**Prostate Cancer Cells Growth Inhibitory Effects by Natural Compound Diosmetin.**

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**Introduction**

Cell growth is a biological cell development and cell division by which cells gather mass and increase in size. This cell growth is a biological process, where mTOR (mammalian target of rapamycin) pathway has its role, regulates cell growth by coordinating energy and nutrient signals with growth factor. mTOR protein kinase have two different complexes; complex-I contains major component raptor; a target of rapamycin and complex II; insensitive to rapamycin, has major component rictor. Growth factors such as insulin and IGF-1 reflect fed status of organism. When food is plentiful, levels of these growth factors are sustained and promote anabolic cell processes such as translation, lipid biosynthesis, and nutrient storage via mTORC1. The binding of insulin to its cognate tyrosine kinase receptor recruits insulin receptor substrate 1 (IRS1) to the receptor and activates phosphoinositide 3-kinase (PI3K), which through the production of phosphatidylinositol (3,4,5)-triphosphate [PtdIns(3,4,5)P3], recruits Akt to the plasma membrane where it becomes activated by direct phosphorylation by PDK1 and mTORC2. Rictor is important component of mTORC2, phosphorylates Ser-473 of Akt/PKB, which is essential for full Akt/PKB activation. mTORC2 plays a crutial role in AKT1 'Ser-473' phosphorylation, which may facilitate the phosphorylation of the activation loop of AKT1 on 'Thr-308' by PDK1 which is a prior condition for full activation. mTORC2 also modulates the phosphorylation of PKCα (protein kinase C-α) on 'Ser-657'. PKC is known to involve in a wide array of cellular processes such as cell proliferation, differentiation and apoptosis. Based on the findings dual-targeting of the compound is needed which could modulate the Rictor induced Akt signaling and also alter PKCα to inhibit increased cell growth. We propose Diosmetin (5,7,3'-trihydroxy-4'-methoxyflavone, is an O-methylated flavone) a natural agent, which modulates AKT and PKCα activity to inhibit progression of prostate cancer in in-vitro and in-vivo model system.

**Materials and Methods:**

We used Diosmetin (≥99% purity) from Sigma –Aldrich, MO. Human prostate cancer cells PC-3 (androgen refractory) and LNCaP (androgen sensitive) cells were cultured in RPMI 1640 medium of 5%, 10% FBS respectively and were treated with Diosmetin5,10, 20 and 40µM for various time intervals. Cell Proliferation were examined by MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-DiphenyltetrazoliumBromide] assay was performed for the cell viability, formed formazan crystals were dissolved in DMSO, and read at 570 nm wavelength. Cells were trypsinized and DNA content/cell cycle analysis was performed by using propidium Iodide (PI). The PI intercalates into the major groove of double-stranded DNA and produces a highly fluorescent adduct that can be excited at 488 nm with a broad emission centered around 600 nm. Cell lysate containing 25µg proteins were resolved on 4-20% Tri-glycine gel, incubated with appropriate primary and secondary antibodies, radiographed using chemiluminescence and hyper-film.

**Results:**

Present study demonstrates, cell growth inhibition and induction of apoptosis in human prostate carcinoma LNCaP (androgen-responsive) and PC-3 (androgen-refractory) cells by diosmetin, however, no significant growth inhibition observed in normal prostate epithelial cells (RWPE1). We observed diosmetin treated prostate cancer cells were able to inhibit growth factor (IGF-1) and cytokine (IL-6) induced rictor and PKCα expressions. Moreover, rictor silencing in PC-3 cells altered Akt (Ser473) and PKCα (Ser657) phosphorylations. Furthermore, diosmetin (20μM) treatment to prostate cancer cells prevented rictor nuclear localization and subsequently inhibited phospho-Akt (Ser-473). These effects were associated with a marked decrease in the protein expression of cyclin D1 and their activating partner, cyclin-dependent kinase (cdk) 2 and 4 with concomitant upregulation of KIP1/p27 and INK4a/p16. Additionally diosmetin treatment to these cells modulated cell survival machinery by down regulating key molecules viz., c-Myc, Survivin and XIAP (X-Linked Inhibitor of Apoptosis). Diosmetin treatment also resulted in alteration in Bax/Bcl2 ratio in favor of apoptosis, which was associated with an increase in cleaved caspase-3. Taken together, our studies demonstrate that diosmetin-mediated growth inhibition and induction of apoptosis in both LNCaP and PC-3 cells were due to deregulated levels of rictor and Akt signaling cascade and modulation of cell-cycle machinery. We are documenting these evidences for the first time that diosmetin acts against potential molecular targets to alter cellular events to elicit anticancer effects in prostate cancer cells.

**Conclusion:**

Our studies demonstrate that diosmetin treatment resulted in cell growth inhibition and induction of apoptosis in prostate cancer cells by altering Rictor signaling cascade. Diosmetin may have potential role to modulate Rictor-AKT–PKCα signaling pathway and induce apoptosis to inhibit the progression of prostate cancer.