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14. ABSTRACT This research study proposed that TNF- family growth factors, BAFF and APRIL play a role in prostate cancer. Our hypothesis proposed that APRIL provides a proliferative signal to normal prostate epithelial cells by means of an unknown receptor. We postulated that TACI is an antagonist receptor which functions to regulate APRIL-mediated proliferation of cells by inducing apoptosis and thereby maintaining the normal glandular structure. The loss of TACI expression in prostate cells therefore conceivably results in an imbalance in homeostasis resulting in the aberrant accumulation of cells which become susceptible to transformation. Our hypothesis anticipated that the addition of APRIL would enhance cell growth whereas the addition of TACI-Ig would either reduce cell growth or induce apoptosis. This did not happen and we did not see any significant changes in the relative numbers of hyperdiploid cells as determined by propidium iodide flow cytometry. Because of the negative results, the study was terminated early and the grant relinquished. Dr. VonBulow no longer works for Indiana University					
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Introduction

The goal of this grant was to gain insight into the molecular basis of prostate cancer. Preliminary evidence suggested that the *taci* gene is expressed in normal prostate tissues, but not in prostate tumor cells. We had proposed the APRIL provides a proliferative signal to normal prostate epithelial cells by means of an unknown receptor.

Body

To determine the role of APRIL and TACI in prostate tumor growth, we (a) cultured LNCaP, PC3 and DU145 prostate cancer cell lines and titrated the effects of recombinant APRIL and TACI-Ig on cell growth as determined by MTT assay, DNA content of cells and Annexin V binding assays; (b) we did not determine the effect of systemic TACI-Ig administration on the growth of LNCaP cells in nude mice because of the negative results obtained in (a); (c) To develop LNCaP clones with tetracycline-inducible TACI expression (Tet-TACI) and determine the effects of TACI activation on cell growth and apoptosis as determined by DNA content and Annexin V binding assay, we utilized the commercial "Tet-on" system from BD-Clontech and were able to derive several inducible TACI-expressing clones for the LNCaP and CWR22 cell lines. In the latter (c), data was repeated at least twice with three inducible clones from each cell line. These experiments were also repeated in conditions of reduced serum.

Key Research Accomplishments

Unfortunately, we have no major research accomplishments to list.

Reportable Outcomes

- (a) According to our hypothesis we anticipated that the addition of recombinant APRIL would enhance cell growth whereas the addition of TACI-IL would either reduce cell growth or induce apoptosis. This did not happen and for all four cell lines tested we did not see any significant changes in relative cell number (as determined by MTT reduction), nor did we see any significant changes in the relative numbers of hyperdiploid cells as determined by propidium iodide flow cytometry. These experiments were repeated several times with similar results. To overcome the possibility that the cells were already maximally stimulated in the presence of fetal calf serum, we repeated the MTT experiments in either serum-free conditions, or in medium with 1% fetal calf serum. Although the MTT values were reduced overall, there was no significant difference observed when titrating in either APRIL or TACI-Ig. We included the titration with BAFF since it is also a TACI ligand, but similar negative results were obtained with this cytokine. When performing the apoptosis assays with propidium iodide and annexin V staining, we encountered a pitfall in that we were not able to separate the cells from the monolayer into individual cells for flow cytometry using EDTA or trypsin without affecting the viability of the cells (and thereby propidium iodide staining). Overall these types of experiments were not very reproducible and were therefore abandoned as unreliable.

- (b) Due to the negative results obtained in (a) and the large amount of resources required, we decided not to perform the experiments to determine the effect of systemic TACI-Ig administration on the growth of LNCaP cells in nude mice.
- (c) Unfortunately no relative change of growth characteristics was observed in the presence of doxycyclin when compared with the parental cell lines. Additionally, induction of TACI expression coupled with treatment with APRIL, BAFF, or TACI-IG also did not manifest any significant differences in cell growth as determined by MTT assay.
- (d) Due to the negative results obtained in (c) we did not perform experiments to determine the effect of docycycline induction of TACI expression of Tet-TACI LNCaP cells implanted in nude mice.

Conclusions

The experiments conducted in this research netted essentially negative results and thus the research project was stopped early and the grant relinquished.

References: None

Appendices: None