IL-6通过STAT3/Oct-1/ATM途径调节人脐静脉平滑肌细胞增殖

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摘要 该研究旨在探究白细胞介素6(interleukin-6, IL-6)对人脐静脉平滑肌细胞(human umbilical vascular smooth muscle cells, HUVSMCs)增殖的影响及具体的调节机制。通过细胞活性检测试剂盒(cell count kit 8, CCK-8)和EdU成像试剂盒检测HUVSMCs增殖的变化, 蛋白质免疫印迹(Western blot)分析细胞的蛋白表达变化, 特异性小RNA抑制八聚体转录因子-1(octamer transcription factor-1, Oct-1)的表达, 免疫荧光技术检测Oct-1在细胞中的位置变化。结果显示, IL-6可以显著促进HUVSMCs的增殖, 激活信号转导子和转录激活子3(signal transducer and activator of transcription 3, STAT3)的Ser727位点磷酸化, 促进Oct-1和共济失调毛细血管扩张突变蛋白(ataxia telangiectasia mutated, ATM)的表达; 此外, IL-6刺激细胞0 h和6 h时, Oct-1主要集中在细胞核中, 12 h和24 h时, Oct-1部分转移至细胞质中。同时, 抑制Oct-1可以有效降低细胞增殖, 减少IL-6和ATM的表达, 加入IL-6可以缓解由Oct-1抑制的HUVSMCs增殖。以上结果表明, IL-6可以通过STAT3/Oct-1/ATM途径影响HUVSMCs的增殖。这一发现为心血管疾病的基础治疗带来新思路。

关键词 IL-6; 人脐静脉平滑肌细胞; 增殖; STAT3; Oct-1; ATM

Interleukin-6 Regulates Proliferation of Human Umbilical Vascular Smooth Muscle cells via STAT3/Oct-1/ATM Pathway

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Abstract This study aimed to explore the effect of IL-6 on the proliferation of HUVSMCs (human umbilical vascular smooth muscle cells) and classify one of its molecular pathway. Cell proliferation was examined with CCK-8 (cell count kit-8) and EdU (5-ethynyl-2-deoxyuracil riboside) imaging assay. The protein expression level was tested with Western blot. Oct-1 (octamer transcription factor-1) expression was inhibited using small interfering RNA, and the position of Oct-1 in cells was detected with immunofluorescence microscopy. It was found that, firstly, IL-6 promoted HUVSMCs proliferation and viability apparently. Secondly, IL-6 activated phosphorylation of STAT3 (signal transducer and activator of transcription 3) at Ser727, and enhanced the expression of Oct-1 and ATM (ataxia telangiectasia mutated). Thirdly, Oct-1 was mainly concentrated in the nucleus when IL-6 stimulated cells at 0 h and 6 h, and part of Oct-1 transferred to the cytoplasm at 12 h and 24 h. Finally, inhibition of Oct-1 re-

收稿日期: 2020-04-08 接受日期: 2020-06-08

国家自然科学基金(批准号: 11372190)资助的课题

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Received: April 8, 2020 Accepted: June 8, 2020

This work was supported by the National Natural Science Foundation of China (Grant No.11372190)

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URL: http://www.cjcb.org/arts.asp?id=5303

duced cell proliferation and decreased the expression of IL-6 and ATM. Adding IL-6 reversed the proliferation of HUVSMCs inhibited by Oct-1. In conclusion, the results classify that IL-6 induces the proliferation of HUVSMCs through the STAT3/Oct-1/ATM pathway. This study provides a new clue for the mechanical treatment of cardiovascular disease.

Keywords interleukin-6; HUVSMC; proliferation; STAT3; Oct-1; ATM

心血管疾病 (cardiovascular disease, CVD)是一种严重危害人类健康的疾病 , 其发病率高 , 同时死亡人数高达艾滋病、疟疾和结核病患病人数总和的两倍 [1]。因此 , 研究 CVD 的发病机理对 CVD 的早期检测、治疗及术后复发检测等均具有重要意义。研究发现 , 在 CVD 病变早期常伴有炎症发生 , 如在动脉粥样硬化中 , 载脂蛋白 B 在血管内皮细胞层下面积累 , 诱导巨噬细胞和树突状细胞等单核细胞募集在斑块处 , 进而引发炎症反应 , 并促进动脉粥样硬化的发展 [2-3]。

白细胞介素-6(interleukin-6, IL-6)也被称为B细胞刺激因子2, 是一种具有多种生物学效应的细胞因子, 最初被认为是T细胞来源的淋巴因子, 其主要作用是诱导活化的B淋巴细胞分化为效应B细胞[4-6]。随后研究发现, 多种细胞均可产生IL-6, 包括心脏黏液瘤细胞[7]、单核细胞[8]、血管内皮细胞[9]等。很多研究报道, IL-6参与多种CVD的发生, 如在高血脂动脉粥样硬化中, 通过原位杂交技术发现兔动脉粥样硬化病变部位的IL-6表达显著上升[10]。对临床肺动脉患者血清检测发现, 肺动脉高压患者血清中IL-6含量较对照组显著提升[11]。此外, IL-6过表达会促进小鼠低氧性肺动脉高压的发展, 与野生型小鼠相比, IL-6过表达会促进小鼠肺动脉血压升高和肺部血管病变[12]。IL-6在心力衰竭患者中表达也发生了变化, 通过检测急性代偿性心力衰竭患者, 包括左心室收缩功能不全与左心室射血功能保留之间的细胞因子表达水平发现, 入院时, 左心室收缩功能不全患者血浆中的IL-6高于左心室射血功能正常的患者, 而治疗后再次检测发现, IL-6在左心室收缩功能不全患者中表达显著降低[13]。虽然研究发现了IL-6在多种心血管疾病中表达升高, 但其具体参与调节疾病的调节机制尚不清楚。据报道, IL-6通过与其可溶性受体sIL-6R及膜受体gp130结合形成复合物IL-6/sIL-6R/gp130激活JAK2/STAT3信号通路来促进肿瘤周围内皮细胞的增殖[14]。由于平滑肌细胞(smooth muscle cells, SMCs)的异常增生通常与心血管疾病的发展相关[15-16], 因此, 进一步探究IL-6在心血管疾病调节过程中对SMCs增殖的影响和分子机制具有重要意义。

八聚体转录因子-1(octamer transcription factor-1, Oct-1)是POU(Pit-Oct-Unc)家族成员, 它可以与目标基因的启动子区特异性结合并影响基因的转录[17]。Oct-1可以调节多种基因表达, 例如免疫球蛋白基因和白介素13[18-19]。作为顺式因子, 编码Oct-1蛋白的*POU2F1*基因在成人组织中广泛表达, 并参与机体发育、细胞增殖和迁移。Oct-1在各种组织器官中表达存在差异性, 其通常在肝脏中高表达, 但在其他组织(如脑、心脏、肾脏、肺、骨骼肌等)和免疫细胞中低表达[20-22]。我们实验室先前的研究表明, Oct-1在SMCs中表达水平较低, 但是外源神经肽类物质促肾上腺皮质激素上调了Oct-1的表达, 从而促进SMCs增殖[23]。许多研究表明, Oct-1在癌症的发展中起着至关重要的作用。如Oct-1在结肠癌组织中呈现高表达, 而抑制Oct-1表达会显著降低结肠癌细胞系的增殖速度[24]。此外, 相似的研究表明, Oct-1在食道癌[25]、胃癌[26-27]、宫颈癌[28]和前列腺癌[29-30]中均出现过表达的现象。这些研究提示, Oct-1在细胞增殖中发挥重要作用。

在本研究中, 我们利用IPA(ingenuity pathway analysis)预测了可能与SMCs增殖及IL-6相关的转录因子, 结果发现, Oct-1与SMCs增殖相关。所以我们假设, IL-6和Oct-1在人脐静脉平滑细胞(human umbilical vascular smooth muscle cells, HUVSMCs)增殖中起重要作用, 研究Oct-1是否参与IL-6介导的HUVSMCs增殖, 以及它参与IL-6诱导的HUVSMCs增殖的作用机制。

1 材料与方法

1.1 材料

HUVSMCs购自美国Sciencell公司。

1.2 试剂及仪器

平滑肌细胞培养基、胎牛血清、平滑肌细胞生长补充剂、青霉素/链霉素购自美国Sciencell公

司; 重组IL-6购自PeproTech公司; CCK-8细胞活性检测试剂盒购自上海圣尔生物科技有限公司; YF®488 Click-iT EdU成像试剂盒购自苏州宇恒生物科技有限公司; Stat3兔单克隆抗体、phospho-Stat3(Ser727)兔单克隆抗体、phospho-Stat3(Tyr705)兔单克隆抗体购自Cell Signaling Technology公司; anti-Oct-1兔单克隆抗体和anti-ATM兔单克隆抗体购自Abcam公司; Anti-IL6兔多克隆抗体购自博士德生物工程有限公司; GAPDH鼠单克隆抗体购自Proteintech公司; Anti-β-tubulin鼠单克隆抗体购自Sigma-Aldrich公司; 碱性磷酸酶标记的山羊抗鼠和山羊抗兔二抗购自康为世纪生物科技有限公司; Alexa Flour 488标记羊抗兔二抗(绿色)购自Cell Signaling Technology公司; RNA干扰片段购自上海吉玛制药技术有限公司。

仪器包括: 6孔、24孔和96孔细胞培养板(美国Costar); 倒置荧光显微镜(Leica DMI3000); 共聚焦扫描显微镜(Leica TCS SP8)。

1.3 细胞培养

HUVSMCs用含有2% FBS、1% SMCGS和P/S的SMCM培养液, 置于37 °C、95%空气和5% CO_2湿润的培养箱中培养。细胞在3~6代之间进行实验。

1.4 IL-6刺激细胞

将HUVSMCs接种在96孔板、24孔板、6孔板中, 24 h后细胞贴壁。使用无FBS的培养基同步化细胞24 h, 然后利用重组人IL-6刺激细胞。设置了3个浓度梯度, 分别为5 ng/mL、10 ng/mL、20 ng/mL, 和3个时间梯度24 h、48 h、72 h。

1.5 细胞增殖检测

1.5.1 CCK-8细胞活性检测 将2-(2-甲氧基-4-硝基苯基)-3-(4-硝基苯基)-5-(2, 4-二磺酸将苯)-2H-四唑单钠盐添加到每个孔中, 然后将细胞培养板置于37 °C培养箱中孵育90 min。其余操作步骤均根据使用说明进行。最后利用酶标仪检测450 nm处的吸光度(*D*)值。

1.5.2 EdU成像试剂盒检测 将EdU添加到细胞中孵育2 h[31], 结束后利用4%的多聚甲醛固定细胞, 0.5%的Triton-X 100用于透膜, 再将Click-iT反应混合物加入细胞中, 室温避光孵育。最后, 用Hoechst33342复染细胞核并在共聚焦显微镜下观察。

以上两种方法用于检测IL-6、Oct-1对HUVSMCs活力及增殖的影响。

1.6 蛋白质印迹(Western blot)分析

将用于Western blot实验的细胞用冰PBS洗涤2次, 然后在冰上用上样缓冲液裂解10 min, 离心, 取上清液用于Western blot分析。一抗稀释后在4 °C下孵育过夜, 一抗的稀释比例分别为: STAT3(1:1 000)、Tyr705(1:1 000)、Ser727(1:1 000)、OCT-1(1:1 000)、IL-6(1:1 000)、ATM(1:2 000)、GAPDH(1:8 000)和β-tubulin(1:400)。将碱性磷酸酶标记的二抗按1:1 000稀释后在室温下孵育1.5 h。实验中以GAPDH和β-tubulin作为对照。

1.7 RNA干扰实验

细胞种在6孔板、24孔板和96孔板中, 24 h内细胞贴壁且密度在60%~80%时进行转染实验, 对照干扰片段和特异性干扰片段与lipo2000混合后孵育细胞。Oct-1的特异性干扰片段序列为5′-CCU UGA ACC UCA GCU UUA ATT-3′[23]。

1.8 数据统计

所有实验都进行了3次以上的独立实验, 实验数据用均值±标准差($\bar{x}\pm s$)表示。利用单因素方差分析和*t*检验方法检测数据之间的差异。$P<0.05$表示数据之间存在显著性差异, $P<0.01$表示数据之间存在非常显著性差异, $P<0.001$表示数据之间存在极显著性差异。

2 结果

2.1 IL-6促进HUVSMCs活性及增殖

为了探究IL-6对HUVSMCs活力及增殖的影响, 首先将IL-6浓度设为3个梯度为5 ng/mL、10 ng/mL和20 ng/mL, 并设置3个刺激时间梯度分别为24 h、48 h和72 h, 然后利用CCK-8检测细胞活性。结果显示, IL-6可以显著提高HUVSMCs的活性(图1A), 通过R语言分析IL-6的浓度和刺激时间与细胞活性的关系, 结果提示, IL-6浓度与细胞活性呈正相关, 而刺激时间与细胞活性呈现微弱负相关关系(图1B), 因此, 我们在后续的实验选择了IL-6的浓度为20 ng/mL刺激细胞。为了进一步证明IL-6促进HUVSMCs增殖, 我们在IL-6刺激细胞48 h后, 通过EdU成像试剂盒分析了细胞增殖情况。结果与CCK-8检测结果相似, 与对照组相比, IL-6刺激组的HUVSMCs增殖增加至2.2倍(图1D)。以上结果表明, IL-6可以显著促进HUVSMCs活力及增殖。

2.2 IPA预测与IL-6及平滑肌细胞增殖相关分子

为了探究IL-6促进HUVSMCs增殖的机制, 利用IPA分析了与IL-6和SMCs增殖相关的分子。结果(图2)显示, 在癌症等细胞增殖相关的常见转录因子信号转导子和转录激活子3(signal transducer and activator of transcription 3, STAT3)也参与其中, 且图中显示转录因子Oct-1(POU2F1)可能间接调节IL-6进而参与到SMCs细胞的增殖中, 所以后续的分子机制探究我们主要关注了Oct-1和STAT3。

2.3 IL-6影响STAT3磷酸化

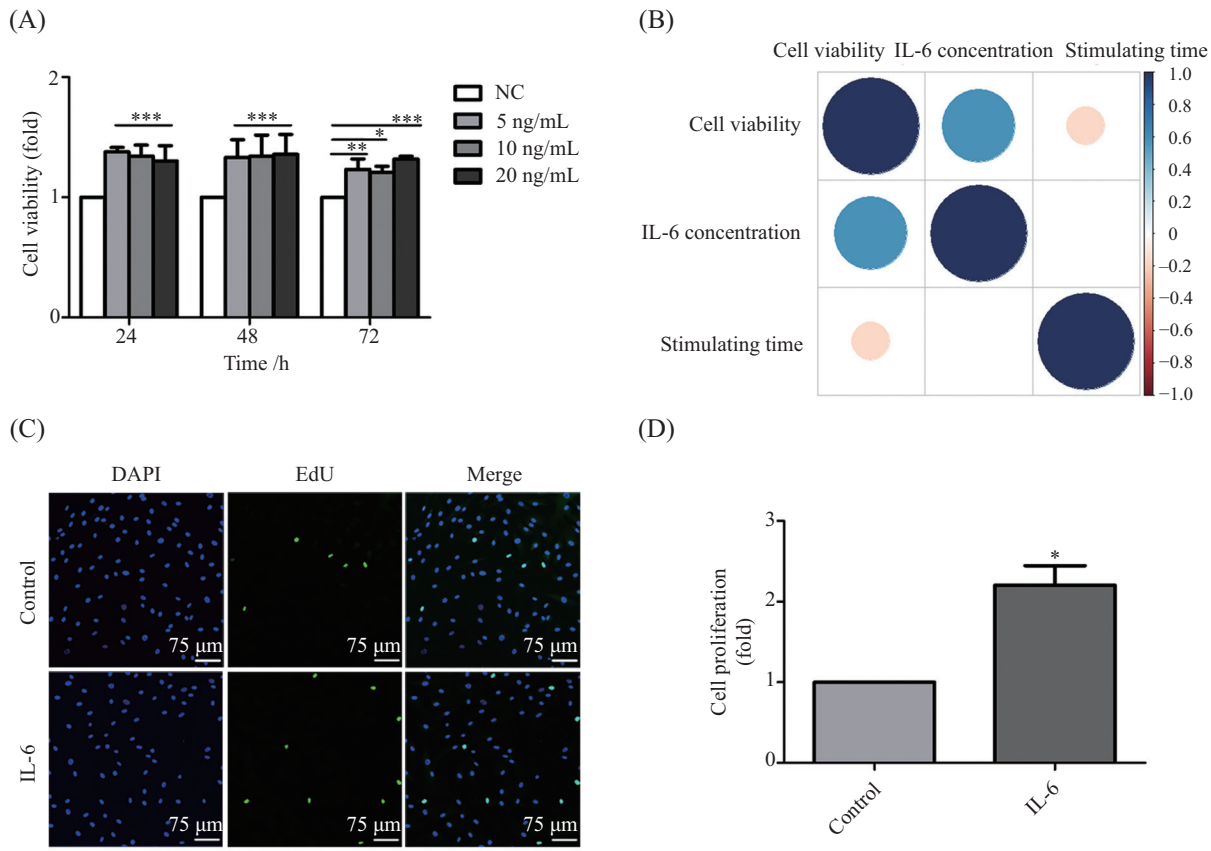
为了探究IL-6对STAT3的影响, 首先利用IL-6刺激细胞15 min、30 min和60 min, 然后用Western blot检测磷酸化位点Ser727。我们发现, p-Ser727在15 min时较0 min显著增加, 在30 min时达到峰值增加至约2.2倍, 随后在60 min时出现下降, 但是Tyr705位点的磷酸化在60 min时被显著地抑制, 分析总STAT3表达没有明显变化(图3)。结果说明, IL-6对STAT3的2个磷酸化位点的影响不同, 且不是通过总STAT3表达影响的。

2.4 IL-6刺激HUVSMCs对Oct-1的影响

接着为了探究IL-6对Oct-1表达和在细胞中分布的影响, 利用IL-6分别刺激细胞6 h、12 h和24 h, 再通过Western blot和免疫荧光技术检测Oct-1的变化。结果显示, IL-6刺激细胞6 h后Oct-1的表达显著增加, 在24 h时Oct-1最显著较0 h增加至约7.9倍(图4A); 免疫荧光结果发现, Oct-1在IL-6刺激细胞0和6 h时集中在细胞核中, 但在12 h和24 h时部分转移到细胞质中(图4B), 这表明IL-6可以增加Oct-1的表达, 调节Oct-1在细胞中的位置。

2.5 抑制Oct-1降低IL-6表达

由于IPA分析结果显示Oct-1可能会调节IL-6,

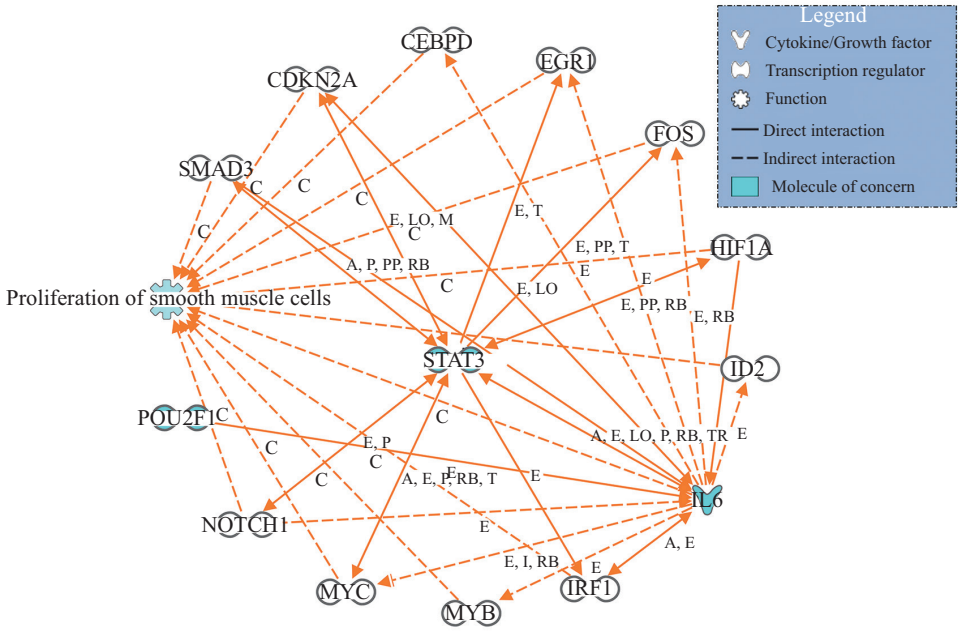


A: CCK-8检测IL-6刺激HUVSMCs的活性变化, 浓度为5 ng/mL、10 ng/mL和20 ng/mL, 时间为24 h、48 h和72 h。n=4; B: 热图显示了IL-6的浓度和刺激时间与细胞活性的相关性。n=4; C: EdU检测IL-6浓度为20 ng/mL, 刺激时间为48 h的细胞增殖荧光图。n=3; D: EdU图像的统计结果。n=3; EdU: 绿色, DAPI: 蓝色; *P<0.05, **P<0.01, ***P<0.001。

A: IL-6 treated cell for 24 h, 48 h, 72 h at 5 ng/mL, 10 ng/mL, 20 ng/mL, respectively, and the cells viability was tested by CCK-8 thereafter. n=4; B: heat map showing correlation of treatment time and IL-6 concentration with cell viability. n=4; C: IL-6 stimulated HUVSMCs for 48 h; the cells were then incubated with EdU and analyzed by confocal microscopy. n=3; D: Statistical result of EdU image. n=3; EdU: green, DAPI: blue; *P<0.05, **P<0.01, ***P<0.001.

图1 CCK-8和EdU试剂盒检测IL-6对HUVSMCs活性及增殖的影响

Fig.1 Effects of IL-6 on the viability and proliferation of HUVSMCs by CCK-8 and EdU kits

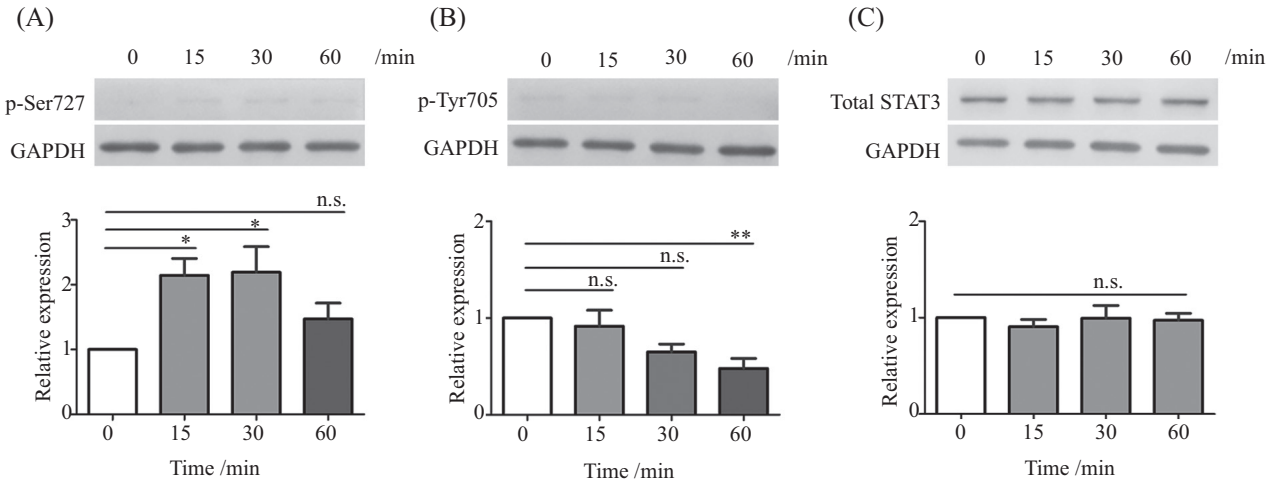


A: 激活; C: 引起/导致; E: 表达; I: 抑制; P: 磷酸化/去磷酸化; RB: 结合调节; T: 转录; TR: 转位; PP: 蛋白结合; LO: 定位; M: 生化修饰。

A: activation; C: causes/leads; E: expression, I: inhibition; P: phosphorylation/de phosphorylation; RB: regulation of binding; T: transcription; TR: translocation; PP: protein-protein binding; LO: localization; M: biochemical modification.

图2 IPA预测与IL-6及平滑肌细胞增殖的相关分子

Fig.2 The moleculars screened by IPA about IL-6 and SMCs proliferation



A、B: Western blot检测IL-6刺激HUVSMCs 15 min、30 min和60 min后Ser727和Tyr705的磷酸化情况; C: Western blot检测IL-6刺激HUVSMCs 15 min、30 min和60 min后总STAT3的表达情况; n= 5, *P<0.05, n.s.: 无显著性差异。

A,B: Western blot was used to detect the phosphorylation of Ser727 and Tyr705 after IL-6 stimulated HUVSMCs at 15 min, 30 min and 60 min; C: Western blot was used to detect the expression of total STAT3 after 15 min, 30 min and 60 min by IL-6 stimulation; n= 5, *P<0.05, n.s.: no significant difference.

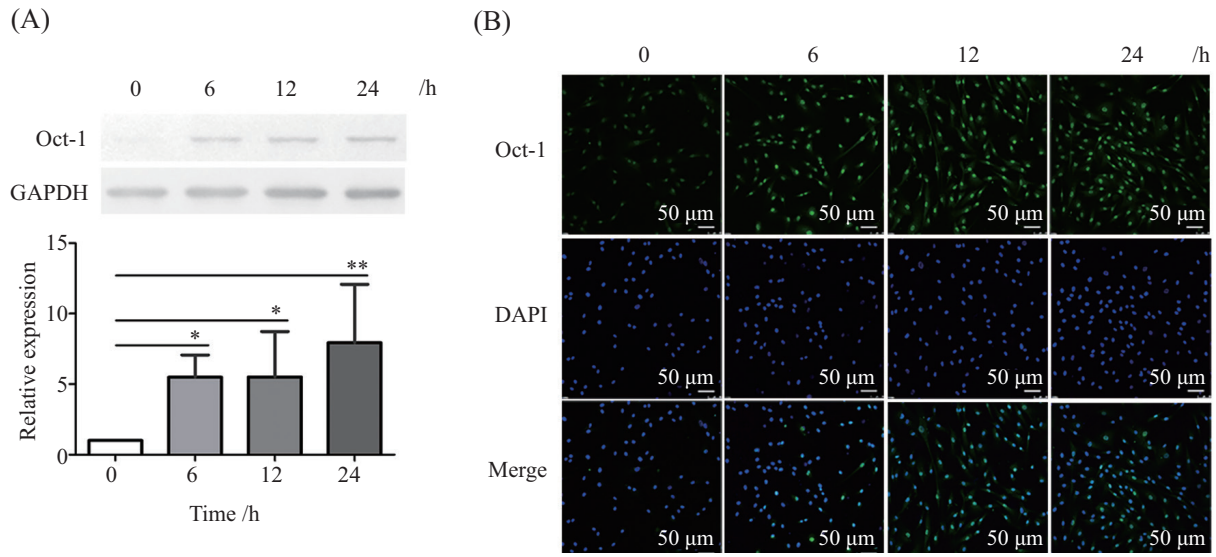
图3 Western blot检测IL-6刺激HUVSMCs对STAT3磷酸化的影响

Fig.3 Western blot detected the effects of IL-6 on phosphorylation of STAT3

所以接下来我们通过小RNA干扰Oct-1表达后检测IL-6的表达, 结果显示, 抑制Oct-1可以有效地降低IL-6的表达, 干扰24 h时IL-6下降最明显, 与对照组相比干扰组的IL-6降低了约50%(图5)。这一结果揭示了IL-6和Oct-1存在相互调节的关系。

2.6 IL-6和Oct-1对ATM表达的影响

为了探究IL-6诱导HUVSMCs增殖的分子机制, 结合我们之前课题组的研究中利用IPA分析Oct-1的下游分子, 筛选出与细胞增殖相关的分子ATM[23]。为了检测IL-6和Oct-1对ATM表达的影响, 我们分别利

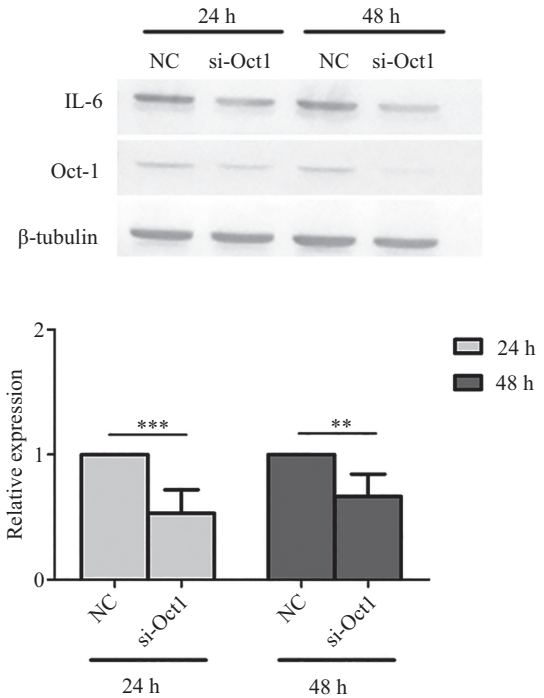


A: Western blot检测IL-6刺激HUVSMCs 6 h、12 h和24 h后Oct-1的表达。 n= 5; B: 免疫荧光技术检测IL-6刺激HUVSMCs 6 h、12 h和24 h后Oct-1的位置; Oct-1: 绿色; DAPI: 蓝色; *P<0.05, **P<0.01。

A: Western blot was used to detect the expression of Oct-1 after IL-6 stimulated HUVSMCs at 6 h, 12 h and 24 h. n=5; B: immunofluorescence technique detected the position of Oct-1 after IL-6 stimulated HUVSMCs for 6 h, 12 h and 24 h; Oct-1: green; DAPI: blue; *P<0.05, **P<0.01.

图4 Western blot检测IL-6对Oct-1表达的影响, 免疫荧光检测IL-6对Oct-1位置的影响

Fig.4 Western blot detected the effect of IL-6 on Oct-1 expression, and immunofluorescence detected the effect of IL-6 on Oct-1 position



Western blot检测干扰Oct-1表达24 h和48 h后IL-6的表达; n= 5, **P<0.01, ***P<0.001.

Western blot was used to detect the expression of IL-6 after inhibiting Oct-1 for 24 h and 48 h. n= 5, **P<0.01, ***P<0.001.

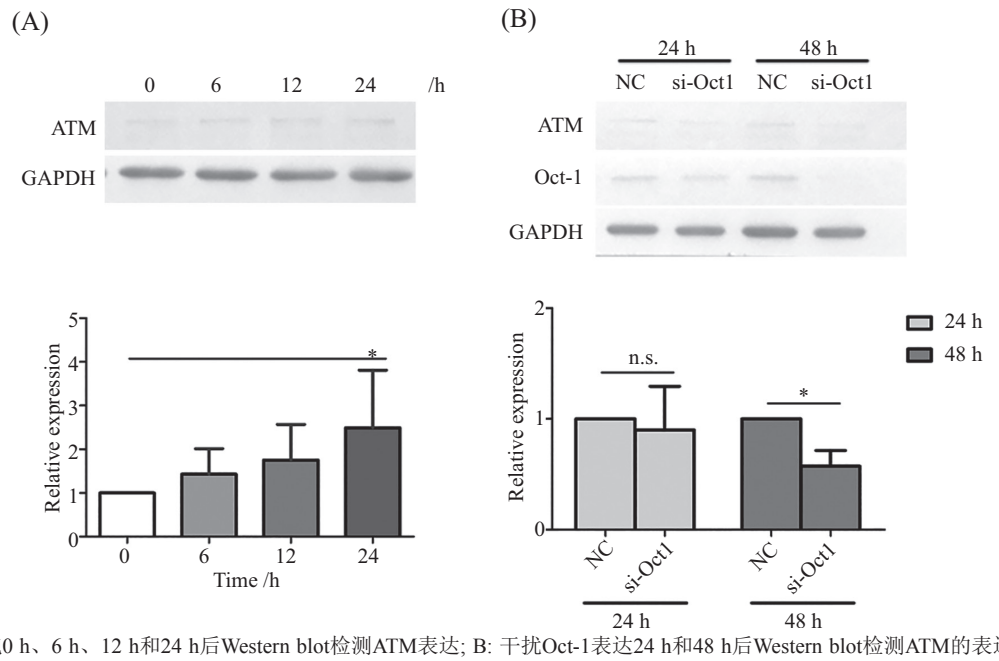
图5 Western blot检测干扰Oct-1对IL-6表达的影响

Fig.5 Effect of interfering Oct-1 on IL-6 expression by Western blot

用IL-6刺激细胞和小RNA干扰Oct-1表达后, Western blot检测ATM的表达。结果显示, IL-6刺激细胞24 h后ATM表达较0 h增加至约2.5倍(图6A); 同时干扰Oct-1表达48 h后可以显著降低ATM的表达(图6B)。

2.7 IL-6诱导HUVSMCs增殖与Oct-1相关

为了确定IL-6通过Oct-1影响HUVSMCs的增殖,



A: IL-6刺激细胞0 h、6 h、12 h和24 h后Western blot检测ATM表达; B: 干扰Oct-1表达24 h和48 h后Western blot检测ATM的表达; n= 5, n.s.代表无显著性差异, *P<0.05。

A: the expression of ATM was detected by western blot after IL-6 stimulated cells at 0 h, 6 h, 12 h and 24 h; B: the expression of ATM was detected by western blot after inhibiting Oct-1 expression at 24 h and 48 h. n= 5, n.s. represents that there is no significant difference, *P<0.05.

图6 IL-6和Oct-1对ATM表达的影响

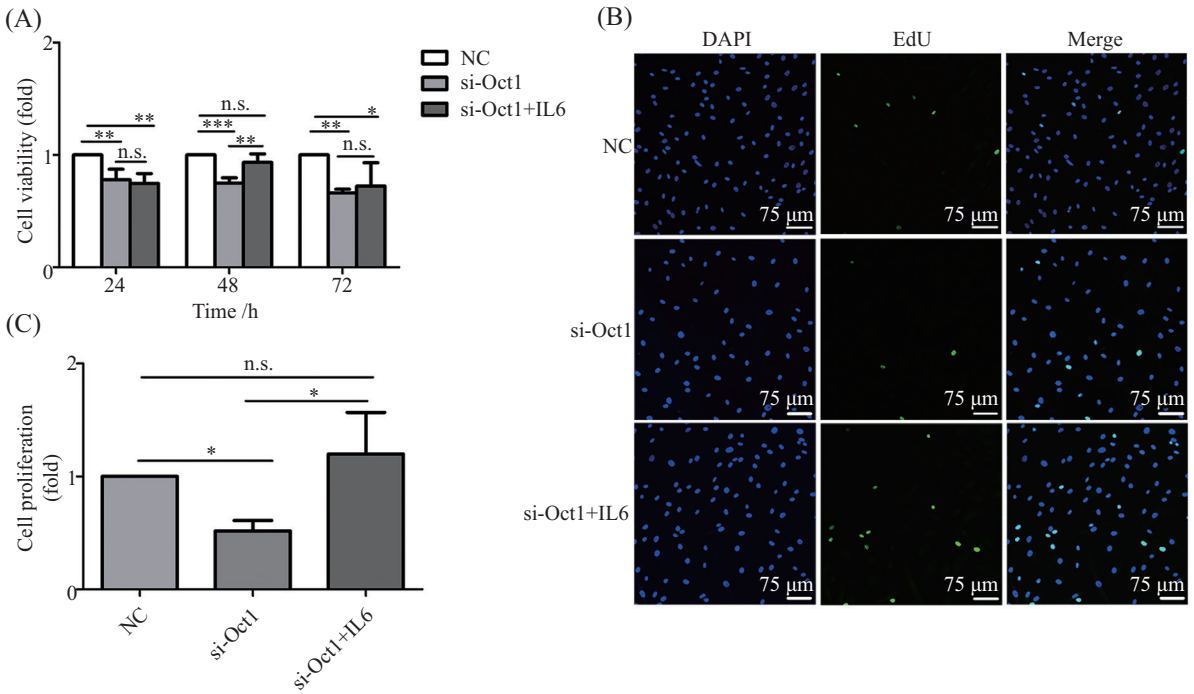
Fig.6 Effects of IL-6 and Oct-1 on ATM expression

我们在抑制Oct-1表达后检测了HUVSMCs的活性和增殖情况, 结果发现, 抑制Oct-1可以降低HUVSMCs的增殖(图7A)。随后, 向干扰Oct-1表达的细胞中加入IL-6, 再检测细胞增殖情况, 结果显示, 加入IL-6 48 h后减轻了由Oct-1表达降低而抑制的细胞增殖作用。EdU成像试剂盒分析显示了相似的结果, 与对照组相比, 干扰Oct-1组细胞增殖降低了50%, 加入IL-6 48 h时细胞增殖得到部分恢复(图7B和图7C), 这表明IL-6通过Oct-1调节HUVSMCs的增殖。

3 讨论

血管壁由内皮细胞、平滑肌细胞和成纤维细胞组成, 血管重塑是血管细胞功能发生变化引发的血管结构改变的过程, 包括细胞增殖、细胞迁移和细胞凋亡等[32]。成熟的SMCs具有较强的收缩能力, 其增殖速率极低[33]。因此, SMCs正常增殖和收缩对于维持血管稳态至关重要。研究发现, 心血管疾病患者的血浆中IL-6显著升高, 例如原发性肺动脉高压患者[2,34]、颈动脉粥样硬化患者[35]。IL-6不但可以通过血液循环运输实现对细胞远距离的调控, 也可以通过自分泌或旁分泌实现对临近细胞的调节作用。在高脂血症兔的主动脉中, 原位杂交分析发现在纤维斑中存在*IL-6*的mRNA, 体外研究发现, 胎牛血清可诱导SMCs的增殖并合成IL-6, 这一结果提示, IL-6是高脂血症兔模型中SMCs增殖和动脉粥样硬化发病机理的重要自分泌或旁分泌调节剂[36]。此外, 研究发现, 血管紧张素II可以促进人微血管内皮细胞的*IL-6*基因表达[37]。血管内皮细胞分泌的细胞因子可以影响临近的SMCs功能, 因此这些研究提示, IL-6可能在某些病理条件下对SMCs的功能起着非常关键的作用。尽管研究发现IL-6与CVD切相关, 但关于IL-6对SMCs增殖作用的研究很少[38]。

为了探索IL-6促进细胞增殖的机制, 我们通过IPA方法预测了在SMCs增殖中与IL-6相关的转录因子, 预测结果显示IL-6可以调节STAT3(图2)。STAT3具有2个磷酸化位点, 可以被磷酸化激活, 从而诱导STAT3二聚化并调节基因的转录[39]。许多研究表明, STAT3的磷酸化与细胞增殖呈正相关。例如, 神经调节蛋白1诱导STAT3和JAK磷酸化, 从而促进肺上皮细胞增殖, 用特定的JAK-STAT抑制剂AG490阻断JAK-STAT途径可抑制由神经调节蛋白1诱导的JAK与STAT3磷酸化和细胞增殖[40]。Arctigenin可以通过抑制STAT3磷酸化诱导人卵巢癌细胞OVCAR3和SKOV3的凋亡[41]。然而, 研究发现在不同细胞中,



A: 干扰Oct-1 24 h后向细胞中加入IL-6, CCK-8检测加入IL-6 24 h、48 h和72 h后细胞活性, n= 4; B: 干扰Oct-1 24 h后向细胞中加入IL-6, 48 h后利用EdU试剂盒并通过共聚焦显微镜进行分析细胞增殖; C: 从图B定量细胞增殖百分比, n=3。EdU: 绿色, DAPI: 蓝色。n.s.代表无显著性差异, *P<0.05, **P<0.01, ***P<0.001。

A: Oct-1 was inhibited by small RNA, next, IL-6 was added to cells after inhibited Oct-1 for 24 h. The cells proliferation was tested by CCK-8 at 24 h, 48 h and 72 h, n= 4. B: Oct-1 was inhibited by small RNA, next, IL-6 was added to cells for 48 h; the cells were then incubated with EdU and analyzed by confocal microscopy. C: quantification of cell proliferation percentage from figure B, n=3. EdU: green; DAPI: blue. n.s. represents that there is no significant difference, *P<0.05, **P<0.01, ***P<0.001.

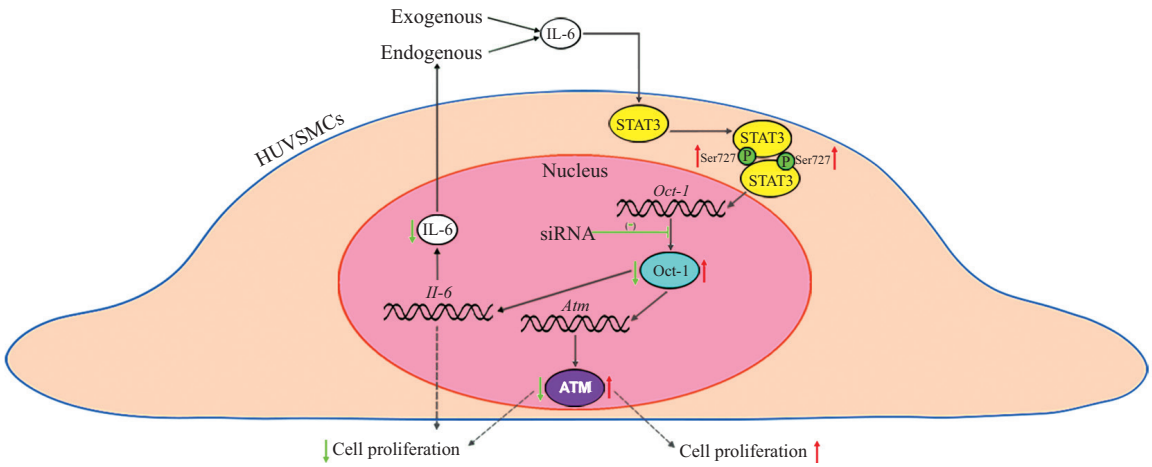
图7 IL-6缓解了Oct-1表达下降对细胞增殖的抑制作用

Fig.7 IL-6 alleviated inhibition of cell proliferation by Oct-1

STAT3的2个磷酸化位点变化可能是不同的。有研究报道, 隐丹参酮可以显著抑制神经胶质瘤细胞系(T98G和U87)的增殖, 研究发现, 隐丹参酮抑制T98G细胞中STAT3 Tyr705位点的磷酸化, 但对Ser727位点的磷酸化无显著影响, 同时发现在U87细胞中, 隐丹参酮不仅可以抑制STAT3 Tyr705的磷酸化, 而且还轻微抑制了Ser727的磷酸化[42]。还有研究表明, 这2个磷酸化位点的变化存在相反的关系。如在非洲绿猴肾细胞系(cercopithecus aethiops kidney fibroblast, COS)的研究中, Ser727和Tyr705 2个磷酸化位点可以独立地被激活, 并且发现IL-6可以激活Ser727位点的磷酸化, 并可以促进激活后的STAT3与其他蛋白相互结合, 再执行其功能; 同时Ser727的磷酸化增加会抑制Tyr705的磷酸化[43]。这表明在相同细胞或不同细胞中, STAT3的Ser727和Tyr705磷酸化变化不同。这与我们研究中STAT3的Ser727和Tyr705 2个磷酸化位点在细胞受到IL-6刺激后的变化是一致的, 即IL-6刺激HUVSMCs首先激活了STAT3 Ser727磷酸化水平, 继而Tyr705的磷酸化水平受到了抑制。

Oct-1在细胞增殖、迁移和凋亡中起到重要的调节作用, IPA的预测结果显示, Oct-1可能通过间接调节IL-6参与SMCs的增殖(图2)。研究表明, 抑制Oct-1表达可显著降低直肠癌细胞系SW620和LoVo的增殖, 并抑制细胞迁移和侵袭[24]。在另一项研究中发现, 抑制Oct-1可以显著降低人直肠癌细胞HCT116和RKO在体内外的增殖[44]。还有研究表明, 正常人食管上皮细胞(human esophageal epithelium cells, HET-1A)中的STAT3过度活化会提高Oct-1的表达, 并促进细胞增殖并降低细胞凋亡[25]。我们的研究发现, IL-6可以激活细胞STAT3的Ser727磷酸化, 并增加了Oct-1的表达, 结合之前的研究结果, 我们推测Oct-1可能是STAT3的下游分子。

Oct-1是核转录因子, 位于细胞核并调节各种基因的表达[25,29]。研究发现, Oct-1在调节基因表达时会与其他蛋白结合为复合物执行其调节功能, 如Oct-1调节人乳腺癌细胞中*CCND1*基因表达时, 与c-



P: 磷酸化; 绿色↓: 分子表达或细胞增殖被抑制; 红色↑: 分子表达或细胞增殖被促进; 实线箭头: 已被实验证实的作用; 虚线箭头: 间接实验证实的作用; 绿色(-), ┤: siRNA抑制Oct-1表达。

P: phosphorylation; green ↓: molecular expression or cell proliferation was inhibited; red ↑: molecular expression or cell proliferation was promoted; solid arrow: the effect had been confirmed by experiment; dotted arrow: the effect had been confirmed by indirect experiment; green (-), ┤: Oct-1 expression was inhibited by siRNA.

图8 IL-6通过STAT3/Oct-1/ATM途径诱导HUVSMCs的增殖

Fig.8 IL-6 induces the proliferation of HUVSMCs by the STAT3/Oct-1/ATM pathway

Jun/c-Fos/雌激素受体α形成复合物再结合到*CCND1*启动子区调节其表达[45]。Oct-1在调节细胞周期蛋白D1表达时也会与甲状腺激素受体β1形成复合物[46]。据报道, Oct-1经神经肽类物质促肾上腺皮质激素处理后可在细胞质和细胞核之间转位[23]。同样, 免疫荧光结果表明, IL-6刺激细胞后Oct-1的位置发生了变化, 这可能与Oct-1调节基因表达需要与其他蛋白质相互作用有关。

为了验证Oct-1参与IL-6诱导的细胞增殖, 我们干扰Oct-1表达后检测细胞增殖变化。结果表明, 抑制Oct-1表达可降低HUVSMCs的增殖。但是, 加入IL-6可逆转由抑制Oct-1减少的细胞增殖, 这表明IL-6可以通过Oct-1促进HUVSMCs的增殖。尽管结果提示, IL-6和Oct-1存在相互调节的关系, 但是尚不清楚Oct-1是如何通过IL-6进而调节HUVSMCs增殖的。利用IPA分析Oct-1及IL-6与高血压的相关分子发现, Oct-1(POU2F1)可以通过下游分子胰岛素基因增强结合蛋白1(insulin gene enhancer protein 1, ISL-1)来调节IL-6的表达。同时, 有关心脏发育的研究报道, Oct-1可以通过影响胰岛素基因增强结合蛋白1表达调节心脏细胞P19CL6的分化, 且敲除*Oct-1*会降低胰岛素基因增强结合蛋白1的表达[47]。进一步探讨Oct-1调控IL-6影响细胞增殖的机制研究将会在我们后续的工作中进行, 旨在为CVD的基础研究提供新思路。

总之, 我们的研究表明, IL-6通过激活STAT3的Ser727位点磷酸化, 诱导Oct-1和ATM的表达来促进HUVSMCs增殖。这些结果表明, IL-6可能通过STAT3/Oct-1/ATM途径调节HUVSMCs增殖(图8)。

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