Silibinin mediated HIF-1α inhibition restores oxidative phosphorylation in highly glycolytic nasopharyngeal carcinoma

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Abstract

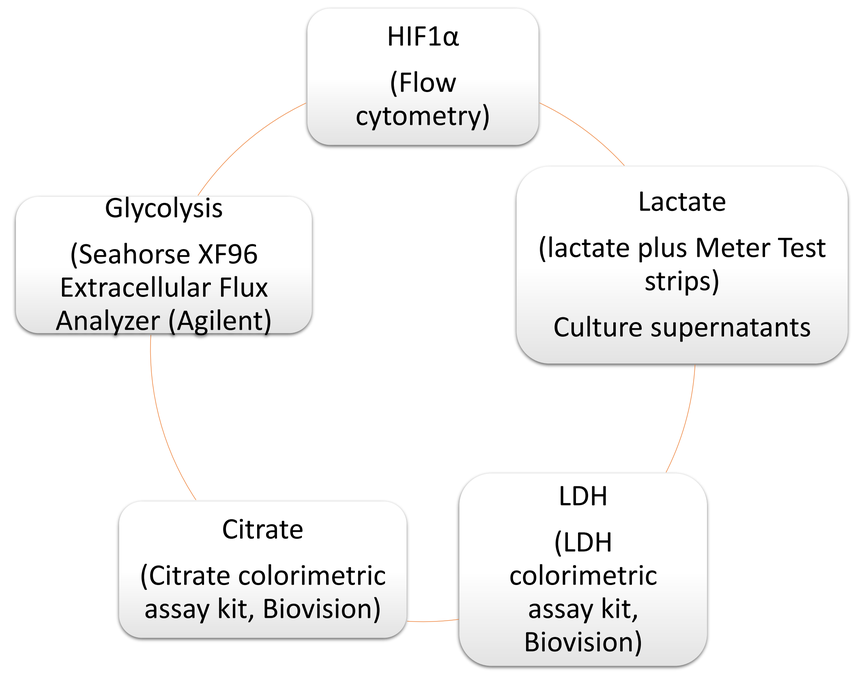
Introduction : Tumor resistance to chemotherapy has been increasingly linked to the installation of a hypoxic phenotype in highly glycolytic tumors. Undifferentiated nasopharyngeal carcinoma (NPC) enhanced aerobic glycolysis, promoted by EBV, has been associated with HIF1-α, LDH-A dysregulation and worse prognosis. Here we investigated the effect of Silibinin (SBN), an emerging antitumor flavonoid, on HIF-1α associated LDH-A activity in High NPC glycolytic tumors.

Materials /Patients and methods : Primary NPC human biopsies (n =20) and C666.1 cells (NPC) were cultured with silibinin (0-100 µM). Glycolysis was tested using Seahorse XF96 Extracellular Flux Analyzer. HIF-1α expression was evaluated by Flow cytometry (C666-1) and IHC (primary NPC biopsies). LDH activity and citrate levels were measured using LDH and citrate colorimetric assay kit, respectively. Lactate levels were evaluated using lactate plus Meter Test strips.

Results : A heat map analysis of LDH-A activity and lactate synthesis derived from patients explants determined that 12 out of 20 analyzed tumors displayed a highly glycolytic phenotype, as indicated by presence of LDH-A^{High}-Lactate^{High} and LDH-A^{High} –Lactate^{Low} profiles. Analysis of the effect of the drug on these tumors, showed that SBN exerted a significant inhibitory effect on the expression and/or release of studied molecules (LDH-A: 53.11%, p = 0.0005; Lactate: 15.41%, p = 0.059). Pearson correlation analysis indicated loss of the link associating LDH-A activity to lactate synthesis in the treated tumor explants compared to untreated controls (r = −0.18, p = 0.57 vs. r = 0.51, p = 0.08). Similar results were obtained with SBN on C666.1 cells. Citrate expression analysis, showed presence of a consistent restoration of OXPHOS at the expense of LDH-A and lactate synthesis in C666-1 cells as demonstrate by a 300% citrate levels increase in presence of SBN. Immunohistological evaluation of HIF-1α expression showed that SBN induced in average a 42.89% reduction (H-score) in hypoxia factor expression in treated tissues compared to control biopsies (p = 0.07).

Conclusion : Overall, we show that silibinin is a potent inhibitor of HIF-1α, with a strong capacity to restore oxidative phosphorylation (OXPHOS) at the expense of aerobic glycolysis in highly glycolytic NPC tumors. These results provide new perspective for silibinin use as a promising anticancer molecule to overcome NPC resistance to chemotherapy.

Study design



Keywords: NPC; LDH-A, OXPHOS; Hypoxia ; Silibinin.

Results

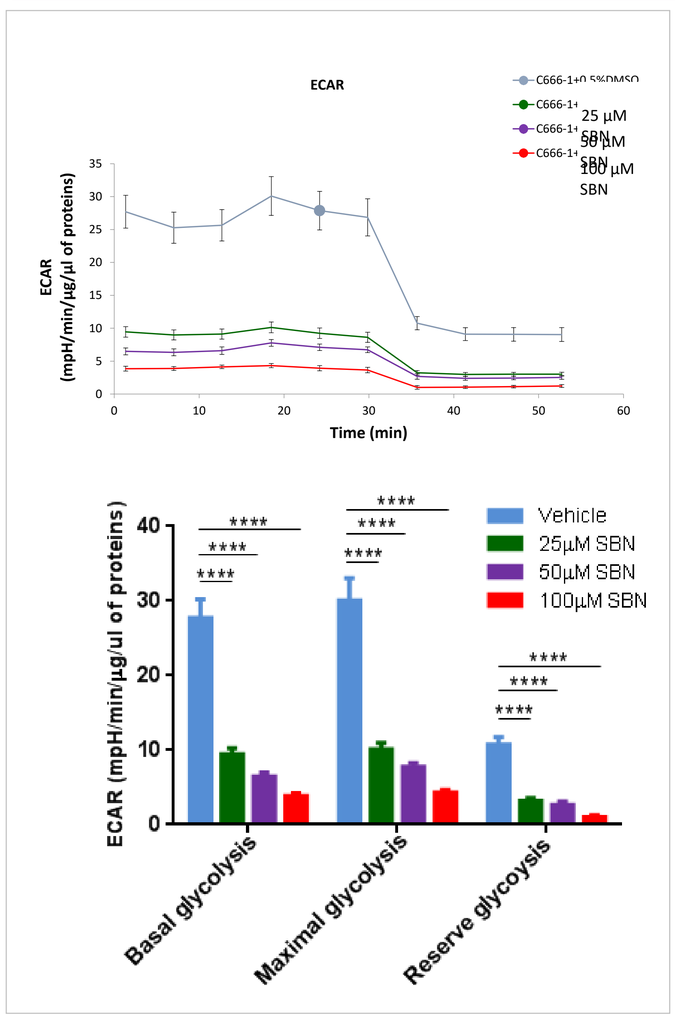


Fig.1 Silibinin reduces glycolysis in a dose dependant manner in C666-1 cells. Differences between control and silibinin-treated cells were statistically significant using ANOVA. (**** p<0,0001).

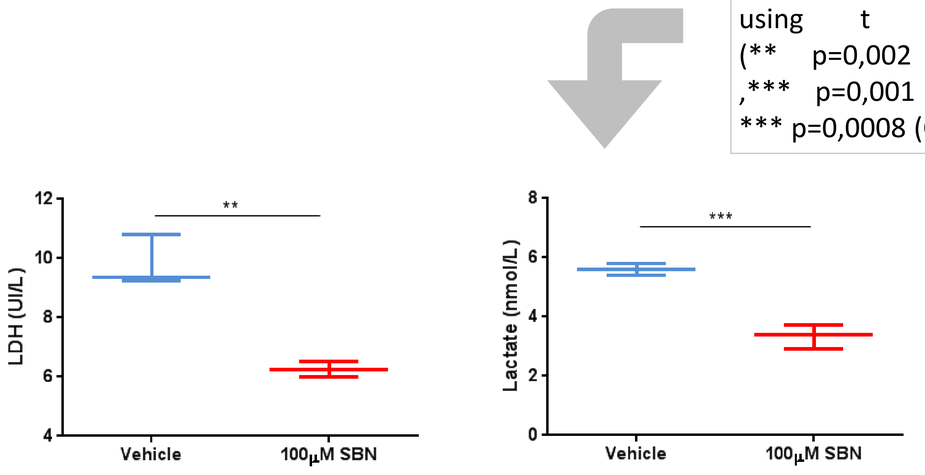


Fig.2 Silibinin reduces LDH activity (A) and lactate production (B) in C666-1 cell.. Differences between groups were analyzed using t test. (** p=0,002 (A) ,*** p=0,001 (B), *** p=0,0008 (C)).

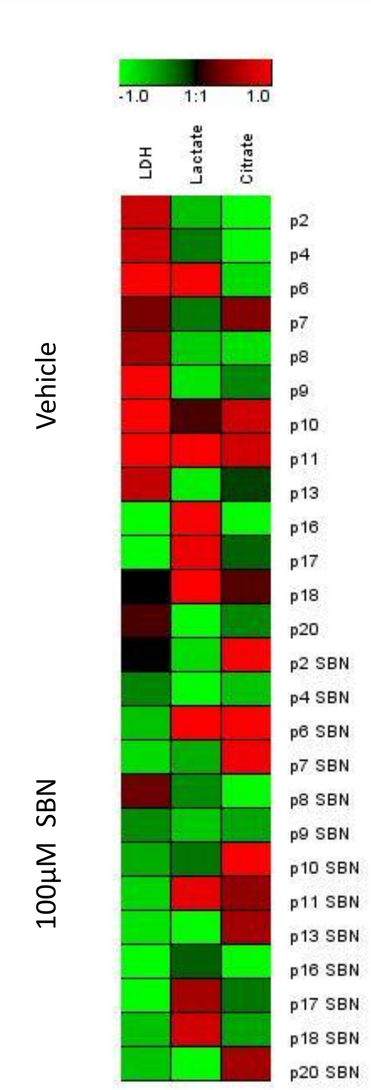
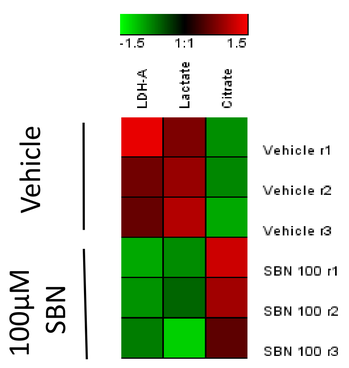


Fig. 3 Silibinin reduces glycolysis in a dose dependant manner in highly glycolytic patients tumors (A) and in C666-1 cells5 (B) . Differences



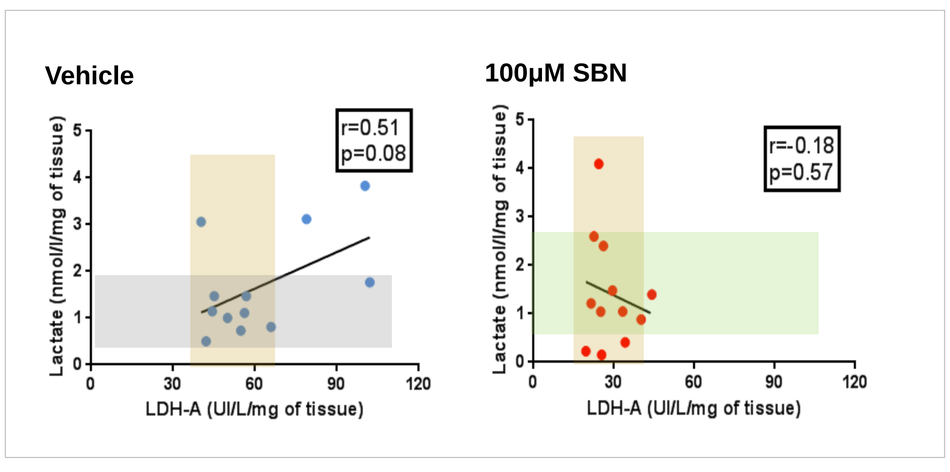


Fig. 4 Silibinin presence deteriorates the link associating LDH-A activity to lactate synthesis in the treated tumor explants compared to untreated controls (r = −0.18, p = 0.57 vs. r = 0.51, p = 0.08). Similar results were obtained with SBN on C666.1 cells

Fig.5 Silibinin decreases citrate expression in C666 cell line and tumor explants. Differences between groups were analyzed using t test. (** p=0,006).

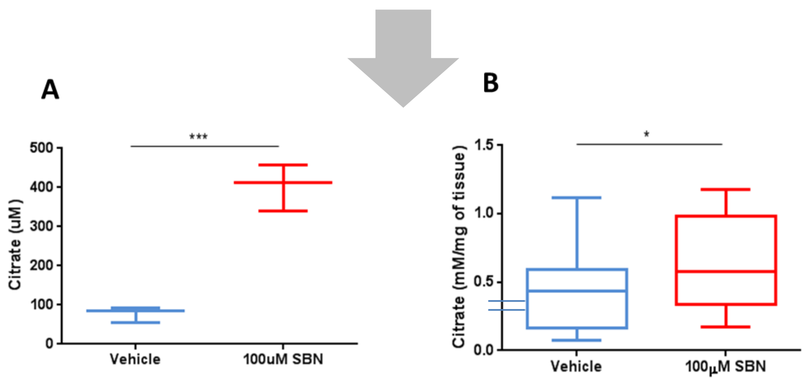
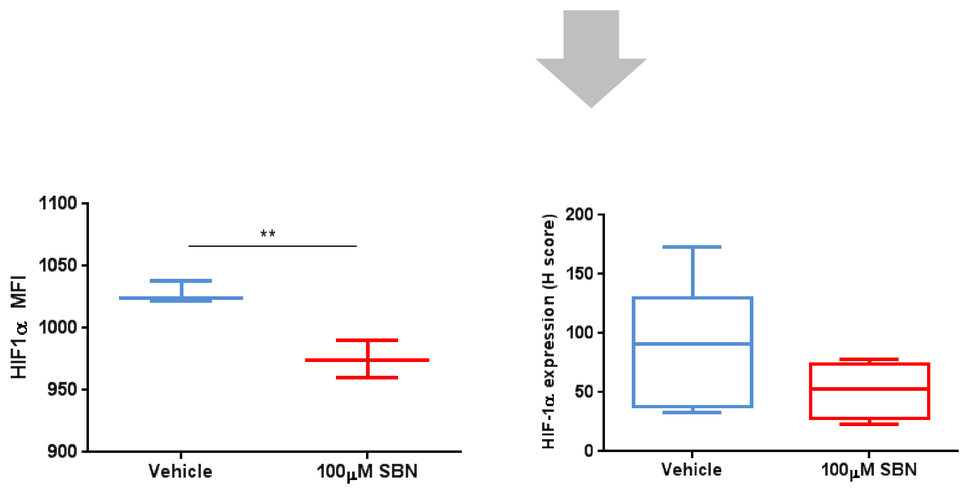


Fig.6 Silibinin decreases HIF1α expression in C666-1 cells and tumor tissus (IHC- H Score). Differences between groups were analyzed using t test. (** p=0,006



Discussion and conclusion

Here we show that silibinin affects NPC tumor cell glycolytic metabolism by interfering with HIF1α signaling and LDH-A activity. A reduction of lactate production has been observed and citrate production has been recovered in highly glycolytic tumors indicating a metabolic rewiring in the treated tumors.

Overall, we show that silibinin is a potent inhibitor of HIF-1α, with a strong capacity to restore oxidative phosphorylation (OXPHOS) at the expense of aerobic glycolysis in highly glycolytic NPC tumors. These results provide new perspective for silibinin use as a promising anticancer molecule to overcome NPC resistance to chemotherapy.