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Letter to the Editor

Expression of AP-2α, AP-2γ and ESDN in primary melanomas: Correlation with histopathological features and potential prognostic value

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To the Editor

Melanoma is characterized by increasing trends of incidence and high metastatic potential [1]. The transition from non-invasive to invasive and metastatic stage is characterized by a multi-step process by which tumour cells acquire the ability to detach and invade adjacent tissues. Loss of the transcription factor activator protein-2α (AP-2α) is an important molecular event in melanoma progression resulting in deregulation of AP-2 target genes involved in tumour growth and metastasis and associated with short Disease-Free-Survival (DFS) [2,3]. Our recent investigations on different tumour cells including melanoma [4–6] provided evidence that not only AP-2α but also AP-2γ play an essential role in tumorigenesis by modulating specific genes such as Endothelial and Smooth muscle cell Derived Neuropilin-like molecule (ESDN) [7]. So far, no data are available regarding AP-2γ and ESDN expression in primary melanoma lesions. Therefore, we analysed the expression of these two genes in primary melanomas, to understand their relationships as well as their connection with AP-2α expression and melanoma clinico-pathological features.

We included 67 patients stage 0 to II (AJCC classification); patients with evidence of metastatic involvement at first diagnosis, including positive sentinel lymph node biopsy (SLNB) were excluded. The study was approved by the Hospital Ethical Committee. Melanoma samples were subdivided according to Breslow thickness based on AJCC classification: 20 in situ and 47 invasive (≤1 mm: 25; >1 – ≤2 mm: 9; >2 – ≤4 mm: 10; >4 mm: 3).

All patients with invasive melanomas had regular follow-up visits (median follow-up 4.9 years, range: 1.1–11.1). One progression localised to regional lymph nodes occurred in a patient with melanoma <1 mm not previously treated by SLNB; 13 progressions occurred in patients with melanoma >1 mm (6 nodes, 5 "in transit" and 2 distant). Four out of 6 patients developed nodal recurrence in the same basin of previously negative SLNB ("false-negative") and 2 in another regional basin.

AP-2α, AP-2γ and ESDN expression was analysed immunohistochemically on paraffin tumour sections (AP-2α, C-18; 1:150; Santa Cruz; DCBLD2-ESDN, 1:50; Sigma; AP-2γ, 1:50; Bioworld Technology). The number of positive melanoma cells were scored in two categories: score 0 ≤ 30% or score 1 > 30% positive nuclei. Univariate/multivariate logistic regression were used to evaluate the significance of the predictor variables' association with DFS. In situ melanomas were excluded from DFS analyses.

Significant differences were found for marker expression according to tumor thickness. In situ melanomas had a high expression of all markers. In invasive melanomas, a progressive down-regulation of AP-2α, AP-2γ and ESDN expression was observed, which inversely correlated with increase thickness. Indeed, the large majority of thin melanomas (≤1 mm) expressed the three markers on more than 30% of tumour cells (AP-2α: 22/2; AP-2γ: 21/25; ESDN: 22/25); on the contrary, the majority of thick melanomas showed significantly lower AP-2α, AP-2γ and ESDN expression (χ^2: $p = 0.022$ for AP-2α, $p < 0.001$ for AP-2γ and $p < 0.001$ for ESDN). (Table 1) AP-2α, AP-2γ and ESDN expression was also related to other histopathological unfavourable features. Univariate logistic regression showed that a low expression of AP-2α, AP-2γ and ESDN was associated with increased vascularity

Table 1
AP-2α, AP-2γ and ESDN expression in primary melanoma and skin metastasis.

Protein analysed	% Positive cells	In situ tumours	Invasive tumours Breslow thickness (mm)				*P* value	Skin metastases
			≤1	1.01–2	2.01–4	>4		
AP-2α	<30%	0	3	3	6	2	0.0022	8
	≥30%	20	22	6	4	1		2
AP-2γ	<30%	3	4	7	10	3	<0.001	8
	≥30%	17	21	2	0	0		2
ESDN	<30%	5	3	4	9	3	<0.001	6
	≥30%	15	22	5	1	0		4

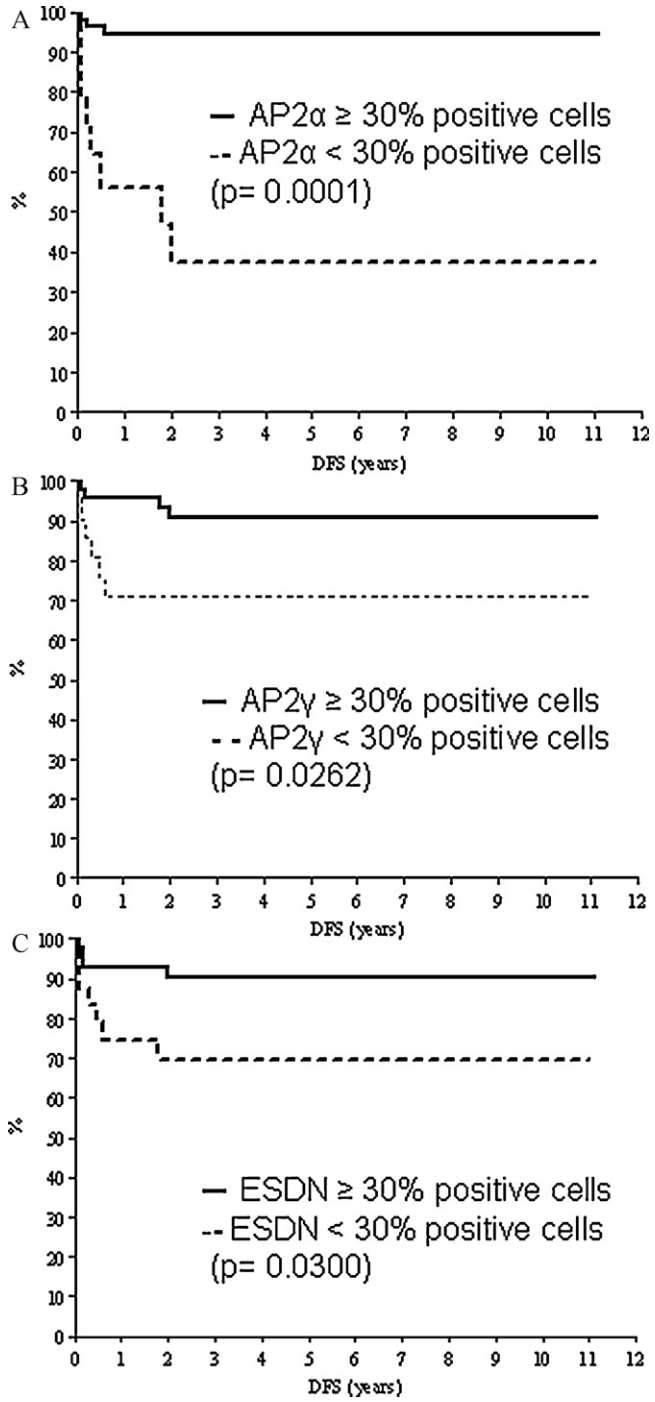

Fig. 1. Correlation of AP-2α (A), AP-2γ (B) and ESDN (C) expression with disease free survival (DFS) in primary melanoma lesions.

(odds-ratio (OR) 5.77 for AP-2α, 5.14 for AP-2γ and 3.14 for ESDN expression <30% positive melanoma cells), vascular invasion (OR 12.8 for AP-2α, 17.34 for ESDN and 52.8 for AP-2γ) and mitotic rate (OR 16.33 for AP-2α, 17.9 for AP-2γ and 14.64 for ESDN expression).

Univariate analyses showed that a shorter DFS is associated with a low expression of AP-2α (5-year DFS 33.3% vs 90.9%, $p < 0.001$), AP-2γ (57.9% vs 85.6%, $p = 0.0233$) and ESDN (57.4% vs 85.6%, $p = 0.0298$) (Fig. 1). The Hazard Rate (HR) was 10.00 for AP-2α ($p = 0.0006$), 3.65 for AP-2γ ($p = 0.0351$) and 3.44 for ESDN <30% positive cells ($p = 0.0439$).

To support the relationship between AP-2 family/ESDN down-regulation and melanoma metastatic potentialities, we investigated the expression of these markers in 10 archival specimens from skin melanoma metastases, 5 corresponding to patients included in the study who developed "in transit" localisations and 5 from other unrelated cases. A low expression of AP-2α, AP-2γ and ESDN was observed in the majority of cases (Table 1).

Conflicting data are reported for AP-2α, AP-2γ and ESDN expression in other cancers. In colorectal carcinoma, cytoplasmic AP-2α expression was reduced in advanced Dukes's stage tumours whilst AP-2α and AP-2γ were inversely related to histological grade [8]. Low AP-2 expression was associated with disease progression in breast cancer [9]. ESDN was up-regulated in the highly metastatic compared with parental cells in lung cancer [7], whereas a study on gastric cancer correlated loss of ESDN expression with increased tumour proliferation [10]. The mechanism of ESDN expression is still poorly understood. We previously identified a direct relationship between AP-2 family and ESDN regulation in both vitro and animal melanomas [4], providing evidences that AP-2α/AP-2γ regulate tumour growth, chemotherapy induced cell death, as well as migration and invasion via specific genetic programs. Furthermore, the analysis of gene expression in HeLa cells lacking AP-2α demonstrated that ESDN was among the major candidates for this molecular mechanism [4].

The concomitant loss of AP-2α, AP-2γ and ESDN in primary melanoma supports preliminary evidence about ESDN regulation by the AP-2 family in human melanomas, suggesting a modulation between these genes and thus confirming our previous findings [4,5]. The relevance of these alterations in the metastatic pathways is underlined by the skin metastasis pattern expression.

In conclusion, despite the relatively low number of patients, this study demonstrates for the first time that ESDN and AP-2γ expression is lower in thick melanomas, it is associated with unfavourable histo-pathological parameters (increased vascularity, vascular invasion and mitoses) and correlates with a shorter DFS like for AP-2α.

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