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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 impacts therapeutic response. Here we discuss recent work that shows that tumor-adjacent cells can respond to genotoxic stress by engaging a paracrine secretory program. While 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 front-line chemotherapy. Thus, local pro-survival 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 occurs in the context of endogenous tissue, with neoplastic cells surrounded by a diverse set of non-transformed 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 engaged during tumor development, including neo-vascularization, immune surveillance and evasion and metastasis. Further, it is well established that non-neoplastic 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 front-line 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 common distal sites of disease dissemination. However, despite this initial tumor clearance, many of these patients will relapse (2). Thus, small cohorts of tumor cells can survive in cryptic anatomical loci following therapy. These surviving cancer cells represent minimal residual disease (MRD) (3). Patients in disease remission can be further sub-classified as MRD positive or negative using high-resolution tumor detection techniques, including flow cytometry or PCR (4). Not surprisingly, patients who are MRD positive have a significantly poorer prognosis than those who are MRD negative. While 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.

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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 multi-drug 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 that 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 pre-clinical models of MRD persistence. Thus, it is unclear whether MRD survives therapy in a stochastic manner, a cell autonomous manner or whether response to therapy is tumor microenvironment specific.

Key findings

To investigate the mechanisms underlying the persistence of MRD, we examined the response of lymphomas in a mouse model of human Burkitt's 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 front-line therapy used as a component of treatment regimens for nearly all B cell lymphomas – tumor cells underwent apoptosis and were cleared from the lymph nodes, spleen and bone marrow rapidly following treatment. Strikingly, however, tumor clearance was not universal, as large population of surviving B lymphoma cells persisted in the post-treatment thymus (10). These findings suggest that drug efficacy can vary among distinct tumor microenvironments.

Surviving thymic lymphoma cells in treated *Eµ-myc* 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. Notably, 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 *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 Map Kinase, which initiates an acute downstream secretory response involving multiple chemokines and cytokines. Two factors secreted by endothelial cells, IL-6 and Timp1, conferred resistance to doxorubicin in lymphoma cells *in vitro* and *in vivo*. Importantly, neither of these proteins affected the growth rate of lymphoma cells, suggesting that this response is pro-survival rather than pro-mitogenic. 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 might play a contributing role in promoting MRD persistence and relapse following therapy. Interestingly, this specialized response to DNA damage is relevant to stress-induced thymic homeostasis in the absence of malignancy, as *IL-6 −/−* mice show impaired regeneration of the T cell compartment following irradiation. Thus, in this setting, chemotherapy engages a secretory response in

endothelial cells that can be co-opted by tumor cells to avoid DNA damage-induced apoptosis (Figure 1).

Stress induced secretory phenotypes

In settings where chemotherapies show efficacy, drug-induced anti-tumor 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) (12). The SASP develops gradually over the course of 5–8 days and occurs only after established markers of senescence are detected. However as apoptosis in treated hematopoietic cancers occurs within 72 hours of treatment, a more rapid release of pro-survival factors would likely be essential to impact therapeutic outcome. This does not preclude a SASP 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. Notably, chemotherapeutics have been shown to engage acute pro-tumorigenic 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 co-opt general stress induced secretory responses that have presumably evolved to promote normal tissue repair and regeneration, to survive and progress after administration of front-line chemotherapy.

The relevance of thymic tumor persistence in the *Eμ-Myc* model to therapeutic response in human tumors remains unclear. Notably, a subset of lymphoma patients present with primary B cell lymphomas in the thymus. Mediastinal (thymic) B-cell lymphoma (Med-DLBCL) is a highly aggressive disease which represents 5–10% of all diffuse large B-cell lymphomas (DLBCL) (16). Med-DLBCL is treated with conventional chemotherapeutic regimens, all of which include anthracyclines such as doxorubicin. While it remains somewhat controversial how Med-DLBCL respond to front-line chemotherapy relative to other DLBCL (17), our data suggest counteracting IL-6 function may improve Med-DLBCL patient outcome.

Pro-survival signals in tissue homeostasis, 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 will focus on physiological pro-survival signals in various anatomical contexts, with an emphasis on the IL-6 pathway.

The concept of pro-survival 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 survival of the organism even when wide spread cell death occurs in a tissue (22). These processes can be engaged by diverse physiological 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 pro-survival 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 pro-survival signal in many contexts. For example, viral IL-6 encoded by Karposi's sarcoma herpes virus (KSHV) promotes B cell survival following KSHV infection (25). IL-6 is also required for liver regeneration, as *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 thrombocythemia, and idiopathic myelofibrosis, most patients contain activating mutations in either gp130, MYD88, or Jak2 which induce high levels of Jak/Stat signaling and drive proliferation (27–29). Furthermore, our data and that of others indicate that IL-6 can induce up-regulation of anti-apoptotic Bcl-2 family members. Thus, IL-6 is a potent pro-survival factor that can affect both tumorigenesis and response to tissue injury.

Enhancing chemotherapeutic efficacy by targeting pro-survival signaling

The idea that tissue damage associated with chemotherapy can activate a paracrine pro-survival secretory program suggests that inhibition of signaling pathways activated by IL-6 might potentiate the therapeutic efficacy of conventional anti-cancer 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. Importantly, mice subjected to IL-6 pathway inhibition showed no tumor regression or difference in overall survival when compared to vehicle control. Thus, simply blocking a pro-survival 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 chemotherapies.

Such combination therapies may be particularly important in cancers like multiple myeloma (30). IL-6 is a tonic pro-survival factor for cultured multiple myeloma cells, such that IL-6 inhibition leads to cell death. However, clinical trials using IL-6 neutralizing antibodies alone show no survival benefit (31). In this malignancy, exogenous stress (culture stress or DNA damage) may be required to reveal a dependency on pro-survival signaling. Thus, while not effective as a single agent, combining IL-6 neutralizing antibodies 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 as well as emerging targeted therapies. For example, recent work has shown improved anti-tumor 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 pro-survival cytokine signaling has been shown to improve the efficacy of the Bcr-Abl inhibitor imatinib in the treatment of B cell Acute Lymphoblastic Leukemia (33).

Clues for additional tumor types that may similarly co-opt IL-6 signaling following systemic DNA damage may come from an examination of non-transformed 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 engage the majority of physiologic responses to IL-6 induction during inflammation (34). Furthermore, in mice, IL-6 promotes the pathogenesis of hepatocellular carcinoma (HCC) in response to chemical carcinogenesis and underlies the gender disparity observed in HCC in humans (35). Notably, hepatocellular carcinomas are highly treatment refractory, yet doxorubicin treatment is the major treatment modality in unresectable disease. Additionally, 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 pro-survival secretory phenotypes can promote the cytotoxic activity of conventional chemotherapeutic agents in a variety of cancers in humans. In the future, one central component of investigating this process is the rapid examination of post-treatment tumor microenvironments. Most studies examining 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 neo-adjuvent treatment prior to surgery may provide key information regarding the impact of chemotherapy on the tumor microenvironment. Additionally, the application of front-line 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 in 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 anti-cancer therapies. Understanding how cancers co-opt developmental survival cues will be essential for the development of combination therapies that can achieve effective and durable treatment outcomes.

Acknowledgments

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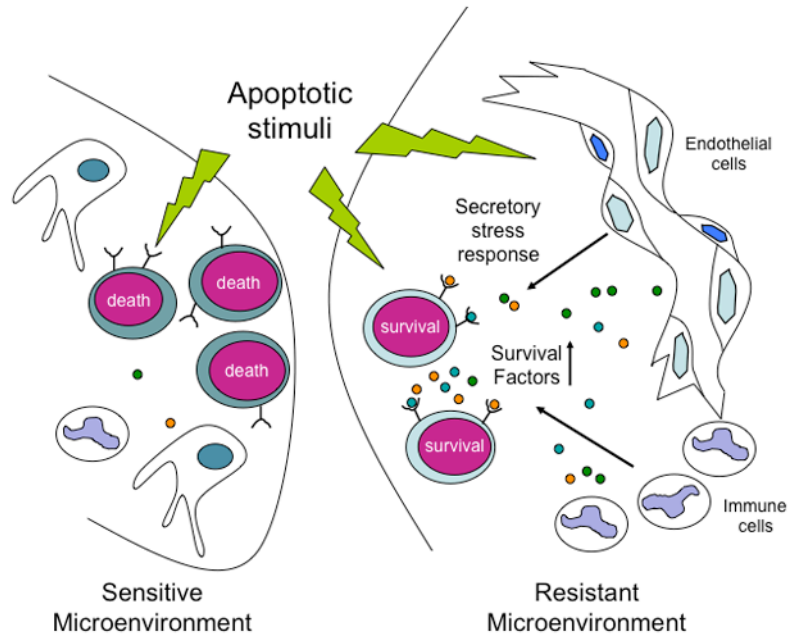
Figure 1.
A diagram showing microenvironment-specific responses to front-line chemotherapy. (Left) Systemic chemotherapy can effectively clear the majority of lymphoid tumor cells. (Right) However, genotoxic damage can also engage organ and cell-type specific stress responses. Paracrine pro-survival signaling in select tumor microenvironments can counter the efficacy of anti-cancer agents, leading to the persistence of minimal residual disease.