Review

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Epithelial plasticity in prostate cancer: principles and clinical perspectives

Rajdeep Das[1,2,3*], Philip A. Gregory[3,4*], Brett G. Hollier[5], Wayne D. Tilley[1,2,3], and Luke A. Selth[1,2,3]

[1] Dame Roma Mitchell Cancer Research Laboratories and Adelaide Prostate Cancer Research Centre, The University of Adelaide, South Australia, Australia
[2] Freemasons Foundation Centre for Men's Health, The University of Adelaide, South Australia, Australia
[3] Discipline of Medicine, The University of Adelaide, South Australia, Australia
[4] Division of Human Immunology, Centre for Cancer Biology, University of South Australia and SA Pathology, South Australia, Australia
[5] Institute of Health and Biomedical Innovation, Australian Prostate Cancer Research Centre – Queensland, Princess Alexandra Hospital, Queensland University of Technology, Queensland, Australia

Over the past decade, the capacity of cancer cells to oscillate between epithelial and mesenchymal phenotypes, termed epithelial plasticity (EP), has been demonstrated to play a critical role in metastasis. This phenomenon may be particularly important for prostate cancer (PC) progression, since recent studies have revealed interplay between EP and signaling by the androgen receptor (AR) oncoprotein. Moreover, EP appears to play a role in dictating the response to therapies for metastatic PC. This review will evaluate preclinical and clinical evidence for the relevance of EP in PC progression and consider the potential of targeting and measuring EP as a means to treat and manage lethal forms of the disease.

Prostate cancer: a major health problem

PC is the second most common solid tumor in men worldwide and a leading cause of cancer-related death [1]. More than 90% of PC-associated mortality is caused by metastasis, which occurs primarily to the bones and lymph nodes, although visceral metastases in liver and lungs, amongst other sites, are also common [2]. The mainstay treatment for men with metastatic PC is androgen deprivation therapy (ADT; see Glossary). ADT exploits the fact that normal and malignant prostate cells require androgens [i.e., testosterone and 5α-dihydrotestosterone (DHT)], which signal through the AR, for growth and survival. ADT typically involves chemical castration to markedly reduce the levels of circulating androgens; this can be combined with AR antagonists. While most men initially respond to ADT, disease progression invariably occurs after a median delay of 18–24 months [3]. Cancer that has progressed following failure of ADT is referred to as castration-resistant PC (CRPC). CRPC is treated with chemotherapy and/or new generation androgen signaling inhibitors (e.g., abiraterone acetate, which inhibits androgen biosynthesis, and enzalutamide, a potent AR antagonist), the latter reflecting the continued reliance of CRPC tumors on AR signaling. However, these treatments only provide marginal survival benefits and palliation, and patients generally die within 2 years [4]. Therefore, novel therapies for CRPC, including those that would prevent and/or inhibit PC metastasis, are urgently required.

Epithelial plasticity and metastasis

Metastasis of carcinomas (epithelial-derived cancers) encompasses a complex series of events whereby epithelial tumor cells invade the surrounding stroma, enter blood or lymphatic circulation, disseminate to distant anatomic sites, exit the vasculature, and colonize a secondary location through metastatic outgrowth. Over the past decade, epithelial–mesenchymal transition (EMT) has been demonstrated to play a critical role in certain phases of this process [5]. EMT is a normal physiological process whereby

Glossary

Androgen deprivation therapy (ADT): a treatment for metastatic or high-risk localized PC that acts by reducing the levels of circulating androgens and inhibiting the activity of the androgen receptor.
Androgen receptor (AR): a nuclear receptor that mediates the actions of androgens (i.e., testosterone and 5α-dihydrotestosterone).
Castration-resistant prostate cancer (CRPC): prostate cancer that has become resistant to ADT.
Cancer stem cell (CSC): cancer cells that possess the ability to give rise to all cell types found in a particular tumor.
Circulating tumor cell (CTC): cells from a primary tumor that have entered the vasculature and circulate in the blood.
Epithelial–mesenchymal transition (EMT): a process whereby epithelial cells convert to mesenchymal cells, characterized by loss of cell–cell adhesion and polarity and gain of migratory and invasive capacity.
Epithelial plasticity (EP): a term used to describe reversible transitions between epithelial and mesenchymal states.
Mesenchymal–epithelial transition (MET): a process whereby mesenchymal cells convert to epithelial cells, characterized by loss of migratory and invasive capacity and gain of cell–cell adhesion and polarity.
Stem-ness: a term used to describe characteristics of stem cells that differentiate them from non-stem cells. Such characteristics include, but are not limited to, pluripotency and capacity for self-renewal.

Corresponding author: Selth, L.A. (luke.selth@adelaide.edu.au).
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*These authors contributed equally to this work.

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sessile epithelial cells lose adhesion and detach from tight junctions, change shape and polarity, and become more migratory and invasive, and it plays a fundamental role during wound healing and morphogenesis. At a molecular level, EMT is associated with loss of epithelial factors, such as E-cadherin, epithelial cell adhesion molecule (EpCAM), zona occludens protein-1 (ZO-1), some cytokeratins (CKs) and miR-200 family members, and gain of mesenchymal factors, such as vimentin, fibronectin, and N-cadherin. Cancer cells hijack EMT to facilitate escape from the primary tumor, migration and invasion into the stroma, and entry/exit from the bloodstream [6]. In tumors, EMT is thought to be primarily triggered by soluble factors secreted from the surrounding stroma and other infiltrating immune cells that impinge on tumor cell signaling pathways, including transforming growth factor-β (TGF-β), Wnt/β-catenin, fibroblast growth factor (FGF), epidermal growth factor (EGF), and Notch, which in turn converge on central transcriptional mediators of the EMT program, for example, members of the Snail, Twist, and zinc finger E-box-binding homeobox (ZEB) families. These EMT transcription factors (EMT-TFs) orchestrate the aforementioned molecular alterations and subsequent underlying changes in cell state [6]. Tumor cells that have undergone an EMT often exhibit other properties that facilitate metastasis and survival within circulation, including suppression of senescence, apoptosis, and anoikis [6].

The role of EMT in cancer metastasis had previously been called into question by the observation that many metastases possess features of the primary tumor, including the expression of epithelial markers (e.g., E-cadherin) [7]. This was clearly inconsistent with the expectation that changes in gene expression associated with EMT would be enriched in metastases. However, this expectation relied on the hypothesis that EMT produced a permanent phenotypic change, whereas it is now clear that cells are capable of reverting back to epithelial phenotypes. This so-called mesenchymal–epithelial transition (MET) would be expected to facilitate the growth of disseminated tumor cells (DTCs) or micrometastases into clinically-relevant metastases, since re-differentiation to an epithelial state is associated with restoration of proliferative capacity [8]. There is now compelling evidence supporting a critical role for MET in metastatic colonization of secondary sites [9–14].

In this review, the term EP will be used to describe reversible transitions between epithelial and mesenchymal states (i.e., EMT and MET). Such plasticity is linked to the frequent appearance of stem cell-like properties during cancer progression [15–18], a concept that will be addressed further later in the review.

Epithelial plasticity and prostate cancer progression

Visualizing EP during cancer progression is inherently difficult because of its transient nature, heterogeneity in tumor cell populations, the scarcity of metastatic tissue cohorts, and the lack of robust EP biomarkers. Nevertheless, the importance of EP during PC progression is now well accepted. Important findings that have led to this realization include, among others: evidence for EMT in circulating tumor cells (CTCs) [19–22]; altered expression of epithelial/mesenchymal markers and EMT-TFs in primary tumors compared to normal tissues [23], including specific changes at the invasive front [24]; and altered expression of epithelial/mesenchymal markers and EMT-TFs in response to ADT and chemotherapy ([25–27], and see below). As is evident from these examples, the preponderance of work in this field has substantiated the role of EMT during disease progression (for excellent recent reviews, see [28,29]). In this section of the review, the focus will be on more recent evidence implicating the reverse process, MET, in PC metastasis and the generation of tumor initiating cells.

Cell line models have afforded extensive and diverse evidence for MET during PC progression. An early study analyzing mesenchymal AT3 cell xenografts in Copenhagen rats found that tumors and lung metastases gained expression of E-cadherin and ZO-1 expression and lost expression of vimentin [30]. Interestingly, in both the primary tumors and the metastases, the cells that had undergone an apparent MET clustered in proximity to stromal components, highlighting potential interplay with microenvironmental cues. Further supporting this concept, DU145 cells co-cultured with hepatocytes exhibited upregulation of E-cadherin and increased chemoresistance [31], while $ARCaP_M$ mesenchymal PC cells gained E-cadherin and lost N-cadherin when grown in the presence of bone marrow stromal cells [32]. The most direct evidence for MET influencing metastasis was provided by a recent study in which repeated rounds of lymph node metastatic selection from mice bearing orthotopic DU145 tumors resulted in cells that had gained epithelial and lost mesenchymal features [9]. This elegant *in vivo* cycling strategy not only provided evidence for spontaneous MET during colonization of lymph nodes but also facilitated the identification of a novel MET-suppressing miRNA, miR-424.

Support for MET in PC progression is further yielded by clinical studies of CTCs and circulating biomarkers. CTCs from 10 patients with metastatic CRPC were found to coexpress vimentin and epithelial CKs in all cases, while other combinations of mixed epithelial/mesenchymal marker expression were also evident [19]. Interestingly, this same study examined bone metastasis biopsies from two of the patients and observed loss of vimentin expression in the CK-positive tumor foci, providing evidence for an MET during metastatic colonization. Studies aimed at assessing the biomarker potential of circulating miRNAs have revealed that serum levels of epithelial miRNAs, including miR-194, miR-200 family, and miR-375, are associated with metastatic PC and/or predict disease progression following surgery for localized disease [33–36]. Interestingly, circulating levels of many of the same miRNAs are prognostic and correlated with CTCs in breast cancer [37]. The frequent elevation of epithelial miRNAs in circulation may reflect upregulation of these factors, and consequent MET, in CTCs/DTCs to enable efficient metastatic colonization. Supporting this concept, miR-194 is expressed at higher levels in metastases compared to primary tumors [34] and is upregulated following lymph node colonization and MET of DU145 cells [9].

The emerging link between epithelial identity and stemness further supports the role of MET in PC metastasis.