



# 基质细胞衍生因子1 $\alpha$ 通过Akt信号通路抑制软骨细胞凋亡并促进自噬\*

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**【摘要】** 目的 探究基质细胞衍生因子1 $\alpha$ (stromal cell-derived factor 1 $\alpha$ , SDF-1 $\alpha$ )对软骨细胞凋亡及自噬的影响及其潜在机制。方法 从新生小鼠的膝关节中分离提取软骨细胞,以0(对照组)、50、100和200 ng/mL的SDF-1 $\alpha$ 刺激软骨细胞,CCK-8实验检测SDF-1 $\alpha$ 刺激24 h、48 h、72 h对软骨细胞活性的影响,划痕实验检测SDF-1 $\alpha$ 刺激12 h、24 h对软骨细胞迁移能力的影响,Western blot实验测定SDF-1 $\alpha$ 作用后软骨细胞内Akt信号通路相关蛋白表达变化。以0(对照组)、200 ng/mL的SDF-1 $\alpha$ 刺激软骨细胞,流式细胞术检测SDF-1 $\alpha$ 对软骨细胞凋亡的影响,透射电镜下检测SDF-1 $\alpha$ 对软骨细胞自噬的影响,并利用免疫荧光染色实验直观显示SDF-1 $\alpha$ 作用后软骨细胞内p-Akt蛋白表达及分布的差异。结果 与对照组相比,50、100和200 ng/mL SDF-1 $\alpha$ 在各时点并不降低软骨细胞活性( $P<0.01$ ),且均能在24 h促进软骨细胞迁移( $P<0.05$ )。Western blot实验结果显示,与对照组相比,50、100和200 ng/mL SDF-1 $\alpha$ 能够显著上调软骨细胞内p-Akt的蛋白表达量,Akt表达量未见明显差异。与对照组相比,流式细胞术示SDF-1 $\alpha$ 能够抑制软骨细胞凋亡( $P<0.05$ ),透射电镜观察到SDF-1 $\alpha$ 促进软骨细胞自噬( $P<0.05$ ),免疫荧光染色实验结果显示,p-Akt在软骨细胞的表达主要集中在细胞核周,SDF-1 $\alpha$ 作用后软骨细胞内p-Akt表达进一步在细胞核周部位增强。结论 SDF-1 $\alpha$ 通过激活Akt信号通路,抑制软骨细胞凋亡,促进软骨细胞迁移和自噬。

**【关键词】** 基质细胞衍生因子1 $\alpha$  软骨细胞 凋亡 Akt 自噬

**Stromal Cell-Derived Factor 1 $\alpha$  Inhibits Chondrocyte Apoptosis and Promotes Autophagy Through the Akt Signaling Pathway** LI Jiazhou, XIE Jing, ZHOU Xuedong $\Delta$ . State Key Laboratory of Oral Diseases & National Center for Stomatology & National Clinical Research Center for Oral Diseases, West China Hospital of Stomatology, Sichuan University, Chengdu 610041, China

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**【Abstract】 Objective** To investigate the effects of stromal cell-derived factor 1 $\alpha$  (SDF-1 $\alpha$ ) on the apoptosis and autophagy of chondrocytes and the underlying mechanisms. **Methods** Chondrocytes were isolated from the knee joints of neonatal mice. The chondrocytes were then stimulated with 0 (the control group), 50, 100, and 200 ng/mL of SDF-1 $\alpha$ . CCK-8 assay was performed to determine the effects of SDF-1 $\alpha$  stimulation for 24 h, 48 h, and 72 h on the viability of the chondrocytes. Wound healing assay was conducted to determine the effects of SDF-1 $\alpha$  stimulation for 12 h and 24 h on chondrocyte migration. The changes in the expression of Akt signaling pathway proteins in chondrocytes were determined by Western blot assay. Chondrocytes were stimulated with 0 (the control group) and 200 ng/mL of SDF-1 $\alpha$ . Flow cytometry was performed to determine the effect of SDF-1 $\alpha$  on the apoptosis of chondrocytes. Transmission electron microscope was used to examine the effect of SDF-1 $\alpha$  on chondrocyte autophagy. Immunofluorescence staining assays were performed to visualize the differences in p-Akt expression and distribution in chondrocytes treated with SDF-1 $\alpha$ . **Results** Compared with the control group, findings for the experimental groups showed that SDF-1 $\alpha$  at the concentrations of 50, 100, and 200 ng/mL did not decrease chondrocyte activity at any time point ( $P<0.01$ ) and it consistently promoted chondrocyte migration at 24 h ( $P<0.05$ ). Western blot results revealed that, in comparison to the control group, SDF-1 $\alpha$  at concentrations of 50, 100, and 200 ng/mL significantly up-regulated the protein expression of p-Akt in chondrocytes, while no significant difference in Akt expression was observed. Flow cytometry demonstrated that SDF-1 $\alpha$  could inhibit chondrocyte apoptosis ( $P<0.05$ ) and transmission electron microscopic observation showed that SDF-1 $\alpha$  promoted chondrocyte autophagy ( $P<0.05$ ). Immunofluorescence staining showed that the expression of p-Akt in chondrocytes was concentrated in the perinuclear area of the cells and this expression was further enhanced in the perinuclear area of the chondrocytes after treatment with SDF-1 $\alpha$ . **Conclusion** SDF-1 $\alpha$  inhibits chondrocyte apoptosis and promotes chondrocyte migration and autophagy through activating the Akt signaling pathway.

**【Key words】** Stromal cell-derived factor 1 $\alpha$  Chondrocyte Apoptosis Akt Autophagy

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骨关节炎(osteoarthritis, OA)是目前最为常见的致残增龄性疾病<sup>[1-2]</sup>。OA一方面给患者带来身体机能上的不便,另一方面,长期的慢性疼痛导致患者心理上的沉重负担<sup>[3]</sup>。近年来,超过2.4亿人罹患OA,且15%以上的老年人群深受困扰<sup>[4]</sup>。然而,目前临床治疗手段局限于保守缓解疼痛,对于逆转OA病程鲜有成效<sup>[5]</sup>。开放性外科手术即使在面向年轻患者时仍表现出术后恢复不佳且只能短暂性延缓恶化的情况。尽管组织工程、免疫治疗和基因治疗等具有创新性的治疗方法逐渐涌现,但离实现目前临床转化相差甚远<sup>[6]</sup>。因此探索OA的发病机制,理清疾病的发展进程具有显著的现实意义。

基质细胞衍生因子1(stromal cell-derived factor 1, SDF-1)又称CXC基序趋化因子配体12(CXC motif chemokine ligand 12)<sup>[7]</sup>。SDF-1作为一种具有遗传高度保守性的因子,在多系统、多器官及多功能的环境中发挥着重要调控作用<sup>[8]</sup>。许多关键的细胞生物学行为如增殖<sup>[9]</sup>、分布<sup>[10]</sup>、分化<sup>[11]</sup>、凋亡<sup>[12]</sup>及自噬<sup>[13]</sup>等过程中,都能发现SDF-1参与其中。目前SDF-1被定义为一种炎症稳态两用因子,以提示其在维稳和促炎中的双面性<sup>[14]</sup>。SDF-1 $\alpha$ 是SDF-1六种亚型中最为常见并被广泛纳入研究的亚型。近期有实验发现,在OA软骨组织周围SDF-1 $\alpha$ 含量显著升高,并伴随了对软骨细胞表型分化和分解代谢等行为的潜在影响<sup>[15]</sup>。但SDF-1 $\alpha$ 对软骨细胞凋亡、自噬的调控及潜在信号通路研究尚不明确。因此本实验旨在探究SDF-1 $\alpha$ 是否调控软骨细胞凋亡和自噬行为以参与OA疾病进展。

## 1 材料和方法

### 1.1 细胞培养

本实验所用动物样本均遵守动物实验伦理原则,符合《实验动物福利伦理审查指南(GB/T 35892—2018)》要求。经四川大学华西口腔医院伦理委员会审查并获批实验资格(No. WCHSIRB-OT-2020-048)。参考文献方法<sup>[16]</sup>,使用眼科剪分离C57BL/6J小鼠(1~3日龄)膝关节,剥离周缘附着纤维,获得小鼠关节透明软骨。将分离组织剪切为小碎块,使用0.25%胰酶溶液在37℃环境中处理30 min。待胰酶消化完成后,将组织转移至0.1% II型胶原酶,持续8~12 h。等体积加入含10%胎牛血清的DMEM(HyClone,美国)完全培养基终止胶原酶消化过程,将悬液转移至15 mL离心管中。以1 100 r/min转速离心5 min,弃去上清液,获得软骨细胞沉淀。加入新鲜培养基并混匀,接种软骨细胞至孔板,在37℃、体积分数5%的湿润二氧化碳环境中培养。

### 1.2 CCK-8实验

为了探究不同浓度的SDF-1 $\alpha$ 对软骨细胞活性的影响,通过CCK-8实验在不同时间点上对其进行检测。将软骨细胞以1 000~2 000细胞/每孔接种至96孔板内,过夜贴壁。以梯度浓度(0、50、100和200 ng/mL)的SDF-1 $\alpha$ (#250-20A, Pepro-tech, 美国)刺激软骨细胞。于24 h、48 h、72 h弃去培养液,按照CCK-8检测试剂盒(LABLEAD, 中国)说明书,加入CCK-8反应溶液,并设置空白组调零孔。在细胞培养箱中避光孵育1 h,通过酶标仪检测各孔450 nm处光密度(OD<sub>450</sub>)值。细胞活性以(SDF-1 $\alpha$ 组OD<sub>450</sub> - 空白组OD<sub>450</sub>)/(对照组OD<sub>450</sub> - 空白组OD<sub>450</sub>)计算。

### 1.3 划痕实验

为了进一步检测SDF-1 $\alpha$ 对软骨细胞迁移能力的影响,采用了划痕实验。将软骨细胞接种于6孔板内,待细胞汇合度达90%~100%。通过梯度饥饿软骨细胞,实现细胞状态同步化后,更换培养基为无血清培养基。使用枪尖于孔板皿底划出直线划痕,洗去细胞碎屑,并加入梯度SDF-1 $\alpha$ (0、50、100和200 ng/mL)处理软骨细胞。显微镜下采集0 h、12 h、24 h图像。各时间点细胞迁移率以(0 h划痕面积 - 检测点划痕面积)/0 h划痕面积计算。

### 1.4 细胞凋亡和自噬的检测

为了检测SDF-1 $\alpha$ 对软骨细胞凋亡及自噬行为的影响,采用了流式细胞仪检测及透射电镜实验(transmission electron microscope, TEM)等技术进行检测。

#### 1.4.1 流式细胞术检测细胞凋亡

接种软骨细胞于6孔板中,过夜贴壁后,加入SDF-1 $\alpha$ (0、200 ng/mL)继续培养48 h。弃去培养液后,PBS洗涤软骨细胞,使用胰酶消化并收集软骨细胞悬液。按照Annexin V-FITC细胞凋亡检测试剂盒(Beyotime, 中国)说明书处理软骨细胞。室温环境中避光孵育20 min,使用流式细胞仪上机检测。

#### 1.4.2 TEM观察软骨细胞内自噬囊泡生成

接种软骨细胞于培养皿上,待细胞融合度达50%~70%,加入SDF-1 $\alpha$ (0、200 ng/mL)持续处理软骨细胞24 h。胰酶消化并收集软骨细胞悬液,离心得到软骨细胞团块。使用0.5%戊二醛固定并重悬软骨细胞,再次离心。3%戊二醛固定液充分覆盖软骨细胞样本,低温保存并运输至成都里来生物技术有限公司进行后续样本处理。采用TEM观察并拍摄软骨细胞内自噬囊泡生成状况。

### 1.5 检测SDF-1 $\alpha$ 对软骨细胞内Akt信号通路

为了观察SDF-1 $\alpha$ 对软骨细胞内Akt信号通路的影响,

利用Western blot实验进行检测。为了进一步观察SDF-1 $\alpha$ 作用后p-Akt在软骨细胞内分布的改变,采用免疫荧光染色实验(immunofluorescence, IF)实验进行验证。

### 1.5.1 Western blot实验检测Akt信号通路

接种软骨细胞于培养皿上,待细胞融合度达50%~70%。以SDF-1 $\alpha$ (0、50、100和200 ng/mL)处理软骨细胞1 h。去除培养液后,将4组软骨细胞样本转移至冰浴环境中,加入RIPA裂解缓冲液及Cocktail蛋白酶抑制剂混合液充分裂解软骨细胞。研磨搔刮皿底后,收集至EP管内。按照BCA检测试剂盒(Beyotime, 中国)指南测定各组样品蛋白浓度。加入Loading buffer缓冲液,100  $^{\circ}$ C高温下水浴变性8~10 min。SDS-PAGE凝胶电泳分离蛋白,转移至PVDF膜。4  $^{\circ}$ C下孵育一抗稀释液(Akt, p-Akt,  $\beta$ -actin, 1:1000, 正能, 中国)过夜。室温孵育对应种属二抗(1:10000)2 h,使用化学发光试剂盒(Millipore, 美国)行蛋白条带可视化。Image J软件分析结果,以内参 $\beta$ -actin的表达量归一化。

### 1.5.2 IF观察p-Akt在软骨细胞内的分布

接种第一代(P1)软骨细胞于共聚焦玻底小皿中,细胞密度约控制在1000个/皿。待软骨细胞贴壁,使用SDF-1 $\alpha$ (0、200 ng/mL)处理1 h。预热4%多聚甲醛固定软骨细胞,0.25% TritonX-100 (Beyotime, 中国)通透软骨细胞。5%BSA封闭后,加入稀释一抗溶液(p-Akt, 1:200, 正能,

中国)4  $^{\circ}$ C过夜孵育。室温避光孵育对应种属Alexa Fluor 647(1:300, Abcam, 英国)荧光二抗2 h。PBS洗涤后,过夜孵育FITC标记鬼笔环肽(1:40稀释, Invitrogen, 美国)特异性染色软骨细胞内骨架, DAPI(1:100稀释, Sigma, 美国)染色软骨细胞核10 min。PBS多次轻柔涤荡软骨细胞,50%甘油封片保存,于共聚焦显微镜下采集细胞图像。

### 1.6 统计学方法

上述实验均独立重复3次,其中透射电镜实验从3次独立重复实验中随机分析每组的软骨细胞,以分别获得3组平均数据。计量数据以 $\bar{x} \pm s$ 的方式呈现,使用GraphPad Prism10软件绘制。多组间比较采用单因素方差分析(ANOVA),组间两两比较采用Tukey法(Tukey's HSD),两两差异用Student's *t* test进行检验比较, $P < 0.05$ 为差异有统计学意义。

## 2 结果

### 2.1 SDF-1 $\alpha$ 处理不降低软骨细胞活性

CCK-8实验结果显示不同浓度的SDF-1 $\alpha$ 处理72 h,仍然不降低软骨细胞活性(图1)。处理24 h及48 h, SDF-1 $\alpha$ 质量浓度为50或100 ng/mL组的细胞活性与对照组相比差异无统计学意义, SDF-1 $\alpha$ 质量浓度为200 ng/mL组的细胞活性与对照组相比的结果出现波动,但3个浓度组在72 h时细胞活性均高于对照组( $P < 0.01$ )。

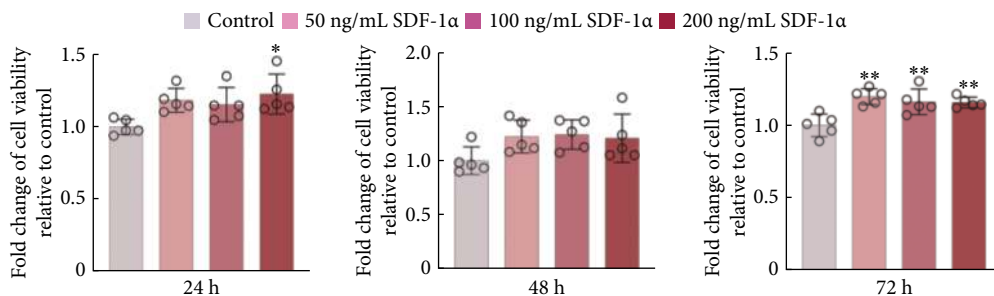


图1 SDF-1 $\alpha$ 处理不同时长后的软骨细胞活力

Fig 1 Chondrocyte viability after SDF-1 $\alpha$  treatment for different durations

\*  $P < 0.05$ , \*\*  $P < 0.01$ , vs. control group.  $n = 5$ .

### 2.2 SDF-1 $\alpha$ 促进软骨细胞迁移

划痕实验结果显示(图2), SDF-1 $\alpha$ 能够显著增加软骨细胞的迁移效率。在12 h时,50及100 ng/mL SDF-1 $\alpha$ 组迁移效率较对照组增高,差异有统计学意义( $P < 0.05$ )。24 h后各浓度SDF-1 $\alpha$ 组均实现了划痕愈合,而对照组尚未完成,50、100、200 ng/mL SDF-1 $\alpha$ 组迁移效率较对照组增高,差异有统计学意义( $P < 0.05$ )。

### 2.3 SDF-1 $\alpha$ 抑制软骨细胞凋亡并促进软骨细胞自噬

结果显示(图3), SDF-1 $\alpha$ 能够抑制软骨细胞凋亡水

平,提高软骨细胞自噬水平。流式细胞术结果提示SDF-1 $\alpha$ 能够有效提高软骨细胞的存活率,降低中晚期凋亡水平。透射电镜实验中观察到,使用200 ng/mL的SDF-1 $\alpha$ 处理后的软骨细胞,自噬囊泡生成量显著增加。

### 2.4 SDF-1 $\alpha$ 激活Akt信号通路

见图4。Western blot实验结果显示, SDF-1 $\alpha$ 能够显著上调软骨细胞内p-Akt的蛋白表达量, Akt表达量未见明显差异。IF实验结果显示, p-Akt在软骨细胞的表达主要集中在细胞核周, SDF-1 $\alpha$ 作用后软骨细胞内p-Akt表达

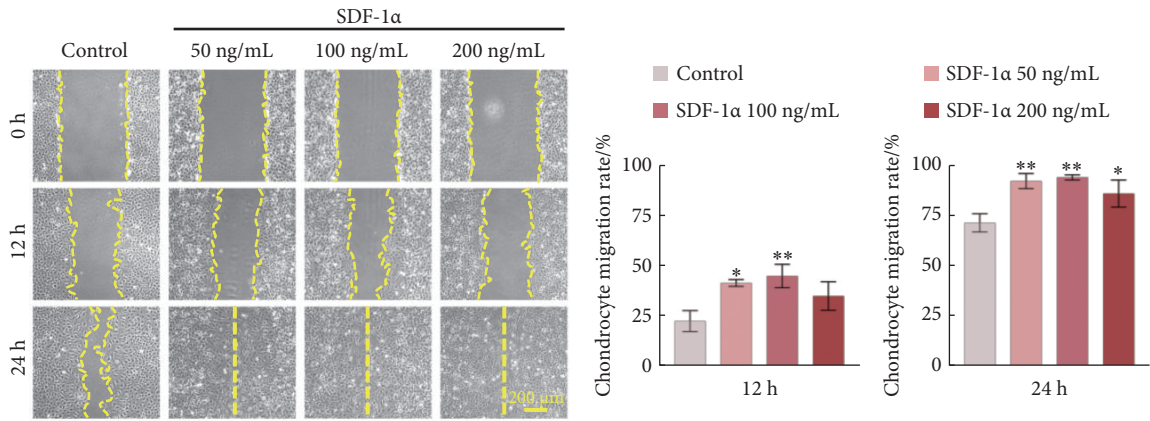


图 2 划痕实验检测SDF-1α对软骨细胞迁移的影响

Fig 2 Effect of SDF-1α on chondrocyte migration examined by wound healing assays

\*  $P < 0.05$ , \*\*  $P < 0.01$ , vs. control group.  $n = 3$ .

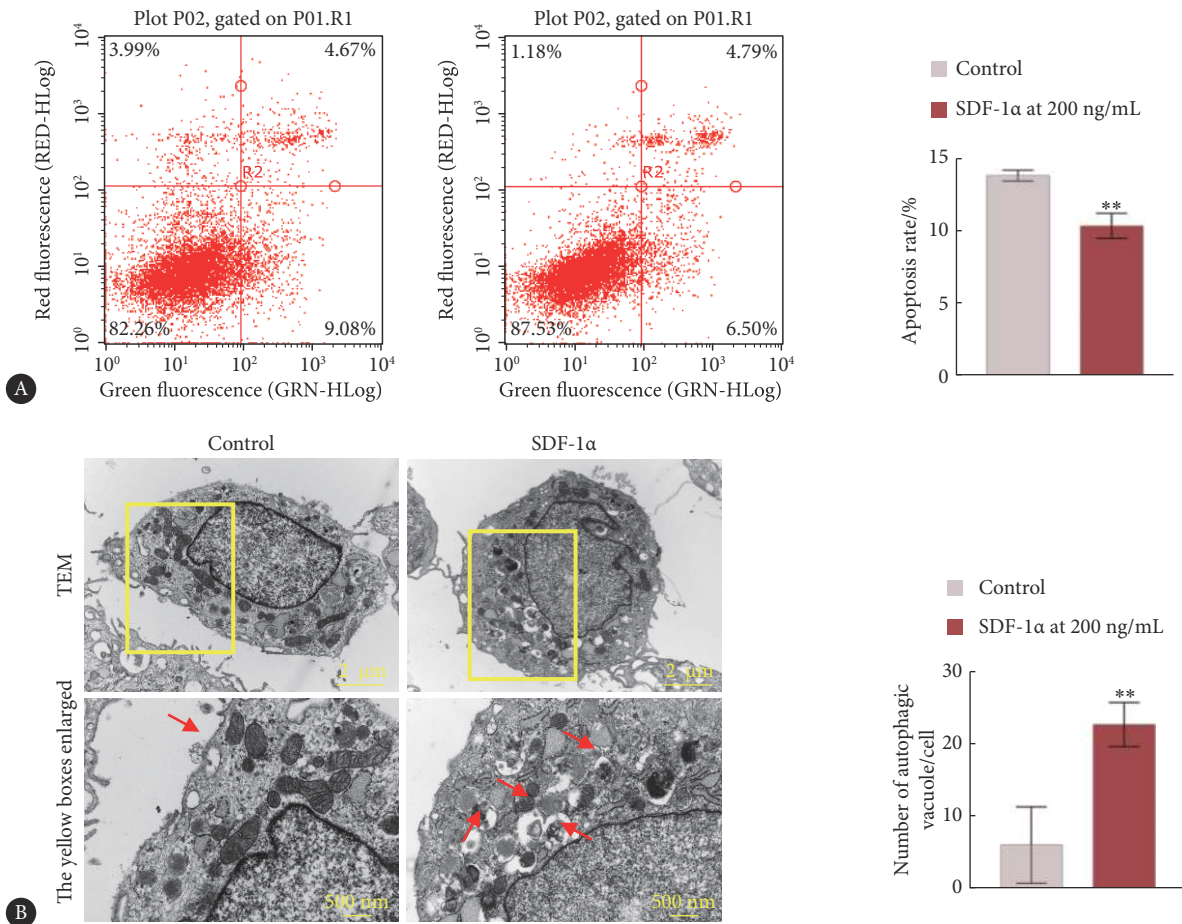


图 3 SDF-1α抑制软骨细胞凋亡并促进软骨细胞自噬

Fig 3 SDF-1α inhibits chondrocyte apoptosis and promotes chondrocyte autophagy

\*\*  $P < 0.01$ , vs. control group.  $n = 3$ . A, Flow cytometry assay; B, transmission electron microscopy observation (red arrows indicate the autophagic vacuoles).

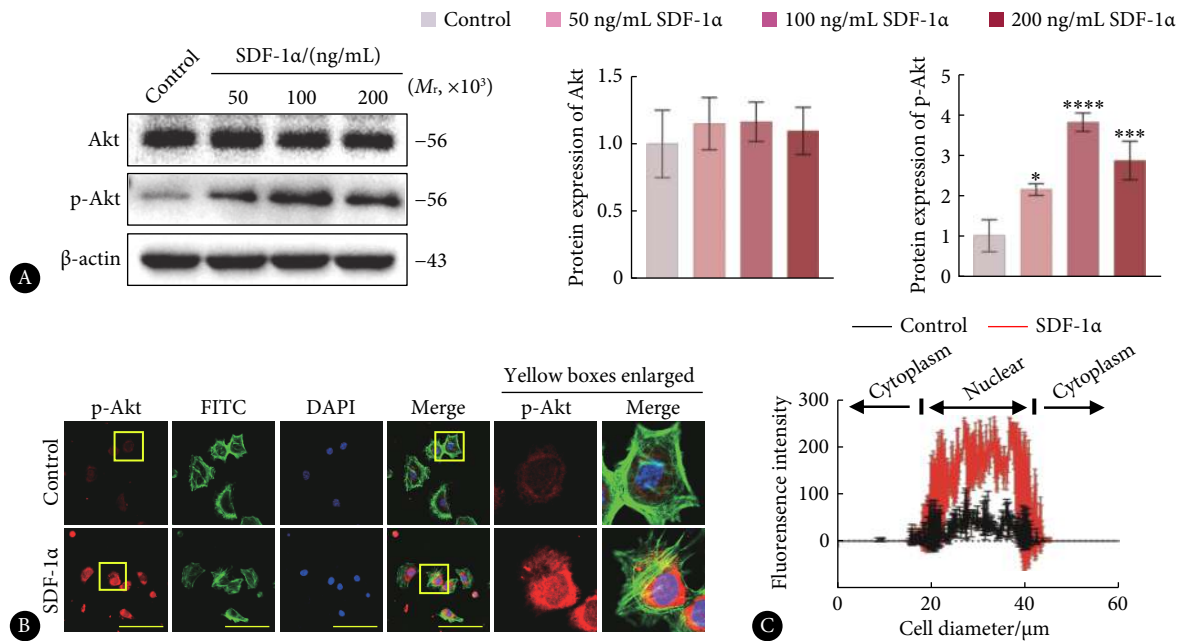
进一步在细胞核周部位增强。以上结果提示, SDF-1α能在软骨细胞内激活Akt信号通路。

### 3 讨论

OA好发于中老年人,且常累及整个关节<sup>[17-18]</sup>,仅在中

国,OA患病率就高达8.1%<sup>[19]</sup>。有研究预测,2030年中国的OA患病人数可能达到4亿<sup>[20]</sup>。遗憾的是目前临床仍未出现能够逆转OA进展的治疗方法,因此探究OA疾病的发病机制及其可能的治疗方法具有现实意义。

OA疾病的发生与软骨组织内环境稳态失衡密切相

图 4 SDF-1 $\alpha$ 激活Akt信号通路Fig 4 SDF-1 $\alpha$  activates the Akt signaling pathway

A, Western blot results ( $^* P < 0.05$ ,  $^{***} P < 0.001$ ,  $^{****} P < 0.0001$  vs. control group,  $n=3$ ); B, representative immunofluorescent images demonstrating the expression and distribution changes of p-Akt (p-Akt: red; cytoskeleton: green; nuclear: blue. bar=50  $\mu$ m); C, linear fluorescent quantification was performed to show the intracellular distribution of p-Akt in Fig B ( $n=3$ ).

关<sup>[21]</sup>。在失衡环境下,软骨细胞的多种生物学行为如增殖、分化、凋亡和自噬等均有显著变化<sup>[22]</sup>。由于软骨组织仅有软骨细胞一种细胞类型,因此软骨细胞的命运转归对疾病的发生发展有着至关重要的影响<sup>[23]</sup>。目前已有研究证明软骨细胞凋亡是OA病理学的潜在发病路径,OA软骨细胞凋亡发生率远高于正常软骨细胞,且凋亡细胞数量与OA软骨组织破坏程度呈正相关<sup>[24-25]</sup>,OA环境下软骨基质降解与软骨细胞程序性死亡也密切相关<sup>[26]</sup>。然而研究受限于时空因素、动力学不一致性等原因,软骨细胞凋亡在OA发病机制中的因果及参与程度难以准确估量<sup>[27]</sup>。在OA疾病进展早期,关节软骨中发现有自噬标志物显著上调,此现象随着疾病进展逐渐消失<sup>[28]</sup>。有研究发现自噬相关蛋白表达降低与软骨细胞凋亡增加及软骨细胞减少有关<sup>[29-30]</sup>。

软骨细胞死亡是OA疾病进展的关键因素,但不同类型的死亡方式在进展过程中发挥着不同的作用<sup>[31]</sup>。软骨细胞凋亡常被软骨组织环境中的炎性物质诱发,进一步加重OA<sup>[32]</sup>。软骨细胞自噬则被认为能够调节软骨细胞活性而抑制OA<sup>[33]</sup>。病变OA组织中SDF-1 $\alpha$ 含量急剧升高<sup>[34]</sup>,但SDF-1 $\alpha$ 是否通过调控软骨细胞凋亡及自噬影响OA进展还尚不清楚。因此本研究采用重组SDF-1 $\alpha$ 刺激软骨细胞,测定各行为变化,以探究其影响及机制。

为了检测SDF-1 $\alpha$ 对软骨细胞活性的影响,本实验采

用不同浓度梯度的SDF-1 $\alpha$ 分别刺激软骨细胞,并在多个时间点进行细胞活力检测。CCK-8结果显示,SDF-1 $\alpha$ 在质量浓度高达200 ng/mL时,也未表现出对软骨细胞的毒性。OA患者的关节滑膜液中,SDF-1质量浓度会升高至100 ng/mL以上<sup>[22]</sup>。该结果提示,SDF-1 $\alpha$ 在OA病理环境中的作用并非以诱导软骨细胞死亡为机制。

为了进一步验证SDF-1 $\alpha$ 对软骨细胞迁移能力的影响,本研究设计了划痕愈合实验进行检测。结果显示SDF-1 $\alpha$ 能够显著提升软骨细胞的迁移速率,加快划痕愈合,提示SDF-1 $\alpha$ 能够促进软骨细胞迁移。而软骨细胞上表达CXC趋化因子受体4(C-X-C chemokine receptor 4, CXCR4)<sup>[35]</sup>,可能是SDF-1 $\alpha$ 对软骨细胞实现趋化的原因。此外,在未来的研究中进一步展开SDF-1 $\alpha$ 趋化软骨细胞的分子机制,能为软骨疾病组织工程提供新的思路<sup>[36]</sup>。

接下来本实验采用流式细胞术及TEM实验等技术,检测SDF-1 $\alpha$ 对软骨细胞凋亡和自噬的影响。流式细胞术分选结果显示SDF-1 $\alpha$ 作用后,软骨细胞的中晚期凋亡水平显著降低,软骨细胞存活率明显升高。TEM实验结果显示,SDF-1 $\alpha$ 刺激能够上调软骨细胞内自噬囊泡的数量,提示软骨细胞自噬水平上升。在OA疾病进展中,软骨细胞死亡是关键因素<sup>[37]</sup>。虽然软骨细胞凋亡是否直接参与OA发病尚不清楚,但软骨细胞凋亡与OA软骨损失程度具有相关性<sup>[38]</sup>。SDF-1 $\alpha$ 能够降低软骨细胞凋亡水平,同

时促进软骨细胞自噬,有助于维持软骨组织稳态,提示SDF-1 $\alpha$ 可能在OA疾病进展中具有一定的保护作用。

Akt信号通路在许多生理活动及病理变化中展现了关键调控作用<sup>[39]</sup>。Akt信号通路一方面在软骨细胞存活和胞外基质合成等方面发挥软骨组织保护作用<sup>[40]</sup>,另一方面又在OA疾病进展中呈现诱导氧化应激反应或抑制软骨细胞自噬等破坏性作用<sup>[13,39]</sup>。因此,为了检测SDF-1 $\alpha$ 是否在软骨细胞内激活Akt信号通路以调控细胞行为,采用Western blot测定SDF-1 $\alpha$ 刺激软骨细胞后Akt和磷酸化Akt蛋白表达量的变化。结果显示,Akt总蛋白未见显著差异,但SDF-1 $\alpha$ 处理后磷酸化Akt蛋白表达量显著上升。进一步通过IF实验验证结果发现,SDF-1 $\alpha$ 刺激后,软骨细胞核周p-Akt表达量上调。以上结果提示SDF-1 $\alpha$ 在软骨细胞内激活Akt信号通路,同时SDF-1 $\alpha$ 可能独立于Akt信号通路以外,激活其他潜在信号通路以调控软骨细胞自噬活动。

综上所述,本实验采用重组SDF-1 $\alpha$ 刺激软骨细胞,结果发现SDF-1 $\alpha$ 不影响软骨细胞活性,并能够抑制软骨细胞凋亡,促进软骨细胞自噬。同时SDF-1 $\alpha$ 能够激活Akt信号通路参与对软骨细胞生物学行为的调控。遗憾的是,在本实验中缺乏了对Akt通路上下游相关信号指标的进一步检测,使得本文未能更加全面地解释Akt信号通路与软骨细胞生物学行为的分子机制。此外,未来的研究应该致力于完善相关体内实验,更加全面地评估SDF-1 $\alpha$ 在OA疾病模型中的功效,以期为OA疾病的发生提供新的视角,为OA疾病的治疗提供新的思路。

\* \* \*

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**Author Contribution** LI Jiazhou is responsible for conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing--original draft, and writing--review and editing. XIE Jing is responsible for project administration, resources, supervision, validation, visualization, writing--original draft, and writing--review and editing. ZHOU Xuedong is responsible for funding acquisition, resources, supervision, and writing--review and editing. All authors consented to the submission of the article to the Journal. All authors approved the final version to be published and agreed to take responsibility for all aspects of the work.

**利益冲突** 所有作者均声明不存在利益冲突

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