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MELANOCYTIC TUMOUR PATHOLOGY

Genomic aberrations in spitzoid melanocytic tumours and their implications for diagnosis, prognosis and therapy

THOMAS WIESNER[1,2], HEINZ KUTZNER[2,3], LORENZO CERRONI[2], MARTIN C. MIHM JR.[4], KLAUS J. BUSAM[5] AND RAJMOHAN MURALI[5,6]

[1]*Human Oncology and Pathogenesis Program, Memorial Sloan-Kettering Cancer Center, New York, United States;* [2]*Department of Dermatology and Venereology, Medical University of Graz, Graz, Austria;* [3]*Dermatopathologie Friedrichshafen, Friedrichshafen, Germany;* [4]*Melanoma Center, Dana–Farber Cancer Institute, Harvard Medical School, Boston,* [5]*Department of Pathology, and* [6]*Marie-Josée and Henry R. Kravis Center for Molecular Oncology, Memorial Sloan Kettering Cancer Center, New York, United States*

Summary

Histopathological evaluation of melanocytic tumours usually allows reliable distinction of benign melanocytic naevi from melanoma. More difficult is the histopathological classification of Spitz tumours, a heterogeneous group of tumours composed of large epithelioid or spindle-shaped melanocytes. Spitz tumours are biologically distinct from conventional melanocytic naevi and melanoma, as exemplified by their distinct patterns of genetic aberrations. Whereas common acquired naevi and melanoma often harbour *BRAF* mutations, *NRAS* mutations, or inactivation of *NF1*, Spitz tumours show *HRAS* mutations, inactivation of *BAP1* (often combined with *BRAF* mutations), or genomic rearrangements involving the kinases *ALK*, *ROS1*, *NTRK1*, *BRAF, RET,* and *MET*. In Spitz naevi, which lack significant histological atypia, all of these mitogenic driver aberrations trigger rapid cell proliferation, but after an initial growth phase, various tumour suppressive mechanisms stably block further growth. In some tumours, additional genomic aberrations may abrogate various tumour suppressive mechanisms, such as cell-cycle arrest, telomere shortening, or DNA damage response. The melanocytes then start to grow in a less organised fashion and may spread to regional lymph nodes, and are termed atypical Spitz tumours. Upon acquisition of even more aberrations, which often activate additional oncogenic pathways or alter cell differentiation, the neoplastic cells become entirely malignant and may colonise and take over distant organs (spitzoid melanoma). The sequential acquisition of genomic aberrations suggests that Spitz tumours represent a continuous biological spectrum, rather than a dichotomy of benign versus malignant, and that tumours with ambiguous histological features (atypical Spitz tumours) might be best classified as low-grade melanocytic tumours. The number of genetic aberrations usually correlates with the degree of histological atypia and explains why existing ancillary genetic techniques, such as array comparative genomic hybridisation (CGH) or fluorescence *in situ* hybridisation (FISH), are usually capable of accurately classifying histologically benign and malignant Spitz tumours, but are not very helpful in the diagnosis of ambiguous melanocytic lesions. Nevertheless, we expect that progress in our understanding of tumour progression will refine the classification of spitzoid melanocytic tumours in the near future. By integrating clinical, pathological, and genetic criteria, distinct tumour subsets will be defined within the heterogeneous group of Spitz tumours, which will eventually lead to improvements in diagnosis, prognosis and therapy.

Key words: Biomarkers; BAP1; BRAF; classification; diagnosis; genetics; genomics; melanocytic tumours; melanoma; molecular biology; pathology; precision oncology; RAS; Spitz tumours; spitzoid neoplasms; targeted therapy.

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INTRODUCTION

Melanocytic neoplasms include several tumour types that are characterised by distinct clinical features, histopathological appearances, genetic aberrations, and clinical behaviour.[1] In most melanocytic tumours, accurate pathological distinction between benign (melanocytic naevus) and malignant (melanoma) is possible based on histological criteria. However, there are diagnostically challenging melanocytic neoplasms with conflicting morphological features, on the basis of which it is difficult to predict clinical behaviour with certainty. This difficulty leads to under-diagnosis as naevus, to over-diagnosis as melanoma, or to a diagnosis of 'melanocytic tumour of uncertain malignant potential' or 'borderline melanocytic tumour'.

Spitzoid melanocytic neoplasms (hereafter designated 'Spitz tumours') are uncommon skin lesions that constitute diagnostic problems for dermatopathologists on a regular basis.[2] These tumours were first described by Sophie Spitz as 'melanomas of childhood', because they occurred predominantly in children and adolescents.[3] The lesions were composed of large epithelioid and/or spindled-shaped melanocytes that contained large nuclei with vesicular chromatin and prominent nucleoli. When it became clear that these tumours may also arise later in life and behave in a benign fashion, they were re-named 'Spitz naevi' to indicate their

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benign nature. Melanocytic tumours with spitzoid features and marked histological atypia that show often an aggressive clinical course similar to conventional melanomas were termed 'spitzoid melanomas'.

The term 'atypical Spitz tumours' (ASTs) refers to melanocytic tumours that exhibit morphological characteristics of Spitz naevi, as well as some features associated with malignancy, but to an insufficient degree as to classify them as spitzoid melanomas. ASTs have the ability to disseminate, but their spread is often limited to regional lymph nodes and has little impact on patient survival.[4–6] Therefore, the histological features of ASTs mirror their clinical behaviour, which is intermediate between benign (no metastasis) and malignant (aggressive clinical behaviour with widespread metastasis) melanocytic tumours, and argues that these lesions might be best classified as low-grade melanocytic tumours.

Pathological classification of Spitz tumours as 'Spitz naevus', 'AST' or 'spitzoid melanoma' and predicting their clinical behaviour can be challenging, even for experts.[2,7,8] This diagnostic uncertainty has stimulated numerous efforts to characterise the underlying genetic and epigenetic aberrations of Spitz tumours with the goal of finding new biomarkers and better explaining their biology. In this review, we summarise the current knowledge of the genetic landscape of Spitz tumours, including Spitz naevi, atypical Spitz tumours and spitzoid melanoma, and describe how genetic aberrations influence their morphological appearance and their clinical behaviour. We also discuss the usefulness and the limitations of ancillary genetic methods in the diagnostic work-up of Spitz tumours.

INTEGRATED CLASSIFICATION OF MELANOCYTIC TUMOURS

The main goals of tumour classification systems are: (1) to distinguish tumours that are malignant and behave aggressively from those that are benign and pose no threat to patients; and (2) to establish relatively distinct disease entities or subsets that can be used for personalised therapeutic decisions. Since the development of drug therapies targeting mitogenic driver aberrations (such as *BRAF* mutations) has improved the treatment of patients with metastatic melanoma,[9] the classification of melanocytic tumours has recently been integrated with genomic data. Thus, melanocytic tumours are currently classified according to their clinical and histological features, their biological behaviour, and their mitogenic driver aberrations.[10]

Several genetic aberrations are associated with specific clinical and histopathological subtypes of melanocytic tumours. For example, blue naevi and Spitz tumours show distinctive clinical and histological appearances, and have very different spectra of genetic aberrations than common acquired naevi and cutaneous melanoma.[1,11] While morphological evaluation often provides clues about the probability of genetic aberrations in a given tumour, genomic or immunohistochemical methods are necessary for confirmation. No histological feature is entirely specific, because other factors, such as additional genetic aberrations or the tumour microenvironment can mask or distort the morphological features associated with specific genetic aberrations.

Common melanocytic naevi arise predominantly in the first three decades of life and normally appear as uniformly brown-pigmented macules with a diameter of less than 6 mm on sun-exposed areas of the skin. The vast majority (>80%) show activating hotspot mutations leading to an amino acid exchange at codon 600 of BRAF ($BRAF^{V600E/K}$).[12] Congenital naevi arise *in utero* or post-natally, tend to be significantly larger than common acquired naevi, and harbour often activating *NRAS* hotspot mutations (~75%), most commonly affecting codon 61 (Fig. 1A).[13]

Blue naevi and related skin neoplasms are heterogeneous melanocytic proliferations that vary in size from a few millimetres in acquired lesions to several centimetres in congenital lesions (e.g., Mongolian spot). Most tumours are histologically characterised by dendritic, spindled, or epithelioid melanocytes without significant epidermal involvement. Most tumours show activating mutations of *GNAQ* or *GNA11*, commonly affecting codon 209 (Fig. 1B).[14–16]

The Cancer Genome Atlas proposed that cutaneous melanoma be classified into four subgroups.[10] The largest subgroup (~50%) of melanoma is characterised by $BRAF^{V600E}$ mutations (*BRAF* subtype).[10] *BRAF* mutations are most frequent in melanoma arising on intermittently sun-exposed skin.[17] Activating mutations in *RAS* genes account for approximately 25% of melanoma (*RAS* subtype), and subsume cases with *NRAS* mutations (~24%), as well as *HRAS* and *KRAS* hot-spot mutations (<1%).[10] The *NF1* subtype shows inactivation of *NF1*, which encodes a negative regulator of *RAS*.[10] *NF1* aberrations are found in approximately 10% of melanoma and more than half of the aberrations lead to complete loss-of-function due to nonsense, frame-shift, or splice-site mutations.[18] *NF1* mutations are associated with co-mutations affecting genes that mildly activate the MAPK/ERK pathway and occur more frequently in desmoplastic melanoma and in melanoma of chronically sun-exposed skin.[19,20] Melanomas lacking *BRAF*, *N/H/KRAS*, or *NF1* mutations comprise the heterogeneous group of 'triple wild-type subtype', which includes tumours with *KIT* mutations (more frequently found in acral and mucosal melanoma[21]), *GNAQ/GNA11* mutations (well established drivers in uveal melanoma[14,15]), or genomic rearrangements involving *BRAF* or *RAF1* (Fig. 1C).

GENETIC ABERRATIONS IN SPITZ TUMOURS

Spitz tumours are a heterogeneous group of melanocytic neoplasms that commonly arise in children and adolescents, but may occur also in older individuals. Spitz tumours may be solitary or have an agminated or eruptive disseminated distribution,[22] have an average diameter of ~10 mm, and are usually well-circumscribed, dome-shaped papules with a homogenous colour ranging from reddish to dark brown. Spitz tumours show genomic aberrations that are rarely observed in the tumours described above, or in other melanocytic neoplasms (Fig. 1D). *BAP1* inactivation occurs in ~5% of Spitz tumours and is associated with an epithelioid phenotype. Spitz tumours with *BAP1* loss are associated with a hereditary tumour predisposition syndrome.[23] *HRAS* mutations occur in ~15% of spitzoid lesions, are often associated with desmoplasia, and are commonly designated as desmoplastic Spitz naevi.[24] Genomic rearrangements (translocations, kinase fusions) of various receptor tyrosine kinases, including *ALK*, *ROS1*, *NTRK1*, *RET*, and *MET*, or the serine-threonine kinase *BRAF* are observed in up to 50% of Spitz tumours.[25,26]