

Perspectives on the mesenchymal origin of metastatic cancer

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Abstract Emerging evidence suggests that many metastatic cancers arise from cells of the myeloid/macrophage lineage regardless of the primary tissue of origin. A myeloid origin of metastatic cancer stands apart from origins involving clonal evolution or epithelial–mesenchymal transitions. Evidence is reviewed demonstrating that numerous human cancers express multiple properties of macrophages including phagocytosis, fusogenicity, and gene/protein expression. It is unlikely that the macrophage properties expressed in metastatic cancers arise from sporadic random mutations in epithelial cells, but rather from damage to an already existing mesenchymal cell, e.g., a myeloid/macrophage-type cell. Such cells would naturally embody the capacity to express the multiple behaviors of metastatic cells. The view of metastasis as a myeloid/macrophage disease will impact future cancer research and anti-metastatic therapies.

Keywords Metastatic cancer · Myeloid/macrophage lineage · Epithelial–mesenchymal transitions · Phagocytosis

1 Introduction

Cancer is a complex disease resulting in tumors composed of multiple cell types with diverse biological characteristics [1, 2]. Cells within the primary tumor can differ with respect to origin, growth rate, karyotype, drug sensitivity,

and metastatic potential [2–4]. While each cell type within the primary tumor contributes to the malignancy of the disease, cells that acquire the abilities to invade and to metastasize are responsible for the majority of cancer related deaths [1, 5]. Despite the clinical importance of metastasis, much remains unknown about the origin and development of this disease.

Metastasis involves the spread of cancer cells from the primary tumor to surrounding tissues and distant organs. The metastatic cascade is a series of sequential and interrelated steps to include cancer cell detachment from the primary tumor, intravasation into the circulation, evasion of immune attack, extravasation at a distant capillary bed, and invasion and proliferation in distant organs [6–10]. Metastatic cells also establish a microenvironment through the release of cytokines and growth factors that facilitate angiogenesis and proliferation, resulting in macroscopic, malignant secondary tumors. In addition, metastatic cells preferentially invade those organs (lymph nodes, lung, liver, brain, bone, pleura, and peritoneum) that promote tumor cell growth and survival consistent with the “seed and soil” hypothesis [8, 11–14]. While the major steps of metastasis are well documented, the process by which a metastatic cell arises from a population of non-metastatic cells in a primary tumor is largely unknown [15–17].

Several mechanisms have been advanced to account for the origin of metastasis. It is widely thought that metastatic cancer cells arise from pre-existing tumor cells that have undergone additional genetic alterations during the later stages of tumor progression, a process known as the clonal origin of metastasis [1, 9, 18–20]. The epithelial–mesenchymal transition (EMT) suggests that metastatic cells arise through a step-wise accumulation of gene mutations that eventually transform an epithelial cell into a tumor cell with mesenchymal features [1, 9, 18–21]. The idea for an EMT origin comes from findings that many cancers generally

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arise in epithelial tissues where abnormalities in cell–cell and cell–matrix interactions occur during tumor progression [22]. Eventually, neoplastic cells emerge that appear as mesenchymal cells, which lack cell–cell adhesion and are dysmorphic in shape [17]. These transformed epithelial cells eventually acquire the multiple effector mechanisms of metastasis [17]. Recent studies suggest that ectopic co-expression of only two genes might be all that is necessary to facilitate EMT in some gliomas [23]. Considerable controversy surrounds the EMT hypothesis of metastasis, as evidence of EMT is not often detected in tumor pathological preparations [22, 24, 25].

Based on numerous similarities between macrophages and metastatic cancer cells, we propose that many metastatic cancers arise from cells of the myeloid lineage. More specifically, metastatic cells arise from resident or infiltrating macrophages or myeloid cells of the tumor stroma that become neoplastic during disease progression. As mesenchymal cells [26, 27], myeloid/macrophage-type cells would naturally embody to the capacity to express multiple behaviors of metastasis. The basis for this hypothesis comes from our recent findings from unique metastatic tumors that arose spontaneously in the inbred VM mouse strain [14, 28]. Two of these highly metastatic/invasive tumors express multiple properties of macrophages. It is unlikely that the metastatic properties of these mouse tumors are unique, as a review of the literature indicates that these mouse tumors share multiple properties with most types of human metastatic cancers. The similarities between the mouse and human metastatic cancers raise the possibility that metastasis is a disease of myeloid cells regardless of tissue origin. Our goal is to highlight the similarities of myeloid cells (especially macrophages) and metastatic cancer cells, and to discuss the possible mechanisms by which these cells could become neoplastic.

2 Linkage of macrophages with metastatic cancer

Macrophages have long been considered the origin of human metastatic cancers; however, this has not been widely accepted or recognized [12, 29–32]. Rather than being considered part of the neoplastic cell population, macrophages are generally considered part of the tumor stroma as macrophages form a large portion of the inflammatory cell infiltrate in most cancer types [33, 34]. Depending on the tumor type, tumor-associated macrophages (TAMs) can constitute up to 80% of the total tumor mass [35]. TAMs are known to facilitate tumor development and progression by establishing a pre-metastatic niche and by enhancing tumor inflammation and angiogenesis [33, 36–39].

Macrophages are among the most versatile cells of the body with respect to their ability to migrate, to change

shape, and to secrete growth factors and cytokines [14, 40–42]. Macrophages are known to have two distinct polarization states: the classically activated (or M1) and the alternatively activated (or M2). Macrophages acquire the M1 phenotype in response to pro-inflammatory molecules and release inflammatory cytokines, reactive oxygen species, and nitric oxide [34, 43–46]. In contrast, macrophages acquire the M2 phenotype in response to anti-inflammatory molecules such as IL-4, IL-13, IL-10, and apoptotic cells [43, 47]. Additionally, M2 macrophages are poor antigen presenters, immunosuppressive, and promote tissue remodeling and repair [34]. Emerging evidence suggests that M1 and M2 macrophages have distinct roles during tumor initiation and malignant progression [48].

M1 macrophages are believed to facilitate the early stages of tumorigenesis through the creation of an inflammatory microenvironment that produces DNA and mitochondrial damage [48, 49]. However, after the establishment of the primary tumor, during tumor progression, TAMs generally undergo a phenotypic switch and acquire the M2 polarization state [43]. The M2 TAMs scavenge debris and promote tumor growth, angiogenesis, and metastasis [34, 43, 44].

Increasing evidence suggests that myeloid/macrophage cells are also part of the malignant cell population. Aichel first proposed over a century ago that tumor progression involved fusion between leukocytes and somatic cells (reviewed in [31]). Several human metastatic cancers are known to express multiple molecular and behavioral characteristics of macrophages to include phagocytosis, cell–cell fusion, and antigen expression (Table 1). We suggest that an origin from myeloid cells can account for many properties of metastatic cancer.

3 The wound-healing M2 macrophage and the metastatic cell: behavioral similarities

Interestingly, macrophages express most hallmarks of metastatic tumor cells when responding to tissue injury or disease. For example, monocytes extravasate from the vasculature and are recruited to the wound via cytokines released from the damaged tissue. Within the wound, monocytes differentiate into alternatively activated macrophages and dendritic cells where they release a variety of pro-angiogenic molecules including vascular endothelial growth factor, fibroblast growth factor, and platelet-derived growth factor [126, 127]. M2 macrophages also actively phagocytose dead cells and cellular debris [42, 128]. On occasion, macrophages undergo homotypic fusion resulting in multinucleated giant cells with increased phagocytic capacity [29, 129]. Following these wound-healing activities, macrophages intravasate back into the circulation where they travel to the lymph nodes to participate in the

Table 1 Tumors expressing macrophage characteristics

Tumor	Phagocytosis	Fusogenicity	Gene expression
Bladder	[50]		
Brain	[14, 51–54]	[55]	[14, 54]
Breast	[56–63]	[64–68]	[69–71]
Carcinoma of unknown primary	[72]	[73]	
Endometrial	[74]		
Fibrosarcoma	[63]		
Gall bladder		[75]	
Liver		[76]	
Lung	[57, 77–80]	[67]	[81–84]
Lymphoma/leukemia	[85–87]	[88–91]	
Melanoma/skin	[92–96]	[32, 97, 98]	[96, 99–101]
Meth A sarcoma	[102]	[102]	[102]
Multiple myeloma	[103]	[104]	
Ovarian	[63, 105]		[106]
Pancreatic	[107, 108]	[109]	[108]
Rectal			[110]
Renal	[111]	[112, 113]	[111]
Rhabdomyosarcoma	[114, 115]		
Reviews	[116–119]	[12, 15, 29–31, 89, 120–125]	

immune response [42, 130, 131]. These findings indicate that normal macrophages are capable of intravasation, tissue invasion, release of pro-angiogenic molecules/cytokines, survival in hypoxic and necrotic environments, and extravasation, i.e., hallmark behaviors of metastatic tumor cells.

4 Phagocytosis: a shared behavior of M2 macrophages and metastatic cells

Phagocytosis, the engulfment and ingestion of extracellular material, is a specialized behavior of M2 macrophages and other professional phagocytes [42]. This process is essential for maintaining tissue homeostasis by clearing apoptotic cells, cellular debris, and invading pathogens [42]. Interestingly, many malignant tumor cells are phagocytic both *in vitro* and *in vivo* (Table 1). Macrophages also express high levels of lysosomal-enriched cathepsins, which facilitate the digestion of proteins ingested following phagocytosis or pinocytosis [132, 133]. This is interesting since lysosomal cathepsins D and B are viewed as prognostic factors in cancer patients [133]. Indeed, a high content of these enzymes in tumors of the head and neck, breast, brain, colon, or endometrium was considered a sign for high malignancy, high metastasis, and overall poor prognosis [133].

The phagocytic behavior of tumor cells was first described over a century ago from histopathological

observations of foreign cell bodies within in the cytoplasm of cancer cells, which displayed crescent-shaped nuclei [134]. This cellular phenotype, commonly referred to as either “birds-eye” or “signet-ring”, is the result of the ingested material pushing the nucleus to the periphery of the phagocytic cell [116]. While this phagocytic/cannibalistic phenomenon is commonly seen in feeding microorganisms, cell cannibalism is an exclusive property of malignant tumor cells in humans [116]. These tumor cell phagocytic/cannibalistic behaviors are not to be confused with autophagy, a cellular self-digestion process often associated with starvation conditions [135, 136]. It has been reported that both human and murine cancers can phagocytose other tumor cells, erythrocytes, leukocytes, platelets, dead cells, as well as extracellular particles (Table 1) [56, 77, 116].

4.1 Phagocytic cancers

Numerous reports have described the phagocytic behaviors seen in aggressive human cancers and in some murine tumors (Table 1). We previously identified two spontaneous invasive/metastatic murine brain tumors (VM-M2 and VM-M3) that express many macrophage behaviors including phagocytosis [14]. While extracranial metastasis of central nervous system tumors is not common, many gliomas, especially glioblastoma multiforme, are highly metastatic if the tumor cells can gain access to extraneural sites [14, 137–141]. Moreover, extracranial metastasis portends an

extremely poor survival, with the vast majority of patients surviving less than 6 months from the diagnosis of metastatic disease [142].

The phagocytic activity of the metastatic VM-M2 and VM-M3 tumor cells was similar to that of the RAW 264.7 macrophage cell line [14]. Similar findings were reported for the methylcholanthrene-induced murine P388 mouse lymphoma cells, which display macrophage morphology, form rosettes, phagocytose latex beads, and strongly adhere to glass and plastic surfaces [85]. These findings indicate that some mouse tumor cell lines can manifest the phagocytic behavior seen in macrophages and in numerous human metastatic cancers.

While phagocytic behaviors have been reported for most forms of human cancer including skin, breast, lymphoma, lung, brain, ovarian, pancreatic, renal, endometrial, rhabdomyosarcoma, myeloma, fibrosarcoma, and bladder, not all cancer cells within a tumor are phagocytes (Table 1). For most of the tumors described, phagocytosis was restricted primarily to those cells that are highly invasive and metastatic [14, 51, 52, 56–58, 78, 79, 92, 93, 107]. Lugini et al. measured the phagocytic behavior of cell lines derived from primary human melanomas ($n=8$) and metastatic lesions ($n=11$) [92]. Interestingly, the phagocytic behavior all of the cell lines derived from metastatic lesions was similar to that of the macrophage controls, whereas phagocytic behavior was not found in any of the cell lines derived from primary melanomas [92]. Histological examination of *in vivo* metastatic melanoma lesions confirmed the presence of phagocytic tumor cells [93]. Similar findings of phagocytosis were reported for human metastatic breast cancer [56]. Numerous phagocytic tumor cells were identified within metastatic breast cancer lesions and were not observed within the primary tumor of the same patient [56]. Additionally, breast cancer malignancy and grade correlates with the number of phagocytic tumor cells present within the tumor stroma [59].

4.2 Targeting phagocytosis

Several investigators suggested that tumor cell phagocytosis could be targeted as a potential therapy for metastatic cancers. For example, Ghoneum et al. showed that MCF-7 breast cancer cells undergo apoptosis after engulfing yeast cells either *in vitro* or *in vivo* [58, 60]. Phagocytosis of yeast cells also effectively induces apoptosis in human cancers of the gastrointestinal tract including tongue, squamous cell carcinoma, and colon adenocarcinoma [143]. These reports suggest that the phagocytic behavior of metastatic tumor cells can be targeted for the development of new anti-metastasis therapies.

Additionally, the phagocytic activity of metastatic melanoma cells is significantly increased when the cells

are grown under low glucose conditions suggesting that metastatic cells use phagocytosis as a way to “feed” when nutrient supplies are low [93, 94]. Therefore, a metastasis targeted therapy could be effective if administered to energy-stressed metastatic tumor cells. Dietary energy restriction (DR) is an effective means to reduce circulating glucose levels and induce energy stress in tumor cells. DR can also reduce inflammation and tumor angiogenesis while increasing tumor cell apoptosis [144–148]. Moreover, DR increases macrophage phagocytosis [149]. Hence, DR administered in combination with an anti-phagocytosis targeted therapy could potentially reduce primary tumor size, vascularity, and the number of metastatic tumor cells.

4.3 Phagocytosis for diagnostics

Effective resection of invasive/metastatic tumors can be improved if the margins between tumor tissue and normal tissue are readily identified. In order to identify rat C6 glioma cells that invaded beyond the main tumor mass, Zimmer et al. used monocrySTALLINE iron oxide nanoparticles that the tumor cells could phagocytose as a contrasting agent [150]. This was able to identify glioma cells that had invaded into the rat parenchyma. Viewed collectively, these studies suggest that the phagocytic behavior of tumor cells can be exploited for therapeutic strategies and development of new diagnostic/imaging protocols.

5 Fusogenicity

Fusogenicity is the ability of a cell to fuse with another cell through the merging of their plasma membranes [129]. This process can be easily induced *in vitro* as is seen with the formation of antibody-producing hybridomas. However, cell fusion in humans is a highly regulated complex process that is essential for fertilization (sperm and egg), and skeletal muscle (myoblasts) and placenta (trophoblast) formation. Outside of these developmental processes, cell-to-cell fusion is normally restricted to differentiated cells of myeloid origin (reviewed in [120]). During differentiation, subsets of macrophages fuse with each other to form multinucleated osteoclasts in bone or multinucleated giant cells in response to foreign bodies [29]. Osteoclasts and giant cells have increased cell volume that facilitates engulfment of large extracellular materials [29]. Macrophages are also thought to fuse with damaged somatic cells during the process of tissue repair [29, 129, 151, 152].

In addition to homotypic fusion, macrophages are known to undergo heterotypic fusion with tumor cells [29, 30, 120, 153]. Aichel first suggested in 1911 that fusion between somatic cells and leukocytes could induce aneuploidy

resulting in tumors with increased malignancy (reviewed in [32]). Nearly 60 years later, Mekler and Warner proposed that fusion of committed tumor cells with host myeloid cells would produce tumor hybrids capable of migrating throughout the body and invading distant organs [121, 154]. Recently, this hypothesis has received more attention with the findings reported by John Pawelek and colleagues [30, 31, 69, 97, 98, 112, 113, 155]. They also suggested that these hybrids could account for the diversity of cell phenotypes within tumors [121, 154]. Fusion between tumor cells and myeloid cells, with subsequent nuclear fusion, could produce new phenotypes in the absence of new mutations, as the hybrids would express genetic and functional traits of both parental cells [32]. These neoplastic hybrids would have the ability of macrophages to intravasate, to extravasate, and to migrate to distant organs while also possessing the unlimited proliferative potential of the cancer cells. Since myeloid cells are part of the immune system, tumor hybrids would also be able to evade immune surveillance [25].

5.1 Fusogenic cancers

Fusogenic tumor cells are found in a wide variety of cancer types including, melanoma, breast, renal, liver, gall bladder, lymphoma, and brain (Table 1). Tumor cell hybrids can form either *in vitro* or *in vivo* from fusions between two tumor cells or between a tumor cell and a normal somatic cell. One of the first reports of tumor cell fusion hybrids showed that human glioma cells, when implanted within the cheeks of hamsters, spontaneously fused with non-tumorigenic host cells, resulting in metastatic hybrid human-hamster tumor cells [55]. Many of the early reports for fusogenic cancers described fusions between lymphomas and myeloid cells. For example, spontaneous *in vivo* fusion between the non-metastatic murine MDW4 lymphoma and host bone marrow cells resulted in aneuploid metastatic tumor cells [88].

Munzarova et al. recognized that numerous traits expressed in macrophages were also expressed in metastatic melanoma cells and suggested that the tumor metastasis could result from fusions between tumor cells and macrophages [12, 122]. Rachkovsky et al. tested this hypothesis by inducing fusions between cultured non-metastatic Cloudman S91 melanoma cells and murine peritoneal macrophages. The majority of the resulting macrophage-melanoma hybrids displayed increased metastatic potential when grown *in vivo* [32]. Further studies revealed that the Cloudman S91 melanoma cells could undergo spontaneous fusion with the murine host cells *in vivo* resulting in secondary lesions that were comprised mostly of tumor-host cell hybrids. The authors concluded that the tumor cells likely fused with host myeloid cells [97]. Artificial

fusions of human monocytes and mouse melanoma cells revealed that the resulting hybrids expressed both human and mouse genes [98]. Other investigators also showed that the macrophage-specific antigens F4/80 and Mac-1 were expressed in murine Meth A sarcoma cells after spontaneous *in vivo* fusion with host cells. Interestingly, latex bead phagocytosis was also expressed in the Meth A sarcoma-host cell fusion hybrids [102]. Since these fusion hybrids expressed genotypes and phenotypes of both parental cells, it appears that the non-metastatic tumor cells could acquire an invasive/metastatic phenotype without new mutations.

Fusion among tumor cells in human solid tumors is difficult to detect. Recent reports, however, have provided direct evidence for fusions between tumor cells and myeloid cells in human bone marrow transplant (BMT) recipients [112, 113]. Both radiation therapy and immunosuppression can increase the incidence of metastatic cancers [156]. DNA analysis of micro-dissected metastatic cells from a child diagnosed with renal cell carcinoma after a BMT revealed DNA from both the BMT donor and the recipient in the metastatic cells [112]. Bone marrow and tumor cell hybrids were also identified in a female who developed renal carcinoma after receiving a BMT from a male donor [113]. These reports provided the first genetic evidence that spontaneous fusions can occur between human myeloid cells and tumor cells.

It is well documented that tumor-associated macrophages promote tumor progression in many cancers through the release of cytokines, and pro-angiogenic and pro-metastatic molecules (reviewed in [33, 38]). However, the fusion of cancer cells with tissue macrophages could also accelerate tumor progression. The tumor hybrid daughter cells could acquire the migratory/invasive behavior from the macrophage genome while still maintaining unlimited proliferative potential. Macrophage-macrophage fusions could also induce aneuploidy resulting in a tumorigenic myeloid cell [30, 123]. The significance of macrophage fusion hybrids in human metastatic cancer requires more attention. The numerous *in vitro* studies and *in vivo* reports suggest that myeloid hybrids are responsible for the metastatic progression of at least a subset of cancers. Multinucleated giant cells, a signature of hybrid formation, are frequently seen in human cancers, suggesting that cell fusions are not rare events (Table 1). Regardless of the mechanism, metastatic cells express numerous behaviors of mesenchymal/myeloid cells and, if exploited, could generate novel therapeutic strategies for managing metastatic cancers.

6 Tumor cell expression of myeloid antigens

Myeloid cells express a wide variety of markers that are unique to their ontogeny and function [157]. Routine

histological and immunohistochemical analyses are often performed to assess tumor type and grade. Since TAMs are often correlated with a poor patient prognosis, tumor biopsies are frequently evaluated for macrophage markers. The macrophage antigen-expressing cells within the tumor stroma are usually classified as TAMs. However, several reports show that macrophage-specific antigens are expressed on a wide variety of human cancer cells (Table 1).

Ruff and Pert demonstrated that several macrophage antigens (CD26, C3bi, and CD11b) were expressed on tumor cells from small cell lung carcinoma (SCLC) [81]. Levels of expression were comparable to that seen in the monocyte controls. It is important to note that the macrophage antigens were expressed in the cultured tumor cells themselves and further confirmed *in vivo*. This eliminated the possibility that the antigen expression was derived from TAMs. These investigators concluded that the SCLC tumor cells in their specimens were not of lung origin, but rather were of myeloid origin. A malignant transformation of recruited myeloid cells, from smoking-related tissue damage, was offered as an explanation for the origin of tumor cells with myeloid/macrophage properties [81]. Although this interpretation was controversial [70, 82], the authors demonstrated additional myeloid properties of these tumor cells [83]. Other investigators confirmed macrophage antigen expression on other SCLC tumors and cell lines [82, 84]. Myeloid-associated antigens (CD14 and CD11b) were also expressed in five metastatic breast cancer cell lines [70]. None of the breast cancer cell lines, however, expressed markers for B or T cells [70]. The authors suggested that common antigen sharing between different cell types could be related to common cellular interactions [70]. In light of these findings and those from Ruff and Pert, we suggest that the shared antigen expression of macrophages and metastatic tumor cells is the result of a common mesenchyme origin.

Further evidence for a mesenchymal origin of metastatic cancer comes from tissue microarray analysis of 127 breast cancer patients [71]. CD163 was expressed on the tumor cells of 48% of the patients, while MAC387 was expressed on the tumor cells of 14% of the patients [71]. Pathology confirmed that the staining was localized to the tumor cells and not solely to the tumor infiltrating macrophages. Interestingly, cancers that contained CD163-expressing tumor cells had a more advanced histological grade, a higher occurrence of distant metastasis, and reduced patient survival [71]. This report demonstrated, for the first time, that tumor cells expressing macrophage antigens could be identified in more than half of breast cancer patients. Similar studies were conducted on 163 patients with rectal cancer [110]. CD163 was expressed in 31% of the rectal tumors from patients in the preoperative irradiation group,

but in only 17% in the non-irradiation group. Prognosis was also worse for those patients with CD163-positive cancer cells than in those patients with CD163-negative cancer cells [110]. These findings are consistent with role of radiation in inducing tumor cell–macrophage fusions and in exacerbating the metastatic properties of some cancers [71, 110, 158]. These studies demonstrated that macrophage antigens, which are associated with enhanced metastasis and poor prognosis, are expressed on the tumor cells of patients with breast and rectal cancers.

7 Carcinoma of unknown primary origin

Carcinoma of unknown primary (CUP) is a systemic metastatic disease without an identifiable primary tumor and is often associated with poor prognosis. Approximately 5% of all newly diagnosed cancers are classified as CUP [159, 160]. Histologically, these cancers are usually classified as adenocarcinomas, squamous cell carcinomas, poorly differentiated carcinoma, and neuro-endocrine carcinomas [160]. It is thought that these aggressive cancers rapidly metastasize before the primary tumor has had time to develop into a macroscopic lesion [160]. Interestingly, aneuploidy was identified in 70% of CUP adenocarcinoma, suggesting that these cancers contain hybrid cells [29, 73]. Reports have also demonstrated that CUP contain signet-ring cells, indicating that a subset of these cancers exhibit phagocytic behavior [72]. Due to the highly aggressive nature of these tumors, we suggest that some CUPs could have a myeloid/macrophage origin.

8 Many cancers express multiple macrophage properties

The evidence presented in this review indicates that many metastatic cancers can express multiple myeloid characteristics (Table 1). For instance, many tumors that were phagocytic or fusogenic also expressed myeloid antigens, further supporting a myeloid origin of these metastatic cancers. It is important to note that the myeloid properties we highlighted were expressed in the tumor cells themselves and should not be confused with myeloid properties expressed by tumor-associated macrophages, which are also present in the tumors but are not tumorigenic.

9 Possible mechanisms

Emerging evidence indicates that cancer is a metabolic disease, regardless of tissue or cellular origin, which arises as a result of impaired cellular energy metabolism and

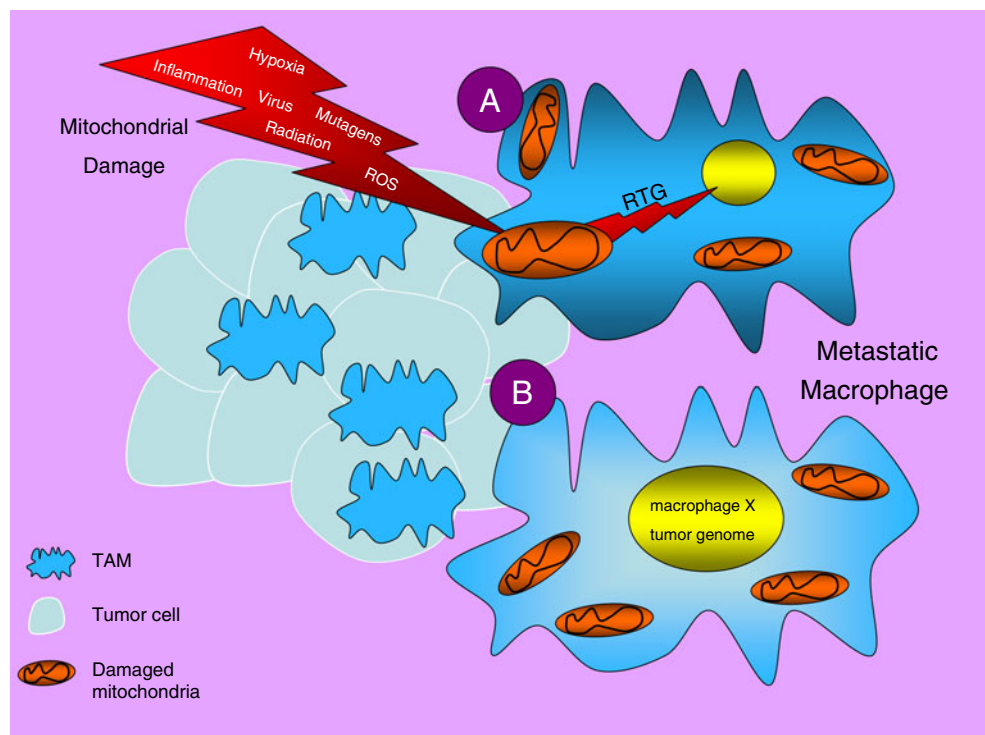
mitochondrial dysfunction (reviewed by [22]). Numerous studies indicate that tumor mitochondria are abnormal and are incapable of generating normal levels of energy [49, 161–166]. Defective mitochondria often arise as the result of damage to mitochondrial membrane lipids, specifically cardiolipin, through mutagens/carcinogens, radiation, hypoxia, inflammation, reactive oxygen species, and inherited mutations that alter mitochondrial energy production [49]. It is currently believed that any impairment in mitochondrial energy formation can lead to genetic instability via the retrograde (RTG) response, a signaling pathway that consists of sensors such as HIF-1 α and Nf κ B that detect mitochondrial stability [167]. Activation of the RTG pathway results in the synthesis of ATP through glycolysis when respiratory function is impaired. Over time, this respiration impairment would result in the upregulation of the TCA cycle and glycolytic substrate level phosphorylation, and induce proliferation and genetic defects, resulting in malignant transformation [22, 167–169]. These findings indicate that the integrity of the nuclear genome is dependent upon the functionality of the mitochondria for all cell types, including cells of myeloid and macrophage origin [22].

Metastatic myeloid tumor cells could arise from resident tissue macrophages or TAM that have suffered mitochondria damage (Fig. 1). The majority of tissues contain macrophages as part of their normal cellular composition and TAM are also a major cell type in most cancers where they can facilitate

tumor progression [33, 42, 157, 158]. In fact, depending on the tumor type, as noted above, up to 80% of the cells within a tumor are macrophages [33, 35]. Macrophages generally hone to mitochondria-damaging environments in response to inflammation, infection, would repair, and tumorigenesis [37, 38, 126, 128, 170]. It seems likely that mitochondria damage would occur resulting in cellular transformation through the RTG response where glucose and glutamine become the primary metabolic fuels for growth and survival [158, 171]. Glucose and glutamine are also major energy metabolites for normal cells of myeloid/macrophage lineage [158, 172]. Damage to myeloid/macrophage respiratory function could lead to a reliance on substrate level phosphorylation for energy, resulting in a malignant macrophage with the highest metastatic potential [22].

Mitochondria are dynamic organelles that undergo regular fusion and fissions [173]; thus, abnormalities in mitochondrial lipid composition would be rapidly disseminated throughout the cell's mitochondrial network and could also be passed along to other cells through cytoplasmic inheritance during cellular fusion events. While cell fusion events are considered rare outside of normal developmental processes, various reports have demonstrated that the frequency of cell fusion can be up to 1% in *in vivo* tumor models [64, 120, 123]. Additionally, chronic inflammation increases fusion events [174, 175]. Considering the large number of cells within a solid tumor, the fusogenic cell population could comprise a significant portion of the tumor.

Fig. 1 Proposed mechanisms of macrophage transformation. The tumor microenvironment consists of numerous mitochondria-damaging elements which would likely result in impaired mitochondria energy production in TAM and tissue macrophages and subsequent genetic instability through the RTG response (A). Macrophage fusion hybrids could result in cells able to express both the tumor and macrophage genomes resulting in cells with metastatic potential (B)



Cell fusion results in the mixing of the two parental cytoplasms [120, 129, 176]. Therefore, the formation of cancer cell and macrophage fusion hybrids would result in the transfer of damaged mitochondria from the cancer cell to the macrophage hybrid daughter cells. The subsequent inheritance of abnormal mitochondria would likely result in the transformation of the hybrid genome through the RTG response [22, 167–169]. In fact, the presence of fusion hybrids has been shown to correlate with tumor malignancy [65].

While it is unknown what triggers formation of macrophage \times tumor hybrids, various steps in the macrophage response to tumor development could provide the opportunity for cell fusion. Within hypoxic tumor areas, macrophages digest apoptotic cells. It has been suggested that macrophages may abort cellular digestion resulting in hybrid formation [31]. Additionally, macrophages could also fuse with somatic cells during tissue repair. Since tumors represent unhealed wounds [33, 177], it is possible that macrophages could fuse with tumor cells in an attempt to “heal” the tissue. Radiation damage during therapeutic intervention will also enhance fusion leading to more aggressive and difficult to manage tumors [110, 158]. Fusion events could also arise through the action of tumor-associated viruses, as several different tumor types (leukemia, lymphoma, Kaposi sarcoma, hepatocellular carcinoma, anogenital cancers, etc.) are associated with viral infection [124, 153]. Viral proteins enable successful cellular infection by fusing biological membranes. Additionally, some viruses utilize cellular fusion as a way to facilitate their spread (reviewed in [124]). Interestingly, several tumor-associated viruses including Epstein–Barr virus, Kaposi’s sarcoma-associated herpes virus, human papilloma virus (HPV), hepatitis B and C viruses, Rous sarcoma virus, and human T cell leukemia virus type 1 localize to the mitochondria compartment of the cell where they could potentially cause mitochondria defects [178–181].

The macrophage fusion hypothesis is an attractive explanation for metastasis. Fusions among macrophages and tumor cells could also account for histological structures in metastatic sites that resemble the histology of the primary tumor tissue as it has been shown, through reprogramming strategies, that macrophages are capable of producing fully functional epithelial cells at secondary sites while retaining histological characteristics of the original primary tissue [123, 182, 183]. Tumor fusions could also explain aneuploidy and chromosomal abnormalities seen in most cancer cells [31, 124]. However, there are conflicting observations showing suppressed tumorigenicity following hybridization between normal cells and tumor cells [22, 184].

Damage to the respiratory capacity of resident tissue phagocytes, TAM, or macrophage hybrids would trigger

a RTG response and over time lead to uncontrolled proliferation and genomic instability. Metastatic behavior would be the expected outcome of impaired mitochondrial function in myeloid or macrophage cells, as these cell types are mesenchymal cells that are able to degrade the extracellular matrix, migrate through local tissues, and enter and exit the circulation.

10 Modeling the myeloid origin of metastasis

The development of more effective therapies for the management of human metastatic cancers can be improved with animal models that accurately reflect the human disease. We recently described two new mouse models of systemic metastatic cancer in the inbred VM mouse strain that express multiple myeloid/macrophages characteristics to include phagocytosis, morphological appearance, and gene and lipid expression [14, 28, 185]. The VM-M2 and the VM-M3 tumors model all major steps of metastasis including, local invasion, intravasation, immune system survival, extravasation, and secondary tumor formation [14, 28, 185]. Moreover, the VM-M2 and the VM-M3 tumor cells are naturally metastatic from any inoculated tissue and, in contrast to most other mouse metastatic models, do not require intravenous injections to initiate the metastatic phenotype. Tumor cells that possess true or natural metastatic potential should not require intravenous injection to demonstrate metastasis. We also showed that the response of the metastatic VM tumors to well described anti-cancer drugs (cisplatin and methotrexate) was similar to that described previously in human cancer patients treated with these drugs [28]. We suggest that these mouse models of systemic metastatic cancer will be useful for developing novel therapies that target the myeloid properties of metastasis.

11 Concluding remarks

A transition from an epithelial-type cell to a mesenchymal-type cell is often considered an underlying characteristic of metastasis. As an alternative to a series of gain-of-function mutation events, we suggest that the metastatic mesenchymal phenotype can arise from malignant myeloid cells either through cell hybrid formation or through direct transformation of tissue macrophages. We think that it is improbable that random mutations acquired through a Darwinian selection process could account for all of the myeloid-cell behaviors necessary for the completion of the metastatic cascade. It is in our opinion that the myeloid origin of metastasis is the most probable explanation of tumor progression to date.

We suggest that future research in this field should focus on the myeloid properties of metastatic cells. The VM mouse model, as well as many of the human and murine cell lines discussed above, will be valuable for the development of novel metastasis therapies. Additionally, we urge others to consider the possibility that some of the cells previously identified as TAM in tumor pathological tissue specimens may in fact be metastatic tumor cells. This is especially true since TAMs are often localized to the leading edge of an invading tumor mass, and high levels of TAM are usually indicative of poor prognosis. We contend that targeting macrophage behaviors (e.g., phagocytosis, fusogenicity, and energy metabolism) would result in therapies that are effective in managing metastatic cancer, regardless of primary tumor origin.

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