Isolation of Bioluminescence Bacterium from Marine Fish and Amplification of Luciferase (lux AB) Gene

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ABSTRACT

Hepatocellular carcinoma is a type of primary liver cancer that accounts for 75% of all liver cancers globally. HCC is said to be the 6th most prevalent cancer and the 3rd major cause of cancer related death accounting for approximately 0.32 million annual deaths across the globe. The increasing incidence of HCC with a very high mortality rate is a major problem encountered by many regions of the world including both developing and developed countries and is a source of increasing economic burden. Therefore, effective treatment strategies for HCC are the need of the hour. Oxidative stress can be referred to as the lack of proportion between generation of reactive oxygen species and their removal by antioxidant defense mechanisms. This imbalance can have deleterious effects and contributes to the pathophysiology of several disorders including inflammation, aging cataract, diabetes and cancer. However, this controversial role of oxidative stress leading to apoptosis can be acquired as a treatment for HCC. The current study aimed to investigate the effect of induced oxidative stress via hydrogen peroxide (H_2O_2) on the proliferation of HCC Huh-7 cells in-vitro. The toxicity generated by H_2O2 produce anti- proliferative effect on HCC Huh-7 cells and up regulates the oxidative stress markers. Proteomic studies were conducted in order to identify differential protein expression as potential therapeutic targets for the treatment of HCC.

Keywords: Hepatocellular carcinoma, Oxidative stress, ROS, Cancer therapy, GSTP-1, PRX-2

INTRODUCTION

Pakistan is one of the many countries where HCC is highly endemic. The incidence rate of HCC in Pakistan is found to be 7.6 per 100,000 persons in men and 2.8 in women annually. 4.8% of the 200 million people in Pakistan are HCV positive while 2.5% are HBV positive hence a big chunk of the population is at a high risk of developing HCC (1). Approximately 782,000 new cases of HCC are reported from across the globe annually. While it accounts for 600,000 deaths worldwide every year. The incidence rate of HCC is almost the same as its mortality rate depicting the fact that the disease is usually identified at a stage where its treatment is not effective. Approximately 782,000 new cases of HCC are reported from across the globe annually. While it accounts for 600,000 deaths worldwide every year (2).

The average developmental period of HCC is said to be 3.2 years, screening and diagnosis during this period has the most impact due to the high potential for the disease to be cured. Despite of the importance of an early diagnosis of HCC, in view of the fact to increase the rate of survival it is very difficult to identify HCC during its subclinical period. Unfortunately, most of the HCC cases are identified after metastasis has occurred due to which the rate of survival for it is very low (3). Some of the vital techniques employed for diagnosing HCC include measuring serum biomarkers, using various imaging modalities and histological analysis. The incidence rate of HCC is almost the same as its mortality rate depicting the fact that the disease is usually identified at a stage where its treatment is not effective. The controversial role of oxidative stress in inducing modifications leading to cell cytotoxicity is the area of increasing interest as a therapeutic approach in various kinds of cancer (4). We aim to investigate the role of induced oxidative stress as a potential therapeutic

approach for HCC and to identify potential biomarkers to be used as therapeutic targets for treating HCC in the future.

OBJECTIVES

a) To analyze the cytotoxic properties of induced oxidative stress on HCC Huh-7 cells as anti-cancerous agent.

b) To investigate the induction of oxidative stress as a promising therapeutic strategy for Hepatocellular Carcinoma.

c) To identify potential molecules for Hepatocellular Carcinoma that could serve as drug targets.

METHODOLOGY

Cell Culture:

HCC Huh-7 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) medium supplemented with 10% Fetal Bovine Serum (FBS) and 1% pen-strep. The cells were incubated in a humid environment with 5% CO2 at 37°C in T25 flasks. The cells were then sub-cultured upon reaching 80% confluency.

Drug Incubation:

The cultured cells were treated with a range of concentrations of H_2O_2 in order to induce oxidative stress.

Cytotoxicity:

After the treatment, the cytotoxicity of induced oxidative stress was determined via MTT Assay. The cells were incubated with 5mg/ml of MTT for 2-3 hours. The plate was then read on 570nm on a microplate reader. The O.D. obtained was used to calculate % cytotoxicity.

qPCR

Total RNA was isolated from cells using TRI Reagent (Sigma Aldrich, Missouri, USA. RNA was reverse transcribed into cDNA by using Revert Aid First-Strand cDNA synthesis kit (Thermo Fisher Scientific). Primers that correspond *Peroxiredoxin2 (Prx2), Glutathione S-transferase p1 (GSTP1)* and *Nuclear factor erythroid 2- related factor 2 (NFE2L2)* genes were used for amplification. The relative expression of the genes were analyzed in treated HCC Huh-7 cells as compared untreated Huh-7 cells.

Immunocytochemistry:

The effect of induced oxidative stress on the expression of proteins of validated through Immunocytochemistry. Untreated and treated Huh-7 cells were immunostained with Rabbit Polyclonal antibodies against PRX2 (PAF757Hu01) and GSTP1 (PAB090Hu01)

(Cloud-Clone Corp. USA) followed by staining with 4,6-diamidino-2-phenylindole (DAPI). The cells were then observed under the NIKON TE 2000 fluorescent microscope.

CONCLUSION

As the treatment given to HCC Huh-7 cells resulted in the significant suppression of their growth and proliferation, from this study, we can conclude that the induction of oxidative stress via hydrogen peroxide treatment could be a substantial therapeutic approach for HCC in the future.

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