Breast Cancer RESEARCH

VIEWPOINT

Sox4, EMT programs, and the metastatic progression of breast cancers: mastering the masters of EMT

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Abstract

Epithelial-mesenchymal transition (EMT) programs require the expression of a variety of so-called master regulators of EMT, including members of the Snail, Zeb, and Twist transcription factor families. Teleologically, the requirement for such a diverse group of 'master regulators' seems evolutionarily cumbersome, and emerging evidence indicates that these transcription factors do in fact mediate unique and specialized functions, suggesting the existence of higher-order 'masters' that truly direct and coordinate EMT programs. Accordingly, Tiwari and colleagues recently delineated an elegant pathway wherein transforming growth factor-beta stimulates Sox4 expression, which induces that of the histone methyltransferase, Ezh2, thereby reprogramming the epigenome to elicit EMT programs and metastasis of breast cancers. This viewpoint highlights Sox4 as a 'new' master of EMT programs and metastatic breast cancer.

Background

Epithelial-mesenchymal transition (EMT) was first described over three decades ago by Greenburg and Hay [1] and referred to the ability of immotile, polarized epithelial cells to 'transform' into highly motile, elongated mesenchymal cells. Initial investigations of EMT programs described their embryonic functions during neural crest, endocardial cushion, and palette formation and during wound healing and tissue remodeling in adult tissues [2]. Interestingly, the inappropriate reactivation of embryonic and developmental programs in adult tissues has been linked to the initiation and progression of human malignancies [3,4]. Despite early alliances between EMT programs and tumorigenesis, the science of EMT failed to advance beyond that of an interesting cell and developmental phenomenon until two major findings propelled EMT programs into scientific prominence: (a) Twist1 expression was found to elicit EMT programs operant in mediating breast cancer metastasis [5], and (b) EMT phenotypes were determined to promote the selection and expansion of 'stem-like' cells [6]. Today, EMT programs are known to be induced by various molecular, cellular, and microenvironmental signals, particularly those engendered by transforming growth factor- β (TGF-β) and its stimulated expression of master EMT regulators, such as members of the Twist, Zeb, and Snail families of transcription factors [4,7]. EMT programs also bestow carcinoma cells with resistance to anoikis and apoptotic stimuli and to chemotherapies and radiation used to treat human malignancies [8]. Moreover, EMT programs are plastic and subject to phenotypic reversion through induction of mesenchymal-epithelial transitions (METs), which are essential in promoting the outgrowth of metastatic foci [9]. Thus, enhancing our knowledge of how EMT programs initiate and resolve, and of how the epigenome regulates these events is critical to alleviating the mortality associated with metastatic disease.

The article

Members of the Sox (Sry-related high-mobility group box) family of transcription factors play instrumental functions during embryonic development and cell fate specification in virtually all cells, tissues, and organ systems [10]. Recently, aberrant Sox4 expression has been observed in many human malignancies [10,11], including breast cancers [12,13]. Indeed, in an intriguing study published in *Cancer Cell*, Tiwari and colleagues [14] performed motif activity response analyses on normal

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mammary epithelial cells (MECs) that were induced to undergo EMT in response to TGF-β. In doing so, Sox4 and Sox9 were the only Sox family members whose expression was coupled to TGF-β and EMT programs, and subsequent Sox4 deficiency proved sufficient (a) to prevent TGF-β stimulation of EMT programs in normal and malignant MECs and (b) to inhibit the growth and metastasis of mammary tumors in mice. Interestingly, whereas Sox4 deficiency significantly reduced the expression of Snail, Zeb, and Twist family members, reciprocal overexpression of these EMT transcription factors had no effect on Sox4 expression, suggesting that Sox4 functions as a higher-order 'master' that governs traditional EMT transcription factors. Finally, genome-wide gene expression profiling of control and Sox4-depleted MECs identified the histone methyltransferase Ezh2 as an essential regulator of epigenome reprogramming operant in facilitating EMT programs. As above, Ezh2 deficiency prevented breast cancer cells from undergoing EMT and colonizing the lungs of mice. Collectively, this intriguing study demonstrates that the foundation of EMT does not reflect mere changes in cell morphology and marker expression, but instead manifests via a coordinated program of global chromatin remodeling and subsequent gene expression changes.

The viewpoint

Recently, Sox4 was identified as a TGF-β gene target in normal and malignant MECs undergoing EMT and as a potential biomarker of triple-negative breast cancers (TNBCs) [12,13]. In extending these findings, Tiwari and colleagues [14] identified a novel epigenomic 49-member gene signature that is governed by aberrant Sox4 and Ezh2 expression and is associated with TNBC development, and with poorer metastasis-free survival rates of lymph node-negative breast cancers. Interestingly, the tumor-suppressing activities of p53, which also functions as a 'gatekeeper' against EMT programs [15], can be augmented or attenuated by Sox4 expression in a cell- and context-specific manner [11]. Because approximately 80% of TNBCs harbor mutated p53 proteins [16], future studies need to explore the functional consequences and molecular connections between p53 and Sox4 in driving TNBC metastasis. Along these lines, TNBCs can be subcategorized into six genetically distinct subtypes [17], raising the question of whether all TNBC subtypes will be equally responsive to Sox4/Ezh2 activity or whether these oncogenic activities will be restricted to specific TNBC subtypes.

Future studies also need to explore the mechanisms whereby TGF-β stimulates Sox4 expression in normal and malignant MECs. Tiwari and colleagues [14] state that although TGF-β can induce Sox4 expression via a Smad2/3/4-dependent pathway [11], Sox4 expression stimulated by TGF-β occurs independently of Smads and relies on concomitant messages activated by the Wnt and Notch signaling systems. Mapping these Sox4-directed pathways is critical to assessing their potential as chemotherapeutic targets or biomarkers for metastatic TNBCs or both. Interestingly, approximately 70% of TNBCs express robust levels of epidermal growth factor receptors (EGFRs), whose clinical targeting has proven to be ineffective [18]. Importantly, Tiwari and colleagues [14] state that administration of EGFR inhibitors was sufficient to induce Sox4 expression, raising the tantalizing possibility that TNBCs escape destruction by EGFR antagonists by acquiring EMT and chemoresistant phenotypes elicited through upregulated Sox4 and Ezh2 expression. As such, initiation of epigenetic therapies targeting Ezh2 (for example, DZNep [19]) may offer new inroads to alleviate TNBCs and their aggressive recurrence.

Finally, questions related to epigenomic reprogramming and its ability to impact the dynamics of EMT/MET cycles deserve additional investigation. For instance, to what extent are Sox4 expression and its associated EMT-based epigenetic marks reversed during subsequent MET programs? Indeed, partial reversion of the epigenome could produce a secondary epithelial state that is genetically distinct from its predecessor, a finding we observe in MECs driven through repeated EMT/MET cycles (MK Wendt and WP Schiemann, manuscript submitted). Indeed, we speculate that breast cancer cells harboring 'tweener' epigenomes contribute to disease severity by providing carcinoma cells that are primed for and/or hypersensitive to EMT-inducing stimuli within the tumor microenvironment (for example, TGF-β, hypoxia, mechanotransduction, and chemotherapies), thereby ensuring disease progression and recurrence. Ultimately, answering these and other questions will provide a foundation to develop EMT-based therapies to alleviate metastatic breast cancers.

Abbreviations

EGFR: Epidermal growth factor receptor; EMT: Epithelial-mesenchymal transition; MEC: Mammary epithelial cell; MET: Mesenchymal-epithelial transition; Sox: Sry-related high-mobility group box; TGF-β: Transforming growth factor-β; TNBC: Triple-negative breast cancer.

Competing interests

The authors declare that they have no competing interests.

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