Effect of Telomerase inhibition on Hexokinase II expression and activity in Hepatocellular carcinoma

Nadine Mahfouz[a], Joe Aoun[b], Roula Tahtouh[a], Nada Alaaeddine[c], George Hilal*[,a]

[a] St. Joseph University, Faculty of Medicine , Laboratory of Cancer and Metabolism,; Beirut, Lebanon; [b] Surgery and Oncology Department, Hotel Dieu de France; [c] St. Joseph University, Faculty of Medicine, Laboratory of Regenerative Medicine, Beirut, Lebanon.

INTRODUCTION

- Hepatocellular carcinoma (HCC) is the third leading cause of cancer deaths worldwide.
- Telomerase is almost universally required for cellular immortality and is expressed in 85% of cancers
- Glycolysis is an anaerobic pathway that generates ATP by oxidizing glucose. This pathway is highly activated in cancer cells.
- The enzyme hexokinase II (HK II), that catalysis the first reaction of glycolysis, has attracted considerable attention because it commits glucose to catabolism and because it has been shown to be upregulated in various malignant tumors and cancer cell lines.
- The purpose of this study was to investigate the effect of Telomerase inhibition on Hexokinase expression and activity; and to assess the implication of Telomerase in glycolysis

MATERIALS AND METHODS

- Hepatocellular carcinoma HepG2/C3A cells) were cultured in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% Penicillin/Streptomycin, 0.1% Non essential amino acids and incubated at 37°c with 5% CO_2
- Cells were treated with three Telomerase inhibitors BIBR-1532 (10 µM), Costunolide (10 µM) and MST-312 (2 µM), Hexokinase inhibitor 3-Bromopyryvate (3BP) at 20 µM , Glycolysis inhibitor 2-Deoxyglucose (2DG) at 4 mM and Fructose at 2 g/l.
- HK II and hTERT (Telomerase catalytic subunit) mRNA levels were assessed using RT-PCR
- Cell viability test was performed using tetrazolium salt.
- Hexokinase activity was measured by a colorimetric assay using Glucose 100 mM, Tris HCl , MgCl2 , ATP 5 mM , Glucose-6-phosphate dehydrogenase (G6PDH) and NADP+

RESULTS

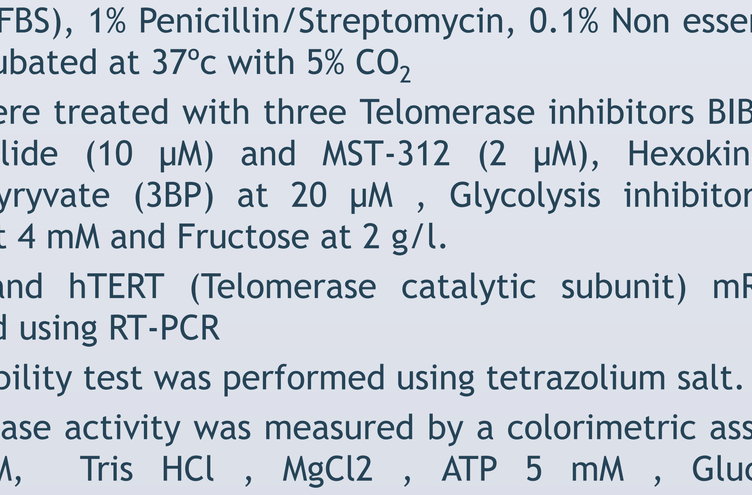

Figure 1: Telomerase inhibition decreased the activity of the total Hexokinase . HepG2/C3A cells were treated for 48 hours with Telomerase inhibitors BIBR-1532 (10µM), Costunolide (10 µM) and MST (2 Mm). Results are expressed as the means ± SD from three experiments. * indicates that there is a significant difference (*: P < 0.05, **: P < 0.005)

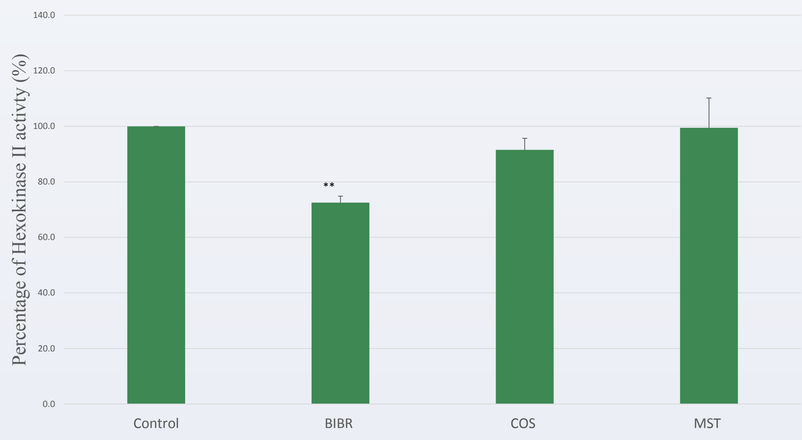

Figure 2: Telomerase inhibition decreased the activity of the Mitochondrial Hexokinase . HepG2/C3A cells were treated for 48 hours with Telomerase inhibitors BIBR-1532 (10µM), Costunolide (10 µM) and MST (2 Mm). Results are expressed as the means ± SD from three experiments. * indicates that there is a significant difference (*: P < 0.05, **: P < 0.005)

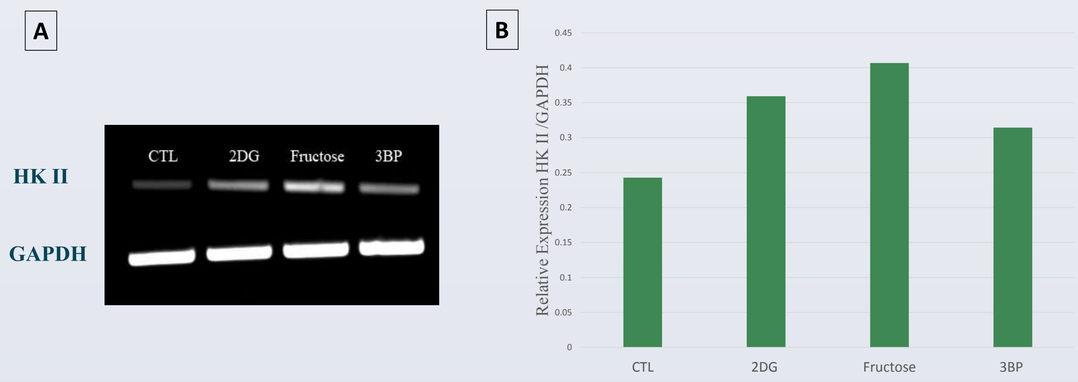

Figure 3: 3BP (20 µM), 2DG (4 mM)) and Fructose (2 g/l) increased HK II mRNA expression levels (A) HepG2/C3A cells were treated for 48 hours. Total RNAs were isolated and analyzed for HK II expression by RT-PCR. GAPDH used as loading control. (B) The HK II mRNA levels were normalized by the GAPDH.

Figure 4: 3BP (20 µM), 2DG (4 mM)) and Fructose (2 g/l) increased hTERT mRNA expression levels (A) HepG2/C3A cells were treated for 48 hours. Total RNAs were isolated and analyzed for hTERT expression by RT-PCR. GAPDH used as loading control. (B) hTERT mRNA levels were normalized by the GAPDH.

CONCLUSION

In this study, we showed that the treatment of HCC cell line HepG2/C3A with Telomerase inhibitor BIBR-1532 (10 µM) decreased total and mitochondrial HK II activity. We further demonstrated that the 3-Bromopyruvate (3BP (20 µM)), 2-Deoxy-D-glucose (2DG (4 mM)) and Fructose (2 g/l) increased mRNA expression levels of Telomerase catalytic component (hTERT) and HK II. These results indicate that telomerase could be involved in glucose metabolism via HK II activity and expression level regulation.

CONTACT INFORMATION

Nadine Mahfouz: nadine.mahfooz@gmail.com

Corresponding Author: george.hilal@usj.edu.lb