

Melatonin, a novel **selective** ATF-6 inhibitor, induces human hepatoma cell apoptosis through COX-2 downregulation

Lijia Bu, Hanqing Yu, Lulu Fan, Xiaoqiu Li, Fang Wang, Jiatao Liu, Fei Zhong, Congjun Zhang, Wei Wei, Hua Wang, Guoping Sun

Corresponding authors:

Dr. Hua Wang or Dr. Guo-Ping Sun, Department of Oncology, the First Affiliated Hospital of Anhui Medical University; Institute for Liver Diseases of Anhui Medical University. Email: wanghua@ahmu.edu.cn or sungp@ahmu.edu.cn.

Tel. +86055162923509, Fax +8605512923509

1 What did this study explore?

This study explores the more detail mechanisms of melatonin enhances ER stress-induced apoptosis in human hepatocellular cell via COX-2 inhibition by selectively targeting ATF-6.

2 How did the authors perform all experiments?

The authors used HepG2 cell in vitro culture system to perform all experiments.

3 How did the authors process all experimental data?

Three or more separate experiments were performed for each experiment. Statistical analysis was performed by Student's t-test or ANOVA. Data are presented as the means \pm standard deviation (S.D.). Significance was noted at $P < 0.05$.

4 How did the authors deal with the pre-study hypothesis?

Our previous studies demonstrated that melatonin sensitizes the human hepatocellular carcinoma cell to ER stress-induced apoptosis and attenuates ER stress-induced doxorubicin resistance. However, the mechanisms involved in the critical ER stress initiating unfolded protein response (UPR) pathway modified by melatonin still need to be clarified. In the present study, we first identified that melatonin selectively blocked activating transcription factor 6

(ATF-6) and then inhibited cyclooxygenase-2 (COX-2) expression, leading to enhanced liver cancer cell apoptosis under ER stress condition. Dramatically increased CCAAT-enhancer-binding protein homologous protein (CHOP) level, suppressed COX-2 and decreased Bcl-2/Bax ratio by melatonin or ATF-6 siRNA contributed the enhanced HepG2 cell apoptosis under tunicamycin (an ER stress inducer) stimulation. In clinical hepatocellular carcinoma (HCC) patients, close relationship between ATF-6 and COX-2 was further confirmed. Our study explores the more detail mechanisms of melatonin enhances ER stress-induced apoptosis in human hepatocellular cell via COX-2 inhibition by selectively targeting ATF-6.

5 What are the novel findings of this study?

These findings indicate that melatonin as a novel selective ATF-6 inhibitor can sensitize human hepatoma cells to ER stress inducing apoptosis.