Review paper

WHAT'S NEW IN MALIGNANT MELANOMA, BIOLOGY AND EPIDEMIOLOGY

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ABSTRACT

The authors relate about the biological pattern of melanoma cells; melanoma cells express melanoma associated antigens (MAA), which are not specific of melanoma cells and melanocytic cells of advanced stages of tumor progression, but express the melanoma progression markers (MPM). It's difficult to assess the expression of MPM mostly because of lack of a large multicenter comparison and different results due to different methods. The demonstration that biological response modifiers (BRMs), in vitro, inhibit the growth of melanoma cell lines and that they modulate the expression of MAA justify their therapeutic application. Some groups of basic scientists have demonstrated the genetic abnormalities coding for dysplastic nevi and melanoma to be expressed on chromosomes 1,6,7,9 and 10. It isn't clear yet, whether these abnormalities involve all melanomas.

The risk factors for cutaneous melanoma: family history of melanoma; previous melanoma; large number of common melanocytic nevi; history of severe burning, tendency of freckling, inability to tan; presence of atypical moles but also the increasing number of common melanocytic nevi are important predictive factors to detect individuals at risk.

KEY WORDS

malignant melanoma, melanoma associated antigens, melanoma progression markers, adhesion molecules, risk factors, epidemiology

In recent years, basic scientists have grown increasingly interested in melanoma cells. The great interest in melanoma depends on three main factors:

1) the steady increase of melanoma incidence over the past 40 years and the resistance of advanced melanoma to conventional chemo- and radiation therapy;

2) benign and malignant lesions of human melanocytes present one of the best characterized model systems for the study of tumor progression in vivo. Thus melanoma is a valuable model to study phenotypic traits that are regulated during cell differentiation and malignant transformation;

3) the easy accessibility of human melanocytic lesions for clinical, pathological and experimental investigations and the good success rate of culturing melanoma cells for experimental studies.

The melanocytic lesions in vivo do represent

sequential steps of tumor progression of the melanocytic system. These main steps are characterized by differences in clinical and histopathological aspects and by different biological markers (1). Some questions arise from previous considerations:

1) do biological markers exist which can differentiate melanocytic lesions from non melanocytic ones and among the melanocytic the benign from the malignant?
2) are the tumoral progression steps and the different evolution times connected with different biological markers?
3) if so, could the tumor biological pattern allow us to foresee the evolution of the disease.

And still, from the knowledge of tumor biology, could we:

4) better understand the function of the biological response modifiers (BRMs)?
5) foresee the efficacy of the treatment with BRMs (and with any other therapeutic weapon)?

Biological properties of melanoma are closely related to the expression of a series of antigens on melanoma cells. Such antigens are not melanoma specific and can be grouped in:

A: antigens specific of melanocyte lineage;
B: antigens shared with normal or tumoral cells of other cell lineage.

It introduces the concept of melanoma associated antigens (MAA), largely expressed on melanoma cells but not specific. In Fig. 1 MAA are grouped according to their biological functions. However, the MAA may have multiple functions and such a division into functional groups will need future revision (2). Among these different groups of antigens only the pigmentation related antigens belong to the antigens specific of melanocytic lineage, all the others belong to the antigens shared with normal or tumoral cells of different lineage. These are the antigens of the I and II MHC, involved in the immune recognition, the growth factors/cytokines and related receptors, the proteins involved in binding and transport of cations, necessary for cellular metabolism, the proteases and proteases inhibitors, allowing the invasiveness of tumoral cells into the surrounding environment, adhesion molecules necessary for cell contacts between cells and substrate, extracellular matrix proteins (ECM) secreted by the cell and gangliosides, important for adhesion processes and immune interactions and the cytoskeletal proteins essential for the cell movement and closely related to the activity of adhesion molecules.

Among these antigens, some of them are used as markers of melanocytic lineage, some others - the majority - are used as markers of melanoma progression. The most commonly used markers of melanocytic lineage are: HMB45 defined antigen (100/7 kDa), specific marker of melanocytes as it is a pigmentation related antigen expressed on premelanosomes, and the S-100 protein which is not a specific marker of melanocytes and is generally used in combination with markers of other cell lineage (like cytokeratins) to identify poorly differentiated amelanotic melanomas in which HMB45 is often non reactive (3).

Among the antigens shared with normal or tumoral

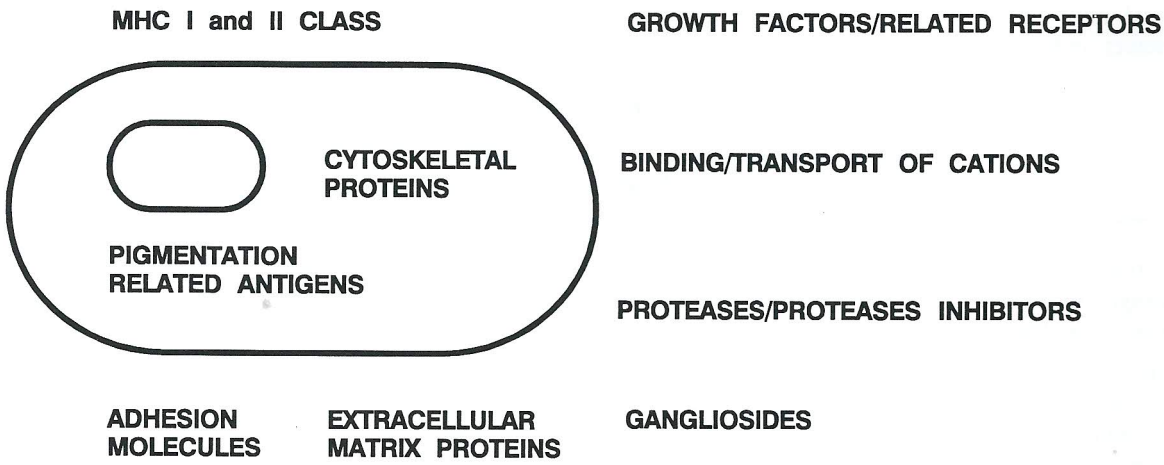

Fig. 1. Antigens of melanocyte/melanoma cells

cells of other cell lineage, there are the antigens related to the tumor progression, belonging to the so-called Melanoma Progression Markers (MPM).

Tumor progression is a term used to describe a set of genotypic and phenotypic traits that characterize neoplastic cells through the biological events that accompany the process of malignant transformation and evolution toward metastatic cells. MPM are biological-antigenic markers that are found more frequently expressed on melanocytic cells of advanced stages of tumor progression. It means that they are found more frequently in vertical growth phase (thick melanomas and metastases), in situ, or on cultured cells obtained from such lesions, in vitro.

Adhesion molecules are surface receptors that may mediate cell-cell and cell-substrate contacts; they include a number of molecules, including integrins and the members of immonoglobulin superfamily.

Integrins are heterodimers consisting of an alpha subunit not covalently associated with a beta subunit. The receptor complex spans the plasma membrane, linking the internal cytoskeleton with the outside environment. Main ligands of integrins are the proteins of the extracellular matrix (ECM); specificity for ligand binding is determined by the particular association of an alpha and beta subunits. The integrins are divided in sub families, each defined by a common beta subunit (4).

Many integrins are expressed on melanoma cells. Of the beta 1 subfamily, alpha 2 beta 1 is expressed on cultured melanoma cells able to metastasize (5); alpfa 4 beta 1 is found on metastatic lesions and on primary melanomas followed by metastatic spreading (6).

The beta 3 subunit (the beta component of the vitronectin receptor, VNR) is strikingly expressed in metastatic and thick primary melanomas (7).

Alpha 6 beta 1 (whose ligand is laminin) is inversely correlated to malignancy because it is more frequently expressed on benign than on malignant lesions (8).

ICAM-1 and MUC 18 are members of the immunoglobulin superfamily and belong to the group of cell-cell adhesion molecules (9).

The ligand of ICAM-1 is LFA-1 (expressed on the surface of T lymphocytes); thus this molecule is involved in both antigen- restricted and antigen independent immune cell contacts which are required for any cellular response (10).

The expression of ICAM-1 in primary melanomas increases with tumor thickness and is higher in metastatic than in primary lesions (11). The functional role of MUC 18 in melanoma is less clear since the ligand for this molecule has not yet been identified. However MUC 18 is remarkably melanoma specific, being present on melanoma but not on other tumors. Expression of MUC 18 correlates with thickness of melanoma, being expressed on at least 1 mm thick primary melanomas (12).

Cells of melanocytic lineage produce a number of extra cellular matrix protein (ECM), but we do not know clearly their role. Melanoma cells produce fibronectin, laminin and collagen IV (13). Melanoma cells also produce higher amounts of tenascin than normal melanocytes but cannot adhere to it (14).

The secretion of Grow Factors (GF) and the expression of GF receptors increase with malignancy. Melanoma production of both GF and GF receptors suggest the possibility of autocrine and paracrine pathways of growth control, allowing cells to escape normal growth regulation (15).

There is a high degree of modulation of HLA expression during melanoma progression, i.e. reduction or loss of HLA class I antigens and appearance of HLA class II (16). The appearance of HLA class II antigens is more frequent in melanoma than in other neoplasias, suggesting a crucial role of the interactions between immune system and tumor. Loss of HLA class I expression may offer a distinct advantage to subpopulations of tumor cells to escape from a cellular immune response against the tumor. In fact, the specific effective immune response by the MHC-I restricted Cytotoxic CD3+ CD8+ lymphocytes of the intratumoral T cell population (TIL) requires the expression of MHC-I (HLA-I class) antigens on target cells (17).

GD2 and GD3 are found in adhesion plaques of melanoma cells, concentrated at the site of cell-matrix interactions (18). In addition to a role in adhesion, GD may also exert effects on immune system (stimulation of suppressor cells?) (19). Among growth - related markers the most important is Ki67, a nuclear antigen whose expression correlates with the thickness of primary melanoma (20). So, the expression of MPM cannot be utilized in order to make a distinction between common nevus and early melanoma. In fact, a marker able to mark malignant transformation of melanocytes has not yet been found. MPM - simply - are more frequently expressed on advanced lesions toward malignancy.

In conclusion, the large majority of MPM are

more often and more largely expressed on melanoma metastases, on primary melanomas thicker than 1 mm, and on primary melanomas with clinically evident metastases. Therefore some of these markers have been proposed as prognostic markers to identify among primary melanomas those with a higher risk of metastases: Alpha 4, beta 3 and HLA-II antigens.

As a matter of fact, in assessing the expression of MPM we meet with difficulties as: A) lack of a large multicenter comparison, B) difficult comparison of results due to different methods, C) difficult availability of suitable tissues; in particular, at present only MHC I and class II can be detected in paraffin embedded, formalin fixed tissues, whereas beta 3, alpha 4, ICAM 1, MUC 18, Ki67 require frozen sections, which are hardly obtained from small lesions.

Advances in biology could perhaps improve our melanoma prevention program. Various groups have attempted to identify the genetic locus or loci for a dominant melanoma-dysplastic nevus trait. Several regions of the genome have been suggested to contain genes associated with dysplastic nevi and melanoma.

Studies on genetic abnormalities have focused on chromosomes 1,6,7,9 and 10 because non-random changes have been found on these chromosomes of melanoma cells. A putative gene related to the "familiar nevus dysplastic syndrome" was localized on chromosome 1p in some families affected by this syndrome (in patients with both dysplastic nevi and melanoma). However, it seems likely that only a subset of familiar melanoma is linked to chromosome 1p (21-22). We hope that in the near future molecular genetics might help us in identifying high risk patients, which is one of the goals of the melanoma prevention programs. According to the National Health Consensus Conference of 1992, the best known risk factors for cutaneous melanoma are listed in Table 1. The family history of melanoma increases the risk for the disease. However, according to a recent report of Aikken (23), there is an heterogeneity of melanoma risk in the families of melanoma patients. Parents, children and siblings of more than one thousand invasive cutaneous melanoma patients were examined to investigate whether the risk increased consistently among the relatives of melanoma patients. A subgroup of families (less than 2%) had a significantly higher risk than others: part of the risk variation was explained by differences in the distribution of phenotipical risk factors (24). Concerning previous cutaneous melanoma it occurs in at least 3-9% of

Table 1. Risk factors for cutaneous melanoma

Risk factors for cutaneous melanoma (NH Consensus Conference, 1992)
• family history of melanoma
• previous melanoma
• large number of common melanocytic nevi
• history of severe burning, tendency of freckling, inability to tan
• presence of atypical moles

melanoma patients (25). Risk factors for multiple primary melanoma are related to the following:

1) age less than 40 at initial diagnosis of melanoma,
2) presence of dysplastic nevi,
3) clinically atypical nevi,
4) familiar history of melanoma and dysplastic nevi,
5) male sex.

Fair skin, blond-red hair color, inability to tan and frequent sunburn, tendency to freckling and number of common melanocytic nevi are predicts of melanoma risk: in some of the studies these variables were independent risk factors (26).

Various studies differ due to criteria of selection different questionnaires, different skills of interviewers, the selection of appropriate controls, etc. In Table 2 the relative risk associated to each variables resulting from studies carried out in different populations are shown. In order to predict the risk of cutaneous melanoma, just recently a great importance has been attributed to the diameter of nevi and to their clinical aspects, more than to their histopathological pattern. As a matter of fact, clinical atypia does not mean histologic atypia (27). In a case control study carried out by Grob and coworkers in France, the presence of at least 5 nevi with a diameter ranging from 5 to 10 mm is associated with a ten-fold increased risk for cutaneous melanoma and the presence of more than one clinically atypical nevi showed a relative risk of 2.7. In the opinion of the authors, when using the number of nevi as risk marker, their size might be more important than other features of clinical atypia (28).

Prospective studies (cohort studies) have shown that clinical assessment of clinically atypical moles - with or without pathological confirmation - is a useful predictor of future melanoma (29).

Table 2. Risk factors for cutaneous melanoma

Risk factors for cutaneous melanoma			
number of CMN	RR	Country	Authors
> 25 (whole body)	24.8	England	Swerdlow, 1984
> 6 (arm)	6.1	Denmark	Osterlind, 1988
> 6 (whole body)	n.s.	Italy	Cristofolini, 1987
> 15 (arm)	6.2	England	Elwood, 1990
> 120 (diam. 1-6 mm)	19.9	France	Grob, 1990
Many (self report)	11.2	Canada	Marret, 1992
Tendency to freckling			
	2.8	Canada	
	6.2	England	
	2.9	Denmark	
Fair skin			
	2.1	Canada	
	1.3	Denmark	
	4.0	Italy	
Blond hair color			
	4.0	Canada	
	1.7	Denmark	
	n.s.	Italy	
Frequent sunburn			
	3.8	England	
	3.4	France	
	2.2	Italy	

CMN = Common Melanocytic Nevi
RR = Relative Risk

In order to evaluate the relative risk associated with the different aspects of acquired melanocytic nevi in the Italian population, we have recently performed a case-control study of the Florentine population. Large nevi are defined as more than 6 mm in diameter independently of other signs of clinical atypia. Clinically atypical nevi are defined as being more than 6 mm in diameter, and having an irregular or ill-defined border and variegated color. Considering as reference category fewer than 10 common melanocytic nevi, a number ranging from 10 to 30 results in a relative risk of 2.6. More than 30 common melanocytic nevi increase the risk by more than 20-fold. Moreover the relative risk is 2.9 for the presence of at least one large nevus and 8.4 for the presence of at least one clinically atypical nevus.

We concluded that also in the Florentine population the increasing number (more than 20) of common melanocytic nevi is the most important risk factor

for cutaneous melanoma. The presence of at least 1 clinically atypical nevus or the presence of large nevi is a risk factor for cutaneous melanoma; however the multivariate analysis showed a strong relation between an high common melanocytic nevi count and such factors.

Individuals at risk should be the main target of the screening programs. As stated by NH Consensus Conference, cutaneous melanoma meets most of the criteria for initiating screening. The goal of screening programs and information campaigns is the early detection.

In any case, in our fight against melanoma major effects should be focused on primary prevention and on identification of the causes of the tumor. We know that the reported incidence of cutaneous melanoma has increased steadily after the past two decades. The total incidence of cutaneous melanoma in 1987 compared with that in 1979-80 almost doubled in males and increased by 50% in females. It is estimated that 1 in 14 men and 1 in 17 women in Queensland will develop melanoma in their life (30). In all countries in which incidence data are available, an increase of the incidence of cutaneous melanoma is shown. In Canada (31), the average annual increases in the age-standardized incidence rate from 1970 to 1986 6.0% among men, and 4.6% among women; the corresponding increases in the death rate were 3.4% and 1.6%. Data from the Connecticut Cancer Registry indicate a rise in incidence as early as 1940 (32). In Scotland between 1979 and 1989 the incidence of melanoma rose by 80% or 7.4% per annum (33). The present doubling time for the incidence of cutaneous melanoma for men in Scotland is 8 years and for women 13.5 years. Similar step rates of increase are observed in other European countries such as Denmark.

Concerning the causes for this epidemiological trend over the past fifteen years, a burst of epidemiological activity - primarily in the form of case control study - has helped to clarify the role of sunlight and the phenotypic factors that modify it. Conflicting data concerning the role of sunlight are:

- melanoma occurs in parts of the body less exposed than others;

- melanoma occurs more frequently in the North than in the South of Europe; indoor workers and higher social class are more affected.

Several findings identify recreational and vacation exposure as being especially hazardous; this has led to the "intermittent exposure hypothesis" (34). Recently there has been increasing evidence that the timing of sun exposure is important, and that events occurring in the first two decades of life may have a special role in determining the risk of melanoma. Studies on immigrants - mainly from England and Ireland - to Australia showed that migration in the first decade of life led to a risk of melanoma similar to that of native born white population, while migration at later ages was associated with a lower risk (35). Data obtained from Mediterranean populations, also in a non-fair-skinned population, the history of sunburn in childhood (36) was associated with a relative risk of 5.9. The data of our Department are in agreement with previous data: after adjustment for age, sex and place of birth a statistically significant trend in risk according to the frequency of sunburn in childhood was found.

However, the history of intense sunexposure in the first two decades seems to be more important than the history of sunburn. Biologically this fact may suggest that melanocytes in children are more sensitive to the sun or that in the context of the multistage model of carcinogenesis may increase the chances of completing the remaining stages in subsequent life (36).

ABBREVIATIONS

BRM	Biological Response Modifiers
MAA	Melanoma Associated Antigens
MPM	Melanoma Progression Markers
MHC	Major Histocompatibility Complex
ECM	Extracelular Matrix Proteins
HMB45	Melanin Marker
VNR	Vitronectin Receptor
ICAM-1	Intercelular Adhesion Molecule
LFA 1	Leukocyte Function Associated Molecule
GF	Growth Factors
TIL	Tumor Infiltrating Lymphocytes
GD2	Gangliosides
GD3	Gangliosides
Ki67	Proliferating Cell Antigen
NHCC	National Health Concensus Conference

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