Histone Deacetylation is the Primary Epigenetic Mechanism for Silencing of Tumor Suppressor Gene - Tissue Factor Pathway Inhibitor-2 in Hepatocellular Carcinoma Cells

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ABSTRACT

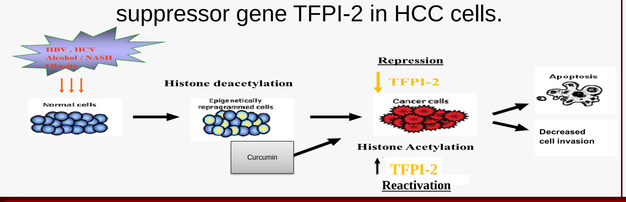
Background: Hepatocellular carcinoma (HCC) is the third leading cause of cancer related mortality worldwide. With a survival rate of less than 5 percent, a therapeutic treatment is desperately needed to manage this disease. Many epigenetic mechanisms that underlay HCC are being identified. A frequently silenced pathway tissue factor pathway inhibitor-2 (TFPI-2) is a critical tumor suppressor gene. In HCC, inactivation of TFPI-2 leads to tumor growth. Trichostatin A (TSA) is a histone deacetylase inhibitor that is known to upregulate TFPI-2 expression in the cells, confirming the epigenetic silencing of TFPI-2.
Objective For the purposes of this study, the phytochemical Curcumin and the HDAC inhibitor TSA were explored to observe its possible effects on the epigenetic mechanisms underlying HCC.
Method: HCC cells underwent curcumin treatment and were examined for gene expression via real-time polymerase chain reaction (Real-time PCR) and chromatin remodeling by chromatin immunoprecipitation (ChIP).
Results: As predicted, curcumin effectively reactivated TFPI-2 and upregulated FasL gene expression. Upon further investigation, histone H3 Lysine 9 (K9Ac) acetylation, a permissive histone mark was seen to be enriched, which actively correlates with gene transcription
Conclusion: Overall, curcumin was found to effectively reduce cell viability and reverse HCC's proliferative nature in mammalian HCC cell lines.

INTRODUCTION

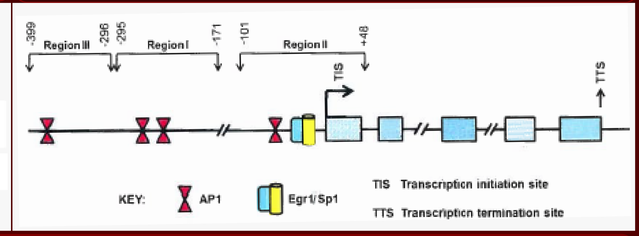
- Hepatocellular carcinoma is the fifth most common cancer in the world and the third most common cause of cancer-related mortality.
- Understanding the epigenetic mechanisms underlying hepatocellular carcinogenesis is critical to development of therapeutics to combat the disease.
- HCC has been linked to mutations, deletions, epigenetic alterations, and histone modifications, which are all known to play a major role in tumorigenesis.
  - The tumor suppressor gene TFPI-2 has been found to be epigenetically silenced frequently in HCC.
  - The HAT-HDAC balance regulated transcriptional activity at gene promoters. This epigenetic mechanism is dysregulated in cancer.
  - K9Ac is a permissive histone modification, which allows for transcription of TFPI-2 gene.
- In previous studies, Curcumin and TSA have been found to have anti-tumerogenic properties and have been linked to causing cell death in HCC.
  - In literature, curcumin and TSA treatment has been shown to cause epigenetic alterations.

HYPOTHESIS

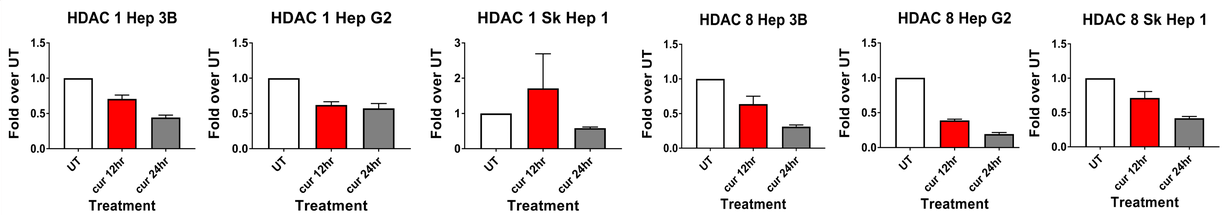
Promoter histone deacetylation silences critical tumor suppressor gene TFPI-2 in HCC cells.



TFPI-2 PROMOTER

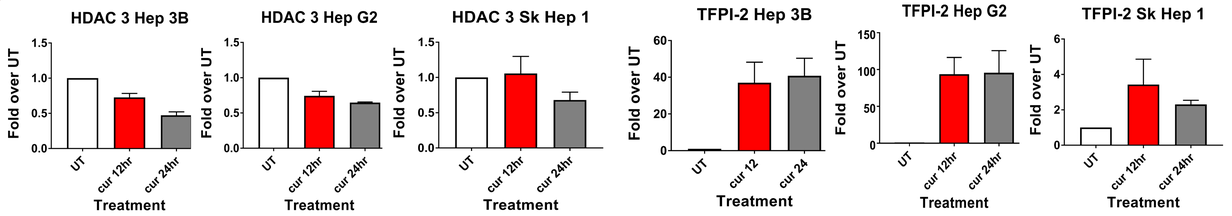


RESULTS



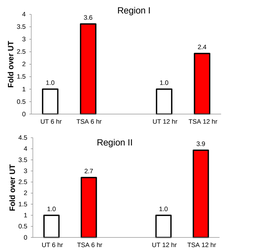
A. HDAC 1

B. HDAC 8

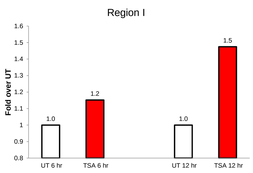


C. HDAC 3

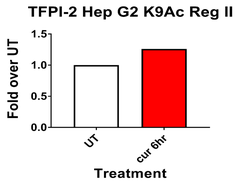
D. TFPI-2



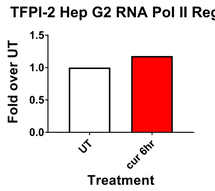
E. H3K9Ac at TFPI-2 promoter in TSA treated HepG2 cells at 24 hrs



F. RNA Pol II at TFPI-2 promoter in TSA created HepG2 cells at 24 hrs



G. H3K9Ac at TFPI-2 promoter in curcumin treatedHepG2 cells at 6 hrs



H. RNA Pol II at TFPI-2 promoter in curcumin treated HepG2 cells at 6 hrs

METHODS

Cell Culture: Three cell lines (Hep G2, Hep 3B, SK HEP 1) were cultured in Eagle's minimum essential medium (EMEM) supplemented with 10% fetal bovine serum (FBS). Cells were plated and incubated overnight at 37°C for all experiments. On the next day, they were exposed to Curcumin at 50uM for different time-points
RNA Isolation and Real time PCR: Total RNA was isolated from cells treated using TRIzol. For real time PCR, the cDNA was synthesized using qScript cDNA SuperMix. Real time PCR was performed with an ABI prism 7500 sequence detection systemizing PerfCta SYBR Green FastMixTM, Low ROX reagents. Each sample was run in duplicates.
Chromatin Immunoprecipitation and ChIP Analysis: The ChIP assay was performed using a protocol standardized within the lab and using in-house buffers.

CONCLUSION

- Curcumin robustly reactivates the epigenetically silenced tumor suppressor gene TFPI-2 in HCC cells.
- Hypoacetylation was found to be a limiting factor of TFPI-2 transcription.
- Histone acetylation is the permissive epigenetic modification which lead to opening up of the TFPI-2 promoter for gene transcription.
- Overall, transcriptional reactivation of TFPI-2 by curcumin correlated with a decrease in cell viability,
- Curcumin worked effectively to reduce cell viability and invert the proliferative nature of the HCC cell lines that were examined.
  - The increase in TFPI-2 in curcumin treated cells indicated a restoration of this gene.
- In addition, TSA was able to upregulate TFPI-2 expression in the cells, confirming the epigenetic silencing of TFPI-2 in our cells.
  - Because TSA reactivated TFPI-2 to a significant degree, histone deacetylation may be the predominant mechanism of TFPI-2 silencing.

FUTURE DIRECTIONS

- To investigate the roles of specific histone acetyltransferases (HATs) in reactivation of TFPI-2.
- To explore the role of repressive histone markers and look into the role of DNA methylation in HCC.
- Investigation into protein interactions through Western Blot
- *In vivo* studies of the influence of curcumin on TFPI-2 cells and its effect on tumor viability and evasiveness would be a further step.

ACKNOWLEDGMENTS

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