

Review

Chemotherapy-exacerbated breast cancer metastasis: A paradox explainable by stress response

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Abstract: An emerging picture in cancer biology is that, paradoxically, chemotherapy can actively induce changes that favor cancer progression. These pro-cancer changes can be either inside (intrinsic) or outside (extrinsic) the cancer cells. In this review, we will discuss the extrinsic pro-cancer effect of chemotherapy; that is, the effect of chemotherapy on the non-cancer host cells to promote cancer progression. We will focus on metastasis, and will first discuss recent data from mouse models of breast cancer. Intriguingly, despite reducing the size of primary tumors, chemotherapy changes the tumor microenvironment, resulting in an increased escape of cancer cells into the blood stream. Furthermore, chemotherapy changes the tissue microenvironment at the distant sites, making it more hospitable to cancer cells upon their arrival. We will then discuss the idea and evidence that these devastating pro-metastatic effects of chemotherapy can be explained in the context of stress response. At the end, we will discuss the potential relevance of these mouse data to human breast cancer and their implication on chemotherapy in the clinic.

Keywords: chemotherapy; breast cancer metastasis; stress response; ATF3; seed and soil theory; cancer-host interaction; tumor microenvironment; immune modulation; tumor immune environment

1. The double-edged sword of chemotherapy – findings from mouse models

1.1. The paradox of chemotherapy

Although tumors can be reduced to undetectable level by modern chemotherapy, in many cases they recur at the original or distant sites. Traditionally, this was thought to be a manifestation of “survival of the fittest”: the chemotherapeutic drugs exert selection pressure that allowed resistant cancer cells to survive, grow, and eventually thrive. However, emerging pictures from cancer research in the last decade showed that, paradoxically, chemotherapy can actively induce changes that favor cancer progression. These pro-cancer changes can be either inside (intrinsic) or outside (extrinsic) the cancer cells. For intrinsic changes, chemotherapeutic drugs have been shown to up-regulate the expression of anti-apoptotic genes [1], and to increase the ability of cancer cells to

migrate/invade [2]. For extrinsic changes, chemotherapeutic drugs have been shown to change the non-cancer cells within the host—the organism that carries the cancer cells (some reviews, [3-7]). Note that the issue at hand here is the pro-cancer effect of chemotherapy, rather than the well-recognized side-effect of chemotherapy such as nausea and hair loss.

Although the field is relatively new, it has made significant advancement by leveraging the extensive knowledge on cancer-host interaction (a few reviews, [8-12]). Intensive research in the past few decades has demonstrated that cancers are not simply autonomous masses of cells. They secrete soluble factors to elicit systemic responses from the host. The host in turn sends soluble factors and hematopoietic precursor cells (from bone marrow) to the tumors and the future metastatic sites to affect cancer progression, forming a loop of cancer-host interaction (above reviews). Relevant to our discussion here is the myeloid-lineage of cells, particularly the macrophages, which play a key role for the host to enhance cancer progression. The ability of these cells to promote cancer progression seems counter-intuitive, since the main function of macrophages is to fight against infection and eliminate damaged cells. A widely accepted explanation is that macrophages in the tumor, called tumor-associated macrophages (TAMs), are educated by cancer cells over time, and are converted from anti-cancer to pro-cancer, at least in part, by changing their gene expression (some reviews, [13-15]). For the complexities and nuances of immune cells in cancer progression, see aforementioned reviews.

It was against this backdrop of cancer-host interaction that various studies showed the pro-cancer effect of chemotherapy. They showed that chemotherapeutic agents (such as paclitaxel, cyclophosphamide, and gemcitabine) can increase the abundance of TAMs in primary tumors. Functionally, inhibition of myeloid cells by small molecule inhibitor, antibodies, or genetic intervention improved the efficacy of chemotherapy as evidenced by the further reduction of tumor size (a few reviews, [4-6]). Thus, chemotherapy can paradoxically elicit pro-cancer effect and counteract its own efficacy. The mechanisms include increasing TAM abundance and altering their bioactivities ([4-6]).

1.2. The effect of chemotherapy on metastasis

Metastasis is a multi-step process composed of cancer cell escape from the primary tumor, survival in circulation, and colonization at the distant site. Since metastasis is the major cause of cancer death, an important question is whether chemotherapy affects metastasis. This was a gap in the field, since the literature above primarily focused on primary tumor growth. Recently, a few reports demonstrated that chemotherapy enhances metastasis [16-21]. In one study [18], paclitaxel was shown to enhance metastasis by activating the TLR4 signaling in cancer cells, which in turn increased systemic inflammation and myeloid cell outgrowth. In the other reports, chemotherapy was shown to enhance metastasis by affecting the non-cancer host cells. Daenen et al. and Gingis-Velitski et al. [16, 17] demonstrated that chemotherapy enhanced metastasis using an experimental metastasis model, where cancer cells are intravenously injected into the mice. This model does not have primary tumor; thus, it only examines the ability of cancer cells (delivered into the venous blood) to colonize the lung, not their ability to escape from the tumors. Alishekevitz et al. [19] used the spontaneous metastasis model, where primary tumor arises from the orthotopic site, allowing

the analyses of both the tumors and metastatic organs. They showed that paclitaxel enhanced metastasis by increasing lymphatic density in the tumors. Chang et al. and Karagiannis et al. [20, 21] also used the spontaneous metastasis model and showed that, despite reducing the tumor size, paclitaxel increased the abundance of a micro-anatomical structure called tumor microenvironment of metastasis (TMEM). This structure is composed of a macrophage and a cancer cell in close proximity at the peri-vascular location [22] as diagramed in Figure 1. Importantly, intravital imaging showed that this is the site where cancer cells enter the blood stream [23]. Consistent with the increase in TMEM, both studies [20, 21] showed increased circulating cancer cells and enhanced metastasis by paclitaxel. Therefore, despite its apparent benefit of reducing tumor size, paclitaxel exacerbated metastasis. We note that, in all the above studies using the spontaneous metastasis models [18-21], chemotherapy was administered while primary tumors were still present. Thus, they mimic the neoadjuvant (pre-operative) chemotherapy, not the adjuvant (post-operative) chemotherapy. This has implication on how to interpret these data in clinical consideration (see below, section 4.1).

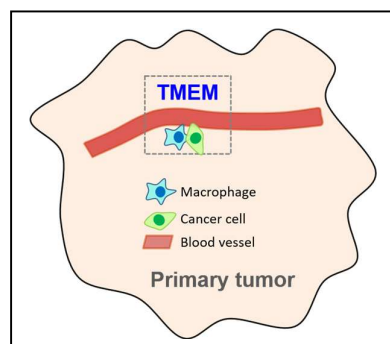


Figure 1 A schematic of TMEM (tumor microenvironment metastasis)

The schematic shows a TMEM composed of a macrophage and a cancer cell at peri-vascular location (first named by Robinson et al. [22]).

Chang et al. also analyzed the lung, the metastatic site of their models [20]. Among other things, paclitaxel increased the abundance of inflammatory monocyte (which is known to differentiate into metastasis-associated macrophages [24]) and suppressed the anti-cancer immune microenvironment. Taken together, paclitaxel enabled more cancer cells (the seeds) to escape from primary tumors, and made the lung microenvironment (the soil) more hospitable to cancer cells, explaining the paradoxical ability of chemotherapy to exacerbate metastasis in the context of the “seed and soil” theory. Of note, this is the first report to address the effect of chemotherapy on both the primary tumor and metastatic site from the same mice.

2. Explaining the pro-cancer effect of chemotherapy from the perspective of stress response

Chang et al. further demonstrated that the pro-cancer effects of paclitaxel (such as increasing TMEM, inflammatory monocyte, and metastasis) were dependent on a stress-inducible gene *Atf3* in the non-cancer host cells [20]. Using a fat pad injection model, they showed that paclitaxel exacerbated the ability of breast cancer cells to metastasize in the wild type mice but not much in the knockout mice deficient of *Atf3*. Since the same breast cancer cells were injected into the mice and the only difference was the host, it means that paclitaxel exerted its pro-metastatic effect by affecting the host cells. The genotype difference between the mice indicates that ATF3-regulated processes in the

host cells are key mediators for paclitaxel. ATF3 is a transcription factor and the expression of its corresponding gene is induced by a variety of stress signals, including chemotherapeutic agents (a review, [25]). One unifying function of ATF3 appears to modulate immune response (a review, [26]). By nature of its critical role in stress response, *Atf3* may also play a role in the ability of non-chemotherapy related stressors to facilitate metastasis, such as traumatic injury and excisional surgery [27-30]. Clearly, more investigation is required to test this supposition. In this context, the following concept is of particular interest: cellular stress response has evolved to promote wound healing and is hijacked by cancer cells to help them survive and progress [3]. A potential mechanistic explanation for this concept is that wound healing and cancer progression—to the first approximation—entail the same biological processes: (i) activation of blood vessels, (ii) remodeling of extracellular matrix, and (iii) recruitment of immune cells. We posit that, *Atf3*, as a key player in the cellular stress response network, may be a linchpin for seemingly different stressors such as chemotherapy and traumatic injury to enhance metastasis.

3. The relevance of the above findings to human breast cancer

Analysis of publicly available datasets from human breast tumors showed that *Atf3* expression was higher in the breast tumor stroma from patients with chemotherapy than those without [20]. Furthermore, analyses of microarray datasets derived from the metastatic organs of human breast cancer patients showed that *Atf3* expression correlated with lower cytotoxic immune cell markers, consistent with the ATF3-associated immune suppression in mouse models. Importantly, Karagiannis et al. analyzed 20 breast cancer tumors before and after neoadjuvant chemotherapy, and found increased TMEM abundance by chemotherapy [21]. Since higher TMEM abundance correlated with worse outcome [22], these results suggest that neoadjuvant chemotherapy may have undesirable long-term consequences. Taken together, data from preclinical research using mouse models support the notion that chemotherapy can enhance metastasis and that this paradoxical effect of chemotherapy is likely to have human relevance.

4. Should findings from mouse models affect clinical practices?

Chemotherapy is a longstanding treatment for cancer patients and has been shown to cure some blood cancers, such as childhood leukemia and adult Hodgkin's lymphoma (a review [31]). Thus, it would not be prudent to change clinical practices without further investigation. However, the data discussed above indicate that it may be possible to improve the efficacy of chemotherapy by inhibiting its paradoxical pro-cancer effect. Below, we discuss neoadjuvant and adjuvant chemotherapy separately.

4.1. Neoadjuvant (pre-operative) chemotherapy

In neoadjuvant setting, chemotherapy is administered before tumor removal. The advantages of this treatment modality include reducing tumor size for operation, increasing breast conservation, providing prognostic information based on tumor's responsiveness to the treatment, and offering optimal setting for research [32]. Importantly, patients have been shown to benefit from neoadjuvant chemotherapy in clinical trials (a review, [33]). This may appear contradictory to the findings discussed above that, in mouse models mimicking neoadjuvant chemotherapy, the treatment enhanced metastasis [18-21]. One potential explanation is that none of those mouse studies removed the tumors before end-point assays. Therefore, the conditions are not the same as those in the clinics. What those studies suggest is that exposing patients to chemotherapy while their tumors are still present could change the biological properties of the tumors and lead to undesirable consequences. These include increased lymphogenesis [19] and higher density of TMEM [20, 21], both of which can allow more cancer cells to escape from the primary tumors. Thus, the benefits of neoadjuvant therapy need to be weighed against the potential undesirable effect. We surmise that the treatment can be

improved by personalized medicine based on individual patients' condition, such as tumor immune-microenvironment. As an example, DeNardo et al. showed that leukocyte complexity can predict patients' response to neoadjuvant chemotherapy [34]. Tumors with low macrophage but high cytotoxic T cells (CD68^{low}/CD8^{high}) responded better to therapy than those with high macrophage but low CD8-T cells (CD68^{high}/CD8^{low}): 27% pathologic complete response versus 7%. Considering the potential detrimental effect of neoadjuvant chemotherapy, careful analysis of individual's conditions and further investigation including clinical trials are warranted.

4.2. Adjuvant (post-operative) chemotherapy

Adjuvant chemotherapy removes tumors first before treating the patients with chemotherapy, and is considered the standard of care, except in the cases of inoperable disease [32]. When patients with adjuvant chemotherapy were compared to those with surgery only, adjuvant chemotherapy has been shown to reduce recurrence and increase overall survival [35-37]. However, as discussed in the section on metastasis, chemotherapy modifies the tissue environment—the soil—at the distant site and makes it more hospitable for cancer cells [20]. Thus, if any cancer cells that disseminated before or during tumor removal can survive chemotherapy, they will have a chance to recur and flourish. This may explain that, in sub-populations of patients, the disease comes back with a vengeance after chemotherapy. Traditionally, this was viewed as the result of “survival of the fittest,” where cancer cells with the most aggressive mutations managed to emerge and succeed. With the insight that chemotherapy can elicit a tissue environment favorable to cancer cells, we now have a new avenue to potentially improve chemotherapy. By elucidating the mechanisms behind this effect, we may be able to dampen the undesirable ability of chemotherapy to modify the soil, thus increasing the therapeutic efficacy of adjuvant chemotherapy.

5. Conclusion

In summary, recent studies in mouse models mimicking the neoadjuvant chemotherapy showed that paclitaxel increases breast cancer metastasis, with the accompany of increased escape of cancer cells (seeds) from the primary tumors and a more favorable tissue environment (soil) at the distant site for cancer cells to colonize. Mechanistic studies showed that paclitaxel does so, at least in part, by inducing a stress-inducible gene *Atf3* in the non-cancer host cells. Many questions remain. Are these findings applicable to other chemotherapeutic agents and other cancers? How will combination chemotherapy affect the data? Is *Atf3*, a key gene in the cellular stress response network, a common element for seemingly different stressors such as chemotherapy and traumatic injury to enhance metastasis? Should the data from mouse models influence clinical practices? Clearly, much more investigation is required before any clinical practice should be changed. However, current literature suggests that the tumor immune microenvironment (leukocyte complexity) may be a useful factor to consider before neoadjuvant chemotherapy. As for adjuvant chemotherapy, it is the standard of care for operable disease. In light of the ability of chemotherapy to promote a favorable soil in mouse models, it is prudent to recognize this undesirable effect and consider its possibility in human. If we can elucidate the mechanisms behind this observation, we may be able to dampen the undesirable effects of chemotherapy and thus improve its efficacy.

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Abbreviations

ATF3	Activating transcription factor 3
CD	Cluster of differentiation
TAM	Tumor associated macrophage
TLR	Toll-like receptor
TMEM	Tumor microenvironment metastasis

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