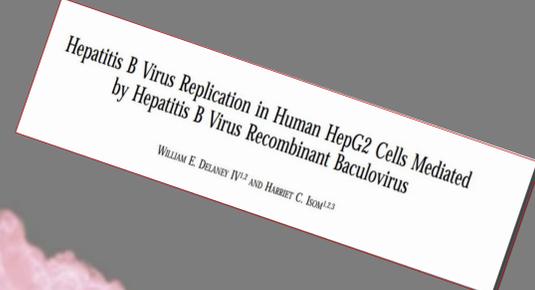
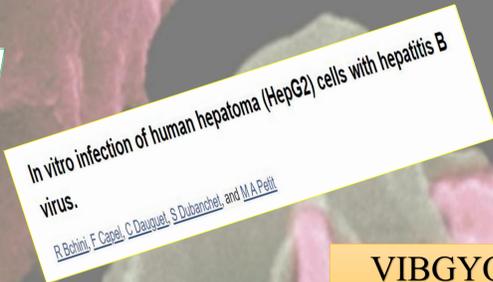
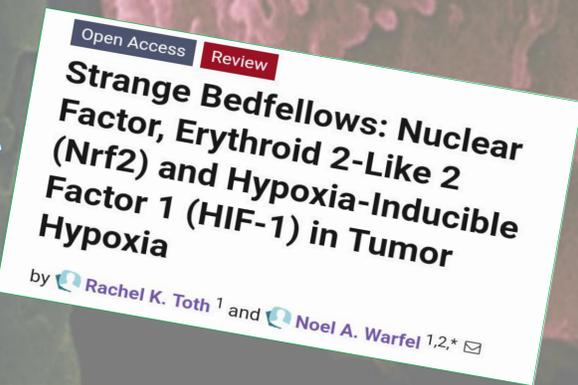
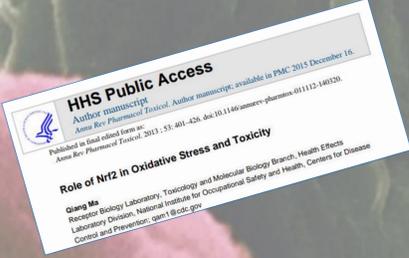


# Inspecting the response of cells from the HepG2 cell line against Hepatitis B virus in the investigation of the oxidative stress derived transcription factor Nrf-2 in the Tumor Microenvironment

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## VIBGYOR OF LITERATURE REVIEW

### TUMOR MICROENVIRONMENT

ROS

???

NRF-2

TUMOR PROGRESSION

HBV

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### OBJECTIVES OF THE RESEARCH

To study the effect of oxidative stress on Nrf-2 expression levels in HepG2 cells under control environment.

To study the effect of oxidative stress on Nrf-2 expression levels in HepG2 cells infected with HBV.

To examine the effect of HepG2 cells infected with HPV in deregulation of the native protective mechanisms and estimating the risk of cancer.

### WHERE DOES THE PROBLEM LIES AND WHAT IS IT

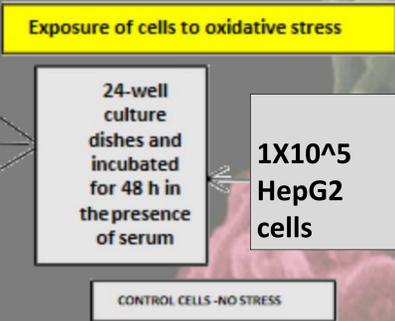
ROS is an established component in the tumor microenvironment, which are related to tumor progression and leads to poor outcome for patients. The transcription factor nuclear factor erythroid 2-related factor 2 (NRF2) is induced in response to oxidative stress. Investigations have linked elevated NRF2 expressions with tumor promotion due to upregulation of angiogenesis, cell survival, and drug resistance by this transcription factor. Therefore, it is apparent that the inhibition of Nrf-2 activity may be a strategy for treating cancer. However only a little was known regarding the HBV and its impact on the levels of the expression of NRF2, and whether HBV infection aggravated the progression of tumor or not.

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### MATERIALS AND METHODS

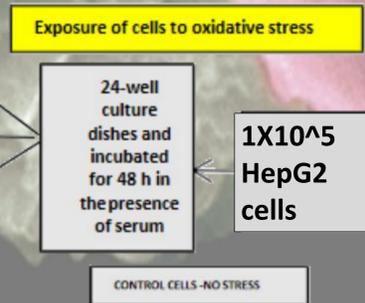
#### UNINFECTED HepG2 CELLS

- Group1: HepG2 cells treated with 100  $\mu$ M H<sub>2</sub>O<sub>2</sub> for 2 hr
- Group2: HepG2 cells treated with 100  $\mu$ M H<sub>2</sub>O<sub>2</sub> for 12 hrs
- Group3: HepG2 cells treated with 100  $\mu$ M H<sub>2</sub>O<sub>2</sub> for 24hrs



#### HBV INFECTED HepG2 CELLS

- Group1: HepG2 cells treated with 100  $\mu$ M H<sub>2</sub>O<sub>2</sub> for 2 hr
- Group2: HepG2 cells treated with 100  $\mu$ M H<sub>2</sub>O<sub>2</sub> for 12 hrs
- Group3: HepG2 cells treated with 100  $\mu$ M H<sub>2</sub>O<sub>2</sub> for 24hrs



#### Nuclear and Cytoplasmic Extract Preparation for immunoblotting and Media collection for HBV antigen detection by RIA

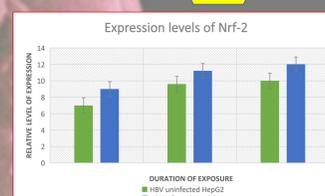
Both the normal uninfected cancer cells and the virus infected cancer cells under oxidative stress, comprising both attached and floating cells were collected and lysed in lysis buffer containing freshly added protease inhibitor cocktail from the Roche Diagnostics. Before being used for immunoblot and RIA analysis, lysates will be precleared by centrifugation and the medium from virus infected HepG2 cells were collected, centrifuged and protein concentrations will be determined by Lowry's method. Nuclear and cytoplasmic proteins will be extracted by using a kit following the manufacturer's instructions. The lysates from the cells were electrophoretically separated using 7.5% or 10% gels. Proteins will be transferred to nitrocellulose membranes. Nrf2 will be detected in the nuclear extracts using anti-Nrf2 polyclonal antibody. The HBV surface antigens were detected by performing RIA.

#### CANCER CELL MIGRATION

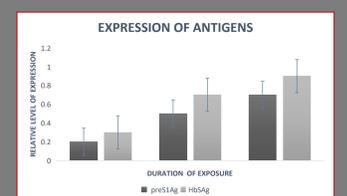
A straight scratch was made in the middle of the monolayer of confluent cells using a sterile pipette. The scratch gap will be recorded and photographed with a light microscope. The cells will then be cultured for 12/24 hrs. The closure of the scratch gap will be photographed.

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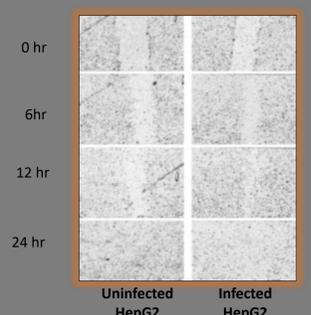
### RESULTS



Plot -1 : After the immunoblot analysis for the uninfected HepG2 cells it was found that under raised conditions of oxidative stress the Nrf-2 expression increased considerably, and the rise in the expression levels were found to increase proportionally with the increased time of exposure. Again the expression levels of Nrf-2 was found to be substantially higher in case of the HepG2 cells infected with HBV over uninfected cells.



Plot - 2 : RIA analysis lead to the detection of two envelop antigens viz. HbsAg and preS1Ag



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## CONCLUSION

Now coming to the conclusion we conclude that oxidative stress was known to induce the expression of cytoprotective TFs like Nrf-2, but the substantial evidence which we found in our research work was very much instrumental in stating that viruses like HBV when infected against cancer cell lines like HepG2, induces the over-expression of Nrf-2 to fight out the risks of cancer, but surely the virus has some immunomodulating properties which ultimately does increase the risk of cancer as the metastatic potential under in vitro conditions were found to be elevated with the HBV infected cells over the uninfected cells. So, we state that infections of HBV in case of cancer patients may be very risky even at the quiescent stage of the infection, if left out without the proper management of Hepatitis B.