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厦门大学

博士学位论文

胆管癌药物的筛选及作用机理的研究

Studies on the Screening of Cholangiocarcinoma Drugs and the Mechanisms

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目　　录

Contents

摘　　要

胆管癌是一种难以治愈的恶性肿瘤。对于胆管癌的治疗，根治性手术是目前唯一有效的办法。但是，胆管癌的早期症状不明显、发现困难，一旦发现多数已进入晚期，很难通过手术方法切除，因此，寻找其他非手术方法治疗胆管癌就变得十分必要。目前临床应用的药物对胆管癌敏感性欠佳，而且存在明显毒副作用。因此，探索更敏感、有效的药物，提高胆管癌药物治疗的疗效就变得十分必要。

本论文以体外培养的人胆管癌 QBC939 细胞为药物筛选模型，选用叶下珠、珠子草、冬青、白英、长春花、三尖杉六种药用植物提取物、淡水贝类河蚬提取物、天然产物化合物、缩氨基硫脲类化合物及常见化疗药物，应用 MTT 测定法，从天然和化学合成物质中筛选具有抗胆管癌效应的药物。结果表明：珠子草和叶下珠的乙酸乙酯和正丁醇提取物、白英的水提物、长春花总碱、河蚬乙酸乙酯提取物、槲皮素、鞣酸、2-氯苯甲醛缩氨基硫脲、2，4-二氯苯甲醛缩氨基硫脲和三苯氧胺等具有体外抗胆管癌细胞增殖的活性。

与胆管癌临床中常用的化疗药物 5-氟尿嘧啶和顺铂相比，三苯氧胺表现出了更强的抑制胆管癌细胞的作用。因此，本研究选择三苯氧胺（TAM）进行下一步的深入研究，探讨三苯氧胺体外抗胆管癌作用的机理。通过 MTT 法、细胞形态观察法、流式细胞术、DNA ladder 等方法研究表明：TAM 呈时间和剂量依赖性抑制胆管癌 QBC939 细胞的增殖、使细胞形态发生明显变化、使细胞周期阻滞于 G_0/G_1 期、且显著诱导细胞凋亡；通过 Western blot 等方法研究表明：TAM 对 Cyclin D1、ERα、C-Myc 蛋白表达的抑制、Caspase-3/Caspase-9 信号通路的激活、Bax 和 p53 蛋白表达的上调，是 TAM 发挥抗胆管癌作用的部分机制。为了进一步的探讨 TAM 抗胆管癌作用的分子机制和药物作用靶点，我们首次应用蛋白质组学技术研究了三苯氧胺对人胆管癌 QBC939 细胞全蛋白表达谱的影响。最终成功鉴定出热休克蛋白、细胞骨架蛋白、膜联蛋白和代谢相关酶等差异表达的蛋白点。结合这些蛋白的功能，全面系统地分析了三苯氧胺抗胆管癌作用的可能的分子机制。为胆管癌的药物治疗提供了新的线索，为三苯氧胺在临床上应用于胆管癌的治疗提供了基础理论支持，也为三苯氧胺抗胆管癌作用新靶点的阐明提供了新的思路和线索。

关键词：胆管癌；药物筛选；三苯氧胺；机理；蛋白质组学

Abstract

Cholangiocarcinoma is a malignant tumor which is difficult to cure. At present, radical surgical removal represents the only curative treatment. However, the tumor often present at an advanced unresectable stage at the time of diagnosis for the majority patients, which eliminates the surgical approach as a curative measure. Thus, it is important to find out a effective therapeutic method excluded surgical. The response of cholangiocarcinoma to current clinical chemotherapeutic regimens is poor. The current clinical drugs for cholangiocarcinoma therapy often have toxic and side effects. Therefore, to improve the curative effect of drug therapy, it is urgent to find more sensitive and effective therapeutic drugs for cholangiocarcinoma.

In this study, cultured human cholangiocarcinoma cell line QBC939 *in vitro* was used as the model for drug screening. Extracts from *Phyllanthus niruri* L., *Phyllanthus urinaria* L., *Ilex chinensis* sims, *Solanum lyratum* Thunb., *Catharanthus rosens*(L.) G. Don, *Cephalotaxus fortunei* Hook. f. which are medicinal plants and *Corbicula Fluminea* which is a kind of freshwater mollusk, natural compounds, thiosemicarbazone compounds and common chemotherapeutic drugs were selected to evaluate the growth inhibitory effects towards cholangiocarcinoma by MTT method. The results showed that ethyl acetate and butanol extrats from *Phyllanthus urinaria* L.and *Phyllanthus niruri* L., water extract from *Solanum lyratum* Thunb., Vinca alkaloids, ethyl acetate extrat from *Corbicula Fluminea*, quercetin, tannic acid, 2-chlorobenzaldehyde thiosemicarbazone, 2,4-dichlorobenzaldehyde thiosemicarbazone and tamoxifen exhibit strong growth inhibitory effects toward cholangiocarcinoma cells.

Compared to 5-fluorouracil and cisplatin, tamoxifen was more sensitive to cholangiocarcinoma cells. Therefore, we did futher studies to investigate the antitumor mechanisms of tamoxifen (TAM) on cholangiocarcinoma cells. By means of MTT method, morphologic observation method, flow cytometry, DNA ladder method and other methods, the results showed that TAM could significantly inhibit the growth of QBC939 cells in a time- and dose-dependent manner, change the morphology of

QBC939 cells, arrest the cell cycle at G_0/G_1 phase and induce apoptosis. By means of western blot and other methods, the results showed that inhibiting the expression of Cyclin D1, ERα, C-Myc, activation Caspase-3/Caspase-9 signal pathway and up-regulating the expression of Bax and p53 were considered to be the partial mechanisms. To understand the possible molecular mechanisms and function targets of inhibitory effect induced by TAM on cholangiocarcionoma cells, proteomics technology was first applied to investigate the effect of TAM on proteomic profile of QBC939 cells. Heat shock proteins, cytoskeletal proteins, membrane associated protein, metabolic related enzymes and other differential expression proteins were successfully identified. According to the functions of these proteins, we analysed the possible molecular anticancer mechanisms of TAM on cholangiocarcinoma cells comprehensive comprehensively and systematically. All our findings provide a new clue for the drug therapy of cholangiocarcinoma, provide theoretical support for application of TAM on cholangiocarcinoma therapy in clinic and provide new targets and strategies for elucidating the anticancer mechanisms of TAM on cholangiocarcinoma.

Key Words: Cholangiocarcinoma; Drug screening; Tamoxifen; Mechanisms; Proteomics.