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Studying cancer metastasis: Existing models, challenges and future perspectives

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ABSTRACT

Cancer metastasis causes most cancer-related deaths. Several model systems to study the complex and multi step process of metastasis exist, including *in vitro* systems, *ex-vivo* organ slices, *Drosophila Melanogaster* and zebrafish models and the use of the chorio allantoic membrane (CAM) of fertilized chicken eggs. These models are relatively easy and cheap but often lack the opportunity to study the complete metastasis cascade. More complex but also more expensive is the use of animal models including the more recently developed patient derived tumor xenografts (PDTX). In this review, we give an overview of the existing metastatic models, discuss the challenges of improving current models to enhance translation from the preclinical to the clinical setting and consider future perspectives.

1. Introduction

Cancer metastasis is a complex and multi-step process, wherein cancer cells detach from the primary tumor, lose their epithelial polarity, degrade and invade the basement membrane and extracellular matrix (ECM) to reach the capillary blood, spread and finally home in distant organs (Kang, 2009; Ramis-Conde et al., 2009; Chambers et al., 2002). It is the most destructive stage of cancer progression and is responsible for nearly all cancer-related deaths (Kang, 2009; Ramis-Conde et al., 2009; Zaman, 2007; Mehlen and Puisieux, 2006). Therefore there is an urgent need to study the metastatic process in appropriate models to find relevant mechanisms and targets for improvement of treatment outcome.

Due to recent progress in technology, possibilities to study the metastatic cascade are rapidly increasing. In this review, we give an overview of existing non-animal and animal models for study-

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ing subsequent steps in the metastatic process. We discuss the challenges of improving current models to enhance translation of promising preclinical results into improved treatment results for cancer patients, and consider future perspectives.

The search strategy in PubMed included the terms cancer, metastasis, preclinical model, animal model, *in vitro* model, *in silico* model, patient derived (tumor) xenograft model, humanized mouse model, clinical trials, 3D bioprinting and organs-on-chips.

2. Existing models

2.1. Non-animal models

2.1.1. In vitro *and* in silico *model systems*

In vitro and *in silico* models are extensively used for studying cancer biology and metastasis since they are easy to establish, cheap and often very reproducible (Van Zutphen and Baumans, 2012). Although these models are simplified model systems with each their advantages and disadvantages (Table 1), they may allow for individual target validation and subsequent selection of suitable treatments. Here, various models are depicted by exploring them along the path of the metastatic process.

Cell cultures, used either in primary cultures or as continuous cell lines (Van Zutphen and Baumans, 2012), contributed to a great extent to the understanding of cancer biology and to the development of rational therapeutic approaches (Bunn and Foss, 1996). They are valuable as a basic model, which can be used as a starting point for testing hypotheses. Increased phenotypic flexibility of tumor cells, for example when they undergo EMT, can be mimicked in vitro. For example, EMT can be examined by immunofluorescent staining for E-cadherin, cytokeratin 18 and vimentin (Bertran et al., 2013). *In vitro* invasion and migration with cell lines identified the role of several proteins in cancer cell metastatic behavior and in the first screening of intervention strategies (Huang et al., 2013; Liu et al., 2013; Barak et al., 1983). Analyses with ovarian cancer cells lines and migration assays revealed a role of the chemokine CXCL12 in cell migration (Jiang et al., 2007) and a role of G protein-coupled estrogen receptor in migration and invasion (Yan et al., 2013).

Probably more than any other cancer cell related process, metastasis is the result of the interplay between the tumor cell and its microenvironment. Coculture systems of tumor cells and stromal cells are the simplest systems to study the interactions between tumor cells and their microenvironment. Coculturing of tumor cells together with mesenchymal stem cells increases the metastatic ability of tumor cells (Luo et al., 2014; Lis et al., 2014).

More complex organoid cell cultures, obtained by growing single stem cells under special conditions supporting the growth of the various cells of an organ, display all hallmarks of the organ in terms of architecture, cell type composition and self-renewal dynamics (Sato et al., 2011). Furthermore, organoids showed to maintain the genomic status of the patients' tumor (Gao et al., 2014). Whole organ explants for various cancer types, such as human colon, adenoma, adenocarcinoma and Barrett's epithelium organoids, organoids for advanced prostate cancer and cystic fibrosis intestinal organoids have been developed (Sato et al., 2011; Dekkers et al., 2013; Nguyen-Ngoc et al., 2012). Human vascular organoids were used to map the extravasation process for human breast cancer metastasis. The organoids were formed by co-implantation of human endothelial cells and mesenchymal cells in basement membrane-like matrix and inoculated subcutaneously into immunodeficient mice. In an orthotopic human breast cancer model, disseminated human breast cancer cells were shown to efficiently colonize organoids, connected to the mouse circulatory system. Human breast cancer cells could be clearly detected at different stages of the metastatic process: initial arrest in the human microvasculature, extravasation, and growth into avascular micrometastases (Fernandez-Perianez et al., 2013).

Organs, pieces of organs or tissues, such as slices of tumor tissue, can represent a mini-model of the organ and contain all cells of the tissue in their natural environment, leaving intercellular and cell-matrix interactions intact (de Graaf et al., 2010). Tumor tissue slices are applicable in invasion and migration assays (Jung et al., 2002).

In silico models are helpful to analyze the metastatic processes with information obtained in using *in vitro* or *in vivo* experiments. Although extrapolation to humans remains difficult, mathematical models significantly contributed to the understanding of tumor cell behavior. Variables are used in formulas to simulate various conditions (Zaman, 2007; Huang et al., 2013). For example, multiscale probabilisitic framework was used to model early steps in tumor metastasis. Cell migration could be studied mimicking the 3D environment of cells. By 'filling' cubic lattices with deformable cells and protein ligands, such as collagen, laminin or fibronectin, movement of cells in the epithelial layer, basal lamina invasion and migration in loose connective tissue could be analyzed (Zaman, 2007).

As a next step in the metastatic cascade, the residence of tumor cells in the circulation can be modeled. Hemodynamic forces in vessels and interactions of tumor cells with the endothelial cells of the vessels can be studied with a parallel-plate flow chamber. Endothelial cell monolayers are cultured on the plates of the system, after which shear stress is measured by applying different flow conditions, or tumor cell adhesion is examined by injecting a tumor cell suspension in the flow loop (Haddad et al., 2010).

Single steps in metastasis, such as the detachment of cells from the primary tumor, degradation and invasion of the basement membrane and extracellular matrix to reach the capillary blood and residence in the circulation can be studied in the models described above, but the investigation of systemic cell spread and preferential homing in distant organs needs more complex model systems.

2.1.2. *In vivo non-animal model systems*

The metastatic process is also studied in model organisms that socially and legally are not considered as animal models (Van Zutphen and Baumans, 2012). Those models overcome the disadvantages of *in vitro* models by containing a microenvironment in which tumor cells are growing. For example, the fruit fly *Drosophila melanogaster* was used to model the initiation of metastasis during the development of cancer *in vivo*. The clonal nature of mammalian cancer cells by using Mosaic analysis was mimicked with a Repressible Cell Marker (MARCM) system. This allows simultaneous manipulation of multiple genes in combination with the incorporation of a marker in small populations of cells (Kango-Singh and Halder, 2004; Lee and Luo, 1999). For example, β-galactosidase-labeled brain tumor cells, induced by mutation of *lgl* or *brat*, transplanted into the abdomens of wild-type flies, were detected in the ovary of the fly (Lee and Luo, 1999; Beaucher et al., 2007). With *D. melanogaster*, detachment of tumor cells from the primary tumor until their distant homing to form metastatic lesions can be studied, although conditions not fully resemble mammalian systems.

In zebrafish, many genes are highly conserved, including genes involved in the oncogenic pathways, such as the Myc family of oncoproteins, and genes encoding for chemokines and their receptors, which are involved in metastasis (Schreiber-Agus et al., 1993; Zlotnik, 2006). Despite this, various non-conserved exceptions limit the recapitulation of the genetic complexity of human tumors in zebrafish. To overcome these limitations, transient genetic modifications of zebrafish can be readily achieved. This is already possible in early embryo stages of the zebrafish (Konantz et al., 2012). One example is the overexpression of genes *via* direct microinjection of messenger ribonucleic acid (mRNA), inducing