOSTAINING
DN-SBCC: High, pale bcl-2 positivity.

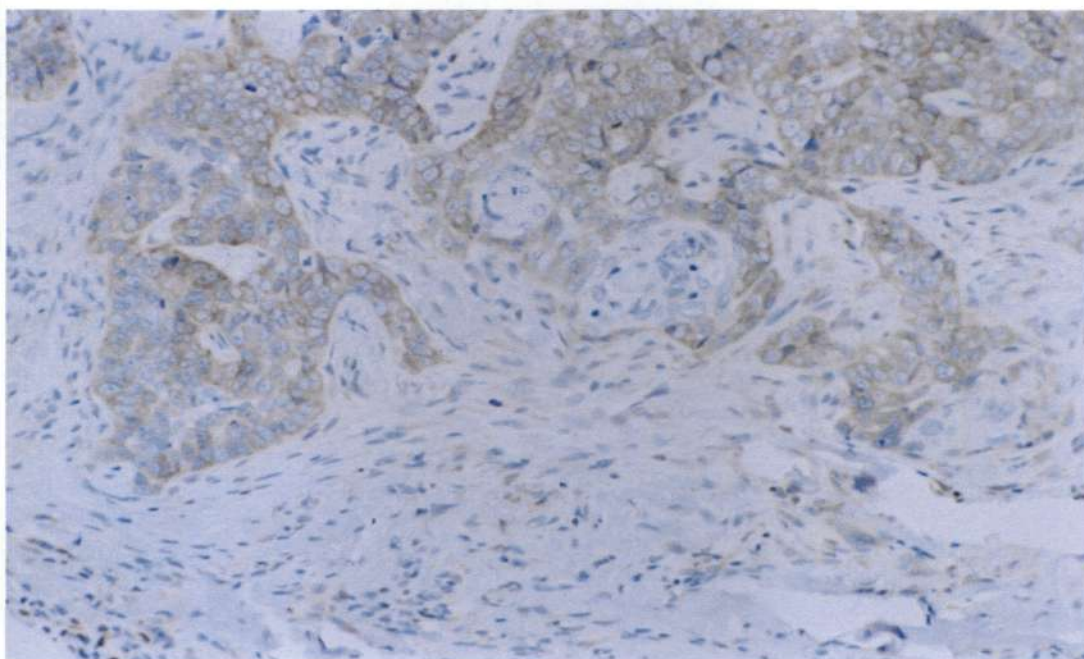


PLATE 107: bcl-2 IMMUNOSTAINING
RG-SBCC: High, bright bcl-2 immunopositivity.

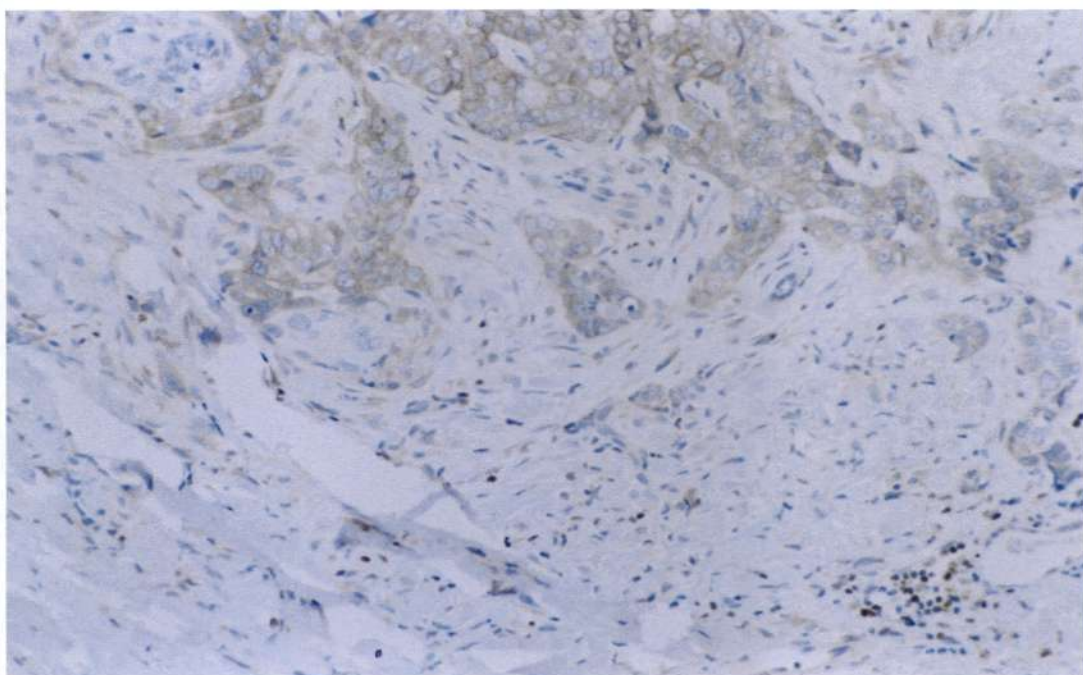


PLATE 108: bcl-2 IMMUNOSTAINING

RG-SBCC: High, bright bcl-2 immunopositivity.

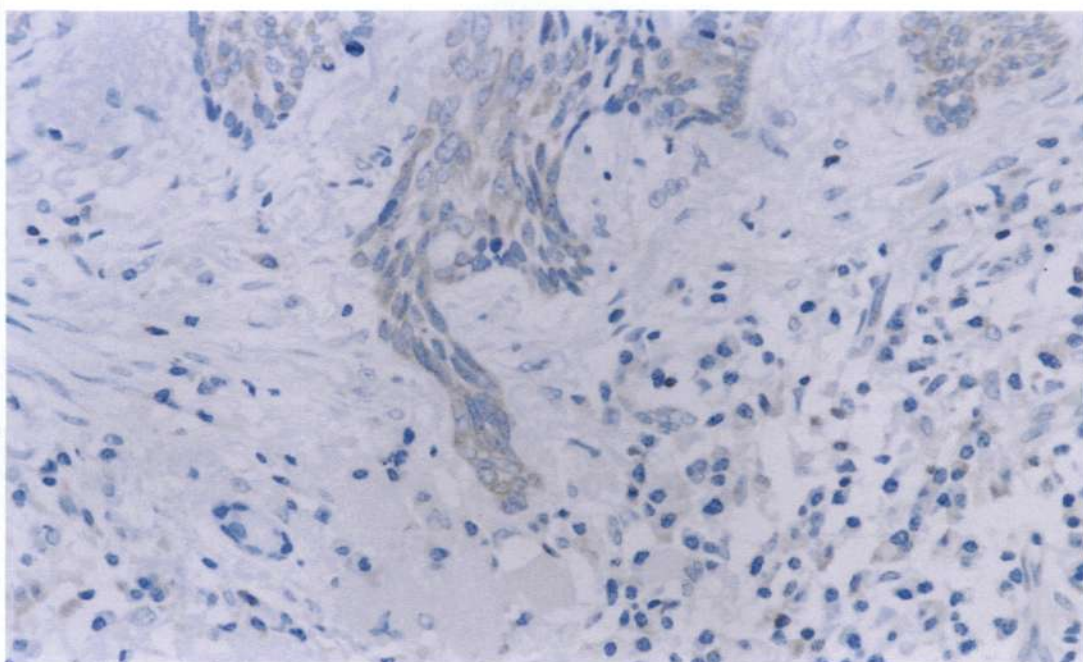


PLATE 109: bcl-2 IMMUNOSTAINING

RG-SBCC: High, pale bcl-2 immunopositivity.

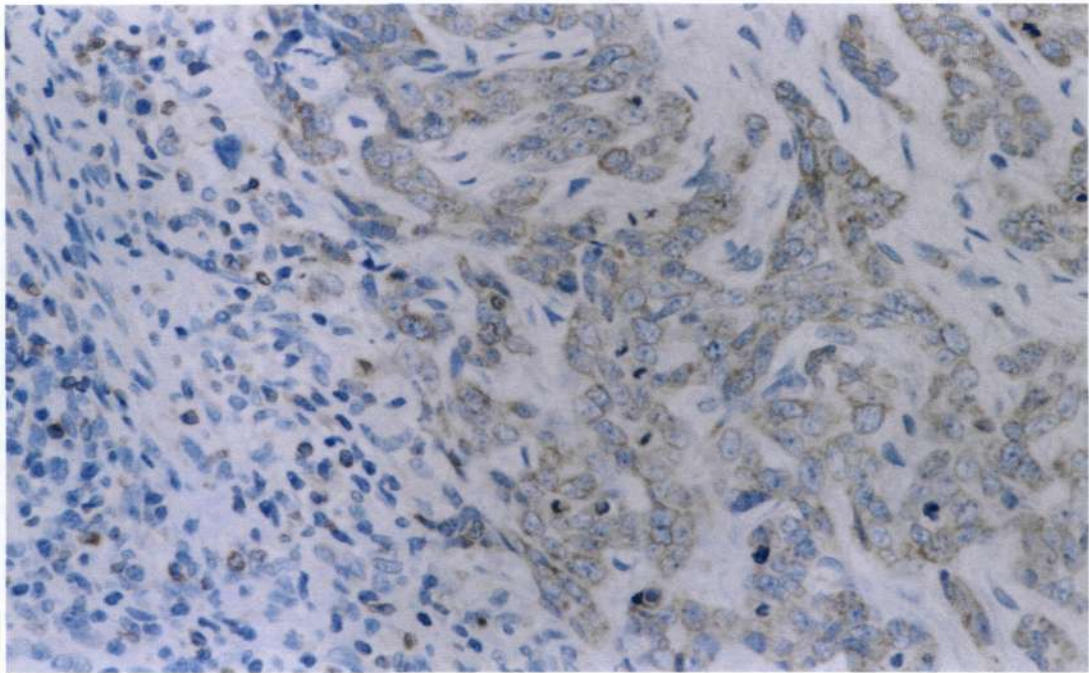


PLATE 110: bcl-2 IMMUNOSTAINING
RG-SBCC: High, bcl-2 immunopositivity.

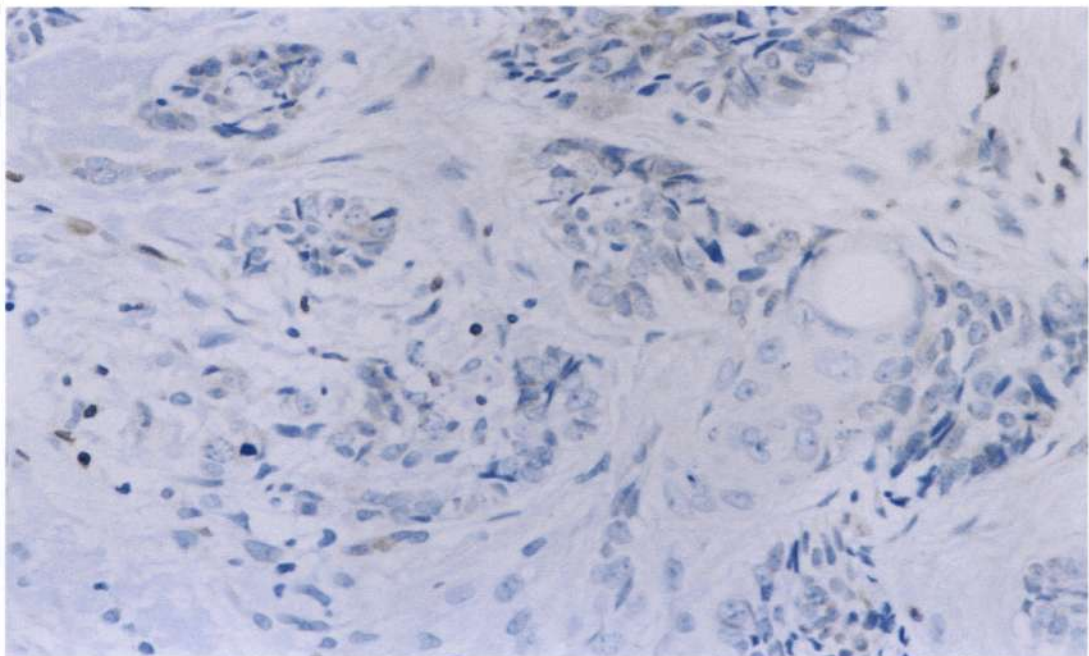


PLATE 111: bcl-2 IMMUNOSTAINING
RG-SBCC: Low, bcl-2 immunopositivity.

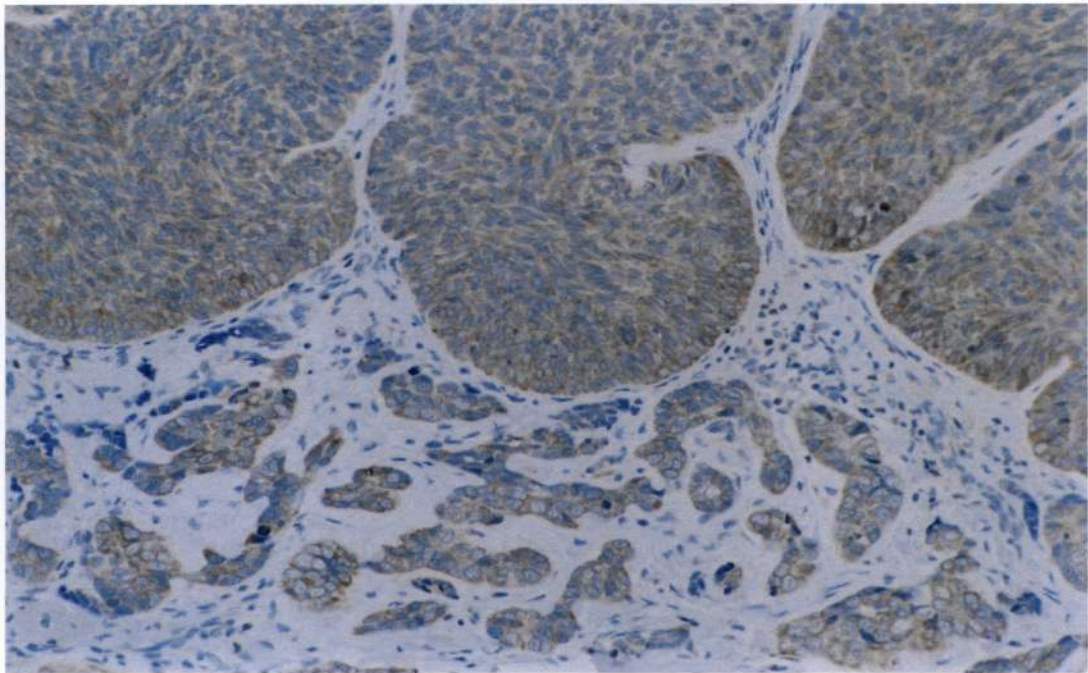


PLATE 112: bcl-2 IMMUNOSTAINING
R-SBCC: High, bright bcl-2 immunopositivity.

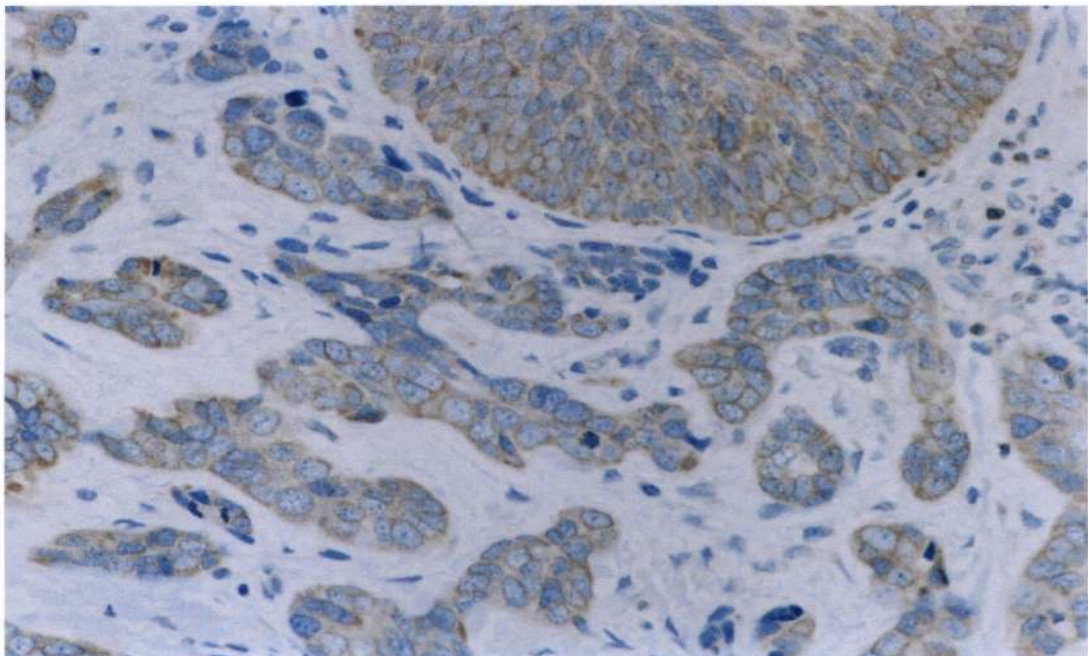


PLATE 113: bcl-2 IMMUNOSTAINING
R-SBCC: High, bright bcl-2 immunopositivity.

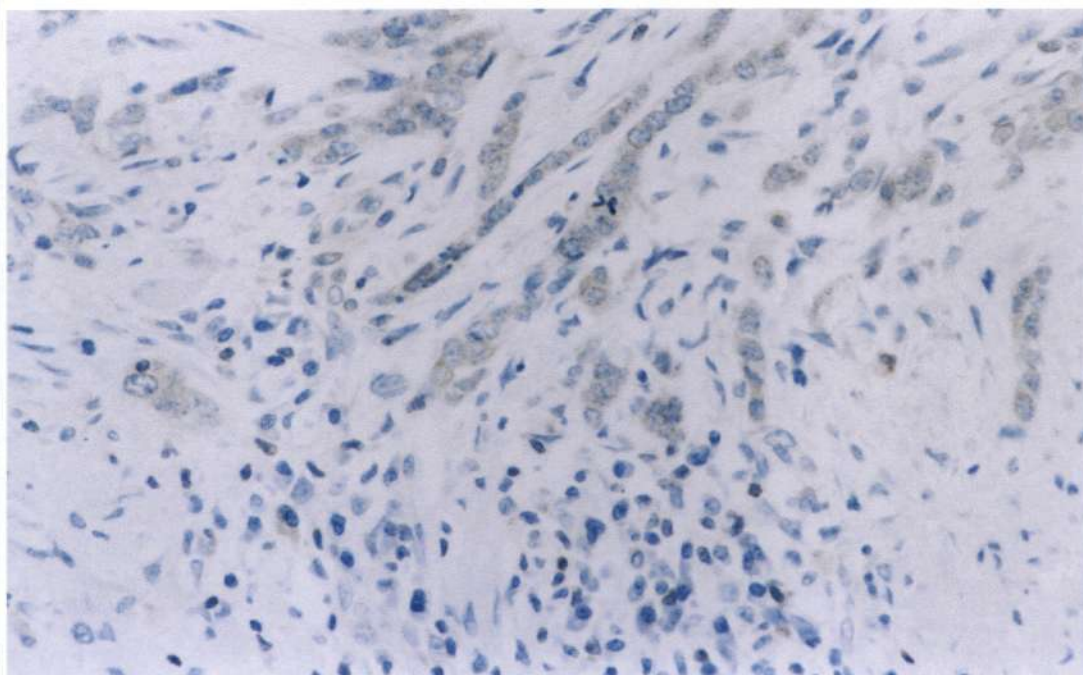


PLATE 114: bcl-2 IMMUNOSTAINING
R-SBCC: High, pale bcl-2 immunopositivity.

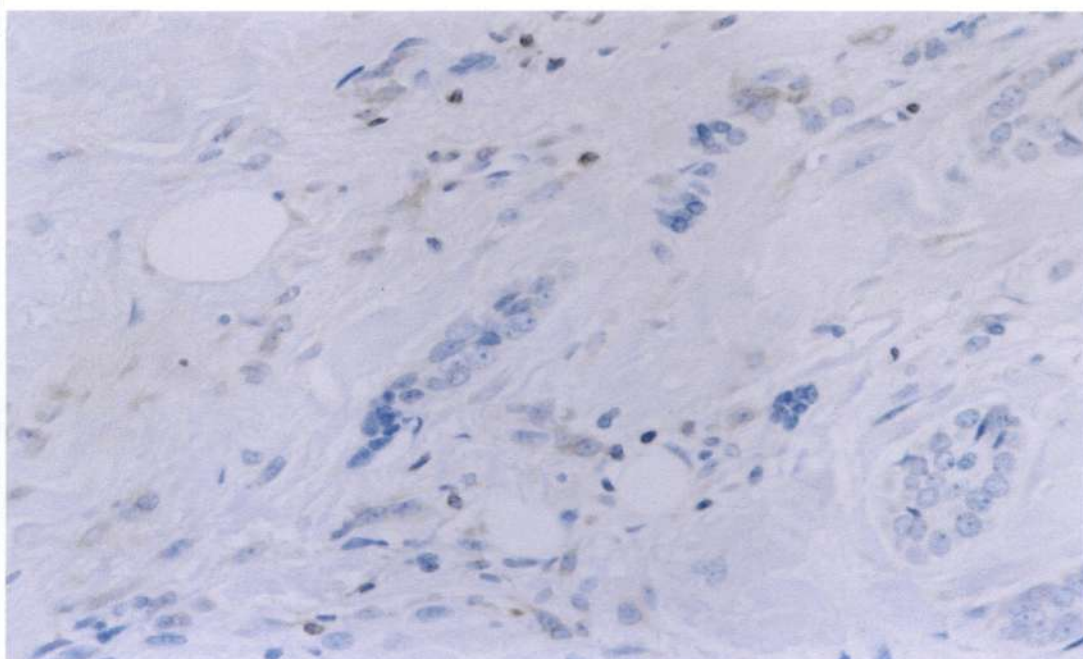


PLATE 115: bcl-2 IMMUNOSTAINING
R-SBCC: Low, bcl-2 immunopositivity.

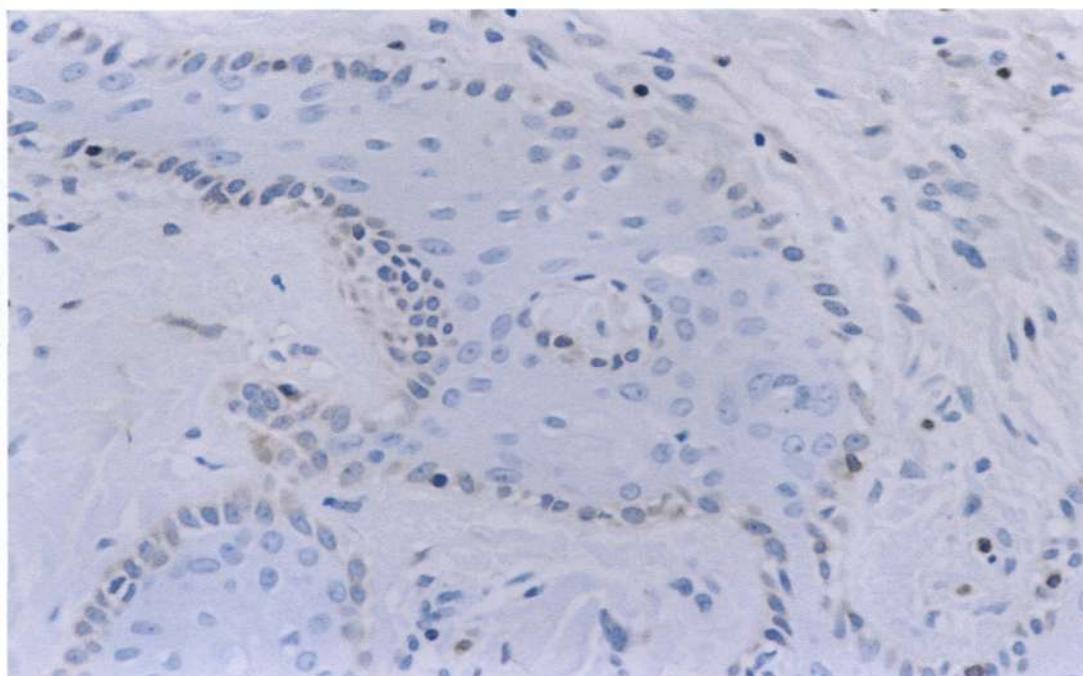


PLATE 116: bcl-2 IMMUNOSTAINING

Epidermal downgrowth: bcl-2 positive basal cells. In-built positive control.

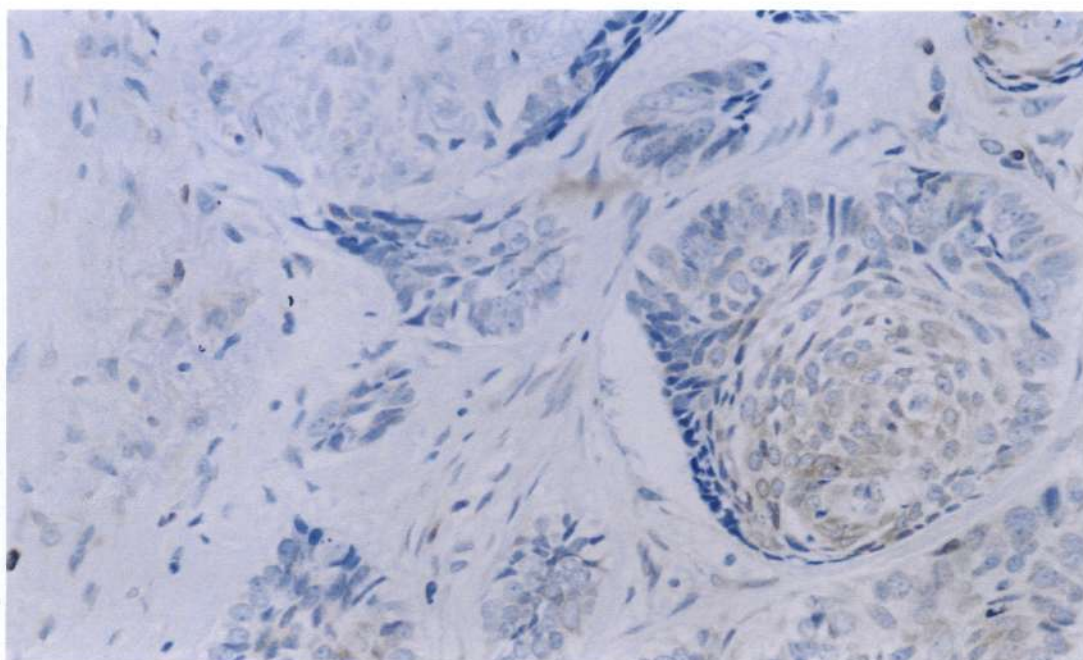


PLATE 117: bcl-2 IMMUNOSTAINING

DN-SBCC: Low bcl-2 immunopositivity.