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Invited Review

Molecular changes in human melanoma metastasis

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Summary. The molecular changes associated with the transition of melanoma cells from radial growth phase to vertical growth phase (metastatic phenotype) are not well defined. Our recent studies have demonstrated that the two tumor suppressor genes, p53 and p16/CDKN2, do not play a major role in the acquisition of the metastatic phenotype in human melanoma. Mutations in p53 are infrequent and do not correlate with the metastatic potential of human melanoma cells while p16/CDKN2 abnormalities are frequent, but are not prerequisite for the acquistion of the metastatic phenotype. On the other hand, the tyrosine-kinase receptor c-KIT and the cell adhesion molecule MCAM/MUC-18 play active roles in the progression of human melanoma. Metastatic melanoma cells overexpress MCAM and do not express the c-KIT receptor. Enforced c-KIT expression in metastatic cells significantly inhibited their growth and metastatic potential in nude mice. Furthermore, exposure of c-KIT-positive melanoma cells *in vitro* and *in vivo* to stem cell factor (SCF), the ligand for c-KIT, triggered apoptosis of these cells but not of normal melanocytes. Ectopic expression of MCAM into primary cutaneous melanoma cells enhanced their tumorigenicity and metastatic ability *in vivo*. We found that both genes, c-KIT and MCAM, are regulated by the transcription factor AP-2 and that metastatic melanoma cells do not express AP-2. We therefore propose that loss of AP-2 might be a crucial event in the progression of human melanoma.

Key words: Malignant melanoma, Metastasis, Tumor suppressor gene, Apoptosis, Transcription factor

Introduction

Malignant melanoma is a common human cancer with high mortality rates. The incidence of this disease is currently increasing faster than any other malignancy. The primary cause of melanoma is thought to be UVR exposure (Fountain et al., 1990). Other risk factors include fair skin, a hereditary predisposition toward the disease, increased age and race (Rhodes et al., 1987). In the Caucasian population, for example, it is increasing rapidly and is projected to be about 1 in 75 by the turn of the century (Ahmed, 1997). The steady overall rise has been attributed to various causes ranging from the reduction of the stratospheric ozone to the changing lifestyles and attitudes that affect sun exposure (Frederick and Shell, 1988; Kerr, 1988; McPhail, 1997). Although it is easily diagnosed and wholly curable if detected early, the high mortality rate (6,700 deaths/yr. in the USA) is due to the propensity for melanoma to metastatize if left untreated (Dooley, 1994).

Most human malignant melanomas develop as a consequence of a lengthy multistep process. This process involves formation of nevi from normal melanocytes, a radial growth phase and a subsequent vertical growth phase (metastatic phenotype, see Fig. 1) which results in decreased dependence on growth factors, reduction of contact inhibition and diminished anchorage dependence (Lu and Kerbel, 1994). The prevalent working model for melanoma metastasis development defines it as a series of interrelated, sequential steps involving the survival and growth of unique subpopulations of cells with metastatic properties that preexisted within the parental neoplasm (Fidler, 1990).

Cytogenetic studies have shown that human melanomas contain a number of frequent chromosomal abnormalities which include translocations, partial deletions or losses that most frequently involve chromosomes 1p, 6p, 6q, 7q, 9p, 10q and 11q. Some of these chromosomal alterations are associated with loss of heterozygosity which strongly indicates that tumor suppressor genes may be present (Fountain et al., 1990). In fact, chromosomal mapping studies eventually led to the identification of the melanoma susceptibility gene, p16/CDKN2 (Kamb et al., 1994).

The molecular steps leading to human malignant melanoma and subsequent metastasis are not well known. Speculation is that they are reminiscent of other more well-defined cancer types like human colorectal cancer that is found to be the result of the cumulative effect of a series of dominant and recessive mutations (Fearon and Vogelstein, 1990; Lu and Kerbel, 1994).

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This brief review will summarize some of our studies concerning the contributions of various genes to the metastatic phenotype of human melanoma.

Relevance of p53 and p16/CDKN2 abnormalities to metastatic phenotype in human melanoma cell lines

Mutations in the p53 tumor suppressor gene are the most common somatic genetic changes associated with human cancer (Nigro et al., 1989; Holstein et al., 1991). The p53 gene codes for a nuclear protein which plays an important role in cell proliferation and tumor progression. Mutations in wild-type p53 inactivate its tumor-suppressor capabilities which leads to uncontrolled cell growth (Levine et al., 1991). Mutant p53 has been noted in various human malignancies including carcinomas of lung, breast, and colon (Weiss et al., 1993). The limited analysis of p53 alterations in cutaneous malignant melanoma have been reported with contradictory results. One study reported that point mutations in the p53 gene were present in only one of nine metastatic melanoma cell lines examined (Volkenandt et al., 1991). In another study 85% of primary and metastatic specimens examined showed detectable evidence of mutant p53 by using immunohistochemical detection of mutant p53 protein (Stretch et al., 1991).

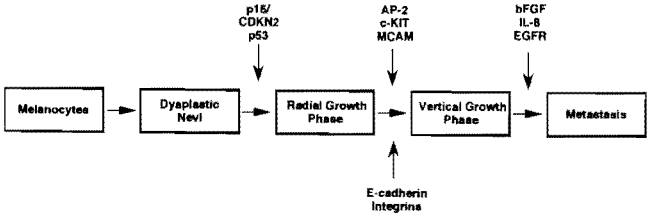

Fig. 1. Molecular changes associated with the progression of human melanoma. The development of malignant melanoma is a multistep process characterized by several genetic alterations. Abnormalities in the p16/CDKN2 gene are usually an early event. Mutations in the p53 gene are infrequent but were observed in early stages. Alterations in the cell-surface receptors c-KIT, MUC-18, E-cadherin and integrins occur in the transition from radial growth phase to vertical growth phase. The transcription factor AP-2 that regulates the expression of c-KIT, MUC-18 and E-cadherin is not expressed in metastatic cell lines. The expression of genes involved in angiogenesis such as bFGF, IL-8 (Luca et al., 1997; Singh et al., 1994, 1995) and EGFR correlates with higher metastatic potential of human melanoma cells.

The purpose of our studies was to determine the frequency of p53 mutations in human melanomas with different *in vivo* metastatic potential (Luca et al., 1993a,b). Eleven human melanoma cell lines derived from cutaneous lesions, lymph nodes, or brain metastases from different patients that ranged from poorly metastatic to highly metastatic were used in the study (see Table 1). The metastatic potential of these melanoma cell lines were determined in nude mice by monitoring experimental lung metastasis formation subsequent to i.v. injection of tumor cell suspensions or subsequent to growth s.c. (spontaneous metastasis) (Li et al., 1989; Schackert et al., 1990; Zhang et al., 1991). DNA was extracted and purified from the cell lines and screened for mutations in exons 5-9 of the p53 gene by SSCP (single-strand conformation poly-morphism) analysis (Murakami et al., 1991). Only the low metastatic DX-3 cell line, established from a subcutaneous melanoma metastasis (McCready et al., 1989), was found to be mutated. SSCP analysis located the mutation in the exon 8-9 region. The DX-3 DNA was then reamplified by PCR and sequenced to confirm and characterize the nature of the mutation. We detected a C to G transition at codon 278 which substitutes arginine for proline. This result was confirmed by immunohistochemical staining for p53. Using the mouse monoclonal antibody Pab 1801, which is immunoreactive with the mutated human p53 protein, only the DX-3 cell line showed positive staining. The cell lines were analyzed

Table 1. Analysis of abnormalities in human melanoma cell lines with different metastatic capabilities

CELL LINE	ORIGIN	METASTATIC PROPENSITY	p53 MUTATIONS	p16/CDKN2 MUTATIONS	MCAM EXPRESSION	c-KIT EXPRESSION
A375 P	Lymph node	Low	-	-	Intermediate	-
A375 Cl-5	*In vitro*	Low	-	-	Low	-
A375 Cl-28	*In vitro*	High	-	n/t	High	-
A375 SM	Selection (*In vivo*)	High	-	-	High	-
DM-4	Lymph node	High	n/t	Deletion	n/t	-
DX-3	Cutaneous	Low	C -> G	-	Low	-
MeWo		High	n/t	-	n/t	n/t
SB-2	Cutaneous	Non-metastatic	-	Deletion	-	+
SB-3	Cutaneous	Low		Deletion	Intermediate	n/t
SK-MEL28	Cutaneous	Low	n/t	CC -> TT	n/t	n/t
TXM-1	Lymph node	High	-	Deletion	n/t	-
TXM-13	Brain metastasis	High	-	Deletion	High	-
TXM-18	Brain metastasis	Low	-	Deletion	n/t	-
TXM-40	Brain metastasis	Low	-	n/t	Low	-
WM-115	Cutaneous	Low	n/t	GAC -> TAC	n/t	n/t
WM-266-4	Metastasis from WM-115	High	n/t	GAC -> TAC	n/t	n/t

n/t : not tested

for p53 allelic loss by Bgl II restriction fragment length polymorphism (RFLP). Only the low metastatic cell line, A375-Cl5, showed a loss of one allele. No mutation was observed in the remaining allele when analyzed by SSCP and direct sequencing.

Our results show that mutations in the p53 gene of human melanomas are infrequent and are not a prerequisite for the acquistion of the metastatic phenotype. This indicates that p53 might not actually play a major role in the pathology of human melanoma and that the observed sporadic p53 mutations may just be a consequence of the genetic instability of the cells.

The p16/CDKN2 tumor suppressor gene which has been associated with malignant melanoma susceptibility has been mapped to chromosomal region 9p21 (Kamb et al., 1994). It was found to encode a previously identified protein, p16, which is known to bind and inhibit the normal activity of cyclin-dependent kinase (CDK 4). The CDK family of proteins promote cell cycle passage and consequently cell proliferation and growth (Serrano et al., 1993). Mutations in p16/CDKN2 are found in both sporadic and familial cases of melanomas (Hussussian et al., 1994; Walker et al., 1995). The p16/CDKN2 gene is also found to be mutated or deleted in a wide variety of other tumors, including breast, lung, and bladder cancer (Kamb et al., 1994).

Our study sought to determine the relevance and contribution of p16/CDKN2 abnormalities to tumorgenicity and metastasis of human melanoma cells as examined *in vivo*. We wanted to know if the loss of p16/CDKN2 expression conferred a growth advantage to the cells and if these abnormalities are a prerequisite for the metastatic phenotype in human melanoma cells (Luca et al., 1995). Fourteen human melanoma cell lines with different metastatic potentials in nude mice were analyzed for possible abnormalities in the p16/CDKN2 gene. Like the previously described p53 study the cells lines were derived from cutaneous lesions, lymph nodes or brain metastases of different patients. The metastatic potentials of the cell lines were determined as in the previous study (Luca et al., 1993).

We tested for homozygous deletions by doing PCR amplification of p16/CDKN2 exons 1 and 2 of cell line genomic DNA. Exon 1 did not reveal abnormalities in any of the cell lines tested. When exon 2 was analyzed, we found homozygous deletions in six of the fourteen cell lines tested. Deletions of the p16/CDKN2 gene were found in the highly metastatic cell lines DM-4, TXM-1 and TXM-13, as well as the low to nonmetastatic cell lines TXM-18, SB-2 and SB-3 (see Table 1). The homozygous deletions observed by PCR amplification were confirmed by Southern blot analysis.

The expression level of the p16/CDKN2 gene in the human melanoma cell lines was examined by Northern blot analysis. As expected p16/CDKN2 was not expressed in any of the six human melanoma cell lines with homozygous deletions. Melanoma cell lines that did not exhibit homozygous deletions were screened by SSCP technique for potential point mutations. SSCP anaylsis of exon 1 did not reveal any mobility shifts. Exon 2, however, showed mobility shifts in three of the eight cell lines tested.

The three cell lines MEWO, WM-226-4 and WM-115 were analyzed by direct sequencing of the PCR product. The MEWO cell line revealed CC to TT transitions at codon 72 which has been characterized as a hallmark of UV induced DNA damage as reported by others for p53 mutations in nonmelanoma skin cancers (Brash et al., 1991). Mutations in the p16/CDKN2 gene with a "UV signature" have been reported in other melanoma cell lines (Liu et al., 1995). Collectively these data may support a possible role for UV light in the development of melanoma.

The other two cell lines WM-115 and WM-226-4 were found to have a GAC to TAC mutation with a tyrosine substituting for aspartic acid at codon 108. These cell lines were derived from the same patient at different stages of the disease. The WM-115 cell line was derived from an epithelioid tumor and the WM-266-4 from a metastasis. Both cell lines having the same point mutation at codon 108, indicates that the metastasis originated from the same primary clone and that the mutation in the p16/CDKN2 gene occurred at the primary stage.

Overall, abnormalities in the p16/CDKN2 gene were found in 9 of the 14 cell lines tested, six with homozygous deletions and three with mutations. However, the biological significance of these abnormalities is not clear. For example, homozygous deletion of p16/CDKN2 was found in the SB-2 melanoma cell line which is nonmetastatic and considered to be poorly tumorigenic in nude mice. The homozygous deletion did not confer a growth advantage to the cells *in vivo*. On the other hand, some highly metastatic cell lines like A375SM had no abnormalities in the p16/CDKN2 gene. This suggests that abnormalities in the p16/CDKN2 gene may not be necessary for the production of metastases by human melanoma cells.

Expression of MCAM/MUC-18 by human melanoma cells increases tumor growth and metastasis

Cell adhesion molecules (CAMs) are cells surface molecules with specific affinities for molecules on the surface of other cells and are thought to play an important role in mediating the interaction of cells with their environment. Changes in CAM expression have been associated with tumor progression and the development of metastasis in a variety of human malignancies (Johnson, 1991). MCAM/MUC-18, a 113-kDa cell surface glycoprotein, is a newly recognized CAM belonging to the immunoglobulin superfamily (IgSf) (Johnson et al., 1996). MCAM was originally identified because of its increased expression on malignant melanomas as compared to normal melanocytes and benign nevi. The majority of advanced and metastatic tumors strongly express the MCAM antigen, while its expression on thin tumors (<0.75), which have

only a low probability of metastasizing, and on benign nevi is weaker and less frequent (Lehmann et al., 1989).

Based on these findings we decided to investigate its role in melanoma metastasis. In our initial study we examined the expression of MCAM in nine human melanoma cell lines with known metastatic abilities in nude mice (see Table 1). The level of MCAM mRNA expression in the different cell lines was determined by Northern blotting. The cell surface expression of the MCAM glycoprotein was assayed by its immunoreactivity with anti-MCAM monoclonal antibodies followed by FACS analysis and direct immunostaining. We concluded from the collective results of our three assays that there is a statistically significant correlation between MCAM expression and the ability to form metastasis *in vivo* (Luca et al., 1993a,b).

In order to provide further and more direct evidence for the involvement of MCAM to the acquistion of the metastatic phenotype we transfected MCAM negative melanoma cells with the MCAM gene and subsequently analyzed their tumorigenic and metastatic abilities in nude mice (Xie et al., 1997). We chose to transfect SB-2 cells that were previously found to be negative for MCAM expression and that are poorly tumorigenic and nonmetastatic in nude mice (Luca et al., 1993a,b). We transfected cells with either an expression vector carrying the full-length human MCAM cDNA or an empty control vector. Following transfection individual neo-resistant colonies were selected and established in culture. Two individual MCAM cell lines were established. Western blot analysis confirmed the expression of the 113-kDa MCAM antigen in both of these cell lines but not in the neo-transfected or parental cell lines.

We determined the tumorigenicity and metastatic potential of our transfected MCAM cell lines by injecting them into BALB/c nude mice. Tumor growth was monitored once a week and the tumors were harvested and analyzed for MCAM expression. The parental and neo-transfected cell lines did not begin to form palpable tumors until 2 months after injection which agreed with previous findings that SB-2 cells are poorly tumorigenic in nude mice (Xie et al., 1997). Conversely, both of our MCAM transfected clones formed detectable tumors after 30 days and grew in all of the injected mice. Metastatic potential was analyzed by experimental lung metastasis assay. Lung metastases were assayed 60 days after cells were injected into the lateral tail vein of BALB/c nude mice. Parental and neo-transfected cells did not metastasize to the lung. The MCAM transfected cells lines formed metastases in 7 of the 10 mice injected. Our results indicate that enforced expression of MCAM in SB-2 cells rendered them highly tumorigenic and increased their metastatic potential in nude mice when compared to parental and transfected control cells.

We continued our study by evaluating the role of MCAM in homotypic aggregation by analyzing the ability of our SB-2 transfected cell lines to grow in a three-dimensional (multicellular spheroid) culture system. We found that coculturing the cells with a MCAM antibody disrupted spheroid formation only in cells expressing MCAM. This data coupled with the observation that melanoma cells can adhere to purified MCAM (Shih et al., 1995) indicates the possible involvement of MCAM in homotypic aggregation.

Further assays performed to support the involvement of MCAM in melanoma metastasis showed that the transfected cells displayed increased attachment to human endothelial cells, a decreased ability to adhere to laminin and increased invasiveness through Matrigel-coated filters. The above changes in functions attributed to the expression of MCAM may underlie the contribution of MCAM to the malignant phenotype.

Enforced c-KIT expression of highly metastatic human melanoma cells inhibits their tumorigenic and metastatic potential and renders them susceptible to apoptosis

The c-KIT protooncogene encodes a transmembrane tyrosine receptor whose expression is lost or diminished in melanoma cells compared to the high levels found in normal melanocytes. About 70% of human melanoma cell lines or metastatic lesions examined do not express detectable levels of the c-KIT receptor (Lassam and Bickford, 1992; Natali et al., 1992). Early studies in mice mapped c-KIT to the dominant white spotting (w) locus (Chabot et al., 1988). Subsequent studies found the c-KIT ligand to be the product of the steel locus (Sl) which encodes the stem cell factor (SCF) (Zsebo et al., 1990). Mutations in the (w) locus and/or (Sl) locus have been found to produce the piebald phenotype manifested as white spotting that has been attributed to a local reduction in the number of cutaneous melanocytes. Injection of neutralizing anti-KIT antibodies into pregnant mice also produced the piebald phenotype (Nishikawa et al., 1991). Mutations in c-KIT have been identified in human piebald patients indictating it may also be required for human melanocyte development (Fleishmann et al., 1991).

Interestingly, activation of c-KIT with its ligand was reported to cause growth inhibition in c-KIT expressing melanoma cell lines which is the opposite effect elicited in normal melanocytes where increased proliferation is detected (Zakut et al., 1993). The seemingly dual nature of c-KIT functionally qualifies it as a quasi suppressor gene in melanoma cells but as a protooncogene in other tumors that express it (Lu and Kerbel, 1994). This discrepancy has been postulated to be due to aberrant signal transduction by c-KIT in melanoma cells in response to the ligand (Zakut et al., 1993) but this has yet to be confirmed.

A previous study from our laboratory had shown that lack of c-KIT expression in malignant melanomas correlates with higher metastatic potential in nude mice (Gutman et al., 1994; see Table 1). In order to further investigate these results, we transfected the c-KIT gene

into highly metastatic A375SM cells and show that their tumorigenic and metastatic abilities are significantly reduced when injected into nude mice. We also propose a mechanism that may explain why the c-KIT ligand, SCF, causes growth inhibition in melanoma cells that express c-KIT (Huang et al., 1996). We chose the A375SM cells for transfection because they do not express either c-KIT or SCF and are highly metastatic in nude mice (Luca et al., 1995). Cells were infected with retroviruses carrying a full-length human c-KIT cDNA (LXSN-KIT) or the parent virus (LXSN-NOT). Following retroviral infection neo-resistant colonies were pooled and established in culture. Cell lines were screened by Northern blot analysis which detected appreciable levels of the expected 3.5 Kb mRNA transcript in the infected cells but not in parental A375SM cells or in cells infected with the control virus LXSN-NOT. The cell lines were checked for stable transduction of c-KIT by analyzing tumors produced by subcutaneous injections in nude mice. The expression of c-KIT in the cell lines was also verified by immunohistochemical staining and FACS analysis using the monoclonal antibody SR-1 which is directed against the extracellular domain of c-KIT. We also assayed for the ability of SCF to stimulate c-KIT autophosphorylation and MAP-kinase phosphorylation in A375SM-KIT cells. We found that SCF induces autophosphorylation of the 145-kDa c-KIT receptor and the phosphorylation of MAP-kinase in A375SM cells but not in the parental and neo-transfected line indicating that the signaling cascade was restored in the A375SM-KIT cells.

The tumorigenicity of the cell lines was determined by injecting $1x10^6$ cells subcutaneously into BALB/c nude mice. Parental and neo-tranduced cell lines showed progressive tumor growth while the c-KIT transduced cells showed significant growth inhibition. The metastatic potential of the cell lines was analyzed by experimental lung metastasis assay. Two different dosages of cells ($5x10^6$ and $1x10^6$) were tested by intravenous injection into the lateral tail vein of BALB/c mice and the number of lung metastases were counted six weeks after injection. Transduction of c-KIT was shown to significantly reduce the potential to form lung metastases. These results were confirmed *in vitro*. A375SM-KIT cells and A375SM parental control cells were grown in culture in the presence of r-h-SCF (200 ng/ml) for three days. Cell death occurred in the c-KIT-transduced cells whereas SCF had no effect on the proliferation and growth of the parental A375SM cell lines.

These results demonstrated that SCF induces apoptosis in melanoma cells expressing c-KIT *in vitro* which may provide a mechanism for its action. To test this hypothesis we examined the ability of SCF to induce apoptosis in A375SM-KIT cells, A375SM-NOT cells and 888-mel (a c-KIT positive control). Addition of SCF (200 ng/ml) to the cell media for three days induced apoptosis in A375SM-KIT and 888-mel cells but not in the A375SM-NOT cells. Studies utilizing the TUNEL method (Gavrieli et al., 1992) for identification of DNA fragmentation in cells undergoing apoptosis *in vivo* confirmed our *in vitro* studies. Subcutaneous tumors were established by injection of A375SM-KIT cells and A375SM-NOT cells. Thirty days later phosphate buffered saline or r-h-SCF was injected daily (1 mg/mouse) into the site of the tumor for five consecutive days. SCF induced apoptosis in tumor cells produced by A375SM-KIT but not in tumor cells produced by A375SM-NOT cells. Since SCF is produced by keratinocytes and other dermal cells in the skin, these results suggest that the loss of c-KIT receptor expression may allow malignant cells to escape SCF/c-KIT-mediated apoptosis, hence contributing to tumor growth and eventually metastasis.

Our data point to the usefulness of SCF as an antitumor and antimetastatic agent in early stages of melanoma while the cells are still expressing c-KIT. Furthermore, c-KIT might be used in gene therapy for advanced tumors and established melanoma metastases. Specific expression of c-KIT in melanoma cells *in vivo* could be achieved by ligating the tyrosinase promoter upstream of the c-KIT gene in the retroviral expression vectors used in our studies.

Conclusions and future directions

Despite improvement in early detection and treatment of primary melanoma, the diagnosis of melanoma is often made after the cancer has already metastasized to the regional and distant lymph nodes, liver, lung, brain and central nervous system (Fidler, 1992). Since current staging systems enable use to identify only some of the melanoma patients who will develop metastases, better prognostic determinants need to be idenitfied. We believe that the two tumor suppressor genes p53 and p16/CDKN2 are not useful staging markers. Abnormalities in p53 are infrequent and do not correlate with the metastatic potential of human melanoma cells (Luca et al., 1993a,b). While p16/CDKN2 abnormalities were observed in 64% of melanoma specimens and cell lines, some of them in early stages of melanoma progression, the biological significance of these abnormalities is not yet clear.

Our data suggest that the production of metastasis by human melanoma cells can occur in the absence of p16/CDKN2 gene abnormalities (Luca et al., 1995). On the other hand, MCAM and c-KIT proteins can serve as true staging markers for human melanoma (see progression model, Fig. 1). Overexpression of MCAM and lack of expression of the c-KIT receptor occur in the vast majority of metastatic lesions and metastatic melanoma cell lines. The molecular basis for up-regulation of MCAM and lack of c-KIT expression in metastatic melanoma cells is unknown.

We have recently demonstrated that both genes are highly regulated by the transcription factor AP-2 and that metastatic melanoma cells do not express AP-2 (Bar-Eli, 1997). Since AP-2 also regulates other genes

that are involved in the progression of human melanoma such as E-cadherin, MMP-2, p21/WAF-1 and HER-2, we therefore propose that loss of AP-2 might be a crucial event in the development of malignant melanoma. This hypothesis is currently under investigation in our laboratory.

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References

Ahmed I. (1997). Malignant melanoma: prognostic indicators. Mayo Clinic Proc. 72, 356-361.

Bar-Eli M. (1997). Molecular mechanisms of melanoma metastasis. J. Cell Physiol. 173, 275-278.

Brash D.E., Rudolph J.A., Simon J.A., Lin A., McKenna G.J., Baden H.P., Halperin A.J. and Ponten J. (1991). A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. Proc. Natl. Acad. Sci. USA. 88, 10124-10128.

Chabot B., Stephenson D.A., Chapman V.M., Besmer P. and Bernstein A. (1988). The proto-oncogene c-kit encoding a transmembrane tyrosine kinase receptor maps to the mouse W locus. Nature 335, 88-89.

Dooley T. (1994). Recent advances in cutaneous melanoma oncogenesis research. Oncol. Res. 6, 1-9.

Fearon E.R. and Vogelstein B. (1990). A genetic model for colorectal tumorigenesis. Cell 61, 759-767.

Fidler I.J. (1990). Critical factors in the biology of human cancer metastasis: twenty-eighth G.H.A. Clowes Memorial Award Lecture. Cancer Res. 50, 6130-6138.

Fidler I.J. (1992). The biology of melanoma metastasis. In: Cutaneous melanoma. Balch C.M., Houghton A.N., Milton G.W., Sober A.J. and Soong S.J. (eds). J.B. Lippincot Company, Philadelphia, PA, pp 112-129.

Fleischman R.A., Saltman D.L., Stastny V. and Zneimer S. (1991). Deletion of the c-kit protooncogene in the human developmental defect piebald trait. Proc. Natl. Acad. Sci. USA 88, 10885-10889.

Fountain J.W., Bale S.J., Housman D.E. and Dracopoli N.C. (1990). Genetics of melanoma. Cancer Surveys 9, 645-671.

Frederick J.E. and Shell H.E. (1988). Ultraviolet radiation levels during the Antartic spring. Science 241, 438-440.

Gavrieli Y., Sherman Y. and Ben-Sasson S.A. (1992). Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J. Cell Biol. 119, 493-501.

Gutman M., Singh R.K., Radinsky R. and Bar-Eli M. (1994). Intertumoral heterogeneity of receptor-tyrosine kinases expression in human melanoma cell lines with different metastatic capabilities. Anticancer Res. 14, 1759-1766.

Holstein M., Sidransky D., Vogelstein B. and Harris C.C. (1991). p53 mutations in cancers. Science 253, 49-53.

Huang S., Luca M., Gutman M., McConkey D.J., Langley K.E., Lyman S.D. and Bar-Eli M. (1996). Enforced c-KIT expression renders highly metastatic human melanoma cells susceptible to stem factor-induced apoptosis and inhibits their tumorigenic and metastatic potential. Oncogene 13, 2339-2347.

Johnson J.P. (1991). Cell adhesion molecules of the immunoglobulin supergene family and their role in malignant transformation and progression to metastatic disease. Cancer Metast. Rev. 10, 11-22.

Hussussian C.J., Struewing J.P., Goldstein A.M., Higgins P., Ally D.S., Sheahan M.D., Clark W.H., Tucker M.A. and Dracopoli N.C. (1994). Germline p16 mutations in familial melanoma. Nature Genet. 8, 15-21.

Johnson J.P., Rummel M.M., Rothbacher U. and Sers C. (1996). MUC18: a cell adhesion molecule with a potential role in tumor growth and tumor cell dissemination. Curr. Top. Mircrobiol. Immunol. 213, 95-105.

Kamb A., Gruis N.A., Weaver-Feldhaus J., Liu Q., Harshman K., Tavtigian S.V., Stockert E., Day R.S. III, Johnson B.E. and Skolnick M.H. (1994). A cell cycle regulator potentially involved in genesis of many tumor types. Science 264, 436-440.

Kerr R.A. (1988). Stratospheric ozone is decreasing. Science 239, 1489-1491.

Lassam N. and Bickford S. (1992). Loss of c-kit expression in cultured melanoma cells. Oncogene 7, 51-56.

Lehmann J.M., Riethmuller G. and Johnson J.P. (1989). MUC18, a marker of tumor progression in human melanoma, shows sequence similarity to the neural cell adhesion molecules of the immunoglobulin superfamily. Proc. Natl. Acad. Sci. USA 89, 9891-9895.

Levine A.J., Momand J. and Finlay C.A. (1991). The p53 tumour-suppressor gene. Nature 351, 453-456.

Li L., Price J.E., Fan D., Zhang R.D., Bucana C.D. and Fidler I.J. (1989). Correlation of growth capacity of human tumor cells in hard agarose with their *in vivo* proliferative capacity at specific metastatic sites. J. Natl. Cancer Inst. 81, 1406-1412.

Liu Q., Neuhausen S., McClure M., Frye C., Weaver-Feldhaus J., Gruis N.A., Eddington K., Allalunis-Turner M.J., Skolnick M.H. and Fujimura F.K. (1995). CDKN2 (MTS1) tumor suppressor gene mutations in human tumor cell lines. Oncogene 10, 1061-1067.

Lu C. and Kerbel R. (1994). Cytokines, growth factors and loss of negative growth controls in the progression of human cutaneous malignant melanoma. Curr. Opin. Oncol. 6, 212-220.

Luca M., Hunt B., Bucana C.D., Johnson J.P., Fidler I.J. and Bar-Eli M. (1993a). Direct correlation between MUC18 expression and metastatic potential of human melanoma cells. Melanoma Res. 3, 35-41.

Luca M., Lenzi R., Lee-Jackson D., Gutman M., Fidler I. and Bar-Eli M. (1993b). p53 mutations are infrequent and do not correlate with the metastatic potential of human melanoma cells. Int. J. Oncol. 3, 19-22.

Luca M., Xie S., Gutman M., Huang S. and Bar-Eli M. (1995). Abnormalities in the CDKN2 (p16 /MTS-1) gene in human melanoma cells: relevance to tumor growth and metastasis. Oncogene 11, 1399-1402.

Luca M., Huang S., Gershenwald J.E., Singh R.K., Reich R. and Bar-Eli M. (1997). Expression of interleukin-8 by human melanoma cells up-regulates MMP-2 activity and increases tumor growth and metastasis. Am. J. Pathol. 151, 1105-1113.

Murakami Y., Hayashi K. and Sekiya T. (1991). Detection of aberrations of the p53 alleles and the gene transcript in human tumor cell lines by single-strand conformation polymorphism. Cancer Res. 51, 3356-3361.

Mc Cready D.R., Balch C.M., Fidler I.J. and Murray J.L. (1989). Lack of comparability between binding of monoclonal antibodies to melanoma cells in vitro and localization in vivo. J. Natl. Cancer Inst. 81, 682-687.

McPhail G. (1997). There's no such thing as a healthy glow: cutaneous malignant melanoma- the case against suntanning. Eur. J. of Cancer Care 6, 147-53.

Natali P.G., Nicotra M.R., Winkler A.B., Caveliere R., Bigotti A and Ullrich A. (1992). Progression of human cutaneous melanoma is associated with loss of expression of c-kit proto-oncogene receptor. Int. J. Cancer 52, 197-201.

Nigro J.M., Baker S.J., Preisinger A.C., Jessup J.M., Hostetter R., Cleary K., Bigner S.H., Davidson N., Baylin S., Devile P., Glover T., Collins F.S., Weston A., Modali R., Harris C.C. and Vogelsteing B. (1989). Mutations in the p53 gene occur in diverse human tumour types. Nature 342, 705-708.

Nishikawa S., Kusakabe M., Yoshinaga K., Ogawa M., Hayashi S., Kunisada T., Era T., Sakakura T. and Nishikawa S. (1991). In utero manipulation of coat color formation by a monoclonal anti-c-kit antibody: two distinct waves of c-kit-dependency during melanocyte development. EMBO J. 10, 2111-2118.

Rhodes A.R., Weinstock M.A., Fitzpatrick T.B., Mihm M.C. and Sober A.J. (1987). Risk factors for cutaneous melanoma. JAMA 258, 3146-3154.

Schackert G., Price J.E., Zhang R.D., Bucana C.D., Itoh K., and Fidler I.J. (1990). Regional growth of different human melanomas as metastases in the brain of nude mice. Am. J. Pathol. 136, 95-102.

Serrano M., Hannon G.J. and Beach D. (1993). A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature 366, 704-707.

Singh R.K., Gutman M., Radinsky R., Bucana C.D. and Fidler I.J. (1994). Expression of interleukin-8 correlates with the metastatic potential of human melanoma cells in nude mice. Cancer Res. 54, 3242-3247.

Singh R.K., Gutman M., Reich R. and Bar-Eli M. (1995). Ultraviolet B irridiation promotes tumorigenic and metastatic properties in primary cutaneous melanoma via induction of interleukin-8. Cancer Res. 55, 3669-3674.

Shih I.M., Elder D.E., Speicher D., Johnson J.P. and Herlyn M. (1995). Isolation and functional characterization of the A32 melanoma-associated antigen. Cancer Res. 54, 2514-2520.

Stretch J.R., Gatter K.C., Ralfkiaer E., Lane D., Harris A.L. (1991). Expression of mutant p53 in melanoma. Cancer Res. 51, 5976-5979.

Volkenandt M., Schlegel U., Nanus D.M. and Albino A.P. (1991). Mutational analysis of the human p53 gene in malignant melanoma. Pigment Cell Res. 4, 35-40.

Walker G.J., Hussussian C.J., Floré J.F., Glendening J.M., Haluska F.G., Dracopoli N.C., Hayward N.K. and Fountain J.W. (1995). Mutations of the CDKN2/p16^{INK4} gene in Australian melanoma kindreds. Human Mol. Genet. 4, 1845-1852.

Weiss J., Schwecheimer K., Cavenee W.K., Herlyn M. and Arden K.C. (1993). Mutation and expression of the p53 gene in malignant melanoma cell lines. Int. J. Cancer 54, 693-699.

Xie S., Luca M., Huang S., Gutman M., Reich R., Johnson J.P. and Bar-Eli M. (1997). Expression of MCAM/MUC18 by human melanoma cells leads to increased tumor growth and metastasis. Cancer Res. 57, 2295-2303.

Zakut R., Perlis R., Eliyahu S., Yarden Y., Givol D., Lyman S.D. and Halaban R. (1993). KIT ligand (mast cell growth factor) inhibits the growth of KIT-expressing melanoma cells. Oncogene 8, 2221-2229.

Zhang R.D., Price J.E., Schackert G., Itoh K. and Fidler I.J. (1991). Malignant potential of cells isolated from lymph node or brain metastases of melanoma patients and implications for prognosis. Cancer Res. 51, 2029-2035.

Zsebo K.M., Williams D.A., Geissler E.N., Broudy V.C., Martin F.H., Atkins H.L., Hsu R.Y., Birkett N.C., Okino K.H. and Murdock D.C. (1990). Stem cell factor is encoded at the Sl locus of the mouse and is the ligand for the c-kit tyrosine kinase receptor. Cell 63, 213-224.