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In vitro models of the metastatic cascade: from local invasion to extravasation

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A crucial event in the metastatic cascade is the extravasation of circulating cancer cells from blood capillaries to the surrounding tissues. The past 5 years have been characterized by a significant evolution in the development of *in vitro* extravasation models, which moved from traditional transmigration chambers to more sophisticated microfluidic devices, enabling the study of complex cell–cell and cell–matrix interactions in multicellular, controlled environments. These advanced assays could be applied to screen easily and rapidly a broad spectrum of molecules inhibiting cancer cell endothelial adhesion and extravasation, thus contributing to the design of more focused *in vivo* tests.

The past four decades were characterized by promising successes in cancer treatment and detection, through the development of devices reducing surgical invasiveness or enabling early diagnosis, and the discovery of drugs blocking primary tumor progression, thus reducing cancer mortality and improving life quality for patients with terminal disease [1]. As discussed in a recent scientific report by the American Cancer Society, the relative 5-year survival rate for all cancers diagnosed between 2002 and 2008 in the USA was 68%, significantly higher compared with the 49% reported for 1975–1977 [2]. However, despite great advances in basic cancer molecular and cell biology with the discovery of oncogenes [3], tumor suppressor mechanisms [4] and cytokines involved in cancer progression [5], the spread of primary tumors toward distant organs and the subsequent metastatic colonization is still responsible for 90% of cancer-associated mortality [6].

In vitro assays can be beneficial to study cancer cell invasion and migration, and for the development of new anticancer drugs [7]. In particular, human 3D models can closely mimic the pathophysiological microenvironment [8], combining multiple cell types and molecular factors in a controlled system, thus bridging the gap between simplified 2D assays, which lack the structural architecture of body tissues and force cells to adapt to an artificial flat and stiff surface [9], and complex, expensive *in vivo* studies, often performed using animal models that might fail to reproduce features of human tumors [10]. Significant advances have been made since the development of soft lithography techniques, which enable microfabrication of structures and channels with poly-dimethyl-siloxane (PDMS) for microfluidic applications, thus replacing traditional plastic surface devices, and patterning of cells and biomolecules [11]. Microfluidic devices with embedded 3D cultures are currently used to study cancer cell behavior within *in vivo*-like microenvironments [12] and new promising applications, including paper-based multilayer constructs, have been developed to control oxygen and nutrient gradients [13].

Modeling the multiple steps of the metastatic cascade represents a challenge that could pave the way for the discovery of new antimetastatic drugs [14]. In particular, the extravasation process represents a crucial point that leads to the invasion of specific secondary sites, with the subsequent growth of metastatic tumors; thus, detailed studies are necessary to clarify the interaction between specific primary tumors and secondary target organs [15].

Following an introductory section on cancer metastases, in this review, we focus on *in vitro* models to study cancer cell invasion, migration and, particularly, extravasation. We also discuss microfluidic applications to investigate extravasation processes and

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other metastasis-related phenomena. Finally, we present *in vitro* and *in vivo* models that can be used to study the effects of therapeutics on cancer cell extravasation, underscoring how highly specific microfluidic models could provide a significant breakthrough in the screening process of antimetastasis drugs.

Cancer cell odyssey in the metastatic cascade

Tumors arising from epithelial tissues represent approximately 80% of life-threatening cancers because of their ability to metastasize in different secondary organs [16]. The complex metastatic process can be conceptually divided into two main phases, namely the physical translocation of cancer cells from the primary tumor to distant sites, and their subsequent colonization (Fig. 1). More specifically, several sequential and interrelated steps can be recognized in the former phase, including loss of cellular adhesion, acquisition of increased invasiveness and motility owing to genetic and epigenetic alterations, and induction of tumor angiogenesis leading to entry into the circulatory or lymphatic systems, a process known as intravasation [6,17]. After intravasation, those cells that survive in the circulation might undergo extravasation, which includes several steps, such as cells becoming trapped in a remote vessel or adhered to its endothelium and transmigrating into tissues, to initiate the development of secondary tumors [18–22].

In one scenario of extravasation, circulating tumor cells (CTCs) showing a leukocyte-like rolling behavior on the vascular walls [23] establish transient, metastable contacts with the endothelium mediated by endothelial cell surface molecules, such as E-selectin and P-selectin, and cancer cell counter-receptors, such as sialyl Lewis-a/x [24,25]. Subsequently, a firmer adhesion is mediated by adhesive molecules on the endothelium, such as vascular cell adhesion molecules (VCAMs), whose expression can be triggered by cancer cells themselves [26], and cancer cell integrins, while chemo-attractant molecules promote *trans*-endothelial migration toward the surrounding tissues [8]. An alternative view is that CTCs, being relatively large, are physically trapped in the small vessels of the microcirculation, become activated, and transmigrate [27].

Steven Paget's 'seed and soil' hypothesis represents a milestone in the study of mechanisms governing metastases, based on the assumption that the interplay between specific cancer cell types and a properly receptive microenvironment guides the metastatic spread of primary tumors to distant organs [28]. However, Paget's theory was challenged by James Ewing, who proposed that the main factor leading to metastases is represented by the anatomy of blood and lymphatic vessels and by circulatory patterns between primary tumors and specific secondary sites [29]. It is now accepted that these theories are not mutually exclusive: scientists have shown how CTCs migrating from the primary tumors target a well-defined subset of organs, specific for each tumor type. This tissue tropism is partially because of the anatomy of the circulatory system, not only leading to physical trapping as described above, but also influenced by the interaction between 'seed cells' and 'receptive soils' [15–17,30].

Endothelial cells in the vasculature of different organs express different surface receptors and specific chemokines are secreted by host cells of individual tissues [31,32]. Moreover, the 'pre-metastatic niche model' states that growth factors secreted by the primary tumor can prime specific tissues for cancer engraftment,

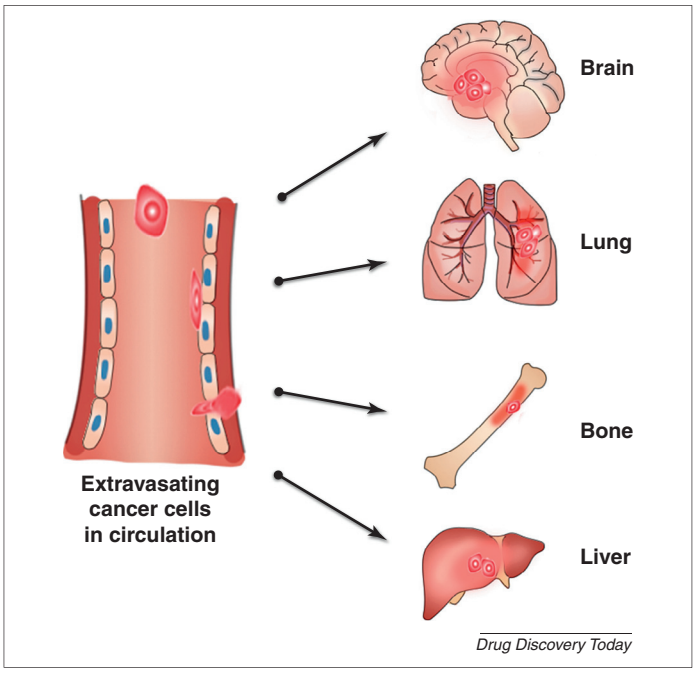

FIGURE 1

Schematic of extravasation cascade. Primary cancer cells travel in the circulatory system and transmigrate across the endothelium to extravasate into secondary sites and colonize at organs such as lung, bone, liver and brain. Predominant primary cancer sites where initial dissemination occurs include breast, pancreas, prostate gland, colon and lung.

determining the attraction of tumor-associated cells, which contribute to the development of a receptive environment [29,33–35] and promoting specific cancer cell homing. Particularly, breast cancer cells often metastasize to the bone and autopsy studies have demonstrated that 70% of patients with breast cancer have skeletal metastases, which represent the major cause of lethality and induce pain, spinal cord compression and fractures, severely compromising quality of life [36,37].

Unraveling the multiple steps of extravasation could enable the identification of new anticancer drugs to inhibit the adhesion and/or transendothelial migration of metastatic cells. *In vitro* testing platforms represent a useful tool, but the lack of organ-specific models, reproducing the human *in vivo* microenvironment and tissue tropism shown by specific cancer cells, constitutes a significant limitation among current systems.

In vivo and *in vitro* cancer models for invasion, migration, extravasation and colonization

Although no *in vivo* or *in vitro* model fully replicates the complex milieu of factors that influence metastasis in humans, there have been numerous studies devoted to understanding cancer cell invasion, migration and interactions with the endothelium, which comprise different stages of cancer metastasis. Conventional studies of metastasis have been mostly limited to *in vivo* mouse models because there is a lack of tumor models and methods to study the associated processes *in vitro*. Mouse models provide a platform to screen for genes involved in metastasis for specific organs or proteins that mediate cancer invasion [38–40]. Roles of chemical factors and different signaling mechanisms that trigger each step of metastasis have also been studied [41–43].