

## Original Article

# Lung microenvironment promotes the metastasis of human hepatocellular carcinoma cells to the lungs

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Received February 28, 2015; Accepted June 1, 2015; Epub June 15, 2015; Published June 30, 2015

**Abstract:** Cancer metastasis is a highly tissue-specific and organ-selective process. It has been shown that the affected tissues and/or organs play a major role in this complex process. The lung is the most common target organ of extrahepatic hepatocellular carcinoma (HCC) metastasis, but the precise molecular mechanism underlying this organ-specific metastasis remains unclear. We hypothesized that lung microenvironment was able to promote the metastasis of HCC cells to the lungs leading to distant metastases. In support of our hypothesis, we provided evidence from targeted metastasis in various types of cancer and contributing factors in the microenvironment of targeted tissues/organs. A better understanding of the steps involved in the interplay between HCC cells and lung microenvironment may offer new perspectives for the medical management of lung metastases of HCC.

**Keywords:** Hepatocellular carcinoma, lung, microenvironment, tissue factors, targeted metastasis

## Introduction

Metastases arise following the spread and subsequent growth of cancer cells from a primary site and a formation of new tumors in distant organs. The metastatic tumor is a major cause of morbidity and mortality associated with human cancers. For some cancer types, biologically active factors have been identified to promote the invasion and metastasis of cancer cells in distant organs [1-3]. Cancer metastasis is a complex process and consists of a large series of interrelated steps. To produce clinically relevant lesions, metastatic cells must survive all steps of this process. The outcome of the metastatic process depends on both the intrinsic properties of cancer cells and their interactions with specific host factors [4-6]. Distant metastases can be located in different organs and in different regions within the same organ. The metastasis of cancer cells is usually organ- and/or tissue-specific, and appears to depend on factors on cancer cells and those in host organ microenvironment, which provides a biological environment supporting the proliferation of cancer cells (i.e., embedding of cancer cells into the capillary system of a target organ,

adhesion of cancer cells to the endothelial cells of a target organ, and production of growth factors for cancer cells), and eventually the formation of distant tumors. The organ microenvironment can modify the response of metastatic cancer cells to the therapy and alter the effectiveness of anti-cancer agents in destroying the tumor cells without producing undesirable side effects. Existing drugs including traditional chemotherapeutics or new antiangiogenic agents have limited efficacy in preventing cancer metastases. A detailed understanding of the mechanisms involved in organ and tissue-specific metastasis may provide new opportunities to improve anti-cancer therapy. Therefore, identifying a factor or factors involved in attracting, for example, hepatocellular carcinoma (HCC) cells to the lungs may be a first step towards providing strategies for anti-metastatic therapy.

## Investigative progress of targeted metastasis of cancer cells

Studies have shown that most cancer cells entering the bloodstream are eliminated rapidly, and thus the presence of cancer cells in the

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circulation does not mean that metastasis will occur [1, 7, 8]. To produce metastases via the systemic circulation, cancer cells must survive from the transportation in the circulation, adherence to the microvascular wall of distal tissues, and growing locally or invading the vessel wall and growing in the organ parenchyma.

### *Early theories*

As early as 1889, Stephen Paget found a non-random pattern of visceral (and bone) metastasis by analyzing autopsy reports of women who died from breast cancer, suggesting that certain cancer cells (the “seed”) have a specific affinity to the microenvironment of certain organs (the “soil”). The paradigm is that metastases occur only when the seed and soil are compatible [9].

In 1928, Ewing [10] challenged the Paget’s “seed and soil” theory and proposed that the dissemination of metastatic cells occurs by purely mechanical factors that result from the anatomical arrangement of the vascular system. However, although hemodynamic and mechanical factors are undeniably important in determining the distribution patterns of several cancers, the mechanical theory does not satisfactorily explain several documented patterns of metastases. For example, choroidal melanoma preferentially metastasizes to the liver and, in doing so, must circumvent several more proximal organs [11]. Metastases of clear cell carcinoma of the kidney frequently arise in the thyroid gland, which cannot be explained by anatomical-mechanical principles. Furthermore, studies on animal models have shown that some cancer cells exhibit specificity for the growth in different regions within a single organ. Schackert and Fidler [12, 13] noted that injection of K-1735 melanoma cells into the internal carotid artery of mice produced metastases only in the brain parenchyma. However, when these investigators repeated the experiment using B16 melanoma cells, only meningeal growths were observed. In a review of clinical studies on site preferences of metastases produced by different human cancers, Sugarbaker et al [14] concluded that common regional metastatic involvements could be attributed to anatomical or mechanical considerations, such as efferent venous circulation or lymphatic drainage to regional lymph nodes, but that metastasis in distant organs from

numerous types of cancers is indeed site-specific. Strong experimental evidence that cancer cells home to and grow in particular distant organs was first reported for Cloudman melanoma [15] and then for murine sarcoma [16]. Targeted metastasis of many solid tumors correlates closely with the microenvironment of targeted organs. For instance, the capillary endothelial cell matrix of mouse lung is chemotactic to lung-targeted metastatic murine large cell lymphoma cell line RAW117, which is mediated by murine JE (equivalent to human monocyte chemoattractant protein-1 [MCP-1]). Distant organ metastasis of cancer cells reveals the specificity of metastatic sites for various cancers.

### *A modern paradigm: chemokines*

More recently, tumor-targeted metastasis is attributed to the interactions among chemokines and their receptors. The paradigm of chemokine function in terms of site-specific homing is apparent in the involvement of these chemokines in several of the key steps of metastasis. For example, prostatic carcinoma cells can secrete a chemokine, the receptor of which is expressed in the bone. Breast cancer cells can secrete another chemokine and its receptor is expressed in the lungs. Many experiments have demonstrated that the binding between chemokines and their receptors leads to the directional movement of cancer cells and thus, causes distant target organ-specific metastasis [17, 18]. Studies also reveal both breast cancer cells and primary breast tumor highly express the chemokine receptors CXCR4 and CCR7. The expressions of specific ligands for these receptors-CXCL12 and CCL21 are increased in the lymph nodes, lungs, liver and bone marrow-organs to which breast cancer often metastasizes. Furthermore, blocking CXCR4 is able to inhibit the metastasis of breast cancer cells in experimental animal models. Because chemokines are involved in the homing of lymphocytes, it is reasonable to suppose that chemokines may also cause the homing of cancer cells to the tissues/organs with high expressions of their receptors, thereby promoting organ-specific metastasis [2].

Melanoma cells often express chemokine receptor type 7 and chemokine receptor type 10. A large number of ligands have been identified in the most common organs that melano-

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**Table 1.** Microenvironment and factors mediating the targeted metastasis of cancer cells

Tumor cells	Microenvironment	Factors
Lymphadenoma RAW 117 [32]	Matrix of endothelial cells in lung microvasculature	JE (mice)/MCP-1 (human)
Prostatic carcinoma cells [33]	Chemokine receptor in bone	Chemokine
Breast cancer cells [34]	Chemokine receptor in lungs	Chemokine
Melanoma cells [35]	Skin and lymph nodes	Chemokine 7, 10
Colon cancer cells [36]	Liver	Sle+
Walker cancer cells [37]	Lung	Neuraminidase
Gastric carcinoma cells [38]	Liver	Chemokine 7
K-1735 melanoma cells [39]	Brain parenchyma	Unknown
B16 melanoma cells [40]	Meninges	Unknown

ma cells easily metastasize to, such as skin and lymph nodes. When a reverse transcription vector with chemokine receptor type 10 is transfected into melanoma cells B16, the metastasis of B16 cells to the lymph nodes is accelerated [19]. In gastric carcinoma cells, 66% of cancer cells are positive for chemokine receptor type 7 and metastatic lymph nodes are strong positive for chemokine receptor type 7. In contrast, unaffected lymph nodes are negative for chemokine receptor type 7 [20]. The microenvironment and factors mediating the targeted metastasis of cancer cells are shown in **Table 1**.

Liver cancer cells express chemokine receptor type 4 which is involved in the metastasis of liver cancer to the lungs [21]. However, chemokine receptor type 4 is not a major receptor in mediating the targeted metastasis of HCC cells, and other important factors with biological activity may participate in modulating the metastasis of HCC to the lungs. We postulated that, in the lungs of HCC patients, there was at least one chemical entity (a “factor”) that may attract HCC cells to the lungs and induce the metastasis of HCC, resulting in metastatic HCC.

### Our hypothesis

We hypothesized that a factor exists in the microenvironment of lungs attracted HCC cells to the lungs resulting in metastatic HCC.

### Evidence supporting our hypothesis

Studies have revealed that unique factors produced in individual tissues of the body may exert different effects on the migration and metastasis of cancer cells [22]. Clinical observations of cancer patients and laboratory stud-

ies on experimental rodent tumors have shown that certain cancers metastasize to specific organs independent of vascular anatomy, blood flow rate, and number of cancer cells delivered to each organ. The distribution and fate of hematogenously disseminated, radiolabeled melanoma cells in experimental animals conclusively demonstrate that cancer cells can reach the microvasculature of many organs, but their growth only occurs in specific organs [4, 23-26].

It has been demonstrated that, once contacting with the specific organs, cancer cells may produce signaling molecules via autocrine (it is a professional term in the field of biology and a regulatory pattern that cytokines and hormones act by, and these cytokines and hormones spread locally and feedback effect on the endocrine cells that produce the above cytokines and hormones after being secreted), paracrine (it is a professional term in the field of biology. The hormones and regulatory factors that tumor cells produce contribute to the other kind of cells through the near intercellular space) or endocrine to influence the targeted metastasis of cancer cells. In *in vitro* studies, Lu et al [27] found that GASL gastric cancer cells exhibited higher adhesiveness and proliferation rate in the liver than in the lungs and pancreas, which suggests that the interstitial matrix is a crucial factor for the specific invasion and metastasis of cancer cells. In cancer metastasis, the affinity of cancer cells to a specific organ is dependent on the adhesion molecules specifically expressed on the endothelial cells of the target organ and on the cancer cells. For instance, melanoma and certain types of lymphomas highly express very late antigen (VLA-4) integrin, and cancer cells may

metastasize to the lungs through specifically binding to VLA-4 and vascular cell adhesion molecule (VCAM). Gastric cancer and colon cancer cells highly express the glycosylated ligand of E-selectin, i.e., Sle+, allowing the hepatic metastasis of gastric cancer and colon cancer cells. Mouse Walker cancer cells can develop lung metastasis. However, cancer cells metastasize to the liver, instead of the lungs, following the removal of the acetylneuraminic acid group at the end of cell surface saccharide chains and subsequent exposure of galactosyl residues by sialidase. Since there are agglutinins that can specifically recognize and bind to galactosyl residues at the end of saccharide chains on the hepatocytes, cancer cells are retained in the liver as a result of specific recognition and binding between galactosyl residues and agglutinins. The metastatic capability of primary HCC cells growing *in situ* in nude mice is related directly to the activity of type IV collagenase. Organ-specific fibroblasts can directly influence the infiltrative capability of cancer cells. For example, infiltrative or metastatic HCC cells can grow on fibroblast monolayers, but fail to penetrate the skin fibroblasts. The microenvironment of target organs may directly promote the induction and maintenance of basic fibroblast growth factor (bFGF) and interleukin-8 (IL-8) production. The mRNA and protein expression of bFGF are influenced by the microenvironment of organs. In various organs of nude mice, human primary kidney cancer cells (HRCC) have distinct metastatic potentials. The cells implanted in the kidney are likely to metastasize to the lungs, but those implanted subcutaneously fail to metastasize to the lungs. In addition, in nude mice, subcutaneous cancers have much less vasculature than kidney cancers [28-30].

### Lung microenvironment and cancer-targeted metastasis

After cancer cells migrate to target organs, their biological activities may change and their responses to paracrine growth factors are enhanced. In many human and animal cancer models, the major paracrine growth factor isolated from conditioned medium of lung tissues is a transferrin-like glycoprotein, which may specifically stimulate the growth of a cancer cell subset with the potential of metastasizing to the lungs or brain. Bone marrow-derived growth factors can promote the growth of pros-

tatic cancer cells with bone metastatic potential. In addition, the organ specificity of cancer metastasis is associated with the selective expression of adhesion molecules by vascular endothelial cells in these organs. For instance, VCAM-1 is excessively expressed in the vascular endothelial cells of the lungs, and melanoma and some lymphomas highly expressing integrin VLA-4 tend to metastasize to the lungs. Lung-derived growth factor-1 (LDGF-1) is a factor able to stimulate the growth of cancer, and can be isolated from the culture medium of lung tissues and lung interstitial cells. LDGF-1 may stimulate the growth of lung cancer cells with the metastatic potential, but is unable to stimulate the growth of cancer cells without metastatic potential or normal kidney cells. Lung specific endothelial cell adhesion factor-1 (Lu-ECAM-1) can promote the binding of cancer cells to the extracellular matrix [31].

### Verification of the hypothesis

Our hypothesis that at least one specific factor involved in attracting HCC cells to the lungs leading to the metastatic HCC may be verified by studying the underlying physiological processes using molecular cloning techniques. We expect the use of a mouse model of HCC, involving a HCC cell line (such as MHCC97-H) suitably transfected by a reporter gene (such as green fluorescent protein, GFP)-modified plasmid, and the GFP is observed by fluorescence microscopy. By using protein technology, biologically active molecules associated with MHCC97-H involved in the formation of metastatic HCC in the lungs may be analyzed, isolated, purified and functionally and structurally identified. Suitable control and inhibition experiments may be carried out to further strengthen the theoretical basis of our hypothesis.

### Conclusion

The lungs are the most common target organ of extrahepatic HCC metastasis; however, the precise molecular mechanisms are poorly understood. We hypothesize that at least one factor exists in the lung microenvironment and may attract HCC cells and induce their metastasis to the lungs to. In support of our hypothesis, we summarize a number of findings related to targeted metastasis of cancer cells provide evidence for the factor-mediated metastasis in various tissues, *inter alia*, in the lungs. In addi-

tion, we also provide a general approach on how to verify our hypothesis. A better understanding of the steps involved in the interplay between HCC cells and lung microenvironment, in particular, the identification of chemical entities involved in the interaction between cancer cells and host tissues leading to preferential metastasis to the lungs, may offer new perspectives for the medical management of metastatic HCC in the lungs. The metastatic process consists of a series of steps, all of which must successfully complete to form a metastatic tumor. The growth of primary HCC requires rich blood supply (also known as angiogenesis) to support its metabolism. These newly generated blood vessels may also provide a route by which cancer cells may leave the primary site and enter the hepatic veins-known as intravasation. The HCC cells may also enter the blood indirectly via the lymphatic system. These cells should survive in the circulation until they can arrest in the lung and initiate and maintain growth to form pre-angiogenic micrometastases; the maintenance of this growth needs the formation of new blood vessels. During the whole process of metastasis, the arresting and maintenance of growth of cancer cells in the lung is the most vital for the organ-specific metastasis. A specific factor (such as chemokine or chemokine receptor) in the lungs may attract or accelerate the arrest and growth of HCC cells in the lungs.

### Acknowledgements

This work was supported by a National Natural Science Foundation (No. 81450045).

### Disclosure of conflict of interest

None.

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