*HOXA10*基因对牛骨骼肌卫星细胞增殖分化的影响

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摘要 该文探讨了*HOXA10*基因对牛骨骼肌卫星细胞(bovine skeletal muscle satellite cells, BSMSCs)增殖分化的作用。采用实时荧光定量PCR(qRT-PCR)检测增殖1、2、3天(P1、P2、P3) BSMSCs中*HOXA10*、*PCNA*基因的表达情况; 将*HOXA10*基因过表达质粒、干扰质粒及对照质粒转染至BSMSCs中, 利用qRT-PCR、蛋白质免疫印迹(Western blot)实验检测过表达及干扰效果; 采用CCK-8、EdU、流式细胞术(Flow CytoMetry, FCM)、免疫荧光、qRT-PCR和Western blot检测细胞活力、细胞增殖、细胞周期、细胞分化以及细胞增殖和分化标志基因的mRNA及蛋白表达情况。结果显示, 在BSMSCs增殖过程中*HOXA10* mRNA水平显著升高(P<0.01, P<0.001); 与对照组相比, 转染24 h后过表达及干扰效果显著(P<0.01, P<0.001); 过表达*HOXA10*基因后细胞活力增强, EdU阳性细胞比例显著增多(P<0.001), G_1期细胞百分比显著降低(P<0.01), S期细胞百分比显著增加(P<0.05), 增殖标志基因PCNA、CCND1 mRNA和蛋白的表达水平显著升高(P<0.05, P<0.01); 肌管形成水平显著降低, 分化标志基因MyoG的mRNA和蛋白表达量显著减少(P<0.01, P<0.001)。与对照组相比, 干扰*HOXA10*基因后细胞活力降低, EdU阳性细胞比例显著减少(P<0.05), G_1期细胞百分比显著升高(P<0.001), S期细胞百分比显著下降(P<0.01), 增殖标志基因PCNA、CCND1 mRNA和蛋白的表达水平显著降低(P<0.05, P<0.001); 肌管形成水平显著上升, 分化标志基因MyoG的mRNA和蛋白表达量显著减少(P<0.001)。综上, *HOXA10*基因促进BSMSCs增殖, 抑制其分化。

关键词 牛骨骼肌卫星细胞; *HOXA10*基因; 增殖; 分化

The Effect of *HOXA10* Gene on Proliferation and Differentiation of Bovine Skeletal Muscle Satellite Cells

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Abstract This study explored the effects of *HOXA10* gene on the proliferation and differentiation of BSMSCs (bovine skeletal muscle satellite cells). qRT-PCR (real-time quantitative PCR) was used to detect the expression of *HOXA10* and *PCNA* genes in BSMSCs proliferating for 1, 2 and 3 days (P1, P2 and P3). *HOXA10* gene overexpression plasmids, interference plasmids, and control plasmids were transfected into BSMSCs. qRT-PCR

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and Western blot were used to detect overexpression and interference effects. CCK-8, EdU, FCM (Flow CytoMetry), immunofluorescence, qRT-PCR and Western blot were used to detect cell viability, cell proliferation, cell cycle, cell differentiation, mRNA and protein expression of genes that mark cell proliferation and differentiation. The results showed that the mRNA of *HOXA10* was significantly increased during the proliferation of BSMSCs ($P<0.01$, $P<0.001$). Compared with the control group, the overexpression and interference effects were significant after 24 hours of transfection ($P<0.01$, $P<0.001$); the cell viability and the proportion of EdU-positive cells in BSMSCs were increased ($P<0.001$) after overexpression of *HOXA10* gene, meanwhile the percentage of cells in G_1 phase was significantly decreased ($P<0.01$); the percentage of cells in S phase was significantly increased ($P<0.05$); and the expression levels of proliferation marker genes PCNA, CCND1 mRNA and protein were significantly increased ($P<0.05$, $P<0.01$). The formation of myotubes was significantly reduced, and the mRNA and protein expressions of the differentiation marker gene MyoG were significantly reduced ($P<0.01$, $P<0.001$). Compared with the control group, after silencing of the *HOXA10* gene, the cell viability decreased, the proportion of EdU-positive cells decreased ($P<0.05$); the percentage of cells in G_1 phase increased ($P<0.001$); the percentage of cells in S phase decreased significantly ($P<0.01$). The levles of PCNA, CCND1 mRNA and protein were decreased significantly after silencing of the *HOXA10* gene ($P<0.05$, $P<0.001$). The formation of myotubes was significantly increased, and the mRNA and protein expressions of the differentiation marker gene MyoG were significantly increased ($P<0.001$). In conclusion, *HOXA10* gene promotes the proliferation and inhibits differentiation of BSMSCs.

Keywords bovine skeletal muscle satellite cells; *HOXA10* gene; proliferation; differentiation

骨骼肌是哺乳动物躯干的主要组成成分，禽畜的产肉量以及肉类的品质离不开骨骼肌的生长发育[1]。动物出生后肌肉的发育主要依赖骨骼肌卫星细胞(skeletal muscle satellite cells, SMSCs)的分裂、分化而导致肌纤维长度增加和周径增粗[2]。当肌肉发生损伤时，SMSCs增殖就会被启动，SMSCs大量分裂，多数细胞融合分化形成再生肌纤维[3]。另外SMSCs的广泛迁移、分裂、分化对于骨骼肌的生长发育及再生具有重要作用[4]。SMSCs增殖分化过程较为复杂，涉及多种调控因子的参与，寻找新的调控途径对骨骼肌损伤后修复及提高家禽肉制品产量具有重要意义。

*HOXA10*是同源框转录因子家族的成员，转录一个全长2 691 bp的mRNA，包含一个183 bp的高度保守序列，被称为同源框[5]；参与子宫发育、细胞分裂、分化、衰老和肿瘤形成等多种生物过程的遗传控制[6]。*HOXA10*基因可通过促进细胞分裂、侵袭和迁移，并抑制细胞凋亡来加速多种癌症的发生和发展[7-9]。此外，*HOXA10*通过识别特定的TTAT位点结合*Runx2*、*ALP*、*OCN*、*BSP*等多种成骨分化基因的启动子区进而影响成骨细胞增殖分化进程[10]。可见，*HOXA10*在细胞的增殖和分化过程中起到重要调节作用，但其在牛骨骼肌卫星细胞(bovine skeletal muscle satellite cells, BSMSCs)增殖、分化中的作用仍不清楚。因此，本实验以BSMSCs为实验材料，探究*HOXA10*基因对BSMSCs增殖分化的影响。

1 材料与方法

1.1 主要试剂与材料

参照兴孝友等[11]的方法，BSMSCs由本实验室分离并冻存。

胎牛血清(FBS)购自Gibco公司；EdU、FCM检测试剂盒购自上海碧云天生物技术有限公司；cDNA反转录试剂盒、TRIzol试剂、qRT-PCR试剂盒购自山东思科捷生物技术有限公司；CCK-8购自美国APExBIO公司；HOXA10一抗(bs-2502R; 1:1 000)购自北京博奥森生物技术有限公司；MyoG一抗(382257; 1:500)购自成都正能生物技术有限责任公司；PCNA(10205-2-AP; 1:15 000)、CCND1(60186-1-Ig; 1:1 000)和β-肌动蛋白(β-actin)(66009-1-Ig; 1:35 000)一抗购自武汉三鹰生物技术有限公司；二

抗(926-32210、926-32211; 1:20 000)购自LI-COR公司。

1.2 细胞培养、转染及诱导分化

用含15% FBS的DMEM培养基在37 °C、5% CO_2及饱和湿度培养箱中培养BSMSCs, 当细胞密度达到90%时进行传代, 当细胞密度达到70%时进行转染。将增殖期细胞用15% FBS继续培养24、48、72 h后收集细胞, 再更换为2%马血清的培养基诱导分化收集分化期细胞。

1.3 载体构建及效果检测

在NCBI中查找*HOXA10*基因CDS(coding sequence)序列, 使用SnapGene在pCMV-3xMyc载体*Eco*R I和*Abs* I之间插入*HOXA10*基因281 bp的CDS区序列。用TRIzol试剂提取BSMSCs总RNA, 反转录试剂盒将0.1 μg总RNA反转录成cDNA, 以cDNA为模板扩增*HOXA10*基因CDS区, 通过同源重组一步克隆法将其克隆到pCMV-3xMyc质粒中, 构建*HOXA10*基因过表达载体。

参照NCBI中*HOXA10*基因CDS序列与短发夹RNA设计原则, 设计*HOXA10*基因的shRNA序列为(5′→3′): GTGTCAAGGCAATTCCAAAGG, 在序列两端加入*Bam*H I和*Hin*d III酶切位点, 序列中间插入loop(CGAA)结构。

将BSMSCs接种至6孔板, 当细胞密度达到70%时, 采用PEI法将*HOXA10*过表达和干扰载体及对照质粒转染至BSMSCs, 培养24 h后利用qRT-PCR、Western blot检测过表达及干扰效果。

1.4 CCK-8检测*HOXA10*基因对细胞活力的影响

将对数生长期的BSMSCs接种至96孔板, 待细胞密度达到70%时, 采用PEI法将*HOXA10*过表达和干扰载体及对照质粒转染至BSMSCs, 培养24、48、72 h后, 每孔加入10 μL CCK-8, 37 °C避光孵育4 h, 孵育完成后, 用酶标仪测定波长在450 nm处吸光度值, 实验重复三次, 检测各组活细胞数, 分析*HOXA10*基因对BSMSCs细胞活力的影响。

1.5 EdU染色检测*HOXA10*基因对BSMSCs增殖的影响

将BSMSCs接种至12孔板, 当细胞密度达到70%时, 采用PEI法将*HOXA10*过表达和干扰载体及对照质粒转染至BSMSCs, 培养24 h后参照EdU试剂盒说明书进行染色。染色完成后使用显微镜(型号Olympus IX71)进行拍照。计数, 统计EdU阳性细胞所占比例, 并分析*HOXA10*基因对BSMSCs增殖的影响。

1.6 FCM检测*HOXA10*基因对BSMSCs细胞周期的影响

将BSMSCs接种至培养瓶, 当细胞密度达到70%时, 采用PEI法将*HOXA10*过表达和干扰载体及对照质粒转染至BSMSCs, 培养24 h后收集细胞, 参照FCM试剂盒说明书进行染色。染色完成后使用流式细胞仪检测细胞周期。

1.7 免疫荧光法检测*HOXA10*基因对BSMSCs分化的影响

将BSMSCs接种至6孔板, 进行爬片。当细胞密度达到70%时, 采用PEI法将*HOXA10*过表达和干扰载体及对照质粒转染至BSMSCs, 培养24 h后换分化培养基继续培养3天, 再用4%多聚甲醛37 °C固定细胞30 min, 用含5% BSA和0.2% TritonX-100的PBS室温封闭1 h, 加入用封闭液稀释的MyoG一抗(1:100), 于4 °C孵育过夜, 洗涤后加入Alexa Fluor 488标记的二抗(1:100), 室温孵育1 h, 洗涤后加入DAPI室温孵育3 min, 洗涤, 拍照。

1.8 qRT-PCR和Western blot检测*HOXA10*基因对BSMSCs增殖分化标志基因表达的影响

将BSMSCs接种至6孔板, 当细胞密度达到70%时, 采用PEI法将*HOXA10*过表达和干扰载体及对照质粒转染至BSMSCs, 培养24 h, 用TRIzol试剂提取各转染组细胞的RNA, 反转录试剂盒将0.1 μg总RNA反转录成cDNA。根据*CCND1*、*PCNA*、*MyoG*、*HOXA10*、*β-actin*基因序列设计特异性引物(表1)。按照qRT-PCR试剂盒说明书进行PCR反应, 扩增条件: 94 °C预变性2 min; 94 °C变性10 s, 60 °C退火10 s, 72 °C延伸30 s, 共40个循环。使用$2^{-\Delta\Delta Ct}$计算增殖和分化标志基因mRNA的表达量。以*β-actin*为内参。

提取各转染组细胞蛋白质, SDS-PAGE电泳后将蛋白转印到PVDF膜上, 一抗(1:10 000) 4 °C孵育过夜, 洗涤后二抗(1:20 000)室温孵育1 h, 使用红外成像系统(Li-cor Odyssey infrared imaging system, Li-cor Biosciences, Lincoln, Nebraska USA)扫描蛋白条带, 检测HOXA10、PCNA、CCND1、MyoG蛋白的表达情况, 以β-actin为内参。

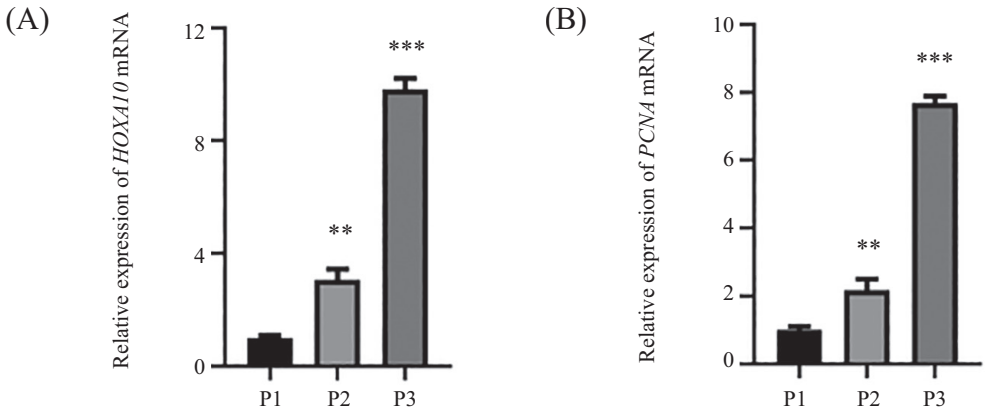
1.9 统计学处理

用 GraphPad Prism 8.0将数据进行处理分析, 实验数据以均数±标准差表示, 采用*t*检验进行两组间

表1 增殖和分化相关基因引物序列

Table 1 Primer sequences of genes related to proliferation and differentiation

基因名称 Gene name	序列(5′→3′) Sequence (5′→3′)
CCND1	F: GGG CAA GTT GAA ATG GAA
	R: TCA TCG ACG GCG GGT AC
PCNA	F: TGA TGG AAC TAA CTA TGC TGG
	R: GCA TAA CAA CGA GAA GGG ATT
MyoG	F: CCA GTG AAT GCA ACT CCC ACA
	R: ATG GAC GTA AGG GAG TGC AGA TT
HOXA10	F: AAC GCA GCC AAC TGG CTC ACT
	R: ACT TGT CTG TCC GTG AGG TGG A
β-actin	F: GAT CAA GAT CAT TGC TCC TCC TGA
	R: CAG CTC AGT AAC AGT CCG CC



A: BSMSCs增殖1、2、3天*HOXA10* mRNA表达量的变化; B: BSMSCs增殖1、2、3天*PCNA* mRNA表达量的变化。**P<0.01, ***P<0.001, 与P1组相比。

A: change in mRNA expression of *HOXA10* at 1, 2, and 3 days of BSMSCs proliferation; B: change in mRNA expression of *PCNA* at 1, 2, and 3 days of BSMSCs proliferation. **P<0.01, ***P<0.001 compared with P1 group.

图1 BSMSCs增殖不同时期*HOXA10*、*PCNA*基因的表达情况

Fig.1 Expression of *HOXA10*, *PCNA* during the proliferation of BSMSCs at different days

比较, P<0.05表示具有显著性差异。

2 结果

2.1 *HOXA10*基因在BSMSCs增殖、分化不同时间的表达

利用qRT-PCR检测BSMSCs增殖不同时间的*HOXA10*、*PCNA*基因的表达情况, 结果显示: 随着天数的增加, *PCNA* mRNA的表达量逐渐升高, 证明此细胞处于增殖阶段, 在细胞增殖过程中, *HOXA10* mRNA的表达量逐渐升高(P<0.01, P<0.001)(图1), 表明*HOXA10*基因可能参与BSMSCs增殖过程。

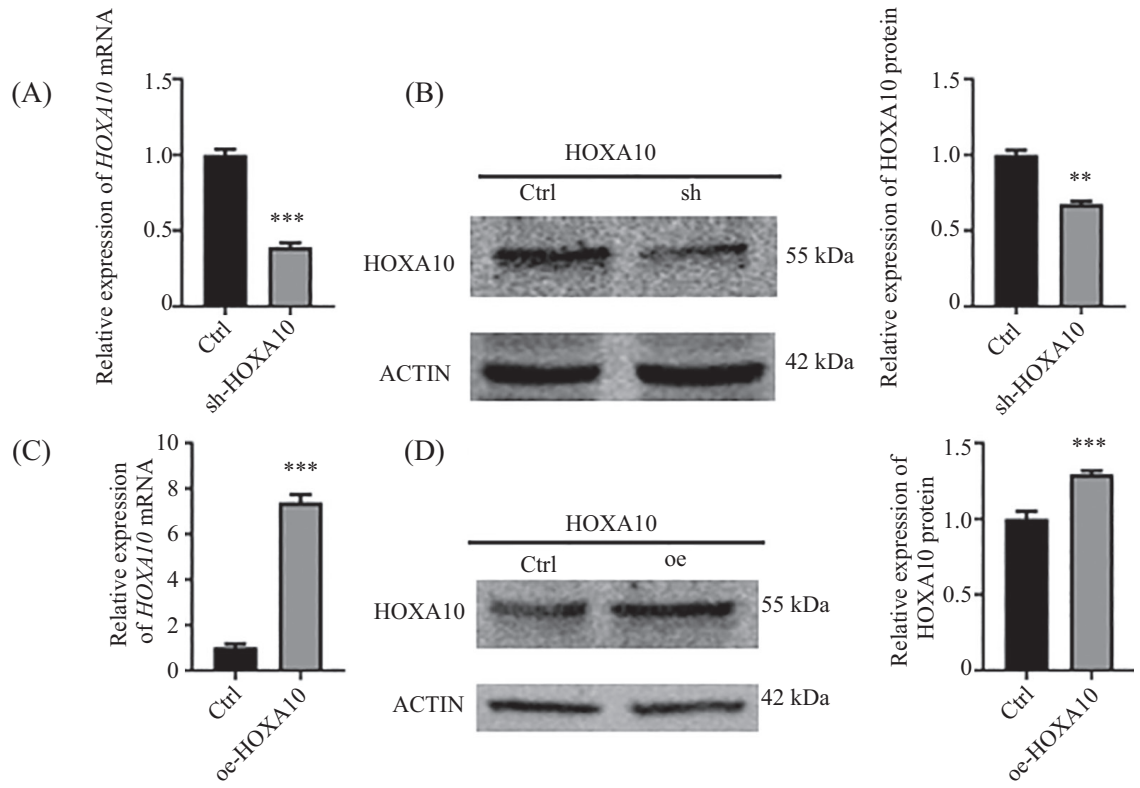
2.2 *HOXA10*过表达载体和干扰载体的效果检测

为了检测*HOXA10*基因的功能, 构建了*HOXA10*基因的干扰和过表达载体, 将*HOXA10*对照质粒及干扰质粒转染至BSMSCs。24 h后利用qRT-PCR、Western blot检测干扰效率。结果显示: 与对照组相比, 转染干扰质粒24 h后细胞中HOXA10 mRNA和蛋白表达量显著降低(P<0.01, P<0.001)(图2A和图2B)。

将*HOXA10*对照质粒及过表达质粒转染BSMSCs 24 h后利用qRT-PCR、Western blot检测过表达效率, 结果显示: 转染24 h后与对照组相比HOXA10 mRNA和蛋白表达量显著升高(P<0.001) (图2C和图2D)。

2.3 *HOXA10*基因促进BSMSCs增殖

为了检测*HOXA10*对BSMSCs增殖的影响, 将构建的*HOXA10*基因的干扰和过表达载体转染



A: 干扰*HOXA10* mRNA表达量; B: 干扰HOXA10蛋白表达量; C: 过表达*HOXA10* mRNA表达量; D: 过表达HOXA10蛋白表达量; Ctrl: 对照组; sh-HOXA10(sh): 干扰*HOXA10*基因; oe-HOXA10(oe): 过表达*HOXA10*基因; ***P*<0.01, ****P*<0.001, 与对照组比较。

A: interferes with *HOXA10* mRNA expression; B: interferes with HOXA10 protein expression; C: overexpression of *HOXA10* mRNA expression; D: overexpression of HOXA10 protein expression; Ctrl: control group; sh-HOXA10 (sh): interferes with the *HOXA10* gene; oe-HOXA10 (oe): overexpression of the *HOXA10* gene; ***P*<0.01, ****P*<0.001 compared with control group.

图2 *HOXA10*干扰载体及过表达载体的效果检测

Fig.2 Detection of the effects of *HOXA10* interference vector and overexpression vector

BSMSCs, 利用CCK-8法、EdU法和FCM检测细胞增殖变化, 结果表明, 与对照组相比, 过表达*HOXA10*后细胞活力升高(图3A)。EdU阳性细胞比例升高(*P*<0.001)(图3B), G_1期细胞百分比显著降低(*P*<0.01), S期细胞百分比显著增加(*P*<0.05)(图3C)。qRT-PCR和Western blot结果显示, PCNA和CCND1增殖标志基因mRNA和蛋白水平显著上升(*P*<0.05, *P*<0.01)(图3E)。干扰*HOXA10*表达后得到相反结果(图4)。综上所述, *HOXA10*基因可促进BSMSCs的增殖。

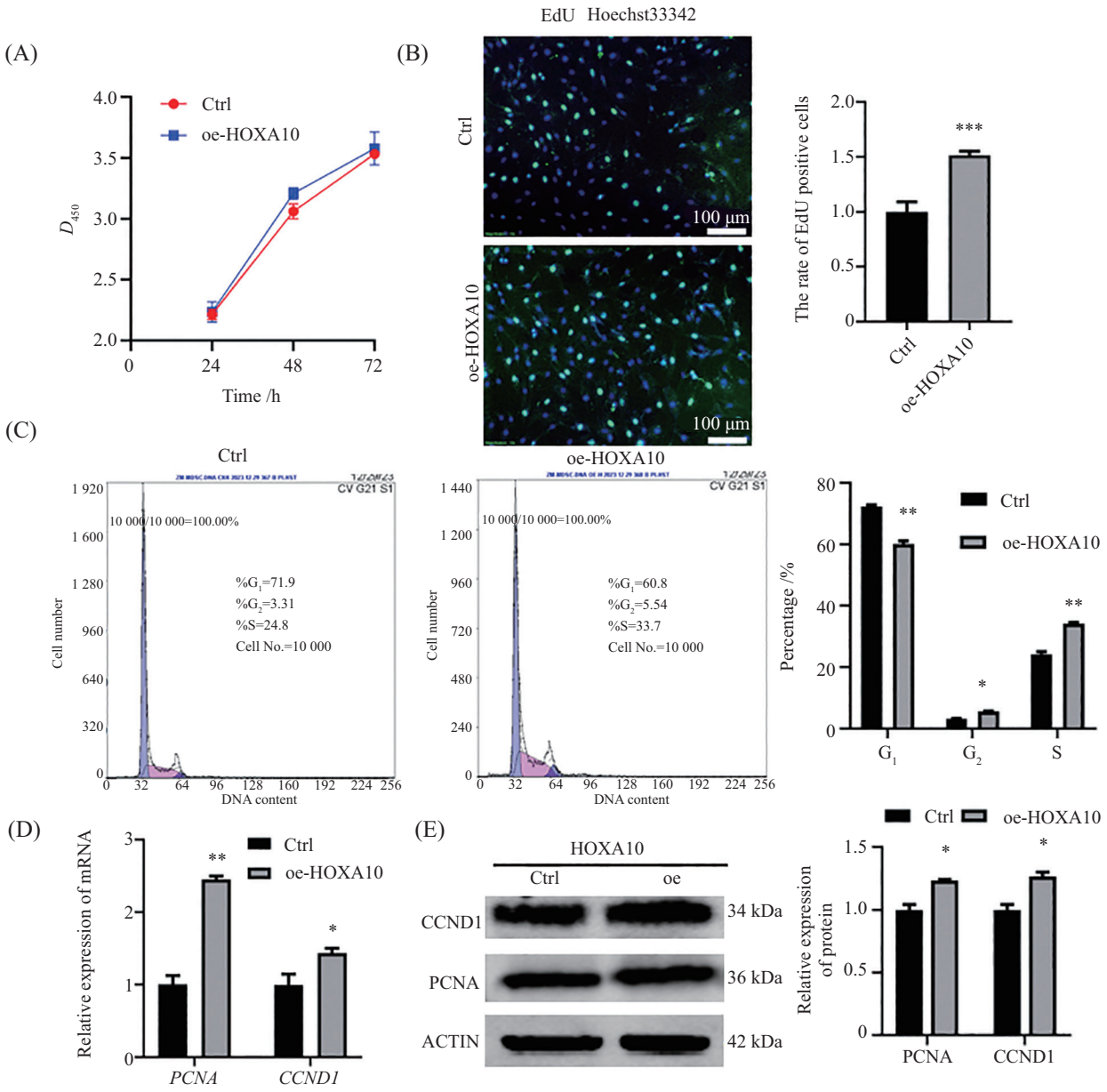
2.4 *HOXA10*基因抑制BSMSCs分化

为了检测*HOXA10*对BSMSCs分化的影响, 将构建的*HOXA10*基因的干扰和过表达载体转染BSMSCs, 利用免疫荧光检测细胞分化情况, 结果表明, 与对照组相比, 过表达*HOXA10*显著抑制BSMSCs肌管的形成(图5A); 成肌分化标志基因MyoG的mRNA和蛋白表达水平显著下调(*P*<0.01, *P*<0.001)(图5C和图5E)。干扰*HOXA10*表达后, 实验得到相反结果(图5B、图5D和图5F)。

3 讨论

骨骼肌的生长与发育是一个十分复杂的过程, 该过程主要包括细胞增殖、退出细胞周期、与肌肉相关的特异性蛋白开始表达、肌源细胞发生融合并形成多核肌管、进一步形成具有一定收缩功能的肌纤维, 最后肌纤维生长形成骨骼肌[12]。研究证实, SMSCs的增殖与分化受到多种转录因子和内在信号通路的影响[13]。miR-142a-3p通过抑制*MEF2A*的表达抑制骨骼肌的增殖和分化[14], Lnc721靶向*MMP9*调控BSMSCs增殖分化[15]。转录因子KLF5、SIX2、FoxO1均被证实对SMSCs增殖分化有重要调控作用[16-18]。然而转录因子HOXA10对BSMSCs增殖分化的影响未见报道。

*HOXA10*作为同源框家族成员之一, 可通过直



A: CCK-8检测过表达*HOXA10*对细胞活力的影响; B: EdU检测过表达*HOXA10*对细胞增殖的影响及统计分析; C: FCM检测过表达*HOXA10*对细胞周期的影响及统计分析; D: 过表达*HOXA10*对增殖标志性基因*PCNA*、*CCND1* mRNA的影响; E: 过表达HOXA10对PCNA、CCND1蛋白的影响及统计图; Ctrl: 对照组; sh-HOXA10(sh): 干扰*HOXA10*基因; oe-HOXA10(oe): 过表达*HOXA10*基因; EdU(绿色), Hoechst33342(蓝色)染色显示细胞核; **P*<0.05, ***P*<0.01, ****P*<0.001, 与对照组比较。

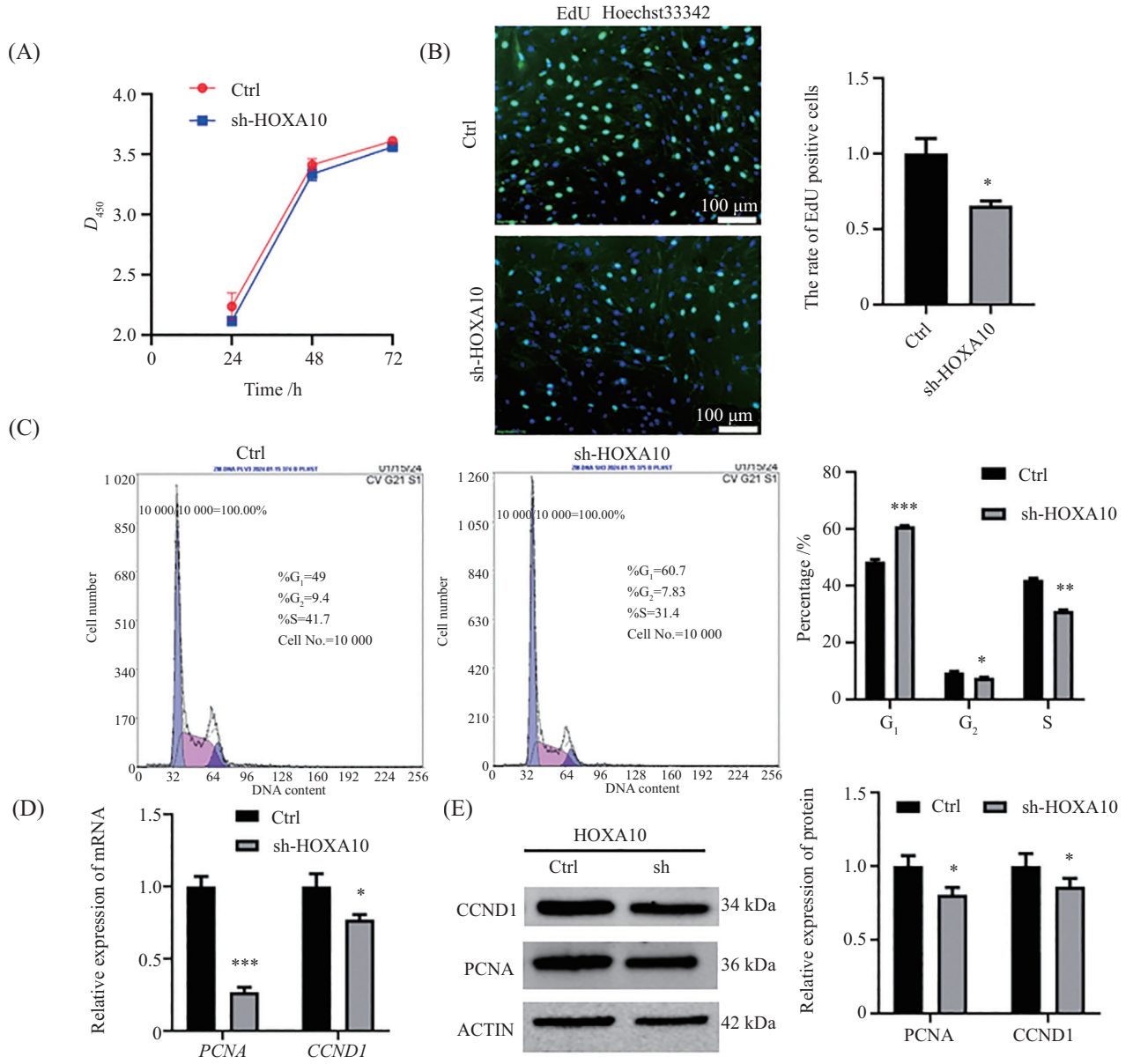
A: CCK-8 was used to detect the effect of overexpression of *HOXA10* on cell viability. B: effect of EdU overexpression of *HOXA10* on cell proliferation and statistical analysis; C: effect of *HOXA10* overexpression on cell cycle detected by FCM and statistical analysis; D: effect of overexpression of *HOXA10* on the proliferation hallmark genes *PCNA* and *CCND1* mRNA; E: effect of overexpression of HOXA10 on the protein of PCNA and CCND1, and its statistical chart; Ctrl: control group; sh-HOXA10 (sh): interferes with the *HOXA10* gene; oe-HOXA10 (oe): overexpression of the *HOXA10* gene; EdU (green), Hoechst33342 (blue) staining showing nuclei; **P*<0.05, ***P*<0.01, ****P*<0.001 compared with control group.

图3 过表达*HOXA10*对BSMSCs增殖的影响

Fig.3 Effect of *HOXA10* overexpression on the proliferation of BSMSCs

接或间接作用对人体多个系统的生长发育起到调节作用[19]。研究报道*HOXA10*可直接激活*RUNX2*及其他成骨细胞表型基因来控制成骨细胞生成[20]。为了研究*HOXA10*基因是否在BSMSCs增殖分化过程中存在调控作用, 本实验首先检测在BSMSCs增殖不同天数时*HOXA10*基因表达水平是否存在变化, 结果表明随着增殖天数的增加, *HOXA10*表达水平显著升高, 提示其可能在BSMSCs增殖过程中发挥作用。

为了研究*HOXA10*基因在BSMSCs增殖过程中的作用, 本实验构建了*HOXA10*基因过表达及干扰



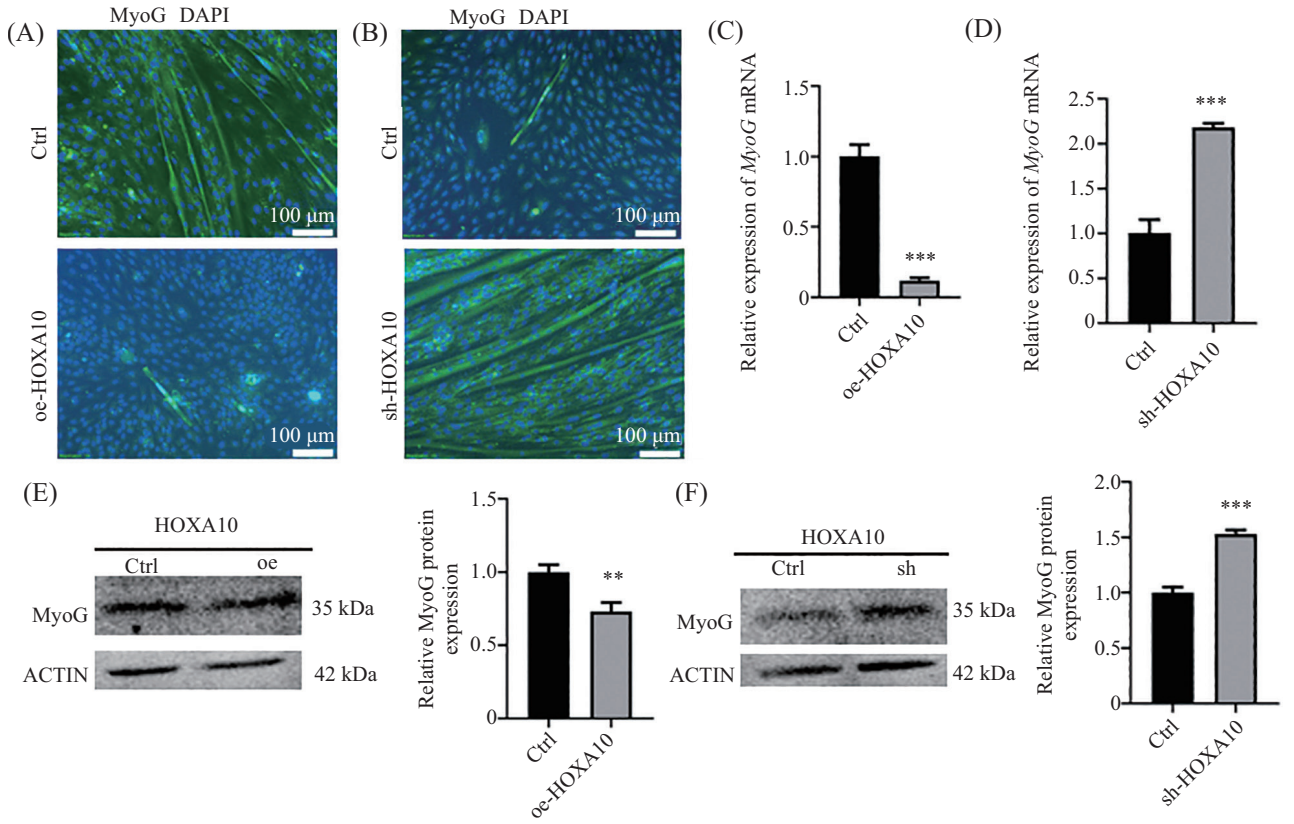
A: CCK-8检测干扰*HOXA10*对细胞活力的影响; B: EdU检测干扰*HOXA10*对细胞增殖的影响及统计分析; C: FCM检测干扰*HOXA10*对细胞周期的影响及统计分析; D: 干扰*HOXA10*对增殖标志性基因*PCNA*、*CCND1* mRNA的影响; E: 干扰HOXA10对PCNA、CCND1蛋白的影响及统计图; Ctrl: 对照组; sh-HOXA10(sh): 干扰*HOXA10*基因; oe-HOXA10(oe): 过表达*HOXA10*基因; EdU(绿色), Hoechst33342(蓝色)染色显示细胞核; **P*<0.05, ***P*<0.01, ****P*<0.001, 与对照组比较。

A: CCK-8 detected the effect of *HOXA10* interference on cell viability; B: EdU detection of the effect of *HOXA10* interference on cell proliferation and statistical analysis; C: effect of *HOXA10* interference on cell cycle detected by FCM and statistical analysis; D: effect of interfering with *HOXA10* on the proliferation hallmark genes *PCNA* and *CCND1* mRNA; E: effect of HOXA10 interference on PCNA and CCND1 proteins, and statistical figures; Ctrl: control group; sh-HOXA10 (sh): interferes with the *HOXA10* gene; oe-HOXA10 (oe): overexpression of the *HOXA10* gene; EdU (green), Hoechst33342 (blue) staining showing nuclei; **P*<0.05, ***P*<0.01, ****P*<0.001 compared with control group.

图4 干扰*HOXA10*对BSMSCs增殖的影响

Fig.4 Effect of *HOXA10* interference on the proliferation of BSMSCs

载体, 检测了*HOXA10*基因变化对BSMSCs增殖的影响, 结果显示, *HOXA10*基因能提高细胞活力, EdU阳性细胞比例显著升高。这与*HOXA10*促进食管癌(esophageal carcinoma, EC)细胞增殖, 抑制细胞凋亡, 从而促进细胞活力升高的机制相似[21]。这一结果在头颈部鳞状细胞癌(head and neck squamous-cell carcinoma, HNSCC)中也得到证实[22]。细胞周期是指能够持续分裂的真核细胞从上一次有丝分裂结



A: 免疫荧光检测过表达*HOXA10*对分化的影响; B: 免疫荧光检测干扰*HOXA10*对分化的影响; C: 过表达*HOXA10*对*MyoG* mRNA的影响; D: 干扰*HOXA10*对*MyoG* mRNA的影响; E: 过表达*HOXA10*对MyoG蛋白的影响; F: 干扰*HOXA10*对MyoG蛋白的影响; Ctrl: 对照组; sh-HOXA10(sh): 干扰*HOXA10*基因; oe-HOXA10(oe): 过表达*HOXA10*基因; MyoG(绿色), DAPI(蓝色)染色显示细胞核。**P<0.01, ***P<0.001, 与对照组比较。
A: effect of overexpression of *HOXA10* on differentiation was detected by immunofluorescence; B: effect of *HOXA10* interference on differentiation was detected by immunofluorescence; C: effect of overexpression of *HOXA10* on *MyoG* mRNA; D: effect of *HOXA10* interference on *MyoG* mRNA; E: effect of overexpression of *HOXA10* on MyoG protein; F: effect of *HOXA10* interference on MyoG protein; Ctrl: control group; sh-HOXA10 (sh): interferes with the *HOXA10* gene; oe-HOXA10 (oe): overexpression of the *HOXA10* gene; MyoG (green), DAPI (blue) staining showing nuclei. **P<0.01, ***P<0.001 compared with control group.

图5 *HOXA10*对BSMSCs分化的影响

Fig.5 Effect of *HOXA10* on the differentiation of BSMSCs

束后到下一次分裂完成时为止的全过程, 包括分裂间期和分裂期, 分裂间期又分为DNA合成前期(G_1期)、DNA合成期(S期)和DNA合成后期(G_2期)[23]。在肝细胞癌((hepatocellular carcinoma, HCC)的研究中发现干扰*HOXA10*基因可导致细胞周期停滞在G_0/G_1期、细胞凋亡加快、*CCND1*和*PCNA*的表达量降低[24]。为验证*HOXA10*是否通过改变细胞周期分布来促进细胞增殖, 采用FCM检测细胞周期。结果显示过表达*HOXA10*基因后BSMSCs细胞G_1期比例降低, S期比例升高, 提示*HOXA10*可使细胞进入细胞周期进程加快。细胞周期进程受到多种因素调控, 细胞周期蛋白D1(cyclin D1, CCND1)可与*CDK4*或*CDK6*结合影响细胞G_1期到S期的转变[25]。为验证*HOXA10*基因是否影响*CCND1*的表达, 通过qRT-PCR和Western blot检测了CCND1 mRNA和蛋白表达量, 结果证实过表达*HOXA10*基因能促进BSMSCs *CCND1*基因的表达。*CCND1*与增殖细胞核抗原*PCNA*是细胞增殖的标志基因[26], 因此, 本实验检测了PCNA mRNA和蛋白的表达, 结果表明, 过表达*HOXA10*基因能促进BSMSCs *PCNA*基因的表达, 综上可知, *HOXA10*基因可促进BSMSCs增殖。

SMSCs成肌分化过程是连续动态的变化过程, 当SMSCs增殖到达一定程度时, 细胞退出细胞周期, 通过相互融合或与已存在的肌纤维融合而分化形成肌管[27]。通过免疫荧光染色检测肌管形成情况, 结果显示*HOXA10*基因能抑制BSMSCs肌管的

形成。*MyoG*是成肌调节因子(Myogenic regulatory factors, MRFs)家族成员之一, 可在所有骨骼肌细胞系中表达[28]。*MyoG*基因可以激活调节肌肉的基因转录, 促进肌细胞分化, 调控肌纤维的融合[29]。本实验通过qRT-PCR和Western blot检测MyoG mRNA和蛋白表达情况, 结果显示过表达*HOXA10*基因抑制*MyoG*基因的表达, 结合肌管形成实验可知, *HOXA10*基因可抑制BSMSCs分化。

综上所述, *HOXA10*基因能促进BSMSCs增殖, 抑制其分化。但*HOXA10*基因促进BSMSCs增殖的机制尚需进一步研究。本研究可为进一步挖掘*HOXA10*基因调控BSMSCs增殖分化机制提供参考。

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