Membrane-bound KIT ligand-targeting DNA vaccination inhibits mammary tumor growth

Patrizia Dentelli¹, Federica Cavallo²,*, and Maria Felice Brizzi¹,*

¹Department of Medical Sciences; University of Torino; Torino, Italy; ²Molecular Biotechnology Center; University of Torino; Torino, Italy

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We have recently demonstrated that a DNA vaccine targeting membrane-bound KIT ligand (KITL) inhibits tumor growth by interfering with vessel stabilization/permeability and by disrupting the recruitment of inflammatory cells and regulatory T cells, the latter being an essential mechanism by which tumors resist available treatments. Combining KITL-targeting vaccines with conventional chemotherapy might avert drug resistance and improve the efficacy of standard-of-care therapeutic interventions.

This is particularly true when the target of such an immune response is expressed not only by cancer cells, but also by genetically stable cells of the tumor stroma, such as TECs. The therapeutic potential of vaccines targeting molecules that are expressed by the tumor-associated endothelium has already been demonstrated.⁷⁻⁹ Based on these considerations, we have recently developed a DNA vaccine that targets human mbKITL and tested its efficacy in a per se non-immunogenic, transplantable model of mammary cancer.10 The choice of using a xenogenic setting was taken based on the need to break the tolerance against a widely expressed, self-antigen. We demonstrated that this DNA vaccine efficiently inhibits the growth of cancer cells administered afterwards in the majority of vaccinated mice. This protective effect became particularly evident in mice in which vaccination promoted a robust anti-mbKITL humoral response. The inability of our vaccine to break the immunological tolerance to mbKITL in some mice was not surprising in view of the crucial role that mbKITL plays in many biological processes.

Impaired tumor growth upon vaccination was associated with a reduction in the number of functional blood vessels. This was mainly caused by a lack of proper pericyte coverage, in turn promoting vessel destabilization and altered vascular permeability. Vessel destabilization coupled to the hyper-dense immature vascular network observed in this setting resulted in a state of “non-functional angiogenesis” (Fig. 1). This was paralleled by an inadequate oxygen supply to malignant cells. Moreover, as in our model mbKITL is also expressed by cancer cells, a direct cytotoxic effect of vaccine-elicited antibodies on the malignant component of the tumor cannot be ruled out. In fact, cancer cells exhibit reduced proliferation rates in mice that produce anti-mbKITL antibodies, as shown by proliferating cell nuclear antigen (PCNA) staining.10 Whereas the limited clinical efficacy of current anti-angiogenic drugs is mainly caused by the expression of hypoxia inducible factor 1 (HIF1) and/or HIF-related genes in response to vascular endothelial growth factor (VEGF) downregulation,5 our mbKITL-targeting vaccine inhibited VEGF production by ECs and malignant cells in the absence of signal transducer and activator of transcription 3 (STAT3) activation and HIF1 expression.10 This suggests that targeting mbKITL with vaccines might confer an additional therapeutic benefit as compared with anti-angiogenic drugs.

The tumor microenvironment is a crucial driver of immunosuppression...
and regulatory T cells (Tregs) robustly contribute to this phenotype by inhibiting effector cell functions. Moreover, the tumor microenvironment contains pro-inflammatory cells that contribute to tumor progression by altering the quality of the local vasculature. Interfering with mbKITL-delivered signals by vaccination limited the recruitment of Tregs and myeloid cells into the tumor microenvironment. Thus, mbKITL-targeting vaccines stand out as valuable strategy for overcoming tumor-mediated immunosuppression while hampering tumor-induced angiogenic responses. Moreover, the presence of mbKITL-specific antibodies failed to affect physiological wound healing and the long-term survival of bone marrow-derived progenitor cells in our pre-clinical model, ruling out major safety concerns about the induction of a long-lasting immune response against mbKITL. This is presumably due to the fact that vaccination is able to induce low-affinity antibodies specific for mbKITL, which is a self protein and hence normally subjected to immunological tolerance. It this therefore likely that these antibodies inhibit mbKITL functions to partial extents, especially in the bone marrow where mbKITL is abundantly expressed together with sKITL. Nevertheless, the interference that these low-affinity, polyclonal antibodies exert on mbKITL is sufficient to alter the tumor microenvironment and inhibit tumor growth.

Unlike targeted anticancer agents, immunotherapeutic...
strategies against tumor-associated antigens face numerous challenges. However, we provide evidence that, as compared with a strategy that only targets tumor-associated antigens, an approach that breaks the tolerance against a protein expressed by both the tumor vasculature and cancer cells might exert an improved therapeutic activity while being well tolerated. Moreover, as pericyte-covered vessels contribute to the resistance of some neoplastic lesions to current chemotherapeutics, targeting the tumor microenvironment in the course of standard-of-care therapeutic regimens might offer an alternative treatment modality that circumvent such a loss in chemosensitivity.

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Disclosure of Potential Conflicts of Interest
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