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Tumors, Genes And Vaccines

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The treatment or prevention of cancer by vaccines is vigorously sought. This approach is based on the qualitative and quantitative differences in antigenic repertoire between tumor cells and normal cells and the knowledge that the immune system can identify these differences. Many tumor associated antigens (TAAs) are recognized by Cytotoxic T Lymphocytes (CTL) as processed peptides bound to MHC class I. Using reverse immunology and a screening system based on D^b x beta2Microglobulin double knockout mice, transgenic for a single-chain HLA-A2-beta2Microglobulin molecule (HHD mice), we have identified novel immunogenic and antigenic TAAs from breast, prostate, bladder and colon carcinomas. Specific CTL were derived by immunization of HHD mice with tumor peptide extracts loaded on antigen presenting cells and with HHD transfected human tumor cell lines. CTL induced against peptides from various tumors recognized tumor peptides more effectively than peptides extracted from normal tissues and also reacted with a serie of peptides derived from overexpressed candidate proteins, identified by differential display methods (SAGE, Microarrays, SSH). Comparison of CTL derived from HHD mice to CTL induced from patient's PBMC showed overlapping recognition of many candidate peptides. Peptide vaccines, derived from the breast carcinoma antigens MUC1 and BA46-1 and from the prostate antigens PAP, PSGR and STEAP induced CTL that retarted growth of human tumors in adoptive transfer and active immunization models (figure 1). Analysis of colon carcinoma differentially expressed genes by SAGE, followed by screening for HLA-A2 binding peptides resulted in 500 candidate peptides for immunogenicity screening. We have identified 22 antigenic peptides of which 7 peptides were found to be immunogenic in HHD mice. Interestingly 3 of these peptides are derived from the same protein, the 1-8D IFN inducible protein. 1-8D derived peptides induce CTL that lyse effectively human colon carcinoma cell lines. Adoptive transfer

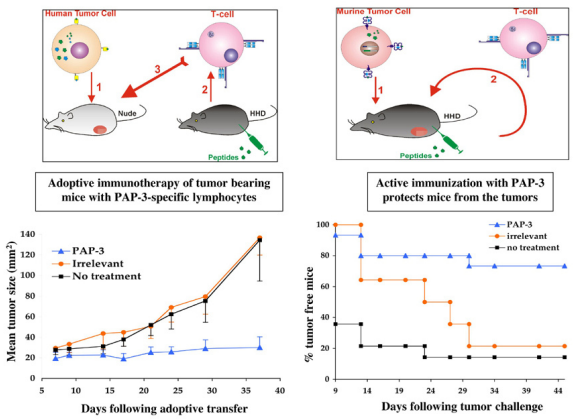
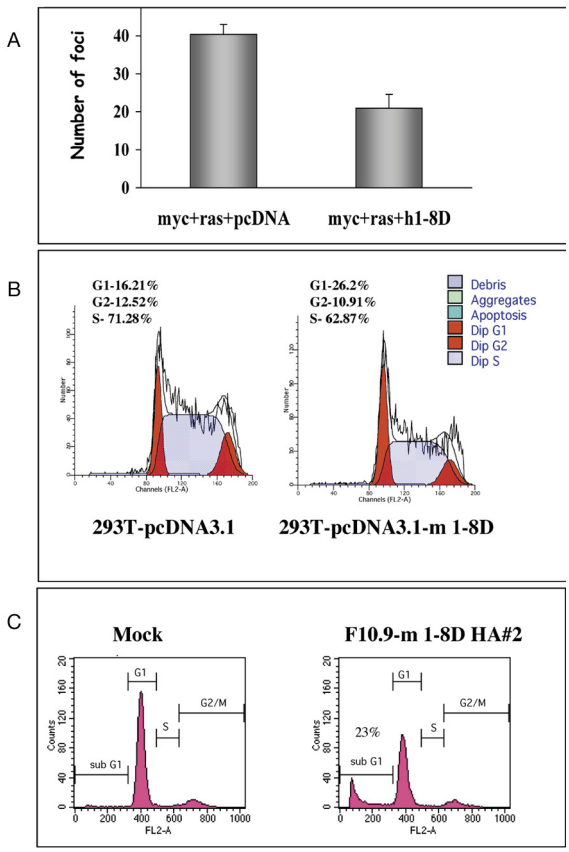


Fig. 1 *Immuntherapy of prostate cancer. Left :Adoptive transfer of Cytotoxic T cells induced against PAP-3 peptide in HHD mice causes rejection of a human prostate tumor xenograft in nude mice. PC-3-HHD tumor-bearing CD1$^{nu/nu}$ mice recieved HHD derived CTL specific to either PAP-3, to an irrelevant peptide or remained untreated. All mice (n=40) had palpable tumor at the time of adoptive transfer (day 7). The mean tumor size (squared millimeters) ± SE of the treatment groups are plotted against the days after tumor challenge. Tumor growth was followed for 40 days. Adoptive transfer of specific CTL significantly inhibited tumor growth. (PAP-3, p < 0.01, by Student's t test). Right:Active immunization with PAP-3 peptide partially protects mice from tumor progression.HHD mice were i.p. vaccinated 3 times, weekly, with 10^6 dendritic cells loaded with either PAP-3 (n=14), an irrelevant peptide (n=15), or injected with PBS (n=12). Seven days after the last immunization, the mice were challenged s.c. with 10^6 D122/HHD/PAP tumor cells. The percentage of tumor-free mice is depicted on y-axis against the days following tumor challenge. The PAP-3 based vaccine protects significantly (p<0.01) against tumor progression.*



of these CTL to nude mice bearing colon carcinoma

Fig. 2 *Tumor suppressive properties of the human and mouse 1-8D genes.A. Transformation assays: Primary rat embryonic fibroblasts (REFs) were transformed with combinations of myc, ras and h1-8D in pcDNA3.1 or empty pcDNA3.1 plasmids (3µg each) Foci were counted 14 days later. Each assay was done in triplicate and the bars represent mean (± standard error) of foci per 9-cm plate.B. Cell cycle analysis of transiently transfected HEK293T cells: HEK293T cells were transfected with m1-8D in pcDNA3.1 or empty pcDNA3.1 and GFP H2B plasmid coding for histone-targeted GFP. After 48hr cells were harvested, washed and fixed in cold methanol. Following RNase A treatment, propidum iodide was added and the cells were subjected to flow cytometric analysis, where GFP-positive cells were gated by their high fluorescence intensity (FL1). C. Cell cycle analysis of m1-8D-F10.9 transfected cells after serum starvation: F10.9 melanoma, that do not express 1-8D, were stabely transfected by m1-8D in pcDNA3.1 or mock transfected with empty vector. Cells were treated for 24hr in medium containing 0.1% FCS and shifted for 24hr to growth medium containing 10% FCS. Cells were fixed and cell cycle analysed as in B.*

xenografts retarded significantly tumor progression. The 1-8D gene was shown to be overexpressed in multiple colon carcinoma samples relative to normal colon tissue from the same patients. The 1-8D gene belongs to a three member family of interferon induced genes. 1-8D, 1-8U and 9-27 are linked on an 18Kb fragment of chromosome 11 and are highly homologous. The murine homologs of 1-8D and 1-8U genes are not tissue specific. Cell cycle analysis of murine 1-8D transfected cells exposed a possible role of these genes in cell growth regulation. The murine 1-8D gene was found to cause growth arrest of transiently transfected HEK293T cells. We also demonstrated that 1-8D stablely transfected F10.9 melanoma cells have an increased fraction of apoptotic cells after starvation. Transformation assays in REF transfected with myc+ras in presence of 1-8D revealed a possible role of 1-8D gene as a cell growth inhibitor (figure 2). These observations suggest a putative role of the 1-8D gene as a cell growth inhibitor, Sequence comparison of the human 1-8D in pairs of tumor/normal colon samples revealed single nucleotides polymorphism, multiple mutations and 3 nucleotide in frame insertions in the tumor derived 1-8D gene. These observation suggest that the 1-8D may function as a classical tumor suppressor gene which is mutated in cancer.

Selected Publications

Carmon, L., Bobilev-Priel, I., Brenner, B., Bobilev, D., Paz, A., Bar-Haim, E., Tirosh, B., Klein, T., Fridkin, M.,Lemonnier, FA., Tzehoval, E., Eisenbach, L. Characterization of novel breast tumor-associated BA46-derived peptides in Db-/-xbeta2microglobulin (beta2m)-/- null mice transgenic for chimeric HLA-A2.1/Db-beta2m single chain. (2002) J.Clin. invest. 110:453-462.

Lee SH, Bar-Haim E, Machlenkin A, Goldberger O, Volovitz I, Vadai E, Tzehoval E, Eisenbach L In vivo rejection of tumor cells dependent on CD8 cells that kill independently of perforin and FasL (2004) cancer Gene Therapy 11:237-248.

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