PRESENTATION TITLE: *PKC-ι and PKC-ζ are heavily responsible of upregulating epithelial-mesenchymal transition (EMT) and activating Vimentin to facilitate cellular motility in prostate cancer cell lines*

ABSTRACT: Prostate carcinoma is the most common cancer among men in terms of number of new cases reported each year. Metastasis is responsible for more than 90% of prostate cancer deaths. Therefore understanding of the cellular mechanisms behind prostate cancer metastasis is very important. Expression of Vimentin is a hallmark of metastatic mesenchymal prostate cancer cells which is initiated by EMT. Our previous studies show that atypical protein kinase C- iota (PKC-ι) and zeta (PKC-ζ) inhibition retards the activation of NF-κB pathway by diminishing NF-κB nuclei translocation. The present study shows that siRNA knockdown of PKC-ι and PKC-ζ downregulate Snail1, PRRX1, Vimentin while upregulating E-cadherin thereby diminishes EMT. In-vitro migration and invasion assays for PC-3 and DU-145 prostate cancer cell lines demonstrated a significant reduction of cellular migration and invasion in PKC-ι and PKC-ζ knocked down samples. Immunoprecipitation experiments suggested direct association of Vimentin with PKC-ι and PKC-ζ separately. Laser stimulated confocal immunofluorescence and immuno-gold transmission electron microscopic techniques were used to further confirm the relationship of Vimentin with aPKCs. Real Time qPCR was used to analyze the mRNA levels of targeted markers to further validate transcriptional downregulation of Vimentin which was observed in Westerns upon aPKC siRNA knockdown. Overall results showed stronger relationship between PKC-ι and Vimentin over PKC-ζ with Vimentin. Microscopic results also show PKC-ι concentrated along the cell membrane together with Vimentin in addition to the abundant distribution throughout the cell. In addition, our results suggest that both aPKCs target multiple activation sites (S33, S39 and S56) on Vimentin thereby playing a crucial role in the regulation of Vimentin dynamics which is essential for increased prostate cancer cell motility. We have used a novel PKC-ι specific inhibitor ICA-1S to conduct in-vivo experiments on murine models. Excised tumors were analyzed for pathways observed in in-vitro experiments. Immunohistochemical and Western blot analysis on tumor samples confirmed the relationship of aPKCs with Vimentin. Overall results suggest that both aPKCs are essential in upregulation of EMT and for the activation of Vimentin to facilitate metastasis of prostate cancer cells. Finally, results suggest that PKC-ι and PKC-ζ can be effectively targeted using specific inhibitors to develop targeted therapeutics for metastatic prostate carcinoma.