

学校编码: 10384

分类号___密级___

学号: 21720021152212

UDC _____

厦 门 大 学

硕 士 学 位 论 文

文蛤多肽的研究与应用

Studies and applicantions of peptide from
Meretrix meretrix Linnaeus

范成成

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专 业 名 称: 生物化学与分子生物学

论文提交日期: 2009年4月29日

论文答辩时间: 2009年5月30日

学位授予日期: 2009年 月 日

答辩委员会主席: 林河通

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2009年4月

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摘 要

癌症是目前死亡率最高的疾病，是名副其实的“头号杀手”。从天然生物中筛选出能与癌症发生、发展、形成等过程中各调节靶点特异结合的活性成分，已成为寻找抗癌药物的新热点。目前各国学者采用现代科学方法与技术从海洋生物中分离和鉴定出了多种具有抗肿瘤活性的天然物质，为研制开发抗肿瘤新药奠定了基础，显示出诱人的研究开发前景，从海洋生物及其代谢产物中筛选和提取具有特异化学结构的天然活性物质成为抗肿瘤药物开发的重要来源。文蛤 (*Meretrix meretrix* Linnaeus) 作为一种深受人们喜爱的海洋软体动物，不仅具有丰富的营养价值，更是有着极高的药用价值。古往今来，文蛤的药用价值一直深受人们的重视，它具有清热利湿、化痰、散结的功效，对肿瘤细胞有明显的抑制作用，还具有降血糖，降血脂，抗衰老等多种生理功能。其有效成分及生理作用受到越来越广泛的关注。

本研究采用破碎、硫酸铵沉淀、乙醇抽提、Sephadex G-25 分子筛层析等方法从文蛤中提取得到四个峰的物质，利用 MTT 法分别检测各峰体外对癌细胞的抑制效应，发现第二峰的物质能抑制癌细胞的生长，将此活性物质命名为 Mer2；发现第一个峰的物质能抑制酪氨酸酶的活性，减少黑素的形成，命名 Mer1。

将 Mer2 处理肝癌 HePG₂ 细胞、胆管癌 QBC939、宫颈癌 Hela 细胞、肺腺癌细胞 SPC-A-1、LTP-a-2，观察研究 Mer2 对癌细胞的生物学效应并进一步探索其抑制癌细胞生长的作用机理。结果表明，Mer2 对五种细胞均有不同程度的抑制作用，但对正常的人肝 chang-liver 细胞抑制作用很小。当浓度大于 10 μg/mL 时，作用时间为 72 h 时，对肝癌 HePG₂ 细胞和胆管癌 QBC939 的抑制率便超过了 50%，其中对肝癌 HePG₂ 细胞的抑制作用更强一些。通过光学显微镜和流式细胞技术等方法，研究了文蛤多肽对体外培养的肝癌 HePG₂ 细胞和胆管癌 QBC939 的细胞形态、细胞周期的影响。结果表明：Mer2 处理后的细胞外形变化明显，贴壁细胞数量减少，出现了单个细胞的悬浮生长，细胞的集落化程度降低，细胞群体数量显著降低，出现了凋亡小体。细胞周期的检测结果说明，经 Mer2 处理的肝癌 HePG₂ 细胞周期发生明显变化，出现明显的凋亡峰，但细胞周期未出现明显阻滞现象。表明文蛤多肽对癌细胞的生长抑制作用与细胞凋亡有关。

进一步研究文蛤抗癌多肽抑制癌细胞的分子机制,我们利用蛋白组学双向电泳技术初步探讨了在经Mer2作用前后肝癌HePG₂细胞蛋白表达的变化,以期为后续的研究提供理论基础。通过图谱分析找到89个差异蛋白,其中66个表达下调,23个表达上调。对其中部分差异较明显的点进行了鉴定,最终鉴定出9种差异蛋白。其中7个蛋白表达下调,2个蛋白表达上调。肝癌细胞HePG₂表达的差异蛋白种类涵盖了从细胞结构到细胞生理调控的各个方面。对差异点进行鉴定后发现,差异蛋白有锌指蛋白(Zinc finger protein 624),膜联蛋白(Annexin A2),细胞骨架蛋白(keratin 1, type II, cytoskeletal),分子伴侣蛋白(TCP-1-beta),线粒体核糖体蛋白(mitochondrial ribosomal protein L27),DNA修复蛋白,肿瘤抑制蛋白以及其他的蛋白等,并对部分差异点的功能进行了初步探讨。

采用动力学研究方法,体外研究了Mer1对蘑菇酪氨酸酶(Tyrosinase)活力的影响。结果表明Mer1对酪氨酸酶有较强的抑制作用,抑制过程呈浓度的关系,抑制作用是一种可逆过程,为反竞争性抑制,使酶活力丧失50%的抑制剂浓度(IC_{50})为95 $\mu\text{g/mL}$,抑制常数(K_{IS})为60.98 $\mu\text{g/mL}$ 。我们还研究了Mer1对小鼠黑素瘤B16生长的影响,发现Mer1不会抑制黑色素瘤细胞的生长,能降低细胞内酪氨酸酶的活性和黑色素的含量,具有清除自由基的能力,具有一定的抗氧化活性是一种安全无毒副作用的美白添加剂,具有潜在的防止褐斑、美白肌肤的作用。

关键词: 文蛤多肽; 抗癌; 抗氧化; 美白

Abstracts

Nowadays, cancer has the highest mortality, is worthy of the name of “the first killer”. Many researches reported that the anticancer components extracted from natural organism could combine with special regulation target during the course of growth, develop and malignance of cancer. Screening out those active substances has been the hotspot in the work of finding new effective anticancer medicaments. Since several years ago, many antineoplastic materials with original structures have been identified from marine organisms. And this presents a marvelous prospect in discovering new drug. Screening and extracting antineoplastic agents with unique chemical constitution from marine lives and their metabolites become more and more important. *Meretrix meretrix* Linnaeus is a kind of popular ocean mollusks, the value of which is not only in the abundant nutrition, but also in the use as a resource of medicine. It can eliminate cyst and detoxify the body, which means its inhibitory effect on the proliferation of some cancer cells. It can also decrease the level of blood sugar and blood fat and protect body cells from mutation or decrepitude. The active components along with its physiological function have aroused popular attention.

In this study, we obtained four peaks by a series of methods including fragmentation, organic precipitation and Sephadex G-25 column chromatogram. We tested the inhibitory effect of four peaks on cancer cells and found that the substance of peak 2 named Mer2 could inhibit the proliferation of cancer cells. And peak1 named Mer1 could inhibit the activity of Tyrosinase.

To investigate the biological effects of Mer2 on cells and functional characteristics, Mer2 was used to treat cell lines including the HePG₂ cells, the human cholangiocarcinoma QBC939 cells, Hela cells and the human lung adenocarcinoma SPC-A-1 LTEP-a-2 cells. The result from MTT showed that Mer2 could inhibit growth of all the cell lines above. And the IC_{50} in 72h to HePG₂ and QBC939 cells were all less than 10 $\mu\text{g}/\text{mL}$. We investigated the effects of Mer2 on the shape and cell-cycle of liver cancer HePG₂ cells by microscope. The results showed that shapes of cells treated by Mer2 had been changed distinctly, which showed an obvious

apoptotic property. It indicated that the inhibitory effect of Mer2 on cancer cell had a relationship with the cell apoptosis.

Proteomics technology was used to study the molecular mechanism of inhibitory effects of Mer2 on the HePG₂. The differentially expressed proteins were analyzed by two-dimensional gel electrophoresis. And totally 89 differentially expressed proteins, 66 of which were down-regulated and 23 were up-regulated, were found on two patterns. One was the control cells proteins pattern, the other was that of cells treated by Mer 2. 9 proteins changed markedly were identified by MALI-TOF. And the result showed that the expression levels of 2 proteins were down-regulated and 7 were up-regulated. These proteins included zinc finger protein, Annexin A2, cytoskeletal protein, mitochondrial ribosomal protein L27, transcription regulation factors and so on. And we discussed the functions of some of these proteins.

By using the method of dynamics, we studied the effect of Mer1 on mushroom Tyrosinase. The peptide showed uncompetitive inhibitory effect on Tyrosinase, the value of IC_{50} was 95 $\mu\text{g/mL}$ and an uncompetitive type inhibitory effect on the mushroom Tyrosinase, the value of inhibition constant (K_{IS}) was 60.98 $\mu\text{g/mL}$. We also studied effect of Mer1 on proliferation of B16. And the result showed that Mer1 did not inhibit growth of B16 cells, but it could inhibit the activities of Tyrosinase and melanin in B16 cells. It was able to clear free radicals without any undesirable damages, which displayed that it had certain activity of anti-oxidant; therefore it's a safe additive with potential function of whitening skin.

Keywords: peptide from *Meretrix Meretrix* Linnaeus; anticancer; activity of anti-lipidperoxidation; depigment.

1 前言

1.1 癌症的发生情况、危害及防治

癌症是一古老的疾病，早在两三千多年前，埃及和我国已有关于癌疾的记载，但不在常见病之列。20 世纪初癌症在各国仍是比较罕见的疾病。我国直到 20 世纪 50 年代初，在死亡人口中癌病占第九位。近半个世纪以来，癌症在医学领域内的地位越来越重要，目前已成为多发病、常见病，占人口死亡原因的第一、二位，严重威胁人民的健康。主要原因有四方面：(1) 随着工业化的发展，人类生存环境中的致癌物越来越多。空气和水的污染，吸烟，不良生活习惯包括膳食的不平衡及食品添加剂和某些药物的滥用，癌疾在全世界范围内有增多趋势。如果不采取有效的措施，这一趋势将继续下去，21 世纪很多国家男性癌症死亡将增加 20%-50%，女性将增加 12%-40%。英国的 R. PeM 教授甚至预言我国如不大力戒烟，到 2025 年将成为肺癌第一大国。(2) 随着医学的发展，过去许多严重威胁人类健康的急性传染病、寄生虫病、营养不良和新生儿死亡等由于找到了病因，采取了适当的预防措施和有效的治疗，因而得到了控制。而一些病因比较复杂，尚无十分有效治疗方法的疾病，如心脑血管疾病和癌症的确在逐年上升。1990-1992 年全国癌症死亡率抽样调查结果表明，我国无论城市还是乡村，癌症均占常见病死因的第二位。1997 年在城市占第一位，农村为第二位。这种相对地位的提高在很大程度上是由于其他疾病死亡率下降。(3) 近半个世纪以来，由于生活水平的提高和医疗卫生工作的发展，人们的平均寿命延长了。以北京为例，1947 年东城区居民平均寿命仅为 35 岁，而目前已超过 70 岁，癌症的发病年龄高峰在 40-45 岁以后。世界卫生组织 1998 年报告，1980-1995 年人口平均年龄提高 4.6 岁，1996 年初人口的预期平均年龄为 65 岁。在 1996-2002 年间 65 岁以上的老龄人口将增加 82%。但这并不是说现在 40 岁以上的人比过去同样年龄的人更容易患肿瘤，癌症病人的数目无疑将会增多^[1,2]。使癌症成为了是当前危害人类健康和生命最为严重的主要常见病、多发病之一，在各种疾病中，可谓“头号杀手”，人们几乎谈癌色变。

攻克癌症一直是人类的梦想，一个多世纪以来，人们不断探索癌症的病因。1858 年，Virchow 就在其《细胞病理学》中指出：“癌是细胞的疾病”。近 40 年来，经过科技工作者的不懈努力，人们日益清楚的看到癌症是有环境、营养、饮食、

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