Nuclear Protein 1 (NUPR1) expression contributes to sorafenib resistance in hepatocellular carcinoma cells through protective autophagy activation and p73 inhibition

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INTRODUCTION

The multikinase inhibitor sorafenib was the first drug approved by the U.S. FDA for treating patients with advanced hepatocellular carcinoma (HCC). However, sorafenib resistance remains a major challenge for improving the effectiveness of HCC treatment. Increasing evidence supports the notion that autophagy may play a pro-survival role in cancer by contributing to tumor resistance during pharmacological treatment. Recent findings have demonstrated that sorafenib induces the activation of protective autophagic responses in HCC cells (1). We recently identified several genes modulated after sorafenib treatment of human HCC cells, including the stress-inducible nuclear protein 1 (NUPR1) gene (2). Multiple studies have shown that NUPR1 regulates autophagy, apoptosis, and chemoresistance (3).

AIMS

The present work aims:

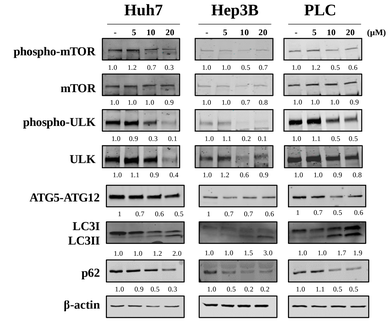
- to clarify the role of NUPR1 in HCC resistance to sorafenib by focusing on its role in modulating autophagy and apoptosis;
- to work toward identifying new potential targets and therapeutic approaches for improving the response to sorafenib in HCC treatment.

METHODS

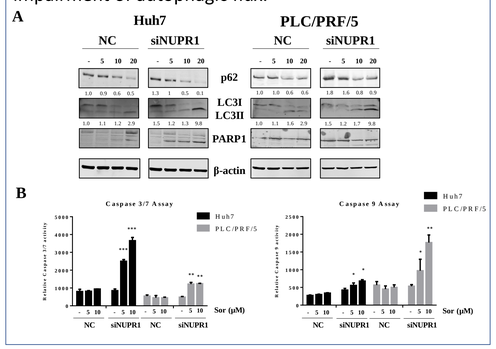
We used different human HCC cell lines (Huh7, Hep3B, and PLC/PRF/5) to investigate the role of NUPR1 in resistance to sorafenib treatment. The expression of autophagic markers and apoptosis-related proteins was detected by western blot. siRNA mediated gene silencing was carried out to downregulate NUPR1 (NUPR1 KD) and p73 (p73 KD) expression levels. Realtime PCR analysis was employed to examine NUPR1, p73, PUMA, NOXA, and p21/WAF1 mRNAs expression. The effect of sorafenib and/or NSC59984 on cell viability was determined by MTS assay. Mouse xenograft models were used to determine the effect of sorafenib and/or NSC59984 on tumor growth *in vivo*.

RESULTS

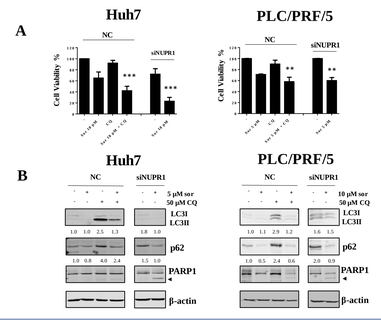
Sorafenib treatment of HCC cells activated autophagic flux, as shown by increased LC3II expression levels, decreased p62 and ATG5/ATG12 complex, and reduced mTOR and ULK1 phosphorylation.



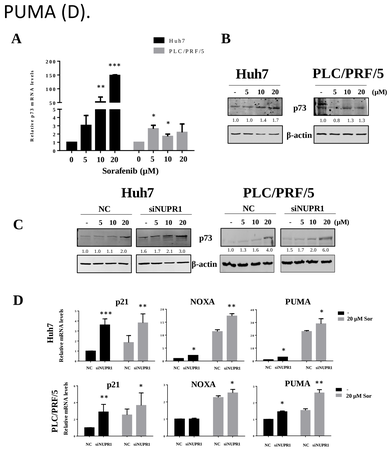
In basal conditions, NUPR1 knockdown (KD) induced an accumulation of p62 (A), suggesting autophagic flux blockage. NUPR1 KD significantly increased tumor cell sorafenib sensitivity, reducing cell viability (data not shown) and increasing apoptosis as shown by PARP1 cleavage (A) and caspase-3/7 and -9 activation (B). Importantly, these effects were associated with increased p62 expression levels, suggesting an impairment of autophagic flux.



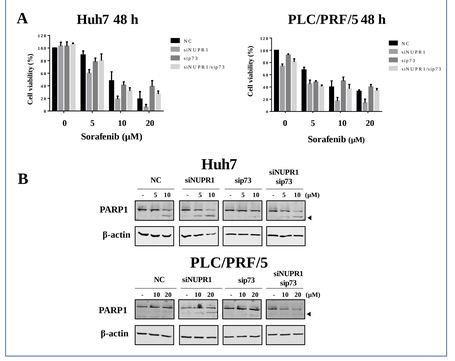
To verify the effects of autophagic flux blockage and apoptosis induction in NUPR1 KD cells, we used chloroquine (CQ) as a pharmacological autophagy inhibitor. CQ treatment or NUPR1 KD significantly increased sensitivity to sorafenib in HCC cells (A) and increased LC3II expression and p62 accumulation, suggesting autophagic flux blockage. Upon CQ treatment or NUPR1 KD, apoptosis was induced after sorafenib treatment, as shown by PARP1 cleavage (B).



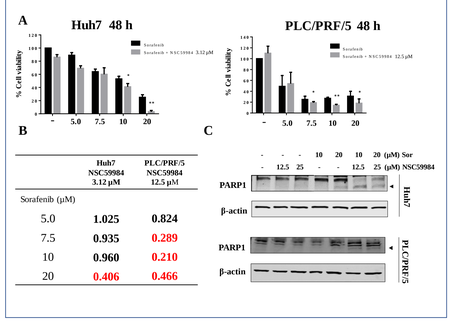
Sorafenib treatment (A-B) and NUPR1 KD (C) were associated with increased expression of p73, along with its downstream transcription targets p21/WAF1, NOXA, and PUMA (D).



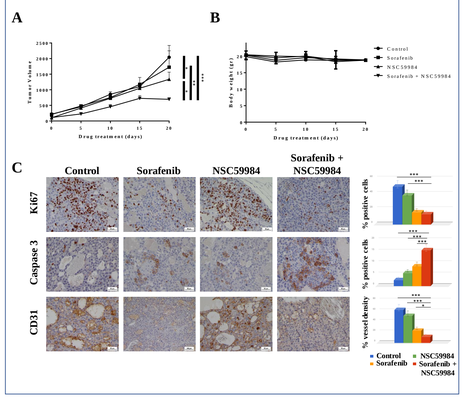
To evaluate the functional role of p73 after NUPR1 KD, p73 and NUPR1 were simultaneously silenced (p73/NUPR1 KD) in HCC cells and then treated with sorafenib. p73/NUPR1 KD HCC cells showed increased resistance to sorafenib treatment (A) and markedly decreased PARP1 cleavage (B) compared to NUPR1 KD cells alone (A).

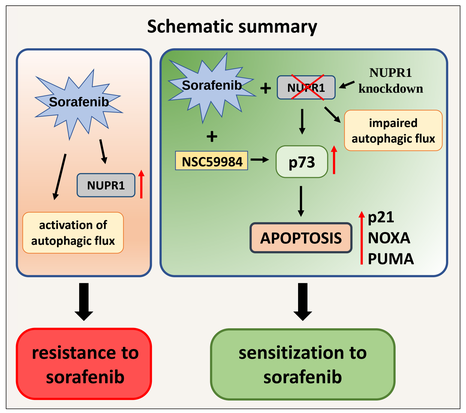


Pharmacological activation of p73 via the novel p73 small molecule activator NSC59984 produced synergistic anti-tumor effects in sorafenib-treated HCC cells (A-B). The NSC59984/sorafenib combination increased apoptosis, as shown by PARP1 cleavage, more than each inhibitor alone (C).



In vivo, the NSC59984/sorafenib combination synergistically suppressed HCC cell tumor growth (A) more than either treatment alone. Moreover, NSC59984/sorafenib-treated mice showed unaltered body weight (B) compared to the control group and either treatment alone. Furthermore, this combination significantly decreased cell proliferation (Ki67) and angiogenesis (CD31) marker expression and increased apoptosis (caspase 3) compared to the control group and either treatment alone (C).





CONCLUSIONS

Our data suggest that the autophagy impairment and increased p73 expression achieved by NUPR1 KD potentiates sorafenib-induced apoptosis of HCC cells. Moreover, combined pharmacological therapy with the p73 activator NSC59984 and sorafenib may be a novel approach for treating HCC.

REFERENCES

1) Zhai B, et al. Inhibition of Akt reverses the acquired resistance to sorafenib by switching protective autophagy to autophagic cell death in hepatocellular carcinoma. Mol Cancer Ther. 2014;13:1589-98.

2) Emma MR, et al. NUPR1, a new target in liver cancer: implication in controlling cell growth, migration, invasion and sorafenib resistance. Cell Death Dis. 2016;7:e2269.

3) Li A, et al. NUPR1 Silencing Induces Autophagy-Mediated Apoptosis in Multiple Myeloma Cells Through the PI3K/AKT/mTOR Pathway. DNA Cell Biol. 2020;39:368-78.

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