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Review

Adverse effects of chemotherapy on cancer progression and outcome

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ABSTRACT

The development of novel strategies to overcome patient mortality is the primary goal of cancer research. Over the past several decades, the clinical outcome of cancer patients has seen improvements owing to the administration of chemotherapy. Despite its systemic cytotoxic effects, chemotherapy improves patient survival short-term. However, the long-term benefits of chemotherapy remain questionable. Tumor drug resistance, manifesting as cancer relapse and progression, is a significant factor that limits sustained chemotherapy effectiveness. Moreover, recent paradoxical evidence suggests that chemotherapy can adversely affect the disease prognosis. This may be not only due to side-effects of chemotherapy but also due to it directly promoting survival and metastatic dissemination of cancer cells. Here, we review the potential mechanisms by which chemotherapy may increase cancer aggressiveness. We discuss the chemotherapy-induced alterations in vital organs, in cancer cells, and the tumor microenvironment. A better understanding of the chemotherapy effects on the tumor-host interaction will aid in the development of improved strategies to intervene in cancer progression.

KEYWORDS: chemotherapy, metastasis, cancer, chemoresistance, genome instability, tumor microenvironment, clonal evolution.

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1. Introduction

Cancer remains modern medicine's most elusive foe until date. With over 100 distinct types, cancer is the most studied disease but regrettably often remains unmanageable. Over the past several decades, advances in cancer treatment have translated into notable improvements in survival rates. However, the current estimate of global cancer incidence predicts that by the year 2020, the number of newly diagnosed cancer cases will increase to a whopping 15 million annually and that the disease will claim the lives of over 12 million individuals worldwide [1]. Cancer cells are endowed with evolutionary advantages that trounce the barriers put in place by the regulatory circuits of the human body. Disseminating tumor cells interact with the host environment, and this tumor-host crosstalk underlies cancer progression [2]. Although localized primary tumors are the original source of malignancy burden, it is the metastases that attribute to over 90% of all cancer-associated morbidity and mortality [3].

Owing to the genetic instability of tumors, their treatment is a daunting challenge. An armamentarium of small molecules, antibodies, viral oncotherapeutics, and other interventions have been amassed to combat malignant progression [4]. These valiant efforts are yet to bear fruit as many of the metastasis-directed therapies are predominantly cytostatic rather than cytotoxic and, hence, have limited clinical efficacy. Currently, the classical standard-of-care employed for both loco-regional and metastatic

disease includes surgery, radiotherapy, and chemotherapy [5]. The use of surgical resection and radiotherapy is limited for some types of cancer, and especially for high-grade cancers, due to the systemic nature of malignant lesions. Thus, chemotherapy often remains the only option to suppress cancer progression [6]. Chemotherapeutic drugs halt the rapidly dividing cancer cells and induce cell death resulting from DNA damage, chromosomal replication prevention, and inhibition of signaling pathways driving cell proliferation. Despite its cytotoxic side effects, chemotherapy benefits the majority of patients diagnosed with primary tumors. Numerous clinical studies have demonstrated increased 5-year survival of patients with breast, ovarian, and pancreatic adenocarcinomas. Moreover, data from clinical trials of anthracycline-based combination regimens for metastatic breast cancer (BC), suggest that a small cohort of patients exhibited a > 10 years disease-free survival [7], making the case that chemotherapy benefits long-term.

While chemotherapy remains a line of defense of choice for most types of cancer, there is a building controversy over its cost/benefit ratio. Although chemotherapy initially causes tumor regression, chemoresistance tends to develop over time. Drug resistance is a major challenge because it often leads to cancer recurrence, dissemination, and subsequent patient mortality [8]. While the adverse side effects of chemotherapeutics have been observed for decades, it is only recently that the possibility of chemotherapy actively triggering cancer aggressiveness has come to light [9]. Chemotherapy acts as a stressor that induces alterations in tumor cells, as well as in the tumor microenvironment (TME). Stemming from initial clinical observations, recent animal studies provide evidence for a tantalizing possibility that chemotherapy selects for cells with the immune-evasive phenotype and may induce metastases [10-12]. While this remains a medical hypothesis, an urgent necessity is to determine if chemotherapy helps or instead hurts patients long-term. It is crucial to gain more insight into cellular and molecular pathways through which chemotherapy might benefit tumor cells. In this review, we first discuss the side effects of chemotherapy. We then focus on genetic instability, clonal evolution, and the skewing of the TME as the putative mechanisms by which chemotherapy can promote cancer cell survival. We also cover recent insights into how chemotherapy may induce metastasis. Lastly, we touch upon the conceptual implications for cancer patients.

2. Off-target effects of chemotherapy

Systemic chemotherapy administration is one of the most efficacious first-line anti-neoplastic treatment options since it targets cancer cells undergoing uncontrolled cell division. Chemotherapeutic agents reduce tumor growth and cause its regression through the induction of autologous cell death pathways secondary to cell-proliferative machinery and DNA damage. However, the non-specific action of chemotherapeutic drugs diminishes their clinical benefit.

2.1. Chemotherapy-induced toxicity

Toxicity of chemotherapeutics results from their deleterious side effects on the cells of the nervous, gastrointestinal, and immune systems, as well as other vital organs, including kidneys, the liver, and the heart (Figure 1). They result in debilitating body conditions, including nausea, vomiting, fatigue, and hair loss, to name a few [13]. The gastrointestinal side-effects are severely distressing and potentially fatal for patients owing to the mucosal injuries. Chemotherapy-induced oral and gastrointestinal mucositis and ulceration often cause malabsorption, pain, anorexia, fatigue, and weight loss [14]. Anticancer chemotherapeutic agents also tend to predispose to anemia and sepsis. Immune suppression results from the effects on leukocyte progenitor populations in the bone marrow. Chemotherapeutic agents repress the expression of genes associated with adaptive immune responses, including components of the MHC class II machinery and T-cell receptor genes. Further, anti-cancer drugs also alter T-cell development and function, suppress the development of T_H1 cells, bias responses towards the T_H2-cell type, and suppress natural killer cell effector functions. All these events may collectively preclude the elicitation of effector and memory anti-tumor immunity that can eventually increase the susceptibility to viral and bacterial infections [15].

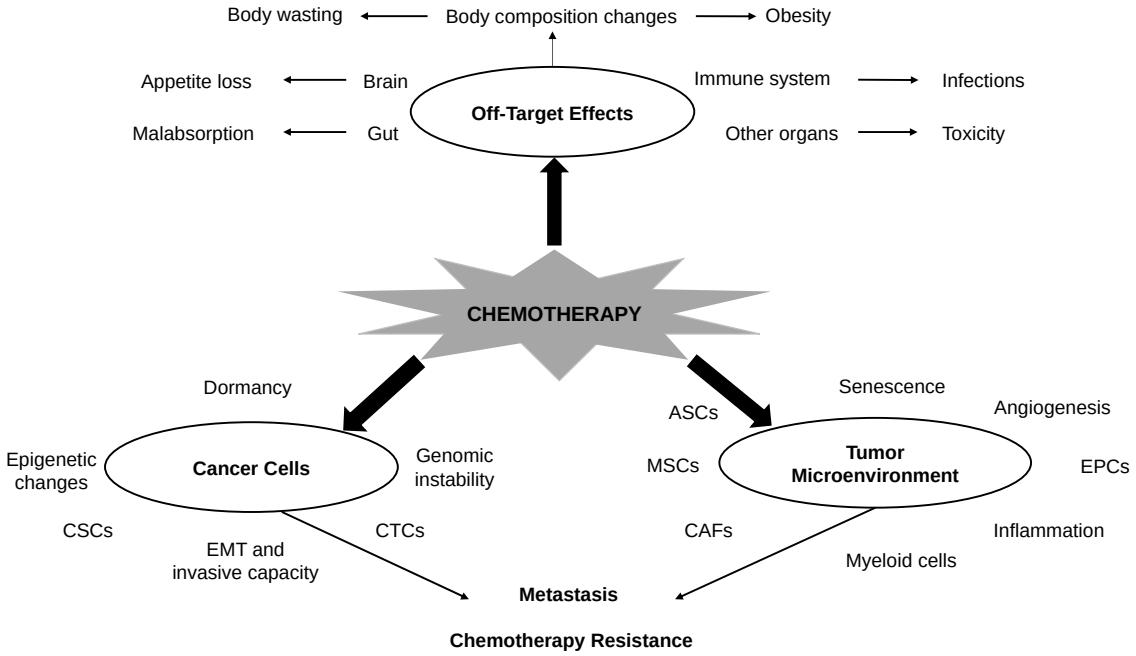

Figure 1. Paradoxical effect of chemotherapy on disease progression. Chemotherapy may promote cancer aggressiveness. In addition to non-specific organ toxicity, chemotherapy induces cancer cell evolution and tumor microenvironment remodeling, which may result in cancer progression and chemoresistance.

With the expanded use of anti-cancer treatments in different patient cohorts, the toxicity profiles associated with established chemotherapeutics continue to broaden. One example is the study that highlighted occurrences of hypersensitivity reactions to platinum-based regimens in children with low-grade glioma. Younger children, girls, and those with allergies were identified to be at a higher risk, and the incidence rate rose with an increased number of infusions rather than just drug dosage [16]. Further, drugs such as cisplatin and doxorubicin were identified to increase the risk of nephrotoxicity and cardiac toxicity in cancer survivors, potentially attributable to vascular damage [17]. Central and peripheral neurotoxicity caused by anti-neoplastic agents can also dramatically reduce the functional capacity and quality of life in cancer survivors. A study identified a plethora of changes in the number of circulating factors and cerebrospinal fluid constituents that were associated with chemotherapy-induced persistent cognitive dysfunctions [18]. The resulting changes in behavior and lifestyle, including feeding and exercise, contribute to the effects on body composition and metabolism discussed below.

2.2. Chemotherapy impact on body composition

Body composition underlies the body's energy balance. Chemotherapeutic agents are known to alter fat and lean body mass, which results in clinical complications such as insulin resistance and skeletal muscle dysfunction [19, 20]. Although body composition is an important prognostic factor in cancer patients, conventional chemotherapeutic regimens are still individualized solely on the basis of body surface area without accounting for lean and fat body mass. The changes in body composition are of clinical relevance, as they predetermine cancer recurrence and mortality.

2.2.1. Chemotherapy-associated obesity

Excess fat mass is known to have repercussions on disease progression in patients with most types of carcinoma [21]. According to clinical studies, significant increases in weight occur in 50–96% of all early-stage BC patients during treatment with adjuvant chemotherapy (ACT), with the median weight gain ranging between 2.5–6.2 kg over the course of treatment and most of the weight gain reportedly occurring during the first year [22]. The degree of chemotherapy-induced weight

gain is contingent on both the nature, as well as the time frame, over which the drugs were administered. Several studies based on a big cohort of patients (n < 3000) showed that weight gain occurred upon receiving the cyclophosphamide, methotrexate, and 5- fluorouracil (5-FU) regimen [23]. The influence of menopausal status on chemotherapy-induced weight gain remains a subject of debate, with several studies finding weight variation to be more pronounced among premenopausal women [24]. Chemotherapy-induced weight variation is an apparent consequence of energy balance dysregulation. During chemotherapy, dietary intake and physical activity are reduced owing to the treatment-related side effects, including fatigue, nausea, and dysgeusia. Chemotherapy-induced alterations in the anorexigenic peptide-YY levels may at least, in part, account for the energy imbalance [25]. Further, chemotherapy treatment is often linked with sleep disorders found in close to 25% of BC patients. Sleep restriction impairs the cross-talk between the brain and peripheral organs, which results in metabolism dysregulation and weight gain [26].

2.2.2. Chemotherapy-associated sarcopenic obesity

Skeletal muscle mass plays a pivotal role in ensuring normal physiology. As a result of chemotherapy, the amount and strength of muscle tend to decline, subsequently resulting in functional impairment and morbidity [27]. Sarcopenia (loss of muscle tissue) and the associated sarcopenic obesity pose a risk in cancer outcomes. Chemotherapeutic agents such as cisplatin, irinotecan, doxorubicin, and etoposide have been shown to induce sarcopenic muscle loss. Chemotherapy induces mitochondrial damage, which cripples myocyte metabolism and results in muscle contractile dysfunction. Myogenesis defects could also result from toxic chemotherapy effects on muscle progenitor cells. The skewing of NF-κB, Akt, and TGFβ pathways by chemotherapy upregulates proteolysis and inflammatory cytokine secretion, which eventually culminates in muscle weakness due to catabolism upregulation [28-30]. Sarcopenic obesity arises apparently to compensate for decreased muscle mass. This condition represents a clinically relevant body composition type as it combines the health risks of obesity and muscle loss. These are only a few studies that have evaluated the extent to which chemotherapy predisposes to sarcopenic obesity. For example, chemotherapy-induced sarcopenia predicted poor survival and an increased chance of mortality in patients undergoing treatment for large B-cell lymphomas (BCL) and colorectal cancer (CRC) [31, 32]. Chemotherapy dosing protocols predominantly use body-surface area in estimating the amount of metabolic target tissue, which is not accurate owing to the heterogeneity in lean and fat tissue distribution across the body [33]. Due to its association with functional impairment and muscle weakness, chemotherapy may promote lifestyle habits such as inadequate dietary nutrient intake, decreased physical activity, weight gain, and tobacco or alcohol use, which independently jeopardize the disease outcome.

2.2.3. Chemotherapy-associated cachexia

Cachexia, defined as a non-intentional weight loss of more than 5% of the normal body weight over six months, has long been recognized as a critical complication of cancer progression [34, 35]. Body wasting is a serious problem in the treatment of patients with advanced cancer. Approximately 50% of cancer patients eventually become affected by cachexia [36]. Both fat and lean body mass are lost in cachexia, which alters the quality of life and reduces survival independently of functional disease severity or age [35]. Patients with cachexia become feeble due to compromised muscle mass and function, and therefore have a poor prognosis and increased mortality [37]. The underlying pathophysiology of cancer cachexia is primarily through the release of tumor cytokines that interfere with host immunity, subsequently leading to paraneoplastic syndromes [38]. However, there is accumulating evidence that chemotherapy-associated side effects can predispose to both fat and lean mass wasting [39]. Further, the effects of cisplatin-induced cachexia were investigated in an engrafted C26 CRC mouse model. While cisplatin reduced tumor burden, it induced a high degree of muscle atrophy in the mice, which was independent of the commonly implicated ubiquitin-proteasome system [40]. These observations suggest that chemotherapeutic drugs trigger multiple muscle tissue responses, which ultimately promote muscle fiber atrophy through the NF-κB pathway affecting both muscle fiber metabolism and muscle stem

cells [41]. Another study examined the skeletal muscle proteome in chemotherapy-treated C26 CRC-tumor bearing mice. They detected a down-regulation of 235 and 345 muscle proteins accompanied by mitochondrial dysfunction and alterations in the TCA cycle, fatty acid metabolism, and Ca^{2+} signaling pathways [42]. Further, a study by the same group on chemotherapy-mediated muscle mitochondria dysregulation indicated that it occurred in a MAPK-dependent fashion [43]. The relevance of these observations to the link between chemotherapy and cachexia in patients remains to be determined.

3. Chemotherapy effects on cancer cells

Tumors are heterogeneous masses composed of both therapy-sensitive and therapy-resistant cell subpopulations. Throughout disease progression, tumor cells undergo spatial and functional changes that manifest in a complex sub-clonal architecture, which is further enhanced by the application of chemotherapeutic drugs [44]. The existence of spatial intra-tumoral heterogeneity within primary tumors accounts for the partial sensitivity to therapy and the initiation of tumor clonal evolution. Tumor cell diversity occurs due to progressive mutational changes that result in distinct cancer cell populations [45]. Relapse and metastatic progression after a positive therapeutic effect often occur due to a resistant population that was present before therapy or originated as a result of chemotherapy. Survival, proliferation, and subsequent dominance of this population could be due to features that were absent before therapy. Below, we discuss the features of cancer cells accompanying the disease progression and the potential mechanisms of their evolution that may be linked with chemotherapy (Figure 1).

3.1. Chemotherapy and tumor dormancy

Despite the initial efficacy of chemotherapy in ‘‘shrinking’’ primary tumors, chemotherapy-resistant tumor cells often survive and contribute to subsequent cancer recurrence. Following chemotherapy administration, chemoresistant tumor cells sometimes exist in a dormant state for several years before resuming proliferation [46]. These dormant cancer cells adapt by entering a quiescent state and undergo alterations in their signaling pathways, protein expression, and modulation to survive chemotherapy-induced cytotoxic stress. An *in vitro* tumor recurrence model demonstrated that short-term exposure of breast and prostate tumor cells to either doxorubicin or docetaxel enriched for slow-cycling, dormant tumor cell populations. The chemo-enriched dormant tumor cells resumed proliferation upon chemotherapy withdrawal, forming colonies that exhibited increased resistance to the original chemotherapy and possessed pro-metastatic potential as a result [47]. Further, a study in preclinical CRC models demonstrated that within the same lineage, individual CRC tumor cells had a spectrum of growth patterns and survival characteristics in response to chemotherapy. Upon oxaliplatin treatment of mice transplanted with CRC tumor cells, the resulting tumors were generated primarily by previously slow-growing and dormant clones. These cells re-initiate tumor growth post-treatment that accounted for disease recurrence after initial response to chemotherapy, indicating that dormant cells survive chemotherapy better than their highly proliferative counterparts [48].

It appears that tumor dormancy is established through mechanisms that in benign cells trigger cell senescence, a stress response resulting in permanent cell cycle arrest without cell death [49]. Senescence-inducing stimuli include tissue injury and remodeling, metabolic perturbations, radiation, and not surprisingly, cytotoxic drugs. In cancer cells, chemotherapy can temporarily promote a senescence-inducing antiproliferative response rather than the activation of the caspase cascade that commits cells to apoptosis. While chemotherapy-induced senescence (CIS) does reduce tumor growth, over time, cancer cells can exit CIS and regain their ability to proliferate. For instance, the treatment of mesothelioma cells with pemetrexed promoted CIS but also enriched a population of clones with increased invasive characteristics and a mesenchymal phenotype [50]. In the Eμ-Myc murine BCL model, doxorubicin-induced senescence in tumor cells promoted an abrogation of p53, which allowed tumor cells to escape senescence and acquire increased tumor-initiating capability [51]. In another study, the escape from CIS by cancer cells was investigated *in vitro* using etoposide- and doxorubicin-treated non-small cell lung carcinoma (NSCLC), CRC, and BC cell lines. While the cells initially acquired CIS, over two weeks, they regained their proliferative potential. Further, to determine the capacity of the senescent tumor cells to generate

tumors *in vivo*, the senescent cells were subcutaneously injected into immunodeficient mice. Within two weeks of injecting the CIS-enriched tumor cell population, the mice showed evidence of tumor development [52]. The effect of chemotherapy on the senescence-linked signaling *via p53, $p16^{lnk4a}$*, *$p21^{Waf1/Cip1}$*, and *$p27^{Kip1}$*, resulting in tumor dormancy, continues to be a hotly debated topic.

3.2. Chemotherapy and cancer cell dissemination

Circulating tumor cells (CTCs) are a cancer cell population detected in peripheral blood and serving as a prognostic factor to predict therapy response and overall survival in patients with different cancer subtypes [53]. CTCs have been implicated in metastatic dissemination. However, only a fraction of CTCs can successfully initiate metastases [54]. Although the effects of chemotherapy on CTCs have been largely underexplored, recent evidence suggests that chemotherapeutic drugs increase CTC mobilization and promote metastasis. For example, while paclitaxel reduces primary tumor size, it increases CTC frequency in blood and consequent pulmonary metastatic burden in BC mouse models [55]. Chemotherapy-mediated tissue damage is known to activate several proteolytic cascades, whose primary purpose is to initiate responses to damaged endothelia. Some of the activated factors, such as the urokinase plasminogen activator receptor and thrombin, are directly or indirectly involved in cancer cell dissemination [56, 57]. Chemotherapy-facilitated colonization may also be mediated by CTCs following the initial interactions of tumor cells within the premetastatic niche, as described in certain cancer models. A critical mediator of this process appears to be the matrix metalloproteinase-9, whose overexpression promoted CTC mobilization, cancer cell extravasation, and the formation of macro-metastatic foci [58].

3.3. Chemotherapy and CSCs

Cancer treatment with standard chemotherapy often promotes the emergence of drug-refractory cell populations that ultimately result in therapy failure [59]. Growing evidence indicates that cancer recurrence is caused by cancer-initiating cells, commonly referred to as cancer stem cells (CSCs). The CSC paradigm has emerged based on the notion that some, but not all, cancer cells have the potential to recapitulate the phenotypic diversity of the original tumor upon transplantation, thus defining CSC as multipotent self-renewing progenitors [60]. The biological features of CSCs largely overlap with those of drug-resistant cancer cells. The molecular events that govern the enrichment for cells with the CSC phenotype after chemotherapy are under investigation. In the presence of chemotherapy, the CSC phenotype could be selected for or directly induced through genetic or epigenetic changes. Drugs like taxol and doxorubicin activate pathways such as the SMAD, TCF/LEF, and STAT3 signaling pathways, which induces the stemness phenotype [61-63]. Further, platinum-based drugs like carboplatin endow self-renewability to hepatocellular carcinoma cells *in vitro* through the induction of pluripotency-related genes such as Sox2 and Oct3/4 [64]. A similar phenomenon was observed in *in vivo* ovarian cancer (OC) xenograft models where the treatment of tumors with either cisplatin or paclitaxel enhanced the expression of CSC markers Oct4 and CD117, which endowed the cells with pro-metastatic traits [65].

Additionally, chemotherapy can alter the non-neoplastic cellular components of the TME that imparts a CSC phenotype to cancer cells. For instance, monocyte chemoattractant proteins (MCP) found in the serum of BC patients are associated with chemotherapy-induced monocytosis. In hormone receptive positive (HR+) BC patients, as well as mouse models, doxorubicin and docetaxel treatment triggered MCP release, which correlated with the increase in CSC-like populations [66]. Another study showed that many types of chemotherapy at the maximum tolerated dose induced STAT1 and NF-κB activity in BC-associated fibroblasts. This stimulated the secretion of ELR^+ chemokines that bound to the CXCR2 receptors expressed on cancer cells, which triggered the induction of both CSC properties and invasive behavior [67].

3.4. Chemotherapy and carcinoma epithelial-mesenchymal transition

Epithelial-to-mesenchymal transition (EMT) is a developmental program that can be hijacked by carcinoma cells [68], which is induced in CSCs [69]. Epithelial cells undergoing EMT have a fibroblastoid phenotype, a characteristic profile of gene expression, enhancement of motility/

invasiveness, and resistance to oxidative damage and cell death. Several recent reports show that chemotherapy can induce EMT. For instance, the treatment of BC cells with paclitaxel promoted EMT gene expression, as well as the formation of invadopodia and cancer cell invasiveness [70]. In a study of MCF-7 mammary carcinoma cells treated with adriamycin and/or 5-fluoro-2'-deoxyuridine (FUdR), increased cellular production of interleukin (IL)-8 was linked with EMT induction. When compared to the untreated cells, the treated cells had enhanced survival and growth potential, which increased their spontaneous metastasis to the lungs in orthotopic BC pre-clinical models [71]. In preclinical patient-derived xenograft mouse model studies, it was shown that paclitaxel treatment promoted the overexpression of $MENA^{INV}$ protein mediating invadopodium maturation and increased invasiveness in BC cancer cells. This phenomenon was also seen in post-chemotherapy-administered patient BC tissue samples [10]. Further, in breast adenocarcinomas, paclitaxel administration promoted tumor cell upregulation of EMT markers such as vimentin, a concerted decrease in epithelial marker E-cadherin, nuclear localization of β-catenin, and induction of lung metastases mediated through a miR-21/Cyclin-dependent kinase (CDK)-5 pathway [72]. Furthermore, BC patients who received a combination of cyclophosphamide, epirubicin plus 5-FU chemotherapy showed suppressed miR-448 levels accompanied by an increased expression of SATB1, Twist1 expression, and acquisition of a mesenchymal phenotype. These findings reveal the involvement of an NF-κB/miR-448 regulatory feedback loop in chemotherapy-induced EMT in human BC [73].

Cell migration and dissemination are linked with the shift to the mesenchymal phenotype, and there is building evidence that the pro-metastatic features of CSCs are in part due to the EMT. Possibly due to reduced metabolic need and increased anti-oxidative machinery, these cells can display an enhanced capacity to survive therapy-induced cell death, adapt to foreign microenvironments, and successfully proliferate in secondary metastatic lesions. Since tumor cell dissemination is a crucial step in the metastatic cascade, it is reasonable to expect that chemotherapy-induced EMT and invasiveness should increase CTC numbers. Indeed, although paclitaxel reduces primary tumor size, it increases CTC number and consequent pulmonary metastatic burden in BC mouse models [9]. A study on BC patients revealed that chemotherapy enriched for CTCs that overexpressed the mesenchymal marker N-cadherin. This was linked with increased invasiveness, and hence metastatic colonization capacity of the tumor cells [74]. In another study, the incidence of EMT-like CTCs in OC patients following platinum-based chemotherapy was analyzed [75]. From a 30% incidence, EMT-like CTC frequency increased to 52% after chemotherapy administration. Further, the authors also observed that this increase was accompanied by the emergence of $PI3K\alpha^{+}$/ $Twist^{+}$ EMT-like CTCs with increased therapy resistance and predisposition to metastatic colonization. A similar phenomenon was observed in patients with HER2-negative BC, who underwent neoadjuvant chemotherapy (NACT) with docetaxel/ doxorubicin/cyclophosphamide ± zoledronic acid before surgery. Following three NACT doses, an increase in CTCs without membrane EpCAM expression, amplified EMT, and elevated stemness potential was observed [76].

3.5. Chemotherapy and epigenetic changes in cancer cells

The mechanism through which cancer cells undergo temporary dormancy or acquire the EMT, CSC, and CTC properties are multifaceted and not well understood [77]. However, it is evident that the gene expression changes underlying these processes are regulated, at least in part, through temporary modification of DNA and chromatin proteins. Chemotherapy may play an important role in modulating epigenetic changes, including DNA methylation, that underlies the resistance to oxidative damage and cytotoxicity. Chemotherapeutic regimens used in the treatment of gastric cancer (GC) make a good case. The application of 5-FU and cisplatin has had limited success due to frequent chemoresistance and subsequent relapses. Importantly, it was discovered that these agents mediated alterations in DNA methylation. In the 43 GC patients that had received oral or intravenous 5-FU-based combination chemotherapy, all the patients showed inactivation of key apoptosis-related genes *PYCARD* and *DAPK1* due to DNA methylation, which may be responsible for chemoresistance and poor prognoses in the GC

patients [78]. Another *in vitro* study involving GC and CRC cell lines revealed a reduced *PCDH17* tumor suppressor expression, which was owing to DNA methylation and accounted for increased resistance to 5-FU [79]. DNA-damaging agents may be particularly relevant in the context of epigenetic changes. Specific CpG methylation changes were observed in OC patients following platinum-based chemotherapy. The authors hypothesized that the DNA damage response (DDR) during platinum-based chemotherapy might change DNA methylation. The authors also used an OC cell line model to investigate the role of DNA mismatch repair (MMR) gene *MLH1* in platinum-induced DNA methylation. They discovered that MMR proteins bind to platinum DNA adducts to recruit the methylating enzyme DNMT1, resulting in aberrant methylation at DNA damage sites [80]. The possible importance of other epigenetic changes, such as histone acetylation and methylation in chemotherapy response, remains to be investigated. Another common epigenetic event is the dysregulation of cell cycle checkpoints regulating the activity of complexes between cyclins and CDKs [81]. A recent study identified the impairment of the CDK5 axis to be implicated in paclitaxel-induced BC metastasis. Upon taxol administration, CDK5 and miRNA-21 were highly expressed in both MDA-MB-231 human BC cancer cell lines and patients with enhanced lymph node metastasis. Paclitaxel bolstered CDK5 activity through the elevation of miR-21 biological target CDK5RAP1, subsequently increasing CDK5 activator p39 and its downstream target p-FAK^{Ser723} expression [72]. This was linked with EMT induction and enhanced cell migration and invasion, the hallmarks of enhanced metastatic potential.

3.6. Chemotherapy-induced mutagenesis and clonal evolution

In addition to temporary epigenetic changes, chemotherapy increases the chance of permanent DNA alterations in tumor cells. The theory of tumor heterogeneity states that cancer cell diversity occurs due to progressive mutational changes that result in distinct tumor cell clones [45]. The cancer genome evolution proceeds gradually through clonal selection. The patterns of mutational processes and the ensuing clonal selection in cancer often show biases at the DNA sequence level, which may reflect prior exposure and selective pressure exerted by chemotherapy [82]. Recent studies in GC have supported the notion that chemotherapeutic agents trigger clonal evolution, which affects tumor heterogeneity [83]. The existence of spatial intra-tumoral heterogeneity may account for the partial sensitivity to therapy. Moreover, clonal evolution may be initiated during therapy administration. Relapse and metastatic progression after a beneficial therapeutic effect may occur due to a resistant clone that was present before therapy or originated as a result of chemotherapy. Survival, proliferation, and subsequent dominance of this clone could be due to features that were likely absent prior to therapy.

Cancer genomes accumulate chromosomal aberrations in the form of point mutations, deletions, minor insertions, as well as large chromosomal rearrangements. Chemotherapy inexorably induces gene mutations in tumor cells, some of which may stimulate cancer aggressiveness. An analysis of patients with acute myeloid leukemia performed before and after chemotherapy revealed that the treatment caused changes in cancer cell composition and triggered an array of mutations. Further, this seminal study also demonstrated differences in aberrations between primary tumors and relapses in cancer patients. The relapses had two possible variants: in three out of eight cases, the dominant sub-clones that were diagnosed in the primary tumor acquired additional mutations after chemotherapy. In the other five cases, a minor sub-clone in the primary tumor developed prior to relapse, survived chemotherapy, and accumulated additional mutations to become the dominant relapsing clone [84]. Similar results were obtained for diffuse BCL. Chemotherapy-induced clonal evolution occurs in primarily two ways. First, a rare clone develops alongside the dominant sub-clone of the tumor and survives chemotherapy to outcompete the sensitive dominant clone. The minor resistant clone then expands to constitute the tumor, becomes dominant, and eventually becomes the initiator of multiple distant malignancies. In the second case, the minor sub-clone develops much later during therapy than the dominant one, survives chemotherapy, and initiates the relapse [85]. Studies in acute lymphoblastic leukemia patients showed that relapse after chemotherapy was due to the

development of a host of *de novo* mutations involving *NRAS*, *KRAS*, and *PTPN11* genes in particular [86]. Another study identified therapy-driven mutations in relapsed glioblastomas from 23 patients. Most of the driver mutations (*TP53*, *ATRX*, *SMARCA4*, and *BRAF*), seen in primary tumors, were not detected in the relapse. Instead, the relapsed tumors acquired mutations in *RB1* and genes of the mTOR pathway following therapy [87]. Further, the microarray analysis study of BC patients (stage IIA to IIIC) subjected to 5-FU, anthracycline, and cyclophosphamide revealed that NACT conferred enhanced metastatic potential to BC tumor clones. 6 out of 26 patients demonstrated the formation of new clones with copy number alterations and amplifications in genes that were implicated in metastasis development [88].

The selection of aggressive sub-clones appears to be an inadvertent aftermath of the genotoxic stress exerted by chemotherapy. Understanding how chemotherapy-exerted selective pressure directs cancer cell evolution and shapes its clonal architecture is critical. Genomic instability (GI) can account for the functional variability of individual cells and thus impart distinct trajectories on the clones in the process. GI can also explain how chemotherapy promotes the clonal selection and subsequent adaptation of tumor cells. GI arises from many different pathways, including telomere damage, centrosome amplification, as well as DNA repair failure. Malfunctioning cell cycle checkpoints can also result in GI and subsequent mutation accumulation. Building evidence indicates that chemotherapy can enable cancer progression by inducing GI through mutations and aneuploidy. Some of the examples and mechanisms underlying the link between chemotherapy and GI are discussed below.

3.6.1. Chemotherapy and DNA damage repair

DNA damage is the mechanism of action of many conventional chemotherapeutics. For instance, by binding to DNA between purine, platinum compounds induce adducts that impair replication and transcription, which leads to the stalling of replication forks and the formation of double-strand breaks. Upon recognizing DNA damage, such as DNA breaks, damaged bases, misalignment, or crosslinks, the cells initiate a plethora of signaling pathways, including MMR and nucleotide excision repair, collectively known as DDR. Cancer cells are notorious for the activation of DDR-related pathways, which may account for their therapy resistance. In several mouse models of advanced NSCLC, the pulmonary tumors initially respond to cisplatin by sensing DNA damage, undergoing cell cycle arrest, and inducing apoptosis, thus leading to a significant tumor burden reduction. However, prolonged cisplatin administration induces the emergence of resistant tumors with enhanced repair capacity. The drug-resistant tumors expressed elevated levels of cell cycle and DNA damage repair genes of the p53 pathway [89]. Not only were these tumors cross-resistant to platinum analogs, but they also exhibited advanced histopathology, and possessed amplified frequency of GI, including whole-chromosomal DNA copy number changes.

In some cases, decreased DDR appears to be pivotal in the evolution of aggressive cancers. Loss of heterozygosity (LOH), highlighted by the loss of a gene and the surrounding chromosomal region, is a common event in cancer. Another common event is mutations in repetitive DNA sequence stretches, termed microsatellites, which is termed microsatellite instability (MSI). Both LOH and MSI arise during replication, at least in part due to insufficient MMR. The consequences of these forms of GI were implicated in increased chemoresistance, recurrence of primary tumors, and secondary malignancies. Studies of MSI and LOH have highlighted the importance of screening patients for GIs after chemotherapy completion [90]. In another seminal study, samples were collected pre-and post-treatment from 117 *de novo* solid tumor patients. The specimens were screened for MSI and LOH in 10 microsatellite sequences in blood, and immunohistochemical analysis of the expression of five MMR proteins was performed on the tissue samples. The authors discovered chemotherapy-induced MSI and LOH in chromosomes 2, 5, 10, and 17 in the tumor patients. This was accompanied by deficiencies in the expression of key MMR proteins such as human mutL homolog 1 (hMLH1), mutS homolog 2 (hMSH2), mutS homolog 6 (hMSH6), post-meiotic segregation increased 2 (hPMS2) and P53. Further, there was a significant association between MSI and LOH in the incidence of secondary tumors [90]. Similar results were seen in a clinical

BC patient study where higher rates of LOH and MSI were observed at multiple allele loci, including Tp53-Alu, Mfd41, and Mfd28, which correlated with deficient hMSH2 protein expression [91]. In an OC setting, the amplification of 10 microsatellite loci and immunohistochemical detection of hMSH2 and hMLH1 expression between primary and secondary resected tumors following patient treatment with cisplatin was performed. The pronounced reduction in hMSH2 and hMLH1 expression suggested the occurrence of MSI [92]. Other studies have supported the fact that a loss in hMLH1 expression due to the hypermethylation of the *hMLH1* gene promoter is a signature of MIS manifestation due to abnormalities in the MMR machinery. MSI has been revealed as a promising diagnostic marker for CRC. In over 15% of CRC tumor xenografts demonstrating high-frequency microsatellite instability (MSI-H), the MMR function is lost, while the remainder of CRC retains DNA MMR function and are called microsatellite stable (MSS). Further, CRC patients with MSI-H have reduced survival and response to 5-FU, compared to patients with MSS tumors [93].

3.6.2. Chemotherapy and chromosomal instability

Chromosomal instability (CIN) is a specific mechanism potentially exploited by chemotherapy to promote tumor heterogeneity and evolution. CIN typically results from aberrant chromosome division during replication, the process affected by most chemotherapeutics. In a pilot study involving 80 pediatric patients, there was a transient increase in chromosomal aberrations induced by anti-tumor regimens, which suggested the existence of increased chromosomal fragilities in specific genomic locations of cancer cells [94]. Similar results were obtained in a study aimed to test the presence of an inherent increase in genetic instability in cancer patients following chemotherapy. An in-depth analysis of 99 pediatric cancer patients with four different tumor types (Ewing's sarcoma, lymphoma, and osteosarcomas) revealed a transiently increased genetic instability in lymphocytes of children exposed to anti-tumor regimens [95]. Whole CIN can also manifest as aberrant kinetochore-microtubule attachment dynamics during cell division. To study this process, an assay using a non-essential human artificial chromosome (HAC) carrying an enhanced green fluorescent protein-expressing transgene was developed. Upon the administration of a combination regimen of paclitaxel and gemcitabine, high-frequency HAC loss indicating chromosome mis-segregation, and hence, CIN was observed [96]. Some chemotherapeutics could be predicted to induce certain types of chromosomal aberrations. Anti-microtubule inhibitors are chemotherapeutic drugs that disrupt normal mitotic function. Through the inhibition of microtubule dynamics, they induce a failure in chromosomal alignment at the metaphase plate and promote spindle assembly checkpoint activation. However, the mitotic arrest induced is countered by a process termed "mitotic slippage" upon excess administration of these drugs. One of the primary mechanisms for mitotic slippage is the degradation of cyclin B1. Upon mitotic slippage, the cells exit mitosis without undergoing cytokinesis and develop tetraploidy, which could drive cancer progression [97].

4. Chemotherapy effects on the tumor microenvironment

Tumors are composed not merely of cancer cells, but also of non-neoplastic cells that constitute the stroma. The TME is a composite niche consisting of mesenchymal, neuro-endocrine, endothelial, and immune cells, as well as the extracellular matrix (ECM) [98]. Since chemotherapy is administered systemically, it exerts an influence on host cells. In a healthy state, the tissue microenvironment plays a protective role by orchestrating repair responses to cytotoxic damage [99]. However, the activation of host-mediated tissue repair programs can similarly protect the tumor cells from chemotherapy and promote the disease. Indeed, accumulating evidence suggests that under certain circumstances, chemotherapy may convert the TME into an accomplice in the evolution of more stubborn and aggressive malignancies. In patients, paclitaxel has been shown to change the expression of specific transcription factors in non-malignant cells, which then activate pro-metastatic signaling cascades in cancer cells [100]. Increased expression of certain stromal proteins has also been associated with metastatic recurrence in patients treated with doxorubicin and docetaxel [101]. The TME features a marked spatial heterogeneity in oxygenation, acidity, the proximity of tumor cells to vessels,

and the presence of stromal and immune cells. This heterogeneity may be responsible for the spatial differences in the tumors' cell response to chemotherapeutic agents. Mesenchymal stroma, endothelial cells, as well as infiltrating immune cells, have been shown to underlie chemotherapy-induced cancer progression (Figure 1).

4.1. Chemotherapy and mesenchymal stromal cells

Mesenchymal stromal cells (MSC) are cells with fibroblast characteristics initially described in the bone marrow. In tumors, their heterogeneous pool is composed of cells derived from organ-resident MSCs, as well as MSCs recruited from surrounding tissues, such as adipose tissue (AT) and the bone marrow. MSCs modulate the TME through the production of ECM, cytokines, growth factors, and other bioactive molecules. The role of MSCs in tumor progression is hotly debated, owing to their context-dependent function as either tumor-promoting or tumor-suppressive. For instance, platinum-analogs, such as cisplatin, stimulate MSCs to release polyunsaturated fatty acids that systemically support tumor growth and metastasis in mouse models of lung, breast, and colon carcinomas [102]. Accumulating evidence indicates that MSCs mediate chemotherapy-induced metastasis. While MSC may signal to tumors through endocrine pathways, their primary function in cancer is within the tumor. Cancer-associated fibroblasts (CAFs) are the MSCs of carcinomas. Recent single-cell RNA sequencing studies identified several CAF subpopulations that were functionally distinct. Collectively, CAFs are known to play a significant role in disease progression. CAFs undergo activation in response to tissue damage, chronic inflammation, and acquisition of epigenetic alterations in cancer. CAFs foster tumor cell proliferation, survival, invasion, and metastasis through both the secretion of paracrine factors such as chemokines and hormones and through ECM remodeling *via* proteases and ECM molecules that they express. Not only they drive tumor desmoplasia, but they also secrete trophic factors that stimulate vascularization. Several studies have highlighted the possible chemotherapy-induced changes in the stromal cell phenotype. For instance, when a co-culture of immortalized human foreskin fibroblasts and MCF7 mammary cancer cells were treated with chemotherapeutic drugs such as azathioprine, carboplatin, cyclophosphamide, doxorubicin, 5-FU and taxol, activation of fibroblasts and the subsequent transformation into the CAF phenotype was observed. However, the effect of these drugs on fibroblasts varied: taxol and doxorubicin induced oxidative stress, while azathioprine reduced IL-6 expression in the fibroblasts. Furthermore, amongst 35 genes differentially expressed in CAFs, chemotherapy promoted the expression of key invasiveness and motility factors such as CXCL2, MMP1, and IL-8 [103]. The co-culture of MDA-MB-231 BC cells with Taxotere-treated patient-derived CAFs conferred highly adhesive, invasive, and proliferative ability to the cancer cells [103]. Treatment of patient-derived CAFs with either cisplatin or paclitaxel induced p53 mutations that altered CAF functionality [104]. The same study also noted a similar observation and increased metastatic burden in pre-clinical mouse xenograft models of breast and lung carcinomas. In a study involving HR+ BC mouse models, doxorubicin administration promoted the recruitment of pro-oncogenic CAFs. These CAFs elevated the ELR^+ chemokine-CXCR2 signaling axis to induce tumor neovascularization, macrophage infiltration, and subsequent metastasis [67]. In another study, it was alluded that chemotherapy-altered fibroblasts trigger the IL-6/STAT3 pathway driving cancer aggressiveness [105]. Clinically, the administration of chemotherapy revealed enrichment of CAF that promoted cancer relapse through the secretion of paracrine factors including IL-17A and Wnt16B in prostate (PRC) and colon cancer patients [106, 107].

The heterogeneity of CAF has made it difficult to establish the potential benefits of their inactivation, and controversial results have been reported [108, 109]. The current challenge is the lack of clarity on the origins and the roles of specific sub-populations of CAF [110]. Our studies indicate that adipose stromal cells (ASC), the MSC of AT, are expanded in obesity, become mobilized, and migrate to tumors, which is linked with poor cancer prognosis. The recruitment of ASCs to tumors is enhanced by obesity [111, 112]. The application of these pre-clinical findings was evidenced by data indicating that obese carcinoma patients have increased tumor CXCL1 expression, as well as ASC in circulation and tumor stroma [113].

ASC infiltrating tumors from adjacent peritumoral AT, undergoing remodeling in cancer, play a particularly important role in BC and PRC [114]. The molecular mechanisms through which these adipose-derived CAF (AD-CAF) promote cancer progression are multifaceted. Some of the cancer-promoting effects of ASC are contact-dependent. In our recent study, CXCL12, a paracrine CAF-secreted chemokine, was discovered as a factor mediating obesity-associated prostate tumor growth and invasiveness [115]. Our recent study demonstrates the role of AD-CAF in EMT induction and PRC aggressiveness [116]. We reported that the interaction of carcinoma cells with ASCs results in EMT and increased invasiveness of cancer cells. Importantly, upon ASC exposure, carcinoma cell had decreased levels of reactive oxygen species and became more resistant to docetaxel, cabazitaxel, and cisplatin, [116]. The apparent cancer-promoting effects of chemotherapy on CAF/MSCs in patients remain to be further investigated.

4.2. Chemotherapy and immune cells

Several studies indicate that chemotherapy facilitates tumor infiltration by immune cells. Myeloid cells are a subclass of leukocytes derived from hematopoietic stem cells in the bone marrow, which have been studied the most in the context of chemotherapy. Monocytes are composed of both mature terminally differentiated cells such as polymorphonuclear neutrophils, granulocytes, macrophages, dendritic cells, as well as relatively immature cells, including granulocytic precursors. A major immunological hallmark of cancer is the abnormal differentiation of the myeloid compartment, which results in the expansion of pathologically active immature myeloid cells with the potent ability to suppress immune responses. Inflammatory monocytes (IM) are recruited to the TME at secondary sites through a CCL2/CCR2 chemotaxis pathway following chemotherapy. The recruitment of these IM promoted the local suppression of cytotoxic $CD8^+$ T-lymphocytes in the lung, thus facilitating metastatic colonization in both spontaneous and experimental metastasis mouse models [117]. Upon the administration of a doxorubicin and cyclophosphamide combination in PyMT-MMTV BC mouse models, the TNF-α-CXCL1/2 axis gets hyperactivated, which recruits granulocytic myeloid cells to tumors that in-turn support cancer cell survival and metastasis [118]. Clinically, the treatment of HER2-positive BC patients with trastuzumab has been shown to increase miRNA-21 levels in dendritic cells, which stimulates EMT in tumor cells through the IL-6 production and also inhibits IL-12 secretion and subsequent differentiation of Th1 lymphocytes [119]. The resulting skewing of T cells from Th1 to Th2 has been linked with decreased anti-tumor immune response and poor cancer prognosis.

Myeloid-derived suppressor cells (MDSC) are an immunosuppressive monocyte population co-expressing lineage differentiation antigens Ly6G and CD11b [120]. Accumulation of MDSCs in both pre-clinical models and clinical patients is accompanied by T cell response inhibition and dendritic cell function defects. Abnormal accumulation of MDSCs is an important mechanism of chemotherapy-mediated cancer progression and metastasis. The effects of chemotherapeutic agents on MDSCs are multifaceted. It has been shown that doxorubicin induces IL-$13R^+$ miR-$126a^+$ MDSCs that promote lung metastasis *via* their effect on angiogenesis [121]. Similarly, the administration of doxorubicin-cyclophosphamide chemotherapy increased the abundance of circulating MDSCs in early-stage BC patients. Further analysis revealed a direct correlation between circulating MDSC levels and metastatic tumor burden among stage IV patients [122]. Another related study demonstrated that paclitaxel and cyclophosphamide might induce cancer cell dissemination and metastatic colonization through the recruitment of myeloid progenitors in the primary tumors in a stress-inducible Atf3-dependent manner. The transcription factor Atf3 is a master regulator of several inflammatory cytokines involved in leukocyte migration and angiogenesis. In both spontaneous and experimental metastasis models of BC, Atf3 played an essential role in cancer cell seeding and the development of distant metastasis [100]. The hypoxic damage of tissues by chemotherapy can cause a surge in chemotactic factors released by tissue-resident cellular players, which, in turn, attracts various bone-marrow-derived stromal cells. It has been documented that MDSCs recruited by cisplatin administration in BC pre-clinical models not only change phosphorylation profiles of PLC-y1, WNK1, RSK1/2/3, and p53 but also increase the

secretion of CXCL1, IL-6, IL-8 and CCL2 cytokines implicated in cancer aggressiveness [123]. Pre-existing chronic inflammatory signatures in the TME may be provoked through chemotherapeutic drugs such as paclitaxel, cisplatin, doxorubicin, paclitaxel, and 5-FU. For example, paclitaxel activates the NF-κB and TLR-4-MyD88-ERK signaling pathways in BC cells. This, in turn, stimulates the production of IL-1β, IL-8, IL-6, and VEGF-A, which regulate inflammation, angiogenesis, proliferation, and invasion [124]. Paclitaxel therapy of TLR4-positive tumor patients also activated inflammatory reactions and mobilized myeloid progenitor cells, which can stimulate angiogenesis and lymphangiogenesis in both the tumor and the premetastatic niches [125]. In studies on orthotopic BC-bearing mice, gemcitabine and 5-FU activated NOD-like receptor protein 3 inflammasome formation in MDSCs. The activation of the inflammasome triggered IL-1β secretion, which induced IL-17 release by $CD4^+$ T cells. Further, in mice with NLRP3/TNF-α type 1 receptor and IL-1 type 1 receptor knockouts, vincristine and doxorubicin synergistically activated NLRP3 inflammasomes and increased expression of IL-1β, IL-6, and CXCL1, which eventually resulted in increased metastatic burden [126].

Tumor-associated macrophages (TAMs) are often specified as a separate myeloid population of the TME. Tumor cells promote the polarization of macrophages into M2-skewed TAMs that promote tumor progression through the Th2-type T lymphocytes [127]. TAMs may serve as the key conduit for chemotherapy to bring about its pro-cancer effects. Chemotherapy not only increases the abundance of TAMs but also aggravates their pro-tumorigenic properties, such as increased bioactivity and inflammasome activation. The mechanisms of chemotherapy-mediated pro-cancer effects of TAMs can be broadly classified into two categories. The first mechanism is macrophage-driven suppression of cytotoxic T cell-mediated immune responses. When murine mammary carcinomas are subjected to paclitaxel treatment, there is an increased infiltration of IL-10-secreting TAMs into primary tumors. These TAMs suppress the IL-12 expression in dendritic cells, leading to the suppression of $CD8^+$ cytotoxic T cells [128]. Alkylating agents such as cyclophosphamide drive the expansion of inflammatory monocytic cells ($F4/80^+$, $Ly6C^+$, $CCR2^+$), which possess immunosuppressive activities [129]. The second mechanism used by TAMs is the pro-metastasis priming of the TME. Treatment with either paclitaxel or cisplatin increases the gradient of chemokine CCL12 that facilitates the recruitment of Tie2-expressing macrophages (TEMs), laying the path to pulmonary metastasis [55]. Upon the administration of paclitaxel and doxorubicin in murine adenocarcinoma models, there is an increased perivascular expression of CXCL12. This chemokine mediates the recruitment of $CXCR4^{hi}$ $MRC1^+$ TAMs that promote tumor vascularization, which also promotes metastasis [130]. Further, paclitaxel also increases the plasma concentration of VEGF-C, which is secreted by macrophages. Through the VEGF-C/VEGFR3 axis, chemotherapy boosts macrophage-induced lymphangiogenesis, which opens the route for metastatic dissemination [131]. Paclitaxel treatment leads to the accumulation of IL-1β-expressing macrophages in blood circulation. Their co-culture with MDA-MB-231 BC cells rendered tumor cells more invasive [132]. Interestingly, while cancer cell motility and primary tumor growth decreased upon IL-1β inhibition, the long-term blockade of IL-1β signaling significantly bolstered spontaneous metastases. It has been concluded that IL-1β blockage results in the differentiation of pro-tumor M2 TAMs in the TME, which promotes an increase in vascular permeability, followed by metastasis [132]. Clinical data linking TAMs and chemotherapy is also building. Further, metastatic relapse in anthracycline-treated BC patients can be associated with the enrichment of $YKL\text{-}39^-$ $CCL18^+$ or $YKL\text{-}39^+CCL18^-$ M2 macrophages in the primary tumor [133].

4.3. Chemotherapy and endothelial cells

In order for tumor cells to survive and metastasize, they require an unremitting supply of nutrients, oxygen, and a route to enter body circulation. Thus, cancer progression depends on the process of tumor angiogenesis, maintained by locally dividing endothelial cells and recruited endothelial progenitor cells (EPCs). When compared to normal endothelium, tumor endothelial cells exhibit an altered phenotype, which is exacerbated upon the administration of chemotherapy. It has been

shown that doxorubicin incites endothelial cells to secrete IL-6, a key player in cancer progression [134]. There is evidence that chemotherapy can induce vascularization through specific angiogenic molecules, released within the TME, that activate endothelial cells [135]. A study in the transgenic PyMT-MMTV BC model revealed that paclitaxel treatment induced VEGF-C expression in TAMs, which in turn facilitated the recruitment of lymphatic endothelial cells and MENA-overexpressing tumor cells to create microscopic structures called the tumor microenvironment of metastasis (TMEM) [10]. Through these TMEM sites, the macrophages and endothelial cells guided cancer cells to enter the body circulation, thus facilitating dissemination to secondary sites. The authors also extended these observations to $ER^+/HER2^-$ BC patients subjected to weekly paclitaxel treatment for 12 weeks, followed by four cycles of doxorubicin and cyclophosphamide. In 20 NACT-administered patients, evaluation of the primary tumor demonstrated changes in TMEM density. TMEM sites were more abundant in malignant tumors than in the tumor biopsy prior to NACT. These results were also corroborated by another group, who demonstrated that TMEM density was greater in patients with distant metastases compared to localized BC [136].

Several studies propose that paclitaxel and gemcitabine mobilize EPCs from the bone marrow and that their engagement at the primary or secondary tumors may promote metastasis. Owing to their augmented angiogenic capacity and production of trophic factors, the EPCs encourage tumor cell colonization in secondary sites. This activity of EPCs is hinged on their membrane expression of vascular endothelial growth factor receptor 1 (VEGFR1). *In vitro* analysis suggests that the membrane expression of VEGFR-1 is upregulated in endothelial cells in response to chemotherapy, which enhances their adhesion to BC tumor cells. Investigations in experimental pulmonary metastasis mouse models also revealed that both cisplatin and paclitaxel promoted lung metastasis in response to enhanced VEGFR-1 expression on endothelial cells [137]. In this study, paclitaxel induced the proangiogenic mobilization of EPCs in BC patients, whereas gemcitabine lacked this ability. The number of EPCs in peripheral blood was found to increase in cancers of breast, ovary, colon, esophagus, cervix, head and neck, and prostate between 7 to 21 days of different chemotherapy regimens regardless of the tumor location.

4.4. Chemotherapy and senescent stromal cells

Senescence induction in tumor stroma is another important mechanism through which chemotherapy can indirectly exert its effect on cancer cells [138]. For instance, senescent stromal cells play an important role in the formation of an immunosuppressive TME, through an IL-6 dependent pathway [139]. Similarly, in the pre-clinical models of Burkitt's lymphoma, paracrine factors such as IL-6 and TIMp-1 increased lymphoma cell survival following chemotherapy. IL-6 was identified to be produced from senescent endothelial cells in the mouse thymus in response to DNA damage. This created a "chemo-resistant niche" that promoted the survival of a minimal residual tumor burden and served as a reservoir for possible tumor relapse [134]. CIS in non-neoplastic host cells could also promote the secretion of factors that disrupt tissue architecture and stimulate neighboring tumor cells to proliferate. It has been shown that paracrine factors such as WNT16B and secreted frizzled-related protein 2 (sFRP2) produced in chemotherapy-treated tumor microenvironments protect cancer cells from chemotherapy in a paracrine manner *in vivo* [107, 140].

4.5. Chemotherapy and extracellular vesicles

Within the TME, the interaction between carcinoma cells and host cells is orchestrated *via* a plethora of signaling networks, ranging from juxtacrine interactions to secreted factors contained within microvesicles (MVs), such as exosomes [141]. MVs have been discovered as an entity pre-conditioning the biology of distant organ niches to enhance the dissemination and seeding of metastatic cancer cells. Cancer cells secrete exosomes in response to a number of stimuli, the most aggressive trigger being anticancer drugs, including carboplatin, paclitaxel, doxorubicin, and irinotecan [142]. The propagation of oncoproteins mediated by chemotherapy-induced exosomes could be responsible for cell transformation and TME modulation that favor metastatic progression. For instance, the exposure to commonly utilized anti-myeloma drugs, carfilzomib or bortezomib, induced

the shedding of exosomes by myeloma cells *in vitro*. These chemotherapy-induced exosomes had a distinct proteome profile, including elevated surface levels of heparinase, which modulates the ECM and tumor/host cell interactions to promote tumor angiogenesis and metastasis [143]. Another study with OC models also revealed exosome release as an outcome of a cisplatin-induced stress response. The secreted exosomes modulated several pathways, including p38 and JNK, which conferred an enhanced invasive capacity to the bystander cancer cells [144]. In a recent study, treatment with taxane and anthracycline in BC models resulted in increased release of exosomes enriched in annexin A6 (ANXA6) by cancer cells. At the secondary site, ANXA6-mediated NF-κB-dependent endothelial cell activation, CCL2 induction, and $Ly6C^{+}CCR2^{+}$ monocyte expansion promoted lung metastasis [145]. Further, the authors also speculated on a potential enrichment of ANXA6-expressing circulating exosomes in BC patients undergoing NACT. A recent study revealed that cancer cells might respond to sublethal chemotherapy doses through the secretion of miRNA encapsulated within MVs. In *in vivo* xenograft tumor models, docetaxel treatment triggered the secretion of circulating MVs that elevated the levels of miR-9-5p, miR-195-5p, and miR-203a-3p, which confer cancer cells' CSC properties [146]. The authors also recorded similar observations in human breast tumors, which indicate a mechanism employed by cancer cells to communicate with each other and self-adapt to survive in response to cytotoxic treatment.

Besides cancer cells, chemotherapy appears to invoke the TME and other cells to secrete pro-metastatic extracellular vesicles. A consequence of chemotherapy is chronic inflammation mediated by MDSCs and $IL\text{-}13^{+}Th2$ cells. Doxorubicin treatment of BC-inflicted mice induces the recruitment of MDSCs that promote lung metastasis through the release of $miR\text{-}126a^{+}$ exosomes that enable $IL\text{-}13^{+}Th2$ cell mobilization and tumor angiogenesis [121]. According to the model in this report, IL-13 released from $IL\text{-}13^{+}Th2$ cells creates a positive-feedback loop that encourages the continued production of MDSCs and $miR\text{-}126a^{+}$ exosomes *via* MDSC IL-13R. Chemotherapy may also prompt blood platelets into releasing platelet-derived MVs, the small membrane fragments enriched for endothelium adhesion receptors, such as CD41 and CD62P [147]. These MVs coat the surface of CTCs and facilitate their attachment to the endothelium, which promotes entrapment and retention of tumor cell emboli within smaller vessels at future sites of metastasis. The coating of CTCs with platelets has multiple functions, all of which enhance the likelihood of future metastasis development [148].

5. Conclusion

The role of chemotherapy in cancer progression is two-sided, owing to its multifaceted short-term and long-term effects. Given the remarkable genomic diversity and instability of tumor cells, chemotherapy alone cannot cure patients with advanced cancer. Combinations of chemotherapies and immunotherapies are expected to significantly improve the efficacy of treatment for patients with many types of metastatic cancer [149, 150]. New strategies to target the TME, used in combination with chemotherapies and immunotherapies, have also given positive results in pre-clinical models [151]. However, irrespective of efficacy shown by combination treatments, it is critical to fully understand the full magnitude of adverse consequences of chemotherapeutics that the patients incur long-term. There is building evidence that post-chemotherapy tumors tend to recur, often with increased aggressiveness that makes them resistant to the previously employed treatment. While clinical evidence for the possible oncogenicity of chemotherapy is still sparse, the body of evidence from animal models is building. Many unanswered questions remain. Which repercussions are shared by all chemotherapeutics and which are unique for individual classes of drugs? Are the consequences of ACT and NACT similar or different? Can we extrapolate data from one cancer type to other cancer types? How much can we learn from mouse models, which fail to emulate the clinical complexities of cancer in patients, especially in regard to long-term effects? Should findings on adverse effects of chemotherapy from animal models be enough to influence clinical practice? Clearly, more research needs to be done before the standard of care is to be reconsidered for any cancer type. A thorough understanding of these novel concepts will promote the development of higher standards in cancer treatment and help to design therapies that are not only effective but also safe long-term.

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CONFLICT OF INTEREST STATEMENT

The authors report no conflict of interest.

ABBREVIATIONS

ACT	Adjuvant chemotherapy
AD-CAF	Adipose-derived cancer-associated fibroblasts
ANXA6	Annexin A6
ASC	Adipose stromal cells
AT	Adipose tissue
BC	Breast cancer
BCL	B-cell lymphoma
CAF	Cancer-associated fibroblast
CDK	Cyclin-dependent kinase
CIN	Chromosomal instability
CIS	Chemotherapy-induced senescence
CRC	Colorectal cancer
CSC	Cancer stem cell
CTC	Circulating tumor cell
DDR	DNA damage response
ECM	Extracellular matrix
EMT	Epithelial to mesenchymal transition
EPC	Endothelial progenitor cell
FUdR	5-fluoro-2'-deoxyuridine
GC	Gastric cancer
GI	Genomic instability
HAC	Human artificial chromosome
HR+	Hormone receptor-positive
IL	Interleukin
IM	Inflammatory monocyte
LOH	Loss of heterozygosity
MCP	Monocyte chemoattractant protein
MDSC	Myeloid-derived suppressor cell
MMR	Mismatch repair
MSC	Mesenchymal Stromal Cell
MSI	Microsatellite instability
MSI-H	High-frequency microsatellite stability
MSS	Microsatellite stable
MV	Microvesicles
NACT	Neoadjuvant chemotherapy
NSCLC	Non-small cell lung carcinoma
OC	Ovarian cancer
PRC	Prostate Cancer
TAM	Tumor-associated macrophage
TEC	Tumor endothelial cell
TEM	Tie2 expressing macrophage
TME	Tumor microenvironment
TMEM	Tumor microenvironment of metastasis
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
5-FU	5-fluorouracil

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