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***Basic Study***

**Antioxidant axis Nrf2--keap1--ARE in inhibition of alcoholic liver fibrosis by IL-22**

## scientific research process

ALF is the turnning point of ALD, so timely discovery and treatment of ALF can successfully reverse ALD, however until now there has no effective way to treat ALF. Acetaldehyde, the most harmful metabolite of alcohol, triggers HSC activation and poliferation by inducing oxidative stress, which forms the key factor of ALF. Nrf2 is the key regulatory factor of anti-oxidative response. Under conditions of oxidative stress, the ubiquitination degradation pathway of Nrf2 is inhibited, resulting in cytoplasmic Nrf2 deposition. Free Nrf2, dissociated from keap1, moves into the nucleus, combines with ARE, and serves as a transcriptional factor to regulate the expression of corresponding downstream genes (e.g. *GSH*, and *HO-1*). IL-22, one member of the IL-10 cytokine superfamily, could delay the progression of liver fibrosis via promoting liver progenitor cell/hepatocyte proliferation, inhibiting hepatocyte apoptosis, and upregulating metallothionein (MTI/II) and glutathione (GSH) expression. But dose it matter with the activity of the antioxidant axis Nrf2--keap1--ARE in HSCs has not yet been elucidated. Therefore, in our present study we used acetaldehyde as the stimulator of HSC-T6 cells to establish a model of ALF in vitro. Different concentrations of IL-22 were added to the culture, and the proliferation rate and activity of HSCs were detected, as well as nuclear translocation of Nrf2. The specific process is as follows：

1. In our study, passaged and activated HSCs were seeded into 25-$cm^2$ sealable flasks with 10% high glucose DMEM complete medium. When the monolayers were

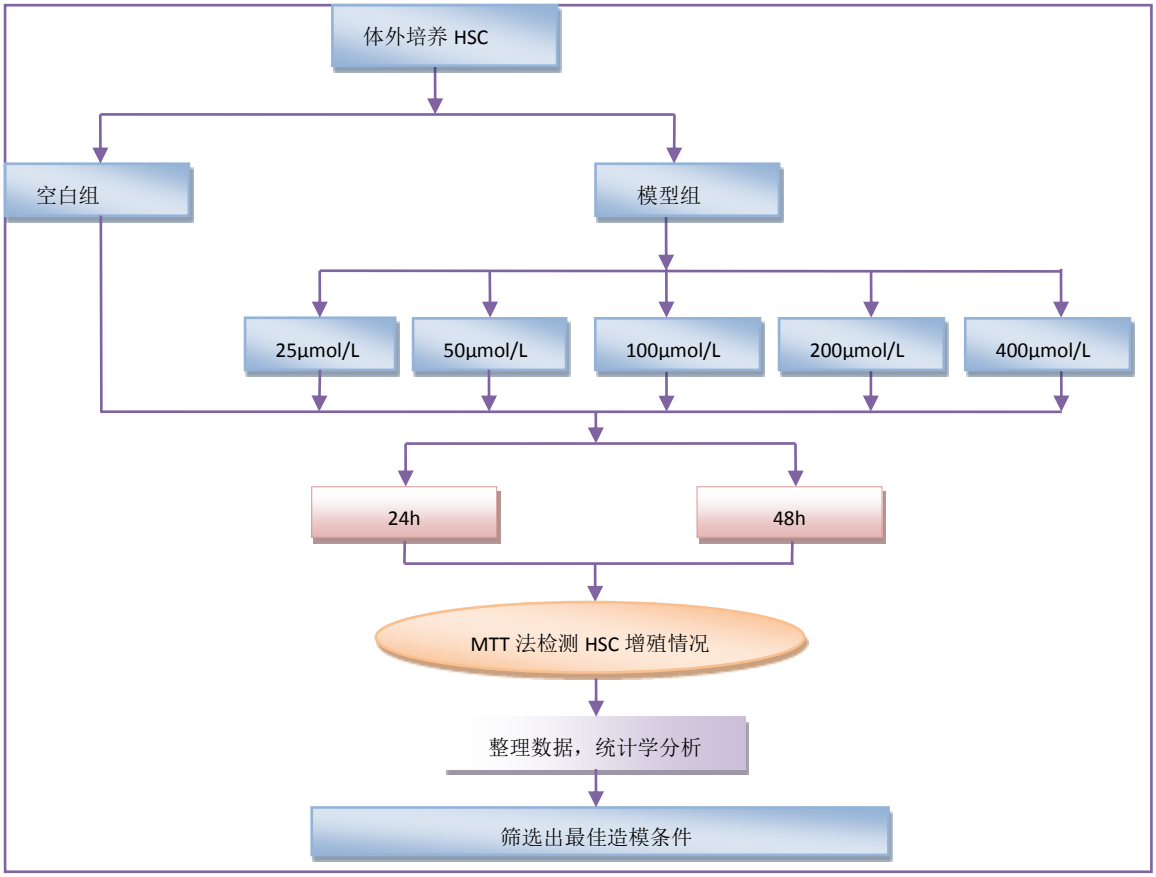
75–80% confluent, cells could used for the following experiments.

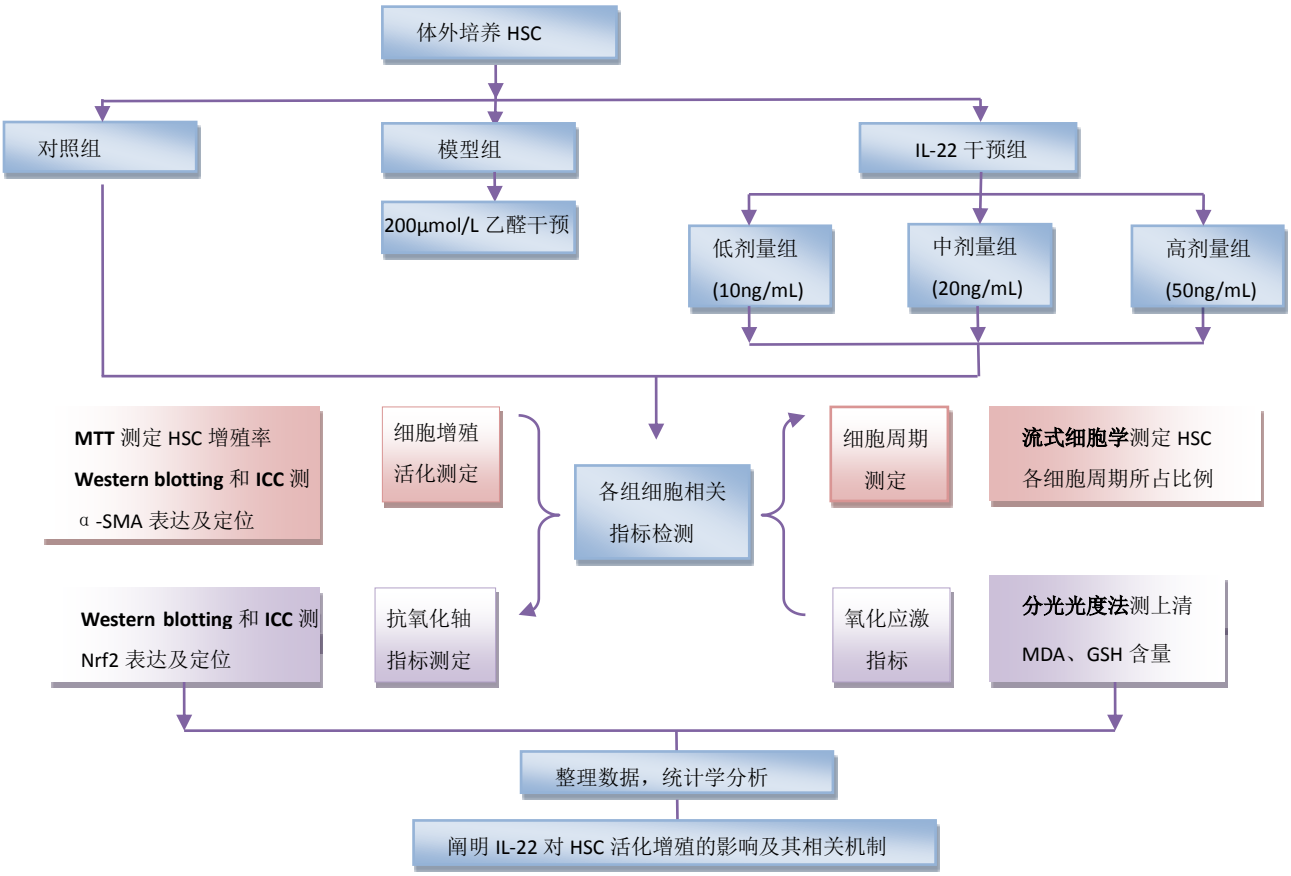
2. HSC-T6 cells were incubated with 25, 50, 100, 200 and 400 μmol/L acetaldehyde. After 24 and 48 h, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to detect proliferation of HSCs to choose the best concentration and action time using for the *in- vitro* model establishment.

3. We used the optimal concentration of acetaldehyde (200 μmol/L) to stimulate HSCs for 24 h, and treated the cells with a final concentration of 10, 20 or 50 ng/mL IL-22. Cell proliferation rate was detected by MTT assay; cell cycle was analyzed by flow cytometry; expression of nuclear-factor-related factor (Nrf)2 and α-smooth muscle antigen (SMA) was detected by western blotting and immunocytochemistry; and levels of malondialdehyde (MDA) and glutathione (GSH) were measured by spectrophotometry.

4. SPSS version 17.0 was used for all statistical analysis and data were expressed as mean ± SD. One-way ANOVA was used to assess the significance of differences between the mean values. Multiple pair-wise comparisons were conducted using the least significant difference (LSD). The difference was statistically significant at $P < 0.05$.

From our research, we successfully established an *in- vitro* model of acetaldehyde-induced ALF. We further demonstrated that IL-22 effectively inhibited activation and proliferation of HSCs, followed by delayed disease progression of ALF. This may partly be related to promoting Nrf2 nuclear translocation and enhancing the activity of the antioxidant axis Nrf2--keap1--ARE. These results could provide an experimental and theoretical basis for new drug development for treatment of ALF.


体外培养 HSC
空白组
模型组
25μmol/L
50μmol/L
100μmol/L
200μmol/L
400μmol/L
24h
48h
MTT 法检测 HSC 增殖情况
整理数据，统计学分析
筛选出最佳造模条件


体外培养 HSC
对照组
模型组
200μmol/L 乙醛干预
IL-22 干预组
低剂量组
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中剂量组
(20ng/mL)
高剂量组
(50ng/mL)
MTT 测定 HSC 增殖率
Western blotting 和 ICC 测
α -SMA 表达及定位
细胞增殖
活化测定
各组细胞相关
指标检测
细胞周期
测定
流式细胞学测定 HSC
各细胞周期所占比例
Western blotting 和 ICC 测
Nrf2 表达及定位
抗氧化轴
指标测定
氧化应激
指标
分光光度法测上清
MDA、GSH 含量
整理数据，统计学分析
阐明 IL-22 对 HSC 活化增殖的影响及其相关机制