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Tumor microenvironment heterogeneity: challenges and opportunities

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Abstract

The tumor microenvironment (TME) has been recognized as an integral component of malignancies in breast and prostate tissues, contributing in confounding ways to tumor progression, metastasis, therapy resistance and disease recurrence. Major components of the TME are immune cells, fibroblasts, pericytes, endothelial cells, mesenchymal stroma/stem cells (MSCs), and extracellular matrix (ECM) components. Herein, we discuss the molecular and cellular heterogeneity within the TME and how this presents unique challenges and opportunities for treating breast and prostate cancers.

Keywords

Mesenchymal Stem Cells (MSCs); Tumor Associated Macrophages (TAMs); Cancer Associated Fibroblasts; Tumor Microenvironment (TME); EMT; Metastasis; Extracellular matrix (ECM); Cancer Progression/Recurrence; Therapy Resistances; Signaling Pathways

I. INTRODUCTION

Breast and prostate cancers are leading causes of cancer-related death in women and men, respectively, world-wide. In fact, prostate cancer is the second most common cancer among men after non-melanoma skin cancer in the United States (<https://www.cdc.gov/cancer/>). While mortality in these reproductive malignancies has been significantly reduced over the past several decades by applying early diagnostic biomarker discovery and targeted therapeutic approaches, therapy resistance, metastasis and/or tumor relapse remain critical hurdles preventing successful treatment of all breast and prostate cancer patients.

Hanahan and Weinberg defined the hallmarks of cancer as a multistep process that includes biological functions such as sustaining proliferative signaling, evading growth suppression, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion/metastasis, acquiring therapy resistance, and escaping immune system control

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Conflict of Interest

F. Runa, S. Hamalian, K. Meade, P. Shisgal, P.C. Gray, and J.A. Kelber each declare no potential conflicts of interest.

Human and Animal Rights and Informed Consent

This article contains no studies with human or animal subjects performed by any of the authors.

(Hanahan and Weinberg). Solid tumors of the breast and prostate usually contain heterogeneous populations of neoplastic cells and collections of tissue-specific resident and recruited stromal cell types, which together form the tumor microenvironment (TME) (Barron and Rowley, 2012; Hanahan and Coussens, 2012; Kalluri, 2016; McAllister and Weinberg, 2014; Shiao et al., 2016; Siegel et al., 2016). The normal breast ductal epithelium consists of a luminal epithelial cell layer surrounded by myoepithelial cells attached to the basement membrane. Several studies demonstrated that components of the TME play critical roles in mammary duct morphogenesis as well (Kalluri, 2016; Place et al., 2011; Soysal et al., 2015). The cellular architecture of the prostate is quite similar to that of the breast in that it also consists of ducts with epithelial luminal and basal layers. As in the breast, the structure of these prostate ductal epithelial layers as they become neoplastic is strongly influenced by the TME (Barron and Rowley, 2012; Chung et al., 2005; Ganguly et al., 2014; Santoni et al., 2015; Shiao et al., 2016). In cancer of both the breast and prostate, components of TME vary greatly between and within each patient contributing to great disease diversity and difficulty in prescribing the optimal treatment plans for each patient.

In this regard, it is well established that bidirectional communication exists between cells and their microenvironment during both normal tissue homeostasis and tumor growth/metastasis. Importantly, the interactions between tumor cells and the associated stromal cells represent an opportune relationship that influences not only disease initiation and progression but also patient prognosis. Historically, cancer prognosis was thought to primarily depend upon the acquisition of aberrant mutations in tumor cells causing them to be governed by genetic and epigenetic alterations of neoplastic cells during the development/progression of a tumor. More recently, however, the TME has emerged as a significant and an equally important determinant of tumor behavior and pathogenesis. It is speculated that the TME also continues to change and evolve as a given tumor evolves in response to systemic changes (e.g., therapy-induced stresses). Further research into the specifics outlined here, in the setting of both breast and prostate cancers, is certain to reveal the dynamic and highly adaptable TME, helping scientists and clinicians to better diagnose and treat these malignancies to improve patient outcomes.

II. TUMOR CELL INTERACTIONS WITH THE EXTRACELLULAR MATRIX

Composition

The extracellular matrix (ECM) is a complex mixture of structural proteins, glycoproteins, and proteoglycans that provide essential physical scaffolds to maintain tissue structure (Xiong and Xu, 2016). The ECM regulates tissue development and homeostasis whereas, dysregulation of ECM functions promote neoplastic progression. Common matrix proteins embedded in breast and prostate cancers include fibrillar collagens, fibronectin, specific laminins and proteoglycans (Insua-Rodríguez and Oskarsson, 2016). Furthermore, the ECM represents an abundant source of soluble factors such as growth factors, angiogenic factors, cytokines and chemokines, proangiogenic platelets and bone marrow-derived cells (BMDCs) (Kuznetsov et al., 2012). Thus, the ECM provides a dynamic and complex network for cancer development, progression and metastasis mediated by extensive reorganization of

ECM and increasing matrix stiffness (Acerbi et al., 2015; Pickup et al., 2014; Wei et al., 2015; Xiong and Xu, 2016).

Several studies (Barcus et al., 2017; Beck et al., 2013; Iijima et al., 2011; Nguyen-Ngoc et al., 2012; Pickup et al., 2014; Wolf and Friedl) suggest that many ECM proteins play a major functional role in breast and prostate cancer progression and metastasis. Further, a number of the induced ECM proteins take part as essential components of metastatic niches and promote stem/progenitor signaling pathways and survival of cells within the metastatic niche. ECM remodeling enzymes also lead to changes in the matrix structure and matrix biomechanical properties (Insua-Rodríguez and Oskarsson, 2016). Collagens are one of the most abundant fibrous proteins in the extracellular matrix. During cancer invasion and metastasis, collagen I fibers are deposited and make tight cross-linked structures with other structural matrix proteins such as elastins, laminins or fibronectin. Crosslinking of collagen I with ECM modifying enzymes create more rigid phenotype of the whole tumor.

In breast tumors, the presence of dense clusters of collagen fibrils indicates increased matrix stiffness, which correlates with poor patient survival. It was found that TWIST1 expression drives essential mechano-mediated changes in the TME that promote epithelial-mesenchymal transition (EMT) in response to increasing matrix stiffness (Wei et al., 2015). High matrix stiffness induces nuclear translocation of TWIST1 and releases TWIST1 from its cytoplasmic binding partner G3BP2 (Wei et al., 2015). The study suggests that TWIST1-G3BP2, a mechanotransduction pathway, actually responds to biomechanical signals from the tumor microenvironment to drive EMT, invasion and metastasis. Another study has reported (Iijima et al., 2011) that stiffness of the ECM potently modulates the repertoire of prolactin signals in human ER α -positive breast cancer cells. In the context of increased tumor invasion, stiff matrices can activate cross-talk signals between Janus Kinase (JAK) 2/STAT5 and pro-tumorigenic extracellular regulated kinase (ERK1/2). Several studies have also shown that extracellular matrix stiffness regulates prostate cancer cell behavior including tumor cell migration and aggressiveness (Baker et al., 2009; Sieh et al., 2012; Zaman et al., 2006)

Physical properties

The ECM extracellular matrix has been shown to confer not only biochemical but also biophysical cues including matrix rigidity to the TME that significantly alters cellular behavior such as cell proliferation, stem cell properties, and metastatic growth (Spill et al., 2016). A recent study has characterized the independent roles of mechanical, structural and adhesion characteristics of 3D hydrogels and found that human breast cancer invasion and aggressive behavior correlate with ECM stiffening and immune cell infiltration. The study undertook a biophysical and biochemical assessment of stromal-epithelial interactions in noninvasive, invasive and normal adjacent human breast tissue and in breast cancers of enhanced aggressive subtype. The data revealed that excessive collagen deposition and progressive linearization/thickening of collagen fibers correlated with human breast cell transformation. When stiffness of the stroma and cellular mechano-signaling were the highest, the linearization of collagen and tissue birefringence were also. Further, the greatest number of infiltrating macrophages and the highest level of TGF- β signaling were also

observed within the cells at the invasive front (Beck et al., 2013). Additionally, it was found that stiffened substrates aberrantly engage cellular mechanosignaling networks in cells, which promotes the assembly of invadosomes and lamellae for cell invasion and migration (Kai et al., 2016). Finally, the impact of mechanotransduction and YAP-dependent matrix remodeling is required for the generation and maintenance of cancer-associated fibroblasts (CAFs) (Calvo et al., 2013).

Remodeling

Several studies have been done to investigate the ECM remodeling within the TME on cancer progression. A study showed that Pleiotrophin (PTN, Ptn), an 18-kDa secretory cytokine, could induce remodeling of the ECM through stimulation of stromal cells and is significantly associated with features of cancer progression (Chang et al., 2007). It was observed that in a co-culture model of equal numbers of NIH 3T3 stromal fibroblasts and MCF-7-*Ptn* cells, PTN secreted from MCF-7-*Ptn* cells could induce a more malignant MCF-7-*Ptn* breast cancer cell phenotype via extensive remodeling of the MCF-7-*Ptn*/NIH 3T3 cell microenvironment. Further, PTN secretion could up-regulate the expression of markers of aggressive breast cancers, including PKC δ and matrix metalloproteinase-9 (MMP-9) in both MCF-7-*Ptn* and NIH 3T3 cells. A growing body of evidence also indicates that interactions between neoplastic cells and tumor-associated macrophages (TAMs) in the TME are crucial in promoting tumor cell invasion and progression through remodeling (Luo et al., 2009). In this study, it was shown that interactions between mouse breast tumor cells and TAMs remodel ECM, leading to the up-regulation of proto-oncogene Fra-1, a member of the FOS family of transcription factors. In turn, Fra-1 initiates activation of the IL-6/JAK/Stat3 signaling pathway, inducing a malignant switch in breast tumor cells and increasing the release of proangiogenic factors MMP-9, vascular endothelial growth factor (VEGF) and transforming growth factor-beta (TGF- β). Another study showed that MMP-9 has different effects on breast cancer progression depending on whether insulin-like growth factor binding proteins (IGFBPs) are expressed (Kong et al., 1995).

Mesenchymal stem cells (MSCs) may also play a critical role in ECM remodeling. It was found that co-culture of MSCs with breast cancer cells causes up regulation of lysyl oxidase (LOX), a collagen crosslinker that promotes breast cancer progression. During invasion/metastasis, cancer cells must navigate and remodel dense ECM for migration through the 3D TME. Two major types of migration (i.e., amoeboid and mesenchymal) are utilized by individual cancer cells. Amoeboid migration is characterized by rounded cells that circumnavigate ECM without the use of adhesion proteins or matrix degradation. On the other hand, through mesenchymal migration, cells elongate, establish integrin-mediated adhesion to the ECM and degrade ECM with MMPs (McAndrews et al., 2015).

Signaling

Matrix stiffness induces integrin clustering to enhance growth factor-dependent ERK activation. Integrin clustering can enhance PI3K signaling to regulate invasion of a premalignant mammary epithelium *in vitro* and tumor progression. Cytochrome P450 (CYP) 4Z1, a novel CYP4 family member, is over-expressed in human mammary carcinoma and associated with high-grade tumors and poor prognosis. It was found that CYP4Z1

overexpression promotes tumor angiogenesis and growth in breast cancer. Increased CYP4Z1 expression promotes tumor angiogenesis and growth in breast cancer, partly via PI3K/Akt and ERK1/2 activation (Yu et al., 2012). ECM stiffness enhances cancer invasion and aggressive tumor cell behavior through TGF- β signaling and immune cell infiltration (Acerbi et al., 2015; Chaffer et al.). ECM signaling with associated immune cells also influences the cancer stem cell niche. It was demonstrated that TAMs could create a CSC-niche via juxtacrine signaling with cancer stem cells (CSCs) (Lu et al., 2014b).

Adhesion

Cancer cell invasion and dissemination are regulated by the matrix adhesion molecules. It was found TME signals can induce dissemination of normal and malignant epithelial cells without requiring the fibrillar structure of collagen I or containing collagen I-specific adhesion sequences (Beck et al., 2013). Further, metastatic tumors preferentially disseminate in specific ECM microenvironments in which breaks in the basement membrane can induce invasion and dissemination through the direct contact between cancer cells and collagen I (Nguyen-Ngoc et al., 2012). Further, alterations in the mechanical environment of the surrounding ECM induce the tumor cells to regulate secretion, which in turn, may influence cell adhesion/dissemination. A recent study reports that extracellular matrix rigidity-dependent Sphingosine-1-Phosphate (SIP) secretion regulates metastatic cancer cell invasion and adhesion in breast cancer (Ko et al., 2016).

Carcinomas commonly invade as a cohesive multicellular unit, usually known as a process of collective invasion (Friedl and Alexander, 2011; Gritsenko et al., 2012). Collective invasion of breast cancer requires a conserved basal and epithelial program and heterotypic interactions between epithelial subpopulations (Cheung et al., 2013). A study was conducted using three-dimensional (3D) organoid assays to identify the most invasive cancer cells in primary breast tumors. It was found that collective invasion was led by specialized cancer cells, which prominently expressed basal epithelial genes, such as cytokeratin-14 (K14) and p63. Further it was found that K14+ cells led collective invasion in the major human breast cancer subtypes. Importantly, luminal cancer cells were observed to convert phenotypically to invasive after induction of basal epithelial genes. On the other hand, during collective invasion, ductal carcinoma cells physically connect with expressed cadherins (Ecadherin and P-cadherin), tight (JAM-A, claudin-3, and -4), and immunoglobulin-based junctions (ALCAM and L1-CAM). But most of these cell-cell junction proteins are down regulated in invading carcinoma cells, with the loss of E-cadherin. Most invasive carcinomas invade collectively as thin multicellular sheets or strands (in single-file patterns) which are parallel to collagen bundles. Although candidate adhesion mechanisms for multicellular cohesion within single file E-cadherin structures include N-cadherin and other cadherins, their mechanical and signaling contribution still need to be investigated (Shree et al., 2011).

III. TUMOR CELL INTERACTIONS WITH THE MESENCHYME

Fibroblasts

Fibroblasts are derived from primitive mesenchyme with elongated, spindle-like morphology and responsible for structural integrity of most connective tissues. Fibroblasts are

predominantly multifunctional cell types involved in the synthesis and degradation of ECM and basement membrane components, epithelial homeostasis, proliferation, differentiation, and modulation of immune responses (Bertos and Park, 2011; Calvo and Sahai, 2011; Marsh et al., 2013; O'Connell et al., 2011; Quail and Joyce, 2013; Tan et al., 2011). Several studies suggest that normal fibroblasts can promote tumor growth, progression and metastasis in breast and prostate cancers (O'Connell et al., 2011; Sharon et al., 2013; Tan et al., 2011). It was reported that tumor-derived osteopontin reprograms normal mammary fibroblasts through paracrine signaling to promote inflammation and breast tumor growth. Further, fibroblasts recruited, tumor-infiltrating regulatory T cells stimulate tumor metastasis through RANKL-RANK signaling. Therefore, fibroblasts are disrupted to promote tumorigenesis and those tumor supportive fibroblasts are defined as CAFs. CAFs are activated sub-populations of stromal fibroblasts, many of which express the myofibroblast marker alpha-smooth muscle actin (α -SMA). As such it has been reported that isolation of normal and CAFs from fresh tissues (mouse and human) by Fluorescence Activated Cell Sorting (FACS) revealed that PDGFR α is abundantly expressed as surface markers by both normal fibroblasts and CAFs (Sharon et al., 2013). Therefore, in the TME, CAFs have been an on-going challenge due to the lack of CAF-specific markers and the vast heterogeneity of these cells. Accumulating evidence support that CAFs facilitate tumor initiation, growth and progression (Calvo and Sahai, 2011; Castaño et al., 2012; Quail and Joyce, 2013) and tumor invasion and metastasis by mediating tumor-enhancing inflammation (Erez et al., 2010). The data suggests that CAFs strongly promote the development of an aggressive cancer cell phenotype (Donnarumma et al., 2017). It was found by differential expression profile analysis that three types of microRNAs (miRs -21, -378e, and -143) increased in exosomes from breast CAFs as compared from normal fibroblasts. In this study, cancer cells (BT549, MDA-MB-231, and T47D lines) exposed to CAF exosomes or transfected with those miRs showed a significant increase in tumorspheres, stem cell and epithelial-mesenchymal transition (EMT) markers, and anchorage-independent cell growth. Current research has focused to understand how CAFs originate and maintain their phenotype in the TME. Studies show that MSCs and matrix remodeling influence the origin and help to maintain the CAF phenotype in the TME of breast cancer (Calvo et al., 2013; Erez et al., 2010; McAndrews et al., 2015). Fibroblasts were isolated from different stages of breast cancer and were analyzed for molecular and biological functions. These analyses revealed that activation of the YAP transcription factor is a signature feature of CAFs. Remodeling of the ECM and promotion of cancer cell invasion requires the actomyosin cytoskeleton. YAP regulates the expression of several cytoskeletal regulators; matrix stiffening further enhances YAP activation, thus establishing a feed-forward self-reinforcing loop that helps to maintain the CAF phenotype (Calvo et al., 2013).

The TME of breast and prostate cancer is regulated by subpopulations of cells including fibroblasts, CAFs, stromal fibroblasts, CSCs, MSCs, immune cells through networks of cytokines and growth factors. Accumulating evidence suggests that both stromal fibroblasts and CSCs mediate tumor growth and metastasis (Benyahia et al., 2017; Elkabets et al., 2011; Ghesquiere et al., 2014; Mao et al., 2013; Tripathi et al., 2012). Since these components have a direct influence in the TME of these reproductive cancers, they represent attractive targets for therapeutic development.

Mesenchymal Stem Cells (MSCs)

An important component of breast TME are mesenchymal stem cells or multipotent mesenchymal stromal cells (MSCs), usually found in the bone marrow and in adipose tissue. They represent the adult stem cells that are able to differentiate into osteogenic, adipogenic, myogenic and chondrogenic lineages (Pittenger et al., 1999). Further, MSCs express stromal cell markers reported as CD73, CD105, and CD90 in the absence of hematopoietic and endothelial cell markers (CD34, CD31, CD45, and CD14) (Dominici et al., 2006). Normally, MSCs are recruited to injured areas or hypoxic tumor microenvironments and this homing of MSCs to tumors is reported as the earliest phenomena of MSC-cancer interactions. Contributions of MSCs within the tumor microenvironment allow tumor cells to exhibit altered biological functions. Accumulating evidence has supported that MSCs play critical roles in tumor development and progression, by increasing stemness of tumor cells, stimulating tumor cell migration, promoting angiogenesis, supporting immune responses, and inducing drug resistance. Additionally, MSCs increase the metastatic potential of tumor cells by advancing their motility and invasiveness and thereby play a role in the nature of a metastatic niche at the secondary site (Bott et al., 2017; Donnarumma et al., 2017; Gonzalez et al., 2017; Luo et al., 2009; McAndrews et al., 2015; Wolfe et al., 2016). Therefore, a comprehensive knowledge on the biology and mechanism of interaction between MSCs and TME of breast and prostate cancer is essential for the development of therapeutics that capitalize on vulnerabilities in these tumor cell-MSC interplay.

A recent study shows that MSCs in the TME has an impact on breast cancer progression by creating a pro-inflammatory milieu within the tumor (Bott et al., 2017). This study identified six putative oncomiRs, strongly correlating with poor overall patient survival. Out of the six candidates, miR-1246 was upregulated in aggressive breast cancer subtypes and expressed at highest levels in MSCs. Functionally, miR-1246 induced the release of pro-inflammatory mediators IL-6, CCL2 and CCL5 in MSCs, and increased NF- κ B activity. Further, incubation with conditioned medium (CM) of MSC overexpressing miR-1246, increased Stat3 phosphorylation in breast epithelial (MCF10A) and cancer cells (SK-BR-3, MCF7, T47D). In human breast cancer cells, metastasis is promoted by mesenchymal stem/multipotent stromal cells (MSC) with increased collagen deposition. It has been reported that the collagen receptor discoidin domain receptor 2 (DDR2) is concordantly up-regulated in metastatic breast cancer and MSCs and also maintains a fibroblastic phenotype by activation of DDR2 signaling in breast cancer cells (Gonzalez et al., 2017).

MSCs also support CSCs through various kinds of interactions and cell fusion to form hybrid tumor cells (Melzer et al., 2017). Since plasticity is a fundamental feature of MSCs, MSCs can also inhibit tumors by activating various MSC-based signaling pathways. Understanding the mechanism of MSC interacted with tumors, TME and inflammatory TME will reveal new targets for cancer therapy.

Signaling

Interactions between tumor cells and mesenchymal cells involves a number of MSC secreted signaling molecules that stimulate various signaling pathways leading to cell growth and regulation of apoptosis in the tumor cells. Cross talk between tumor-MSC in the TME is

complex in the case of both normal development and tumor pathogenesis. This change in the cell phenotype is induced by contextual signals that epithelial cells receive from their microenvironment. Signals from tumor cells can stimulate MSCs as well as MSCs secreted factors involved in tumor initiation and early tumor growth. A study shows that paracrine and autocrine signals induce and maintain mesenchymal and stem cell states in the breast (Scheel et al., 2011). Spontaneously arising mesenchymal subpopulations (MSP) of cells were isolated from immortalized human mammary epithelial cells (HMECs) and were analyzed to understand how signals coordinate in the breast TME. It was revealed that a set of extracellular signals operated in a paracrine manner to induce entrance of HMLE cells into the mesenchyme/SC state and subsequently function as autocrine factors. A recent study, however, suggests that paracrine NF- κ B activation promotes the initiation of Notch in basal-type breast cancer cells. Several studies identified MSCs as signal-sending cells of Notch signaling whereas breast cancer cells were able to receive the signals. Blocking the Notch signal during co-culture could reduce the expression of MSC marker CD90 and thus suggest a functional role of this pathway in interaction of MSC-tumor cells (Zhang and Grivennikov, 2013).

Increased collagen deposition by breast cancer (BC)-associated MSCs promotes metastasis, but the exact mechanisms still need to be investigated. A recent study has reported that the collagen receptor Discoidin Domain Receptor 2 (DDR2) is essential for stromal-breast cancer communication. In MSCs isolated from human BC metastasis, DDR2 induces activation of DDR2 signaling and maintains a fibroblastic phenotype with collagen deposition. Loss of DDR2 in MSCs inhibits DDR2 phosphorylation in BC cells, alignment, migration, and metastasis (Gonzalez et al., 2017).

MSCs support CSC development and enhance the proportions of CSC in tumors and promote EMT by activation of various signaling pathways. Further, EMT confers on cancer cells mesenchymal traits and an ability to enter the CSC state. Recent studies show that breast tumor progression and EMT are activated by ERK/GSK-3 β /Snail (Li et al., 2016) and both NF- κ B and β -catenin signaling (Luo et al., 2016) mediated by MCP-1 and VEGF/NPR respectively. In this context, it may speculate that ERK/GSK-3 β /Snail and NF- κ B and β -catenin signaling may induce MSCs to secrete tumor initiating growth factors that stimulates sphere formation or growth of CSC-like cell. Although the interactions between MSCs, CSCs and their surrounding microenvironments are poorly understood, it has been shown by quantitative proteomic profiling that tumor-associated monocytes and macrophages (TAMs) create a CSC-niche via juxtacrine signaling with CSCs (Lu et al., 2014a). The EMT program up-regulates the expression of CD90/Thy1 and EphA4 receptors, which allow the physical interactions of CSCs with TAMs by directly binding with their respective counter-receptors on these cells. Further, the EphA4 receptor on the carcinoma cells activates Src and NF- κ B in which the signaling of NF- κ B induces the secretion of a variety of cytokines. Cytokines from CSCs and MSCs may help to maintain the stem-cell state.

Exosomes

Exosomes are small vesicles formed in vesicular bodies with a diameter of 30–100 nm with a distinctive “cup” or “dish” morphology that play an important role in the TME of breast

cancer. Exosomes in the TME are considered as potential transporters and biomarkers since they can contain microRNAs, mRNAs, DNA fragments and proteins, and transfer from a donor cell to recipient cells [95]. Exosome derived tumor cells are called Tumor-Derived (TD) exosomes and accumulating evidence suggest that TD exosomes can construct a suitable TME to support tumor proliferation, angiogenesis, invasion and metastasis (Bobrie et al., 2012; Cho et al., 2012; King et al., 2012; Luga et al., 2012) through exosome mediated signaling. Breast cancer-derived exosomes can directly suppress T-cell proliferation and inhibit NK cell cytotoxicity, and thereby suppress the anticancer immune response in premetastatic organs (Wen et al., 2016). Studies show that the small GTPases RAB27A and RAB27B regulate exocytosis of multivesicular endosomes, which lead to exosome secretion, in human HeLa cells. Further, Rab27a maintains both exosome dependent and independent mechanisms, modifies TME and promotes tumor progression (Bobrie et al., 2012). A recent study examined the influence of breast cancer derived exosomes on MSCs by treating adipose tissue-derived MSCs (ADSCs) with TD exosomes. The exosome-treated ADSCs exhibited the phenotypes of tumor-associated myofibroblasts with enhanced expression of α -SMA, tumor-promoting factors SDF-1, VEGF, CCL5 and TGF β . This study suggests that tumor-derived exosomes can promote tumor progression and metastasis by switching MSCs within tumor stroma into tumor-associated myofibroblasts. Another study showed that fibroblast-secreted exosomes mobilize autocrine Wnt-PCP signaling to drive breast cancer cell invasive behavior (Luga et al., 2012). Exosomes can deliver invasion-potentiating microRNA (miRNA) and thereby decrease sensitivity of breast cancer cells to chemotherapeutic drugs (Mao et al., 2016; Yang et al., 2011). A study has shown that the enrichment of a specific cargo secreted from breast cancer derived exosomes can promote cell survival and thereby potentially provide a marker of chemotherapy resistance (Kreger et al., 2016). Exosomes can be mutually exchanged by tumor cells and adjacent cell populations in the tumor microenvironment (Kahlert and Kalluri, 2013). Exosomes levels in the serum of breast cancer patients are higher when compared to normal subjects. Further, breast cancer cells secrete exosomes with specific capacity for cell-independent miRNA biogenesis, which is absent in normal cell derived exosomes (Melo et al., 2014). Several studies show that some miRNAs such as miR-222/223 from MSC-released exosomes promote dormancy/quiescence and drug resistance in a subset of breast cancer (Bliss et al., 2016; Ono et al., 2014; Santos et al., 2016). It is reported that internalization of MSC-derived exosomes by breast and ovarian cancer cells provide tumor cells with new properties and functions through the expression of MMP2 and ecto-5'-nucleotidase (CD73, a MSC surface marker) that are potentially reorganized in the TME (Melzer et al., 2017). On the other hand, exosomes derived from tumor cells are able to transport tumor factors and appear to be involved in tumor invasion, angiogenesis, chemoresistance, immune evasion, and circumventing cell death (Kahlert and Kalluri, 2013). By proteomic profiling analysis it has been identified that Annexin II is one of the most highly expressed proteins in exosomes that causes macrophage-mediated activation of the p38MAPK, NF- κ B, and STAT3 pathways and increased secretion of IL6 and TNF α [128]. TD-exosomes can provide autocrine, paracrine, endocrine, and other signals that promote cancer growth and maintain its invasion and metastasis (Yu et al., 2015). In this context, since Annexin II plays an important role in angiogenesis and breast cancer metastasis, it can

be exploited as a potential biomarker as well as a therapeutic target for diagnosis and treatment of metastatic breast cancer.

IV. METASTATIC NICHES

Local and Systemic Dissemination

Dissemination of tumor cells is an essential step in metastasis. The TME is an important supporter of tumorigenesis in all forms of breast and prostate cancers and has been associated with the risk of metastasis and disease relapse. Resident CAFs and MSCs modulate early dissemination, tumorigenesis, and metastasis through alteration of ECM characteristics (Dumont et al., 2013). Further, several studies support the notion that tumor cell dissemination is predominantly affected by endothelial adhesion and related to functional changes of adhesion receptors. Therefore, the contribution of tumor-secreted factors to tumor-adhesion represents a therapeutic opportunity for preventing cancer metastasis. A recent study shows a network of paracrine signals between carcinoma, myeloid, and endothelial cells that drives both metastasis and chemoresistance in breast cancer (Acharyya et al., 2012). In this study, cancer cells that overexpress CXCL1 and 2 are attracted by CD11b+ Gr1+ myeloid cells into the tumor and enhance cancer cells survival by producing chemokines (S100A8/9). TNF is produced by endothelial or other stromal cells via NF- κ B which amplifies the CXCL1/2 expression, thus strengthening the CXCL1/2-S100A8/9 loop, which causes chemoresistance in the TME. Another study demonstrated a novel mechanism to control tumor-cell dissemination. NEDD9 is a recognized marker of invasive and metastatic cancers that is required for matrix metalloproteinase 14 (MMP14). MMP14 secretion recycles through late endosomes and allows the release of tissue inhibitors of matrix metalloproteinase 2 (TIMP2) for tumor invasion. This finding illustrates a novel mechanism to control tumor-cell dissemination through NEDD9/Arf6-dependent regulation of MMP14/TIMP2 trafficking (Loskutov et al., 2015). Several studies support that other factors such as BRD4, IL-30, serpin -E2, VEGF-A and Tenascin-C regulates breast cancer dissemination and metastatic colonization (Airoldi et al., 2016; Andrieu et al., 2016; O'Connell et al., 2011; Smirnova et al., 2016) in the TME. Using real time imaging it was observed that for tumor cell dissemination, vascular permeability is transient, restricted only to TME of metastasis (Harney et al., 2015). VEGF-A signaling from Tie2Hi TMEM macrophages causes local loss of vascular junctions, transient vascular permeability and tumor cell intravasation, which explains a role of TME within the primary mammary tumor.

Composition

Metastasis is the systemic dissemination of tumor cells that profoundly depends on its microenvironment or “niche” interactions with resident components. It is a multistep process whether primary tumors detach and invade into the blood vessels or lymphatic system and finally develop as secondary tumors (Rahman and Mohammed, 2015). Emerging research is illuminating the underlying cellular and molecular events controlling the metastatic cascade from onset to colonization. Now it is defined that a reciprocal interplay between tumor cells, stromal cells and the ECM mediate metastasis in epithelial cancers (Khamis et al., 2012). The essential components of metastatic niches include ECM proteins, secreted enzymes, growth factors, cytokines and other molecules that carry information to cancer cells. The

sources of this molecular milieu are multiple cell types including cancer cells themselves, which are local or recruited to the site of metastasis (Descot and Oskarsson, 2013). Further, the stromal compartment contains various bone-marrow-derived cells (BMDCs) such as macrophages, mast cells, neutrophils, and lymphocytes recruited by the primary tumor cells to increase tumor cell migration, angiogenesis, and invasion. Observation of immune infiltrates at sites of focal disruptions of the myoepithelial cell layer in breast tissues suggests a potential role of these cells in tumor malignancy and metastatic spread (Khamis et al., 2012; Kuznetsov et al., 2012). Collective dissemination is a frequent mechanism for metastatic spread and whether the most invasive, keratin 14+ (K14+) expression in breast cancer cells regulate cell–cell adhesion, cell–matrix adhesion, and immune evasion (Cheung et al., 2013; Cheung et al., 2016). Understanding the molecular basis of collective dissemination will provide novel prognostics and therapies to improve patient outcomes.

Research has demonstrated that metastatic niches are shaped by the interactions of stroma and tumor and is mediated by monocarboxylate metabolism (Patel et al., 2017). This study suggests that stromal carcinoma-associated fibroblasts (CAFs), generated from bone marrow-derived MSCs, may (i) recycle tumor-derived lactate for their own energetic requirements and thus sparing glucose for neighboring glycolytic tumor cells, and also (ii) discharge extra valuable metabolites of lactate oxidation, such as pyruvate for supporting tumor growth. Breast cancer metastasizes to the lung, bone, liver, and brain whether an emerging paradigm suggests that primary tumor cells may secrete factors capable of inducing a productive microenvironment, termed the “pre-metastatic niche.” Further, pre-metastatic niches favor the seeding and proliferation of metastatic cells at unique sites. A study has shown that breast cancer-secreted miR-122 reprograms glucose metabolism in the pre-metastatic niche to promote metastasis. High miR-122 levels in the circulation have been associated with metastasis in breast cancer patients. This study demonstrated that by modifying glucose utilization by recipient pre-metastatic niche cells, cancer-derived extracellular miR-122 can reprogram energy metabolism and facilitate disease progression (Fong et al., 2015). Another study has revealed the role of TAMs within stroma of the pre-metastatic niche. It was found that human breast cancer specimens exhibited significant association between mRNA expression levels of MIP-1 β and MYO3A and both, whether MIP-1 β and MYO3A showed positive correlation with an established molecular determinant of cancer cell invasion, MMP9. Higher expression of these genes correlated with poor survival of breast cancer patients (Baghel et al., 2016).

Recently, O’Connell and colleagues found that S100A4-positive fibroblasts provide the suitable metastatic niche to support metastatic colonization. Fibroblasts can promote angiogenesis and protect against apoptosis, respectively through production of VEGF-A and tenascin-C. This study of the metastatic microenvironment of breast cancer in respect of clinical implication revealed that postmenopausal women treated with adjuvant bisphosphonates showed an 18% improvement in DFS (hazard ratio [HR] = 0.82; 95%CI 0.74–0.92, 2P = <0.001) by meta-analysis data. Reductions in relapse rates not only in bone but also at extra-skeletal and local-regional sites indicate that bone-targeted treatments may modify the course of the disease through both direct and indirect inhibitory effects on niche components and signalings between tumor and bone cells (Coleman et al., 2013).

Plasticity

Both tumor microenvironmental signals and cell-intrinsic modifications contribute to epigenetic changes that can induce cancer cells to adapt under challenging conditions. Successful invasion of the stroma, allow them to survive in lymphatic or blood vessels, and colonization of distant/different organs, as well as become resistant to cytotoxic drugs. Common adaptive responses include enhanced plasticity in cell motility, resistance to apoptosis and survival in a hostile environment characterized by hypoxia. Moreover, several components of the TME including tumor mesenchyme such as fibroblasts, MSCs, CAFs, and macrophages are directly or indirectly linked with oxidative stress (OS), and serve as drivers of adaptive strategies (Taddei et al., 2013).

To understand plasticity and the metastatic niche, the interplay between cancer cells and the microenvironment of intact xenograft tumors at distinct stages of metastasis is determined using a dual species-specific microarray platform. The study focused on three organ sites (brain, bone and lung) to which breast cancer commonly metastasizes. It was found stage-specific changes happen in tumor-cell-derived proteases and their inhibitors in expression during metastasis. However, stromal-derived genes are primarily regulated in a tissue specific manner. It was found that cathepsin S expression enhances breast-to-brain metastasis and is regulated in both a cell-type-specific and stage-dependent manner (Sevenich et al., 2014). Another study showed that microenvironmental factors and recurrence rates are not strictly related to cell intrinsic properties. Outgrowth of the same population of incipient tumors is accelerated in mice with triple-negative breast cancer (TNBC) relative to those with luminal breast cancer (LBC). A tumor supportive microenvironment enriched for EGF and IGF-1 is created by systemic signals provided by overt TNBCs at distant indolent tumor sites. Further, EGF and IGF-1 enhances expression of transcription factors associated with pluripotency, proliferation, and EMT (Castano et al., 2013). Macrophage phenotypic subtypes completely regulate epithelial-to-mesenchymal plasticity in breast cancer cells (Yang et al., 2016). Co-culturing of epithelial and mesenchymal cell lines with macrophages revealed that M2 macrophages might impart outgrowth and M1 macrophages may contribute to dormancy behaviors in metastatic breast cancer cells. A study has reported that embryonic stem cells precondition microenvironments through signal transducer activator of transcription 3 (Stat3) and suppress tumorigenic properties in breast cancer (He et al., 2016). This study may act as a platform to better characterize tumor cell plasticity within the metastatic niche, providing new therapeutic strategies to inhibit breast and/or prostate cancer progression.

V. CONCLUDING REMARKS & PROSPECTS

The complexity of the tumor microenvironment and its evolution throughout the course of breast and prostate cancer progression presents significant challenges to how basic scientists and translational clinicians collaborate to best identify effective strategies for curing patients. As such, it is essential that future work carefully study and characterize the complex cellular states that exist within the primary and metastatic tumor microenvironments of these malignancies, and more specifically, how these cellular states change in response to surgical or pharmaceutical intervention. Understanding these changing cellular states will likely

unveil new tumor and microenvironment cell vulnerabilities that can further be exploited to adjust treatment strategies – even possibly in real time.

Importantly, large data efforts have begun to make progress along this avenue, but more work is needed in this area. The Human Protein Atlas project is an online repository providing large quantities of subcellular immunofluorescence and clinical immunohistochemistry (IHC) imaging data from normal and malignant cells and patient tissues (Uhlen et al., 2005; Uhlen et al., 2015; Uhlen et al., 2010). This resource provides a wealth of information for oncology researchers. Figures 1 and 2 represent IHC images from breast and prostate malignancies that were extracted from the Human Protein Atlas and show markers of both tumor and stromal cell compartments in these malignancies. While helpful to query the relevance of specific markers in various cancer types – no doubt instructing follow-up experiments, these data do not provide co-labeling of these various markers across multiple patient samples or in heterogeneous co-cultures. Furthermore, this resource does not enable access to patient samples pre-/post- resection or therapeutic intervention.

Examples of new methodologies that are opening distinct avenues for the study tumor cell state evolution within the complex microenvironment in response to treatment regimens are the cyclic immunofluorescence (CycIF) (Lin et al., 2016; Lin et al., 2015) and tissue-based CycIF (t-CycIF) (Lin et al., 2017) techniques. These open-source platforms enable high-resolution, multi-plexed, sub-cellular immunofluorescence analysis of cells in culture (CycIF) and in frozen or fixed tissues (t-CycIF). Thus, researchers can now profile over 30 unique antigens simultaneously and obtain single-cell information about the spatial heterogeneity of tumor and stroma cells within their native or more defined microenvironments. Achieving such resolution will further inform research efforts aimed at identifying vulnerabilities for better treatment strategies, ultimately leading to better breast and prostate cancer patient outcomes.

VI. REFERENCES

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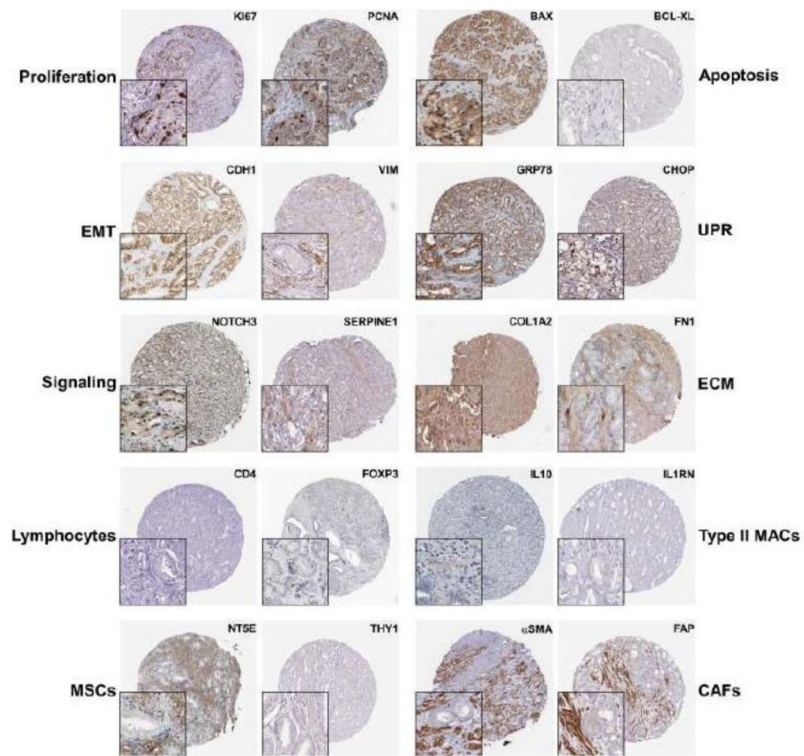


Figure 1. Immunohistochemistry of cell state markers indicating prostate cancer heterogeneity. Patient samples were retrieved from the Human Protein Atlas.

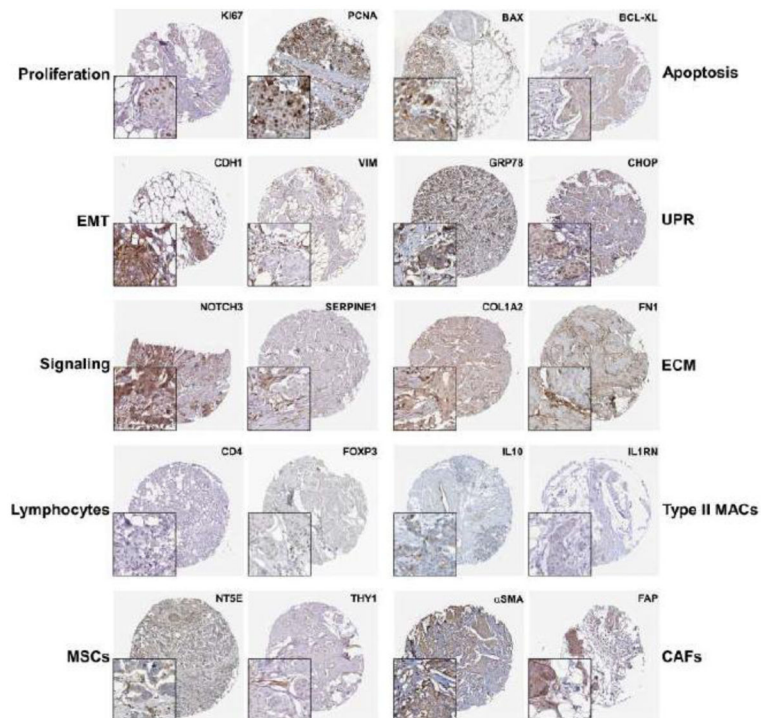


Figure 2. Immunohistochemistry of cell state markers indicating breast cancer heterogeneity Patient samples were retrieved from the Human Protein Atlas.