

Letter to the Editor

Apaf-1 expression in malignant melanoma

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Dear Editor,

We have previously reported that 10 out of 19 metastatic melanoma cell lines and 10 out of 24 melanoma specimens expressed low levels of Apaf-1, and that Apaf-1 expression inversely correlated with the chemosensitivity of melanoma cell lines *in vitro*.¹ Based on these observations, we proposed that Apaf-1 downregulation contributes to chemoresistance in malignant melanoma. In that study, we also suggested that Apaf-1 downregulation may be a late event during tumor progression (four out of five *in situ* melanomas were positive for Apaf-1). However, two recent Letters to the Editor published in this journal by Allen *et al.* and Peltenburg *et al.* imply a lower rate of downregulation of Apaf-1 in melanoma. In their panel of metastatic melanoma cell lines, only 1/11 and 1/10, respectively, were negative for Apaf-1.^{2,3}

What could explain the dissimilar rates of Apaf-1 repression between studies? One possibility is methodological. However, the antibody used in our study is specific – it recognizes a polypeptide that has the electrophoretic mobility of Apaf-1, associates with caspase-9 in cell free systems, and is lost from cells transfected with two different siRNAs against Apaf-1.^{4,5} Moreover, our experiments correlating Apaf-1 expression with chemosensitivity were conducted blindly – one laboratory (YL) measured Apaf-1 expression and the other (SWL) assayed apoptosis and the results were compared later. Finally, we confirmed the immunoblotting results by Northern blotting and *in situ* hybridization¹ and, more recently, other investigators corroborated our observations using three different antibodies,^{6–9} and by LOH analyses and quantitative RT-PCR.^{10,11}

Given that technical issues are unlikely to account for the differences between data sets, it is possible that the studies by Peltenburg and Allen did not examine enough samples to make reliable conclusions. In fact, six recent studies that analyzed over 400 pigmented lesions support the downregulation of Apaf-1 during melanoma progression. Fujimoto *et al.* concluded that one copy of the locus containing the *Apaf-1* gene is lost in 37% of metastatic melanomas, and this deletion can also be detected in circulating cells.^{10,11} mRNA expression studies supported the impact of deletions in this locus on Apaf-1 expression.¹¹ Baldi *et al.*, Dai *et al.*, Zannon *et al.* and Mustika *et al.* report a heterogeneous expression of Apaf-1, highly positive in benign nevi but weak or undetectable in 35–50% *in situ* melanomas and lymph node and visceral metastases.^{6–9} In addition, Qin *et al.* have also independently reported low Apaf-1 expression in metastatic melanoma cell lines used in our initial study.¹² These results, along with recent reports of low Apaf-1 expression in other melanoma cell lines,¹³ are consistent with our initial findings.

Still, it is conceivable that there are *bona fide* differences in Apaf-1 expression between melanoma cell lines depending on their site of origin or culture conditions. This would not be surprising, as melanoma is notorious for its heterogeneity⁷ and, accordingly, such discrepancies have been reported before. For example, *BRAF* was originally found mutated in 66% of malignant melanomas, but subsequently rates varying from 0 to 100% have been observed depending on the anatomical site and association with sun exposure.¹⁴ Also, most studies find that *p53* is rarely mutated in melanoma;¹⁵ however, Peltenburg and co-workers have reported that *p53* mutations can occur in up to 44% of melanomas derived from chronically sun-exposed sites.¹⁶ Alternatively, the mechanisms repressing Apaf-1 in primary tumor specimens can be occasionally altered through genetic or epigenetic changes that accompany passaging in culture (e.g., by potentiation of the Rb/E2F-1 pathway, which can control *Apaf-1* mRNA levels¹⁷). The fact that certain leukemia cells^{18,19} as well as AML, ALL, and CML blasts¹⁸ and cells from glioblastoma²⁰ and cervical cancer²¹ may also inactivate Apaf-1 expression, in part by methylation, suggests a broader impact of tumor-related events modulating Apaf-1 expression.

We agree with Allen and Peltenburg that Apaf-1 downregulation is not the only determinant of chemoresistance in melanoma, and highlighted a drug-resistant line (SK-Mel-173) that retained high Apaf-1 expression in our initial report.¹ Nonetheless, several studies suggest that Apaf-1 levels can affect apoptosis in melanoma cells. We showed that re-expression of Apaf-1 in cells with low Apaf-1 levels restored the ability of doxorubicin (adriamycin) to efficiently induce apoptosis, and this observation was independently confirmed and extended to cisplatin and vinblastin in isogenic settings.⁶ Similarly, Furukawa *et al.*¹³ showed that melanoma cells expressing low levels of Apaf-1 are resistant to apoptosis induced by E2F-1. In contrast, Peltenburg *et al.* did not find a direct correlation between Apaf-1 levels and caspase-9 activity in response to etoposide. Intriguingly, these results of Peltenburg *et al.* are in contrast to a recent report showing a delayed or increased resistance of low Apaf-1-expressing melanoma cells to high doses of etoposide (as well as doxorubicin or 5FU).⁸ In this context, it should be noted that the Peltenburg group has already reported mechanistic variability in the response of melanoma cells to etoposide.²²

In summary, while it is clear that Apaf-1 loss does not universally promote cell survival and is not the sole determinant of melanoma chemoresistance,²³ we think it premature to minimize its role in melanoma without further functional studies. Like other apoptotic regulators, its role in

tumor suppression and drug response may depend on context.^{24,25} An open question, therefore, is to what extent apoptosis can influence melanoma response²⁶ and patient survival. At least for cutaneous lesions, LOH at 12q22–23 (including, although certainly no limited to the Apaf-1 locus) correlates with disease progression and poor patient prognosis.¹¹ Moreover, allelic imbalances at 12q22–23 in serum circulating DNA of melanoma patients can predict disease outcome in response to biochemotherapy.¹⁰ How Apaf-1 repression and other apoptotic lesions affect melanoma progression, and how to bypass these defects, remain key challenges in cutaneous oncology, particularly because no evidence has been provided yet for the induction of apoptotic pathways *in vivo* by standard chemotherapeutic agents.

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