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评 述 多细胞器之间的离子稳态调控专题

内质网-线粒体互作与钙稳态

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摘要 内质网(endoplasmic reticulum, ER)是细胞中最大的细胞器之一, 是细胞内的钙库, 也是蛋白质合成与运输、蛋白质折叠、脂肪以及类固醇合成主要部位. 线粒体是细胞内的能量工厂, 参与细胞内的凋亡调控、氧化还原平衡、生物合成和信号转导, 同时线粒体在钙稳态维持中也发挥着关键作用. 内质网和线粒体外膜存在物理上的接触结构, 这一结构被称为线粒体相关内质网膜(mitochondria-associated endoplasmic reticulum membranes, MAMs). MAMs在多种细胞通路中发挥着核心作用, 参与包括线粒体动态、自噬、炎症反应、脂质代谢、钙稳态及内质网应激等多种生物学功能, 并与多种疾病的发生发展密切相关. 本文主要介绍MAMs的结构和分子组成, 并重点围绕MAMs在钙稳态中的作用进行综述.

关键词 线粒体, 内质网, 线粒体相关内质网膜, 钙稳态

细胞的膜结构可以将细胞内不同区域分割为相对独立的区域, 由此产生各种不同的细胞器. 细胞器的功能并不是孤立的, 而是形成动态的相互连接的网络, 因此细胞器之间存在着紧密的相互作用, 这种互作主要体现在细胞器之间的物质交换及信号传递, 以及物理上的接近或者直接接触. 内质网(endoplasmic reticulum, ER)与线粒体之间存在动态的膜接触位点(ER-Mito contact sites), 它们密切联系, 形成一个动态的平台, 称为线粒体相关内质网膜(mitochondria-associated endoplasmic reticulum membranes, MAMs). 钙(Ca^{2+})是细胞内稳态所必需的第二信使, 通过MAMs结构内质网能将适当的钙信号传递给线粒体, 线粒体将其解码为特定的输入信号, 以调节细胞的基本功能, 包括新陈代谢、能量产生和细胞凋亡等. 本文主要立足于讨论Ca^{2+}在内质网-线粒体之间的转运, 以及在内质网-线粒体互作情况下的钙离子在细胞内动态调节的机制. 并进一步介绍了参与内质网-线粒体之间钙信号转运的关键蛋白及信号通路(图1).

1 MAMs简述

在20世纪50年代, 科学家们就发现了内质网和线

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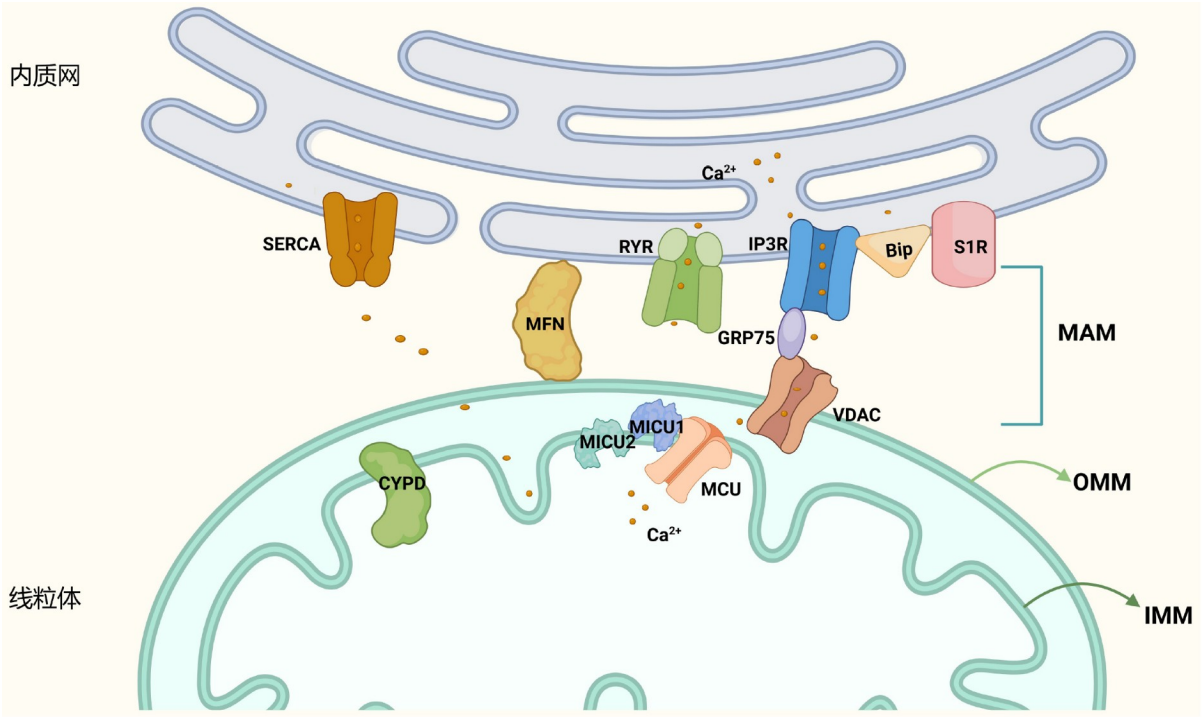

图 1 参与内质网-线粒体钙离子转运的蛋白质和通道(Created with BioRender.com)
Figure 1 Proteins and channels involved in ER-mitochondrial Ca^{2+} transfer (Created with BioRender.com)

粒体之间的相互连接，这是第一个发现的两种细胞器间接触部位[1]. 20世纪90年代初，Vance研究小组分离出了与线粒体外膜(outer mitochondrial membrane, OMM)密切接触的内质网亚细胞部分，称为MAMs. 根据电子显微镜的分类，MAMs分为三种类型: (ⅰ) 覆盖约50%线粒体表面的内质网突起; (ⅱ) 包裹整个线粒体的内质网小管; (iii) 延伸至线粒体的内质网小管，形成覆盖约10%线粒体表面的单一接触点[2]. 随着从不同的哺乳动物组织和培养细胞中生化分离MAMs的技术不断优化，MAMs相关领域的研究取得了很大的进展[3,4]. 最初，MAMs被认为是脂质合成、运输和代谢的重要平台，主要是因为在MAMs发现了数种脂质合成相关蛋白质，其中包括参与胆固醇酯形成的酰基辅酶A，胆固醇酰基转移酶1(ACAT1/SOAT1)[5]和二酰基甘油酰基转移酶2(DGAT2)[6]，以及磷脂酰丝氨酸(PS)合酶[7]. 其中ACAT1能将长链脂肪酸转移到胆固醇从而通过酯化作用形成胆固醇酯，并结合成胞质脂滴; DGAT2负责各组织及皮肤基本脂肪的平衡; 磷脂酰丝氨酸合酶则主要参与PS的合成. 研究人员从酵母细胞中分离出被称为内质网-线粒体接触位点结构(ER-mitochondria encounter structure, ERMES)和内质网-膜蛋白复合体(ER membrane protein complex, EMC)的特殊蛋白质复合体，并对该复合体的所有蛋白质组分进行了详细的鉴定和研究，发现ERMES由胞质蛋白Mdm12、ER膜蛋白Mmm1以及线粒体外膜蛋白Mdm34和Mdm10形成[8,9]，并且通过对比发现大多数酵母的ERMES蛋白质缺乏哺乳动物的同源物. 研究表明在哺乳动物细胞中，有一些内质网蛋白和一些相应的线粒体蛋白存在互作，并且这些蛋白都有MAMs的定位，可能是MAMs的组成部分[10]. 例如，位于内质网的丝裂原蛋白2(MFN2)，与线粒体丝裂原蛋白1和2(MFN1和MFN2)形成同型和异型接触[11]; 内质网定位的B细胞受体相关蛋白31(BAP31)，与线粒体裂变蛋白1(FIS1)结合[12]; 内质网囊泡相关蛋白B(VAPB)，与线粒体蛋白酪氨酸磷酸酶蛋白51(PTPIP51)存在相互作用[13]; 线粒体FUN14结构域包含蛋白1(FUNDC1)，在缺氧条件下通过与ER驻留蛋白钙联蛋白(CANX)相互作用而在MAMs处富集[14]. 此外，有研究人员通过生物信息预测的方法在哺乳动物神经元中发现了一种内质网-线粒体互作的拴系蛋白PDZD8，并证明了PDZD8为

酵母ERMES复合体组分Mmm1的结构同源物[15]. 研究人员通过蛋白质组学方法鉴定出整个MAMs蛋白质组可能包括900~1200个蛋白质[16,17]. 不同的细胞被认为具有不同的线粒体-内质网接触(mitochondria-ER contacts, Merc)图谱和不同的MAMs数量, 从而赋予相应细胞特定的分子和物理特征. MAMs占HeLa细胞线粒体膜总面积的5%~20%[18], 占小鼠肝细胞线粒体膜总面积的4%~11%[19]. 膜区域的部分的MAMs含量取决于细胞的能量状态, 当线粒体的呼吸量减少20%时, 膜区域的MAMs会增加一倍[19]. 这些研究表明MAMs是一种动态结构, 同时MAMs的可塑性也是调节细胞功能的一个非常重要的因素. 许多研究表明, MAMs蛋白的功能是多方面的, 涉及钙信号转导、线粒体动力学、内质网应激、细胞凋亡等多种功能(图2). 本文将主要讨论MAMs与钙稳态调控相关的机制与通路.

2　内质网中的钙调控

内质网中主要存在两种参与钙调控的转运体, 第一种是肌浆/内质网钙-ATPase型钙泵(sarco/endoplasmic reticulum Ca^{2+} ATPase, SERCA), 它将钙离子从胞浆主动地泵入内质网进而维持内质网钙水平稳定. 其中SERCA以三种不同的SERCA基因(*ATP2A1*, *ATPA2*和*ATP2A3*)存在, 每个基因都产生不同亚型, 其中*SERCA2b*和*SERCA2c*亚型丰度较高, 而*SERCA3*表达较少. 此外, SERCA蛋白也表现出组织特异性的分布; 例如,

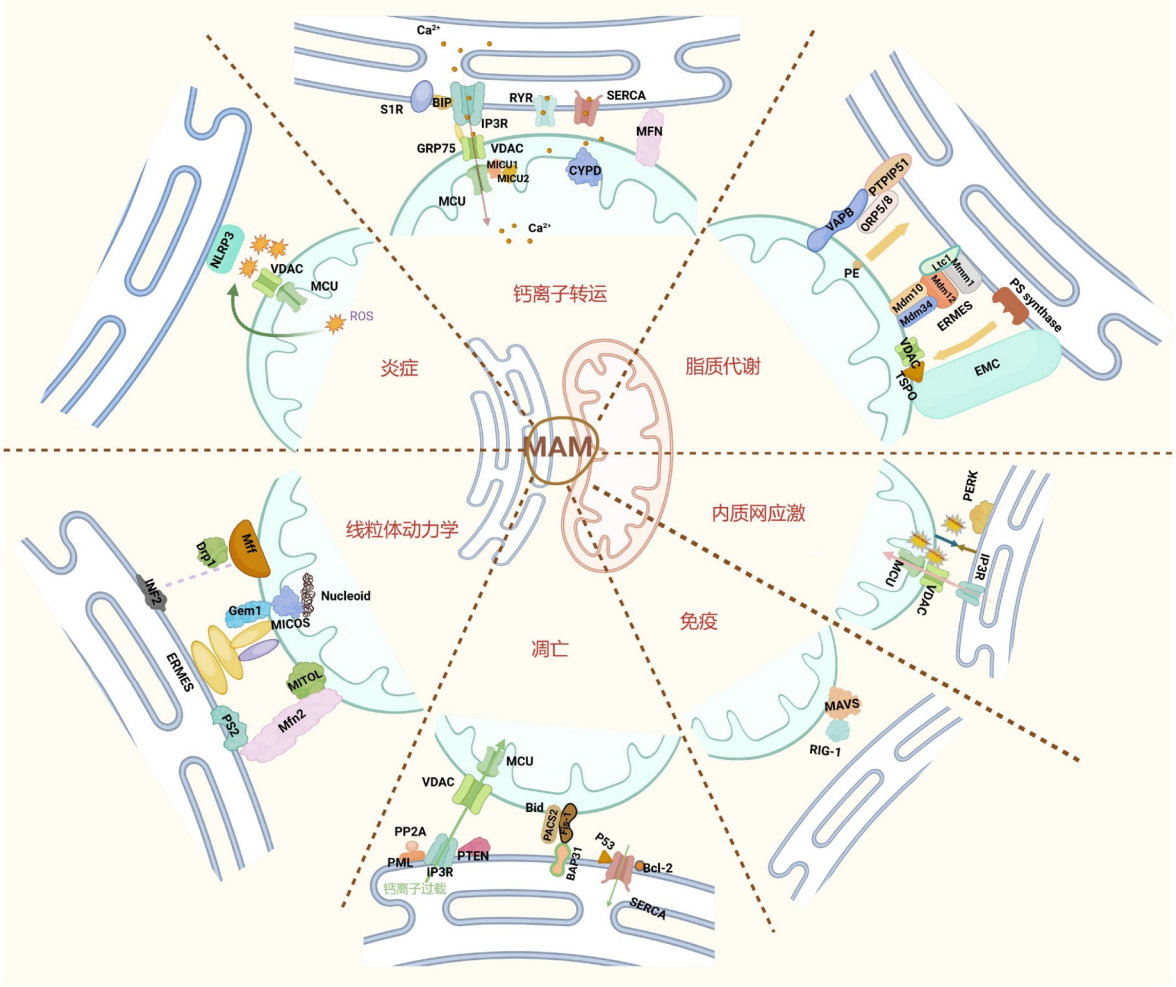

图 2　内质网与线粒体间的互作与多种细胞生命过程(Created with BioRender.com)

Figure 2　Interactions between endoplasmic reticulum and mitochondria in various cellular life processes (Created with BioRender.com)

SERCA1a和SERCA1b主要在骨骼肌中表达，SERCA2a主要在心肌和骨骼肌慢纤维中表达[20]. 其中具有最高的钙亲和力亚型为SERCA2b，它的正确表达对于内质网钙摄取和细胞死亡机制的调控至关重要. 在转基因小鼠中，编码*SERCA2b*基因的杂合缺失会导致上消化道鳞状细胞癌[21]，甲状腺细胞中*SERCA2b*基因的表达显著减少会诱发甲状腺肿瘤[22]. 截短的*SERCA1*亚型的过度表达会引起内质网应激，并通过增加MAMs数量和线粒体钙积累进而促进凋亡的发生[23]. 有氧化还原活性形式的硒蛋白N1(SEPN1)在MAMs处与SERCA相互作用，从而调节ER Ca^{2+}水平[24]. 同时，SEPN1可以拮抗内质网应激过程中发生的氧化损伤，并触发未折叠蛋白反应(unfolded protein response, UPR)，在UPR诱导后活性氧(reactive oxygen species, ROS)的数量增加，而氧化衍生物会使SERCA失活[24]. 内质网膜蛋白(TMCO1)可以作为内质网钙过载的漏流通道, 其能感知内质网中过高的钙浓度, 并形成活性的四聚体钙通道, 将内质网中过多的钙离子排出, 有研究发现, TMCO1缺乏会引起患者颅面胸畸形以及神经发育异常[25,26]. 同时大量研究表明, 机体发生各种疾病与内质网上的SERCA的调控相关. 例如, SERCA2的表达与结直肠癌的大小和转移呈正相关[27]; 调节SERCA可以抑制跨膜受体蛋白Notch-1所驱动的癌症[28]. 在肿瘤浸润的$CD8^+$ T细胞中, MFN2通过与SERCA2相互作用, 刺激了SERCA2的ER Ca^{2+}回收活性, 从而防止过多的线粒体Ca^{2+}积累和细胞凋亡，因此可以通过增加$CD8^+$ T细胞中的MFN2来提高线粒体-ER接触，从而提高癌症免疫疗法的效果[29].

第二个主要参与内质网钙调控的转运体是一个蛋白质家族, 该家族主要分为两个亚组: 兰尼碱受体(ryanodine receptors, RyRs)和三磷酸肌醇受体(inositol 1,4,5-trisphosphate receptors, IP3Rs), 它们是ER上主要的钙释放通道[30]. RyRs主要在肌浆网中表达，而IP3R是一种大电导非选择性阳离子通道，在所有类型的细胞中普遍表达. 与RyRs相比，IP3Rs的研究更为广泛. IP3R通道以三种不同的亚型存在(IP3R1，IP3R2和IP3R3)，它们的氨基酸序列显示出大约60%~80%的同源性，并且都能被第二信使IP3、钙、钙结合蛋白和ATP修饰以及被几种癌基因和肿瘤抑制因子的磷酸化激活[31]. 这些异构体在不同类型的细胞中表达特定的亚型：IP3R1主要在神经细胞中表达，IP3R2主要在肌肉和肝脏细胞中表达，IP3R3在大多数培养细胞类型中均表达[32]. 研究表明，肿瘤抑制因子早幼粒细胞白血病蛋白(PML)可以与IP3R3互作调控ER的钙释放[33]. 同时也有研究表明，PML可以通过影响IP3R3的活性，进而减少从内质网向线粒体的钙释放以此来降低线粒体的新陈代谢和ATP的产生，最终引起自噬发生[34,35]. 此外有研究发现，癌细胞依赖IP3R介导的钙离子以维持线粒体功能，抑制IP3R可导致癌细胞大量死亡，而正常细胞却不会发生死亡，为癌症的治疗提供了新的思路[36].

3 线粒体钙调控

从内质网释放的钙离子通过线粒体膜外膜上的电压依赖性阴离子通道(voltage-dependent anion channel, VDAC)到达膜间隙. VDAC具有三种不同的亚型, 这三种亚型在几乎所有的哺乳动物组织中都有表达，且均表现出类似的通道特性. 其中，VDAC1位于内质网线粒体接触位点, 选择性地与IP3R3相互作用, 从而加强低幅度的凋亡钙信号向线粒体的转移[37]. 研究表明在MAMs处，葡萄糖调节蛋白75(GRP75)参与IP3R-VDAC1的互作, 并且会形成VDAC1-GRP75-IP3R复合物，敲低GRP75则会抑制IP3R和VDAC1之间的互作, 进而减少线粒体的钙摄取[38]. 髓系细胞白血病因子1(Mcl-1)与VDAC1相互作用, 可以通过增加线粒体钙摄取和ROS生成进而促进肺癌细胞迁移[39]. 不同于VDAC1，VDAC2与B淋巴细胞瘤-2(Bcl-2)家族成员Bcl-xs相互作用并导致促凋亡蛋白(Bak)的释放，VDAC2的过表达则会选择性地抑制Bak的激活，进而抑制线粒体的凋亡途径[40,41].

膜间隙内的钙离子要到达线粒体基质，必须通过线粒体钙单向转运蛋白(mitochondrial calcium uniporter, MCU). MCU的表达严格依赖于钙离子, 核因子环磷酸腺苷反应元件结合蛋白(cAMP responsive element bind protein, CREB)会直接结合MCU启动子并促进其转录[42~45]. 研究人员通过冷冻电子显微镜技术发现，MCU在人体内主要由四个蛋白亚基组成，包括形成离子通道的MCU、对MCU活性非常关键的EMRE以及调节MCU活性的MICU1和MICU2. MICU1和MICU2对MCU活性的调节涉及一种门控机制：当细胞处于静息状态、胞质内钙离子(Ca^{2+})浓度较低时，MICU1-

MICU2抑制Ca^{2+}通过MCU进入线粒体; 而当细胞受到信号刺激、胞质内Ca^{2+}浓度升高并超过一定阈值时(约大于1 μmol/L), MICU1-MICU2则允许Ca^{2+}通过MCU进入线粒体[46]. 此外, 有研究人员通过果蝇全基因组RNA干扰(RNA interference, RNAi)筛选, 鉴定出线粒体内膜上的另一个具有钙调控的含亮氨酸拉链/EF手的跨膜蛋白1(LETM1), LETM1进化保守, 在内膜上普遍表达, 是一种钙离子/氢离子的反向转运体[47]. 含有LETM1的染色体区域的杂合缺失会引发以颅面缺陷、生长和智力低下、肌张力低下、先天性心脏缺陷和癫痫发作为特征的Wolf-Hirschhorn综合征, 其中LETM1的缺失会损伤机体的葡萄糖氧化功能, 进而进一步促发Wolf-Hirschhorn综合征中的癫痫发作[48,49].

线粒体内Ca^{2+}升高会导致两种可能的相反效果: 刺激Krebs循环, 增加NADH的形成和加强呼吸链活性, 从而增加ATP的产生; 或者由于线粒体Ca^{2+}超载, 线粒体通透性转换孔(mitochondrial permeability transition pore, MPTP)打开, 细胞色素c的释放增加, 进而激活体内的细胞凋亡途径[50~52]. 内质网Ca^{2+}释放, 导致线粒体基质的Ca^{2+}浓度瞬时增加, 可以刺激NADH的产生和ATP的合成进而刺激柠檬酸循环[53,54]. 在这个过程中丙酮酸脱氢酶(PDH), 可以将丙酮酸转化为乙酰辅酶A, 进入柠檬酸循环; 异柠檬酸脱氢酶(ICDH)和氧化戊二酸脱氢酶(OGDH)会受到钙离子的直接结合和变构调节, 进而导致对底物的亲和力增加[55]. 研究表明, 通过IP3Rs抑制剂Xestospongin B或通过IP3Rs缺失可以阻断ER-线粒体钙离子的转运进而抑制线粒体的ATP合成, 导致细胞内AMP/ATP比率增加, AMPK激活而后诱导自噬的发生[35]; 此外, 稳定的MCU基因敲除也会抑制线粒体钙的摄取, 降低细胞的氧耗率, 激活AMPK并诱导自噬[56]. 同时研究表明, 线粒体Ca^{2+}过载会导致线粒体的功能发生变化, 例如, ATP生成减少和ROS生成增加[57~59]. 导致MPTP开放的主要因素包括Ca^{2+}、亲环素D(CYPD)和ROS, MPTP开放会导致线粒体结构肿胀进而导致含半胱氨酸的天冬氨酸蛋白水解酶(Caspase)辅助因子释放到胞浆中, 其中主要包括凋亡诱导因子(apoptosis-inducing factor, AIF)、细胞色素c、半胱氨酸激活剂(Smac/DIABLO)、Caspase9原和核酸内切酶G. 其中, 细胞色素c与凋亡蛋白激活因子1(Apaf-1)相互作用诱导产生的“凋亡体”在细胞质中可以激活caspase9原和效应caspase(caspase-3/6/7); AIF和内切酶G互作会导致DNA片段化和染色质凝集[60,61].

4　MAMs与钙稳态的调控

Ca^{2+}在细胞正常生理功能维持中发挥着关键作用, 其能调节线粒体产生ATP的能力, 同时也是细胞代谢调节和细胞膜完整性及通透性维持所必需的. 同时, Ca^{2+}也是细胞内重要信号转导和细胞生存所必需的细胞内第二信使, 在体内, Ca^{2+}参与各种组织的生理功能的调控, 如神经调节、肌肉收缩、凝血、免疫调节等. MAMs通过调节细胞内钙信号, 在细胞代谢和细胞命运决定的各个方面发挥着关键作用[62]. 内质网是细胞内主要的钙存储细胞器, 线粒体内钙离子的积累主要依赖于内质网, 受内质网泵和通道的控制. 内质网蛋白肌醇/内质网钙ATPase(SERCA)和三磷酸肌醇受体(IP3R)参与钙离子的转移, 并且在MAMs处大量富集. SERCA定位于ER膜, 通过将胞浆中的Ca^{2+}泵入内质网来调节ER的Ca^{2+}水平, 从而在胞浆(0.1 μmol/L)和ER(100~1000 μmol/L)之间形成一个Ca^{2+}梯度. SERCA2b亚型表现出最高的钙亲和力, 并在MAMs中富集. SERCA2b的活性也受到其他蛋白的调控, 比如CANX和硫氧还蛋白相关跨膜蛋白1(TMX1), CANX通过棕榈酰化增强SERCA2b活性, 诱导其功能从蛋白质折叠的质量控制向ER-Ca^{2+}信号的转变, 而TMX1则通过抑制SERCA2b活性进而促进钙离子内流到线粒体[63]. IP3Rs和RyRs是内质网钙释放的主要通道. 在MAMs组分中富含大量的IP3R3, IP3R3参与钙离子从内质网到线粒体的转运, 并与线粒体电压依赖性阴离子通道1(VDAC1)相互作用. Ca^{2+}通过VDAC1跨过线粒体外膜, 进入线粒体膜间隙(intermembrane space of mitochondria, IMS), 再由线粒体内膜(inner mitochondrial membrane, IMM)上的MCU转移到线粒体基质中[42,43]. MAMs中的σ1R(sigma-1 receptor)可以通过调控内质网和线粒体间的钙离子转运进而调控铁死亡[64]. 此外还有研究表明, 在内质网和线粒体之间的界面上存在高钙微域, 若两者间的距离超过9 μm, 胞质Ca^{2+}浓度会大幅上升, 这表明内质网和线粒体之间的距离是有效地将钙离子输送到低钙亲和力MCU所必需的[65~67].

此外, MAMs中还存在各种不同的癌基因和抑癌基因编码的蛋白, 这些蛋白也参与钙稳态的调控. 例如, 定位于MAM s的磷酸酶和张力蛋白同源物(PTEN),

是一种具有脂质和蛋白质双重特异性活性的磷酸酶, 其缺失或突变会诱发癌症发生. 在MAMs中, Akt能够磷酸化IP3R3, 并通过抑制IP3R3介导的Ca^{2+}从ER向线粒体的流出进而抵抗凋亡; PTEN通过IP3R以蛋白磷酸酶依赖的方式调节ER钙释放, PTEN敲除会抑制ER Ca^{2+}释放, 降低胞质和线粒体Ca^{2+}瞬变, 并抑制Ca^{2+}介导的凋亡的发生[68,69]; 细胞内的关键抑癌蛋白P53被证明定位于内质网和MAMs区域且与SERCA泵存在相互作用, 而增加内质网的钙释放导致细胞凋亡[70]. BcL-2是Bcl-2家族的成员, 在MAMs中富集, 可以减少线粒体的钙释放, 从而抑制细胞的凋亡[71,72]. 癌基因*H-RAS*被证明定位于MAMs和脂质相关膜(plasma membrane-associated membranes, PAM), 其所调控的钙信号在促进肿瘤的形成和维持中具有重要作用[73]; 此外, 在结肠癌细胞系中观察到致癌K-RAS抑制内质网钙离子释放, 降低内质网钙离子水平, 抑制钙离子流入线粒体[74]. 胎儿和成人睾丸表达的蛋白(fetal and adult testis-expressed transcript protein, FATE1)在多种癌症中过度表达, 同时也被发现定位于MAMs中, FATE1参与调节ER-线粒体距离、线粒体对钙的摄取以及癌细胞中药物依赖性的凋亡[75]. MAMs也与多种疾病的发生发展相关, 例如, 定位于MAMs的蛋白Beclin1与PINK1会在自噬过程中发生互作, 而诱导帕金森病的发生[76]; 在糖尿病心脏病中, 高血糖导致小鼠心肌细胞中AMPK活性降低, FUNDC1表达增加, 进而增加MAMs的含量, 线粒体中Ca^{2+}浓度增加, 从而诱发糖尿病心肌病[77].

5 总结

综上所述, 在生理和病理条件下, 内质网和线粒体在钙信号的传递中起着核心作用, 而MAMs是钙离子转运的关键区域. 在MAMs处SERCA将钙离子从细胞质转运到内质网, IP3R和RyRs将钙离子从内质网释放, 导致细胞内钙离子迅速增加. GRP75将负责内质网钙外流的通道IP3R与线粒体电压依赖性离子通道VDAC1联系起来以调节线粒体对钙的摄取. 从内质网流出的钙离子通过VDAC1通道通过线粒体外膜, 到达线粒体膜间隙, 并通过MCU复合体再转入线粒体基质中.

内质网与线粒体间的互作在钙稳态的调控中具有十分重要的生理意义. 二者间的钙稳态失衡与很多正常生理活动和病理变化相关, 很多癌症相关因子和MAMs定位的钙调控相关蛋白有关. 研究内质网和线粒体间的互作与钙稳态间的关系, 对于理解相关疾病的发病机制、提出有效的防治策略也有积极意义.

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Endoplasmic reticulum-mitochondrial interaction and calcium homeostasis

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The endoplasmic reticulum (ER) is one of the largest organelles in the cell, and is the main site of protein synthesis and transport, protein folding, lipid and steroid synthesis, as well as the intracellular calcium reservoir. Mitochondria are intracellular energy factories involved in apoptosis regulation, redox homeostasis, biosynthesis, and signal transduction, and they also play a key role in the maintenance of calcium homeostasis. The endoplasmic reticulum and the outer mitochondrial membrane are in physical contact with each other in a structure known as mitochondria-associated endoplasmic reticulum membranes (MAMs). MAMs play a central role in a variety of cellular pathways, including mitochondrial dynamics, autophagy, inflammation, lipid metabolism, calcium homeostasis etc,and are closely related to the development of many diseases. This article introduces the structure and molecular composition of MAMs and focuses on the role of MAMs in calcium homeostasis.

mitochondrial, endoplasmic reticulum, mitochondrial related endoplasmic reticulum, calcium homeostasis

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