Chemotherapeutic Resistance: Surviving Stressful Situations

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Abstract

Chemotherapeutic regimens involve the systemic administration of genotoxic compounds that induce cancer cell death via well-established DNA damage response signaling networks. Less understood is how the treatment of other cell types within the tumor microenvironment affects the therapeutic response. Here we discuss recent work that shows that tumor-adjacent cells can respond to genotoxic stress by activating a paracrine secretory program. Although this secretory response serves to protect progenitor cells and promote tissue regeneration in conditions of cellular stress, it can also be coopted by tumor cells to survive frontline chemotherapy. Thus, local prosurvival signaling may present a fundamental barrier to tumor clearance by genotoxic agents, suggesting that effective treatments need to target both cancer cells and the tumor microenvironment.

Introduction

Tumor development and treatment occur in the context of endogenous tissue, with neoplastic cells surrounded by a diverse set of nontransformed cells and a heterogeneous stromal compartment (1). In fact, for many tumors, the stromal tissue constitutes the majority of the overall tumor mass. Tumor cells interact with normal cells in the tumor microenvironment through secreted and surface-bound proteins, and these interactions are critical for tumor progression. For example, tumor–stromal interaction is essential for numerous processes that occur during tumor development, including neovascularization, immune surveillance and evasion, and metastasis. Further, it is well established that nonneoplastic cells in the tumor microenvironment secrete a variety of factors that promote tumor cell survival and growth during various stages of tumor development. Here we discuss the emerging idea that the tumor microenvironment modulates the response to frontline cancer therapy.

Effective cancer therapy using surgery, radiotherapy, or chemotherapy results in the absence of macroscopic disease either at the site of the primary tumor or at common distal sites of disease dissemination. However, despite this initial tumor clearance, many patients who have undergone such therapy will relapse (2). Thus, small cohorts of tumor cells can survive in cryptic anatomic loci following therapy. These surviving cancer cells represent minimal residual disease (MRD) (3). Patients in disease remission can be further subclassified as MRD-positive or -negative with the use of high-resolution tumor detection techniques, including flow cytometry and PCR (4). Not surprisingly, patients who are MRD-positive have a significantly poorer prognosis than those who are MRD-negative. Although the persistence of residual disease is a well-established contributor to disease recurrence and treatment failure, preclinical models of cancer therapy have generally failed to interrogate how these cancer cells survive and relapse.

The mechanisms by which MRD survives chemotherapy despite the effective elimination of bulk tumor cell populations remain unclear (5). Tumor drug resistance is classically associated with cell-intrinsic processes, including apoptotic defects, upregulation of multidrug efflux pumps, decreased proliferation rate, and defects in DNA damage recognition (6, 7). More recently, it has been suggested that cancer stem or initiating cells are more resistant to conventional chemotherapy, and it is this population of tumor cells that fuels disease relapse (8). However, these putative resistance mechanisms for MRD have not been examined in relevant therapeutic settings, largely due to the absence of established preclinical models of MRD persistence. Thus, it is unclear whether MRD survives therapy in a stochastic or cell-autonomous manner, or if response to therapy is specific to the tumor microenvironment.

Key Findings

To investigate the mechanisms underlying the persistence of MRD, we examined the response of lymphomas in a mouse model of human Burkitt lymphoma, the Eμ-myc mouse, to conventional chemotherapy (9). In this model, transplanted lymphoma cells disseminate to all lymphatic tissues, including the bone marrow, spleen, thymus, and peripheral lymph nodes. Following administration of doxorubicin [a frontline therapy used as a component of treatment regimens for nearly
all B-cell lymphomas (BCLs), the tumor cells underwent apoptosis and were rapidly cleared from the lymph nodes, spleen, and bone marrow. Strikingly, however, tumor clearance was not universal, as a large population of surviving BCL cells persisted in the posttreatment thymus (10). These findings suggest that drug efficacy can vary among distinct tumor microenvironments.

Surviving thymic lymphoma cells in treated Eμ-\textit{mhc} mice were critically important for tumor relapse and disease progression, as mice with physical or genetic ablation of the thymus exhibited significantly longer tumor-free and overall survival following therapy. Thus, in this model the thymus represents a treatment-refractory tumor microenvironment that supports the survival of a subset of lymphoma cells, a phenomenon that parallels the persistence of MRD following therapy. Of note, the thymic microenvironment promoted lymphoma-cell drug resistance in a non–cell-autonomous manner. Specifically, soluble factors in conditioned media derived from the thymus, but not other primary lymphatic tissue, conferred resistance to doxorubicin \textit{in vitro}.

Studies in this system revealed an unexpected mechanism for surviving genotoxic stress. Briefly, endothelial cells in the tumor microenvironment responded to high levels of DNA damage induced by conventional chemotherapy by activating the p38 mitogen-activated protein kinase, which initiates an acute downstream secretory response involving multiple chemokines and cytokines. Two factors secreted by endothelial cells, interleukin 6 (IL-6) and Timp1, conferred resistance to doxorubicin in lymphoma cells \textit{in vitro} and \textit{in vivo}. Of importance, neither of these proteins affected the growth rate of lymphoma cells, suggesting that this response is prosurvival rather than promitogenic. The mechanism underlying the specific induction of a DNA damage–induced secretory response in endothelial cells in the thymus but not the peripheral lymph nodes or spleen remains unclear. One explanation may lie in the organ-specific heterogeneity and plasticity of endothelial cells in both the vasculature and lymphatics (11). Additionally, other resident cell types in the thymus may play a contributing role in promoting MRD persistence and relapse following therapy. Of interest, this specialized response to DNA damage is relevant to stress-induced thymic homeostasis in the absence of malignancy, as IL-6\textsuperscript{−/−} mice show impaired regeneration of the T-cell compartment following irradiation. Thus, in this setting, chemotherapy induces a secretory response in endothelial cells that can be coopted by tumor cells to avoid DNA damage–induced apoptosis (Fig. 1).

### Stress-Induced Secretory Phenotypes

In settings where chemotherapies show efficacy, drug-induced antitumor activity occurs rapidly following therapeutic administration. Thus, for a secretory response to affect therapeutic response, it must occur acutely. This is particularly true for hematopoietic malignancies, where tumor clearance frequently occurs within 24–48 hours of treatment. In the thymus, release of IL-6 from both human and mouse endothelial cells occurs within 24 hours of treatment, suggesting this secretory response is engaged rapidly enough to influence tumor response to DNA damage. This acute stress–associated phenotype (ASAP) is distinct from reported secretory phenotypes that are more indirectly engaged in response to DNA damage, such as the senescence-associated secretory phenotype (SASP; ref. 12). The ASAP develops gradually over the course of 5 to 8 days and occurs only after established markers of senescence are detected. However, because apoptosis in treated hematopoietic cancers occurs within 72 hours of treatment, a more rapid release of prosurvival factors would likely be essential to affect therapeutic outcome. This does not preclude a ASAP from influencing therapeutic efficacy, but its relevance may be restricted to settings such as metronomic chemotherapy, in which therapy is applied in an ongoing manner over a period of days (13).

Thus, the ASAP represents a microenvironment-specific stress response in which endothelial cells sense DNA damage and acutely activate a cytoprotective secretory program, protecting both normal and tumor cells in the thymus from apoptosis. Of note, chemotherapeutics have been shown to engage in acute protumorigenic processes in other settings. For example, treatment with paclitaxel, but not gemcitabine, can promote tumor angiogenesis through the mobilization and recruitment of bone marrow–derived endothelial cells to tumors (14, 15). This process is mediated by an acute drug-mediated release of systemic SDF-1 and G-CSF. Thus, tumor cells can coopt general stress-induced secretory responses that presumably have evolved to promote normal tissue repair and regeneration, to survive and progress after administration of frontline chemotherapy. The relevance of thymic tumor persistence in the Eμ-\textit{Myc} model to therapeutic response in human tumors remains unclear. Of note, a subset of lymphoma patients present with primary BCLs in the thymus. Mediastinal (thymic) diffuse large BCL (Med-DLBCL) is a highly aggressive disease that accounts for 5% to 10% of all DLBCLs (16). Med-DLBCL is treated with conventional chemotherapeutic regimens, all of which include anthracyclines such as doxorubicin. Although the question of how Med-DLBCLs respond to frontline chemotherapy relative to other DLBCLs (17) remains somewhat controversial, our data suggest that counteracting IL-6 function may improve Med-DLBCL patient outcome.

### Prosurvival Signals in Tissue Homeostasis and Development, and Cancer

The tumor microenvironment can present multiple barriers to effective cancer treatment. Perhaps the best established of these mechanisms are physical barriers to drug delivery. These include the classic problem of delivering drugs across the blood–brain barrier, as well as decreased drug accessibility in solid tumors due to negative interstitial fluid pressure in the tumor, aberrant tumor vasculature, or fibrosis (18). Less understood is how paracrine factors from stromal, immune, or endothelial cells promote cancer cell resistance to apoptosis (19). In this section, we focus on physiologic prosurvival signals in various anatomic contexts, with an emphasis on the IL-6 pathway.
The concept of prosurvival signaling is well described in developmental biology and occurs during both adult and embryonic development. For example, during B-cell development, IL-7 is critically required for cell survival during the transition of pre-pro to pro B cells (20). Other paracrine signals, such as the Notch, Wnt, and Hedgehog pathways, similarly support self-renewal and repopulation of stem or progenitor populations in the skin, blood, gut, and nervous system (21). In fact, metazoans have developed many evolutionarily conserved processes to modulate and repair tissues, ensuring the survival of the organism even when widespread cell death occurs in a tissue (22). These processes can be activated by diverse physiologic stresses, including ischemia, wounds, and pathogens. For example, Notch signaling from endothelial cells within the bone marrow is required for hematopoietic stem cell renewal and repopulation following irradiation (23). Additionally, it has long been appreciated that wounds or infections induce inflammation in which numerous cytokines are secreted locally and systemically (24). Indeed, this cytokine release is required for immune and stromal cell recruitment and activation of processes required for physiologic tissue restoration. Here, IL-6 is a critical prosurvival signal that is induced acutely following tissue injury and acts primarily to activate immune cells.

Emerging literature suggests that IL-6 can act as a potent prosurvival signal in many contexts. For example, viral IL-6 encoded by Kaposi sarcoma herpes virus (KSHV) promotes B-cell survival following KSHV infection (25). IL-6 is also required for liver regeneration, as shown by the fact that IL-6−/− mice die due to massive necrosis following partial hepatectomy (26). Here again, survival signaling must be acute, as mortality occurs within 24 hours of liver damage in IL-6−/− animals. In cancer, the IL-6/Jak/Stat signaling pathway is frequently activated by overexpression or activating mutations. In hepatocellular adenomas, lymphomas, and the myeloproliferative disorders polycythemia vera, essential thrombocytemia, and idiopathic myelofibrosis, most patients contain activating mutations in gp130, MYD88, or Jak2, which induce high levels of Jak/Stat signaling and drive proliferation (27–29). Furthermore, our data and those of others indicate that IL-6 can induce upregulation of antiapoptotic Bcl-2 family members. Thus, IL-6 is a potent...
prosurvival factor that can affect both tumorigenesis and response to tissue injury.

Enhancing Chemotherapeutic Efficacy by Targeting Prosurvival Signaling

The idea that tissue damage associated with chemotherapy can activate a paracrine prosurvival secretory program suggests that inhibition of signaling pathways activated by IL-6 might potentiate the therapeutic efficacy of conventional anticancer agents. In the Eμ-myc model, we tested whether chemical inhibition of Jak kinase activity, a downstream mediator of both IL-6 and Timp-1 signaling, could potentiate the action of doxorubicin. Mice treated with a pan-JAK inhibitor and doxorubicin showed significantly longer tumor-free and overall survival than mice treated with doxorubicin alone. Of importance, mice subjected to IL-6 pathway inhibition showed no tumor regression or difference in overall survival when compared with vehicle control. Thus, simply blocking a prosurvival signal may not be an effective therapy in the absence of DNA damage. Consequently, determining whether a microenvironment-specific cytokine functions as a mitogen or a survival factor is critical for determining whether a targeted agent should be used as a monotherapy or applied in combination with conventional chemotherapeutics.

Such combination therapies may be particularly important in cancers such as multiple myeloma (30). IL-6 is a tonic prosurvival factor for cultured multiple myeloma cells, such that IL-6 inhibition leads to cell death. However, clinical trials that used IL-6-neutralizing antibodies alone showed no survival benefit (31). In this malignancy, exogenous stress (culture stress or DNA damage) may be required to reveal a dependency on prosurvival signaling. Thus, although IL-6-neutralizing antibodies are not effective as single agents, combining them with high-dose chemotherapeutic regimens could improve tumor clearance in a variety of tumor types. The value of such combination regimens may hold true for both conventional chemotherapeutics and emerging targeted therapies. For example, recent work showed improved antitumor activity when IL-6 inhibition was combined with the administration of targeted therapy for the treatment of a mouse model of lung cancer (32). Additionally, inhibition of prosurvival cytokine signaling was shown to improve the efficacy of the Bcr-Abl inhibitor imatinib in the treatment of B-cell acute lymphoblastic leukemia (33).

Clues to additional tumor types that may similarly coopt IL-6 signaling following systemic DNA damage may come from an examination of nontransformed tissues that respond to IL-6 signaling. The IL-6 receptor is only expressed on hematopoietic cells and hepatocytes, and it is these two cell types that activate the majority of physiologic responses to IL-6 induction during inflammation (34). Furthermore, IL-6 promotes the pathogenesis of hepatocellular carcinoma (HCC) in response to chemical carcinogenesis in mice and underlies the gender disparity observed in HCC in humans (35). Of note, HCCs are highly treatment-refractory, yet doxorubicin treatment is the major treatment modality in unresectable disease. Additionally, a poor prognosis in HCC is strongly associated with a paracrine stromal IL-6 signature (36). These data suggest that perhaps, as in the Eμ-myc model, inhibition of IL-6 signaling could promote drug sensitivity in this tumor type.

Thus, it remains to be tested whether inhibition of acute prosurvival secretory phenotypes can promote the cytotoxic activity of conventional chemotherapeutic agents in a variety of cancers in humans. In the future, a central component of investigating this process will be rapid examination of posttreatment tumor microenvironments. Most studies that examine cytokine and chemokine levels in tumor biopsies report on steady-state concentrations in the absence of treatment, an environment that may be drastically altered in the presence of chemotherapy. Here, the analysis of tumor samples subjected to neoadjuvant treatment prior to surgery may provide key information regarding the impact of chemotherapy on the tumor microenvironment. Additionally, the application of frontline therapies to a range of existing genetically engineered mouse models of cancer would allow for a temporal analysis of dynamic changes in the tumor microenvironment that accompany drug treatment.

Conclusions

Tumors can relapse despite months to years of sustained remission following therapy. Thus, understanding how subsets of cancer cells survive treatment and where they persist during this period of remission are fundamental questions in cancer biology. It has long been appreciated that tumor initiation and progression involve a complex set of interactions between tumor cells and their associated stroma. The studies described in this review suggest that the tumor microenvironment also plays an integral role in overall therapeutic response. This is perhaps not surprising given the striking difficulties of treating tumors in their native setting versus treating tumor cells in culture. Nevertheless, this work highlights the emerging relevance of developmental biology and tissue homeostasis to the response to anticancer therapies. Understanding how cancers coopt developmental survival cues will be essential for the development of combination therapies that can achieve effective and durable treatment outcomes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Gilbert and Hemann

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