TFEB介导的自噬溶酶体通路影响角质形成细胞分泌TGF-β1的机制研究

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摘要 该研究探讨了转录因子EB(transcription factor EB, TFEB)介导的自噬溶酶体通路对角质形成细胞(keratinocyte, KC)分泌转化生长因子β1(transforming growth factor β1, TGF-β1)的影响及机制。以人皮肤KC为研究对象, 分为对照组、血清刺激组、血清刺激+磺酸去氧胆酸(tauroursodeoxychloic acid, TUDCA)组、血清刺激+TFEB siRNA组、血清刺激+NC siRNA组和血清刺激+氯喹组, 再用KC条件培养基培养成纤维细胞(fibroblast, FB), ELISA检测KC上清液中TGF-β1含量, Western blot检测KC内质网应激相关蛋白(GRP78、p-PERK)、自噬相关蛋白(LC3、LAMP1、TFEB)、凋亡相关蛋白(p-elF2α、CHOP、caspase-3)的表达和FB平滑肌动蛋白α(smooth muscle actin α, α-SMA)、I型胶原(collagen I, COL I)的表达。血清刺激后, 免疫荧光染色检测KC内TGF-β1与LAMP1、LC3共定位。加用氯喹后, 免疫荧光染色检测KC内Rab8a与TGF-β1、LAMP1共定位。与对照组比较, 血清刺激能诱导KC分泌TGF-β1增多($P<0.01$), 上调细胞内质网应激(增加GRP78、p-PERK表达, $P<0.01$)和细胞自噬水平(增加TFEB、LC3 II、LAMP1表达, $P<0.01$)并增加FB α-SMA、COL I蛋白表达($P<0.01$)。加用磺酸去氧胆酸后, p-PERK和GRP78($P<0.05$)表达降低, TFEB、LC3 II、LAMP1($P<0.05$)表达降低。与血清刺激组比较, siRNA敲低TFEB表达后, KC分泌TGF-β1明显下降($P<0.01$), 内质网应激下游凋亡相关蛋白p-elF2α、CHOP、caspase-3表达增强($P<0.01$), FB的α-SMA、COL I蛋白表达减弱($P<0.01$)。血清刺激后, 免疫荧光显示, KC细胞内TGF-β1与LAMP1($P<0.01$)、LC3($P<0.01$)共定位程度明显增加。而与血清刺激组比较, 加用氯喹后, 膜分泌蛋白Rab8a与TGF-β1($P<0.05$)、LAMP1($P<0.01$)共定位程度显著减少, TGF-β1细胞外分泌减少($P<0.05$)。TFEB介导的自噬不仅通过降解途径清除错误折叠蛋白, 还通过参与TGF-β1分泌来降低内质网内蛋白负荷、抑制凋亡相关的caspase激活, 从而减少KC损伤。

关键词 角质形成细胞; TGF-β1; 内质网应激; 自噬; TFEB

The Effect of TFEB-Induced Autophagy on TGF-β1 Secretion of Keratinocyte

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Abstract In present study, We investigated the effect of TFEB-induced autophagy on TGF-β1 secretion of keratinocytes and its possible mechanism. Human cutaneous KC was divided into control group, serum stimulation group, serum stimulation+TUDCA group, serum stimulation+TFEB siRNA group, serum stimulation+NC siRNA

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group and serum stimulation+chloroquine group. The secretion of TGF-β1 in keratinocytes was detected by ELISA kit. The expression of p-PERK, GRP78, TFEB, LC3, LAMP1, p-elF2α, CHOP and caspase-3 in keratinocytes was studied by Western blot. And the expression of α-SMA and COL I in firboblasts cultured with conditioned culture medium was also studied by Western blot. Inhibiting TFEB expression of keratinocytes by RNA interference, the secretion of TGF-β1 and the expression of p-elF2α, CHOP and caspase-3 was detected. And the expression of α-SMA and COL I in firboblasts was also studied by Western blot. After serum stimulation, co-localization of TGF-β1 and LAMP1, TGF-β1 and LC3 in keratinocytes was detected by immunofluorescence staining. After the addition of chloroquine, an autophagic lysosome pathway inhibitor, the co-localization of Rab8a and TGF-β1, Rab8a and LAMP1 in keratinocytes was detected by immunofluorescence staining. Compared with control group, serum stimulation can induce TGF-β1 secretion of keratinocytes, causing cell endoplasmic reticulum stress (increase GRP78, p-PERK expression, P<0.01), increase cell autophagy level (increase TFEB, LC3 II, LAMP1 expression, P<0.01) and increase the α- SMA, COL I protein expression of fibroblasts (P<0.01). After the addition of Sulfonic acid deoxycholic acid, an endoplasmic reticulum stress inhibitor, compared with serum stimulus group, the expression of GRP78 and p-PERK (P<0.05) decreased and the expression of TFEB, LC3 II and LAMP1 (P<0.05) in keratinocytes also decreased. After inhibiting TFEB expression of keratinocytes by RNA interference, compared with serum stimulus group, the secretion of TGF-β1 decreased obviously (P<0.01), and the expression of p-elF2α, CHOP and caspase-3 (P<0.01) in keratinocytes increased and the expression of α- SMA, COL I (P<0.01) in fibroblasts decreased significantly. Immunofluorescence showed that the co-localization level of TGF-β1 and LAMP1 (P<0.01), TGF-β1 and LC3 (P<0.01) in KC was significantly enhanced after serum stimulation. After addition of chloroquine, the co-localization level of Rab8a and TGF-β1 (P<0.05), Rab8a and LAMP1 (P<0.01) was significantly reduced, and the secretion of TGF-β1 was reduced (P<0.05). We concluded that TFEB-mediated autophagy reduces protein load in the endoplasmic reticulum and inhibits apoptosis-related caspase protein activation by clearing misfolded proteins and participating in TGF-β1 secretion, thereby reducing keratinocytes damage.

Keywords keratinocyte; TGF-β1; endoplasmic reticulum stress; autophagy; TFEB

增生性瘢痕(hypertrophic scar, HS)是一种创伤后的病理愈合状态, 通常发生在手术、创伤和烧伤之后, 对患者精神和身体带来严重影响。HS的病理特点是伤口愈合过程中成纤维细胞过度增殖并导致细胞外基质生成过多, 而转化生长因子β1(transforming growth factor β1, TGF-β1)促进皮肤纤维细胞(fibroblast, FB)活化和增加细胞外基质生成, 被认为是与增生性瘢痕形成关系最密切, 也是最具代表性的重要细胞因子[1-2]。分化的角质形成细胞(keratinocyte, KC)是转化生长因子的重要来源[3]。但目前对影响KC分化和TGF-β1合成及分泌的具体分子生物学机制还缺乏足够的了解。有研究结果暗示, 自噬可能对KC增殖和分化有着影响[4]。但自噬在瘢痕形成过程中影响KC TGF-β1分泌的确切机制还不清楚, 更好地理解这个问题可能为瘢痕诊治提供新的治疗靶点。因此, 我们通过KC的血清刺激模型[5], 研究TFEB介导的自噬溶酶体通路对其分泌功能的影响。

1 材料与方法

1.1 材料

兔抗LC3购自美国Sigma公司; 鼠抗COL I及兔抗TFEB、抗LAMP-1、抗α-SMA、抗GRP78、抗caspase-3、抗eIF2α、抗p-eIF2α(ser52)、抗PERK、抗p-PERK(T982)购自美国Abcam公司; 鼠抗CHOP购自美国Santa cruz公司; 山羊抗Rab8a购自美国Abnova公司; 鼠抗β-actin抗体购自碧云天生物技术公司; 兔抗鼠及羊抗兔IR Dye 700 CW及800 CW购自LI-COR Biosciences公司; 驴抗鼠IgG-Alexa Fluor 488、驴抗兔IgG-Alexa Fluor 555、羊抗兔IgG-Alexa Fluor 647、驴抗山羊IgG-Alexa Fluor 647购自Invitrogen公司; 人TGF-β1 ELISA试剂盒购自美国R&D公司; TFEB siRNA和siRNA阴性对照购自武汉枢密科技公司; KSFM培养基、DMEM培养基及小牛血清购自美国Gibco公司; 其余试剂购自武汉博士德生物工程有限公司。PVDF膜购自美国Millipore公

司; SDS凝胶电泳及转印装置购自美国BioRad公司; Odyssey凝胶图像处理系统购自基因有限公司; 小型台式冷冻离心机购自德国Eppendorf公司; 激光共聚焦显微镜购自德国Zeiss公司。

1.2 方法

1.2.1 细胞培养、细胞分组及干预 KC原代培养和传代: 健康男性包皮环切术后遗弃的皮肤组织, 消毒、剪碎后用0.25%真表皮分离酶于4 °C消化24 h, 分离表皮用0.25%胰酶于37 °C消化、过滤、离心、重悬, 接种于培养皿中, 培养4 h后, 换液去除未贴壁细胞, 置于KSFM培养基中培养, 每3天换液。实验入选患者均知情并同意, 且本实验获得武汉大学中南医院伦理委员会批准。

FB原代培养和传代: 健康男性包皮组织, 消毒、剪碎后用0.25%真表皮分离酶于4 °C消化24 h, 分离表皮用0.25%胰酶与37 °C消化、过滤、离心、重悬, 接种于培养瓶中, 加入含10%小牛血清的DMEM培养基, 每3天换液。待细胞80%融合后用0.25%胰酶消化传代, 实验用2~4代FB。

细胞分组: 原代培养的KC分为对照组、血清刺激组(简称血清组)、血清+磺酸去氧胆酸(tauroursodeoxychloic acid, TUDCA)组、血清+TFEB siRNA组、血清+NC siRNA组、血清+氯喹组。血清组加用10% FCS, 刺激12 h; 血清+TUDCA组在10% FCS刺激同时, 加入终浓度为2.5 μmol/L的TUDCA; 血清+TFEB siRNA组和血清+NC siRNA组在血清刺激前24 h加入50 nmol/L的TFEB siRNA或NC siRNA; 血清+氯喹组在血清刺激同时, 加入终浓度为10 μmol/L的氯喹。

1.2.2 FB条件培养基的获取 贴壁后的KC第2天更换新鲜的KSFM培养基, 继续培养24 h, 然后加入10% FCS, 刺激12 h后, 收集培养上清液, 用来培养FB。FB培养24 h后, 检测相关指标。

1.2.3 ELISA实验检测TGF-β1含量 取正常培养、血清刺激、转染TFEB siRNA后的KC培养基入1.5 mL EP管中, 2 500 r/min离心5 min, 取上清置于新的1.5 mL EP管中, 按ELISA说明书步骤倍比稀释标准品、加样、孵育及显色。加入终止液100 μL/孔, 混匀后立即450 nm处测定*D*值。

1.2.4 蛋白印迹试验 每孔蛋白上样量30 μg, 电泳、转膜后, 5%脱脂奶粉室温封闭2 h; 一抗(抗体1:1 000稀释; 抗β-actin抗体1:10 000稀释) 4 °C孵育过夜; 1× TBST液洗3次, 每次5 min; 荧光二抗(羊抗兔1:10 000, 兔抗鼠1:10 000)室温摇床孵育1 h; 1× TBST液洗3次, 每次10 min; 将PVDF膜置于Odyssey凝胶图像处理系统显影。

1.2.5 免疫荧光染色 KC爬片, 2%多聚甲醛固定10 min, 0.2% Triton X-100透膜10 min, 0.01 mol/L PBS洗3次, 每次5 min; 5% BSA室温封闭1 h; 0.01 mol/L PBS洗2次, 每次5 min; 一抗鼠抗COL I(1:100)、兔抗LAMP-1(1:150)、兔抗LC3(1:150)、羊抗Rab8a(1:100) 4 °C孵育过夜, 室温复温1 h; 0.01 mol/L PBS洗3次, 每次10 min; 驴抗IgG-Alexa Fluor荧光二抗(1:250)室温避光保湿2 h; 0.01 mol/L PBS洗4次, 每次5 min; DAPI封片, 置于激光共聚焦显微镜采集图像。Image pro plus 6.0软件进行线性相关性分析。不加一抗的封闭液作为阴性对照。

1.3 统计学方法

采用SPSS 20.0软件进行分析, 数据采用$\bar{x}\pm s$表示。组间用*t*检验分析, $P<0.05$为差异有统计学意义。

2 结果

2.1 血清刺激对KC分泌TGF-β1、FB表型转化和胶原生成的影响

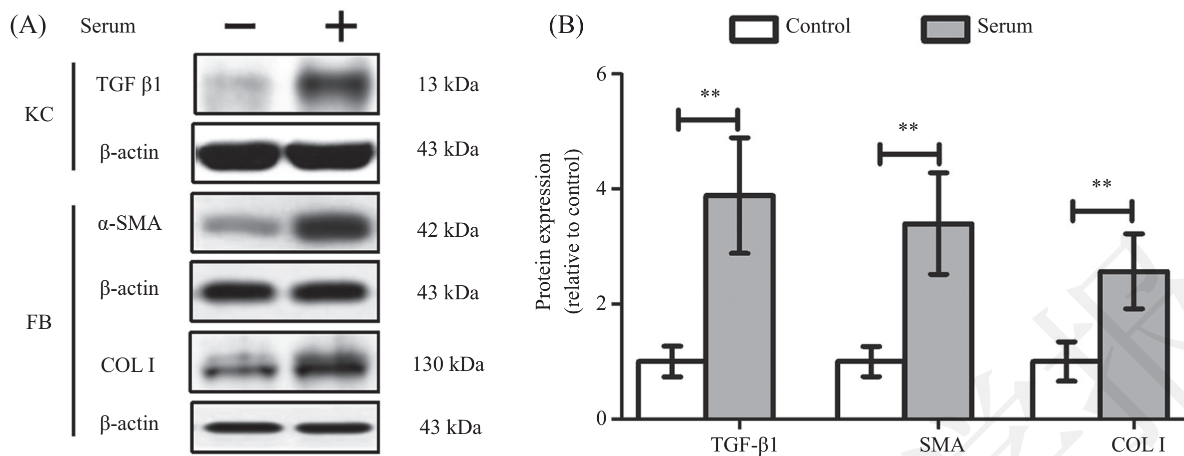
与对照组比较, 血清刺激后, KC表达(t=9.24, P<0.01)和分泌TGF-β1增多(t=10.30, P<0.01), 用条件培养基培养的FB, 其α-SMA(t=5.72, P<0.01)、COL I(t=4.77, P<0.01)蛋白表达增强(图1), 提示KC分泌的TGF-β1促进FB向肌成纤维细胞表型转化, 增加 I型胶原生成。

2.2 血清刺激通过内质网应激上调自噬相关蛋白的表达

与对照组比较, 血清刺激后, KC内质网应激相关蛋白GRP78(t=5.87, P<0.01)和p-PERK(t=7.13, P<0.01)表达增高(图2), 自噬相关蛋白TFEB(t=5.12, P<0.01)、LC3 II(t=4.37, P<0.01)和LAMP1(t=7.31, P<0.01)表达增加(图3)。加用内质网应激抑制剂TUDCA后, 与血清刺激组比较, KC内质网应激相关蛋白GRP78(t=3.21, P<0.05)和p-PERK(t=6.60, P<0.01)表达降低(图2), 自噬相关蛋白TFEB(t=4.42, P<0.01)、LC3 II(t=3.05, P<0.05)和LAMP1(t=3.62, P<0.05)表达降低(图3)。提示血清刺激分化的KC通过内质网应激上调自噬, 抑制内质网应激可下调自噬水平。

2.3 敲低TFEB对KC凋亡、TGF-β1分泌及FB表型转化、胶原生成的影响

与血清刺激组相比, 通过转染siRNA敲低KC

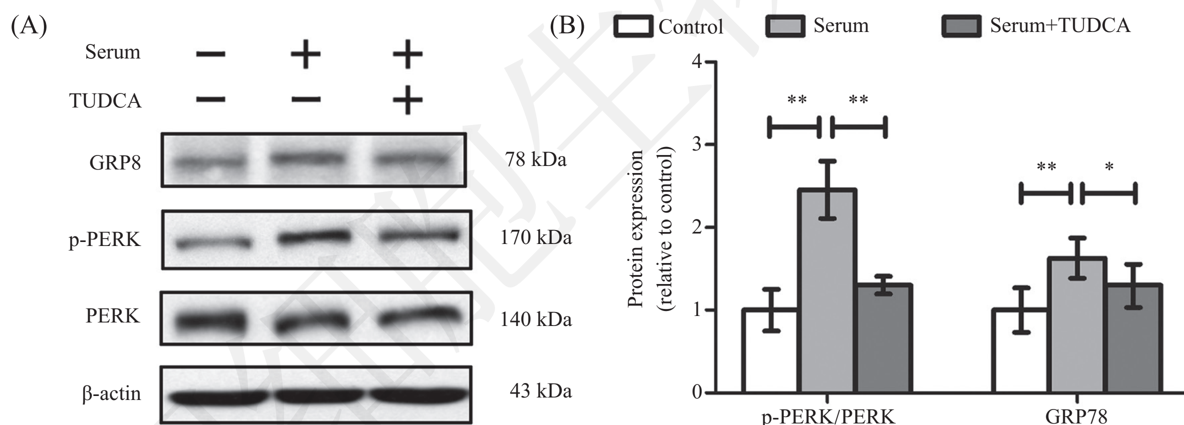


A: Western blot检测10%FCS刺激KC 12 h后TGF-β1蛋白表达量; KC培养上清液培养FB, 24 h后α-SMA和COL I蛋白表达量; B: TGF-β1、α-SMA和COL I条带灰度值半定量分析, 柱条为各蛋白与其对应的β-actin条带灰度值相比, 并以对照组做标准化(n=6, **P<0.01)。

A: Western blot was used to detect the expression of TGF-β1 in keratinocytes after serum stimulation for 12 hours. Expression of α-SMA and COL I in fibroblasts cultured in conditioned medium for 24 hours. B: semi-quantitative analysis of band gray value of TGF-β1, α-SMA and COL I bands. The bars were compared with the gray values of each protein and its corresponding β-actin bands, and standardized with the control group (n=6, **P<0.01).

图1 血清刺激增加KC表达TGF-β1, 增加FB表达α-SMA和COL I

Fig.1 Serum stimulation increases the expression of TGF-β1 in KC and the expression of α-SMA and COL I in FB



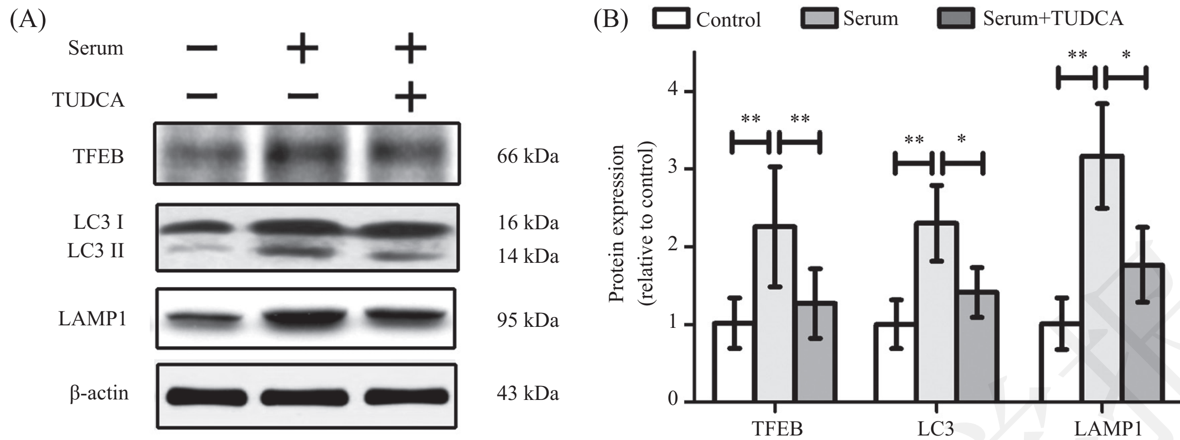
A: Western blot检测KC经不同处理(10% FCS或2.5 μmol/L的TUDCA) 12 h后GRP78、p-PERK、PERK蛋白表达量; B: GRP78、p-PERK/PERK条带灰度值半定量分析, 柱条为各蛋白与其对应的β-actin条带灰度值相比, 并以对照组做标准化(n=6, *P<0.05, **P<0.01)。

A: Western blot was used to detect the expression of GRP78, p-PERK and PERK in keratinocytes after serum stimulation or TUDCA treatment for 12 hours; B: semi-quantitative analysis of band gray value of GRP78 and p-PERK/PERK bands. The bars were compared with the gray values of each protein and its corresponding β-actin bands, and standardized with the control group (n=6, *P<0.05, **P<0.01).

图2 KC内质网应激相关蛋白表达

Fig.2 The expression of proteins related to ER stress in KC

TFEB表达, KC内质网应激下游凋亡相关通路蛋白p-elF2α(t=6.81, P<0.01)、CHOP(t=9.06, P<0.01)、cleaved caspase-3(t=5.78, P<0.01)表达增强(图4), 显示当TFEB介导的自噬通路被抑制后, 内质网应激通过p-PERK/p-p-elF2α/CHOP触发细胞凋亡。同时KC内TGF-β1含量(图5A和图5B, t=0.72, P>0.05)虽无明显下降, 但KC细胞外分泌TGF-β1水平显著降低(图5C, t=6.45, P<0.01), 条件培养基培养的FB中α-SMA(图5A和图5B, t=3.15, P<0.05)、COL I(图5A和图5B, t=6.61, P<0.01)蛋白表达减少。与血清刺激组相比, NC-siRNA转染KC后p-elF2α(t=0.04, P>0.05)、CHOP(t=0.04, P>0.05)、cleaved caspase-3(t=0.32, P>0.05)表达无明显变化。提示TFEB介导的自噬可抑制凋亡相关的caspase激活, 减少细胞损伤, 维持KC的分化和细胞因

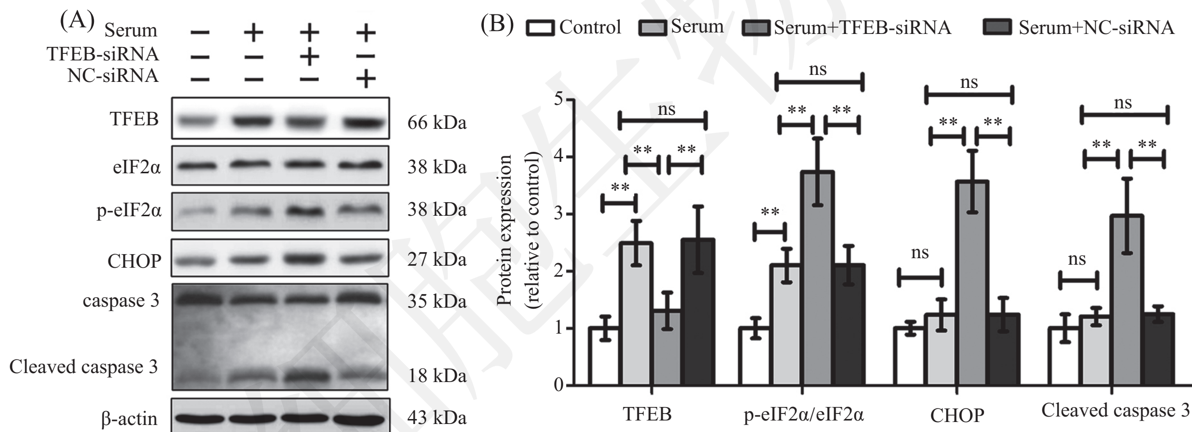


A: Western blot检测KC经不同处理(10% FCS或2.5 μmol/L的TUDCA) 12 h后TFEB、LC3和LAMP1蛋白表达量; B: TFEB、LC3 II/I和LAMP1条带灰度值半定量分析, 柱条为各蛋白与其对应的β-actin条带灰度值相比, 并以对照组做标准化(n=6, *P<0.05, **P<0.01)。

A: Western blot was used to detect the expression of TFEB、LC3 and LAMP1 in keratinocytes after serum stimulation or TUDCA treatment for 12 hours. B: semi-quantitative analysis of band gray value of TFEB, LC3 II/I and LAMP1 bands. The bars were compared with the gray values of each protein and its corresponding β-actin bands, and standardized with the control group (n=6, *P<0.05, **P<0.01).

图3 KC自噬相关蛋白表达

Fig.3 The expression of proteins related to autophagy in KC



A: KC经siRNA转染(50 nmol/L TFEB siRNA或NC siRNA) 24 h, 继续予以10% FCS刺激12 h, Western blot检测TFEB、eIF2α、p-eIF2α、CHOP、caspase 3蛋白表达量; B: TFEB、p-eIF2α/eIF2α、CHOP和cleaved caspase 3条带灰度值半定量分析, 柱条为各蛋白与其对应β-actin条带灰度值的相对表达量, 并以对照组做标准化(n=6, **P<0.01)。

A: Western blot was used to detect the expression of eIF2α, p- eIF2α, CHOP and caspase 3 in keratinocytes after siRNA transfection for 24 hours and continued serum stimulation for 12 hours; B: semi-quantitative analysis of band gray value of p-eIF2α/eIF2α, CHOP and cleaved caspase 3 bands. The bars were compared with the gray values of each protein and its corresponding β-actin bands, and standardized with the control group (n=6, **P<0.01).

图4 抑制TFEB介导的自噬促进内质网应激介导的细胞凋亡

Fig.4 Inhibition of TFEB-mediated autophagy promotes ER stress-mediated apoptosis

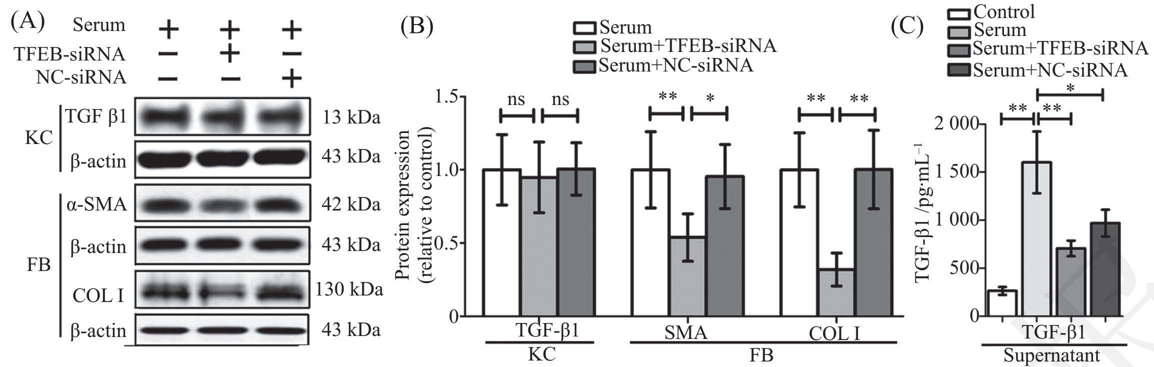
子分泌。

2.4 TFEB介导的自噬溶酶体通路参与KC分泌TGF-β1

免疫荧光(图6)显示, 与对照组比较, 血清刺激后, KC细胞内TGF-β1与LAMP1(t=7.89, P<0.01)、TGF-β1与LC3(t=9.23, P<0.01)共定位程度明显增强。而加用氯喹抑制自噬溶酶体通路后(图7), 与血清刺激组比较, Rab8a与TGF-β1(t=3.97, P<0.05)、Rab8a与LAMP1(t=6.73, P<0.01)共定位程度显著减少, TGF-β1分泌减少(t=3.56, P<0.05), 提示自噬参与TGF-β1的胞外分泌。

3 讨论

尽管目前许多学者对病理性瘢痕的发病机制

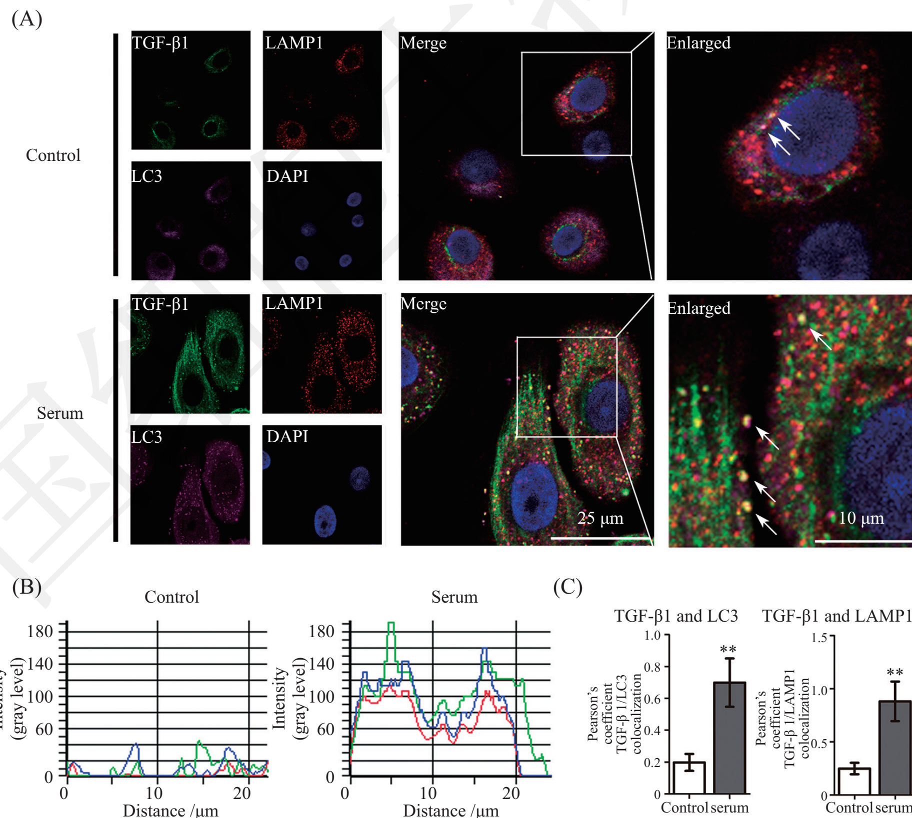


A: KC经siRNA转染(50 nmol/L TFEB siRNA或NC siRNA) 24 h, 继续予以10% FCS刺激12 h, Western blot检测KC TGF-β1蛋白表达量; KC培养上清液培养FB 24 h后Western blot检测FB α-SMA和COL I蛋白表达量; B: TGF-β1、α-SMA和COL I条带灰度值半定量分析, 柱条为各蛋白与其对应的β-actin条带灰度值相比, 并以对照组做标准化; C: 50 nmol/L TFEB siRNA转染KC 24 h, 分别予以10% FCS和/无10 μmol/L氯喹处理12 h后, ELISA检测不同组上清液TGF-β1含量(n=6, *P<0.05, **P<0.01)。

A: Western blot was used to detect the expression of TGF-β1 in keratinocytes after siRNA transfection for 24 hours and continued serum stimulation for 12 hours. Western blot was used to detect the expression of α-SMA and COL Iin fibroblasts cultured in conditioned medium for 24 hours. B: semi-quantitative analysis of band gray value of TGF-β1, α-SMA and COL I bands. The bars were compared with the gray values of each protein and its corresponding β-actin bands, and standardized with the control group. C: KC were transfected with 50 nmol/L TFEB siRNA for 24 hours and treated with 10% FCS and /or 10 μmol/L chloroquine for 12 hours respectively. The content of TGF-β1 in supernatant of different groups was detected by ELISA (n=6, **P<0.05, **P<0.01).

图5 抑制TFEB介导的自噬减少KC TGF-β1的分泌及FB表型转化、胶原生成

Fig.5 Inhibition of TFEB-mediated autophagy decreases the secretion of TGF-β1 in KC and reduces the phenotypic transformation and collagen production of FB

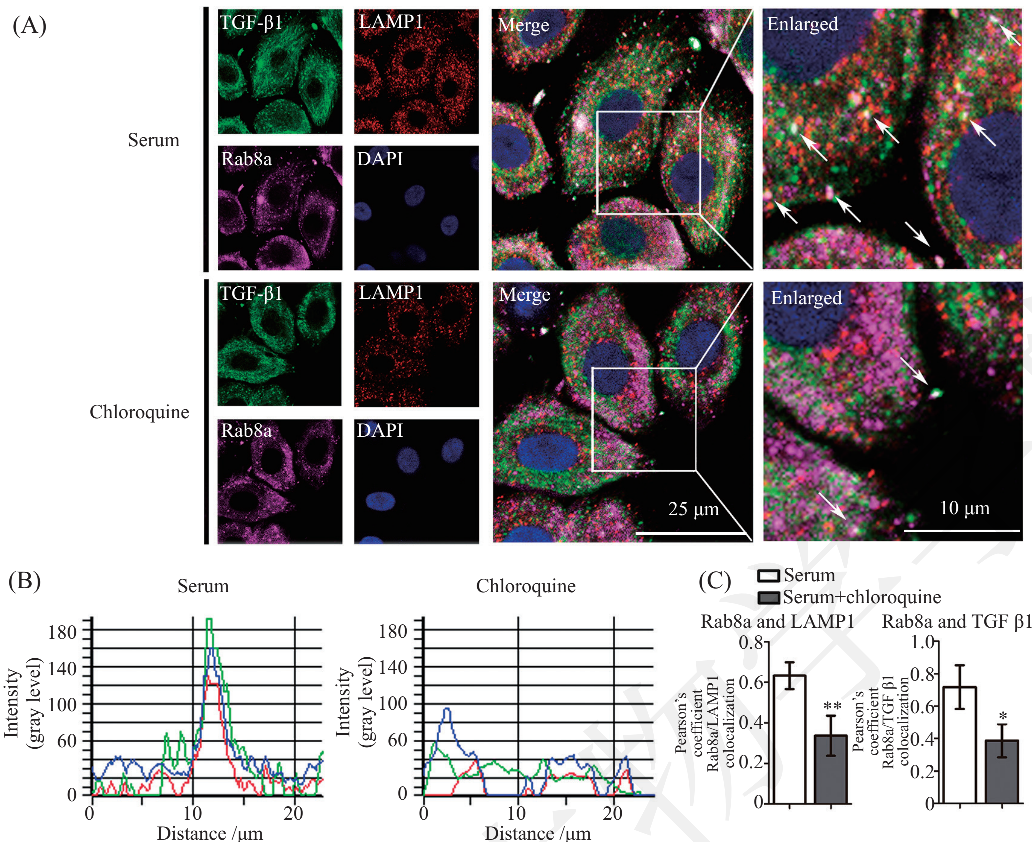


A: 细胞免疫荧光检测10% FCS刺激KC 12 h后KC TGF-β1、LC3和LAMP1的分布; B: A图TGF-β1分别与LC3、LAMP1共定位线性色度分析; C: Pearson's相关性分析计算TGF-β1分别与LC3、LAMP1共定位系数(n=6, **P<0.01)。

A: distribution of TGF-β1, LC3 and LAMP1 in KC was detected by cellular immunofluorescence after serum stimulation for 12 hours; B: linear chromaticity analysis for the co-location of TGF-β1 with LC3 and LAMP1. C: the Pearson's colocalization coefficient for TGF-β1 with LC3 and LAMP1 (n=6, **P<0.01).

图6 KC细胞内TGF-β1与LAMP1、TGF-β1与LC3共定位

Fig.6 The colocalization of TGF-β1 and LAMP1, TGF-β1 and LC3 in KC



A: 细胞免疫荧光检测10% FCS和/无10 μmol/L氯喹处理12 h后, KC Rab8a和TGF-β1、LAMP1的分布; B: A图Rab8a和TGF-β1、LAMP1共定位线性色度分析; C: Pearson's相关性分析计算Rab8a分别与TGF-β1、LAMP1共定位系数(n=6, *P<0.05, **P<0.01)。

A: distribution of TGF-β1, LAMP1 and Rab8a in KC was detected by cellular immunofluorescence after serum with /without 10μmol/L chloroquine stimulation for 12 hours. B: linear chromaticity analysis for the co-location of Rab8a with TGF-β1 and LAMP1. C: the Pearson colocalization coefficient for Rab8a with TGF-β1 and LAMP1 (n=6, *P<0.05, **P<0.01).

图7 氯喹减少KC内Rab8a与TGF-β1、Rab8a与LAMP1共定位

Fig.7 Chloroquine decreases the colocalization of Rab8a and TGF-β1, Rab8a and LAMP1 in KC

进行了研究, 但其分子机制仍未完全阐明且缺乏理想的防治手段。以往对于瘢痕的研究主要集中于FB, 但最近的研究表明, 分化的KC可通过产生多种细胞因子, 包括TGF-β1, 影响FB的增殖和基质积累, 在病理性纤维化发展中发挥重要作用[3]。我们结果也显示, 通过血清刺激模拟创面愈合, KC分泌TGF-β1增加, 并促进FB表型转化及胶原合成。

TGF-β1作为前蛋白单体产生, 需要在内质网中适当折叠、形成二聚体, 并输出至高尔基体剪接形成复合体[6]。内质网负责分泌蛋白和跨膜蛋白的合成、折叠、质量控制和降解, 而随着分化的KC合成和分泌细胞因子增多, 会增加对内质网折叠能力的需求。当错误折叠或未折叠蛋白的积累超过内质网处理能力时, 通过磷酸化PERK等启动信号触发内质网应激及激活未折叠蛋白反应(unfolded protein response, UPR)[7-8]。近年来在肺和肝脏纤维化研究中发现, UPR通过下游信号可继发自噬水平改变[10], 我们研究结果显示, 血清刺激后KC内质网应激指标上调的同时, KC自噬水平上调, 而加用内质网应激抑制剂TUDCA后, 其自噬水平降低。提示血清刺激分化的KC通过内质网应激上调自噬。

UPR通过重新平衡蛋白质的负载和折叠, 减缓压力并促进细胞内稳态的重建; 然而, 在长时间的内质网应激下, UPR的激活也可通过CHOP触发细胞凋亡程序[9]。因此, 我们进一步通过TFEB siRNA转染KC下调自噬, 观察细胞凋亡和TGF-β1分泌变化, 以确定KC血清刺激后继发的自噬在这一过程中扮演的角色。TFEB是一种新近发现的转录因子。在正常条件下, TFEB被磷酸化并隔离在细胞质中, 呈失功能状态; 在饥饿、氧化等损伤或刺激下, TFEB去磷酸化并转运至细胞核, 激活自噬和溶酶体生物发生相关基因的转录[11], 在调节溶酶体生物发生和自

噬等基本细胞过程中起着关键作用[12]。结果显示, 敲低*TFEB*表达后, KC内质网应激相关指标进一步上升, 并进而导致CHOP和凋亡效应蛋白cleaved caspase 3表达上调。提示在内质网应激条件下, 自噬通过清除错误折叠/未折叠的蛋白和受损的细胞器, 可降低内质网压力, 抑制凋亡相关的caspase激活, 减少细胞损伤, 维持KC的分化和存活。

同时, 我们进行自噬指标的免疫荧光染色过程中发现, 血清刺激后, TGF-β1与LC3、LAMP1存在高度共定位现象。自噬是一个膜单位形成的过程, 用于形成自噬体膜的来源仍不清楚, 包括内质网、高尔基体、循环内质体等都涉及其中[13]。TGF-β1在内质网–高尔基体体系输送并向细胞外分泌过程中, 是否同样有自噬体的参与？最近研究也表明, 自噬除了降解功能外, 在非自噬过程中也发挥作用, 特别是在分泌途径中的角色引起研究者的兴趣[14]。由此, 我们猜测自噬也参与TGF-β1的分泌。我们进一步检测了TGF-β1、LAMP1与Rab8a的共定位。Rab8a是细胞膜极化分选的调节因子, 被证明对分泌性自噬至关重要[15]。结果显示血清刺激后, KC细胞存在TGF-β1、LAMP1与Rab8a共定位。而加用自噬溶酶体抑制剂氯喹后, 共定位程度显著减少, 且培养液中TGF-β1分泌量显著减少, 这些结果均提示自噬溶酶体通路参与了TGF-β1向细胞外的分泌过程, 但Rab8a阳性的自噬囊泡如何到达细胞表面, 以及控制释放到细胞外环境的因素仍有待确定。

综上所述, 分化的KC内质网应激增加, 继发上调的自噬不仅通过降解途径清除错误折叠/未折叠的蛋白和受损的细胞器, 还通过参与TGF-β1分泌, 降低内质网内蛋白负荷; 抑制凋亡相关的caspase激活, 减少细胞损伤。我们的结果能帮助更好地理解自噬在瘢痕形成中的角色及其具体作用机制, 并且提示以调节自噬和溶酶体生物发生的途径为靶点, 可能为瘢痕的预防和药物治疗提供新的方向。

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