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Letter to the Editor

Is Apaf-1 expression frequently abrogated in melanoma?

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Dear Editor,

Melanoma is highly resistant to chemotherapy, other systemic therapies and radiotherapy. Explants and derived cell lines have proved resistant to a variety of proapoptotic stimuli *in vitro*, leading to suggestions that the resistance to nonsurgical treatments reflects defects in apoptosis pathways, or their downregulation, in melanoma cells.[1] Unlike many other tumours, melanomas rarely exhibit defects in p53 function and they also express little *BCL2*; thus, if there are common blocks in apoptosis pathways in melanoma cells, they must lie elsewhere.

In 2001, Soengas *et al*[2] reported that the gene locus of the caspase-9 coactivator Apaf-1 is subject to loss-of-heterozygosity in approximately 40% of primary melanomas. In addition, fully half of a panel of cell lines derived from metastatic melanomas had abrogated expression of Apaf-1 and this correlated with resistance to the cytotoxic drug doxorubicin. Apaf-1 has been considered central to the activation of caspases through the BCL2-regulated (also called 'mitochondrial' or 'intrinsic') apoptosis pathway; consequently, these results offered an explanation for chemoresistance in a large fraction of metastatic melanomas. More broadly, it was evident that this mechanism of multidrug resistance is pertinent to recalcitrant solid tumours, which had been questioned.[3] Since then, immunohistochemical studies suggested that downregulation of Apaf-1 was associated with melanoma progres-

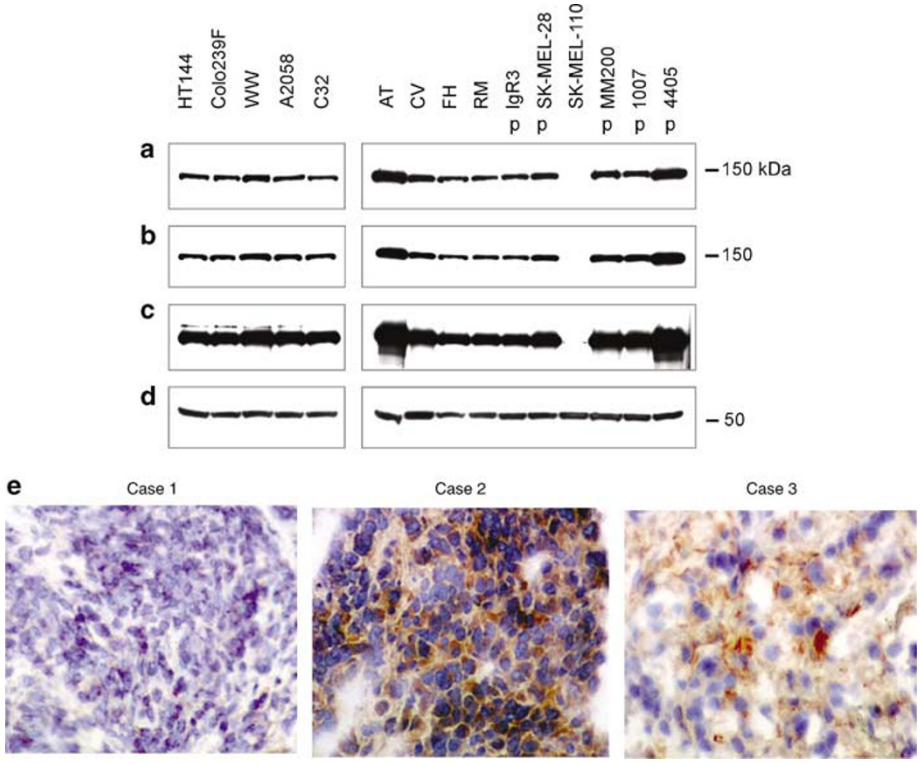

Figure 1 Immunoblot analysis of Apaf-1 levels in melanoma cell lines. Lines derived from primary as opposed to metastatic melanomas are marked 'p'. Apaf-1 was detected with monoclonal antibodies (**a**) 18H2 or (**b**) and (**c**) 2E12 (Hausmann *et al.*[13] and Alexis, Lausen, Switzerland), using short (10 s, **b**) and long (2 min, **c**) ECL exposures, respectively. Each lane contained 20 μg of whole-cell proteins as determined by the Bradford assay. (**d**) An identical blot was probed with anti-tubulin monoclonal antibody YL1/2 (Abcam, Cambridge, UK) as a loading control. Gels and blots corresponding to left and right panels were run, blotted, probed and exposed in tandem. (**e**) Immunohistochemical analysis of Apaf-1 expression in metastatic melanoma. Frozen tumour sections were stained by the avidin—biotin–peroxidase complex (ABC) method using the 2E12 antibody. Representative negative (case 1) and positive sections (case 2 and case 3) are shown

sion and metastasis[4] and allelic imbalance at the Apaf-1 locus was associated with adverse treatment outcome.[5]

However, recent work in one of our groups (by CS and AS) indicated that the absence of Apaf-1 or the associated caspase-9 did not affect either apoptosis or drug sensitivity in a lymphoid tumour model[6] or in primary lymphocytes,[7] raising doubts that Apaf-1 is crucial to apoptosis. Indeed, a recent study concluded that Apaf-1 is dispensable for normal apoptosis of human melanoma cells.[8] We therefore re-examined Apaf-1 expression in 15 melanoma cell lines by SDS-PAGE and immunoblot analyses. Five of the lines originated from primary melanomas and the other 10 from metastatic tumours. Some were obtained from public sources and the others derived from patient biopsies (by PH or GB). Apaf-1 was readily detected in all but one of the 15 lines examined (SK-Mel-110), with either of two monoclonal antibodies (Figure 1). Variation in Apaf-1 levels between the other 14 cell lines was modest given their diverse origins and, with the exception of the one negative line, there was no systematic difference between lines derived from primary or metastatic melanomas. In addition, of the 20 frozen metastatic melanoma sections examined by immunohistochemistry, 17 were clearly positive for Apaf-1; 13 of these showed strong staining and the rest weak to intermediate staining. Thus, although we do find inactivation of Apaf-1 in metastatic melanoma, it appears to occur far less frequently than that previously reported. Our conclusions appear to be supported by a recent analysis of the NCI Cell line Panel, all of which, including the eight melanoma lines therein, express Apaf-1.[9] Collectively, these results do not support the view that inactivation of Apaf-1 occurs frequently in the progression from primary to metastatic melanoma, or that such changes could account for the resistance of many melanomas to nonsurgical treatments. We believe that Apaf-1 LOH data should be treated with caution until a role for nearby genes, such as the proapoptotic IKIP,[10] is ruled out.

The observation by Soengas *et al*[2] of a relationship between Apaf-1 levels and chemosensitivity in their melanoma lines remains of great interest as evidence that disruption of apoptosis pathways is pertinent to melanoma drug resistance, at least *in vitro*. Crucially, it remains to be seen whether the same is true of melanomas *in vivo*, given that cytotoxic drugs, doxorubicin included, are rarely effective in that context. We are also left with the question of what other genetic or epigenetic changes might contribute to the refractory character of melanomas. Recent attention has focused on overexpression of certain caspase-inhibitory IAPs[11] and on other members of the Bcl-2 family of pro- and anti-apoptotic proteins.[12] We therefore await with interest the results of further studies relating the expression of such genes, including Apaf-1, to treatment responses and survival of patients with primary or metastatic melanoma.

JD Allen[1], XD Zhang[2], CL Scott[3], GM Boyle[4], P Hersey[2] and A Strasser[*,3]*

[1] Centenary Institute of Cancer Medicine and Cell Biology, Sydney, Australia
[2] Immunology and Oncology Unit, Mater Misericordiae Hospital, Newcastle, Australia
[3] The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia
[4] Queensland Institute of Medical Research, Brisbane, Australia
[*] Corresponding author: A Strasser, WEHI, 1G Royal Parade, Parkville, Victoria 3050, Australia. Tel: +61 3 9345 2555; Fax: +61 3 9347 0852; E-mail: strasser@wehi.edu.au

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