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Bcl-2 and p53 Immunoprofile in Kaposi's Sarcoma

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Seventy three cases of Kaposi's sarcoma (KS) from the 3 histological subtypes (patch, plaque and nodular) were assessed for bcl-2 and p53 protein expression. The aim was to determine the level of expression of these proteins in KS and in the different subtypes. Commercially available antibodies to bcl-2 and p53 were applied after both microwave and pressure cooking antigen retrieval. Bcl-2 immunoexpression increased from the patch stage (36%) to the plaque stage (45%) to the nodular stage (70.83%). Better immunostaining for bcl-2 was obtained after pressure cooking. p53 on the other hand, was not expressed in the patch or plaque stages, but 54.16% of cases in the nodular stage were immunopositive. These results show a progression of immunoexpression of both bcl-2 and p53 from the early histological stages to the late tumor stage, implying that these proteins are upregulated late in the evolution of KS. (Pathology Oncology Research Vol 5, No 1, 17–20, 1999)

Key words: bcl-2, p53, and Kaposi's sarcoma

Introduction

Apoptosis is the programmed elimination of cells with DNA damage or growth dysregulation that could become precursors of a malignant clone. This process plays a role in embryogenesis, aging, reproduction and many disease processes and represents a stereotyped sequence of structural damage.[1,2] Bcl-2 and p53 play important roles in apoptosis. *Bcl-2* was first recognized in non-Hodgkin's lymphomas with the (t:14;18) translocation which places the *bcl-2* gene in close proximity to the enhancer element of the immunoglobin heavy chain at 14q32 resulting in deregulated expression of the protein in B cells.[1] The *bcl-2* gene codes for a 26 kDa protein and it has been demonstrated that insertion into membranes is closely associated with the ability of *bcl-2* to regulate apoptosis.[1]

*p53 i*s the most commonly mutated gene in human neoplasms.[1,3] The loss of p53 dependent apoptosis is believed to be critical in carcinogenesis.[3] The *p53* gene codes for a 53 kDa phosphoprotein that can act as a negative regulator of cell proliferation.[4] The expression of p53 and bcl-2 proteins has been investigated in great detail in many neoplasms. Thus far only a few studies have explored the expression of p53 and bcl-2 in Kaposi's sarcoma. Some authors believe that the expression of bcl-2 protein plays no role in the prognosis of KS,[5] others suggest that bcl-2 is expressed at increased levels in the later stages,[6] and that p53 may be expressed in the more aggressive lesions.[3,4] Due to these slightly conflicting results, the present study was performed to investigate the expression of these two proteins in the various histological stages of KS.

Materials and Methods

73 cases of Kaposi's sarcoma were retrieved from the archives in the Department of Pathology, University of Natal. These cases were categorized into the various histological stages as set out by Chor and Cruz.[7] Accordingly 24 cases were placed in the nodular stage, 24 into the plaque stage and 25 into the patch stage. Formalin fixed paraffin embedded sections were stained with bcl-2 (clone 124, 1:20 dilution) and p53 (clone D07, 1:50 dilution) after pressure cooking and microwave antigen retrieval using streptavidin biotin complex technique with DAB as chromogen. In addition CD34 (Immunotechnology, dilution 1:4) and CD31 (Dako, dilution 1:40) were also performed.

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A gastric carcinoma known to be positive for p53 and a follicular lymphoma, which is positive for bcl-2, were used as controls. Resident lymphoid cells within the tumor were also used as an inbuilt control. The results were scored as follows: 0-5% of positive tumor cells = negative, 6-25% = +1, 26-50% = +2, 51-75% = +3 and >75% = +4.

Results

Bcl-2 immunostaining – In the patch stage, 9 cases were positive for bcl-2 only. Eleven cases were positive for bcl-2 in the plaque stage (see *Tables 1 and 2)*. The staining in the nodular stage was more intense for bcl-2 *(Figure 1);* 17 cases were positive (see *Tables 1 and 2)*. We found that pressure cooking yielded better results and the results tabulated below are those after pressure cooking.

p53 immunostaining – Immunoreactivity was noted only in the nodular stage *(Figure 2)*. Thirteen of the cases showed p53 positivity.

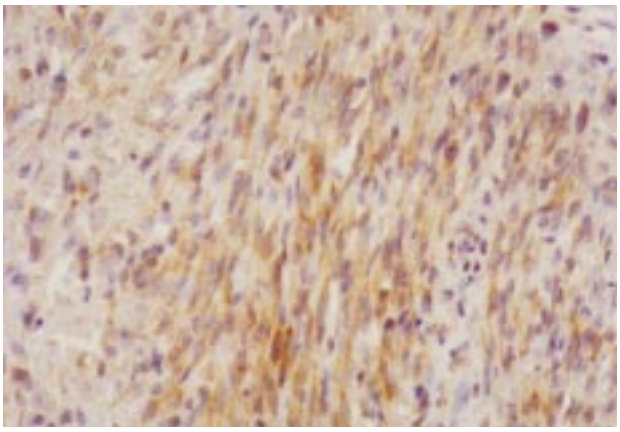

Figure 1. *bcl-2 immunoexpression in nodular Kaposi's sarcoma. In this case, the majority of tumor cells are immunolabelled.*

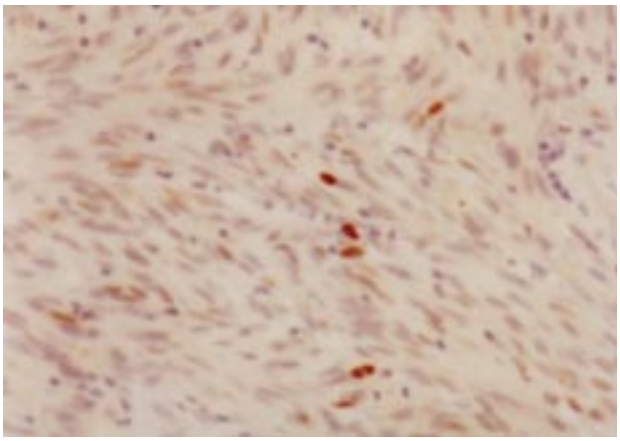

Figure 2. *p53 in the nucleus of several tumor cells in a nodular stage of Kaposi's sarcoma. Not all the cells stain positively.*

Table 1. **Immunohistochemical results on the cases studied**

Stage	*bcl-2*	*p53*
Patch stage	8 cases +1 1 case +2 **36%**	No positive staining
Plaque stage	8 cases +1, 2 cases +2 1 case +4 **45%**	No positive staining
Nodular stage	4 cases +1 2 cases +2 2 cases +3 9 cases +4 **70.83%**	11 cases +1 2 cases +2 **54.16%**

Discussion

Dei Tos et al found that 3 of 7 cases of KS showed 1–2% of the tumor cells positive for p53.[3] Subsequent to this study, Bergman et al investigated the accumulation of p53 in classical KS and correlated this with the histological subtypes.[8] In this study only a small percentage of tumor cells in the later stage of KS expressed p53 protein, which prompted the authors to suggest that p53 plays no significant role in the progression and evolution of KS. Dada et al proposed that p53 may be upregulated in the more aggressive stages.[5] This correlates with opinion of Li et al who suggested that although the rate of p53 mutations is low in KS the alteration of p53 in AIDS–related KS may lead to an increase in tumor progression and hence to aggressive behavior.[4] Noel et al found that only a minority of the cases in the patch stage displayed p53 immunopositivity whereas all the cases in the plaque and nodular stages were positive for p53.[9,10] As alluded to in the introduction, it is clear that the expression of p53 in KS, unlike other tumors, is a controversial issue. In our study we found that 54.16% of the cases in the nodular stage stained positively for p53, and that there was no staining in the earlier histological lesions. Kaposi's sarcoma-associated herpesvirus (Human Herpesvirus 8, HHV8) is now recognized as the likely infectious cause of KS in all the different epidemiological varieties of KS.[11] With regard to bcl-2, an open reading frame (ORF 16) of HHV8 is thought to have homology with cellular bcl-2.[11] The bcl-2 immunostaining seen in the more advanced stages of KS may possibly be due to the viral bcl-2 homologue being overexpressed and hence mimicking cellular bcl-2 overexpression. Cheng et al did not find specific interactions between the HHV8-bcl-2 homologue and other bcl-2 proteins using co-immunoprecipitation.[12] It is interesting to note that both the viral and cellular bcl-2 proteins share anti-apoptotic functions. It is possible that a degree of syn-

Table 2. **Clinical data of the patients studied**

Case	*Age*	*Sex*	*Histological subtype*	*Epidemiological subtype*	*Case*	*Age*	*Sex*	*Histological subtype*	*Epidemiological subtype*
1	27	Female	Patch	HIV related	38	36	Male	Plaque	HIV related
2	32	Female	Patch	HIV related	39	42	Male	Plaque	classic
3	33	Male	Patch	HIV related	40	22	Female	Plaque	HIV related
4	26	Female	Patch	HIV related	41	31	Female	Plaque	HIV related
5	66	Male	Patch	classic	42	55	Male	Plaque	classic
6	36	Male	Patch	HIV related	43	23	Male	Plaque	HIV related
7	32	Male	Patch	HIV related	44	36	Male	Plaque	HIV related
8	36	Male	Patch	HIV related	45	25	Female	Plaque	HIV related
9	28	Male	Patch	HIV related	46	49	Female	Plaque	HIV related
10	36	Female	Patch	HIV related	47	36	Male	Plaque	HIV related
11	45	Male	Patch	HIV related	48	27	Male	Plaque	HIV related
12	42	Male	Patch	classic	49	16	Male	Plaque	HIV related
13	44	Male	Patch	HIV related	50	35	Male	Nodular	HIV related
14	22	Male	Patch	HIV related	51	65	Female	Nodular	classic
15	40	Male	Patch	classic	52	24	Male	Nodular	HIV related
16	30	Female	Patch	HIV related	53	52	Male	Nodular	classic
17	27	Female	Patch	HIV related	54	44	Male	Nodular	HIV related
18	36	Male	Patch	HIV related	55	25	Male	Nodular	HIV related
19	40	Female	Patch	HIV related	56	26	Female	Nodular	HIV related
20	60	Male	Patch	classic	57	65	Female	Nodular	classic
21	27	Male	Patch	HIV related	58	58	Male	Nodular	classic
22	74	Male	Patch	aids related	59	42	Male	Nodular	classic
23	24	Female	Patch	HIV related	60	45	Female	Nodular	classic
24	41	Male	Patch	classic	61	26	Male	Nodular	HIV related
25	41	Male	Patch	HIV related	62	48	Female	Nodular	HIV related
26	42	Male	Plaque	HIV related	63	41	Female	Nodular	classic
27	22	Female	Plaque	HIV related	64	23	Female	Nodular	HIV related
28	78	Male	Plaque	classic	65	22	Female	Nodular	HIV related
29	34	Male	Plaque	HIV related	66	34	Male	Nodular	HIV related
30	27	Female	Plaque	HIV related	67	28	Male	Nodular	HIV related
31	52	Female	Plaque	classic	68	32	Male	Nodular	HIV related
32	35	Female	Plaque	HIV related	69	32	Male	Nodular	HIV related
33	33	Male	Plaque	HIV related	70	56	Female	Nodular	HIV related
34	30	Male	Plaque	HIV related	71	23	Male	Nodular	HIV related
35	40	Female	Plaque	classic	72	28	Male	Nodular	HIV related
36	29	Male	Plaque	HIV related	73	32	Male	Nodular	HIV related
37	24	Female	Plaque	HIV related					

ergy exists between the viral and cellular forms of bcl-2 in maintaining longevity of the KS tumor cells. With respect to the staining for bcl-2, it is clear that there is an increased expression of this protein as the lesion progresses from the early lesions to the nodular stage. In the progression of KS the upregulation of bcl-2 facilitates the survival of the tumor cells by the inhibition of apoptosis. These results correlate with those of Morris et al.[6] The expression of both p53 and bcl-2 proteins follow a similar pattern of progression from the early to the advanced stages. From our study, we conclude that if one believes in the progression of KS from the patch, to the plaque and finally to the nodular stage, there is an increased expression of bcl-2 and p53 proteins in the later histological stage as compared to the earlier stages. In addition to this, we recommend pressure cooking as the method of choice for antigen retrieval of bcl-2 protein.

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