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硕 士 学 位 论 文

苦参碱对人食管癌细胞株 Eca-109 增殖和凋亡的影响

The effects of matrine on the proliferation and apoptosis of human esophageal carcinoma Eca-109 cell line

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摘 要

食管癌（carcinoma of the esophagus）是一种常见的消化系统恶性肿瘤，它严重威胁着人类的健康。全世界每年有20余万人死于食管癌，我国每年死亡达15万人，占据世界食管癌死亡人数的大部分。在我国，食管癌发病率男性居各种恶性肿瘤第四位，女性为第七位，而死亡率男女均居第四位。食管癌的发病率有明显的地区差异，高发地区食管癌的发病率可高达150/10万以上，低发地区则只有3/10万左右。国外以中亚、非洲、法国北部和中南美洲为高发区，我国以太行山地区、秦岭东部地区、大别山区、四川北部地区、闽南和广东潮汕地区、苏北地区为高发区。其中河南林县，食管癌死亡率男性为161.33/10万，女性为102.88/10万，其死亡率居各种恶性肿瘤首位[1]。尽管国内外医学工作者经过几十年的探索实践，在食管癌的防治方面取得很大的成绩，但食管癌的具体发病机制仍然不十分清楚，以手术、化疗、放疗为主的综合治疗手段仍不尽如人意，尤其在药物治疗方面，食管癌对化疗药物敏感性很差，化疗效果有限。食管癌的预后仍较差，虽然早期食管癌术后五年生存率可高达80%--95%，但中晚期病人仅为10%左右，后者也正是临床最多见的[2]。如果能开发出一种新的、效果明显的食管癌化疗药物或者新的辅助化疗药物以减轻化疗副作用，则可有望提高食管癌患者的生存率和生存质量，造福广大食管癌患者。

苦参碱（Matrine MA）是传统中药苦参根提纯的化学单体之一，广泛存在于豆科槐属苦参、苦豆子及广豆根中，在我国有丰富的资源，它是白金雀儿碱(lupanine)的异构体， 属于四环的喹诺里西啶类（quinolizidine），分子式：$C_{15}H_{24}N_2O$ 分子量：248.37，化学结构式如下图。多年来对其大量的药理和临床研究发现，MA具有免疫抑制[3]、抗炎、抗肝纤维化[4]、抗心律失常[5]等多种药理作用。广泛应用于临床各科，具有长效、持久、副作用少等特点，容易为广大患者所接受，新近的研究发现，该药还具有抑制多种肿瘤细胞增殖与诱导其凋亡等生物活性[4, 5]。研究证实，MA对肝癌、肺癌、胃癌及前列腺癌等多种肿瘤细胞具有抑制作用。文献报道，一定浓度的苦参碱在体外可诱导胃腺癌SGC-7901

细胞、肺癌 A549 细胞和肝癌 HepG2 细胞凋亡，且其诱导凋亡的作用随苦参碱的浓度增加而增加[6]。体外 0.8g/L 苦参碱处理 3d 就能明显抑制人肝癌细胞株（HepG2）的增殖，1.5g/L 可明显诱导其凋亡[7]。有研究提示苦参碱可克服肿瘤的多药耐药现象， 有可能成为一种理想的中药抗肿瘤制剂[8]。还有的学者试图从基因水平来阐述苦参碱对白血病细胞诱导分化作用的机制，认为从中药和天然药物中寻找和提取诱导分化剂来进行肿瘤细胞的诱导分化治疗可能是一种新的抗肿瘤治疗途径[9]。有研究表明，苦参碱可明显抑制肿瘤细胞与内皮细胞粘附后 CD44、CD49 粘附因子的表达，从而抑制了肿瘤细胞与内皮细胞的粘附，减少了肿瘤的转移[10]。王涌等[11]研究发现苦参碱可上调细胞粘附调节基因 CARmRNA 在人肝癌细胞系 Bel-7404 中的表达，进而影响细胞信号转导通路，最终降低肿瘤细胞的侵袭转移能力，可能是苦参碱诱导分化的重要机制；另外，对肿瘤细胞周期变化的影响也是苦参碱诱导分化的可能机制之一。林洪生等[12]研究表明，一定浓度苦参碱可明显抑制 Tiaml 基因的表达，通过这种控作用可以降低肿瘤细胞的粘附和迁移，从而对一些肿瘤的浸润和转移产生一定的抑制作用。虽然苦参碱对肺腺癌、前列腺癌、肝癌、乳腺癌、胃癌、白血病细胞的体外抑制作用都有相应的研究，并有部分阐述了一定的分子机制，但是苦参碱对食管癌细胞的抑制作用研究以及分子机制的探讨在国内却并不多见，氧化苦参碱对食管癌细胞的作用研究相对多一些。

本论文以人食管癌细胞株（Eca-109）为研究对象，采用 MTT 比色法、Hoechst33342 染色法以及流式细胞分析来探讨中药提取物苦参碱单体对 Eca-109 的诱导凋亡和抑制增殖作用。首先采用 MTT 法分别测定 Eca-109 经不同浓度苦参碱（0.5、1.0、2.0、4.0、8.0mg/ml）处理 24 h 和经固定浓度苦参碱（2.0 mg/ml）处理不同时间（0、12、24、36、48 h）后的细胞增殖存活率。结果表明，苦参碱在体外对 Eca-109 具有明显的存活抑制作用，它们的增殖存活率与苦参碱呈剂量-时间依赖关系。随着苦参碱的浓度增加和处理时间的延长，其对 Eca-109 的抑制增殖作用越强，细胞增殖存活率越低。为了探讨苦参碱是否具有诱导 Eca-109 发生凋亡的能力，本实验采用 Hoechst33342 染色后经荧光显微镜观察和 Annexin V/PI 双染经流式细胞仪检测相结合的方法，研究证实苦参碱可以有效诱导 Eca-109 发生凋亡，而且凋亡细胞的数量随苦参碱处理

时间和作用浓度的增加而增加，表现出明显的时间-剂量依赖关系。同时，本实验还采用 PI 单染经流式细胞仪检测的方法，研究证实苦参碱可以有效诱导 Eca-109 发生细胞周期 G2 期阻滞。

综上所述，本论文的研究结果证实了苦参碱具有抑制 Eca-109 存活增殖及诱导凋亡发生的能力，以及苦参碱诱导 Eca-109 发生细胞周期 G2 期阻滞，表明苦参碱抑制 Eca-109 细胞增殖以及诱导其发生凋亡的机制可能与其诱导 Eca-109 发生细胞周期 G2 期阻滞有关，这为本实验研究苦参碱诱导人食管癌细胞株发生凋亡的分子机制提供了一个新的思路和方向。通过这些研究，阐明了苦参碱在体外对 Eca-109 存活增殖的抑制作用和诱导凋亡作用，从而为其作为抗食管癌药物开发奠定了一定的理论基础。

关键词：苦参碱　食管癌细胞　增殖　凋亡　细胞周期

Abstract

Carcinoma of the esophagus is one of common malignant tumors from human digestive system, and it is a serious threat to human health. Every year, more than 20 million people died of esophageal cancer, in China 15 million, occupy the majority of the world deaths of esophageal cancer. In China, the incidence rate of esophageal cancer in a variety of malignant tumors, male fourth place, women seventh place, while the mortality of men and women were fourth. Significant regional differences in the incidence of esophageal cancer, in ares of high incidence of esophageal cancer can be as high as 150/100 000, low-prone areas, only about 3/100 000. The high incidence of esophageal cancer was Central Asia, Africa, Nothern France and Central and South America in foreign, while in China was Taihang mountains, the Qinling regions, Dabie mountain area, northern Sichuan region, Fujian Province, Guangdong Chao-Shan area, northern Jiangsu region. Linxian, in Henan Province, which, esophageal cancer mortality in men 161.33/100 000 and 102.88/100 000 for women, their mortality rates in a variety of malignant tumors first. Although medical workers in the world, after decades of exploration and practice in the prevention and treatment of esophageal cancer achieved great success, but the specific pathogenesis of esophageal cancer is still not very clear, and the combined therapy with surgery, chemotherapy, radiation therapy, a comprehensive treatment is still not satisfactory, especially in terms of drug treatment, esophageal cancer is poor sensitivity to chemotherapeutic drugs, the effect of chemotherapy is limited. The prognosis of esophageal cancer remains poor, early esophageal cancer postoperative five-year survival rate can be as high as 80%-95%, in patients with advanced only about 10%, the later is also the most common in clinical. If we can be able to develop a new effect of esophageal cancer drugs or new adjuvant chemotherapy drugs to alleviate the side effects of chemotherapy, it can be expected to improve survival and quality of life of patients with esophageal cancer.

Matrine is one of the traditional Chinese medicine Sophora flavescens root purified monomers, widely present in the legume Cassia genus Sophora, Sophora alopecuroides and Sophora root, has rich resources in China, it is the lupanine isomers, belonging to the Fourth Ring of the quinolizidine, the formula: $C_{15}H_{24}N_2O$, molecular weight: 248.37, chemical structure as shown below. Their pharmacological and clinical studies found that over the years, matrine has immunosuppressive, anti-inflammatory, anti-liver fibrosis, anti-arrhythmic and a variety of pharmacological effects. Widely used in clinical subjects, with the characteristics of long-lasting, durable, and less side effects and so on, easily accepted by the majority of patients, recent studies have found that the drug also inhibit cell proliferation and induce apoptosis and other biological activity. Some studies confirmed that matrine can inhibit a variety of tumors of the liver, lung, stomach and prostate cancer cells. Reported in the literature, a certain concentration of matrine in vitro can induce apoptosis of gastric adenocarcinoma SGC-7901 cells, A549 cells and HepG2 cells, and induction of apoptosis increasing with matrine concentration increases. In vitro 0.8g/L matrine handle 3d will be able to significantly inhibit the proliferation of human HepG2 cell, 1.5g/L can significantly induce apoptosis. Some studies suggest that matrine can overcome tumor multidrug resistance phenomenon, may be an ideal traditional Chinese medicine anti-tumor agents. Some scholars tried to elaborate the mechanism of matrine on leukemia cells to induce differentiation gene level, and believe that from the traditional Chinese medicine and natural medicine to find and extract the differentiation inducer of tumor cells induced differentiation therapy may be a new anti-cancer treatment pathway. Studies have shown that matrine can inhibit tumor cells and endothelial cells adhesion, CD44, CD49 adhesion molecule expression, thus inhibiting the adhesion of tumor cells and endothelial cells, reduced tumor metastasis. Wang Yong and other study found that matrine can increase the expression of the cell adhesion and regulate gene CARmRNA in the human hepatoma cell line Bel-7404, thereby affecting the cellular signal transduction pathways, and ultimately reduce the ability of tumor cell invasion and metastasis, may be matrine important mechanism to

induce differentiation. In addition, the impact of changes in the cell cycle is one of the possible mechanisms of matrine induced to differentiate. Lin Hongsheng and other studies have shown that certain concentration of matrine can significantly inhibit Tiaml gene expression, and through this control role can reduce tumor cell adhesion and migration, in order to produce a certain number of tumor invasion and metastasis inhibition. Matrine on lung adenocarcinoma, prostate cancer, liver cancer, breast cancer, gastric cancer, leukemia cell in vitro inhibition has a corresponding study, and some described the molecular mechanisms, but matrine on esophageal carcinoma cells inhibition studies and molecular mechanism of the country are few and far, relatively more of the effect of oxymatrine on esophageal carcinomacells. Human esophageal carcinoma cell line(Eca-109) was studied in this thesis. MTT assay, Hoechst 33342 staining and flow cytometry analysis were used to examine the extract of Chinese medicine matrine monomer induce apoptosis and inhibit the proliferation of Eca-109. First of all, we determined the viabilities of the cell lines treated by different concentration of matrine for 24 h and 2.0 mg/ml matrine for different time periods. The results show that matrine can significantly inhibit the viabilities of human esophageal carcinoma cell lines Eca-109 in a dose-time dependent manner. With the increased of matrine concentration and the processing time, inhibit the proliferation of Eca-109 was stronger and the survival rate of cell proliferation was lower. We combined Hoechst33342 staining and Annexin-V/PI staining assays to investigate whether matrine can induce human esophageal carcinoma cells apoptosis. The study confirmed that matrine could effectively induce apoptosis of Eca-109, and the number of apoptotic cells increased with matrine processing time and the role of concentration increases, showing a dose-time dependent manner. We also found that matrine induced Eca-109 cell cycle arrest in G2 by PI staining.

Conclusions: our studies confirm that matrine can inhibit the viabilities and induce apoptosis of Eca-109 cell, and induced Eca-109 cell cycle arrest in G2. Prove that the mechanism of matrine inhibit Eca-109 cell proliferation and induce their apoptosis may be related to induced Eca-109 cell cycle arrest in G2 phase. This may

be a new idea and direction for our study about the molecular mechanism of matrine induced Eca-109 cell apoptosis. These studies clarify the inhibitory effect and induction of apoptosis of matrine in vitro to proliferation of Eca-109, and cell cycle arrest provides a new approach to our future research, so its anti esophageal cancer drugs the development has lead a theoretical basis.

Key Words: matrine; esophageal carcinoma cell; proliferation; apoptosis; cell cycle;

英文缩略词表

英文缩写	英文名称	中文名称
Eca-109		人食管癌细胞株
Cyclin		细胞周期蛋白
HUVECs		人脐静脉内皮细胞
CDK	cyclin dependent kinases	细胞周期蛋白依赖性激酶
MMP	matrix metalloproteinase	基质金属蛋白酶
EGFR	epidermal growth factor receptor	上皮生长因子受体
VEGF	vascular endothelial growth factor	血管内皮生长因子
ERK	extracellular regulated protein kinases	细胞外调节蛋白激酶
MAPK	mitogen-activated protein kinases	促分裂素原活化蛋白激酶
E-cad	E-cadherin	E-钙粘附素
LRP	lung resistance protein	肺耐药蛋白
TOPOⅡ	topoisomerase Ⅱ	拓扑异构酶Ⅱ
APL	acute promyelocytic leukemia	急性早幼粒细胞白血病
ATRA	all transretinoic acid	全反式维甲酸
DSB	double strand break	双链 DNA 断裂

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