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Epigenetic remodeling combined with photodynamic therapy elicits anticancer immune responses

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Abbreviations: 5-aza-dC, 5-aza-2'-deoxycitidine; CTL, cytotoxic T cells; DAMP, damage-associated molecular pattern; DC, dendritic cell; IL, interleukin; MHC, major histocompatibility complex; PDT, photodynamic therapy; PS, photosensitizer; TAA, tumor-associated antigens; TNF, tumor necrosis factor

Photodynamic therapy has been shown to induce strong immunity against tumor cells expressing exogenous tumor-associated antigens (TAAs), including P1A antigen. Cancer cells can evade the immune system by epigenetic silencing of TAAs, while DNA methyltransferase inhibitors, such as 5-aza-2'-deoxycytidine (5-aza-dC) can restore the expression of silenced or downregulated TAA. Thus, epigenetic remodeling with 5-aza-dC combined with PDT can elicit robust and durable antitumor immunity.

Photodynamic therapy (PDT) is a minimally invasive therapeutic modality used in the treatment of several types of solid tumors including skin, bladder, esophagus as well as head and neck cancers.[1] The treatment consists of administration of a photosensitizer (PS) followed by irradiation of the lesion with visible light typically delivered by thin fiberoptic systems from infrared laser sources. Light triggers PS-based photochemical reaction resulting in the formation of highly reactive singlet oxygen causing oxidative damage to intracellular structures and subsequent tumor cell death.[2] Antitumor effects of PDT result from its direct cancer cell cytotoxicity as well as from disruption of tumor vasculature and induction of local inflammation. The latter is a consequence of interdependent phenomena that include oxidative stress-associated cancer cell death leading to the release of damage-associated molecular patterns (DAMPs) and acute ischemia of the tumor area due to the destruction of tumor-associated vasculature. This initial wave of tumor cell death is accompanied by activation of the complement system, which not only acts as a mediator of antitumor effects but is also involved in stimulation of recruited leukocytes to release robust inflammatory mediators, including IL-1β, IL-6, tumor necrosis factor α (TNFα), prostaglandins, leukotrienes and histamine.[3]

PDT-elicited immunogenic cell death facilitates activation of dendritic cells (DCs) attracted to the tumor lesion by local inflammatory signals. DCs ingest and process tumor-associated antigens (TAAs) and migrate to draining lymph nodes to present TAA-derived peptides to $CD8^+$ cytotoxic T cells (CTLs) and $CD4^+$ helper T cells. This series of events should result in activation followed by homing of effector T cells to both primary and metastatic tumor lesions and elimination of remaining tumor cells. Indeed studies in immunodeficient mice revealed that PDT regimens that are curative in normal mice produce incomplete responses followed by tumor relapses.[4] Similarly, selective depletion of $CD8^+$ or $CD4^+$ T cells showed no demonstrable effect on the initial ablation of PDT-treated tumors but promoted delayed tumor re-growth.[5]

A series of studies revealed that PDT can induce antigen-specific antitumor immunity against tumor cells genetically engineered to express model TAAs, including β-galactosidase, green fluorescent protein or P1A, a mouse homolog of human melanoma-associated antigen (MAGE).[6] However, in syngeneic tumor models PDT-induced immune responses are usually insufficient for successful tumor eradication and control over metastatic foci. A number of mechanisms allow tumor cells to escape from immune surveillance, including epigenetic modifications that downregulate both MHC class I and TAA expression.[7] Conspicuously, patients with vulval intraepithelial neoplasia that failed to respond to PDT were more likely to

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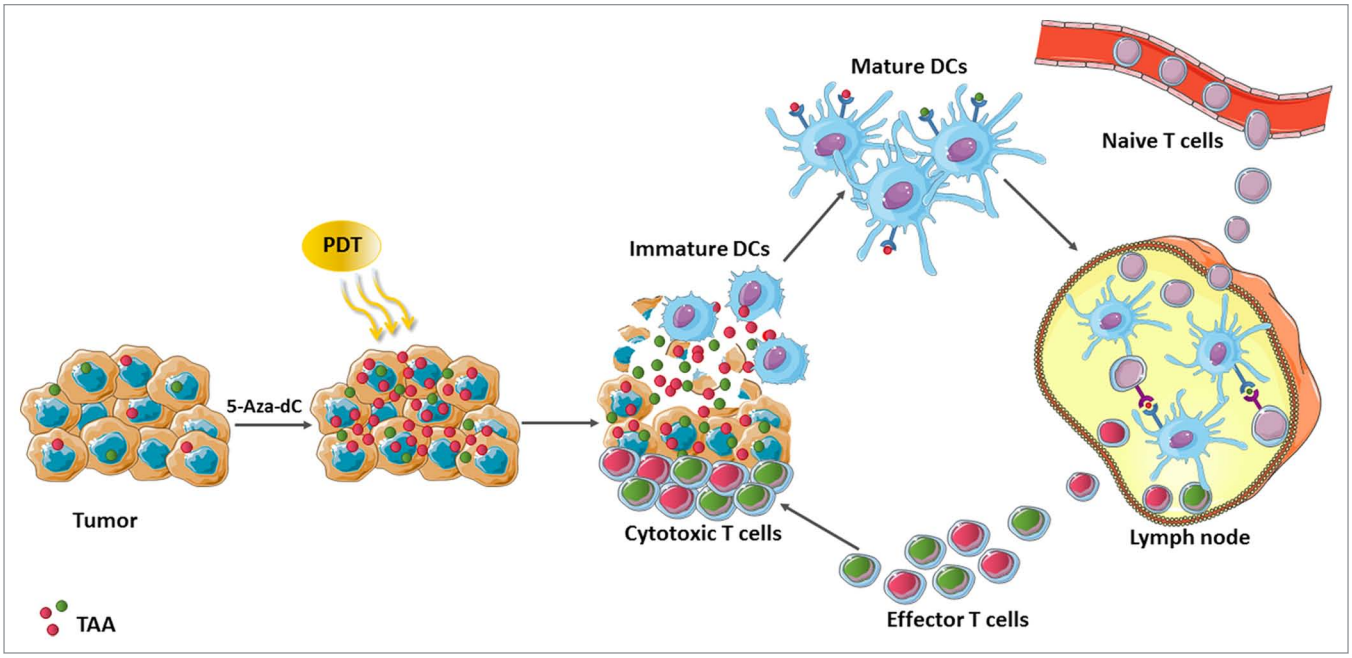

Figure 1. Photodynamic therapy-induced activation of antigen-specific antitumor immune response. 5-aza-2′-deoxycitidine (5-aza-dC) restores expression of silenced tumor-associated antigens (TAAs). After photodynamic therapy (PDT), dying tumor cells release TAAs and are phagocytosed by immature dendritic cells (DCs) subsequently attracted to the tumor site. DCs become activated and migrate to local lymph nodes, where they present TAA-derived peptides in association with major histocompatibility complex (MHC) molecules to T lymphocytes. Activated T cells differentiate into effector cells homing back to the tumor where they destroy residual cancer cells.

lack MHC class I molecules as compared with those who responded well to the treatment.[8]

Decitabine (5-aza-2′-deoxycitidine, 5-aza-dC) is a clinically approved methyltransferase inhibitor used in the treatment of myelodysplastic syndrome and acute myeloid leukemia. This cytidine analog is incorporated into DNA and irreversibly binds the methyltransferase enzymes resulting in DNA demethylation followed by reactivation of epigenetically silenced or downregulated genes including tumor suppressor or differentiation-associated genes. Coincidentally, it also restores expression of cancer-testis antigens, including P1A, as well as MHC molecules.[9] Therefore, we decided to study the effects of the combined treatment involving administration of 5-aza-dC to induce expression of P1A and MHC molecules subsequent to PDT in order to effectively initiate local cytotoxic responses associated with the release of DAMPs, TAA and induction of local inflammation. Using four different murine tumor models and two strains of mice, we confirmed that treatment with 5-aza-dC alone restores expression of MHC class I molecules, as well as that of P1A antigen. However, the antitumor activity of the epigenetic modifying therapy proved to be rather insignificant. Only the combinatorial therapy of PDT together with 5-aza-dC prolonged survival of mice bearing 4T1 and LLC tumors, and further, induced complete responses in mice harboring EMT6 and CT26 tumors.[10] Experiments with lymphocyte-depleting antibodies revealed that the tumor-inhibitory effects of the combination treatment were strongly dependent on the presence of $CD8^+$ CTLs, whereas $CD4^+$ T cells played only a supportive role. In these regards, a noteworthy expansion of IL-17-producing $CD4^+$ T cells population, a subset of $CD4^+$ cells known to stimulate $CD8^+$ cells, was observed in mice treated with 5-aza-dC and PDT. Intriguingly, determination of P1A-specific $CD8^+$ T cell population in draining LNs and spleens revealed no significant changes between experimental groups and all tumor-free mice rejected re-inoculated tumor cells in a re-challenge assay, despite the fact that the cancer cells were not pre-incubated with 5-aza-dC and were thus P1A-negative. These observations suggest that expression of P1A is not essential for the maintenance of durable antitumor immunity. However, when combined with PDT, 5-aza-dC may lead to increased expression and release of TAA in the PDT-treated microenvironment providing better antigen presentation and tumor recognition by immune cells that can further improve the immunogenic outcome of PDT. Thus, it can be hypothesized that PDT of tumors expressing TAAs upregulated by 5-aza-dC can induce concomitant immunity to other subdominant and possibly weakly immunogenic antigens. Immunity to the latter could sustain antitumor activity in mice.

To summarize, our findings demonstrate that combinatorial treatment with the epigenetic modifier 5-aza-dC along with PDT stimulates local tumor destruction concordant with the release of induced TAAs, thereby leading to antitumor immune response cascades and long-term survival (**Fig. 1**). Based on these observations we surmise that inhibition of DNA methylation via treatment with 5-aza-dC unleashes stronger immune responses against TAA in tumor-bearing

mice undergoing PDT, an effect that could be translated to the clinical treatment of malignant disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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