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RESEARCH PAPER

Chemotherapy-induced metastasis: mechanisms and translational opportunities

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Abstract

Tumors often overcome the cytotoxic effects of chemotherapy through either acquired or environment-mediated drug resistance. In addition, signals from the microenvironment obfuscate the beneficial effects of chemotherapy and may facilitate progression and metastatic dissemination. Seminal mediators in chemotherapy-induced metastasis appear to be a wide range of hematopoietic, mesenchymal and immune progenitor cells, originating from the bone marrow. The actual purpose of these cells is to orchestrate the repair response to the cytotoxic damage of chemotherapy. However, these repair responses are exploited by tumor cells at every step of the metastatic cascade, ranging from tumor cell invasion, intravasation and hematogenous dissemination to extravasation and effective colonization at the metastatic site. A better understanding of the mechanistic underpinnings of chemotherapy-induced metastasis will allow us to better predict which patients are more likely to exhibit pro-metastatic responses to chemotherapy and will help develop new therapeutic strategies to neutralize chemotherapy-driven prometastatic changes.

Keywords TMEM · Cancer cell dissemination · Mena^{Calc} · Bone marrow-derived cells · Mesenchymal stem cells · Macrophages

Introduction

Current standard of cancer care for the loco-regional disease commonly includes surgery, radiotherapy and/ or chemotherapy. Depending on cancer type and stage of the disease, these treatments may be curative. However, a subset of patients will develop distant metastases and face high mortality despite achieving complete control of the local disease. The conventional belief is that metastases represent growth of clinically and radiographically undetectable foci of cancer already present at the time of initial treatment [1–3]. However, accumulating evidence now suggests that chemotherapy itself may under certain circumstances induce intratumoral or systemic changes, which can paradoxically exacerbate cancer cell proliferation and dissemination in certain patients [3]. For example, preoperative or neoadjuvant chemotherapy (NAC) may not only select for chemoresistant tumor clones, as traditionally suggested, but it may also drive the development of novel mutant clones which directly correlate with the development of metastatic disease [4]. In addition to inducing novel mutant clones, NAC may induce pro-metastatic changes in the microenvironment of the primary tumor. These pro-metastatic changes represent consequences of host-repair mechanisms in response to cytotoxic tissue damage [5], and are typically triggered by the systemic release of cytokines and chemokines, resembling those found during wound healing and inflammation. The systemic release of cytokines can also occur during postoperative or adjuvant chemotherapy and may render distant organs more prone to metastatic seeding [6, 7].

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Thus, an increasing body of evidence indicates that chemotherapy in certain instances could increase the metastatic potential of cancers. Therefore, it is crucial to gain more thorough understanding of the contextual prerequisites under which chemotherapy induces or exacerbates metastasis. Consistent with the idea that treatment of tumors that induce an injury-like response can contribute to metastasis, it has been noted that increased circulating tumor cells may rise in cancer patients and preclinical animal models of cancer, as a consequence of radiotherapy, surgery and surgical biopsy, besides chemotherapy [3]. However, here, we provide a comprehensive review specifically on the pro-metastatic effects of chemotherapy and not of other treatment modalities (for the later, please see an excellent review by Martin et al. [3]). Understanding the relationship of tumor injury to metastasis will help improve treatment of metastatic disease, and help stratify cancer patients according to their potential response to chemotherapy, to achieve the highest standards in personalized medicine.

Revisiting the metastatic cascade

The metastatic cascade has been described as a sequence of events leading to the development of metastatic tumors in organs and tissues distant from the primary tumor site. The knowledge of the molecular and cellular events involved in individual steps of the metastatic cascade has expanded over the past years revealing complexity beyond what was originally thought [8, 9]. This review focuses on the recent conceptual advancements on the biology of metastasis, critical for understanding how chemotherapy could paradoxically induce the progression of metastasis.

Epithelial-to-mesenchymal transition (EMT), cell invasion and migration

In most epithelial cancers, tumor cells undergo epithelial-to-mesenchymal transition (EMT), a biological program that allows the cells to gain mesenchymal phenotype and invade blood or lymphatic vessels. Hence, the evidence of EMT in many tumors has been associated with increased metastasis and worse prognosis [9–15]. During EMT cancer cells typically downregulate epithelial-specific cadherin, E-Cadherin, and upregulate mesenchymal-specific cadherin, N-Cadherin [16, 17] Detailed molecular mappings of multiple EMT markers and pathways have been explored in detail to better understand how mesenchymal plasticity conveys metastatic behavior in tumor cells [18]. Although EMT is a crucial hallmark of the metastatic cascade [9], the extent of EMT contribution during metastasis is debated. For instance, an early study by Wicki et al. suggested that podoplanin-based filopodia can enhance cancer cell invasion in the absence of EMT in breast and pancreatic beta-cell cancers [19]. Another study by Fischer et al. that utilized an EMT lineage-tracing system which examined the expression of a mesenchymal-specific fluorescent reporter whose expression becomes irreversible after EMT induction, suggested that EMT is only partial during metastasis [20].

Markers of EMT and its associated tumor cell dissemination have emerged were shown to be clinically useful in the assessment of metastatic risk in breast cancer patients. During EMT, the activity of Epithelial Splicing Regulatory Protein 1 (ESRP1) reduces the expression of MENA11a, an isoform of the actin-regulatory protein MENA that promotes cellular cohesiveness [21]. The decreased level of Mena11a is frequently accompanied by concurrent increase in the expression of invasive MENA isoforms, such as $MENA^{INV}$ among others, which promote invasion and migration of tumor cells [22–25]. This MENA expression pattern, $MENA11a^{low}$ and $MENA^{INV\text{-}Hi}$, also known as $MENA^{Calc}$, is associated with increased cancer cell invasiveness, metastasis and poor prognosis in breast cancer patients [26–28]. Mechanistically, $MENA^{INV\text{-}Hi}$ expression induces up to a 50-fold enhanced chemotactic response to EGF, HGF and IGF ligands by sequestering PTB1B away from the receptor tyrosine kinases (RTKs) [29–32]. In addition, $MENA^{INV\text{-}Hi}$ cells have increased haptotaxis on fibronectin via interaction with integrin α5 [33], and generate mature invadopodia by enhancing the phosphorylation of cortactin in the invadopodium core structure [34]. Invadopodia are actin-polymerization driven protrusions that focally degrade extracellular matrix (ECM) and are required for transendothelial migration during tumor cell dissemination [35, 36]. Within the tumor microenvironment, $MENA^{INV\text{-}Hi}$ tumor cells migrate on collagen fibers as a stream paired with tumor associated macrophages (TAMs) towards HGF-secreting endothelial cells [31, 37–41]. This pairing behavior is maintained though the EGF/CSF1 paracrine loop (Fig. 1), which keeps macrophages and cancer cells in close proximity [22, 38, 42, 43]. Interestingly, a recent study showed that the direct contact between cancer cells and macrophages induces $Mena^{INV}$ expression in cancer cells via a juxtacrine loop, (Fig. 1) involving Notch-Jagged mediated signaling [44]. It is plausible that macrophage-cancer cell contact during streaming is required for $MENA^{INV}$ expression in tumor cells in vivo. Thus, the directional streaming of $MENA^{INV\text{-}Hi}$ tumor cells towards the underlying vasculature may represent the major route for cancer cell dissemination and thus a prerequisite for metastasis [45].

Regulation of vascular permeability and intravasation by $TIE2^{HI}$ macrophages

The EMT and the directional streaming of $MENA^{INV\text{-}Hi}$ tumor cells towards blood vessels are not sufficient to cause

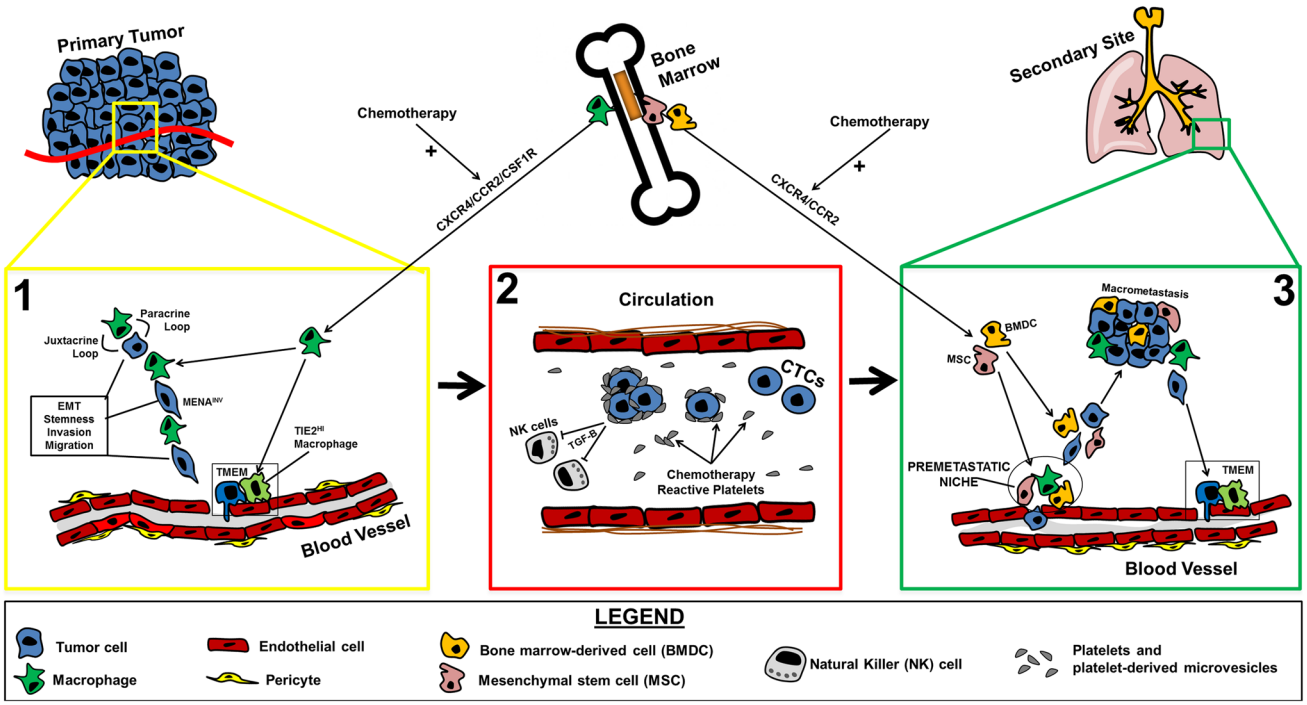

Fig. 1 Chemotherapy-induced metastasis. A working model depicting critical molecular and cellular events of the metastatic cascade, including those in **1** primary tumor site (yellow box), **2** blood circulation (red box), and **3** secondary tumor site (green box). Illustrations of chemotherapy-induced cellular and molecular events that facilitate the metastatic cascade are shown for each compartment individually. Chemotherapy treatment induces the infiltration of a wide variety of bone marrow-derived cells (BMDCs) and mesenchymal stem cells (MSCs), mostly including proangiogenic and intratumoral macrophages, by altering the tumor chemokine network (including CXCR4/CCR2/CSF1R), thus amplifying all prometastatic pathways which involve the TMEM dissemination machinery (primary and secondary tumor sites) and the premetastatic niche formation (secondary tumor site). In addition, chemotherapy treatment may induce a platelet-mediated prometastatic response in blood circulation, as evidenced by the aggregation of platelets and platelet-derived macrovesicles around circulating tumor cells (CTCs). Cartoon abbreviations: *EC* endothelial cell, *M* macrophage, *TC* tumor cell, *TMEM* tumor microenvironment of metastasis. (Color figure online)

cancer cell dissemination, since a specialized cancer cell intravasation mechanism is also required for the entry of cancer cells into the circulation [45]. Intravital imaging of breast cancer in live mice has demonstrated that intravasation does not occur throughout the entirety of the cancer-associated endothelium, but instead is localized in specific microanatomical structures, known as "tumor microenvironment of metastasis" (TMEM) (Figs. 1, 2A). TMEM is composed of three cell types, a Mena^{Hi} tumor cell, a perivascular macrophage and an endothelial cell, all in direct contact with each other [46]. TMEM density in the primary tumor predicts metastatic risk in breast cancer patients [45–48].

Although many macrophage subtypes may be present in perivascular regions, only macrophages expressing high levels of the angiopoietin receptor TIE2 (designated as TIE2^{Hi} macrophages), are capable of assembling functional TMEM structures [49]. It is known that tumor cells intravasating via TMEM express Mena^{INV}, which is required for transendothelial migration and express an invasion signature that is characteristic of cell migration during embryonic development [44, 45, 50, 51].

TMEM-associated endothelial cells have not been explored with regards to their gene and protein expression profiles and characteristics essential for TMEM function. However, the role of the perivascular TIE2^{Hi} macrophages has been recently evaluated in this context [49]. It was shown that TMEM function depends on the release of vascular endothelial growth factor (VEGF) from the TIE2^{Hi} macrophage. Indeed, the conditional knockout of the VEGF gene specifically in macrophages blocks TMEM-dependent paracellular cancer cell intravasation without affecting TMEM assembly [49].

VEGF can cause intra-tumoral endothelial cell permeability [52] via three distinct mechanisms: (a) pinocytosis paired with transcytosis, (b) endothelial fenestration, and (c) tight-junction-regulated (also known as "paracellular") permeability [53–56]. Although the tumor neovasculature generated under the control of VEGF is almost always fenestrated, tumor cells cannot cross through the fenestrae of endothelia [56]. Cancer cell transendothelial migration requires paracellular permeability which involves disruption of tight junctions by relatively high concentrations of VEGF

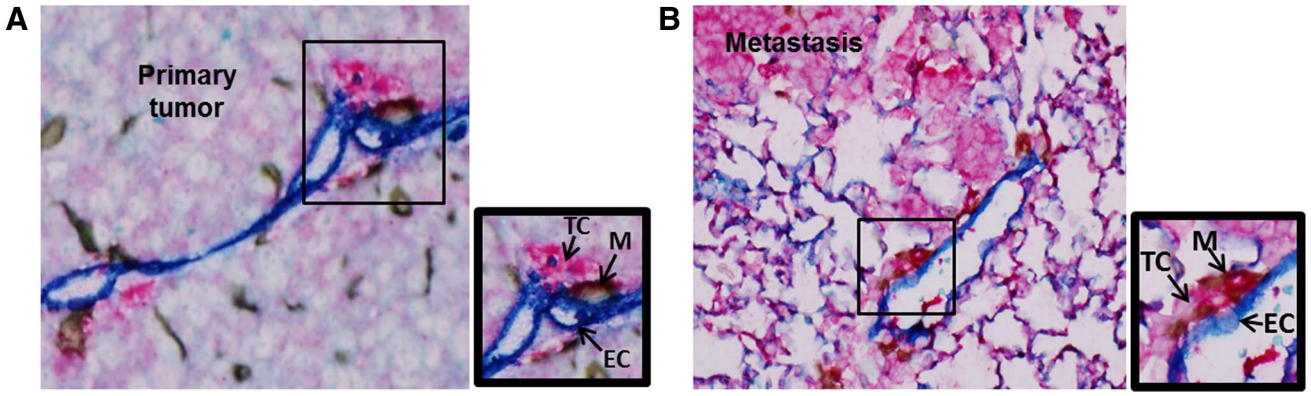

Fig. 2 TMEM in primary and secondary tumor sites. Tissue images from primary mammary tumor (**a**), and lung metastatic tumor (**b**), showing TMEM sites as visualized by triple-stain immunohistochemistry. The black boxes represent magnified inserts of the black squared areas to display TMEM structures in higher detail. EC, blue, endothelial cell; M, brown, macrophage; TC, red, MENA-expressing tumor cell. Figure from Entenberg et al. [161]. (Color figure online)

[55–58]. Only endothelium around TMEM sites can achieve high enough VEGF concentrations through the function of $TIE2^{Hi}VEGF^{Hi}$ macrophages, for the disruption of endothelial junctions and "paracellular" cancer cell intravasation as observed at TMEM [49]. The proangiogenic-perivascular $TIE2^+$ macrophages, which comprise a functional constituent of TMEM, are derived from $TIE2^+$ monocyte progenitors from the bone marrow [59–61]. TIE2 macrophage recruitment in breast and pancreatic tumors and vascular permeability at TMEM and cancer cell dissemination in breast tumors can be blocked by TIE2 inhibitor rebastinib, offering promising treatment options targeting TMEM associated vascular permeability and cancer cell dissemination [49, 62].

Monocyte infiltration could be achieved by the expression of three chemotactic receptors, the colony stimulating factor receptor (CSF1R), the C-X-C chemokine receptor type-4 (CXCR4), and the C-C chemokine receptor type-2 (CCR2), on the $TIE2^+$ monocyte surface [60, 61, 63]. Tumors and tumor-associated stromal cells often upregulate and release systemically the respective ligands for the aforementioned chemotactic receptors, namely CSF1, CXCL12 (SDF-1) and CCL2, resulting in increased monocyte and myeloid cell chemotaxis [64–66] (Fig. 1). In addition, the CSF1/CSF1R axis also promotes macrophage maturation and survival in the tumor microenvironment, thus increasing the overall yield through maturation and/or macrophage repolarization [67]. Moreover, the expression of TIE2 can be upregulated in both endothelial cells and macrophages under the control of hypoxia-inducible factor-1a (HIF1A) [68]. Therefore, $TIE2^+$ macrophages may be generated over time from tumor-resident macrophages undergoing hypoxic stress [68]. Furthermore, TIE2 signaling can suppress apoptosis and promote survival of $TIE2^+$ macrophages, $TIE2^+$ endothelial cells and even $TIE2^+$ hematopoietic stem cells in the bone marrow niche [60, 69–73]. Therefore, it is possible that the perivascular $TIE2^+$ macrophages have more prolonged lifespan than classically-activated inflammatory macrophages, due to enhanced TIE2-mediated retention in the perivascular niche. In conclusion, in many solid carcinomas the aforementioned molecular pathways may contribute to an increasing population of $TIE2^+$ macrophages, which are known to promote angiogenesis, TMEM assembly, and TMEM-dependent cancer cell dissemination [45, 59, 60, 68, 73].

An important microanatomical element for vascular permeability and intravasation during cancer progression is the pericyte coverage of the post-capillary venules [9]. Pericytes mediate antiproliferative and stabilizing paracrine signaling to the adjacent endothelium, mainly through the secretion of angiopoietin-1 (ANG1), which promotes blood vessel quiescence and basement membrane synthesis [74]. The cancer-associated endothelium, and especially the neoplastic neovasculature, frequently presents with low pericyte coverage [75] (Fig. 1). Although pericyte-TMEM interactions have not been described in detail, it has been noted that functional TMEM sites are depleted of pericytes [49].

Biological programs in circulating tumor cells

Once tumor cells escape the primary site, they need to establish mechanisms of survival or resistance against immunological destruction, lack of adhesion to ECM substrates, as well as physical hazards, such the increased shear stress of the blood flow (Fig. 1). These are accomplished through signaling pathways that convey immune evasion, resistance to anoikis, and possibly cluster formation mediated by circulating platelets [9, 76]. Only a fraction of CTCs survive, extravasate and initiate metastatic growth [77, 78] which implies that not all CTCs have tumor-initiating capability [45]. In addition, it appears that stem-like properties are

required for successful metastatic colonization [79, 80]. Since "stemness" is triggered in specific niches within the primary tumor mostly involving juxtacrine signaling from tumor-associated macrophages (TAMs) and other myeloid-derived cells [81–83], the composition of the primary tumor microenvironment may play a pivotal role in metastatic capabilities of the primary tumor. Thus, areas of tumor enriched for tumor cell-macrophage pairing during chemotaxis and haptotaxis towards blood vessels and TMEM sites [31, 33, 37, 38], may represent niches for cancer cell education required for successful metastatic colonization.

CTCs have been extensively analyzed using high-throughput approaches, such as RNA-sequencing at the single-cell level and it has been shown that they can carry mutational and even epigenetic information from the primary tumor. However, there are certain discrepancies arising from the comparison of these profiles, making the origin of CTCs more debatable. In particular, it is not certain whether CTCs exclusively originate from the primary tumor, or also disseminate from clinically undetectable secondary sites [45]. Thus, we theorize that the microenvironment of the secondary sites may also contribute to programming of tumor cells and further cancer progression (Fig. 2b).

Cancer–stroma interactions in the metastatic microenvironment

While CTCs could potentially access all tissues in the body, metastatic disease usually develops in selected tissues and organs, depending on the type of cancer. Recent evidence suggests that the organotropic properties of disseminating cancer cells may be dictated by the exosomes, small membrane vesicles ranging in size from 40 to 100 nm, secreted from primary tumors [84]. The exosome function has been described as a major pathway contributing to the formation of a metastasis-receptive niche [85–87]. In particular, primary tumors may secrete exosomes that exert specific tropism for particular secondary sites, based on the integrin profile of the tumor-derived exosomal cargo and that of the tissue-specific stromal cells [84].

Metastasizing tumor cells frequently home to tissues in which tumor-promoting stromal cells offer a supportive microenvironment, also known as the "premetastatic niche" [88] (Fig. 1). Recent evidence suggests that myeloid-derived suppressor cells (MDSCs), which have been traditionally considered components of the immunosuppresive tumor microenvironment, can promote premetastatic niche formation, as well as increase tumor angiogenesis and invasion [89]. Furthermore, it seems that neutrophils may contribute to successful formation of metastatic foci. In particular, neutrophils recruited to the premetastatic niche produce a set of leukotrienes which specifically expand cancer cell subpopulations with high tumorigenic potential, such as cancer stem cells (CSCs) [90]. Tissue-resident stromal cells may also contribute to premetastatic niche formation. For instance, periostin secreted locally by recruited fibroblasts facilitates metastatic initiation and colonization of breast cancer cells in the lung [91]. Therefore, a variety of bone marrow-derived cells (BMDC) and tissue-resident stromal cells may contribute to the formation of micrometastatic foci in the secondary site.

The last critical step in the metastatic cascade is the transition of micrometastatic foci into clinically overt metastases; a process that involves awakening of disseminated tumor cells (DTCs) from dormancy [92, 93] (Fig. 1). Dormancy represents a specialized biological program exploited by metastasizing cancer cells to convey survival advantages in the secondary tumor site, until they become capable of further expansion and colonization. Recently proposed scenarios suggest that DTCs may activate stress signals in the secondary tumor microenvironment which may help them to resume growth. DTCs may also carry specific gene signatures triggered by the hypoxic niche of the primary tumor microenvironment and these signatures may be associated with tumor progression at the secondary sites [92–94]. Thus, both tumor cell intrinsic factors and the tumor microenvironment (involving both tissue-resident stromal cells and recruited BMDCs) are involved in the formation of the premetastatic niche, as well as in the regulated entry/escape of DTCs from the dormancy program and parallel initiation of the colonization step.

Chemotherapy-induced systemic and tissue-specific prometastatic effects

The metastatic process is controlled by a delicate balance between promoting and suppressive factors in the tumor microenvironment. The dominance of the former over the latter increases the efficiency of metastasis. A body of evidence presented over the past several years indicates that chemotherapy treatment may tilt this balance in favor of cancer cell dissemination. Chemotherapy may lead to tissue damage and subsequent activation of host-mediated tissue repair program, which involves plethora of cytokines and chemokines [5] that can affect the metastatic susceptibility of distant organs [6, 7]. If chemotherapy is given preoperatively, significant changes occur in the composition of the primary tumor microenvironment which may favor the metastasis-promoting rather than the metastasis–suppressing components of the tumor microenvironment. Since chemotherapy treatment may, under certain circumstances, shift the balance towards favoring metastases it is important to elucidate the exact contextual prerequisites for metastasis induction and identify risk factors which could potentiate those effects in certain patient subpopulations.

Chemotherapy may provide systemic support for metastasis through the induction of pro-inflammatory circuits

It has been shown that certain chemotherapeutic drugs, such as paclitaxel, may initiate prometastatic responses in the primary tumor microenvironment by directly activating specific inflammatory signaling pathways [95]. Paclitaxel structurally resembles a pattern recognition receptor called toll-like receptor-4 (TLR4), which is mainly expressed on the surface of antigen-presenting cells and responds to lipopolysaccharide (LPS), a component of the bacterial membrane [96]. Macrophages with activated TLR4 pathway, mediated by either LPS or paclitaxel, quickly migrate to a site of infection, or tissue repair induced by chemotherapy and/or radiotherapy [97] to either destroy the invaders or restore homeostasis in the affected tissue [96]. However, overexpression of TLR4 has been observed on tumor cells as well. Thus, TLR4 positive cancer cells can be activated by paclitaxel which can exacerbate proinflammatory tumor microenvironment. These host-initiated proinflammatory responses are frequently accompanied by increased angiogenesis and cancer cell invasion which may promote metastatic dissemination [97–101].

However, it should be noted that most of the described proangiogenic and prometastatic effects of chemotherapy are not a direct result of specific signal transduction pathways, as in the case of paclitaxel-TLR4 axis, but a more generic response to cytotoxic tissue damage, hypoxic stress and a prolonged wound healing-like process [99, 102, 103]. For instance, Chang et al. [104] have shown that paclitaxel and cyclophosphamide may induce cancer cell dissemination and metastatic colonization by recruiting myeloid progenitors in both the primary and the secondary sites in a stress-inducible Atf3-dependent manner. The transcription factor Atf3, a member of the ATF/CREB family of transcription factors activated upon stress, is a master regulator of a plethora of inflammatory cytokines involved in leukocyte migration and angiogenesis [105]. In both spontaneous and experimental metastasis models, Atf3 was shown to be required for cancer cell seeding and the development of distant metastasis [104], indicating that proinflammatory circuits are necessary for chemotherapy-induced metastasis.

Chemotherapy mediates the mobilization of bone marrow progenitors to primary and secondary sites to promote metastasis

It has been proposed that metastasis is regulated through an incipient host repair mechanism initiated by the mobilization of bone marrow-derived cells (BMDCs), such as hemangiocytes, endothelial progenitor cells (EPCs), $TIE2^{+}$ monocytes and myeloid-derived suppressor cells (MDSCs), all known to regulate angiogenesis and blood vessel homeostasis in damaged tissues [65, 106–108]. Indeed, it has been shown that chemotherapy can trigger the mobilization of various BMDCs to primary tumor site, as well as to the lung [106, 109, 110]. Chemotherapy may promote the formation of the premetastatic niche, by creating a stress response and amplifying the chemotactic signals and the proinflammatory circuits to which all these BMDCs may respond. Once recruited, BMDCs can then promote metastatic dissemination by producing and systemically releasing chemokines, bioactive lipids, alarmines and growth factors [102]. Depending on the adaptive characteristics and the expression profiles of the respective receptors on the tumor cell surface, the dissemination and homing of tumor cells at secondary sites can be exacerbated [102]. Moreover, chemotherapy may induce metastasis by mechanisms that do not involve myeloid and/or endothelial progenitors. For instance, Roodhart et al. (2011) demonstrated that platinum analogs, such as cisplatin, may stimulate mesenchymal stem cells (MSCs) to release polyunsaturated fatty acids, which may, in turn, systemically support tumor growth, resistance to chemotherapy and metastasis in mouse models of breast, lung and colon carcinomas [111, 112]. The role of MSCs in tumor progression is debatable, and depending on the context may be either tumor-promoting or tumor-suppressive [113–116]. However, it has been documented that MSCs are recruited to chemotherapy-damaged tissues, where they can also exert certain prometastatic effects [111, 112, 117, 118]. Since these bone marrow-derived progenitor cells are principal mediators of chemotherapy-induced metastasis in virtually all steps of the metastatic cascade (Fig. 1), and are released from the bone marrow in response to proinflammatory circuitries, we will refer to them as the BMDC/MSC infiltrate.

Chemotherapy may promote EMT and increased cancer cell invasiveness

The remainder of this chapter describes chemotherapy-induced pro-metastatic changes in the sequential steps of the metastatic cascade, as described in the previous sections.

As mentioned before, the acquisition of mesenchymal phenotype through EMT is considered the initial step in the metastatic cascade [11, 12, 80]. Although there are studies on chemotherapy-mediated EMT suppression and/or MET induction [119, 120], several reports have also linked chemotherapy treatment with an induction of EMT in the primary tumor microenvironment. For instance, continued treatment with paclitaxel or vincristine promoted EMT and contributed to the formation of lung metastasis in mice bearing hematopoietic malignancies [121]. Likewise, in breast carcinoma, paclitaxel was shown to promote the expression of EMT markers in cancer cells, including the concerted decrease of

E-cadherin, increase of vimentin and nuclear localization of β-catenin, as well as induced lung metastases through a miR-21/Cyclin-dependent kinase-5 (CDK5) pathway [122]. Moreover, high-dose paclitaxel treatment, which could be achievable in the clinical setting, significantly increased the formation of invadopodia in breast cancer cells in vitro [123]. Chemotherapy may also affect EMT in an indirect fashion. For example, it has been reported that miRNA, miR-488, inhibits EMT in breast cancer cells [124]; however chemotherapy treatment frequently suppresses miRNA-488 in an NF-kB-dependent manner which relieves miR-488 EMT inhibition and thus indirectly stimulates EMT. In particular, cancer patients who received cyclophosphamide, epirubicin plus taxotere, or epirubicine plus 5-fluororacil had significantly suppressed levels of miR-488 [124], thus indicating potential chemotherapy-mediated EMT induction. Chemotherapy-induced EMT has also been reported in non-epithelial cancers, for instance, in cisplatin-treated osteosarcomas [125]. However, it still remains unclear whether the relative increase of mesenchymal-like tumor cells observed upon chemotherapy is a result of direct chemotherapy mediated EMT induction or a consequence of selection of chemoresistant cancer cells [80, 126].

Chemotherapy can also increase the proportion of invasive cancer cells. It was noted that paclitaxel treatment promotes the expression of MENA^{INV} in the PyMT mouse model of breast carcinoma, a metastatic patient-derived xenograft (PDX) model and post-chemotherapy breast cancer tissue samples from patients [26]. Since MENA^{INV} promotes invadopodium maturation [34], the increase in MENA^{INV} expression upon chemotherapy may be mechanistically linked to the observation that chemotherapy induces invadopodia [123]. As described earlier, MENA^{INV} sensitizes cancer cells to RTK ligand-dependent chemotaxis and ITGA5B1/FN-dependent haptotaxis [33], enhancing the migratory behavior of tumor cells. In addition, MENA^{INV} increases tumor cell transendothelial migration at TMEM [44, 51]. Thus, chemotherapy-induced MENA^{INV} expression may be responsible for recently reported observation of chemotherapy-induced increase in CTCs [26, 104]. Interestingly, mice lacking both functional copies of the *MENA* gene (i.e. MENA$^{-/-}$) developed no CTCs and DTCs, even after receiving a metastasis-exacerbating dose of neoadjuvant chemotherapy, which indicates that MENA orchestrates a cell motility/invasion program in cancer cells, irrespective of chemotherapy treatment [26]. Although it is not clear how chemotherapy causes an upregulation of MENA^{INV} expression in primary breast tumors [26], recent evidence has shown that MENA^{INV} can be upregulated in cancer cells as a result of Notch1-mediated juxtacrine signaling upon contact of cancer cells with macrophages [44]. Thus, chemotherapy-induced BMDC/MSC recruitment may be mechanistically associated with the induction of EMT and/or invasive cancer cell phenotypes (i.e. MENA^{INV-Hi}) in the primary tumor microenvironment.

Chemotherapy may affect cancer cell intravasation and dissemination

As outlined in "Regulation of vascular permeability and intravasation by TIE2^{HI} macrophages" section, the highly-invasive MENA^{INV} cancer cells are required but are not sufficient for cancer cell dissemination, unless they utilize functional intravasation sites, called TMEM [44, 51]. Accumulating evidence now demonstrates that a wide variety of chemotherapy regimens promote the mobilization of BMDCs/MSCs to the primary tumor microenvironment to repair the cytotoxic tissue damage, which in turn facilitate tumor regrowth and TMEM formation [26, 59, 61, 110, 127]. In particular, in the process of eliciting this chemotherapy-driven tissue repair response, new blood vessel formation (angiogenesis) frequently takes place, and encourages residual cancer cells that survived chemotherapy to resume growth [60, 66, 68, 73, 110, 127–132]. Recent experimental work by Hughes et al. suggested that cancer cell death and chemotherapy-induced hypoxia/necrosis could potentially promote the expression and systemic release of chemotactic factors, such as CXCL12, which in turn signals to CXCR4^{+} EPCs and monocyte progenitors, naturally residing in the bone marrow to home into primary tumors [132]. Indeed, cyclophosphamide treatment resulted in an influx of perivascular CXCR4^{+}TIE2^{+} macrophages, which accelerated neoangiogenesis and tumor regrowth [132].

In addition, at least two different chemotherapy regimens given in the neoadjuvant setting, either paclitaxel alone or the doxorubicin-cyclophosphamide combinatorial treatment, were both capable of promoting TIE2^{Hi} macrophage infiltration and increasing TIE2^{+} macrophage-associated TMEM assembly in multiple immunocompetent or immunodeficient mouse models of breast cancer [26]. Chemotherapy-induced TMEM assembly was subsequently corroborated independently by another research group [104]. Moreover, TMEM score increased in post-neoadjuvant breast cancer tissue samples from patients with ER^{+}/HER2^{-} breast cancer, who were treated with weekly paclitaxel for up to 12 weeks followed by four cycles of doxorubicin plus cyclophosphamide [26]. This observation may at least in part explain why long term survival of patients who do not achieve pathologic complete response (pCR) after neoadjuvant chemotherapy is worse than in patients who do achieve pCR [133]. The most concerning observation however was that in 10 out of 20 patients neoadjuvant chemotherapy increased TMEM score over the threshold that separates low-medium risk from high risk score for developing distant metastasis [26], as determined in a retrospective case-control study which demonstrated that TMEM is prognostic for metastasis in

$ER^+/HER2^-$ breast cancer [47]. In conclusion, chemotherapy-mobilized $TIE2^+$ macrophages may not only elicit proangiogenic but also prometastatic effects, since the $TIE2^+$ macrophage subpopulation is a prerequisite for function of TMEM sites.

The studies discussed above [26, 104], also documented chemotherapy-induced increase in CTCs, a result of increased TMEM assembly and function in chemotherapy-treated animal tumors. Indeed, although CTC count measured by U.S. Food and Drug Administration-approved CellSearch System is a strong prognostic factor in both primary and metastatic breast cancer in humans, there is no conclusive evidence that chemotherapy significantly reduces CTCs [134]. On the contrary, several reports have indicated that CTC counts in post-chemotherapy blood samples actually increase in some patients and decrease in others, yet they all correlate with distant metastasis-free survival [135, 136]. Collectively, these observations demonstrate that neoadjuvant chemotherapy may induce prometastatic changes in the primary tumor microenvironment, which may promote TMEM assembly and TMEM-dependent cancer cell dissemination. These findings indicate that chemotherapy-treated tumors do not use de novo mechanisms of cancer cell dissemination, but rather amplify the already established ones through the recruitment of BMDCs/MSCs (Fig. 1).

Chemotherapy may convey prometastatic properties on circulating tumor cells

The effects of chemotherapy treatment on CTCs have been rather underexplored. It has been demonstrated that chemotherapy-mediated tissue damage may also activate proteolytic cascades, including the complement cascade, the coagulation cascade and the fibrinolytic cascade, whose primary purpose is to initiate responses in damaged endothelia, but some of their activated proteolytic cleavage products are directly or indirectly involved in the ability of CTC to form metastases [102]. For example, the upregulation of urokinase plasminogen activator receptor (uPAR) [137, 138] and thrombin [139, 140], have been both linked to increased metastatic capacity. Furthermore, it has been demonstrated that chemotherapy may activate blood platelets into releasing platelet-derived microvesicles in form of small membrane fragments containing platelet-endothelium cell adhesion receptors (Fig. 1), such as CD41 and CD62P [102, 141–143]. These platelet-derived membrane fragments can subsequently coat the surface of CTCs, facilitating their attachment to the endothelium at the site of future metastasis [144, 145]. In addition, the coating of CTCs with platelets may shield tumor cells from violent shear forces [146], as well as promote the aggregation and formation of tumor cell emboli that can be more easily entrapped and retained in small vessels [147]. Finally, tumor cells within platelet-mediated aggregates are significantly protected from immunological destruction, mainly through the release of platelet-derived transforming growth factor-beta1 (TGF-β1), which inactivates NK cells through downregulation of the NK cell receptor NKG2D [148] (Fig. 1). This pathway could represent one of the multiple mechanisms of NK cell evasion by cancer cells and metastatic subversion of NK cell surveillance [149].

Chemotherapy may facilitate cancer cell seeding and colonization at distant sites

Chemotherapy may inflict hypoxic damage in tissues other than the primary tumor site, thus causing the release of chemotactic factors by tissue-resident leukocytes, fibroblasts and endothelial cells, and these chemotactic factors in turn, attract various BMDCs/MSCs [150]. The recruitment of BMDCs/MSCs to the secondary sites (either triggered by chemotherapy or not) initiates the formation of the premetastatic niche [88]. Once homed in the premetastatic niche (Fig. 1), BMDCs/MSCs may then regulate the development and progression of metastasis through paracrine interactions with the newly arrived metastasizing tumor cells. For example, Daenen et al. showed that mice treated with paclitaxel or cisplatin had significantly increased tumor cell retention in the lung vasculature with consequent metastatic colonization. This phenotype was explained by chemotherapy-induced expression of the vascular endothelial growth factor receptor 1 (VEGFR1) by the endothelial cells which enhanced endothelial-tumor cell adhesion and paracrine interactions [6]. This result was obtained with different tumor types, including breast and colon carcinoma as well as melanoma cells, suggesting that creation of the premetastatic niche is a more generalized, rather than a tumor cell-dependent effect of chemotherapy [6].

Furthermore, certain chemotherapies were shown to either increase the production and release of exosomes or to alter the composition of tumor-specific exosomes, also described as chemotherapy-induced exosomes or "chemoexosomes" [151]. However, the evidence of a direct effect of chemoexosomes on premetastatic niche formation is currently lacking, although these observations certainly warrant further investigations.

The final step of the metastatic cascade involves the survival of disseminated cells and micrometastatic foci in the microenvironment of the secondary site, and, following the exit from a dormancy program, the subsequent cancer cell proliferation at the secondary sites [8, 93, 152]. Chemotherapy-facilitated colonization has been described in certain cancer models, following the initial interactions of tumor cells within the premetastatic niche. A critical mediator of this step was shown to be matrix metalloproteinase-9 (MMP9), which was significantly overexpressed

in VEGFR1$^+$ EPCs or in other BMDCs following chemotherapy [109, 153]. Indeed, the local release of MMP9 in the metastatic niche eventually supported metastatic colonization of CTCs in an experimental metastasis mouse model, and was reversed by specific inhibition of MMP9 [109]. In these studies, chemotherapy-induced MMP9 overexpression had a distinct effect on cancer cell extravasation and the formation of micrometastatic foci, which increased the overall rate of macrometastasis formation [109, 153]. A different study demonstrated that inflammatory monocytes (iMs) could be recruited to the tumor microenvironment at the secondary sites through a CCL2/CCR2 chemotaxis pathway following chemotherapy [104]. Recruitment of these iM promoted the local suppression of cytotoxic CD8$^+$ T-lymphocytes in the lung, thus facilitating metastatic colonization in mouse model of either spontaneous or experimental metastasis [104]. These observations collectively suggest that chemotherapy induces recruitment of BMDCs/MSCs to the premetastatic niche, which in turn, facilitate tumor cell seeding and subsequent colonization of the secondary site (Fig. 1).

Therapeutic reversal of the chemotherapy-induced prometastatic effects

Chemotherapy increases survival in patients with a variety of localized and advanced cancers [133, 154]. However, there are many patients who do not draw full benefit from chemotherapy, and according to recent findings (as described in "Chemotherapy-induced systemic and tissue-specific prometastatic effects" section), chemotherapy may induce more aggressive disease in some patients. Therefore, new treatment modalities for preventing chemotherapy-induced metastasis as well as new markers that can predict which patients will likely develop more advanced disease due to chemotherapy are needed. It should be noted that certain biological programs, such as chemotherapy-induced MENA^{INV} expression as described in "Chemotherapy may promote EMT and increased cancer cell invasiveness" section, are particularly attractive candidates to eliminate chemotherapy-driven metastasis. However, given the current challenges of intracellular drug delivery in vivo [155], in this section, we focus on basic principles and rationale for designing approaches based on extracellular targets.

Burning off the "catalyst" of the metastatic cascade

Various cells within the tumor microenvironment, such as leukocytes, macrophages, endothelial cells, fibroblasts as well as tumor cells release chemokines and create the so called tumor "chemokine network" [150]. Chemotherapy induces cytotoxic tissue damage and hypoxia and subsequent recruitment of myeloid and/or mesenchymal cells from the bone marrow. These bone marrow-derived BMDC/MSC infiltrates modify the chemokine network of the primary tumor and shift the balance towards the prometastatic phenotype. Thus, the BMDCs/MSCs act as "catalysts" in the progression of the metastatic cascade (Fig. 1). In view of this working hypothesis, various pharmacological interventions in the chemokine network could theoretically prevent the accumulation of BMDC/MSC infiltrates in the primary and secondary tumor microenvironments, thus eliminating the prometastatic effects of chemotherapy.

As already explained in "Revisiting the metastatic cascade" section, chemotherapy may support the infiltration, maturation and increased retention of metastasis-promoting BMDCs/MSCs in a context-dependent manner, mainly through the induction of the CXCL12/CXCR4, CCL2/CCR2 and CSF1/CSF1R chemotactic pathways [61, 132]. Therefore, pharmacological inhibition of CXCR4 paired with chemotherapy can significantly suppress primary tumor growth [156], chemotherapy-induced angiogenesis [132], and metastatic burden of chemoresistant tumors [157], as shown in preclinical models of ovarian, breast and small cell lung cancer. Therefore, the pharmacological inhibition of the chemokine receptor CXCR4 in conjunction with chemotherapy could potentially counteract the chemotherapy-exacerbated CXCR4-mediated prometastatic effects. Similarly, the selective antagonists of the chemokine receptor CCR2 (or small molecule inhibitors of CCL2) have been used quite efficiently in this context, since they are capable of disrupting M2-like macrophage recruitment, macrophage-mediated immunosuppression and metastatic efficiency in preclinical models of prostate, liver and pancreatic cancers [64, 158, 159]. Finally, the specific blockade of the CSF1/CSF1R pathway can also efficiently reprogram the immunosuppressive responses of myeloid cells in the primary tumor microenvironment [67], thus reducing tumor growth and metastasis [160].

The few examples discussed above provide a proof-of-principle that the therapeutic modulation of the chemokine network in many types of solid carcinomas could pose an effective strategy for preventing chemotherapy-induced metastasis. However, the chemotactic pathways leading to recruitment of BMDCs/MSCs in the tumor microenvironment are promiscuous. In other words, there are many different types of BMDCs/MSCs that respond to a variety of chemotactic stimuli [9, 150], and therefore, the selective targeting of one such pathway may promote the selection of alternative pathways achieving similar metastatic potential through different BMDC/MSC "catalysts". The chaotic nature of the chemokine network along with our limited knowledge behind the overall chemokine repertoire of

individual human cancer types [150] make this therapeutic approach quite challenging, but worth-pursuing.

Sealing the "doorways" to cancer cells

Another approach to eliminate the chemotherapy-induced prometastatic effects would be blocking cancer cell intravasation and extravasation, the two "vulnerable" steps of the metastatic cascade with regards to the efficiency of the metastatic process (Fig. 1). Indeed, since tumor cells disseminate via an intravasation mechanism involving TMEM [49] and TMEM are present in both primary tumors and secondary metastatic sites (Fig. 2) [161], the pharmacological inhibition of the TMEM doorways to seal them to tumor cell intravasation would eliminate CTCs, irrespective of the chemotherapeutic that induced prometastatic changes. In addition, since TMEM are present in both the primary tumor and its metastatic sites, TMEM inhibition would be beneficial in all stages of treatment.

The crucial BMDC involved in TMEM-dependent cancer cell dissemination is the perivascular TIE2$^+$ macrophage, which in fact, is a proangiogenic M2-like macrophage [49, 132]. TIE2$^+$ macrophages are tethered in the perivascular niche of primary tumors, because they respond to angiopoietin signals or other non-canonical ligands, such as integrins and lysyl oxidase, originating from the cancer-associated endothelium [162–166]. Recently, the TIE2 kinase switch pocket inhibitor rebastinib, was shown to inhibit TIE2, and subsequently reduced tumor growth, angiogenesis and metastasis in orthotopic mouse models of metastatic mammary carcinoma and pancreatic neuroendocrine tumors [62]. In particular, rebastinib inhibited TMEM function by inhibiting the angiopoietin receptor TIE2 on the TMEM macrophage and prevented VEGF-dependent vascular permeability [62]. Furthermore, rebastinib significantly reduced the number of TMEM-dependent CTCs in the blood and the number of DTCs in the lungs [26], and significantly increased the overall survival of paclitaxel-treated mice even after resection of the primary tumor [62]. These observations indicate that TIE2 inhibition inhibits the chemotherapy-induced prometastatic tumor microenvironment associated with TMEM [26].

Another therapeutically "vulnerable" step of the metastatic cascade is tumor cell extravasation, as it is also required for effective colonization. Previous studies have documented that tumor cell adherence and retention in the intraluminal side of blood vessels in the metastatic organs may persist for a varying period of time, which dictates tumor cell survival probability and their clearance by cytotoxic immune cells, such as NK cells [167]. Therefore, the interactions of CTCs with the premetastatic niche are of utmost importance for successful seeding, and the therapeutic intervention of those interactions may pose another attractive strategy for counteracting chemotherapy-induced metastasis. For instance, Daenen et al. demonstrated that chemotherapy-recruited VEGFR1$^+$ EPCs in the lung endothelium can significantly promote the early retention and survival of tumor cells, eventually facilitating the formation of metastasis, as already described in "Chemotherapy may facilitate cancer cell seeding and colonization at distant sites" section. Indeed, the targeted inhibition of VEGFR1 with neutralizing antibodies, but not that of other VEGF receptors such as VEGFR2, completely eliminated the chemotherapy-mediated tumor cell retention and subsequent lung colonization [6]. These observations suggest that the disruption of critical tumor-host cell interactions during cancer cell extravasation may profoundly affect the fate of metastasis in the presence of chemotherapy.

Conclusions and future directions

Our understanding of cancer progression has been rapidly evolving and it has moved in somewhat unexpected directions in the past several years. We have been reminded that tissues and organ systems in complex metazoan organisms operate in harmony, and that any insult, even if introduced with the intention to cure, may have complex consequences that we only now have started to unravel. As a large body of preclinical evidence indicates, cytotoxic chemotherapy in the process of destroying tumor cells activates host reparatory mechanisms that may sabotage our intentions to cure cancer. We now need to move our focus from studying cancer cells in isolation to studying cancer cells not only in the context of their immediate tumor microenvironment, but also in the context of the whole organism. As our understanding of the effect of chemotherapy on complex host repair mechanisms feedback grows, so will our efforts to develop novel therapeutic combinations. We are already witnessing the use of combined cytotoxic chemotherapy with the TMEM inhibitor rebastinib in clinical trials. In the years to come, we expect to see many more clinical trials focused on combining cytotoxic therapies with therapies targeting chemotherapy-induced pro-metastatic changes. Thus, we expect that in the future our efforts will refocus from treating cancer towards treating the cancer patient as a harmonious system.

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Compliance with ethical standards

Conflict of interest MHO and JSC are inventors on a patent application (#96700/2505) submitted by the Albert Einstein College of Medicine that covers methods detecting and reducing chemotherapy-induced prometastatic changes in breast tumors. GSK declares no competing interests.

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