Abstract

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

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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 showed 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, XMD8-92, as a therapeutic agent.

Materials and Methods

- Surgically resected ccRCC specimens
- Human RCC cell lines
- ERK5 inhibitor (XMD8-92)
- Proteasome inhibitor (MG132)
- BALBC/c-nu/nu mice
- IHC (Immunohistochemical staining)
- Western blotting
- MTS assay
- Flowcytometry
- Luciferase reporter assay
- siRNA transfection
- miRNA transfection
- Quantitative RT-PCR

Results

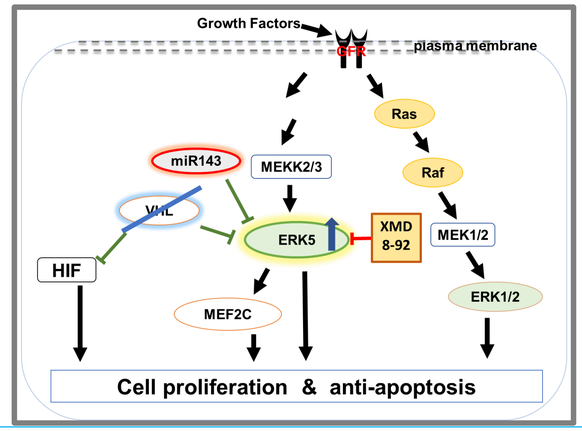
A total of 168 surgical specimens taken from patients with localized RCC were analyzed using immunohistochemistry (IHC). Of these, 82 (48.8 %) were ERK5-positive and 20 (11.9 %) were strongly ERK5-positive. Proteasome inhibition appeared to enhance ERK5 expression in wild type cell lines, but not in *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 (n=48, Fisher's exact test p=0.0625). Furthermore, ERK5 inhibition with XMD8-92 resulted in an increase of 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, XMD8-92 showed anti-tumor activity in tumor xenograft mice model and downregulated Ki67 and CD34 expression.

Conclusion

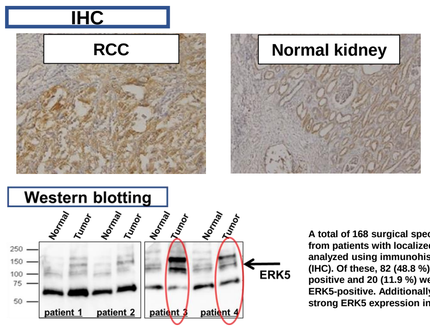
An inverse correlation between ERK5 expression and miR143 quantification was observed in human RCC specimens, and ERK5 expression in RCC was found to be suppressed by miR143.

In renal cancer cell lines, ERK5 inhibition by XMD8-92 indicated the induction of apoptosis.

ERK5 is a promising therapeutic target for ccRCC.

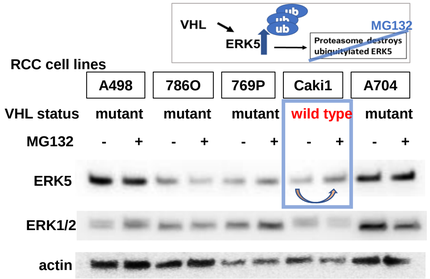


Results 1 ERK5 expression in patients with RCC



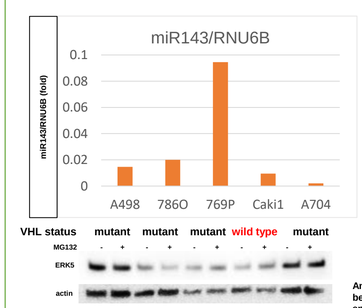
A total of 168 surgical specimens taken from patients with localized RCC were analyzed using immunohistochemistry (IHC). Of these, 82 (48.8 %) were ERK5-positive and 20 (11.9 %) were strongly ERK5-positive. Additionally, there was strong ERK5 expression in some tumors.

Results 2 Effect of proteasome inhibition on ERK5



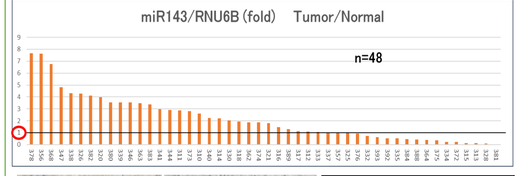
Proteasome inhibition appeared to enhance ERK5 expression in wild type cell lines, but not in *VHL*-mutant cell lines as shown by western blot (WB) analysis.

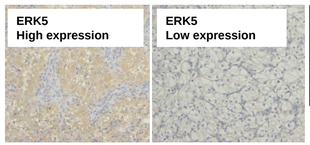
Results 3 Quantification of miR143 and expression of ERK5 in cell lines



An inverse correlation between ERK5 expression and miR143 quantification was observed in cell lines.

Results 4 Quantification of miR143 and expression of ERK5 in RCC cases



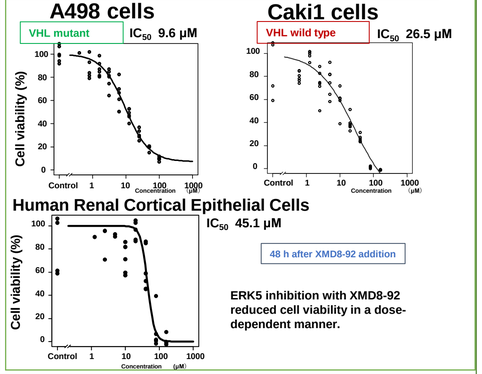


	ERK5 High expression	ERK5 Low expression
miR143 less than 1	6	12
miR143 1 or more	3	27

Fisher's test p value 0.0625

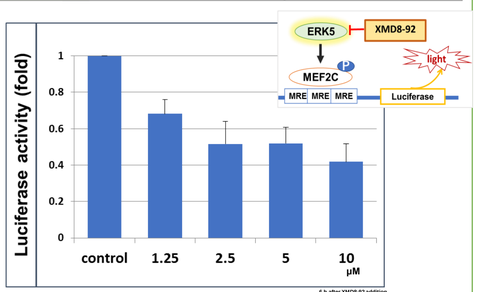
In 48 RCC specimens, an inverse correlation between ERK5 expression and miR143 quantification was observed (Fisher's exact test p=0.0625).

Results 5 Suppression of cell proliferation by ERK5 inhibitors



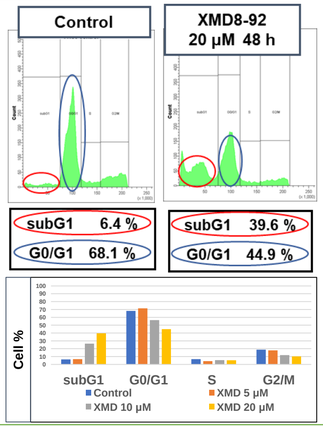
ERK5 inhibition with XMD8-92 reduced cell viability in a dose-dependent manner.

Results 6 A luciferase reporter assay for XMD8-92 with a MEF2C promoter



A498 cells were treated with XMD8-92 at different concentrations. MEF2C activity was assessed using the luciferase reporter assay after 6 h. XMD8-92 inhibited MEF2C activity in a dose-dependent manner.

Results 7 Effect of ERK5 inhibition on cell cycle

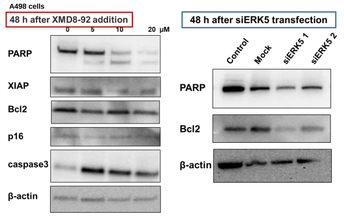


A498 cells

48 h after XMD8-92 addition

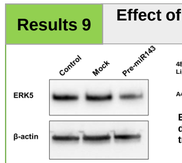
ERK5 inhibition with XMD8-92 resulted in an increase in the sub-G1 population as observed by flow cytometry.

Results 8 ERK5 inhibition and ERK5 knockdown



Cleaved PARP expression indicated an increase in apoptotic cells as seen by WB. ERK5 knockdown downregulated PARP and Bcl-2 expression.

Results 9 Effect of miR143 transfection on ERK5



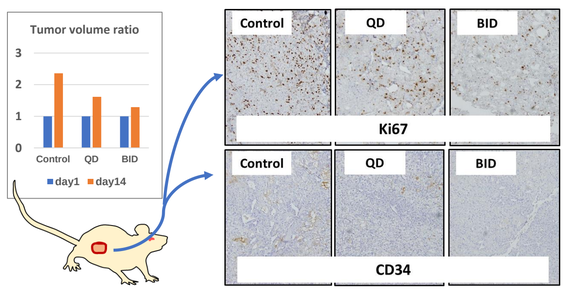
48 h after Pre-miR143 introduction by Lipofectamin 3000

A498 cell line

ERK5 protein expression level was decreased in the A498 cells transfected with pre-miR-143.

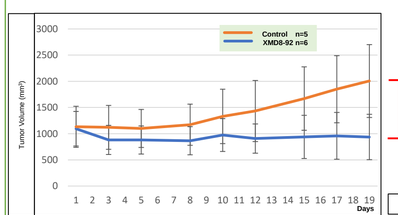
Results 10 ERK5 inhibition in tumor xenografted mice

Mice were injected 8.5×10^5 A498 cells subcutaneously (SC). In case of the appearance of a palpable tumor, mice were treated with XMD8-92 at a dose of 50 mg/kg once daily (QD), twice daily (BID), or with carrier solution intraperitoneally. After 2 weeks, the tumors were harvested, sectioned, and stained with Ki67 and CD34.



XMD8-92 showed anti-tumor activity in tumor xenograft mice model and downregulated Ki67 and CD34 expression.

8.5×10^5 A498 cells were resuspended in DMEM and injected SC into 11 athymic mice. Mice were randomized into two groups at the appearance of a palpable tumor. The XMD8-92 group was treated with XMD8-92 at a dose of 50 mg/kg once daily on weekdays, intraperitoneally. The control group received injections of the same volume of carrier solution with the same frequency.



* p value 0.0027

The tumor growth in athymic mice was measured every 2 or 3 days within a 3 week timeframe. After 19 days, treatment of the mice with XMD8-92 significantly inhibited the growth of the xenografted tumors.

COI Disclosure Information

Lead Presenter/Responsible Researcher: Hidenori Kanno

I have no financial relationships to disclose.

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