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Review

Paradoxical effects of chemotherapy on tumor relapse and metastasis promotion

Crescenzo D'Alterio[a], Stefania Scala[a], Gabriella Sozzi[b], Luca Roz[b,1], Giulia Bertolini[b,*,1]

[a] Functional Genomics Unit, Istituto Nazionale Tumori - IRCCS - Fondazione "G. Pascale", Naples, Italy
[b] Tumor Genomics Unit, Department of Research, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

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ABSTRACT

Several lines of compelling pre-clinical evidence identify chemotherapy as a potentially double-edged sword: therapeutic efficacy on the primary tumor may in fact be counterbalanced by the induction of tumor/host reactive responses supportive for survival and dissemination of cancer cell subpopulations. This paradoxical effect of chemotherapy can affect different districts such as the primary tumor, the circulation and distant organs by simultaneously shaping properties and composition of tumor and stromal cells.

At the primary tumor site, chemotherapy has been reported to promote selection of chemoresistant and disseminating tumor cells endowed with properties of cancer stem cells (CSCs) through activation of autocrine and paracrine self-renewing/survival pathways promoted jointly by therapy-selected tumor and stromal cells. Resistant CSCs represent seeds for tumor relapse and increased infiltration by immune cells, together with enhanced vascular permeability induced by chemotherapy, facilitates tumor cells intravasation, the first step of the metastatic cascade. As a consequence of primary tumor/metastasis re-shaping induced by chemotherapy, circulating tumor cells (CTCs) detected during therapy can display a shift towards a more mesenchymal and stem-like phenotype, conductive to increased ability to survive in the circulation and seed distant organs. At the metastatic site, host responses to therapy activate inflammatory pathways that ultimately facilitate tumor cells extravasation and metastatic colonization. Finally, cooperation of immune cells and endothelial cells at perivascular niches favors the extravasation of tumor cells endowed with high potential for metastasis initiation and protects them from chemotherapy.

This review highlights the paradoxical pro-metastatic effects of chemotherapy linking reactive responses to treatment to tumor relapse and metastasis formation through primary tumor remodeling and generation of a favorable pro-metastatic niche.

1. Introduction

Metastatic disease represents the main cause of cancer related mortality [1]. Although the advent of novel therapies based on immune checkpoint inhibitors (ICIs) is rapidly changing the treatment of metastatic patients in several settings [2–4] the current standard of cancer care for loco-regional disease is still commonly based on surgery, chemotherapy and/or radiotherapy [5]. In addition, combination therapies with ICIs and standard chemotherapy are gaining increased attention and are being actively evaluated [6,7]. Clear understanding of the underlying mechanisms of action of different treatments both on tumor cells and on host responses is therefore needed to design novel rational therapeutic strategies.

Despite its effectiveness in disease control at the primary tumor site, in several cases standard chemotherapy does not impact on metastatic disease resulting in the unfortunately common clinical evidence of patients developing distant metastases despite efficient control of local disease [5]. This might not only be an inherent shortcoming of therapies originally designed and clinically validated to control primary tumors, but may also conceal wider detrimental effects generated by chemotherapy. Accumulating pre-clinical evidence suggests in fact that chemotherapy induces intra-tumoral and systemic changes that can paradoxically promote cancer cell survival/proliferation ultimately fostering dissemination to distant organs [8,9].

At the primary tumor site, selection induced by chemotherapy operates in the context of intra-tumor heterogeneity with specific clones or cellular subsets possessing intrinsic resistance properties [10,11]. In particular, specific subsets of cancer stem cells (CSC) endowed with self

* Corresponding author at: Fondazione IRCCS Istituto Nazionale dei Tumori, Via G. Venezian 1, 20133 Milan, Italy.
E-mail address: giulia.bertolini@istitutotumori.mi.it (G. Bertolini).
[1] These authors contributed equally to this work.

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renewal ability, have been shown to have increased ability to initiate and sustain primary tumors [12–14]. The chemoresistant properties of CSCs have been extensively reported in several tumor types and are based both on intrinsic properties, such as the high expression of drug efflux transporters and their relative quiescence [15–19]. Moreover, extrinsic resistance can also occur, frequently related to microenvironment stimuli that directly activate CSC self-renewal pathways and/or induce epithelial mesenchymal transition (EMT) providing tumor cells with enhanced disseminating properties, CSC features and chemoresistance [20,21]. In this context EMT can be considered the link between chemoresistance and metastatic potential although this connection might be more complex than originally imagined and several aspects still need to be thoroughly investigated [22].

The fundamental role of the EMT program in tumor dissemination and acquisition of chemoresistance properties is very well established [23,24]; however the formal proof of concept that EMT is necessary to establish metastasis is still debated and some experimental evidence suggest that overt metastases are generated only by tumor cells with an epithelial phenotype [25,26]. A balance between these two visions is provided by the recent notion that tumor cells in an hybrid phenotype (maintaining both mesenchymal and epithelial phenotypes) might indeed possess the highest chance to complete all the steps of the metastatic cascade [24,26,27].

Besides the direct effect on tumor cells, chemotherapy also induces host-mediated pro-metastatic changes through systemic release of cytokines and chemokines, mimicking an injury-like response as typically detected in wound healing and inflammation processes [7]. Chemokine/cytokines release may in turn foster the expansion of the subset of CSCs sustaining tumor relapse as well as stimulate the generation of metastasis-receptive niches by recruiting both tumor cells and supportive stromal cells at distant sites [8,28–30].

Understanding the mechanisms through which chemotherapeutic drugs model tumor cells and the microenvironment to promote metastatic dissemination could represent an important step towards development of effective treatments for metastatic disease. At the same time identification of clinical settings in which patients may be at risk for hidden pro-metastatic effects of standard chemotherapy is a compelling research priority to devise novel strategies for more accurate therapeutic choice and personalized treatments.

Here we will summarize some of the main concepts in the field by highlighting experimental evidence on the paradoxical role of chemotherapy in metastasis in different settings:selection/generation of disseminating CSCs through both tumor-mediated (paragraph 2.1) and stromal-mediated mechanisms (2.2); mobilization and recruitment of pro-tumorigenic immune cell subsets (3); regulation of CTCs phenotype (4) and contribution to generation of pro-metastatic niches favoring extravasation (5.1) or survival and proliferation at distant sites (5.2).

2. Chemotherapy-induced selection/generation of resistant and disseminating cancer stem cells at the primary tumor site

At the primary tumor site, conventional chemotherapies can induce tumor shrinkage while simultaneously eliciting stress responses able to protect specific subsets of tumor cells from drug activity. Drug resistance could be driven by intrinsic cancer cell properties and extrinsic mechanisms mediated by signals from the tumor microenvironment (TME) protecting cancer cell from drugs insults (extrinsic resistance) [31–34]. Moreover, such stimuli can also promote EMT activation in cancer cells thereby increasing chemoresistance and cancer cell invasiveness [35].

The subset of cancer stem cells responsible for tumor initiation and maintenance has been extensively reported to play a primary role in drug resistance [15,36]. Several studies have demonstrated that targeting key pathways essential for CSC survival and/or differentiation can overcome therapeutic resistance [16,37–41]. CSCs show inherent drug-resistance properties which allow them to escape the effects of chemotherapies, including the expression of drugs efflux pumps or their quiescence [19,36]. CSCs spared by therapy may be then responsible for tumor re-growth and tumor recurrence and metastasis [37,42]. Signals from the tumor microenvironment can also indirectly mediate the chemoresistance of tumor cells by generating/expanding subsets of CSCs [43].

2.1. The origin of chemoresistant CSCs

Tumor heterogeneity and cellular plasticity have emerged as important determinants of drug resistance and cancer therapies failure [11]. The concept of Cancer Stem Cells, which posits the existence of minor subpopulations of tumor cells uniquely capable of seeding tumors, can in part explain tumor cell heterogeneity in term of phenotypic diversity and has been helpful in conceptualizing some of the observed differences in therapy response in different cellular subsets [11,12]. Chemotherapy, a pivotal treatment for solid tumors, in fact often fails to completely eradicate cancer cells and can result in the emergence of drug-resistant cells which ultimately lead to tumor regrowth and therapy failure [44]. The origin of such populations is still investigated and multiple pathways have been documented: resistant cells can already exist in the tumor bulk and be selected by drug treatments due to their intrinsic resistance or alternatively they can be generated by drugs through phenotypic transitions from more proliferative to more quiescent and resistant phenotypes [44,45]. Such conversions are triggered by epigenetic changes in treated cells often mediated by microenvironment signals that sustain and maintain the resistant phenotype [44,46]. Specific subsets of drug-tolerant cancer cells entering a state of quiescence (persister cells) can also act as a reservoir for the emergence of resistant clones often in the context of targeted therapies [45]. Interestingly, regardless their origin, resistant cells features are largely overlapping with phenotype and properties of CSCs including self-renewal ability, quiescence/ dormancy, tumor-initiating capacity and cell plasticity [15,44,46]. This highlights the relevance of a better understanding of the effect of chemotherapy on modulation of stemness phenotype.

In particular chemotherapy has also been reported to generate/expand CSCs through induction of epithelial to mesenchymal transition [15,21]. Accumulating evidence indicates that conventional therapies often fail to eradicate carcinoma cells that have entered the CSC state via activation of the EMT program, thereby permitting CSC-mediated clinical relapse [21]. Induction of EMT in tumor cells is generally accompanied by apoptotic tolerance, expression of resistance-genes, reduced proliferation, increased dissemination and stemness phenotypes leading to chemotherapy survival and increased metastatic properties [21,22,47]. Rather than a binary process EMT is now regarded as a continuum and the existence of cancer cells endowed with hybrid epithelial/mesenchymal phenotype has been documented and associated with an increased ability to acquire CSC traits, disseminate and to initiate metastasis [48–50]. Recently, Pastushenko et al have elegantly demonstrated that EMT in a genetic mouse model of skin squamous cell carcinoma proceeds through distinct intermediate states with different invasive, metastatic and cellular plasticity characteristics [51]. Cancer cells with hybrid phenotype are more prone to escape primary tumor and develop metastases. Each transient EMT state is strictly controlled by specific chromatin conformation and gene expression signatures. Finally, according to the different state, cancer cells are localized in specific microenvironmental compartments in direct contact with different stromal cells that support the maintenance of such phenotypic state highlighting once more the functional significance of specific niches [51]. This is highly relevant as the CSC state can be acquired under selective pressure of drug treatments triggered by microenvironmental stimuli that dictate acquisition of epithelial/mesenchymal hybrid state associated with stem-like features, migration and slowly proliferative properties.

In this scenario, even if a genetically-defined chemoresistant

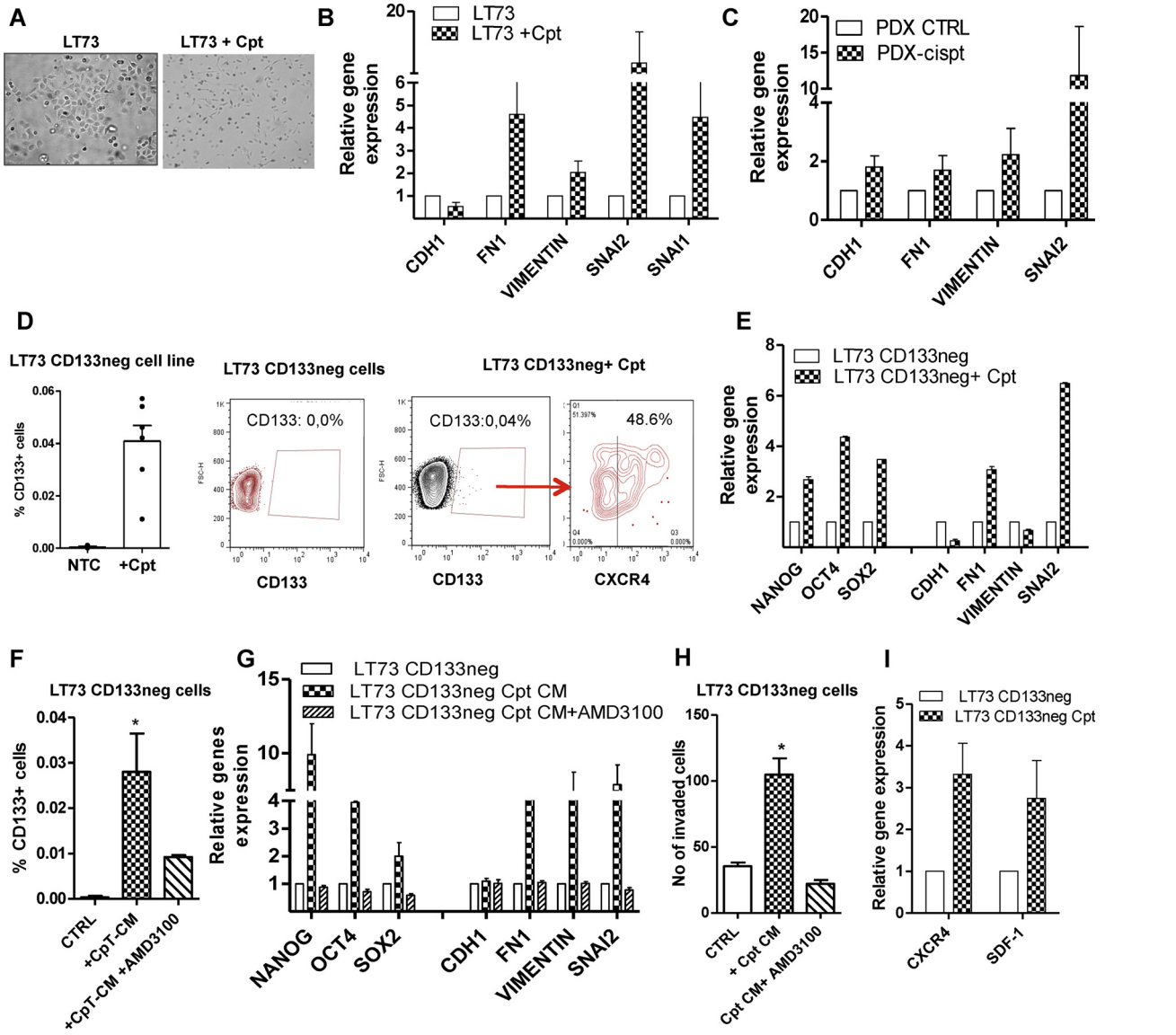

Fig. 1. Cisplatin induces de novo generation of CD133 + CSC.
A) Representative imagines of LT73 primary cell line before and after treatment with cisplatin (10 μM) showing the acquisition of spindle-like morphology. **B–C)** Real Time PCR analysis for EMT-related genes in LT73 cell line and primary cultures from PDX models (n = 4), 72 h after *in vitro* cisplatin treatment (10 μM). Untreated cells were used as calibrator. **D)** FACS evaluation of CD133 (AC133/1-PE Miltenyi) in control LT73 CD133neg cell line (completely negative for CD133 expression) and after treatment with cisplatin (10 μM) for 72 h. Representative gating strategy for identification of CD133 + cells and their relative expression of CXCR4 (CXCR4-APC, Clone 12G5 BD Bioscience) is reported on the right. **E)** Real Time PCR analysis for stemness (Nanog, OCT4/5, SOX2) and EMT-related genes in LT73 CD133neg cell line 72 h after cisplatin treatment (10 μM). Untreated cells were used as calibrator. **F)** FACS analysis for CD133 in control LT73 CD133neg cell line and after 48 h in culture with conditioned medium (CM) derived from LT73 CD133neg cells treated with cisplatin (10 μM), alone or in presence of AMD3100-CXCR4 inhibitor (10 μM). Data are the mean of n = 4 experiment * = $p < 0.05$. **G)** Real Time PCR analysis for stemness and EMT-related genes in the same cells as in F. Untreated cells were used as calibrator. **H)** Invasion assay performed on transwells coated with matrigel with control LT73 cell line and after exposure to cisplatin-CM o cisplatin-CM + AMD3100 (10 μM). After 48 h, invaded cells, chemoattracted by 10%FBS, were counted in 4 random fields of the inserts; data are the mean value ± SD. * = $p < 0.05$. **I)** Gene expression of CXCR4 and SDF-1 in LT73 CD133neg cell line72 h after cisplatin treatment relative to untreated cells.

population of CSC can already pre-exist in the tumor bulk, the concept of CSC is changing toward a more dynamic and epigenetically-determined phenotype, associated with distinct properties such as chemoresistance and enhanced ability to disseminate.

Of note the existence of intermediate hybrid epithelial/mesenchymal states associated with increased metastatic dissemination and initiation and propensity to acquire a stem-like phenotype can lead to a new understanding of tumor heterogeneity and drug resistance mechanisms to be exploited for novel anti-resistance strategies [52].

2.2. Contribution of chemotherapy–treated tumor cells in CSC modulation

Chemotherapy-induced damages foster the release of 'survival' signals by cancer cells that in turn activate a feedback loop resulting in CSC selection and/or expansion [8,30].

In breast cancer, cytokines released by tumor cells after chemotherapy can activate both Wnt/β-catenin and NF-κB pathways that amplify the secretion of further cytokines able to establish an autocrine inflammatory forward-feedback loop enriching for chemoresistant CSCs. This feedback loop can be interrupted targeting the IL8/CXCR1/2 axis resulting in tumor inhibition and prevention of generation of paclitaxel-enriched CSCs [53].

In bladder cancer, Kurtova and co-authors demonstrated that CSCs actively contribute to therapeutic resistance by promoting cell division of a quiescent pool of CSCs that ultimately re-populate residual tumors between chemotherapy cycles. This effect is driven by the release prostaglandin E_2 (PGE_2) by apoptotic cells after chemotherapy, able to paradoxically promote neighboring CSC repopulation. in vivo co-treatment with a PGE_2 inhibitor enhances chemotherapeutic response of bladder xenograft models by abrogating tumor repopulation [54].

Chemotherapy may also induce stromal and tumor release of cytokines able to trigger EMT induction and CSC formation [30]. For instance, cisplatin treatment of lung cancer cell lines and xenografts triggers an increased release of the pro-inflammatory cytokine IL-6, that contributes to chemoresistance of cancer cells by activating EMT and up-regulating anti-apoptotic proteins and DNA repair associated molecules [55].

Notably, the association between EMT induction and acquisition of CSC phenotype caused by chemotherapy was also reported in breast cancer patients. Creighton and colleagues have shown that residual breast cancer cells surviving after conventional treatments are enriched for subpopulations of cells co-expressing stemness and EMT features associated with a strong enrichment in tumor-initiating ability [56].

As introduced before recent evidence has however deepened the understanding of the EMT role in acquisition of aggressive features, identifying the functional relevance of hybrid phenotypes characterized by a partial EMT induction retaining both mesenchymal and epithelial futures [27,57]. In this respect we have shown that lung cancer cells characterized with a hybrid phenotype have the highest ability to sense TME stimuli (see below), to modulate the CSC subset and ultimately to colonize distant organs [48]. We also previously reported that cisplatin treatment is able to select and enrich for the chemoresistant fraction of CSC that also expresses the chemokine receptor CXCR4 and the drug efflux transporter ABCG2. Concomitant up-regulation of stemness genes was observed with cisplatin-induced CSC enrichment [58]. We then verified that the subset of CD133 + CXCR4 + CSCs possessed indeed increased metastatic potential in *in vivo* pre-clinical models and that their detection in primary lung cancers correlated with poor patient prognosis and metastatic recurrence. Therefore the chemoresistant subset of CD133 + CXCR4 + cells represent the fraction of metastatic lung CSCs [59]. Interestingly, despite primary tumor shrinkage, cisplatin-selection and priming of chemoresistant and metastatic CD133 + CXCR4 + cells results in increased distant metastasis formation and this paradoxical pro-metastatic effect of chemotherapy is prevented by co-treatment with a small molecule inhibitor of CXCR4 able to prevent the increase of metastatic CSC and coherently to prevent lung metastasis formation [59].

Recently we obtained new experimental evidence showing that cisplatin is able to directly trigger a partial EMT in lung cancer cell lines and primary cultures from patient derived xenografts (PDXs) as demonstrated by the acquisition of a more mesenchymal spindle-shaped morphology phenotype, up-regulation of mesenchymal genes and EMT-induced transcription factors, coupled with retention of E-cadherin expression (Fig. 1A-C). We next investigated whether the CSC increase observed after cisplatin treatment may be consequential to the expansion of intrinsically chemoresistant pre-existing CSCs [58] or induced by EMT activation able to endow non-CSCs with CSC properties. To elucidate this point, using flow cytometry we depleted CD133 + CSCs from a primary adenocarcinoma cell line (LT73), generating the LT73-CD133neg cell line that stably shows a CD133neg phenotype during in vitro culturing. This cell line was already used to demonstrate that microenvironment stimuli eliciting EMT, including signals from cancer associated fibroblasts (CAFs), are able to generate the subset of metastatic and chemoresistant CD133 + CXCR4 + CSC [48,59]. *In vitro* cisplatin treatment of LT73 CD133neg resulted in effective *de novo* generation of CD133 + cells that also express high levels of CXCR4 (Fig. 1D). This effect was associated with the up-regulation of stemness genes and induction of EMT-related genes (Fig. 1E). Therefore, we prove the ability of chemotherapy to convert non-CSC into CSC possibly through the activation of an EMT process. Culturing of LT73 CD133neg cells with conditioned medium (CM) derived from LT73 CD133NEG cells treated with cisplatin was also able to efficiently generate CD133 + CSC, to induce stemness and EMT-related genes and to provide cancer cells with increased invasive ability (Fig. 1 F–H). These results indicate that *de novo* generation of EMT-induced CSC is mediated by soluble factors released from cisplatin-treated cancer cells. Based on our previous results pointing at SDF-1, the ligand of CXCR4 receptor, as one of the crucial mediators of tumor-stroma crosstalk [59], we envisioned a possible involvement of SDF-1/CXCR4 axis in mediating the conversion of non-CSC into EMT-induced CSC. We confirmed that cisplatin treatment of LT73 CD133NEG cell line indeed induced up-regulation of both CXCR4 and SDF-1 expression (Fig. 1 I). Consistently, SDF-1/CXCR4 axis blockade in LT73 CD133NEG cell line by AMD3100 CXCR4 inhibitor strongly impaired the ability of cisplatin-CM to generate novel CSCs, modulate EMT-related genes and increase invasiveness of tumor cells, overall indicating SDF-1/CXCR4 axis as one of the central players in the non-CSC to CSC conversion triggered by chemotherapy (Fig. 1 F–H).

Further preliminary evidence demonstrates that also in colon cancer chemotherapy or chemo/radiotherapy induces mesenchymal transition and CXCR4 antagonism is able to reverse it. The new developed CXCR4 antagonist Peptide R [60,61] potentiates the efficacy of 5-Fluorouracil (5FU) or 5-Fluorouracil plus Radiotherapy (5FU-RT) in HCT116 human colon cancer cells and xenografts reducing 5FU and 5FU-RT induced EMT. 5FU and 5FU-RT induces Zeb-1, Twist and CXCR4 expression while reducing E-Cad expression. Interestingly Peptide R treatment reverses this transcriptional induction (D'Alterio C et al, manuscript in preparation).

Similar results, proving the ability of chemotherapy to induce *de novo* generation of CSCs, were also reported by Auffinger B et al in glioma cell lines. They demonstrated that expansion of CSC pool caused by temozolomide is mainly driven by the interconversion of non-CSCs into CSCs, through HIF axis targeting. This newly formed CSCs demonstrate *in vivo* an infiltrative phenotype, enhanced chemoresistance and ability to regenerate tumors following chemotherapy [62].

Overall, our data together with evidences from other groups contribute to the formal demonstration that CSC is a dynamic status that can be acquired by non-CSCs through epigenetic changes. Chemotherapy forces such transition promoting release of tumor and stromal-derived factors able to trigger EMT.

A deeper understanding of this mechanism can offer the possibility to interrupt this cross-talk and prevent CSC generation which may represent a novel combination strategy to improve chemotherapy effectiveness.

2.3. Contribution of chemotherapy-treated stromal cells to generation of disseminating CSCs

Cancer cells can also resist treatment through supportive interactions with the tumor microenvironment (TME) [43]. Different cells within the TME have profound effects on tumor cell phenotype, properties and resistance to therapy thus representing a potential attractive target to modulate responses to standard chemotherapy [63,64].

Stromal cells can in fact also respond to chemotherapy insults by releasing protective factors that stimulate epithelial–mesenchymal transition and generation of CSCs. This may be the result of a direct drug activity on stromal cells or follow more complex mechanisms mediated by interactions within the TME or even following death of drug-sensitive cancer cells [64]. Under selective pressure from chemotherapy, the dynamic interplay between tumor cells and the surrounding stroma is constantly evolving [65]. Among the different cell types in the TME, key roles in mediating response to therapy have been described in particular for fibroblasts and macrophages.

In prostate cancer, it has been demonstrated that stromal fibroblasts

secrete Wnt16b ligand in response to chemotherapy. In turn Wnt pathway activation in tumor cells promotes the acquisition of mesenchymal and stem-like characteristics that can influence the migratory and invasive behavior of cancer cells overall resulting in attenuation of cytotoxic effects of therapy [66]. Growth factors such as IGF-II, HGF and SDF-1 released by cancer associated fibroblast (CAFs) can also promote the conversion of lung primary non-CSC cells into CSCs through the paracrine activation of EMT and WNT, Notch and Hedgehog signaling. Thus CAFs also promote tumor cells drug chemoresistance and tumor dissemination by microenvironment-generated CSC subset [67]. Moreover, non-small cell lung cancer (NSCLC) CAFs were demonstrated to contribute to EMT induction and chemoresistance through a paracrine loop based on IL-6. Treatment of lung cancer cells with cisplatin induced secretion of TGF-β leading to activation of fibroblasts. Activated fibroblast increased IL-6 production that in turn can activate EMT in cancer cells and increase resistance to chemotherapy [68].

We also demonstrated that CAFs isolated from primary lung cancers can promote the acquisition of CSC phenotype through the induction of EMT, driven by different pathways including TGFβ, IGFII and SDF-1 [59]. Interestingly the proclivity to CSC-compartment modulation appears to be controlled by the balance between epithelial and mesenchymal features with 'partial EMT cells' being more susceptible to TME mediated conversion from non-CSC to CSC [48].

A direct proof of the pro-metastatic effects of chemo-treated CAFs has been provided by Kanzaki R et al showing that up regulation of Gas6 ligand release by CAFs during chemotherapy increases tumor cells migration by activating receptor tyrosine kinase Axl. In NSCLC primary tumors, stromal Gas6 expression increases after chemotherapy. Moreover the detection of Axl and stromal Gas6 positive cells is correlated with worse disease-free survival rates, suggesting that CAFs promote migration of Axl-expressing lung cancer cells during chemotherapy fostering distant metastasis and resulting in unfavorable patient outcomes [69]. Similarly, in pancreatic cancer, chemotherapy treated CAFs are able to increase tumor cell viability, migration, and invasion and to foster tumor growth when co-injected with tumor cells. Factors released by treated CAFs are similar to the previously described senescence-associated secretory phenotype (SASP). Inhibition of stress-associated MAPK signaling (P38 MAPK or JNK) are able to attenuate SASP induction in chemotherapy-treated CAFs and consequently to reduce their support to tumor cell growth and invasiveness [70].

Recent evidence also indicates that CAFs can contribute to tumor progression, modulation of CSC phenotype and chemoresistance by secreting extracellular vesicles, including exosomes. In pancreatic ductal adenocarcinoma (PDAC), CAFs secreted exosomes can promote cancer cell proliferation and chemoresistance by increasing the expression of Snail [71]. Interestingly, gemcitabine treatment causes a significant increase in exosome release from CAFs that in turn exacerbate chemoresistance of recipient tumor cells mediated by Snail overexpression [71].

Among the different factors mediating tumor-stromal interactions, miRNAs, small non-coding RNAs loaded into exosomes, can be functionally delivered to recipient cells where they can regulate expression of genes involved in acquisition of stemness phenotype, chemoresistance and metastatic behavior [72,73].

In colorectal carcinoma (CRC), CAFs transfer exosomal miR-92a-3p to tumor cells inducing EMT, resistance to apoptosis and acquisition of stemness properties through the targeting of FBXW7 and MOAP1, inhibitors of Wnt/β-catenin pathway. Thus, CAFs exosomal miR-92a-3p promotes metastatic properties and chemotherapy resistance of CRC cells. Notably, detection of exosomal miR-92a-3p in CRC patient plasma predicts metastasis occurrence and fluorouracil (5-FU)/oxaliplatinum (L-OHP) chemotherapy resistance [74].

Tumor associated macrophages (TAMs) are also well known to support cancer cell survival and progression. TAMs may contribute to chemoresistance by releasing chemoprotective factors and promoting invasion and metastasis [34,75–77].

In PDAC, $CD163^+$ TAMs and myofibroblasts directly support chemoresistance of pancreatic cancer cells by secreting insulin-like growth factors (IGF) 1 and 2 that activate the insulin/IGF1 receptor survival signaling pathway in pancreatic cancer cells, enhancing their chemoresistance. *In vivo* blockade of IGF can sensitize pancreatic tumors to gemcitabine suggesting that combination with IGF inhibition might potentiate chemotherapy effectiveness in PDAC patients [78].

Hypoxic epithelial ovarian cancers (EOC) are also able to recruit macrophages at primary site and to induce their conversion into TAMs. In turn, hypoxic TAMs release exosome enriched in miR-223 that directly targets PTEN, enhancing EOC chemoresistance and metastatic properties. In the clinical setting, high levels of circulating exosomal miR-223 in EOC patients are closely related to tumor recurrence [79].

Also in myeloma chemotherapy considerably stimulates secretion of exosomes and modifies exosome composition. Tumor 'chemoexosomes' contain high levels of heparanase that mediates ECM degradation, thus facilitating tumor cells dissemination. Moreover 'chemoexosomes' promote macrophage recruitment and enhance their secretion of TNF-α, an important myeloma growth factor [80].

Notably, TAMs provide pivotal signals that ensure CSC survival, maintenance and migratory ability enhancing tumor progression, chemoresistance and metastasis [81].

In pancreatic tumors, TAMs can support CSC maintenance by activating the transcription factor STAT3. Targeting TAMs or inflammatory monocytes through the inhibition of myeloid cell receptors colony-stimulating factor-1 receptor (CSF1R) or chemokine receptor 2 (CCR2) decreases the number of CSCs and also overcomes macrophage-induced $CD8^+$ cytotoxic T lymphocytes suppression, overall resulting in increased efficacy of chemotherapy and prevention of metastasis [82].

De Nardo et al reported that breast tumors with high TAM counts and low numbers of cytotoxic T cells poorly respond to neo-adjuvant chemotherapy. They demonstrated in MMTV-PyMT transgenic mouse model of breast cancer that this effect is mediated by tumor release after chemotherapy treatment of CSF1, a potent chemokine acting as an attractant for macrophages. TAMs can limit responses to therapy, at least in part, through the suppression of the antitumor functions of cytotoxic T cells [83].

Another elegant proof of concept of the pro-survival and metastatic effect resulting from tumor-stroma cross-talk under chemotherapy stress was reported by Acharyya and co-workers. They showed that chemotherapeutic drugs (doxorubicin, cyclophosphamide and paclitaxel) induce the release of TNF-α from stromal cells boosting the expression of the pro-metastatic cytokine CXCL1 by treated breast cancer cells. CXCL1 release is able to attract myeloid cells into the tumors. Myeloid cells infiltrating tumors can release several chemokines favoring cancer cell survival and fostering their dissemination. Finally, in this context, CXCL1/CXCR2 blockade was shown to increase the efficacy of chemotherapy against breast tumors and particularly against metastasis [84].

3. Chemotherapy-induced recruitment of immune cells favoring primary tumor dissemination

At primary tumor site, chemotherapy may both favor CSC selection/generation, supportive of chemoresistance and tumor relapse, and generate a microenvironment promoting neo-angiogenesis, tumor regrowth and tumor cell intravasation.

In both mouse and human breast cancer models, chemotherapy can determine the increase of a specific subset of tumor associated macrophages ($CD206 + TIE2^{High}$ $CXCR4^{high}$) that are preferentially detected around blood vessels. This TAM subset was demonstrated to possess pro-angiogenic activity and to be implicated in tumor progression [85,86]. $CD206 + TIE2^{High}$ $CXCR4^{high}$ TAM subset appears particularly prone to foster tumor revascularization and tumor relapse after chemotherapy. The suggested mechanism implies chemotherapy-induced

up-regulation of CXCL12, (the ligand for CXCR4) at perivascular sites followed by recruitment of CD206 + TIE2^{High} CXCR4^{high} TAMs expressing VEGFA, supporting the revascularization process. Consistently, pharmacologic blockade of TAMs by CXCR4 inhibition selectively impairs tumor revascularization and re-growth after chemotherapy enhancing chemotherapy effectiveness [87].

Another report in breast cancer models highlighted the primary role of vascular permeability and innate immune cell infiltration in mediating drug response. An association between vascular leakage and decreased response to doxorubicin was observed. Doxorubicin treatment of breast tumors *in vivo* induces necrotic cancer cell death that increases CCL2-dependent tumor infiltration by CCR2+ inflammatory monocytes/TAMs. This subset of bone marrow-derived myeloid cells recruited at primary tumor site, expresses high level of metalloproteinase (MMP)-9 that increases vascular permeability by diminishing pericyte-coverage of the vasculature and increasing phosphorylation of VE-cadherin (vascular endothelial cadherin) thus affecting endothelial cell-cell adhesion. Vascular leakage limits drug delivery that in turn is associated with decreased therapy response, tumor cell escape and recurrence [88].

The intravasation process, that allows tumor cells to escape from the primary tumor and enter the circulation, has been demonstrated to occur at micro-anatomical structures within the tumors, named tumor microenvironment of metastasis (TMEM), consisting of direct physical contact of a tumor cell expressing the invasive isoforms of the actin-regulatory protein Mammalian-enabled (MENA), a perivascular macrophage and an endothelial cell [89]. The presence of TMEM has been associated with metastasis in both murine mammary tumors and human breast cancer [90]. *In vivo* treatment with paclitaxel of both mouse breast cancer models and human breast PDXs has been shown to increase TMEM assembly by promoting the mobilization and accumulation of myeloid derived TIE2^{hi}/VEGF^{hi} macrophages at perivascular regions. Chemotherapy also enhanced localized areas of transient vascular permeability specifically associated with TMEM sites that facilitate cancer cell intravasation. Finally, TAMs associated to TMEM are able to promote the up-regulation of Mena expression in tumor cells, through NOTCH pathway activation, thus promoting intravasation and tumor cells dissemination. Overall chemotherapy induced increase in density and activity of TMEM sites, ultimately enhancing the number of circulating tumor cells and promoting distant metastasis formation. These effects can be reversed by either blocking TIE2 receptor or knockdown of the *MENA* gene preventing TMEM assembly and tumor escape [91]. Interestingly, the recruitment of Tie2+ TAMs associated with TMEM in pre-malignant lesions, through a tumor-derived CCL2 gradient, has also been demonstrated to act as gateway for tumor cells intravasation at very early time points in tumor progression. This might indicate a primary role for macrophages in early dissemination that affects long-term metastasis development [92].

In preclinical models of mouse and human breast cancer it has been shown that acquired resistance to paclitaxel can be mediated by activation of the Toll-like receptor TLR4 in cancer cells. TLR4 activation by paclitaxel in cancer cells up regulates a plethora of inflammatory mediators able to recruit myeloid cells that in turn promote angiogenesis, lymphangiogenesis and metastasis. Paclitaxel-mediated activation of TLR4-positive tumors induced *de novo* generation of intratumoral lymphatic vessels, highly permissive for tumor invasion by malignant cells. These findings indicate that TLR4 targeting in combination with chemotherapy may significantly improve therapy effectiveness by simultaneously preventing local and systemic inflammatory [93].

4. Mobilization and modulation of CTC heterogeneity induced by chemotherapy

Circulating tumor cells (CTCs) are ultimately the seeds of metastasis and may also represent an attractive source of biomarkers to dissect tumor heterogeneity and monitor tumor response to therapy [94–96]. Accumulating evidence has provided proof for great heterogeneity within CTCs across patients and even within the same individual [95,97]. The existence of a partial EMT cell state, showing hybrid phenotype with both epithelial and mesenchymal features, has been postulated to provide tumor cells with the highest ability to colonize distant organs [27]. This plastic phenotype is also more prone to respond to microenvironmental cues and acquire stem-like features thus increasing metastatic ability [98]. Indeed within the heterogeneous CTC population it has been demonstrated through *in vivo* functional studies that only the subset of CTCs endowed with mesenchymal traits and stemness features were able generate metastases after xenotransplantation in immunodeficient mice [99–101]. Therapeutic pressure may prime cells at the primary tumor to acquire an hybrid phenotype conductive to high disseminating and seeding properties thereby generating 'chemoresistant' and highly metastatic CTCs [49,49].

In breast cancer patients, hybrid CTCs were identified through the expression of pooled epithelial (E) or mesenchymal (M) markers by the use of dual-colorimetric RNA–in situ hybridization (ISH) assay. Monitoring of CTCs during treatment revealed a reversible shift between epithelial and mesenchymal features associated to response to therapy and disease progression. The patients who experienced progressive disease during therapy showed an increased number of M^+ CTCs in the post-treatment sample. This suggests an impact of chemotherapy on CTC phenotypic switching and prove that CTCs which have undergone EMT during treatment correlate with resistance to chemotherapy more accurately that the absolute CTC count [102]. In this prospect, CTCs can be considered as a mirror to evaluate chemotherapy shaping of primary tumors to be exploited as a biomarker to assess therapy effectiveness in real-time.

In breast cancer clinical setting, the detection of a fraction of mesenchymal and stem-like cells has been observed and correlated with increased tumor incidence and poor response to chemotherapy. A study performed in 130 breast cancer patients evaluated the incidence and the prognostic significance of CTCs characterized for CSC marker ALDH1 and partial EMT features (evaluated by co-expression of cytokeratins and nuclear TWIST1, a master EMT-inducing transcription factor) during first-line chemotherapy treatment. The authors found that detection of CSC/partial-EMT CTCs is correlated to lung metastases and decreased progression-free survival and represents an independent predictive factor for increased risk of relapse. Notably, chemotherapy resulted in a significant increase in the incidence of CSC/partial-EMT CTCs in contrast to the other CTC subsets (CSC/epithelial-like, non-CSC/epithelial-like or non-CSC/partial-EMT CTCs) which were reduced post-chemotherapy. These findings suggest that CSC/partial-EMT CTCs are resistant to conventional chemotherapy and coherently the enrichment of this CTC subset is associated with lack of long term efficacy [103]. Another study in 27 breast cancer patients undergoing neoadjuvant chemotherapy confirmed a significant change in the fraction of CTCs endowed with stem like phenotype (CD44 + CD24-) and EMT properties (indicated by the low expression of EpCAM and high expression of N-Cadherin) after the 3rd cycle therapy [104].

Similarly, in NSCLC patients receiving platinum based treatment (n = 43), CTCs were analyzed by multi-immunofluorescence to assess phenotypic changes in epithelial (pan-CK), mesenchymal (N-cadherin) and stem cell-like (CD133) composition. The authors observed that the presence of N-cadherin + mesenchymal CTCs and an increased ratio of CD133 + CSC vs pan-CK epithelial cells were associated to poor treatment response and shorter progression free survival, thus confirming the association between EMT-like CSCs, drug resistance and cancer metastasis [105].

Overall, CTCs detected after chemotherapy confirmed an enrichment in the fraction of chemoresistant, tumor initiating cells showing EMT/partial EMT status that might be predictive of worst clinical outcomes. Modifications induced by chemotherapy in tumor cells (i.e.

selection of chemoresistant CSCs, induction of EMT, increased intravasation process) can be monitored using CTC-based biomarkers to predict therapy effectiveness and potentially avoid unnecessary and toxic treatments [106].

5. Chemotherapy-induced generation of the metastatic niche

In parallel with the shrinkage of the primary tumor, chemotherapy can favor the dissemination of cancer cells (the seeds) from primary tumors and promote a favorable microenvironment generation (the soil) to receive cancer cells, thereby explaining the paradoxical pro-metastatic effect of chemotherapy also through generation of metastatic niches. Microenvironment 'educated' by chemotherapy plays therefore a pivotal role in dictating all the steps of the metastatic cascade, from facilitating primary cancer cell intravasation into the circulation, to extravasation to secondary metastatic sites and fostering metastatic growth [76,107,108].

5.1. Chemotherapy-induced pro-metastatic niche favoring tumor cells extravasation

Different stromal cell types have been described to guide and affect metastatic properties of tumor cells [108]. However, in the context of chemotherapy, several reports indicated a preferential activation of the bone-marrow derived population of CCR2+ inflammatory monocytes (IM) that are mobilized and recruited towards tissue injury caused by chemotherapy. Within primary tumors or at metastatic sites CCR2+ monocytes can differentiate into the subsets of tumor or metastasis associated macrophages (TAMs or MAMs) [109]. Interestingly, different reports demonstrated that the recruitment of CCR2 + IM to metastatic sites during chemotherapy is dependent on CCL2 ligand over-expression by both tumor and stromal cells in response to chemotherapy. The interaction of IM with cancer cells at metastatic sites favors the extravasation process mediated by the release of monocyte-derived vascular endothelial growth factor (VEGFA) and promotes metastasis growth. As a result, inhibition of CCL2-CCR2 signaling can block the recruitment of inflammatory monocytes and prevent metastasis formation in *in vivo* models of murine breast cancer [110,111].

Additional recent work also underlined the primary role of IM in fostering metastasis, uncovering a novel mechanism for their recruitment to the metastatic site mediated by chemotherapy-elicited tumor-derived extracellular vesicles (EV). In a mouse model of breast cancer, neoadjuvant treatment with taxanes and anthracyclines promoted tumor-derived extracellular vesicles (EV) that show pro-metastatic capacity. Indeed chemotherapy-elicited EVs contain high amount of annexin A6 (ANXA6), a Ca2+-binding membrane-associated protein, able to promote NF-κB-dependent endothelial cell activation. Activated endothelial cells increased CCL2 expression and consequent recruitment of $CCR2^{+}$ inflammatory monocytes at the pulmonary pre-metastatic niche thereby promoting lung metastasis establishment [112]. The authors confirmed these findings in the clinical setting, demonstrating that neo-adjuvant chemotherapy augments ANXA6 levels in the circulating EVs of patients with breast cancer [112].

Chemotherapy has been proved to have a profound impact in the regulation of vascular permeability and angiogenesis that may strongly influence tumor response and metastasis progression. Liu et al demonstrated in *in vivo* pre-clinical models of different tumor types that a single dose of paclitaxel and carboplatin was sufficient to enhance lung metastasis in tumor-bearing mice. Host response to chemotherapy induced an increased release of cytokines and angiogenic factors, among which CXCR2, CXCR4, S1P/S1PR1, PlGF and PDGF-BB that were able to promote angiogenesis, and augment vascular permeability in turn favoring early retention of tumor cells in the lungs. Moreover, chemotherapy-induced cytokines also have a direct effect on tumor cells, by promoting their viability, migration and induction of EMT which led to increased metastasis formation. *In vivo* inhibition of these factors, especially the SDF-1/CXCR4 or S1P/S1PR1 axes, was able to prevent chemotherapy-enhanced metastasis in tumor-bearing mice. Notably, the repertoire of chemotherapy-induced cytokines and angiogenic factors indentified in pre-clinical models was also confirmed in gene expression repositories from human patients following chemotherapy treatment [113].

The importance of chemotherapy-induced modifications in vascular permeability/function in the establishment of metastasis was also underlined in a study proving that cisplatin and paclitaxel significantly augmented lung metastasis by enhancing adhesion of tumor cells to endothelial cells. Chemotherapy was shown to enhance early retention of tumor cells in the lungs by up-regulating endothelial cells expression of VEGFR-1. Therefore, treatment with an antibody targeting VEGFR-1 reversed the early retention of tumor cells in the lungs and prevented the formation of chemotherapy-induced pulmonary metastases [28,29].

Overall these evidence clearly demonstrate that host response to chemotherapy has a profound impact on metastasis formation mainly exerted by increased vascular permeability/density and recruitment to the metastatic site of bone marrow derived myeloid cells, in particular of inflammatory monocyte subset that favors tumor cells extravasation.

5.2. Stromal contribution to metastatic cell survival and proliferation during chemotherapy

Interaction with chemotherapy-induced reactive stromal cells can also favor disseminated tumor cells (DTCs) extravasation at distant tissues [106]. It has been recognized that a localized anatomical region, named perivascular niche (PVN), can protect DTCs from chemotherapy allowing their persistence and possibly reactivation. In particular PVNs can specifically support the survival of disseminated tumor cells with stem-like features and quiescent phenotype [114–116]. Reactivation of quiescent DTCs able to generate overt metastasis years after complete primary tumor removal followed by chemotherapy is a very relevant clinical issue which still needs to be fully addressed [117,118]. DTC niches can support the maintenance of tumor initiating properties and quiescent state fostering the ability of DTCs to resist standard chemotherapy targeting dividing cells [37,119].

Naumov et al., showed that doxorubicin treatment was able to effectively reduce the metastatic tumor burden but it was ineffective in reducing the number of disseminated dormant cells responsible for late metastasis formation [120]. However, quiescence might not be the only mechanism responsible for DTCs chemoresistance. Recently, Carlson et al demonstrated in an experimental model of breast cancer that chemotherapy was in fact able to substantially reduce DTCs number but the population of DTCs localized within bone-marrow (BM) vasculature was instead doubled, showing that chemoresistant DTCs were protected from therapy by endothelial cells at the perivascular niche. Inhibiting integrin-mediated interactions between DTCs and PVN, sensitized DTCs to chemotherapy without inducing DTC proliferation or exacerbating chemotherapy-associated toxicities, ultimately resulting in bone metastasis prevention [116].

Remarkably, the phenotype and fate of DTCs is also closely dependent on primary tumor microenvironment [121]. Interestingly, hypoxic microenvironments have been shown to control heterogeneity of disseminated tumor cells in head & neck and breast cancer through an NR2F1-mediated mechanism that generates dormant DTCs able to evade chemotherapy. This evidence supports the hypothesis that priming at the primary tumor site might be a crucial determinant of metastatic fitness [122].

Notably, the existence of a chemo-resistant niche that dictates therapeutic response has also been demonstrated in hematopoietic malignancies. In a mouse model of Burkitt's lymphoma, chemotherapy caused the release of IL-6 and Timp-1 by thymus in response to DNA damage creating a protective and chemo-resistant niche supportive for survival of minimal residual disease responsible for tumor relapse [123].

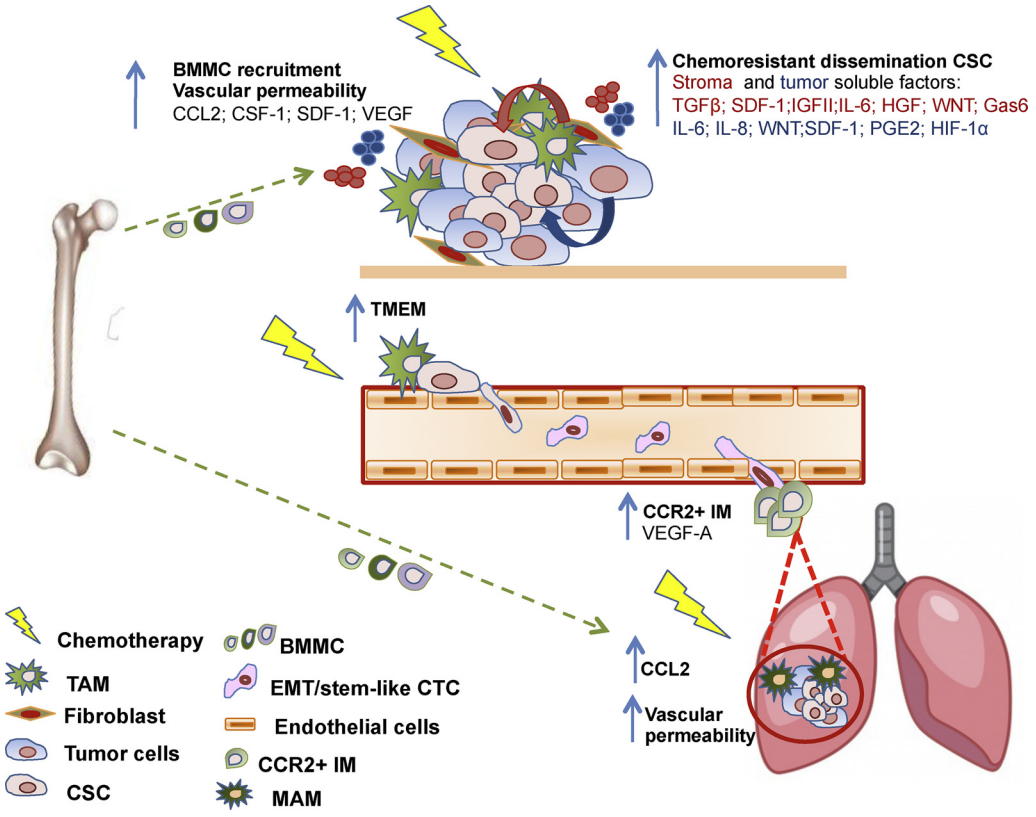

Fig. 2. Mechanisms mediating pro-metastatic effects of chemotherapy at different tumor districts.
At primary tumor chemotherapy damages cause the release of tumor and stroma soluble factors able to trigger the expansion/generation of the pool of chemoresistant CSC or to induced EMT process enabling cells with CSC and motility properties. Chemotherapy-induced release of cytokines/chemokines from treated tumor/stroma cells able to recruit bone marrow derived myeloid cells (BMMC) at tumor site that can support tumor cells survival and angiogenesis. Chemotherapy also increases vascular permeability at primary site and enhances the formation of tumor microenvironment of metastasis (TMEM) (comprising of tumor associated macrophages (TAM), tumor cells and endothelial cell) that favours tumor cells intravasation.
Chemotherapy can select for CTCs enriched in CSC and hybrid phenotype that are more prone to survive in circulation, seed distant organs and initiate distant metastasis.
At distant site, chemotherapy can trigger the release of stroma and tumor soluble factors, among which CCL2, able to recruit CCR2+ inflammatory monocyte (IM). IM can foster CTC extravasation, mediate by VEGF-A along with increased chemotherapy-induced vascular permeability. IM at the metastasis site can differentiate into metastasis associated macrophages (MAM) that promote metastatic cells growth.

Thus, conventional chemotherapies, despite inducing tumor regression, can simultaneously promote in selected anatomical locations the creation of chemo-resistant niches able to protect tumor cells competent for tumor recurrence.

6. Concluding remarks

In several tumor types, chemotherapy has so far demonstrated an overall unsatisfactory potential to counteract and control metastatic dissemination even when a partial or complete response has been achieved at the primary tumor site [4]. This clinical issue could be in part related to host responses to chemotherapy activating reactive and reparatory mechanisms able to foster generation of CSCs, primary tumor cells escape and distant site colonization [7] (Fig. 2).

CTCs isolated in patient's blood represent a great window of opportunity to assess the changes caused by chemotherapy in primary tumors/metastasis without the need of serial biopsies [95]. Liquid biopsies can offer the unique possibility to monitor in real time the selection/*de novo* generation of more aggressive mesenchymal/stem-like population possibly induced by chemotherapy, in association with chemotherapy-induced systemic release of inflammatory cytokines promoting the recruitment and/or activation of pro-metastatic stromal cells (Fig. 2). The microenvironment plays a fundamental role in tumor promotion and strongly affects all different steps of metastasis formation as well as chemoresistance [55–57] (Fig. 2). Reactive changes in stromal cells after chemotherapy might therefore represent another aspect of the paradoxical metastasis promoting activity of chemotherapy.

A more comprehensive analysis of tumor stromal cross-talk, a deeper understanding of the mechanisms by which chemotherapy can select and/or generate the subset of persister and metastatic CSCs and the identification of different chemotherapy-activated microenvironment mediators of pro-metastatic effects may result in novel therapeutic targets to increase chemotherapy efficacy.

According to individual host response, the possibility to select those patients that may have a higher risk to develop chemotherapy-induced metastasis could be clinically relevant to improve the concept of a tailored-made therapeutic strategy, avoiding ineffective and potentially toxic treatments for the patients and proposing more beneficial therapeutic strategies.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest

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References

[1] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2019, CA Cancer J. Clin. 69 (1) (2019) 7–34.
[2] F.P. Madorsky Rowdo, A. Baron, M. Urrutia, J. Mordoh, Immunotherapy in cancer: a combat between tumors and the immune system; you win some, you lose some, Front. Immunol. 6 (2015) 127.
[3] L.E. Giroux, C. Dumenil, C. Julie, V. Giraud, J. Dumoulin, S. Labrune, T. Chinet, Immunotherapy revolutionises non-small-cell lung cancer therapy: results, perspectives and new challenges, Eur. J. Cancer 78 (2017) 16–23.
[4] A. Inno, R.G. Lo, M. Salgarello, G. Corrao, R. Casolino, G. Galli, A. Modena, L. Romano, S. Pusceddu, F.G. Greco, M.C. Garassino, S. Gori, The evolving landscape of criteria for evaluating tumor response in the era of cancer immunotherapy: from Karnofsky to iRECIST, Tumori 104 (2) (2018) 88–95.

[5] K.D. Miller, R.L. Siegel, C.C. Lin, A.B. Mariotto, J.L. Kramer, J.H. Rowland, K.D. Stein, R. Alteri, A. Jemal, Cancer treatment and survivorship statistics, 2016, CA Cancer J. Clin. 66 (4) (2016) 271–289.

[6] M.S. Chatwal, T. Tanvetyanon, Combination chemotherapy and immunotherapy in metastatic non-small cell lung cancer: a setback for personalized medicine? Transl. Lung Cancer Res. 7 (Suppl 3) (2018) S208–S210.

[7] J.A. Wargo, A. Reuben, Z.A. Cooper, K.S. Oh, R.J. Sullivan, Immune effects of chemotherapy, radiation, and targeted therapy and opportunities for combination with immunotherapy, Semin. Oncol. 42 (4) (2015) 601–616.

[8] G.S. Karagiannis, J.S. Condeelis, M.H. Oktay, Chemotherapy-induced metastasis: mechanisms and translational opportunities, Clin. Exp. Metastasis 35 (4) (2018) 269–284.

[9] O.A. Martin, R.L. Anderson, K. Narayan, M.P. MacManus, Does the mobilization of circulating tumour cells during cancer therapy cause metastasis? Nat. Rev. Clin. Oncol. 14 (1) (2017) 32–44.

[10] R.A. Burrell, C. Swanton, Tumour heterogeneity and the evolution of polyclonal drug resistance, Mol. Oncol. 8 (6) (2014) 1095–1111.

[11] H. Easwaran, H.C. Tsai, S.B. Baylin, Cancer epigenetics: tumor heterogeneity, plasticity of stem-like states, and drug resistance, Mol. Cell 54 (5) (2014) 716–727.

[12] J.A. Magee, E. Piskounova, S.J. Morrison, Cancer stem cells: impact, heterogeneity, and uncertainty, Cancer Cell 21 (3) (2012) 283–296.

[13] J.P. Sullivan, J.D. Minna, J.W. Shay, Evidence for self-renewing lung cancer stem cells and their implications in tumor initiation, progression, and targeted therapy, Cancer Metastasis Rev. 29 (1) (2010) 61–72.

[14] J.E. Visvader, G.J. Lindeman, Cancer stem cells: current status and evolving complexities, Cell Stem Cell 10 (6) (2012) 717–728.

[15] T.B. Steinbichler, J. Dudas, S. Skvortsov, U. Ganswindt, H. Riechelmann, I.I. Skvortsova, Therapy resistance mediated by cancer stem cells, Semin. Cancer Biol. 53 (2018) 156–167.

[16] V. Adorno-Cruz, G. Kibria, X. Liu, M. Doherty, D.J. Junk, D. Guan, C. Hubert, M. Venere, E. Mulkearns-Hubert, M. Sinyuk, A. Alvarado, A.I. Caplan, J. Rich, S.L. Gerson, J. Lathia, H. Liu, Cancer stem cells: targeting the roots of cancer, seeds of metastasis, and sources of therapy resistance, Cancer Res. 75 (6) (2015) 924–929.

[17] Y. An, W.M. Ongkeko, ABCG2: the key to chemoresistance in cancer stem cells? Expert Opin. Drug Metab. Toxicol. 5 (12) (2009) 1529–1542.

[18] R. Januchowski, K. Wojtowicz, M. Zabel, The role of aldehyde dehydrogenase (ALDH) in cancer drug resistance, Biomed. Pharmacother. 67 (7) (2013) 669–680.

[19] W. Chen, J. Dong, J. Haiech, M.C. Kilhoffer, M. Zeniou, Cancer stem cell quiescence and plasticity as major challenges in cancer therapy, Stem Cells Int. 2016 (2016) 1740936.

[20] A. Singh, J. Settleman, EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer, Oncogene 29 (34) (2010) 4741–4751.

[21] T. Shibue, R.A. Weinberg, EMT, CSCs, and drug resistance: the mechanistic link and clinical implications, Nat. Rev. Clin. Oncol. 14 (10) (2017) 611–629.

[22] L. Ombrato, I. Malanchi, The EMT universe: space between cancer cell dissemination and metastasis initiation, Crit. Rev. Oncog. 19 (5) (2014) 349–361.

[23] S. Lamouille, J. Xu, R. Derynck, Molecular mechanisms of epithelial-mesenchymal transition, Nat. Rev. Mol. Cell Biol. 15 (3) (2014) 178–196.

[24] T. Brabletz, R. Kalluri, M.A. Nieto, R.A. Weinberg, EMT in cancer, Nat. Rev. Cancer 18 (2) (2018) 128–134.

[25] X. Zheng, J.L. Carstens, J. Kim, M. Scheible, J. Kaye, H. Sugimoto, C.C. Wu, V.S. LeBleu, R. Kalluri, Epithelial-to-mesenchymal transition is dispensable for metastasis but induces chemoresistance in pancreatic cancer, Nature 527 (7579) (2015) 525–530.

[26] M.K. Jolly, K.E. Ware, S. Gilja, J.A. Somarelli, H. Levine, EMT and MET: necessary or permissive for metastasis? Mol. Oncol. 11 (7) (2017) 755–769.

[27] M.K. Jolly, M. Boareto, B. Huang, D. Jia, M. Lu, E. Ben-Jacob, J.N. Onuchic, H. Levine, Implications of the hybrid Epithelial/Mesenchymal phenotype in metastasis, Front. Oncol. 5 (2015) 155.

[28] L.G. Daenen, J.M. Houthuijzen, G.A. Cirkel, J.M. Roodhart, Y. Shaked, E.E. Voest, Treatment-induced host-mediated mechanisms reducing the efficacy of antitumor therapies, Oncogene 33 (11) (2014) 1341–1347.

[29] L.G. Daenen, J.M. Roodhart, M. van Amersfoort, M. Dehnad, W. Roessingh, L.H. Ulfman, P.W. Derksen, E.E. Voest, Chemotherapy enhances metastasis formation via VEGFR-1-expressing endothelial cells, Cancer Res. 71 (22) (2011) 6976–6985.

[30] D. Vyas, G. Laput, A.K. Vyas, Chemotherapy-enhanced inflammation may lead to the failure of therapy and metastasis, Onco. Ther. 7 (2014) 1015–1023.

[31] G. Bertolini, L. Gatti, L. Roz, The "stem" of chemoresistance, Cell Cycle 9 (4) (2010) 628–629.

[32] C. Holohan, S.S. Van, D.B. Longley, P.G. Johnston, Cancer drug resistance: an evolving paradigm, Nat. Rev. Cancer 13 (10) (2013) 714–726.

[33] K.H. Paraiso, K.S. Smalley, Fibroblast-mediated drug resistance in cancer, Biochem. Pharmacol. 85 (8) (2013) 1033–1041.

[34] L.R. Sanchez, L. Borriello, D. Entenberg, J.S. Condeelis, M.H. Oktay, G.S. Karagiannis, The emerging roles of macrophages in cancer metastasis and response to chemotherapy, J. Leukoc. Biol. (2019).

[35] A.C. Obenauf, Y. Zou, A.L. Ji, S. Vanharanta, W. Shu, H. Shi, X. Kong, M.C. Bosenberg, T. Wiesner, N. Rosen, R.S. Lo, J. Massague, Therapy-induced tumour secretomes promote resistance and tumour progression, Nature 520 (7547) (2015) 368–372.

[36] C. DI, Y. Zhao, Multiple drug resistance due to resistance to stem cells and stem cell treatment progress in cancer (Review), Exp. Ther. Med. 9 (2) (2015) 289–293.

[37] T. Oskarsson, E. Batlle, J. Massague, Metastatic stem cells: sources, niches, and vital pathways, Cell Stem Cell 14 (3) (2014) 306–321.

[38] S.M. McAuliffe, S.L. Morgan, G.A. Wyant, L.T. Tran, K.W. Muto, Y.S. Chen, K.T. Chin, J.C. Partridge, B.B. Poole, K.H. Cheng, J. Daggett Jr, K. Cullen, E. Kantoff, K. Hasselbatt, J. Berkowitz, M.G. Muto, R.S. Berkowitz, J.C. Aster, U.A. Matulonis, D.M. Dinulescu, Targeting Notch, a key pathway for ovarian cancer stem cells, sensitizes tumors to platinum therapy, Proc Natl Acad Sci U S A 109 (43) (2012) E2939–E2948.

[39] M.D. Friedman, D.S. Jeevan, M. Tobias, R. Murali, M. Jhanwar-Uniyal, Targeting cancer stem cells in glioblastoma multiforme using mTOR inhibitors and the differentiating agent all-trans retinoic acid, Oncol. Rep. 30 (4) (2013) 1645–1650.

[40] M. Moro, G. Bertolini, U. Pastorino, L. Roz, G. Sozzi, Combination treatment with all-trans retinoic acid prevents cisplatin-induced enrichment of CD133+ tumor-initiating cells and reveals heterogeneity of Cancer stem cell compartment in lung Cancer, J. Thorac. Oncol. 10 (7) (2015) 1027–1036.

[41] M.T. Shekhani, A.S. Jayanthy, N. Maddodi, V. Setaluri, Cancer stem cells and tumor transdifferentiation: implications for novel therapeutic strategies, Am. J. Stem Cells 2 (1) (2013) 52–61.

[42] Y. Li, H.A. Rogoff, S. Keates, Y. Gao, S. Murikipudi, K. Mikule, D. Leggett, W. Li, A.B. Pardee, C.J. Li, Suppression of cancer relapse and metastasis by inhibiting cancer stemness, Proc Natl Acad Sci U S A 112 (6) (2015) 1839–1844.

[43] V. Plaks, N. Kong, Z. Werb, The cancer stem cell niche: how essential is the niche in regulating stemness of tumor cells? Cell Stem Cell 16 (3) (2015) 225–238.

[44] F.M. Vallette, C. Olivier, F. Lezot, L. Oliver, D. Cochonneau, L. Lalier, P.F. Cartron, D. Heymann, Dormant, quiescent, tolerant and persister cells: four synonyms for the same target in cancer, Biochem. Pharmacol. 162 (2019) 169–176.

[45] A. Recasens, L. Munoz, Targeting Cancer cell dormancy, Trends Pharmacol. Sci. 40 (2) (2019) 128–141.

[46] M.L. De Angelis, F. Francescangeli, T.F. La, A. Zeuner, Stem cell plasticity and dormancy in the development of Cancer therapy resistance, Front. Oncol. 9 (2019) 626.

[47] J.P. Thiery, C.T. Lim, Tumor dissemination: an EMT affair, Cancer Cell 23 (3) (2013) 272–273.

[48] F. Facchinetti, F. Andriani, G. Bertolini, E. Baldoli, M. Moro, P. Casalini, R. Caserini, M. Milione, G. Leone, G. Pelosi, U. Pastorino, G. Sozzi, L. Roz, Conversion to stem-cell state in response to microenvironmental cues is regulated by balance between epithelial and mesenchymal features in lung cancer cells, Mol. Oncol. 10 (2) (2016) 253–271.

[49] M.K. Jolly, J.A. Somarelli, M. Sheth, A. Biddle, S.C. Tripathi, A.J. Armstrong, S.M. Hanash, S.A. Bapat, A. Rangarajan, H. Levine, Hybrid epithelial/mesenchymal phenotypes promote metastasis and therapy resistance across carcinomas, Pharmacol. Ther. 194 (2019) 161–184.

[50] M.K. Jolly, B. Huang, M. Lu, S.A. Mani, H. Levine, E. Ben-Jacob, Towards elucidating the connection between epithelial-mesenchymal transitions and stemness, J. R. Soc. Interface 11 (101) (2014) 20140962.

[51] I. Salmon, I. Pastushenko, A. Brisebarre, A. Sifrim, M. Fioramonti, T. Revenco, S. Boumahdi, K.A. Van, D. Brown, V. Moers, S. Lemaire, C.S. De, E. Minguijon, C. Balsat, Y. Sokolow, C. Dubois, C.F. De, S. Scozzaro, F. Sopena, A. Lanas, N. D'Haene, J.C. Marine, T. Voet, P.A. Sotiropoulou, C. Blanpain, Identification of the tumour transition states occurring during EMT, Nature 556 (7702) (2018) 463–468.

[52] P.G. Santamaría, G. Moreno-Bueno, A. Cano, Contribution of epithelial plasticity to therapy resistance, J. Clin. Med. 8 (5) (2019).

[53] D. Allan, D. Jia, L. Li, S. Andrew, X. Li, J. Lee, G. Ji, Z. Yao, S. Gadde, D. Figeys, L. Wang, An autocrine inflammatory forward-feedback loop after chemotherapy withdrawal facilitates the repopulation of drug-resistant breast cancer cells, Cell Death Dis. 8 (7) (2017) e2932.

[54] A.V. Kurtova, J. Xiao, Q. Mo, S. Pazhanisamy, R. Krasnow, S.P. Lerner, F. Chen, T.T. Roh, E. Lay, P.L. Ho, K.S. Chan, Blocking PGE2-induced tumour repopulation abrogates bladder cancer chemoresistance, Nature 517 (7533) (2015) 209–213.

[55] S. Duan, Y. Tsai, P. Keng, Y. Chen, S.O. Lee, Y. Chen, IL-6 signaling contributes to cisplatin resistance in non-small cell lung cancer via the up-regulation of anti-apoptotic and DNA repair associated molecules, Oncotarget 6 (29) (2015) 27651–27660.

[56] C.J. Creighton, X. Li, M. Landis, J.M. Dixon, V.M. Neumeister, A. Sjolund, D.L. Rimm, H. Wong, A. Rodriguez, J.I. Herschkowitz, C. Fan, X. Zhang, X. He, A. Pavlick, M.C. Gutierrez, L. Renshaw, A.A. Larionov, D. Faratian, S.G. Hilsenbeck, C.M. Perou, M.T. Lewis, J.M. Rosen, J.C. Chang, Residual breast cancers after conventional therapy display mesenchymal as well as tumor-initiating features, Proc Natl Acad Sci U S A 106 (33) (2009) 13820–13825.

[57] C. Kroger, A. Afeyan, J. Mraz, E.N. Eaton, F. Reinhardt, Y.L. Khodor, P. Thiru, B. Bierie, X. Ye, C.B. Burge, R.A. Weinberg, Acquisition of a hybrid E/M state is essential for tumorigenicity of basal breast cancer cells, Proc Natl Acad Sci U S A 116 (15) (2019) 7353–7362.

[58] G. Sozzi, G. Bertolini, L. Roz, P. Perego, M. Tortoreto, E. Fontanella, L. Gatti, G. Pratesi, A. Fabbri, F. Andriani, S. Tinelli, E. Roz, R. Caserini, S. Lo Vullo, T. Camerini, L. Mariani, D. Delia, E. Calabro, U. Pastorino, Highly tumorigenic lung cancer CD133+ cells display stem-like features and are spared by cisplatin treatment, Proc Natl Acad Sci U S A 106 (38) (2009) 16281–16286.

[59] G. Sozzi, G. Bertolini, L. D'Amico, M. Moro, E. Landoni, P. Perego, R. Miceli, L. Gatti, F. Andriani, D. Wong, R. Caserini, M. Tortoreto, M. Milione, R. Ferracini, L. Mariani, U. Pastorino, I. Roato, L. Roz, Microenvironment-modulated metastatic CD133+/CXCR4+/EpCAM- lung cancer-initiating cells sustain tumor dissemination and correlate with poor prognosis, Cancer Res. 75 (17) (2015) 3636–3649.

[60] L. Monfregola, L. Portella, R. Vitale, L.S. De, C. D'Alterio, C. Ierano, M. Napolitano, A. Riccio, M.N. Polimeno, A. Barbieri, A. Luciano, A. Ciarmiello, C. Arra, G. Castello, P. Amodeo, S. Scala, Preclinical development of a novel class of CXCR4

antagonist impairing solid tumors growth and metastases, PLoS One 8 (9) (2013) e74548.
[61] M.S. Di, F.S. Di Leva, A.M. Trotta, D. Brancaccio, L. Portella, M. Aurilio, S. Tomassi, A. Messere, D. Sementa, S. Lastoria, A. Carotenuto, E. Novellino, S. Scala, L. Marinelli, Structure-activity relationships and biological characterization of a novel, potent, and serum stable C-X-C chemokine receptor type 4 (CXCR4) antagonist, J. Med. Chem. 60 (23) (2017) 9641–9652.
[62] B. Auffinger, A.L. Tobias, Y. Han, G. Lee, D. Guo, M. Dey, M.S. Lesniak, A.U. Ahmed, Conversion of differentiated cancer cells into cancer stem-like cells in a glioblastoma model after primary chemotherapy, Cell Death Differ. 21 (7) (2014) 1119–1131.
[63] N.K. Altorki, G.J. Markowitz, D. Gao, J.L. Port, A. Saxena, B. Stiles, T. McGraw, V. Mittal, The lung microenvironment: an important regulator of tumour growth and metastasis, Nat. Rev. Cancer 19 (1) (2019) 9–31.
[64] F. Runa, S. Hamalian, K. Meade, P. Shisgal, P.C. Gray, J.A. Kelber, Tumor microenvironment heterogeneity: challenges and opportunities, Curr. Mol. Biol. Rep. 3 (4) (2017) 218–229.
[65] M.R. Junttila, F.J. de Sauvage, Influence of tumour micro-environment heterogeneity on therapeutic response, Nature 501 (7467) (2013) 346–354.
[66] Y. Sun, J. Campisi, C. Higano, T.M. Beer, P. Porter, I. Coleman, L. True, P.S. Nelson, Treatment-induced damage to the tumor microenvironment promotes prostate cancer therapy resistance through WNT16B, Nat. Med. 18 (9) (2012) 1359–1368.
[67] W.J. Chen, C.C. Ho, Y.L. Chang, H.Y. Chen, C.A. Lin, T.Y. Ling, Y.S.S. Yu SL, Y.J. Chen, C.Y. Lin, S.H. Pan, H.Y. Chou, Y.J. Chen, G.C. Chang, W.C. Chu, Y.M. Lee, J.Y. Lee, P.J. Lee, K.C. Li, H.W. Chen, P.C. Yang, Cancer-associated fibroblasts regulate the plasticity of lung cancer stemness via paracrine signalling, Nat. Commun. 5 (2014) 3472.
[68] Y. Shintani, A. Fujiwara, T. Kimura, T. Kawamura, S. Funaki, M. Minami, M. Okumura, IL-6 secreted from cancer-associated fibroblasts mediates chemoresistance in NSCLC by increasing epithelial-mesenchymal transition signaling, J. Thorac. Oncol. 11 (9) (2016) 1482–1492.
[69] R. Kanzaki, H. Naito, K. Kise, K. Takara, D. Eino, M. Minami, Y. Shintani, S. Funaki, T. Kawamura, T. Kimura, M. Okumura, N. Takakura, Gas6 derived from cancer-associated fibroblasts promotes migration of Axl-expressing lung cancer cells during chemotherapy, Sci. Rep. 7 (1) (2017) 10613.
[70] P.A. Toste, A.H. Nguyen, B.E. Kadera, M. Duong, N. Wu, I. Gawlas, L.M. Tran, M. Bikhchandani, L. Li, S.G. Patel, D.W. Dawson, T.R. Donahue, Chemotherapy-induced inflammatory gene signature and protumorigenic phenotype in pancreatic CAFs via Stress-Associated MAPK, Mol. Cancer Res. 14 (5) (2016) 437–447.
[71] K.E. Richards, A.E. Zeleniak, M.L. Fishel, J. Wu, L.E. Littlepage, R. Hill, Cancer-associated fibroblast exosomes regulate survival and proliferation of pancreatic cancer cells, Oncogene 36 (13) (2017) 1770–1778.
[72] O. Fortunato, P. Gasparini, M. Boeri, G. Sozzi, Exo-miRNAs as a new tool for liquid biopsy in lung Cancer, Cancers (Basel) 11 (6) (2019).
[73] M. Wang, F. Yu, H. Ding, Y. Wang, P. Li, K. Wang, Emerging function and clinical values of exosomal MicroRNAs in cancer, Mol. Ther. Nucleic Acids 16 (2019) 791–804.
[74] J.L. Hu, W. Wang, X.L. Lan, Z.C. Zeng, Y.S. Liang, Y.R. Yan, F.Y. Song, F.F. Wang, X.H. Zhu, W.J. Liao, W.T. Liao, Y.Q. Ding, L. Liang, CAFs secreted exosomes promote metastasis and chemotherapy resistance by enhancing cell stemness and epithelial-mesenchymal transition in colorectal cancer, Mol. Cancer (2019) 18.
[75] C.E. Lewis, J.W. Pollard, Distinct role of macrophages in different tumor microenvironments, Cancer Res. 66 (2) (2006) 605–612.
[76] P.M. De, C.E. Lewis, Macrophage regulation of tumor responses to anticancer therapies, Cancer Cell 23 (3) (2013) 277–286.
[77] P.M. De, C.E. Lewis, Cancer: macrophages limit chemotherapy, Nature 472 (7343) (2011) 303–304.
[78] L. Ireland, A. Santos, M.S. Ahmed, C. Rainer, S.R. Nielsen, V. Quaranta, U. Weyer-Czernilofsky, D.D. Engle, P.A. Perez-Mancera, S.E. Coupland, A. Taktak, T. Bogenrieder, D.A. Tuveson, F. Campbell, M.C. Schmid, A. Mielgo, Chemoresistance in pancreatic cancer is driven by stroma-derived insulin-like growth factors, Cancer Res. 76 (23) (2016) 6851–6863.
[79] X. Yin, X. Zhu, H. Shen, M. Yang, H. Wei, Q. Chen, F. Feng, Y. Liu, W. Xu, Y. Li, Macrophages derived exosomes deliver miR-223 to epithelial ovarian cancer cells to elicit a chemoresistant phenotype, J. Exp. Clin. Cancer Res. (2019) 38.
[80] S.K. Bandari, A. Purushothaman, V.C. Ramani, G.J. Brinkley, D.S. Chandrashekar, S. Varambally, J.A. Mobley, Y. Zhang, E.E. Brown, I. Vlodavsky, R.D. Sanderson, Chemotherapy induces secretion of exosomes loaded with heparanase that degrades extracellular matrix and impacts tumor and host cell behavior, Matrix Biol. 65 (2018) 104–118.
[81] B. Sainz Jr., E. Carron, M. Vallespinos, H.L. Machado, Cancer stem cells and macrophages: implications in tumor biology and therapeutic strategies, Mediators Inflamm. 2016 (2016) 9012369.
[82] J.B. Mitchem, D.J. Brennan, B.L. Knolhoff, B.A. Belt, Y. Zhu, D.E. Sanford, L. Belaygorod, D. Carpenter, L. Collins, D. Piwnica-Worms, S. Hewitt, G.M. Udupi, W.M. Gallagher, C. Wegner, B.L. West, A. Wang-Gillam, P. Goedegebuure, D.C. Linehan, D.G. DeNardo, Targeting tumor-infiltrating macrophages decreases tumor-initiating cells, relieves immunosuppression, and improves chemotherapeutic responses, Cancer Res. 73 (3) (2013) 1128–1141.
[83] D.G. DeNardo, D.J. Brennan, E. Rexhepaj, B. Ruffell, S.L. Shiao, S.F. Madden, W.M. Gallagher, N. Wadhwani, S.D. Keil, S.A. Junaid, H.S. Rugo, E.S. Hwang, K. Jirstrom, B.L. West, L.M. Coussens, Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy, Cancer Discov. 1 (1) (2011) 54–67.
[84] S. Acharyya, T. Oskarsson, S. Vanharanta, S. Malladi, J. Kim, P.G. Morris, K. Manova-Todorova, M. Leversha, N. Hogg, V.E. Seshan, L. Norton, E. Brogi, J. Massague, A CXCL1 paracrine network links cancer chemoresistance and metastasis, Cell 150 (1) (2012) 165–178.
[85] M.A. Venneri, P.M. De, M. Ponzoni, F. Pucci, C. Scielzo, E. Zonari, R. Mazzieri, C. Doglioni, L. Naldini, Identification of proangiogenic TIE2-expressing monocytes (TEMs) in human peripheral blood and cancer, Blood 109 (12) (2007) 5276–5285.
[86] M.L. Squadrito, M. De Palma, Macrophage regulation of tumor angiogenesis: implications for cancer therapy, Mol. Aspects Med. 32 (2) (2011) 123–145.
[87] R. Hughes, B.Z. Qian, C. Rowan, M. Muthana, I. Keklikoglou, O.C. Olson, S. Tazzyman, S. Danson, C. Addison, M. Clemons, A.M. Gonzalez-Angulo, J.A. Joyce, P.M. De, J.W. Pollard, C.E. Lewis, Perivascular M2 macrophages stimulate tumor relapse after chemotherapy, Cancer Res. 75 (17) (2015) 3479–3491.
[88] E.S. Nakasone, H.A. Askautrud, T. Kees, J.H. Park, V. Plaks, A.J. Ewald, M. Fein, M.G. Rasch, Y.X. Tan, J. Qiu, J. Park, P. Sinha, M.J. Bissell, E. Frengen, Z. Werb, M. Egeblad, Imaging tumor-stroma interactions during chemotherapy reveals contributions of the microenvironment to resistance, Cancer Cell 21 (4) (2012) 488–503.
[89] B.D. Robinson, G.L. Sica, Y.F. Liu, T.E. Rohan, F.B. Gertler, J.S. Condeelis, J.G. Jones, Tumor microenvironment of metastasis in human breast carcinoma: a potential prognostic marker linked to hematogenous dissemination, Clin. Cancer Res. 15 (7) (2009) 2433–2441.
[90] T.E. Rohan, X. Xue, H.M. Lin, T.M. D'Alfonso, P.S. Ginter, M.H. Oktay, B.D. Robinson, M. Ginsberg, F.B. Gertler, A.G. Glass, J.A. Sparano, J.S. Condeelis, J.G. Jones, Tumor microenvironment of metastasis and risk of distant metastasis of breast cancer, J. Natl. Cancer Inst. 106 (8) (2014).
[91] G.S. Karagiannis, J.M. Pastoriza, Y. Wang, A.S. Harney, D. Entenberg, J. Pignatelli, V.P. Sharma, E.A. Xue, E. Cheng, T.M. D'Alfonso, J.G. Jones, J. Anampa, T.E. Rohan, J.A. Sparano, J.S. Condeelis, M.H. Oktay, Neoadjuvant chemotherapy induces breast cancer metastasis through a TMEM-mediated mechanism, Sci. Transl. Med. 9 (397) (2017).
[92] N. Linde, M. Casanova-Acebes, M.S. Sosa, A. Mortha, A. Rahman, E. Farias, K. Harper, E. Tardio, T.I. Reyes, J. Jones, J. Condeelis, M. Merad, J.A. guirre-Ghiso, Macrophages orchestrate breast cancer early dissemination and metastasis, Nat. Commun. 9 (1) (2018) 21.
[93] L. Volk-Draper, K. Hall, C. Griggs, S. Rajput, P. Kohio, D. DeNardo, S. Ran, Paclitaxel therapy promotes breast cancer metastasis in a TLR4-dependent manner, Cancer Res. 74 (19) (2014) 5421–5434.
[94] C. Alix-Panabieres, K. Pantel, Clinical applications of circulating tumor cells and circulating tumor DNA as liquid biopsy, Cancer Discov. 6 (5) (2016) 479–491.
[95] D.T. Ting, D.T. Miyamoto, M. Toner, S. Maheswaran, D.A. Haber, Single-cell analysis of circulating tumor cells as a window into tumor heterogeneity, Cold Spring Harb. Symp. Quant. Biol. (2017).
[96] T.A. Yap, D. Lorente, A. Omlin, D. Olmos, J.S. de Bono, Circulating tumor cells: a multifunctional biomarker, Clin. Cancer Res. 20 (10) (2014) 2553–2568.
[97] T.M. Gorges, A. Kuske, K. Rock, O. Mauermann, V. Muller, S. Peine, K. Verpoort, V. Novosadova, M. Kubista, S. Riethdorf, K. Pantel, Accession of tumor heterogeneity by multiplex transcriptome profiling of single circulating tumor cells, Clin. Chem. 62 (11) (2016) 1504–1515.
[98] C. Agnoletto, F. Corra, L. Minotti, F. Baldassari, F. Crudele, W.J.J. Cook, d'Adamo A.P. Di LG, P. Gasparini, S. Volinia, Heterogeneity in circulating tumor cells: the relevance of the stem-cell subset, Cancers (Basel) 11 (4) (2019).
[99] I. Baccelli, A. Schneeweiss, S. Riethdorf, A. Stenzinger, A. Schillert, V. Vogel, C. Klein, M. Saini, T. Bauerle, M. Wallwiener, T. Holland-Letz, T. Hofner, M. Sprick, M. Scharpff, F. Marme, H.P. Sinn, K. Pantel, W. Weichert, A. Trumpp, Identification of a population of blood circulating tumor cells from breast cancer patients that initiates metastasis in a xenograft assay, Nat. Biotechnol. 31 (6) (2013) 539–544.
[100] L. Zhang, L.D. Ridgway, M.D. Wetzel, J. Ngo, W. Yin, D. Kumar, J.C. Goodman, M.D. Groves, D. Marchetti, The identification and characterization of breast cancer CTCs competent for brain metastasis, Sci. Transl. Med. 5 (180) (2013) 180ra48.
[101] C.J. Miller, C.J. Morrow, F. Trapani, R.L. Metcalf, G. Bertolini, C.L. Hodgkinson, G. Khandelwal, P. Kelly, M. Galvin, L. Carter, K.L. Simpson, S. Williamson, C. Wirth, N. Simms, L. Frankliln, K. Frese, D.G. Rothwell, D. Nonaka, G. Brady, F.H. Blackhall, C. Dive, Tumourigenic non-small cell lung cancer mesenchymal circulating tumour cells – a clinical case study, Ann. Oncol. (2016).
[102] M. Toner, M. Yu, A. Bardia, B.S. Wittner, S.L. Stott, M.E. Smas, D.T. Ting, S.J. Isakoff, J.C. Ciciliano, M.N. Wells, A.M. Shah, K.F. Concannon, M.C. Donaldson, L.V. Sequist, E. Brachtel, D. Sgroi, J. Baselga, S. Ramaswamy, D.A. Haber, S. Maheswaran, Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition, Science 339 (6119) (2013) 580–584.
[103] M.A. Papadaki, G. Stoupis, P.A. Theodoropoulos, D. Mavroudis, V. Georgoulias, S. Agelaki, Circulating tumor cells with stemness and epithelial-to-mesenchymal transition features are chemoresistant and predictive of poor outcome in metastatic breast cancer, Mol. Cancer Ther. 18 (2) (2019) 437–447.
[104] E.V. Kaigorodova, O.E. Savelieva, L.A. Tashireva, N.A. Tarabanovskaya, E.I. Simolina, E.V. Denisov, E.M. Slonimskaya, E.L. Choynzonov, V.M. Perelmuter, Heterogeneity of circulating tumor cells in neoadjuvant chemotherapy of breast cancer, Molecules 23 (4) (2018).
[105] I. Nel, U. Jehn, T. Gauler, A.C. Hoffmann, Individual profiling of circulating tumor cell composition in patients with non-small cell lung cancer receiving platinum based treatment, Transl. Lung Cancer Res. 3 (2) (2014) 100–106.
[106] S. Kasimir-Bauer, A.K. Bittner, L. Konig, K. Reiter, T. Keller, R. Kimmig, O. Hoffmann, Does primary neoadjuvant systemic therapy eradicate minimal residual disease? Analysis of disseminated and circulating tumor cells before and after therapy, Breast Cancer Res. 18 (1) (2016) 20.

[107] D. Hanahan, L.M. Coussens, Accessories to the crime: functions of cells recruited to the tumor microenvironment, Cancer Cell 21 (3) (2012) 309–322.
[108] J.A. Joyce, J.W. Pollard, Microenvironmental regulation of metastasis, Nat. Rev. Cancer 9 (4) (2009) 239–252.
[109] B. Qian, Y. Deng, J.H. Im, R.J. Muschel, Y. Zou, J. Li, R.A. Lang, J.W. Pollard, A distinct macrophage population mediates metastatic breast cancer cell extravasation, establishment and growth, PLoS One 4 (8) (2009) e6562.
[110] B.Z. Qian, J. Li, H. Zhang, T. Kitamura, J. Zhang, L.R. Campion, E.A. Kaiser, L.A. Snyder, J.W. Pollard, CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis, Nature 475 (7355) (2011) 222–225.
[111] T. Kitamura, B.Z. Qian, D. Soong, L. Cassetta, R. Noy, G. Sugano, Y. Kato, J. Li, J.W. Pollard, CCL2-induced chemokine cascade promotes breast cancer metastasis by enhancing retention of metastasis-associated macrophages, J. Exp. Med. 212 (7) (2015) 1043–1059.
[112] I. Keklikoglou, C. Cianciaruso, E. Guc, M.L. Squadrito, L.M. Spring, S. Tazzyman, L. Lambein, A. Poissonnier, G.B. Ferraro, C. Baer, A. Cassara, A. Guichard, M.L. Iruela-Arispe, C.E. Lewis, L.M. Coussens, A. Bardia, R.K. Jain, J.W. Pollard, P.M. De, Chemotherapy elicits pro-metastatic extracellular vesicles in breast cancer models, Nat. Cell Biol. 21 (2) (2019) 190–202.
[113] G. Liu, Y. Chen, F. Qi, L. Jia, X.A. Lu, T. He, Y. Fu, L. Li, Y. Luo, Specific chemotherapeutic agents induce metastatic behaviour through stromal- and tumour-derived cytokine and angiogenic factor signalling, J. Pathol. 237 (2) (2015) 190–202.
[114] C.M. Ghajar, H. Peinado, H. Mori, I.R. Matei, K.J. Evason, H. Brazier, D. Almeida, A. Koller, K.A. Hajjar, D.Y. Stainier, E.I. Chen, D. Lyden, M.J. Bissell, The perivascular niche regulates breast tumour dormancy, Nat. Cell Biol. 15 (7) (2013) 807–817.
[115] C. Calabrese, H. Poppleton, M. Kocak, T.L. Hogg, C. Fuller, B. Hamner, E.Y. Oh, M.W. Gaber, D. Finklestein, M. Allen, A. Frank, I.T. Bayazitov, S.S. Zakharenko, A. Gajjar, A. Davidoff, R.J. Gilbertson, A perivascular niche for brain tumor stem cells, Cancer Cell 11 (1) (2007) 69–82.
[116] P. Carlson, A. Dasgupta, C.A. Grzelak, J. Kim, A. Barrett, I.M. Coleman, R.E. Shor, E.T. Goddard, J. Dai, E.M. Schweitzer, A.R. Lim, S.B. Crist, D.A. Cheresh, P.S. Nelson, K.C. Hansen, C.M. Ghajar, Targeting the perivascular niche sensitizes disseminated tumour cells to chemotherapy, Nat. Cell Biol. 21 (2) (2019) 238–250.
[117] M.S. Sosa, P. Bragado, J.A. Aguirre-Ghiso, Mechanisms of disseminated cancer cell dormancy: an awakening field, Nat. Rev. Cancer 14 (9) (2014) 611–622.
[118] J.A. Aguirre-Ghiso, P. Bragado, M.S. Sosa, Metastasis awakening: targeting dormant cancer, Nat. Med. 19 (3) (2013) 276–277.
[119] D. Paez, M.J. Labonte, P. Bohanes, W. Zhang, L. Benhanim, Y. Ning, T. Wakatsuki, F. Loupakis, H.J. Lenz, Cancer dormancy: a model of early dissemination and late cancer recurrence, Clin. Cancer Res. 18 (3) (2012) 645–653.
[120] G.N. Naumov, J.L. Townson, I.C. MacDonald, S.M. Wilson, V.H. Bramwell, A.C. Groom, A.F. Chambers, Ineffectiveness of doxorubicin treatment on solitary dormant mammary carcinoma cells or late-developing metastases, Breast Cancer Res. Treat. 82 (3) (2003) 199–206.
[121] C.A. Grzelak, C.M. Ghajar, Metastasis' systems' biology: how are macro-environmental signals transmitted into microenvironmental cues for disseminated tumor cells? Curr. Opin. Cell Biol. 48 (2017) 79–86.
[122] G. Fluegen, A. vivar-Valderas, Y. Wang, M.R. Padgen, J.K. Williams, A.R. Nobre, V. Calvo, J.F. Cheung, J.J. Bravo-Cordero, D. Entenberg, J. Castracane, V. Verkhusha, P.J. Keely, J. Condeelis, J.A. guirre-Ghiso, Phenotypic heterogeneity of disseminated tumour cells is preset by primary tumour hypoxic microenvironments, Nat. Cell Biol. 19 (2) (2017) 120–132.
[123] L.A. Gilbert, M.T. Hemann, DNA damage-mediated induction of a chemoresistant niche, Cell 143 (3) (2010) 355–366.