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Molecular Characterization of Spitz Tumors: An Approach to Improve Disease Diagnosis and Classification

Giubellino A[1,2*] and Zhou Y[1,2*]

[1]Department of Laboratory Medicine and Pathology, University of Minnesota, 420 Delaware St. SE, C516 Mayo Minneapolis, USA
[2]Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota, USA

Abstract

Spitz neoplasms are relatively uncommon melanocytic lesions characterized by an epithelioid or spindled cell morphology and present more commonly in younger age. They represent a spectrum from benign to malignant lesions which sometime represent a diagnostic conundrum. Recent advances in the genetic characterization of these lesions allow a better classification with consequent improvement in diagnostic accuracy and patient management. This review aims at summarizing our recent understanding of these lesions at the molecular level.

Keywords: Spitz tumor; Spitz nevus; Spitzoid neoplasm; Atypical spitz tumor; Spitzoid melanoma

Introduction

Spitz tumors represent a distinct group of uncommon melanocytic lesions characterized by large epithelioid and/or spindled melanocytes. They typically arise in children and adolescents, although can also occur in adults [1,2]. The clinical behaviors range widely from benign to lesions with uncertain malignant potential to clearly malignant neoplasms. Currently, Spitz tumors can be classified as benign Spitz nevus (SN), atypical Spitz tumor (AST) and spitzoid melanoma based on their biological potential [3]. However, accurate diagnoses of these lesions, especially reliable distinction between ASTs and spitzoid melanomas, remain very challenging. One major reason is that the morphologic features of spitzoid neoplasms do not usually correlate with their clinical behaviors [4-6]. The histopathologic criteria that reliably predict the clinical course of conventional melanomas do not apply to Spitz tumors. For example, some ASTs involving locoregional lymph nodes do not show further progression or lethal metastasis; while other subset of ASTs with ambiguous morphologic features present with lethal clinical course [7,8].

The limitation in using histomorphologic features to classify Spitz neoplasms outlines the need for development of ancillary testing to better understand the molecular features for a more accurate and reproducible diagnosis of these lesions. Since introduced in 1990s, comparative genomic hybridization (CGH) has long been used for efficient detection of copy number alterations throughout the entire genome of solid tumors including melanomas (Figure 1). CGH analysis of Spitz tumors revealed that the number of copy number aberrations increases progressively from benign Spitz nevi to spitzoid melanomas, with AST having an intermediate amount of alterations. In addition, several relatively unique copy number aberrations were identified with possible diagnostic and prognostic values in Spitz nevi and ASTs respectively [9-11]. These findings also promoted the development of FISH assays as an ancillary diagnostic tool for diagnostically challenging spitzoid lesions [11,12]. Recent advances in next generation sequencing (NGS) and mass spectrometry permitted the discovery of molecular signatures useful for identifying subsets of Spitz neoplasms. As the genomic landscape of spitzoid neoplasms is rapidly emerging, the diagnostic algorithm for Spitz tumors, especially diagnostically challenging ones, evolves into a comprehensive approach that includes

the combination of morphologic recognition and stepwise applications of diagnostic techniques such as immunohistochemistry (IHC), FISH, array-based CGH and NGS.

Literature Review

In the present review we will give an overview of the molecular features of several clinically important subtypes of spitzoid lesions and discuss recent advances in this field.

Molecular-genetic classification scheme for Spitz tumors

Molecular investigations based on CGH, FISH and RNA sequencing analyses have identified certain subgroups of Spitz tumors with characteristic histologic/molecular signatures and relatively predictable clinical outcome. These include Spitz tumors with *HRAS* mutations, BAP1 loss and BRAFV600E mutation, TERT promoter mutations, certain chromosomal alterations (such as homozygous deletion of 9p21 and isolated loss of 6q23), and kinase gene rearrangements [10,13-18]. To be noted, the driver mutations that are typically present in conventional melanomas, such as BRAF, NRAS, and NF1, are usually not present in Spitz and spitzoid nevi and melanomas (Table 1).

Spitz tumors with *HRAS* mutations

This subtype was the first to be described with specific molecular features. A gain in chromosome 11p, which harbors the *HRAS* gene, is recurrently found in around 20% of Spitz nevi, but not in other nevi and it is extremely rare in melanomas [9]. Most of these tumors with 11p copy number gains present with activating *HRAS* mutations and exhibit characteristic histopathology: relatively low cellularity and

*Corresponding authors: Dr. Alessio Giubellino, Department of Laboratory Medicine and Pathology, University of Minnesota, 420 Delaware St. SE, C516 Mayo Minneapolis, MN, 55455, USA, Tel: 612 273 5920, Fax: 612 273 1142; E-mail: agiubell@umn.edu

Dr. Yan Zhou, Department of Laboratory Medicine and Pathology, University of Minnesota, 420 Delaware St. SE, C516 Mayo Minneapolis, MN, 55455, USA, Tel: 612 273 5920, Fax: 612 273 1142; E-mail: zhou1237@umn.edu
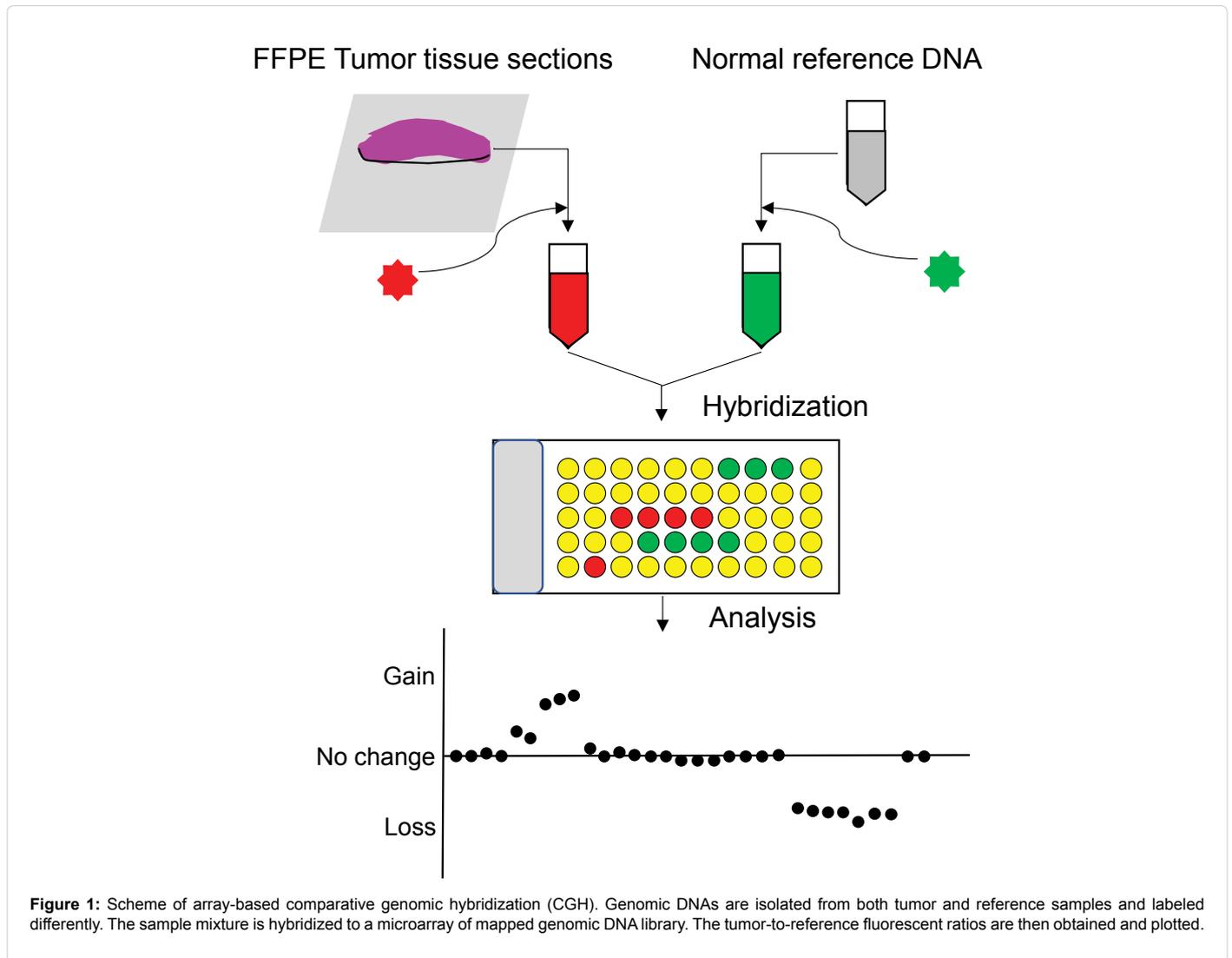
Figure 1: Scheme of array-based comparative genomic hybridization (CGH). Genomic DNAs are isolated from both tumor and reference samples and labeled differently. The sample mixture is hybridized to a microarray of mapped genomic DNA library. The tumor-to-reference fluorescent ratios are then obtained and plotted.

prominent desmoplastic stroma, and infiltrative single-cell growth in the background of collagen bundles [10,13]. Despite the infiltrative growth pattern and prominent nuclear pleomorphism and frequent deep mitoses, no lesions have been reported to undergo malignant progression to melanomas and no spitzoid melanomas have been found to harbor *HRAS* mutations [19]. Thus, *HRAS* mutation analysis may help differentiate between benign Spitz nevi and spitzoid lesions with malignant potential.

BAP1-inactivated Spitz nevi

BAP1 protein, localized in the cell nuclei, functions as a de-ubiquitination enzyme. A hereditary autosomal dominant tumor syndrome predisposed by germline inactivating mutations of the *BAP1* gene (3p21) was first described by Wiesner et al. in two unrelated families [14]. The affected individuals in these families presented with numerous epithelioid melanocytic neoplasms resembling Spitz nevi and an increased risk of developing other malignancies including uveal and cutaneous melanoma. In the same and subsequent studies, Wiesner et al. also found a subset of sporadic ASTs (28% in a series of 32 ASTs) with epithelioid morphology and loss of *BAP1* expression [15].

Both familial and sporadic lesions are predominantly intradermal. Histologically, they are mainly composed of epithelioid melanocytes with abundant amphophilic cytoplasm and clearly-demarcated cytoplasmic borders. Cytologic atypia is often present, with enlarged vesicular nuclei, prominent nucleoli and substantial nuclear pleomorphism [14,15]. However, these lesions clearly lack other characteristic features of a typical Spitz nevus, such as epidermal hyperplasia and hypergranulosis, clefting around junctional nests, and the presence of Kamino bodies.

Another feature distinct from typical Spitz nevi is that both familial and sporadic lesions harbor the *BRAF* V600E mutation [14,15,20]. Furthermore, in a few studies involving melanocytic tumors with combined morphologies, loss of *BAP1* expression was restricted to the epithelioid cells, but BRAFV600E mutation were found in all melanocytes within the lesion. This phenomenon suggested that tumors with *BAP1* loss might progress from common acquired nevi [14,21]. Although these *BAP1*-inactivated tumors were considered a subset of spitzoid neoplasms, they may not represent true Spitz nevi based on these distinct histologic and molecular features [22]. Nevertheless, these unique features facilitate the diagnostic algorithm for these tumors, and more importantly, malignancy risk assessment for these patients.

Molecular alteration	Sample types	Investigation technique	Demographic features	Presence of distinctive Histologic features	Clinical behaviors	References
HRAS mutation/ Chr. 11p gains	SN, AST	Array-CGH, FISH, NGS	--	Yes	Benign/favorable outcome	[10,13]
BAP1 loss (+BRAF V600E mutation)	AST	IHC, CGH, NGS	Familial and sporadic	Yes	Mostly benign	[14,15]
TERTp mutation	AST, Spitzoid melanoma	PCR, Sequencing	--	No	Associated with hematogenous dissemination	[16]
Chromosomal alterations						
Homozygous deletion in 9p21	AST, Spitzoid melanoma	CGH, FISH	More frequent in children	No	High risk for advanced disease	[11]
6p25 gain	AST	CGH, FISH	--	No	Intermediate risk	[11]
11q13 gain	AST, Spitzoid melanoma	CGH, FISH	--	No	Intermediate risk	[11]
6q23 loss	AST	CGH, FISH	--	No	Favor benign	[11,17]
8q24 gain	AST	CGH, FISH	--	No	Limited data	[11]
Tyrosine kinase fusions						
ALK	SN, AST, Spitzoid melanoma	IHC, FISH, NGS	Generally, of a younger age than fusion-negative Spitz tumors	Yes	No predictive value found, can be present in all spectrums of Spitz tumors	[36-39]
ROS1		IHC, FISH, NGS		No		[18]
NTRK1		IHC, NGS		Yes		[39,43]
NTRK3		NGS		No		[44,45]
RET		NGS		No		[18]
MET		NGS		No		[18,32]
BRAF		FISH, NGS		No		[18,39]

Table 1: Molecular-genetic classifications of Spitz tumors and their clinical features.

Overall, the clinical behavior of *BAP1*-deficient nevi with coexisted *BRAF* V600E mutation is indolent, although malignant progression to melanoma may occur in rare cases [21,23,24]. A conservative complete excision with possible close follow-up is indicated for an isolated lesion, whereas genetic counseling and testing for germline BAP1 mutation may be indicated if patients present with multiple melanocytic lesions.

TERT promoter (TERT-p) mutation

TERT-p mutations have been found in aggressive cutaneous conventional melanomas, and recently identified in spitzoid melanomas [16]. The largest study so far evaluating its prognostic value in Spitz tumors (56 patients) demonstrated that the TERT-p mutations were present in 4 patients who died of hematogenous metastasis, but not in any of the other 52 patients alive and with favorable outcomes [16]. However, another recent study including 24 patients with all spectrum of Spitz tumors did not find the association of TERT-p mutations with disseminated disease and mortality [25]. Further validation of using TERT-p mutations as an indicator for spitzoid tumors with high-risk behaviors is needed.

Spitzoid lesions with specific chromosomal alterations

Assessment of genomic instability has been used in distinguishing benign and malignant spitzoid lesions [26,27]. Array-CGH studies showed that the majority of Spitz nevi have a normal karyotype (except for gains in 11p and rare isolated loss of chromosome 3), whereas malignant spitzoid lesions reveal several chromosomal alterations [11,27]. Some of these chromosomal aberrations in diagnostically ambiguous sptizoid lesions have been observed to correlate with certain clinical behavior, and thus provide diagnostic and prognostic value.

Homozygous deletion in 9p21 was found to be associated with "high-risk" lesions with tumor spreading beyond the sentinel lymph nodes or patient death from melanoma in a study of 75 ASTs [11]. In another prospective study in children, homozygous 9p21 deletion was strongly associated with recurrence [28]. Most of the current evidence support that this aberration can be employed as a useful indicator for aggressiveness and poor prognosis of spitzoid neoplasms, although this is not specific and controversial results have been reported [29,30]. The significance might be related to the deletion of CDKN2A gene located at 9p21 that encodes for both p16INK4a and p14ARF [31]. Due to its clinical significance, a diagnosis of "ASTs or spitzoid melanoma with homozygous deletion of 9p21" should be considered in the diagnostic report. Moreover, chromosomal gains in 6p25 or 11q13 are associated with poor prognosis, whereas isolated deletion in 6q23 predicts a more favorable behavior with no tumor extension beyond sentinel lymph nodes [11,17].

Spitz tumors with kinase fusions

Recent studies demonstrated that Spitz tumors with no *HRAS* or other mutations frequently show gene rearrangements of kinases, resulting in in-frame kinase fusions [18,32]. These events may represent a critical mechanism of oncogene activation early in tumorigenesis of spitzoid neoplasms. In general, patients with fusion-positive Spitz tumors are younger than those with fusion-negative ones. Wiesner et al. were the first to identify kinase fusion events in 51% of 140 spitzoid neoplasms, including 55% of Spitz nevi, 56% of ASTs and 39% of spitzoid melanomas, respectively [18]. So far, the translocations were found in *ROS1, ALK, NTRK1, NTRK3, RET, MET, BRAF* and *MAPK,* and the presence of fusions are mutually exclusive with each other [18,33]. These translocation activating kinases may represent promising therapeutic targets for certain subsets of spitzoid tumors with malignant potential, as selective inhibitors have been already developed or are currently under investigation [34,35]. However, their use as diagnostic tools is limited due to the inability to differentiate benign and malignant lesions.

Spitz nevi with *ALK* gene fusion: *ALK* fusion was found in 10% of Spitz tumors in the cohort of 140 patients above described [18]. Spitz tumors with *ALK* gene rearrangement typically occur as dome-shaped or exophytic lesions in children or adolescents but are also found in older adults occasionally. According to a review of recent studies, the majority of them were classified as ASTs (69%), followed by Spitz nevi in 23% of cases, and only 8% were classified as spitzoid melanoma [36]. It seems that the age of onset does not correlated with biological potential [37]. While they may be located anywhere in the body, more than half of cases are found on extremities [37-40].

Spitz tumors with *ALK* fusions demonstrate distinctive histologic features among all tumor types with kinase gene rearrangements [37-40]. Their unique histologic features, together with easy and accurate identification of *ALK* translocations by IHC, aid in the improved diagnosis of this subset of tumors. *ALK* translocations can also be confirmed by FISH or NGS. Histologically, they can be compound or intradermal, and exhibit characteristic plexiform growth pattern with large fusiform melanocytes arranged in fascicles. Cytologic features are fairly bland, with fibrillar cytoplasm, enlarged nuclei and prominent nucleoli. The majority of the lesions shows infiltrative pattern at the periphery and dermal mitoses are fairly common, however, these seem not to be predictive of aggressive behaviors [37-40].

Various N-terminal partners have been reported to fuse with the 3' portion of the intact *ALK* kinase domain, including TPM3, DCTN1, NPM1, TPR, CLIP1 and GTF3C2. In addition, a novel fusion MLPH-*ALK*, which has not been reported in tumorigenesis of this and other types of tumors was identified recently [41]. In terms of chromosomal alterations, frequent chromosomal 2 (where *ALK* resides) changes and loss of 1p have been identified by array-CGH analysis [37]. *ALK* fusion-positive Spitz tumors are generally considered free from CNVs in 6p, 9p or 11q that are associated with aggressive clinical behavior. However, exceptions have been reported [42]. Two cases of *ALK* fusion ASTs were found to harbor 9p21 homozygous deletion and gains of 6p25; long-term follow-up would be informative to further define their clinical course.

Spitz nevi with *NTRK1* gene fusion: *NTRK1* fusions have been found in up to 16% of Spitz tumors, of which the majority were Spitz nevi and ASTs. Similar to *ALK* or other fusions, the resulting chimeric protein expression may be associated with the activation of *MAPK/ERK* and *PI3K/Akt/mTOR* pathways. The histopathologic features are those of classic spitzoid features, with frequent epidermal hyperplasia and presence of Kamino bodies [39]. A recent study identified that filigree-like rete ridges, markedly smaller melanocytes arranged in rosette-like structures, and exaggerated maturation are distinct features suggestive of a diagnosis of *NTRK1* fusion-positive Spitz tumors [43]. IHC showing strong staining for *NTRK1* are usually highly sensitive and specific for diagnosis.

Spitz nevi with *NTRK3* gene fusion: *NTRK3* fusions are relatively uncommon in Spitz tumors, but they can lead to constitutive activation of the *MAPK, PI3K* and *PLCγ1* pathways in melanocytes [44]. Molecular characterization of these fusion events in Spitz tumors provided useful information for targeted therapies of rare melanomas harboring *NTRK3* fusions [44]. Spitz nevi and ASTs positive for ETV6-*NTRK3*, MYO5A-*NTRK3* and MYH9-*NTRK3* fusions have been identified in previous studies [44,45]. They tend to occur in young female patients younger than 18 years old, on the head and neck. The histologic features are non-specific, and no recurrence were found after complete excision of lesions based on current available data [44,46-49].

Conclusion and Future Perspectives

Ancillary diagnostic techniques based on molecular characteristics of Spitz tumors have been playing increasingly important roles in diagnosis, prognostic evaluation and clinical management of spitzoid neoplasms. However, due to the complex nature of the pathogenesis and pathology of spitzoid lesions, current molecular-genetic classifications remain to be improved. Recent advances in NGS, microRNA or mRNA profiling, and mass spectrometry may provide more opportunities to identify potential specific biomarkers for better risk stratification of Spitz tumors.

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Disclosure Statement

The authors have no conflict of interest.

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