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Mini Review

A Twist in the Tale: TWIST1-SOX2 Axis Governs ABCG2-Mediated Paclitaxel Resistance of Breast Cancer Stem Cells

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Abstract

Epithelial-mesenchymal transition (EMT) is an important process during development by which epithelial cellsb acquire mesenchymal, fibroblast-like properties and show reduced intercellular adhesion and increased motility.The identification of epithelial-mesenchymal plasticity of breast cancer stem cells provided another level of complexity regarding development of strategies to eliminate these lethal seeds of breast cancer. In determining the association between Sox2, cell migration and expression of EMT markers, we found a persistently high expression of Twist1 and its apparent lack of EMT-like properties during migratory arrest of MDA-MB-231 cells, even after paclitaxel treatment of Sox2-silenced cells. The role of Sox2-dependent Twist1 in maintaining stemness was more prominent when Sox2 expression was high in brCSCs. It can be presumed now that Twist1 expression in presence or absence of Sox2 defines the precise mechanism underlying the possible role of Twist1 in crossroads of pluripotency and EMT of breast cancer stem cells.

Keywords: Breast cancer; Breast cancer stem cells; Pluripotency;Epithelial-to-mesenchymal transition;Chemoresistance; SOX2; Chemotherapy; TWIST1; ABCG2;Gene silencing

Introduction

The major caveat in treatment of breast cancer patients is the possibility of recurrence, even afterimplementation of successful therapeutic strategies. Asmall population of relatively quiescent chemo-resistant cancer stem cells(CSCs) have been implicated for disease relapse. CSCs can escape the deleterious effects of chemotherapy through a variety of mechanisms and thus mediate chemoresistance. Therefore, targeting CSCs may prove to be a useful approach for exploiting key mechanisms that sustain overall tumor survival and overcoming drug treatment failures that ultimately lead to recurrence and death [1]. Different factors mediate chemoresistance in CSCs, like ATP-binding cassette (ABC) transporters [2,3]. Aldehyde dehydrogenase (ALDH1) activity [4,5]. Bcl-2 protein family members [6-8]. Altered DNA damage responses [9-11] and CSC-related signaling components [12-15].

Epithelial-Mesenchymal Traits and Survival of Cancer Stem Cells

Advances in cancer research have focused on the role of epithelial-to-mesenchymal transition (EMT) and CSCs in tumor progression, metastasis and treatment resistance [16,17]. Epithelial-to-mesenchymal transitioned tumor cells have been reported to possess increased motility and invasiveness, tumor-propagating potential, and resistance to apoptosis and anti-tumor drugs [18,19]. EMT is triggered by a number of distinct molecular processes including the expression of specific cell-surface proteins and the activation of transcription factors (TFs). EMT-TFs belong to different families, including SNAI1 (or Snail) and SNAI2 (or Slug), two ZEB factors, ZEB1 and ZEB2, and Twist [20,21]. The biological link between EMT phenotypes and CSCs has recently been studied in many types of cancer including prostrate, pancreatic, ovarian and breast [22-24]. In breast cancer, it has been reported that the overexpression of Twist, Snail or FOXC2 not only rendered the breast cancer cells with more mesenchymal properties, but also an increased expression of $CD44^{+}/CD24^{-/low}$breast CSC markers and an increased mammosphere forming efficiency [16,25]. Prostate cancer cells with an EMT phenotype showed increased expressions of Sox2, Nanog, Pou5F1, lin28B and Notch1, concomitant with an enhanced sphere-forming ability [26]. In pancreatic cancer, hypoxia induced upregulation of FOXA2, accompanied by EMT, down-regulation of E-cadherin and upregulation of mesenchymal markers, viz., Vimentin, Slug, Snail and Twist [27].

The Dual Role of Twist in Cancer Stem Cells

The role of EMT during early development and differentiation is critical to the survival of the embryo, as it is for survival of tumors. However, the main difference between normal development and pathological processes is that cellular and molecular events follow highly regulated spatial and temporal plans during development, whereas during transformation the order of events may be stochastic or bypassed. During tumorigenesis, EMT increases

the motility and invasiveness of cancer cells, and malignant transformation is oftentriggered by specific signaling pathways which promote EMT [28]. Oncogenic factors such as Notch, Wnt, Hh, TGF-β, peptide growth factors, Src, Ras, Ets, and integrin act as EMT-initiating signaling components inducing well-differentiated epithelial cells to convert into motile mesenchymal cells via the activation of multiple transcription factors, including Twist, Snail, Slug and ZEB [29].Interestingly, these factors have also been shown to regulate both self-renewal and oncogenesis of cancer stem cells from different tumor sites [30-33]. A strong relationship thus exists between stemness and EMT which ultimately governs the aggressive nature of cancer stem cells and their chemoresistance[16,34].

Over the past decade, the reactivation of Twist has been described as a marker of poor prognosis in an impressive array of human cancers [35]. Growing evidencesubstantiates that these cancers modulate the embryonic functions of Twist, and grant them oncogenic and metastatic properties. Twist up-regulation is observed in many cancers such as melanoma, T-cell lymphoma, prostate cancer, gastric carcinomas, rhabdomyosarcomas, and breast cancer [36-41]. Mechanistically, overexpression of Twist has been shown to inhibit apoptosis and interfere with p53 tumor suppressor functions [42].

Although Twistplays a major role duringEMT [43,44], the emerging role which Twistplays at the crossroads of EMT and maintenance of CSCs is a recent breakthrough.Twist has been associated with stem cell compartments during embryogenesis [45]. Overexpression of Twist in an in vitromouse embryonic stem cell model system prevents premature muscle cell differentiation, indicating its functions within the stem cell context [46]. During development, Twist has been shown to be upregulated through the Sonic hedgehog pathway and in turn upregulates Gli-1 [47]. In pathological conditions, Twist is overexpressed in more than 60% of breast tumors and is associated with loss of E-cadherin expression and increased genetic instability [48]. Overexpressing Twist in breast cells increased invasiveness, motility, angiogenesis, and resistance to radiation [43], indicating its active and dual role in both EMT and maintenance of stem cell characteristics. Studies are presentlyfocussing onidentification and characterization of the role of Twist in generating cancer stem cells. It has been shown that overexpression of Twist in MCF7 cells could significantly increase the stem cell population, as identified by an increase in the number of $CD44^{+}CD24^{-/low}$ and $ALDH^{+}$ cells [49].Several evidences now support that there is a direct role of Twist1 in regulating and maintaining stemness of cancer stem cells together with some major pluripotency genes independent of its role in EMT.

The Twist1-Sox2 Axis Governs Chemoresistance of Cancer Stem Cells

CSCs have been operationally defined through their ability to generate tumors with high efficiency when injected in limiting dilutions into immune-compromised mice [50-55]. Cancer recurrence has also been attributed to thisfiend population of CSCs which are not eliminated by conventional chemotherapy, primarily because of elevated expression of drug efflux pumps [56,57]. In addition, the fact that chemotherapy enriches the CSC population within a tumor poses a greater threat for the patient [57,58]. Therefore, modulation of factors responsible for elevating expression of drug transporters points toward a more effective and complete cure for cancer patients.

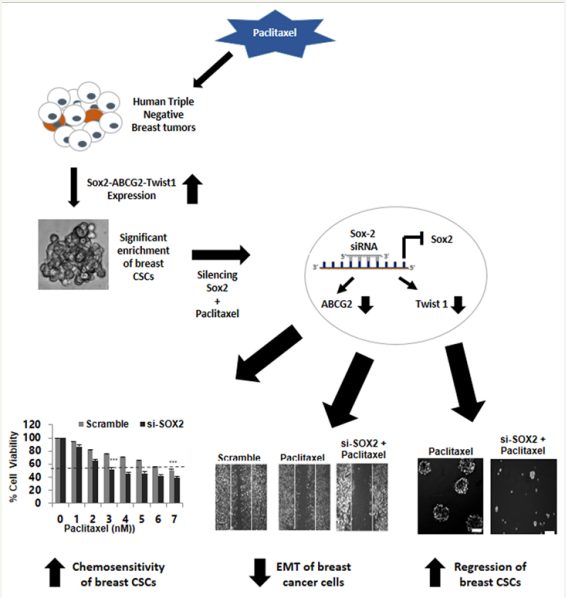

Figure 1: Paclitaxel treatment of human triple negative breast tumors targeted the bulk epithelial cancer cells (shown in white) leaving behind the cancer stem cells (CSCs; shown in orange) that are drug resistant. Chemo-treatment alters the gene expression profile of cancer stem cells in comparison to their normal counterpart, such as SOX2, TWIST1 and ABCG2, which are significantly upregulated in paclitaxel-treated triple negative breast tumors. This cascade contributes topluripotency and stemness properties of breast CSCs, with enhanced mammosphere formation and migratory propensity. Silencing SOX2 with siRNA leads to the down regulation of ABCG2 and TWIST1, confirming that SOX2 governs the expression of these two major CSC markers. Further, paclitaxel treatment in conjunction to si-SOX2, led to i) increased chemosensitivity of breast CSCs to the drug, ii) regulates epithelial-to-mesenchymal transition (EMT) and induces migratory arrest that sustains even after drug removal, simulating a post-chemo treatment condition in patients, and iii) reduces the sphere forming efficiency of CSCs, thus rendering them more susceptible to the anti-cancer effects of paclitaxel.

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Among the several genes that belong to the survival and maintenance family of CSCs, Sox2 stands out to be a major contributor for tumor initiation and cancer stem cell functions. This transcription factor plays an essential role in cell fate determination, thereby regulating developmental processes [59]. Aberrant expression of Sox2 has been reported in many types of cancers and is correlated with the persistent presence of CSCs[60,61]. Sox2 has also been linked to drug resistance in several studies.In a recent studyon breast cancer, we have demonstrated that paclitaxel treatment confers aTwist1-independent reduction of invasiveness in triple negative breast cancer cells. Reduction in invasiveness was more prominent when Sox2-silenced cells were treated with paclitaxel which continued to remain retarded even after drug removal[58]. Expression of Twist1 however remained significantly high in the Sox2-silenced MDA-MB-231 cells, both during and post paclitaxel treatment. There was an apparent lack of Twist1 involvement in migrationof both paclitaxel-treated MDA-MB-231 cells and Sox2-silenced MDA-MB-231 cells. Subsequently, clarification of its role in mammospheresswas necessary. Therein we found that paclitaxel-treated mammospheres re-grown as secondary spheres retained a high expression of Twist1 suggesting its major contribution in maintaining stem-like nature and chemoresistance of mammospheres rather than being a controller of EMT in cancer stem cells[58]. However, silencing Sox2 inmammospheres not only reduced their capacity to form secondary spheresbut enhanced chemosensitivity to paclitaxel (Figure 1). This was accompanied bydown regulation of Twist1 expression in both secondary spheres and cells cultured under adherent conditions, and was sustained even after drug removal, corroborating a post-chemotherapy condition. Previous literature and current data suggest that Sox2 and Twist1 are major regulators of CSC features in cancers. Specifically, based on Sox2-silencing experiments and chemosensitivity assays, it may be concluded that the Sox2-ABCG2-Twist1 axis plays a key role in regulating chemoresistance and tumorigenicity in triple negative brCSCs. Therefore, obliterating Sox2-Twist1 expression specifically in brCSCs before or during chemotherapy is a possible approach to eliminate the brCSC population within a tumor, with a promise to prevent post-chemotherapy recurrences in future.

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