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Master thesis

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Inception of the metastatic phenotype by macrophage-cancer cell fusion.

Do cancer cells live a macrophage dream?

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Abstract

It is now well-established that patient outcome are good if the primary tumor has not spread; can be surgically removed; treated locally with radiation; or preventive chemotherapy can be applied. In contrast, when cancer cells have disseminated, the mortality rates increase significantly and therapeutic treatment strategies are eventually switched from curative to palliative. Although responsible for the majority of cancer deaths, knowledge about the emergence of metastatic cells remains scarce. Nevertheless, *in vivo* evidence in animal models and humans is accumulating and supportive of the century-old macrophage-cancer cell fusion theory. The theory implies that the metastatic cells emerge from a fusion event and express the phenotypes of the migratory macrophage and the proliferative cancer cell. Although the theory is steadily gaining approval from cancer researchers, it still lacks essential attention from the broader research community. We will review the current knowledge surrounding the macrophage-cancer cell fusion theory. It is our believe that work on the fusion theory and in the research fields related to it will enhance our understanding of metastasis, and ultimately lead to the development of novel and effective strategies for the treatment of metastatic cancers.

Introduction

Cancer is a common disease that represents a major worldwide public health problem. Cancer is an uncontrolled proliferation of cells in a primary lesion that can spawn metastatic cells. Those are capable of invading surrounding tissues, spreading systemically and seeding secondary lesions. The disease is thought to take its cellular origin in the accumulation of somatic mutations in certain proto-onco- and tumor suppressor-genes. The mutations are mainly caused by environmental (90 – 95% of cases), but also hereditary factors (1). The initial mutations together with epigenetic changes give rise to an uncontrolled proliferating cell that is resistant to apoptosis and displays intrinsic genomic instability. It has been estimated that about 7-8 oncogenic mutations are required for a malignant transformation in sporadic colon cancer (182).

Further gain-of-function mutations award the cell clones with higher and higher proliferative indices and selective advantages over normal cells.

After unraveling numerous genetic alterations and pathways involved in the process of oncogenesis, of developing animal models that mimic the human disease phenotypes and of successfully implementing targeted therapies, the focus lies more than ever on cancer metastasis research. The importance of studying the genesis of metastatic cells and defining therapeutic targets of their spread reflects in the clinical fact that of approximately 7.9 million cancer deaths annually, 90% are due to metastasis (2,3). Knowledge of the intrinsic processes and microenvironmental interactions involved in cancer metastasis is accumulating, but in contrast to cancerogenesis however, only hypothetical models exist, as to what the initiating mechanisms of metastasis are.

The hypothesis that cell fusion underlies the emergence of metastatic cells was first proposed by Otto Aichel in 1911 and based on observations with the light microscope (4). Meanwhile, plenty of studies have outlined numerous genotypic and phenotypic similarities between migratory bone marrow-derived cells (BMDCs), such as macrophages, and metastatic cells. *In vitro*, *in vivo* and clinical data from recent studies is pointing towards a fusion event between macrophages and cancer cells as the origin of metastatic cells (4). Coinciding, research on tumor-associated macrophages (TAMs) has revealed direct roles for TAMs in every single step of the malignant progression (5). The fusion theory therefore comes closer to a unifying explanation of tumor progression than any yet proposed model, and we take it as an imperative to examine its clinical relevance, especially since there is accumulating evidence.

In the following chapters the widely accepted models for the emergence of metastatic cells (metastatogenesis) and the general macrophage and TAM biology will be discussed. Thereafter, the current understandings of macrophage and cancer cell fusion mechanisms are going to be reviewed and the macrophage-cancer cell fusion theory will be introduced. At last, the most important common traits between macrophages, macrophage-cancer cell hybrids and metastatic cells will be highlighted and a hypothetical model of fusion-dependent metastasis is going to be presented.

Metastasis

Metastasis is a multi-step process that depends on special cancer-cell properties there is no need for in the primary tumor site, nor in secondary lesions (6). At some point, cancer cells from the primary lesion manage to disseminate and proliferate in foreign tissues until they cause the dysfunction of vitally essential organs. The most frequently seeded metastasis sites of solid tumors are the nearby lymph nodes, the lungs, the bones, the liver and the brain (7). Malignant cells must succeed in the following cascade of sequential and interrelated steps to form metastasis: separate from the primary tumor, invade surrounding tissue stroma, intravasate the blood and lymphatic vasculature, survive and traffic in the circulation, arrest in distant capillary beds, extravasate and proliferate in foreign tissues (8). It is important to note that the cascade of events varies between metastatic cancers of different tissue origins.

One of the cancer cells earliest metastatic phenotypes is characterized by the abilities to detach from neighboring epithelial cells and to disrupt its actin cyto-skeleton, allowing the cell to invade through the basement membrane into the extracellular matrix (ECM;11). This migratory phenotype resembles characteristic features of mesenchymal progenitor-cells. The acquisition of the metastatic phenotype is referred to as epithelial-to-mesenchymal transition (EMT;12). EMT is a developmental process during which the expression of genes involved in cell adhesion, mesenchymal differentiation, cell migration and invasion are upregulated. E-cadherin downregulation is associated with cancer progression and poor prognosis in human and mouse cancers and is therefore a hallmark of EMT (13). The activation of the EMT program requires transforming growth factor (TGF)-β-, Wnt-, integrin, NFκB or other tyrosine kinase receptor-signaling (MNNG HOS transforming gene [Met], fibroblast growth factor [FGF], insulin-like growth factor-binding protein [IGF], epithelial growth factor [EGF] and platelet-derived growth factor [PDGF]) pathways to activate EMT-inducing transcription factors, such as SNAIL, SLUG, δ crystalline enhancer binding factor (EF)-1, smad interacting protein (SIP)1, TWIST1, forkhead box (FOX)-C2, and Goosecoid (12). Gene expression studies on human tumor samples and experimental animal models delivered numerous indications for EMT to be essentially involved in the metastatic transformation (14-16). Even so, the involvement of EMT in metastatic colonization is highly controversial. This

is in part due to clinical studies that have found the majority of human breast carcinoma metastases to express E-cadherin and appear to maintain their epithelial rather than mesenchymal cell morphology (26,27). On this basis, there is no direct clinical evidence for EMT in tumor dissemination.

Whether the processes underlying the emergence of metastatic cells are based on genetic selection, reversible adaptation to the microenvironment and/or other mechanisms is still under debate (181). Nowell was the first to propose the widely accepted "clonal evolution" model for cancer. In this model tumor cells acquire genetic changes, which are selected for in a Darwinian process and lead to clonal expansion of the fittest tumor cells. Metastatic cells are considered to arise from pre-existing cancer cells through a step-wise accumulation of gain-of function mutations (9) and emerge in the latest phase of the malignant progression (10). Notably, the common ancestry of primary cancer cell and metastasis cell clones is evident from comparative gene expression profiles (185). According to the "clonal evolution" model, research in the cancer metastasis field has defined major hallmarks for cancer cells to successfully metastasize. Extrinsically and/or intrinsically caused DNA alterations and a lack of DNA-checkpoint proteins, collectively causing intrinsic genomic instability, are thought to be the ultimate prerequisites for metastasis. They form a heterogeneous cell population within the primary tumor that in conjunction with metastasis promoting cells of the microenvironment spawns clones with a metastatic phenotype.

Studies on breast cancer patients and mouse models have demonstrated that disseminated cancer cells display different and fewer aberrations than their primary tumors and that subsets of cancer cells disseminate before the onset of chromosomal instability and microscopic invasion (20-23,184,186). Furthermore, five percent of cancer patients are diagnosed with metastatic cancers of unknown primary origin. If ever, small, well-differentiated lesions can be found during autopsy, which according to the "clonal evolution" model harbor far too less cells to spawn clones with a high metastatic potential (24). Together with the discovery of cancer gene-expression signatures in early primary cancers that are predictive of metastases (183) these findings implied that early primary cancer clones harbor a global metastasis-promoting genetic make-up and evolve independently (or parallel) of the primary tumor. Moreover, common mutations in

primary cancers and their metastasis were suggested to be less a product of linear progression, but to be metastasis-promoting polymorphisms in germline susceptibility genes (187). Hence, the "clonal evolution" model was in great parts refined by the so-called "parallel evolution" model, which explained the lack of translational success in cancer therapy, since treatment was traditionally designed according to primary tumor diagnosis. Studies in animal models have revealed that an intriguingly low number of about 0.01% of invasive cancer cells entering the circulation actually develop into metastases (17,18). In line with the parallel evolution model, this implies a rather dynamic view of metastasis, wherein metastasis-propagating cells are rare and exist in a metastatic compartment that would seed metastasis in distant organs as a result of contextual interactions with the microenvironment. This notion together with findings that certain cancer cell subpopulations were capable of self-renewal, to propagate a tumor following serial transplantation and reconstitute the cellular heterogeneity of an initial tumor mass lead to the discovery of cancer stem cells (CSCs, 188). In support of the existence of CSCs, gene-signatures specific for CSCs can be detected in the bulk tumor mass and correlate with poor prognosis in breast cancer patients (189,190). Nevertheless, with regard to the metastatic competence, considerable heterogeneities in the tumor's CSC compartments have been observed in melanoma and pancreatic cancers (191-194). Thus, the question remains as to what the fundamental mechanisms are that regulate the inception of metastasis-founding cells.

In summary, it is indeed tempting to point at gene mutation as the major driving force behind cancer, unifying the processes of cancerogenesis, malignant progression, and metastatogenesis in a reductionistic disease model that resembles Darwinian selection. However, in the light of recent evidence, it is reasonable to refine the current models and propose that additional mechanisms are at work during metastatogenesis, to cause high metastasis incidence rates, such as 10% for breast cancer patients (19).

Macrophages

Macrophages are leucocytes found in virtually all tissues including bone (osteo-clasts), brain (microglia), liver (Kupffer cells), kidney (mesangial cells) and lung (alveolar

macrophages). They directly descend from blood monocytes, which originate from a mesenchymal myeloid progenitor cell in the bone marrow. The engagement in different organs and migration throughout the body demands abilities to invade tissue, change shape and adapt to new environments. Thus, macrophages are amongst the most versatile cells with respect to their migratory potential.

Along with their broad tissue specificity comes an exceptionally wide array of cellular functions that are crucial to immune responses, development and tissue homeostasis. To name a few, macrophages are empowered to engulf and digest cellular debris and pathogens, to regulate innate and adaptive immune cells via the secretion of cytokines and chemokines, to fuse homo- or heterotypic for the development of specialized cells, to trans-differentiate and to degrade the extra-cellular matrix via the secretion of proteases (28-32).

Depending on the environmental clues, macrophages generally have two distinct activation states: the classically activated (M1, effector) phenotype and the alternatively activated (M2) phenotype (33). The M1 phenotype is usually induced by a combination of a priming inflammatory cytokine, such as interferon-gamma (IF-γ) and a secondary stimulus, usually provided by toll-like receptor activation (33). Effector macrophages are instructed to kill microbes and are marked by their production of nitric oxide (NO), reactive oxygen species, tumor necrosis factor (TNF), interleukin (IL)-6 and other inflammatory cytokines. In contrast, the M2 phenotype is activated by exposure to anti-inflammatory cytokines of a helper T-2 cell (TH2) immune response, such as IL-4, IL-13 and IL-10. Alternatively activated macrophages are generally instructed to promote wound healing or to secrete anti-inflammatory cytokines that regulate and terminate inflammation (33). They are characterized by their poor antigen presentation, secretion of immunosuppressive IL-10, their secretion of angiogenic factors, their ability to phagocytose dead cells and cellular debris, to remodel damaged tissue and to fuse homotypic to form phagocytic giant cells (34-37).

Tumor-associated macrophages

Besides being present in tissues under physiologic conditions, macrophage involvement has been implicated in several pathogenic processes, such as cancer, atherosclerosis and rheumatoid arthritis. Macrophages are found to reside in the stroma of cancers, and can constitute up to 80% of the total tumor mass (38). Clinical studies of human cancers demonstrate a positive correlation between macrophage infiltration and tumor progression (5,39,40). Furthermore, genes that are associated with macrophage infiltration have been identified as part of expression signatures that predict poor prognosis in breast carcinomas (41). Due to these facts, substantial cancer research was dedicated to understanding the role of TAMs.

TAMs have been characterized as $CD11b^{+}F4/80^{+}CSF1R^{+}GR1^{-}$ cells that have a high constitutive expression of IL-1 and IL-6, and a low expression of TNF-α (42). They were found to promote the tumor angiogenic switch, to be essential to cancer cell invasion, migration and intravasation, to suppress anti-tumor immune responses and appear not only to set up preferred sites for metastatic cell seeding, but to engage in cancer cell extravasation and the establishment and growth of metastatic lesions (5). That being the case, they have a direct role in every critical step of metastasis. Important to note, work by Mantovani and colleagues has suggested that exposure to IL-4 and IL-10 in the tumor microenvironment actually generates alternatively activated macrophages, which engage in wound healing or immune-regulation (35,43).

Macrophage fusion mechanisms

Cell fusion is a common biological process that produces viable cells involved in mammalian development and differentiation. Among mammalian fusion-competent cell types, macrophages harbor a special niche, since they do not undergo fusion as an essential part of their developmental program. Macrophages fuse rather rarely to form osteoclasts or multinucleated giant cells (MGCs) and pertain their nuclear integrity within a shared cytoplasm (heterokaryons; 44). The fusion of macrophages increases their size and allows them to resorb larger targets/foreign bodies that cannot be internalized by a single cell. Instead of degrading the target in the lysosomes, MGCs acquire the ability to

degrade it in an extracellular compartment, which has a low pH and contains lysosomal enzymes (45).

In vitro, macrophages and monocytes form osteoclasts or MGCs in the presence of macrophage colony stimulating factor (MCSF)-1 and receptor activator of nuclear factor kappa-B ligand (RANKL) or granulocyte macrophage colony stimulating factor (GM-CSF), IL-4, IL-13, and monocyte chemoattractant protein (CCL-2/MCP)-1, respectively (46-48). The capacity to form MGCs depends on the primary macrophage population and the microenvironmental conditions. Especially thioglycollate-elicited peritoneal macrophages show the highest induction of fusion competence in response to IL-4 (49). IL-4 has been suggested to signal through the IL-4 receptor α-chain and the signal transducer and activator of transcription 6 (STAT6) to upregulate E-cadherin and the dendritic cell-specific transmembrane protein (DC-STAMP;50,51).

Prior to fusion macrophages are instructed to undergo aggregation, which is most likely regulated by chemotactic factors, such as CCL2 (48,52,53). It has been hypothesized that the DNAX activating protein (DAP)-12 and its associated triggering receptor expressed on myeloid cells (TREM)-2 are activated by homotypic macrophage interactions via an intrinsically expressed, uncharacterized ligand. This activation leads to the induction of DC-STAMP, matrix metallo-protease (MMP)-9 and E-cadherin, all of which involved in macrophage fusion (54).

At least one of the cells designated to fuse is required to enter a "prefusion" state by signaling through DC-STAMP (55). The seven-transmembrane receptor (TM7) DC-STAMP is identical to the viral TM7s C-X-C chemokine receptor type (CXCR)-4 and C-C chemokine receptor (CCR)-5, which are involved in viral membrane fusion, and is therefore thought to either regulate or mediate fusion via as yet unknown mechanisms (55).

Subsequently, membrane proteins of the immuno globulin super family (IgSF) form adhesive interactions between the plasma membranes of the fusion partners and initiate intracellular signaling (56-58). The myeloid specific macrophage fusion receptor (MFR/SIRPα) is highly expressed before fusion and binds the ubiquitously expressed thrombospondin 2 receptor (CD47). The ligation downregulates phagocytosis. It is therefore assumed that phagozytic macrophages, recognize CD47 as a reciprocal signal of

self – leading to multinucleation rather than lysosomal degradation of the internalized cell (59). In addition, the finding that overexpression of CD47 leads to cell death, raised the hypothesis that CD47 may creates a membrane pore that triggers macrophage fusion once the opposite cell membranes are apposed and stable, as has been observed during myoblast fusion (60,61).

The expression of the hyaluron receptor (CD44) is elevated at the onset of fusion. The cleavage of CD44 by membrane type (MT)-1-MMP is assumed to entertain a closer interaction of fusing cells and its concomitant nuclear translocation of the intracellular domain was found to activate nuclear factor kappa B (NF-κB), which is required for osteoclast differentiation (62,63).

Lamellipodia formation precedes the fusion of macrophages and is induced by the activation of RAC1 (64). The activation of RAC1 is mediated by the guanine nucleotide exchange factor (GEF) dedicator of cytokinesis (DOCK)-180 and is essential for cytoskeletal rearrangements prior to fusion. Interestingly, the cytoplasmic tail of MT1-MMP was found to be involved in protease-independent RAC1 activation and deficiency for MT1-MMP attenuated macrophage fusion and giant cell formation *in vitro* and *in vivo* (65). It is known from other studies that the cytoplasmic tail of CD44 interacts with the actin cytoskeleton and as such directs MT1-MMP to lamellipodia by association with MT1-MMPs hemopexin-like domain (66). These observations suggest that complex formation of CD44, MT1-MMP, RAC1 and DOCK-180 is essential to induce lamellipodia formation and fusion.

E-cadherin depletion lowers the number of IL-4 induced MGC formations, suggesting its involvement in setting fusion competence in alternatively activated macrophages (67). E-cadherin/catenin (p120-,α-,β-catenin) protein complexes form at the plasma membrane and represent in vivo markers of alternatively activated macrophages. Complex formation is induced by IL-4 and IL-13 via STAT6, the expression of arginase-(Arg)-1 and production of polyamines. Fluorescence microscopy revealed that IL-4 induced E-cadherin/catenin complex formation is preferentially located at regions of cell contacts and is involved in homo- and heterotypic cell interactions with CD103 and killer cell lectin-like receptor (KLR)-G1 (67). On that account, we hypothesize that E-cadherin is involved in mediating adhesion prior to homo- and heterotypic fusion.

Additional cell surface molecules, which are involved in macrophage fusion, are the mannose receptor and beta- 1 and -2 integrins (68-70).

The lipid composition of fusing cells is important for hybridization. At the contact area of fusing macrophages phospatidylserine (PtdSer), which is normally confined to the inner leaflet, integrates into the outer membrane leaflet. During IL-4 induced MGC formation PtdSer is exposed and then recognized by CD36 for fusion (71). Interestingly, exposure and recognition of PtdSer are also involved in the phagocytosis of apoptotic cells (72).

Although knowledge of the mechanisms involved in macrophage fusion is accumulating, a lot of unknowns remain to be elucidated. It seems likely that research on macrophage fusion will not only lead to an advanced understanding in the field of metastatogenesis, but will undoubtedly result in new therapeutic targets of metastasis and the metastatic spread.

Cancer cell fusion

Fusogenic cells have been found in a variety of cancers including melanoma, breast, renal, liver, gall bladder, lymphoma and brain (73). Cancer cells fuse spontaneously with several types of somatic cells, including stromal, epithelial, endothelial cells and bone-marrow derived cells (74-79). The hybrid cells are viable and capable of forming mitotic figures containing mixed parental cell chromosomes. Initially, fusing cells form tetraploid binucleated hybrids with synchronous or asynchronous nuclei and a doubled amount of centrosomes. The nuclei eventually fuse, creating a mononucleated cell. Fused nuclei are characterized by nuclear subcompartmentation, displaying both parental cells chromosomes that do not intermingle (80). Nuclear subcompartmentation is a phenomenon also found in mouse preimplantation embryos and is preserved through several rounds of cellular division.

The generation of tetraploid cells and supernumerary centrosomes results in aneuploidy and chromosomal instability (CIN;81). Notably, aneuploidy can lead to cancer, given the existence of defective tumor suppressor mechanisms (82,83). CIN promotes diversity among cancer cell populations by causing chromosomal aberrations and through cascading changes in genome-wide gene expression. However, supernumerary

centrosomes do not always lead to chromosomal instability. By as yet unknown mechanisms, extra centrosomes can be coalesced, discarded or orphaned, which restores a robust chromosomal stability over long-term proliferation *in vitro* and *in vivo* (84). It has even been hypothesized that mechanisms suppressing spindle multipolarity are inactivated during the early stage of tumorigenesis, which creates heterogeneous populations of cancer cells and are activated randomly during tumor progression, which allows the clonal outgrowth and survival of a dominant population (85).

Numerous studies have reported *in vivo* cancer cell fusion (86-95). Although fusion is a rather rare and controlled event that takes place under specific conditions, cancer cell fusion occurs frequently in experimental tumor models (96). Several reasons might account for the increased fusogenicity of cancer cells. First, deregulation of exogenous and/or endogenous fusogenic proteins promotes the fusion of cancer and host cells. Second, an inflammatory wound healing microenvironment, which is frequently found in primary cancers, has been shown to favor cell fusion between hematopoeitic and various somatic cells during tissue repair and regeneration (97-101). Third, cell fusion might represent a by-product of phagocytosed cancer cells by macrophages, which are highly abundant in the tumor stroma.

Chakraborty and colleagues have delivered the first direct evidence of cancer cell fusion as an initiating mechanism of metastasis in a mouse model of cancer. Cloudman S91 mouse melanoma cells that are wildtype for the melanogenesis rate-limiting enzyme tyrosinase (C/C) were implanted s.c. into the tail of Balb/c mice, which are homozygous for a tyrosinase loss-of-function mutation (c/c; 93). Spontaneous lung metastasis formed rarely, were fairly small and amelanotic. One of the mice however formed melanin-producing metastasis in the proximity of the implantation site. In the following weeks a highly pigmented pulmonary metastasis formed. Interestingly, metastatic cells had a 30-40% increased DNA content, showed increased *in vitro* chemotaxis, activation of N-acetylglucosaminyltransferase V (GnT-V), produced β1,6-branched oligosaccharides, contained malanosomes (melanin-containing granules), adapted dendritic cell morphologies and were heterozygous for tyrosinase (C/c), indicating they were hybrids between cancer and host cells. Since C/c hybrids producing melanosomes were also

found in the macrophage infiltrated implantation site it was proposed that fusion had occurred in the original implant, likely with TAMs.

According to the cell fusion theory, BMDC-cancer cell hybrids acquire genetic and phenotypic features from both mother cells. In support of this notion, *in vitro* hybridization of a mouse melanoma cell line with human monocytes resulted in the expression of human genes in the metastatic hybrids (102). Furthermore, a recent study by Powell and colleagues revealed that cancer cells primarily fuse with blood-derived macrophages as a natural course of tumor progression in a parabosis mouse model of intestinal cancer (101). The hybrids retained a transcriptome identity characteristic of both, the macrophage and epithelial cell parent, while also expressing a unique subset of transcripts, distinguishing them from their parental lineages. Thus, metastatic macrophage-cancer cell hybrids are thought to co-express traits of motility and homing from the macrophage parent, coupled to the background of deregulated growth and apoptosis resistance from the cancer cell parent.

In order to develop such phenotypic expression upon fusion, genetic reprogramming of the nuclei is a critical step during metastatic hybrid formation. Hybrid-reprogramming activity has been studied in liver regeneration. BMDCs have fused with hepatocytes in order to regenerate liver parenchyma that lacked the fumarylacetoactetate hydrolase (Fah) gene. Upon fusion of $Fah^{+/+}$ bone-marrow derived donor cells with $Fah^{-/-}$ hepatocytes, genes, including Fah, that were otherwise silenced in the solitary donor cell became activated and vice versa, to promote the regenerative process (97). This implicates, that a similar genetic reprogramming activity takes place upon macrophage-cancer cell fusion and may play a critical role during metastatogenesis. If this assumption holds true, genetic reprogramming of hybrids likely poses an attractive target for anti-metastatic therapies.

In contrast to cell cultures and animal models of cancer, which allow for an easy detection of hybrid cells, the lack of applicable genetic tracking tools makes it difficult to detect and analyze fusion events in human cancers. The three most striking direct observations of BMDC-cancer cell fusion in humans were made in patients that received bone-marrow transplants (BMT) prior to the development of clinical cancers. The first reported case was found in a child following a BMT from its cancer-free brother.

Analyses of lymph node metastasis revealed that the metastatic cells contained the donor-specific A allele of the ABO blood group, indicating that BMDCs might have fused with renal carcinoma cells (74). In the second case, a woman received a BMT from her son two years prior to detection of a renal cell carcinoma. Fluorescence *in vitro* hybridization (FISH) analyses in formalin-fixed tissue samples revealed that 10% of the cancer population consisted of Y chromosome-containing cells, indicating that fusion events occurred between cancer cells and BMDCs (103). However, due to technical reasons both studies lack definitive proof for BMDC-cancer cell fusion (reviewed in 4). Thus, the as yet sole confirmation of *in vivo* cell fusion in humans was observed in multiple myeloma patients (104). Multiple myeloma is a B-cell neoplasm that is nearly always associated with bone destruction and an increased numbers of bone-resorbing osteoclasts. The study revealed that osteoclasts contained nuclei with translocated chromosomes of myeloma B-cell clone origin, suggesting that fusion events have taken place between myeloma cells and macrophages/osteoclasts. The nuclei were transcriptionally active and contributed to more than 30% of the osteoclast population.

Collectively, fusion of cancer cells with somatic cells represents a frequent event during cancer progression in mouse models and has been observed in human primary cancers and metastasis. Nevertheless, there is great need for tracking tools, to investigate the exact impact of fusion events on cancer progression in human patients.

Macrophage-cancer cell fusion

As noted earlier, developing a metastatic phenotype requires cancer cells to become invasive, adapt a higher motility, intravasate the vasculature, survive in the circulation, extravasate at distant capillary beds and survive and grow in unfamiliar hypoxic microenvironments. The discrepancy with the development of such phenotypic abilities, solely by gain-of-function mutations as suggested by the "clonal evolution" model, hails from the fact that all of the properties are rarely of selective advantage in the primary tumor site.

Wound-healing M2 macrophages share significant characteristics with metastatic cells. Besides their tissue tropism, the resemblance is evident during the following

inflammatory response. Monocytes are recruited to extravasate the blood circulation and invade the wounded tissue, where they differentiate into macrophages. Macrophages survive in necrotic and hypoxic areas to phagozytose dead cells and cellular debris. To increase their phagocytic capacity they occasionally fuse with each other and become giant cells. After their wound healing activity, macrophages intravasate into the lymphatic vasculature and home to the lymph nodes, where they participate in immune responses (105). Thus, the macrophage-cancer cell fusion theory suggests M2 macrophages to pose the ideal fusion partners of cancer cells, to acquire a metastatic phenotype without the need for additional mutations.

In several studies fusion between cancer cells and somatic cells of the tumor microenvironment was induced *in vitro* and the hybrids were analyzed for their metastatic potential (4,106,107). When transplanted into mice, hybrids of cancer cells or of cancer cells with epithelial cells or fibroblasts had a reduced tumorigenicity, compared to the parental cancer cell. In contrast, macrophage-cancer cell hybrids displayed the most aggressive metastatic phenotype and *in vivo* studies revealed that macrophages represent the primary fusion partner of cancer cells (88,101). Consistently, Rachkovsky and colleagues found that ~50% of *in vitro*-induced macrophage-cancer cell hybrids displayed an increased metastatic phenotype, when grown *in vitro* in the absence of any host-selective pressure (94). The great extent of hybrids developing a metastatic phenotype is remarkable, considering the chaotropic nature of aneuploidy. Together, these findings indicate that metastatic cancer cell hybrids that form *in vivo* are most likely of myeloid origin.

Several studies have investigated the expression of myeloid specific markers in breast cancer, rectal cancer and small cell lung carcinoma (SCLC) cells. Amongst the analyzed markers were CD26, C3bi, CD11b, CD14 and CD163 (108-111). In clinical rectal and breast cancer studies, between 30 and 50% of the patients had cancers with CD163-positive cancer cells (110,111). Notably, CD163-positive cancer cell patients had a more advanced histological grade, a higher occurrence of distant metastasis and reduced patient survival.

Below are outlined a few important phenotypic analogies of macrophages, macrophage-cancer cell hybrids and metastatic cancer cells that are supportive of the macrophage-cancer cell fusion theory.

Fusogenicity

According to the fusion theory, a rate-limiting step of metastatogenesis is the activation of fusion-promoting pathways and the subsequent fusion of cancer cells and macrophages.

As discussed earlier, it is hypothesized that interaction of CD44, MT1-MMP, RAC1 and DOCK180 induces lamellipodia formation and is essentially involved in macrophage fusion (64-66). Interestingly, CD44, MT1-MMP and RAC1 play critical roles in tumor progression. CD44 is highly expressed in various cancer types and overexpression of a CD44 variant in non-metastasizing rat cancer cell lines is sufficient to establish full metastatic behavior (112). RAC1 overexpression correlates with testicular, gastric and breast cancer progression. Knock-down of RAC1 in a xeno-transplanted human colorectal adenocarcinoma cell line suppresses tumor progression in a mouse model, whereas over-expression accelerates the tumorigenic process (113-116). Increased MT1-MMP expression in human primary tumors correlates with advanced tumor stages and poor outcomes in breast and colorectal cancer patients (117,118). Furthermore, MT1-MMP localizes to the protruding lamellipodia of migrating cancer cells and degrades the extracellular matrix. In contrast to the knock-downs of MMP1 and MMP2, which are secreted by fibrosarcoma and breast cancer cells during migration, silencing of MT1-MMP virtually blocked all invasive activities (119). On this basis, it appears that the complexation of CD44, MT1-MMP and RAC1 promotes macrophage-cancer cell fusion and consequently drives metastatogenesis. Intriguingly, the protease-independent recruitment of RAC1 by MT1-MMP may serve as an explanation for the failure of peptidomimetic MMP inhibitors to exert beneficial effects in previously reported clinical cancer trials (120).

$CD44^+$ bladder cancer cells, which efficiently induce tumorigenesis in xenograft models and predict poor prognosis in cancer patients, were found to highly express CD47 (121).

CD47 and SIRPα expressions correlate and are upregulated in the bone marrow of breast cancer patients with poor prognosis (122). In a mouse model of bone metastasis CD47 deficiency significantly reduced the metastasis burden without affecting the primary tumor and caused osteoclast defects (180). *In vitro* blocking of CD47-SIRPα interaction with an antibody induced phagocytosis of cancer cells by macrophages (121). Moreover, *in vivo* blocking of CD47 prior to radiation primes tumor cells more sensitive to radiotherapy (123). Since CD47 and SIRPα interactions have been implicated in macrophage fusion, we suggest that CD47 inhibition minimizes the "oncogenic resistance" (reviewed in the following chapters) of cancer cells and promotes their phagocytosis, via the suppression of macrophage-cancer cell fusion.

Another putative mediator of macrophage-cancer cell fusion is the chemotactic factor CCL2. Clinical studies on colorectal cancers have found that CCL2 expression is higher in primary tumors of patients with hepatic metastasis and can serve as a prognostic marker for predicting hepatic metastasis (124). Furthermore, CCL2 expression was shown to indirectly promote prostate tumor growth and metastasis, by recruiting macrophages to the primary lesion (125).

The upregulation and active involvement of fusion-promoting proteins in different cancers support a key role for cell fusion in cancer progression. We propose that macrophage-cancer cell fusion underlies the process of metastatogenesis and therefore poses an important therapeutic target of cancer metastasis.

Invasion and migration

ECMs represent steric barriers to cell migration. To overcome these heterogeneous barriers and transmigrate through endothelial walls, basal membranes and interstitial tissues cells have acquired various modes of cellular migration, such as amoeboid, mesenchymal and collective cell migration. Amoeboid migration is characterized by a rounded cell shape and the absence of both, proteolytic matrix degradation and strong adhesive interactions (focal adhesion). Mesenchymal migration is slow and characterized by an elongated cell shape, with long membrane protrusions, the presence of strong integrin-mediated adhesion sites and proteolytic degradation of the ECM (126).

Collective cell migration requires a group of cells to retain cell-cell contacts by adhesion junctions, to exhibit group polarization with a defined front-rear asymmetry and the secretion of proteases by the leading tip cells. Migration results either by adhesion of the leading edge to the ECM and contractile pulling, or from forward pushing by proliferation (127).

Leucocyte and especially macrophage migration were commonly associated with amoeboid migration, whereas invasive cancer cell migration was associated with amoeboid, mesenchymal and collective migration (128,129). Even so, a recent study on solitary cell migration through 3D matrices of different rigidity concluded that macrophages opt their mode of migration in dependence of the environmental architecture and geometry (32). They perform amoeboid migration in relatively porous fibrillar collagen 1 matrices, which mimic stromal/interstitial tissues. In contrast, they switch to mesenchymal migration in the rather densely cross-linked, gel-structured networks of a collagen 1, which mimic basement membrane matrices beneath epithelia and endothelia. The mesenchymal migration of macrophages can be grouped into two distinct movements: a slow, directional movement and a fast, bi-directional movement. Importantly, the later is very typical for cancer cells and forms persisting tunnels that have been suggested to facilitate the progression of following cancer cells through anatomic barriers. In fact, cancer cells migrating in a collective mode, such as in lobular breast cancer, epithelial prostate cancer, large cell lung cancer, melanoma and most prominently in squamous cell carcinoma follow a leading cell that generates microtracks (130,131). Thus, the leading cell of collectively migrating cancer cells likely has a macrophage-like mesenchymal migration mode. Both, cancer cell invadopodia and macrophage podosomes were found to have an identical co-localization of F-actin and β1-integrin and to secrete proteases at the interface to the ECM. Although the data cannot provide an explanation as to why cancer cells have a mesenchymal migration mode in porous fibrillar collagen-1, rather than an amoeboid such as macrophages, its certain that mesenchymal migration has an evolutionary advantage considering the formation of persisting microtracks for following cancer cells.

Integrins are transmembrane glycoproteins that besides conferring cell migration, are involved in cell adhesion, growth and survival. Control of cell migration requires the

redistribution of certain integrin molecules at the cell surface. Directional motility is achieved in part by integrin internalization at the retracting margins and their redistribution to the protruding lamellipodia. Interestingly, one of the most frequently observed macrophage traits in metastatic cancer cells is the elevated expression and/or activation of motility-associated integrins $\alpha 2$, $\alpha 3$, $\alpha 5$, $\alpha 6$, αv, $\beta 1$ and $\beta 3$ (132).

A bone marrow-derived $CD11b^+$ macrophage population, with dendritic cell morphology, has been found to express lymphatic vessel endothelial hyaluronan receptor (LYVE)-1, prospero-related homebox (PROX)-1 and podoplanin. Via a collective migration mode, similar to cancer cells, this macrophage population contributes to the formation of de novo lymphatic vessels from cell aggregates upon irritation of the cornea (133-135). Intrinsic podoplanin expression has been shown to mediate collective cancer cell invasion *in vitro* and *in vivo* in the absence of epithelial-to-mesenchymal transition, by inducing lamellipodia formation (136). Since another *in vitro* study revealed that the leading cells of collectively migrating carcinoma cells can be of a distinct cellular lineage (137), we propose a role for macrophage-cancer cell hybrids as heterotypic tip cells during collective cancer cell migration.

Furthermore, unpublished data by Kerjaschki and colleagues shows 15-Lipoxygenase (LOX)-1 expression by metastatic cancer cell lines to mediate cancer cell intravasation via the formation of circular defects in the lymphatic vasculature. Importantly, 15-LOX1 is strongly upregulated in alternatively activated macrophages that have been stimulated with the fusion-promoting TH2 cytokines IL-4 and IL-13 (138-140).

Conclusively, metastatic cancer cells and macrophages share identical invasive and migratory abilities, suggesting that the origin of these features in metastatic cells may stems from a macrophage-cancer cell fusion event.

Tissue tropism and homing

Another commonality of macrophages and metastatic cancer cells, supportive of the fusion-theory, is their tissue tropism to lymph nodes (LNs), lung, bone marrow, liver and brain. LNs represent the first and most frequently seeded sites of metastatic cancer cells and lymph node metastasis serve as valuable prognostic markers for cancer patients

(141). Although LN metastases per se are not life threatening, evidence is accumulating for their active involvement in the systemic spread of cancer cells (142). Typically, 20% of cells in the lymphatic vasculature are of the mononuclear phagocytic lineage. A great body of knowledge has already been accumulated of how cells of the immune system home through the lymphatic vasculature towards the LNs. With regard to the macrophage-cancer cell fusion theory, LNs pose an ideal model system to answer whether identical cytokines, chemokines and receptors involved in the homeostatic and inflammatory emigration of immune cells to the LNs, are critical for seeding metastasis in the LNs (143).

Recent work has revealed that the CXCR3, CXCR4 and CCR7 play critical roles in LN metastasis (142). The same chemokine receptors are involved in the homing and patterning of leukocytes to the LNs, suggesting that cancer cells expressing these receptors are recruited by their ligands in a similar manner as immune cells. In human colon cancer specimens CXCR3 was expressed in 33% of patient samples, and its expression correlated with LN metastasis and marked poor prognosis (144,145). CXCR3 expression is also associated with poor prognosis in breast cancer patients, compared to CXCR3-negative patients (146). Moreover, knock-down of CXCR3 in transplanted melanoma cells significantly reduces their metastatic potential to the LNs, without affecting the hematogenous metastasis route (144,145)

Whereas responsiveness to most chemokines is downregulated in dendritic cells (DCs) upon activation and maturation, the CCR7 and CXCR4 receptors are upregulated. DAP12 activation, which is also involved in macrophage fusion, has been shown to induce CCR7 expression (143). CCR7 and CXCR4 are essential for DCs and macrophages to migrate to the LNs. CCR7 and CXCR4 bind C-C motif ligand (CCL)-21-Leu/-Ser, and CCL-19 or stromal-derived factor-1 (SDF-1/CXCL-12), respectively (147-149). CCL-21-Leu is expressed in lymphatic endothelial cells and CCL-21-Ser in high endothelial venules (HEV) of the LNs (150). CCL-19 is constitutively expressed by stromal cells scattered within the T-cell areas of secondary lymphoid tissues (151). Important to note, CCR7 and CXCR4 are amongst the most widely expressed chemokine receptors in various human cancers. Elevated expressions of CCR7 and CXCR4 have been shown to correlate with (LN) metastasis and predict poor prognosis for cancer patients (152-154). Furthermore,

CXCR4 and CCR7 not only promote metastasis by mediating homing to CXCL-12, CCL-19 and CCL-21, but by inhibiting detachment-induced cell death in murine cancer models (anoikiis; 155,156).

Macrophages have a high expression of Sialyl Lewis X (sLex) antigen, which is a tetrasaccharide that is involved in selectin-mediated adhesion to the vascular endothelium. Upon capture of sLex by L-, E- and P-selectin leukozytes are brought into close proximity of the endothelial vessels (157-159). The close apposition leads to rolling along the vessel wall and permits the engagement of secondary adhesion receptors, such as β1-, β2-integrins and leukocyte function-associated antigen (LFA)-1 that bind to intercellular adhesion molecules (ICAMs) expressed on the endothelium. The firm attachments between integrins and ICAMs are usually initiated via the engagement of chemokine receptors (CCR7, CXCR4) and result in the cessation of rolling. Notably, the expression of sLex is significantly elevated in human carcinoma cells and several studies have shown the association of metastasis and sLex expression (160,161). In this context, sLex has been used clinically as a prognostic marker in several human cancers (161).

N-Acetylglucosamintransferase (GNTV) is a Golgi complex enzyme that is responsible for branching asparagine-linked (β1,6-branched) oligosaccharides. It is highly expressed in metastatic cancer cells and is upregulated in *in vitro*- and *in vivo*-formed macrophage-melanoma and BMDC-cancer cell hybrids (103,74,162). Furthermore, β1,6-branched oligosaccharides are the direct carriers of sLex. Prominent substrates of GNTV, such as LAMP1, are also upregulated in macrophages and in macrophage-melanoma hybrids.

The activation of certain integrins has been revealed to control tumor cell arrest in the vasculature, in a similar manner as leukocytes (163,164). In addition, integrins have been shown to mediate tissue tropism of melanoma, breast and prostate carcinoma cells to the bone and lymph nodes (165).

A recent study focused on the role of the macrophage mannose receptor (MR) in the migration of leukocytes to the draining lymph nodes through the afferent lymphatics. The MR is expressed by tissue macrophages and its expression on the vasculature is restricted to the lymphatic endothelia. In the vasculature it supports lymphocyte adherence by binding to L-selectin. In a mouse tumor model deficient for the MR the primary tumors grew larger compared to Wt mice. In contrast, adhesion of normal leukocytes and cancer

cells to lymphatic vessels was significantly decreased and regional lymph node metastases grew markedly smaller compared to Wt mice (166).

Together, these findings suggest that cancer cells home through the vasculature to distant sites by adopting abilities of immune cells. We speculate that fusion with macrophages confers cancer cells with the phenotypic expression of CXCR3, CXCR4, CCR7, GNTV, sLex, MR and other multi-tropism-conferring factors, which are essential for metastasis.

"Resistance to radiation and chemotherapy"

A major problem during chemotherapeutic and/or radiotherapeutic treatment of clinical cancers is the malignant cells acquisition of resistance to treatment (107). *In vitro* fusion of cancer cells and of cancer cells with stromal cells, such as fibroblasts, leads to increased drug resistance (81,96). Furthermore, *in vitro* fusion between breast cancer cells and breast stem cells gives rise to hybrids with elevated expression levels of ABC multi-drug transporters and anti-apoptotic proteins, compared to the parental cells (107). These findings indicate that cancer cell fusion increases drug resistance and that both parental cells can contribute (107). Paradoxically, by causing tissue destruction in malignant lesions, cancer therapies potentiate the inflammatory processes in tumor tissues and lead to an increased recruitment of inflammatory cells. Amongst the inflammatory infiltrates are M2 macrophages that are recruited to the lesions to engage in tissue repair and feature a fusogenic phenotype. We hypothesize that macrophage-cancer cell fusion contributes to the increased drug resistance of metastatic cancer cells and that current cancer therapies represent two-edged swords, in that they not only kill cancer cells, but induce the development of more aggressive metastatic phenotypes by setting a fusion-promoting microenvironment.

Phagocytosis

As discussed earlier, phagocytosis is a special ability of alternatively activated macrophages and other professional phagocytes. It describes the cellular process of engulfing and ingesting extracellular material (167). Interestingly, phagocytosis and

autophagy, which is linked to phagocytosis in macrophages, are characteristic features of most human cancers (4,168-171,73). The phagocytic behavior is restricted primarily to invasive and metastatic cells, and Zimmer and colleagues exploited this fact to tag primary tumor margins for imaging (172). Accordingly, cathepsins D and B that are enriched in lysosomes facilitate the digestion of proteins following phagocytosis and are prognostic markers for high malignancy, metastasis and poor patient outcome (172). In addition, the phagocytic behavior of metastatic melanoma cells has been found to increase significantly under low glucose conditions or dietary energy restriction (DR), similar to macrophages (173,174).

These facts indicate that the phagocytic behavior of metastatic cells resembles that of macrophages. We believe that the phagocytic abilities of metastatic cells are a reflection from the fusion of cancer cells with macrophages. Furthermore, it has been hypothesized that the linkage of phagocytosis and autophagy in metastatic cells may actually serve as an alternative energy source when nutrient supplies are low (4,73). Thus, the phagocytic pathways of metastatic cells may be essential for dissemination and pose potential targets for anti-metastatic therapies.

Conclusions

Heterogenic cell fusion represents an evolutionary conserved mechanism of most single-celled and multi-cellular organisms. In humans life begins with the fusion between a sperm and an egg cell. During ensuing development more cell fusions occur, including fusions of cytotrophoblasts to form syncytiotrophoblasts, of myoblasts to form muscle fibers and of macrophages to form osteoclasts, multinucleated giant cells and liver-regenerating stem cells (175). Cell fusion in cancer cell populations rapidly produces complex new genotypes by combining and recombining genomes. In this sense, cell fusion is a powerful engine for creating and sustaining diverse cell populations, without the necessity of new mutations. A primary function of somatic cell fusion is to reprogram the developmental fates of fusing cells via a multi-potent stem cell-like phenotype (176). Interestingly, a recent study has hypothesized that macrophages, which are capable to restore degenerated tissue function by fusion, represent the ideal fusion partners of cancer

Fig. 1a 1b

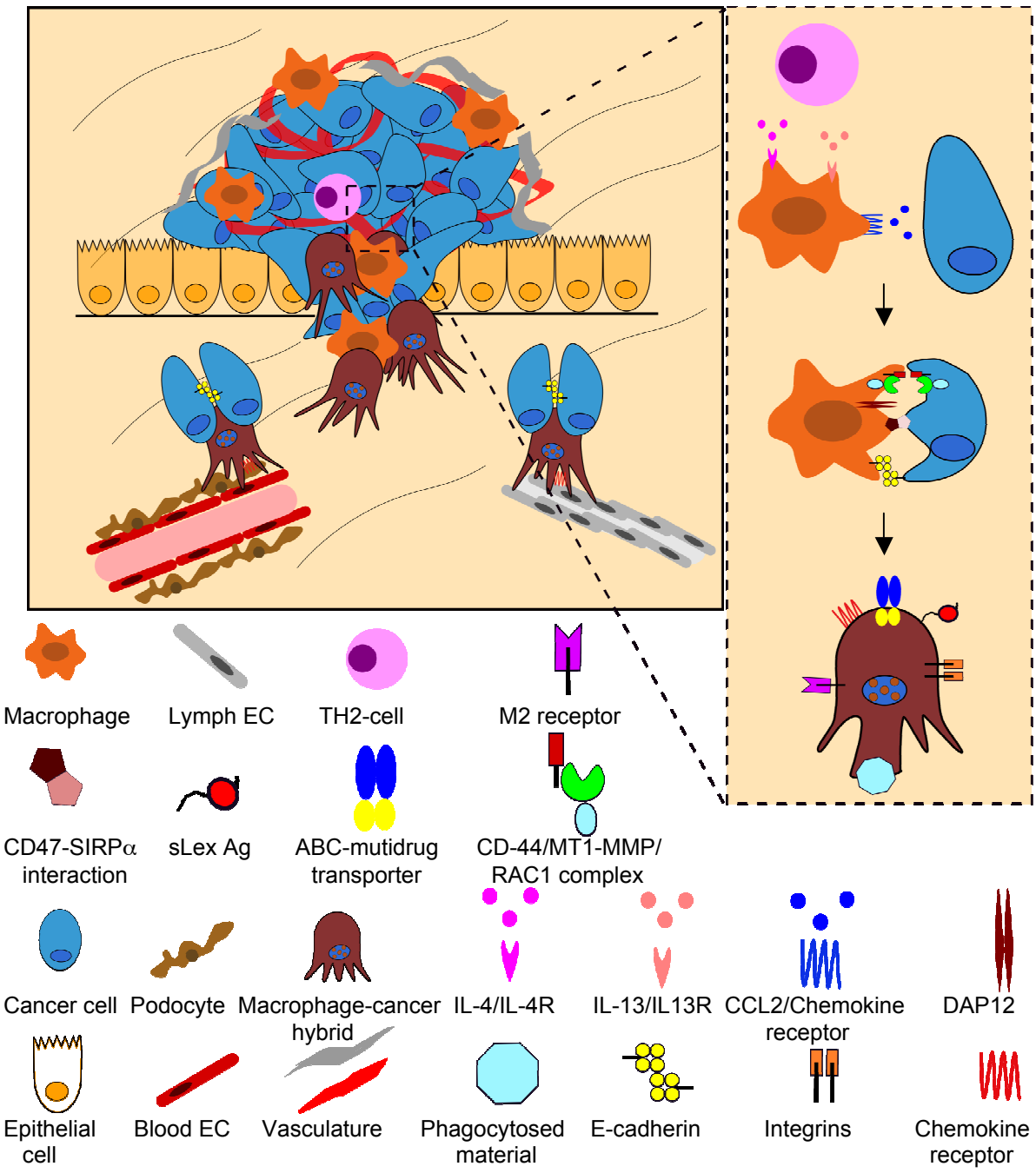

Figure 1. (a) Primary tumors form in the epithelial cell layer. In the course of tumor progression blood- and lymph angiogenesis are induced in the tumor parenchyma and/or stroma. Amongst other cells, macrophages are recruited into the tumor stroma to interact with cancer cells and the tumor microenvironment. Upon entering a fusion-competent state macrophages and cancer cells fuse to form hybrids. Some hybrids adopt migratory and proliferative traits from their parental cells. This metastatic phenotype enables them to invade the surrounding tissue trough the basement membrane and migrate towards the vasculature. The hybrids will then intravasate the vasculature, and due to their broadened tissue tropisms home to other tissues, in a similar fashion as macrophages. The macrophage-conferred ability to adapt to different microenvironments, allows the hybrids to survive in distant organs and seed metastasis. (b) In cooperation with cancer cells, TH2-cells create a fusogenic microenvironment, via the secretion of CCL2, IL-4 and IL-13. CCL2 further mediates the aggregation of macrophages and tumor cells. Signaling via DAP12, sets the macrophages and cancer cell s into a fusion-competent state, which is marked by upregulated E-cadherin, MMP-9 and DC-STAMP expressions. Association of CD44, MT1-MMP, RAC1 and DOCK180 induces lamellipodia formation at fusion sites. Interaction of CD-47 on cancer cells and SIRPα on macrophages inhibits the macrophages to phagocytose the cancer cells and thus is essential to fusion. E-cadherin mediates a closer apposition between the cancer cells and the macrophages, which eventually leads to fusion. After genetic reprogramming the hybrids are marked by the elevated expression of the ABC-multidrug transporter, motility-associated integrins, the sLex Ag (antigen), various chemokine receptors, the mannose 2 receptor, phagocytosis-, proliferation-, and anti-apoptosis-associated genes.

cells for the formation of cancer stem cells (107). In fertilization, when cell fusion reprograms gametes into a totipotent fertilized egg, similar reprogramming could take place in macrophage-cancer cell hybrids to award them with stem cell-like features.

For cancer cell dissemination, cell fusion is a strategy to home to and proliferate in tissues whose particular properties they did not possess prior to fusion. Based on the facts reviewed here and current knowledge surrounding the macrophage-cancer cell fusion theory, we propose the following hypothetical model for metastatogenesis (Figure 1).

Monocytes home to the primary tumor stroma via chemokine and cytokine gradients produced by the cancer cells and stromal cells of the tumor microenvironment. A key player in the recruitment of monocytes and macrophages is CCL-2, but other chemokines and cytokines, such as CCL-5, CCL-7, CXCL-8 and CXCL-12, VEGF, PDGF and M-CSF additionally enhance myeloid cell recruitment (177). Due to IL-4 and IL-13 production of TH2 cells in the tumor stroma TAMs adopt the alternative wound-healing M2 activation status, which primes them for promoting angiogenesis, immune regulation and most importantly fusion (44,69,175). Signaling via DAP12, DC-STAMP and the IL-4R dictates one or both fusion partners to enter a prefusion state, which is marked by an elevated expression of E-cadherin, MMP-9 and DC-STAMP (50,51,54). In combination with as yet unknown factors CCL-2 promotes the aggregation of macrophages and cancer cells. The aggregation allows them to establish intercellular interactions via cell adhesion molecules (CAMs), such as E-cadherin, CD103, KLRG1 (48,52,67). IL-4 induced signaling via STAT-6, expression of ARG-1 and polyamines triggers E-cadherin/catenin complex formation and promotes intercellular interaction of the fusion partners (67). Subsequently, interactions of IgSF members with other plasma-membrane receptors pertain an even closer association of fusion partners and initiate signaling pathways involved in cellular fusion. The signaling initiated upon interaction of SIRPα and CD47 specifically promotes cell fusion and inhibits phagocytosis of cancer cells by macrophages. Furthermore, CD47 is involved in the membrane pore formation for cell fusion (46-48,55-57,79). Eventually, CD44 in concert with MT1-MMP, RAC1 and DOCK180 induces lamellipodia formation, which is essential at sites of membrane fusion (61,68,69). After genetic reprogramming of the nuclei some of the newborn hybrids are equipped with advantageous metastatic features from both parental cells, allowing them

to disseminate from the primary tumor throughout the body and seed metastasis in distant organs (97,102). Whereas the high, uncontrolled proliferation index and the apoptotic resistance stem from the parental cancer cell, the abilities to invade and migrate through tissue, to intravasate, home and survive in the vasculature, to phagozytose and display increased drug resistance stem from the parental macrophage.

As discussed earlier, a recent study has revealed that fusion of macrophages and foreign body giant cell formation is preceded by lamellipodia formation in a RAC1 dependent manner (64). Another study found that RAC1 inhibition via specific GEF inhibition with NSC23766, NSC23766 derivatives and RNA knockdown not only inhibited macrophage fusion and giant cell formation *in vitro* and *in vivo*, but also reversed the metastatic phenotype of breast cancer cells (64,178). The NSC23766 derivatives were not toxic to normal mammary epithelial cells and reduced cancer cell migration and spreading. Increased RAC1 activity in breast cancer cells has been found to contribute to drug resistance towards Trastuzumab by as yet unknown mechanisms (179). With these findings in mind, we hypothesize that RAC1 promotes fusion between macrophages and cancer cells and thereby conveys oncogenic resistance and the migratory metastasis phenotype. Furthermore, we consider macrophage-cancer cell fusion mechanisms to be ideal targets for the inhibition of metastatic cancer progression.

In my opinion, the macrophage-cancer cell fusion theory poses an elegant and appealing explanation for the emergence of metastatic cancer cells, unifying the many facets of cancer metastasis. Yet an overwhelming amount of research needs to be undertaken to answer the complex questions the theory imposed. We think that a collaborative research attempt between the clinics and the fields of cell fusion, tumor microenvironment, metastasis, macrophages and immunology has to be formed to evaluate the impact of macrophage-cancer cell fusion on metastatic cancer cell dissemination. By focusing metastasis research on analogies between metastatic cancer cells and macrophages we may gain the biological insight to therapeutically recreate a suppressive microenvironment that can fully revert the malignant disease phenotype to a controllable benign phenotype. Hence, the view of metastatogenesis as a macrophage-cancer cell fusion event will impact future cancer research and anti-metastatic therapies.

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Dedicated to the memory of Adolf Tschuertz.

References

1 Kinzler K.W., Vogelstein B., Introduction, The genetic basis of human cancer. (2nd, illustrated, revised ed.). New York: McGraw-Hill, 2002

2 Weigelt B., Peterse J. L., van 't Veer L. J., Breast cancer metastasis: markers and models. Nature Rev. Cancer 5:591–602, 2005

3 WHO, "Cancer". World Health Organization. Retrieved 2007-06-25.

4 Pawelek J..M., Chakraborty A.K., Fusion of tumour cells with bone marrow-derived cells: a unifying explanation for metastasis., 377-386, Nat Rev Cancer 2008

5 Qian B., Pollard J.W., Macrophage diversity enhances tumor progression and metastasis., Cell. 141(1): 39–51, 2010

6 Lu X., Kang Y., Organotropism of breast cancer metastasis. J Mammary Gland Biol Neoplasia 12:153–62, 2007

7 National Cancer Institute: Metastatic Cancer: Questions and Answers.Retrieved on 2011-02-15

8 McGee S.F., Lanigan F., Gilligan E., Groner B., Mammary gland biology and breast cancer. Conference on Common Molecular Mechanisms of Mammary Gland Development and Breast Cancer Progression., EMBO Rep. 7(11):1084-8, 2006

9 Nowell P.C., The clonal evolution of tumor cell populations. Science. 194(4260):23-8,1976

10 Van Noorden C. J. F., Meade-Tollin L. C., Bosman F. T., Metastasis. Am. Sci. 86:130-141, 1998

11 Bacac M., Stamenkovic I., Metastatic cancer cell. Annu Rev Pathol. 3:221-47, 2008

12 Kalluri R., Weinberg R.A., The basics of epithelial mesenchymal transition. J. Clin. Invest. 119(6):1420, 2009

13 Vincent-Salomon A., Thiery J.P., Host microenvironment in breast cancer development: epithelial-mesenchymal transition in breast cancer development. Breast Cancer Res. 5:101–106, 2003

14 Peinado H., Olmeda D. and Cano A., Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? Nat. Rev. Cancer 7:415–428, 2007

15 Frixen U.H., Behrens J., Sachs M., Eberle G., Voss B, Warda A., Lochner D., Birchmeier W., E-cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. J. Cell Biol. 113:173–185,1991

16 Perl A.K., Wilgenbus P., Dahl U., Semb H., Christofori G., A causal role for E-cadherin in the transition from adenoma to carcinoma. Nature 392:190–193, 1998

17 Chambers A.F., Groom A.C., MacDonald I.C., Dissemination and growth of cancer cells in metastatic sites. Nat Rev Cancer 2:563-572, 2002

18 Luzzi K.J., MacDonald I.C., Schmidt E.E., Multistep nature of metastatic inefficiency: dormancy of solitary cells after successful extravasation and limited survival of early micrometastases. Am J Pathol 153:865-873,1998

19 American Cancer Society, Breast Cancer Facts and Figures 2009-2010.

20 Klein C.A., The systemic progression of human cancer: A focus on the individual disseminated cancer cell - the unit of selection. Adv. Cancer Res. 89, 35–67, 2003

21 Schmidt-Kittler O., Ragg T., Daskalakis A., Granzow M., Ahr A., Blankenstein T.J., Kaufmann M., Diebold J., Arnholdt H., Muller P., From latent disseminated cells to overt metastasis: Genetic analysis of systemic breast cancer progression. Proc. Natl. Acad. Sci. USA 100:7737–7742, 2003

22 Schardt J.A., Meyer M., Hartmann C.H., Schubert F., Schmidt-Kittler O., Fuhrmann C., Polzer B., Petronio M., Eils R., Klein C.A., Geno- mic analysis of single cytokeratin-positive cells from bone marrow reveals early mutational events in breast cancer. Cancer Cell 8:227–239, 2005

23 Husemann Y., Geigl J.B., Schubert F., Musiani P., Meyer M., Burghart E., Forni G., Klein C.A., Systemic Spread Is an Early Step in Breast Cancer. Cancer Cell 13:(1)58-68, 2008

24 Riethmuller G., Klein C.A., Early cancer cell dissemination and late metastatic relapse: clinical reflections and biological approaches to the dormancy problem in patients. Semin Cancer Biol. 11(4):307-11, 2001

25 Weiss L., Metastatic inefficiency. Adv Cancer Res 54:159-211, 1990

26 Tarin D., Thompson E.W., Newgreen D.F., The fallacy of epithelial mesenchymal transition in neoplasia. Cancer Res. 65:5996–6000, 2005

27 Thompson E.W., Newgreen D.F., Tarin D., Carcinoma invasion and metastasis: a role for epithelial-mesenchymal transition? Cancer Res. 65:5991–5995, 2005

28 Kerjaschki D., The crucial role of macrophages in lymphangiogenesis, J Clin Invest. 115(9): 2316–2319, 2005
29 Metchnikoff I.I., On the present state of the question of immunity in infectious disease, 1908
30 Cavaillon J.M., Cytokines and macrophages. Biomed Pharmacother. 48(10):445-53, 1994
31 Sica A., Mantovani A., Macrophage fusion cuisine. Blood 114(21):4609-10, 2009
32 Van Goethem E., Poincloux R., Gauffre F., Maridonneau-Parini I., Le Cabec V., Matrix architecture dictates three-dimensional migration modes of human macrophages: differential involvement of proteases and podosome-like structures. J Immunol. 184(2):1049-61, 2009
33 Mantovani A., Macrophage polarization: Tumor- associated macrophages as a paradigm for polarized M2 mononu- clear phagocytes. Trends in Immunology 23(11):549–555, 2002
34 Gordon S., Alternative activation of macrophages., Nat. Rev. Immunol. 3:23–35, 2003
35 Mantovani A., Sica A., Sozzani S., Allavena P., Vecchi A., Locati M., The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol. 25:677–686, 2004
36 Noel W., Raes G., Hassanzadeh Ghassabeh G., De Baetselier P., Beschin A., Alternatively activated macrophages during parasite infections., Trends Parasitol. 20:126–133, 2004
37 Wynn T.A., Fibrotic disease and the T(H)1/T(H)2 paradigm. Nat. Rev. Immunol. 4:583–594, 2004
38 Morantz R.A., Wood G.W., Foster M., Clark M., Gollahon K., Macrophages in experimental and human brain tumors. Part 1: Studies of the macrophage content of experimental rat brain tumors of varying immunogenicity. J Neurosurg. 50(3):298-304, 1979
39 Coussens L.M., Werb Z., Inflammation and cancer. Nature 420(6917):860–7, 2002
40 Mantovani A., Sozzani S., Locati M., Allavena P., Sica A., Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol 23(11):549–55, 2002
41 Paik S., Shak S., Tang G., Kim C., Baker J., Cronin M., A multigene assay to predict recurrence of tamoxifen-treated, node- negative breast cancer. N Engl J Med 351(27):2817–26, 2004
42 Lewis C.E., Pollard J.W., Distinct role of macrophages in different tumor microenvironments. Cancer Res. 66(2):605-12, 2006
43 Mantovani A., Sica, A., Macrophages, innate immunity and cancer: Balance, tolerance, and diversity. Curr Opin Immunol, 22(2):231–237, 2010
44 Chen E. H., Grote E., Mohler W., Vignery A., Cell–cell fusion. FEBS Lett. 581:2181–2193, 2007
45 Bruzzaniti A., Baron R., Molecular regulation of osteoclast activity. Rev. Endocr. Metab. Disord. 7:123–139, 2006
46 McNally A.K., Anderson J.M., Interleukin-4 induces foreign body giant cells from human monocytes/macrophages. Differential lymphokine regulation of macrophage fusion leads to morphological variants of multinucleated giant cells. Am. J. Pathol. 147:1487–1499, 1995
47 Boyle W.J., Simonet W.S., Lacey D.L., Osteoclast differentiation and activation. Nature 423:337–342, 2003
48 Kyriakides T.R., Foster M.J., Keeney G.E., Tsai A., Giachelli C.M., Clark-Lewis I., Rollins B.J., Bornstein P., The CC chemokine ligand, CCL2/MCP1, participates in macrophage fusion and foreign body giant cell formation, Am. J. Pathol. 165:2157–2166, 2004
49 Helming L. and Gordon S., Macrophage fusion induced by IL-4 alternative activation is a multistage process involving multiple target molecules. Eur. J. Immunol. 37:33–42, 2007
50 Moreno J.L., IL-4 promotes the formation of multinucleated giant cells from macrophage precursors by a STAT6-dependent, homotypic mechanism: contribution of E-cadherin. J. Leukoc. Biol. 82:1542–1553, 2007
51 Yagi M., Induction of DC-STAMP by alternative activation and downstream signaling mechanisms, J. Bone Miner. Res. 22:992–1001, 2007
52 Helming L., Gordon S., The molecular basis of macrophage fusion, Immunobiology 212:785–793, 2008
53 Jay S.M., Foreign body giant cell formation is preceded by lamellipodia formation and can be attenuated by inhibition of Rac1 activation. Am. J. Pathol. 171:632–640, 2007
54 Helming L., Tomasello E., Kyriakides T.R., Martinez F.O., Takai T., Gordon S., Vivier E.. Essential role of DAP12 signaling in macrophage programming into a fusion-competent state. Sci Signal. 1(43):11, 2008

55 Yagi M., Miyamoto T., Sawatani Y., Iwamoto K., Hosogane N., Fujita N., Morita K., Ninomiya K., Suzuki T., Miyamoto K., Oike Y., Takeya M., Toyama Y., Suda T., DC-STAMP is essential for cell–cell fusion in osteoclasts and foreign body giant cells. J. Exp. Med. 202:345–351, 2005
56 Saginario C., Qian H.-Y., Vignery A., Identification of an inducible surface molecule specific to fusing macrophages. Proc. Natl. Acad. Sci., USA 92:12210–12214, 1995
57 Saginario C., Sterling H., Beckers C., Kobayashi R.-J., Solimena M., Ullu E., Vignery A., MFR, a putative receptor mediating the fusion of macrophages. Mol. Cell Biol. 18:6213–6223, 1998
58 Vignery A., Osteoclasts and giant cells: macrophage–macrophage fusion mechanism, Int. J. Exp. Pathol. 81:291–304, 2000
59 Oldenborg P.A., Gresham H.D., Lindberg F.P., CD47-signal regulatory protein alpha (SIRPalpha) regulates Fcgamma and complement receptor-mediated phagocytosis. J. Exp. Med. 193:855–862, 2001
60 Doberstein S.K., Fetter R.D., Mehta A.Y., Goodman C.S., Genetic analysis of myoblast fusion: blown fuse is required for progression beyond the prefusion complex, J. Cell Biol. 136:1249–1261, 1997
61 Nishiyama Y., Tanaka T., Naitoh H., Mori C., Fukumoto M., Hiai H., Toyokuni S., Overexpression of integrin-associated protein (CD47) in rat kidney treated with a renal carcinogen, ferric nitrilotriacetate, Jpn. J. Cancer Res. 88:120–128, 1997
62 Sterling H., Saginario C., Vignery A., CD44 occupancy prevents macrophage multinucleation, J. Cell Biol. 143:837–847, 1998
63 Cui W., Ke J.Z., Zhang Q., Ke H.Z., Chalouni C., Vignery A., The intracellular domain of CD44 promotes the fusion of macrophages. Blood 107:796–805, 2006
64 Jay S.M., Skokos E., Laiwalla F., Krady M.M., Kyriakides T.R., Foreign body giant cell formation is preceded by lamellipodia formation and can be attenuated by inhibition of Rac1 activation. Am J Pathol. 171(2):632-40, 2007
65 Gonzalo P., Guadamillas M.C., Hernández-Riquer M.V., Pollán A., Grande-García A., Bartolomé R.A., Vasanji A., Ambrogio C., Chiarle R., Teixidó J., Risteli J., Apte S.S., del Pozo M.A., Arroyo A.G. MT1-MMP is required for myeloid cell fusion via regulation of Rac1 signaling. Dev Cell. 18(1):77-89, 2010
66 Mori H., Tomari T., Koshikawa N., Kajita M., Itoh Y., Sato H., Tojo H., Yana I., Seiki M., CD44 directs membrane-type 1 matrix metalloproteinase to lamellipodia by associating with its hemopexin-like domain. EMBO J. 21(15):3949-59, 2002
67 Van den Bossche J., Bogaert P., van Hengel J., Guérin C.J., Berx G., Movahedi K., Van den Bergh R., Pereira-Fernandes A., Geuns J.M., Pircher H., Dorny P., Grooten J., De Baetselier P., Van Ginderachter J.A., Alternatively activated macrophages engage in homotypic and heterotypic interactions through IL-4 and polyamine-induced E-cadherin/catenin complexes. Blood. 114(21):4664-74, 2009
68 Mbalaviele G., Chen H., Boyce B.F., Mundy G.R., Yoneda T., The role of cadherin in the generation of multinucleated osteoclasts from mononuclear precursors in murine marrow, J. Clin. Invest. 95:2757–2765, 1995
69 McNally A.K., DeFife K.M., Anderson J.M., Interleukin-4-induced macrophage fusion is prevented by inhibitors of mannose receptor activity, Am. J. Pathol. 149:975–985, 1996
70 McNally A.K., Anderson J.M., Beta1 and beta2 integrins mediate adhesion during macrophage fusion and multinucleated foreign body giant cell formation, Am. J. Pathol. 160:621–630, 2002
71 Helming L. The scavenger receptor CD36 plays a role in cytokine-induced macrophage fusion, J. Cell Sci. 122:453–459, 2009
72 Greenberg M.E., Oxidized phosphatidylserine–CD36 interactions play an essential role in macrophage-dependent phagocytosis of apoptotic cells. J. Exp. Med. 203:2613–2625, 2006
73 Huysentruyt L.C., Seyfried T.N., Perspectives on the mesenchymal origin of metastatic cancer.Cancer Metastasis Rev. 29(4):695-707, 2010
74 Chakraborty A., Lazova R., Davies S., Bäckvall H., Ponten F., Brash D., Pawelek J., Donor DNA in a renal cell carcinoma metastasis from a bone marrow transplant recipient. Bone Marrow Transplant. 34:183–186, 2004
75 Rizvi A. Z., Swain J.R., Davies P.S., Bailey A.S., Decker A.D., Willenbring H., Grompe M., Fleming W.H., Wong M.H., Bone marrow-derived cells fuse with normal and transformed intestinal stem cells. Proc. Natl Acad. Sci. USA 103:6321–6325, 2006
76 Mortensen K., Lichtenberg J., Thomsen P.D., Larsson L.I., Spontaneous fusion between cancer cells and endothelial cells. Cell. Mol. Life Sci. 61:2125–2131, 2004
77 Bjerregaard B., Holck S., Christensen I.J., Larsson L.I., Syncytin is involved in breast cancer-endothelial cell fusions. Cell. Mol. Life Sci. 63:1906–1911, 2006

78 Alison M.R., Lovell M.J., Direkze N.C., Wright N.A., Poulsom R., Stem cell plasticity and tumour formation. Eur. J. Cancer 42:1247–1256, 2006
79 Kerbel R.S., Lagarde A.E., Dennis J.W., Donaghue T.P., Spontaneous fusion in vivo between normal host and tumor cells: possible contribution to tumor progression and metastasis studied with a lectin-resistant mutant tumor. Mol. Cell. Biol. 3:523–538, 1983
80 Mortensen K., Lichtenberg J., Thomsen P.D., Larsson L.I., Spontaneous fusion between cancer cells and endothelial cells. Cell Mol Life Sci 61:2125-31, 2004
81 Duelli D., Lazebnik Y.. Cell-to-cell fusion as a link between viruses and cancer. Nat Rev Cancer 7:968–76, 2007
82 Fujiwara T., Bandi M., Nitta M., Ivanova E.V., Bronson R.T., Pellman D., Cytokinesis failure generating tetraploids promotes tumorigenesis in p53-null cells. Nature 437:1043–7, 2005
83 Heselmeyer K., Gain of chromosome 3q defines the transition from severe dysplasia to invasive carcinoma of the uterine cervix. Proc Natl Acad Sci USA 93:479–84, 1996
84 Lu X., Kang Y., Efficient acquisition of dual metastasis organotropism to bone and lung through stable spontaneous fusion between MDA-MB-231 variants. Proc Natl Acad Sci U S A 106:9385–90, 2009
85 Lu X., Kang Y., Cell fusion as a hidden force in tumor progression. Cancer Res. 69(22):8536-9, 2009
86 Pawelek J.M., Tumour cell hybridization and metastasis revisited. Melanoma Res. 10:507–514, 2000
87 Pawelek J.M., Tumour-cell fusion as a source of myeloid traits in cancer. Lancet Oncol. 6:988–993, 2005
88 Pawelek J., Chakraborty A., Lazova R., Yilmaz Y., Cooper D., Brash D., Handerson T., Co-opting macrophage traits in cancer progression: a consequence of tumor cell fusion? Contrib. Microbiol. 13:138–155, 2006
89 Rizvi A.Z., Swain J.R., Davies P.S., Bailey A.S., Decker A.D., Willenbring H., Grompe M., Fleming W.H., Wong M.H., Bone marrow-derived cells fuse with normal and transformed intestinal stem cells. Proc. Natl Acad. Sci. USA 103:6321–6325, 2006
90 Alison M.R., Lovell M.J., Direkze N.C., Wright N.A., Poulsom R., Stem cell plasticity and tumour formation. Eur. J. Cancer 42:1247–1256, 2006
91 Herzog E.L., Van Arnam J., Hu B., Zhang J., Chen Q., Haberman A.M., Krause D.S., Lung-specific nuclear reprogramming is accompanied by heterokaryon formation and Y chromosome loss following bone marrow transplantation and secondary inflammation. FASEB J. 21:2592–12601, 2007
92 Kerbel R.S., Lagarde A.E., Dennis J.W., Donaghue T.P., Spontaneous fusion in vivo between normal host and tumor cells: possible contribution to tumor progression and metastasis studied with a lectin-resistant mutant tumor. Mol. Cell. Biol. 3:523–538, 1983
93 Chakraborty A.K., Sodi S., Rachkovsky M., Kolesnikova N., Platt J.T., Bolognia J.L., Pawelek J.M., A spontaneous murine melanoma lung metastasis comprised of host tumor hybrids. Cancer Res. 60:2512–2519, 2000
94 Rachkovsky M.S., Sodi S., Chakraborty A., Avissar Y., Bolognia J., McNiff J.M., Platt J., Bermudes D., Pawelek J., Melanoma x macrophage hybrids with enhanced metastatic potential. Clin. Exp. Metastasis 16:299–312, 1998
95 Jacobsen B.M., Harrell J.C., Jedlicka P., Borges V.F., Varella-Garcia M., Horwitz K.B., Spontaneous fusion with, and transformation of mouse stroma by, malignant human breast cancer epithelium. Cancer Res. 66:8274–8279, 2006
96 Duelli D., Lazebnik Y., Cell fusion: A hidden enemy? Cancer Cell 3:445–8, 2003
97 Vassilopoulos G., Wang P.R., Russell D.W., Transplanted bone marrow regenerates liver by cell fusion. Nature 422:901-4, 2003
98 Johansson C.B., Youssef S., Koleckar K., Holbrook C., Doyonnas R., Corbel S.Y., Steinman L., Rossi F.M., Blau H.M., Extensive fusion of haematopoietic cells with Purkinje neurons in response to chronic inflammation. Nat Cell Biol 10:575–83, 2008
99 Nygren J.M., Liuba K., Breitbach M., Stott S., Thorén L., Roell W., Geisen C., Sasse P., Kirik D., Björklund A., Nerlov C., Fleischmann B.K., Jovinge S., Jacobsen S.E., Myeloid and lymphoid contribution to non-haematopoietic lineages through irradiation-induced heterotypic cell fusion. Nat Cell Biol 10:584–92, 2008
100 Troester M.A., Lee M.H., Carter M., Fan C., Cowan D.W., Perez E.R., Pirone J.R., Perou C.M., Jerry D.J., Schneider S.S., Activation of host wound responses in breast cancer microenvironment. Clin Cancer Res. 15(22):7020-8, 2009

101 Powell A.E., Anderson E.C., Davies P.S., Silk A.D., Pelz C., Impey S., Wong M.H., Fusion between Intestinal Epithelial Cells and Macrophages in a Cancer Context Results in Nuclear Reprogramming. Cancer Res. 71(4):1497-505, 2011
102 Chakraborty A.K., de Freitas Sousa J., Espreafico E.M., Pawelek J.M., Human monocyte mouse melanoma fusion hybrids express human gene. Gene 275:103–106, 2001
103 Yilmaz Y., Lazova R., Qumsiyeh M., Cooper D., Pawelek J., Donor Y chromosome in renal carcinoma cells of a female BMT recipient: visualization of putative BMT-tumor hybrids by FISH. Bone Marrow Transplant 35:1021–4, 2005
104 Andersen T.L., Boissy P., Sondergaard T.E., Kupisiewicz K., Plesner T., Rasmussen T., Haaber J., Kølvraa S., Delaissé J.M., Osteoclast nuclei of myeloma patients show chromosome translocations specific for the myeloma cell clone: a new type of cancer-host partnership? J Pathol 211:10-7, 2007
105 Bellingan G.J., Caldwell H., Howie S.E., Dransfield I., Haslett C., In vivo fate of the inflammatory macrophage during the resolution of inflammation: Inflammatory macrophages do not die locally, but emigrate to the draining lymph nodes. Journal of Immunology. 157(6):2577–2585, 1996
106 Stanbridge E.J., Suppression of malignancy in human cells. Nature 260:17–20, 1976
107 Dittmar T., Nagler C., Schwitalla S., Reith G., Niggemann B., Zänker K.S., Recurrence cancer stem cells--made by cell fusion? Med Hypotheses. 73(4):542-7, 2009
108 Ruff M.R., Pert C.B., Small cell carcinoma of the lung: macrophage-specific antigens suggest hemopoietic stem cell origin.Science. 225(4666):1034-6, 1984
109 Calvo F., Martin P.M., Jabrane N., De Cremoux P., Magdelenat H., Human breast cancer cells share antigens with the myeloid monocyte lineage.Br J Cancer. 56(1):15-9, 1987
110 Shabo I., Stål O., Olsson H., Doré S., Svanvik J., Breast cancer expression of CD163, a macrophage scavenger receptor, is related to early distant recurrence and reduced patient survival. International Journal of Cancer. 123(4):780–786, 2008
111 Shabo I., Olsson H., Sun X.F., Svanvik J., Expression of the macrophage antigen CD163 in rectal cancer cells is associated with early local recurrence and reduced survival time. International Journal of Cancer. 125(8):1826–1831, 2009
112 Günthert U., Hofmann M., Rudy W., Reber S., Zöller M., Haussmann I., Matzku S., Wenzel A., Ponta H., Herrlich P., A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cells.Cell. 65(1):13-24, 1991
113 Espina C., Céspedes M.V., García-Cabezas M.A., Gómez del Pulgar M.T., Boluda A., Oroz L.G., Benitah S.A., Cejas P., Nistal M., Mangues R., Lacal J.C., A critical role for Rac1 in tumor progression of human colorectal adenocarcinoma cells.Am J Pathol. 172(1):156-66, 2008
114 Kamai T., Yamanishi T., Shirataki H., Takagi K., Asami H., Ito Y., Yoshida K., Overexpression of RhoA, Rac1, and Cdc42 GTPases is associated with progression in testicular cancer. Clin Cancer Res 10:4799-4805, 2004
115 Pan Y., Bi F., Liu N., Xue Y., Yao X., Zheng Y., Fan D., Expression of seven main Rho family members in gastric carcinoma. Biochem Biophys Res Commun 315:686-691, 2004
116 Schnelzer A., Prechtel D., Knaus U., Dehne K., Gerhard M., Graeff H., Harbeck N., Schmitt M., Lengyel E. Rac1 in human breast cancer: overexpression, mutation analysis, and characterization of a new isoform, Rac1b. Oncogene 19:3013-3020, 2000
117 Sardinha T.C., Nogueras J.J., Xiong H., Weiss E.G., Wexner S.D., Abramson S., Membrane-type 1 matrix metalloproteinase mRNA expression in colorectal cancer. Dis Colon Rectum 43: 389–395, 2000
118 Tetu B., Brisson J., Wang C.S., Lapointe H., Beaudry G., Blanchette C., Trudel D., The influence of MMP-14, TIMP-2 and MMP-2 expression on breast cancer prognosis. Breast Cancer Res 8: R28, 2006
119 Sabeh F., Shimizu-Hirota R., Weiss S.J., Protease-dependent versus -independent cancer cell invasion programs: three-dimensional amoeboid movement revisited. J Cell Biol. 185(1):11-9, 2009
120 Overall C.M., Kleifeld O., Tumour microenvironment - opinion: validating matrix metalloproteinases as drug targets and anti-targets for cancer therapy. Nat. Rev. Cancer. 6:227–239, 2006
121 Chan K.S., Espinosa I., Chao M., Wong D., Ailles L., Diehn M., Gill H., Presti J.Jr., Chang H.Y., van de Rijn M., Shortliffe L., Weissman I.L., Identification, molecular characterization, clinical prognosis, and therapeutic targeting of human bladder tumor-initiating cells. Proc Natl Acad Sci U S A. 106(33):14016-21, 2009
122 Nagahara M., Mimori K., Kataoka A., Ishii H., Tanaka F., Nakagawa T., Sato T., Ono S., Sugihara K., Mori M., Correlated expression of CD47 and SIRPA in bone marrow and in peripheral blood predicts recurrence in breast cancer patients. Clin Cancer Res. 16(18):4625-35, 2010

123 Maxhimer J.B., Soto-Pantoja D.R., Ridnour L.A., Shih H.B., Degraff W.G., Tsokos M., Wink D.A., Isenberg J.S., Roberts D.D., Radioprotection in normal tissue and delayed tumor growth by blockade of CD47 signaling. Sci Transl Med. 1(3):3ra7, 2009
124 Hu H., Sun L., Guo C., Liu Q., Zhou Z., Peng L., Pan J., Yu L., Lou J., Yang Z., Zhao P., Ran Y., Tumor cell-microenvironment interaction models coupled with clinical validation reveal CCL2 and SNCG as two predictors of colorectal cancer hepatic metastasis. Clin Cancer Res. 15(17):5485-93, 2009
125 Loberg R.D., Ying C., Craig M., Yan L., Snyder L.A., Pienta K.J., CCL2 as an important mediator of prostate cancer growth in vivo through the regulation of macrophage infiltration Neoplasia. 9(7):556-62, 2007
126 Friedl P., Weigelin B., Interstitial leukocyte migration and immune function. Nat. Immunol. 9: 960–969, 2008
127 Friedl P., Gilmour D., Collective cell migration in morphogenesis, regeneration and cancer.Nat Rev Mol Cell Biol. 10(7):445-57, 2009
128 Sahai E., Mechanisms of cancer cell invasion. Curr. Opin. Genet. Dev. 15:87–96, 2005
129 Sabeh F., Shimizu-Hirota R., Weiss S.J., Protease-dependent versus -independent cancer cell invasion programs: three-dimensional amoeboid movement revisited. J. Cell Biol. 185:11–19, 2009
130 Wolf K., Wu Y.I., Liu Y., Geiger J., Tam E., Overall C., Stack M.S., Friedl P., Multi-step pericellular proteolysis controls the transition from individual to collective cancer cell invasion. Nat Cell Biol 9:893–904, 2007
131 Friedl P., Wolf K., Tube travel: the role of proteases in individual and collective cancer cell invasion. Cancer Res 68:7247–9, 2008
132 Felding-Habermann B., Integrin adhesion receptors in tumor metastasis. Clin Exp Metastasis. 20(3):203-13, 2003
133 Maruyama K., Ii M., Cursiefen C., Jackson D.G., Keino H., Tomita M., Van Rooijen N., Takenaka H., D'Amore P.A., Stein-Streilein J., Losordo D.W., Streilein J.W., Inflammation-induced lymphangiogenesis in the cornea arises from CD11b-positive macrophages. J Clin Invest. 115:2363–2372, 2005
134 Religa P., Cao R., Bjorndahl M., Zhou Z., Zhu Z., Cao Y., Presence of bone marrow-derived circulating progenitor endothelial cells in the newly formed lymphatic vessels. Blood. 106:4184–4190, 2005
135 Schledzewski K., Falkowski M., Moldenhauer G., Metharom P., Kzhyshkowska J., Ganss R., Demory A., Falkowska-Hansen B., Kurzen H., Ugurel S., Geginat G., Arnold B., Goerdt S., Lymphatic endothelium-specific hyaluronan receptor LYVE-1 is expressed by stabilin-1+, F4/80+, CD11b+ macrophages in malignant tumours and wound healing tissue in vivo and in bone marrow cultures in vitro: implications for the assessment of lymphangiogenesis. J Pathol. 209:67–77, 2006
136 Wicki A., Lehembre F., Wick N., Hantusch B., Kerjaschki D., Christofori G., Tumor invasion in the absence of epithelial-mesenchymal transition: podoplanin-mediated remodeling of the actin cytoskeleton. Cancer Cell. 9(4):261-72, 2006
137 Gaggioli C., Hooper S., Hidalgo-Carcedo C., Grosse R., Marshall J.F., Harrington K., Sahai E., Fibroblast-led collective invasion of carcinoma cells with differing roles for RhoGTPases in leading and following cells. Nat Cell Biol. 9(12):1392-400, 2007
138 Nassar G.M., Morrow J.D., Roberts J.L., Lakkis F.G., Badr K.F., Induction of 15-lipoxygenase by interleukin-13 in human blood monocytes, J. Biol. Chem. 269:27631–27634, 1994
139 Chaitidis P., Billett E.E., O'Donnell V.B., Bermudez-Fajardo A., Fitzgerald J., Kuban R.J., Ungethuem U., Kuhn H., Th2 response of human peripheral monocytes involves isoform-specific induction of monoamine oxidase-A, J. Immunol. 173:4821–4827, 2004
140 Xu B., Bhattacharjee A., Roy B., Xu H.M., Anthony D., Frank D.A, Feldman G.M., Cathcart M.K., Interleukin-13 induction of 15-lipoxygenase gene expression requires p38 mitogen-activated protein kinase-mediated serine 727 phosphorylation of Stat1 and Stat3, Mol. Cell. Biol. 23:3918–3928, 2003
141 Tanis P.J., Nieweg O.E., Valdes Olmos R.A., Rutgers E.J., Kroon B.B., History of sentinel node and validation of the technique. Breast Cancer Res 3:109-12, 2001
142 Kawada K., Taketo M.M., Significance and mechanism of lymph node metastasis in cancer progression. Cancer Res. 71(4):1214-8, 2011
143 Randolph G.J., Angeli V., Swartz M.A., Dendritic-cell trafficking to lymph nodes through lymphatic vessels. Nat Rev Immunol. (8):617-28, 2005

144 Kawada K., Sonoshita M., Sakashita H., Takabayashi A., Yamaoka Y., Manabe T., Inaba K., Minato N., Oshima M., Taketo M.M., Pivotal role of CXCR3 in melanoma cell metastasis to lymph nodes. Cancer Res 64: 4010–7, 2004
145 Kawada K., Hosogi H., Sonoshita M., Sakashita H., Manabe T., Shimahara Y., Sakai Y., Takabayashi A., Oshima M., Taketo M.M., Chemokine receptor CXCR3 promotes colon cancer metastasis to lymph nodes. Oncogene 26:4679–88, 2007
146 Ma X., Norsworthy K., Kundu N., Rodgers W.H., Gimotty P.A., Goloubeva O., Lipsky M., Li Y., Holt D., Fulton A., CXCR3 expression is associated with poor survival in breast cancer and promotes metastasis in a murine model. Mol Cancer Ther 8:490–8, 2009
147 Randolph G.J., Ochando J., Partida-Sánchez S., Migration of dendritic cell subsets and their precursors. Annu Rev Immunol. 26:293–316, 2008
148 Ohl L., Mohaupt M., Czeloth N., Hintzen G., Kiafard Z., Zwirner J., Blankenstein T., Henning G., Förster R., CCR7 governs skin dendritic cell migration under inflammatory and steady-state conditions. Immunity. 21:279–288, 2004
149 Kabashima K., Shiraishi N., Sugita K., Mori T., Onoue A., Kobayashi M., Sakabe J., Yoshiki R., Tamamura H., Fujii N., Inaba K., Tokura Y., CXCL12-CXCR4 engagement is required for migration of cutaneous dendritic cells. Am J Pathol. 171:1249–1257, 2007
150 Sallusto F., Lanzavecchia A., Mobilizing dendritic cells for tolerance, priming, and chronic inflammation. J Exp Med 189: 611, 1999
151 Caux C., Vanbervliet B., Massacrier C., Ait-Yahia S., Vaure C., Chemin K., Dieu-Nosjean A. M.C., Vicari A., Regulation of dendritic cell recruitment by chemokines. Transplantation. 73(1 Suppl):S7-11, 2002
152 Kim M., Koh Y.J., Kim K.E., Koh B.I., Nam D.H., Alitalo K., Kim I., Koh G.Y., CXCR4 signaling regulates metastasis of chemoresistant melanoma cells by a lymphatic metastatic niche. Cancer Res. 70(24):10411-21, 2010
153 Otsuka S., Bebb G.J., The CXCR4/SDF-1 chemokine receptor axis: a new target therapeutic for non-small cell lung cancer. Thorac Oncol. 3(12):1379-83, 2008
154 Zlotnik A., New insights on the role of CXCR4 in cancer metastasis. J Pathol. 215(3):211-3, 2008
155 Zlotnik A., Chemokines and cancer. Int J Cancer 119:2026–2029, 2006
156 Kochetkova M., Kumar S., McColl S.R., Chemokine receptors CXCR4 and CCR7 promote metastasis by preventing anoikis in cancer cells. Cell Death Differ. 16(5):664-73, 2009
157 von Andrian U.H., Mempel T.R., Homing and cellular traffic in lymph nodes. Nat Rev Immunol. 3(11):867-78, 2003
158 Varki A., Selectin ligands. Proc. Natl. Acad. Sci. U.S.A. 91:7390-7397, 1994
159 Robert C., Klein C., Cheng G., Kogan A., Mulligan R.C., von Andrian U.H., Kupper T.S., Gene therapy to target dendritic cells from blood to lymph nodes. Gene Therapy 10:1479–1486, 2003
160 Kannagi R., Molecular mechanism for cancer-associated induction of sialyl Lewis X and sialyl Lewis A expression-The Warburg effect revisited. Glycoconjugate J 20:353-364, 2004
161 Dabelsteen E., Cell surface carbohydrates as prognostic markers in human carcinomas. J Pathol 179:358-369, 1996
162 Chakraborty A.K., Pawelek J., Ikeda Y., Miyoshi E., Kolesnikova N., Funasaka Y., Ichihashi M., Taniguchi N., Fusion hybrids with macrophage and melanoma cells up-regulate N-acetylglucosaminyltransferase V, 1–6 branching, and metastasis. Cell Growth Differentiation 12:623–630, 2001
163 Pilch J., Habermann R., Felding-Habermann B., Unique ability of in- tegrin alpha(v)beta 3 to support tumor cell arrest under dynamic flow conditions. J Biol Chem 277(24): 21930–8, 2002
164 Felding-Habermann B., O'Toole T.E., Smith J.W., Fransvea E., Ruggeri Z.M., Ginsberg M.H., Hughes P.E., Pampori N., Shattil S.J., Saven A., Mueller B.M., Integrin activation controls metastasis in human breast cancer. Proc Natl Acad Sci USA 98(4):1853–8, 2001
165 Byzova T.V., Kim W., Midura R.J., Plow E.F., Activation of integrin al- pha(V)beta(3) regulates cell adhesion and migration to bone sialo- protein. Exp Cell Res 254(2):299–308, 2000
166 Marttila-Ichihara F., Turja R., Miiluniemi M., Karikoski M., Maksimow M., Niemelä J., Martinez-Pomares L., Salmi M., Jalkanen S., Macrophage mannose receptor on lymphatics controls cell trafficking. Blood. 112(1):64-72, 2008
167 Burke B., Lewis C.E., editors. The macrophage. 2. Oxford University Press: New York; 2002

168 Marin-Padilla M., Erythrophagocytosis by epithelial cells of a breast carcinoma. Cancer. 39(3):1085–1089, 1977
169 DeSimone P.A., East R., Powell R.D., Phagocytic tumor cell activity in oat cell carcinoma of the lung. Human Pathology. 11(5 Suppl):535–539, 1980
170 Fais S., Cannibalism: A way to feed on metastatic tumors. Cancer Letters. 258(2):155–164, 2007
171 Amer A.O., Byrne B.G., Swanson M.S., Macrophages rapidly transfer pathogens from lipid raft vacuoles to autophagosomes. Autophagy 1:53–58, 2005
172 Stehle G., Sinn H., Wunder A., Schrenk H.H., Stewart J.C., Hartung G., Maier-Borst W., Heene D.L., Plasma protein (albumin) catabolism by the tumor itself—Implications for tumor metabolism and the genesis of cachexia. Critical Reviews in Oncology/Hematology. 26(2):77–100, 1997
173 Lugini L., Matarrese P., Tinari A., Lozupone F., Federici C., Iessi E., Gentile M., Luciani F., Parmiani G., Rivoltini L., Malorni W., Fais S., Cannibalism of live lymphocytes by human metastatic but not primary melanoma cells. Cancer Research. 66(7):3629–3638, 2006
174 Dong W., Selgrade M.K., Gilmour I.M., Lange R.W., Park P., Luster M.I., Kari F.W., Altered alveolar macrophage function in calorie-restricted rats. American Journal of Respiratory Cell and Molecular Biology. 19(3):462–469, 1998
175 Witze E., Rothman J. H., Cell fusion: an EFFicient sculptor. Curr. Biol.12:R467 – R469, 2002
176 Podbilewicz B., White J.G., Cell fusions in the developing epithelia of C. elegans, Dev. Biol. 161:408–424, 1994
177 Solinas G., Germano G., Mantovani A., Allavena P., Tumor-associated macrophages (TAM) as major players of the cancer-related inflammation. J Leukoc Biol. 86(5):1065-73, 2009
178 Hernández E., De La Mota-Peynado A., Dharmawardhane S., Vlaar C.P., Novel inhibitors of Rac1 in metastatic breast cancer. P R Health Sci J. 29(4):348-56, 2010
179 Dokmanovic M., Hirsch D.S., Shen Y., Wu W.J., Rac1 contributes to trastuzumab resistance of breast cancer cells: Rac1 as a potential therapeutic target for the treatment of trastuzumab-resistant breast cancer. Mol Cancer Ther. 8(6):1557-69, 2009
180 Uluçkan O., Becker S.N., Deng H., Zou W., Prior J.L., Piwnica-Worms D., Frazier W.A., Weilbaecher K.N., CD47 regulates bone mass and tumor metastasis to bone. Cancer Res. 69(7):3196-204, 2009
181 Scheel C., Onder T., Karnoub A., Weinberg R.A., Adaptation versus selection: the origins of metastatic behavior. Cancer Res. 67(24):11476-9; 2007
182 Calabrese P., Mecklin J.P., Järvinen H.J., Aaltonen L.A., Tavaré S., Shibata D., BMC Numbers of mutations to different types of colorectal cancer. Cancer. 3;5:126, 2005
183 Bernards R., WeinbergR.A., A progression puzzle.Nature 418: 823, 2002
184 Gray J.W., Evidence emerges for early metastasis and parallel evolution of primary and metastatic tumors. Cancer Cell 4:4–6, 2003
185 Ramaswamy S., Ross K.N., Lander E.S., Golub T.R., A molecular signature of metastasis in primary solid tumours. Nat Genet 33:49–54, 2003
186 Stoecklein N.H., Klein C.A., Genetic disparity between primary tumours, disseminated tumour cells, and manifest metastasis. Int J Cancer 126: 589–598, 2010
187 Crawford N.P., Ziogas A., Peel D.J., Hess J., Anton-Culver H., Hunter K.W., Germline polymorphisms in SIPA1 are associated with metastasis and other indicators of poor prognosis in breast cancer. Breast Cancer Res 8:R16, 2006
188 Shackleton M., Quintana E., Fearon E.R., Morrison S.J., Heterogeneity in cancer: cancer stem cells versus clonal evolution. Cell 138:822–829, 2009
189 Shipitsin M., Campbell L.L., Argani P., Weremowicz S., Bloushtain-Qimron N., Yao J., Nikolskaya T., Serebryiskaya T., Beroukhim R., Hu M., Halushka M.K., Sukumar S., Parker L.M., Anderson K.S., Harris L.N., Garber J.E., Richardson A.L., Schnitt S.J., Nikolsky Y., Gelman R.S., Polyak K., Molecular definition of breast tumour heterogeneity. Cancer Cell 11: 259–273, 2007
190 Liu R., Wang X., Chen G.Y., Dalerba P., Gurney A., Hoey T., Sherlock G., Lewicki J., Shedden K., Clarke M.F., The prognostic role of a gene signature from tumorigenic breast-cancer cells. N Engl J Med 356: 217–226, 2007
191 Pang R., Law W.L., Chu A.C., Poon J.T., Lam C.S., Chow A.K., Ng L., Cheung L.W., Lan X.R., Lan H.Y., Tan V.P., Yau T.C., Poon R.T., Wong B.C., A subpopulation of CD26+ cancer stem cells with metastatic capacity in human colorectal cancer. Cell Stem Cell 6: 603–615, 2010

192 Quintana E., Shackleton M., Sabel M.S., Fullen D.R., Johnson T.M., Morrison S.J., Efficient tumour formation by single human melanoma cells. Nature 456:593–598, 2008
193 Boiko A.D., Razorenova O.V., van de Rijn M., Swetter S.M., Johnson D.L., Ly D.P., Butler P.D., Yang G.P., Joshua B., Kaplan M.J., Longaker M.T., Weissman I.L., Human melanoma-initiating cells express neural crest nerve growth factor receptor CD271. Nature 466: 133–137, 2010
194 Roesch A., Fukunaga-Kalabis M., Schmidt E.C., Zabierowski S.E., Brafford P.A., Vultur A., Basu D., Gimotty P., Vogt T., Herlyn M., A temporarily distinct subpopulation of slow-cycling melanoma cells is required for continuous tumour growth. Cell 141: 583–594, 2010