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Superficial spreading and nodular melanoma are distinct biological entities: a challenge to the linear progression model

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Abstract

The classification of melanoma subtypes into prognostically relevant and therapeutically insightful categories has been a challenge since the first descriptions of melanoma in the 1800s. One limitation has been the assumption that the two most common histological subtypes of melanoma, superficial spreading and nodular, evolve according to a linear model of progression, as malignant melanocytes spread radially and then invade vertically. However, recent clinical, pathological, and molecular data indicate that these two histological subtypes might evolve as distinct entities. We here review the published data that support distinct molecular characterization of superficial spreading and nodular melanoma, the clinical significance of this distinction including prognostic relevance, and the therapeutic implications.

Keywords

melanoma; histological subtype; nodular melanoma; superficial spreading melanoma; linear progression

Background

Since the first descriptions of melanoma in the 1800s, pathologists and clinicians have been trying to categorize it in meaningful ways. In 1912, Dubreuilh, a French dermatologist, provided the first classification system by dividing melanomas according to their precursor lesion such as those arising in a nevus, those arising in pigmented skin lesions that appeared in adult life and those arising in previously blemish-free skin [1]. Fifty-five years later, Australian pathologists published their own recommendations for categorization based on both histological and clinical features [2]. However, Wallace Clark and colleagues' seminal publication in 1969 on the histopathological classification of melanoma has retained its importance and remains the basis of the World Health Organization (WHO) classification for melanoma [3, 4]. The current four major clinical-histopathological subtypes are superficial spreading melanoma (SSM), nodular melanoma (NM), acral-lentiginous melanoma (ALM) and lentigo maligna melanoma (LMM) [3, 5].

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To date, the impact of histologic subtypes on melanoma prognosis and treatment has been relatively limited. Staging is determined by the American Joint Committee on Cancer (AJCC) system that incorporates tumor thickness, ulceration, mitotic index and the lymph node status but not histological subtype in the recurrence/metastases risk assessment of patients with primary, localized melanoma [6]. Yet, recent discoveries in other solid tumor types have highlighted the potential of histology driven molecular characterization to impact clinical decision making [7–10]. For example, in lung adenocarcinomas mutational analysis identified correlations between major histopathological subtypes and genetic aberrations that were responsive to targeted therapies [11–13].

The clinical utility of histological classification in melanoma has been exemplified by acral-lentiginous melanoma (ALM). ALM, which makes up roughly 10% of primary melanomas, has been redefined as a distinct and useful sub-classification of melanoma based on the prevalence of the c-kit mutation. The identification of distinct molecular alterations characterizing ALM and melanoma arising in sun protected areas [14–17] has prompted the initiation of phase II trials evaluating the efficacy of the c-KIT inhibitor imatinib as part of a subtype-specific melanoma treatment strategy for metastatic melanoma [8, 9]. Preliminary results from one such trial reported that the patient whose metastatic tumor had the most robust expression of KIT had an almost complete response, emphasizing the importance of delineating and targeting molecular pathways that define specific melanoma histologic subtypes [8].

Though over 80% of melanomas are histologically classified as either superficial spreading (SSM) or nodular melanoma (NM) (or alternatively referred to as radial growth phase (RGP) and vertical growth phase (VGP), respectively), the clinical relevance of these subtypes have been largely overlooked. This is in part due to the current understanding of the linear model of melanoma progression, which entails that melanoma begins with the transformation of epidermal melanocytes that initially undergo radial growth (SSM) and subsequently transition to vertical growth (NM) [18]. It is currently accepted that the speed of dermal invasion is the only aspect that separates the NM and SSM subtypes [19, 20]. Nevertheless, evidence has emerged challenging this linear model of progression and suggesting that these two subtypes are distinct entities [16, 21–28].

Here we review current data on the clinical and biologic heterogeneity of SSM and NM, and discuss its potential implications for treatment options and melanoma outcome.

Clinical Differences of Superficial Spreading and Nodular Melanoma

The incidence of melanoma has increased 3–7% per year, faster than any other cancer, and is predicted to double every 10–20 years [29]. This increase is in part due to widespread awareness of melanoma and the “ABCD” rule for self-monitoring of moles. This criteria is used to raise consciousness about mole Asymmetry, Border irregularity, Color (uneven), and Diameter (>6mm) as warnings signs for melanoma.

Although the incidence of melanoma is increasing, SSM and NM make up steady rates of about 65% and 20%, respectively [20], of all primary melanomas diagnosed. However, different clinical presentations suggest divergent characteristics of SSM and NM. Studies comparing melanoma cohorts over time have supported that there have been changes in the clinical presentation of SSM without corresponding differences in NM. Specifically, our work at New York University found a 25% increase in the proportion of Stage I SSM when comparing a cohort from 1972–82 to a cohort from 2002–07 [22]. This was consistent with our finding that the median SSM thickness had decreased over time by nearly 50% from 1.10 mm to 0.6 mm. However, there was not a significant shift in NM tumor thickness at diagnosis and stage at presentation remained stable with a trend towards an increase in Stage

I [22]. Population based studies in Queensland, Australia, and Southern Germany found a similar trend of thick NM compared to SSM [24, 30]. More recently, a population based study in Italy that examined melanoma patients diagnosed between 1984 and 2004 also found no change in median thickness for NM over time, while SSM thickness significantly decreased [23]. The data from the 4 studies are summarized in Table 1. These findings are consistent with non-hospital-based prospective databases such as the Surveillance, Epidemiology, and End Results (SEER) cancer registry [31–37], and illustrate stage migration of SSM, likely due to increased surveillance, while there is a lack of progress with NM.

The lack of improvement toward a more prognostically favorable stage at diagnosis in NM may be explained by its unique phenotype that often fails the ABCD categorization of suspicious moles, as they are more likely to exhibit border regularity and homogeneous color [22]. However, the majority of nodular melanoma patients do report a history of change in their mole, typically shorter in duration than for SSM [38]. Notably, our group has previously reported that the median duration of change in a lesion before treatment, as assessed by patient recall, was only 5 months for NM compared to 9 months for SSM [22, 38, 39]. This suggests that it is not late presentation and delayed diagnosis that accounts for advanced thickness of NM at presentation, but rather indicates a more clinically aggressive biology of NM and supports the importance of adding ‘E’ for ‘evolution’ to the ABCD criteria [22, 30, 40, 41].

There are consistent pathological differences between SSM and NM, lending further support for inherent differences in the tumor types. For example, NMs have been more invasive, ulcerated, and have more mitoses than SSMs [22]. Taken together, these differences lend support to the postulate that SSM and NM are distinct entities and suggest that biological heterogeneity might play a role in driving different phenotypic and clinical presentation of SSM and NM.

Biologic Heterogeneity of Superficial Spreading and Nodular Melanoma

Different Alterations in the MAPK Pathway in Superficial Spreading and Nodular Melanoma

Discoveries related to the disruption of normal functioning of the mitogen activated protein kinase (MAPK) pathway have driven breakthroughs in understanding the biology of melanoma. Constitutive activation of the pathway by NRAS mutations on chronically sun-exposed skin and BRAF on intermittently sun-exposed skin has launched the development of a variety of targeted therapies [42]. It is crucial to consider and incorporate known the molecular aberrations involved in the MAPK pathway in order to make histological subtypes more meaningful distinctions, but there is conflicting evidence regarding the association of melanoma subtypes and these mutations [43, 44].

As the first oncogenic mutation defined in melanoma, NRAS mutations make up almost virtually all RAS mutations in melanoma and occur in about 15% of primary melanomas [45–49]. These mutations occur at codons 12, 13, and 61 and transform the GTPase into a constitutively active state [50]. Several groups found no difference in NRAS mutation rates among SSM and NM [51, 52], although other studies report higher rates in NM [43, 53, 54]. These mixed associations with histopathological subtype need to be further explored in larger cohorts that take the degree of sun damage into account in a standardized way [17, 44, 55, 56].

BRAF somatic missense mutations occur in about 66% of melanomas, and tumors almost never harbor both NRAS and BRAF mutations simultaneously [17, 57–59]. Studies have shown that the BRAF mutation is not sufficient on its own for malignant transformation [60]

although the recent FDA approval of a BRAF specific inhibitor has made the testing for BRAF mutations part of the standard of care both to identify patients who might benefit from targeted therapy and to utilize in clinical management.

Several studies have demonstrated that BRAF mutation is less common in NM than SSM [17, 44, 53, 55, 56, 61–63] and is more frequently mutated in AML melanomas [64]. Of note, this study was conducted in a Japanese population, which has been shown to have higher frequencies of ALM (41.9% vs. 10.0% in US), and lower frequency of SSM (11.8% vs. 69.6% in US), bringing the general applicability of these results in other populations into discussion. Other studies have found similar BRAF mutation rates of SSM and NM, but all the tumors included were relatively thick [51, 65]. The lack of consensus on histologic-genetic correlations in known genetic alterations in melanoma underlies the importance of utilizing unbiased approached and new technologies to explore other alterations.

Differences in Superficial Spreading and Nodular Melanoma Identified using Integrative Genomics

Uncovering biologic distinctions between SSM and NM is integral to understanding the phenotypic differences between these histological subtypes. High throughput techniques such as comparative genomic hybridization (CGH), single nucleotide polymorphism (SNP) arrays and microarrays have potential as unbiased approaches to identifying subtype specific alterations. Although both CGH and SNP array can detect chromosomal amplification and loss as well as cytogenic abnormalities, SNP array can also identify regions of loss of heterozygosity [66]. CGH has been extensively utilized in melanoma in the last 10 years, but it has mainly been used for three purposes: 1. to distinguish nevi (usually Spitz nevi) from melanoma [67, 68]; 2. to identify the genomic alterations that characterize uveal melanoma (subtypes) and correlate with prognosis [69–72]; and 3. to identify the differences between primary cutaneous melanoma (irrespective of the histologic subtype) and metastasis [73, 74]. While CGH has also been used to identify the genomic alterations that distinguish melanomas with different degrees of sun damage as measured by the presence or absence of solar elastosis, it has not been applied to identify the differences between SSM and NM. As such, the CGH characterization of SSM and NM is still missing.

Microarray technology has enabled gene expression profiling to provide better understanding of tumorigenesis via simultaneous evaluation of the expression levels of thousands of genes in a single experiment [75–77]. Our group recently examined genomic and gene expression alternations using integrative genomics to reveal molecular differences in SSM and NM. In a comparison of normal melanocytes, primary NM, primary SSM, metastatic NM and other metastatic melanoma cell lines, single nucleotide polymorphism (SNP) array data indentified 408 SSM-specific regions with significant genomic alteration and 543 that were NM-specific [21]. Moreover, 114 differentially expressed genes were identified using gene expression array, over half of which, including *MTAP* and *ALDH7A1*, fell into the category of metabolic processes. *MTAP*, *ALDH7A1* and erythrocyte membrane protein *EPB41L3* displayed the highest fold changes in gene expression, and at the same time showed lower expression in SSM compared to NM and nevi, with results verified by genomic PCR and qRT-PCR [21]. The linear progression model predicts that all the genomic differences would follow step-wise transformations from SSM to NM. However, the dip in gene expression levels in SSM compared to nevi and NM cannot be explained by a step-wise transformation. These alterations suggest underlying genetic differences and support the hypothesis of separate biological entities. When the SNP array data and the gene expression array data were analyzed for interaction, five genes showed significant copy number loss that correlated with lower gene expression in SSM compared to NM. The presence of recurrent SSM-specific deletions that are present or even amplified NM cannot be reconciled with the linear progression model, even taking epigenetic modifications into

account. To further support the fidelity of these findings, external validation was performed using two publically available gene expression data sets [16, 26]. Jaeger et al. identified 67 genes differentially expressed in SSM and NM, including SSM-specific down regulation of the five genes validated by using quantitative PCR and qRT-PCR in our study. Whole genome microarrays performed by Scatoloini and colleagues only included 3 of the genes (*MTAP*, *SEC23IP* and *G3BP2*) but all showed the same downregulation in SSM-like RGP cell lines compares to VGP cell lines for the three genes [26]. These results represent a discordance with the current paradigm of linear progression and introduce the existence of differentially expressed genetic alterations that can potentially be exploited in the search for targeted, subtype specific therapies.

Not only has genetic evidence emerged supporting discrete pathways of development from the transformed melanocyte to invasive melanoma for SSM and NM that is in contrast to the theory of sequential progression, but gene expression patterns also suggest that there are subtype-specific alterations. The studies reporting differentially expressed genes are summarized in Table 2.

microRNA Differential Expression in Superficial Spreading and Nodular Melanoma

Improvements in our understanding of the functional relevance of microRNAs and advances in array based technology have allowed for high-throughput profiling of these genetic regulators to further uncover tumor biology. microRNAs have consequently emerged as a key regulations in melanomagenesis. As short, endogenous, non-coding RNA molecules, they negatively regulate gene expression at the post-transcriptional level. microRNAs can also accurately distinguish different cell types [78, 79]. Moreover, since each microRNA can potentially modulate several hundred genes, changes in expression of a single microRNA can have deep pathological consequences [80–82]. Their ability to successfully discriminate between cancer tissues and normal tissues, subtype, age at diagnosis and mutational status in other cancers suggests that microRNA expression can also yield insight into distinctions among melanoma histopathological subtypes [83–86]. ALM, already distinguished as a unique histologic subtype, has been shown to exhibit an ALM-specific microRNA pattern [87].

A study by our group found that among primary melanoma samples and congenital nevi (CN), the majority of microRNAs (n=31) were downregulated in SSM compared to both CN and NM, and real time PCR on genomic DNA revealed that the genomic loci corresponding 7 microRNAs selectively lost in SSM, supporting the results obtained by microRNA array [25]. Only about half of the microRNAs showed stepwise differential expression from CN to SSM to NM. This data also suggests that genomic differences cannot be reconciled with the linear model of progression.

Additional Studies Supporting Differences between Superficial Spreading and Nodular Melanoma

Several recent studies have aimed to undercover relevant molecular trends among the current histologic subtypes. Kanoh and colleagues examined 15 NM and 12 SSM formalin-fixed, paraffin-embedded (FFPE) tissues for levels of nestin, a marker or neural stem cells that has recently been suggested to be a marker of melanoma cells of origin [88]. They found nestin immunoreactivity in all amelanotic and 80% of the melanotic NMs while expression was only seen in 17% of the SSM cases [28]. However, this small sample size was not matched for thickness and previous reports have shown that nestin expression was significantly correlated with the stage of melanomas [89].

CDKN2A is a gene known to be important in both sporadic and hereditary melanoma. A mutation in this gene, which codes for p16INK4A (p16), in melanoma prone families is more common in SSM compared to NM and other histological subtypes [90]. In examining p16 protein levels, one group found decreased expression among NM compared to SSM [91] although previous studies report mixed expression levels [92–95]. However, Pavey and colleagues do not control for mitosis and thickness in their analysis, both of which have been shown to vary with p16 expression [91, 96].

In another analysis of subtype differences, our group investigated the clinicopathological relevance of cryptic epitope HU177 in serum from 291 melanoma patients. Extracellular matrix remodeling has been shown to generate protein fragments, such as Hu177 and other cryptic collagen peptides, which may function in tumor migration and angiogenesis [97–100]. After controlling for time of blood draw and tumor thickness, multivariate analysis showed a significant relationship between higher HU177 levels and NM. Elevated levels were trending towards association with ulcerated melanomas and recurrence but remained insignificant [101]. HU177 has also been shown to correlate with tumor response in a phase II trial of sorafenib (BAY 43-9006) in melanoma, suggesting future development of histotype specific biomarkers might have clinical utility [102].

Future Steps

New technologies using high throughput sequencing have provided an opportunity to gain a more complex understanding of the biology underlying melanomagenesis. Present evidence suggests that NM and SSM cannot arise sequentially via the linear model of progression and that they are two distinct biologic entities. This shift in the understanding of how melanoma develops dictates a revision of clinical care procedures based on histologic-genetic correlations. Building on the expanding foundation of knowledge regarding the biology of melanoma, alterations defining these two subtypes can be utilized moving forward to improve prognosis and develop novel therapeutic approaches. Histological subtypes can be integrated into prognostic decision trees or added to the AJCC staging as a complement to existing schemes.

While there have been new classifications proposed based solely on molecular alterations and sun exposure and ignoring histopathological subtype [103], the existing subtypes have a wealth of molecularly relevant information. The ALM subtype in melanoma as well histological subtypes in other cancers have effectively transformed their molecularly enhanced classification into individualized targeted treatments. In addition to ALM, mucosal and uveal melanoma, which are less common melanoma histologic subtypes, have also utilized specific molecular alterations for treatment [104–106]. The experimental evidence that we have summarized offers clear rationale for extending this approach to SSM and NM through the identification of new, and hopefully more effective, subtype-specific therapeutic strategies.

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Table 1
Changes in clinical presentation of superficial spreading and nodular melanoma over time

Location	Sample Source	Time Period	Histological Subtype Proportions	Changes in Thickness	Trends Noted	Limitations	References
New York, USA	Distinct cohorts from the New York University Medical Center	1971–1982 & 2002–2007	1119 SSMs 253 NMs 283 Others	SSM: median of 1.1 mm to 0.6 mm ($p<0.001$) NM: median of 3.2 mm to 2.4 mm ($p=0.10$)	SSM thickness decreased while NM stayed constant	Academic referral centers tend to see more aggressive melanoma cases	Warycha et al 2008
Queensland, Australia	Population based cancer registry in Queensland, Australia	2000–2003	369 NM	NM: Median: 1.90 mm Range: 0.40 mm– 9.30 mm		Only examined nodular melanoma over 4 years	Geller et al 2009
Munich, Germany	Cohort from Ludwig-Maximilian-University of Munich	1977–2000	3660 SSMs 1703 NMs 1112 Others	Female: SSM: –1.5 AAPC ($p<0.001$) NM: –0.04 AAPC ($p=0.379$) Male: SSM: –1.7 AAPC ($p<0.001$) NM: –0.7 AAPC ($p=0.090$)		No thickness values reported. Values reported based on gender.	Baumert et al 2009
Tuscany, Italy	Population based cancer registry in providences of Florence and Prato, Italy	1985–2004 (1985–1989 & 2000–2004)	1655 SSMs 238 NMs 1009 Others	SSM: median of 1.20 mm to 0.68 mm ($p<0.001$) NM: median of 3.33 mm to 4.00 mm ($p=0.517$)		Included in-situ cases.	Crocetti et al 2010

Key: SSM: superficial spreading melanoma; NM: nodular melanoma; AAPC: average annual percentage change

Table 2

Summary of studies reporting differential gene and microRNA expression found between superficial spreading and nodular melanoma

Sample Size	Identified Alterations between SSM and NM		Technologies Used		References
	Array Based	Validated Targets	Discovery	Validation	
Cell Lines: 1 NHM 2 Melanocytes 3 SSM 2 NM 2 VGPM 2 Metastatic NM 12 Met UP Tissues: 20 NM 20SSM	114 differentially expressed genes	DIS3, FGFR1OP, G3BP2, GALNT7, MTAP, SEC23IP, USO1, and ZNF668 with NM/SSM copy number gain/loss correlated to altered gene expression Deleted in SSM: USO1, G3BP2, DIS3, and SEC23IP	<ul style="list-style-type: none"> Single Nucleotide Polymorphism DNA array Gene Expression Array 	<ul style="list-style-type: none"> qRT-PCR Genomic PCR Western Blot Immunohistochemistry on tissue microarrays 	Rose et al 2011
Tissues: 13 SSM 5 NM 1 ALM	60 differentially expressed genes	Lower in SSM: MMP16, BCL2A1, ICAM1 and CEACAM1	<ul style="list-style-type: none"> Oligonucleotide microarrays with 22,283 probe sets 	<ul style="list-style-type: none"> Immunohistochemistry Immunoblotting 	Jaeger et al 2007
Screening: 18 CN 11 DN 8 RGPM 15 VGPM 5 MTS Validation: 5 CN 5 RGPM 5 VGPM 5 MTS	540 differentially expressed sequences	Lower in RGPM: AKT3, CTNNB1, NRAS, APL5, BCL2A1, HSPA5, BIRC2, ANXA5, NPM1, TNS3, HSP90AB1, PAIP1 and DAPL1	<ul style="list-style-type: none"> Whole Genome Microarrays for 41,000 human unique genes and transcripts 	<ul style="list-style-type: none"> qRT-PCR 	Scatolini et al 2010
Tissues: 26 SSM 56 NM 9 CN Cell Lines: 4 RGPM 6 VGPM	134 microRNAs differentially expressed	Genomic loss in SSM: let-7g, miR-15a, miR-16, miR-138, miR-181a, miR-191 and miR-933	<ul style="list-style-type: none"> miRCURY LNA microRNA Arrays 	<ul style="list-style-type: none"> qRT-PCR 	Poliseno et al 2011

Key: SSM: superficial spreading melanoma; NM: nodular melanoma; ALM: acral-lentiginous melanoma; CN: common nevi; DN: dysplastic nevi; RGPM: radial growth phase melanomas; VGPM: vertical growth phase melanomas; MTS: melanoma metastases; qRT-PCR: quantitative Reverse Transcriptase Polymerase Chain Reaction; NHM: normal melanocytes cultured from infant foreskin; Met UP: metastatic melanoma of unknown primary histologic subtype