The Role of TLR4 in Chemotherapy-Driven Metastasis
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Abstract

Tumor resistance to cytotoxic drugs is one of the main obstacles to successful cancer therapy. Emerging evidence suggests that chemoresistance is promoted by substances released from dead and damaged cells that activate the host repair program orchestrated by Toll-like receptor-4 (TLR4). TLR4 is often overexpressed in malignant and tumor-infiltrating immune cells. In addition to endogenous ligands released by therapy-induced tumor destruction, TLR4 is directly activated by paclitaxel, one of the most commonly used chemotherapeutic drugs against various human cancers. TLR4 activation promotes local and systemic inflammation, leading to induction of multiple circuits that create a regenerative environment favoring local recurrence and metastasis. Of particular importance is TLR4-mediated recruitment of endothelial progenitors derived from immature myeloid cells. These cells play a major role in rebuilding tumor-associated lymphatic and blood vessels, thereby promoting lymphatic and hematogenous metastasis. The latter is further enhanced by the premetastatic niche generated by mobilization of myeloid provascular cells to distant organs. This review summarizes the recent evidence demonstrating that paclitaxel and other clinically used anticancer drugs actively induce metastasis even while shrinking the primary tumor. Better understanding of the mechanisms underlying TLR4-dependent chemotherapy-driven metastasis might be the key to overcoming challenges of cancer eradication.

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Introduction

Cytotoxic drugs remain the main, and sometimes the only, therapeutic modality for advanced, refractory, and metastatic tumors as well as for those cancers lacking a specific molecular target. The main drugs used clinically for anticancer therapy are anthracyclines, platin-based, and various taxanes. Although these drugs are highly efficacious in killing cultured tumor cells, application of chemotherapy to cancer patients results in up to 40% of recurrence at a primary, locoregional, or distant site (1). The underlying mechanisms of recurrence are multifaceted. Recent studies suggest that, in addition to genetic alterations in malignant cells, the local tumor environment and systemic host responses to injury strongly contribute to reducing drug efficacy (2–4). Moreover, some studies show that chemotherapy by itself can instigate metastatic spread while simultaneously restraining growth of the primary tumor (5–7). Such counterintuitive outcomes of cytotoxic therapies might be explained by two synergistic events: the natural tendency of the host to protect and repair sites of injury in order to maintain continuous communication with the host. This is achieved by expanding the pools of provascular progenitors in the bone marrow (BM) and spleen followed by their recruitment to injured and remodeled tissue. In the context of cancer, these normal host responses to injury promote metastasis because they endow the tumor cells surviving chemotherapy with increased invasive capacity while simultaneously providing the means for their transportation. To make things worse, one of the most commonly used drugs, paclitaxel, directly stimulates TLR4 (9). Coupled with tumor-protective host mechanisms, paclitaxel therapy might be a driving force for metastasis, particularly in patients with advanced tumor burden or genetic predisposition to evasion of apoptosis. This review will summarize the current understanding of the mechanisms mediated by host immune cells and cytotoxic drugs that cumulatively promote rather than inhibit metastatic behavior. Validation of this concept could fundamentally change the clinical paradigms for cancer therapy to account for unwanted consequences of an activated TLR4 pathway and the host responses to tissue damage.

Biologic Modifiers of the Tumor Response to Chemotherapy In Vivo

Initially, overexpression of drug-excluding pumps in tumor cells was thought to be the main reason for resistance to cytotoxic therapy (10). However, after decades-long exploration, pharmacologic inhibition of these pumps failed to eliminate the problem. This suggested that alternative powerful mechanisms in vivo may
Contribute to limitations of cytotoxic therapies for cancer treatment. This conclusion is supported by extensive evidence showing the protective effect of the tumor environment (11), including upregulation of prosurvival proteins in tumor and tumor-associated cells. Superficially, the idea that cytotoxic drugs enhance survival of malignant cells appears counterintuitive. It is consistent, however, with well-known physiologic responses to the organ damage, necrosis, and hypoxia that invariably occurs due to collapse of blood vasculature during tissue loss. Such outcomes of successful chemotherapy are typically compensated by massive influx of progenitors programmed to restore homeostasis by rebuilding epithelial, stromal, and vascular structures in damaged tissue. Therefore, tumor eradication in vivo may depend not only on genetically dictated sensitivity of malignant cells to cytotoxic drugs but also on an inflammation-amplifying host response caused by drug-induced damage (11).

Paclitaxel as a prototype of anticancer drugs with functionally opposing effects on tumor growth
Paclitaxel is an excellent example of an anticancer drug that both efficiently kills tumor cells and promotes their survival. Paclitaxel cytotoxicity is mediated by binding to β-tubulin, an event that overstabilizes microtubules, leading to interruption of the cell cycle, blockade of mitosis, and eventually, cell death (12). Although early studies demonstrated undeniable efficacy of paclitaxel against many types of metastatic and refractory cancers (13), they also showed drug-specific activation of the NF-κB pathway leading to transcriptional induction of numerous inflammatory genes (14). The shift to proinflammatory phenotype induced by paclitaxel was detected in mouse macrophages (9) and human tumor cells (14) as well as in the blood of breast cancer patients receiving paclitaxel monotherapy (15). Studies in vitro showed that the kinetics and expression profile of paclitaxel-activated genes strongly resemble those induced by lipopolysaccharide (LPS; refs. 16, 17), a pathogen-derived molecule with strong inflammatory properties. Initially, paclitaxel-mediated inflammatory response was thought to enhance the tumoricidal effects of immune cells, and therefore, was regarded as beneficial for cancer patients (14). Subsequent studies showed that paclitaxel-induced inflammatory mediators promote tumor cell survival (18), suggesting that drug-associated inflammation blunts tumor sensitivity to anticancer drugs (19) rather than helping to mount the antitumor defense. Although many questions in this area of investigation are still open, multiple studies using human clinical cancers favor the hypothesis that paclitaxel-induced inflammation leads to progression of tumor growth. Studies in breast, ovarian, prostate, and other tumors showed that TLR4, a natural LPS receptor, is highly upregulated in malignant epithelial cells (20–22), and paclitaxel signals through TLR4 (21, 23). Because paclitaxel is a functional mimetic of LPS (9), both compounds elicit nearly identical responses in TLR4-positive myeloid cells and in cancer cells with upregulated TLR4 expression. Paclitaxel similarly to LPS causes dimerization of TLR4, which, in turn, activates the NF-κB pathway (23), leading to upregulation of numerous inflammatory, migratory, and prosurvival proteins (e.g., VEGF-A, COX2, IL6, IL8, MMP9, XIAP, and Bcl-2; refs. 24–26). These proteins cumulatively confer resistance to cell death (26), evasion from immunosurveillance (27), and increased metastasis (6). Consistently, silencing TLR4 expression restores tumor cell sensitivity to paclitaxel in vitro (21, 23) and correlates with significant increase in tumor-free animals after chemotherapy in breast tumor models in vivo (6, 23). These studies suggest that while paclitaxel is highly toxic to TLR4-negative or low-expressing tumor cells, it is cytotoxic or even growth-promoting for cancer cells expressing high TLR4 levels. Therefore, TLR4 could be an important discriminating marker to distinguish between paclitaxel-responsive and -resistant cancers.

Mechanisms by which paclitaxel therapy promotes metastasis
If cytotoxic drugs could be administrated at the unlimited dose and time required for tumor eradication, the ability of these drugs to kill tumor cells would probably prevail over tumor-promoting actions. However, since all cytotoxic therapies are restricted by toxicities, the drug pro-oncogenic effects on surviving tumor cells can translate into local and distant recurrence. Paclitaxel, specifically, has been shown to promote metastasis by multiple mechanisms. The primary effects on TLR4-positive tumor cells are: (i) enhancement of tumor inflammation (23), the hallmark of the activated TLR4 pathway and a well-known promoter of tumor growth; (ii) induction of a migratory/invasive phenotype in tumor cells (28), an essential prerequisite for escaping the primary tumor site; and (iii) upregulation of prosurvival Bcl-2, XIAP, and Bcl-XL proteins (29) that promote anoxia and successful establishment of metastatic lesions. Although all these activities promote metastatic behavior, paclitaxel’s ability to increase tumor cell invasion might be of particular significance. In the screen using 1,300 compounds and a variety of human cancer lines, paclitaxel was shown as one of the most potent inducers of invadopodia, i.e., cell protrusion required for invasion (28). This prometastatic effect of paclitaxel is exponentially magnified by a regenerative environment of the primary tumor created by inflamed tumor cells and BM-recruited monocytes (23).

Paclitaxel-induced inflammation in tumor cells is phenotypically identical to that induced by LPS in normal immune cells. However, the magnitude of the response might be much higher in the context of cancer because particularly large tumors can generate inflammatory conditions greatly surpassing the outcome of tissue-recruited mononuclear infiltrates. Qualitatively, activation of TLR4 in either situation aims at restoration of homeostasis by remodeling the damaged tissue while protecting the residual cells during repair. Consistent with this concept, we recently demonstrated in breast cancer models that paclitaxel supports TLR4-mediated proinflammatory response and recruiting them to the damaged site (6) as well as by the local upregulation of inflammatory cytokines and their receptors in tumor cells, thus creating autocrine tumor-promoting loops (23).

One of the most deleterious consequences of tumor activation of TLR4 results is mobilization of provascular progenitors to the damaged site. BM-derived cells (BMDC) that include hematopoietic, myeloid, and endothelial progenitors have been repeatedly implicated in promoting the growth of primary tumors and priming the distant sites to favor extravasation and metastatic outgrowth (30). Human BMDC are significantly increased in paclitaxel-treated tumors as shown, for instance, by a 5-fold increase of CD14+ myeloid progenitors in breast cancers of treated women relative to untreated patients (31). Paclitaxel can also directly mobilize BMDC to the lungs as shown in non-tumor-bearing mice with a significantly higher number of lung homing tumor cells in treated animals relative to controls (7). Consequently, drug-treated hosts had a significantly higher number of pulmonary metastases relative to untreated mice (7). Paclitaxel can also activate TLR4 in blood vascular endothelial
cells, which may explain the direct effect of paclitaxel on pulmonary vessel remodeling (4). The exposure of endothelial cells to paclitaxel in vivo impaired the vascular barrier, leading to increased tumor cell lodging and consequent metastasis (4). We recently showed that these effects of paclitaxel on preparing the distant organs for potential tumor invasion are amplified by systemic inflammation recorded in the blood, lungs, spleen, lymph nodes, and BM. Induction of these systemic inflammatory circuits substantially increases pools of myeloid-derived provascular progenitors that subsequently are recruited to the tumor and distant organs and promote vascular outgrowth (6).

One of the earliest events in either physiologic tissue repair or tumor recovery after chemotherapy is regeneration of blood vasculature. Supporting this concept, multiple studies show that chemotherapy-induced provascular BMDC play a paramount role in induction of tumor angiogenesis and consequent hematogenous metastasis (32). We recently expanded this concept by demonstrating that paclitaxel therapy of TLR4-positive (but not negative) breast tumors also increases the number of lymphatic vessels that were highly permissive for invasion by malignant cells (6). The preferential invasion of the lymphatics might be due to their defective formation deep inside the recurring tumor—an unusual site for tumor-induced lymphatics even in metastatic cancers. Albeit deformed, the new intratumoral lymphatics (in mice with TLR4-positive tumors treated with paclitaxel) increased the burden of lymph node metastases by more than 250-fold relative to untreated animals or tumors lacking TLR4 (6). These studies attest to the alarming potential of paclitaxel to augment metastasis of TLR4-positive tumors by direct upregulation of prometastatic genes in neoplastic cells, and indirectly, through recruitment of immature BMDC that contribute to formation of new tumor vasculature.

Other cytotoxic drugs with prometastatic activities

The pro-oncogenic effects of chemotherapy are not limited to paclitaxel by virtue of the fact that TLR4 can be activated by 20 or more endogenous ligands released from damaged or dead cells (33). The list of TLR4-activating factors includes many danger-sensing proteins known as “alarmins” such as HMGB1, S100A8/A9, and various heat-shock proteins (33). It should be mentioned that host response to alarmins is very complex, and the endogenous ligands of TLR4 can generate pro-oncogenic effects via various TLRs (34) as well as additional homeostasis-maintaining receptors (35). In fact, it has not been firmly established that functional effects of paclitaxel are mediated through physical binding to TLR4. Alternatively, paclitaxel might activate TLR4 indirectly by alarmins released from dead cells. If alarmins are ultimately responsible for activation of TLR4, any cytotoxic drug can potentially generate a proinflammatory response, which might be sufficient to rescue residual cells from apoptosis, thereby initiating tumor regrowth.

This idea is strongly supported by mounting evidence indicating that several structurally unrelated drugs induce tumor and host responses that strikingly resemble the effects of paclitaxel. Activation of NF-κB and resultant inflammatory response have been noted after tumor or host cell exposure to at least five drugs other than paclitaxel, including doxorubicin, 5-fluoracil (5-FU), oxaliplatin, cyclophosphamide, and cisplatin (36). Similarly to paclitaxel, stimulation of the NF-κB pathway by these drugs led to activation of the MAPK pathway, upregulation of the proinflammatory AP-2 transcription factor (37), and overexpression of inflammatory (36), prosurvival (29), and migratory genes (38). The TLR4-dependent inflammatory response induced by various chemodrugs was also implicated in promoting epithelial–mesenchymal transition (36) and selecting tumor cells with the stem-like phenotype (39). Acquisition of these traits is strongly associated with metastatic behavior reflecting the diversity of mechanisms by which TLR4, in conjunction with chemotherapy, can promote metastasis.

Paclitaxel and other cytotoxic agents share an additional functional aspect: all chemodrugs prompt BM to dispatch massive amounts of myeloid progenitors to the tumor. Studies with various chemodrugs also showed that the circulating BM-derived progenitors can be absorbed by the lungs (4, 7, 37). This event may initiate the preparation of the premetastatic niche because lung-recruited BMDC secrete inflammatory and vascular remodeling factors that increase extravasation of circulating tumor cells (4, 40), and create a tissue-regenerative environment (4). Thus, inflammatory factors produced by tumor TLR4 initiate the development of the niche while cytokines produced by lung-recruited BMDC advance its formation.

Prometastatic effects of several cytotoxic drugs have recently been reported in multiple animal models and clinical studies. Doxorubicin injected into non–tumor-bearing mice has been shown to induce the formation of the inflammasome (a multi-protein complex that generates mature IL1β) in BM cells (41). This is significant because fully processed IL1β induces a secondary inflammatory wave by upregulating IL6, G-CSF, and other mediators (41). An unrelated drug, cyclophosphamide, has been shown to promote the growth of primary tumors (42), BMDC homing to the lungs (43), enhancement of bone (5) and lung (43) metastases in mice, and increased circulating myeloid-derived suppressor cells in cancer patients (44). The impact of the cyclophosphamide-induced and host-propagated prometastatic effect can be as high as a 1,000-fold increase in pulmonary lesions formed by circulating tumor cells in drug-pretreated mice relative to controls (45). Similarly, pretreatment with cisplatin increased influx of provascular BMDC to primary tumors and the burden of pulmonary metastases by 6- to 10-fold (4). Cisplatin also significantly shortened tumor growth doubling time in lung cancer patients (46). A similar compound, oxaliplatin, increased spontaneous metastasis in an orthotopic mouse cancer model (38). Collectively, these studies suggest that damage-sensing receptors might be ultimately responsible for induction of the cytoprotective and provascular inflammatory responses that negate the clinical benefits of cytotoxic therapies.

Summary and Perspectives—How to Eliminate Tumor-Promoting Effects of Chemotherapy

Emerging evidence from multiple tumor models and clinical studies suggests a distinct possibility that commonly used anticancer drugs promote tumor cell survival and facilitate metastasis in addition to their cytotoxic effects. The main culprit underlying the prometastatic effects of chemotherapy appears to be drug-induced inflammation (Fig. 1). This response is induced by TLR4 and similar damage-sensing receptors that bear natural responsibility for restoring homeostasis to the injured tissue. In the context of TLR4-positive tumors, this local response is rapidly translated to systemic inflammation through excessive production of mediators released to the blood that expand the pools of
BM and spleen progenitors. While drug-induced apoptosis shrinks the tumor, upregulated products of the activated TLR4 pathway protect the residual tumor cells and enhance their dissemination. This prometastatic trend is strongly amplified by the host "misreading" the damage signals from the drug-destroyed tumor. The host translates these signals into a massive regenerative effort led by provascular and immunosuppressive BMDC. The widely documented immunosuppressive phenotype of tumor BMDC (47) is congruent with the late immune cell function of rebuilding the injured tissue rather than their early role in attacking undesirable cells. Once mobilized to the tumor, BMDC promote tumor vasculature that connects new blood and lymphatic vessels to systemic circulation. Access to new vasculature might be a deciding event in posttherapy tumor spread, also...
favored by the cytoprotective atmosphere of the remodeled tumor mass. BMDC also support distant metastasis because circulating immature progenitors lodge into the lungs and other organs where their vascular and tissue remodeling properties enhance extravasation of tumor cells and sustained expansion of metastatic colonies, respectively [Fig. 1].

Although TLR4 activation in malignant cells clearly benefits the tumor, stimulation of this pathway in immune cells might have both pro- and anticancer consequences. For instance, immune cells depend on TLR4 activation for effective antigen presentation and recruitment of cytototoxic T cells. This may result in decreased growth of primary tumors due to chemotherapy-induced TLR4 and recruitment of cytotoxic T cells. This may result in decreased cytotoxic therapies as well as to intercept lymphatic and distant metastasis. Here, translational science should benefit from the plethora of existing TLR4 antagonists generated to combat sepsis that have already demonstrated an acceptable safety profile in human subjects. Cumulatively, exploration of these questions should provide the mechanistic details of TLR-mediated resistance to cytotoxic therapies and be a significant step forward in addressing current challenges to eradicating cancer.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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