

## ESSENTIAL ROLE OF $G\alpha 2$ PROTEIN IN MIGRATION OF PROSTATE CANCER CELLS

**Shafiq A. Khan**

*Center for Cancer Research and Therapeutic Development, Clark Atlanta University, Atlanta, GA, USA*

**Abstract:** Prostate cancer is the most diagnosed and the second leading cause of cancer deaths among American men. Early stage prostate cancer which is localized in the prostate gland is treatable by surgery and radiation therapy and the prognosis in these patients is very good. However, the prostate cancers in later stages of disease metastasize and the majority of prostate cancer deaths are due to metastatic disease. Tumor cell motility is the initial step in the process of invasion and metastasis and is an essential component of dissemination of tumor cells from the primary tumor to local and distant sites. Growth factors and chemokines, acting through both G-protein coupled receptors (GPCRs) protein tyrosine kinase receptors (PTKRs) signal through their intracellular substrates to induce cell migration in target cells. Our recent studies have shown that  $G\alpha 2$  protein is essential for migration and invasion of prostate cancer cells induced by ligands acting via both GPCR-dependent and GPCR-independent pathways. Studies from our laboratory showed that oxytocin (OXT) induces cell migration in prostate cancer cells (PC3) and this effect is mediated by pertussis toxin (PTX) sensitive Gi/Go $\alpha$ -protein coupled receptor. Induction of migration in response to EGF was not affected by PTX pretreatment indicating that it did not require activation of GPCR. We also showed  $G\alpha 2$  as the only PTX sensitive isoform required for OXT effects on prostate cancer cell migration. Interestingly, the knockdown of endogenous  $G\alpha 2$  protein led to inhibition of both OXT and EGF induced cell migration. The essential role of  $G\alpha 2$  in EGF effects on cell migration was also confirmed in DU145 cells. To determine the possible role of  $G\alpha 2$  protein in the activation of PI3-kinase pathway, we investigated the effects of PTX pre-treatment or knockdown of endogenous  $G\alpha 2$  on pAKT levels in PC3 cells in response to various ligands. Pretreatment with PTX increased basal levels of p-AKT in PC3 cells; however, it blocked the stimulatory effects of OXT on p-AKT levels. PTX pretreatment had no effect on EGF induced p-AKT levels. In the cells transfected with  $G\alpha 2$  siRNA, the basal levels of p-AKT were slightly higher than in control cells; however, OXT failed to cause any increase in p-AKT levels. Interestingly, the induction of PI3-kinase activity and increased p-AKT levels induced by EGF were not affected by the absence of  $G\alpha 2$  protein. These results suggested that the essential role of  $G\alpha 2$  in EGF effects on cell migration and invasion is either independent or down-stream of PI3-kinase/AKT/mTOR activation. Rac1 is activated at the leading edge of motile cells and induces the formation of actin-rich lamellipodia. Basal and EGF induced activation of Rac1 was not affected after knockdown of endogenous  $G\alpha 2$  in both PC3 and DU145 cells. Over-expression of constitutively active Rac1 in PC3 cells resulted in significantly increased cell migration which was not further increased by exogenous EGF; selective knockdown of  $G\alpha 2$  in these cells resulted in attenuation of cell migration. These results suggested that  $G\alpha 2$  acts down-stream of Rac1 to induce cell migration. Next, we determined if  $G\alpha 2$  is involved in the formation of lamellipodia, a key structure for cancer cell migration and invasion. Transfection of  $G\alpha 2$  siRNA resulted in decrease in cell size, rounding of the cells which failed to produce a leading edge and lamellipodia compared with control siRNA transfected cells. These morphological changes were also seen in PC3 cells after selective knockout of  $G\alpha 2$  by CRISPR/Cas9 with or without treatment with EGF. On the basis of these results, we conclude that  $G\alpha 2$  protein is essential for cell migration in prostate cancer cells and acts down-stream of PI3-kinase/AKT/mTOR and Rac1 in cell stimulated with EGF and is required for lamellipodia formation at the leading edge of migrating cell. This novel function of  $G\alpha 2$  is distinct and independent of its role in GPCR signaling.

**Keywords:** Prostate cancer, cell migration and invasion,  $G\alpha 2$ , metastasis, cell signaling

**Acknowledgments:** This study was supported by grants from NIH/NIMHD/RCMI grant #G12MD007590 and NIH/NIMHD grant #5P20MD002285.