雷公藤甲素对结肠癌细胞 SW480 的基因表达谱的影响 *

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摘要 目的:探讨雷公藤甲素对结肠癌 SW480 细胞的基因表达谱的影响。方法:雷公藤甲素处理结肠癌 SW480 细胞 24h 后,分别提取给药组和空白对照组 SW480 细胞总 RNA,纯化并逆转录成用 Cy3 和 Cy5 标记的 cDNA 探针,经全基因芯片杂交,洗涤,通过生物信息学方法分析雷公藤甲素处理组和空白对照组 SW480 细胞基因表达谱的差异。结果:与空白对照组比较,共发现了 902 个差异基因,雷公藤甲素处理组有 196 个基因上调,706 个基因下调。上调基因主要涉及细胞代谢。下调基因主要涉及 wnt 通路、细胞周期通路、Toll 样受体通路以及 MAPK 等通路。结论:雷公藤甲素能导致结肠癌细胞基因表达谱的改变,这些基因改变可能参与了细胞增殖、分化、凋亡等过程。这些信息可能为探讨雷公藤抗结肠癌作用机制提供线索。

关键词:雷公藤甲素;SW480 细胞;基因表达谱;基因芯片

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Effect of Triptolide on Gene Expression Profile of Colon Cancer Cells SW480*

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ABSTRACT Objective: To investigate the effect of triptolide on the gene expression of human colon cancer SW480 cells. Methods: incubate SW480 cells with or without triptolide for 24 hours, and then the total RNAs were extracted. All RNA were purified and then reversely transcribed to cDNA. The cDNAs were tagged with fluorescence dye Cy3/Cy5, and then hybridized with whole-genome chip. After washing, the gene expression profile of SW480 cells was analyzed by bioinformatics to screen the differential expressed genes among the cells with or without triptolide. Results: Compared with the control group, a total of 902 differentail genes in triptolide-treated group were found, among which, 196 were up-regulated and 706 were down-regulated. The up-regulated genes were mainly involved in metabolism, and down-regulated genes mainly involved in Wnt signaling pathway, cell cycle pathway, Toll-like pathway and MAPK pathwayand so on. Conclusion: Triptolide alters the gene expression profiles of colon cancer cells SW480, and these genes are mainly related to cell proliferation, differentiation, and apoptosis. The screening of the genes may be meaningful for study the mechanisms of triptolide treat colon cancer.

Key words: Triptolide; SW480 cells; Gene expression profile; Micro-array

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前言

雷公藤甲素(triptolide)是从植物雷公藤中提取的环氧二萜内酯类化合物,是雷公藤的主要有效成分之一。具有较强的抗风湿,免疫抑制,抗生育作用[1]。近年发现雷公藤甲素具有较好的抗肿瘤作用,能够抑制多种肿瘤细胞的增殖,包括乳腺癌、前列腺癌、子宫内膜癌和卵巢癌等细胞[2-4]。我们之前的研究表明雷公藤甲素能够抑制结肠癌 SW480 细胞的增殖[5],但其作用机制尚不清楚。本实验应用全基因组表达谱基因芯片,从基因水平检测雷公藤甲素作用前后结肠癌 SW480 细胞基因表达谱的变化,为揭示雷公藤甲素抗结肠癌的作用机制提供线索。

1 材料与方法

1.1 材料

雷公藤甲素(Triptolide)购自深圳市牌牌科技有限公司。胎牛血清、RPMI1640 培养基(Hyclone 公司);Cy3 NHS 标记物、Cy5 NHS 标记物(美国 GE Healthcare 公司);Human 4x44k 全基因芯片、aaUTP(美国 Ambion 公司);Low RNA Input Linear Amplication kit、Gene Expression Hybridization Kit、Gene Expression Wash Buffer Kit、Stabilization and Drying Solution、Gasket slide、Hybridization Chamber(美国 Agilent 公司);RNeasy Mini kit(德国 QIAGEN 公司);TRIzol 试剂(美国 Invitrogen 公

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司）；PTC-100 PCR 仪（MJ 公司，美国）；G25245BA 扫描仪、G2534A 型杂交炉（美国 Agilent 公司）；ND1000 紫外分光光度计（美国 Nanodrop 公司）。

1.2 方法

1.2.1 细胞培养 SW480 细胞用含有 10 %胎牛血清的 RMPI-1640 培养液培养。实验分为两组，实验组细胞加入终浓度为 150 nM 的雷公藤甲素，在 37 ℃、5 % CO_2 饱和湿度条件下培养 24 h。对照组不加入雷公藤甲素。

1.2.2 总 RNA 提取 按照 Trizol 试剂说明书提取细胞总 RNA，并使用 QIAGEN RNeasy Kit 对总 RNA 进行纯化，纯化过程按照试剂盒操作说明进行。

1.2.3 探针制备和杂交 探针制备和杂交由上海伯豪生物技术有限公司完成，简述如下：分别取两组 RNA 2μg 加入到 20μL 转录反应体系中，合成 cDNA。用 Cy5-dNTP 标记对照组 cRNA，用 Cy3-dNTP 标记雷公藤甲素组 cRNA。先将 cRNA 样品在 60 ℃片段化，后加入等体积 2× GEx Hybridization Buffer 混匀，高速离心，取样品上芯片，65 ℃，10 rpm 滚动杂交 17 h。取出芯片在洗涤液 1 和洗涤液 2 中依次洗涤 1 min 并干燥。

1.2.4 芯片扫描和结果分析 Agilent 扫描仪进行芯片扫描，Agilent Feature Extraction 软件读取数据，最后采用 Feature Extration 进行 Normalize 处理分析，计算基因 log_2ratio 值（Cy3/Cy5），将 log_2Ratio≥ 1 或≤ -1 分别作为显著上调和显著下调的标准。

2 结果

2.1 总 RNA 提取质量分析

雷公藤甲素组和对照组总 RNA 经紫外分光光度计检测其 A260 /A280 均为为 1.95，证明所提取 RNA 无蛋白质污染。电泳分析结果证明雷公藤甲素组和对照组 RNA28S/18S 分别为 1.5 和 1.7，说明 RNA 无降解，能满足后续实验。

2.2 DNA 芯片实验结果

按照筛选标准，共筛选出 902 个基因，其中上调基因 196 个，下调基因 706 个。上调的基因多为代谢相关基因（见表 1）。下调的基因中 36 个归属于细胞周期，32 个基因归属于 Wnt 通路，42 个归属于 MAPK 通路，16 个归属于 Toll 样受体通路（见表 2）。

表 1 雷公藤甲素作用于 SW480 细胞后上调的基因通路

Table 1 The up-regulated signal transduction genes after treated with triptolide

KEGG pathway	Numbers of related genes	%
Complement and coagulation cascades	20	1.9
Lysosome	19	1.8
Valine, leucine and isoleucine degradation	10	0.9
Complement Pathway	6	0.6
Classical Complement Pathway	5	0.5
Propanoate metabolism	7	0.7
Type I diabetes mellitus	8	0.8
Retinol metabolism	9	0.8
Systemic lupus erythematosus	13	1.2
Oxidative phosphorylation	15	1.4
Metabolism of xenobiotics by cytochrome P450	9	0.8
Histidine metabolism	6	0.6
Tryptophan metabolism	7	0.7
Drug metabolism	9	0.8
Lectin Induced Complement Pathway	4	0.4
Alanine, aspartate and glutamate metabolism	6	0.6
Phenylalanine metabolism	5	0.5
Beta-Alanine metabolism	5	0.5
Nitrogen metabolism	5	0.5
Tyrosine metabolism	7	0.7
Butanoate metabolism	6	0.6
Viral myocarditis	9	0.8
Fatty acid metabolism	6	0.6

表 2 雷公藤甲素作用于 SW480 细胞后下调的基因通路

Table 2 The down-regulated signal transduction genes after treated with triptolide

KEGG pathway	Numbers of related genes	%
Cell cycle	36	1.8
Pathways in cancer	56	2.8
Wnt signaling pathway	32	1.6
Acetylation and Deacetylation of RelA in The Nucleus	9	0.5
SODD/TNFR1 Signaling Pathway	7	0.4
Ubiquitin mediated proteolysis	26	1.3
Ribosome	19	1.0
MAPK signaling pathway	42	2.1
Spliceosome	24	1.2
TGF-beta signaling pathway	18	0.9
NFkB activation by Nontypeable Hemophilus influenzae	10	0.5
p53 signaling pathway	15	0.8
hsa05210:Colorectal cancer	17	0.9
Apoptosis	17	0.9
NF-kB Signaling Pathway	9	0.5
TSP-1 Induced Apoptosis in Microvascular Endothelial Cell	5	0.3
Regulation of transcriptional activity by PML	7	0.4
HIV-I Nef: negative effector of Fas and TNF	15	0.8
Neurotrophin signaling pathway	21	1.1
Oocyte meiosis	19	1.0
Acute myeloid leukemia	12	0.6
Prostate cancer	16	0.8
Chronic myeloid leukemia	14	0.7
Melanogenesis	17	0.9
Small cell lung cancer	15	0.8
Endocytosis	27	1.4
Heparan sulfate biosynthesis	7	0.4
Basal cell carcinoma	11	0.6
RIG-I-like receptor signaling pathway	13	0.7
Regulation of MAP Kinase Pathways Through Dual Specificity Phosphatases	5	0.3
Pathogenic Escherichia coli infection	11	0.6
Oxidative Stress Induced Gene Expression Via Nrf2	7	0.4
RNA polymerase	7	0.4
Adipocytokine signaling pathway	12	0.6
Thyroid cancer	7	0.4
TNFR1 Signaling Pathway	9	0.5
Toll-like receptor signaling pathway	16	0.8
Adherens junction	13	0.7
FAS signaling pathway (CD95)	9	0.5
Progesterone-mediated oocyte maturation	14	0.7
Notch signaling pathway	9	0.5
Cytosolic DNA-sensing pathway	10	0.5
Keratinocyte Differentiation	10	0.5
Role of Mitochondria in Apoptotic Signaling	7	0.4
Gap junction	14	0.7
RNA degradation	10	0.5

3 讨论

雷公藤甲素是植物雷公藤的主要有效成分之一，具有免疫抑制、抗炎、抗肿瘤的功效。基因芯片具有高敏感、高通量的特性，可快速获得大量基因 mRNA 水平的表达信息。本实验观察给予结肠癌 SW480 细胞 150 nM 雷公藤甲素 24 h 后，细胞的基因变化情况。结果提示雷公藤甲素显著影响了与结肠癌发生发展相关的多条通路。现将与结肠癌发生发展密切相关的四条通路列举如下：

首先，显著影响了 Wnt 通路。Wnt 通路在结肠癌的发生发展过程发挥着重要作用。正常情况下，细胞内的糖原合成激酶 3β (GSK3β)，肿瘤抑制因子 APC 以及 Axin 形成一个复合物，促进 β-catenin 的降解；而当细胞受到 wnt 配体刺激时，细胞质蛋白 Dishevelled (DVl) 向细胞膜聚集，并与蛋白 Axin 结合，抑制 Axin 复合物的形成，使得 β-catenin 的降解减少，大量 β-catenin 进入细胞核，与转录因子 TCF/LEF 结合，促进细胞的转录、增殖[6,7]。文献报道称，85 %的结肠癌患者都发生 Wnt/β-catenin 通路的激活，激活的 Wnt/β-catenin 将会促进结肠癌的发展[8]。

其次，影响了细胞周期通路。完整的细胞周期包括分裂间期和分裂期，它是一个有序、紧凑的过程，受到多个因素的调控，包括细胞外的生长因子，细胞的大小，以及 DNA 的完整性等。分裂间期分为 G1 期，S 期和 G2 期，是细胞生长和 DNA 合成时期。E2F 是重要的转录因子，能够促进 G1/S 的转化[9,10]。Myc 基因也是细胞周期通路中的重要分子，在多种肿瘤形成过程中起到关键作用，研究表明，抑制 Myc，能够明显的抑制细胞的生长增殖。c-Myc 在促进 G1/S 转化以及 G2/M 转化过程中均起到关键作用[11,12]。我们的结果提示，加入雷公藤甲素后，E2F 和 Myc 这两个基因均显著下调。我们之前的研究也发现雷公藤甲素能够降低结肠癌 SW480 细胞 Cyclin D1 以及 CDK4 的表达，引起细胞周期 G1 期阻滞[2]。

第三，影响了 Toll 样受体通路。Toll 样受体属于模式识别受体，为重要的天然免疫分子，目前发现的共有九个亚型，为 Toll like receptor 1-9，其中 toll-like receptor 1-4 均与结肠癌的发生发展相关[13,14]。实验结果提示雷公藤甲素下调了 Toll 样受体通路中关键分子 TLR4 基因的表达。Toll like receptor-4 (TLR-4) 是 Toll 样受体家族中非常关键的一员，TLR-4 通过促进 Akt 的磷酸化，调节结 / 直肠癌细胞的生长 [15]；通过促进 COX-2 表达及升高 EGFR 途径活性来促进小鼠结肠炎相关的结 / 直肠癌发展[16]。部分报道指出 结 / 直肠癌组织的 TLR-4 表达过量[17]。

第四，影响了 MAPK 通路。MAPK 通路与细胞的增殖、凋亡、转移均密切相关，影响着结肠癌的发生发展[18,19]。报道指出：在结肠癌细胞中，通常能检测到 MAPK 的活化[20]。有 56.4%的结肠癌患者都发生 MAPK 通路的活化，且发生 MAPK 通路活化的结肠癌患者其三年的存活率明显降低[21]。MAPK 属于丝氨酸酪氨酸激酶家族，分为三个亚家族，其中 Ras/Raf/MEK/ERK 是 MAPK 家族中重要的组成部分，抑制 ERK 的活性，结肠癌细胞的生长将会受到抑制[22,23]。

实验结果提示：雷公藤甲素可能主要通过以上四条通路影响结肠癌细胞的增殖、分化、凋亡和转移，显示出良好的结肠癌治疗前景。

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