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Effect of emodin on c-*myc* proto-oncongen expression in cultured rat mesangial cells

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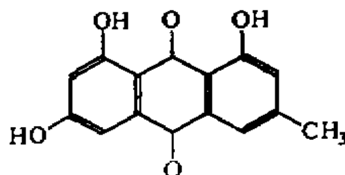
KEY WORDS emodin; cultured cells; glomerular mesangium; proto-oncogen proteins c-*myc*

AIM: To explore the possible molecular mechanism of emodin on the inhibition of glomerular mesangial cells (MC). **METHODS**: In rat MC culture, c-*myc* mRNA level was detected by dot-blotting as expressed by the dilutions of the total RNA on the visible dots. **RESULTS**: Low level c-*myc* mRNA was found in serum-free cultured quiescent MC. Addition of lipopolysaccharide (LPS 10 mg · L^{-1}) induced a higher level expression of c-*myc* mRNA within 30 min, maximal expression at 2.5 h and persisted for 6 h. This over-expressing of c-*myc* mRNA was markedly suppressed by emodin (25 mg · L^{-1}), the inhibition of emodin on c-*myc* mRNA expression was still seen at 6 h, and the greatest suppressive effect was at 2.5 h. **CONCLUSION**: Emodin participating in its down-regualtory effect of c-*myc* mRNA over-expression contributes to its inhibitory action on MC.

Emodin (3-methyl-1,6,8-carboxyl-anthraquinone), a component isolated from of the roots of *Rheum palmatum* L, inhibits the proliferation of cultured rat mesangial cells (MC) and may be beneficial of retarding the progression of chronic renal failure[1]. Proto-oncongens play a major role in the control of cell proliferation[2]. They encode for proteins which act in cell cycle regulation, signal transduction, and cellular gene transcription[3]. In the present study, we investigated whether emodin could suppress the over-expression of c-*myc* proto-oncongen in cultured rat MC, just to define its possible molecular basis on MC.



Emodin

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MATERIALS AND METHODS

Materials Emodin was kindly gifted by Professor CHEN Qiong-Hua (from China Pharmaceutical University). cDNA probe for c-*myc* was a 6.5 kb Hind III/EcoRI fragment from plasmid pPE123. Photobiotin (Academy of Military Medical Sciences) was used to prepare a biotin-labeled non-radioactive probe as described[4]. RPMI-1640 culture medium and lipopolysaccharides (LPS) were obtained from Gibco, USA.

Mesangial cell culture Kidney glomeruli were isolated from 6-8 wk old SD rats, mesangial cells were cultured as previously described[5]. Culture medium consisted of RPMI-1640 supplemented by 15 % fetal calf serum (FCS), penicillin 170 mmol · L^{-1}, streptomycin 70 mmol · L^{-1}, and 2-mercaptoethanol 50 mmol · L^{-1}, once confluent, mesangial cells were trypsinized and subcultured. The 4th to 6th passages were used in this experiment.

RNA preparation and dot blot hybridization Mesangial cells were washed twice in RNase-free PBS (0.12 mol · L^{-1}, pH 7.2) and lysed with 1 % SDS. Total RNA was then extracted by the guanininum thiocyanated/phenol chloroform single-step method[6]. Same amount of measured RNA (20 mg/dot) was dissolved in 100 mL H_2O_2, and five serial dilutions were spotted onto nitrocellulose filters. The blots were dried at 80 ℃ in a vacuum oven for 2 h, then soaked in 2×SSC and prehybridized at 42 ℃ for 3 h. Hybridization was performed in fluid containing photobiotin-labeled cDNA probes for c-*myc* at 42 ℃ for 24 h. The filters were washed with 0.1×SSC, 0.1 % SDS at 20 ℃ for 14 min, and three washes with 0.2×SSC, 0.1 % SDS for 20 min each time at 68 ℃. An avidin-alkaline phosphatase conjugate was added at 37 ℃ for 30 min after using bovin serum albumin to block the non-specific binding. The filters were washed and NBT-BCIP were added, allowing the filter to show the visible formed hybrids in dark place. The mRNA level was recorded as maximal dilution of visible dot.

Experimental design In preparation for dot-blot analysis, 1 × 10^5 mesangial cells were added to 5-well plates and cultured for 48 h, the cells were made quiescent in serum-free culture medium containing insulin (0.5 mmol · L^{-1}) and transferrin (5 mg · L^{-1}) for 48 h.

Then fresh medium with/without emodin and LPS were added to plates as follows: Group A: medium with 5 % FCS as a control. Group B: 5 % FCS medium with LPS 10 mg · L^{-1}. Group C: 5 % FCS together with LPS 10 mg · L^{-1} and emodin 25 mg · L^{-1}. The mesangial cells were harvested at various incubation periods.

RESULTS

Mesangial cells in culture showed typical spindle-like apperance and stained positively for desmin and Thy-1 antibody (Fig 1, Plate 3).

In quiescent mesangial cells, a low based expression of c-*myc* mRNA was detected. However, in response to LPS, a rapid induction of c-*myc* mRNA was observed within 30 min, which peaked at 2.5 h and persisted for 6 h (Tab 1).

Tab 1. c-*myc* mRNA level under different experiment conditions.

5 % FCS-1640	LPS 10 mg · L^{-1}	Emodin 25 mg · L^{-1}	c-*myc* mRNA level 0.5 h	1.5 h	2.5 h	6 h
+			—	1 : 1	—	1 : 1
+	+		1 : 10	1 : 20	1 : 40	1 : 40
+	+	+	1 : 5	1 : 10	1 : 5	1 : 5

To evaluate the effects of emodin underlying these response, we added emodin in the presence of LPS. The c-*myc* mRNA level was markedly suppressed by emodin, showing a 200 % reduction in dilution at 30 min and a maximal reduction of 400 % at 2.5 h. The reduction of c-*myc* mRNA level persisted for 6 h after the addition of emodin with LPS (Fig 2).

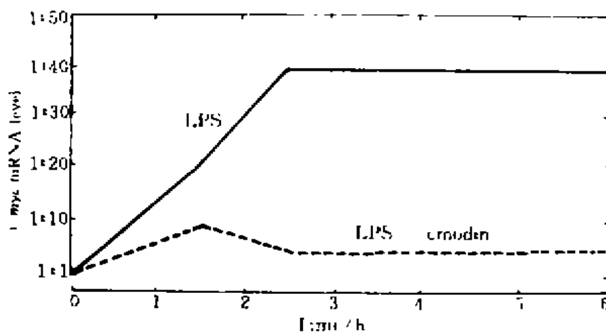

Fig 2. Effect of emodin on c-*myc* mRNA expression.

DISCUSSION

Our previous study has demonstrated that *in vitro* emodin significantly inhibited thymidine incorporation into mesangial cells[1] and we also found that emodin could suppress PCNA/cyclin expression in mesangial cells[7]. It may be speculated that emodin inhibited mesangial cell growth through its inhibitory effect on the transition of mesangial cells from G_1 to S phase. To test this hypothesis further, we chose c-*myc* proto-oncogen as a target gene. As we know, c-*myc* is an immediate early gene encoding transcription factors expressed in G_1-phase of the cell cycle and has a DNA-binding property. It has become clear that the proto-oncogen is an important regulator of cell growth and is activated in numerous cell types in response to growth-promoting agents such as LPS[2]. It was known to be mandatory from the transition of cells from G_1 to S phase of the cell cycle[5]. Furthermore, the inhibition of c-*myc* protein by anti-sense oligomers has been shown to inhibit cell transition to the S phase[9]. So altered expression of c-*myc* gene is an important event in the cell transformation and proliferation. In present study we found LPS could raise c-*myc* mRNA level of mesangial cells, but this over-expression of c-*myc* mRNA was markedly suppressed by the adding emodin. From this experiment, it is well established that emodin has a down-regulatory effect on the over-expression of c-*myc* mRNA in mesangial cells. It will be of considerable interest to further study the molecular mechanisms of emodin on the inhibition of mesangial cell proliferation, especially in the light of the possible regulatory effect on the cell-cycle progress.

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大黄素对肾小球系膜细胞 c-*myc* 原癌基因表达的影响

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关键词　大黄素；培养的细胞；肾小球系膜；原癌基因蛋白 c-*myc*

R286.91　R7

目的：观察大黄素对肾小素系膜细胞（MC）c-*myc* mRNA 表达的影响，探讨大黄素抑制 MC 生长的分子机理. **方法**：网筛法分离大鼠肾小球，培养肾小球 MC. AGPC 一步法提取细胞总 RNA，c-*myc* mRNA 水平用斑点杂交法测定，以显影斑点最大的 RNA 稀释度表示 mRNA 水平. **结果**：生长相对静止状态的 MC 有低水平 c-*myc* mRNA 表达，而细菌脂多糖（LPS）显著增高 MC c-*myc* mRNA 表达. 于 2.5 h 达最高峰，并持续 6 h. LPS 诱导的 MC 高表达 c-*myc* mRNA 可被大黄素（25 mg · L^{-1}）所抑制. **结论**：大黄素对 MC c-*myc* mRNA 表达的抑制效应与其抑制 MC 的生长相关.

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Effects of egtazic acid and calcimycin on synthesis of DNA and collagen in cultured human lung fibroblasts

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KEY WORDS　egtazic acid；calcimycin；DNA；collagen；fibroblasts；cultured cells

AIM：To study the effects of egtazic acid（EA）and calcimycin（Cal）on the synthesis of DNA and collagen in cultured human lung fibroblasts（HLF）. **METHODS**：The synthesis of DNA and collagen was determined by measuring the incorporation of [^{3}H]TdR and [^{3}H]proline of HLF respectively. **RESULTS**：The collagen synthesis increased markedly 24 h after exposure to both EA（0.05－4 mmol · L^{-1}）and Cal（0.25－20 μmol · L^{-1}），and that there was no obvious change in DNA synthesis. After 36－48-h exposure，both DNA and collagen syntheses decreased in the groups of EA（1，2，and 4 mmol · L^{-1}）；the DNA synthesis was also suppressed in Cal groups in a concentration-dependent manner，whereas collagen synthesis decreased only in Cal（10 and 20 μmol · L^{-1}）. **CONCLUSION**：Extracellular Ca^{2+} influx into fibroblasts increased collagen production. However，the DNA synthesis was suppressed when the cytosolic Ca^{2+} was too high or too low.

Ca^{2+} plays an important role in modulating cell proliferation and functional activities[1,2]. Most studies focused on the effects of Ca^{2+} on nerve cells，myocardium cells，smooth muscle cells，and tumor cells[3－5]. Few articles dealt with the influences of Ca^{2+} on DNA and collagen synthesis of fibroblasts. Organ fibroses，such as pneumonocirrhosis and hepatocirrhosis，result from an overproliferation of fibroblasts and excessive collagen synthesis. To determine the effects of Ca^{2+} influx on the DNA and collagen syn-

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