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Sorafenib-Triggered Stress Granules Promote Resistance in Renal Cancer Cells by Recruiting Anti-apoptotic Cyclooxygenase 2

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Introduction

Tyrosine kinase inhibitors (TKIs) represent the mainstay of therapeutic options for advanced renal cell cancer (RCC) patients. However, a significant proportion of RCC patients are inherently refractory to sorafenib, and most of the remaining patients develop resistance. We found sorafenib triggered the formation of stress granules (SGs) in renal cancer cells. Several studies have reported SGs promote resistance to chemotherapeutic agents. The SGs induced by sorafenib contributing to renal cancer cell resistance is unknown.

Methods

Sorafenib induced SGs were shown by immunofluorescence (IF) using canonical SGs markers HuR, TIA-1 and G3BP1. We then confirmed the function of SGs by using specific siRNAs. The activation of GCN2 and the phosphorylation of its downstream eIF2α were detected by western blotting (WB). Cells apoptosis percentages were measured by AnnexinV-PI staining. Cox2 mRNA level was evaluated by real-time quantitative polymerase chain reaction (RT-qPCR). To examine whether the SGs participate in Cox2 mRNA turnover, RNA-FISH was performed using specific Cox2 mRNA probes, and SG-IF was performed using HuR antibody. The combination of celecoxib plus sorafenib was investigated in vitro and vivo.

Results

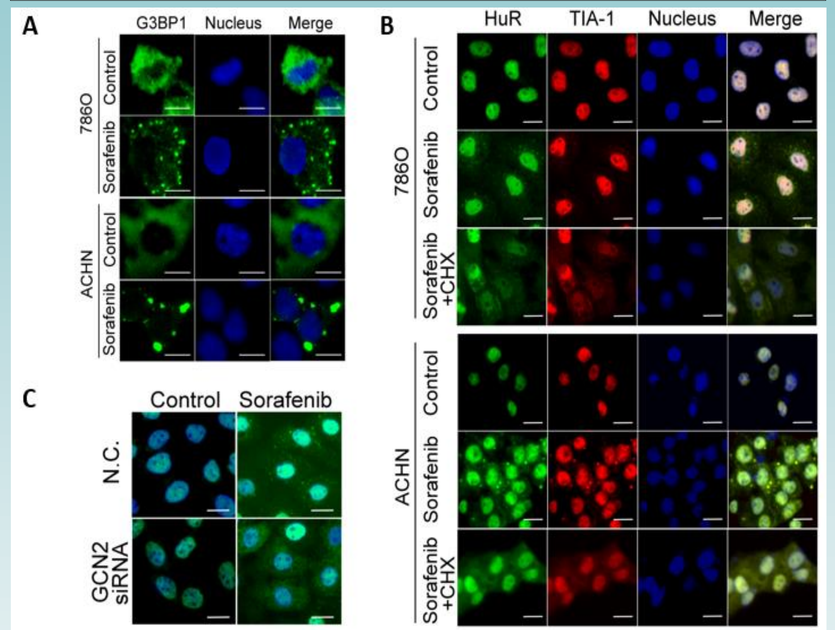

Fig.1 Stress granules (SGs) triggered by sorafenib were found to be dependent on GCN2 in renal cancer cells. (A) Sorafenib can trigger GTPase activating protein (SH3 domain) binding protein 1 (G3BP1) accumulation and formation many small cytoplasmic foci in renal cancer cells. (B) These particles-like foci were nicely co-localized with SG markers TIA-1 and HuR. (C) Sorafenib-triggered SGs were significantly decreased in GCN2 siRNA transfected cells.

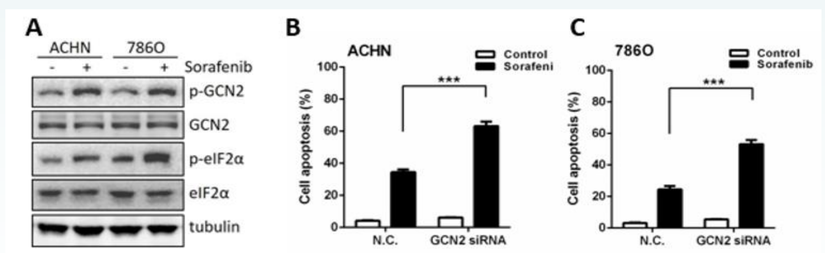

Fig.2 GCN2 has a protective role for renal cancer cells.(A)In ACHN and 786O cells, sorafenib increased the phosphorylation of GCN2 and eIF2α without influencing the total protein expression. (B)Silencing of GCN2 expression also promoted sorafenib-induced cell apoptosis.

Results

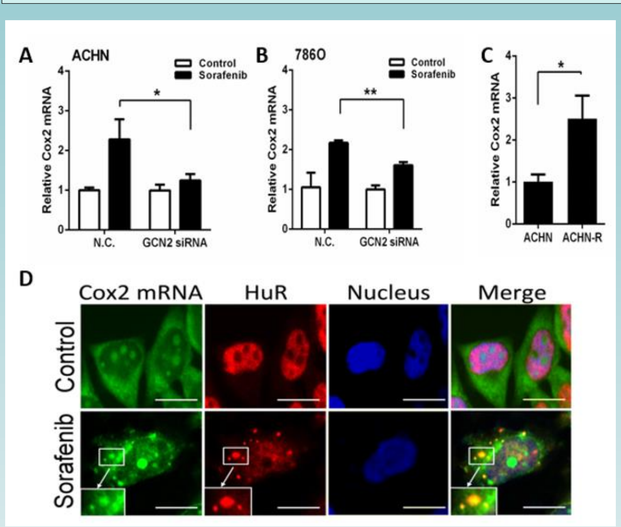

Fig.3 Anti-apoptotic gene cyclooxygenase 2 (cox2) was recruited into SGs.(A,B)Silencing of GCN2 expression alleviated sorafenib-increased cox2.(C)The expression of cox2 was higher in sorafenib-resistant cells.

(D) RNA-based fluorescence in-situ hybridization (RNA-FISH) experiment was performed to observe the cellular distribution of cox2 mRNA before and after sorafenib treatment, while anti-sense RNA probe was used to detect intracellular cox2 mRNA.

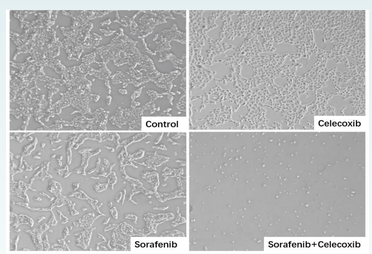

Fig.4 analgesic sTo override sorafenib resistance, we inactivated cox2 using celecoxib, a clinical pecific cox2 inhibitor. A combination treatment with low dosage of celecoxib and sorafenib induces ACHN cells death more efficient than sorafenib alone.

Conclusions

The SGs triggered by sorafenib were dependent on GCN2-eIF2α signaling. Notably, the protective effect of SGs was probably dependent on cox2 expression. Our findings suggest that a combination therapy of cox2 inhibitor celecoxib may represent a novel therapeutic approach for TKI resistant RCC treatment.