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Mini-review

Immunogenic chemotherapy: Dose and schedule dependence and combination with immunotherapy

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ABSTRACT

Conventional cytotoxic cancer chemotherapy is often immunosuppressive and associated with drug resistance and tumor regrowth after a short period of tumor shrinkage or growth stasis. However, certain cytotoxic cancer chemotherapeutic drugs, including doxorubicin, mitoxantrone, and cyclophosphamide, can kill tumor cells by an immunogenic cell death pathway, which activates robust innate and adaptive anti-tumor immune responses and has the potential to greatly increase the efficacy of chemotherapy. Here, we review studies on chemotherapeutic drug-induced immunogenic cell death, focusing on how the choice of a conventional cytotoxic agent and its dose and schedule impact anti-tumor immune responses. We propose a strategy for effective immunogenic chemotherapy that employs a modified metronomic schedule for drug delivery, which we term medium-dose intermittent chemotherapy (MEDIC). Striking responses have been seen in preclinical cancer models using MEDIC, where an immunogenic cancer chemotherapeutic agent is administered intermittently and at an intermediate dose, designed to impart strong and repeated cytotoxic damage to tumors, and on a schedule compatible with activation of a sustained anti-tumor immune response, thereby maximizing anti-cancer activity. We also discuss strategies for combination chemo-immunotherapy, and we outline approaches to identify new immunogenic chemotherapeutic agents for drug development.

1. Introduction

Cancer is a disease of malignant cells that interact with and co-opt their environment in complex ways, stimulating tumor growth, angiogenesis, invasion and metastasis and fostering an immune suppressive environment that counters the tumoricidal effects of many cytotoxic anti-cancer agents [1]. To be most effective, anti-cancer therapies need to take into account drug effects on the tumor microenvironment. This environment is dynamic and can be remodeled through interventions that alter the interactions between tumor cells and stromal cells, creating new therapeutic opportunities [2]. Certain conventional tumor cell cytotoxic and cytostatic cancer chemotherapeutic drugs have the potential to increase tumor cell immunogenicity by activating immunogenic cell death (ICD), an immunostimulatory form of cell death that activates innate immune responses and also elicits a tumor-specific adaptive immune response [3–5], with an increase in overall anti-tumor efficacy compared to tumor cell cytotoxicity alone [3,6]. In practice, however, the toxicity of these and many other cancer chemotherapeutic drugs to T cells, natural killer (NK) cells and dendritic cells (DCs) limits the extent of immune stimulation and can lead to immunosuppression [7,8]. Here we review studies on the actions of drugs that induce ICD, focusing on the dose and schedule dependence of conventional chemotherapy-activated immune responses and on combinations with immunotherapy, both in mouse models and in the clinic. We propose that anti-cancer chemo-immunotherapeutic responses to drugs that induce ICD can be optimized by using a modified metronomic schedule for drug delivery, which we term MEDIC, medium-dose intermittent chemotherapy. Finally, we outline approaches to identify novel lead immunogenic chemotherapeutic agents for drug development.

Abbreviations: DC, dendritic cell; ICD, immunogenic cell death; IFN, interferon; MEDIC, medium-dose intermittent chemotherapy; MTD, maximum tolerated dose; NK, natural killer; TLR, toll-like receptor; Treg, regulatory T cells.

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2. Chemotherapy-induced ICD

Doxorubicin, cyclophosphamide and several other cancer chemotherapeutic drugs have the capacity to induce ICD. Key events in this cell death pathway include: early translocation to the tumor cell surface of the endoplasmic reticulum chaperone protein calreticulin, which generates an essential "eat-me" signal for DC engulfment and tumor antigen uptake [9,10]; secretion of ATP from lysosomal stores, which stimulates macrophage recruitment and maturation [11], induces NK cell proliferation, and stimulates IFNγ production [12]; and post-apoptotic release of the nuclear chromatin binding protein HMGB1, which activates toll-like receptor 4 (TLR4) and mediates nucleic acid-activation of TLRs 3, 7 and 9 [13,14]. Certain ICD drugs can also activate type-I interferon signaling pathways in tumor cells, which may contribute to the downstream activation of host antitumor immunity [15].

ICD-induced translocation of HMGB1 from the nucleus to the cytoplasm is followed by HMGB1 release into the extracellular matrix of dying tumor cells. This release enables HMGB1 to interact with TLR4 expressed on DCs, thereby stimulating antigen presentation by DCs as well as DC production of IL1β, which activates $CD8^+$ T cells [16,17]. ATP secreted from dying tumor cells can act on DC purinergic P2RX7 receptors to activate $CD8^+$ T cells [16,18]. The importance of chemotherapy-induced ICD is highlighted by the low efficacy of chemotherapy in cells with loss-of-function alleles of TLR4 and P2RX7 [7,17,18]. Tumor cell surface molecules that present "don't eat me" signals for DCs, including CD31, CD46, and CD47, are down regulated during ICD, allowing the eat-me signals to prevail and phagocytosis of apoptotic corpses to occur [19]. Molecular chaperones such as HSP90 appear on the tumor cell surface, enhancing DC-tumor cell adhesion and stimulating DC maturation [20]. Factors that inhibit ICD include: CD39/ENTPD1, which hydrolyzes extracellular ATP [21]; CD73/NT5E, which converts AMP into adenosine and is highly immunosuppressive of macrophages, NK cells and T cells [12]; and CD47, which counters the phagocytic signal of surface-expressed calreticulin [22].

Chemotherapy can also increase tumor cell immunogenicity by inducing expression of MHC-I molecules and tumor-specific antigens on the tumor cell surface [23]. Chemotherapy-induced stress may also activate NK cells by inducing expression of NK cell stimulatory ligands, such as NKG2D activating ligands [24,25] and by decreasing tumor cell surface levels of NK cell inhibitory ligands [26,27]. Death receptors present on the tumor cell surface, such as TRAIL receptor and mannose-6-phosphate receptor, can also be induced by chemotherapy, rendering tumor cells susceptible to immune cell attack [28,29].

Some of the stimulatory immune responses to cytotoxic anticancer drug treatment may result from the transient lymphopenia that many of these drugs induce, as seen in both animal models and in the clinic [30,31]. Lymphopenia is associated with up regulation of host danger-sensing and repair mechanisms, which lead to a "storm" of cytokines and chemokines, DC differentiation, maturation and homeostatic proliferation, T cell activation, and antitumor immune cell recruitment into tumors [32,33]. Depletion of chemotherapy-sensitive immune suppressive cells, such as myeloid-derived suppressor cells and circulating Tregs [34], can lead to restoration of NK cell effector function and T cell proliferation in patients [35] and contribute to the immune stimulatory effects of chemotherapy.

3. Dependence of ICD on choice of chemotherapeutic drug and tumor model

Anticancer drugs that induce ICD include cyclophosphamide, doxorubicin, epirubicin, idarubicin, mitoxantrone, and oxaliplatin [36–39]. The impact of ICD can be seen when immune competent mice are injected with tumor cells treated *ex vivo* with mitoxantrone, doxorubicin or idarubicin, which confers immunity against live tumor cell challenge on the opposite flank. Thus, the ICD drug-treated tumor cells immunize the host to the tumor and thus serve as an anti-cancer vaccine [9]. Other DNA-damaging agents, such as etoposide and mitomycin C, are non-immunogenic, and show little such vaccine activity when tested in the same experimental setting [9]. However, the immunogenicity of etoposide and mitomycin C becomes apparent when calreticulin is overexpressed or when protein phosphatase-1/GADD34 complex, a negative regulator of calreticulin exposure, is inhibited [9]. Poor calreticulin exposure is thus a critical determinant of the inability of these two drugs to induce ICD. While oxaliplatin and cisplatin both trigger HMGB1 release in colon cancer cells, oxaliplatin, but not cisplatin, stimulates calreticulin exposure and induces anticancer immunity in mice *in vivo* [40]. In other studies, the ICD drugs doxorubicin and idarubicin, but not the non-ICD drugs gemcitabine and etoposide, activate markers of ICD and stimulate various immune responses, including tumor cell uptake by DCs, DC maturation, and T cell activation [41]. Thus, non-ICD chemotherapeutic drugs may be non-immune stimulatory because of their inability to activate one or more of the cellular responses required to elicit ICD. Cell-based assays for the classic features of ICD (calreticulin exposure, HMGB1 release, etc.) can therefore be very useful, both from a mechanistic perspective and for their utility in screening for candidate ICD drugs (see below). However, evidence for a functional immunogenic response *in vivo* is ultimately required, for instance, by testing for the ability of *ex vivo* drug treated tumor cells, when injected on one flank of a mouse, to induce the rejection of live tumor cells injected on the opposite flank (vaccine activity assay) [9]. Such an assay can distinguish drugs (or drug-tumor cell combinations; see below) that show one or more hallmarks of ICD (e.g., calreticulin translocation or HMGB1 release) from those that additionally show a *bona fide* ICD response.

Cyclophosphamide, when given on a 6-day repeating schedule, induces robust innate anti-tumor immune responses leading to major tumor regression in glioma-bearing *scid* immunodeficient mice [42–45]. Tumor regression is abolished in NSG mice, where NK cells are absent and macrophages are dysfunctional, highlighting the essential role of the innate immune system in the overall anti-tumor response [42]. KM12 colon cancer xenografts given the same cyclophosphamide regimen do not show these responses, despite the intrinsic chemo-sensitivity of KM12 tumor cells to activated cyclophosphamide [46]. In C57BL/6 mice, which are fully immune competent, the every 6-day cyclophosphamide schedule cures GL261 gliomas by an NK cell- and $CD8^+$ T cell-dependent mechanism. In contrast, the same treatment regimen effects only modest growth delay and little or no immune responses in LLC lung carcinoma and B16F10 melanoma models, despite their intrinsic sensitivity to cyclophosphamide cytotoxicity [47]. Thus, immune effects of an ICD drug, such as cyclophosphamide, can differ dramatically between tumor models and/or tumor types, and most likely, between individual cancer patients as well. Tumors unresponsive to the immunogenic actions of cyclophosphamide may be deficient in factors essential for ICD, such as stress ligands like MHC class I [23], or may express factors that confer resistance to ICD, such as PD-L1 [48]. Tumor mutational burden and the presence of neo-antigens [49] may also be a factor in the responsiveness of a tumor to an ICD drug. Tumor vascularity may also be a factor, as poorly perfused tumors could present a barrier to drug access and/or immune cell infiltration [50]. Given this tumor model dependence of ICD, it is important to identify biomarkers that distinguish ICD immune responsive from non-responsive tumors and patients [47].