Growth inhibition of human hepatocellular carcinoma cells by overexpression of G protein-coupled receptor kinase 2

Zhengyu Wei, Reginald Hurtt, and Cataldo Doria

Division of Transplantation, Department of Surgery, Thomas Jefferson University, Philadelphia, PA 19107

Introduction:

G protein-coupled receptor kinase 2 (GRK2) is a ubiquitously expressed serine/threonine kinase. It is a unique member of GRK family with diverse functions (Metaye et al., 2005; Ribas et al., 2007). The role of GRK2 was first discovered in the desensitization of G protein-coupled receptors (GPCR) signaling by phosphorylating agonist-activated 7-transmembrane receptors. The phosphorylated receptor enhances the binding of β-arrestins to form a molecular complex which prevents further coupling of the receptor from its G protein, leading to attenuation of the receptor mediated signalings (Aragay et al., 1998; Ribas et al., 2007). Despite of its traditional function as a kinase in receptor desensitization, a growing body of evidence has been documented that GRK2 interacts with a variety of other cytosolic proteins involved in signaling pathways relevant to essential cellular processes such as proliferation/apoptosis, migration, trafficking, cell cycle, and development (Guo et al., 2009; Jiang et al., 2009; Kahsai et al.; Penela et al., 2008; Penela et al.) . Some of these physiological functions of GRK2 are achieved through kinase-independent mechanisms by directly binding to other proteins (Chen et al.; Cipolletta et al., 2009; Jiang et al., 2009; Namkung et al., 2009). Altered expression levels of GRK2 have been reported in many human diseases including heart failure, hypertension, rheumatoid arthritis, cystic fibrosis, and cancer (Lombardi et al., 1999; Lymperopoulos et al., 2007; Mak et al., 2002; Metaye et al., 2005; Vroon et al., 2004; Vroon et al., 2005)

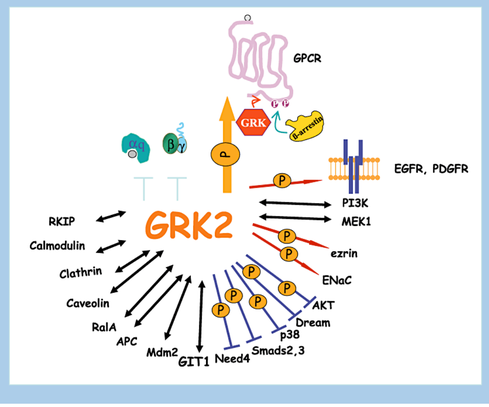
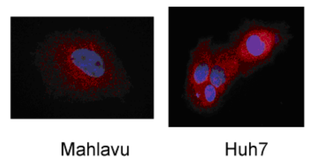


Fig. 1. The complex interactome of GRK2 in different cellular signaling pathways and processes. Adapted from Penela, P. et al. Br J Pharmacol. 2010.

Methods and Results:

2A



2B

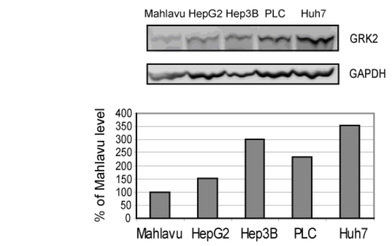
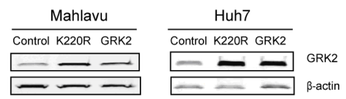


Fig. 2. Basal GRK2 protein levels in HCC cell lines. A. Cytosolic distribution of GRK2. B. Basal GRK2 protein levels in all five HCC cell lines detected by Western blotting. Cell lysates (25 ug total protein) were subjected to immunoblotting with antibodies as indicated. GRK2 levels were normalized to GAPDH. The immunoblot shown is a representative of two independent experiments.

3A



3B

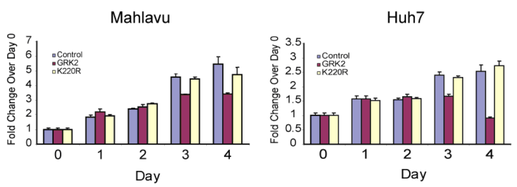
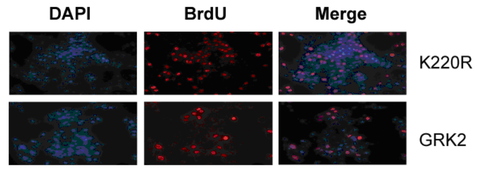


Fig. 3. Overexpression of GRK2 reduces cell viability in HCC cells. A. Western blots showing overexpression of wildtype GRK2 and its kinase-dead K220R mutant in Mahlavu and Huh7 cells transduced with recombinant adenovirus containing WT GRK2 or GRK2 K220R mutant. β-actin was used as total protein loading control. B. MTT method was used to detect cell viability with overexpression of GRK2 and K220R mutant during 4 day growth period in 10% FBS medium.

4A



4B

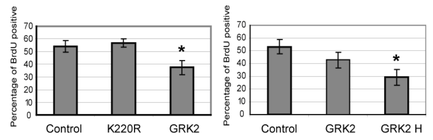
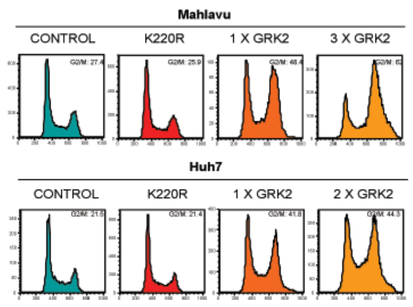


Fig. 4. Overexpression of GRK2 suppresses cell proliferation in HCC cells. A. Immunofluorescent staining of BrdU incorporation in Huh7 cells. DAPI was used for staining nucleus. Antibody against BrdU was immunoblotted with a secondary antibody with labeled with Tritc. Overexpression of GRK2 and K220R mutant was achieved by adenovirus transduction as indicated in Figure 3. B. BrdU incorporation in Huh7 cells as shown in A and in Mahlavu cells, respectively. GRK2 H represents at least two fold higher of MOI of adenovirus used for achieving GRK2 overexpression. *, P < 0.01.

5A



5B

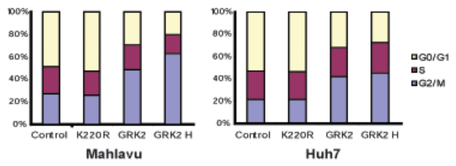


Fig. 5. Overexpression of GRK2 leads to G2/M phase arrest in HCC cells. A. Representative cell cycle histograms with overexpression of GRK2 and K220R mutant two days post adenovirus transduction. Cells were analyzed by FACS for DNA content by PI staining. 2 × GRK2 represents at least two fold higher of MOI of adenovirus used for achieving GRK2 overexpression. B. Cells distributed in different phases as shown in A.

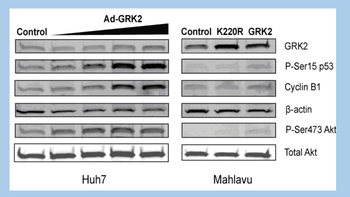
Summary:

In this study, we investigated a novel approach for HCC treatment by inducing overexpression of GRK2 in human HCC cells. We found that overexpression of GRK2 through recombinant adenovirus transduction inhibits the growth of human HCC cells. BrdU incorporation assay showed that the growth inhibition caused by elevated GRK2 level was due to reduced cell proliferation but not apoptosis. To examine the anti-proliferative function of increased GRK2 level, we performed cell cycle analysis using propidium iodide staining. We found that the proliferation suppression was associated with G2/M phase cell cycle arrest by the wild type GRK2 but not its kinase-dead K220R mutant. Furthermore, increased levels of wild type GRK2 induced upregulation of phosphor-Ser15 p53 and cyclin B1 in a dose dependent manner. Our data indicate that the anti-proliferative function of elevated GRK2 is associated with delayed cell cycle progression and is GRK2 kinase activity-dependent. Given the public importance of HCC, it is likely that the enforced expression of GRK2 in human HCC by molecular delivery may offer a potential therapeutic approach for the treatment of human liver cancer.

Acknowledgements:

We thank Dr. Walter Koch laboratory for providing GRK2 viruses and other reagents. This work was supported by the Department of Surgery and the Dean's Office of the Jefferson Medical College.

6A



6B

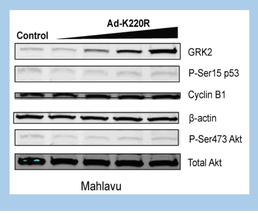


Fig. 6. Overexpression of GRK2 induces upregulation of phosphor-p53, cyclin B1, and phosphor-Akt. A. Overexpression of GRK2 was achieved by increasing MOI of adenovirus for examining dose dependent effects. Cell lysates were subjected to immunoblotting with antibodies as indicated on the penal. B. Western blotting in Mahlavu with overexpression of GRK2 K220R mutant in a dose-dependent manner.