Molecular histology on the diagnostic cutting edge between malignant melanomas and cutaneous melanocytomas (Review)

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Abstract. In recent years, the screening accuracy of clinical dermoscopy has increased in the early detection of evolving atypical melanocytic neoplasms. However, the most dramatic cases of human malignant melanomas (MM), i.e. the fast-growing neoplasms, usually escape the classical clinical criteria of MM. As a result, a number of puzzling cases exhibit uncertain clues for MM. Thus, the risk of microscopic misdiagnosis is likely on the rise if the histological criteria are not fine-tuned. This review summarizes a conceptual classification of atypical melanocytic neoplasms grouped under the heading of melanocytomas. Some immunohisto-chemical markers are tentatively used as discriminators between fast-growing MM and melanocytomas. However, some differences seem to be more statistically significant than clinically useful due to extensive overlap in immunoreactivity from any case to case. A multipronged immunohistological screening is therefore welcome.

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1. Introduction

Over the past decades, the worldwide incidence of human malignant melanoma (MM) has considerably increased in Caucasian populations (1-4). In this perspective, there is growing need to improve diagnostic procedures supporting early treatment and decreasing MM morbidity and mortality.

Currently, MM is in part classified according to its clinical growth rate. Fast-growing MM are typically characterized by a vertical growth pattern. They have a worse prognosis compared to slow-growing MM which are commonly confined superficially (5-7). In fact, this concept is related to the histo-logical distinction between the radial (superficially spreading) MM and the vertical growth phase (invasion of the dermis).

2. Histological distinction between atypical melanocytic neoplasms

In clinical practice a clear-cut distinction is required between benign and malignant melanocytic neoplasms (8-10). In most instances, the histological diagnosis of MM is straightforward for experts in the field (11,12). MM are commonly classified according to the total thickness in millimeters, the mitotic activity, the presence of ulceration, the penetration depth, and the identification of metastases (13). However, due to the wide spectrum of histological features the situation may occasionally appear ambiguous (11,12). For instance, in small size melanocytic lesions, all the classical histological criteria for borderline MM or undisputable MM are not always met, or they fail to make the distinction confidently. Those neoplasms are tentatively classified in a spectrum going from 'benign' to 'not-so-benign', 'not-so-malignant', and 'malignant' (10). In the literature, the two intermediate categories are variously referred to as melanocytomas, melanocytic dysplasias, minimal deviation melanomas, borderline melanomas, melanocytic tumors of uncertain malignant potential (MELTUMP) and spitzoid melanocytic tumors of uncertain malignant potential (STUMP) (10,14-22).

Although conventional histology is the mainstay for diagnosing atypical melanocytic neoplasms, clinical features, in particular the dermoscopic aspects remain of central importance. Cutaneous melanocytoma in its strict etymological sense, refers to benign tumor of melanocytes (10,14). This term encompasses melanocytic neoplasms which do not meet

the classical histological criteria of any type of common melanocytic naevi and MM (10,18,21,22). They often develop singly but occasionally, multiple melanocytomas occur. Some of these lie grouped together (agminate type) and potentially recur after removal of a solitary lesion (23). The term melanocytoma is similarly used in other fields of human pathology (leptomeninges, eye) and animal pathology (skin) for distinguishing peculiar and usually benign melanocytic neoplasms (24,25).

The recognition of the 'newer' intermediate set of atypical melanocytic neoplasms and a better molecular staging of MM types has benefited in recent years from progress in immunohistochemistry (9,10,15,18,26-31). Using such an approach, most uncommon MM variants are also identified with confidence (9,17,19,32). It is acknowledged that MM molecular alterations and their respective immunological responses accompany the neoplastic progression from incipient to advanced stages (9,18). In this field, it is possible to use markers of proliferation, melanocytic differentiation and immunomodulation. In addition, the identification of signalling molecules, nerve growth factors and receptors is potentially useful, particularly in spindle cell variants of MM.

3. Triggered melanocytic naevi and cutaneous melanocytomas

In a series of endogenous and exogenous conditions, melanocytic naevi are triggered and they may appear as melanocytomas. Some specific internal messages and environmental factors have been identified in this field. Examples are melanocytomas developed on congenital naevi, dysplastic naevi (33), naevi modified by pregnancy or oral contraception (34), naevi of subjects on growth hormone therapy (15), naevi under ultraviolet-light irradiation (35,36). It should be mentioned that some of these lesions mimic MM or are at risk for MM development (37). The possibility of a melanocytoma should be evoked in each single case. In this review, the term melanocytoma encompasses a broad category of melanocytic neoplasms that can further be distinguished according to the identified origins and the clinico-pathological confrontation.

Globally, the melanocytoma class is histologically recognized by the combination of some criteria including architectural disorganisation and asymmetry, discrete nuclear atypia and anisokaryocytosis as well as the eventual juxtaposition of an ancillary focal or diffuse inflammatory cell reaction (10). However, the variable combination and extent of such signs in different lesions preclude establishing any straightforward set of major criteria identifying distinct specific subsets of melanocytomas, with, however, the exception of the common type of Spitz melanocytoma (naevus) (10).

Another special type of melanocytoma presents as an atypical dermal nodule in an otherwise normal-looking melanocytic naevus. Such atypical nodules may suggest an intralesional transformation recognized by some pathologists as a sign of malignancy, although it does not exhibit other features of aggressive behaviour. The increase in size of this type of melanocytic lesion is mainly due to more abundant, pale cytoplasm in each individual cell. The nuclei show only a marginal increase in size and do not exhibit pleomorphism. There may be invagination of the nucleus by cytoplasm, giving a vacuolated appearance. Mitoses are hardly ever seen.

After excluding the pregnancy-related changes in melanocytic lesions, the overall melanocytoma gender ratio (F/M) reaches 1.6 (10). The age distribution is similar in both gender groups. The prevalence of all melanocytomas peaks during the 3rd and 4th decades of life and a sharp decrease occurs after the age of 50 years on. Such age and gender distributions resemble that of MM (2,4).

The distinction between melanocytomas and MM ideally expects that all members of each group behave either completely benign or fully malignant. This concept probably does not fully hold true. The variability in the histological presentations of MM and melanocytomas poses diagnostic difficulties and the clinical attributes may remain disturbing. Any error in this differential diagnosis has profound consequences including mutilating overtreatment or, conversely, life-threatening undertreatment. At the present time, controversies exist as to the diagnoses to be given for certain neoplasms and their predictive evolution leading to potential implication in legal liability (38).

4. Phenotypic presentations of cutaneous melanocytomas

Some differentiation markers are routinely used when facing an atypical melanocytic neoplasm (9,10,18,26). Immunohistochemistry is used as an adjunct for distinguishing MM and melanocytomas or other neoplasms. The common antibodies are directed to the S100 protein, the gp100/HMB45, the melan A/MART-1, the CD63/NKi-C3 and tyrosinase.

The S100 protein is a 21 kDa acidic calcium-binding protein which is abundant in the nucleus and cytoplasm of melanocytic cells. HMB45 is a marker of the cytoplasmic premelanosomal glycoprotein gp100. The melanoma antigen recognized by T-cells-1 (MART-1) is similar to Melan-A. The CD63/NKi-C3 is a 25-110 kDa glycoprotein located on the inner membrane of cytoplasmic vesicles in melanocytes. Tyrosinase is an enzyme that hydrogenates tyrosine in the process of melanin synthesis.

In general, the common benign melanocytic neoplasms are structured in an orderly manner showing symmetry and so-called maturation with deeper location of smaller cells toward the base of the lesion. The immunohistochemical labelling of differentiation markers including S100 protein and melan-A is often intense, diffuse and uniform in these lesions and in most melanocytomas as well (18). Tyrosinase immunoreactivity decreases toward the base of most melanocytomas.

In benign melanocytic naevi, the HMB-45 and CD63 immunoreactivities are negative or restricted to the superficial part of the lesions inside the papillary dermis. Some lesions show trailing off with progressive descent in the deeper portion of the melanocytic naevus. These two markers are quite often expressed in tandem in melanocytomas. Lateral asymmetry and spotty or patchy patterns of immunohistochemical differentiation are typically found in MM and in melanocytomas including MELTUMP and STUMP (10,18,19,27). According to the antibody, the immunohistochemical pattern may be diffuse throughout the melanocytomas.

In general, the sensitivity of the differentiation markers decreases or becomes heterogeneous in MM with increasing clinical stage including metastatic lesions (39). Such a feature is not found with maturation of melanocytomas. It should be mentioned that immunohistochemistry proved to be very useful in tracking MM microsatellites (40) which are predictors of sentinel lymph node metastases and relapse-free survival (41).

5. Growth fraction, proliferation and apoptosis in cutaneous melanocytomas

The disturbance of autonomous growth regulatory pathways in melanocytomas and MM appears to be of prime importance. Earlier observations relied on the assessment of the number of mitotic figures and AgNOR (Nuclear Organizing Region) staining. This proved to be fastidious giving only a rough estimate of the proliferative rate. Mitoses observed in the deepest parts of the melanocytic neoplasm appeared to have greater significance for MM diagnosis than those more superficially located. However, there were no absolute thresholds for the mitotic index being indicative of malignancy. Atypical tripolar mitotic figures were indicative for MM rather than melanocytomas.

Twenty-five years ago, a breakthrough was obtained using autoradiography after incorporation of tritiated thymidine (42). A correlation was found between the ^{3}H-TdR labelling index and the MM thickness. Metastases remained with a high immunolabelling index while benign melanocytic lesions showed a very low proportion of cells in S phase. As the radioautography procedure was almost impossible to apply routinely, the method was switched to the PCNA and Ki67 immunolabellings which brought similar information. There are two clinical applications for which these markers have been studied, namely the distinction of melanocytic naevi from MM and the assessment of clinical prognosis for patients with MM.

The most widely used proliferation marker is Ki67, a nuclear antigen present in all active phases of cell cycle proliferation (G_1, S, G_2 and M), but absent in the quiescent phase (G0). Ki67 immunolabelling has been shown to be positive in <5% of cells in most melanocytic naevi. Up to 15% positivity may be found in melanocytomas (10,43-47). The quantitative loss of Ki67 expression with depth correlates with maturation and less atypical lesions (19,48). A brisk mitotic rate or Ki67 index is not a common feature of melanocytomas, but rather suggests MM. Most thin MM appear to be in a growth-stunted phase exhibiting an accretive rather than proliferative formation of nests. Thicker MM and their metastases have a higher Ki67 index. On the overall, the Ki67 index reaches 15-30% of MM cells although some individual cases show much more nuclear positivity. The Ki67 index alone is not a reliable discriminator between MM and melanocytomas when it ranges in the overlap 10-20%. The presence of MM stem cells (low Ki67 index) (49) and clonal events (50) further complicate the process (51).

In our experience, the Ki67 index of keratinocytes is often higher than that of tumoral cells in melanocytomas. The reverse situation is seen in MM thicker than 0.4 mm.

Apoptosis is quite different from necrotic cell death and represents one major mechanism involved in reducing the expansible growth of melanocytic neoplasms. As a functional counterpart of mitosis, apoptosis plays a crucial role and is normally firmly regulated. Apoptosis is deranged in melanocytic neoplasms when the components and regulators of the cellular apoptotic machinery are mutated or present in inappropriate amounts. The pro-apoptotic factors include Bax, Bid, Fas/FasL, IFN, c-Kit/SCF, Noxa, p53, PITSLRE, PUMA, TNF and TRAIL. The anti-apoptotic factors include Bcl-2, Bcl-XI, livin, Mcl-1, ML-LAP, NFκB and survivin. Alternatively, other molecules including endothelins, integrins, c-Myc and TRAF-2 may show either pro- or anti-apoptotic effects (52).

Apoptosis may appear quite active in melanocytomas. The so-called Kamino bodies in Spitz melanocytoma likely result from such a process. At present the complex machinery of apoptosis has not been thoroughly studied in melanocytomas.

6. Microvasculature of cutaneous melanocytomas

In thin MM, the microvascular density shows a wide range of development among distinct lesions. An overall stochastic relationship appears to be present between the microvasculature size and the size of the germinative compartment.

Microvessels have been reported to be fewer in melanocytomas than in MM. The vascular endothelial growth factor (VEGF) is frequently detectable in MM, contrasting with the usual negativity in melanocytomas (10).

The extent of angiogenesis may help in distinguishing melanocytomas from MM (10). However, it should be noted that some growth-stunted MM show weak angiogenesis (53). Conversely, angiomatoid melanocytomas have been described (54-57). Therefore, the differences in MM and melanocytoma angiogenesis seem to be more statistically significant than clinically useful due to the extensive overlap in extent from any case to case.

7. Microscopic satellites and MM stem cells

The definition of malignancy is disputed in the field of melanocytic neoplasms. Indeed, distant locations including nodal infiltration by melanocytic cells is not synonymous with a metastatic spread. There may be melanocytomas, particularly with a spitzoid aspect, that do not remain confined to the primary site. They exhibit the propensity to spread regionally in the skin (agminate type) and in the lymph nodes, but do not reach more distant sites. The interpretation of regional spread is subject to controversy. Some authors regard it as formal proof of malignancy and consider the secondary lesions as satellitosis or in-transit metastases (40). Others argue that this stance constitutes an over-interpretation. Nonetheless, it appears that the regional cutaneous and nodal spread of melanocytomas neither equals distant metastasis nor constitutes sufficient proof of malignancy. All these considerations are relevant in orienting the choice of systemic therapy for metastatic MM (58).

It is possible that cells at the origin of these distant locations in MM and melanocytomas are different in nature. MM stem cells are possibly involved in MM metastases (59,60). By contrast, melanocytoma satellitosis do not apparently involve stem cells.

8. Conclusion

Melanocytic neoplasms of the skin are heterogeneous in nature and in aspect. A vast number of genetic changes are described in malignant melanoma, but the primary ones are not clearly defined. Melanocytomas represent benign melanocytic neoplasms showing atypical features at the histological inspection. However, there is no single criterion defining melanocytomas. The distinction between malignant melanoma and melanocytomas is of utmost importance for the management of patients. The combination of moderately increased cell proliferation and heterogeneous patchy differentiation is a clue for melanocytomas irrespective of their type. Most melanocytomas appear to result from the effect of some internal or extrinsic triggering factors.

Traditional classification systems for MM have centered on clinicopathological correlations. Although some particular MM subtypes are clinically and histologically distinct, a wealth of studies indicates that current parameters for classification are without independent prognostic value. Indeed, some markers of potential clinical utility looking promising in small-scale studies fail to prove clinically useful in larger-scale studies. Such disappointing features appear to stem from the heterogeneous nature of MM during its evolution. A vast number of molecular changes are indeed present in advanced MM compared with melanocytes. Various mutations are disclosed from comparative gene expression profiling of MM from different stages. It is difficult to sort out which are central to malignancy and typical for MM. In the wide range of molecular changes it is therefore difficult to sharply define when a given melanocytic neoplasm becomes malignant. In this context, melanocytomas are part of an evolving paradigm for classifying melanocytic neoplasms.

Melanocytomas form an intermediate category of melanocytic neoplasms between common naevi and MM. They behave as benign lesions but their histological presentations may be troublesome or worrying. Immunohistochemistry evaluating differentiation markers and proliferation markers helps distinguishing melanocytomas from MM. A multipronged immunohistochemical analysis should be performed in order to reach a high level of diagnostic accuracy.

We underscore recent studies that have provided insights into the diversity of melanocytic neoplasms. We expect these studies and subsequent analogous studies will inform the melanoma research community and the dermatologist, and provide novel ways to manage patients with atypical melanocytic neoplasms.

Acknowledgements

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