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14. ABSTRACT As described in the 2008 progress report, the revised hypothesis is that agonist DR5 Ab induced by DNA vaccination will trigger tumor cell apoptosis without compromising T cell activity. The specific aims are to (1) Construct and test DR5 vaccines to induce anti-DR5 Ab, (2) Test the agonist activity of vaccine-induced anti-DR5 Ab, and (3) Amplify anti-tumor activity of DR5 vaccination with novel chemotherapeutics. During the funding period, we established that immune sera to human DR5 exhibit agonist activity to induce apoptosis in triple negative breast cancer cells and to inhibit tumor growth in vivo. To enable the testing of human DR5 vaccine in tolerant hosts, hDR5 transgenic mice have been generated and they are being back-crossed into BALB/c background. These mice will continue to be useful for studies targeting hDR5. To test the principle of inducing greater immunity with heterologous DR5 DNA vaccine, we have cloned DR5 cDNA from rat and two species of wild mice. These new reagents can be tested in future studies. To complement DR5 vaccination with additional therapies, we showed Sp-1 mediated TRAIL induction by HDAC inhibitor and that Akt survival pathway contributes to TRAIL resistance. These findings provide strong basis for combining DR5 vaccination with targeted therapy.					
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INTRODUCTION

As described in the 2008 and 2009 progress report, the revised hypothesis is that agonist DR5 Ab induced by DNA vaccination will trigger tumor cell apoptosis without compromising T cell activity. The specific aims are to

- (1) Construct and test DR5 vaccines to induce anti-DR5 Ab,
- (2) Test the agonist activity of vaccine-induced anti-DR5 Ab,
- (3) Amplify anti-tumor activity of DR5 vaccination with novel chemotherapeutics.

BODY

Specific Aims 1-2

- (1) Construct and test DR5 vaccines to induce anti-DR5 Ab
- (2) Test the agonist activity of vaccine-induced anti-DR5 Ab

Human DR5 vaccine constructs induce agonist DR5-targeted immunotoxicity

As described in 2008 and 2009 progress reports, we constructed and tested two DR5 vaccines in pVax derived from human DR5 cDNA (isoform 2: NCBI RefSeq # NM_147187). phDR5 is the full-length wt form. phDR5ectm contains the N-terminal half of hDR5; it is deleted after the first 18 aa's of the intracellular domain. phDR5Δ contains a nonsense mutation that inactivates the conserved death domain via deletion of the C-terminal 57 aa's (a mutant hDR5 from a head and neck tumor. All three constructs express human DR5 protein in transfected cells as judged by flow analysis with mAb A631 to the extracellular domain.

We demonstrated that wt mice electrovaccinated with each of the phDR5 constructs produced potent agonist anti-hDR5 Abs that elicited apoptotic cell death in human triple negative breast cancer (TNBC) SUM 159 cells in vitro, and blocked tumor growth in vivo. This anti-tumor activity was also seen with two other DR5-positive TNBC lines MDA-MB231 and BT474, but not with DR5-negative/ER+ PR+ Her-2+ human breast cancer lines. The apoptosis-triggering mechanism of immune sera induced by the hDR5 DNA vaccines was analogous to that of the death-receptor ligand TRAIL, but did not compete for TRAIL binding sites in the ecd of DR5. Importantly, the viability of activated host T cells was not affected by the agonist aDR5 immune sera, thus alleviating concern about deleterious side effects on DR5+ immune cells. This is in line with the lack of serious effects on normal cells following administration of recombinant TRAIL in recent clinical trials.

A manuscript describing these results received very positive comments from the reviewers of "Cancer Research" journal, but the editor felt the immune response should be measured when human DR5 is expressed as a self antigen. Therefore, we have developed a transgenic hDR5 mouse strain in which phDR5 is driven by whey acidic protein promoter. Founder hDR5 transgenic lines are still stabilizing, but early results indicate reduced host response to the phDR5 vaccines. These mice could become powerful tools for testing immunotherapeutic agents against human DR5.

To enhance the immunogenicity of phDR5, we have generated fusion constructs with adjuvant moieties such as the recall-antigen Tetanus toxin for further testing as needed. An alternative strategy is to vaccinate the host with allo-DR5, i.e., DR5 homologs from closely related species with minimal difference in amino acid sequence. This hypothesis was tested in wild type mice using mouse or rat DR5 (cloned in our labs: Accession # FJ515907). However, the death receptors are highly divergent evolutionarily (~60% sequence identity between mouse and rat DR5), and while the ratDR5 was highly immunogenic in mice, no cross reactivity to mouse DR5 was seen. We have taken to develop a panel of wild mouse pDR5 vaccines that differ only 5 to 10% in aa sequence from lab mouse. We have cloned DR5 cDNAs from *Mus gentilulus* (93% similarity to Balb/c) and *Mus caroli* (88%). This

approach has been notably successful in breaking tolerance to human Her-2 (Jacob et al. 2009; Quagliano et al. 2010). The tools are now available for establishing this type of DR5 vaccines.

Specific aim 3

(3) Amplify anti-tumor activity of DR5 vaccination with novel chemotherapeutics.

Amplification of DR5 agonist activity combinations of chemotherapeutic agents

TRAIL significantly enhances cytotoxicity of DR5 immune sera, as do IgG crosslinking antibodies (2009 annual report). We found that the HDAC inhibitor MS275 markedly upregulates DR5-mediated death in the breast cancer cell lines T47D, MDA231 and MCF7 via Sp1 positive control of TRAIL gene transcription (Xu et al 2008). Chemotherapeutics targeting DNA such as adriamycin and cell stress agents induce cell death by the mitochondrial pathway. We show that adriamycin can synergize with death receptor pathway activation (Xu et al 2008).

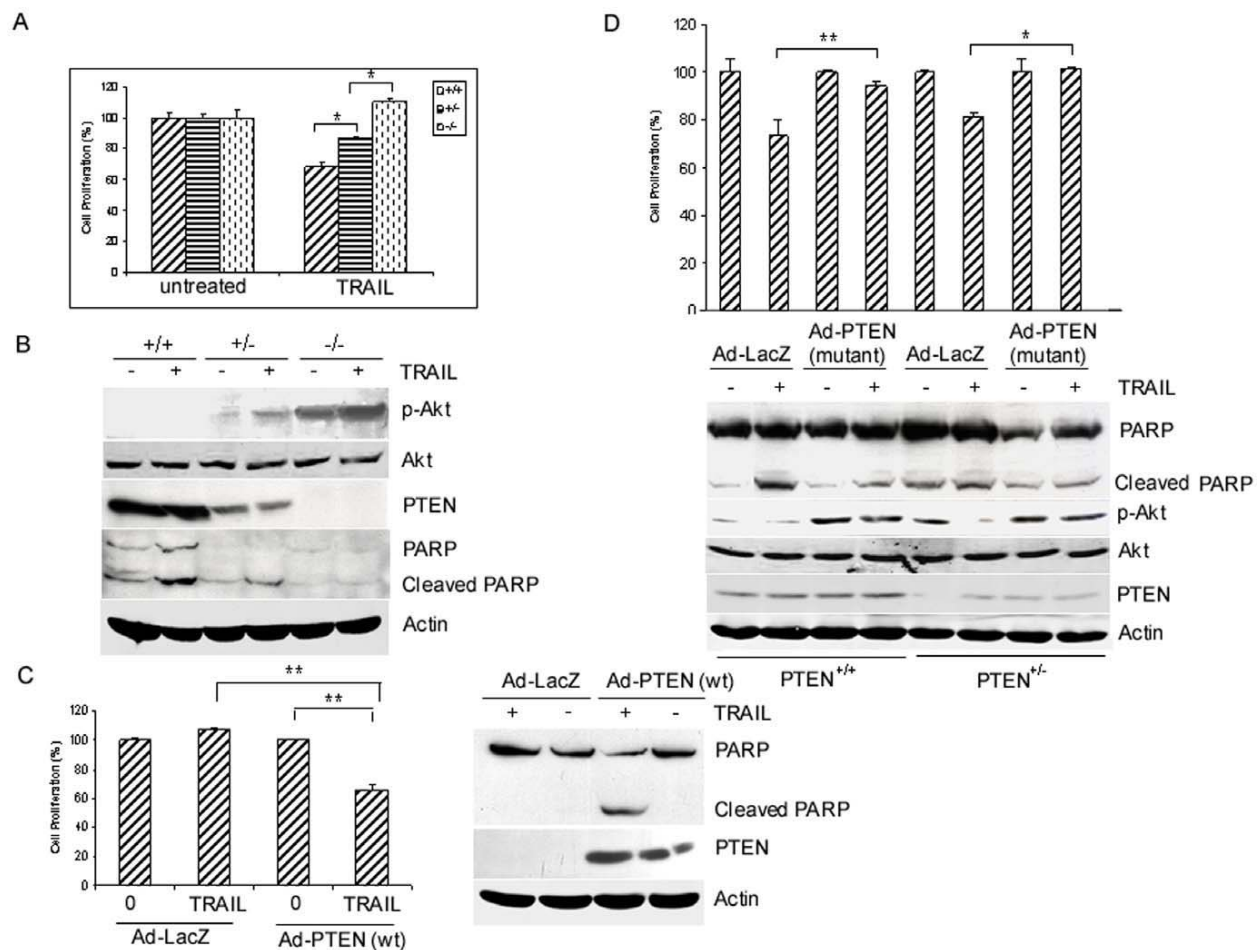


Figure 1. The role of PTEN in TRAIL sensitivity. A, effect of loss of PTEN in TRAIL-induced growth inhibition. Mouse prostate epithelial cells with wild type PTEN (PTEN^{+/+}), PTEN knockout (PTEN^{-/-}) and heterozygous (PTEN^{+/-}) were plated in 96-well plates, and then treated with 100 ng/ml TRAIL for 48 h. Cells proliferation was determined by MTT assays and expressed as percentage of untreated cells. *, P<0.01. B, effect of PTEN loss on TRAIL-induced apoptosis. PTEN^{+/+}, PTEN^{+/-}, and PTEN^{-/-} cells were treated with 100 ng/ml TRAIL for 48 h as described in (A). Total protein was extracted for assaying total and phosphorylated Akt (p-AKT), PTEN and PARP by Western blot analysis. b-actin was used as a loading control. C, restored PTEN expression sensitizes PTEN^{2/2} cells to TRAIL. PTEN^{2/2} cells were infected with adenoviruses

expressing wild-type PTEN (Ad-PTEN wt) or expressing LacZ (Ad-lacZ). At 72 h after infection, cells were trypsinized and plated. The next day, cells were left untreated or treated with TRAIL. Cells were harvested for MTT assays (Upper panel) and Western blot analysis was employed for detecting the levels of PARP and PTEN proteins (lower panel), respectively. Cell proliferation data were expressed as percentage of untreated cells. **, P<0.001. D, inhibition of PTEN function by a dominant negative form of PTEN confers TRAIL resistance. PTEN+/+ and PTEN+/- cells were infected with adenoviruses expressing a dominant negative form of PTEN for 72 h and then treated with TRAIL. Cell growth inhibition and the levels of PARP, PTEN, and total and phosphorylated Akt were analyzed (lower panel). *, P<0.01, **, P<0.001.

Mechanisms of TRAIL resistance: modulation of the Akt survival pathway by PI3K and PTEN

The development of TRAIL-resistance frequently compromises the durability of protocols using DR5-targeted therapies and complementing agents that enhance apoptosis. The Akt survival pathway plays an important role in TRAIL resistance in human cancer cells. We found that TRAIL treatment activates the Akt survival pathway and that inhibition of this pathway by the PI3K inhibitor LY294002 or knockdown of Akt sensitizes resistant cancer cells to TRAIL (2009 annual report). Since Akt is negatively regulated by the tumor suppressor PTEN, we examined the TRAIL sensitivity in PTEN knockdown mouse prostate epithelial cells (Fig. 1). PTEN(-/-) cells are more resistant than PTEN(+/+) cells, while PTEN(+/-) were intermediate in sensitivity. Over expression of mutant PTEN confers TRAIL resistance in PTEN(+/+) cells, supporting a role of PTEN in TRAIL sensitivity. In TRAIL resistant breast T47D cells, overexpression of the mutant PTEN further increased their resistance to TRAIL. Taken together, our data indicate that inactivation of functional PTEN and the consequent activation of the Akt pathway prevents TRAIL-induced apoptosis, leading to TRAIL resistance. Therefore, our results suggest that an approach to overcoming TRAIL resistance may be targeting PTEN or the Akt pathway in cancer cells.

KEY RESEARCH ACCOMPLISHMENTS

1. Establish the agonist activity of DR5 immune sera
2. Demonstrate the activity of DR5 immune sera in triple negative breast cancer cells.
3. Generate hDR5 transgenic mice for studies targeting human DR5.
4. Cloned rat DR5 cDNA and DR5 of two wild mouse species
5. Demonstrate Sp1-mediated TRAIL induction by HDAC inhibitor
6. Demonstrate that Akt survival pathway contributes to TRAIL resistance in cancer cells

REPORTABLE OUTCOMES

Xu, J., Zhou, J.Y., Wei, WZW., Philipsen, S., and Wu G.S. Sp1-mediated TRAIL induction in chemo-sensitization. *Cancer Res* 68(16):6718-26, 2008.

Xu, J., Zhou, JY, Wei, WZ, and Wu, GS, Activation of the Akt survival pathway contributes to TRAIL resistance in cancer cells? *PLoS One*, 5:e10226, 2010.

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GenBank Accession FJ515907, Zhou, J.Y., Wei, W.Z. and Wu, G.S. "Rattus norvegicus tumor necrosis factor receptor superfamily member", 2008

US patent pending “Cancer vaccines targeting TRAIL death receptors”

Human DR5 transgenic mice have been generated and they are being back-crossed into BALB/c background.

CONCLUSIONS

Agonist antibodies induced by hDR5 DNA vaccine in wild type mice inhibit the growth of triple negative breast cancers, showing potential efficacy of DR5 vaccination. To test hDR5 vaccines in a tolerant model system, hDR5 transgenic mice have been generated and they are being back-crossed into BALB/c background. Anti-tumor activity of DR5 vaccination may be enhanced by combined treatment with HDAC inhibitor to increase TRAIL expression. One mechanism of tumor resistance to TRAIL or DR5 agonist is via activation of Akt survival pathway, which is modulated by PTEN, indicating potential benefit of combining hDR5 vaccination with therapies targeting Akt, PTEN and/or PI3K.

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Xu J, Zhou JY, Wei WZ, Philipsen S, Wu GS. [Sp1-mediated TRAIL induction in chemosensitization.](#) *Cancer Res.* 2008; 68(16):6718-26.