Cell cycle protein expression in AIDS-related and classical Kaposi's sarcoma

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Statement of originality

The work presented in this thesis was carried out in the Departments of Radiation Oncology and Anatomical Pathology at Royal Prince Alfred Hospital, Sydney. All the experiments were designed, analysed and interpreted by the candidate, except where due acknowledgement is given in the text.

This thesis contains no material which has been submitted for any other degree at any university and complies with the stipulations set out for the degree of Doctor of Philosophy by the University of Sydney. It contains no material previously published by another researcher, except where due reference is made.

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Summary

Kaposi's sarcoma (KS) is a peculiar vascular neoplasm that occurs mainly in elderly Mediterranean men and patients with acquired immunodeficiency syndrome (AIDS). The current literature indicates that KS is initiated by the human herpes virus 8 (HHV8) as a reactive polyclonal process but with deregulation of oncogene and tumour suppressor genes, it can progress to a true malignancy with monoclonality. Clinically, classical KS often presents as an indolent disease affecting mainly the lower extremities whereas AIDS-related KS has no site predilection and can progress rapidly with systemic involvement. Histologically, KS can be classified into patch, plaque and nodular stages. Interestingly, classical and AIDS-related KS are indistinguishable histologically and this suggests that AIDS-related KS and classical KS might be initiated by a common aetiology but given their different clinical courses, they may progress through different mechanisms. In view of the importance of the cell cycle proteins in the development and progression of many human malignancies, this thesis aims to examine the role of these proteins in the progression of the two main clinical subtypes of KS.

The cell cycle protein expressions in a cohort of 47 patients with KS with well-documented clinical and histological features were studied. Using a monclonal antibody against the latent nuclear antigen-1 molecule of HHV8, HHV8 was detected in 78% of the cases. The more advanced nodular lesions were found to have a higher level of proliferative activity as measured by the proliferation

marker, Ki-67. This suggests it is valid to use the histological specimens as a tumour progression model of KS.

The role of the Rb/cyclin D1/p16 pathway was examined. The more advanced nodular stage KS lesions were more likely to be positive for cyclin D1, suggesting that cyclin D1 is important in the progression from patch stage to nodular stage. p16 acts as a tumour suppressor and it has an inhibitory effect on cyclin D1. The p16 expression rate was low in early stage KS but high in the more advanced lesions. It seems that reduced p16 expression occurs early in KS and may be important in its development. The rate of Rb expression, on the other hand, did not differ significantly among the histological subtypes. The results revealed the significant role of the Rb/cyclin D1/p16 pathway in the progression of KS.

Of the mitotic cyclins examined, cyclin A expression was correlated with the advanced tumor stage. The rate of $p34^{cdc2}$ expression was high in the lesions and there was no correlation with histological stage. This suggests that $p34^{cdc2}$ is important in the early development of the tumour but not necessarily in its progression.

Along the p53-apoptotic pathway, mutant p53 expression was significantly more common in the nodular stage. The cyclin G1 (a protooncogene, one of the target genes of p53) expression also paralleled that of mutant p53 with the majority of the KS lesions showing cyclin G1 expression and significant

correlation between advanced histological stage and increasing rate of cyclin G1 expression. These findings suggest that progression along the p53 pathway may be important in the advanced stage development of KS. On the other hand, expression of the CDK inhibitor, p27, a protein that normally negatively regulates cyclin G1, was reduced in nodular KS. These findings suggest that some KS lesions may progress through a deregulated or abnormal p53 pathway.

There were correlations between cyclin D1, cyclin A, cyclin G1, mutant p53 and negative HIV status. The findings suggest that components of both the Rb/cyclin D1/p16 and p53-apoptotic pathways are important in the progression of classical KS.

Rb protein was the only cell cycle protein whose rate of expression correlated significantly with HHV8 status in KS. The majority of HHV8 positive lesions were also positive for Rb protein, unlike HHV8 negative lesions. This suggests that some of the HHV8 negative lesions can progress through a defective Rb pathway whereas the role of Rb in the progression may not be as important in the HHV8 positive lesions. This was an unexpected finding given that one of the postulated mechanisms of tumour initiation by the HHV8 virus is via the viral cyclin it produces. The viral cyclin produced by HHV8 acts through the Rb pathway much the same as cyclin D1 and one would have expected that HHV8 positive cases are less likely to be positive for the Rb protein.

In summary, the majority of the KS lesions examined in this thesis show HHV8 infection. The Rb/cyclin D1/p16 pathway appears to be important in the progression of the different stages of KS and expression of the proteins involved in the p53 pathway were found to be important in the advanced stages of the development of KS. There were differential expressions of cell cycle proteins between AIDS-related and classical KS, and between HHV8 positive and HHV8 negative lesions. The findings also provided some clues to the possible mechanisms of development in KS lesions that were not initiated by HHV8.

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Publications and presentations

1. Hong A and Lee CS (2001). The emerging role of the human herpesvirus 8 (HHV8) in human neoplasia. Pathology 33: 460-467.

2. Hong A and Lee CS (2002). Kaposi's sarcoma: clinico-pathological analysis of human immunodeficiency virus (HIV) and non-HIV associated cases. Pathology Oncology Research 8(1): 31-5.

3. Hong A, Davies S, Lee CS (2003). Immunohistochemical detection of the human herpes virus 8 (HHV8) latent nuclear antigen-1 is a useful adjunct for the diagnosis of Kaposi's sarcoma. Pathology. 35(5): 448-50.

4. Hong A, Stevens G, Davies S, Lee CS (in press). Cyclin D1 overexpression AIDS-related and classical KS. Applied Immunohistochemistry.

5. Hong A, Sarris M, Lee CS. Expression of cyclin G1, mutant p53, p16 and rentinoblastoma protein in classical and AIDS-related Kaposi's sarcoma. (Submitted for publication)

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1. Hong A, Stevens G, Lee CS (2000). Kaposi's sarcoma: a clinicopathological analysis of 42 patients. Path International Suppl. A.11

2. Hong A, Sarris M, Lee CS (2001). Cyclin A expression In Kaposi's sarcoma. Pathology International 51: A3

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2. Hong A, Stevens G, Lee CS (2000). Kaposi's sarcoma: A clinicopathological analysis of 42 patients. Poster presentation at the 26th Annual Scientific Meeting of International Academy of Pathology

3. Hong A, Davies S, Lee CS (2001). Cyclin D1 expression in Kaposi's sarcoma. Oral presentation at the 51st Annual Meeting of the Royal Australian and New Zealand College of Radiologists

4. Hong A, Sarris M, Lee CS (2001). Overexpression of cyclin A in Kaposi's sarcoma. Poster presentation at the 27th Annual Scientific Meeting of International Academy of Pathology

5. Hong A, Stevens G, Lee CS (2001). Cyclin A expression in different clinical subtypes of Kaposi's sarcoma. Oral presentation at the 52nd Annual Meeting of the Royal Australian and New Zealand College of Radiologists

6. Hong A, Sarris M, Lee CS (2002). Expression of the cyclin dependent kinase, $p34^{cdc2}$, occurs early in the development of Kaposi's sarcoma. Poster presentation at the 28th Annual Scientific Meeting of International Academy of Pathology

List of specific abbreviations

Avidin Biotin Complex	ABC
Acquired Immunodeficiency Syndrome	AIDS
Epstein Barr Virus	EBV
Gray	Gy
Human Immunodeficiency Virus	HIV
Human Herpes Virus 8	HHV8
Immunoperoxidase	IPX
Kaposi's Sarcoma	KS
Retinoblastoma	Rb