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厦门大学

博士学位论文

视黄酸受体γ在胆管癌发生发展中的作用及其机理

The Role and Mechanism of RARγ in the Development of Cholangiocarcinoma

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缩略语表

英文缩写	英文全称	中文全称
MTT	methyl thiazolyltetrazolium	噻唑蓝
PBS	phosphate buffered saline	磷酸盐缓冲液
DMSO	dimethyl Sulfoxide	二甲亚砜
AO	acridine orange	吖啶橙
EB	ethidium bromide	溴乙锭
PI	propidine iodide	碘化丙啶
FCM	flow cytometry	流式细胞术
FITC	fluorescein isothiacyanate	异硫氰酸荧光素
CDK	cyclin-dependent kinase	细胞周期素依赖激酶
RARγ	retinoic acid receptor γ	视黄酸受体γ
MDR	multidrug resistance	多药耐药
CCA	cholangiocarcinoma	胆管癌
ICCA	interhepatic cholangiocarcioma	肝内胆管癌
ECCA	extrahepatic cholangiocarcioma	肝外胆管癌
CDDP	cis-diaminodichloroplatinum	顺铂
5-FU	5-fluorouracil	5-氟尿嘧啶
TAM	tamoxifen	三苯氧胺
VCR	vincristine	长春新碱
MMC	mitomycin C	丝裂霉素 C
MMP-9	matrix metallopeptidase 9	金属蛋白酶-9
P-gp	permeability glycoprotein	P-糖蛋白
PCNA	proliferating cell nuclear antigen	增殖细胞核抗原
CA19-9	carbohydrate antigen 19-9	糖类抗原 19-9
CEA	carcinoembryonic antigen	癌胚抗原
MRP	muitidrug resistant associated protein	多药耐药相关蛋白
RA	retinoic acid	视黄酸
CDI	coefficient of drug interaction	药物相互作用指数

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TPA	12-O-Tetradecanoylphorbol-13-Acetate	12-O-十四烷酰佛波醋酸酯-13
IC_{50}	50% inhibiting concentration	半抑制浓度
IHC	immunohistochemistry	免疫组织化学
IF	immunofluorescence	免疫荧光学
HRP	horseradish peroxidase	辣根过氧化物酶
ECL	enhanced chemilumienescence	增强型化学发光
PSC	primary sclerotic cholangitis	原发性硬化性胆管炎
LRP	1ung resistance related protein	肺耐药相关蛋白
ABCC	ATP-binding cassette superfamily C	三磷酸腺苷结合盒转运体基因
Gal-3	galectin-3	半乳凝素-3
TS	thymidylate synthase	胸腺嘧啶核酸合酶
GST-π	glutathione S transferase	谷胱甘肽 S 转移酶 π
BCRP	breast cancer resistance protein	乳腺癌耐药蛋白
RARE	RA responsive element	视黄酸应答元件
NLS	nuclearlocalization sequence	核定位序列
NES	nuclear export sequence	出核序列

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中文摘要

胆管癌是一种起源于胆管上皮细胞的肝胆系统恶性肿瘤。由于解剖位置特殊、临床症状隐匿、早期诊断困难、易以多种方式发生转移、术后容易复发以及放化疗不敏感，胆管癌预后较差。因此，深入研究胆管癌发生发展、侵袭转移以及耐药性产生的分子机制具有重要的意义。视黄酸受体与肿瘤的关系一直是研究的关注点，然而，RARγ 参与肿瘤发生发展的作用及其分子机制尚不清楚。本论文以胆管癌为研究对象，以 RARγ 为切入点，研究 RARγ 在胆管癌发生发展中的作用及其分子机制，并寻找新的有效的抗胆管癌效应物。

本论文研究发现，RARγ 在胆管癌组织中基因和蛋白水平表达明显高于配对癌旁组织，以及胆管炎和正常胆管组织，且 RARγ 异常表达于胞浆。RARγ 高表达与胆管癌病理分级的低分化、淋巴结转移、血清 CA19-9 高值以及预后差密切相关。采用基因转染技术下调胆管癌细胞 RARγ 的表达，结果发现：RARγ 表达下调导致胆管癌细胞 QBC939、SK-ChA-1 和 MZ-ChA-1 生长明显缓慢，体外细胞集落形成和体内裸鼠皮下异种成瘤受到抑制，这与其表达下调介导 p21 表达上调而诱导细胞周期阻滞以及 PCNA 表达下调有关；RARγ 表达下调导致 MMP-9 表达与活性下降，从而抑制胆管癌细胞的迁移与侵袭；RARγ 表达下调介导胆管癌细胞耐药性相关蛋白 P-gp 表达下调从而提高胆管癌细胞对 5-氟尿嘧啶、顺铂、长春新碱和丝裂霉素 C 的敏感性及逆转本实验构建的胆管癌细胞耐药模型 QBC939/5-FU 的耐药性。进一步研究发现，RARγ 表达下调后降低 AKT 和 IκBα 的磷酸化水平，PI3K/Akt 和 NF-κB 信号通路抑制剂可以影响 p21、PCNA、MMP-9 和 P-gp 的表达。由此表明，RARγ 是通过胞浆转移调控 PI3K/AKT/NF-κB 信号通路从而介导与胆管癌发生发展密切相关的 p21、PCNA、MMP-9 和 P-gp 等基因表达改变，最终实现其癌基因作用。

本文在筛选抗胆管癌效应物研究中发现，七叶皂苷钠通过诱导胆管癌细胞周期抑制和细胞凋亡发挥其抗胆管癌作用，其药效明显高于其他临床胆管癌化疗药物；七叶皂苷钠还可逆转胆管癌细胞耐药性，提高其他抗肿瘤药物的抗癌活性。七叶皂苷钠这种生物学效应是通过抑制 RARγ 在胆管癌细胞的胞浆转移，从而达到抑制 PI3K/Akt/NF-κB 信号通路的活性而实现的。

关键词：胆管癌；RARγ；七叶皂苷钠

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Abstract

Cholangiocarcinomas (CCA) are rare malignant tumors arising from the biliary tract. Because of special anatomical position, conceal anatomical position, difficulty in early diagnosis, metastasis by a variety of ways, easy to relapse after surgery, and insensitivity to insensitivity, its prognosis is poor. Therefore, it is important to intensively study the mechanisms of the development, metastasis, and the multidrug resistance of CCA. The relationship of retinoic acid receptors and tumor is always the research focus. However, the roles and mechanism of RARγ in the development of CCA were unclear. In this study, as the object of study in CCA and the breakthrough point with RARγ, we study the the roles and mechanism of RARγ in the development of CCA, and search for new effective drug to CCA.

In the current study, we found that the expression of RARγ mRNA and protein were abnormal in cell cytoplasm and elevated in CCA tissues compared to paired noncancerous tissues, cholangitis tissues, and normal bile duct tissues. Its overexpression was closely associated with pathology of low differentiation, lymphatic metastasis, serum CA19-9 high value and poor prognosis. It was found that after downregulation of RARγ in CCA cells by gene transfer techniques: Inhibiton of cells growth in vitro, cells clone formation, and xenograft tumor in nude mice in vivo, which were associated with cell cycle arrested by upregulation of p21 and downregulation of PCNA. Inhibiton of cells migration and invasion by decreased the expression of MMP-9 and activity. Enhancement of drug susceptibility of CCA cells to 5-FU, CDDP, VCR, and MMC, reversion of QBC939/5-FU multidrug resistance, which was associated with the downregulation of P-gp. Furthermore, the phosphorylation level of AKT and IκBα were decreased after downregulation of RARγ, and the inhibitor of PI3K/Akt or NF-κB signal passway could change the expression of p21, PCNA, MMP-9, and P-gp. It was showed that RARγ exerted its oncogene activation by mediating the expression of p21, PCNA, MMP-9, and P-gp in way of regulating the PI3K/Akt/NF-κB signal passway.

In the screening of CCA effectors, we found that β-escin inhibited the

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proliferation of CCA cells by inducing cell cycle arrested and cell apoptosis, and the antiproliferative effect was stronger than all the other chemotherapeutic agents of the same concentration. Furthermore, β-escin not only reversed the multidrug resistance of QBC939/5-FU, but also enhanced the drug susceptibility of CCA cells. The functions of β-escin were exhibited by regulation of PI3K/Akt/NF-κB signal passway through inhibiting RARγ cytoplasmic translocation.

Keywords: cholangiocarcinoma; RARγ; β-escin.

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