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Shock block for improved immunotherapy

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Cancer cells use heat shock proteins (HSP) to stabilize growth/survival-associated client proteins such as receptor tyrosine kinases (RTKs), in vivo. Our recent work suggests that chemical HSP90 inhibitors combined with a vaccination strategy targeting HSP90 client proteins that are (over)expressed in the tumor microenvironment yields superior therapeutic benefit.

Receptor tyrosine kinases (RTKs) mediate various processes that are critical for cell growth, differentiation and survival. Altogether, there are 20 distinct families of RTKs, including epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR) and Eph receptor families. In normal cells, RTK expression on the plasma membrane is regulated by ligand binding, which induces the phosphorylation of tyrosine residues in the cytoplasmic (kinase) domain of the receptor and hence promotes the internalization of the ligand/RTK complex. Subsequently, the ligand/RTK complex can either undergo ubiquitin/proteasome-dependent degradation or heat-shock protein (HSP)-facilitated recycling to the cell surface.[1] RTK signaling is a tightly-regulated process that frequently becomes dysfunctional in tumor cells. Defects in RTK internalization and degradation are often observed in cancer cells, leading to the accumulation of RTKs and/or sustained signaling through these molecules, ultimately resulting in uncontrolled cell growth, proliferation and survival commonly associated with tumor progression. Indeed, a substantial number of RTKs have been reported to be overexpressed by tumor cells and/or the tumor-associated vasculature in situ, indicating a role for these signaling molecules in tumorigenesis and angiogenesis.[1] Such a differential expression/function in the tumor microenvironment makes RTKs attractive targets for anticancer therapeutic interventions.

A number of therapeutic approaches have been used that target RTKs in tumors. Most of these approaches involve either blocking signaling via RTKs (by means of antagonistic antibodies or small chemical inhibitors), or stimulating their degradation (by means of recombinant ligands).[2] These strategies as exemplified by trastuzumab (and anti-HER2 monoclonal antibody), bevacizimab (antibody monoclonal antibody targeting the vascular endothelial growth factor, VEGF), sunitinib (a small molecule that inhibit multiple RTKs) and Ephrin-1-Fc recombinant ligand, have been widely successful in pre-clinical, as well as clinical, studies.[3] However, RTKs, like most oncoproteins, are frequently expressed by tumors as well as by normal tissues, giving rise to concerns about the off-target impact and safety of anti-RTK agents. In addition, there are concerns about the duration of the therapeutic effects mediated by these drugs, linked to the generation of escape (resistant) variants that arise from long-term usage.[4] Therefore, instead of just blocking RTK signaling or inducing RTK degradation in cancer cells, a more desirable situation would be to have drugs that activate the degradation of RTK proteins via the proteasome, leading to the generation of RTK-derived peptides that may be presented on the tumor cell surface in MHC Class I/peptide complexes. Such a paradigm would conditionally allow for treated tumor cells to become more "visible" to the host immune system. In particular, this intervention would allow for anti-RTK $CD8^+$ T cells of modest functional avidity to recognize cancer cells and mount a response against them, thus inhibiting tumor growth. Interestingly, some recombinant ligands and agonistic antibodies against tumor RTKs have been observed to result in this situation.[5] Furthermore, we have recently shown that transient inhibition of HSP90 function in tumor cells and/or tumor blood vascular endothelial cells in vivo improves protective antitumor immunity.[6]

HSP90 plays an important chaperoning/salvage role in intrinsic protein (re) folding, and tumor cells commonly overexpress HSP90 (as compared with their normal counterparts). HSP90 has been reported to interact with an array of overexpressed wild-type and mutated proteins in tumor cells, operating to stabilize and sustain the tumor-promoting function of an increasingly large number of client proteins. Due to the large number of client proteins HSP90 interacts with and the various functions these proteins mediate, HSP90 is now considered to play a central function in tumorigenesis, making it an attractive target for therapeutic interventions.[7] 17-Dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG) is a small-molecule HSP90 inhibitor that is currently being evaluated in Phase II

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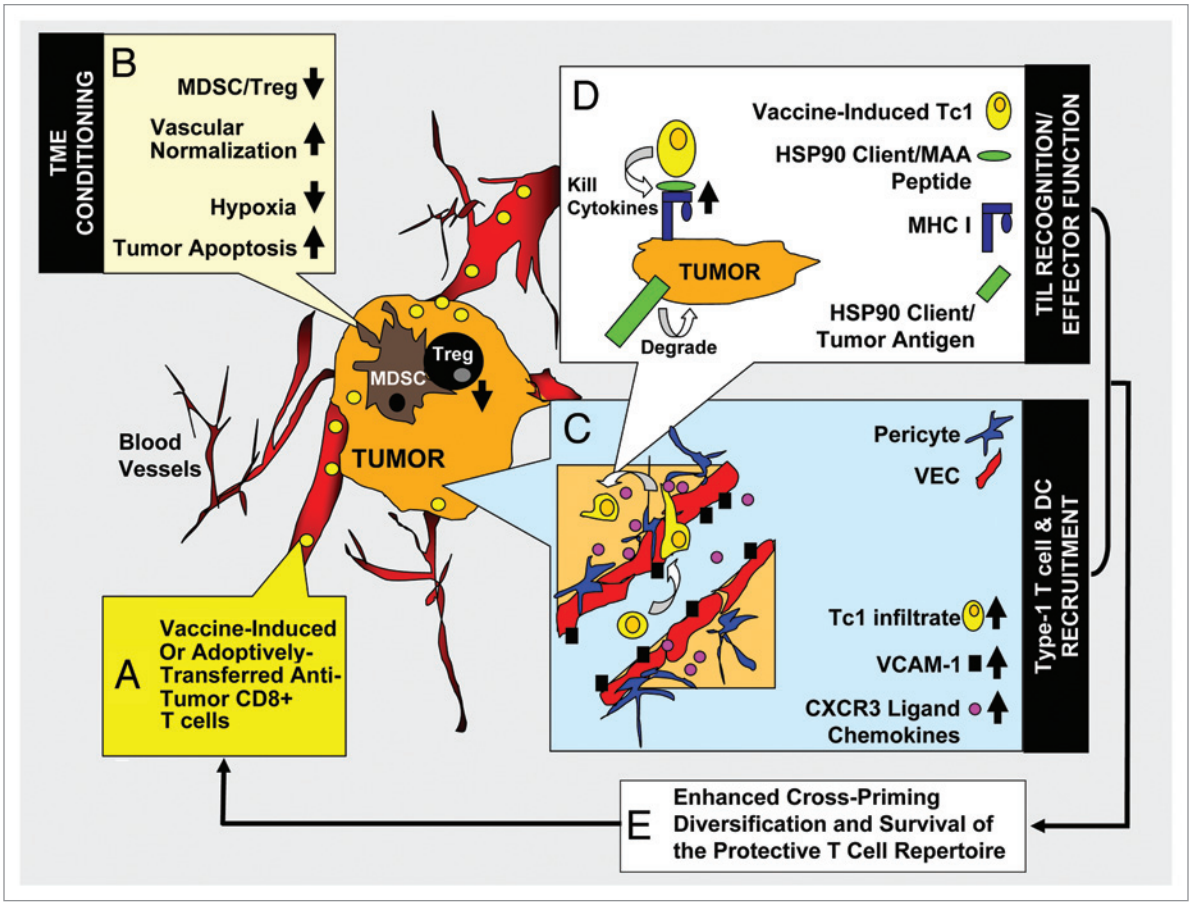

Figure 1. Combination chemoimmunotherapy for vascularized solid cancers using HSP90 inhibitors. Based on our published results[8] we suggest the following paradigm associated with improved anti-tumor efficacy for combination chemoimmunotherapy using the HSP90 inhibitor 17-DMAG. (**A**) Patients are treated with vaccines incorporating antigens differentially overexpressed by (tumor or stromal) cells within the tumor microenvironment (TME) or they receive adoptive transfer of autologous $CD8^+$ T cells specific for tumor/stromal antigens; (**B**) HSP90 inhibitor is applied on a daily schedule for up to 5 consecutive days to condition the TME for "receptivity" to circulating T effector cells (based on vascular normalization and the removal of hypoxia and regulatory immune subsets); (**C**) In a coordinate manner, the tumor-associated vasculature may become activated ($VCAM\text{-}1^+$) and CXCR3 ligand chemokine production is induced within the tumor stroma, thereby recruiting Type-1 $VLA\text{-}4^+CXCR3^+$ Tc1 into the TME; (**D**) By conditionally driving overexpressed client proteins into the proteasomal degradation pathway, HSP90 inhibitors allow for derivative peptide epitopes to be presented at high stochastic levels to moderate-to-low avidity, MHC Class I-restricted Tc1 leading to the immunogenic death of tumor cells and tumor-associated stromal cells; (**E**) Secondary uptake of locoregional antigens by mature DC allows for the longitudinal cross-priming of an expanded therapeutic Tc1 repertoire that may again be recruited into the TME upon HSP90 readministration. VEC, Vascular endothelial cell.

clinical trials. This drug is specific for the "active" protein-bound conformation of HSP90 that is preferentially found in tumor cells. As 17-DMAG is sequestered/retained preferentially within tumor lesions in vivo,[8] this drug may exhibit a respectable safety and efficacy profile.

Cancer is a complex multifactorial disease, perhaps explaining why single therapeutic interventions so far have had limited success. Combinational therapy strategies have frequently been observed to be more effective in treating progressive disease.[9] These approaches are often based on the simultaneous targeting of non-overlapping pathways that are required for tumor cell survival/growth, making the emergence of drug-resistant variants from heterogeneous populations of cancer cells more difficult. Our recent findings suggest that a short course of low-dose 17-DMAG leads to improved recognition of tumor cells or tumor-associated vascular endothelial cells expressing EphA2 (an HSP90 client protein) by specific $CD8^+$ T cells in vivo. Administration of 17-DMAG for 5 d also results in beneficial "off-target" effects, including an increased infiltration of therapeutic inflammatory cells and a decreased incidence of immunosuppressive cells within the tumor microenvironment. The antitumor effects of 17-DMAG alone (**Figure 1**) were significantly potentiated when the drug was combined with anti-EphA2 immunotherapy, leading to complete tumor regression in treated mice and the generation of antitumor immunological memory.[6] Our results suggest that the (metronomic) administration of HSP90 inhibitors like 17-DMAG act as an immune "adjuvant," thereby favoring the elicitation of immune responses directed against HSP90 client proteins within the tumor microenvironment. Thus, the combination of HSP90 inhibitors with immunotherapeutic strategies targeting HSP90 client proteins may constitute a superior approach for the treatment of cancer patients.

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