**Abstract**

ERK5 inhibition as a potential therapeutic target for clear cell renal cell carcinoma

**Introduction and Objective**

Clear-cell renal cell carcinoma (ccRCC) is characterized by a mutation in the von Hippel-Lindau tumor suppressor gene (VHL). A recent study revealed that extracellular signal-regulated kinase 5 (ERK5) is degraded through the ubiquitin-proteasome system, in a process mediated by VHL and hypoxia-inducible factor (HIF). The involvement of ERK5 in cell proliferation, angiogenesis, and anti-apoptosis has been reported in various carcinomas. Several studies have shown that microRNA 143 (miR143), which displays decreased expression in RCC cells, regulates ERK5 expression. Our objective was to examine the effect of ERK5 in ccRCC and to investigate the potential of ERK5 inhibitor, XMD10-92, as a therapeutic agent.

**Materials and Methods**

- Surgically resected ccRCC specimens
- Human RCC cell lines
- ERK5 inhibitor (XMD10-92)
- Proteasome inhibitor (MG132)
- BALB/c-nu nu mice
- IHC (Immunohistochemical staining)
- Western blotting
- MTS assay
- Flow cytometry
- Luciferase reporter assay
- siRNA transfection
- miRNA transfection
- Quantitative RT-PCR

**Results**

A total of 148 surgical specimens taken from patients with localized RCC were analyzed using immunohistochemistry (IHC). Of these, 82 (48.8%) were ERK5-positive and 20 (11.9%) were strongly ERK5-positive. Proteasome inhibition appeared to enhance ERK5 expression in wild type cell lines, but not in VHL-mutant cell lines as shown by western blot (WB) analysis. An inverse correlation between ERK5 expression and miR143 quantification was observed in RCC specimens (p=4. Fisher's exact test p=0.0625). Furthermore, ERK5 inhibition with XMD10-92 resulted in an increase in the sub-G1 population as observed by flow cytometry, and cleaved PARP expression indicated an increase in apoptotic cells as seen by WB. ERK5 inhibition reduced cell viability and ERK5 knockdown downregulated PARP and Bcl-2 expression. Additionally, XMD10-92 showed anti-tumor activity in tumor xenograft mouse model and downregulated Ki67 and CD34 expression.

**Conclusion**

An inverse correlation between ERK5 expression and miR143 quantification was observed in human RCC specimens, and ERK5 expression in RCC was found to be suppressed by miR143.

In renal cancer cell lines, ERK5 inhibition by XMD10-92 indicated the induction of apoptosis.

ERK5 is a promising therapeutic target for ccRCC.