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Hot Topic

Epithelial-to-mesenchymal transition: What is the impact on breast cancer stem cells and drug resistance

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ABSTRACT

There is increasing interest in cancer stem cells (CSCs) and their role in cancer progression. Recently, CSCs have been identified in brain, skin, and intestinal tumors and it has been suggested that these CSCs are responsible for tumor growth and metastasis. In breast cancer fatality is often due to the development of metastatic disease (MBC). Almost 30% of early breast cancer patients eventually develop MBC and in 90% of these multi-drug resistance (MDR) occurs. This could be attributed to the presence of breast cancer stem cells (BCSCs). Epithelial-to-mesenchymal transition (EMT) is a process known to contribute to metastasis in cancer and it is mainly characterized by loss of E-cadherin expression. The TGF-β signaling pathway has an established role in promoting EMT by down-regulating E-cadherin via a number of transcription factors, such as Twist, Snail and Slug. EMT has also been reported to produce cells with stem cell-like properties. Definition of the exact molecular mechanisms that are involved in the generation of stem cells through EMT could lead to the identification of new potential therapeutic targets and enable the development of more efficient strategies for particular patient groups. In this review we discuss what is known about the relationship between EMT, BCSCs and MDR.

Introduction

Breast cancer is the most commonly diagnosed type of cancer in the UK. It is also the second most prevalent cause of death from cancer in women after lung cancer, accounting for approximately 11,556 deaths in women and 77 in men in the UK in 2010 [1]. Metastasis is the main cause of fatality in breast cancer. The metastatic process is facilitated by a number of steps; after a tumor mass has expanded at the primary site some cells can acquire increased motility and detach from the primary tumor. They achieve this by breaking natural barriers, such as the extracellular matrix (ECM) and gaining access to the blood system, which "supplies" them with essential growth factors. These cells have then the ability to migrate and colonize at remote sites, such as bone, lungs, brain and liver where they grow in order to form new tumors [2].

Two models have been suggested to explain the cellular origin of cancer, although it is more likely that the two models coexist: (1) *The Stochastic Theory*; which claims that every single cell can potentially become cancerous in the appropriate microenvironment. However, differentiated cells have a shorter life span and are unlikely to accumulate a sufficient number of mutations in order to become neoplastic. (2) *The Hierarchy* (*Cancer Stem Cell*) *Theory*; which suggests that CSCs are more likely to generate a tumor, because they have a longer life span and ability to self-renew above that of non-stem cells. Consequently, fewer mutations are required for neoplastic transformation [3]. Additionally, CSCs have an increased migratory and proliferative potential and advanced DNA repair mechanisms. Conventional chemotherapy targets the bulk of the tumor cells but fails to target slow cycling cells, such as CSCs, which are also resistant to apoptosis. In many cases CSCs also express elevated levels of ABC transporters that enable them to efflux some in use chemotherapeutic drugs. Therefore, it has been suggested that CSCs might be responsible for tumor regrowth and the development of drug resistance [4].

Although there is an increasing number of studies that support the role of CSCs in cancer, their origin remains poorly understood. It is not yet known whether they originate from the transformation of normal stem cells or the acquisition of multiple mutations in cells that become dedifferentiated. It has also been suggested that EMT is involved in the generation and function of CSCs. This field has gained attention over the last years, since it provides the rationale for developing new

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therapeutic approaches for the management of metastasis. This review focuses on recent research findings related to EMT and CSCs in breast cancer.

EMT and metastasis in breast cancer

In addition to the role of EMT in normal embryonic development, it is also involved in pathological conditions, such as fibrosis and cancer metastasis [5]. In epithelial cancers, including breast cancer, metastasis is thought to occur by EMT. During this process, the epithelial cells lose their epithelial characteristics and acquire more mesenchymal properties by cytoskeleton rearrangements and alterations in adhesion, cellular structure and morphology. In fact, cell surface proteins, such as E-cadherin or integrins are replaced by mesenchymal markers, such as N-cadherin, vimentin or fibronectin. As a result, epithelial cells are detached from the basal membrane and they are then more capable of migrating to other sites or they become more invasive and enter the blood and lymphatic systems [6].

The molecular mechanisms involved in metastasis are not clearly understood, but it has been suggested that there is deregulation of the signaling pathways that control normal EMT. The most critical and well studied EMT molecule is E-cadherin (CDH1). It belongs to the family of genes coding calcium-dependent cell adhesion molecules (CAMs) and plays an important role in the maintenance of epithelial tissues. Loss of E-cadherin expression is associated with increased invasiveness in cancer and is considered a hallmark in the process of EMT. It has also been shown that E-cadherin expression is re-established in cells that form secondary tumor colonies and undergo MET. A recent study demonstrated that the levels of E-cadherin were significantly higher in these cells compared to the cells from the primary tumor. Additionally, more than 50% of metastatic organs in breast ductal carcinoma showed increased expression of E-cadherin [7]. The underlying mechanisms controlling E-cadherin re-expression are not clear, but it may occur at the metastatic sites if the appropriate microenvironment and signals are provided, so that the migrating tumor cells can connect and incorporate with the target organs. For instance, E-cadherin promoter methylation was reversed in some breast cancer cells when co-cultured with normal hepatocytes [8].

TGF-β is thought to play an essential role in the induction of EMT not only during embryogenesis, but also during cancer progression. TGF-β is a cytokine that activates multiple signaling pathways by binding to the TGF-βR I and TGF-βR II receptors which have a serine/threonine kinase activity. These can phospholyrate downstream cytoplasmic molecules e.g., Smad 2 and 3 and activate them. Phosphorylated Smad 2 and 3 can in turn bind to Smad 4 and enter the nucleus, where they form complexes with other factors and promote the expression of several target genes related to proliferation, differentiation, apoptosis and cell migration. TGF-β can also regulate EMT via the activation of additional molecules, such as MAPK, PI3K or GTPases belonging to the Rho family of proteins [9].

TGF-β has a direct effect on EMT by down-regulating epithelial markers and by up-regulating mesenchymal markers [10]. Although, TGF-β has tumor promoting effects in almost all types of cancer, in some types, including breast cancer, it seems to have a dual role. It acts as a tumor suppressor at early stages, whereas at later stages of the disease it drives invasion and metastasis. Tumor suppression activity can be seen in breast cancer due to the presence of particular mutations at genes encoding either TGF-β receptors or the three Smad molecules that participate in the TGF-β signaling pathway. For instance, abnormal signaling is found in advanced breast cancers because of point mutations in the kinase domain of TGF-β I or II receptor [11].

The transcription factors Snail, Slug and Twist are known to regulate the down-regulation of E-cadherin. In fact, Snail can bind with strong affinity to the E-boxes in the promoter of the E-cadherin gene and repress its expression [12]. It has also been shown that ectopic expression of Snail in different types of epithelial cells caused a mesenchymal-like phenotype and in these cells E-cadherin expression is significantly reduced. Furthermore, Snail expression is abundant in highly tumorigenic and invasive areas in both murine and human carcinomas, while it is very low or absent in non carcinogenic regions [13]. Additionally, when Snail levels were estimated by immunohistochemical analysis in human breast cancer tissue, it was found that there was a significant correlation of elevated expression levels with infiltrating ductal carcinomas (IDCs) with a poor grade of differentiation, but that do not develop lymph node metastases. Therefore, Snail was suggested as a prognostic marker for the metastatic potential in breast cancer [14]. Microarray analysis of human breast cancer samples also revealed that Snail was overexpressed in patients who had decreased relapse-free survival [15].

Slug has also been shown to directly repress E-cadherin in breast cancer cell lines. In fact, both Snail and Slug down-regulated the expression of wild-type E-cadherin genes, whereas they failed to do so when the E-cadherin gene contained mutated E-box elements [16]. High expression of Snail and Slug were inversely correlated with E-cadherin expression in a large number of cancerous cell lines, but the same was not observed in breast cancer samples. Both increased Slug and Snail levels were detected in breast tumors associated with lymph node metastases, but Slug was also overexpressed in semi-differentiated tubules of ductal carcinoma [17].

Twist is a basic helix-loop-helix transcription factor expressed during embryonic development. It is also overexpressed in many cancers, including breast cancer. MCF-7 cells overexpressing Twist exhibited loss of E-cadherin and gain of vimentin expression. These cells also acquired increased motility and invasive potential [18]. Twist has been found to directly repress E-cadherin in a dose-dependent manner. Increased expression of Twist and decreased expression of E-cadherin have been associated with grade III tumors in human breast cancer [19].

Identification of putative BCSCs populations

In recent years it has been suggested that metastasis occurs early in primary tumor development [20]. This led to the identification of cells with stem cell-like properties, which expressed high levels of CD44 and low levels of CD24 and were found to be present in eight out of nine patients with breast cancer. The tumorigenic ability of these CD44+/CD24− cells was demonstrated in immunocompromised mice in which a few cells were sufficient to form new tumors, while a high number of cells with alternative profiles failed to do so. Al-Hajj et al. also showed that CD44+/CD24− cells were able to give rise to new tumorigenic and non tumorigenic cells [21].

Aldehyde dehydrogenase 1 (ALDH1) is an enzyme responsible for the oxidation of intracellular aldehydes. It has been suggested that it is involved in self-renewal of cells at early differentiation stages by oxidizing retinol and thus converting it to retinoic acid [22]. Moreover, ALDH1 activity has been identified in murine and human hematopoietic and neural stem cells, suggesting it has a role in stem cell function [23]. ALDH1 expression has been detected in both normal and cancer human mammary stem cells [24]. While ALDH1+ tumor cells derived from human breast cancer cell lines have a higher ability of mammosphere formation in culture and increased tumorigenicity *in vivo* compared to ALDH1− cells [25]. Mammospheres are clusters of cells formed from a single