Review

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Current concepts of ectopic nodal inclusions with special emphasis on nodal nevi

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Summary

Nodal inclusions of ectopic tissue within lymph nodes are seen comparatively often in dermatopathology and general pathology. Glandular and nonglandular epithelium, as well as melanocytic nevi can be observed within lymph nodes and represent mostly incidental findings without any relevance. The main challenge in reporting these morphologic features is to differentiate such benign inclusions from metastatic settlements of distinct organ tumors. As sentinel node biopsy and lymph node dissection have become standard procedure in clinical oncology and have an immense clinical impact, the correct evaluation of these nodal inclusions is indispensable to avoid undertreatment or overtreatment of patients. In addition, the genesis of these inclusions has not yet been satisfactorily clarified. Two concepts have been laid out: the theory of benign metastases and the migration arrest theory. However, neither theory has so far been able to answer the following questions: Why do we find more nodal nevi in patients with melanoma who had a sentinel node biopsy than in patients without melanoma, and why do we not find nodal nevi in deep visceral lymph nodes? We present a comprehensive review of the current knowledge on nodal inclusions, proposing a concept for the pathogenesis of nodal nevi, to answer these questions.

Introduction

Both terms, ectopia and heterotopia, are used interchangeably; they include all tissues and organs that are located distant from their correct localization within the body. Examples are gastric mucosal ectopia, endometriosis, deciduosis, and extrauterine ectopic pregnancy [1]. The lymph nodes are common sites for ectopic tissue. Over the past few decades, lymph node excision and sentinel lymph node examination have become crucial for microstaging of malignant tumors. Therefore, knowledge of these aberrant inclusions is indispensable to differentiate between micrometastases and benign ectopic inclusions. The following article provides an overview of the various nodal inclusions and defines the characteristics relevant to a differential diagnosis, here with a special emphasis on dermatopathologic considerations. The article is mainly intended as a discussion of pathophysiological aspects of nodal inclusions and in particular of nodal melanocytic nevi from a morphological and immunohistochemical perspective, in order to shed light on the topic in a later step using molecular genetic methods.

General aspects of aberrant intranodal inclusions – What can be found within lymph nodes?

In general, a distinction between epithelial, glandular, and nonepithelial nodal inclusions can be made. Nonglandular inclusions are much less common. Depending on the lymph node station, there are a number of typical tumors that can colonize a lymph node station. Nevus cells

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are typically observed in cervicoaxillary and inguinal lymph nodes [2]; decidua, which is mainly found in intra-abdominal lymph nodes, can be confused with metastatic squamous carcinoma [3–5], and nodal leiomyomatosis in abdominal lymph nodes of patients can be mistaken for disseminated peritoneal leiomyomatosis or uterine leiomyomas [6, 7]. Benign inclusions in axillary lymph nodes are the most common cause of false positive diagnoses in sentinel lymph node biopsies (SNLB) or lymph node dissections [8].

The following inclusions are typically found within axillary lymph nodes:

- Nodal melanocytic nevi (NMN) in 0.33 % of lumpectomy specimens and up to 6.2 % of lymph node resections performed for malignant melanoma [9–12]
- Epithelial cysts [13, 14]
- Heterotopic mammary glands [8]

In addition to glandular or nonglandular lymph node inclusions, miscellaneous materials can be found within the lymph node. Pulitzer et al. described the occurrence of solar elastotic material in dermal lymphatics and lymph nodes; this material was localized within capsules, subcapsular sinuses, and parenchyma of lymph nodes of nine patients, from which eight had melanoma and one Merkel cell carcinoma. They found this elastotic material within the nodes in metastatic melanoma, in nodal nevi, and in nodes without any extrinsic cell [15].

Interestingly, the following distinct intrinsic and extrinsic materials can be found within lymph nodes:

- Cosmetic and tattoo inks [15–17]
- Colloid material used in lymphology studies of flow rate and capacitance [16, 18, 19]
- Polyethylene particles remaining after primary joint replacement [20, 21]
- Silicone after breast implants [22]

Pulitzer et al. have already critically discussed the paradigm of general pathology: the existence of extrinsic cells or heterotopic tissue within lymph nodes is inseparable from the metastatic stage of malignant tumors. Hence, they proposed the existence of some benign and clinically irrelevant passive nodal "metastases" [15].

The main diagnostic challenge is to differentiate benign nodal inclusions from metastatic settlements of primary solid tumors such as melanoma or breast cancer. Misdiagnosis can lead to extensive surgery, inadequate staging, and overtreatment [8]. Morphologic differentiation in the case of glandular nodal inclusions can be made because of the absence of carcinoma in the primary gland of origin, the appearance of normal epithelium, the absence of atypia and mitotic activity, the largely intracapsular location of a few glands, and the absence of desmoplasia [2].

Endosalpingiosis, most commonly observed in glandular inclusions, is an example of localization-typical glandular inclusions in lymph nodes. It is frequently (5–41 %) seen in the pelvic and retroperitoneal lymph nodes of women, whereas it occurs only rarely in supradiaphragmatic lymph nodes [8]. Cases of endosalpingiosis within axillary and inguinal lymph nodes have been observed very rarely [2, 8]. Sampson introduced the term endosalpingiosis in 1930 [23]; it is defined as the heterotopic occurrence of glandular proliferation of the scattered remnants of the epithelium of the Müllerian ducts, with differentiation into fallopian tube epithelium [8]. The terms "paramesonephric" or "Müllerian inclusions with tubal-type epithelium" are used interchangeably [24]. Associated stromal tissue is seen in endometriosis, and the coincident occurrence of smooth muscle tissue is defined as endomyometriosis [24, 25].

The inclusion of mesothelial cells is exceedingly rare and most often seen in infradiaphragmatic lymph node stations such as mediastinal, para-aortic, pelvic, and inguinal lymph nodes [2], while salivary glands and thyroid follicles occur within cervical lymph nodes [14, 26], colonic glandular tissue, and renal tissue [15, 27, 28]. The intranodal inclusions of mesothelial cells tend to be associated with hyperplasia and inflammation of the associated serous membranes, as evidenced by the presence of associated effusions. Mesothelial cells imitate sinus histiocytosis or metaplastic carcinoma but lack in impingement on the nodal structure [2].

Differential diagnoses of diverse heterotopic nodal inclusions in the context of mesothelial tissue in general pathology differ because of the underlying histogenesis of original tissue. A distinction is possible by using a larger panel of immunohistochemical stains [2]. Differential diagnoses of nodal mesothelial inclusions include metastatic melanoma (S100+, HMB45+), nodal melanocytic nevi (S100+, HMB45–), and carcinoma, especially in the case of serious borderline tumors with subcapsular sinusoidal pattern of involvement (cytokeratin+, MOC-31 antibody+, also known as epithelial-specific antigen/Ep-CAM) [2, 29, 30]. Further differential diagnoses include anaplastic large cell lymphoma (which can in turn also be stained with epithelial markers) [31, 32] and – the most challenging – metastatic mesothelioma [33]. Because immunohistochemistry cannot distinguish between benign and malignant, the differentiation of benign mesothelial cells and metastatic mesothelioma depends on distinct criteria, and this distinction is sometimes extremely difficult. Intriguingly, mesothelioma usually does not show marked atypia, whereas benign inclusions may show atypia and mitotic activity [34, 35]. A cytological evaluation or biopsy of the

suspected mesothelial surfaces may distinguish between the two types [2].

Nodal melanocytic inclusions – nodal melanocytic nevi

Nodal melanocytic nevi (NMN) were first recognized in 1931 by Stewart and Copeland and defined as melanocytic nevocellular inclusions within lymph nodes; they noted that NMN were always incidental findings [36]. Mostly, these are bland nevus cell aggregates that resemble the nevocellular cells found in the intradermal part of cutaneous nevi. Homogeneous eosinophilic cytoplasm and unremarkable melanin pigment, indistinct cytoplasmic borders, and nearly no mitotic activity are the accepted morphologic characteristics of NMN [11] (Figure 1a–c). The morphologic similarities between cutaneous melanocytic nevi and NMN are the main reasons why most authors believe that these are basically benign [11]. Recently, Gonzàlez-Farré et al. proposed a classification of NMM nevi into three types and explained their associated diagnostic difficulties [37]:

- Intraparenchymal nevus in a patient with invasive melanoma
- Nodal nevus adjacent to a melanoma micrometastasis within the same sentinel node
- Nodal blue nevus in a patient with malignant melanoma

In our opinion, a fourth type of nodal melanocytic inclusion is missing from this list: those that are found in the context of radical lymph node dissection because of other

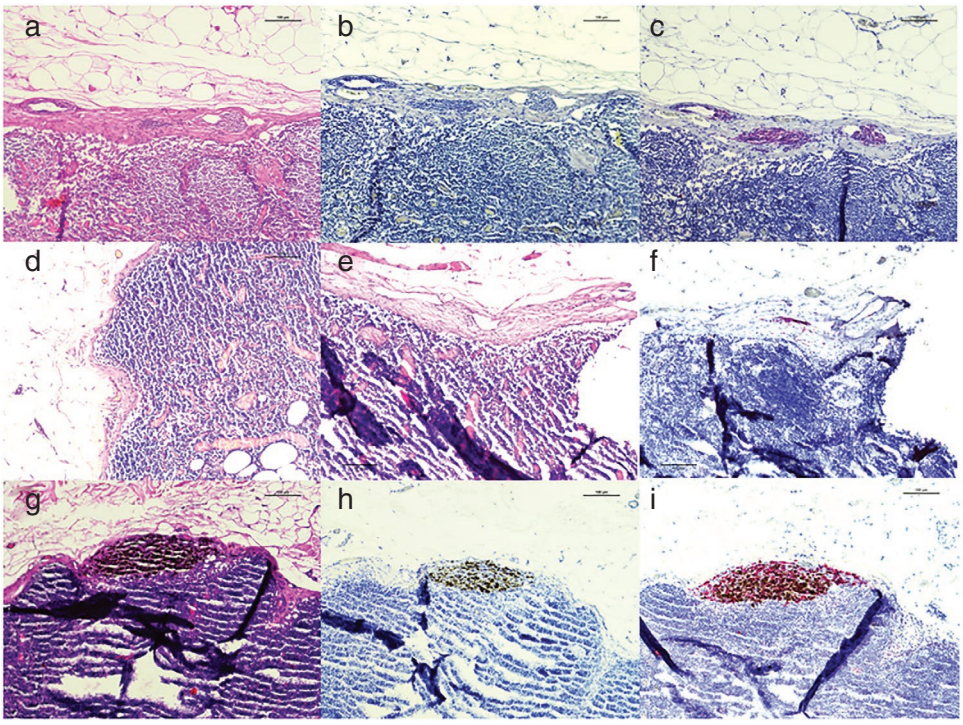

Figure 1 Small nodal nevus aggregates typically located within the fibrous capsule of a lymph node. Small nests consisting of monomorphous melanocytic cells are seen (hematoxylin-eosin stain [HE], original magnification x 100) (a). Nodal melanocytes usually do not express HMB45; this can be used as adjunct in distinction between benign and malignant (HMB45 stain, original magnification x 100) (b). Melan A expression proving melanocytic differentiation (Melan A stain, original magnification x 100) (c). Regular capsule area of a small subcutaneous lymph node with tender fibrous capsule and some histiocytes within subcapsular sinus (HE, original magnification x 100) (d). Extremely small aggregates of nodal nevus presenting as a small layer of spindle shaped melanocytes within a fibrous capsule (HE, original magnification x 100) (e). Please note, extensive adjunct immunohistochemistry is not possible due to very limited morphologic correlate (Melan A stain, original magnification x 100) (f). Same lesion of nodal nevus (g–i). Heavily pigmented nodal nevus resembling nodal blue nevus (HE, original magnification x 100) (g). Missing expression argues against the diagnosis of nodal blue nevus (HMB45 immunohistochemistry, original magnification x 100) (h); Melan A stain, original magnification x 100 (i).

tumor entities, such as breast cancer or squamous cell carcinoma of the head and neck area. Lymph nodes with nevus complexes are also found in these tumors, albeit much less frequently than in sentinel lymph nodes from patients with melanoma [38, 39].

Despite well-defined morphological criteria and modern immunohistochemical staining techniques, the differentiation of NMN from metastatic melanoma remains difficult or even impossible in individual cases. This is especially true in the context of nodal inclusions of blue nevi, where the distinction between benign and malignant is already challenging concerning the primary cutaneous tumor, so a residual uncertainty will remain. In such cases, it is imperative that the dermatopathologist obtains a second opinion report. The aim is to avoid patient overtreatment and undertreatment because of an incorrect interpretation of nodal melanocytic inclusions. To be able to assess the nodal cell proliferation in terms of their dignity reliably, it is recommended to compare the nodal cell morphology with the primary tumor lesion. If morphology and immune phenotype in the primary and nodal infiltrate are the same, there is a high probability that the same lesion is present in the lymph node. This applies to both metastases of melanoma and "relocations" of a congenital nevus cell nevus [39, 40].

Location of nodal melanocytic nevi

The regular capsule area of a subcutaneous lymph node with a tender fibrous capsule and some histiocytes within the subcapsular sinus is shown in Figure 1d. Nodal melanocytic nevi are predominantly located within this area of fibrous capsule and trabeculae and rarely within nodal parenchyma and sinusoids of superficial lymph nodes drained from skin (Figure 1a–c and g–i). The occurrence of nodal nevus within perinodal vessels is also a known phenomenon [41, 42]. Ogawa et al. detected parts of NMN partly within a lymph node hilus [43]. An axillary lymph node extirpation in the context of breast cancer was required and melanoma was unknown at the time of the axillary lymph node operation. In this respect, on the one hand, the complex of melanocytic cells, which initially appeared to be intraparenchymal, was diagnosed as a colonization of melanoma, and only further diagnostic steps, including immunohistochemistry and extended dermatological assessment of the patient, allowed for the diagnosis of a nodal nevus, which was presented in the step sections in the lymph node hilus [43].

Characteristically, NMN are found in superficial lymph nodes; the deep abdominal and pelvic lymph node stations do not have these inclusions [10, 11, 39, 44]. The location of NMN is a "soft" but very important criterion to differentiate between benign nevus or metastatic nodal melanoma [37]. Although many studies consider trabecular or capsular location as a criterion for benignity, if melanocytic cells appear within the lymph node parenchyma, it is mandatory to consider metastatic melanoma.

A study by Biddle et al. described 13 patients who underwent neck dissection and axillary and inguinal lymph node extirpation in the context of a malignant disease [39]. The patients suffered partly from melanoma and partly from breast cancer, tonsil carcinoma, and adnexal carcinoma. In all patients, individual lymph nodes could be detected in all the stations mentioned, which had nevus cell aggregates within the lymph node parenchyma. Mitoses could not be detected, and there were no prominent vacuoles or lymphovascular invasion [39].

Incidence of nodal melanocytic nevi (NMN) and their association to congenital cutaneous nevi

For benign and malignant diseases, NMN are observed with an incidence of 1–22 % [9–12], while the occurrence of NMN is higher in lymph nodes from patients with melanoma compared with those who have breast or prostate cancer [11]. In a huge data analysis of 11,274 patients by de Beer et al., an incidence of intranodal nevi within sentinel nodes of 5 % was found [45]. Additionally, Holt et al. found a different incidence in sentinel nodes from melanoma patients compared with non-sentinel nodes from melanoma patients (1 %) with higher rates of NMN in sentinel nodes (3.9 %) [11]. The occurrence of NMN correlates significantly with an increase of Breslow's thickness of malignant melanoma greater than 2.5 mm and is also associated with the existence of associated cutaneous nevi (75 %), especially nevi with congenital features (19 %) [11]. Nodal melanocytic nevi are usually not observed in non-sentinel lymph nodes of melanoma patients [11].

The occurrence of melanoma associated with congenital melanocytic nevi adjacent to primary melanoma leads to higher incidences of NMN in these cases [9–11]. Additionally, these NMN are more frequent in cases of melanoma arising from a preexisting nevus than *de novo* melanoma, as well as in cases where small congenital nevi are located in the corresponding draining area of the skin [9, 10].

In the first description by Stewart and Copeland, a nodal nevus was described in a patient whose melanoma occurred within a large congenital nevus and who also had neurofibromatosis [36]. In 1974, McCarthy et al. published a case series with NMN associated with cutaneous nevi, presenting an extremely high percentage of patients who displayed this association (21 out of 22 patients with congenital nevi displayed NMN in corresponding regional skin areas) [44]. In both series from Fontaine et al. (2002) and Carson et al. (1996), this association of NMN and congenital nevi of the skin adjacent to primary melanoma was examined, and a significant

association between both was found [9, 10]. Carson et al. demonstrated that 40 % of all lymph nodes from patients with melanoma arising in a nevus showed nodal nevus inclusions, while in cases of *de novo* melanoma, only 20 % of the lymph nodes had nodal nevus complexes [9]. Fontaine was able to show a significant association between the presence of cutaneous nevi in corresponding zones of the skin with nodal nevi and a significant association of the presence of congenital nevi with nodal nevi [10].

Lymph node extirpation represents a special situation in patients with large to giant congenital nevi; these patients have a significantly increased risk of developing malignant melanoma within the congenital nevus [46, 47]. A few reports of the occurrence of nodal nevi in patients with melanoma arising in a giant congenital nevus have been described [40, 48, 49]. However, all published cases have different starting situations (none of the children had a nevus-associated melanoma, and some balloon cell-like nevus cell populations were described, which could also be found in the associated lymph nodes) [40]. Furthermore, prophylactic lymphadenectomies and sentinel extirpations were carried out [48, 49]; one year after diagnosis, all the nodal inclusions were present, and the children were still alive and well. Therefore, the cases are only comparable to a limited extent, especially because the immunohistochemical findings were not congruent. Hara et al. described a two-year-old boy with a giant congenital nevus of the right abdomen, buttocks, and legs; this was surgically removed together with three subcutaneous lymph nodes, which were accidentally recorded and assessed. These showed large pericapsular, capsular, and trabecular, perisinusoidal nevus aggregations that were positive for HMB45 [48]. Thus, this case must remain ambiguous regarding the dignity of the lymph node inclusions.

Impact of nodal melanocytic nevi on the survival of melanoma patients

The ectopic tissue within lymph nodes raises several concerns, especially for a dermatopathologist and general pathologist, because most nodal inclusions are incidentally found. After the correct interpretation of these tissue inclusions within the clinical and oncologic context, the fear remains that on the one hand, confusion of benign and malignant nodal inclusions may occur, greatly impacting patients in their further treatment (over- and undertreatment). Additionally, there is the fear of overlooking metastatic settlements because of deflections by nodal inclusions. An impressive example of this is reflected by nodal pigmentation because of ornamental tattoos. Recently, we showed that nodal pigmentation by tattoo pigment does not influence the survival of melanoma patients [17]. Meanwhile, cases of NMN have also been checked several times to determine whether they may affect the survival of melanoma patients.

Gambichler et al. showed that nodal nevus cell aggregates do not affect the prognosis of melanoma patients, with a 5-year disease-free survival of these patients corresponding to that of those without nodal nevi [50]. Smith et al. confirmed these data, showing that patients with intranodal nevi can be treated just like sentinel node negative patients. Furthermore, they observed a significant survival benefit for patients with intranodal nevi compared with sentinel node-positive patients or patients with isolated nodal tumor cells [51]. Finally, de Beer et al. performed an evaluation of data from the Dutch Nationwide Network and Registry of Histopathology and Cytopathology (PALGA) and confirmed that there seems to be no difference in survival between patients with intranodal nevi and those who are sentinel negative [45].

Nodal blue nevus

In the meantime, numerous reports on the appearance of nodal blue nevi have been published. Begum et al. showed a case of combined blue nevus and benign common nevus within a sentinel node of a patient with invasive duct carcinoma of the breast but with no evidence for cutaneous melanoma in her history. Two of the four excised sentinel nodes did show nodal blue nevus within the extension in the nodal fibrous capsule and trabeculae [52]. The authors justified their diagnosis of nodal blue nevi with the sparse proliferative activity with Ki67 and the benign cytomorphology. HMB45 was positive in metastatic cells and in nodal blue nevus cells [52]. Most of the cases published are difficult to interpret because they describe blue nevus cells with expression of HMB45 within the sentinel nodes of melanoma patients. Because of the localization of melanocytic cells to the capsule and nodal trabeculae, a lack of atypia and of resemblance to primary tumor lesions, these tumors are assessed as nodal blue nevi. This must at least be critically examined.

Interpretation of nodal melanocytic inclusions and its impact on diagnosis

False-negative sentinel lymph node interpretation has been reported in less than 5 % of cases and probably occurs because of pre-laboratory processes from the performance of prior biopsies altering the lymphatic drainage including the inexperience of the surgeon and failure of the detection technique used [38]. The misinterpretation of immunohistochemical stains must be noted because inadvertent immunostaining issues can occur [38], and nonspecific antibody stains can also lead to interpretation errors. Scognamiglio et al. attached

great importance to careful interpretation of sentinel nodes in clinical oncology, calling it "the need for vigilance in the pathologic evaluation of sentinel lymph node" [53].

"False positive" cells in sentinel nodes of melanoma patients are also possible. This term describes the case when the inclusions of melanocytic cells are found in sentinel lymph nodes from non-melanoma patients. Thus, not all melanocytic cells with a characteristic lineage differentiation in immunohistology correspond to metastatic melanoma. For example, Melan A–positive cells have also been detected in sentinel lymph nodes from patients with breast cancer without a history of melanoma [54]. Hypothetically, these "false positive" cells can be explained as nodal nevus cells, non-melanocytic cells with cross-reacting antigenic determinants, melanophages that present melanocytic antigens, or melanocytes or melanocytic stem cells that are released on the skin at the time of the surgical intervention and are enriched within the lymph node via lymphatic vessels [54].

Hopeful adjuncts for differential diagnostics of nodal melanocytic nevi

Although cytomorphology seems to be the most helpful tool to distinguish between benign nevus cell inclusions and nodal metastatic cells of malignant melanoma [37], abundant immunostains are available to support conventional morphology in doubtful cases. Immunohistochemistry for S100, Tyrosinase, SOX10, and Melan A/MART-1 cannot distinguish between nodal nevus and nodal melanoma.

Ambiguous situations in which the nodal nevi are difficult to distinguish from melanoma on morphologic grounds are the following:

- Intraparenchymatous or sinusoidal proliferations of melanocytic cells, mainly in single cell formation
- Atypical cytomorphologic criteria (mitoses, vacuolated cells)
- Atypical immunohistochemistry (for example HMB45 positivity) without the possibility of comparison with primary melanoma

Detailed knowledge of the staining evidence of the various antibodies is imperative to avoid diagnostic pitfalls in the analysis of sentinel lymph node biopsy from melanoma patients in the context of NMN. One of the biggest secondary problems in the diagnosis of nodal nevi is tissue loss during technical processing of tissue due to the variety of necessary immunostains and the mostly small lesion size of NMN (Figure 1e, f).

Table 1 enumerates selected promising stains that may help differentiate between nevus and metastatic cells due to differential expression in nevus cells and metastatic melanoma: Primary and metastatic melanoma display diffuse cytoplasmatic expression of PRAME, whereas NMM does not express PRAME [55]. For a detailed summary of the specific expression characteristics of PRAME in the various melanocytic lesions, we refer to the comprehensive work of Cecilia Lezcano from 2018 [56]. PRAME expression was found in 87 % of metastatic melanoma and 83.2 % of primary melanoma whereas 86.4 % of all nevi examined were negative for PRAME [56]. PRAME expression is seen in all subtypes of malignant melanoma (nodular, superficial spreading as well as desmoplastic melanoma). It should be mentioned, however, that Lezcano also found that 13.6 % of cutaneous nevi including all subtypes (common acquired, traumatized, Spitz and dysplastic nevi) did show immunoreactivity for PRAME [56]. Moreover, Raghavan et al. showed diffuse expression of PRAME in benign and atypical Spitz nevi [57]. These possible pitfalls, as well as the expression of PRAME in many other solid organ tumors and sarcoma severely limit the use of this antibody for diagnosis of malignancy and especially for distinction of benign nodal melanocytic deposits in the context of malignant melanoma.

Mib1 is generally expressed at a much lower level in nodal nevi than in melanoma (ranging from 0–5 %), although metastatic melanoma is consistently positive for Mib1 [39, 58]. A distinct difference in staining pattern was shown by Kanner et al. with reticulin staining groups of nodal metastatic melanoma but surrounding individual nodal melanocytic cells of nodal nevus inclusions [59]. NM23, a metastasis-suppressor gene, did not show any distinct staining that could distinguish between benign and malignant types [59].

The relevance of HMB45 in the context of NMN is not straightforward, and there are important limitations. Forty percent of nodal melanoma metastases did not express HMB45 [60]; thus, negative staining does not exclude melanoma [60, 61]. Conversely, a positive stain with HMB45 is not automatically a sign for malignancy because diverse benign cutaneous nevi and blue nevi express HMB45 [62]. Thus, concerning the differentiation of nodal nevus and metastatic melanoma, there is an urgent need to use a panel of distinct immunostains. Chen et al. proved that nestin and Sox2 are useful markers to differentiate between nodal nevi and metastatic melanoma [63]. Nestin is expressed in melanoma and much less in nodal nevi. SOX2 is not expressed in nodal nevi but is expressed in melanoma [63]. Expression of fatty acid synthase (FASN) in malignant melanoma and melanoma metastases (cutaneous and nodal) has been described by Innocenzi et al. in 2003. They proposed FASN as a reliable prognostic marker in human melanomas [64]. But even in this work, FASN expression was not specific for melanoma or metastasis [64]. Additionally, Kapur et al. reported FASN expression as being highly specific for melanoma and

Table 1 Characteristics of selected immunohistochemical markers that may help differentiate between benign and malignant.

Antibody	Expression characteristics	Special comments on nodal nevus nests vs. metastatic nodal melanoma deposits	Restrictive information	References
PRAME (preferentially expressed antigen in melanoma)	– Member of the family of cancer testis antigens – Expression in normal tissue: testis, ovary, placenta, adrenals, endometrium – Expression in cutaneous melanoma, ocular melanoma, various non-melanocytic malignant neoplasms (for example uterine carcinosarcoma, synovial sarcoma, and leiomyosarcoma, non-small cell lung cancer, breast carcinoma, renal cell carcinoma and many others)	– Malignant melanoma displays diffuse nuclear labeling for PRAME – Cutaneous nevi are mainly negative for PRAME	– Spitz nevi and blue nevi were not examined in Lezcano et al. (1) – *But:* Benign and atypical Spitz nevi do express PRAME (2)	[87–92]
FASN (fatty acid synthase) ACC (acetyl-CoA carboxylase)	– FASN expression is observed in some normal human tissues and highly proliferative lesions in carcinoma, for example colon, breast and ovary carcinoma	– FASN/ACC expression: observed in malignant melanoma and melanoma metastases – No expression in NMN	– Congenital nevi also express FASN	[93] [93–95]
5-hydroxymethylcytosine	– Loss of 5-hydroxymethylcytosine is known as *epigenetic hallmark* of human malignancy – Independent predictor of worse prognosis in melanoma, gastric cancer, hepatocellular carcinoma and more – Due to dysfunction of the tumor-suppressive ten-eleven translocase (TET) family of enzymes and active DNA demethylation pathway	– Expression in melanocytic nevi – Metastatic melanoma does not express 5-hydroxymethylcytosine	– Dysplastic nevi and melanoma do not express 5-hydroxymethylcytosine	[96–98]
p16	– Tumor suppressor protein involved in the regulation of cell cycle and senescence – Loss of p16 expression is seen in several human malignancies, for example melanoma	– Nuclear and cytoplasmatic expression in dermal and nodal nevi – Melanoma metastases are mostly negative or display different staining pattern than nevi	– Staining pattern (nuclear vs. cytoplasmatic vs. weak nuclear and diffuse cytoplasmatic) in nevi or melanoma metastases hamper interpretation of staining results	[99, 100]

melanoma metastases with stronger expression in melanoma with a higher Clark level. Nevertheless, they also observed an expression of FASN in benign congenital nevi [65]. In the work by Saab et al., FASN and ACC (acetyl-CoA carboxylase) were reliably positive in metastatic melanoma and negative in nodal nevi [60]. In this work, NMN displayed negativity for FASN and ACC in all cases examined with a sensitivity and specificity of 100 % while HMB45 showed sensitivity of 60 % and specificity of 100 % in identification of melanoma metastases and distinction of nodal melanocytic nevi [60]. Lee et al. introduced 5-hydroxymethylcytosine expression as an adjunct marker for differentiation because it stained positive in nodal nevi and negative in all melanoma cases examined [66]. p16 also can serve as a useful marker for differentiation because it is expressed mainly in nevi of all kinds, cutaneous as well as nodal, while cells of melanoma metastases lacked nuclear staining for p16 [67, 68]. Mihic-Probst et al. examined the staining pattern of p16 in nodal and dermal melanocytic nevi and melanoma metastasis. They propose p16 as a useful additional marker for differentiation of nevi and melanoma metastases based on its staining pattern [67]. However, in our opinion, the staining pattern is difficult to assess (nuclear vs. cytoplasmatic vs. weak nuclear and diffuse cytoplasmatic) and it is far from unambiguous. p16 cannot be used on its own, but only in a panel of different antibodies.

In summary, the expression of p16 and 5-hydroxymethylcytosine is seen in cells of nodal nevi and lacks expression in metastatic melanoma. In contrast, FASN, ACC, Mib1, PRAME, Nestin, and SOX2 are expressed mainly in melanoma, but not in nodal nevi.

For everyday histopathology we use and recommend the regular application of an antibody panel made from an antibody cocktail (Dako DuoFLEX Cocktail, anti-S100, anti-tyrosinase, anti-Melan A; Code IC001) and HMB45 for routine stains of lymph nodes in a setting of known cutaneous melanoma. Herewith primarily melanocytic deposits that are most often seen in hematoxylin-eosin stain, can be visualized as well. The differential expression of HMB45 facilitates the primary differentiation of metastatic deposits. Further immunohistochemical stains are carried out individually. Most nodal deposits can be characterized with this approach.

To distinguish between metastatic melanoma and nodal nevus, a fluorescence *in-situ* hybridization (FISH) examination, in addition to conventional and immunohistochemical examinations, seems ideal. Although a FISH examination cannot replace a histomorphological assessment, it is a helpful adjunct with a sensitivity of 83 % [69]. Dalton et al. confirmed that vigorous expression of HMB45 indeed shows a high specificity of melanoma or metastatic nodal inclusions of the same primary type. A significantly improved interpretation of these findings can be achieved if, in addition to the nodal melanocytic inclusions, the primary melanoma can also be examined when searching for mutations. Cases in which the nodal deposit shows no genetic aberration that corresponds to the primary melanoma can be regarded as nodal nevus inclusions [69].

Theories on the histogenesis of nodal inclusions

Several theories on the genesis of ectopic tissue in lymph nodes are currently being discussed, but none of them is ultimately satisfactory, especially for nodal melanocytic inclusions. For endosalpingiosis, the following modes of genesis are being discussed: surgical displacement [70], peritoneal implantation of sloughed tubal epithelium [70], dissemination of tubal mucosa via lymphatics [23], Müllerian metaplasia of pelvic peritoneal mesothelium and submesothelium and the subsequent formation of benign glands (endosalpingiosis), or carcinomas resembling those of the ovary and fallopian tube [8]. Entrapment of remnants during embryologic development and Müllerian-type inclusions that are thought to arise from embryologic remnants of coelomic epithelium are also possible modes of pathogenesis [2].

Pathophysiology of nevus aggregates within lymph nodes

There are currently two different theories addressing the question of how nevus cells reach lymph nodes. Both are unsatisfactory and explain by no means all clinical variations of nodal nevi [10, 11, 71]:

- The theory of benign metastasis: embolic transfer of cutaneous melanocytic cells via lymphatics from skin to the corresponding lymph node [9, 72].
- Migration arrest theory: Nodal nevomelanocytes arise from a melanocyte precursor cell with aberrant embryologic migration *en route* from the neural crest to destined cutaneous location via the dorsolateral pathway [10, 72, 73].

In our opinion, it is likely that there is more than one correct answer and more than one explanation for nodal nevus inclusions. In our view, there is only a handful of convincing arguments for or against one theory or the other. Hence, the theory of migration arrest does not explain why nodal nevi are seen with higher frequency in sentinel nodes from melanoma patients than in other primary tumors.

In general, causes for nodal inclusions of any kind can be explained by the transport of all manner of cells through lymphatics to the draining lymph node; this is mainly favored

for colonic glands, renal epithelium, urothelium, some melanocytic nevi, and endometriosis [25]. The occasional appearance of extrinsic tissue within lymph nodes is well documented. Even acellular to paucicellular solar elastotic material can be found within lymph nodes [15]. Passive mechanical transport or trauma may play an important role in many cases of ectopic and heterotopic nodal tissue inclusions.

Regarding the occurrence of nodal inclusions of breast tissue, two options are discussed: the spreading of tumor cells, because of prior surgery, by mechanical passage of tumor cells via the lymphatic system and collection in the lymph nodes, and breast massage as part of the sentinel node biopsy [74]. A trauma-associated genesis because of an earlier surgery to remove a primary melanoma has also been discussed regarding nodal melanocytic cell aggregates.

Although this rather mechanistic theory appears logical it has considerable weaknesses. It is highly unlikely that the time between surgery of a primary malignant melanoma and the subsequent sentinel lymph node biopsy is long enough that any mechanically washed out cutaneous melanocytic cells can be flushed into the lymph nodes via the lymphatic system, settle there, and form nodal nevus aggregates. This theory would only explain scattered solitary tumor cells within the sentinel lymph node. This would, however, be the opposite of a benign process (no "benign metastases"); instead, it would – by paradigmatic definition – be the start of tumor cell dissemination by the underlying malignant tumor within the corresponding lymphatic area.

The flushing out of benign nevomelanocytes through lymphatic channels is well described. Sood et al. described the case of a young boy with an intradermal melanocytic nevus and with evidence of intralymphatic nevus cell emboli, and they discussed the concept of a nevus resulting from benign metastasis [75]. As early as 1979, Bell et al. described a series of cases of dermal nevus cell nevi with evidence of intralymphatic nevus cell nests [76]. Although this phenomenon was rarely observed and published for common dermal nevi, it was repeatedly observed in Spitz nevi, with Howat et al. reporting a frequency of 14.3 % [77]. According to the thesis of benign metastasis, and the frequent observation of lymphatic nevus cell embolism by Spitz nevi, one would expect increased nodal inclusions of melanocytic cells in sentinel lymph nodes or lymph node extirpation in the context of spitzoid cutaneous lesions. Surprisingly, this is not the case. On the contrary, Caraco et al. studied 40 cases of atypical Spitz nevi retrospectively. In none of the cases melanocytic inclusions were found in the lymph node, either benign in nature as a nodal Spitz nevus, or as metastatic settlement [78]. The theory of benign metastases is supported by the finding of circulating benign nevus cells in the blood displaying the dissemination of nevus cells via lymphatics as well as with blood [79]. Leblebici et al. supported the mechanical theory of benign metastasis in lymph nodes; they observed intralymphatic nevus cell aggregates in 13 out of 369 (3.5 %) benign cutaneous nevi. All nevi examined displayed protrusions of nevus cells inside lymphatics independently of the existence of intralymphatic nevus cell aggregates [80]. Protrusions of the nevus cells inside lymphatics may be the precursor step to intralymphatic nevus cell aggregates because these findings closely depend on the tissue sections investigated. However, for solitary nevus cells to settle in the lymph node, it will most likely take more time than a few days or weeks.

Change of a paradigm?

To make matters worse, the theory of benign metastasis, attacks one fundamental pathological paradigm, namely the paradigm that the sowing of tissue via lymphatic tissue in lymph nodes represents a classic defining criterion of malignancy. The theory of benign and clinically irrelevant metastasis, a thesis that Pulitzer et al. proposed against the background of the sowing of elastotic material in lymph nodes [15], is more than plausible from a mechanistic point of view, but it must consequently lead to a paradigm shift in clinical-pathological oncology.

Spatial and temporal aspects speak against the theory of benign metastasis in the case of nodal nevi without cutaneous nevi in lymphatic corresponding skin areas and against the occurrence of the traumatically or mechanically induced settlement of disrupted cutaneous nevus cells within lymph nodes in the setting of sentinel lymph node biopsy. The concept of benign metastases may be the leading explanation for non-melanocytic ectopic tissues and the incidental occurrence of nodal nevi in non-melanoma patients, but it is not for NMN in the context of sentinel node biopsy because of malignant melanoma.

A simple explanation for this could be the fact that melanoma patients show on average more than twice as many melanocytic nevi compared to control subjects of the same age and sex [81]. Thus, it can be discussed that a higher frequency of melanocytic nevi in the context of melanoma justifies a higher probability for melanocytic nevus cells being transported to lymph nodes.

The occurrence of primary extracutaneous melanoma (for instance, mucosal melanoma of the head and neck region or genitalia, urinary tract, or esophagus and biliary tract) and meningeal or choroidal melanoma [82] provoke the question why NMN do not occur in the deeper visceral lymph node stations as well. Higher frequencies of nodal nevi in the superficial lymph nodes of patients with malignant cutaneous melanoma are historically explained by the concept of benign metastases or migration arrest. In consequence, nodal nevi would also be possible within the lymph nodes of the deep visceral compartment. However, there have been no reports on this up to now.

If melanoblastic cells are able to migrate also to the small intestine (ileum) *via* the omphalomesenteric canal [82] and represent the cells of origin of primary melanomas of the gastrointestinal tract [83], one should have expected many more case reports on nodal nevi in visceral lymph nodes to have been published. Ectopic melanocytes are one possible explanation for development of melanoma of an unknown primary origin [83, 84]. However, the differentiation of melanocytes from pre-existing pluripotent stem cells of distinct sites (e.g., lymph nodes, visceral organs) could also be imagined.

The "sleeping ectopic melanoblast"

Here, we propose another, more extraordinary unifying concept of nevogenesis in lymph nodes in patients with melanoma, which we would like to name sleepy ectopic melanoblast theory. This means the hypothetical coexistence of primary ectopic melanocytes – via migration arrest – and ectopic pluripotent stem cells in all the compartments mentioned above (in which extracutaneous melanoma may occur) would explain all clinical and histomorphological aspects of nodal nevi, especially their higher frequency of occurrence within sentinel nodes. The concept of the lymph node as a premetastatic niche supports our theory [85]. Consequently, in melanoma, there may be certain circumstances involving a kind of occult induction of the expression of prometastatic growth factors and cytokines, along with factors that induce remodeling of the extracellular matrix within lymph nodes. These conditions might favor pathways leading to altered plasticity of melanoblasts and ectopic single cells that have settled within the lymph node since early development and beyond, inducing proliferation and differentiation toward nodal nevus or – beyond the lymphatic transport of tumor cells – also malignant transformation of these resident melanoblastic cells.

Based on the widely accepted fact that precursor cells of melanoblasts and of the neurogenic population are glial-melanocytic bipotent progenitors with the ability to differentiate into distinct cell lines [86], it is, in our opinion, in the range of the conceivable that these nodal ectopic and/or pluripotent melanocytic precursor cells are activated in a differential mode. This may be either via distinct driver mutations characteristic for cutaneous melanomas or by factors released by the primary cutaneous melanoma, resulting in subsequent differentiation towards nodal nevoid aggregates in all types of nodal compartments independently of afferent lymphatics.

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