Adoptive Gene Immunotherapy Using Redirected Lymphocytes for Disseminated Cancer, and Diversity of Prostate Cancer

Zelig Eshhar
Tova Waks
Eran Elinav
Amit Maliar
Dinorah Friedmann-Morvinski
Vica Malina
Lilach Agemy
Itai Kela
Assaf Marcus
Orly Aziz-Boaron

The immune system has been evolved not only to selectively recognize foreign from self-antigens but also to differentiate self from modified-self, such as occurs following viral infection and neoplastic transformation. Accordingly, the immune system is equipped with a versatile arsenal to identify and eliminate malignant cells that express tumor associated antigens (TAA). T cells are the major mean of the immune system that recognize and efficiently and completely eradicate both large tumors as well as disseminated metastasis in remote sites. Yet, functional, specific T cells are rare in most malignancies due to the fact that many spontaneous tumors develop a host of mechanisms to escape and evade T cells attack. Anti-tumor antibodies that can be more readily prepared are less effective, especially in the elimination of solid tumors. Based on our ability to genetically program and redirect the recognition of T cells using chimeric receptors, we have endowed T cells with antibody-type anti-tumor specificity (nicknamed T-Bodies) and thereby combined the advantages of both cellular and humoral arms of the immune response to combat cancer. These studies have been performed in animal models using human breast and prostate cancer xenografts attempting to treat advanced disseminated cancer, a disease stage that is by-and-large incurable. The various prostate cancer xenografts that we have established are also being used to study the patho-physiology of this disease.

Redirecting effector lymphocytes using chimeric receptors with antibodies’ specificity

To expand the recognition spectrum of effector lymphocytes and redirect them to predefined targets we expressed in T and NK cells chimeric receptor (CR) genes with antibody specificity. The modular structure of the CR allowed it’s engineering to fit a desired specificity and task. A most useful configuration is the tripartite CR (TPCR) that uses a scFv of a given antibody, the extracellular hinge region, transmembrane and cytoplasmic domains of CD28 linked to either the FcRγ or the CD3ζ intra-cellular moieties that include the ITAM activation motif (Figure 1). This tripartite CR combines the stimulatory and co-stimulatory signals needed for full activation of T cells, as we confirmed using TPCR transgenic mice. Naïve, unprimed T cells of such transgenic mice underwent non-MHC restricted stimulation for proliferation, high IL-2 production, rescue from apoptosis upon encountering plastic-bound antigen as well as specific killing of target cells in-vitro. In-vivo application of the antigen elicited DTH responses without priming. Interestingly, transgenic mice expressing TPCR that cross reacts with thymic antigens have been demonstrated unexpected cellularity, reflecting the power of usage of CR to perturb the normal homeostasis.

To determine the clinical applicability of the ’T-Body’ approach we have used erbB2 as target TAA of choice because it is associated with the uncontrolled growth and is commonly expressed in human adenocarcinomas, while its expression is elevated in advanced metastatic stages of the disease. We have efficiently expressed erbB2-specific TPCR in human T and NK cells using retrovectors and demonstrated their functionality in-vitro against tumor targets expressing erbB2. Following to their intratumoral administration, the engineered lymphocytes were able to eliminate pre-established orthotopically transplanted human breast and prostate cancer xenografts in SCID mice. To mimic bone metastasis (the favorite dissemination site of these malignancies) we have generated bone lesions by injection of tumor cells into the tibia of SCID mice. Systemic administration of the T-bodies arrested the growth of prostate cancer bone lesions and prolonged the life span of the mice, yet, only after mild lympho-reductive pretreatments that enhanced the migration of the T-bodies to the bones. Similar observations were obtained using breast cancer models. We found that at least part the mechanism beyond these pre-treatments is related to enhanced migration of the T-bodies to the tumor site. We currently further optimize the in-vivo performance of the T-bodies towards their clinical application as a new strategy for adoptive gene-immunotherapy of cancer. Towards this end, new strains of transgenic mice harboring two different erbB2- specific TPCR that we have been generated are extremely helpful.
Prostate cancer (PC) is a slow growing tumor that appears in adult men and is the second cause of death in men in the Western World. At its early stages, surgery, irradiation and androgen ablative therapies are quite effective and curative. However, at its advanced stages PC is incurable. We took advantage of several PC cancer xenografts that we established (together with Prof. J. Ramon Sheba Medical Center and Dr I. Leibovich, Meir Hospital) to probe several features that are involved in the transition from androgen dependent to androgen refractory, from primary to metastatic tumor and from irradiation sensitive to resistant growth. The PC xenografts were derived from samples taken from patients at various stages of their disease and represent different types of PC— from the common adenocarcinoma, the less common and more aggressive prostatic small cell carcinoma (with neuroendocrine (NE) cell features) to the very rare clear-cell carcinoma. We characterized these xenografts and could derive variants that differ in their sensitivity to ionizing irradiation (single dose or fractionated) and variants that could grow in castrated SCID mice with no external supply of androgens. Interestingly we found that the NE tumors can facilitate, in a paracrine manner, the transition of PC adenocarcinomas from androgen-dependent to androgen independent, both in vitro and in-vivo. We characterize now the NE molecules involved in the biochemical pathways relevant to this process. To determine whether there is genetic predisposition in prostate cancer for resistance to irradiation and for metastasis, we compare the transcription pattern and gene amplification of the different xenografts and their variants (with Prof. E. Domany, Physics of Complex Systems and Prof. Avi Orr, Tel Aviv Medical Center) Figure 2. Hopefully these studies will help to understand the mechanism(s) involved in the aforementioned processes and will lead to the definition of the genetic signature underlying these diverse phenotypes and to development of new therapies.

**Selected publications**


**Acknowledgements**

These studies were supported in part by the US Army DoD for prostate and breast cancer research, The Israel Science Fund, The Prostate Cancer Foundation. ZE is incumbent of the Renette and Marshal Ezralow Chair of Chemical and Cellular Immunology; Moross Institute for Cancer Research, Wolfson Center for cancer diversity, The Weizmann Institute-TLV-Sourasky Medical Center Foundation and the estate of Irene Kuhn and Lotte Stern UK.