P2-P170 Development of resistance to sorafenib, a multikinase inhibitor, in hepatocellular carcinoma is mediated by SIRT

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Background

Sorafenib is a multi-kinase inhibitor and one of the few systemic treatment options for patients with advanced hepatocellular carcinomas (HCCs). Resistance to sorafenib develops frequently and could be mediated by the NAD dependent deacetylase sirtuin (SIRT) 1, a master regulator of cellular energy metabolism and stress responses. We aimed to find out if sorafenib effects depend on changes in cellular NAD levels as well as activity of SIRT1 and the cellular energy sensor adenosine monophosphate kinase (AMPK).

Results

Sorafenib decreases phosphorylation of ERK and induces apoptosis in hepatocarcinoma cell lines

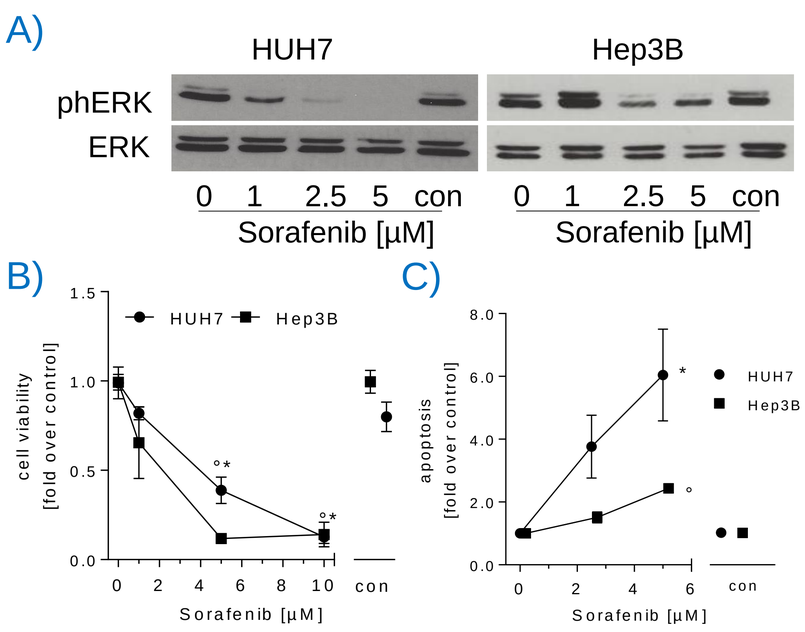

Figure 1 For all experiments, HUH7 and Hep3B cells were stimulated with sorafenib [0 -10 µM] for 24h. A) Western Blot analysis of ph-ERK and total ERK after stimulation with sorafenib for 24h (n=3). B) WST Assay was perfomed to measure cell viablitaty. C) Apoptotic cell number was measured by flow cytometry (n=3). Serum free medium [0 µM] was set one. Data are shown as mean ± SEM. con: solvent control (DMSO); *p<0.05 (HUH7); °p<0.05 (Hep3B)

Sorafenib regulates phosphorylation of the AMPK/mTOR pathway in HUH7 cells

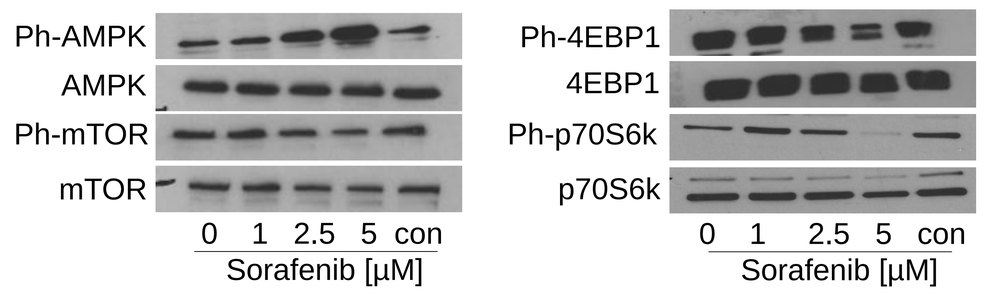

Figure 4 HUH7 cells cells were stimulated with sorafenib [1 µM; 2.5µM and 5 µM] for 24h. Western blot analysis was performed to determine phosphorylation status of AMPK, mTOR, 4EBP1 and p70S6K.

Inhibition of SIRT1 does not sensitize hepatocarcinoma cells to sorafenib (24h)

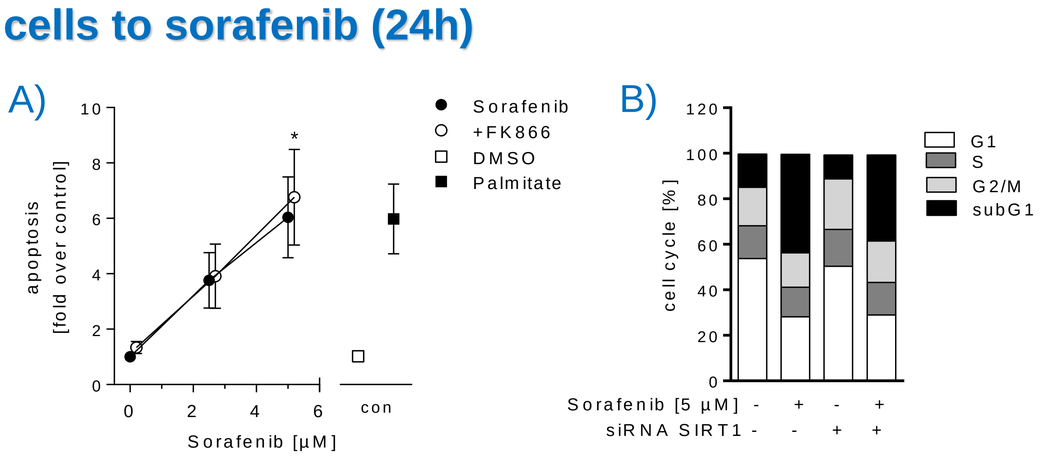

Figure 5 A) Cells were stimulated with sorafenib alone or in combination with FK866 [10 nM] for 24h. A) Apoptosis was determined by flow cytometry (n=3). DMSO served as solvent control and palmitate [0.5 mM] as positive control. Serum free medium [0 µM] was set one. B) SIRT1 protein levels were downregulated by SIRT1 siRNA using electroporation. Cell cycle distribution was analyzed by PI staining and flow cytometry. Data are shown as mean ± SEM. *p<0.05.

Sorafenib decreases NAD levels in HUH7 cells which is not mediated by NAMPT or SIRT1.

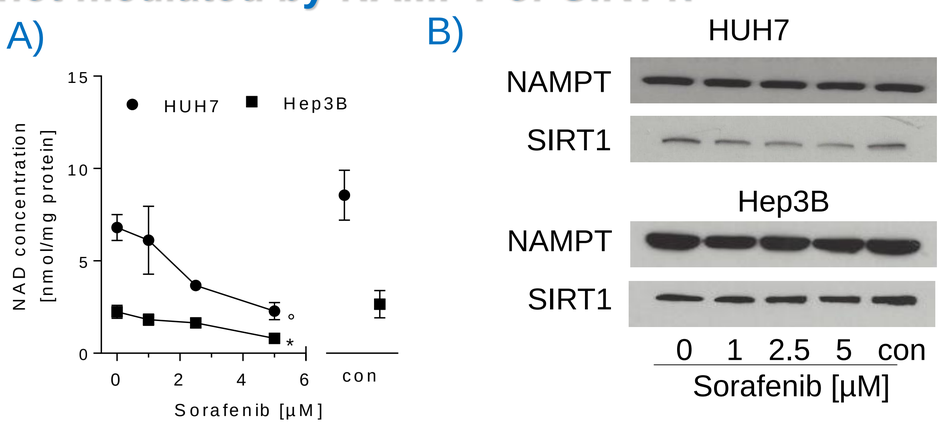

Figure 2 For all experiments, HUH7 and Hep3B cells were stimulated with sorafenib [1 µM; 2.5µM and 5 µM] for 24h A) Intracellular NAD levels were measured by gradient HPLC /UV analysis (n=3). B) NAMPT and SIRT1 protein were measured by Western blot analysis (n=3). Data are shown as mean ± SEM. con: solvent control (DMSO°p<0.05 (HUH7); *p<0.05 (Hep3B).

Sorafenib incubation induces mitochondrial dysfunction

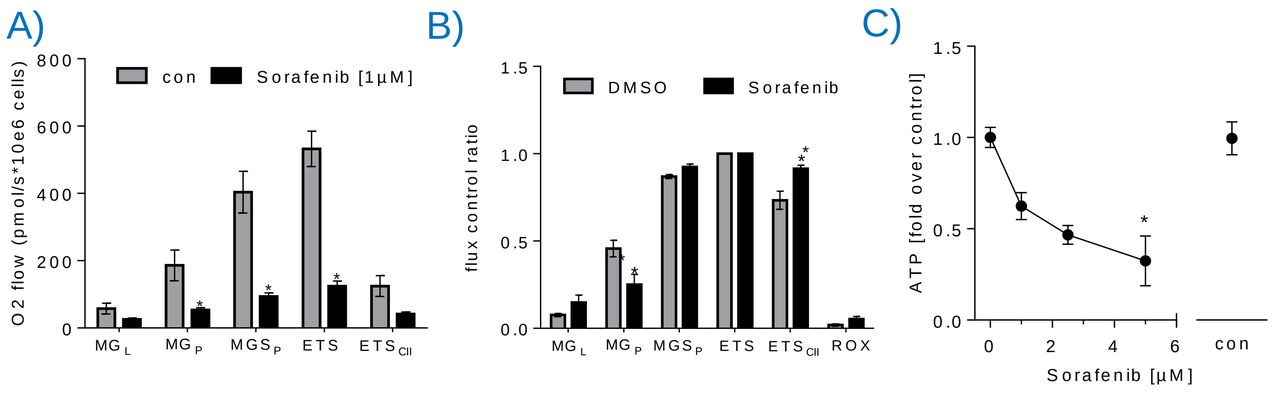

Figure 3 Oxygen flow measured by high resolution respirometry (Oxygraph2K, Oroboros) in A) permeabilised HUH7 cells after stimulation with 1 µM sorafenib and B) after direct addition of sorafenib to isolated mitochondria (10 µM). Values are normalised to maximal O_2 flux (uncoupling conditions). O_2 consumption is measured after addition of substrates malate and glutamate (MG_L), ADP (MG_P), succinate (MGS_P), uncoupling by FCCP, complex I inhibitor rotenone and complex III inhibitor Antimycin A. C) ATP levels were measured by CellTiter-Glo Luminescent Cell Viability Assay after stimulation with sorafenib [1µM; 2.5µM and 5µM] for 24h. Serum free medium [0 µM] was set one. Data are shown as mean ± SEM (n≥3). con: solvent control (DMSO); *p<0.05

Sorafenib® - induced apoptosis is counteracted by SIRT1 overexpression and NAD supplementation

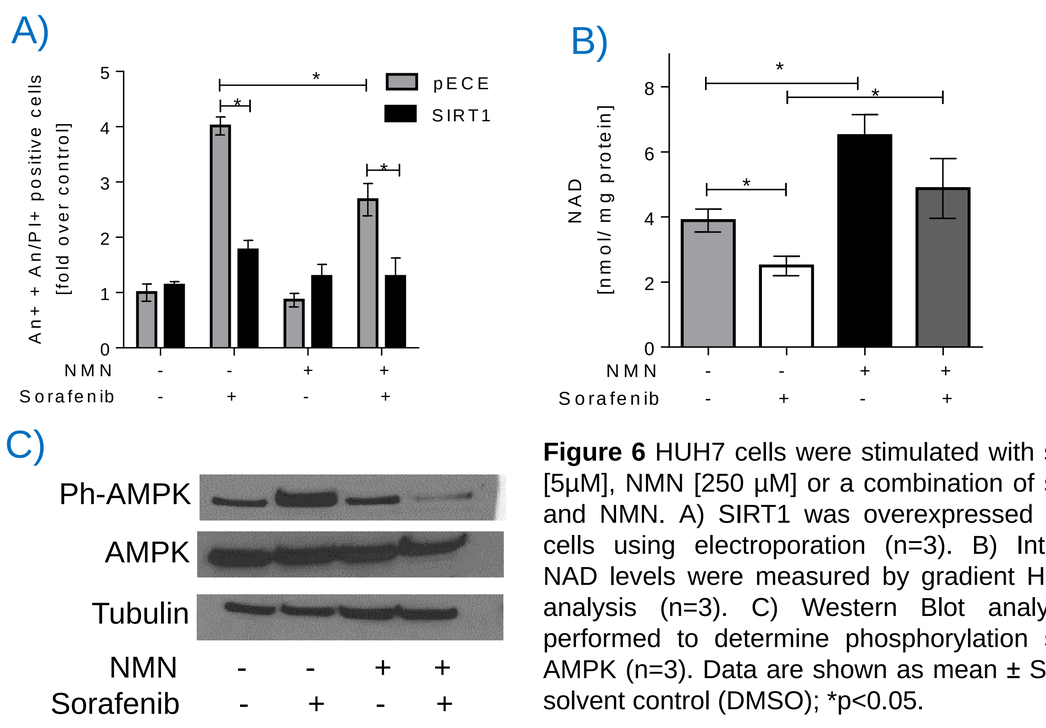

Figure 6 HUH7 cells were stimulated with sorafenib [5µM], NMN [250 µM] or a combination of sorafenib and NMN. A) SIRT1 was overexpressed in HUH7 cells using electroporation (n=3). B) Intracellular NAD levels were measured by gradient HPLC /UV analysis (n=3). C) Western Blot analysis was performed to determine phosphorylation status of AMPK (n=3). Data are shown as mean ± SEM. con: solvent control (DMSO); *p<0.05.

Conclusion

Sorafenib® targets multiple cellular pathways including the SIRT1/AMPK axis and that overexpression of SIRT1 could be an underlying mechanism of resistance to sorafenib treatment in HCC.

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