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Isolated from Prostate Tumor and Normal Microenvironment

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INTRODUCTION

The focus of this project is to evaluate the effects of calcitriol (1,25-dihydroxycholecalciferol), the active form of vitamin D and/or dexamethasone on endothelial cells found in both tumor and non-tumor or normal microenvironments. Calcitriol has significant anti-tumor effects in LNCaP, PC-3, and MLL Dunning rat prostate models. Dexamethasone potentiates the antitumor effects of calcitriol. In mice, we have demonstrated that calcitriol's effect *in vivo* could contribute to its action on the endothelial cells (EC) found in the tumor (tumor-derived endothelial cells, TDEC). Preclinical data indicate that these effects were more profound in TDEC when compared to the endothelial cells isolated from matrigel plugs implanted in normal animals (matrigel-derived endothelial cells, MDEC). The differential effects observed could be due to differences in the availability of vitamin D receptor (VDR) to interact with the transcription machinery. To dissect the mechanisms underlying these differences, three specific aims were proposed:

- I. To determine the effects of calcitriol and/or dexamethasone on TDEC and MDEC**
- II. To elucidate the molecular mechanism(s) of the differential effect of calcitriol and/or dexamethasone in TDEC as compared to MDEC,**
- III. To evaluate oral calcitriol (QDx3) and/or dexamethasone (QDx4) in a phase II trial of localized prostate cancer using a pre-prostatectomy model.**

BODY

I. To determine the effects of calcitriol and/or dexamethasone on TDEC and MDEC

TDEC and MDEC expressed similar basal levels of vitamin D receptor (VDR) protein and responded to 10 nM calcitriol by increasing VDR protein expression in a time dependent manner, as analyzed using Western blot analyses. Despite similar VDR content, substantial variation is seen in response to the anti-proliferative effects of calcitriol. 10 nM calcitriol treatment for 48 hours inhibits the growth of TDEC (47% inhibition), with much less effect in MDEC (12 % inhibition) ($P = 0.00017$). Calcitriol-mediated growth inhibition in TDEC is mediated by G₀/G₁ cell cycle arrest and apoptosis. In TDEC, calcitriol increases the proportion of G₀/G₁ phase cells (11 % increase vs. vehicle control) and a reduction of S phase cells (39 % decrease vs. vehicle control), as assessed by propidium iodide (PI) staining and BrdU incorporation, respectively. Minimal changes in these parameters were seen in MDEC. Modulation of p21 and p27 protein expression is observed in calcitriol-treated TDEC but not in calcitriol-treated MDEC. Calcitriol also promotes apoptosis in TDEC as determined by annexin V binding. There is a significant increase of total cell death (39 %) after 48 hours treatment of 10 nM calcitriol observed in TDEC, but only minimal apoptosis (4 %) in MDEC. The apoptosis induced involves the mitochondria pathway; Bcl-2 is decreased and caspase-3 and PARP cleavage are increased. In addition, the survival signaling pathways in calcitriol treated-TDEC are also affected; we observed a downregulation of phospho-Erk and phospho-Akt, with no changes in the total Akt.

II. To elucidate the molecular mechanism(s) of the differential effect of calcitriol and/or dexamethasone in TDEC as compared to MDEC

Differential response to calcitriol in TDEC and MDEC could largely attribute to the functional activity of the receptor (VDR). At the genomic level, there is no difference between the coding regions of VDR in these cells. However, single point saturation of receptor binding assay reveals that the total VDR content is different; 31 fmol/mg protein and 24 fmol/mg protein in TDEC and MDEC, respectively ($P = 0.001$). Using Scatchard plot, VDR in TDEC shows a higher ligand binding affinity (K_d , 0.26 nM) compared to MDEC (K_d , 0.65 nM) ($P = 0.0016$). Although the VDR in these cells have different ligand binding characteristics, in both cell types, VDR is phosphorylated upon treatment with calcitriol, as shown by a dephosphorylation assay using alkaline phosphatase. Furthermore, immunocytochemical staining in combination with confocal microscopy visualization shows that the VDR in both cell types accumulates in the nucleus after treatment with calcitriol. The function of the VDR in TDEC and MDEC was evaluated with a luciferase reporter construct containing the 24-hydroxylase promoter: in both cell types, VDR transactivates the 24-hydroxylase promoter at a similar rate. These findings indicate that the VDR signaling is intact and functional in both TDEC and MDEC, though somewhat more efficient ligand binding is observed in TDEC.

25-D-24-hydroxylase (CYP24), encoded by the CYP24A1 gene, is a key enzyme in calcitriol metabolism. CYP24-mediated hydroxylation at C-24 leads to the formation of a much less potent vitamin D form. CYP24 hydroxylation is thus a major pathway of attenuation of calcitriol effect. There is differential induction of CYP24 expression by calcitriol in TDEC and MDEC. In MDEC, CYP24 mRNA is significantly induced by 10 nM calcitriol as early as 3 hours post treatment. However, in TDEC, only a slight mRNA induction is observed only after 48 hours of treatment. Similar expression profiles are also observed with CYP24 protein and enzyme activity. In MDEC, treatment with a CYP24 selective inhibitor (RC-8800) in combination with calcitriol resulted in partial restoration of MDEC responsiveness to calcitriol. This suggests that induction of CYP24 could explain the lack of response to calcitriol-mediated anti-proliferative effects in MDEC.

III. To evaluate oral calcitriol (QDx3) and/or dexamethasone (QDx4) in a phase II trial of localized prostate cancer using a pre-prostatectomy model.

We proposed to examine clinical specimens following treatment with calcitriol/dex to evaluate the biological effect of these agents on TDEC, tumor vasculature in the prostate of men who received the treatment and then undergo a prostatectomy. Out of 80 patients evaluated, to date, we have accrued 7 patients to the trial. Of those seven, studies continue to isolate and evaluate endothelial and other cell types in the prostate specimens.

KEY RESEARCH ACCOMPLISHMENTS

- Selective anti-proliferative effects of calcitriol were observed in TDEC when compared to MDEC via induction of cell cycle arrest and apoptosis.
- There is no mutation found in VDR of TDEC and MDEC; however, VDR in TDEC has a higher binding affinity to calcitriol than MDEC.
- The VDR signaling axis in TDEC and MDEC were intact and functional when the receptors were able to translocate and transactivate DNA.
- Differential induction of 24-hydroxylase in TDEC and MDEC may be the mechanism of selectivity of calcitriol in these cells.
- 7 patients have been recruited to date.

REPORTABLE OUTCOMES

- Poster presentation at AACR Special Conference in Cancer Research: Anti-Angiogenesis and Drug Delivery to Tumors: Bench to Bedside and Back at Waltham-Boston, Massachusetts in December 2005. Title: Calcitriol-mediated differential anti-proliferative effects in tumor-derived endothelial cells as compared to non-tumor microvascular endothelial cells.
- Poster presentation at 96th Annual Meeting American Association Cancer Research at Washington in April 2006. Title: Epigenetic silencing of CYP24 in tumor-derived endothelial cells (TDEC) contributes to selective growth inhibition by calcitriol.
- Poster presentation at 13th Workshop on Vitamin D at Victoria, BC, Canada in April 2006. Title: Differential anti-proliferative effects of calcitriol on tumor-derived endothelial cells as compared to non-tumor microvascular endothelial cells.
- Oral presentation at 13th Workshop on Vitamin D at Victoria, BC, Canada in April 2006. Title: Epigenetic silencing of CYP24: the mechanism to selective growth inhibition by calcitriol in TDEC.
- Publication:
 1. Ivy Chung, Michael K. Wong, Geraldine Flynn, Wei-dong Yu, Candace S. Johnson and Donald L. Trump. Differential Antiproliferative Effects of Calcitriol on Tumor-Derived and Matrigel-Derived Endothelial Cells (2006) Cancer Research 66 (17): 8565-8573.

CONCLUSIONS

Endothelial cells derived from a tumor microenvironment (TDEC) are more sensitive to calcitriol-mediated anti-proliferative effects, while those from non-tumor microenvironment (MDEC) are relatively resistant. Although a higher ligand binding affinity VDR species was found in TDEC, both TDEC and MDEC have intact VDR signaling axis. Such differential responsiveness can be explained by the lack of CYP24 induction in TDEC. If similar selectivity exists *in vivo*, this will provide a therapeutic window that allows inhibition of tumor angiogenesis without adversely affecting normal vasculature. Lack of calcitriol-induced CYP24 expression in endothelial cells isolated from tumor microenvironment provides a rationale for using vitamin D therapy, even in resistant epithelial tumors.

REFERENCES

None.

APPENDICES

None.