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Laboratory-Clinic Interface

The role of the tumor-microenvironment in lung cancer-metastasis and its relationship to potential therapeutic targets ☆

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ABSTRACT

Non-small cell lung cancer (NSCLC) accounts for >80% of lung cancer cases and currently has an overall five-year survival rate of only 15%. Patients presenting with advanced stage NSCLC die within 18-months of diagnosis. Metastatic spread accounts for >70% of these deaths. Thus elucidation of the mechanistic basis of NSCLC-metastasis has potential to impact on patient quality of life and survival.

Research on NSCLC metastasis has recently expanded to include non-cancer cell components of tumors-the stromal cellular compartment and extra-cellular matrix components comprising the tumor-microenvironment. Metastasis (from initial primary tumor growth through angiogenesis, intravasation, survival in the bloodstream, extravasation and metastatic growth) is an inefficient process and few released cancer cells complete the entire process. Micro-environmental interactions assist each of these steps and discovery of the mechanisms by which tumor cells co-operate with the micro-environment are uncovering key molecules providing either biomarkers or potential drug targets.

The major sites of NSCLC metastasis are brain, bone, adrenal gland and the liver. The mechanistic basis of this tissue-tropism is beginning to be elucidated offering the potential to target stromal components of these tissues thus targeting therapy to the tissues affected. This review covers the principal steps involved in tumor metastasis. The role of cell–cell interactions, ECM remodeling and autocrine/paracrine signaling interactions between tumor cells and the surrounding stroma is discussed. The mechanistic basis of lung cancer metastasis to specific organs is also described. The signaling mechanisms outlined have potential to act as future drug targets minimizing lung cancer metastatic spread and morbidity.

Introduction

Lung cancer is characterized by uncontrolled proliferation of cells within the lung, most commonly epithelial cells (carcinomas). Lung cancer is the leading cause of cancer-related death within Europe and the USA with metastasis responsible for >70% of deaths. The majority of late stage lung cancer patients die within 18-months of diagnosis [1]. Currently out of 1050 clinical/preclinical trials registered on the ClinicalTrials.gov registry, 350 address advanced stage lung cancer (86 dealing with metastasis). The predominant form of lung cancer is non-small-cell-lung-cancer (NSCLC), responsible for 85% of cases encompassing adenocarcinoma (AC) (~40% of cases), squamous cell carcinoma-SCC-(~25–30%) and large-cell carcinoma (~10–15%). These subtypes differ in terms of site of origin and patient characteristics, SCC being associated with smoking and origin from bronchial epithelial cells, whilst AC is mainly derived from alveolar/bronchial cells [1]. Irrespective of histological subtype the principal sites for NSCLC metastasis are bone, brain, adrenal gland and the liver with evidence these sites of preferential metastasis are determined by interactions between cancer-cell-surface proteins and capillary-lining endothelial-cell receptors at distant sites.

Key stages in lung cancer metastasis

Lung cancer originates within a microenvironment characterized by high degrees of vascularization and oxygenation. Uniquely

Abbreviations: LC, lung cancer; AC, adenocarcinoma; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; LCC, large cell carcinoma; SCC, squamous cell carcinoma; miRNA, micro-RNA; CSC, cancer stem cell; RANK, receptor activator of nuclear factor κB; RANKL, receptor activator of nuclear factor κB-ligand; CXCL12/SDF1α, stromal derived factor-1α; PTHrP, parathyroid hormone related peptide; EMT, epithelial-to-mesenchymal transition; M2-MØ, M2-macrophage; MDSC, myeloid-derived suppressor cells; M2-N, M2-neutrophil; MC, mast-cell; MSC, mesenchymal stem cell; M1-MØ, M1-macrophage; NK, natural killer cell; N1-N, N1-neutrophil; DC, dendritic cell.

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lungs are exposed to inhaled toxic insults. Cigarette smoking is the major risk factor for lung cancer development. Cigarette smoke causes an inflammatory reaction within the lungs recruiting inflammatory cells and the consequent altered cytokine secretion predisposes towards lung cancer [2]. Evidence suggests that interactions between tumor cells and stroma mediate development of lung cancer and tissue-preferences for metastasis. The latter phenomenon may reflect migration of cancer cells to released chemo-attractants from distant tissues or alternatively disseminated cancer cells may have differing abilities to survive in specific tissue-microenvironments. One consequence of these cancer cell intrinsic responses to externally released cytokines and pro-/ anti-apoptotic signals is that differing cancers have distinct preferences for the tissues to which they metastasize (“tissue-tropism”).

Metastasis of epithelial cancers, involves several stages [3]:

(1) Local tumor invasion through the basement membrane and stroma frequently involving matrix-metalloproteinase action [4].
(2) Intravasation into the lymphatic system [5] or blood vessels [6].
(3) Tumor cells survival within the circulation [7].
(4) Arrest at the distant tumor site [8], facilitated by formation of circulating tumor cell microemboli [9].
(5) Extravasation into the distant tissue microenvironment, facilitated by tumor-cell secreted factors [10].
(6) Initial survival of tumor cells in the distant tumor stromal environment, possibly involving formation of a “pre-metastatic niche” [11,12] as well as cancer-cell intrinsic events modifying growth at distant sites [13].
(7) Eventual formation of macrometastases [14].

Each of these steps presents a barrier to dissemination rendering the overall metastatic process inefficient [9]. A common observation within epithelial carcinomas is an extensive latency period between primary diagnosis and subsequent development of distant metastasis. Lung cancer does not display this latency period suggesting that cells leaving the primary tumor are sufficiently well adapted to survival at distinct metastatic sites [15], although this may also reflect the relatively late stage of most lung cancer diagnosis.

The role of microenvironment in development of metastatic ability within the primary tumor

(a) Cancer cell-intrinsic-responses

One of the earliest stages of tumor metastasis is local tumor invasion, in which carcinoma cells breach the basement membrane coming into contact with tumor stroma and tissue parenchymal cells. Degradation of the basement membrane is a key event in establishment of tumor–stroma interactions, however there is considerable evidence that tumors also recruit stromal components (e.g. immune cells) from the circulation. Observation of cancer cell metastasis reveals two distinct modes by which carcinoma cells invade the local stroma-(1) a “collective mode” of cancer invasion in which tumor cells move as clusters/sheets and single-cell invasion involving movement of individual tumor cells [16]. The latter mode of metastasis occurs via a protease-, integrin and stress-fiber mediated pathway or via an alternative Rho/ROCK-mediated pathway. Several proteins have been identified as promoting NSCLC invasion including: (1) L1-cell adhesion molecule (L1CAM) [17] and (2) specific isoforms of collapsin-response mediator protein-1 (CRMP-1) [18].

The switch to a more motile phenotype involves loss of polarized, epithelial morphology and acquisition of a spindle-shaped, mesenchymal phenotype-a process termed epithelial-to-mesenchymal transition (EMT). EMT involves the switch from E-cadherin to N-cadherin expression, mediated by the transcriptional co-repressors Slug, snail 1/2, twist, Zeb1, SIP1 and E47 [19]. Low oxygen levels (hypoxia) also increase the malignant behavior of cancer cells, in part via hypoxia-inducible factor (HIF) family transcription factors. HIFs regulate the expression of EMT-genes as well as promoting angiogenesis, cell-proliferation and tissue remodeling [20]. Lung cancer metastasis after chemotherapeutic treatment may also involve a lung-cancer-stem-cell population [21].

(b) Tumor-stromal cell-based interactions

The tumor stroma consists of extracellular matrix (ECM) and cellular components. The ECM includes secreted proteoglycans playing both a structural and cell-signaling role. The cellular components includes immune cells, cancer-associated fibroblasts-CAFs, endothelial cells, adipocytes, bone marrow-derived cells, myofibroblasts and fibroblasts [22] (Fig. 1). Functional genomic studies have identified gene signatures prognostic for NSCLC survival including genes encoding ECM-proteins [23], highlighting the role of stroma in survival. Growth factors involved in malignant development include endothelin-1 (EDN1), VEGF-A, PDGF-C, osteopontin, IL-8 and CXCL1 [22].

When tumor cells penetrate through the thin layer of fibers underneath the epithelium of organs/blood vessels (the basement membrane) they encounter a variety of cells including endothelial cells, adipocytes, bone marrow derived cells, myofibroblasts, fibroblasts, and immune cells [24]. Tumor-associated stroma displays a phenotype similar to that of stroma associated with wound healing [25]. The predominant cells within NSCLC stroma are fibroblasts. Cancer associated fibroblasts (CAFs) promote lung cancer development and local tissue invasion via promoting tumor growth and modulating drug response [26,27]. CAFs display elevated expression of α-smooth muscle actin (αSMA) and down-regulation of cell-cycle/stress-response genes i.e. p53 and p21 [28–30] as well as induction/secretion of pro-tumorigenic growth factors/cytokines [31]. It has been proposed that CAFs may arise via transformation of normal fibroblasts, migration to tumors from bone marrow, EMT or from pre-existing fibroblasts [32].

CAFs are the major cells implicated in protease-mediated degradation of the ECM [33]. Furthermore CAF secreted factors (e.g. TGFβ) alter protein expression within cells at the leading edge of invading tumors [34]. Thus CAF/NSCLC-tumor cell interactions promote invasive development. CAFs also secrete pro-metastatic factors: hepatocyte growth factor (HGF) [31] and SDF-1/CXCL12 [35]. Altered ECM-properties can re-programme CAFs facilitating metastasis via mechano-transduction sensing [36].

Gene-expression studies comparing CAFs with normal fibroblasts identified altered proteins including components regulated by the EMT-regulator TFGβ1 [37]. TGFβ is a pleiotropic growth factor exerting a tumor-suppressive function within pre-malignant cells but promotes tumor-growth at later stages of cancer [38]. TGFβ is secreted by tumor cells and stromal cells and induces expression of anti-apoptotic proteins (i.e. Bcl2), cell-adhesion molecules and integrins within cancer cells at the leading edge of invasive lung cancer [34]. Bcl2 and integrin-expression levels are predictive of metastasis and survival [39]. Adenocarcinoma-associated CAFs also secrete immunomodulatory cytokines including TGF-β and VEGF inducing Forkhead box P3 expressing regulatory T-cells, immunosuppressive lymphocytes associated with significantly poorer outcome and disease-free survival in AC [40].

Mesenchymal stem cells (MSCs) are also present at tumor sites [41] due to homing downstream of cytokine/cytokine-receptor pairs including: monocyte-chemotactic protein (MCP)/CCR2, HMGB1/RAGE, SDF-1(CXCL12)/CXCR4, SCF/c-Kit, VEGF/VEGFR and HGF/c-Met [22,42]. MSCs release pro-angiogenic mediators includ-