匹美克莫司使线粒体受损并激活AMPK信号通路与线粒体自噬

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摘要 自噬是真核生物中普遍存在的现象, 它可以降解细胞中堆积的错误折叠蛋白和衰老蛋白或者破损细胞器, 从而维持细胞稳态平衡。研究表明, 钙调神经磷酸酶能调节自噬, 但其具体分子机制未阐明, 尚有待研究。研究发现, 钙调神经磷酸酶的抑制剂匹美克莫司通过腺苷酸活化蛋白激酶[adenosine 5′-monophosphate (AMP)-activated protein kinase, AMPK]信号通路诱导自噬。进一步的研究表明, 匹美克莫司使线粒体受损, 并使线粒体内膜移位酶复合物23(mitochondrial inner membrane translocase complex, subunit 23, Tim23)下调。自噬特异抑制剂3-甲基腺嘌呤(3-methyladenine, 3MA)和shRNA稳定敲低*AMPK*基因表达能抑制匹美克莫司引起Tim23的下调。由此可见, 匹美克莫司通过AMPK信号通路诱导线粒体自噬发生。该研究阐明了钙调神经磷酸酶调节线粒体自噬的机制。

关键词 自噬; 匹美克莫司; 线粒体自噬

Pimecrolimus Impaired Mitochondrion and Induced Mitophagy via AMPK Signaling Pathway

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Abstract Autophagy is a ubiquitous phenomenon in eukaryotes, which maintains the homeostasis of cells by degrading the misfolded and aging proteins or damaged organelles. It has been reported that calcineurin regulates autophagy, but its molecular mechanism has not been elucidated. It was reported that calcineurin inhibitors pimecrolimus induced autophagy via AMPK [adenosine 5′-monophosphate (AMP)-activated protein kinase] signaling pathway. Further study showed that pimecrolimus impaired mitochondrion and downregulated mitochondrial membrane gene Tim23 (mitochondrial inner membrane translocase complex, subunit 23) and this effect was inhibited by the autophagy inhibitor 3-MA (3-methyladenine) or stable knock down of AMPK expression. These results indicate that calcineurin inhibitor pimecrolimus regulates mitophagy via AMPK signaling pathway. The molecular mechanism of how calcineurin regulates mitophagy has been elucidated.

Keywords autophagy; pimecrolimus; mitophagy

收稿日期: 2017-02-28 接受日期: 2017-05-03

国家自然科学基金(批准号: 31601125)和湖南农业大学人才引进基金资助的课题

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Received: February 28, 2017 Accepted: May 3, 2017

This work was supported by the National Natural Science Foundation of China (Grant No.31601125) and the Foundation of Science and Technology Talents in Hunan Agriculture University

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网络出版时间: 2017-06-08 11:13:48 URL: http://kns.cnki.net/kcms/detail/31.2035.Q.20170608.1113.002.html

匹美克莫司(pimecrolimus)属于免疫调节剂, 可特异抑制T细胞的活化[1], 其靶标是钙调磷酸酶(calcineurin, CaN), 能抑制此酶活性[2]。钙调神经磷酸酶在胞内Ca^{2+}信号转导中起着重要作用, 是T细胞活化的关键信号酶, 它能使T细胞激活核转录因子(nuclear factor for activated T cells, NF-AT)入核。随着胞内Ca^{2+}水平的升高, 钙调神经磷酸酶被激活, 进而催化胞质中NF-AT去磷酸化, 使NF-AT进入核内, 促进白介素-2(interleukin-2, *IL-2*)基因的转录[3]。钙调神经磷酸酶可调节线粒体融合, 调控细胞死亡和细胞周期[4-6]。研究表明, 钙调神经磷酸酶能调节自噬[7-10]。前期我们利用基于网络药理学的药物新功能计算预测方法发现, 匹美克莫司可诱导细胞自噬, 但匹美克莫司如何调节自噬并未阐明[11]。

自噬是真核细胞中的一种普遍的生命现象, 分为选择性和非选择性自噬两种类型。非选择性自噬包括大自噬、小自噬和分子伴侣自噬。选择性自噬包括线粒体自噬、过氧化物酶体自噬、内质网自噬等。自噬水平的变化是否影响人体健康以及是否可以通过调节细胞的自噬水平从而预防和控制相关疾病、保持人体身体健康已成为科学家目前最关心的问题。因此, 对细胞自噬的研究不仅具有理论意义, 而且还有重要的应用价值。

自噬形成机制非常复杂。简单地说, 自噬的生物学过程可以分为欧米茄体(omegasome)/自噬前体形成、自噬形成、自噬体同溶酶体的融合这三个步骤。迄今为止, 已鉴定超过40个自噬相关基因, 其分子机制大致可以分为以下几个过程: (1)雷帕霉素靶蛋白(the mechanistic target of rapamycin, mTOR)活性下降或AMPK活性增强; (2)自噬相关蛋白1(autophagy related gene 1, ATG1)复合体的形成促使自噬前体形成; (3)磷脂酰肌醇3激酶[phosphatidylinositol 3-kinase catalytic subunit type 3, PI3K, 又称为Vps34(vacuolar protein sorting 34)]复合体形成介导自噬膜延伸; (4)泛素样连接系统的激活介导自噬膜延伸和成熟[12-14]。3-MA为特异的自噬抑制剂, 通过抑制Vps34活性来抑制自噬[15]。

我们通过细胞免疫荧光法发现, 匹美克莫司使自噬体数目增多。经典的自噬信号通路主要有两条: (1)依赖mTOR活性下降诱导自噬; (2)依赖AMPK[adenosine 5′-monophosphate (AMP)-activated protein kinase]活性增强诱导自噬。核糖体S6蛋白激酶(ribosomal protein S6 kinase B1, S6K)是mTOR的底物, 其磷酸化水平可以显示mTOR活性的变化[16-17]。我们采用细胞免疫荧光法、Western blot和shRNA稳定敲低*AMPK*基因的表达证实, 匹美克莫司不依赖mTOR信号通路, 而是依赖AMPK信号通路诱导自噬。进一步研究表明, 匹美克莫司能破坏线粒体, 使线粒体标志蛋白质Tim23水平下降, 自噬特异抑制剂3-MA和shRNA稳定敲低*AMPK*基因的表达, 抑制匹美克莫司引起的Tim23水平下降。本研究结果证实, 匹美克莫司能诱导线粒体自噬的发生, 为探究匹美克莫司在免疫调节的作用机制提供新的线索, 揭示了线粒体自噬新的分子机制。

1 材料与方法

1.1 材料

3-MA(货号: M9281)购自Sigma-Aldrich公司。Lysosensor Green DND-189(货号: L-7535)购自Invitrogen公司。电转缓冲液Solution T购自Amaxa公司。Primerstar PCR试剂盒购自TaKaRa公司。免疫印迹化学发光底物购自Thermo Scientific公司。PCR所需引物全部购自生工生物工程(上海)股份有限公司。抗体Tim23(货号: 9206)购自BD公司。Actin抗体购自Sigma公司。绿色荧光二抗Goat anti-Rabbit IgG (H+L) Alexa 488(货号: A-11034)购自Invitrogen公司。抗体P70S6K和P-P70S6K抗体购自Cell Signaling Technology公司。抗体AMPKα和Phospho-AMPKα (Thr172)购自Cell Signaling Technology公司。转染试剂X-tremeGENE HP DNA Transfection Reagent购自Roche公司。

1.2 方法

1.2.1 *细胞培养* 细胞在37 ℃、5% CO_2、10%的小牛血清和100 U/mL青霉素和100 μg链霉素的DMEM培养基中培养。细胞长至80%汇合时进行细胞传代。

1.2.2 *活细胞观察* 细胞传至10 cm培养皿中, 长至80%汇合后, PBS清洗1次, 再用胰蛋白酶消化30 s, 取5 μL放入到玻璃底的容器(PeCon公司), 处理后放入37 ℃、5% CO_2的活细胞工作站用激光共聚焦荧光显微镜拍照。

1.2.3 *细胞转染* 细胞传至10 cm培养皿中, 长至80%汇合后, PBS清洗1次后, 胰酶消化30 s, 取10%的细胞放至1.5 mL的离心管。1 000 ×*g*离心5 min。弃

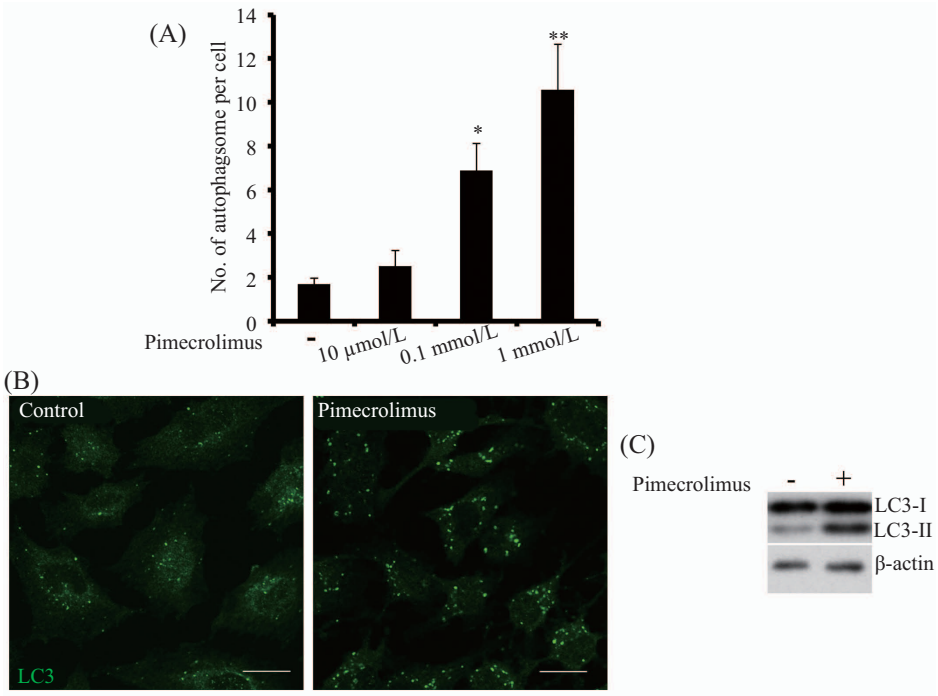
上清, 加入100 μL电转缓冲液和质粒, 用移液器混匀后放入电转杯, 用电转仪进行电转。电转后向电转混合液中加入完全培养基1 mL, 取混合物100 μL放入玻璃器皿或10 cm的培养盘中培养24 h。

1.2.4 细胞免疫荧光 一定量的细胞传入已放入圆形玻片的12孔板中, 24 h后, 进行各种处理。PBS洗3次, 用4%多聚甲醛固定10 min。PBS清洗3次后10%的小牛血清室温封闭20 min。用封闭液和0.1%皂素按照1:500稀释抗体LC3, 将抗体稀释液在玻片上室温孵育1 h。然后, 用PBS清洗3次, 每次10 min。用封闭液和0.1%皂素按照1:500稀释二抗, 将抗体稀释液避光室温孵育1 h后用PBS清洗3次, 每次10 min。最后, 将封片剂滴在载玻片上, 圆形玻璃片倒扣在上。避光室温放置1 h后, 用激光共聚焦显微镜观察。

1.2.5 Western blot 正常大鼠肾细胞系(normal rat kidney cell line, NRK)细胞传至6孔板中, 24 h后, 不同处理后用PBS洗2次, 每孔加200 μL 2% SDS裂解液, 收样后, 95 ℃加热10 min, 用4×上样缓冲液稀释样品, 95 ℃加热10 min, 再进行SDS-PAGE, 然后, 转到硝酸纤维素膜上。5%脱脂奶室温封闭1 h, PBST清洗3次, 每次10 min。一抗孵育1 h或过夜。然后用PBST清洗3次, 每次10 min。室温孵育二抗1 h, PBST洗3次, 每次10 min。加入化学发光液后用超敏化学发光检测仪拍照。

1.2.6 电镜样品制备 NRK细胞用10%胰蛋白酶消化后, 2.5%戊二醛固定1 h, 切片机(Lecia EM UC6)切片, 醋酸铀和柠檬酸铅双染后, 在透射电镜下观察。

1.2.7 稳定表达荧光融合蛋白细胞系的构建 将Lamp1-pmCherry或Tom20-GFP质粒2 μg转染入NRK细胞后。将1 μL终浓度为1 μg/mL嘌呤霉素或2 mg/mL G418加入细胞培养基中培养细胞1周, 挑取单克隆, 待细胞长满后传入玻璃底的容器(PeCon公司), 不同处理后放入37 ℃、5% CO_2的活细胞工作站用激光



A: 10 μmol/L、0.1 mmol/L或1 mmol/L的匹美克莫司处理细胞4 h, 用抗LC3抗体, 采用细胞免疫荧光检测自噬体数目的变化, 通过激光共聚焦拍摄后对自噬体数目进行统计, 至少分析30个细胞, *P<0.05, **P<0.01, 与对照组比较; B: 1 mmol/L的匹美克莫司处理细胞4 h, 细胞免疫荧光检测自噬体的变化, 标尺=10 μm; C: 1 mmol/L的匹美克莫司处理细胞4 h, 用LC3抗体通过Western blot检测NRK细胞中LC3-I和LC3-II蛋白质水平变化, β-actin为内参。

A: NRK cells were treated with or without 10 μmol/L, 0.1 mmol/L or 1 mmol/L pimecrolimus for 4 h, respectively, stained with an anti-LC3 antibody, then observed under confocal microscopy. The numbers of autophagosome were quantified. At least 30 cells were counted. *P<0.05, **P<0.01 compared to control group; B: NRK cells were treated with or without 1 mmol/L pimecrolimus for 4 h and stained with anti-LC3 antibody, then observed under confocal microscopy. Scale bars=10 μm; C: cells either cultured in nutrient-rich medium for 4 h, or cultured under 1 mmol/L pimecrolimus treatment for 4 h, then harvested and subjected by Western blot analysis with anti-LC3 antibody. β-actin was served as loading control.

图1 匹美克莫司增加NRK细胞中自噬体数目

Fig.1 Pimecrolimus increases the number of autophagosome in NRK cells

共聚焦荧光显微镜拍照。

1.2.8 shRNA稳定敲低*AMPK*基因表达 NRK细胞传入6孔板中, 生长至80%汇合后, 用转染试剂将NC shRNA或AMPK shRNA 1 μg质粒转入细胞中, 24 h后加入1 μL终浓度为0.5 μg/mL嘌呤霉素, 持续加药2周进行筛选, 待长出单克隆后挑选单克隆并传至另外的6孔板, 长满后冻存细胞或进行不同处理。

1.2.9 自噬和线粒体共定位分析 将1 μg GFP-LC3和Ds-red-mito用转染试剂转入NRK细胞, 24 h后进行相应处理, 用共聚焦观察。

1.3 统计分析

采用GraphPad Prism 5软件处理, 数据采用mean±S.D.表示, 单因素方差分析采用*t*检验, $P<0.05$为差异具有统计意义, 实验数据至少重复3次。

2 结果

2.1 匹美克莫司促进自噬体数目的增多

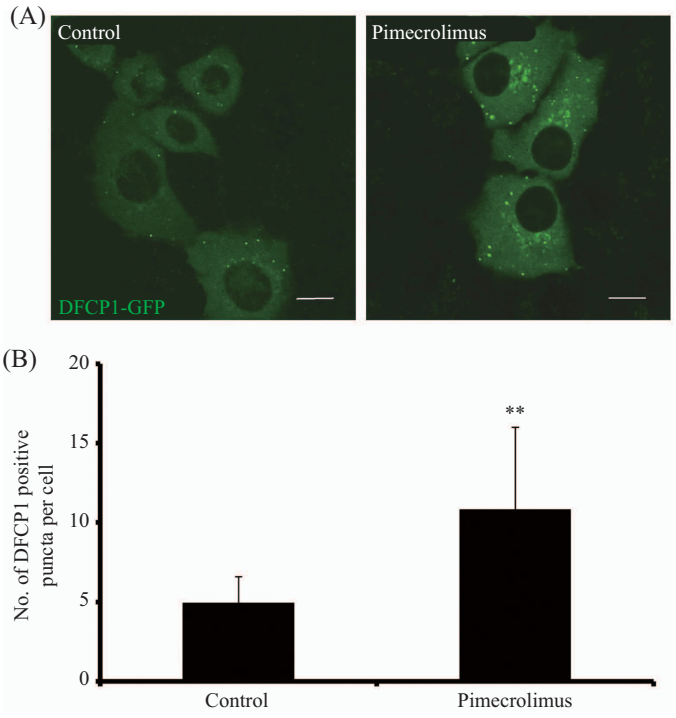
为了验证匹美克莫司是否诱导自噬发生, 我们分别用10 μmol/L、0.1 mmol/L和1 mmol/L的匹美克莫司处理NRK细胞4 h, 统计结果表明, 0.1 mmol/L和1 mmol/L能显著提高自噬体数目(图1A)。 细胞免疫荧光结果显示, 1 mmol/L匹美克莫司使自噬体数目增多(图1B)。Western blot结果也表明, 1 mmol/L匹美克莫司能显著提高LC3-II与LC3-I蛋白质比例(图1C)。

2.2 匹美克莫司诱导自噬体前体的产生

自噬体数目增多并不能说明匹美克莫司能诱导自噬发生, 改变溶酶体pH值或抑制自噬与溶酶体融合也能增加自噬体数目。为进一步验证匹美克莫司是否诱导自噬, 我们用1 mmol/L的匹美克莫司处理GFP-DFCP1 NRK细胞4 h。活细胞结果显示, 匹美克莫司使自噬体前体(omegasome)的数目增多(图2A)。统计结果表明, 匹美克莫司处理组的自噬体前体数目比对照组多($P<0.01$, 图2B)。

2.3 匹美克莫司通过AMPK信号通路诱导自噬

为验证匹美克莫司是否会诱导自噬, 以及通过哪条信号通路诱导自噬, 我们用1 mmol/L的匹美克莫司处理NRK细胞。Western blot结果表明, 1 mmol/L的匹美克莫司不影响mTOR的活性(图3A和图3B), 但能提高AMPK α亚基第172位苏氨酸磷酸化水平(图3C和图3D), 通过活细胞检测发现, 匹

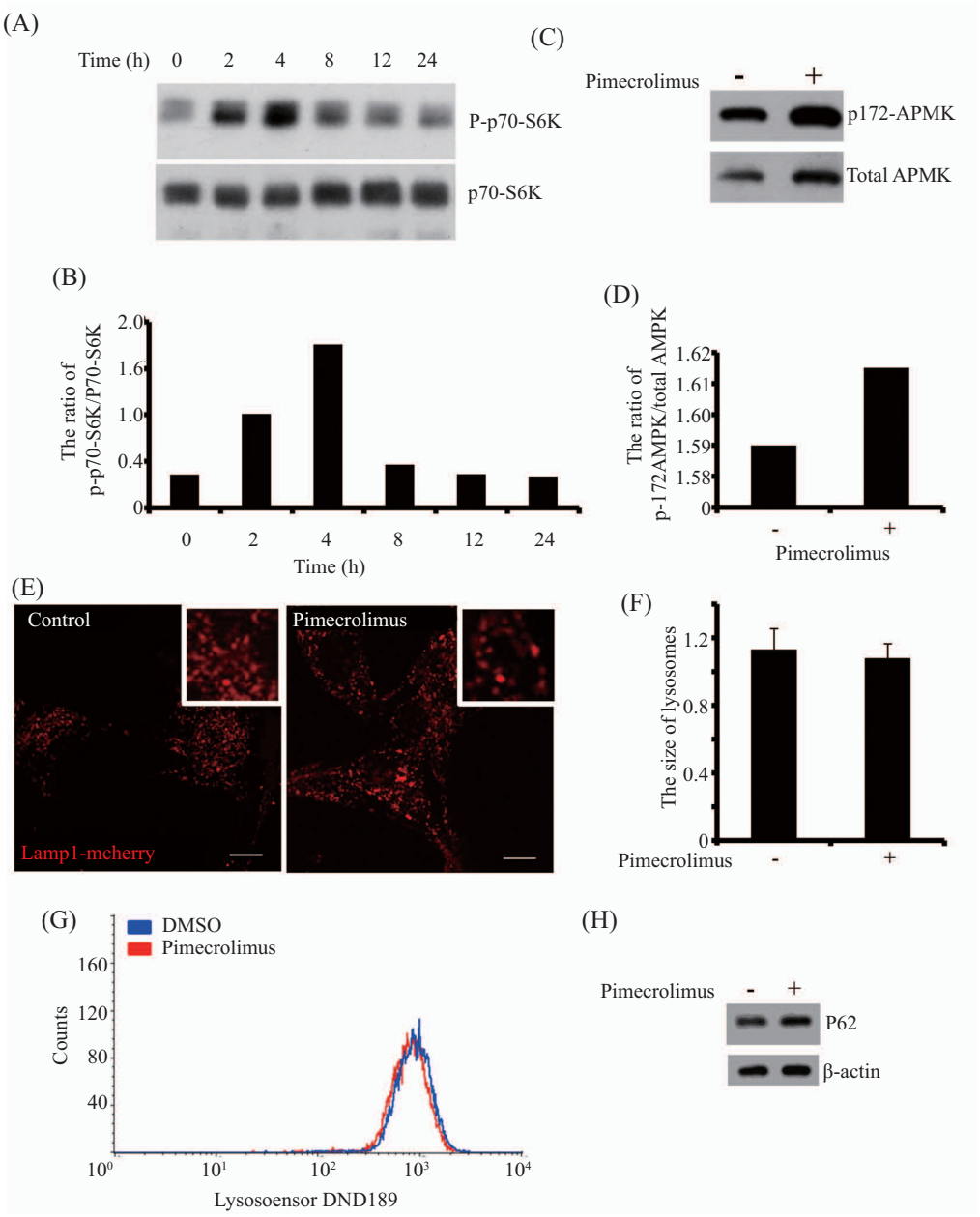


A: 1 mmol/L的匹美克莫司处理稳定表达自噬体前体标记蛋白DFCP1-GFP细胞4 h, 活细胞观察细胞中DFCP1-GFP点状数目的变化, 标尺=10 μm; B: A图自噬体前体数目的统计结果, 至少分析30个细胞, **$P<0.01$, 与对照组比较。

A: GFP-DFCP1 NRK cells were treated for 4 h with or without 1 mmol/L pimecrolimus, and imaged by confocal microscopy. Scale bars=10 μm; B: cells from Figure A were quantified for the number of omegasomes. At least 30 cells were counted. **$P<0.01$ compared to control group.

图2 匹美克莫司促进NRK细胞中自噬前体数目增加

Fig.2 Pimecrolimus increases the number of omegasome formation in NRK cells

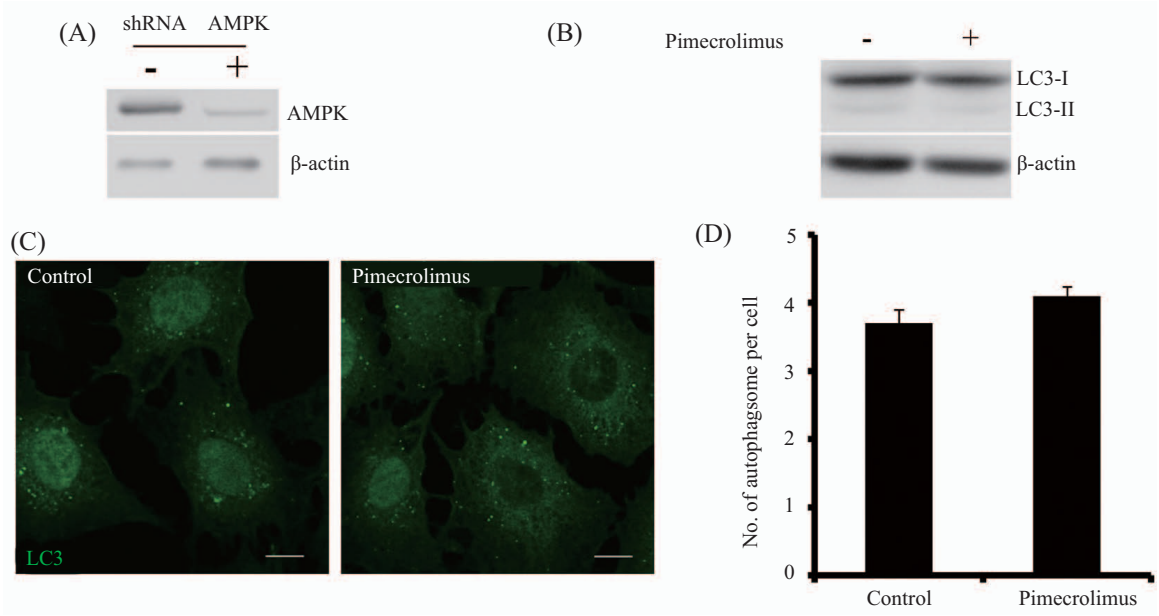


A: 1 mmol/L的匹美克莫司处理NRK细胞2、4、8、12、24 h后, 用抗S6K和p-S6K的抗体检测S6K和p-S6K蛋白水平的变化; B: 采用Image pro-plus 6.0软件对A图统计分析总的光密度变化; C: 1 mmol/L的匹美克莫司处理NRK细胞4 h, 用抗AMPKα和p-172 Thr AMPKα的抗体检测AMPKα和p-172 Thr AMPKα蛋白水平的变化; D: 采用Image pro-plus 6.0对C图统计分析总的光密度变化; E: 1 mmol/L的匹美克莫司处理稳定表达溶酶体标记蛋白Lamp1-pmcherry细胞4 h, 观察活细胞中溶酶体大小的变化, 标尺=10 μm; F: 采用Image pro-plus 6.0对A图统计分析溶酶体大小的变化, 至少分析30个细胞; G: 1 mmol/L的匹美克莫司处理NRK细胞4 h, lysosensor DND 189染色10 min后, 流式细胞仪分析细胞内荧光强度的变化; H: 1 mmol/L的匹美克莫司处理NRK细胞4 h后, 用抗P62和β-actin的抗体检测P62蛋白水平的变化。

A: NRK cells were incubated with 1 mmol/L pimecrolimus for the indicated time and total cell extracts were immunoblotted with antibodies against S6K and phospho-70 S6K; B: IOD (integrate optical density) of Figure 3A were analyzed by Image pro-plus 6.0; C: NRK cells were incubated with 1 mmol/L pimecrolimus for 4 h and total cell extracts were immunoblotted with antibodies against AMPKα and phospho-172 Thr AMPKα; D: IOD (integrate optical density) of Figure 3C were analyzed by Image pro-plus 6.0; E: Lamp1-pmcherry NRK cells were incubated for 4 h with 1 mmol/L pimecrolimus and imaged by confocal microscopy, scale bar=10 μm; F: lysosomal size was analyzed with Image pro-plus 6.0 software, at least 30 cells were analyzed; G: representative fluorescent pictures of NRK cells exposed for 10 min to 1 μmol/L lysosensor DND-189 after 4 h pimecrolimus treatment; H: NRK cells were incubated with 1 mmol/L pimecrolimus for 4 h and total cell extracts were immunoblotted with antibodies against P62 and β-actin.

图3 匹美克莫司诱导自噬并激活AMPK信号通路

Fig.3 Pimecrolimus induces autophagy and activates AMPK pathway



A: NRK细胞转入shRNA AMPK和NC shRNA, 用抗AMPK的抗体检测AMPK蛋白质水平; B: 细胞免疫荧光检测经1 mmol/L匹美克莫司处理4 h shRNA AMPK细胞中LC3和β-actin的抗体检测LC3-I和LC3-II水平; C: 通过细胞免疫荧光检测经1 mmol/L匹美克莫司处理4 h shRNA AMPK细胞中自噬数目, 标尺=10 μm; D: 对C图的自噬体数目进行统计分析, 至少分析30个细胞。

A: NRK cells were transfected with shRNA AMPK or NC shRNA, the protein level of AMPK were tested by Western blot. B: shRNA AMPK NRK cells were incubated with 1 mmol/L pimecrolimus for 4 h and total cell extracts were immunoblotted with antibodies against LC3 and β-actin. C: shRNA AMPK NRK cells were incubated with 1mmol/L pimecrolimus for 4 h and stained with an anti-LC3 antibody, then observed under confocal microscopy, scale bars=10 μm. C: the number of autophagosome were quantified. $n \geqslant 30$.

图4　匹美克莫司通过AMPK信号通路诱导自噬

Fig.4　Pimecrolimus induces autophagy via AMPK pathway

美克莫司不影响溶酶体的形态大小(图3E和图3F)。Lysosensor DND189可以用来检测溶酶体的酸碱度。Lysosensor在细胞内绿色荧光越强则表示酸性越强, 反之碱性越强。结果发现, 匹美克莫司不影响溶酶体pH值(图3G)。P62(sequestosome 1)为自噬特异降解的底物之一, 我们的结果表明, 1 mmol/L的匹美克莫司并不能使自噬特异降解的底物P62水平下降(图3H)。

2.4　敲低*AMPK*基因对匹美克莫司诱导自噬的影响

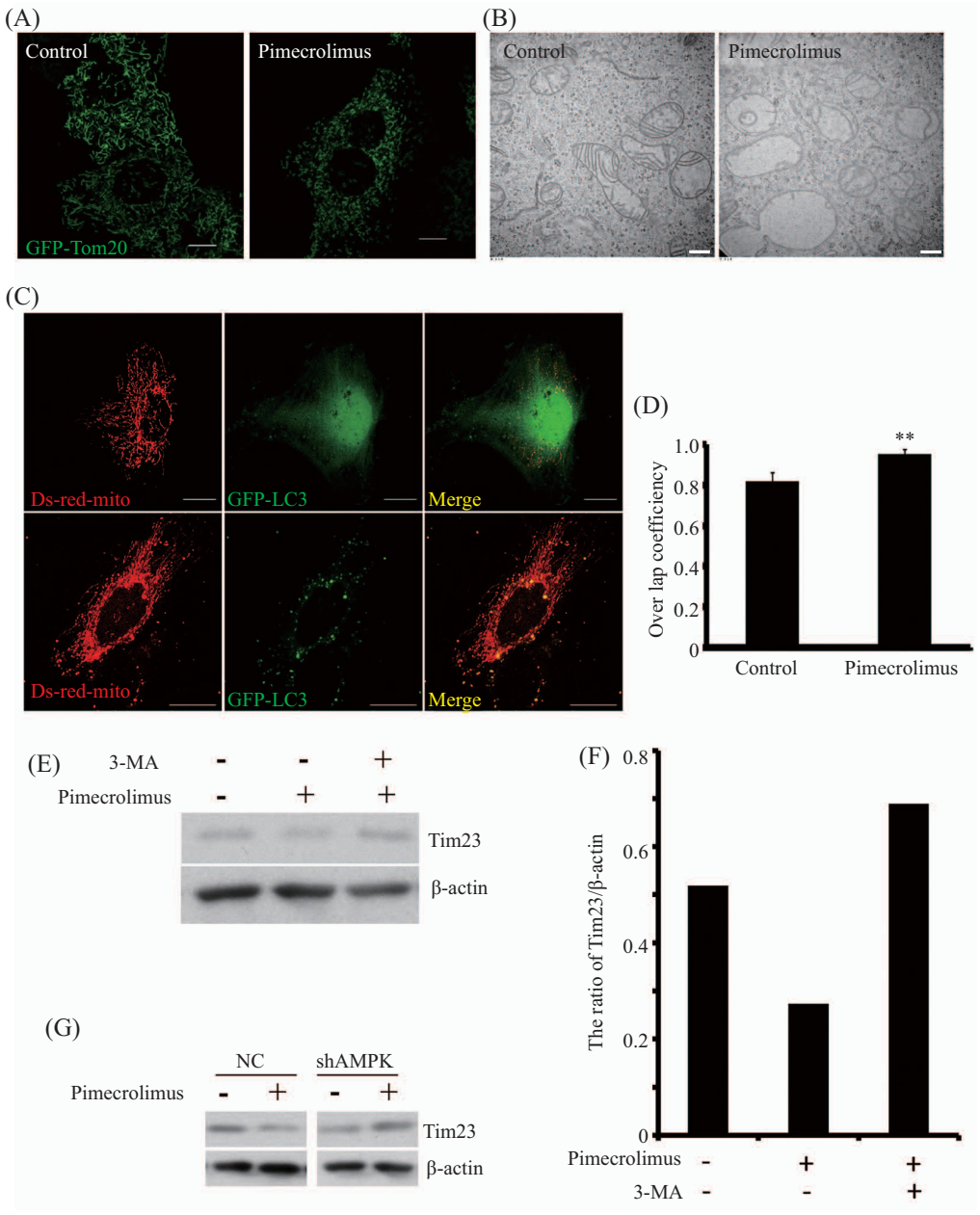
shRNA AMPK或随机序列的shRNA转入NRK细胞后, 经Western blot检测AMPK蛋白质水平的变化, 结果表明, 成功敲低*AMPK*基因(图4A)。Western blot结果也表明, shRNA敲低*AMPK*处理组不能提高LC3-II/LC3-I型蛋白的比例(图4B)。1 mmol/L的匹美克莫司处理shRNA AMPK或NC NRK细胞4 h, 细胞免疫荧光检测发现, shRNA敲低*AMPK*能显著抑制自噬形成(图4C), 统计结果显示, 野生型处理组与shRNA敲低*AMPK*处理组自噬体数目无明显差异(图4D)。

2.5　匹美克莫司诱导线粒体自噬发生

1 mmol/L的匹美克莫司处理Tom20-GFP NRK细胞4 h, 活细胞和投射电镜的检测结果表明, 匹美克莫司使线粒体受损(图5A和图5B), 并且能使自噬与线粒体共定位比例增加(图5C和图5D)。Tim23为线粒体内膜转位酶亚基, 进一步研究表明, 匹美克莫司降低细胞内Tim23蛋白质水平, 加入3-MA后, 匹美克莫司不影响细胞内Tim23蛋白质水平(图5E和图5F)。1 mmol/L的匹美克莫司处理shRNA AMPK NRK细胞4 h, 结果表明, *AMPK*敲低抑制Tim23水平的下调(图5G)。以上结果显示, 匹美克莫通过AMPK信号通路诱导线粒体自噬的发生。

3　讨论

钙调神经磷酸酶是迄今所知唯一受Ca^{2+}/钙调蛋白调节的丝氨酸/苏氨酸蛋白磷酸酶。CaN广泛分布于机体内各种组织中, 参与多种受Ca^{2+}离子调节的信号转导通路, 并作用于不同的底物而产生不同的生物学效应。线粒体活性氧类(reactive oxygen species, ROS)水平增多, 能激活溶酶体膜上的钙离子通道蛋白IV型粘脂质贮积症关键蛋白1(transient receptor potential mucolipin 1, TRPML1), 使溶酶体内的Ca^{2+}外流, 从而激活CaN, CaN通过调节转录因



A: 1 mmol/L的匹美克莫司处理稳定表达线粒体标记蛋白GFP-Tom20细胞4 h, 活细胞观察细胞中线粒体形态的变化, 标尺=10 μm; B: 1 mmol/L的匹美克莫司处理NRK细胞4 h, 透射电镜观察细胞中线粒体形态的变化, 标尺=500 nm; C: 1 mmol/L的匹美克莫司处理转入GFP-LC3和Ds-red-mito的NRK细胞4 h, 用激光共聚焦观察, 标尺=10 μm; D: Image J软件分析C图的共定位比例, 至少分析30个细胞, $^{**}P<0.001$, 与对照组比较。E: 1 mmol/L 的匹美克莫司和10 mmol/L 3-MA分别处理NRK细胞4 h, 用Tim23抗体采用Western blot方法检测Tim23水平; F: 采用Image pro-plus 6.0对图5E统计分析总的光密度变化; G: 1 mmol/L的匹美克莫司处理野生型或稳定敲低*AMPK*的NRK细胞, Western blot方法检测Tim23水平, β-actin作为内参。

A: the GFP-Tom20 cells were treated for 4 h with or without 1 mmol/L pimecrolimus, then imaged by confocal microscopy, scale bars=10 μm; B: NRK cells were treated for 4 h with or without pimecrolimus, then imaged by transmission electron microscope (TEM), scale bars=500 nm; C: NRK cells were transfected with GFP-LC3 and Ds-red-mito, then treated with or without pimecrolimus for 4 h and imaged by confocal microscopy, scale bars=10 μm; D: the overlap coefficency of GFP-LC3 puncta colocalizing with Dsred-mito per cell. At least 30 cells were examined for 1 mmol/L pimecrolimus treatment. These data were analyzed by Image J, $^{**}P<0.001$ *vs* control group; E: NRK cells were treated for 4 h with pimecrolimus or 1 mmol/L pimecrolimus plus 10 mmol/L 3-MA, total cell extracts were immunoblotted with antibodies against Tim23, β-actin served as loading control; F: IOD (integrate optical density) of Figure 5E were analyzed by Image pro-plus 6.0; G: wild type or stable knock down of AMPK NRK cells were treated with 1 mmol/L pimecrolimus for 4 h, total cell extracts were immunoblotted with antibodies against Tim23 and β-actin.

图5 匹美克莫司诱导线粒体自噬发生

Fig.5 Pimecrolimus induces mitophagy

子(transcription factor EB, TFEB)的磷酸化调节自噬[7,9-10]。也有研究表明, 抑制CaN的活性可促进自噬的形成, 但CaN活性变化如何调节自噬并未阐明[8]。匹美克莫司的靶标是钙调神经磷酸酶, 能抑制钙调神经磷酸酶的活性[2-3]。本研究发现, 匹美克莫司诱导线粒体自噬发生。线粒体自噬(mitophagy)作为一种重要的线粒体质量控制的方式是指细胞通过自噬机制选择性清除受损伤或不必需的线粒体。线粒体自噬可以清除细胞内受损线粒体产生的活性氧类, 对维持细胞的稳态十分重要。ROS蓄积会导致细胞内DNA发生突变和蛋白质发生错误折叠, 也会使线粒体变大, 通透性改变并释放促凋亡因子, 最终导致细胞死亡。因此, 这些功能异常或受损线粒体是缺氧应激状态下细胞是否存活的因素。及时清除这些线粒体对维持线粒体质量、数量及细胞稳态具有重要意义。线粒体自噬能防止线粒体DNA突变, 在癌症发生和发展、神经退行性疾病以及皮肤病中具有保护作用[18-21]。研究表明, 线粒体自噬为选择性自噬的一种。迄今已发现有多种基因参与识别自噬特异降解底物, 如P62、哺乳动物自噬受体(autophagy cargo receptor, NBR1)、核点蛋白52(nuclear dot protein 52, NDP52)、Nix/BNIP3L(BCL2 interacting protein 3 like)和FUNDC1(FUN14 domain containing 1)的C-端都具有一个UBA(carboxy-terminal ubiquitin-associated domain)结构域, 并能够同LIR基序与LC3/ATG8直接相互作用实现对靶标蛋白或细胞器的降解[22-23]。本研究发现, 匹美克莫司诱导的线粒体自噬并不参与调节P62水平, 匹美克莫司诱导的线粒体自噬中起识别作用的蛋白质及具体机制有待进一步阐明。匹美克莫司用于治疗皮肤病, 自噬在炎症反应和免疫中有着非常重要的作用: 一方面, 细胞自噬的缺损是诱发炎症反应和炎症性疾病的一个重要因素; 另一方面, 自噬又能抑制炎症小体形成或调节细胞因子的转录、合成和分泌以抑制炎症信号[24]。本研究为揭示线粒体自噬的新分子机制奠定基础, 并为探究其在皮肤病治疗中的作用机制提供一些帮助。

致谢——

感谢清华大学生命科学学院俞立教授实验室提供质粒GFP-LC3、Ds-red-mito、NC shRNA、shRNA AMPK质粒以及Tom20-GFP NRK和DFCP1-GFP NRK稳定细胞株。

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