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论著

依托度酸诱导SMMC7721细胞凋亡的分子机理研究

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摘要 目的：探讨选择性环氧合酶抑制剂依托度酸（etodolac）诱导肝癌SMMC7721细胞凋亡的分子机理。方法： 采用流式细胞术、DNA琼脂糖凝胶电泳法测定细胞凋亡情况；Western blotting法检测不同浓度etodolac处理后凋亡相关蛋白Bcl-2、Bax表达的变化；流式细胞术检测半胱氨酸酶-3 (caspase-3)活性的变化；TransAMTM NF-κB p65/p50核转录因子活性检测试剂盒检测核因子-κB (NF-κB)活性变化。 结果： 流式细胞术显示etodolac（0.25、0.50、1.0、2.0 mmol/L）作用SMMC7721细胞48 h后，与对照组（0 mmol/L）相比，出现明显凋亡峰（$P<0.01$ vs control）；高浓度etodolac处理后DNA琼脂糖凝胶电泳出现明显的DNA Ladder，凋亡相关蛋白Bcl-2表达下降，Bax表达增加；与对照组相比，低浓度组（0.25 mmol/L）caspase-3活性未明显活化（$P>0.05$），NF-κB活性也未受明显抑制（$P>0.05$），随着etodolac浓度的增大（0.50、1.0、2.0 mmol/L），caspase-3活性明显活化（$P<0.05$ *vs* control）； NF-κB活性明显受到抑制（$P<0.05$ *vs* control）。经Pearson 相关分析，caspase-3活性和NF-κB活性呈显著负相关（$r=0.919$, $P<0.01$）。 结论： 选择性COX-2抑制剂etodolac可能通过抑制NF-κB结合活性，调节Bcl-2、Bax蛋白表达，活化caspase-3，从而诱导肝癌SMMC7721细胞凋亡。

关键词 环加氧酶-2 NF-κB； 细胞凋亡； 半胱氨酸天冬氨酸蛋白酶3

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Molecular mechanism of etodolac-induced apoptosis in SMMC7721 cell line

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Abstract

<FONT face=Verdana>AIM: To investigate the possible role of nuclear transcription factor kappa B (NF-κB), Bcl-2, Bax and caspase-3 in etodolac-induced apoptosis of liver tumor SMMC7721 cell line. METHODS: Cell apoptosis was determined by flow cytometry analysis with PI staining and DNA laddering. Expression of Bcl-2 and Bax protein was measured by Western blotting. Caspase-3 activity was evaluated by active caspase-3 apoptosis kit with flow cytometry. NF-κB activation was detected by ELISA-based TransAMTM NF-κB p65/p50 kit. RESULTS: Etodolac, a selective COX-2 inhibitor, stimulated apoptosis in liver tumor SMMC7721 cell line significantly. Flow cytometry showed that the apoptotic rate was 16.3%±3.1%, 19.9%±3.6%, 22.9%±3.2%, 31.2%±3.3% with different concentrations of etodolac (0.25, 0.50, 1.0 or 2.0 mmol/L), while the apoptotic peak did not appear in the control group (0 mmol/L) (<EM>P</EM><0.01 vs control). Expression of Bax protein was up-regulated while Bcl-2 protein was down-regulated, and cells with caspase-3 activation was 3.61%±0.32%, 2.93%±0.15%, 10.29%±0.39%, 27.33%±1.28%, 57.40%±1.69%， respectively (<EM>P</EM><0.05, 0.50, 1.0, 2.0 mmol/L vs control). Compared with the control group, NF-κB activation was inhibited significantly as etodolac concentration increased (<EM>P</EM><0.05, 0.50, 1.0, 2.0 mmol/L vs control). Caspase-3 activation and NF-κB activity was negatively correlated (<EM>r</EM>=0.919, <EM>P</EM><0.01). CONCLUSION: Selective COX-2 inhibitor etodolac induces SMMC7721 cells apoptosis, possibly via inhibition of NF-κB activity and regulation of Bcl-2, Bax protein expression， which ultimately activate caspase-3. </FONT>

Key words Cyclooxygenase-2 NF-kappa B Apoptosis Caspase-3

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通讯作者