Attacking malignant cells that survive therapy
Exploiting immunogenic modulation

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We have recently defined “immunogenic modulation,” a mechanism whereby malignant cells that survive anticancer therapy, due to sublethal delivery or development of treatment resistance, become nonetheless more sensitive to killing by cytotoxic T lymphocytes. This mechanism can be exploited to identify which therapies will best synergize with immunotherapy, potentially maximizing patient clinical benefit.

Introduction

Anticancer therapies aim at eradicating malignant lesions through the direct killing of neoplastic cells. However, achieving a complete and sustained clinical remission is elusive for most patients receiving standard-of-care therapeutic regimens. Striking clinical observations in recent years indicated that patients harboring certain malignancies achieved higher clinical benefit with immunotherapy if previously treated with certain anticancer therapies. These observations are now supported by findings demonstrating that some conventional chemotherapeutic as well as emerging anticancer agents modulate the tumor to become an immunostimulatory milieu. The immunological consequences of anticancer therapy stem from the modulation of the immune system as well as from direct effects on malignant cells.1 Anticancer therapies can induce a continuum of immunogenic alterations in malignant cells that can manifest in dying neoplastic cells, in the surviving cancer cell population, or on both (Fig. 1). These changes can be harnessed to improve the clinical benefits of subsequent or concomitant immunotherapy.

Elegant studies by the Zitvogel and Kroemer laboratories have established that malignant cells dying upon exposure to radiation or selected chemotherapeutics can elicit strong antitumor immune responses. Such a cell death modality has been designated “immunogenic cell death” (ICD).2 We have examined the immunological consequences of multiple anticancer treatment modalities on the population of malignant cells that survive therapy, either because of sublethal delivery or upon the development of treatment resistance, and demonstrated the importance of combining conventional treatments with therapeutic anticancer vaccines and other immunotherapeutic platforms.3–7

Molecular Mechanisms of Immunogenic Modulation

Our investigations have highlighted a novel mechanism, complementary to ICD, whereby anticancer therapies alter the biology of surviving cancer cells to render them more sensitive to antigen-specific CD8+ T cell-mediated lysis.1–7 We defined this mechanism “immunogenic modulation” (IM). IM encompasses a spectrum of molecular alterations in the biology of cancer cells that, independently or collectively, render neoplastic cells more susceptible to cytotoxic T lymphocyte (CTL)-mediated destruction. These include (1) changes in the surface phenotype of cancer cells, including the exposure of calreticulin on the outer leaflet of the plasma membrane,3–6 (2) the downregulation of anti-apoptotic and/or pro-survival genes,1,3 and (3) the modulation of components of the antigen-processing machinery (APM).6,7 (Fig. 1).

Various treatment modalities have been shown to induce IM in tumors of diverse origin by increasing the levels of immunologically relevant proteins on the surface of cancer cells, including multiple tumor-associated antigens (TAAs), adhesion molecules, e.g., intercellular adhesion molecule 1 (ICAM1), heat-shock 70 kDa protein (HSP70), and MHC class I molecules.3–5 These phenotypic changes, as triggered by exposure to sublethal doses of irradiation, chemotherapy with cisplatin plus vinorelbine, or androgen deprivation with enzalutamide, have been shown to translate into an increased sensitivity of murine and/or human cancer cells to CTL-mediated lysis in vitro. In a murine colon carcinoma model, the use of radiofrequency ablation (RFA) confirmed and expanded the spectrum of phenotypic changes induced by this treatment modality in vivo, resulting in sequential T-cell responses to multiple TAAs.3 Importantly, combining RFA with a therapeutic vaccine targeting...
Figure 1. Rationale behind immunogenic modulation. Multiple immunogenic effects elicited by radiation therapy, conventional chemotherapeutics, small molecule inhibitors, and androgen deprivation can be exploited to improve the efficacy of anticancer immunotherapy.
carcinoembryonic antigen (CEA) resulted in synergistic and effective antitumor immunity.  

Enzalutamide, a second generation androgen receptor antagonist, has recently been approved by the US Food and Drug Administration (FDA) for the treatment of castration-resistant prostate carcinoma (CRPC). Enzalutamide has been shown to modulate the phenotype of murine prostate carcinoma cells, augmenting their sensitivity to CTL-mediated lysis. Notably, the combination of enzalutamide with a therapeutic anticancer vaccine encoding twist family bHLH transcription factor 1 (TWIST1), a transcription factor involved in the metastatic process, translated into a significant increase in the overall survival (27.5 vs. 10.3 weeks) of mice bearing spontaneous prostate adenocarcinomas (TRAMP model).  

Accumulating evidence suggests that the downregulation of anti-apoptotic and/or pro-survival genes may be an important component in the molecular signature of IM. A robust downregulation of multiple pro-survival genes, including anti-apoptotic members of the Bcl-2 gene family, has been observed in murine and/or human carcinoma cells exposed to sublethal doses of chemotherapy (cisplatin plus vinorelbine), chemoradiation (5-fluorouracil, cisplatin and radiation), and a small molecule BCL-2 inhibitor.  

Successful IM and the consequent CTL-mediated lysis of cancer cells is critically dependent upon the proper presentation of CD8-restricted peptide/MHC class I complexes on the surface of tumor cells, a process that is controlled by the cooperative action of multiple components of the APM. Defects in the APM of cancer cells limit T-cell recognition and correlate with poor clinical prognosis. Recent investigations have uncovered a potential role of anticancer therapy in modulating the activity of the APM. Malignant cells recovering from radiation therapy exhibit increased sensitivity to CTL-mediated killing while displaying an altered antigenic repertoire, and show increased transporter 1, ATP-binding cassette, sub-family B (TAP1) activity. We have recently demonstrated that docetaxel induces IM by upregulating the expression of various APM components, in vitro and in vivo. Furthermore, the exposure of human carcinoma cells to docetaxel, while not eliciting ICD, increased the sensitivity of surviving cancer cells to the cytotoxic activity of CTLs, an effect that was largely mediated by the translocation of calreticulin to the cancer cell surface. This process highlights a novel role for this component of the APM with potential implications for immunotherapy.  

**Clinical Implications**  

The preclinical findings described above have translated into various clinical trials, some of which have already generated encouraging preliminary data. In a randomized Phase II study, metastatic breast cancer patients who received docetaxel combined with the therapeutic anticancer vaccine PANVAC (a poxvirus-based vaccine encoding 2 TAAs, namely, CEA and mucin 1, along with a TR1ad of C0stimulatory Molecules, designated TRICOM) attained a superior progression-free survival (PFS) as compared with individuals receiving docetaxel alone (6.6 vs. 3.8 mo). In an ongoing multi-center Phase II clinical trial in which metastatic CRPC patients were randomized to receive 153Sm-EDTMP (Quadramet®; from Cytygen Corp, Princeton, NJ), an FDA-approved radiopharmaceutical targeting bone metastases) alone or in combination with PSA-TRICOM, an interim analysis demonstrates improved PFS among patients receiving the combination regimens as compared with subjects treated with 153Sm-EDTMP alone, warranting continued accrual. Thus, one can envision that the immunogenic consequences of anticancer therapy, ranging from ICD to IM, can be harnessed to maximize the clinical benefits provided by cancer vaccines and other immunotherapeutic regimens.  

**Disclosure of Potential Conflicts of Interest**  

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