Melanoma Tumor Cell Heterogeneity: A Molecular Approach to Study Subpopulations Expressing the Embryonic Morphogen Nodal

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As the frequency of melanoma increases, current treatment strategies are struggling to significantly impact patient survival. One of the critical issues in designing efficient therapies is understanding the composition of heterogeneous melanoma tumors in order to target cancer stem cells (CSCs) and drug-resistant subpopulations. In this review, we summarize recent findings pertinent to the reemergence of the embryonic Nodal signaling pathway in melanoma and its significance as a prognostic biomarker and therapeutic target. In addition, we offer a novel molecular approach to studying the functional relevance of Nodal-expressing subpopulations and their CSC phenotype.
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The incidence and mortality rates of melanoma have been increasing over the last few decades with the most recent statistics reporting the highest lifetime risk of developing melanoma as 1 in 50 for Caucasians (American Cancer Society; http://www.cancer.org/cancer/skincancer-melanoma/detailedguide/melanoma-skin-cancer-key-statistics). Especially noteworthy is that metastatic melanoma, a disease of poor prognosis, is highly resistant to conventional chemotherapies, with a poor clinical outcome.[1] Despite significant efforts devoted to advancing new therapies to improve patient survival, including the recent development of inhibitors to RAS, RAF, and MAP-ERK kinase (MEK), not all melanoma cells respond in a predictable manner—primarily due to the conundrum of tumor cell heterogeneity.[2] Thus, a primary focus of cancer researchers is to better understand cellular heterogeneity within tumors, such as melanoma, with a special emphasis on identifying and ultimately targeting tumor cells exhibiting stem cell properties, known as the cancer stem cell phenotype, generally associated with drug resistance.[3–5]

Recognizing that cancer cells can exploit normally dormant embryonic pathways to promote tumorigenicity and metastasis presents a unique therapeutic opportunity.[6–7] Studying embryonic signaling pathways in melanoma has led to the discovery that the embryonic morphogen Nodal is re-expressed in the aggressive phenotype.[6,8–10] Interestingly, Nodal is a member of the transforming growth factor-beta (TGF-β) superfamily and is a critical factor in normal embryonic development, including maintenance of pluripotency in human embryonic stem cells (hESCs), initiation of mesoderm formation, and regulation of left–right asymmetry.[11] In humans, Nodal expression is largely restricted to embryonic tissues, including the trophoblast and the developing mammary gland, but is generally lost in most normal adult tissues. Therefore, studies addressing the role of Nodal in cancer progression have focused on the mechanisms underlying its re-expression in tumor cells and the translational relevance of targeting Nodal-positive tumor cells as a novel therapy.[12,13]

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Recent findings have revealed that Nodal is a critical regulator of melanoma growth, plasticity, and tumorigenicity, and holds promise as a new biomarker for metastatic potential.[9,10,14] Similar observations have been reported in gliomas and carcinomas of the breast, prostate, endometrium, liver, and pancreas.[15–20] However, with any new discovery, there are associated challenges. In the case of Nodal, research in this field for human cells and tissues has been hampered by inconsistencies and sometimes incorrect information available in public databases, and by lackluster commercially available reagents and the associated disparate results.[21] Furthermore, because Nodal is a secreted protein that can influence cellular behavior in an autocrine and a paracrine manner via a diffusion gradient,[22] it has been particularly challenging to assess the extent and influence of Nodal-expressing tumor cells versus tumor cells where Nodal is simply adherent to the cell surface, thus compromising our ability to measure directly the functional relevance of Nodal-expressing subpopulations of melanoma cells. Fortunately for this avenue of discovery, molecular detection probes have been developed recently that allow live cell sorting, imaging, and assessment of Nodal (both independently and together with other critical biomarkers) in tumor cells,[23,24] thereby advancing our ability to understand the significance of Nodal-expressing subpopulations in heterogeneous melanomas.

MELANOMA TUMOR CELL PLASTICITY

The aggressive melanoma phenotype has been described as plastic and multipotent, similar in many respects to embryonic stem cells.[10] However, dissimilar to normal embryonic progenitors, these tumor cells lack critical regulators, resulting in the aberrant activation of embryonic signaling pathways, which maintains their plastic phenotype and promotes unregulated growth.[6,25] Of special note are the phenotypes expressed by aggressive melanoma cells that are associated with hESCs and endothelial cells/progenitors, in which their respective molecular signatures correlate with functional plasticity.[26] An example of an endothelial phenotype found in advanced-stage melanoma cells is demonstrated by the expression of vascular endothelial cadherin (CD144) and de novo formation of vascular perfusion networks, in vitro and in vivo, revealing a transendothelial functionality resulting in tumor perfusion.[27] Interestingly, only subpopulations of melanoma cells express CD144, specifically the tumor cells forming vasculogenic networks.[28,29]

The definition for cancer stem cells (CSCs) has expanded to include the capacity for differentiation and self-renewal for subpopulations of tumor cells originally described as having stem cell-like properties.[30] However, unlike many solid tumors that consist of defined, isolatable subpopulations of CSCs, melanoma cells can generate reversible stem-like cells through "phenotype switching".[5] In this manner, melanoma cells can switch between a migratory, stem-like state that may be resistant to therapy, and a highly proliferative state that leads to tumor growth. Further support for the phenotype switching paradigm includes a recent study showing at least 25% of tumor cells isolated from melanoma patients were able to establish new tumors.[31] Moreover, similar to normal developmental events where cell fate determination is profoundly influenced by the microenvironment, phenotypic changes in tumor cells can be similarly influenced. Indeed, an excellent example of this phenomenon has been shown in studies where metastatic melanoma cell subpopulations have been reprogrammed by the embryonic microenvironments of hESCs and chick neural crest to assume a more differentiated melanocyte phenotype.[32]

TARGETING EMBRYONIC SIGNALING IN AGGRESSIVE MELANOMA

The significance of Nodal expression in melanoma, as well as other tumor models, is of special interest to researchers, pathologists, and oncologists, based on the emerging body of evidence supporting its potential as a biomarker for tumor progression and a viable target for therapy.[9–10,14–20] Immunohistochemical studies have shown that Nodal protein is absent in normal skin, is seen in only a few cells in radial growth phase (RGP) melanomas, and is robustly detected in vertical growth phase (VGP) melanomas and metastases,[6,9,10,14] as demonstrated in representative tissues shown in Figure 1. Additional findings have noted the localization of Nodal in vascular networks in advanced stages of melanoma progression, and have associated the expression with therapeutic resistance.[1] Thus, these expression studies suggest that Nodal may be a promising biomarker for not only melanoma progression but also responsiveness to therapy.

In vitro studies have provided some clarity to the Nodal signaling cascade employed by aggressive melanoma cells, which differs from that described for hESCs.[6,25] Nodal propagates its signal by binding to and activating the heteromeric complex composed of the epidermal growth factor (EGF)-like glycoprotein co-receptor Cripto-1 and types I and II activin-like kinase receptors, leading to activation and nuclear translocation of the Smad2/3/4 complex, where it regulates the expression of