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石河子大学

硕 士 学 位 论 文

异甘草素在高血压血管重构中的作用及机制研究

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专 业 名 称	病理学与病理生理学
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The Role and Mechanism of Isoliquiritigenin in the Prevention of Hypertension-Induced Vascular Remodeling

A Dissertation Submitted to

Shihezi University

In Partial Fulfillment of the Requirements

for the Degree of

master of Medicine

By

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May, 2026

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摘要

目的：高血压是慢性心血管疾病发生发展的独立危险因素，而血管重塑（Vascular Remodeling，VR）是高血压形成过程中的核心环节。在这一过程中，血管平滑肌细胞（Vascular Smooth Muscle Cells, VSMCs）由收缩表型转变为合成表型，并表现出过度增殖和迁移的能力，是驱动高血压 VR 的关键机制。异甘草素（Isoliquiritigenin, ISL）是一种提取自甘草根部的天然异黄酮类化合物，已有研究表明 ISL 具有降压的特性，但它在高血压 VR 中的作用及相关机制尚不明确。本研究拟探讨 ISL 对高血压 VR 的保护作用及其调控机制，进而为高血压 VR 的临床防治提供新的理论依据和潜在药物靶点。

方法：1. 体内动物实验：观察 ISL 对高血压 VR 的影响。选用 6-8 w 龄的雄性京都 Wistar 大鼠（Wistar-Kyoto rats, WKY）12 只和自发性高血压大鼠（Spontaneously Hypertensive rats, SHR）18 只，随机分为 5 组，分别为 WKY 组、WKY + ISL（40 mg/kg/d）组、SHR 组、SHR + ISL（40 mg/kg/d）组以及 SHR + 缬沙坦（Valsartan, Val，10 mg/kg/d）组。各组连续灌胃给药 10 w，期间记录体重，每隔 2 w 测量一次血压。给药结束后采集主动脉组织，采用 H&E 染色观察血管中膜厚度、管腔直径并计算二者比值；通过 Masson 染色评估纤维化程度；用免疫荧光双标法检测 α-SMA 和 OPN 的表达；免疫组化法检测 PCNA、MMP2 和 MMP9 的表达；并利用 Western blot 检测相关蛋白的表达水平。2. 体外细胞实验：探讨 ISL 对原代 VSMCs 异常增殖、迁移及表型转换的影响。通过酶消化法分离并鉴定 WKY 和 SHR 大鼠来源的原代 VSMCs；利用 CCK-8 法检测 ISL 对细胞活力和增殖的抑制作用，并据此确定给药剂量，将细胞分为 WKY 组、WKY + ISL-H（60 μM）组、SHR 组、SHR + ISL-L（40 μM）组、SHR + ISL-M（50 μM）组、SHR + ISL-H（60 μM）组；通过 EDU 染色评估细胞增殖情况；结合划痕实验和 Transwell 实验检测细胞迁移能力；采用免疫荧光染色检测 α-SMA 和 OPN 的表达；利用 Western blot 检测与增殖、迁移及表型转换相关蛋白的表达水平。3. 结合生物信息学与实验验证，探讨 ISL 改善高血压 VR 的潜在靶点和机制。通过网络药理学和分子对接预测关键靶点及调控通路，进一步利用分子动力学模拟和细胞热迁移实验（Cellular Thermal Shift Assay, CETSA）验证靶点与药物的结合情况，并在体内外实验中通过 Western blot 检测相关蛋白的表达，明确其调控作用。

结果：1. 体内动物实验结果显示，ISL 能够改善高血压引起的 VR。各组大鼠体重无明显差异，给药 10 w 后，ISL（40 mg/kg/d）能明显降低 SHR 大鼠的血压，降压效果与阳性药 Val 接近；H&E 染色结果显示，SHR 组出现明显的 VR，ISL 干预后可减少主动脉中膜厚度，改善 VSMCs 的排列情况；Masson 染色结果表明，ISL 和 Val 均能减轻血管壁的增厚和胶原沉积，同时改善 VSMCs 的排列紊乱；免疫荧光双标染色及成像结果显示，SHR 组中 OPN 表达升高、α-SMA 表达降低，ISL 和

Val 干预后可逆转这一表型转换，而 WKY + ISL 组与 WKY 组相比无明显变化；免疫组化结果显示，SHR 组中 PCNA、MMP2 和 MMP9 的表达均显著升高，ISL 和 Val 干预后这些指标明显下降，WKY + ISL 组与 WKY 组之间未见明显差异。2. 体外细胞实验显示：ISL 可抑制 SHR 大鼠的 VSMCs 的增殖、迁移和表型转换：CCK-8 结果显示，10-70 μM ISL 无明显细胞毒性，且可浓度依赖性抑制 SHR-VSMCs 的增殖；EDU、划痕及 Transwell 实验表明，ISL 能显著抑制 VSMCs 的增殖与迁移；免疫荧光及 Western blot 结果显示，ISL 可上调 α-SMA、下调 OPN、PCNA、MMP2、MMP9 蛋白的表达，促进 VSMCs 由合成表型向收缩表型逆转。3. 网络药理学分析显示：EGFR/SRC/STAT3 信号通路可能为 ISL 改善高血压 VR 的关键机制：共筛选靶点 583 个，分子对接、GO 及 KEGG 富集分析显示 EGFR、SRC、STAT3 为核心靶点；分子动力学模拟与 CETSA 证实 ISL 与 EGFR 具有高亲和力；体内外 Western blot 显示，SHR 组 p-EGFR、p-SRC、p-STAT3 表达升高，ISL 可降低其磷酸化蛋白水平而不影响总蛋白；加入 EGFR 抑制剂 Gef 与激动剂 EGF 发现，ISL 或 Gef 可逆转 SHR 组 VSMCs 的异常增殖、迁移及表型转换，EGF 则加重该现象，证实 ISL 可能通过调控 EGFR/SRC/STAT3 通路发挥相关作用。

结论：ISL 可能通过抑制 EGFR/SRC/STAT3 信号通路的异常激活，进而抑制高血压状态下 VSMCs 的增殖、迁移及表型转化，从而改善高血压 VR。

关键词：高血压；血管重塑；异甘草素；网络药理学；EGFR 信号通路

研究类型：A（基础研究）

Abstract

Objectives: Hypertension is an independent risk factor for the occurrence and development of chronic cardiovascular diseases, and vascular remodeling (VR) is a core link in the formation of hypertension. During this process, vascular smooth muscle cells (VSMCs) transform from a contractile phenotype to a synthetic phenotype and exhibit excessive proliferation and migration capabilities, which are the key mechanisms driving VR in hypertension. Isoliquiritigenin (ISL), a natural isoflavone compound extracted from the roots of licorice, has been shown to have antihypertensive properties. However, its role and related mechanisms in VR of hypertension remain unclear. This study aims to explore the protective effect of ISL on VR in hypertension and its regulatory mechanism, thereby providing new theoretical basis and potential drug targets for the clinical prevention and treatment of VR in hypertension.

Methods: 1. In vivo animal experiments: To observe the effect of ISL on vascular remodeling (VR) in hypertension. Twelve 6-8-week-old male Wistar-Kyoto rats (WKY) and eighteen spontaneously hypertensive rats (SHR) were randomly divided into five groups: the WKY group, the WKY + ISL (40 mg/kg/d) group, the SHR group, the SHR + ISL (40 mg/kg/d) group, and the SHR + valsartan (Val, 10 mg/kg/d) group. Each group was given intragastric administration for 10 weeks. During this period, body weight was recorded and blood pressure was measured every two weeks. After the administration, aortic tissues were collected. H&E staining was used to observe the thickness of the vascular media and the diameter of the lumen and calculate their ratio. Masson staining was used to assess the degree of fibrosis. The expression of α-SMA and OPN was detected by immunofluorescence double labeling. The expression of PCNA, MMP2 and MMP9 was detected by immunohistochemistry. The expression levels of related proteins were detected by Western blot. 2. In vitro cell experiments: To explore the effect of ISL on abnormal proliferation, migration and phenotypic transformation of primary vascular smooth muscle cells (VSMCs). Primary VSMCs from WKY and SHR rats were isolated and identified by enzymatic digestion. The inhibitory effect of ISL on cell viability and proliferation was detected by CCK-8 assay, and the dosage was determined accordingly. The cells were divided into the WKY group, the WKY + ISL-H (60 μM) group, the SHR group, the SHR + ISL-L (40 μM) group, the SHR + ISL-M (50 μM) group, and the SHR + ISL-H (60 μM) group. Cell proliferation was evaluated by EDU staining. Cell migration ability was detected by scratch assay and Transwell assay. The expression of α-SMA and OPN was detected by immunofluorescence staining. The expression levels of related proteins were detected by Western blot to clarify their regulatory effects. 3. Combining bioinformatics and experimental verification to explore the potential targets and mechanisms of ISL in improving hypertension VR. Key targets and regulatory pathways were predicted by network pharmacology and molecular docking. The binding of targets and drugs was further verified by

molecular dynamics simulation and cellular thermal shift assay (CETSA). The expression of related proteins was detected by Western blot in in vivo and in vitro experiments to clarify their regulatory effects.

Results: 1. The results of in vivo animal experiments showed that ISL could improve VR caused by hypertension. There was no significant difference in body weight among the groups of rats. After 10 weeks of administration, ISL (40 mg/kg/d) could significantly reduce the blood pressure of SHR rats, and the antihypertensive effect was close to that of the positive drug Val. H&E staining results showed that obvious VR occurred in the SHR group, and ISL intervention could reduce the thickness of the aortic media and improve the arrangement of VSMCs. Masson staining results indicated that both ISL and Val could alleviate the thickening of the vascular wall and collagen deposition, and simultaneously improve the disordered arrangement of VSMCs. Immunofluorescence double staining and imaging results showed that the expression of OPN was increased and the expression of α-SMA was decreased in the SHR group, and ISL and Val intervention could reverse this phenotypic transformation. There was no significant change in the WKY + ISL group compared with the WKY group. Immunohistochemical results showed that the expressions of PCNA, MMP2 and MMP9 were significantly increased in the SHR group, and these indicators were significantly decreased after ISL and Val intervention. There was no significant difference between the WKY + ISL group and the WKY group. 2. In vitro cell experiments showed that ISL could inhibit the proliferation, migration and phenotypic transformation of VSMCs from SHR rats: CCK-8 results indicated that 10-70 μM ISL had no obvious cytotoxicity and could inhibit the proliferation of SHR VSMCs in a concentration-dependent manner. EDU, scratch and Transwell experiments demonstrated that ISL could significantly inhibit the proliferation and migration of VSMCs. Immunofluorescence and Western blot results showed that ISL could up-regulate the expression of α-SMA, down-regulate the expressions of OPN, PCNA, MMP2 and MMP9 proteins, and promote the phenotypic transformation of VSMCs from the synthetic phenotype to the contractile phenotype. 3. Network pharmacology analysis indicated that the EGFR/SRC/STAT3 signaling pathway might be the key mechanism by which ISL improves VR in hypertension: A total of 583 targets were screened. Molecular docking, GO and KEGG enrichment analysis showed that EGFR, SRC and STAT3 were the core targets. Molecular dynamics simulation and CETSA confirmed that ISL had high affinity for EGFR. Western blot results in vivo and in vitro showed that the expressions of p-EGFR, p-SRC and p-STAT3 were increased in the SHR group, and ISL could reduce the phosphorylation levels of these proteins without affecting the total protein levels. The addition of EGFR inhibitor Gef and agonist EGF revealed that ISL or Gef could reverse the abnormal proliferation, migration and phenotypic transformation of VSMCs in the SHR group, while EGF aggravated this phenomenon, confirming that ISL might exert its effects by regulating the EGFR/SRC/STAT3 pathway.

Conclusions: ISL may inhibit the abnormal activation of the EGFR/SRC/STAT3 signaling pathway,

thereby suppressing the proliferation, migration and phenotypic transformation of VSMCs under hypertension conditions, and thereby improving hypertension-induced vascular remodeling.

Key words: Hypertension; Vascular Remodeling; Isoliquiritigenin; Network Pharmacology; EGFR signaling pathway

Research Type: A (Basic Research)

目录

英文缩略词

(LIST OF ABBREVIATIONS)

英文缩写	英文全名	中文译名
α-SMA	α-smooth muscle actin	α-平滑肌肌动蛋白
Acr	Acrylamide	丙烯酰胺
AP	Ammonium persulfate	过硫酸铵
BSA	Albumin from bovine serum	牛血清白蛋白
BP	Biological process	生物学过程
CCK-8	Cell counting kit-8	细胞计数试剂盒
CETSA	Cellular Thermal Shift Assay	细胞热迁移实验
ddH2O	Distillation-Distillation H2o	双蒸水
DBP	Diastolic Blood Pressure	舒张压
DMSO	Dimethyl sulfoxide	二甲基亚砜
DMEM	Dulbecco’smodified Eagle’s medium	基础培养基
EGFR	Epidermal Growth Factor Receptor	表皮生长因子受体
EGF	Epidermal Growth Factor	表皮生长因子
FBS	Fatal bovine serun	胎牛血清
Gef	Gefitinib	吉非替尼
GO	Gene ontology	基因本体
H&E	Hematoxylin and eosin	苏木素-伊红

英文缩写	英文全名	中文译名
IF	Immunofluorescence	免疫荧光
ISL	Isoliquiritigenin	异甘草素
KEGG	Kyoto encyclopedia of genes and genomes	京都基因和基因组百科全书
MF	Molecular function	分子功能
MMP2	Matrix Metallopeptidase 2	基质金属蛋白酶 2
MMP9	Matrix Metallopeptidase 9	基质金属蛋白酶 9
MBP	Mean Blood Pressure	平均动脉压
Nrf2	Nuclear factor erythroid 2-related factor 2	核因子-红细胞 2 相关因子 2
OPN	Osteopontin	骨桥蛋白
PBS	Phosphate buffered solution	磷酸盐缓冲液
PMSF	Phenylmethanesulfonyl fluoride	苯甲基磺酰氟
PVDF	Polyvinylidene fluoride	聚偏二氟乙烯膜
PPI	Protein-protein interaction	蛋白-蛋白相互作用
PCNA	Proliferating cell nuclear antigen	增殖细胞核抗原
p-EGFR	Phosphorylation epidermal growth factor receptor	磷酸化表皮生长因子受体
p-STAT3	Phosphorylation signal transducer and activator of transcription 3	磷酸化信号转导与转录激活因子 3
STAT3	Signal Transducer and Activator of Transcription 3	信号转导与转录激活因子 3
SDS	Sodium dodecyl sulfate	十二烷基硫酸钠
SBP	Systolic Blood Pressure	收缩压
SHR	Spontaneous hypertension rat	自发性高血压大鼠

英文缩写	英文全名	中文译名
Tris	Tris（hydroxymethyl）aminomethane	三羟甲基氨基甲烷
TEMED	Tetram Ethylethylenediamine	四甲基乙二胺
TBS	triethanolamine buffered saline solution	Tris-Hcl 缓冲盐溶液
VSMCs	Vascular smooth muscle cells	血管平滑肌细胞
Val	Valsartan	缬沙坦
WKY	Wistar-Kyoto rat	京都 Wistar 大鼠

第 1 章 前言

高血压（Hypertension）是临床最为常见的慢性心脑血管疾病，我国患病人数已达约3.3 亿[1]，患病率呈持续上升趋势，已成为亟待解决的重大公共卫生问题[2, 3]。该病危害严重，可诱发脑卒中、心肌梗死等严重并发症，显著增高患者致死率与致残率[4]，高血压相关灾难性卫生支出在贫困家庭中占比达 72.67%[5]，给社会带来了沉重经济负担[6]。长期高血压可损伤主动脉等弹性大动脉，诱导血管硬化，其核心病理改变为血管重构（Vascular Remodeling，VR），即血管壁在结构和功能层面发生的异常重塑[7, 8]。大动脉中膜主要由血管平滑肌细胞（Vascular Smooth Muscle Cells，VSMCs）构成[9]，生理状态下 VSMCs 呈收缩表型，参与维持血管张力及血流量稳态[10]。在高血压等病理刺激下，VSMCs 发生表型转化，增殖、迁移能力变强，并大量合成细胞外基质，进而导致血管中膜增厚、基质沉积，促进 VR 进展[11]。当前临床高血压防治以生活方式干预与五类一线降压药物为主[12]，虽可有效控制血压，但难以逆转已形成的 VR，且存在药物抵抗、患者依从性差等问题[13, 14]。VSMCs 的异常增殖、迁移及表型转化是介导血管壁增厚、管腔狭窄的关键环节，以 VSMCs 为干预靶点，探索新型治疗策略，对改善高血压相关血管病变具有重要的临床研究价值与转化意义[15]。

在探寻高血压新型治疗靶点的过程中，研究者发现表皮生长因子受体（Epidermal Growth Factor Receptor, EGFR）家族，因其在细胞功能调控中的核心地位备受关注，该家族也是目前研究最为透彻的配体依赖性受体酪氨酸激酶之一[16]，其核心成员为表皮生长因子（Epidermal Growth Factor, EGF）的特异性受体 EGFR，共包含 ErbB1-4（人类同源物为 HER1-4）四个亚型[17-19]，EGFR 家族成员均包含胞外结合域、跨膜域及胞内激酶域，可通过同源/异源二聚化激活下游信号通路，进而调控多种细胞类型的增殖、迁移、分化、侵袭及组织修复等关键生理病理过程[19-27]。与此同时，多篇综述文章表明，EGFR 信号通路能够激活 MAPK-ERK、PI3K-AKT、JAK-STAT、JNK 和 PKC-PLCγ1 等信号通路[28, 29]，这些通路是调控细胞增殖、迁移与表型转化的核心轴[30-33]，与高血压状态下 VSMCs 异常活化及 VR 的病理进程高度相关，为后续机制探讨提供重要依据。近年研究发现，该通路在高血压 VSMCs 表型转换及 VR 中扮演关键角色，Singh 等人用 15(S)-HETE 以时间依赖性方式刺激 VSMCs 中 EGFR 的酪氨酸磷酸化的研究发现，15(S)-HETE 可时间依赖性诱导 VSMCs 的 EGFR 酪氨酸磷酸化，阻断 EGFR 激活能抑制 SRC/STAT3 磷酸化、MCP-1 表达及 VSMCs 迁移，SRC 可与 EGFR、Jak2 形成复合物，其抑制可完全阻断 Jak2/STAT3 磷酸化等过程，15-Lox1-15(S)-HETE 轴通过氧化还原依赖的 EGFR