The Biology of Tumour Metastasis

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Metastasis is a life-threatening disease that accounts for as much as 90% of cancer-related mortality. Carcinoma cells have often spread to distant organs at the time patients present with cancer. Routine clinical examinations have produced significant progress in detecting metastasis but existing methods for screening cancer patients are incapable of detecting micrometastasis and disseminated tumour cells (DTCs) in distant organs. Adjuvant chemotherapy and adjuvant radiotherapy are anticipated to prevent relapse and death. However, over periods of time ranging from years to decades, these metastatic cells residing in distant organs often relapse, corrupt the local microenvironment and acquire the ability to develop into macrometastases. Metastatic nodules are known to be formed by carcinoma cells harboring increased numbers of epigenetic alterations conferring aggressive and drug-resistant propensities. In addition, more recently emerging evidence supports the notion that the tumour-associated stroma, consisting of endothelial cells, leukocytes, macrophages, myofibroblasts, bone marrow–derived progenitors and abundant extracellular matrix (ECM), significantly facilitates tumour metastasis. The molecular signalling underlying the complexity of heterogeneous stromal–tumour interactions that is relevant to tumour metastasis is the subject of intensive research. The tumour–associated stroma, in addition to tumour cell-autonomous alterations plays significant roles to instigate and support progression of the multi-step processes of tumour metastasis.

Key words: Invasion-metastasis cascade, Carcinoma-associated fibroblasts (CAFs), Tumour-associated stroma, Disseminated tumour cells (DTCs), Circulating tumour cells (CTCs)

The invasion-metastasis cascade

It has been widely accepted that carcinoma cells disseminate into distant organs via blood or lymphatic vessels. Recent studies support that the blood circulation plays clearly pivotal roles in metastatic dissemination. The contribution of the lymphatic vessels to the dispersion of cancer cells is, however, considered to be less obvious. It is documented that functional lymphatic vessels are rarely found through tumour masses due to their little internal hydrostatic pressure resulting in their collapse.

The depiction of the invasion–metastasis cascade ascribes several distinct steps to the overall process, as shown in Figure–1. The initial step of localised invasiveness enables in situ carcinoma cells to breach the basement membrane followed by their intravasation into either lymphatic or blood microvessels. The latter may then transport these cancer cells to distant anatomical sites, where they may be trapped and subsequently extravasate and form dormant micrometastases. Eventually some of the micrometastases may acquire the ability to colonise the tissue in which they have landed, enabling them to form a macroscopic metastasis. The last step so-called colonisation seems to be the most inefficient of all. The small probability of completing all steps of this cascade supports the notion that any single cancer cell leaving a primary will rarely succeed in becoming the founder of a distant, macroscopic metastasis.

In spite of the fact that recent studies extensively investigated tumour metastasis at molecular levels, a number of questions still remain unanswered. For example, it is unclear whether the multi-step processes of metastasis including colonisation are mediated by one or other distinct signalling pathways.
pathways and whether the metastasis-promoting signal pathway differs from the primary tumour-promoting signal pathway. Notably, the tumour-associated stroma is also assumed to promote metastasis by influencing each step of the invasion-metastasis cascade (Figure-1). Studying the tumour-stroma interaction during progression and dissemination of carcinoma cells would therefore be crucial for understanding the biology of tumour metastasis that allows the development of novel diagnostic and therapeutic approaches.

Spread of distinct cancer cell populations and evolution of metastasis

Cellular origins of carcinoma cells in a primary tumour have been extensively studied for the past three decades, however, those of DTCs begin to be investigated very recently. The long standing question is whether metastasis is formed by cancer cells that were present at the late or early stage of primary tumours. Figure-2 shows two existing models supporting "late dissemination" or "early dissemination" that allows stepwise progression or parallel progression of DTCs, respectively, during the series of tumourigenesis. It has been believed that dominant or minor clones present in the late stage of tumours could give rise to metastasis (Figure-2). In contrast, carcinoma cells disseminated during relatively early tumourigenesis, are also indicated to form metastasis.

Genetic alterations harboured by carcinoma cells have long been considered to play major roles in promoting the invasion-metastasis cascade. Recent studies using whole genome sequencing and copy number analyses examined genetic alterations in detail in carcinomas, including those of the colon, pancreas, breast and prostate. For these studies, matched pairs of primary tumours and metastases were employed. Considerable sharing of somatic mutations identified in metastases with those found in the corresponding primary tumours was revealed in several cases. It was concluded that
metastases had originated from predominant populations of primary carcinoma cells harboring alterations late in the genetic evolution of carcinomas. This conclusion supporting a stepwise progression model of carcinoma metastasis contradicts a parallel progression model (Figure-2). The latter proposes that carcinoma cells, which disseminate to distant organs early during tumour progression, may acquire genetic alterations independently of those present in primary tumour cells. In recent years, it has been shown that tumour cell dissemination is often an early event, suggesting that primary and metastatic sites evolve in parallel and independently. These two events—stepwise and parallel progression models—would not be mutually exclusive and the both may occasionally occur during the series of tumour progression. Molecular and biological characteristics of tumour cells metastasised should therefore be assessed in each patient based on the comparison with those in cancer cells in primary tumour, in order to develop individually the most suitable therapeutic approaches against the metastatic recurrence.

**Metastasis-promoting signal from the tumour-associated stroma**

Various types of non-neoplastic stromal cells are frequently present within human primary carcinomas, including heterogeneous populations of immune cells, endothelial cells, fibroblasts, myofibroblasts and bone marrow-derived progenitors (Figure-3). Immune cells are represented by those of innate immunity, including macrophages, neutrophils, mast cells, myeloid-derived suppressor cells, dendritic cells and natural killer (NK) cells, and cells of adaptive immunity, such as T and B lymphocytes. The immune cells which infiltrate the tumour (excluding NK cells) produce tumour-promoting cytokines including tumour necrosis factor-α (TNF-α), IL-1β, IL-6 and IL-8, which increase NFκB and STAT3 signalling in nearby premalignant cells.

Tumour-associated macrophages (TAMs), which are mature myeloid cells, can be found within the tumour microenvironment in high numbers. TAMs, when educated by microenvironmental cues within the primary tumour, adopt the M2/trophic phenotype. M2 type TAMs produce paracrine factors promoting neo-angiogenesis, immunosuppression and local inflammation, all of which facilitate the invasion-metastasis cascade. For example, IL-4 and colony-stimulating factor-1 (CSF-1) cytokines produced by carcinoma cells and/or T-lymphocytes stimulate recruitment and
activation of TAMs, which promote cancer cell invasion by producing epidermal growth factor (EGF) and cathepsin B and S proteinases (Figure-3). TAMs also play essential roles in promoting colonisation of DTCs in distant organs. Prior to their extravasation, carcinoma cells which enter the circulation need to overcome anchorage-indepen-dent growth conditions, i.e., survive sheer forces
and resist anoikis. The subsequent colonisation is thought to be a rate-limiting step of metastasis, as it has been estimated that less than 0.01% of circulating tumour cells (CTCs) which survive in the circulation, extravasate and give rise to micrometastases.

The presence of large numbers of stromal cells, and the high density and stiffness of ECM are characteristic features of the tumour stroma, which is often referred to as a “desmoplastic” stroma. Fibroblasts and myofibroblasts, collectively designated carcinoma-associated fibroblasts (CAFs), have been shown to substantially contribute to the development of desmoplastic stroma. Myofibroblasts are \( \alpha \)-smooth muscle actin (\( \alpha \)-SMA)-positive fibroblasts which are a hallmark of activated fibroblasts. CAFs interact with cancer cells and collaborate with other components of the stroma through their production and secretion of various growth factors, cytokines and chemokines. These signalling molecules effectively mediate neoangiogenesis, as well as proliferation, survival, motility and invasion of cancer cells.

Cancer stem cells (CSCs) and epithelial mesenchymal transition (EMT)

The cells of origin for metastasis are known to play major roles influencing the invasion-metastasis cascade. The concept of cancer stem cells (CSCs), whereby rare populations of carcinoma cells are capable of forming a tumour, derives from the well-established characteristics of normal tissue stem cells, including their self-renewal and multi-potency. Recent studies notably showed de novo induction of the stem cell state in various normal and carcinoma cells which underwent epithelial mesenchymal transition (EMT). The latter is a well-characterised process of cellular trans-differentiation through which epithelial cells acquire the mesenchymal phenotype. This trans-differentiation program is also reversible as judged by the cells undergoing mesenchymal-epithelial transition (MET). Furthermore, the bidirectional nature of the CSC phenotype (transition between CSC- and non-CSC-states) was often observed as associated with the reversible EMT trait and this encouraged revision of the definition of CSCs in human breast carcinoma cells.

A recent observation, however, conflicted with the notion supporting the EMT–induced CSC state. It was shown that an E-cadherin-expressing human prostate carcinoma epithelial cell line, which was originally isolated from liver metastasis experimentally generated in nude mice, indeed exhibited stronger CSC and increased metastatic traits, compared to those in their mesenchymal counterpart. Moreover, overexpression of Snail, an EMT transcription factor, induced mesenchymal phenotypes and suppressed their epithelial trait in these cells, although their CSC state and metastatic potential were significantly inhibited in the resultting Snail-expressing mesenchymal cancer cells. These findings therefore indicate the association of CSC state and mesenchymal trait may depend on the cellular context. Further investigation requires for clarifying how the EMT program influences CSC state promoting metastatic potential also in the different cellular context.

CTCs, DTCs and metastasis

CTCs found in the bloodstream, and DTCs that have already spread and localised in distant organs, are believed to be precursors of metastatic nodules. Importantly, increased CTC numbers and the presence of DTCs in bone marrow predict a poor outcome in breast cancer patients, indicating that these cells can serve as an independent prognostic
factor for this disease. Notably, particular genetic alterations harboured in CTCs, which are responsible for drug-resistance, such as those of the epidermal growth factor receptor (EGFR) gene, have been detected in non-small-cell lung cancer patients. This may serve as a new approach for monitoring the genetic changes which develop de novo in carcinoma cells during application of systemic therapy.

A newly emerging hypothesis suggests that CTCs that are capable of giving rise to distant metastases are enriched for CSCs, and as such show increased invasiveness, anoikis-resistance, tumour-initiating potential and the ability to avoid cellular dormancy during metastatic colonisation. Indeed, it has been demonstrated that several CSC-enriched cancer cell populations show increased ability to form metastases when implanted intravenously into recipient mice. It is however unknown whether the emergence of spontaneous metastases observed in cancer patients is mediated by CTCs enriched for CSCs. Technical challenges of existing methods for handling and culturing CTCs make proving this assumption experimentally difficult. Low numbers of CTCs present in circulating blood, their short half-life and a paucity of functional markers available for their identification, render isolating CTCs very challenging. Interestingly, it has been shown that distinct populations of CTCs can be found in blood samples of cancer patients, including those circulating as single cells which show a mesenchymal or ameboid-like appearance and those found in the circulation in the form of epithelial sheets, indicative of collective movement of a group of epithelial cells. Moreover, CTCs appear to contain clusters of heterogeneous cell populations composed of platelets, leukocytes and mesenchymal cells.

The half-life of CTCs in the circulation is short, i.e., measured in hours, which is partially related to their fast clearance from the circulation and/or apoptosis. To date, CTCs and DTCs extracted from either patients or mouse tumour models are incapable of efficiently forming metastases when introduced into recipient mice. These cells may require niche support to exert their potential to initiate and develop metastasis. Cells of primary tumours are known to be capable of inducing both local and systemic changes in the microenvironment that prime and facilitate their metastatic spread and promote colonisation of distant organs. A variety of soluble factors secreted by tumour cells and supporting stromal cells contribute to increased vascular permeability and penetration of blood vessels by cancer cells, immune evasion, and vascular adhesion which allow cancer cells to intravasate, survive in the circulation and extravasate into sites of metastatic nodule formation. Support provided by a local, organ-specific metastatic niche is also thought to play a critical role in the abilities of DTCs to initiate and develop metastasis. Further improvements in the techniques allowing detailed characterisation of CTCs and DTCs with regard to their epi/genetic status and gene expression profiles is needed to advance our knowledge of the metastasis-forming ability and CSC phenotype of these cells in future studies.

**Closing Remark**

Carcinoma cells possess the ability to instigate changes in the surrounding stroma in both primary and metastatic sites, which upon activation stimulate local invasion, dissemination and metastatic colonization of tumour cells. Various cell-autonomous alterations within carcinoma cells resulting in corruption of the stroma have increasingly reported. The tumour-associated stroma is also shown to support CSC-niche formation and retarded immune surveillance, both of which facilitate survival and growth of cancer cells within primary tumours and metastatic nodules. Exploration of the signalling which underpins the formation of the niche for CSCs suggests its dependence on complex interactions between tumour and stromal cells, and their functional cooperation with cell-autonomous alterations within carcinoma cells. The development of therapeutic interventions targeting stromal-tumour interactions is therefore crucial and combining novel approaches with conventional therapies should be considered for increasing the efficacy of these treatments.

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