中国科学: 生命科学 2023 年 第 53 卷 第 10 期: 1345 ~ 1360
SCIENTIA SINICA Vitae lifecn.scichina.com
《中国科学》杂志社 SCIENCE CHINA PRESS
评 述 南华大学衡阳医学院65周年庆专辑

线粒体蛋白酶与人类疾病

郑斌娇[1,2], 张煜[1], 杨佳钰[1], 吕斌[1,3*]

1. 南华大学衡阳医学院基础医学院细胞生物学与遗传学教研室, 衡阳 421001;
2. 温州医科大学检验医学院(生命科学学院), 温州 325035;
3. 南华大学衡阳医学院附属南华医院消化内科, 衡阳 421002
* 联系人, E-mail: lubinmito@usc.edu.cn

收稿日期: 2023-06-01; 接受日期: 2023-09-12; 网络版发表日期: 2023-10-08

摘要 线粒体是真核细胞的重要细胞器, 在能量转换、细胞应激、脂质合成以及细胞凋亡中具有调节作用. 许多线粒体蛋白酶参与蛋白质运输、加工激活和降解过程. 其中, ATP依赖性的线粒体蛋白酶通过其AAA$^+$结构域(ATP associated multiple activity domain, AAA domain)利用ATP水解来执行线粒体蛋白质质量控制和调节蛋白降解. 线粒体蛋白酶活性的改变会导致线粒体功能障碍, 从而导致多种人类疾病, 包括心血管疾病、神经退行性疾病、衰老和肿瘤等. 本文重点综述线粒体蛋白酶1(Lon protease 1, LONP1)、酪蛋白水解蛋白酶P(caseinolytic protease, ClpP)、m-AAA(IMM-embedded AAA face to matrix)和i-AAA(IMM-embedded AAA face to intermembrane space)蛋白酶四种ATP依赖性线粒体蛋白酶及其功能, 并阐述其与人类疾病的相关性和临床意义.

关键词 线粒体, 线粒体蛋白酶, 线粒体蛋白质质量控制

线粒体有一套严格的蛋白质质量控制(protein quality control, PQC)系统, 其调控功能的发挥依赖于线粒体蛋白质组的完整性和线粒体稳态的维持. 线粒体蛋白质组主要分布在四个区域: 外膜(outer mitochondrial membrane, OM)、膜间隙(intermembrane space, IMS)、内膜(inner mitochondrial membrane, IM)和基质[1~4], 主要参与线粒体动力学、有丝分裂、凋亡、脂质生物合成、钙缓冲、氧化磷酸化和蛋白质运输相关底物的蛋白降解[5,6]. 这些蛋白质组的功能失衡会导致线粒体缺陷, 对细胞生存产生潜在的不利影响[7~10]. 其中, 线粒体蛋白酶就是一组重要的功能调节酶, 这些酶不仅将转运至线粒体的前体蛋白去除前体序列, 帮助其正确折叠, 而且能够清除错误折叠或损伤的蛋白质, 以维持线粒体正常功能. 哺乳动物线粒体45种蛋白酶中约有25种蛋白酶完全定位于线粒体内, 其余蛋白酶在线粒体与细胞质之间穿梭[11]. 研究发现, 多数线粒体蛋白酶参与蛋白质运输、加工、激活和降解过程. 大部分线粒体基因组编码的线粒体蛋白被线粒体内膜加工肽酶(processing peptidases mitochondrial inner membrane protease, IMMP)加工成熟[12,13]. 其中一些蛋白被IMMP去除疏水信号转移至线粒体膜间隙[14]. 还有部分蛋白由细胞质转运至线粒体基质后, 被线粒体基质加工肽酶(mitochondrial processing peptidase, MPP)识别并降解前体序列, 再被线粒体中间肽酶(mitochondrial intermediate peptidase, MIP)和X-脯氨酰氨肽酶3(X-Pro aminopeptidase 3,

引用格式: 郑斌娇, 张煜, 杨佳钰, 等. 线粒体蛋白酶与人类疾病. 中国科学: 生命科学, 2023, 53: 1345–1360
Zheng B J, Zhang Y, Yang J Y, et al. Mitochondrial protease and human disease (in Chinese). Sci Sin Vitae, 2023, 53: 1345–1360, doi: 10.1360/SSV-2023-0102

XPNPEP3)进一步加工成熟[12,13]. 蛋氨酸氨基肽酶1D (met aminopepti-dase 1D, METAP1D)参与去除一些线粒体编码蛋白的N端蛋氨酸残基, 从而产生功能性的线粒体蛋白[15]. 此外, 一些线粒体蛋白酶能够降解和清除错误折叠和装配的蛋白质及线粒体胁迫而损伤的蛋白质. 例如, 在胁迫条件下, LONP1(Lon protease 1)能够降解线粒体内错误折叠、氧化以及受损的蛋白质, 从而维持细胞活力. 研究发现, LONP1缺失小鼠线粒体呼吸和氧化磷酸化系统异常[16]. 线粒体基质ClpP (caseinolytic protease)与伴侣Clp蛋白酶亚单位ClpX形成复合体. 研究表明, 小鼠*ClpP*基因突变后, ClpX和mtDNA大量积累, 导致小鼠不育和生长迟缓[17]. 此外, IM上两种ATP依赖的AAA蛋白酶, i-AAA(IMM-embedded AAA face to intermembrane space)和m-AAA(IMM-embedded AAA face to matrix), 能够降解受损膜蛋白和未装配的呼吸链亚基, 维持呼吸链和线粒体嵴组织系统等复合体装配及功能, 是IM蛋白质量控制的关键酶[18]. 这些蛋白酶的缺失导致线粒体有缺陷的氧化磷酸化复合体不断积累[19]. 此外, AAA蛋白酶还具有促进线粒体蛋白质合成, 调节线粒体动力学和钙稳态的功能[20~22]. 研究发现, ATP依赖蛋白酶降解的肽进一步由前导序列蛋白酶1(presequence protease 1, PITRM1)分解为氨基酸. 如, PITRM1参与线粒体β淀粉样蛋白降解, 从而维持正常的线粒体功能[23]. 线粒体蛋白酶活性的改变会导致线粒体功能障碍, 从而导致多种人类疾病, 包括心血管疾病、神经退行性疾病、衰老和肿瘤等. 例如, LONP1蛋白酶在结肠癌和宫颈癌中高表达[24,25], ClpP蛋白酶敲除会引起Perrault综合征(Perrault syndrome, PRLTS)等[17]. 本文将重点介绍LONP1, ClpP, m-AAA和i-AAA蛋白酶四种ATP依赖性线粒体蛋白酶, LONP1和ClpP通过基于ATP水解的AAA^+结构域(ATP associated multiple activity domain, AAA domain)来执行线粒体蛋白质质量控制和调节蛋白降解(图1). 所有这些蛋白酶都形成同源或异寡聚环状结构, 具有内部依赖于ATP的蛋白水解腔[5]和线粒体早老素相关菱形样蛋白(presenilin associated thomboid like protein, PARL)[26,27].

1 LONP1

1.1 LONP1基本结构

LONP1蛋白含有963个氨基酸, 由*LONP1*(也称为*PRSS15*)基因编码, 该基因定位于人类染色体19p13.3. *LONP1*基因有三种剪切体形式, 其中剪切体2和剪切体1有共同的转录起始位点, 但比剪切体1少编码64个氨基酸; 剪切体3缩短了N端的长度(图2)[28]. LONP1蛋白主要由N端结构域、AAA^+结构域和P结构域三部分功能区域组成(图3)[29~31]. MTS(mitochondrial targeting

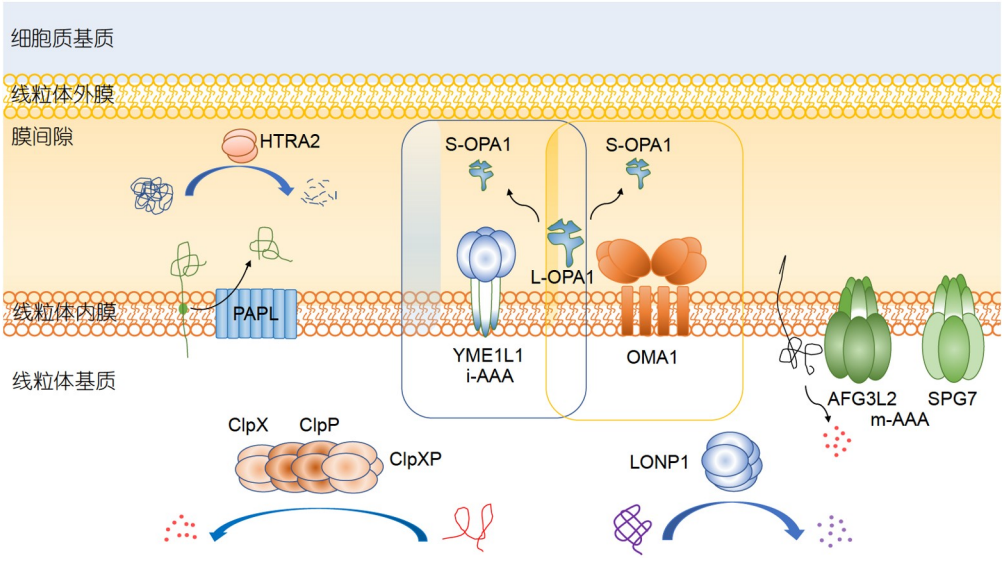

图 1 线粒体蛋白酶在线粒体蛋白质质量控制中的作用
Figure 1 The role of mitochondrial proteases in the quality control of mitochondrial proteins

sequence)是由67个氨基酸编码的线粒体前导肽. 在核糖体中翻译成蛋白后, 由MTS序列引导LONP1蛋白穿过线粒体双层膜从细胞质转移至线粒体基质, 继而MTS序列被切除, 形成成熟的LONP1蛋白[31]. N结构域是底物蛋白结合区域, 参与不同底物蛋白的识别. AAA^+结构域具有ATP酶活性, 可与ATP结合或参与ATP水解. P结构域是一个含有丝氨酸-赖氨酸二联体的水解酶活性区域[32,33]. 因此, LONP1具有ATP依赖性的蛋白质降解作用, 即通过N结构域识别蛋白底物, 依赖AAA^+结构域与ATP特异性结合, 使底物进入蛋白水解部位, 从而发生降解.

1.2　LONP1生物学功能

90%的LONP1蛋白位于线粒体基质, 剩余10%镶嵌在线粒体内膜上[28]. LONP1蛋白在线粒体基质中形成一种同源六聚体复合物, 具有蛋白水解酶活性、分子伴侣功能, 以及调节线粒体基因表达, 维持线粒体基因稳定等作用[28]. LONP1的蛋白水解酶活性促进ATP依赖蛋白的降解, 包括未折叠蛋白、氧化损伤蛋白和修饰蛋白等, 如顺乌头酸酶2(aconitase 2, Aco2)、谷氨酰胺酶(Cglutaminase C, GAC)、类固醇生成急性调控蛋白(steroidogenic acute regulatory protein, StAR)、氨乙酰丙酸δ合酶1(aminolevulinate Delta synthase 1 ALAS1)、胱硫醚-β-合成酶(cystathionine-β-synthase, CBS)和线粒体转录因子A(mitochondrial transcription factor A, TFAM)等[34~40]. Aco2在线粒体内容易受氧化损伤, 氧化变性或失活的Aco2可以被LONP1降解[41~43]. LONP1通过降解mtDNA转录必需的TFAM来调节mtDNA拷贝数, 保持必要的TFAM/mtDNA比率来调控复制和转录[44~46]. LONP1可以作为mtDNA结合蛋白, 与高GT的单链DNA(single-stranded DNA, ssDNA)以及RNA结合[28]. 还通过与mtDNA聚合酶γ及Twinkle解旋酶相互作用, 参与mtDNA的复制[47,48]. mtDNA调控区域(control region, CR)包含线粒体重链启动子区和轻链启动子区, LONP1蛋白还能与CR结合, 从而影响mtDNA的复制及转录[49]. LONP1是参与mtDNA复制和ATP依赖的有丝分裂蛋白酶(ClpX)成熟所必需的[50,51]. 低氧条件下, 小鼠神经元细胞中LONP1

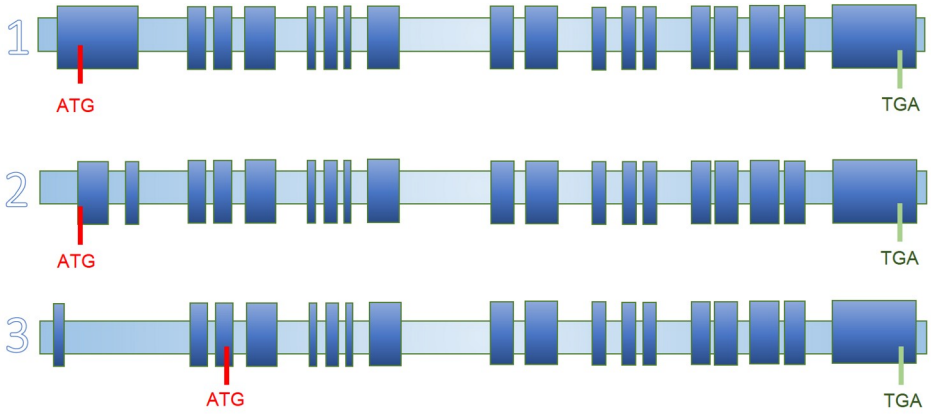

图 2　LONP1的三种剪切体结构
Figure 2　Three splicing structures of LONP1

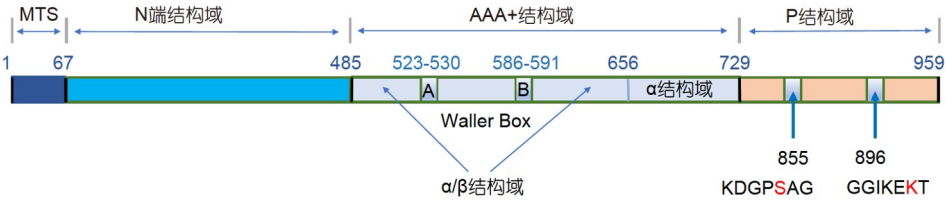

图 3　LONP1蛋白功能结构域
Figure 3　Functional domain of LONP1

降解细胞色素c氧化酶Ⅳ亚型1(cytochrome c oxidase subunit 4 isoform 1, COX4-1), 促进末端电子传递链(electron transport chain, ETC)细胞色素c氧化酶Ⅳ亚型2(cytochrome c oxidase subunit 4 isoform 2, COX4-2)的组装, 从而优化电子转移和耗氧[52]. *LONP1*基因突变的患者在线粒体疾病中往往表现出氧化磷酸化缺陷[53]. 在伴有脑、眼、齿、耳和骨异常的多系统发育障碍的CODAS(cerebral, ocular, dental, auricular, skeletal)综合征患者中发现有*LONP1*基因突变[54,55]. 突变聚集在ATP结合和蛋白水解结构域中, 导致底物蛋白水解缺陷, 以及线粒体超微结构异常和呼吸链复合体缺陷. 纯合的*Lonp1*基因缺失会导致小鼠胚胎死亡, 可能是由于无法满足胚胎发育的能量需求[56], 这也进一步证明了LONP1的重要性. 另一方面, LONP1蛋白酶的过表达与小鼠心脏功能的改善以及柄孢壳菌属细胞寿命的延长有关[57].

2 ClpP

2.1 ClpP基本结构

ClpP是一种包含丝氨酸/组氨酸/天冬氨酸蛋白酶催化三联体结构域的ATP依赖蛋白水解酶, 是细胞内一种重要的热休克蛋白, 在物种进化过程中高度保守[58,59]. 作为蛋白酶亚基的ClpP常与其ATP酶亚基伴侣(如ClpA, ClpC, ClpX)结合形成Clp复合物, 共同行使蛋白酶功能. Clp复合物是一种桶状的异质寡聚体结构[60], 由两个堆积的同质寡聚ClpP七聚体环面对面形成十四聚体, ATP酶分子伴侣(ClpX等)形成的六聚体环聚合在十四聚体的一端或两端组合为Clp全酶复合体[61,62].

2.2 ClpP生物学功能

ClpP蛋白酶在体内主要发挥蛋白水解作用, 降解异常蛋白或短寿命蛋白. ClpP蛋白酶更多的是作为Clp复合物组成之一, 与ATP酶亚基伴侣一起参与蛋白高级结构的正确折叠, 降解体内受损的蛋白, 维持机体代谢平衡. 尽管ClpP能够在没有ATP的情况下切割小肽, 但需要分子伴侣来识别特定的底物[41]. Clp复合物的ATP酶亚基伴侣是一种功能性的去折叠蛋白酶, 一方面参与蛋白底物的识别, 如大肠杆菌SsrA蛋白的C端11个氨基酸残基; 另一方面水解ATP提供能量, 将大分子底物蛋白的高级结构展开, 输送至ClpP蛋白酶的水解腔[63]. 同质性十四聚体的ClpP蛋白酶水解腔中含有14个蛋白水解活性位点, 每个水解活性位点都含有Ser/His/Asp催化三联体氨基酸[64]. 蛋白底物在水解腔中被降解为5~10个氨基酸残基的产物, 随后被发生构象改变的ClpP十四聚体指环释放[65].

研究表明, 缺失N端前段10~17个氨基酸的ClpP能够不依赖于分子伴侣ClpA或ClpX的辅助, 快速降解去折叠的大分子底物[66]. 因此, 在没有ATP酶伴侣存在的情况下, ClpP的N端区域维持闭合状态才能有效地阻止蛋白底物进入水解腔. Clp复合物分子伴侣能诱导N端构象改变, 有利于去折叠大分子蛋白底物的转移. 有研究发现, ClpA和ClpX等分子伴侣结合到ClpP后, 诱导十四聚体轴向孔开放, 允许大分子底物进入水解腔[64~67]. ClpP蛋白酶N端区域的Glu8, Arg12, Glu14和Arg15带电残基之间的相互作用对ClpP闭合构型的稳定十分重要. 另外, 相邻的两个ClpP七聚体之间形成的疏水簇也能自我稳定十四聚体的构型[68]. 因此, ClpP蛋白酶分子间的相互作用共同控制着ClpP轴向孔的开关.

然而, 目前ClpP调节蛋白残基释放的机制还有待进一步的研究, X射线下所观测到的ClpP蛋白酶N端结构域高度可变, 所以ClpP蛋白酶N端构型的变化与轴向孔开关之间的关系仍难以明确. 关于底物具体如何被降解以及降解产物通过怎样的途径来释放还存在争议. ClpP全身敲除小鼠重现了Perrault综合征的主要特征, 包括炎症和T细胞活化[17]. 线粒体ClpP的缺失可以改善真菌的健康状况, 延长其寿命[69]. ClpP蛋白酶的缺失与酿酒酵母和葡萄球菌中呼吸复合物Ⅰ功能的丧失以及它们作为兼性厌氧菌的能力相吻合[70].

3 AAA蛋白酶

AAA蛋白酶是线粒体中普遍存在的一种ATP依赖性蛋白酶家族, 它们形成的六聚蛋白酶体水解复合物嵌入线粒体内膜. 这些六聚蛋白酶体水解复合物的亚基包含一个AAA家族的ATP酶结构域和一个M48家族的金属肽酶[22]. 催化结构域分别面向线粒体基质(m-AAA)和间隙(i-AAA).

蛋白酶体复合物能够特异性地识别底物蛋白(未组装的线粒体复合物亚基或错误折叠的跨膜蛋白多肽

链)[71,72]，底物蛋白与AAA蛋白酶的外表面结合后，从膜双分子层中易位到蛋白水解腔中进行降解[72~74]. 底物易位是通过ATP酶结构域的中心孔环直接与底物蛋白结合来介导ATP水解的[75]. AAA蛋白酶具有简并的底物特异性，可以介导蛋白质完全降解为多肽，也可以因对底物蛋白的结构限制(如紧密折叠的结构域)，限制性地允许蛋白质水解[76]. 这种功能可塑性使得AAA蛋白酶成为线粒体内的多功能蛋白水解机器.

3.1　m-AAA蛋白酶

m-AAA蛋白酶是催化结构域面向线粒体基质的AAA蛋白酶，负责对基质内的常驻蛋白和面对基质的整体IMM蛋白进行蛋白水解[77]. m-AAA蛋白酶控制着线粒体蛋白质质量并调节核糖体装配，具有多效性功能[58]. m-AAA蛋白酶是肝脏和大脑线粒体中的线粒体编码蛋白合成所必需的[77,78].

在酵母中，m-AAA蛋白酶可通过其底物线粒体核糖体蛋白L32(mitochondrial ribosomal protein L32, MRPL32)调节线粒体核糖体的组装，来维持能量生产[78]. 但m-AAA蛋白酶如何调节哺乳动物线粒体中的核糖体组装和基因表达仍有待证实. m-AAA蛋白酶亚单位, 人AFG3样蛋白2(AFG3-like protein 2, AFG3L2)缺失的神经元, 会影响线粒体蛋白合成和呼吸, 造成线粒体碎片化和轴突运输缺陷[79]. m-AAA蛋白酶亚单位SPG15(也称spastizin)的突变会导致遗传性痉挛性截瘫(hereditary spastic paraplegia, HSP)[80]. 不同组织中线粒体m-AAA蛋白酶组装具有可变性. 例如, AFG3L2亚基可以与AFG3L1形成同型寡聚体或异型寡聚体[81]. 在酵母中AFG3L2亚基也可以与SPG7组装成杂合低寡聚体，替代酵母m-AAA蛋白酶[81,82]. 啮齿动物中m-AAA蛋白酶亚基AFG3L1仅在小鼠大脑中低水平表达. 因此在小鼠中，杂合低寡聚体中的一个AFG3L1亚基可以替代SPG7或AFG3L2[5,27,28]. HSP截瘫患者的SPG缺失不会导致脑线粒体中m-AAA蛋白酶活性的丧失，可能是由于形成具有改变底物特异性的m-AAA蛋白酶导致[83].

此外，m-AAA蛋白酶还可调节线粒体钙单向转运体(mitochondrialcalcium uniporter, MCU)复合物的组装[84]. 在AFG3L2缺陷的线粒体中，MCU调节亚基(essential MCU regulator, EMRE)的降解受损，导致MCU复合物的形成失控，线粒体钙超载，造成IM中线粒体通透性转换孔(mitochondrial permeability transition pore，mPTP)开放和程序性细胞死亡[85]. 在AFG3L2缺陷的小鼠模型中, 减少Purkinje细胞质钙流入可以挽救共济失调[86]. 在没有m-AAA蛋白酶的情况下，错误折叠的线粒体编码蛋白积累可以激活金属蛋白酶相关蛋白1(metalloprotease-related protein 1, OMA1)并诱导线粒体碎片化[19,87,88].

3.2　i-AAA蛋白酶

i-AAA蛋白酶是催化结构域面向线粒体膜间隙的AAA蛋白酶, 由细菌同源物FtsH进化而来[29]. i-AAA蛋白酶由酵母Yme1的6个亚基组成，在人体中被称为Yme1样蛋白酶1(YME1 like 1 ATPase, YME1L1)[89]. i-AAA蛋白酶由支架蛋白样蛋白2(stomatin-like protein2, SLP2)锚定(该蛋白也锚定线粒体加工肽酶OMA1和PARL)，并影响与其相互作用蛋白酶的蛋白水解功能[90].

i-AAA蛋白酶降解受损和错误折叠的蛋白质[71]，并对IMS常驻蛋白(如线粒体动力学、脂质转移和蛋白运输相关的蛋白)进行调控切割[91]. YME1L1与OMA1共同调节视神经萎缩蛋白1(optic atrophy 1, OPA1)，从而控制线粒体融合和分裂之间的平衡. OPA1最初在导入线粒体后由线粒体加工蛋白酶(mitochondrial processing protease, MPP)处理，产生的L-OPA1整合到内膜中. L-OPA1可通过YME1L或OMA1进行二次切割，将OPA1从L-OPA1裂解为可溶的S-OPA1，从而调节线粒体融合和裂变之间的平衡[27,92]. OMA1因其与m-AAA蛋白酶的重叠活性而得名，是一种在多种应激条件下被过度激活的金属蛋白酶，但在基本条件下也能对IMS和IM底物进行裂解. 在酵母中，*OMA1*参与mtDNA编码的突变体MT-CO1的周转[93,94]. 在哺乳动物中, OMA1将L-OPA1切割到S-OPA1以调节线粒体动力学, 当线粒体受到胁迫时, 例如使用去极化剂[87,95,96]、呼吸链抑制剂、酰基甘油激酶[97]、人高温需求因子2(high temperature requirement factor A 2, HTRA2)[98]或线粒体基因突变[87,99~101]，OMA1活性增加导致蛋白水解和L-OPA1降解，从而阻断线粒体融合. YME1L还参与细胞膜蛋白质组的蛋白质重塑，部分是通过活性氧物种调节因子1(reactive oxygen species modulator 1, ROMO1)的结构改变来实现的[91,102,103].

此外, i-AAA蛋白酶控制磷脂转移蛋白(PRELI domain containing 1, PRELID1)的调节, PRELID1的作用是将心磷脂(cardiolipin, CL)脂质前体磷脂酸(phosphatidic acid, PA)从OM穿梭到IM. PRELID1缺陷导致HeLa细胞中CL缺乏、线粒体碎片化和凋亡敏感性[104], 这也证实了IM中维持一定的磷脂组分对线粒体完整性十分重要.

在人体中, YME1L突变会导致一种神经发育疾病, 其特征为发育迟缓、听力损失、脑和视神经萎缩[97]. 像LONP1一样, 小鼠*Yme1L*缺失在胚胎阶段会致命, *Yme1L*的神经元特异性缺失导致轴突变性和眼功能障碍[105]. 小鼠心肌细胞特异性缺失*Yme1L*会导致心肌病和心力衰竭[106]. *Yme1L*的缺失增强了OPA1对OMA1的依赖性, 使平衡向线粒体代谢增加、线粒体网络断裂倾斜, 这种情况可以通过*Oma1*的缺失得以挽救[106]. 这也证明了线粒体形态对心脏功能的重要性. 然而, 在脑特异性*Yme1L* KO小鼠中, *Oma1*缺失改善了线粒体形态, 但未能预防轴突变性和运动功能障碍, 说明线粒体动力学调节组织特异性的重要性[106]. 重新平衡线粒体动力学对器官功能是有益的, OMA1缺失可预防神经退行性疾病[107]、急性肾损伤[108]和心肌病[106,109]. 最近, OMA1被确定为线粒体蛋白DAP3结合细胞死亡增强子1(DAP3 binding cell death enhancer 1, DELE1)的关键调节因子, OMA1切割DELE1促进其从线粒体输出, 以协调哺乳动物细胞系中ATF4介导的应激反应[110,111].

线粒体形态取决于OPA1的数量及其长短链的比例[5]. 在一个有四名儿童患者的家庭中, 发现YME1L中的一个纯合突变导致其序列内的错义突变, 使这些儿童患有早发性线粒体病, 伴有发育迟缓、肌肉无力、共济失调和视神经萎缩的症状[100]. 突变体YME1L快速降解, 并由于OPA1加工过程的变化导致线粒体网络的增殖缺陷和断裂.

4 线粒体蛋白酶与人类疾病

4.1 线粒体蛋白酶与肿瘤

线粒体功能障碍是癌症生物学的标志. 肿瘤线粒体代谢的特征是在缺氧条件下糖酵解(Warburg效应)功能异常[112], 遗传性肿瘤和散发性癌症都存在缺陷mtDNA的积累[113]. 线粒体呼吸异常会直接或间接引起许多癌细胞中体细胞突变或线粒体蛋白质质量控制系统成员表达水平的改变, 导致蛋白毒性和线粒体功能失衡[114,115]. 线粒体蛋白酶作为线粒体蛋白质质量控制系统中重要的调节因子, 在肿瘤中发挥癌症驱动因子或抑制因子的作用[58].

LONP1在侵袭性肿瘤中高表达[116], 其氧气和营养供应不足引起广泛性区域缺氧, 是侵袭性肿瘤的标志[117], 如结肠癌、黑色素瘤、膀胱癌、肺癌等, 并与患者的低生存率有关[49,118]. LONP1蛋白酶在肿瘤中的作用不仅体现在缺氧生存和侵袭迁移, 还可能与肿瘤细胞的抵抗凋亡及治疗耐药性有关[119]. LONP1缺失的癌细胞表现出增殖能力降低、细胞凋亡增加、生物能量降低和耐药性降低[119~121]. 在体外3D培养中, LONP1表达下调的细胞表现出增殖能力下降, 这可能与细胞周期停止在G2/M期有关[119], 也可能与LONP1沉默后产生的ROS毒性有关[120]. 而过表达LONP1则促进癌细胞的存活、增殖和迁移, 从而触发上皮到间充质的转化[121].

一些研究人员认为ClpP在癌细胞中的主要作用是增强对氧化应激和毒性应激的抵抗力, 也有人认为ClpP可能对呼吸链的质量控制和线粒体基因表达发挥更大的调节作用, 从而导致更低的损伤水平. ClpP参与氧化磷酸化过程, 因为它的底物之一是琥珀酸脱氢酶亚基A(呼吸链复合体Ⅱ的组成部分)[119,122]. ClpP也参与线粒体转录和翻译, 其底物之一线粒体GTPase ERAL1(Era (G-protein)-like 1)可以抑制线粒体活性[123]. ClpP蛋白酶的活性似乎对某些肿瘤(并非所有)很重要. 前列腺癌细胞的增殖和集落形成能力非常依赖ClpP的表达, 但ClpP活性的丧失对非转移性乳腺癌的增殖能力几乎没有影响[124]. 最近的一项研究报告称, ClpP蛋白酶可以作为急性髓系白血病(acute myeloid leukemia, AML)潜在的癌症治疗靶点[122]. 在511个AML患者样本中, 有45%的患者ClpP过度表达. 在ClpP高表达的AML细胞系中敲低ClpP可以降低细胞活力, 增加ROS产生, 同时氧化磷酸化受损[122]. ClpP过表达会诱导致死性白血病和淋巴瘤[125], 表明维持细胞稳态需要严格调节ClpP的活性, 特别是在严重依赖线粒体呼吸作用的AML细胞中.

目前没有m-AAA蛋白酶或其伴侣蛋白与癌症相关的报道[126]. 与LONP1和ClpP不同, i-AAA蛋白酶与癌症之间的联系尚不完全清楚. 有数据表明它可能抑

制癌细胞的生长, 例如, 大鼠嗜铬细胞瘤细胞PC12中, 原癌基因产物c-Myc可以下调i-AAA蛋白酶的表达[127], 也有报道称i-AAA过表达可以抑制人肝癌细胞SMMC7721的生长[128]. *YME1L1*是原发性人胶质瘤细胞发生遗传改变的基因之一, 与预后不良有关[129]. 线粒体蛋白质组重编程对存在于恶劣微环境中(如缺氧或缺营养)的实体瘤十分重要, 如胰腺导管腺癌就是最缺氧和营养最匮乏的癌症类型之一[130], PDAC细胞通过重编程谷氨酰胺代谢和HIF信号来响应这些条件[131,132]. PDAC患者活检显示, HIF1α伴随着PDAC肿瘤组织中YME1L底物的消耗而稳定[91]. 刺激YME1L蛋白水解可以优化线粒体, 以支持PDAC发育过程中的代谢适应. 但是, 肝癌细胞系的生长不依赖于YME1L, 并且在高度血管化的HCC组织中未观察到YME1L底物的消耗[91]. 因此, 在肿瘤重编程中, YME1L对蛋白水解的需求可能取决于每个肿瘤的环境和代谢需求. YME1L在人类结直肠癌和其他癌症中经常发生较小程度的突变, 这些突变的功能意义尚不清楚[133]. mTORC1抑制剂治疗刺激YME1L介导的蛋白水解, 并可能通过代谢重编程促进癌细胞存活. 另外, 用mTORC1抑制剂处理的细胞激活了YME1L, 使之发挥了线粒体拉伸的细胞保护作用[134]. YME1L的抑制已被证明可促进体外和体内细胞死亡, 这也进一步支持其作为mTORC1抑制剂的联合靶标[106,135]. 目前尚没有开发可以特异性靶向或调节i-AAA蛋白酶的化学试剂, 也没有利用i-AAA蛋白酶作为癌症药物治疗靶标的相关报道[126].

4.2　线粒体蛋白酶与衰老

线粒体功能障碍也是衰老的标志之一, 许多与年龄有关的疾病与线粒体功能障碍有关[136]. 维持线粒体功能的方式有很多种, 其中包括通过线粒体AAA蛋白酶家族选择性降解非功能性线粒体蛋白[137]. 一般认为, 细胞损伤控制酶的活性降低会导致过早衰老, 反之亦然, 蛋白质质量控制系统的活性上调有助于延长寿命[138~140]. 位于线粒体基质、内膜和膜间隙的蛋白酶在维持线粒体蛋白平衡和衰老过程中具有重要功能[140]. LONP1蛋白酶作为ROS相关线粒体蛋白质质量控制系统中的重要酶也与衰老有关. LONP1突变患者的淋巴母细胞样细胞系显示线粒体形态改变和线粒体呼吸活动减少, 导致线粒体蛋白质平衡和功能受损[141]. 敲除SPG7的果蝇寿命缩短, 与衰老相关的神经肌肉功能恶化, 并增加对热/机械/氧化应激的敏感性[142]. 在真菌中, 线粒体蛋白质质量控制效率的改善有助于提高健康和寿命[57]. 小鼠的长寿模型显示, 线粒体蛋白质质量控制系统成员(mtHsp60和LONP1)水平的增加, 与寿命延长相关[143]. 敲低Lon蛋白酶的果蝇, 整体寿命缩短, 呼吸效率降低, 并且在老年果蝇中更为明显[141]. 然而, LONP1很可能本身不是一个明显的“衰老因素”, 而是通过其作为维持线粒体功能所需的线粒体蛋白质质量控制系统成员而间接影响衰老[143]. 运动可以有效地延缓衰老导致的肌肉中LONP1下降, 同时增加线粒体生物发生[102]. 表明在衰老过程中, LONP1转录、翻译和酶活性的下降可能是由于年龄和代谢物的综合因素, 至少在骨骼肌中是这样. ClpP/ClpX对于线粒体未折叠蛋白反应(unfolded protein response, UPR)至关重要, ClpP与伴侣ClpX一起降解线粒体基质中的错误折叠蛋白质[144]. YME1L和OMA1通过对OPA1的蛋白水解和加工来调节线粒体形态, YME1L对OPA1的加工导致OPA1-d亚型的形成, 促进管状线粒体形态的维持或恢复. 相反, OMA1介导的OPA1加工导致c和e亚型的产生, 促进线粒体碎裂. YME1L和OMA1相互降解以应对不同类型的毒性挑战: YME1L降解OMA1使线粒体膜去极化但不消耗ATP. 相反, YME1L被OMA1降解, 既使线粒体膜去极化又消耗ATP. 通过这种方式, 这两种蛋白酶可以差异调节线粒体动力变化以响应不同类型的压力[145].

再生能力下降和干细胞功能障碍也是衰老的标志. 线粒体蛋白酶LONP1是小鼠卵母细胞存活所必需的, 条件性*Lonp1*敲除小鼠的卵母细胞, LONP1丢失导致进行性卵母细胞死亡, 卵巢储备下降和不孕, 还导致Aifm1介导的卵母细胞凋亡[140]. 同样, 致病性LONP1变异导致女性不孕和卵巢早衰[146]. 线粒体蛋白酶YME1L调节神经干细胞/祖细胞(neural stem/progenitor cell, NSPC)的静止和激活, 调节多种线粒体蛋白的丰度来保持NSPC的自我更新, YME1L 缺失可减少脂肪酸β氧化, 诱导NSPC过早分化, 最终导致NSPC池耗尽[147]. 线粒体蛋白酶还可以在全局范围内使蛋白质组适应细胞的营养和能量需求. 例如, 线粒体蛋白酶YME1L在营养匮乏后重塑线粒体蛋白质组. mTORC1活性降低和磷脂酸磷酸酶LIPIN1的调节启动线粒体蛋白水解, 降解线粒体转运蛋白和代谢酶, 以减少线粒

体生物发生并储备资源以支持细胞生长[91]. 这些研究说明, 蛋白酶在营养感知中的作用是抗衰老措施(如饮食限制)所必需的[140].

4.3 线粒体蛋白酶与罕见病

ROS的过量产生会促进LONP1蛋白酶表达, 以应对氧化损伤. 线粒体受损后, 不断产生并积累大量有毒的ROS, 引起帕金森病(Parkinson disease, PD)[148]. 但是, 活化的蛋白酶无法降解所有在线粒体中积累的羰基化蛋白质, 所以当氧化应激增强时, LONP1蛋白酶可能容易失活, 从而导致线粒体功能障碍和神经细胞死亡[149]. 线粒体损伤不会持续产生大量的ROS, 线粒体能够通过LONP1-ClpP蛋白质量控制轴降解线粒体呼吸链复合物Ⅰ的外周臂来抑制ROS的生成[150], 例如PD患者脑线粒体复合物Ⅰ的选择性活性缺失[151].

脑、眼、牙、耳、骨骼异常的CODAS综合征是一种罕见的常染色体隐性遗传的多系统发育异常[152]. CODAS综合征的遗传基础是LONP1中的复合杂合或纯合突变(错义、无义点突变或小片段缺失)[54,55]. CODAS患者的淋巴母细胞样细胞系表现为线粒体肿胀, 伴有电子致密包涵体和IM形态异常, 线粒体呼吸能力改变, 能量生成受损[54]. LONP1的c.2161C>G纯合子突变表型非常严重, 存在致密的双侧核性白内障和听力损失、肌张力低下、发育迟缓、智力障碍, 并且有声带萎缩、声门狭窄、慢性流涎和吞咽功能障碍, 证实了LONP1调节线粒体活性在人体发育中的重要作用[54]. 与ClpP相关的疾病包括弗里德赖希共济失调(Friedreich ataxia, FRDA), 这是一种由线粒体铁伴侣缺陷引起Fe-S簇组装失败的神经退行性疾病, 引发共济失调[153]. 在FRDA小鼠模型中, ClpP上调表达, 这种上调伴随着ClpP靶标Fe-S蛋白的损失[154]. 人类Perrault综合征以感音神经性听力损失和卵巢衰竭为特征, 其致病因素被认为是ClpP突变[155]. ClpP敲除小鼠也呈现了Perrault综合征相关表型, 具有严重的生长迟缓, 并在几种组织中表现出生物能量和呼吸缺陷[27]. ClpXP代谢相关途径的发现与这些观察结果非常一致, 表明

表 1 本文提及的线粒体蛋白酶与人类疾病汇总表

Table 1 A summary of mitochondrial proteases and human diseases mentioned in this review

线粒体蛋白酶	基因	蛋白底物	生物学功能	相关的人类疾病	参考文献
LONP1	*LONP1*	SLIRP MRPL32 FASTKD2 TFAM	PQC 蛋白水解酶活性 分子伴侣 mtDNA结合蛋白 核糖体组装	CODAS综合征 宫颈癌结肠癌 黑色素瘤 膀胱癌 肺癌 PD	[28,58,116,152,148]
CLpP	*CLpP*	ETC NDUFV1 ERAL1	PQC 蛋白底物识别 水解ATP	Perrault综合征 AML 前列腺癌 共济失调	[17,58,63,122,124,153]
CLpX	*CLpX*	ERAL2	PQC 核糖体组装	红细胞生成性原卟啉病	[27,58]
AFG3L2	*AFG3L2*	MRPL32	PQC 核糖体装配	SCA28 SPAX5	[86]
YME1L1	*YME1L1*	L-OPA1 ROMO1 TIMM17A PR1LID1	PQC 线粒体生物发生	共济失调 视神经萎缩 PDAC 心肌病	[58,71,91,98,106]
PARL	*PARL*	PINN1	PQC	PD Leber遗传性视神经病变 增加麻风病风险	[27]
OMA1	*OMA1*	DELE1	调节因子	肌萎缩侧索硬化症 神经退行性疾病 急性肾损伤 心肌病	[27,58,106~111]

Perrault综合征可能部分受ClpP功能突变后线粒体能量代谢失调驱动[156].

AAA蛋白酶亚基的基因突变与人体神经元丢失和神经变性有关. YME1L纯合突变可引起近亲系中线粒体疾病与视神经萎缩[100]. 编码m-AAA蛋白酶亚基的SPG7隐性突变导致HSP, 这种线粒体畸形的主要临床特征是皮质和小脑萎缩, 肌萎缩和智力迟钝[79]. 另一个m-AAA蛋白酶亚基AFG3L2突变与脊髓小脑性共济失调28型(spinocerebellar ataxia 28, SCA28)有关[157]. SCA28是一种罕见的常染色体显性遗传的共济失调, 常在青少年发病, 其特征是进行性步态, 肢体共济失调和小脑异常导致的眼球运动异常[142]. SPG7和AFG3L2突变与神经退行性疾病的关联可以通过两个亚基形成不同的同工酶来解释: 虽然SPG7的丢失不利于异寡聚m-AAA蛋白酶的形成, 在没有AFG3L2的情况下, 同源和异寡聚形式都会受到影响. 由于两种同工酶在很大程度上具有重叠的底物特异性, 因此SPG7和AFG3L2的相对表达可能与疾病的细胞类型特异性有关[142]. 这与AFG3L2与SPG7相互作用作为痉挛性共济失调5型(hereditary spastic ataxia 5, SPAX5)的致病因素一致. SPAX5是一种严重的早发性常染色体隐性的共济失调, 其特征是行走障碍、小脑性共济失调和肌张力障碍, 与HSP7和SCA28的临床特征类似[158].

在沙特阿拉伯血统中发现因YME1L纯合突变而导致的各种神经系统症状和线粒体网络碎片化相关的多系统线粒体病[108]. 同样, OPA1的切割增加和线粒体碎裂是小鼠心脏或神经系统中特异性缺乏YME1L的早期表型[99,105]. 值得注意的是, YME1L缺陷心肌细胞和神经元中的线粒体嵴形态正常[99,105].

5　结论和展望

近年来研究发现, 线粒体蛋白酶对于维持线粒体稳态有重要作用, 线粒体蛋白酶的缺陷对于人类疾病也有着重要影响(表1). 本文总结的四种常见的ATP依赖性蛋白酶(LONP1, ClpP, m-AAA和i-AAA蛋白酶), 及其在人类肿瘤、衰老与罕见病中的病理机制, 有助于其他线粒体蛋白酶功能和作用机制的深入研究. 以线粒体蛋白酶为潜在靶点的相关机制研究, 或将为肿瘤的治疗提供新的思路.

参考文献

1 Sickmann A, Reinders J, Wagner Y, et al. The proteome of *Saccharomyces cerevisiae* mitochondria. Proc Natl Acad Sci USA, 2003, 100: 13207–13212
2 Pagliarini D J, Calvo S E, Chang B, et al. A mitochondrial protein compendium elucidates complex I disease biology. Cell, 2008, 134: 112–123
3 Vögtle F N, Burkhart J M, Gonczarowska-Jorge H, et al. Landscape of submitochondrial protein distribution. Nat Commun, 2017, 8: 290
4 Morgenstern M, Stiller S B, Lübbert P, et al. Definition of a high-confidence mitochondrial proteome at quantitative scale. Cell Rep, 2017, 19: 2836–2852
5 Deshwal S, Fiedler K U, Langer T. Mitochondrial proteases: multifaceted regulators of mitochondrial plasticity. Annu Rev Biochem, 2020, 89: 501–528
6 Shpilka T, Haynes C M. The mitochondrial UPR: mechanisms, physiological functions and implications in ageing. Nat Rev Mol Cell Biol, 2018, 19: 109–120
7 Topf U, Wrobel L, Chacinska A. Chatty mitochondria: keeping balance in cellular protein homeostasis. Trends Cell Biol, 2016, 26: 577–586
8 Andréasson C, Ott M, Büttner S. Mitochondria orchestrate proteostatic and metabolic stress responses. EMBO Rep, 2019, 20: e47865
9 Suomalainen A, Battersby B J. Mitochondrial diseases: the contribution of organelle stress responses to pathology. Nat Rev Mol Cell Biol, 2018, 19: 77–92
10 Eisenberg-Bord M, Schuldiner M. Ground control to major TOM: mitochondria-nucleus communication. FEBS J, 2017, 284: 196–210
11 Kunová N, Havalová H, Ondrovičová G, et al. Mitochondrial processing peptidases—structure, function and the role in human diseases. Int J Mol Sci, 2022, 23: 1297
12 Wiedemann N, Pfanner N. Mitochondrial machineries for protein import and assembly. Annu Rev Biochem, 2017, 86: 685–714
13 Vögtle F N, Wortelkamp S, Zahedi R P, et al. Global analysis of the mitochondrial N-proteome identifies a processing peptidase critical for protein stability. Cell, 2009, 139: 428–439

14 Nunnari J, Fox T D, Walter P. A mitochondrial protease with two catalytic subunits of nonoverlapping specificities. Science, 1993, 262: 1997–2004

15 Serero A, Giglione C, Sardini A, et al. An unusual peptide deformylase features in the human mitochondrial N-terminal methionine excision pathway. J Biol Chem, 2003, 278: 52953–52963

16 Xu Z, Fu T, Guo Q, et al. Disuse-associated loss of the protease LONP1 in muscle impairs mitochondrial function and causes reduced skeletal muscle mass and strength. Nat Commun, 2022, 13: 894–913

17 Gispert S, Parganlija D, Klinkenberg M, et al. Loss of mitochondrial peptidase *Clpp* leads to infertility, hearing loss plus growth retardation via accumulation of CLPX, mtDNA and inflammatory factors. Hum Mol Genet, 2013, 22: 4871–4887

18 Li H, Ruan Y, Zhang K, et al. Mic60/Mitofilin determines MICOS assembly essential for mitochondrial dynamics and mtDNA nucleoid organization. Cell Death Differ, 2016, 23: 380–392

19 Hornig-Do H T, Tatsuta T, Buckermann A, et al. Nonsense mutations in the COX1 subunit impair the stability of respiratory chain complexes rather than their assembly. EMBO J, 2012, 31: 1293–1307

20 Richter U, Ng K Y, Suomi F, et al. Mitochondrial stress response triggered by defects in protein synthesis quality control. Life Sci Alliance, 2019, 2: e201800219

21 Consolato F, Maltecca F, Tulli S, et al. m-AAA and i-AAA complexes work coordinately regulating OMA1, the stress-activated supervisor of mitochondrial dynamics. J Cell Sci, 2018, 131: jcs213546

22 Patron M, Sprenger H G, Langer T. *m*-AAA proteases, mitochondrial calcium homeostasis and neurodegeneration. Cell Res, 2018, 28: 296–306

23 Falkevall A, Alikhani N, Bhushan S, et al. Degradation of the amyloid β-protein by the novel mitochondrial peptidasome, PreP. J Biol Chem, 2006, 281: 29096–29104

24 Smolarz B, Skalski D, Rysz A, et al. Polymorphism of the multidrug resistance 1 gene *MDR1* G2677T/A (rs2032582) and the risk of drug-resistant epilepsy in the Polish adult population. Acta Neurol Belg, 2017, 117: 849–855

25 Nie X, Li M, Lu B, et al. Down-regulating overexpressed human Lon in cervical cancer suppresses cell proliferation and bioenergetics. PLoS ONE, 2013, 8: e81084

26 Spinazzi M, De Strooper B. PARL: the mitochondrial rhomboid protease. Semin Cell Dev Biol, 2016, 60: 19–28

27 Gomez-Fabra Gala M, Vögtle F N. Mitochondrial proteases in human diseases. FEBS Lett, 2021, 595: 1205–1222

28 Zanini G, Selleri V, Malerba M, et al. The role of Lonp1 on mitochondrial functions during cardiovascular and muscular diseases. Antioxidants, 2023, 12: 598

29 Pinti M, Gibellini L, Liu Y, et al. Mitochondrial Lon protease at the crossroads of oxidative stress, ageing and cancer. Cell Mol Life Sci, 2015, 72: 4807–4824

30 Rotanova T V, Botos I, Melnikov E E, et al. Slicing a protease: Structural features of the ATP-dependent Lon proteases gleaned from investigations of isolated domains. Protein Sci, 2006, 15: 1815–1828

31 Wang N, Maurizi M R, Emmert-Buck L, et al. Synthesis, processing, and localization of human Lon protease. J Biol Chem, 1994, 269: 29308–29313

32 Park S C, Jia B, Yang J K, et al. Oligomeric structure of the ATP-dependent protease La (Lon) of *Escherichia coli*. Mol Cells. 2006, 21: 129–134

33 Duman R E, Löwe J. Crystal structures of bacillus subtilis Lon protease. J Mol Biol, 2010, 401: 653–670

34 Bota D A, Davies K J A. Lon protease preferentially degrades oxidized mitochondrial aconitase by an ATP-stimulated mechanism. Nat Cell Biol, 2002, 4: 674–680

35 Kita K, Suzuki T, Ochi T. Diphenylarsinic acid promotes degradation of glutaminase C by mitochondrial Lon protease. J Biol Chem, 2012, 287: 18163–18172

36 Granot Z, Kobiler O, Melamed-Book N, et al. Turnover of mitochondrial steroidogenic acute regulatory (StAR) protein by Lon protease: the unexpected effect of proteasome inhibitors. Mol Endocrinol, 2007, 21: 2164–2177

37 Tian Q, Li T, Hou W, et al. Lon peptidase 1 (LONP1)-dependent breakdown of mitochondrial 5-aminolevulinic acid synthase protein by heme in human liver cells. J Biol Chem, 2011, 286: 26424–26430

38 Teng H, Wu B, Zhao K, et al. Oxygen-sensitive mitochondrial accumulation of cystathionine β-synthase mediated by Lon protease. Proc Natl Acad Sci USA, 2013, 110: 12679–12684

39 Lu B, Lee J, Nie X, et al. Phosphorylation of human TFAM in mitochondria impairs DNA binding and promotes degradation by the AAA^+ Lon

protease. Mol Cell, 2013, 49: 121–132
40 Ugarte N, Petropoulos I, Friguet B. Oxidized mitochondrial protein degradation and repair in aging and oxidative stress. Antioxid Redox Signal, 2010, 13: 539–549
41 Bota D A, Davies K J A. Mitochondrial Lon protease in human disease and aging: including an etiologic classification of Lon-related diseases and disorders. Free Radic Biol Med, 2016, 100: 188–198
42 Gardner P R, Nguyen D D, White C W. Aconitase is a sensitive and critical target of oxygen poisoning in cultured mammalian cells and in rat lungs. Proc Natl Acad Sci USA, 1994, 91: 12248–12252
43 Grune T, Blasig I E, Sitte N, et al. Peroxynitrite increases the degradation of aconitase and other cellular proteins by proteasome. J Biol Chem, 1998, 273: 10857–10862
44 Matsushima Y, Goto Y, Kaguni L S. Mitochondrial Lon protease regulates mitochondrial DNA copy number and transcription by selective degradation of mitochondrial transcription factor A (TFAM). Proc Natl Acad Sci USA, 2010, 107: 18410–18415
45 Bogenhagen D F. Mitochondrial DNA nucleoid structure. Biochim Biophys Acta, 2012, 1819: 914–920
46 Kukat C, Wurm C A, Spåhr H, et al. Super-resolution microscopy reveals that mammalian mitochondrial nucleoids have a uniform size and frequently contain a single copy of mtDNA. Proc Natl Acad Sci USA, 2011, 108: 13534–13539
47 Fu G K, Markovitz D M. The human LON protease binds to mitochondrial promoters in a single-stranded, site-specific, strand-specific manner. Biochemistry, 1998, 37: 1905–1909
48 Liu T, Lu B, Lee I, et al. DNA and RNA binding by the mitochondrial Lon protease is regulated by nucleotide and protein substrate. J Biol Chem, 2004, 279: 13902–13910
49 Liu Y, Lan L, Huang K, et al. Inhibition of Lon blocks cell proliferation, enhances chemosensitivity by promoting apoptosis and decreases cellular bioenergetics of bladder cancer: potential roles of Lon as a prognostic marker and therapeutic target in baldder cancer. Oncotarget, 2014, 5: 11209–11224
50 Zurita Rendón O, Shoubridge E A. LONP1 is required for maturation of a subset of mitochondrial proteins, and its loss elicits an integrated stress response. Mol Cell Biol, 2018, 38: e00412-17
51 Lagouge M, Morier A, Lee H J, et al. SLIRP regulates the rate of mitochondrial protein synthesis and protects LRPPRC from degradation. PLoS Genet, 2015, 11: e1005423
52 Hao Y H, Zhang J, Wang H, et al. HIF-1α regulates COXIV subunits, a potential mechanism of self-protective response to microwave induced mitochondrial damages in neurons. Sci Rep, 2018, 8: 10403
53 Peter B, Waddington C L, Oláhová M, et al. Defective mitochondrial protease LonP1 can cause classical mitochondrial disease. Hum Mol Genet, 2018, 27: 1743–1753
54 Strauss K A, Jinks R N, Puffenberger E G, et al. CODAS syndrome is associated with mutations of LONP1, encoding mitochondrial AAA$^+$ Lon protease. Am J Hum Genet, 2015, 96: 121–135
55 Dikoglu E, Alfaiz A, Gorna M, et al. Mutations in *LONP1*, a mitochondrial matrix protease, cause CODAS syndrome. Am J Med Genet, 2015, 167: 1501–1509
56 Quirós P M, Español Y, Acín-Pérez R, et al. ATP-dependent lon protease controls tumor bioenergetics by reprogramming mitochondrial activity. Cell Rep, 2014, 8: 542–556
57 Luce K, Osiewacz H D. Increasing organismal healthspan by enhancing mitochondrial protein quality control. Nat Cell Biol, 2009, 11: 852–858
58 Ng M Y W, Wai T, Simonsen A. Quality control of the mitochondrion. Dev Cell, 2021, 56: 881–905
59 Bross P, Andresen B S, Knudsen I, et al. Human ClpP protease: cDNA sequence, tissue-specific expression and chromosomal assignment of the gene. FEBS Lett, 1995, 377: 249–252
60 Aljghami M E, Barghash M M, Majaesic E, et al. Cellular functions of the ClpP protease impacting bacterial virulence. Front Mol Biosci, 2022, 9: 1054408
61 Liu K, Ologbenla A, Houry W A. Dynamics of the ClpP serine protease: a model for self-compartmentalized proteases. Crit Rev Biochem Mol Biol, 2014, 49: 400–412
62 Lowth B R, Kirstein-Miles J, Saiyed T, et al. Substrate recognition and processing by a Walker B mutant of the human mitochondrial AAA$^+$ protein CLPX. J Struct Biol, 2012, 179: 193–201
63 Kress W, Maglica Ž, Weber-Ban E. Clp chaperone-proteases: structure and function. Res Microbiol, 2009, 160: 618–628

64 Gribun A, Kimber M S, Ching R, et al. The ClpP double ring tetradecameric protease exhibits plastic ring-ring interactions, and the N termini of its subunits form flexible loops that are essential for ClpXP and ClpAP complex formation. J Biol Chem, 2005, 280: 16185–16196

65 Yu A Y H, Houry W A. ClpP: a distinctive family of cylindrical energy-dependent serine proteases. FEBS Lett, 2007, 581: 3749–3757

66 Jennings L D, Bohon J, Chance M R, et al. The ClpP N-terminus coordinates substrate access with protease active site reactivity. Biochemistry, 2008, 47: 11031–11040

67 Martin A, Baker T A, Sauer R T. Distinct static and dynamic interactions control ATPase-peptidase communication in a AAA^+ protease. Mol Cell, 2007, 27: 41–52

68 Lee M E, Baker T A, Sauer R T. Control of substrate gating and translocation into ClpP by channel residues and ClpX binding. J Mol Biol, 2010, 399: 707–718

69 Fischer F, Weil A, Hamann A, et al. Human CLPP reverts the longevity phenotype of a fungal *ClpP* deletion strain. Nat Commun, 2013, 4: 1397

70 Abdrakhmanova A, Zickermann V, Bostina M, et al. Subunit composition of mitochondrial complex I from the yeast *Yarrowia lipolytica*. Biochim Biophys Acta, 2004, 1658: 148–156

71 Leonhard K, Stiegler A, Neupert W, et al. Chaperone-like activity of the AAA domain of the yeast Yme1 AAA protease. Nature, 1999, 398: 348–351

72 Leonhard K, Guiard B, Pellecchia G, et al. Membrane protein degradation by AAA proteases in mitochondria: extraction of substrates from either membrane surface. Mol Cell, 2000, 5: 629–638

73 Graef M, Seewald G, Langer T. Substrate recognition by AAA^+ ATPases: distinct substrate binding modes in ATP-dependent protease Yme1 of the mitochondrial intermembrane space. Mol Cell Biol, 2007, 27: 2476–2485

74 Tatsuta T, Augustin S, Nolden M, et al. *m*-AAA protease-driven membrane dislocation allows intramembrane cleavage by rhomboid in mitochondria. EMBO J, 2007, 26: 325–335

75 Augustin S, Gerdes F, Lee S, et al. An intersubunit signaling network coordinates ATP hydrolysis by m-AAA proteases. Mol Cell, 2009, 35: 574–585

76 Bonn F, Tatsuta T, Petrungaro C, et al. Presequence-dependent folding ensures MrpL32 processing by the *m*-AAA protease in mitochondria. EMBO J, 2011, 30: 2545–2556

77 Almajan E R, Richter R, Paeger L, et al. AFG3L2 supports mitochondrial protein synthesis and Purkinje cell survival. J Clin Invest, 2012, 122: 4048–4058

78 Nolden M, Ehses S, Koppen M, et al. The *m*-AAA protease defective in hereditary spastic paraplegia controls ribosome assembly in mitochondria. Cell, 2005, 123: 277–289

79 Kondadi A K, Wang S, Montagner S, et al. Loss of the m-AAA protease subunit AFG3L2 causes mitochondrial transport defects and tau hyperphosphorylation. EMBO J, 2014, 33: 1011–1026

80 Casari G, De Fusco M, Ciarmatori S, et al. Spastic paraplegia and OXPHOS impairment caused by mutations in Paraplegin, a nuclear-encoded mitochondrial metalloprotease. Cell, 1998, 93: 973–983

81 Koppen M, Metodiev M D, Casari G, et al. Variable and tissue-specific subunit composition of mitochondrial *m*-AAA protease complexes linked to hereditary spastic paraplegia. Mol Cell Biol, 2007, 27: 758–767

82 Kremmidiotis G, Gardner A E, Settasatian C, et al. Molecular and functional analyses of the human and mouse genes encoding AFG3L1, a mitochondrial metalloprotease homologous to the human spastic paraplegia protein. Genomics, 2001, 76: 58–65

83 Martinelli P, La Mattina V, Bernacchia A, et al. Genetic interaction between the m-AAA protease isoenzymes reveals novel roles in cerebellar degeneration. Hum Mol Genet, 2009, 18: 2001–2013

84 Sancak Y, Markhard A L, Kitami T, et al. EMRE is an essential component of the mitochondrial calcium uniporter complex. Science, 2013, 342: 1379–1382

85 König T, Tröder S E, Bakka K, et al. The *m*-AAA protease associated with neurodegeneration limits MCU activity in mitochondria. Mol Cell, 2016, 64: 148–162

86 Maltecca F, Baseggio E, Consolato F, et al. Purkinje neuron Ca^{2+} influx reduction rescues ataxia in SCA28 model. J Clin Invest, 2015, 125: 263–274

87 Ehses S, Raschke I, Mancuso G, et al. Regulation of OPA1 processing and mitochondrial fusion by *m*-AAA protease isoenzymes and OMA1. J Cell Biol, 2009, 187: 1023–1036

88 Richter U, Lahtinen T, Marttinen P, et al. Quality control of mitochondrial protein synthesis is required for membrane integrity and cell fitness. J Cell Biol, 2015, 211: 373–389

89 Wai T, Langer, T. The i-AAA protease. In: Rawlings N D, Salvesen G, eds. Handbook of Proteolytic Enzymes. 3rd ed. New York: Academic Press, 2013

90 Wai T, Saita S, Nolte H, et al. The membrane scaffold SLP2 anchors a proteolytic hub in mitochondria containing PARL and the *i*-AAA protease YME1L. EMBO Rep, 2016, 17: 1844–1856

91 MacVicar T, Ohba Y, Nolte H, et al. Lipid signalling drives proteolytic rewiring of mitochondria by YME1L. Nature, 2019, 575: 361–365

92 MacVicar T, Langer T. OPA1 processing in cell death and disease—the long and short of it. J Cell Sci, 2016, 129: 2297–2306

93 Bohovych I, Donaldson G, Christianson S, et al. Stress-triggered activation of the metalloprotease Oma1 involves its C-terminal region and is important for mitochondrial stress protection in yeast. J Biol Chem, 2014, 289: 13259–13272

94 Khalimonchuk O, Jeong M Y, Watts T, et al. Selective Oma1 protease-mediated proteolysis of Cox1 subunit of cytochrome oxidase in assembly mutants. J Biol Chem, 2012, 287: 7289–7300

95 Baker M J, Lampe P A, Stojanovski D, et al. Stress-induced OMA1 activation and autocatalytic turnover regulate OPA1-dependent mitochondrial dynamics. EMBO J, 2014, 33: 578–593

96 Head B, Griparic L, Amiri M, et al. Inducible proteolytic inactivation of OPA1 mediated by the OMA1 protease in mammalian cells. J Cell Biol, 2009, 187: 959–966

97 Vukotic M, Nolte H, König T, et al. Acylglycerol kinase mutated in sengers syndrome is a subunit of the TIM22 protein translocase in mitochondria. Mol Cell, 2017, 67: 471–483.e7

98 Oláhová M, Thompson K, Hardy S A, et al. Pathogenic variants in *HTRA2* cause an early-onset mitochondrial syndrome associated with 3-methylglutaconic aciduria. J Inher Metab Disea, 2017, 40: 121–130

99 Anand R, Wai T, Baker M J, et al. The *i*-AAA protease YME1L and OMA1 cleave OPA1 to balance mitochondrial fusion and fission. J Cell Biol, 2014, 204: 919–929

100 Hartmann B, Wai T, Hu H, et al. Homozygous *YME1L1* mutation causes mitochondriopathy with optic atrophy and mitochondrial network fragmentation. eLife, 2016, 5: e16078

101 Song Z, Chen H, Fiket M, et al. OPA1 processing controls mitochondrial fusion and is regulated by mRNA splicing, membrane potential, and Yme1L. J Cell Biol, 2007, 178: 749–755

102 Rainbolt T K, Saunders J M, Wiseman R L. YME1L degradation reduces mitochondrial proteolytic capacity during oxidative stress. EMBO Rep, 2015, 16: 97–106

103 Richter F, Dennerlein S, Nikolov M, et al. ROMO1 is a constituent of the human presequence translocase required for YME1L protease import. J Cell Biol, 2019, 218: 598–614

104 Potting C, Tatsuta T, König T, et al. TRIAP1/PRELI complexes prevent apoptosis by mediating intramitochondrial transport of phosphatidic acid. Cell Metab, 2013, 18: 287–295

105 Sprenger H, Wani G, Hesseling A, et al. Loss of the mitochondrial *i*-AAA protease YME1L leads to ocular dysfunction and spinal axonopathy. EMBO Mol Med, 2019, 11: e9288

106 Wai T, García-Prieto J, Baker M J, et al. Imbalanced OPA1 processing and mitochondrial fragmentation cause heart failure in mice. Science, 2015, 350: aad0116

107 Korwitz A, Merkwirth C, Richter-Dennerlein R, et al. Loss of OMA1 delays neurodegeneration by preventing stress-induced OPA1 processing in mitochondria. J Cell Biol, 2016, 212: 157–166

108 Xiao X, Hu Y, Quirós P M, et al. OMA1 mediates OPA1 proteolysis and mitochondrial fragmentation in experimental models of ischemic kidney injury. Am J Physiol Renal Physiol, 2014, 306: F1318–F1326

109 Acin-Perez R, Lechuga-Vieco A V, del Mar Muñoz M, et al. Ablation of the stress protease OMA1 protects against heart failure in mice. Sci Transl Med, 2018, 10: eaan4935

110 Guo X, Aviles G, Liu Y, et al. Mitochondrial stress is relayed to the cytosol by an OMA1-DELE1-HRI pathway. Nature, 2020, 579: 427–432

111 Fessler E, Eckl E M, Schmitt S, et al. A pathway coordinated by DELE1 relays mitochondrial stress to the cytosol. Nature, 2020, 579: 433–437

112 Kato K, Ogura T, Kishimoto A, et al. Critical roles of AMP-activated protein kinase in constitutive tolerance of cancer cells to nutrient deprivation and tumor formation. Oncogene, 2002, 21: 6082–6090

113 Bayley J P, Devilee P. Warburg tumours and the mechanisms of mitochondrial tumour suppressor genes. Barking up the right tree? Curr Opin Genet Dev, 2010, 20: 324–329
114 Warburg O. On respiratory impairment in cancer cells. Science. 1956, 124: 269–270
115 Seyfried T N. Cancer as a mitochondrial metabolic disease. Front Cell Dev Biol, 2015, 3: 43
116 Gibellini L, Pinti M, Boraldi F, et al. Silencing of mitochondrial Lon protease deeply impairs mitochondrial proteome and function in colon cancer cells. FASEB J, 2014, 28: 5122–5135
117 Gillies R J, Gatenby R A. Adaptive landscapes and emergent phenotypes: why do cancers have high glycolysis? J Bioenerg Biomembr, 2007, 39: 251–257
118 Jorissen R N, Gibbs P, Christie M, et al. Metastasis-associated gene expression changes predict poor outcomes in patients with dukes stage B and C colorectal cancer. Clin Cancer Res, 2009, 15: 7642–7651
119 Bulteau A L, Bayot A. Mitochondrial proteases and cancer. Biochim Biophys Acta, 2011, 1807: 595–601
120 Ghosh J C, Seo J H, Agarwal E, et al. Akt phosphorylation of mitochondrial Lonp1 protease enables oxidative metabolism and advanced tumor traits. Oncogene, 2019, 38: 6926–6939
121 Cheng C W, Kuo C Y, Fan C C, et al. Overexpression of Lon contributes to survival and aggressive phenotype of cancer cells through mitochondrial complex I-mediated generation of reactive oxygen species. Cell Death Dis, 2013, 4: e681
122 Cole A, Wang Z, Coyaud E, et al. Inhibition of the mitochondrial protease ClpP as a therapeutic strategy for human acute myeloid leukemia. Cancer Cell, 2015, 27: 864–876
123 Szczepanowska K, Maiti P, Kukat A, et al. CLPP coordinates mitoribosomal assembly through the regulation of ERAL1 levels. EMBO J, 2016, 35: 2566–2583
124 Seo J H, Rivadeneira D B, Caino M C, et al. The mitochondrial unfoldase-peptidase complex ClpXP controls bioenergetics stress and metastasis. PLoS Biol, 2016, 14: e1002507
125 Ishizawa J, Zarabi S F, Davis R E, et al. Mitochondrial ClpP-mediated proteolysis induces selective cancer cell lethality. Cancer Cell, 2019, 35: 721–737.e9
126 Wong K S, Houry W A. Recent advances in targeting human mitochondrial AAA^+ proteases to develop novel cancer therapeutics. Adv Exp Med Biol, 2019, 1158: 119–142
127 Guo Q M, Malek R L, Kim S, et al. Identification of c-myc responsive genes using rat cDNA microarray. Cancer Res, 2000, 60: 5922–5928
128 Wan D, Gong Y, Qin W, et al. Large-scale cDNA transfection screening for genes related to cancer development and progression. Proc Natl Acad Sci USA, 2004, 101: 15724–15729
129 Bredel M, Scholtens D M, Harsh G R, et al. A network model of a cooperative genetic landscape in brain tumors. JAMA, 2009, 302: 261
130 Vaziri-Gohar A, Zarei M, Brody J R, et al. Metabolic dependencies in pancreatic cancer. Front Oncol, 2018, 8: 617
131 Guillaumond F, Leca J, Olivares O, et al. Strengthened glycolysis under hypoxia supports tumor symbiosis and hexosamine biosynthesis in pancreatic adenocarcinoma. Proc Natl Acad Sci USA, 2013, 110: 3919–3924
132 Son J, Lyssiotis C A, Ying H, et al. Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. Nature, 2013, 496: 101–105
133 Srinivasainagendra V, Sandel M W, Singh B, et al. Migration of mitochondrial DNA in the nuclear genome of colorectal adenocarcinoma. Genome Med, 2017, 9: 31
134 Morita M, Gravel S P, Chénard V, et al. mTORC1 controls mitochondrial activity and biogenesis through 4E-BP-dependent translational regulation. Cell Metab, 2013, 18: 698–711
135 Stiburek L, Cesnekova J, Kostkova O, et al. YME1L controls the accumulation of respiratory chain subunits and is required for apoptotic resistance, cristae morphogenesis, and cell proliferation. Mol Biol Cell, 2012, 23: 1010–1023
136 López-Otín C, Blasco M A, Partridge L, et al. The hallmarks of aging. Cell, 2013, 153: 1194–1217
137 Quirós P M, Langer T, López-Otín C. New roles for mitochondrial proteases in health, ageing and disease. Nat Rev Mol Cell Biol, 2015, 16: 345–359
138 Labbadia J, Morimoto R I. The biology of proteostasis in aging and disease. Annu Rev Biochem, 2015, 84: 435–464
139 Ozkurede U, Miller R A. Improved mitochondrial stress response in long-lived Snell dwarf mice. Aging Cell, 2019, 18: e13030
140 Rai M, Curley M, Coleman Z, et al. Contribution of proteases to the hallmarks of aging and to age-related neurodegeneration. Aging Cell, 2022,

21: e13603
141 Pareek G, Thomas R E, Pallanck L J. Loss of the *Drosophilam*-AAA mitochondrial protease paraplegin results in mitochondrial dysfunction, shortened lifespan, and neuronal and muscular degeneration. Cell Death Dis, 2018, 9: 304
142 Cagnoli C, Mariotti C, Taroni F, et al. SCA28, a novel form of autosomal dominant cerebellar ataxia on chromosome 18p11.22–q11.2. Brain, 2006, 129: 235–242
143 Voos W, Pollecker K. The mitochondrial Lon protease: novel functions off the beaten track? Biomolecules, 2020, 10: 253
144 Haynes C M, Petrova K, Benedetti C, et al. ClpP mediates activation of a mitochondrial unfolded protein response in C. *elegans*. Dev Cell, 2007, 13: 467–480
145 Rainbolt T K, Lebeau J, Puchades C, et al. Reciprocal degradation of YME1L and OMA1 adapts mitochondrial proteolytic activity during stress. Cell Rep, 2016, 14: 2041–2049
146 Sheng X, Liu C, Yan G, et al. The mitochondrial protease LONP1 maintains oocyte development and survival by suppressing nuclear translocation of AIFM1 in mammals. Ebiomedicine, 2022, 75: 103790
147 Wani G A, Sprenger H G, Ndoci K, et al. Metabolic control of adult neural stem cell self-renewal by the mitochondrial protease YME1L. Cell Rep, 2022, 38: 110370
148 Pryde K R, Hirst J. Superoxide is produced by the reduced flavin in mitochondrial complex I: a single, unified mechanism that applies during both forward and reverse electron transfer. J Biol Chem, 2011, 286: 18056–18065
149 Bulteau A L, Mena N P, Auchère F, et al. Dysfunction of mitochondrial Lon protease and identification of oxidized protein in mouse brain following exposure to MPTP: implications for Parkinson disease. Free Radic Biol Med, 2017, 108: 236–246
150 Pryde K R, Taanman J W, Schapira A H. A LON-ClpP proteolytic axis degrades complex I to extinguish ROS production in depolarized mitochondria. Cell Rep, 2016, 17: 2522–2531
151 Parker Jr W D, Parks J K, Swerdlow R H. Complex I deficiency in Parkinson′s disease frontal cortex. Brain Res, 2008, 1189: 215–218
152 Shebib S M, Reed M H, Shuckett E P, et al. Newly recognized syndrome of cerebral, ocular, dental, auricular, skeletal anomalies: CODAS syndrome—a case report. Am J Med Genet, 1991, 40: 88–93
153 Puccio H, Kœnig M. Friedreich ataxia: a paradigm for mitochondrial diseases. Curr Opin Genet Dev, 2002, 12: 272–277
154 Guillon B, Bulteau A L, Wattenhofer-Donzé M, et al. Frataxin deficiency causes upregulation of mitochondrial Lon and ClpP proteases and severe loss of mitochondrial Fe-S proteins. FEBS J, 2009, 276: 1036–1047
155 Jenkinson E M, Rehman A U, Walsh T, et al. Perrault syndrome is caused by recessive mutations in *CLPP*, encoding a mitochondrial ATP-dependent chambered protease. Am J Hum Genet, 2013, 92: 605–613
156 Fischer F, Langer J D, Osiewacz H D. Identification of potential mitochondrial CLPXP protease interactors and substrates suggests its central role in energy metabolism. Sci Rep, 2015, 5: 18375
157 Di Bella D, Lazzaro F, Brusco A, et al. Mutations in the mitochondrial protease gene *AFG3L2* cause dominant hereditary ataxia SCA28. Nat Genet, 2010, 42: 313–321
158 Pierson T M, Adams D, Bonn F, et al. Whole-exome sequencing identifies homozygous *AFG3L2* mutations in a spastic ataxia-neuropathy syndrome linked to mitochondrial *m*-AAA proteases. PLoS Genet, 2011, 7: e1002325

Mitochondrial protease and human disease

ZHENG BinJiao[1,2], ZHANG Yu[1], YANG JiaYu[1] & LU Bin[1,3]

1 Department of Cell Biology and Genetics, School of Basic Medical Sciences, Hengyang Medical School, University of South China, Hengyang 421001, China;
2 School of Laboratory Medicine and Life Sciences, Wenzhou Medical University, Wenzhou 325035, China;
3 Department of Gastroenterology, The Affiliated Nanhua Hospital of Hengyang Medical School, University of South China, Hengyang 421002, China

Mitochondria are important organelles of eukaryotic cells and confer regulatory roles in energy conversion, cellular stress, lipid synthesis, and cell death. A plethora of mitochondrial proteases are involved in protein transport, processing activation and degradation. Among them, adenosine triphosphate ATP-dependent mitochondrial proteases, based on ATP hydrolysis through their Adenosine triphosphatases (ATPases) associated with diverse cellular activities (AAA^+) structural domain, the quality control of mitochondrial protein and regulate protein degradation. Alterations in mitochondrial protease activity can lead to mitochondrial dysfunction, thus leading to a variety of human disease, including cardiovascular disease (CVDs), neurodegenerative disease, aging, and tumor. This study provides a systematic review of several well-studied ATP-dependent mitochondrial proteases and explores their functional relevance and clinical implications to human disease.

mitochondria, mitochondrial protease, mitochondrial protein quality control

doi: 10.1360/SSV-2023-0102