

Biomedical Sciences Featured Investigator - September 2004

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Project Title: Novel Therapies for the Treatment of Colon Cancer

Final Research Progress

The major accomplishments of our 3-year colon carcinoma research effort supported by the California Cancer Research Program can be divided into 4 parts. First, we demonstrated proof of concept that DNA vaccines encoding the human carcinoembryonic antigen (CEA) gene or selected minigens, delivered orally by attenuated *Salmonella typhimurium*, evoked a T cell-mediated immune response, breaking peripheral T cell tolerance to the CEA self-antigens, and eradicating pulmonary colon carcinoma metastases in CEA-transgenic mice. We also showed for the first time that a combined boost with a plasmid encoding CD40 ligand trimer and small, non-curative doses of recombinant Ab-cytokine (IL-2) fusion protein increased vaccine efficacy to the point where 100% of CEA-transgenic mice completely rejected a lethal challenge of murine colon carcinoma cells. Second, we established that the immunological mechanisms governing the DNA vaccines' action involved primarily activation of CD8+ T cells and dendritic cells (DCs) indicated by increased secretion of proinflammatory cytokines, IFN-gamma, IL-12 and GM-CSF, upregulation of T cell activation markers CD25, CD28, CD-69, LFA-1 and co-stimulatory molecules on DCs such as B7.1, B7.2 and ICAM-1. Third, we established that a DNA vaccine encoding murine VEGF receptor 2 (FLK-1) prevents effective blood flow to tumor cells (angiogenesis) in the tumor neovasculature and inhibits growth of pulmonary colon cancer metastases in prophylactic and therapeutic settings via a long-lived memory T cell response. Fourth, we established a novel paradigm indicating that a DNA vaccine encoding survivin, an inhibitor of programmed death of cancer cells (apoptosis) can effectively protect mice against the onset of pulmonary colon cancer metastases. This vaccine is especially effective in killing colon cancer cells when it also encodes a natural killer cell ligand (NKG2D) H60.

Importantly, we not only accomplished all the major aims of our 3-year grant proposal, but also extended its original objectives. Thus, we established a new paradigm for DNA-based cancer vaccines. Specifically, we changed the target for cytotoxic T cells (CTLs) induced by a vaccine against vascular growth factor receptor 2 (VEGFR2) from difficult to kill, mutating tumor cells to genetically stable proliferating endothelial cells in the tumor vasculature. In contrast to tumor cells, these endothelial cells overexpress this growth factor receptor and are effectively killed by cytotoxic T cells induced by our vaccine. This creates a breakdown in the tumor vasculature and consequently stops blood supply to the tumor causing it to die from starvation. This strategy indeed accomplished this blockage of the tumor's blood supply and resulted in the eradication of colon cancer metastases.

It is anticipated that this approach, once translated to clinical application, will contribute to the treatment of colon cancer in a minimal residual disease setting and possibly prevent or retard cancer recurrence.

Publications resulting from CRP funding:

1. **Xiang R, Silletti S, Lode HN, Dolman CS, Ruehlmann JM, Niethammer AG, Pertl U, Gillies SD, Primus FJ, Reisfeld RA.** Protective immunity against human carcinoembryonic antigen (CEA) induced by an oral DNA vaccine in CEA-transgenic mice. *Clin Cancer Res*, 2001; **7**: 856s-864s.
2. **Xiang R, Primus FJ, Ruehlmann JM, Niethammer AG, Silletti S, Lode HN, Dolman CS, Gillies SD, Reisfeld RA.** A dual-function DNA vaccine encoding carcinoembryonic antigen and CD40 ligand trimer induces T cell-mediated protective immunity against colon cancer in carcinoembryonic antigen-transgenic mice. *J Immunol*, 2001; **167**: 4560-5.
3. **Ruehlmann JM, Xiang R, Niethammer AG, Ba Y, Pertl U, Dolman CS, Gillies SD, Reisfeld RA.** MIG (CXCL9) chemokine gene therapy combines with antibody-cytokine fusion protein to suppress growth and dissemination of murine colon carcinoma. *Cancer Res*, 2001; **61**: 8498-503.
4. **Dummer W, Niethammer AG, Baccala R, Lawson BR, Wagner N, Reisfeld RA, Theofilopoulos AN.** T cell homeostatic proliferation elicits effective antitumor autoimmunity. *J Clin Invest*, 2002; **110**: 185-92.
5. **Niethammer AG, Xiang R, Becker JC, Wodrich H, Pertl U, Karsten G, Eliceiri BP, Reisfeld RA.** A DNA vaccine against VEGF receptor 2 prevents effective angiogenesis and inhibits tumor growth. *Nat Med*, 2002; **8**: 1369-75.
6. **Luo Y, O'Hagan D, Zhou H, Singh M, Ulmer J, Reisfeld RA, James Primus F, Xiang R.** Plasmid DNA encoding human carcinoembryonic antigen (CEA) adsorbed onto cationic microparticles induces protective immunity against colon cancer in CEA-transgenic mice. *Vaccine*, 2003; **21**: 1938-47.
7. **Zhou H, Luo Y, Mizutani M, Mizutani N, Becker JC, Primus FJ, Xiang R, Reisfeld RA.** A novel transgenic mouse model for immunological evaluation of carcinoembryonic antigen-based DNA minigene vaccines. *J Clin Invest*, 2004; **113**: 1792-8.