Review

Cancer Research

Chemotherapy-Induced Metastasis: Molecular Mechanisms, Clinical Manifestations, Therapeutic Interventions

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Abstract

Chemotherapy offers long-term clinical benefits to many patients with advanced cancer. However, recent evidence has linked the cytotoxic effects of chemotherapy with the *de novo* elicitation of a prometastatic tumor microenvironment. This "modified" tumor microenvironment is triggered by a chemotherapy-driven cytokine storm or through direct effects of certain chemotherapeutics on stromal and/or immune cells, the most critical being tumor-associated macrophages. These chemotherapy-educated cells act as facilitators in tumor–host cell interactions promoting the establishment of distant metastasis. Certain clinical studies now offer substantial evidence that prometastatic changes are indeed identified in the tumor microenvironment of certain patient subpopulations, especially those that do not present with any pathologic response after neoadjuvant chemotherapy. Deciphering the exact contextual prerequisites for chemotherapy-driven metastasis will be paramount for designing novel mechanism–based treatments for circumventing chemotherapy-induced metastasis.

Introduction

A number of recent preclinical and clinical observations indicate an unexpected involvement of all major cancer treatment modalities in enhancing the number of circulating tumor cells, and as such, potentially inducing distant metastasis (1). With an ever increasing understanding of the molecular complexities and the intertwining circuitries governing the effect of tumor microenvironment on disease progression, it is not surprising that research efforts have focused on uncovering the mechanisms of therapy-driven tumor progression, which may obfuscate the long-term benefits of anticancer therapeutic interventions (2). Therapeutic procedures previously linked to paradoxical promotion of the prometastatic machinery include chemotherapy (3–5), radiotherapy (6–8), surgery (9), or even perioperative anesthesia (10). A common paradigm of all these therapies is their capacity to induce systemic host responses that, in addition to providing antitumoral effects, paradoxically induce a proinflammatory milieu that supports critical hallmarks of cancer, including cancer cell survival, stemness, dissemination, angiogenesis, and metastatic colonization, resulting in local and/or distant recurrence (3, 11). A detailed overview of the abovementioned treatment modalities is beyond the scope of the current review. Here, we rather focus on delineating the status quo of chemotherapy-driven prometastatic mechanisms, their clinical importance and potential strategies for eliminating them.

Mechanistic Principles of Chemotherapy-Induced Metastasis

The overarching paradigm of how chemotherapy generates a metastasis-favorable tumor microenvironment is illustrated in Fig. 1A. Cytotoxic chemotherapy acts as a critical "stressor" in primary tumors that inflicts tissue damage, hypoxia, and cancer cell apoptosis, enforcing the release of proinflammatory cytokines and chemokines locally and systemically, collectively known as the "cytokine storm." These cytokines are principally secreted by "stress-reading" host cells, such as tissue-resident macrophages, endothelial cells, fibroblasts and others, although tumor cell–secreting factors may also contribute to the overall cytokine milieu (3). Locally, the cytokine storm induces immunosuppression and T-cell exhaustion, and reeducates macrophages and tumor cells, by altering or enhancing their prometastatic properties. Systemically, the cytokine storm mobilizes bone marrow–derived progenitors to primary or secondary tumor sites, where they can, in turn, regulate and facilitate the acquisition of hallmarks of the metastatic cascade. Taken together, these modifications conspire to create changes within the microenvironment of the primary tumor that promote systemic cancer cell dissemination to secondary sites, as well as metastasis-receptive niches at the secondary sites.

Chemotherapy induces a cytokine storm

Several interpretations have been proposed with regards to mechanisms underlying the chemotherapy-elicited prometastatic responses (3, 4, 11–14). A common denominator of most studies is the induction of the so-called "cytokine storm," that is, a surge of proinflammatory cytokines/chemokines and bioactive lipids

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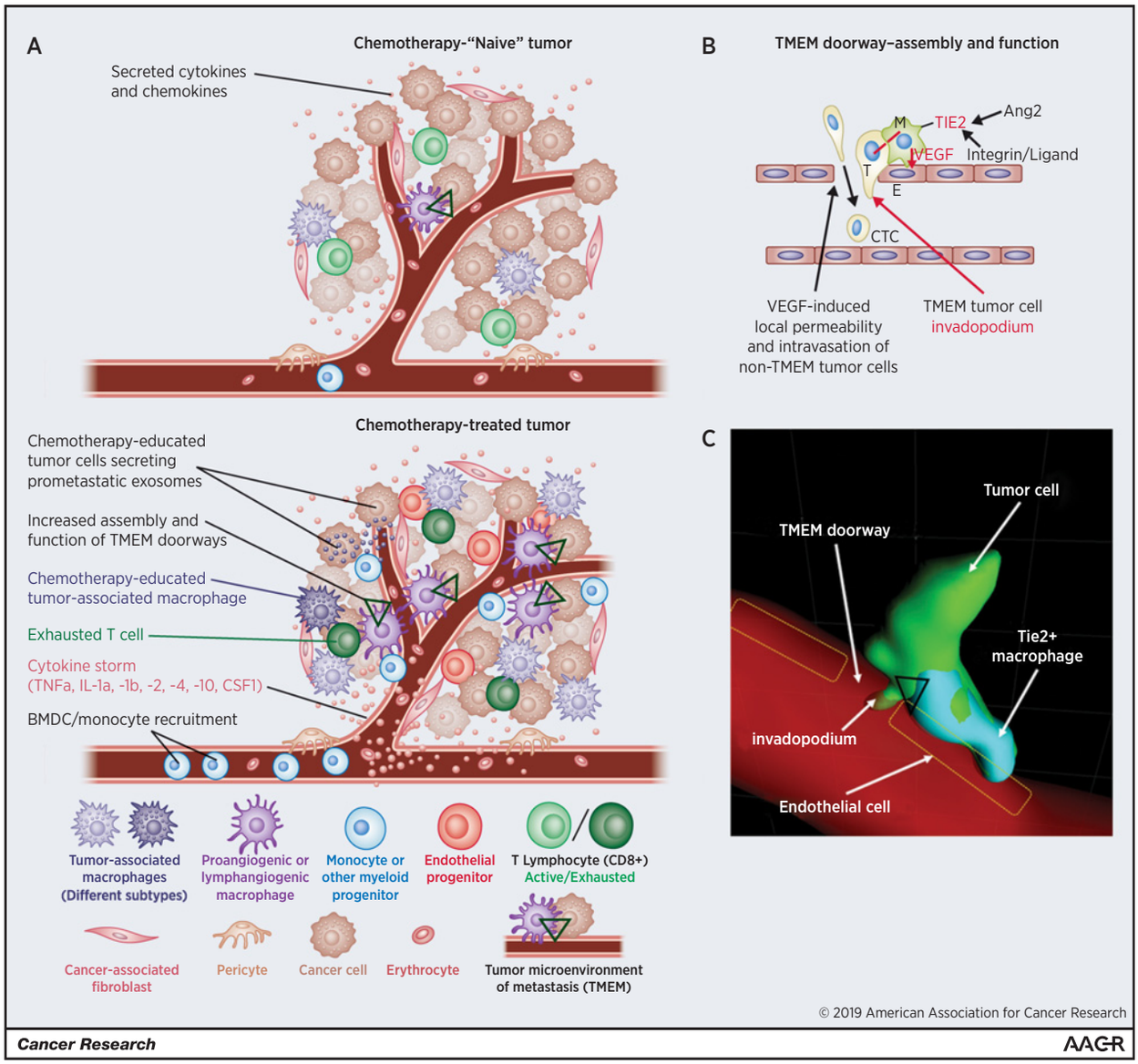

Figure 1.

Hallmarks of chemotherapy-induced metastasis. **A,** An illustrative model of chemotherapy-"naïve" (top) and -treated (bottom) tumor microenvironments, associated with the process of metastasis. Chemotherapy acts as a "cell stressor" that inflicts cytotoxic tissue damage and severe cancer cell apoptosis and hypoxia, resulting in the extensive release of proinflammatory cytokines and chemokines, collectively known as the "cytokine storm." The cytokine storm promotes metastasis through distinct mechanisms, including, but not limited to (i) immunosuppression and T-cell exhaustion, (ii) macrophage repolarization, (iii) tumor cell education into releasing prometastatic factors (exosomes and extracellular vesicles), (iv) mobilization of endothelial progenitors, contributing to tumor angiogenesis, and (v) recruitment of bone marrow–derived myeloid cells, including perivascular $Tie2^{High}$ macrophages assembling TMEM doorways (triad cells shown in green triangles), and $Vegfr3^{High}$ macrophages supporting tumor lymphangiogenesis. At the bottom, there is a legend showing all the different types of cells involved in chemotherapy-induced metastasis, as explained in the figure and the manuscript text. Illustration created by BioRender.com. **B,** Detailed illustration of TMEM doorway assembly and function in both chemotherapy-"naïve" and chemotherapy-treated primary tumors. TMEM doorway function is a two-step process. First, the TMEM tumor cell inserts a stable invadopodium between endothelial cells to define the site of vascular weakness and intravasation. Second, the TIE2 receptor on the TMEM macrophage has multiple stimulatory inputs, including Ang2 and integrin ligands, and controls VEGF production by the TMEM macrophage, resulting in VEGF-induced vascular permeability at the site of vascular weakness and intravasation defined by the TMEM tumor cell. **C,** High resolution 3D image projection of a TMEM doorway, prepared from intravital imaging of a mammary tumor *in vivo,* showing the three cell types stably bound together, located as points of a black triangle. The TMEM tumor cell invadopodium is inserted between two endothelial cells of the blood vessel to define the site of vascular weakness and intravasation.

secreted by chemotherapy-treated tumors (tumor and nontumor cells) and released locally in the primary tumor microenvironment and systemically into circulation, affecting bone marrow and lungs among other sites (12, 13, 15–20). Among the most prominent cytokines/chemokines included in this repertoire are factors, formerly demonstrated to regulate angiogenesis and metastasis, such as TNFα, granulocyte-colony stimulating factor (G-CSF), C-X-C motif chemokine ligand-12 (CXCL12), chemokine C-C motif ligand-2 (CCL2), and -4 (CCL4), and intercellular adhesion molecule-1 (ICAM1; ref. 15). Importantly, these factors have been independently or collectively linked to inflammation related to cancer progression and metastasis (21–26).

The emergence of the cytokine storm largely reflects to the combined direct and indirect results of cytotoxic tissue damage, elicited by administration of chemotherapy. For instance, Gartung and colleagues demonstrated that chemotherapy-generated debris can directly stimulate macrophages to initiate the tumor- and metastasis-promoting cytokine surge, through inflammatory pathways, such as the COX-2, and the soluble epoxide hydrolase (sHE) pathways (15). Along the same lines, Chang and colleagues reported that chemotherapy-exacerbated metastasis is dependent on the presence of stress-inducible gene *Atf3* in stromal cells (17). *Atf3* is member of the ATF/CREB family of transcription factors (27, 28). Of note, the *Atf3* gene is expressed at low or basal levels in normal cells, but its expression is significantly augmented by a wide variety of stress signals (28), and it binds to a large number of sites on the genome (29). As an intermediate/early-response factor, ATF3 can encode transcription factors that regulate the expression of other transcription factor genes, resulting in a cascade of changes in the transcriptional milieu; not surprisingly then, these target genes encode for cytokines and chemokines, irrespective of the signals that induce it, suggesting that one coalescing function of ATF3 could be to modulate the cytokine storm that sets off the prometastatic tumor microenvironment (17, 27, 28).

Interestingly, a third group previously demonstrated that certain chemotherapeutics, such as the microtubule-stabilizing agent paclitaxel, can directly bind to and activate toll-like receptor-4 (TLR4), which is frequently overexpressed in the surface of breast cancer cells, besides that of innate immune cells, and by doing so, upregulates a variety of proinflammatory regulators, which promote lymphangiogenesis and distant metastasis (16, 30, 31). Of all known pattern recognition receptors belonging to the toll-like receptor family, TLR4 is among those recognizing the bacterial lipopolysaccharide (LPS) as the primary ligand (32). Interestingly, it has been shown that paclitaxel can engage TLR4 in an analogous fashion to the canonical agonist LPS, resulting in production and release of similar proinflammatory cytokines from macrophages and other cells (33–35). These observations bring forward a hypothesis that paclitaxel, in particular, may be capable of initiating the chemotherapy-induced cytokine storm by acting as an LPS-mimetic in TLR4-expressing tumors (13).

Chemotherapy mediates prometastatic (re)-education of the tumor microenvironment via the cytokine storm

Certain groups identified distinct modifications in the tumor microenvironment during chemotherapy treatment that are otherwise not seen in chemotherapy-"naïve" tumors, suggesting that the cytokine storm is capable of re-educating tumor and immune cells, by altering or even enhancing their well-reported functions. For instance, chemotherapy alters either the bioactivity or the polarization status of intratumoral macrophages toward an M2-like status (36–39) that supports tumor progression. Karagiannis and colleagues suggested that in certain mouse models of breast carcinoma (i.e., the MMTV-PyMT), chemotherapy does not necessarily foster a prominent increase in the burden of infiltrating, intratumoral macrophages, but it definitely enhances the density of peri-vascular prometastatic $Tie2^{HIGH}$ macrophage subpopulation, capable of assembling functional tumor microenvironment of metastasis (TMEM) doorways for tumor cell intravasation (40). In another study, Liu and colleagues showed that neoadjuvant chemotherapy modifies/educates TAMs into secreting higher levels of the chitinase-like protein YKL-39 (possibly as a result of chemotherapy-mediated release of TGFβ and IL4 levels in the tumor microenvironment), which facilitates monocyte chemotaxis, angiogenesis, and distant metastasis in breast cancer (41). These studies collectively suggest a possible chemotherapy-driven repolarization of TAMs, cultivating an immunosuppressive, proangiogenic, and prometastatic blueprint in the tumor microenvironment.

It should not be surprising that the major cell type that undergoes such phenotypic modifications following chemotherapy is the macrophage, a cell type with incredible functional plasticity, as well as pleiotropic roles and interactions with other cell types under different physiologic and pathophysiologic contexts, including cancer (22, 42–46). Besides macrophages, endothelial cells have also been demonstrated to be reshaped following chemotherapy to adapt to and accommodate a novel prometastatic microenvironment. For example, Daenen and colleagues demonstrated that chemotherapy treatment increases VEGFR1 expression in lung endothelial cells, a phenotypic change that facilitates circulating tumor cell retention and interactions with the normal vasculature in the metastatic site (47). Interestingly, the authors additionally demonstrated that such VEGFR1-facilitated cancer cell retention and metastasis in the lung vasculature could be achieved through chemotherapy treatment in the absence of a primary tumor (47). Further analyses excluded the possibility that these VEGFR1-overexpressing endothelial cells were chemotherapy-mobilized VEGFR1-expressing hematopoietic cells from the bone marrow (47), which have also been documented to play a role in the induction of the premetastatic niche (48), clearly indicating that chemotherapy may elicit prometastatic responses in both primary and secondary microenvironments by *in situ* educating host cells interacting with tumor cells.

Besides macrophages and endothelial cells, mesenchymal cells and their progenitors may also undergo functional skewing to promote cancer cell development and progression upon chemotherapy. Through a recent study, Timaner and colleagues showed that mesenchymal stem cells (MSC), which frequently home along with tumor-initiating cancer cells in pancreatic adenocarcinomas, can be reeducated upon treatment with gemcitabine, to locally secrete C-X-C motif chemokine ligand-10 (CXCL10), to support and enrich the $CXCR3^{+}$ cancer stem cell compartment (49). In general, MSCs are known to support epithelial-to-mesenchymal transition and cancer cell stemness (50, 51), thus observations that link their functional plasticity to the metastatic cascade during chemotherapy treatment warrant further investigations.

Chemotherapy may also reeducate tumor cells by altering and enhancing their prometastatic properties. In a profound study by Keklikoglou and colleagues, it was demonstrated that chemotherapy mediates *bona fide* production and release of prometastatic annexin-6 (ANXA6)-expressing exosomes, capable of

interacting with endothelial cells in the premetastatic niche and initiating a proinflammatory cascade of events, leading up to the secretion of macrophage-specific chemotactic factors, such as CCL2 from endothelial cells (52). It has been previously demonstrated that CCL2 acts as the principal chemoattractant of $Ly6C^{+}CCR2^{+}$ macrophages, which are quite notorious for promoting local immunosuppression and facilitating metastatic seeding and eventually cancer cell colonization (53, 54). In an older study, Fremder and colleagues similarly demonstrated that paclitaxel-treated mammary carcinoma cells could readily secrete tumor-derived microparticles with higher potential of recruiting bone marrow-derived progenitor cells (BMDC), supporting tumor angiogenesis and metastasis (55). Although, it has been well- and long-known that extracellular vesicles (and other microparticles) can support metastasis by inducing the homing of bone marrow–derived progenitors to tumor microenvironment (56–60), the studies by Keklikoglou and colleagues and Fremder and colleagues, have both uniquely demonstrated that chemotherapy can further enhance metastases by modulating the composition of tumor cell–secreted extracellular vesicles (52, 55).

Chemotherapy mediates the mobilization of metastasis-promoting cells from the bone marrow via the cytokine storm

Regardless of the origin or the mechanisms via which the cytokine storm is induced upon treatment with chemotherapy, the most critical question is how it supports a tumor microenvironment that favors the metastatic process. Although many cellular meditators respond to the same chemotactic pathways, it has been suggested that, the main responders to the chemotherapy-driven cytokine storm are BMDCs of monocyte or endothelial origin (3, 11, 12, 18, 19, 42, 61, 62). Indeed, many chemotherapeutic agents can increase the abundance of myeloid cells in primary tumors, which obfuscates the overall efficacy of treatment (38, 63–68). As a consequence, the specific targeting, ablation or reprogramming of myeloid cells may significantly improve the efficacy of chemotherapy or even of other targeted therapies (69–72). Many studies have also reported the rapid mobilization of endothelial progenitors upon chemotherapy to the primary tumor microenvironment, which can eventually turn the angiogenic switch on and support tumor regrowth, thus eliminating the long-term beneficial effects of chemotherapy (18, 19, 61, 62). However, most of these studies focused, as their primary endpoint, on the ability of chemotherapy to regulate tumor growth and local relapse, but not metastatic dissemination. On the contrary, Gingis-Velitski and colleagues demonstrated that BMDCs claiming residence in paclitaxel-treated tumors can produce elevated levels of matrix metalloproteinase-9 (MMP9), which, in turn, enables epithelial-to-mesenchymal transition (EMT) and cancer cell invasion and dissemination in Lewis lung carcinoma (73). Interestingly, a similar phenotype was not recapitulated when tumors were treated with the nontaxane chemotherapeutic, gemcitabine (73), further suggesting that distinct drug categories stimulate the cytokine storm via disparate mechanisms.

Several groups have demonstrated that treatment with paclitaxel, or the combination of doxorubicin/cyclophosphamide, induces infiltration of $Tie2^{HIGH}$ macrophages in primary tumors, which eventually reside in the perivascular niche to regulate angiogenesis and metastasis (18, 40, 65). Although mostly notorious for their proangiogenic and immunosuppressive properties (65, 74–77), recent evidence shows that $Tie2^{HIGH}$ macrophages interact with tumor cells expressing the actin-regulatory protein Mammalian enabled (Mena), and an endothelial cell to form a stable three-cell complex that functions as a doorway for tumor cell entry into the blood. This doorway is called TMEM (Fig. 1B and C; refs. 78, 79). TMEM doorways are active sites of cancer cell dissemination in the tumor microenvironment, in both primary and metastatic tumors, as shown by intravital imaging (80, 81). The number of TMEM doorways present in primary tumor tissue is prognostic for metastatic recurrence in patients with breast cancer (82–84). Recently, it was shown that transendothelial migration by tumor cells at TMEM doorways is regulated via localized and transient release of VEGFA from the $Tie2^{HIGH}$ macrophage, disrupting endothelial cell adherens and tight junctions, and creating a passageway for the invasive/migratory tumor cells to enter the peripheral circulation (Fig. 1B; ref. 80). Pharmacologic suppression of TMEM function via Tie2 inhibition, or the conditional ablation of the *Vegfa* gene in the macrophage lineage, both result in inhibition of TMEM-dependent cancer cell dissemination and significant reduction of circulating tumor cells (CTC; ref. 80). A principal prometastatic mechanism elicited by chemotherapy in breast cancer, is the induction of *de novo* TMEM assembly, as a result of increased $Tie2^{HIGH}$ macrophage levels, apparently the direct outcome of the chemotherapy-triggered cytokine storm attracting monocytes that mature into $Tie2^{HIGH}$ macrophages (17, 40, 85).

Besides the prominent mobilization of $Tie2^{HIGH}$ monocytes, previous evidence demonstrated that endothelial cell progenitors are also rapidly mobilized during chemotherapy treatment, and that they are mainly responsible for triggering an angiogenic microenvironment supporting tumor growth and local relapse (18, 19, 61). However, the recruitment of endothelial progenitors derived from immature myeloid cells plays a major role in rebuilding tumor-associated lymphatic and blood vessels, promoting lymphatic and hematogenous metastasis, respectively (13). In addition, the tumor neovasculature is often characterized by a branching morphology (86, 87), and it has been previously observed by intravital imaging studies that blood vessel branching points represent the preferable spots for TMEM assembly and TMEM-dependent cancer cell intravasation (80, 88–90). Therefore, the rapid mobilization/recruitment of endothelial progenitors in the primary tumor microenvironment following treatment with cytotoxic chemotherapy cannot only be viewed as a proangiogenic, but also as a prometastatic response.

Besides triggering the hematogenous route of metastasis, it has also been proposed that chemotherapy stimulates lymphatic metastasis, via the recruitment of a lymphangiogenic macrophage subtype, expressing the VEGFR3 (20). Indeed, tumor cells disseminating via lymphatics from the primary tumor to regional lymph nodes can first lodge and subsequently metastasize (hematogenously) to tertiary/peripheral sites (91–93). Macrophages have been previously shown to promote lymphangiogenesis under various contexts (94, 95). Indeed, Alishekevitz and colleagues demonstrated that infiltration of $VEGFR3^{+}$ macrophages in the primary tumor microenvironment following treatment with paclitaxel results in the induction of new lymphatic vessel formation via the VEGFR3/VEGFC axis (20). In particular, the prolymphangiogenic activities of $VEGFR^{+}$ macrophages are dependent on the secretion of cathepsins and the cathepsin-dependent cleavage of latent

heparanase into its active form, leading to the increased availability of VEGFC (20). In most solid carcinomas, functional lymphatic vessels are located in the periphery of tumors and in adjacent normal tissues, and they likely serve as primary channels for seeding of metastases into the draining lymph nodes (91, 96–99). Interestingly, the described VEGFR3/VEGFC interactions upon chemotherapy lead to the development of lymphatic neovasculature inside the breast cancer parenchyma (20), and as such, the functionality and metastatic capacity of these chemotherapy-induced intratumoral lymphatics remains to be further explored.

Although research on this field has mostly focused on macrophage and endothelial cell progenitors as outlined above, the contributions of the overall tumor microenvironment and the complicated paracrine signaling conversations with other stromal cells should not be neglected in the context of chemotherapy-driven metastasis. In an exemplary study by Acharyya and colleagues (2012), the authors unraveled a complicated paracrine network supporting tumor cell survival in primary and metastatic sites, which involves cancer, myeloid, endothelial, and other stromal cells (100). Tumor cell–secreted CXCL1 attracts $Cd11b^{+}Gr1^{+}$ myeloid cells into the tumor, which, in turn, support tumor cell growth and metastasis (100). Interestingly, chemotherapeutic drugs trigger a parallel stromal reaction in endothelial and other stromal cells, which results in NFκB-dependent secretion of TNFα, a pleiotropic cytokine that further heightens CXCL1 production by tumor cells, exacerbating the paracrine network loop and supporting a tumor- and metastasis-promoting microenvironment (100).

Therapeutic Interventions to Eliminate/Suppress Chemotherapy-Induced Metastasis

Preoperative (neoadjuvant) chemotherapy provides long-term clinical benefits to certain patients, especially those whose primary tumor fully regresses before surgery (101–107). Although the therapeutic benefits of chemotherapy may be hindered by tumor-promoting host responses induced by cytotoxic drugs (3, 11), as described in this review, there are hardly many alternatives in clinical practice. Thus, the current challenge is to identify and propose novel approaches of maximizing the clinical benefits derived from chemotherapy, while simultaneously restricting the side-effects that limit its maximal efficacy.

Low-dose metronomic chemotherapy

LDM refers to the administration of low doses of a chemotherapeutic drug (compared with the conventional dose) on a frequent or continuous schedule with no extended interruptions (108–111). Although it was initially proposed that LDM chemotherapy exclusively exerts its antitumor effects by targeting tumor angiogenesis (112–114), recent evidence has underscored that LDM chemotherapy cultivates a tumor microenvironment that impairs tumor growth, modulates the activities of certain subtypes of immune and inflammatory cells, and induces dormancy on tumor cells (111, 115, 116). Although little progress has been made in directly comparing LDM chemotherapy and standard or MTD chemotherapy regimens with regards to the induction of the cytokine storm and the associated prometastatic effects described above, there are strong indications that LDM chemotherapy may induce a less metastasis-favorable tumor microenvironment. For example, Chan and colleagues has demonstrated that although both LDM and MTD chemotherapy can equally kill a fraction of tumor cells, MTD chemotherapy may additionally induce persistent STAT1 and NFκB activation in cancer-associated fibroblasts (CAF), eventually leading to the expression and secretion of chemokines signaling through CXCR2 on cancer cells, further exacerbating cancer progression (116). Interestingly, LDM chemotherapy, using the exact same chemotherapeutics did not provoke any tumor- and metastasis-favorable stromal responses in this context (116).

LDM chemotherapy can systemically downregulate angiogenesis and therefore not only suppress local tumor relapse, but also distant tumor cell dissemination (117). For instance, Bertolini and colleagues demonstrated that MTD cyclophosphamide increased the number of circulating endothelial progenitors in the blood of lymphoma-bearing mice, while the equivalent LDM regimen suppressed their levels for the entire duration of treatment (118). As already explained, tumor endothelial cells and endothelial cell progenitors, besides being proangiogenic, have significant prometastatic properties (119–122), further indicating that LDM regimens have a direct antiangiogenic and an indirect antimetastatic effect.

Furthermore, it has been reported that LDM chemotherapy results in significant local activation and release of prominent inhibitors of the angiogenic switch, such as thrombospondin-1 (TSP1; ref. 123). Of note, a seminal study by Ghajar and colleagues (2013) suggested that TSP1 is a critical perivascular niche factor that promotes breast cancer cell quiescence and dormancy, thus suppressing the progression of metastatic disease (124), further implying that LDM chemotherapy-induced TSP1 functions as a prominent antimetastatic signature in the tumor microenvironment. Along the same lines, LDM chemotherapy has been shown to cause the decrease of proangiogenic factors, such as VEGF and platelet-derived growth factor-BB (PDGF-BB; ref. 125). PDGF-BB, depending on the context, may also have prometastatic properties. For instance, Hsu and colleagues has recently demonstrated that CXCL17-mediated chemotaxis of myeloid-derived suppressor cells (MDSC) facilitates angiogenesis and breast cancer cell colonization of the lung via the aberrant secretion of PDGF-BB in the lung microenvironment (126). In aggregate, the aforementioned observations postulate that the antiangiogenic effects of LDM chemotherapy are linked to circumvention of the chemotherapy-induced prometastatic mechanism, although more studies are needed to confirm the efficiency of this therapeutic strategy.

Targeted antimetastatic approaches

Nowadays, targeted treatment strategies using small-molecule inhibitors are at the frontier of cancer therapeutics, mainly due to their small size that allows them to pass through the plasma membrane and interact with their intracellular targets (127). Small-molecule inhibitors, including, but not limited to small-molecule kinase inhibitors, extracellular protease inhibitors, and proteasome inhibitors, have been previously used to target mediators of multiple hallmarks of cancer, including dissemination and metastasis (127, 128). In the context of chemotherapy-mediated metastasis, counteracting the prometastatic properties of chemotherapy-recruited macrophages and BMDCs using small-molecule inhibitors has

been documented to enhance the long-term clinical benefits of preoperative chemotherapy, as well as to improve clinical outcome of metastatic disease. In preclinical models of breast carcinoma for instance, targeted disruption of $Tie2^+$ macrophage function using pharmacologic Tie2 inhibition significantly improves survival, by suppressing TMEM-mediated vascular permeability and its associated cancer cell intravasation, even in the context of increased TMEM assembly during paclitaxel treatment (40, 129). Along the same lines, inhibition of MMP9 along with chemotherapy suppresses chemotherapy-driven invasion, EMT, and metastatic dissemination, as mediated by MMP9-producing BMDCs (73). Furthermore, pharmacologic strategies of eliminating prometastatic functions of $CCR2^+$ macrophages, especially critical in secondary sites, also disrupt the establishment and progression of metastatic colonization (53, 54, 130).

Although TAMs are known to be involved in a broad spectrum of cancer hallmarks, their prometastatic potential appears to also be, in part, due to their immunosuppressive functions in the primary and secondary tumor microenvironment (42, 64, 70, 131–133). These immunosuppressive functions typically include the secretion of suppressive cytokines, such as IL10 and TGFβ, or the expression of ligands for immune checkpoint receptors, such as programmed death ligand 1 (PDL1), eventually leading to T-cell dysfunction and exhaustion (134–137). Therefore, in addition to disrupting the macrophage-dependent prometastatic responses, the targeting of TAMs would also enhance the therapeutic efficacy of chemotherapy, by additionally restoring T-cell functions (130, 138). In a prominent example, Salvagno and colleagues demonstrated that CSF1R blockade in a poorly immunogenic transgenic mouse model of breast cancer stimulates intratumoral type I IFN signaling, which significantly enhances the anticancer efficacy of platinum-based chemotherapy (139). Whether chemotherapy-driven metastasis would be totally rescinded by eliminating the immunosuppressive tumor microenvironment remains to be properly investigated and elucidated in the future.

Besides macrophages, targeting the mesenchymal stem cells supporting the cancer stem cell niche may also be an efficient strategy for eliminating MSC-driven tumor progression during chemotherapy treatment. For instance, based on the previously established CXCL10-CXCR3 paracrine loop between MSCs and CSCs during chemotherapy, Timaner and colleagues proposed that nanovesicles derived from MSC membranes loaded with an CXCR3 antagonist were able to produce enhanced therapeutic outcomes in a pancreatic tumor model receiving gemcitabine (49). Although this study specifically investigated the local relapse and regrowth of the tumors (49), it is now well-known that metastatic stem cells are responsible for seeding distant metastases, and are supported by the stem cell niche in the primary tumor microenvironment (140). Collectively the aforementioned studies all suggest that the pharmacologic pursuit of the cellular mediators of chemotherapy-driven metastasis, whether those are prometastatic macrophages, mesenchymal stem cells, or others may be a prominent strategy for complementing currently established chemotherapy regimens.

Alternative targeted approaches would be to disrupt/suppress central mediators of the cytokine storm and proinflammatory mediators critical for the metastatic cascade (16, 17). However, it should always be kept in mind that chemokines/cytokines are generally compensatory to each other in their phenotypic responses (21, 25, 141, 142), a circumstance that often makes their individual contributions in the dynamic interplay within the tumor microenvironment quite redundant. Even so, a thorough understanding of the molecular prerequisites for chemotherapy-induced metastasis may render such approaches efficient in certain contexts; for instance, Shaked and colleagues demonstrated that a neutralizing antibody against CXCL12 efficiently prevents the rapid mobilization of endothelial progenitors with proangiogenic and prometastatic functions in primary tumors, following chemotherapy treatment (61). Other researchers have proposed the targeted inhibition of central hubs of inflammation, such as the COX2/sHE pathway (15), or the stress response pathway ATF3 (17), both alleviated the protumoral and prometastatic effects of chemotherapy.

The Clinical Importance of Chemotherapy-Induced Metastasis

Besides evidence from preclinical animal models of cancer, several groups have documented the increase of prometastatic features in patients receiving preoperative chemotherapy, also known as neoadjuvant chemotherapy (NAC), regardless of drug categories used. For instance, Karagiannis and colleagues investigated the assembly of TMEM doorways and $Mena^{INV}$ expression in a cohort of 20 patients with ER^+ breast cancer who had residual disease after NAC, and found a significant increase in both TMEM and $Mena^{INV}$ post-NAC treatment with doxorubicin/cyclophosphamide plus paclitaxel (40). Furthermore, Keklikoglou and colleagues demonstrated that extracellular vesicles, capable of nurturing a $CCR2^+$ macrophage–dependent prometastatic niche, were significantly increased in the blood of patients with breast cancer receiving doxorubicin/cyclophosphamide and paclitaxel in a neoadjuvant setting (52). Notably, in the patients who achieved partial or complete pathologic response following NAC, the concentration levels of the prometastatic extracellular vesicles in the posttreatment samples reverted back to the levels of the pretreatment samples (52), further suggesting that chemotherapy-induced metastasis may only reflect to a certain subgroup of patients who do not respond well to NAC treatment.

The aforementioned studies shed light on a long-seen clinical problem, in which NAC-treated patients with residual disease have an increased risk of developing distant metastasis compared with patients with pathologic complete response (pCR; ref. 143). Because patients who achieve pCR upon NAC typically derive full clinical benefit from NAC treatment, it is critically important to predict who the responders are so that they are offered NAC treatment (101–107). However, consistent with prior clinical observations (143), patients who respond with prominent prometastatic changes upon NAC treatment likewise need to be identified (40, 52), and the exact characteristics of these patients need to be explored. Along these lines, a recent study by Pastoriza and colleagues in a large breast cancer patient cohort treated with NAC demonstrated that black patients have worse distant recurrence-free survival (DRFS) compared with white patients (144), suggesting that racial features define patient subpopulations at risk of developing chemotherapy-driven prometastatic changes. Underlying this racial difference might be the density of $Tie2^+$ macrophages (145), and microvascular density in the tumor microenvironment (146), both higher in blacks compared with whites, raising the possibility that worse DRFS post-NAC in

blacks compared with whites may be the consequence of higher TMEM activity (144). However, further studies, including modern high-throughput (-omics) approaches, are required to identify the exact patient subpopulations at risk, in the context of chemotherapy-induced metastasis.

It has also been discussed that chemotherapy increases the efficiency of metastasis, by directly influencing the premetastatic niche (47, 147). This observation suggests that even in the adjuvant (AC) setting, the clinical benefits of chemotherapy may be hindered by modifications in the tumor microenvironment. This may be true because after the surgical removal of the primary tumor, the already disseminated tumor cells could develop metastatic disease, which may be facilitated from chemotherapy-mediated prometastatic events, irrespective of primary tumor effects (47, 147). Indeed, the clinical and preclinical data discussed in this review may help explain why a direct comparison of metastasis-free survival between patients receiving NAC and AC does not show significant difference (101). AC and NAC can both elicit prometastatic responses, albeit via different molecular mechanisms. Therefore, the aforementioned data support the notion that counteracting (or circumventing) the chemotherapy-driven prometastatic changes may improve the clinical outcome in both AC- and NAC-treated patients.

Conclusion

The main challenge remains to weigh the benefit versus detriment of all cancer treatment modalities, including chemotherapy. Chemotherapy is a frontier therapy, essential for cancer control, but unfortunately, it may present with negative consequences in some patients. Overall, more clinical studies are needed to confirm a causative link between the prometastatic responses of chemotherapy and development of metastatic disease in patients with cancer and to develop biomarkers to distinguish patients who would derive the most benefit from chemotherapy from those who would not. Prospective studies are not available at this point, because the phenotype has only been recently recognized at the molecular and cellular level, and prospective studies in breast and other solid cancers typically require long follow-up of patients, which may reach a decade or more. These studies, however, will be paramount for evaluating the current therapeutic approaches in the management of cancer, and possibly recommending alternative ones, as has been discussed in the current review. Furthermore, a clear mechanistic understanding of how chemotherapy is able to assist in tumor cell escape and metastasis will enable more efficient therapeutic strategies to minimize these side-effects and improve patient outcomes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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