Review

The Inherent Premise of Immunotherapy for Cancer Dormancy

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Abstract

Clinical cancer dormancy is evident from the detection of circulating tumor cells in the blood and tissue-residing disseminated tumor cells in the bone marrow of cancer survivors who have been clinically disease free. Emerging evidence from clinical and preclinical studies suggests that tumor dormancy is a critical step in the development of both primary cancer and advanced-stage disease. In this review, it is shown that (i) naturally occurring tumor dormancy precedes occurrence of primary cancer, and (ii) conventional cancer therapies result in treatment-induced tumor dormancy, which in turn could lead to distant recurrence of cancer or permanent tumor dormancy, depending on immunogenic status of dormancy. Given that cellular dormancy is an evolutionary conserved survival mechanism in biologic systems, any stress or cytotoxic therapy could trigger cellular dormancy. Therefore, a successful cancer therapy is likely to be achieved by establishing permanent tumor dormancy and preventing distant recurrence of cancer or by eliminating dormant tumor cells. This could be accomplished by cancer immunotherapy because of the establishment of long-term memory responses. Cancer Res; 74(23); 6745-9. ©2014 AACR.

Introduction

In general, primary cancers, breast and prostate cancers in particular, tend to be responsive to conventional therapies and do not usually cause mortality in patients. Mortality is a result of disease recurrence at a distant site to which the disease has metastasized. This metastatic recurrent disease develops from residual tumor cells that emerge during conventional cancer therapies and lay dormant for months, years, or even decades. There is no effective therapy that could eliminate dormant tumor cells; neither is there an effective therapy that could prevent the establishment of tumor dormancy during the treatment of primary cancer. This is because of the existence of an evolutionary conserved survival mechanism in the cells that protects them from environmental hazards, including radiation, pollution, toxic compounds, and oxidative damage. There are striking similarities between the concept of tumor dormancy and the cancer stem cell theory of tumor progression. The tumor dormancy hypothesis similarly predicts that cellular homeostasis controls cell proliferation by orchestrating the activity of oncogenes and tumor-suppressor genes. They also overlap in predicting drug resistance and cell-cycle modifications in tumor cells. However, they differ in that dormant tumor cells are not limited to stem-like spheroid forming or nonadherent cells, and that cancer stem cells are not always quiescent and most often continuously give rise to daughter tumor cells to form cancer. In other words, cancer stem cells could become dormant, but not all dormant tumor cells exhibit markers of cancer stem cells. Therefore, dormancy and stemness could be mutually exclusive. Controversial reports in the field as to specific markers for the stemness in different types of cancer and for the characterization of dormant tumor cells in the circulation of healthy individuals make a comparative analysis of dormant tumor cells and cancer stem cells difficult. Advances in our understanding of tumor dormancy could lead to the development of an effective therapeutic strategy that will maintain residual tumor cells in permanent dormancy or eliminate dormant cells without causing toxicity to normal tissues. In this review, it is suggested that immunotherapy is a promising strategy that could target chemo-refractory and radiation-refractory dormant tumor cells to reduce cancer mortality.

Tumor dormancy precedes the establishment of primary cancer

Biologic systems have an evolutionary conserved regulatory equipment to keep organs at a precise size such that certain size is restored if the organ is injured or altered in size. For instance, if part of the liver is removed, liver cells begin to divide to the extent that the liver reaches normal size (1). This cellular homeostasis can be regulated by a balanced cell growth and apoptosis, which could also protect individuals from progression of nascent transformed cells to cancer. Without such cellular homeostasis human beings could have become extinct because environmental hazards continually induce cell transformation. Therefore, malignant cells will not necessarily establish cancer as long as cellular homeostasis keeps them...
in dormant state. In fact, clinically evident cancers result from the loss of cellular homeostasis in dormant tumor cells. Dormant tumor cells have been detected in autopsies of individuals who were cancer free and died from noncancer causes. For example, 39% of women autopsied between the ages 40 and 50 years had indolent malignant cells, whereas the clinical incidence of breast cancer in the same age range is only 1% (2). Similar occult tumors have been detected for other types of cancers, including thyroid, prostate, pancreatic cancers, and follicular lymphoma. This seems to be a general phenomenon, which is called “cancer without disease” (3). This homeostasis-associated tumor dormancy can be disrupted by epigenetic changes that regulate angiogenesis. It has been shown that human tumors can survive in the absence of angiogenic activity and lay dormant for years without establishing cancer (3). Awakening of dormant tumor cells to establish primary cancer is associated with downregulation of angiogenesis inhibitors, which is a late event during carcinogenesis (4). Long-term tumor dormancy for at least 300 days has also been reported in a mouse model of pregnancy-dependent mammary carcinoma (5). We also reported the presence of malignant mammary cells before the development of spontaneous mammary carcinoma in FVB/N202 transgenic mice (6). An elegant review article by Bissell and Hines (7) gathered histologic evidence in support of the existence of tumor dormancy in cancer-free individuals, some of whom were in their twenties.

Naturally occurring tumor dormancy does not seem to be controlled by the immune surveillance only, but rather by an intrinsic cellular homeostasis that controls cell proliferation. Tumor immune surveillance is a major component of long-lasting tumor dormancy, only when tumor cells are highly immunogenic, for example, following immunogenic chemotherapy or radiotherapy of primary cancers, as discussed below, or in virally induced malignancies. This could explain an increased incidence of viral-driven cancers compared with spontaneous cancers in immunocompromised patients or in transplant recipients.

Presence of dormant malignant cells before a cancer is clinically apparent suggests that many cancer-free individuals may not be cancer-free per se rather they could be cancer survivors because of harboring dormant malignant cells, which could establish a primary cancer at any time. High-risk individuals, including heavy smokers, those with a family history of cancer or hereditary cancer as well as those who are exposed to carcinogens in workplace perhaps, harbor dormant malignant cells that could be detected in the blood and targeted by an effective therapeutic strategy to prevent cancer development.

**Treatment-induced tumor dormancy and metastatic recurrence of cancer**

Clinical relevance of cancer dormancy following a successful treatment of primary cancer has been proven by the observation that recipients of organ transplantation developed cancer that could be tracked back to the donors who were cancer survivors. In addition, circulating tumor cells (CTC) that are found in the blood as well as tissue-residing disseminated tumor cells (DTC) that are found in the bone marrow or other organs have been detected in cancer survivors. In the case of carcinomas, DTC are capable of adhering to the tissue culture flask, *in vitro*, whereas CTCs are floating cells that are incapable of adherence unless they can reawaken during tumor relapse.

It has been shown that conventional cancer therapies can concurrently induce apoptosis and inhibit tumor cell proliferation. This dual function of cancer therapies is apparent because of the heterogeneity of the tumor cells differentially responding to treatment, that is, cell death versus growth inhibition. In fact, residual surviving tumor cells that escape from chemotherapy- or radiotherapy-induced apoptosis are likely to be in a state of indolent proliferation and dormancy, as long as the treatment regimen is continuous. Such dormant tumor cells become resistant to apoptosis induced by chemotherapy or radiotherapy, because of being in a quiescent state (8, 9). CTC have been detected in the blood of breast cancer survivors several years after successful treatment of primary breast cancer (10). In an *in situ* model of tumor recurrence, chemotherapy of breast and prostate tumors established chemo-resistant dormant tumor cells that resumed proliferation after drug removal (11). Treatment-induced tumor dormancy is also evident in patients with platinum-resistant and platinum-responsive ovarian cancer who eventually develop tumor relapse. In humans, DTCs that were found to be quiescent were isolated from the bone marrow after surgical removal of the primary lesion (12). Sixty-three percent of prostate cancer survivors harbor DTC in their bone marrow (13). A comparative analysis of dormant cells in patients with lung cancer (14) and breast cancer (15) shows that breast CTC and DTC are more resistant to chemotherapy than dormant lung cancer cells, because the former contains higher fraction of Ki67-negative indolent cells. DTCs that can resume proliferation and establish distant metastasis have been recently reported by Ghajar and colleagues (16). Using a mouse model of human breast cancer metastasis, they showed that DTCs reside on the endothelium of the microvasculature in the lung, bone marrow, and brain, which are common metastatic destinations of breast cancer.

Detection of CTC and DTC in patients with cancer is not limited to those with metastatic disease, as patients with nonmetastatic cancer or early-stage breast cancer also harbor CTC (10, 17). Most of these methods for detecting CTC are based primarily on epithelial markers, which miss out CTCs that are undergoing epithelial–mesenchymal transition (EMT). Mesenchymal CTCs are also correlated with poor prognosis and resistance to chemotherapy. CellSearch is the only CTC detection method approved by the FDA in patients with advanced breast, prostate, and colorectal cancers. A baseline detection of five CTC per 7.5 mL blood or higher can predict prognosis. In patients with hepatocellular carcinoma, high incidence of recurrence after hepatectomy or orthotopic liver transplantation was suggested to be due to the presence of CTC (18).

Not all patients with dormant CTC or DTC develop recurrence and metastasis. In fact, 50% of DTC-positive patients...
Immunotherapy of Cancer Dormancy

Immunotherapy is a promising approach for tumor dormancy and elimination of cancer-related mortality

Immune-mediated tumor dormancy has been documented during unintentional transplantation of cancer into immunocompromised recipients from organ donors who were in clinical remission (19) or with no clinical history of cancer (20). Immune-mediated tumor dormancy has also been demonstrated in the animal model of methylcholanthrene-induced sarcoma (21). This elegant study showed that immunity can induce and maintain tumor dormancy by cytostatic and cytolytic functions. This phenomenon was termed "equilibrium," which is not limited to cancer. Immunity against Mycobacterium tuberculosis is also of cytostatic nature that keeps the infectious agent in dormant or latent state, thus protecting the host from active disease. Long latency before the appearance of AIDS is also evident in the presence of the immune response. In fact, HIV latency is influenced by the innate immune response producing cell restriction factors that inhibit the postintegration steps in the virus replication cycle. HLA class I–restricted CD8⁺ T cells can also modulate HIV reservoirs during natural infection. Sustaining such host-protective immune responses by booster immunization or novel strategies that could give rise to memory cells is feasible and safe.

It has been shown that T cells can prevent spontaneous tumor metastasis by maintaining malignant cells in permanent dormancy (22). In a murine model of B-cell lymphoma, Farrar and colleagues reported that IFN-γ–producing CD8⁺ T cells can establish and maintain tumor dormancy (23). Preclinical studies show that indolent tumor cells in the bone marrow are the source of antigenic stimulation that could maintain T-cell memory pool (24) to keep indolent tumor cells in check. Cancer vaccines that target tumor blood vessel antigens could also induce and maintain CD8⁺ T-cell–dependent tumor dormancy (25). Immune-mediated tumor dormancy has been suggested to be the major mechanism for keeping residual tumor cells in check after the completion of conventional cancer therapies. Immune-mediated maintenance of tumor dormancy or "equilibrium" could be due to "adaptation," which is the mechanism by which tumor cells and tumor-reactive immune cells modulate each other without being eliminated, ignored, suppressed, or dominated. In a recent perspective article, the adaptation model of immunity has been introduced and discussed (26). In fact, anticancer chemotherapies are efficient primarily when they elicit immunogenic cell death. Some drugs that induce immunogenic cell death include but are not limited to anthracyclines, oxaliplatin, and cyclophosphamide. Immunogenic cell death or dormancy is characterized by a pre-mortem endoplasmic reticulum stress–associated exposure of calreticulin to the cell membrane and secretion of adenosine triphosphate (ATP), as well as a postmortem release of high-mobility group box 1. Immune-mediated dormancy can be established by cancer therapies that induce caspase-dependent apoptosis rather than caspase-independent mitochondrial apoptosis. This is because ATP redistribution from lysosomes to autophagolysosomes and its secretion require the lysosomal protein LAMP1, which translocate to the cell membrane in a caspase-dependent manner. In addition, ATP release requires the opening of pannexin 1 (PANX1) channel, which is triggered by caspases. Taxol has been shown to induce caspase-independent apoptosis in human cancer cells, yet its antitumor efficacy can be enhanced when combined with additional treatments that induce an immune response. A clinically relevant fractionated dose of radiotherapy has been also shown to render tumor cells immunogenic by upregulating tumor MHC class I expression, cross-priming of T cells, and enhancing the immunotherapy of cancer (27). Very recently, we made a striking observation that dormant mammary tumor cells established by chemotherapy, in vitro, became chemo-refractory but remained sensitive to apoptosis induced by tumor-sensitized T cells (unpublished data). However, in some types of cancer, tumor antigen loss and/or HLA loss could emerge as a result of immunoediting, and eventually lead to tumor escape and relapse. In the FVB mouse model of breast carcinoma, we showed that neu-specific IFN-γ–producing CD8⁺ T cells concurrently induced apoptosis and indolent tumor dormancy that eventually led to tumor relapse because of neu antigen loss (28). Subsequent analysis revealed that such tumor immunoediting was associated with EMT and expression of breast cancer stem cell markers in the relapsed tumor cells. This tumor immunoediting was facilitated through the IFN-γ/IFN-γ R axis such that low, but not high, levels of IFN-γ R expression in tumor cells established tumor dormancy, which in turn led to tumor escape and relapse (29). In these types of cancer that are prone to immunoediting, tumor escape and relapse could be overcome by designing immunotherapeutic strategies that could eliminate rather than maintain dormant tumor cells. It was reported that natural killer (NK) cells can kill dormant tumor cells that resist T-cell–induced apoptosis (30). Thus, a potentially effective immunotherapeutic strategy should incorporate NK T cells, NK cells and T cells in the formulation of adoptive cellular therapy rather than focusing only on CD8⁺ T cells. Tumor escape from the immune surveillance and subsequent cancer progression could also occur as a result of the reduced immune function in the elderly or following transplantation, when immunogenic dormancy is present. Dormant tumor cells could also lead to active cancer if they express PD-L1 or other immune checkpoint ligands that promote T-cell exhaustion. There is no evidence in support of the role of myeloid-derived–suppressor cells (MDSC) or regulatory T cells (Treg) in compromising T-cell–mediated maintenance of tumor dormancy. In fact, quiescent tumor cells are not capable of promoting MDSC or Tregs, as no elevation of
these suppressor cells was reported in individuals who harbor dormant tumor cells.

Conclusion

A hypothetical model of treatment-induced tumor dormancy and recurrence is depicted in Fig. 1. According to this model, conventional cancer therapies concurrently induce apoptosis and growth inhibition in tumor cells. The latter effect of therapies establishes tumor dormancy. Awakening of dormant tumor cells will depend on treatment-induced immunogenic dormancy such that nonimmunogenic dormancy leads to early recurrence after the completion of conventional therapies whereas immunogenic dormancy induces antitumor immune responses and leads to prolonged tumor dormancy. Such prolonged dormancy could be permanent or may lead to tumor escape and late recurrence as a result of immunoediting, altered immune responses during aging, or immune suppression following organ transplantation. Levels of tumor IFN-γ Rα are a key determinant of tumor escape and relapse or relapse-free survival during immunogenic tumor dormancy, which is usually the case in viral-driven malignancies or following immunogenic chemotherapy or radiotherapy of primary cancers.

Because dormant tumor cells are resistant to chemotherapy or radiotherapy because of being quiescent, they remain sensitive to immunotherapy because of becoming immunogenic and also being outnumbered by antitumor T cells. Reprogramming of endogenous antitumor immune responses toward memory phenotypes could keep dormant tumor cells in check without continuous and potentially toxic therapies, thereby inducing permanent tumor dormancy and overcoming

Figure 1. A hypothetical model of treatment-induced tumor dormancy and recurrence. Heterogeneity of tumor cells results in differential responses to the treatment by cell death or growth inhibition and dormancy. Outcome of cancer treatment will depend on nonimmunogenic dormancy, leading to early tumor relapse, or immunogenic dormancy, leading to the elimination of tumor dormancy, or tumor escape and relapse, depending on the status of tumor IFN-γ Rα.
tumor escape. Such immunotherapeutic strategy could also be used in high-risk individuals who harbor CTC or DTC without a clinically apparent cancer, to prevent development of a primary cancer. In the long-term, cancer immunotherapy could be further improved to eliminate dormant tumor cells when combined with chemotherapy along with the blockade of pathways involved in tumor dormancy. However, breaking tumor dormancy to render them sensitive to chemotherapy is a risky approach compared with establishing permanent dormancy.

Disclosure of Potential Conflicts of Interest
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