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ARTICLES

Expression of AP-2 Transcription Factor and of Its Downstream Target Genes c-Kit, E-Cadherin and p21 in Human Cutaneous Melanoma

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Abstract The AP-2 transcription factor plays a pivotal role in regulating the expression of several genes involved in tumor growth and progression of melanoma. We determined, by Western blot, variation in the level of expression of AP-2 and three of its downstream targets, c-kit, E-cadherin, and p21 in several human melanoma cell lines and, by immunohistochemistry, in a group of 99 histological samples including benign and malignat melanocytic lesions. A significant negative correlation between AP-2 expression level and tumor thickness was found. Moreover, AP-2 expression was positively associated with E-cadherin and c-kit expression. In contrast, there was a significant negative association between AP-2 and p21 expression levels. These findings suggest that p21 is independent of AP-2 transactivator function during the latest phases of melanoma progression. Finally, AP-2, c-kit, E-cadherin, and p21 expression levels did not show to be able to distinguish between dysplastic nevi and nevi without dysplasia. We conclude that changes in the expression of these proteins are involved in the later phases of melanoma progression, and may be responsible for the transition from local invasive melanoma to metastasis. J. Cell. Biochem. 83: 364–372, 2001.

Key words: human skin melanoma; AP-2; p21; c-kit; E-cadherin; immunohistochemistry

Incidence and mortality of human melanoma has increased considerably this century [Parker et al., 1997]. Conversely, the increasing incidence rate exceeds the mortality rate, apparently because of detection of biologically early primary melanomas, which are curable through surgery [Newton Bishop, 1997]. Based on clinical and histopathological features, several steps of melanoma progression have been proposed [Clark et al., 1984; Clark, 1991]. The normal melanocytes in the common acquired or congenital nevi give rise to dysplastic nevi, which, upon further evolution become radial growth phase melanoma (RGP). Tumor cell proliferation and invasion of the dermis are characteristic of the vertical growth phase melanoma (VGP). Finally, local invasion is necessary but not sufficient to enable metastasis. In recent years, concern has arisen about

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this model of progression, since either individual melanomas can skip steps in their development, appearing without identifiable intermediate lesions, or histologically borderline lesions not invariably progress to invasive melanomas [Mancianti et al., 1990; Barnhill et al., 1993].

It is, therefore, clear that access to biological markers of malignancy or invasion potential would be very useful. Nevertheless, the molecular changes responsible for the development and stepwise progression of melanoma are still unclear [Welch and Goldberg, 1997]. Many candidates oncosuppressor genes and oncogenes have been proposed. Among these the AP-2 transcription factor, a 52-kDa DNA-binding protein, which has been shown to control gene expression in neural crest and epidermal cell lineages both during embryonic morphogenesis and adult cell differentiation [Imagawa et al., 1987; Williams et al., 1988; Mitchell et al., 1991]. Several data indicate that AP-2 play a mayor role in tumor growth and metastasis of human melanoma. AP-2 chromosomal location (6p24-p22.3) has been reported to be a frequent site of loss of heterozygosity in melanomas, occurring around the RGP–VGP conversion [Gaynor et al., 1991; Robertson et al., 1996]. In fact, the progression of human cutaneous melanoma is associated with loss of expression of AP-2 [Bar-Eli, 1997]. Moreover, it has been reported that overexpression of AP-2 in highly metastatic melanoma cell lines inhibited their tumor growth and metastatic potential in nude mice [Luca et al., 1997]. Furthermore, decreased immunohistochemical AP-2 expression was independently associated with an elevated risk of subsequent metastatic behavior of stage I cutaneous melanoma [Karjalainen et al., 1998]. Since several in vitro studies have shown that AP-2 is involved in the transcription regulation of various genes, it has been proposed that AP-2 controls melanoma growth and progression through the activation or inhibition of several downstream target genes [Bar-Eli, 1999]. Among these targets, the proto-oncogene c-kit is a transmembrane tyrosine-protein kinase receptor, whose normal function is required for human melanocyte development [Yarden et al., 1987]. Indeed, several recent studies have demonstrated that the progression of human cutaneous melanoma is associated with loss of expression of c-kit [Lassam and Bickford, 1992; Natali et al., 1992]. Furthermore, c-kit expression is highly regulated by AP-2 in human melanoma cell lines and transfection of AP-2 in melanoma cell lines down regulates the expression of c-kit [Huang et al., 1996, 1998]. The adhesion molecule E-cadherin is involved in determining melanocyte positioning in the skin [Hennig et al., 1996]. It has been reported that loss of E-cadherin is directly correlated with melanoma progression and that AP-2 regulates its transcription level [Cowley and Smith, 1996]. Finally, the cyclin-dependent kinase inhibitor p21 has been suggested to have tumor-suppressor activity in melanoma cell lines and its transcription is also regulated by AP-2 [Zeng et al., 1997]. Based on this observation, it has been hypothesized that AP-2 may control cell cycle and tumor growth through p21 activation [Bar-Eli, 1999].

To the best of our knowledge, no previous reports exist concerning the correlation between the expression of AP-2 and its downstream targets in in vivo material. We therefore, analyzed several human melanoma cell lines using comparative Western blot, and a group of 99 samples, spanning from benign lesions to melanoma metastases, using immunohistochemistry, to quantify the expression of AP-2, c-kit, E-cadherin, and p-21. The detected expression levels were correlated to each other, as well as with clinicopathological data, to address the functional role of AP-2 in melanoma growth and progression.

MATERIALS AND METHODS

Cell Lines

Human melanoma cell lines (BRNTT, MG3, CAP, AQB, and IR-1) were established from human melanoma metastases at the Regina Elena Cancer Institute, and maintained in culture in Dulbecco's modified Eagle medium (DMEM) containing 0.5 mg/ml gentamicin and complemented with heat inactivated 10% fetal calf serum at 37°C in a 5% CO_2-containing atmosphere.

Western Blot

Western blotting on cell lysates was performed, using a working dilution of 1:500 for all the antibodies, as described previously [Raschellà et al., 1998]. HSP70 was detected using a mouse monoclonal antibody (HSP01, Oncogene Science).

Patients

This retrospective study consists of a consecutive series of 53 clinical stage I–II–III cutaneous malignant melanoma, 10 metastases, and 36 cutaneous naevi with complete histopathologic data available. The patients were diagnosed and treated at the Second University of Naples between 1990 and 1999. The clinical staging of all tumors was performed according to the International Union Against Cancer. There were 99 valid immunostainings for AP-2, E-cadherin, c-kit, and p21. According to tumor thickness, cutaneous melanoma samples were divided as follows: <0.76 mm (22 samples), 0.76–3 mm (15 samples), and >3 mm (16 samples). According to presence or absence of dysplasia, naevic lesions were divided as follows: dysplasic lesions (26 samples), lesions without dysplasia (10 samples).

Histology

The formalin fixed, paraffin-embedded samples were sectioned at 5 μm and stained with hematoxylin and eosin. The histologic diagnosis as well as tumor thickness, according to Breslow [1970] were re-examined.

Immunohistochemistry

Sections from each specimen were cut at 5–7 μm, mounted on glass, and dried overnight at 37°C. All sections were then deparaffinized in xylene, rehydrated through a graded alcohol series, and washed in phosphate-buffered saline (PBS). PBS was used for all subsequent washes and for antibody dilution. Endogenous peroxidase activity was blocked by 5% hydrogen peroxide. Then the sections were immunostained with the streptavidin-biotin system (Dako), using 3-amino-9-ethylcarbazide (AEC) as the final chromogen and hematoxylin as the nuclear counterstain. The primary antibodies (Santa Cruz Biotechnology, CA) were applied at room temperature for 1 h at the following dilutions: AP-2α (C-18) at 1:200; E-cadherin (H-108) at 1:150; c-kit (C-19) at 1:100; and p21 (F-5) at 1:100. The optimal working dilutions were defined on the basis of a titration experiment. Negative controls for each tissue section were prepared by leaving out the primary antibody. All samples were processed under the same conditions.

Scoring and Quantitation of the Immunoreactivity

Three observers (A.B., R.R., and F.B.) evaluated separately the staining pattern of the proteins separately and scored each specimen for the percentage of positive cells ($1 = <5\%$ of positive cells; $2 = 5–15\%$ of positive cells; $3 = 15–30\%$ of positive cells; $4 = >30\%$ of positive cells). The level of concordance, expressed as the percentage of agreement between the observers, was 91% (90 of 99 specimens). In the remaining specimens, the score was obtained after collegial revision and agreement. For all immunostainings, the immunopositivity was assessed as the percentage of positively stained tumor cells within the entire tumor area.

Statistical Analyses

Descriptive statistics and analysis of frequency where used to describe patients' characteristics, tumor thickness, tumor site, and expression of immunostaining indicators. Spearman's rank correlation was used to assess relationship between ordinal data (correlation matrix between immunostaining indicators, correlation between AP-2, E-cadherin c-kit, p-21, and tumor thickness). P values <0.05 was regarded as statistically significant. All statistical evaluations were performed using STATA® statistical software (Stata Corporation, College Station, TX. http://www.stata.com).

RESULTS

Expression of AP-2 and Its Downstream Targets in Human Melanoma Cell Lines

To study whether AP-2 expression level was related to c-kit, E-cadherin, and p21 expression in human melanoma cells, we first examined the status of these proteins in several established human melanoma cell lines, derived from melanoma metastases (see Materials and Methods). Western blot analysis showed that in four out of five of the cell line tested, an evident overexpression of p21 was visible compared to the expression level of AP-2, c-kit, and E-cadherin (Fig. 1).

Immunohistochemical Expression Pattern of AP-2 and Its Downstream Targets in Human Melanoma Samples

It is important to investigate whether the correlation found in established cell lines grown

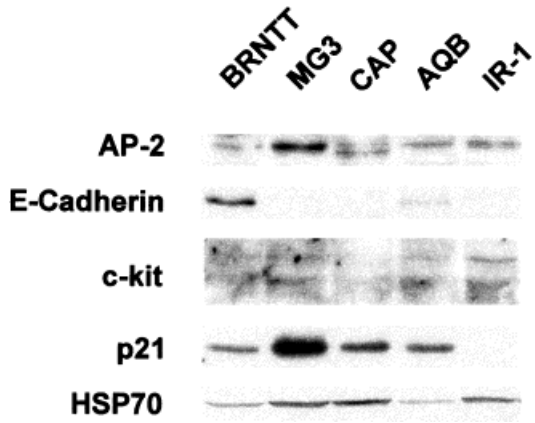

Fig. 1. Western blot analysis of a representative panel, showing AP-2, E-cadherin, c-kit, and p21 differential expression in several human melanoma cell lines (1 = BRNTT; 2 = MG3; 3 = CAP; 4 = AQB; 5 = IR-1). The amount of Heat Shock Protein 70 (HSP70) was used for normalization.

TABLE I. Melanoma Patients Characteristic

	Absolute frequency	Percentage frequency	Cumulative frequency
Sex	27		
Male		51	
Female	26	49	
Anatomic Site			
Head and neck	4	7.5	
Trunk	26	49	
Upper limbs	8	15	
Lower limbs	15	28.5	
Tumor thickness			
<0.75	22	41.5	41.5
0.75–3	15	28.3	69.8
>3	16	30.2	100

in vitro can also be observed in clinical melanoma tumor specimens. To this end we performed immunohistochemical staining for all the four examined proteins in a group of 99 clinical samples ranging from common nevi to melanoma metastases. Melanoma patient characteristics and histopathologic data have been reviewed and listed in Table I.

AP-2 Expression

AP-2 staining was either nuclear or cytoplasmatic. Compared to histologically normal adjacent epidermis, the lesions generally showed much lower AP-2 expression levels (Fig. 2A–D). The distribution of AP-2 expression into different categories is listed in Table II. According to AP-2 scoring, there was a significant negative correlation between AP-2 index and tumor thickness ($r = -.6212$; $P < .001$) (Table III). AP-2 index had a significantly positive association with E-cadherin expression levels ($r = +.7464$; $P < .001$) and with c-kit expression levels ($r = +.7846$; $P < .001$) (Table IV). Moreover there was a significant negative association between AP-2 index and p21 expression levels ($r = -.5887$; $P < .001$) (Table IV). AP-2 index correlated inversely in metastases ($P = <.001$). According to presence or absence of malignant lesions, AP-2 index correlated inversely with presence of malignant lesions ($P = <.001$). AP-2 index did not show to be able to distinguish between dysplastic nevi and nevi without dysplasia ($P = .852$). Finally, according to tumor thickness, AP-2 index did not correlate with sex, edge, and anatomic site.

E-Cadherin Expression

E-cadherin staining was consistently associated with the plasma membrane. Compared with histologically normal adjacent epidermis, all the lesions generally showed much lower E-cadherin expression levels (Fig. 2E–F). The distribution of E-cadherin expression into different categories is listed in Table II. Also for E-cadherin expression, there was a significant negative correlation between E-cadherin index and tumor thickness ($r = -.7558$; $P = <.001$) (Table III). The statistical analysis showed that E-cadherin index had a significantly positive

Fig. 2. Immunohistochemical analysis of AP-2 and its downstream targets in human melanoma. All the sections have been stained as described in the Materials and Methods section: the specific antibody stain is red. **A**: High expression of AP-2 in the superficial portion of a melanoma (Breslow > 0.75 mm) (original magnification ×100). **B**: Low expression of AP-2 in the deep portion of the same tumor (original magnification ×100). **C**: Very low expression of AP-2 in a nodular melanoma (Breslow > 3 mm) (original magnification ×100). **D**: Undetectable level of expression of AP-2 in lymph node metastasis of a melanoma (original magnification ×200). **E**: High expression level of E-cadherin in a melanoma (Breslow < 0.75 mm) (original magnification ×200). **F**: Undetectable level of expression of E-cadherin in a melanoma metastasis (original magnification ×400). **G**: High expression level of c-kit in a melanoma (Breslow < 0.75 mm) (original magnification ×100). **H**: Undetectable level of expression of c-kit in a melanoma metastasis (original magnification ×200). **I**: High expression level of p21 in the deep portion of a nodular melanoma (Breslow > 3 mm) (original magnification ×200). **J**: High expression of p21 in a melanoma metastasis (original magnification ×400). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]

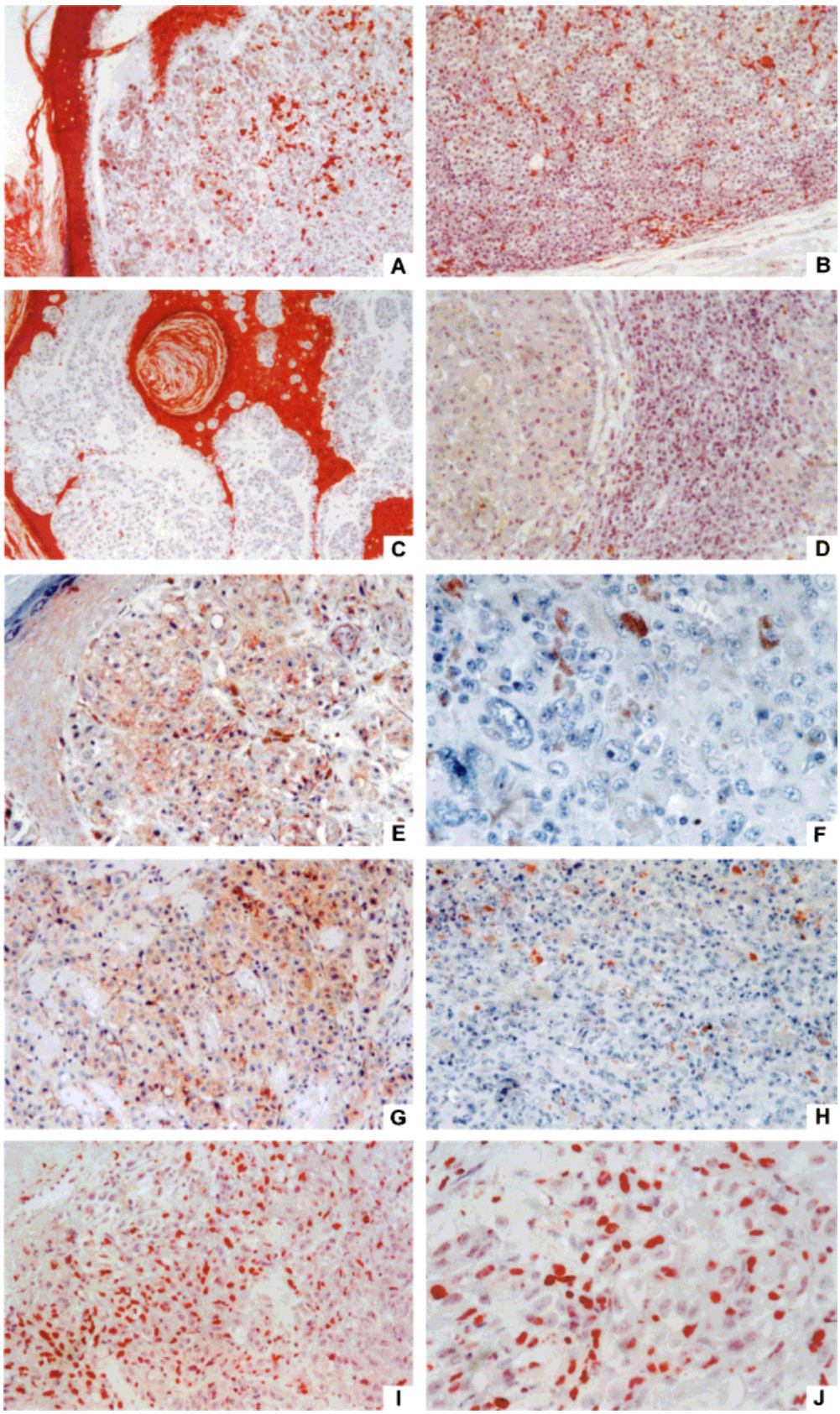
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TABLE II. Expression of AP-2, E-Cadherin, c-kit, p21 in Cutaneous Malignant Melanomas (Stratified by Thickness) and in Metastases

Immuno-staining	<0.75 mm			0.75–3 mm			>3 mm			Metastases		
	Abs φ	Perc φ	Cum φ	Abs φ	Perc φ	Cum φ	Abs φ	Perc φ	Cum φ	Abs φ	Perc φ	Cum φ
AP-2												
0–5 (%)	0	0	0	0	0	0	6	37.5	37.5	8	80	80
6–10 (%)	10	45.5	45.5	12	80	80	10	62.5	100	2	20	100
11–20 (%)	11	50	95.5	3	20	100	0	0	100	0	0	100
>20 (%)	1	4.5	100	0	0	100	0	0	100	0	0	100
E-cadherin												
0–5 (%)	0	0	0	3	20	20	10	62.5	62.5	9	90	90
6–10 (%)	6	27.3	27.3	10	66.7	86.7	6	37.5	100	1	10	100
11–20 (%)	14	63.6	90.9	2	13.3	100	0	0	100	0	0	100
>20 (%)	2	9.1	100	0	0	100	0	0	100	0	0	100
c-kit												
0–5 (%)	0	0	0	9	60	60	12	75	75	10	100	100
6–10 (%)	17	72.3	72.3	6	40	100	4	25	100	0	0	100
11–20 (%)	5	22.7	95	0	0	100	0	0	100	0	0	100
>20 (%)	0	0	95	0	0	100	0	0	100	0	0	100
p21												
0–5 (%)	2	9	9	0	0	0	0	0	0	0	0	0
6–10 (%)	18	82	91	3	20	20	2	12.5	12.5	2	20	20
11–20 (%)	2	9	100	9	60	80	7	43.75	56.25	6	60	80
>20 (%)	0	0	100	0	20	100	7	43.75	100	2	20	100

φ = frequency; abs = absolute; perc = percentage; cum = cumulative.

association with AP-2 expression levels and with c-kit expression levels ($r = +.7114$; $P < .001$) (Table IV). Moreover there was a significant negative association between E-cadherin index and p21 expression levels ($r = -.6377$; $P < .001$) (Table IV). E-cadherin index correlated inversely in metastases ($P = <.001$). According to the histological diagnosis, E-cadherin index correlated inversely with presence of malignant lesions ($P = <.001$). E-cadherin index was not able to distinguish between dysplatic nevi and nevi without dysplasia ($P = .861$). Finally, according to tumor thickness, E-cadherin index did not correlate with sex, edge, and anatomic site.

c-Kit Expression

The staining of c-kit was always around the plasma membrane. Also for this molecular marker, compared with histologically normal adjacent epidermis, the cutaneous lesions generally showed much lower c-kit expression levels (Fig. 2G–H). The distribution of c-kit expression into different categories is listed in Table II. The statistical analysis showed a significant negative correlation between c-kit expression and the thickness of melanoma samples ($r = -.6918$; $P = < .001$) (Table III). c-kit index was significantly associated with AP-2 and E-cadherin expression levels (Table IV), but showed a significantly negative association with p21 expression levels ($r = -.6512$; $P < .001$) (Table IV). c-kit index correlated inversely in metastases ($P < .001$) and, according to presence or absence of malignant lesions, correlated inversely with presence of cutaneous melanoma ($P < .001$). c-kit index was unable to distinguish between dysplastic and non-dysplastic nevi ($P = .872$). Finally, according to tumor thickness, c-kit index did not correlate with sex, edge, and anatomic site.

TABLE III. Spearman's Rank Correlation Between AP-2, E-Cadherin, c-Kit, p21, and Tumor Thickness

Parameter	Spearman's rank correlation coefficient	*P*
AP-2	−0.6212	<0.001
E-cadherin	−0.7558	<0.001
c-kit	−0.6918	<0.001
p21	+0.7126	<0.001

TABLE IV. Spearman's Rank Correlation Matrix (and Statistical Significance) for Molecular Markers. All Patients

	AP-2	E-cadherin	c-kit
E-cadherin	$R = +0.7464$ $P < 0.001$		
c-kit	$R = +0.7846$ $P < 0.001$	$R = +0.7114$ $P < 0.001$	
p21	$R = +0.5887$ $P < 0.001$	$R = -0.6377$ $P < 0.001$	$R = -0.6512$ $P < 0.001$

p21 Expression

The staining of p21 was always nuclear. In contrast with the other molecular markers, the cutaneous lesions generally showed higher p21 expression levels, if compared with histologically normal adjacent epidermis (Fig. 2I–J). The distribution of p21 expression into different categories is listed in Table II. The statistical analysis performed showed a significant positive correlation between p21 expression levels and tumor thickness of melanoma samples ($r = +.7126$; $P < .001$) (Table III). p21 index was significantly negatively associated with the other molecular markers (AP-2, E-cadherin, and c-kit) in all samples (Table IV). Moreover, p21 expression level did not show any significant correlation in metastases ($P = .706$), but it was significantly positive correlated with presence of cutaneous malignant lesions ($P < .001$). p21 index could not distinguish between dysplastic and non-dysplastic nevi ($P = .470$). Finally, according to tumor thickness, p21 expression level did not correlate with sex, edge, and anatomic site.

DISCUSSION

Several in vitro studies have shown that AP-2 is involved in the progression and metastasis of human cutaneous melanoma through the regulation of several downstream targets [Bar-Eli, 1999]. Thus, it has been suggested that loss of AP-2 expression is a crucial event in the development of malignant melanoma, since the suppression of endogenous AP-2 transactivator function may inhibit melanoma cells to respond to growth- and differentiation-regulatory signals [Buettner et al., 1993]. According to this hypothesis, screening of human melanoma cells in vitro for AP-2 protein expression has shown that the majority of highly metastatic cells express low to undetectable levels of AP-2 [Bar-Eli, 1997]. Furthermore, studies on the expression of AP-2 on clinical samples have shown that loss of AP-2 expression is associated with malignant transformation and tumor progression in stage I cutaneous melanoma [Karjalainen et al., 1998]. Despite a wealth of data about the correlation between expression of the AP-2 transcription factor and expression of several target genes in human melanoma cell lines, (to the best of our knowledge) studies looking at this correlation in clinical samples have not been performed.

We focused our attention on c-kit, E-cadherin, and p21 genes, whose transcription levels have been shown to be, at least, in part, regulated by AP-2 [Hennig et al., 1996; Huang et al., 1996; Bar-Eli, 1997]. In the preliminary screening we performed on several melanoma cell lines derived from metastatic melanoma, we found that AP-2, as well as E-cadherin and c-kit were consistently down-regulated. On the contrary, p21 was up-regulated. Starting from this observation, we looked at the immunohistochemical expression of these four proteins in a group of 99 clinical samples, ranging from normal nevi to melanoma metastases. Statistical analyses performed on the immunohistochemical scores, showed that AP-2 index was inversely correlated with tumor thickness and metastases. This result confirms previous data [Bar-Eli, 1997; Karjalainen et al., 1998]. When we compared the AP-2 index with the expression levels of its putative targets in the same group of clinical cases, we found that it had a significantly positive association with E-cadherin expression levels and with c-kit expression levels. On the other hand, there was a significant negative association between AP-2 index and p21 expression levels. This trend confirmed the pattern we have observed in the cell lines. Down-regulation of c-kit and E-cadherin during the progression of human cutaneous melanoma is a well known phenomenon [Natali et al., 1992; Cowley and Smith, 1996]. Our results strengthen the link between AP-2 and c-kit and E-cadherin and gives further evidence for AP-2 function as a tumor suppressor. Although a positive correlation between AP-2 and p21 immunostaining in human malignant melanoma has been described, we found a significant correlation between p21 expression levels and tumor thickness of melanoma samples and consequently an inverse correlation between AP-2 and p21 expression. The overexpression of the cyclin-dependent kinase inhibitor p21 in malignant melanoma compared to common acquired nevi has been already described in several studies. It has been suggested that high levels of p21 may confer upon melanoma tumors their known characteristic resistance to therapies [Trotter et al., 1997; Sparrow et al., 1998; Bales et al., 1999]. During progression of human melanoma, the expression of p21 may become dependent on other up-stream regulatory genes such as p53 [El-Deiry et al., 1993]. However, immunohistochemistry evaluates only the end

product of gene expression. Comparative studies at protein, mRNA and DNA levels are warranted in order to further address the functional link between AP-2 and the other proteins investigated.

Finally, the index of each of the four proteins did not show to be able to distinguish between dysplastic nevi and nevi without dysplasia. However, it is reasonable to think that changes in the expression of these proteins are involved in the later phases of melanoma progression, and possibly responsible for the transition from local invasive melanoma to metastasis.

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REFERENCES

Bales ES, Dietrich C, Bandyopadhyay D, Schwahn DJ, Xu W, Didenko V, Leiss P, Conrad N, Pereira-Smith O, Orengo I, Medrano EE. 1999. High levels of expression of p27KIP1 and cyclin E in invasive primary malignant melanomas. J Invest Dermatol 113:1039–1046.

Bar-Eli M. 1997. Molecular mechanisms of melanoma metastasis. J Cell Physiol 173:275–278.

Bar-Eli M. 1999. Role of AP-2 in tumor growth and metastasis of human melanoma. Cancer Metas Rev 18:377–385.

Barnhill RL, Mihm MC. 1993. The histopathology of cutaneous malignant melanoma. Semin Diag Pathol 10:47–75.

Breslow A. 1970. Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma. Ann Surg 12:902–908.

Buettner R, Kannan P, Imhof A, Bauer R, Yin SO, Glockshuber R, Van Dyke MW, Tainsky MA. 1993. An alternatively spliced mRNA from the AP-2 gene encodes a negative regulator of transcriptional activation by AP-2. Mol Cell Biol 13:475–480.

Clark WH. 1991. Tumor progression and the nature of cancer. Brit J Cancer 64:631–644.

Clark WH, Elder DE, Guerry D, Epstein MN, Greene MH, van Horn M. 1984. A study of tumor progression: the precursor lesions of superficial spreading and nodular melanoma. Hum Pathol 15:1147–1165.

Cowley GP, Smith ME. 1996. Cadherin expression in melanocytic naevi and malignant melanoma. Am J Pathol 179:183–187.

El-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kirzler KW, Vogelstein B. 1993. WAF1 a potential mediator of p53 tumor suppression. Cell 75:817–825.

Gaynor RB, Muchardt C, Xia YR, Klisak I, Mohandas T, Sparkes RS, Lusis AJ. 1991. Localization of the gene for the DNA-binding protein AP-2 to human chromosome 6p22.3-pter. Genomics 10:1100–1102.

Hennig G, Lowrick O, Birchmeier W, Behrens J. 1996. Mechanisms identified in the transcriptional control of epithelial gene expression. J Biol Chem 271:595–602.

Huang S, Luca M, Gutman M, McConkey DJ, Langley KE, Lyman SD, Bar-Eli M. 1996. Enforced c-kit expression renders highly metastatic human melanoma cells susceptible to stem cell factor-induced apoptosis and inhibits their tumorigenic and metastatic potential. Oncogene 13:2339–2347.

Huang S, Jean D, Luca M, Tainsky MA, Bar-Eli M. 1998. Loss of AP-2 results in downregulation of c-KIT and enhancement of melanoma tumorigenicity and metastasis. EMBO J 17:4358–4369.

Imagawa M, Chiu R, Karin M. 1987. Transcription factor AP-2 mediates induction by two different signal-transduction pathways: protein kinase C and cAMP. Cell 51:251–260.

Karjalainen JM, Kellokoski JK, Eskelinen MJ, Alhava EM, Kosma VM. 1998. Downregulation of transcription factor AP-2 predicts poor survival in stage I cutaneous malignant melanoma. J Clin Oncol 16:3584–3591.

Lassam M, Bickford S. 1992. Loss of c-kit expression in cultured melanoma cells. Oncogene 7:51–56.

Luca M, Huang S, Gershenwald JE, Singh RK, Reich R, Bar-Eli M. 1997. Expression of IL-8 by human melanoma cells upregulates MMP-2 activity and increases tumor growth and metastasis. Am J Pathol 151:1105–1113.

Mancianti ML, Clark WH, Hayes FA, Herlyn M. 1990. Malignant melanoma stimulants arising in congenital melanocytic nevi do not show experimental evidence for a malignant phenotype. Am J Pathol 4:817–829.

Mitchell PJ, Timmons PM, Hebert JM, Rigby PW, Tjian R. 1991. Transcription factor AP-2 is expressed in neural crest cell lineages during mouse embryogenesis. Genes Dev 5:105–119.

Natali PG, Nicotra MR, Winkler AB, Cavaliere R, Bigotti A, Ullrich A. 1992. Progression of human cutaneous melanoma is associated with loss of expression of c-kit protoncogene receptor. Int J Cancer 52:197–201.

Newton Bishop JA. 1997. Molecular pathology of melanoma. Cancer Metas Rev 16:141–154.

Parker SL, Tong T, Bolden S, Wingo PA. 1997. Cancer statistics. Cancer Clin 47:5–27.

Raschellà G, Tanno B, Bonetto F, Negroni A, Claudio PP, Baldi A, Amendola R, Calabretta B, Giordano A, Paggi MG. 1998. Retinoblastoma family genes induce differentiation in neuroblastoma cells and downregulate B-myb promoter. Cell Death Differ 5:401–407.

Robertson GP, Coleman AB, Lugo TG. 1996. Mechanisms of human melanoma cell growth and tumor suppression by chromosome 6. Cancer Res 56:1635–1641.

Sparrow LE, Eldon MJ, English DR, Heenan PJ. 1998. P16 and p21/WAF1 protein expression in melanocytic tumors by immunohistochemistry. Am J Dermatopathol 20:255–261.

Trotter MJ, Tang L, Tron VA. 1997. Overexpression of the cyclin-dependent kinase inhibitor p21 (WAF1/CIP1) in human cutaneous malignant melanoma. J Cutan Pathol 24:265–271.

Welch DR, Goldberg SF. 1997. Molecular mechanisms controlling human melanoma progression and metastasis. Pathobiology 65:311–330.

Williams T, Admon A, Luscher B, Tjian R. 1988. Cloning and expression of AP-2, a cell-type- specific transcription factor that activates inducible enhancer elements. Genes Dev 2:1557–1569.

Yarden Y, Kuang WJ, Yang-Feng T, Coussens L, Munemitsu S, Dull TJ, Chen E, Schlessinger J, Francke U, Ullrich A. 1987. A human proto-oncogene c-KIT: a new cell surface receptor tyrosine kinase for an unidentified ligand. EMBO J 6:3342–3351.

Zeng YX, Somasundaran K, El-Deiry WS. 1997. AP-2 inhibits cancer cell growth and activates p21/WAF/CIP1 expression. Nat Genet 15:78–82.