

Targeting epithelial–mesenchymal transition: therapeutic reversal of the cancer stem cell phenotype

Malignant tumors are composed of various cell types, including heterogeneous mixtures of neoplastic cells. Epithelial tissues that undergo transformation exist in an adverse environment where oxygen and nutrient supply is low, resulting in the upregulation of survival pathways, many of which are driven by hypoxia inducible factors-1 and -2. The intersection between downstream survival pathways driven by hypoxia inducible factors, stem cell characteristics of cancer and epithelial–mesenchymal transition has recently come to light. Insights into these processes are beginning to yield exciting new avenues for targeted therapies that promise to overcome treatment resistance.

KEYWORDS: cancer stem cells ■ epithelial–mesenchymal transition
■ hypoxia inducible factor

Mechanisms of cancer cell drug resistance & cancer stem cells

One of the most difficult problems facing clinicians who take care of cancer patients is treatment resistance. Therapeutic advances now depend on the development of new insights into cancer biology. Recently, a deeper understanding of cancer signaling pathways has generated paradigm shifting models of how cancer cells become immortalized and survive the onslaught of high doses of chemotherapy. Targeting these pathways has led to significant improvements in therapeutic outcomes. Insight into how chemotherapy mediates programmed cell death (apoptosis) via activation of mitochondrial pore opening was a major step forward [1]. DNA damage repair activated by DNA damaging chemotherapeutics has been found to trigger apoptosis by upregulating mitochondrial pore opening proteins, such as BAX and p53. The subsequent discovery that oncogene cell signaling networks cause drug resistance by promoting the release of pore closing proteins, such as BCL-xL, has particularly enabled the development of targeted therapies, such as imatinib, herceptin, erbitux, tarceva, vemurafinib and crizotinib [2,3]. Progress in cancer genomics has recently led to the development of another key unifying concept: cancer 'stem cells' [4]. These cells express a particularly drug-resistant phenotype [5,6]. The current view holds that stem cell-like features are turned on in a subset of cells within a tumor that provide a never ending source of somewhat more differentiated cancer cells that are treatment sensitive [7]. However, the cancer stem cell pool is unfazed by

chemotherapy, just as the stem cells residing in the bone marrow are resistant enough to chemotherapy to allow for repopulation of a patient's peripheral blood elements a few weeks after each cycle of treatment. Generation of cancer stem cells in the laboratory provides a useful model for the potential discovery of their 'Achilles heel' [7,8]. Recently, gene knock-in experiments using human telomerase have been carried out that yield cancer stem cells [9–11]. Of particular interest is the finding that cancer stem cells also express a mesenchymal 'fibroblast-like' phenotype associated with invasion and migration, behaviors critical for the generation of metastases [12]. Insight into the mechanisms responsible for the transition from an epithelial to a mesenchymal 'stem cell' phenotype, or the epithelial–mesenchymal transition (EMT) is providing a new set of potential therapeutic targets.

Role of hypoxia & hypoxia inducible factor in EMT

Tumor hypoxia appears to be a critical determinant in the development of cancer stem cells and the EMT [12–16]. As few as 300 malignant cells within a tissue microenvironment can produce a hypoxic and hyponutrient environment associated with an angiogenic response, suggesting that this selective pressure occurs early in tumor development [17]. Evidence suggests that hypoxia causes mitochondrial signaling via efflux of hydrogen peroxide that blocks hypoxia inducible factor (HIF)-1 α binding to von Hypel–Lindau protein, preventing HIF-1 α ubiquitination and degradation [18]. This leads to accumulation and

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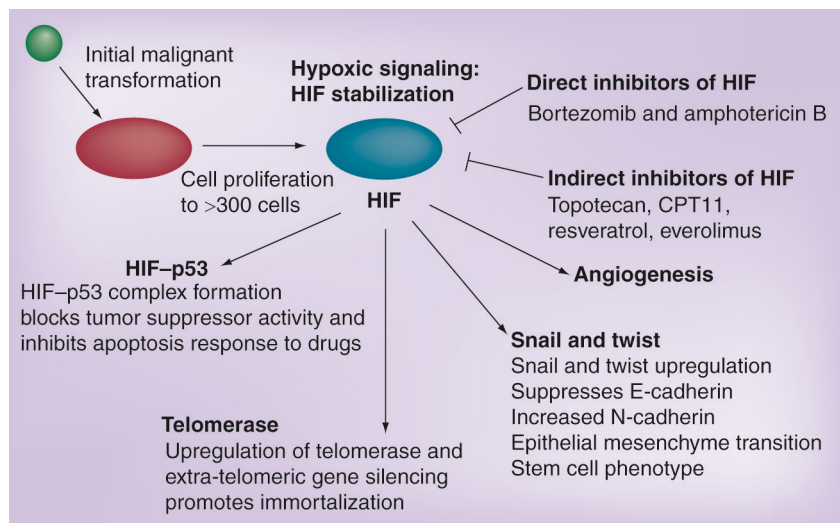


Figure 1. Role of hypoxia inducible factor in cancer cell epithelial-mesenchymal transition and stem cell phenotype.
HIF: Hypoxia inducible factor.

migration into the nucleus where it can partner with several other cotranscription factors leading to the upregulation of numerous pro-survival pathways [19]. Of particular interest with respect to the stem cell phenotype and EMT is the observation that HIF upregulates two genes of central importance to the mesenchymal phenotype, *SNAIL* and *TWIST* [20–22]. *SNAIL* encodes Snail, a zinc-finger transcription factor that belongs to a family of repressor proteins that block E-cadherin expression. E-cadherin plays an important role in cellular adhesion (selective stickiness) [23]. Snail normally promotes migration and prevents terminal senescence in keratinocytes, functions that can lead to metastatic events when overexpressed in cancer cells [24]. Twist, a protein involved in mesoderm development, also acts to suppress E-cadherin and to upregulate N-cadherin, another characteristic of the EMT phenotype [25]. HIF-1 accumulation is thus an important modulator of EMT via upregulation of Snail and Twist. HIF has also been noted to be stabilized in circulating hematopoietic stem cells under normoxic conditions via peroxide signaling mediated by NADPH oxidase, to upregulate stem cell factor and to increase the transcription of human embryonic stem cell markers in hypoxic cancer cells [26–28].

HIF & telomerase

Another key feature of the cancer stem cell is immortalization. HIF-1 binding motifs lay in the human telomerase reverse transcriptase promoter region gene, and various reports suggest that hypoxia upregulates human telomerase reverse transcriptase via HIFs [19,29–31]. Human

telomerase reverse transcriptase is a critical component of telomerase, playing a key role in the maintenance of telomere lengthening. This enables relatively unlimited cell division by malignant cells. Recently, telomerase functions distinct from telomere lengthening have been described. Telomerase has been found to participate in RNA transcription in complex with RNA polymerase [32] and to bind to the WNT promoter and upregulate its transcription [33]. Perhaps of even greater interest with respect to the extra-telomeric roles of telomerase was the finding that methyltransferase enzymes are associated with the telomerase complex in tumor cells, but not in untransformed cells, suggesting that extra-telomere functioning of telomerase may be altered in cancer to participate in gene methylation and possibly gene silencing [34]. Telomerase inhibitors are under development, and include veronistat and imetelstat [35,36]. One wonders about the double-edged sword of these agents, which could lead to telomere shortening in normal tissues. CDA2, an agent that disrupts telomerase associations with methyltransferase, may be cancer specific in its actions [34].

Therapeutic targeting of HIF in cancer

Taken together, these recent findings suggest that HIF-1 upregulates many pathways associated with the stem cell phenotype and EMT, leading us to speculate that targeting HIF may be pivotal in the reversal of drug resistance and the metastatic behavior of cancer (FIGURE 1). Screening compound libraries for agents that target HIF has revealed that some agents can directly block HIF, while others indirectly modulate its function [37]. Direct inhibitors that are clinically available include bortezomib and amphotericin B [38,39]. Indirect inhibitors in clinical use include antitopoisomerase-I agents such as topotecan and camptothecin-11, and mTOR targeting agents such as everolimus [40]. The nutritional supplement transresveratrol has also been found to accelerate HIF degradation, while simultaneously increasing the levels of the tumor suppressor protein p53 [41–44]. One of the potentially damaging effects of HIF upregulation in hypoxic cancer cells may be its direct binding to p53, blocking its tumor suppressor function [45,46]. Agents that antagonize HIF function may therefore have the added benefit of reverting the cancer stem cell to an epithelial phenotype with renewed p53 tumor suppressor function, potentially rendering the cancer far more sensitive to chemotherapy.

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Executive summary**New models of cancer biology**

- Cancer cells undergo transformation into stem cells that are drug resistant.
- Cancer stem cells remain after chemotherapy to repopulate the tumor.
- Stem cells may result from cancer cell transition from epithelial to mesenchymal programming.
- Mesenchymal cells behave like fibroblasts and exhibit high metastatic potential.

Underpinnings of epithelial to mesenchymal programming & stem cell character

- Culturing cell under low oxygen conditions potentiates the transition to a stem cell character.
- Hypoxia inducible factor (HIF)-1 α may be a central transcription factor promoting these changes.

Targeting HIF

- Drug screening programs have identified agents that block HIF function and are being explored in the clinic.

Future perspective

- New treatment approaches that capitalize on combining agents that target HIF and inhibit telomerase may lead to reversal of epithelial to mesenchymal programming and promote chemosensitivity and improved outcomes for cancer patients.

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