

内质网应激调控细胞自噬和凋亡

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摘要 内质网是真核细胞中蛋白质合成、折叠与分泌的重要膜性细胞器。当内源或外源性的刺激导致内质网的蛋白质折叠功能发生紊乱时, 内质网腔内累积大量未折叠或错误折叠的蛋白质, 并引起一系列后续反应称为内质网应激。此时, 细胞启动未折叠蛋白反应, 以清除未折叠蛋白并恢复内质网稳态。当内质网应激持续时, 未折叠蛋白反应并不足以清除越积越多的未折叠蛋白, 也无法去除受损伤的细胞器, 细胞自噬被激活。当内质网应激过强或持续时间过长时, 过度激活的自噬最终引起细胞死亡。该文就近年来内质网应激调控细胞自噬和细胞凋亡机制的研究进行综述, 以期对相关领域的研究者提供新的思路。

关键词 内质网应激; 未折叠蛋白反应; 自噬; 细胞凋亡

Endoplasmic Reticulum Stress Regulates Cell Autophagy and Apoptosis

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Abstract Endoplasmic reticulum (ER) is an important membranous organelle for protein synthesis, folding and secretion in eukaryotic cells. Endogenous or exogenous stimuli, however, can lead to an imbalance between the ER protein folding capacity and protein load, giving rise to an accumulation of unfolded or misfolded proteins in the ER lumen, a condition dubbed as ‘ER stress’. In an attempt to meet the increased folding demand, cells utilize a conserved signaling pathway, the unfolded protein response (UPR), which is initially charged to restore the homeostasis of endoplasmic reticulum. If this mechanism fails, persistent ER stress will eventually cause this cytoprotective UPR to switch into a autophagy pathway or even a cell death pathway. In this paper, we reviewed recent studies on mechanisms of ER stress-induced autophagy and apoptosis, which may provide relevant information for the researchers.

Keywords endoplasmic reticulum stress; unfolded protein response; autophagy; apoptosis

1 内质网应激

内质网(endoplasmic reticulum, ER)是真核细胞中蛋白质合成、折叠与分泌的重要膜性细胞器。大约30%的细胞蛋白质在内质网中经过折叠、加工和

修饰, 形成有活性的功能性蛋白质, 包括绝大多数分泌蛋白、膜结合蛋白和整合膜蛋白^[1-3]。新合成的蛋白质包裹在囊泡中离开内质网, 并被运送到高尔基体, 进而进入内膜系统或被分泌到细胞外。此

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外,内质网亦可参与代谢过程,例如钙离子储存、糖异生、脂质和胆固醇的合成以及自噬空泡的形成等^[3]。当受到内源或外源性刺激时(如缺乏分子伴侣或细胞能量、 Ca^{2+} 缺乏、氧化还原稳态破坏、蛋白质变异和二硫键减少等),内质网的蛋白质折叠功能发生紊乱。内质网腔内累积大量未折叠或错误折叠的蛋白质,并引起一系列后续反应称为内质网应激(endoplasmic reticulum stress, ER stress)。

2 内质网应激反应的生存途径——未折叠蛋白反应

为了应对内质网应激,能感应复杂信号网络的未折叠蛋白反应(unfolded protein response, UPR)对内质网的折叠和降解能力做出动态调整。UPR由位于内质网膜的特殊应力传感器和下游重新编码基因表达使得细胞能够适应应激或诱导凋亡的转录因子两个部分组成。UPR信号通路有三个典型分支,分别由三种内质网应激传感器介导:PERK(protein kinase RNA-like ER kinase)、IRE1(inositol-requiring enzyme 1)和ATF6(activating transcription factor 6)。此外,葡萄糖调节蛋白78(78 kDa glucose regulated protein, GRP78)或Bip(immunoglobulin heavy chain binding protein)、GRP94(94 kDa glucose-regulated protein)、Calreticulin和PDI(protein disulfide-isomerase)等ER分子伴侣在促进膜蛋白及分泌蛋白的折叠、组装和运输等过程也发挥重要作用。

2.1 ATF6介导的信号通路

ATF6是一种被锚定在未应激细胞ER膜上的转录因子。内质网应激时,内质网腔内的未折叠蛋白能使GRP78/BiP和ATF6分离,ATF6以囊泡转移的方式从内质网膜转移到高尔基体,依次被两个位点的蛋白酶切割。丝氨酸蛋白酶位点-1(serine protease site-1, S1P)切割ATF6的管腔结构域,而N-端部分随后被金属蛋白酶位点-2蛋白酶(metalloprotease site-2 protease, S2P)切割。切割后形成的只有50 kDa的N-末端胞质结构域(the cytosolic ATF6 fragment, ATF6f)转移到细胞核中,激活具有内质网应激反应元件(ER stress element, ERSE)基因的转录,这些基因包括分子伴侣如BiP和GRP94、折叠酶PDI以及C/EBP同源蛋白(C/EBP-homologous protein, CHOP)等,同时活化的ATF6f也能激活转录因子X盒结合蛋白1(X box-binding protein 1, XBP1)和内质网相关蛋白降解(ER-

associated protein degradation, ERAD)基因的转录^[4]。ATF6f可以与XBP1形成异源二聚体,以控制特异性基因表达模式的诱导^[5]。

2.2 PERK信号通路

PERK是负责内质网应激下mRNA翻译衰减的主要蛋白质,防止新合成的蛋白质进入已经发生应激的内质网腔。这种翻译减弱是由真核起始因子2 α (eukaryotic initiation factor 2 α , eIF2 α)的磷酸化介导的。非应激时,PERK二聚体位点被GRP78遮盖,N-末端位于内质网腔,C-末端位于细胞质中,C-端有丝/苏氨酸蛋白激酶活性,但是无核酸内切酶活性。应激时,GRP78与PERK解离,PERK形成寡聚体且发生自身磷酸化而被激活,活化的PERK能特异性的磷酸化eIF2 α 的51位丝氨酸。eIF2 α 的磷酸化抑制eIF2 α 再循环到其活性GTP结合形式(多肽链合成起始阶段所必需的),从而使eIF2 α 失去启动蛋白质翻译的能力,蛋白翻译水平下降,下调细胞内蛋白合成的整体水平。

除了抑制总体蛋白的翻译外,eIF2 α 磷酸化促进编码转录因子ATF4(activating transcription factor 4)mRNA的选择性翻译^[4]。ATF4直接上调转录因子C/EBP同源蛋白(CHOP)。随后,ATF4和CHOP协同诱导涉及凋亡、自噬、氨基酸代谢和抗氧化反应各种基因的表达^[6]。

2.3 IRE1信号通路

最保守的UPR信号转导分支和唯一存在于酵母中的信号由IRE1启动。IRE1是一种跨膜蛋白,由N-末端内质网腔传感器结构域、单个跨膜结构域和C-末端胞质效应器组成,负责蛋白激酶和内切核糖核酸酶活性。内质网应激使IRE1腔内结构域与GRP78解离,导致IRE1位于内质网腔的结构域发生二聚化,激活胞质区的蛋白激酶域,进而发生自身磷酸化,IRE1的二聚化及其自身磷酸化激活其内切核糖核酸酶活性,以催化编码转录因子XBP1的mRNA的非常规剪接。该事件切除了一个26个核苷酸的内含子,其改变了mRNA的编码阅读框。剪接的XBP1(spliced X box-binding protein 1)是一个活跃的转录因子,其上调多重折叠酶、氧化还原酶、细胞内运输组分、内质网相关的降解机制和糖基化酶,控制与蛋白质折叠相关的UPR目标基因、蛋白质转运入内质网、脂质合成等过程以校正内质网稳态^[1,5-7]。IRE1还通过调节的IRE1依赖性mRNA降

解(regulated IRE1-dependent mRNA decay, RIDD)以组织特异性方式降解特定mRNA, 并通过衔接蛋白的结合激活相关激酶, 包括c-Jun氨基末端激酶(c-Jun-N-terminal kinase, JNK)和凋亡信号调节激酶1(apoptosis signal-regulating kinase 1, ASK1)途径^[7]。

XBP1不是IRE1 α 的RNA酶活性靶向的唯一RNA, IRE1 α 还通过切割自身的mRNA来控制它自身的表达^[4]。此外, IRE1 α 切割控制Caspase家族细胞死亡蛋白酶水平的微小RNA。XBP1蛋白结合涉及UPR和ERAD的若干基因的启动子以恢复蛋白质内稳态并促进细胞保护。

2.4 分子伴侣GRP78

GRP78又称为热休克蛋白家族A成员5[heat shock protein family A (Hsp70) member 5, HSPA5]属于热休克蛋白70(heat shock protein 70, Hsp70)家族的一员, 是内质网应激发生、发展的标志因子。GRP78是一种多功能蛋白, 传统的GRP78作为内质网腔内的分子伴侣, 主要功能是折叠和处理内质网内的蛋白质, 结合内质网内的Ca²⁺并保持内质网稳态^[8]。作为UPR的主调节器, GRP78在非应激情况下与内质网的三个传感器直接结合并保持其非活性形式; 在内质网应激的情况下, GRP78被累积的未折叠蛋白结合, 释放UPR传感器, 允许UPR信号跨过ER膜激活和转导到细胞质和细胞核^[9]。

在特定的细胞类型或受到应激时, GRP78可游离于内质网外, 包括细胞膜表面、细胞质、线粒体及细胞核。此外, 它也可分泌后在细胞外与互相作用的分子伴侣结合, 从而对细胞生长及信号转导产生影响^[10]。GRP78的一个主要功能是保护细胞免受应激诱导的凋亡。GRP78阻断细胞凋亡的能力包括结合和失活促细胞凋亡组分, 如定位于内质网上的BIK(BCL2 interacting killer)和Caspase-7, 以及抑制介导UPR细胞凋亡的CHOP的诱导^[11]。GRP78也可能通过与BIK、NOXA(phorbol-12-myristate-13-acetate-induced protein 1)和BCL-2(B-cell lymphoma protein-2)之间的交互作用起到抗凋亡作用^[12]。GRP78能够抵消由BIK诱导引起的凋亡, 即使BIK被另一种仅含BH-3结构域的蛋白(如NOXA)辅助。GRP78通过其氨基部分以BH-3结构域独立的方式结合BIK, 与先前的发现一致, GRP78的亚分子可以以跨膜构象存在, 其中氨基部分暴露于胞质溶胶^[13]。有意思的是, GRP78和BCL-2与BIK的不同结构域形成分离

的复合物, 并且在细胞中表达量增加的GRP78导致BCL-2与BIK的结合降低, 反之亦然^[12]。

3 内质网应激与自噬

自噬(autophagy)是细胞自食的过程, 也是重要的蛋白质降解途径, 特别是在饥饿或应激过程中。与泛素-蛋白酶体系统(ubiquitin-proteasome system, UPS)介导的ERAD相反(其以单个短寿命蛋白为靶标), 自噬能够降解大量的长寿命蛋白和细胞器如内质网、线粒体、过氧化物酶体、细胞核和核糖体^[14-16]。自噬与ERAD一起也是蛋白质稳态和质量控制的关键机制。事实上, 细胞内基础性的自噬对于损伤和功能失调的蛋白质和细胞器的降解很重要, 有研究表明, 自噬缺陷小鼠表现出错误折叠的受损蛋白质的累积^[17]。

自噬最初通过将胞质部分隔离成超微结构特征的双层膜结合的囊泡, 称为自噬体。自噬体随后与溶酶体融合, 进而对隔离胞质蛋白和细胞器进行降解^[18]。对酵母的研究已经确定了超过30种由自噬相关基因(autophagy-related genes, ATGs)编码的蛋白, 其中许多已经被认定为哺乳动物直系同源物^[19-20]。自噬体形成包括三个主要步骤: 分隔膜的起始、成核和扩增。自噬体形成的复杂过程始于杯状分隔膜组装位点(也称为隔离膜), 在该位点蛋白质UNC51样激酶(UNC51-like kinase, ULK)复合物[由ULK1或ULK2和ATG13、FAK家族激酶相互作用蛋白质(FAK family kinase interacting protein of 200 kDa, FIP200)以及ATG101组成]组装启动自噬体形成^[18]。接下来, 在成核阶段, 活化的ULK复合物靶向由Beclin1(酵母中的Atg6)、液泡蛋白分选15(vacuolar protein sorting 15, VPS15)、VPS34和ATG14组成的III类PI3K(phosphatidylinositol 3-kinase)复合物, 以促进局部产生自噬特异性的磷脂酰肌醇3-磷酸。最近的一项研究表明, ATG14也促进自噬体与内溶酶体隔室的融合^[21]。在扩增阶段, 将ATG12-ATG5-ATG16复合物引入自噬体膜, 促进微管相关蛋白1轻链3(microtubule-associated protein 1 light chain 3, MAP1LC3, 也称为LC3)与磷脂酰乙醇胺的脂化。LC3是酵母ATG8的主要哺乳动物同源物, 其是扩增分隔膜所必需的。在饥饿诱导的自噬过程中, 核LC3的脱乙酰化和胞质易位对于LC3与磷脂酰乙醇胺的脂化是必需的^[22]。

内质网应激介导自噬的分子机制主要有内质网应激激活PERK/eIF2 α 通路、内质网应激通过IRE1调控细胞自噬、细胞内Ca²⁺浓度介导内质网应激和自噬的相互作用等三种。

3.1 内质网应激激活PERK/eIF2 α 通路

UPR信号通路PERK/eIF2 α 诱导ATG12表达^[23]。在细胞自噬过程中,两个相互依赖的泛素样结合系统即ATG12与ATG5连接系统、ATG8(LC3)脂化系统起着极为重要的作用。其中,前者通过ATG12的泛素样活性将LC3-II募集到分离膜上,该活性形式的ATG12与ATG5共价结合,并与ATG16L1物理相关以形成复合体,进而促进自噬体的成熟^[24-26]。已有研究表明,PERK通过ATF4转录调节ATG基因,如ATG7、ATG10和ATG5调控介导自噬^[27]。应激依赖性转录因子ATF4和CHOP在涉及自噬体形成、延长和功能的基因转录激活中是必需的^[6]。通过eIF2 α -ATF4途径激活的这些自噬基因可以分为三个功能组:(1)编码参与自噬体形成和成熟的蛋白质的Beclin1;(2)编码泛素样蛋白(ubiquitinlike protein, Ubl)系统蛋白质的基因,包括四种Ubl蛋白[MAP1LC3B(microtubule associated protein 1 light chain 3 beta)、GABARAP(GABA type A receptor-associated protein)、GABARAPL2(GABA type A receptor associated protein like 2)和ATG12]、ATG7、ATG5和ATG16L1;(3)参与泛素化底物特异性降解[p62和NBR1(autophagy cargo receptor)]的转运受体的基因。MAP1LC3B和ATG5也被认定为通过PERK/eIF2 α 途径的ATF4和CHOP靶标^[28-29]。eIF2 α -ATF4途径在补充自噬体相关蛋白库,使细胞维持自噬状态方面可能起重要作用^[30-31],从而使细胞在长时间的应激状态下能生存下去。

3.2 内质网应激通过IRE1调控细胞自噬

非应激状态下,细胞凋亡抑制蛋白BCL-2与Beclin1结合并抑制后者的活性。内质网应激状态下,IRE1/XBP1可以直接促进BCL-2的表达,从而抑制Beclin1的活性,抑制细胞自噬。IRE1 α 与肿瘤坏死因子受体相关因子2(tumor necrosis factor receptor associated factor 2, TRAF2)和凋亡信号调节激酶1(ASK1)形成复合物,其引起促进自噬或凋亡的应激激酶c-Jun氨基末端激酶(JNK)下游信号的激活^[32-33]。在内质网应激的早期阶段,JNK介导的BCL-2的磷酸化导致Beclin1/BCL-2的解离和自噬的激活^[34-35]。此

外,由IRE1 α 介导的转录因子XBP1通过Beclin1的转录激活引发自噬^[36]。

3.3 细胞内Ca²⁺浓度介导内质网应激和自噬的相互作用

内质网是细胞钙库,在应激条件下,内质网中的Ca²⁺释放到细胞质,也可诱导自噬和凋亡。哺乳动物雷帕霉素靶蛋白(mammalian target of rapamycin, mTOR)是一种进化上相对保守的丝/苏氨酸蛋白激酶,它通过磷酸化ATG13抑制自噬前体的形成而对自噬发挥关键负调控作用。各种Ca²⁺动员剂如离子霉素、ATP(通过嘌呤受体)和毒胡萝卜素(内质网Ca²⁺ ATP酶的不可逆抑制剂)均能抑制mTOR的活性,并诱导大量的自噬体以Beclin1和ATG7依赖的方式积累^[37]。内质网释放的Ca²⁺促进(calcium/calmodulin dependent protein kinase kinase/AMP-activated protein kinase, CamKK/AMPK)依赖性途径而抑制mTOR,进而诱导自噬^[38]。此外,Ca²⁺从内质网释放还能诱导蛋白激酶C(protein kinase C, PKC)激活,通过不依赖mTOR的机制诱导自噬^[39]。死亡相关蛋白激酶(death-associated protein kinase, DAPK)通过钙释放而被激活参与Beclin1磷酸化和随后的破坏Beclin1/BCL-2复合物^[40]。

4 内质网应激与凋亡

虽然UPR激活可能维持细胞存活,但是在严重或持久的内质网应激条件下,UPR的适应性反应不能消除内质网应激,细胞无法恢复内质网稳态,最终导致凋亡信号通路的激活^[41-42]。参与细胞凋亡的途径包括:(1)诱导PERK/eIF2 α 依赖的促凋亡转录因子CHOP激活;(2)IRE1介导的肿瘤坏死因子受体相关因子2(TRAF2)激活,其刺激凋亡信号调节激酶1(ASK1)/c-Jun氨基末端激酶(JNK)激酶级联;(3)Caspase-12通路的激活。

4.1 CHOP/GADD153通路

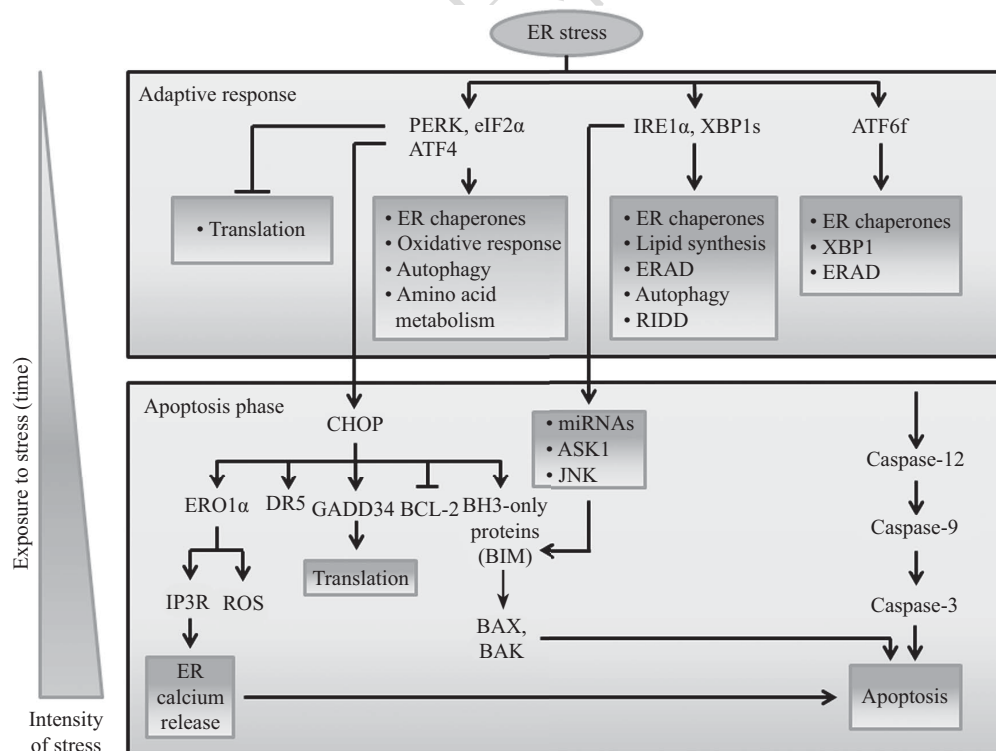
由基因DDIT3(DNA damage inducible transcript 3)编码的C/EBP同源蛋白(C/EBP-homologous protein, CHOP),又称为生长阻滞和DNA损伤诱导蛋白153(growth arrest and DNA damage-inducible protein, GADD153)诱导凋亡基因如DR5(death receptor-5)、TRB3(tribbles-related protein 3)、BIM(Bcl2-interacting mediator of cell death)和PUMA(p53 upregulated modulator of apoptosis)的表达。研究表

明, CHOP在未折叠蛋白反应中发挥会聚性的作用, 已被确定为内质网应激诱导凋亡最重要的调控因子之一^[43], PERK、ATF6以及IRE1都能诱导CHOP的转录。如果*DDIT3*从细胞中敲除, 则内质网应激导致内质网中蛋白质聚集较少, 并降低氧化应激和细胞凋亡水平^[44-47]; 如果任何其他UPR基因被敲除, 则不会发生上述情况。细胞凋亡相关靶向CHOP的转录因子包括: GADD34、DR5(一种肿瘤坏死因子受体家族的Caspase激活的细胞表面死亡受体)、内质网氧化还原酶1 α [(endoplasmic reticulum oxidase 1 α , ERO1 α), 其高度氧化内质网并促进细胞死亡]。ERO1 α 是一种氧化还原酶, 可介导二硫键形成期间电子转移到分子氧以产生过氧化氢。此反应增加了活性氧类(reactive oxygen species, ROS)的产生和肌醇-1,4,5-三磷酸受体(inositol-1,4,5-trisphosphate receptor, IP3R)介导的Ca²⁺从内质网的流出^[48]。从内质网释放的Ca²⁺通过线粒体相关的内质网膜被线粒体吸收, 可通过不同的机制促进线粒体产生ROS。这些机制包括细胞色素c的释放、三羧酸循环脱氢

酶的刺激和一氧化氮合酶的激活。内质网与线粒体间Ca²⁺的交换使蛋白质折叠所需的ATP增加, 而整个过程所需的ATP由线粒体产生。因此, 内质网应激诱导氧化应激并破坏线粒体功能^[49], 导致CHOP依赖的细胞凋亡。CHOP介导的GADD34的激活促进了eIF2 α 的蛋白质去磷酸化逆向翻译抑制^[50]。翻译抑制的释放有助于应激细胞中蛋白质合成的恢复及未折叠蛋白质在内质网腔中的积累, 同时允许编码促凋亡蛋白的mRNA翻译, 导致ATP耗尽, 氧化应激和细胞死亡的增加^[51]。CHOP诱导凋亡的另一种可能的机制是通过诱导BCL-2家族抗凋亡蛋白的表达下调^[52]和多种促凋亡蛋白的转录上调, 如上调仅含BH3结构域的蛋白BIM, 导致BAX(BCL-2 associated X protein)和BAK(BCL2 antagonist/killer 1)依赖性细胞凋亡^[1-53]。

4.2 Caspase-12通路

Caspase-12是内质网特异性的细胞凋亡信号通路。Caspase-12缺陷的细胞仅能部分抵抗内质网应激诱导的细胞凋亡^[54]。此外, 研究发现, Caspase-12



在经历内质网应激的细胞中随着时间的推移, 观察到不同的未折叠蛋白反应(UPR)相关的反应。早期UPR通过抑制翻译以及增强RIDD、ERAD和自噬来减弱蛋白质合成并降解折叠错误的蛋白质。严重的或持续的内质网应激诱导细胞凋亡发生。

Different unfolded protein response (UPR)-related reactions were observed over time in cells undergoing endoplasmic reticulum stress. Early UPR signaling events attenuate proteins synthesis and degrade the misfolding proteins by inhibiting translation and enhancing RIDD, ERAD and autophagy. Severe or sustained endoplasmic reticulum stress induced apoptosis.

图1 内质网应激对细胞命运的影响(根据参考文献[1]修改)

Fig.1 Effects of endoplasmic reticulum stress on cell fate (modified from reference [1])

缺陷小鼠能抵抗内质网应激引起的凋亡,而其他死亡刺激仍可促使细胞凋亡。这说明, Caspase-12与内质网应激介导凋亡的机制有关,而非内质网应激介导的凋亡无关^[54-55]。Caspase-12定位于内质网膜上,在正常生理状态下, Caspase-12与肿瘤坏死因子受体相关因子2(TRAF2)结合。而持续的内质网应激时, TRAF2与Caspase-12分离,促进Caspase-12的激活,活化的Caspase-12激活Caspase-9进而激活Caspase-3等凋亡效应酶,最终诱导细胞凋亡的发生^[56-57]。

4.3 JNK通路

除了细胞保护功能外, IRE1 α 还刺激凋亡信号激酶1(ASK1)的激活,进而诱导下游促进凋亡的应激激酶c-Jun氨基末端激酶(JNK)和p38的激活^[33]。JNK凋亡诱导底物BCL-2和BIM分别被JNK磷酸化抑制和激活。此外, p38磷酸化并激活转录因子CHOP,引起凋亡基因表达的改变,包括增加BIM和DR5的表达,同时降低BCL-2的表达^[53-58]。

然而,在严重的内质网应激下, IRE1 α 也可以在促细胞凋亡过程中,低聚并序列特异性地降解多种内质网上的mRNA和microRNA,这称为调节IRE1 α 依赖性降解(regulated IRE1-dependent mRNA decay, RIDD)^[59-60]。独立于其内切核糖核酸酶功能,药物性的内质网应激发生时磷酸化的IRE1 α 招募TRAF2,以促进JNK和核转录因子NF- κ B(nuclear factor-Kappa B)的活化^[61-62]。

5 总结与展望

轻度的内质网应激引起的未折叠蛋白反应是一种防御机制,以此维护细胞和机体的稳态。然而,长时间或过强的内质网应激则诱导细胞自噬或凋亡,最终导致疾病的发生。近年来,内质网应激与疾病病理生理的相关性研究取得了很大的进展,人们并以此开展了多种治疗药物的研发。然而,内质网应激参与疾病病理生理过程中仍存在许多需要迫切回答的问题,比如,内质网应激介导的细胞生存和死亡平衡点在哪里?内质网如何与其他细胞器相互配合来调节细胞死亡?对相关疾病中内质网应激的分子机制的更好理解将为许多疾病的治疗提供新的有效策略。

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