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Rosette-like structures in the spectrum of spitzoid tumors

Background: Spitz nevi demonstrate a diverse spectrum of morphologies. Recently, there have been two reported examples of Spitz nevi with rosette-like structures similar to Homer-Wright rosettes. Rosettes have also been described in melanomas and in a proliferative nodule arising in a congenital nevus.
Methods: A retrospective review of 104 cases of Spitz nevi and variants (n = 51), pigmented spindle cell nevi (n = 26), combined melanocytic nevi with features of Spitz (n = 8), atypical Spitz tumor (AST, n = 9), and spitzoid melanoma (n = 10).
Results: Rosette-like structures were present in 3 of the 104 cases (2.9%), including a compound Spitz nevus, a desmoplastic Spitz nevus, and an AST. All three cases demonstrated several foci of small nests of epithelioid cells with peripherally palisaded nuclei arranged around a central area of fibrillar eosinophilic cytoplasm. Immunohistochemical staining of the three spitzoid lesions demonstrated that the rosette-like structures express S100 protein, Melan-A, and neuron specific enolase (NSE) and lacked expression of neurofilament, glial fibrillary acidic protein and synaptophysin.
Conclusions: While uncommon, rosette-like structures can occur as a focal feature in Spitz nevi and AST. Rosette-like structures may represent a normal morphologic finding in Spitz nevi, and awareness of them may prevent misdiagnosis as a neural tumor or melanoma.

Keywords: melanoma, rosette, Spitz nevi

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Spitz nevi typically present as pink or flesh-colored papules or nodules most commonly in children and young adults. Histopathologically, such nevi are composed of spindled or epithelioid cells, or both. Many variants, including pagetoid, angiomatoid, desmoplastic, myxoid and tubular Spitz nevi, have been described.[1] Although generally considered a distinct entity, pigmented spindle cell nevus of Reed often has some histopathologic features overlapping with Spitz nevi and may be considered a related lesion occupying the spindled end of the spectrum. Recognition of these variants helps to avoid diagnostic confusion that may lead to overdiagnosis of melanoma.

The term 'rosette' is used to describe a ring of cells arranged around a central lumen or cytoplasmic space, and is often regarded as evidence of neuronal differentiation.[2] Homer-Wright rosettes are characterized by radially arranged cells with central fibrillary cell processes. There is no true lumen formation, unlike Flexner-Wintersteiner rosettes. Homer-Wright rosettes, while nonspecific, are typically associated with neural tumors such as neuroblastoma and peripheral neuroectodermal tumor.

Two cases of compound Spitz nevi with rosette-like structures have been previously reported: a nodule arising on the knee of a 36-year-old female[3] and a pigmented lesion on the lower back of a 29-year-old female.[4] Rosette-like structures have also been infrequently described in other melanocytic lesions which, to our knowledge, are limited to several reports of primary and metastatic melanomas, and a proliferative nodule in a congenital nevus.[5–12] The purpose of this study was to determine the incidence of rosette-like structures in the spectrum of spitzoid lesions, ranging from benign Spitz nevi to spitzoid melanomas.

Methods

Using the keywords 'Spitz', 'spitzoid melanoma', and 'pigmented spindle cell' 104 cases of various spitzoid lesions were identified from the University of Michigan pathology database from 2009 to 2012. The cases included Spitz nevi (n = 51; compound, dermal, junctional, angiomatoid, desmoplastic and pagetoid variants), pigmented spindle cell nevi (n = 26), combined nevi with features of Spitz (n = 8), atypical Spitz tumors (AST, n = 9) and spitzoid melanomas (n = 10). Cases were previously diagnosed based on established histopathologic criteria for Spitz nevi,[1,13,14] angiomatoid Spitz nevi,[15] desmoplastic Spitz nevi,[16] pagetoid Spitz nevi,[17,18] pigmented spindle cell nevi,[19,20] AST[21–25] and spitzoid melanoma.[26,27]

Patient demographics for benign spitzoid lesions showed a M : F of 1 : 2, average age 25-years-old, occurring on the lower extremity (31%), upper extremity (28%), trunk (26%), head/neck (13%) and unknown site (2%). AST showed a M : F of 1.25 : 1, average age 24-years-old, and most commonly occurred on the lower extremity (56%), followed by head/neck (33%) and upper extremity (11%). Patients with spitzoid melanoma were M : F of 1 : 9, average age of 27-years-old, with the most common site being the upper extremity (40%), followed by lower extremity (20%), head/neck (20%), trunk (10%) and unknown site (10%).

Hematoxylin and eosin (H&E) stained slides were reviewed by two pathologists (D.F. and D.A.) to assess histomorphology. Immunohistochemistry was performed on the three spitzoid lesions containing rosette-like structures using the following antibodies: S100 (predilute; rabbit polyclonal), Melan-A (predilute; mouse clone A103), neuron specific enolase (NSE, predilute; mouse clone E27), synaptophysin (predilute; rabbit clone SP11), neurofilament (predilute; mouse clone 2F11) from Ventana Medical Systems (Tucson, AZ, USA), and glial fibrillary acid protein (dilution 1/3200; rabbit polyclonal) from Dako (Glostrup, Denmark). Staining was performed on the Ventana Benchmark Ultra automated immunostainer according to standard protocols validated by the Immunoperoxidase Laboratory within the Department of Pathology at University of Michigan Health System.

Results

Rosette-like structures most similar to Homer-Wright rosettes were present in the dermal component of 3 of the 104 cases (2.9%), including a compound Spitz nevus, desmoplastic Spitz nevus and an AST. The three lesions were remarkable for several foci of nests of epithelioid cells with peripherally placed nuclei and central fibrillar eosinophilic cytoplasm lacking discernible lumen formation. These lesions contained a background of epithelioid and spindled cells, with the AST being predominantly epithelioid. None of the pigmented spindle cell nevi or spitzoid melanomas demonstrated rosettes. The microscopic and immunohistochemical findings are described below.

Case 1

An excisional biopsy was performed on a lesion arising on the forearm of an 8-year-old female. Upon histopathologic examination, the lesion demonstrated an overall wedge-shaped, symmetric growth pattern with sharply demarcated lateral borders (Fig. 1). The lesion was composed of both epithelioid and spindle cell nests, with involvement of the dermal–epidermal junction. There was some cleft retraction between the nests and the hyperplastic rete ridges. Rare Kamino bodies were observed in the epidermis. A single dermal mitosis was appreciated in the mid portion of the lesion. The lesion lacked significant maturation of the dermal component, with large nests present at the deep aspect. These findings led to the diagnosis of compound Spitz nevus. Notably, scattered throughout the dermal component were occasional rosette-like structures. These structures were most prominent in the upper aspect; however, one was noted at the base of the lesion as well. The rosette-like structures were composed of small nests of epithelioid cells with peripherally placed nuclei and central fibrillar eosinophilic cytoplasm. There was no lumen formation. Cytologically, the cells of the rosette-like structures showed vesicular nuclei and central nucleoli, similar to the epithelioid cells seen in the remainder of the lesion. No melanin pigment was appreciated.

Immunohistochemical stains were performed on the excisional biopsy to further characterize the rosette-like structures (Fig. 2). The structures were immunoreactive for S100, Melan-A and NSE with

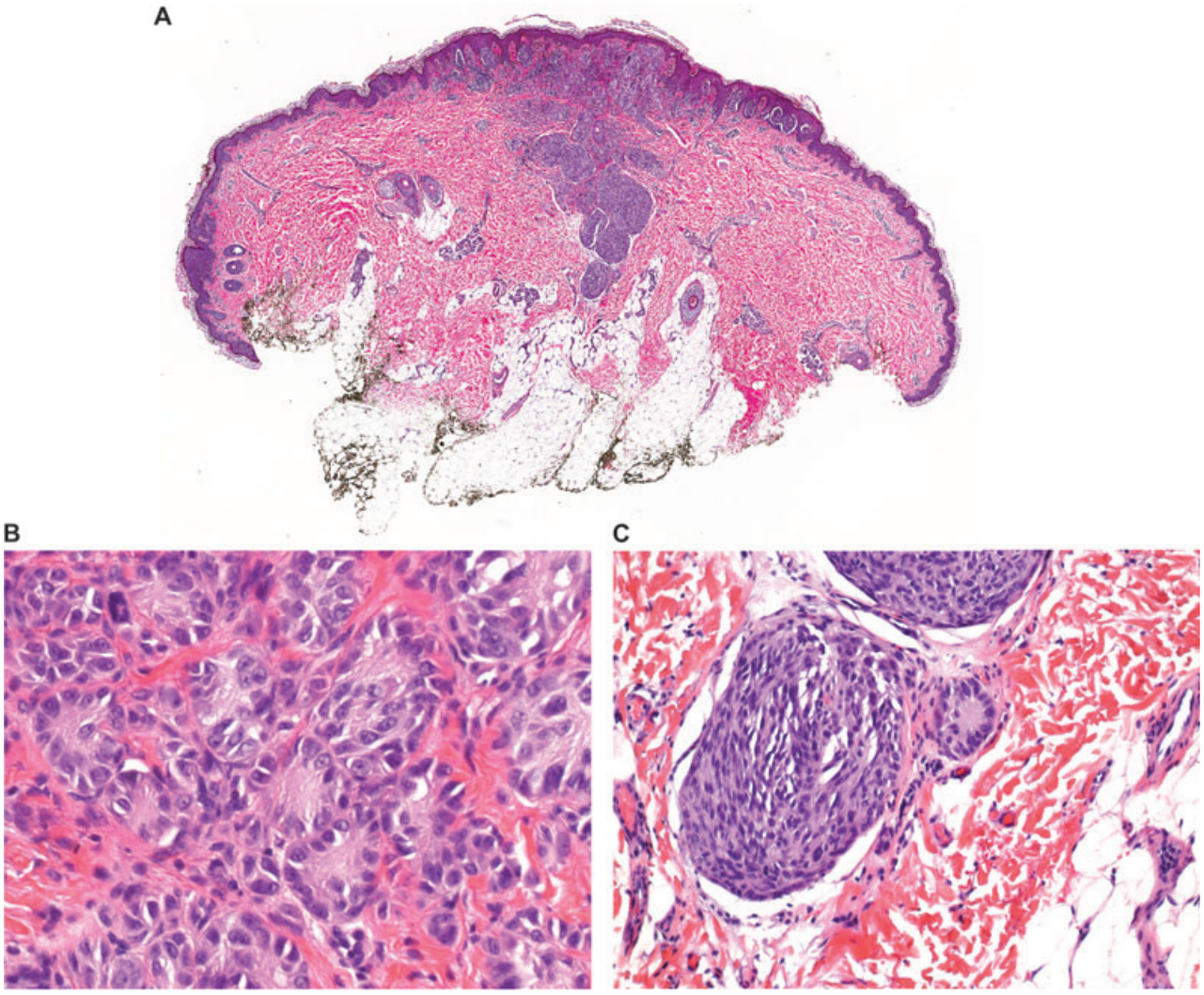

Fig. 1. Case 1. Compound Spitz nevus with rosette-like structures from the forearm of an 8-year-old female, H&E. A) Wedge-shaped, symmetric compound lesion, ×10. B) The dermis contains numerous rosette-like structures admixed with epithelioid and spindle cell nests, ×400. C) Rosette-like structure at the base of the lesion, with no evidence of maturation with decent, ×200.

all three stains highlighting both the melanocytes and the central fibrillary cytoplasm. The rosette-like structures were negative for neuroendocrine (synaptophysin) and neural (neurofilament, glial fibrillary acid protein) markers.

Case 2

A 20-year-old male presented with a flesh-colored papule on the cheek. Excisional biopsy demonstrated a symmetric, wedge-shaped lesion composed of epithelioid to spindled cells arranged in loose nests and cords within a background of desmoplastic stroma (Fig. 3). The lesion contained focal nuclear pleomorphism of the spitzoid cells and lacked significant maturation with dermal descent. No expansile growth or dermal mitoses were noted. The diagnosis of an intradermal desmoplastic Spitz nevus was rendered. On closer inspection, several rosette-like structures limited to the dermal papillae were appreciated. The nests were relatively large with peripheralized cells, one to three cells in thickness, arranged around a central fibrillary core. Similar to the dermal component, the cells of the rosette-like structures exhibited occasional nuclear pleomorphism. Focal perinuclear melanin pigment was also appreciated within the rosette-like structures, but absent in the central fibrillary material. Immunohistochemical stains demonstrated that the rosettes were immunoreactive for S100, Melan-A, and NSE and negative for synaptophysin, neurofilament and glial fibrillary acid protein.

Case 3

An 18-year-old female presented with an asymptomatic pink papule arising on her inner thigh. An initial punch biopsy of the lesion was diagnosed

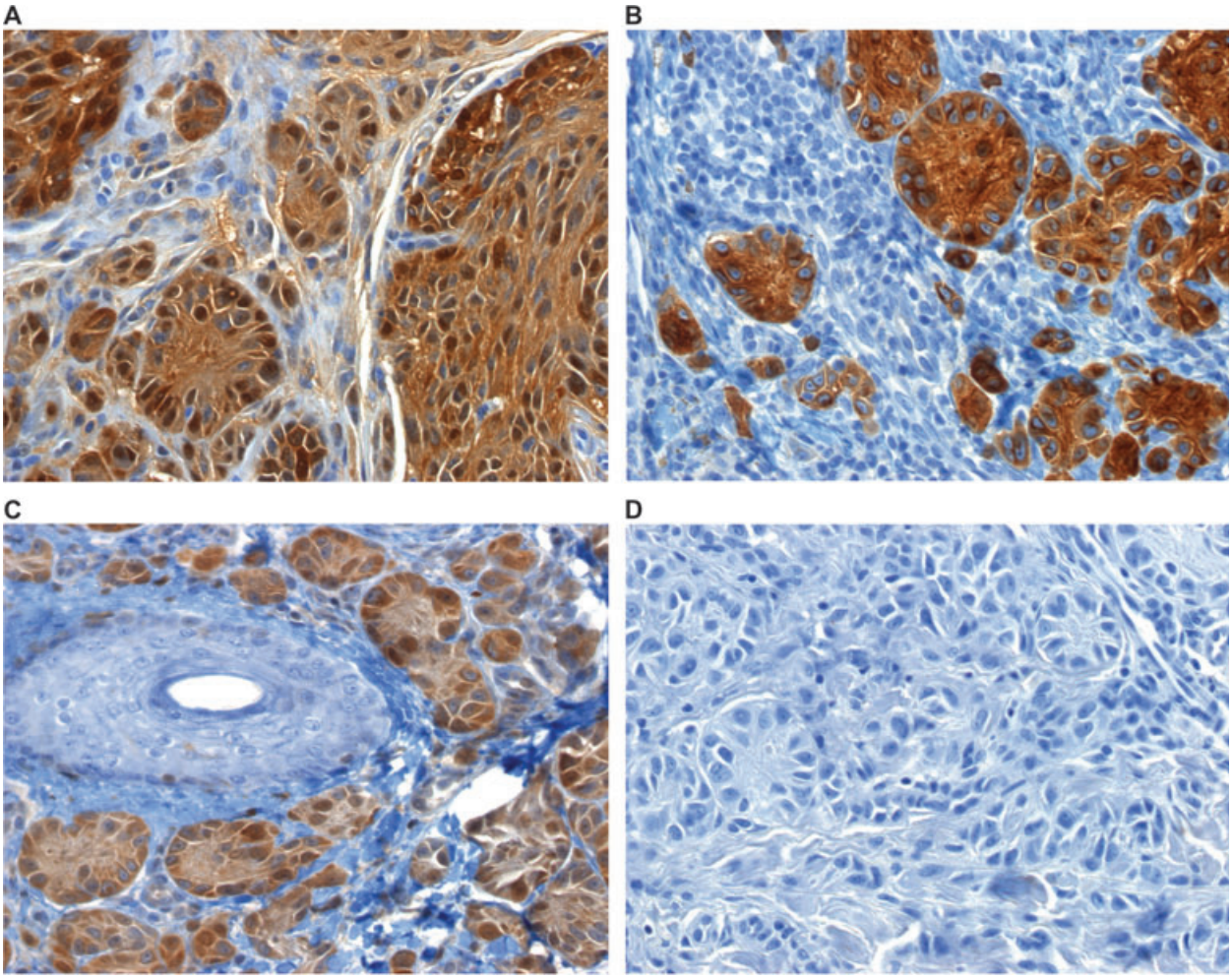

Fig. 2. Case 1. Immunohistochemical stains performed on the compound Spitz nevus with rosette-like structures, ×400 (A) S100. B) Melan-A. C) Neuron specific enolase (NSE). D) Glial fibrillary acidic protein (GFAP).

as superficial spreading melanoma. Examination of the subsequent excisional biopsy demonstrated a symmetric, relatively well-circumscribed lesion composed predominantly of epithelioid nests with occasional spindle cell morphology (Fig. 4). Rare Kamino bodies were appreciated. Concerning features included incomplete maturation of the lesion with dermal descent, large expansile nests and compact dermal growth. The cell morphology was relatively monotonous with vesicular chromatin and central nucleoli. Dermal mitoses were appreciated, up to 3/mm^2, with most in the superficial portion of the lesion and rare mitoses in the deeper half. A diagnosis of atypical Spitz tumor of uncertain biologic potential was rendered. Rosette-like structures were scattered throughout the dermal component, admixed between the larger expansile nests. These rosette-like structures were most prominent in the upper portion of the lesion, however there were several seen at the base of the lesion. Interestingly, the rosette-like structures at the base of the lesion did show some degree of maturation, with nests being relatively smaller and composed of cells containing less cytoplasm and smaller nuclei than those in the superficial dermis. Immunohistochemical stains demonstrated that the rosettes were positive for S100, Melan-A, NSE and negative for synaptophysin, neurofilament and glial fibrillary acid protein. A sentinel lymph node biopsy demonstrated nodal nevi, with no rosette-like structures appreciated.

Discussion

Rosette-like structures are infrequently described in melanocytic lesions (Table 1). Focal rosette-like formation has been described in several reports of malignant melanoma and melanoma metastases.[5–11] However, descriptions of rosette-like structures in benign melanocytic lesions are quite limited. Hoang et al.[12] described rosette formation in a proliferative nodule of an atypical combined melanocytic nevus arising on the ear of a 59-year-old male.

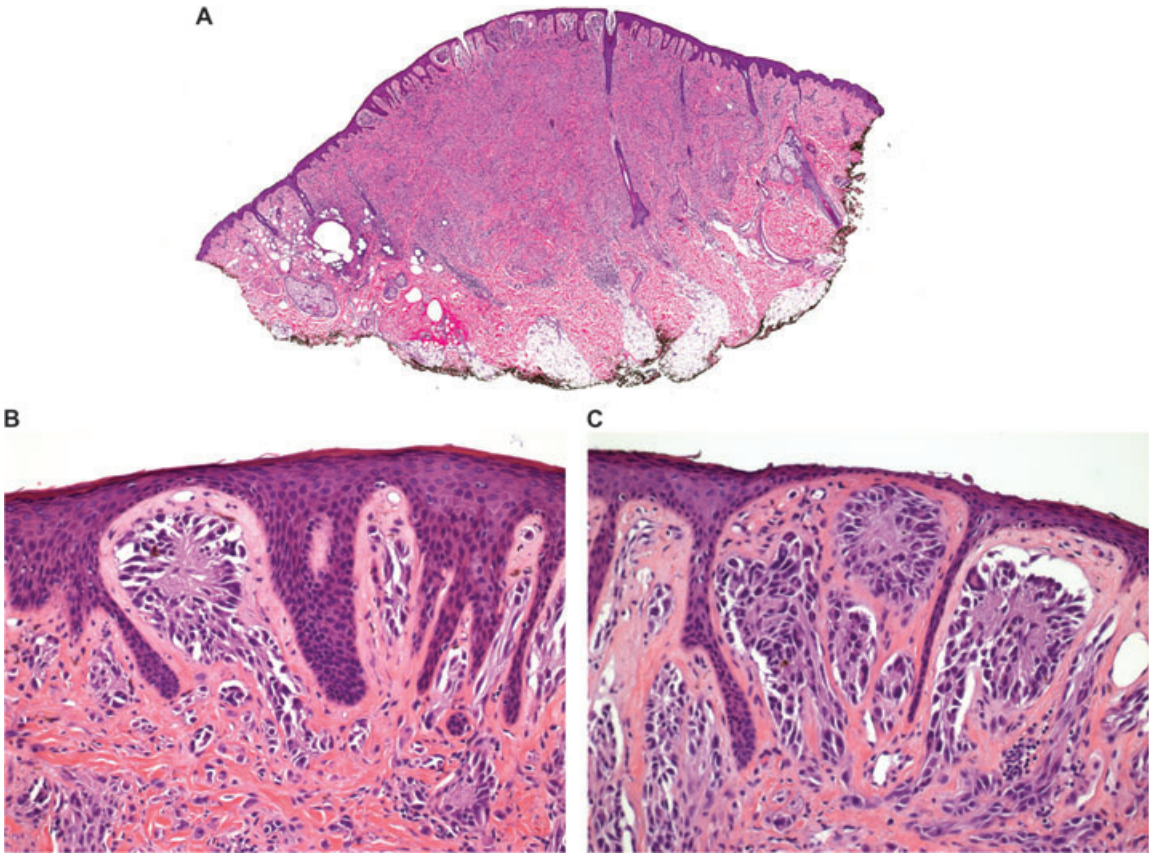

Fig. 3. Case 2. Intradermal desmoplastic Spitz nevus from the cheek of a 20-year-old male, H&E. A) Wedge-shaped, symmetric lesion composed of epithelioid to spindled cells within a desmoplastic stroma, ×10. (B and C) Several large rosette-like structures limited to the dermal papillae, composed of peripheralized cells, one to three cells in thickness, arranged around a central fibrillary core, ×200.

The authors described Homer-Wright-like rosettes with central coarse cell processes. Melanin pigment was appreciated in both the cells of the rosette and the central cell processes. In the same report, no rosette-like structures were found in a retrospective review of 78 congenital nevi of pediatric patients.

There are two previous case reports of Spitz nevi with rosette-like structures most similar to Homer-Wright rosettes. Miller et al.[4] reported a Spitz nevus arising on the lower back of a 29-year-old female. They noted the 'florid rosette-like morphology was present throughout the thickness of the lesion, making assessment of maturation with depth difficult'. However, the lesion lacked significant mitotic activity, expansile growth, nuclear pleomorphism or hyperchromasia. Those findings, together with a benign staining pattern for MIB-1 and HMB-45, led the authors to the diagnosis of a Spitz nevus. The case reported by Miller and colleagues is similar to our *Case 1*, in which a rosette-like structure was found at the base of the lesion, with no apparent maturation. Kantrow et al.[3] reported a Spitz nevus with rosette-like structures arising on the knee of a 36-year-old female. The rosette-like structures were present diffusely throughout the lesion. While the authors did not comment on dermal maturation, they noted 'the absence of dermal mitotic figures and low proliferation rate with Ki67 support the diagnosis of nevus'.

Our study also identified rosette-like structures in one case that had several histopathologic features that sufficiently deviated from a Spitz nevus yet were not entirely diagnostic of spitzoid melanoma (*Case 3*). Such 'gray zone' lesions represent a significant diagnostic challenge, although several reports have proposed diagnostic criteria.[21–25] In this particular case, the cellular density, confluence of melanocytes, mitotic activity and the lack of zonation and significant maturation were the most concerning features. Given the uncertainty of how such features may impact biologic potential, we reached the diagnosis of AST. This is the first report, to our knowledge, to describe rosette-like structures in an AST. Although we did not identify rosette-like structures in any spitzoid melanomas in our series, the number of cases evaluated was low and was a limitation of this study.

The pathogenesis of rosette-like structures in melanocytic lesions is not known.[12] It has been postulated that rosette-like structures may represent a form of neurotization in nevi, similar to neuroid

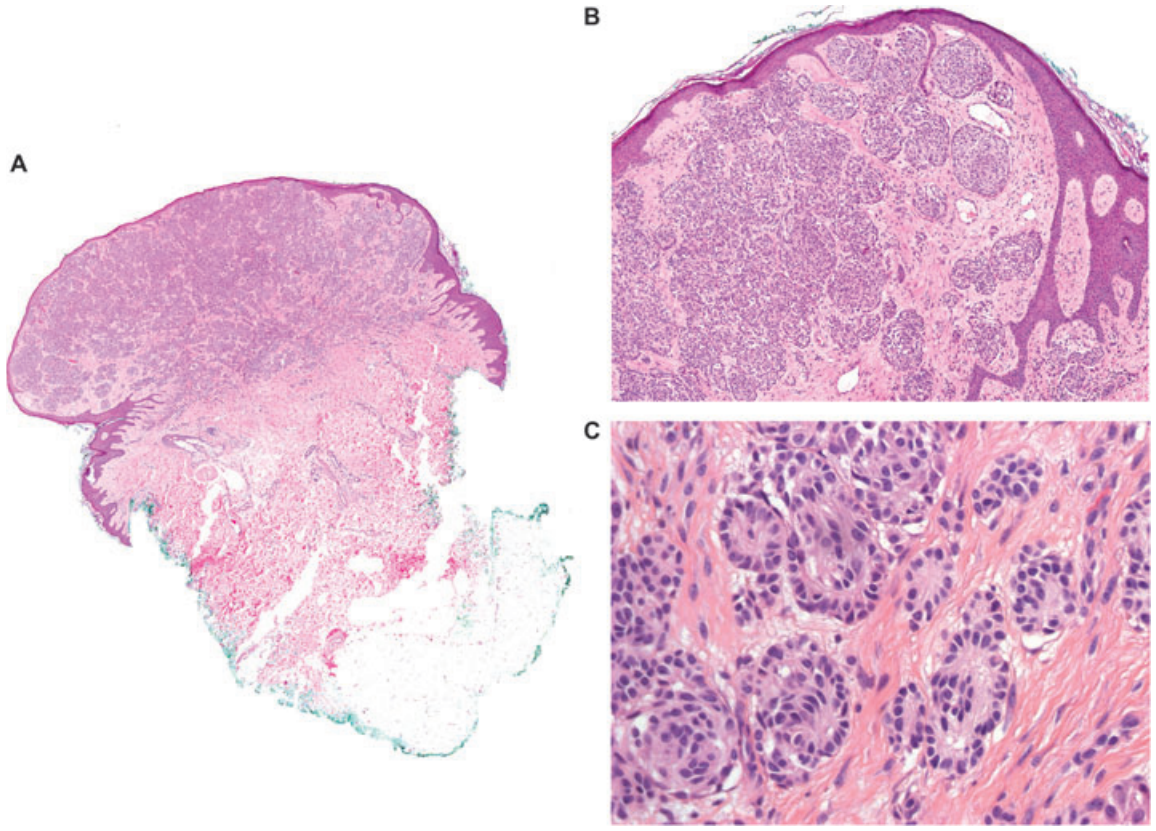

Fig. 4. Case 3. Atypical Spitz tumor with rosette-like structures from the thigh of an 18-year-old female, H&E. A) Symmetric, relatively well-circumscribed lesion composed predominantly of epithelioid nests with occasional spindle cell morphology, ×10. B) Atypical features included compactly arrayed expansile nests within the dermal component, ×60. C) Rosette-like structures scattered throughout the dermal component, ×400.

cords and pseudomeissnerian bodies. Alternatively, primitive cells of neural crest origin which give rise to melanocytes may also play a role in the formation of rosette-like structures. Neural crest cells give rise to a variety of cellular phenotypes including neurons and neuroendocrine cells, both of which can produce tumors displaying rosette formation. The two previous reports of rosette-like structures in Spitz nevi[3,4] mention only melanocytic immunohistochemical markers. Our study expands on the immunohistochemical characterization of these structures in spitzoid lesions. Of our three rosette-containing cases, there was no immunophenotypic evidence of neural (neurofilament, glial fibrillary acidic protein) or neuroendocrine (synaptophysin) differentiation.

Cutaneous lesions with rosette formation raise several diagnostic considerations. Neuroblastoma is the most common extracranial tumor in children, with 32% of neonatal cases and 3% of all cases presenting with cutaneous metastases.[28,29] The neuroblasts of the tumor may demonstrate a spectrum of neuronal differentiation, including Homer-Wright rosette formation. Primitive neuroectodermal tumor (PNET), part of the Ewing sarcoma spectrum, can also contain Homer-Wright rosettes. While primary cutaneous PNET is rather uncommon, it is associated with improved prognosis versus visceral PNET.[30] Malignant peripheral nerve sheath tumor (MPNST) is an aggressive neoplasm which most commonly arise from neurofibromas in the setting of neurofibromatosis type I. Homer-Wright rosette-like structures formed by epithelioid tumor cells may be focally present.[28,31] Although rare, cutaneous neuroblastoma-like schwannoma have also been reported in which small lymphocyte-like Schwann cells arrange around collagen nodules forming rosette-like structures.[32,33] Pseudorosettes have also been described in Merkel cell carcinoma.[34] In such cases with microscopic overlap, an immunohistochemical panel of melanocytic, neuroendocrine and neural markers may be helpful. In our cases, the presence of more conventional spitzoid features distinguished the lesions from other tumors that may contain rosettes or pseudorosettes.

The morphologic spectrum of Spitz nevi is exceedingly diverse, with many variants described. Of particular relevance to this study are Spitz nevi with epithelioid nests containing central 'tubules', referred to by some as tubular Spitz nevi.[35,36] The origin of such structures is debatable, having been attributed

Table 1. Summary of melanocytic tumors containing rosette-like structures

Diagnosis	Gender, age (years)	Site	Histopathology	Authors
Combined nevus	Male, 59	Ear	Rosette-like structures with in a deep proliferative nodule	Hoang et al.[12]
Spitz nevus	Female, 29	Back	Florid rosette-like structures throughout the lesion	Miller et al.[4]
Spitz nevus	Female, 36	Knee	Rosette-like structures diffusely present throughout the lesion	Kantrow et al.[3]
Spitz nevus	Female, 8	Forearm	Rosette-like structures scattered throughout the dermal component	This report
Desmoplastic Spitz nevus	Male, 20	Cheek	Rosette-like structures limited to the dermal papillae	This report
Atypical Spitz tumor	Female, 18	Thigh	Rosette-like structures scattered throughout the dermal component	This report
Melanoma	Female, 75	Arm	Focal pseudorosette-like	Mirzabeigi et al.[5]
Melanoma	Female, 51	Scalp	Focal rosette-like pseudomeissnerian alveolar nests	Monteagudo et al.[6]
Melanoma	Female, 15	Scalp	'Carcinoid-like pattern' with rosette-like structures	Kacerovska et al.[7]
Melanoma	Male, 85	Back	'Carcinoid-like pattern' with rosette-like structures	Kacerovska et al.[7]
Nevoid melanoma	Female, 43	Back	Dermal Homer-Wright-like rosettes	Falconieri et al.[8]
Recurrent melanoma	Male, 61	Shoulder	'Carcinoid-like pattern' with rosette-like structures present at recurrence	Kacerovska et al.[7]
Metastatic melanoma	Male, 76	Back	'Carcinoid-like pattern' with rosette-like structures limited to the metstasis	Kacerovska et al.[7]
Metastatic melanoma	Female, 29	Arm	Rosette-like structures limited to the metastasis	Pfohler et al.[9]
Metastatic melanoma	Male, 61	Trunk	Homer-Wright-like rosettes limited to the metastasis	Alonso et al.[10]
Metastatic melanoma	Female, 41	Thigh	Homer-Wright-like rosettes limited to the metastasis	Banerjee et al.[11]

to central apoptosis, tubular metaplasia and fixation artifact. On examination, our cases did not contain tubular or microcystic structures with optically clear central spaces. Rather, our cases demonstrated central fibrillary cytoplasm with no lumen formation. No apoptotic bodies were appreciated, and it is unlikely that rosette-like structures represent an artifact of tissue processing. Additionally, multinucleate giant nevus cells are commonly appreciated in ordinary Spitz nevi. While we regard the nuclear palisading and central fibrillary cytoplasm seen in rosette-like structures to be quite distinctive, we cannot exclude the possibility they represent a morphologic variant of multinucleated giant nevus cells at least in a subset of cases. Furthermore, we did not observe eosinophilic cytoplasmic inclusion bodies, which can be seen in multinucleated spitzoid melanocytes as recently described by Shon et al.[37]

In summary, we report rosette-like structures in three spitzoid lesions from a retrospective review of 104 cases spanning the spectrum of spitzoid lesions. Although uncommon, rosette-like structures may occur as a focal finding in Spitz nevi and atypical Spitz tumors. Awareness of this morphologic feature in a small subset of non-malignant spitzoid lesions may prevent overdiagnosis as melanoma or misdiagnosis as a neural tumor.

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