**Diosmetin: A natural compound for inhibition of prostate cancer progression.**

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**Introduction:**

Cell growth is a fundamental biological process whereby cells accumulate mass and increase in size. The mammalian TOR (mTOR) pathway regulates growth by coordinating energy and nutrient signals with growth factor-derived signals. mTOR is a large protein kinase with two different complexes. One complex contains mTORC1, GβL and raptor, which is a target of rapamycin. The other complex, insensitive to rapamycin, includes mTORC2, GβL and Rictor. mTORC2 seems to function upstream of Rho GTPases to regulate the actin cytoskeleton, probably by activating one or more Rho-type guanine nucleotide exchange factors. mTORC2 plays a critical 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 prerequisite for full activation. mTORC2 regulates the phosphorylation of SGK1 at 'Ser-422'. mTORC2 also modulates the phosphorylation of PKCα on 'Ser-657'. Additionally, PKC is also involved in a wide array of cellular processes such as cell proliferation, differentiation and apoptosis. Furthermore, reports suggest dual-targeting mTOR and phosphatidylinositol 3-kinase/Akt signaling prevents mTOR inhibition-initiated Akt activation and enhances anti-tumor effects both in cell culture and animal xenograft models, suggesting this is an effective anti-cancer therapeutic strategy. Therefore, the utmost requirement is for an effective Rictor inhibitor as an anticancer preventive agent having a capacity to further inhibit AKT and PKCα kinase activity. 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 and were treated with Diosmetin 5,10, 20 and 40µM for various time intervals. Cell Proliferation were examined by MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] assay was performed for the cell viability, formed formazan crystals were dissolved in DMSO, and read at 570 nm wavelength. 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. Luciferase tag PC-3 cells (0.5 million cells per mice) were orthotopically implanted in ventral prostate after midline incision in lower abdomen. There were five groups of animals (Control, Diosmetin 20µg, Diosmetin 50µg, Rictor-/- and Rapamycin) and each group had 8-10 mice. After 8 weeks of drugs feeding, tumors were harvested, weighed and total lysate, cytosolic and nuclear fractions were made to analyze the involved signaling cascade. Whole body X-ray scan performed for the possible bone metastasis.

**Results:**

Anti-proliferative effects of diosmetin on human prostate cancer LNCaP and PC-3 cells were observed in dose and time dependent fashion by MTT assay. Photomicrographs of LNCaP and PC-3 cells after 24hrs represented apoptotic phenotype after diosmetin treatment.

Rictor silencing in androgen-refractory PC-3 cells down modulated phospho-Akt (Ser476), whereas, phospho-PKCα levels were increasing. Photomicrographs of rictor silenced PC-3 cells represented altered phenotype. Moreover, we observed diosmetin treatment to these cells decreased kinases activity AKT, PKCα.

After 8 weeks of orthotopic PC-3 cells implant, we observed mice body weight and after mice sacrifice we observed significant decrease in prostate tumor weight and uro-genital apparatus weight. Orthopically implanted (PC-3 cells in mouse prostate) tumor diosmetin fed mice represented decreased mTOR, Rictor, p-AKT (Ser-473), p-PKCα (Ser-657), p-ERK1/2, c-Myc, Cyclin D1 and CDK4, conversely FOXO3a was increasing, which promotes apoptosis.

Orthotopically implanted luciferase-PC-3 cells represented more luciferase activity in control mice than diosmetin treated mice. PC-3 (Rictor-/-) cells and Rapamycin treated mice represented various organ metastasis. However diosmetin (20µg/Kg/day) treated mice represented no metastasis.

**Conclusion:**

Our studies demonstrate that diosmetin-fed animals resulted in growth inhibition and induction of apoptosis 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.