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活化T淋巴细胞核因子5在高盐诱导小鼠平滑肌细胞衰老中的作用及其机制

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[摘 要] **目的：**探讨活化T淋巴细胞核因子5（NFAT5）抑制剂KRN5在高盐诱导小鼠血管平滑肌细胞（VSMCs）衰老中的作用，并阐明其作用机制。**方法：**30只8周龄雄性ApoE$^{-/-}$小鼠分为正常组、衰老组和高盐处理衰老组，每组10只，衰老组和高盐处理衰老组构建小鼠自然衰老模型；分离培养小鼠VSMCs，将VSMCs分为正常组、衰老组、高盐处理衰老组和高盐处理衰老＋KRN5组。采用β-半乳糖苷酶（Sa-β-gal）染色法检测各组小鼠主动脉组织和VSMCs衰老情况，免疫荧光法检测各组小鼠主动脉组织和VSMCs中NFAT5和磷酸化的组蛋白H2A变异体X（γ-H2AX）蛋白表达情况，实时荧光定量PCR（RT-qPCR）法检测各组细胞中NFAT5、γ-H2AX、细胞周期依赖性激酶抑制剂2A（P16）和细胞周期依赖性激酶抑制剂1A（P21）mRNA表达水平，Western blotting法检测各组VSMCs中NFAT5、γ-H2AX、P16和P21蛋白表达水平。**结果：**Sa-β-gal染色法，与正常组比较，衰老组和高盐处理衰老组小鼠主动脉组织衰老阳性面积比例均明显增加（$P<0.05$），小鼠VSMCs衰老细胞阳性比例均明显增加（$P<0.05$）。与衰老组比较，高盐处理衰老组小鼠VSMCs衰老细胞阳性比例明显增加（$P<0.05$）；与高盐处理衰老组比较，高盐处理衰老＋KRN5组小鼠VSMCs衰老细胞阳性比例明显减少（$P<0.01$）。免疫荧光法，与正常组比较，衰老组小鼠VSMCs中γ-H2AX蛋白表达量明显增加（$P<0.05$）；与衰老组比较，高盐处理衰老组小鼠主动脉组织中SA-β-gal染色和NFAT5蛋白表达量均明显增加（$P<0.05$）；与正常组比较，衰老组和高盐处理衰老组小鼠VSMCs中NFAT5蛋白表达量明显增加（$P<0.05$）；与衰老组比较，高盐处理衰老组小鼠VSMCs中NFAT5蛋白表达量明显增加（$P<0.05$）。RT-qPCR法，与正常组比较，衰老组和高盐处理衰老组小鼠VSMCs中NFAT5、γ-H2AX、P16及P21 mRNA表达水平均明显升高（$P<0.05$）；与衰老组比较，高盐处理衰老组小鼠VSMCs中NFAT5、γ-H2AX、P16和P21 mRNA表达水平均明显升高（$P<0.05$）；与衰老组比较，高盐处理衰老组和高盐处理衰老＋KRN5组小鼠VSMCs中NFAT5、γ-H2AX、P16及P21 mRNA表达水平均明显升高（$P<0.05$）；与高盐处理衰老组比较，高盐处理衰老＋KRN5组小鼠VSMCs中NFAT5、γ-H2AX、P16和P21 mRNA表达水平均明显降低（$P<0.05$）。Western blotting法，与正常组比较，衰老组和高盐处理衰老组小鼠VSMCs中NFAT5、γ-H2AX、P16及P21蛋白表达水平均明显升高（$P<0.05$）；与衰老组比较，高盐处理衰老组小鼠VSMCs中NFAT5、γ-H2AX、P16和P21蛋白表达水平均明显升高（$P<0.05$）；与衰老组比较，高盐处理衰老组和高盐处理衰老＋KRN5组小鼠VSMCs中NFAT5、γ-H2AX、P16及P21蛋白表达水平均明显升高（$P<0.05$）；与高盐处理衰老组比较，高盐处理衰老＋KRN5组小鼠VSMCs中NFAT5、γ-H2AX、P16和P21蛋白表达水平均明显降低

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（$P<0.05$）。**结论**：NFAT5对高盐诱导小鼠VSMCs衰老可能具有一定促进作用。
[关键词] 血管衰老；活化T淋巴细胞核因子5；KRN5；血管平滑肌细胞；β-半乳糖苷酶
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Effect of nuclear factor of activated T lymphocytes 5 on senescence of smooth muscle cells of mice induced by high-salt and its mechanism

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ABSTRACT **Objective**: To discuss the role of nuclear factor of activated T-cells 5 (NFAT5) inhibitor KRN5 in high salt-induced senescence of mouse vascular smooth muscle cells (VSMCs), and to clarify its mechanism. **Methods**: Thirty 8-week-old male $ApoE^{-/-}$ mice were divided into normal group, senescence group and high-salt treatment senecence group, with 10 mice in each group; the mice in senescence group and high-salt treatment senecence group were used to establish natural senecence mouse models; the mouse VSMCs were isolated and cultured, and divided into normal group, senescence group, high-salt treatment senecence group and high-salt treatment senecence+KRN5 group. β-galactosidase (Sa-β-gal) staining was used to detect the senescence of aortic tissues and VSMCs in various groups; immunofluorescence method was used to detect the expressions of NFAT5 and phosphorylated histone H2A variant X (γ-H2AX) proteins in mouse aortic tissues and VSMCs in various groups; real-time fluorescence quantitative PCR (RT-qPCR) method was used to detect the mRNA expression levels of NFAT5, γ-H2AX, cyclin-dependent kinase inhibitor 2A (P16) and cyclin-dependent kinase inhibitor 1A (P21) in the cells in various groups; Western blotting method was used to detect the protein expression levels of NFAT5, γ-H2AX, P16 and P21 in VSMCs in various groups. **Results**: The Sa-β-gal staining results showed that compared with normal group, the proportions of senescence-positive area in aortic tissues of the mice in senescence group and high-salt treatment senecence group were significantly increased ($P<0.05$), and the proportion of senescence-positive cells in the VSMCs of the mice was significantly increased ($P<0.05$); compared with senecence group, the proportion of senescence-positive cells in the VSMCs mice in high-salt treatment senecence group was significantly increased ($P<0.05$); compared with high-salt treatment senecence group, the proportion of senescence-positive cells in the VSMCs of the mice in high-salt treatment senecence+KRN5 group was significantly decreased ($P<0.01$). The immunofluorescence results showed that compared with normal group, the expression level of γ-H2AX protein in mouse VSMCs of the mice in senescence group was significantly increased ($P<0.05$); compared with senescence group, the expression levels of SA-β-gal staining and NFAT5 protein in aortic tissue of the mice in high-salt treatment senecence group were significantly increased ($P<0.05$); compared with normal group, the expression level of NFAT5 protein in the VSMCs of the mice in senecence group and high-salt treatment senecence group was significantly increased ($P<0.05$); compared with senecence group, the expression level of NFAT5 protein in the VSMCs of the mice in high-salt treatment senecence group was significantly increased ($P<0.05$). The RT-qPCR results showed that compared with normal group, the expression levels of NFAT5, γ-H2AX, P16, and P21 mRNA in the VSMCs of the mice in senescence group and high-salt treatment senecence group were significantly increased ($P<0.05$); compared with senecence group, the mRNA

expression levels of NFAT5, γ-H2AX, P16, and P21 mRNA in the VSMCs of the mice in high-salt treatment senecence group were significantly increased ($P<0.05$); compared with senecence group, the expression levels of NFAT5, γ-H2AX, P16, and P21 mRNA in the VSMCs of the mice in high-salt treatment senecence group and high-salt treatment senecence+KRN5 group were significantly increased ($P<0.05$); compared with high-salt treatment senecence group, the mRNA expression levels of NFAT5, γ-H2AX, P16, and P21 in the VSMCs of the mice in high-salt treatment senecence+KRN5 group were significantly decreased ($P<0.05$). The Western blotting results showed that compared with normal group, the expression levels of NFAT5, γ-H2AX, P16, and P21 proteins in the VSMCs of the mice in senescence group and high-salt treatment senecence group were significantly increased ($P<0.05$); compared with senescence group, the expression levels of NFAT5, γ-H2AX, P16, and P21 proteins in the VSMCs of the mice in high-salt treatment senecence group were significantly increased ($P<0.05$); compared with senecence group, the expression levels of NFAT5, γ-H2AX, P16, and P21 proteins in the VSMCs of the mice in high-salt treatment senecence group and high-salt treatment senecence+KRN5 group were significantly increased ($P<0.05$); compared with high-salt treatment senecence group, the expression levels of NFAT5, γ-H2AX, P16, and P21 proteins in the VSMCs of the mice in high-salt treatment senecence+KRN5 group were significantly decreased ($P<0.05$). **Conclusion**: NFAT5 may play a promoting role in high salt-induced senescence of the mouse VSMCs.

KEYWORDS Vascular aging; Nuclear factor of activated T-cells 5; KRN5; Vascular smooth muscle cells; β-galactosidase

随着全球饮食习惯的变化，高盐饮食已成为普遍问题，特别是在中国，长期高盐摄入与心血管疾病等衰老相关疾病密切相关。高盐饮食被认为是高血压、心力衰竭和冠心病等心血管事件的独立危险因素[1]。然而，高盐饮食与血管衰老之间的关系尚未完全明确。衰老是指随着年龄增长，机体损伤不断累积，各组织和器官发生退行性变化的过程[2]。血管衰老是导致多种心血管疾病的重要因素之一[3]。研究[4-5]表明：高盐饮食可能通过增加氧化应激和炎症反应，加速血管平滑肌细胞（vascular smooth muscle cells，VSMCs）衰老，从而增加心血管疾病的发生风险。活化T淋巴细胞核因子5（luclear factor of activated T-lymphocytes 5，NFAT5）是一种重要的转录因子，在高盐环境中发挥关键的调控作用[6]。NFAT5可通过调节一系列基因的表达，促使细胞适应高盐条件[7-8]。然而，高盐环境对VSMCs衰老的影响及NFAT5在其中的具体作用机制尚未完全阐明。本研究探讨NFAT5抑制剂KRN5对高盐诱导小鼠VSMCs衰老的抑制作用，并阐明其作用机制，为相关疾病的预防和治疗提供新的策略。

1　材料与方法

1.1　实验动物、主要试剂和仪器　8周龄雄性ApoE$^{-/-}$小鼠30只，体质量25～28 g，购自江苏集萃药康生物科技股份有限公司，实验动物生产许可证号：SCXK（苏）2018-0008，所有动物实验经江苏大学实验动物伦理委员会批准；所有小鼠给予正常饲料和高盐饲料喂养，饲料购自广东琪康实业发展有限公司，高盐饲料为含4%氯化钠（NaCl）饲料和含1%NaCl自来水[9]。NFAT5购自美国Santa Cruz公司，组蛋白磷酸化的组蛋白H2A变异体X（phosphorylated H2A histone family member X，γ-H2AX）、细胞周期依赖性激酶抑制剂2A（cyclin-dependent kinase inhibitor 2A，P16）、细胞周期依赖性激酶抑制剂1A（cyclin-dependent kinase inhibitor 1A，P21）和Senescence Detection试剂盒购自美国Abcam公司，肌动蛋白（β-actin）抗体购自上海碧云天生物技术有限公司，细胞衰老检测试剂盒购自东仁化学科技（上海）有限公司，RNA快速提取试剂盒和实时荧光定量PCR（real-time fluorescence quantitative PCR，RT-qPCR）试剂盒购自上海弈杉生物科技有限公司，博来霉素（Bleomycin，BLM）购自美国Medchemexpress生物科技公司，焦磷酸二乙酯水（DEPC-treated water）购自北京兰杰柯科技有限公司，BCA蛋白定量试剂盒购自北京康为世纪生物科技公司。Western blotting电泳仪购自美国Bio-Rad公司，荧光倒置显微镜购自日本Olympus公司，37 ℃细胞培养箱购自美国ThermoFish公司，动物氧浓度控制

系统购自上海塔望生物科技有限公司。

1.2 实验动物分组及小鼠衰老模型的制备 取30只8周龄雄性ApoE$^{-/-}$小鼠，随机分为正常组（8周龄小鼠直接购买）、衰老组（正常饲料喂养2年）和高盐处理衰老组（高盐饲料喂养2年），每组10只，衰老组和高盐处理衰老组构建小鼠自然衰老模型。取各组小鼠主动脉组织，制备主动脉组织冰冻切片。

1.3 小鼠VSMCs分离培养、细胞衰老模型制备及分组 ApoE$^{-/-}$小鼠采用高浓度CO_2麻醉处死，75%乙醇浸泡消毒，固定后剪开胸部组织后剪下胸主动脉，磷酸盐缓冲液（phosphate buffer saline，PBS）漂洗3次，主动脉取出并用0.1% Ⅱ型胶原酶在37 ℃孵育7 min后消化血管外膜，剥离血管外膜将血管切成小段，长约1 mm，以滴管递送组织块，使组织块均匀附着于培养瓶底面，并向其中加入含20%胎牛血清（fetal bovine serum，FBS）的DMEM培养液，将培养瓶倒置于37 ℃、5% CO_2的恒温细胞培养箱中培养，2 h后平放培养瓶，待VSMCs由组织块爬出后，每隔3 d换液。

将VSMCs分为正常组、衰老组、高盐处理衰老组和高盐处理衰老＋KRN5组。正常组细胞由8周龄小鼠血管组织提取后培养3 d，衰老组、高盐处理衰老组和高盐处理衰老＋KRN5组建立细胞早衰模型。衰老组细胞使用5 mg·L^{-1} BLM刺激3 d后[10]，更换为正常培养基继续培养10 d；高盐处理衰老组细胞采用5 mg·L^{-1} BLM和350 mosmol·kg^{-1} NaCl共同处理3 d[11]，换液后加入含NaCl培养基继续培养10 d，构建VSMCs高盐衰老模型；高盐处理衰老＋KRN5组细胞采用1 μmol·L^{-1} KRN5、5 mg·L^{-1} BLM和350 mosmol·kg^{-1} NaCl共同处理3 d，之后换液，继续用KRN5和NaCl培养10 d。所有独立实验重复3次。

1.4 Sa-β-gal染色法检测各组小鼠主动脉组织和VSMCs衰老情况 将血管冰冻切片常温放置20 min，PBS缓冲液洗涤10 min，甩干切片，圈出组织。按照SA-β-gal衰老试剂盒说明书操作，固定组织，染色剂37 ℃过夜，核红染核10 min。PBS缓冲液洗涤10 min，甘油封固后显微镜观察。采用Image J软件计算主动脉组织衰老阳性面积比例和衰老细胞阳性比例，以主动脉组织衰老阳性面积比例和衰老细胞阳性比例代表小鼠主动脉组织和VSMCs衰老情况，Sa-β-gal染色中衰老小鼠主动脉组织和VSMCs呈蓝色染色。主动脉组织衰老阳性面积比例＝阳性面积/总面积，衰老细胞阳性比例＝衰老细胞数/总细胞。

1.5 免疫荧光法检测各组小鼠主动脉组织和VSMCs中NFAT5蛋白及γ-H2AX蛋白表达情况 将小鼠血管冰冻切片常温放置20 min，PBS缓冲液洗涤10 min，甩干切片，圈出组织。4%多聚甲醛溶液固定，0.1% Triton X-100打孔，滴加一抗，4 ℃过夜后滴加荧光标记的二抗，室温孵育1 h，PBS缓冲液洗涤3次后，DAPI染细胞核，甘油封固，荧光显微镜镜检。主动脉组织中红色荧光为NFAT5，绿色荧光为SA-β-gal，蓝色荧光为DAPI染色后的细胞核；将VSMCs于培养箱中取出，PBS缓冲液洗涤3次，每次约5 min，4%多聚甲醛溶液固定，0.1% Triton X-100打孔，滴加一抗，4 ℃过夜后滴加荧光标记的二抗，室温孵育1 h，PBS缓冲液洗涤3次后，DAPI染色细胞核，鬼笔环肽染色细胞骨架，甘油封固，荧光显微镜镜检。VSMCs中红色荧光为NFAT5和γ-H2AX蛋白，绿色荧光为鬼笔环肽细胞骨架染色，蓝色荧光为DAPI染色后的细胞核。Image J软件计算NFAT5和γ-H2AX与细胞核重叠阳性面积比例，代表VSMCs中NFAT5和γ-H2AX蛋白表达情况。阳性面积比例＝阳性面积/总面积。

1.6 RT-qPCR法检测各组细胞中NFAT5、γ-H2AX、P16和P21 mRNA表达水平 每孔细胞或每100 mg组织加入1 mL TRIzol，充分吹打后室温静置10 min。将裂解液转移至EP管中，每管加入200 μL氯仿，充分振荡混匀后室温静置10 min。4 ℃、12 000 g离心15 min后转移上清液至新EP管，并加入500 μL异丙醇轻轻混匀。室温静置10 h后4 ℃、12 000 g离心10 min，弃上清后用75%乙醇轻轻洗涤沉淀。4 ℃、7 500 g离心5 min，弃上清。加入适量焦碳酸二乙酯水溶解沉淀。获得RNA经浓度测定后使用采用Superscript Ⅲ逆转录酶合成cDNA并进行后续qPCR检测。以β-actin为内参。引物序列：NFAT5上游引物，5′-AGCAGCTGGTGCTTTGAGT-3′，NFAT5下游引物，5′-AGCCAGTCGTTTTCATTGCTTT-3′；γ-H2AX上游引物，5′-CTCCCAGGCCTCTCAGGAGAGA-3′，γ-H2AX下游引物，5′-GTGGCTCAGCTCTTTCTGTGA-3′；P16上游引物，5′-AAAGAGGAGGGGTTGGTTGGTTATTA-3′，P16下游

引物，5′-TACCTGATTCCAATTCCCCTGCAA-ACT-3′；P21上游引物，5′-CCACATGGTCTTC-CTCTGCTG-3′，P21下游引物，5′-GATGTCCG-TCAGAACCCATG-3′；β-actin上游引物，5′-GTG-GGGCGCCCCAGGCACCA-3′，β-actin下游引物，5′-CTCCTTAATGCACGCACGATTTC-3′。采用$2^{-\triangle\triangle Ct}$法计算目的基因表达水平。

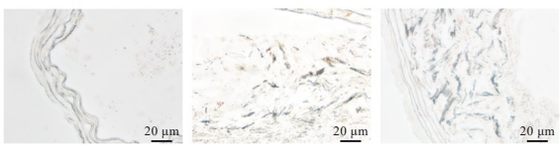
1.7　Western blotting法检测各组细胞中NFAT5、γ-H2AX、P16和P21蛋白表达水平　使用RIPA裂解液，按照步骤提取总蛋白后使用BCA法测定蛋白浓度，每孔50 μg上样加入胶孔中。蛋白到达分离胶下缘后，采用湿转法转移蛋白至PVDF膜上。经封闭、洗涤后分别用NFAT5、γ-H2AX、P16、P21和β-actin一抗于4 ℃孵育过夜，次日使用TBST溶液洗膜后，加入二抗（1∶5 000）室温孵育1 h，使用增强化学发光体系进行曝光，Image J软件分析蛋白条带灰度值，计算目的蛋白表达水平。目的蛋白表达水平＝目的蛋白条带灰度值/内参蛋白条带灰度值。

1.8　统计学分析　采用SPSS 22.0统计软件进行统计学分析。各组小鼠主动脉组织和VSMCs衰老阳性面积比例，主动脉组织和VSMCs中NFAT5蛋白表达量，VSMCs中γ-H2AX蛋白表达量，VSMCs中NFAT5、γ-H2AX、P16和P21 mRNA及蛋白表达水平均符合正态分布，以$\bar{x}\pm s$表示，多组间样本均数比较采用单因素方差分析，组间样本均数两两比较采用SNK-q检验。以$P<0.05$为差异有统计学意义。

2　结　果

2.1　各组小鼠主动脉组织和VSMCs衰老情况　与正常组比较，衰老组和高盐处理衰老组小鼠主动脉组织衰老阳性面积比例均明显减少（$P<0.05$），衰老组和高盐处理衰老组小鼠VSMCs衰老细胞阳性比例均明显增加（$P<0.05$）。见图1、表1、图2和表2。经KRN5处理后，与衰老组比较，高盐处理衰老组小鼠VSMCs衰老细胞阳性比例明显增加（$P<0.05$），高盐处理衰老＋KRN5组小鼠VSMCs衰老细胞阳性比例均明显减少（$P<0.05$）；与高盐处理衰老组比较，高盐处理衰老＋KRN5组小鼠VSMCs衰老细胞阳性比例明显减少（$P<0.01$）。见图3和表3。

2.2　各组小鼠主动脉组织和VSMCs中γ-H2AX及NFAT5蛋白表达情况　与正常组（0.03±0.01）比较，衰老组小鼠VSMCs中γ-H2AX蛋白表达量（0.47±0.05）明显增加（$P<0.05$）。见图4。与衰老组（48.33±4.16）比较，高盐处理衰老组小鼠主动脉组织中SA-β-gal染色和NFAT5蛋白表达均明显增强，SA-β-gal染色联合NFAT5蛋白表达



A　　B　　C

A: Normal group; B: Senescence group; C: High-salt treatment senescence group.

图1　各组小鼠主动脉组织SA-β-gal染色情况

Fig. 1　SA-β-gal staining in aorta tissue of mice in various groups

表1　各组小鼠主动脉组织衰老阳性面积比例

Tab. 1　Proportions of positive area of aortic tissue senescence of mice in various groups　（$n=10$，$\bar{x}\pm s$）

Group	Proportion of positive area
Normal	1.00±0.00
Senescence	0.22±0.04*
High-salt treatment senescence	0.33±0.05*

*$P<0.05$ *vs* normal group.

A　　B　　C

A: Normal group; B: Senescence group; C: High-salt treatment senescence group.

图2　各组小鼠VSMCs中SA-β-gal染色情况

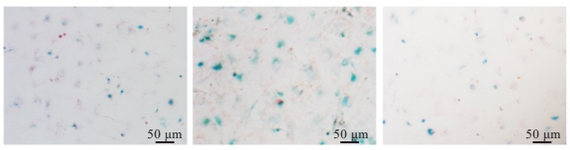
Fig. 2　SA-β-gal staining in VSMCs of mice in various groups

表2　各组小鼠VSMCs衰老细胞阳性面积比例

Tab. 2　Proportions of positive area of VSMCs senescence cells of mice in various groups　（$n=6$，$\bar{x}\pm s$）

Group	Proportion of positive area of senescence cells
Normal	0.00±0.00
Senescence	0.44±0.04*
High-salt treatment senescence	0.80±0.02*

*$P<0.05$ *vs* normal group.



A B C

A：Senescence group；B：High-salt treatment senescence group；C：High-salt treatment senescence＋KRN5 group.

图3 KRN5处理后各组小鼠VSMCs中SA-β-gal染色情况

Fig. 3 SA-β-gal staining in VSMCs of mice in various groups after treated with KRN5

表3 KRN5处理后各组小鼠VSMCs衰老细胞阳性面积比例

Tab. 3 Proportions of positive area of VSMCs senescence cells of mice in various groups after treated with KRN5

（$n=10$，$\bar{x}\pm s$）

Group	Proportion of positive area
Senescence	0.42±0.02
High-salt treatment senescence	0.78±0.07*
High-salt treatment senescence＋KRN5	0.27±0.03*△

*$P<0.05$ *vs* senescence group；△$P<0.05$ *vs* high-salt treatment senescence group.

量（83.33±7.02）明显增加（$P<0.05$）。见图5。与正常组比较，衰老组和高盐处理衰老组小鼠VSMCs中NFAT5蛋白表达量明显增加（$P<0.05$）；与衰老组比较，高盐处理衰老组小鼠VSMCs中NFAT5蛋白表达量均明显增加（$P<0.05$）。见图6和表4。

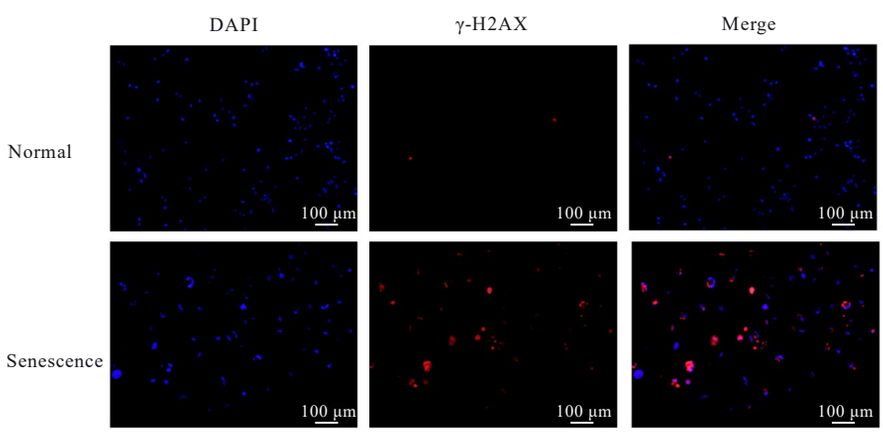


图4 免疫荧光法检测各组小鼠VSMCs中γ-H2AX蛋白表达情况

Fig. 4 Expression of γ-H2AX protein in VSMCs of mice in various groups detected by immunofluorescence assay

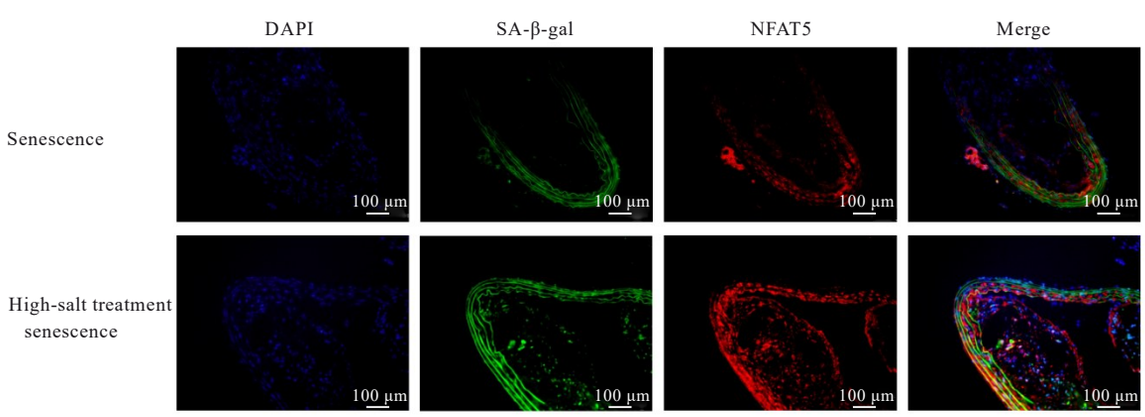


图5 免疫荧光法检测各组小鼠主动脉组织中SA-β-gal染色和NFAT5蛋白表达情况

Fig.5 SA-β-gal staining and expression of NFAT5 protein in aortic tissue of mice in various groups detected by immunofluorescence assay

2.3 各组小鼠VSMCs中NFAT5、γ-H2AX、P16和P21 mRNA表达水平 与正常组比较，衰老组和高盐处理衰老组小鼠VSMCs中NFAT5、γ-H2AX、P16及P21 mRNA表达水平均明显升高（$P<0.05$）；

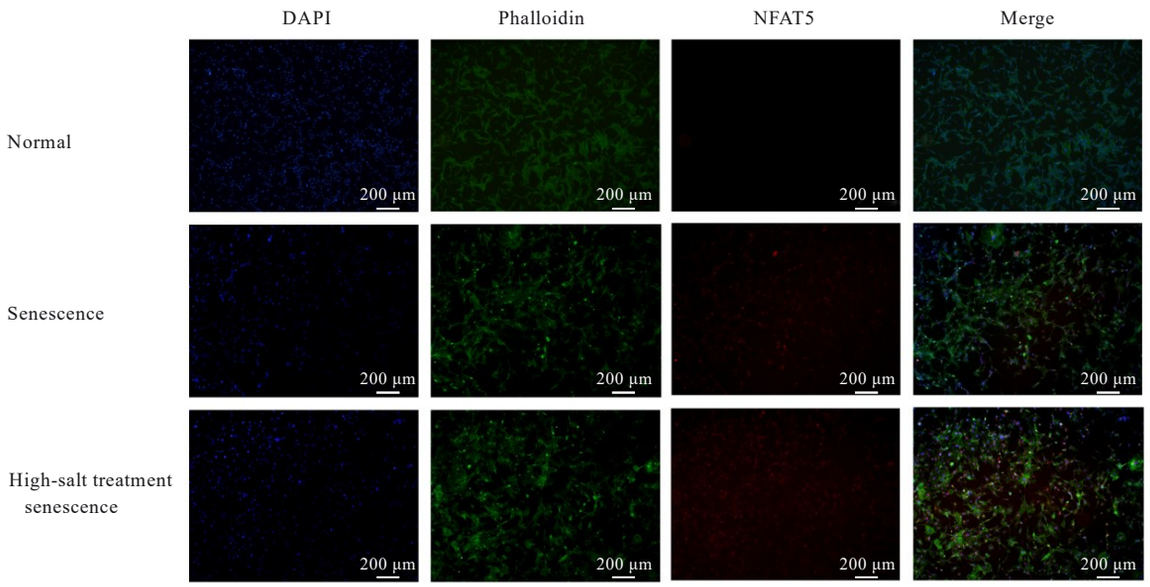


图6　免疫荧光法检测各组小鼠VSMCs中NFAT5蛋白表达情况

Fig. 6　Expression of NFAT5 protein in VSMCs of mice in various groups detected by immunofluorescence assay

表4　各组小鼠VSMCs中NFAT5蛋白表达情况

Tab. 4　Expressions of NFAT5 protein in VSMCs of mice in various groups　（$n=6$，$\bar{x}\pm s$）

Group	Expression of NFAT5
Normal	0.00±0.00
Senescence	0.52±0.04*
High-salt treatment senescence	0.70±0.02*△

*$P<0.05$ *vs* normal group；△$P<0.05$ *vs* senescence group.

与衰老组比较，高盐处理衰老组小鼠VSMCs中NFAT5、γ-H2AX、P16和P21 mRNA表达水平均明显升高（$P<0.05$）。见表5。经KRN5处理后，与衰老组比较，高盐处理衰老组和高盐处理衰老＋KRN5组小鼠VSMCs中NFAT5、γ-H2AX、P16及P21 mRNA表达水平均明显升高（$P<0.05$）；与高盐处理衰老组比较，高盐处理衰老＋KRN5组小鼠VSMCs中NFAT5、γ-H2AX、P16和P21 mRNA表达水平均明显降低（$P<0.05$）。见表6。

表5　各组小鼠VSMCs中NFAT5、γ-H2AX、P16和P21 mRNA表达水平

Tab. 5　Expression levels of NFAT5，γ-H2AX，P16，and P21 mRNA in VSMCs of mice in various groups　（$n=6$，$\bar{x}\pm s$）

Group	NFAT5	γ-H2AX	P16	P21
Normal	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
Senescence	2.34±0.08*	2.69±0.22*	1.91±0.18*	1.84±0.13*
High-salt treatment senescence	3.07±0.15*△	3.42±0.09*△	2.18±0.08*△	2.35±0.19*△

*$P<0.05$ *vs* normal group；△$P<0.05$ *vs* senescence group.

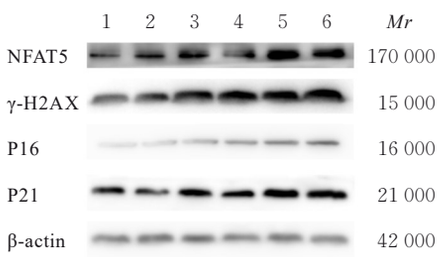
表6　KRN5处理后各组小鼠VSMCs中NFAT5、γ-H2AX、P16和P21 mRNA表达水平

Tab. 6　Expression levels of NFAT5，γ-H2AX，P16，and P21 mRNA in VSMCs of mice in various groups after treated with KRN5　（$n=6$，$\bar{x}\pm s$）

Group	NFAT5	γ-H2AX	P16	P21
Senescence	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
High-salt treatment senescence	2.91±0.14*	2.40±0.09*	3.25±0.05*	3.15±0.16*
High-salt treatment senescence＋KRN5	2.27±0.32*△	1.96±0.20*△	2.41±0.16*△	2.84±0.04*△

*$P<0.05$ *vs* senescence group；△$P<0.05$ *vs* high-salt treatment senescence group.

2.4 各组小鼠VSMCs中NFAT5、γ-H2AX、P16和P21蛋白表达水平 与正常组比较，衰老组和高盐处理衰老组小鼠VSMCs中NFAT5、γ-H2AX、P16及P21蛋白表达水平均明显升高（$P<0.05$）；与衰老组比较，高盐处理衰老组小鼠VSMCs中NFAT5、γ-H2AX、P16和P21蛋白表达水平均明显升高（$P<0.05$）。见图7和表7。经KRN5处理后，与衰老组比较，高盐处理衰老组和高盐处理衰老＋KRN5组小鼠VSMCs中NFAT5、γ-H2AX、P16及P21蛋白表达水平均明显升高（$P<0.05$）；与高盐处理衰老组比较，高盐处理衰老＋KRN5组小鼠VSMCs中NFAT5、γ-H2AX、P16和P21蛋白表达水平均明显降低（$P<0.05$）。见图8和表8。



Lane 1,2: Normal group; Lane 3,4: Senescence group; Lane 5,6: High-salt treatement senescence group.

图7 Western blotting法检测各组小鼠VSMCs中NFAT5、γ-H2AX、P16和P21蛋白表达电泳图

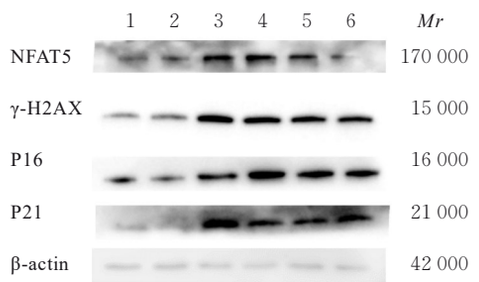
Fig. 7 Electrophoregram of expressions of NFAT5, γ-H2AX, P16, and P21 proteins in VSMCs of mice in various groups detected by Western blotting method

表7 各组小鼠VSMCs中NFAT5、γ-H2AX、P16和P21蛋白表达水平

Tab. 7 Expression levels of NFAT5, γ-H2AX, P16, and P21 proteins in VSMCs of mice in various groups ($n=6$, $\bar{x}\pm s$)

Group	NFAT5	γ-H2AX	P16	P21
Normal	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
Senescence	1.25±0.05*	1.98±0.08*	1.42±0.12*	2.39±0.09*
High-salt treatment senescence	1.63±0.14*△	2.34±0.07*△	1.79±0.26*△	3.68±0.78*△

*$P<0.05$ *vs* normal group; △$P<0.05$ *vs* senescence group.



Lane 1,2: Senescence group; Lane 3,4: High-salt treatment senescence group; Lane 5,6: High-salt treatment senescence＋KRN5 group.

图8 Western blotting法检测KRN5处理后各组小鼠VSMCs中NFAT5、γ-H2AX、P16和P21蛋白表达电泳图

Fig. 8 Electrophoregram of expressions of NFAT5, γ-H2AX, P16, and P21 proteins in VSMCs of mice in various groups after treated with KRN5 detected by Western blotting method

3 讨 论

研究[12-14]显示：高血压和血管衰老具有相关关系，摄入过量的钠盐会增加VSMCs内钠离子水平，间接增加VSMCs内的钙离子浓度，从而引起血管平滑肌收缩，血管僵硬度增加。同时VSMCs内钠离子水平增加，导致VSMCs渗透压升高，细胞肿胀，进而使肱动脉腔直径减小，外周血管的阻力增大，引起高血压患者发生血管衰老[15]。转录因子NFAT5在细胞适应高渗透压环境中发挥重要作用[16]。NFAT5通过调节一系列基因的表达，促使细胞适应高盐条件[17-18]。

本研究结果显示：与衰老组比较，高盐处理衰老组小鼠主动脉组织中SA-β-gal阳性细胞和衰老标志物（γ-H2AX、P16和P21）表达水平升高，

表8 KRN5处理后各组小鼠VSMCs中NFAT5、γ-H2AX、P16和P21蛋白表达水平

Tab. 8 Expression levels of NFAT5, γ-H2AX, P16, and P21 proteins in VSMCs of mice in various groups after treated with KRN5 ($n=6$, $\bar{x}\pm s$)

Group	NFAT5	γ-H2AX	P16	P21
Senescence	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
High-salt treatment senescence	2.95±0.12*	1.27±0.02*	2.34±0.06*	3.45±0.05*
High-salt treatment senescence＋KRN5	2.23±0.35*△	1.08±0.03*△	1.83±0.26*△	2.54±0.09*△

*$P<0.05$ *vs* senescence group; △$P<0.05$ *vs* high-salt treatment senescence group.

提示高盐饮食是导致血管衰老的重要因素；高盐处理衰老组小鼠VSMCs中NFAT5蛋白表达水平明显升高，使用NFAT5抑制剂KRN5可以减少相关衰老标志物的表达，提示NFAT5在这一过程中的潜在作用。

衰老的过程需要多种信号通路的调控，其中P21和P16信号通路均是影响细胞周期的关键机制，在细胞衰老过程中发挥重要作用[19]。本研究结果显示：高盐饮食通过激活NFAT5信号通路加速VSMCs衰老，进而导致血管老化，提示NFAT5可能作为干预血管衰老的潜在治疗靶点。

综上所述，NFAT5对高盐诱导小鼠平滑肌细胞衰老可能具有一定促进作用，本研究结果为开发基于NFAT5抑制剂的新型心血管疾病预防和治疗策略提供了科学依据。

利益冲突声明：

所有作者声明不存在利益冲突。

作者贡献声明：

仲威参与研究设计、数据分析和论文撰写，戴芝银、崔星钢和李波参与论文撰写指导，姜瑜参与研究设计和论文审阅。

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