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[编委会](#)

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[投稿指南](#)

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297-301.含AFP基因调控序列的pAFP-P53-EGFP质粒诱导AFP阳性肝癌细胞凋亡[J].张焕铃,王俊霞,尤红煜,刘健敏,郑龙,连伟光,刘福英.中国肿瘤生物治疗杂志,2010,17(3)

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[张焕铃](#) [王俊霞](#) [尤红煜](#) [刘健敏](#) [郑龙](#) [连伟光](#) [刘福英](#)

河北医科大学 分子生物学研究室,河北省实验动物重点实验室,河北 石家庄 050017;河北医科大学 分子生物学研究室,河北省实验动物重点实验室,河北 石家庄 050017;河北医科大学 分子生物学研究室,河北省实验动物重点实验室,河北 石家庄 050017;河北医科大学 分子生物学研究室,河北省实验动物重点实验室,河北 石家庄 050017;河北医科大学 分子生物学研究室,河北省实验动物重点实验室,河北 石家庄 050017;河北医科大学 分子生物学研究室,河北省实验动物重点实验室,河北 石家庄 050017;河北医科大学 分子生物学研究室,河北省实验动物重点实验室,河北 石家庄 050017

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摘要:

目的:观察含 AFP 基因调控序列的载体对AFP阳性肝癌细胞的靶向致凋亡作用。方法:将 AFP 启动子、沉默子和远端增强子III组合为1.2 kb的 AFP 基因调控序列,构建pAFP EGFP载体,分别转染人肝癌HepG2 (AFP阳性)、人肝癌SMMC7721 (AFP阴性)和人宫颈癌HeLa (AFP阴性)细胞,荧光显微镜下观察EGFP荧光蛋白表达强度。引入 P〔STBX〕53 〔STBZ〕基因片段,构建pAFP P53 EGFP重组质粒,转染HepG2、SMMC7721和HeLa细胞,Western blotting检测各组细胞P53蛋白的表达,流式细胞术分析各组细胞凋亡率及细胞周期。结果:成功构建了pAFP EGFP和pAFP P53 EGFP重组质粒。pAFP EGFP转染后,AFP阳性的HepG2细胞中EGFP荧光蛋白表达显著高于AFP阴性的SMMC7721和HeLa细胞。pAFP P53 EGFP转染后,HepG2细胞中P53蛋白的表达量明显高于SMMC7721和HeLa细胞;HepG2细胞的G₁期细胞及细胞凋亡率明显高于SMMC7721和HeLa细胞〔(66.7±0.25)% vs (50.5±0.18)%, (51.0±0.20)%, P <0.05; (2.65±0.08)% vs (0.42±0.03)%, (0.39±0.02)%, P <0.05〕,但S期细胞明显低于转染后SMMC7721和HeLa细胞〔(20.1±0.22)% vs (29.8±0.18)%, (37.8±0.21)%, P <0.05〕。结论:含 AFP 基因调控序列的pAFP P53 EGFP载体可专一性地作用于AFP阳性肝癌细胞,引起肝癌细胞周期阻滞和凋亡。

关键词: [AFP 基因](#) [肝癌细胞](#) [表达调控](#) [P53](#) [细胞周期阻滞](#) [细胞凋亡](#)

pAFP P53 EGFP plasmid containing AFP regulation sequence induces apoptosis of AFP positive hepatoma cells [Download Fulltext](#)

[ZHANG Huan-ling](#) [WANG Jun-xia](#) [YOU Hong-yu](#) [LIU Jian-min](#) [ZHENG Long](#) [LIAN Wei-guang](#) [LIU Fu-ying](#)

Molecular Biology Laboratory of Hebei Medical University, Key Laboratory of Laboratory Animal of Hebei Province, Shijiazhuang 050017, Hebei, China; Molecular Biology Laboratory of Hebei Medical University, Key Laboratory of Laboratory Animal of Hebei Province, Shijiazhuang 050017, Hebei, China; Molecular Biology Laboratory of Hebei Medical University, Key Laboratory of Laboratory Animal of Hebei Province, Shijiazhuang 050017, Hebei, China; Molecular Biology Laboratory of Hebei Medical University, Key Laboratory of Laboratory Animal of Hebei Province, Shijiazhuang 050017, Hebei, China; Molecular Biology Laboratory of Hebei Medical University, Key Laboratory of Laboratory Animal of Hebei Province, Shijiazhuang 050017, Hebei, China; Molecular Biology Laboratory of Hebei Medical University, Key Laboratory of Laboratory Animal of Hebei Province, Shijiazhuang 050017, Hebei, China; Molecular Biology Laboratory of Hebei Medical University, Key Laboratory of Laboratory Animal of Hebei Province, Shijiazhuang 050017, Hebei, China

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Abstract:

Objective: To observe the targeting pro-apoptotic effect of expression vector containing AFP regulation sequence on AFP positive hepatoma cells. Methods: AFP promoter, silencer and the most remote enhancer III were ligated to construct a 1.2 kb AFP regulation sequence, which was then used to construct a pAFP EGFP plasmid. The pAFP EGFP was used to transfect human hepatoma HepG2 (AFP positive), human hepatoma SMMC7721 (AFP negative) and human cervical carcinoma HeLa (AFP negative) cells; the fluorescent protein expression intensities were observed under fluorescence microscope. A pAFP P53 EGFP plasmid was further constructed by inserting P53 gene into pAFP EGFP, which was then transfected into HepG2, SMMC7721 and HeLa cells. P53 protein expressions were detected by Western blotting analysis in different groups; apoptosis rates and cell cycles were examined by flow cytometry. Results: pAFP EGFP and pAFP P53 EGFP recombinant plasmids were successfully constructed. The expression of EGFP fluorescent protein in pAFP EGFP transfected AFP positive HepG2 cells was significant higher than those in AFP negative SMMC7721 and HeLa cells; P53 protein expression in HepG2 cells transfected with pAFP P53 EGFP was also significantly higher than those in SMMC7721 and HeLa cells. The G₁ phase proportion and apoptosis rate of pAFP P53 EGFP transfected HepG2 cells were significantly higher than those of SMMC7721 and HeLa cells [(66.7±0.25)% vs (50.5±0.18)%, (51.0±0.20)%, P <0.05; (2.65±0.08)% vs (0.42±0.03)%, (0.39±0.02)%, P <0.05], but S phase proportion of pAFP P53 EGFP transfected HepG2 cells was significantly lower than those of SMMC7721 and HeLa cells [(20.1±0.22)% vs (29.8±0.18)%, (37.8±0.21)%, P <0.05]. Conclusion: The pAFP P53 EGFP plasmid containing AFP regulation sequence can specifically target AFP positive hepatoma cells, inducing cell cycle arrest and apoptosis of hepatoma cells.

Keywords: [AFP gene](#) [hepatoma cell](#) [expression and regulation](#) [P53](#) [cell cycle arrest](#) [apoptosis](#)

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