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MELANOCYTIC TUMOUR PATHOLOGY

Melanoma progression

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Summary

Tumours progress to fully malignant neoplasms through stages of development in a stepwise process. A carcinogenic stimulus such as UV light typically results in a large number of lesions, most of which are benign. Most such lesions will remain stable or regress, while a few will develop cytological and architectural atypia, placing them in a morphologically 'intermediate' category between wholly benign and fully malignant. It is important to categorise intermediate lesions, as they may be simulants, risk markers, and potential precursors of malignancy. Although many but not all malignancies arise in an evident precursor lesion, the vast majority of 'potential precursors' will not progress, as is evidenced by their vastly greater numbers in populations. Progression continues with the onset of malignancy including in metastatic disease. In melanoma as in other tumours, progression has been clearly related to the stepwise acquisition of genetic abnormalities. The first step is the activation of a single 'driver' oncogene, which is sufficient to induce a benign neoplasm whose growth is limited by oncogene-induced senescence driven by activated suppressors. In the intermediate lesions, additional genetic alterations occur such as additional driver mutations or heterozygous loss of suppressors due to copy number variation or other mechanisms. Fully malignant lesions are characterised by complete loss of relevant suppressors and by additional abnormalities, which together account for attributes of malignancy such as invasion and metastasis. Any of the steps of progression can be 'skipped', potentially due to telescoped progression or to alternative pathways. Although stages of progression might simply be viewed as markers of an individual's risk for developing subsequent stages, genetic associations that have been demonstrated among contiguous stages of progression in complex primary tumours and in their metastases would argue against this. For example, complex primary melanomas can be associated with remnants of earlier stage lesions both clinically and histologically. These include small symmetrical benign naevi and/or morphologically atypical dysplastic naevi ('precursor naevi'), and/or radial growth phase melanomas many of which may be inexorably progressive but lack competence for metastasis. Vertical growth phase is the stage of melanoma progression in which risk for metastasis may be acquired. This risk can be characterised statistically using prognostic attributes which at present are mostly clinicopathological, although in the future molecular profiling may contribute or even supplant these attributes. In metastatic disease, tumours can continue to progress, acquiring resistance to various therapeutic strategies which presents a considerable challenge to the efficacy of current promising therapeutic strategies.

Key words: Melanoma; progression; naevi; dysplastic naevi; radial growth phase; vertical growth phase; metastatic melanoma; genomics.

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CHARACTERISTICS OF TUMOUR PROGRESSION

Tumour progression was perhaps first described by Rous, the discoverer of the eponymous sarcoma virus, who in 1935 defined the concept as one of tumours 'going from bad to worse'.[1] Progression in this sense is essentially a clinical concept, emphasising the increasing lethal potential of a given tumour over time, and providing a basis for early diagnosis and excision of tumours at a stage when they are more likely to be curable. Experimental studies in mice by Foulds and others demonstrated that the initial response to a carcinogen is not usually a fully developed malignancy, but rather a benign tumour, such as a papilloma.[2] These benign tumours are often multiple, and most of them are inert. Over time, a small subset of these lesions may develop changes in their morphology and behaviour, including increasing size, atypical gross and microscopic morphology, and ultimately the ability to metastasise. These changes do not represent a global change in the pre-existing lesion, but rather the development of new patterns of proliferation. These patterns tend to occur in discrete steps, progressing from the initial benign lesion to a fully malignant lesion through intermediate steps characterised by increasing atypia. At some point, an intermediate lesion may acquire potential for inexorable growth, and then for metastasis. At this point, cure becomes more difficult or impossible. Although the initial lesions can be thought of as 'precursors', progression in any one lesion is not inevitable, but represents a rare event, with most lesions remaining stable or regressing. With increasing understanding of the genetic basis of cancer, it has become clear that these 'rare events' represent somatic changes in the genome of the lesional cells, such as activating mutations of oncogenes, inactivating mutations of suppressor genes, copy number variation, and so on.[3] These changes will be reviewed in more detail below.

TUMOUR PROGRESSION IN THE EPIDERMAL MELANOCYTIC SYSTEM

In the epidermal melanocytic tumour system, benign, intermediate and fully malignant steps of progression have long

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been recognised, perhaps because these are easily visualised in the skin and easily biopsied. Clark described six clinically and histologically evident lesional steps of tumour progression in this system: (1) the common acquired melanocytic naevus (CMN); (2) a melanocytic naevus with lentiginous melanocytic hyperplasia, i.e., aberrant differentiation, lentiginous melanocytic naevus (LMN); (3) a melanocytic naevus with aberrant differentiation and melanocytic nuclear atypia, i.e., melanocytic dysplasia or dysplastic melanocytic naevus (DMN); (4) the radial growth phase (RGP) of primary malignant melanoma (MM), which may be *in situ* or invasive but is non-tumorigenic; (5) the vertical growth phase (VGP) of primary MM, which is invasive and tumorigenic; and (6) metastatic melanoma.[4] The 'wholly benign' and 'fully malignant' ends of the spectrum, CMN and advanced VGP primary or metastatic melanoma, generate little diagnostic or conceptual controversy. The intermediate lesions, almost by definition, present diagnostic difficulty because they contain attributes of both benign and malignant lesions. They can be placed on a spectrum from low to higher grades of atypia. At one end of the spectrum, mildly DMN differ only slightly from CMN and likely should be considered as such. At the other end, severely DMN are difficult to distinguish clinically and histologically from *in situ* and superficially invasive (non-tumorigenic) RGP MM.

GENETIC BASIS OF TUMOUR PROGRESSION IN THE MELANOCYTIC SYSTEM

As briefly discussed above, tumour progression in the melanocytic and other cancer systems is now known to involve genetic events. Bastian's group has recently published a landmark study of genetic alterations in the process of evolution of melanoma from precursor lesions.[5] In this study, 293 melanoma and cancer-related genes were sequenced in 150 different areas of 37 primary melanomas and their adjacent precursor lesions. The histological spectrum of these areas included unequivocally benign and intermediate lesions, as well as *in situ* and invasive melanomas. The precursor lesions included benign and dysplastic naevi. These lesions were found to contain 'driver' mutations known to activate the MAP kinase pathway. There were significant differences in genetic alterations across the stages of progression. The unequivocally benign lesions exclusively contained the $BRAF^{V600E}$ mutation that is common in melanomas. The lesions characterised as intermediate were enriched for *NRAS* mutations as well as additional mutations. The various driver mutations were also seen in the *in situ* and invasive melanomas. In addition, a majority of intermediate and melanoma *in situ* areas harboured TERT (telomerase) promoter mutations. Loss of suppressor genes occurred progressively, with bi-allelic inactivation of the *CDKN2A* gene that codes for the p16 and p14 tumour suppressor proteins being identified predominantly in invasive melanomas. The intermediate lesions exhibited heterozygous loss in some instances only. Mutations of the tumour suppressors PTEN and TP53 were found only in advanced primary melanomas. The burden of point mutations increased from benign through intermediate lesions to melanoma, with a strong UV radiation signature at all evolutionary stages. Copy number alterations were prevalent only in invasive melanomas. As the lesions progressed, tumour heterogeneity became apparent in the form of genetically distinct subpopulations.[5] These results describe a predictable series of genetic alterations paralleling the progression stages described by Clark. As already noted, these steps can be skipped; for example, a melanoma arising in a $BRAF^{V600E}$ mutated naevus would presumably skip the intermediate step of dysplasia.

Common melanocytic naevi

As mentioned above, in experimental studies of carcinogenesis, the most common response to a carcinogen is a benign lesion. Common melanocytic naevi are benign localised proliferations of melanocytes present in large numbers among a high proportion of individuals across ethnic groups. A naevus is a lesion comprised of naevus cells[6] which are defined by three major properties compared to normal melanocytes: loss of the inhibition that maintains them as isolated cells in the basal layer of the epidermis and a tendency to proliferate and to form clusters or 'nests'; loss of dendrites; and a tendency to retain pigment in their cytoplasm. Naevi evolve through stages of evolution from a junctional naevus which is a localised proliferation of naevus cells in the epidermis, usually comprised of single cells and nests, the latter present by definition, to a compound naevus in which the lesional cells are also present in the dermis. In a dermal naevus, the cells are confined to the dermis and pigmentation is usually diminished or lost. Therefore, naevus cells have the property of being able to migrate from the epidermis to the dermis, a process similar to the property of 'invasion' in radial growth phase melanomas. Naevus cells also have the property of being able to survive in the dermis, but they lack the property of continuous proliferation, which on the other hand is an important property of a melanoma cell. Naevus cells generally have small nuclei without marked irregularity, prominent nucleoli or hyperchromasia. Mitotic figures are observed only very rarely. The growth of naevi has been modelled in a computer simulation program, wherein naevus cells that have the properties of invasion and survival, but not continuous proliferation, can reproduce patterns of infiltration of the reticular dermis seen in acquired and congenital pattern naevi and in spindle and epithelioid cell naevi.[7]

Most naevus cells in the dermis appear to be in a state of senescence, typically strongly expressing the tumour suppressor p16,[8,9] although some are capable of occasional proliferation and transformation.[10] In the seminal genomic studies of Shain *et al*. of Bastian's group, the benign naevus precursor lesions were characterised by simple changes typically limited to an activating mutation of a single driver oncogene that leads to activation of the MAP kinase pathway. Remarkably, in 13 of 150 areas of lesions that were unanimously considered benign (i.e., not intermediate or malignant), a $BRAF^{V600E}$ mutation was the only apparent pathogenic mutation. Copy number abnormalities (CNA), such as loss of the CDKN2A locus of the p16 suppressor, were not observed.[5]

Dysplastic naevi

The lesions termed dysplastic naevi (or dysplastic melanocytic naevi, DMN) were first recognised in members of hereditary melanoma-prone families as lesions characterised by clinical and histological atypia, the latter including architectural and cytological features.[11] They were later