

1. Scientific Abstract

Hepatocellular carcinoma (HCC) is one of the most common fatal tumors^{1,2} with an annual global incidence of 1.2 million³. In the United States, approximately 13,000 new cases are diagnosed each year and the median survival is generally less than 6 months^{1,4}. Resection, transplantation chemoembolization, alcohol injection and cryoablation are potentially curative, but only in small, localized tumors⁵⁻⁷. Unfortunately, most patients have advanced disease at diagnosis and current systemic therapies are largely ineffective. The development of novel treatment strategies is greatly needed.

AFP is expressed during fetal development, but transcriptionally repressed shortly after birth⁸. Certain tumors, principally HCC and germ cell tumors, express AFP and its measurement in serum plays an important role in diagnosis and monitoring responses to treatment⁹. The normal function of AFP is unknown. It has been hypothesized to play a role in serum component transport since AFP has been shown to bind fatty acids, steroids and heavy metals¹⁰⁻¹².

The idea that AFP can serve as a target for immunotherapy is not new. Efforts were reported in earlier tumor immunology literature that involved attempts to generate antibody responses¹³⁻¹⁵. These were unsuccessful, in part due to high circulating levels of AFP that neutralized antibody. However, AFP-producing tumors would be expected to process and present on their cell surface AFP-derived peptide fragments in the context of major histocompatibility molecules, thereby becoming potential targets for cellular immune responses. These MHC-restricted AFP peptides could potentially be recognized by the immune system provided that these T cells were not clonally deleted during the ontogeny of the immune system. Both murine and human T cell repertoires appear to contain "self" reactive T cell clones for such proven and putative tumor-rejection antigens as MART-1^{16, 17}, MAGE^{18, 19}, gp100^{16, 20}, carcinoembryonic antigen²¹⁻²⁴ and others. It would be surprising if potential AFP-reactive clones could not be marshaled with an appropriate set of activation signals in an immunostimulatory environment.

Dendritic cells (DC) are uniquely equipped to initiate immune responses due to high major histocompatibility (MHC) class I and II expression together with costimulatory molecule expression. Our strategy in examining human T cell responses to AFP was guided by our parallel studies of human T cell responses to the well characterized melanoma antigen MART-1²⁵. Robust responses could be generated *in vitro* by DC genetically engineered to express MART-1. DC transduced with a recombinant MART-1 adenovirus expressed this melanoma antigen at high levels and correctly processed and presented the immunodominant HLA-A*0201-restricted MART-1₂₇₋₃₅ peptide. MART-1 engineered human DC could be used to generate specific human T cell responses *in vitro*. We have reported a murine

MART-1 model in which potent CTL, cytokine-producing T cells and protective immunity are generated after immunization with MART-1-engineered DC²⁶⁻²⁹. A trial using DC genetically engineered to express MART-1 using a replication defective adenoviral vector (AdVMART1) has been approved by the ORDA, and the clinical grade vector is being produced for us by Molecular Medicine, San Diego, CA (see letter of cross-reference in Appendix ii). Additionally, another clinical trial using adenoviral transduction of DC is underway in Dana Farber Institute (see abstract by Kaplan et al in Appendix vii). We have exploited these potent antigen presenting cells, as well as other genetic immunization strategies to investigate human T cell responses to AFP.

In a murine model, two genetic immunization strategies were employed to determine if AFP could serve as a target for T cell immune responses. We utilized the AdVmAFP to transduce murine DC, and used these DC expressing high levels of mAFP to immunize C57 BL/6 mice. This immunization strategy generated potent antitumor responses. The growth of tumors expressing mAFP was significantly delayed, with three vaccinations being marginally better than one. Tumor growth retardation was dependent upon both CD4+ and CD8+ T cell subsets. The immunization was able to generate mAFP specific CTL which lysed mAFP+ targets in a cytotoxicity assay. In addition, mAFP antigen-specific IFN γ producing splenocytes were also generated. The second genetic immunization strategy utilized was i.m. plasmid immunization comparing the mAFP cDNA in an expression vector with the backbone vector only or the human MART-1 cDNA in the same backbone. Naked DNA was also able to generate antigen-specific antitumor responses, splenic CTL and IFN γ -producing cells, but the response was far less impressive than with AdVmAFP transduced DC³⁰.

We have investigated potential HLA-A*0201-restricted epitopes from human AFP both *in vitro*, in human T cell cultures, and *in vivo*, in HLA-A*0201/K^b transgenic mice. We have collected compelling evidence that the immunodominant epitope is hAFP₅₄₂₋₅₅₀. Human T cells stimulated with DC transduced with AdVhAFP recognize hAFP₅₄₂₋₅₅₀ in both cytotoxicity assays and by secretion of IFN γ in ELISPOT assays. Conversely, hAFP₅₄₂₋₅₅₀-specific cultures generated from peptide-pulsed PBMC recognized AFP+ cells (both autologous lymphoblastoid cells stably transfected with hAFP and HLA-A*0201+ melanoma cells stably transfected with hAFP, compared to untransfected parental cells) in both cytotoxicity and cytokine-release assays³¹.

HLA-A*0201/K^b transgenic mice are an excellent animal model for immunotherapy, since these mice are able to present the same antigenic epitopes as HLA-A*0201 human subjects. HLA-A*0201/K^b transgenic mice that were immunized with syngeneic DC transduced with AdVhAFP likewise recognized hAFP₅₄₂₋₅₅₀ in both cytotoxicity and cytokine release assays. Also, transgenic mice immunized with hAFP₅₄₂₋₅₅₀ peptide emulsified in IFA recognized AFP+ cells in both assays. Taken together, this body of data indicates that hAFP₅₄₂₋₅₅₀ is a naturally processed and presented epitope of AFP restricted by HLA-A*0201,

which is sufficiently immunogenic *in vitro* and *in vivo* to generate CTL against AFP-expressing tumors³¹. The use of AdVhAFP transduced DC should enable the DC to process and present all of the potentially immunogenic AFP epitopes to the immune system, including hAFP₅₄₂₋₅₅₀, generating a polyclonal anti-AFP response.

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