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From genomics to immuno-therapy: Identifying tumor associated antigen peptides for anti-tumor vaccines

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Prevention or treatment of tumor metastasis is a major problem in clinical oncology. Active immunization protocols, directed to induce cytotoxic T lymphocytes (CTL) are explored as possible adjuvant treatments. The discovery of distinct antigens of neoplastic cells has suggested that it may be possible to create anti-tumor vaccines. Previously, vaccine strategies have used tumor-derived cells or cellular material, containing cryptic or unidentified antigens to induce specific immune recognition of these Tumor-Associated Antigens (TAAs). Recently, several newly discovered tumor-associated antigens have been tested directly as immunogens in vaccine formulations and have provided the first demonstrations that immune reactions against cancer antigens can lead to the regression of invasive tumors in selected patients. A diversity of mechanisms can result in the generation of antigenic epitopes recognized by tumor specific T cells. These epitopes come from normal nonmutated genes whose expression is limited to selected normal tissues (differentiation antigens), from uniquely expressed (embryonal reexpressed) or overexpressed proteins, from mutated proteins and from viral proteins in viraly associated tumors.

Identification of TAA genes or peptides depends on availability of anti-tumor CTL. CTL lines were isolated from peripheral blood (PBL) or tumor infiltrating lymphocytes (TiL) from melanoma patients. Yet, CTL to other types of malignancies are often hard to derive. We establish ways to isolate and test human Tumor Associated Antigen (TAA) peptides from breast, colon, prostate and bladder cancer. In these studies, anti-tumor CTL were induced in MHC knockout mice (β-2-microglobulin-/-xDb-/-), transgenic for a human single chain HLA-A2.1 - β-2-microglobulin molecule (HHD mice). HHD mice express a T cell repertoire which is highly homologous to the repertoire of HLA-A2.1 positive human (50% of the population). Immunization of HHD mice were performed with peptides extracted from fresh human tumors loaded on Antigen Presenting Cells (APCs), like Dendritic Cells (DC) or HHD and B7.1 transfected, TAP deficient RMA-S cells that can be loaded efficiently with exogenous peptides. Alternatively HHD mice were immunized with HHD transfected human tumor cell lines or synthetic peptides loaded on APCs. Immunized mice develop anti-tumor CTL which can be isolated from the spleen. These CTL were used to lyse target cells pulsed with putative synthetic TAA peptides derived from protein sequences by an algorithm that scored the peptides according to their ability to stabilize MHC, utilizing programs for independent binding of individual peptide side chains. Peptides that confer lysis on the target cells are then used to immunize HHD mice and the CTL are tested for their ability to lyse human tumor cells *in vitro* and to reject human tumor explants in nude mice. Putative TAA peptides are also tested for their ability to stimulate human peripheral blood lymphocytes (PBL) from HLA-A2.1 positive donors. Using these procedures we define

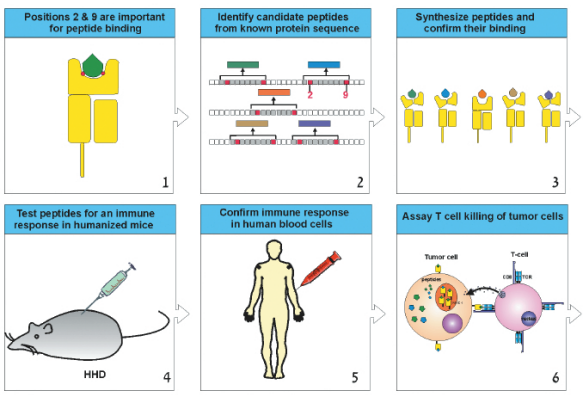
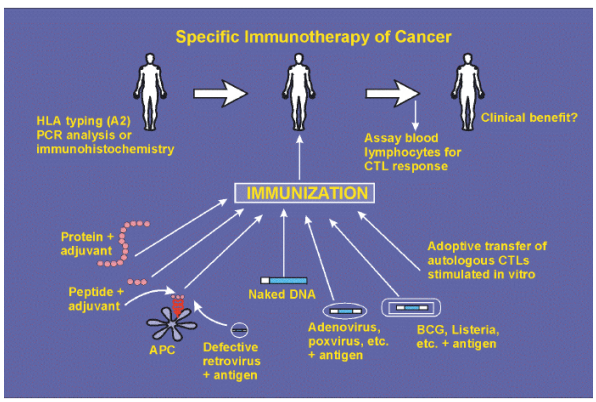


Fig. 1 *REVERSE IMMUNOLOGY STRATEGY?*
1. Choose candidate tumor-associated antigens by differential display of genes (microarrays, SAGE) or proteins (proteomics) overexpressed in cancer vs. normal tissue.
2. Identify putative HLA-A2.1 binding peptide epitopes, derived from candidate antigens, insilico.
3. Verify binding to HLA-A2.1.
4. Test antigenicity and immunogenicity in HHD mice. Select epitopes that induce CTL that specifically lyse HLA-A2.1 positive and antigen positive human tumor cells.
5. Test whether peptides immunogenic in HHD mice, induce CTL in Peripheral Blood Lymphocytes (PBL) of HLA-A2.1 positive patients or normal donors.
6. Develop vaccine formulation(s) based on these epitopes and establish animal models of tumors to evaluate vaccine strategies.

novel immunogenic peptides from the breast cancer TAAs, MUC-1 and BA46-1. Rather than testing single protein sequences for prediction of putative TAA peptides we utilized differential display data that screen for overexpressed or uniquely expressed genes in tumors versus normal tissue. SAGE analysis of colon carcinoma/colon tissue, microarray analysis of Transitional Cell Carcinoma (TCC) versus transitional bladder cells, and data base screens of Prostate carcinoma versus normal prostate/BPH tissue enabled screens of multiple genes and peptides. We defined 7 novel immunogenic peptides in colon carcinoma (out of 503 tested), 3 of these derived from the 1-8D gene from a family of interferon inducible genes. Novel peptides were defined also from PAP and STEAP for prostate cancer and from MAGE-8 and Uroplakins for TCC. Tumor rejection of breast and colon carcinoma transplants was achieved by adoptive transfer of anti-peptide CTL from MUC1 and BA46-1 (breast) and 1-8D (colon). Other studies include mechanism in vaccine design.



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