综述

天冬氨酸介导的细胞增殖调控

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摘要 细胞生长与增殖是一个复杂的调控过程, 需要大量营养物质、能量、辅因子及代谢中间物。该文着重讨论了天冬氨酸与细胞呼吸过程对细胞增殖的影响及其作用机制。首先, 介绍了电子传递链(electron transport chain, ETC)的作用原理, 并讨论与细胞增殖之间的关系; 其次, 从天冬氨酸的合成代谢开始, 探讨了天冬氨酸对ETC缺陷型细胞的影响, 与三羧酸循环、核苷酸代谢的相互关系, 进而探讨了天冬氨酸作为一个重要氨基酸对细胞周期的协调作用。总结已有研究成果发现, 天冬氨酸不同于丙酮酸作为电子受体发挥功能, 而是通过其在体内的合成与转化参与胞内三羧酸循环及核苷酸代谢等生物途径进而促进细胞增殖, 为后续细胞增殖调控研究提供了新思路。

关键词 天冬氨酸; 细胞呼吸; 细胞增殖

The Aspartate-Mediated Coordination of Cell Proliferation

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Abstract Regulation of the cell proliferation and cell growth is complex, which requires a large number of nutrient, energy, cofactor and metabolic intermediates. The paper discussed the roles of aspartate and cell respiration in the cell proliferation. We described the ETC (electron transport chain) working mechanisms and discussed its relationship with cell proliferation. We also summarized the functions of aspartate in the cell cycle progression by reviewing the aspartate metabolism, effects of aspartate in ETC defective cell. The review finds that aspartate accelerates the cell cycle progression through participating in the Krebs cycle and nucleotide metabolism instead of serving as an electron acceptor like pyruvate. The review may provide a clue for further work in regulation of the cell proliferation.

Keywords aspartate; cell respiration; cell proliferation

快速增殖细胞在每一个细胞周期都需要将胞内的全部组分进行合成以实现细胞增殖[1]。细胞增殖需要额外的能量以维持其生长及分裂需要[2]。细胞增殖通过将胞外的营养物质如葡萄糖、谷氨酰胺转变为前体物质以供生物大分子合成, 进而促进细胞生长[3]。细胞增殖是一个全局性的调控过程, 需要增加葡萄糖、谷氨酰胺等营养物质的摄取, 提高核苷酸、脂质、蛋白质的生物合成, 维持6-磷酸葡萄糖、3-磷酸甘油醛、乙酰辅酶A等代谢中间物的含量, 不断再生辅因子为反应提供自由能(adenosine triphosphate, ATP)或还原当量NADH(nicotinamide adenine dinucleotide)等[2]。

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2015年7月发表于同期*Cell*的两篇文章中, Sullivan等[4]和Birsoy等[5]两组研究者都提出, 天冬氨酸促进呼吸作用缺陷型细胞的增殖。2014年, 本实验室Liu等[6]发现, 天冬氨酸促进大肠杆菌细胞周期进程。以两篇*Cell*文章为主线, 结合本实验室研究结果, 本文简要介绍了天冬氨酸对细胞增殖及细胞周期的影响及其作用机制。

1 ETC与细胞增殖

呼吸作用是一个细胞利用营养物质释放能量并为生命活动供能的过程, 位于线粒体内膜上的电子传递链(electron transport chain, ETC)是呼吸作用非常重要的组成部分。营养物质氧化与ADP(adenosine diphosphate)磷酸化生成ATP的过程相偶联, 即经过一系列氧化还原反应电子供体逐步传递电子至最终的电子受体——氧分子, 而消耗的营养物质发生氧化, 氧分子还原生成水。这一系列氧化还原过程使线粒体内外产生电化学梯度并释放自由能进一步驱动ATP合成[7]与物质跨膜转运[8]等过程。

1.1 ETC工作模型

氧化磷酸化过程中的ETC包括复合体I、II、III、IV[9-10]。复合体I、II与复合体III之间有脂溶性电子载体辅酶Q; 复合体III与复合体IV之间有水溶性电子载体细胞色素c。

电子可以通过复合体I或复合体II两种途径进入ETC进行传递。复合体I是电子进入ETC的主要位点, 也是ETC的第一个电子受体, 为NADH:辅酶Q氧化还原酶, 也称为NADH脱氢酶。哺乳动物复合体I由45个不同亚基组成, 分子量980 kDa, 一个非共价结合的黄素单核苷酸(flavin mononucleotide, FMN)以及8个铁硫簇作为复合体I的辅基成分嵌于其亲水部分[11-12]。细菌的NADH脱氢酶则较小, 含有13~14个亚基, 分子量550 kDa[13-14]。复合体I可分为三个主要功能模块: (1)脱氢酶模块, 将NADH氧化为NAD^+; (2)氢化酶模块, 将2个电子传递至辅酶Q(ubiquinone, UQ)使其还原为UQH_2; (3)质子转运模块, 使线粒体基质内的4个质子通过线粒体内膜, 转运至线粒体膜间隙(inter-membrane space, IMS)[15-17]。则复合体I催化的总反应如下: $NADH+H^++UQ+4H^+(matrix)\rightarrow NAD^++UQH_2+4H^+(IMS)$。

复合体II是电子进入ETC的另一个位点, 也是ETC中唯一既参与三羧酸循环又参与电子传递的酶, 即琥珀酸:辅酶Q氧化还原酶, 其主要成分是琥珀酸脱氢酶(succinate dehydrogenase, SDH)。SDH存在于所有好氧生物中, 位于线粒体内膜内侧参与三羧酸循环(tricarboxylic acid cycle, TCA), 这是氧化磷酸化过程中最小的复合体, 仅包含4个亚基[9-10,18]。该复合体使琥珀酸(succinate)脱氢生成延胡索酸(fumarate), 并在线粒体基质产生2个质子, 而1分子琥珀酸提供的2个电子通过复合体II的辅基FAD(flavine adenine dinucleotide)、铁硫簇转移至辅酶Q, 并消耗基质中的两个质子使其还原为UQH_2[17]。复合体II催化琥珀酸氧化的电子传递过程并不改变基质内的质子浓度, 也不会对膜两侧的质子梯度产生影响, 即该复合体是电子受体而非质子泵。复合体II的总反应式如下: 琥珀酸+UQ→延胡索酸+UQH_2。

由复合体I、II进入ETC的电子, 都经过复合体III进一步向复合体IV进行传递。复合体III是ETC的中心, 即辅酶Q:细胞色素c氧化还原酶, 可催化电子由辅酶Q向细胞色素c传递, 且与质子跨膜转运相偶联[9-10,19]。复合体III单体形式包括11个亚基, 天然状态下以二聚体形式存在[9-10,20-21]。复合体III有两个辅酶Q结合位点, 近基质侧的称为Qi位点, 催化辅酶Q还原为UQH_2; 近膜间隙侧的称为Qo位点, 使UQH_2发生氧化。复合体I、II产生的UQH_2在Qo位点被氧化, 产生的2个电子, 一个通过铁硫蛋白及血红素c1传递至细胞色素c, 另一个通过血红素b_L及b_H传递至Qi位点。在Qi位点, 辅酶Q被还原为UQH_2后重新在Qo位点被氧化, 此过程被称为辅酶Q循环[22-24]。每一轮循环中, Qi位点还原一个辅酶Q需要Qo位点消耗基质中的2个质子并氧化UQH_2, 释放4个质子至膜间隙中, 即通过辅酶Q循环可使线粒体内膜两侧产生质子浓度梯度, 而每一轮循环中有2个电子传递至2分子细胞色素c, 因此净产生4个质子的浓度差[17]。总反应式如下: 2细胞色素c(氧化型)+UQH_2+$2H^+$(基质)→2细胞色素c(还原型)+UQ+$4H^+$(IMS)。

复合体IV是ETC的最后一步, 即细胞色素c:氧氧化还原酶, 它是一个多亚基的酶复合体, 接受来自4个细胞色素c分子传递而来的四个电子并将其传递至1个氧分子, 消耗基质侧的4个质子最终生成2分子水, 与此同时将基质侧的4个质子泵至膜间隙[25], 是细胞呼吸逐步氧化营养物质并传递至分子氧的最后一个电子受体[9-10]。电子从细胞色素c经过一系列辅

基传递至细胞色素a3, 而氧分子也在此位点被铁–铜中心所捕获并还原[26-27]。基质侧的质子一方面通过细胞色素a3被消耗用于氧气还原, 另一方面被转运至细胞间隙, 前者称为K途径, 后者称为D途径, K途径通过赖氨酸354将质子转运, 用于H_2O生成; D途径通过天冬氨酸124将基质侧的质子转运至谷氨酸278进而转运至膜间隙[28-30]。复合体IV的总反应式如下: 2细胞色素c(还原型)+8H^+(基质)+O_2→4细胞色素c(氧化型)+4H^+(IMS)+2H_2O。

复合体V为ATP合酶(F_0F_1 ATP酶), 与ETC无关, 但由于该复合体可利用由复合体I、II、IV产生的质子梯度驱动ATP合成, 因此被认为是氧化磷酸化过程中的一员[9-10]。电子供体NADH、琥珀酸产生的电子分别通过复合体I、II进入ETC, 经复合体III、IV传递至最终的电子受体氧分子进而还原成水。在传递过程中伴随着线粒体基质侧质子被泵至膜间隙形成质子浓度梯度, 最后通过复合体V的F_0亚基将质子转运至基质中并由F_1亚基驱动ATP形成(图1)。

1.2　ETC与细胞增殖的关系

糖酵解、三羧酸循环、脂肪酸β-氧化等多种代谢途径都可以产生电子供体进入ETC。ETC除参与氧化磷酸化为细胞供能外, 还可以影响许多生命进程, 如活性氧类的产生[31-32]、氧化还原状态[33-34]、线粒体膜电位[35]、线粒体蛋白转运[36]、细胞凋亡[37]及衰老[38]等。当ETC的一个或多个复合体发生问题时, 最直接的影响为ATP产量降低, 更严重的为具细胞毒性的活性氧类累积、凋亡诱导因子释放等, 最终导致细胞死亡及组织退化[39]。ETC遗传缺陷会引起许多不同疾病, 如神经退行性疾病[40-41]、肌肉疾病[42]和失聪[43-44]等。

ETC作为线粒体结构与功能上的重要组成部分, 在调控细胞增殖及凋亡方面发挥着重要作用进而影响肿瘤发生, 已有许多遗传学及药理学实验证明了这一说法[45]。如遗传学方面, 敲除*Tfam*(mitochondrial transcription factor A)使ETC缺失后, 增加小鼠心肌细胞凋亡[46]; 而过表达*Tfam*则提高ETC酶活性和细胞存活率并减少凋亡[47]。药理学方面, 鱼藤酮等特异性抑制剂抑制复合体I会增加ETC产生的活性氧类(reactive oxygen species, ROS)而改变线粒体膜通透性、释放细胞色素c、激活细胞凋亡蛋白并使DNA片段化等, 出现一系列细胞凋亡特征[48-50]。特异性抑制复合体II、复合体III同样也会引起细胞凋亡[51-53]。大量研究结果表明, ETC功能紊乱会抑制细胞增殖, 如ETC复合体I[54-55]或复合体III[53-56]受到药理学或遗传性抑制会阻滞人细胞生长; 同样, 呼吸作用抑制剂也会抑制癌细胞增殖[56-59]与肿瘤生长[55,60]。

ETC功能紊乱可增加AMP(adenosine monophosphate)水平并产生ROS, 而AMP与ROS这两种活性信号分子可以协调线粒体生物能量与细胞增殖[61]。ETC中复合体I和复合体IV突变引起的G_1/S期阻滞, 既可以通过AMP介导的AMPK[adenosine 5′-monophosphate (AMP)-activated protein kinase]而激活也可以通过ROS介导的JNK(c-Jun N-terminal kinase)而激活[61-62]。这些结果进一步说明, ETC对于细胞增殖是十分必要的。

ETC生物合成及其功能的上调或下调, 与细胞凋亡调控有着非常紧密的关系。ETC生物合成与(或)功能上调时, 会抑制细胞凋亡并刺激细胞生长与增殖; 而当其下调或抑制时, 会诱导细胞凋亡并抑制细胞生长与增殖[45]。

2　ETC缺陷型细胞的增殖

2.1　丙酮酸挽救ETC缺陷型细胞增殖

虽然ETC如何促进细胞增殖并不十分清楚, 但科学家们很早已发现, 丙酮酸可以使ETC功能缺陷的细胞增殖[63]。丙酮酸还可以缓解抗线粒体药物引起的生长抑制作用[56-57,64]并刺激线粒体突变体细胞的生长[64-65]。用线粒体丙酮酸转运抑制剂处理细胞可抑制其呼吸作用[66]。那么, 丙酮酸是如何挽救ETC缺陷型细胞增殖的呢? 丙酮酸的碳骨架有多种代谢归宿, 如通过丙酮酸羧化酶转变为草酰乙酸, 通过苹果酸酶转变为苹果酸, 通过丙酮酸脱氢酶复合体转变为乙酰辅酶A。显然, 丙酮酸可能作为生物合成中间物的碳源底物而发挥作用。此外, ETC功能缺失导致细胞无法充分氧化细胞内的NADH, 而丙酮酸作为一种外源电子受体可以通过乳酸脱氢酶的作用再生NAD^+[4,57,67]。Sullivan等[4]发现, α-酮丁酸(alpha-ketobutyric acid, AKB)与丙酮酸有类似作用, 均可促进ETC缺陷型细胞的增殖, α-酮丁酸与丙酮酸在体内的碳骨架代谢归宿并不一致, 他们证实加入丙酮酸或α-酮丁酸都会增加细胞内NAD^+/NADH的比值, 说明ETC缺陷型细胞中丙酮酸或α-酮丁酸并非作为碳源底物, 而是作为电子受体来促进细胞

增殖。Cardacil等[68]研究发现, 复合体II活性缺失时, 细胞需要消耗胞外的丙酮酸以维持细胞糖酵解并促进细胞增殖, 丙酮酸羧化酶可以维持该细胞增殖及肿瘤发生的能力, 并通过促进天冬氨酸生物合成而促进细胞生长。

2.2 天冬氨酸挽救ETC缺陷型细胞增殖

2.2.1 *天冬氨酸合成代谢* 快速增殖细胞中, 谷氨酰胺代谢水平高于其他非必需氨基酸[69], 可以作为碳源合成脂肪酸、转变为谷氨酸经谷氨酸脱氢酶或转氨基作用转变为α-酮戊二酸(alpha-ketoglutarate, AKG)进入三羧酸循环和合成天冬氨酸[70-71]。

由谷氨酸产生α-酮戊二酸继而合成天冬氨酸有氧化和还原两种途径。氧化途径: 经TCA循环进行三个氧化反应, 即α-酮戊二酸经过氧化反应生成琥珀酸, 后者再次氧化生成延胡索酸并转变为苹果酸, 苹果酸进一步氧化生成草酰乙酸[72-73]。还原途径: α-酮戊二酸首先羧化产生异柠檬酸, 再转变为柠檬酸, 之后脱掉乙酰辅酶A生成草酰乙酸[4,73]。显然, α-酮戊二酸经过氧化和还原途径都会产生草酰乙酸, 而草酰乙酸则是合成天冬氨酸的底物。在谷氨酸草酰乙酸转氨酶[glutamate oxaloacetate transaminase, GOT, 也称天冬氨酸氨基转移酶(aspartate aminotransferase, AST)]的作用下, 草酰乙酸经转氨基作用生成天冬氨酸(图2)。大肠杆菌中, 催化天冬氨酸氨基转移的酶称为AspC, 在细菌、古细菌、酵母、植物、哺乳动物中都相对保守[6]。

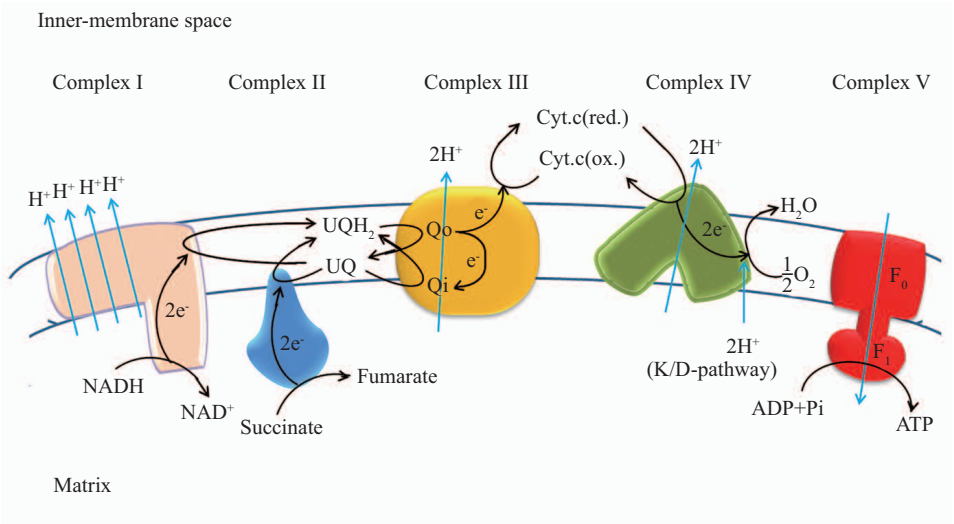
上述氧化途径和还原途径间的转变主要依赖于细胞内α-酮戊二酸与柠檬酸的比例[72]。当线粒体功能紊乱(ETC或TCA循环异常)时, 细胞合成代谢主要依赖谷氨酰胺, 此时谷氨酰胺代谢产生的α-酮戊二酸主要由还原性途径的羧化反应产生柠檬酸[73-75]。正常情况下, 天冬氨酸主要由谷氨酰胺的氧化途径生成, 而当ETC抑制时则主要由还原途径合成[5]。由于两条途径合成天冬氨酸都需要电子受体的存在。因此, 当呼吸作用受到抑制而细胞缺乏电子受体时, 天冬氨酸合成也受到抑制。

2.2.2 *天冬氨酸合成代谢挽救ETC缺陷型细胞* 癌细胞培养时, 通常需要谷氨酸经过线粒体分解代谢产生α-酮戊二酸以维持细胞快速生长[3]。在没有外源电子受体的情况下, 加入高于生理浓度的天冬氨酸也可使ETC缺陷型细胞进行增殖[4]。ETC所必需的NADH进入线粒体时, 由于线粒体内膜对NADH的不透过性而受到阻碍[76], 苹果酸–天冬氨酸穿梭在转移还原当量通过线粒体膜由胞质进入线粒体的过程中起着非常重要的作用[77-78]。苹果酸–天冬氨酸穿梭由两对既存在于胞质也存在于线粒体的关键酶催化完成, 即谷氨酸草酰乙酸转氨酶(GOT)和苹果酸脱氢酶(malate dehydrogenase, MDH), 二者共同作用转移还原当量通过线粒体膜且不引起碳与氮原子的净移动[50,79-80]。

GOT催化谷氨酸与草酰乙酸的转氨基作用生成α-酮戊二酸与天冬氨酸, α-酮戊二酸可进一步进入三羧酸循环, 天冬氨酸则是嘌呤与嘧啶合成中重要的中间物(图3)[81-82]。因此, 抑制GOT作用会降低α-酮戊二酸与天冬氨酸产量。MDH利用NAD^+、NADH为辅酶催化苹果酸与草酰乙酸的转化, 为天冬氨酸的转氨合成提供反应底物[83]。

线粒体基质中, 通过GOT2催化草酰乙酸与谷氨酸转变为天冬氨酸和α-酮戊二酸[84-85], 反之, 二者进入细胞质后在GOT1催化作用下形成草酰乙酸与谷氨酸[86]。胞质中, MDH1还原草酰乙酸为苹果酸并氧化NADH参与苹果酸–天冬氨酸穿梭, 苹果酸进入线粒体后由MDH2催化氧化为草酰乙酸, 并产生NADH, 后者进入ETC产生ATP[79]。MDH不仅参与苹果酸–天冬氨酸穿梭作用, 而且也是三羧酸循环中的主要酶。作为别构调节酶, MDH可以调节苹果酸–草酰乙酸的浓度平衡, 即高浓度苹果酸会刺激草酰乙酸生成, 而高浓度草酰乙酸则会抑制这一反应[87-88]。

GOT1缺失会影响肿瘤细胞生长[85], 抑制ETC过程会使GOT1缺失细胞阻滞或死亡。的确, GOT1缺失细胞对ETC抑制剂更加敏感[5]。有趣的是, 额外的天冬氨酸能够使ETC受阻的GOT1缺失细胞增殖, 而且GOT1缺失细胞中, 抑制ETC后, 几乎检测不到天冬氨酸。这些研究结果说明, 抑制ETC后, 细胞通过GOT1依赖的模式产生天冬氨酸。所以, GOT1的功能是双向的, 天冬氨酸水平降低时, GOT1逆向产生天冬氨酸而并非消耗天冬氨酸[5]。当ETC功能紊乱时, GOT1缺失使细胞彻底丧失天冬氨酸合成途径。然而, MDH1缺失并不完全阻止天冬氨酸合成, 细胞依然可以通过还原途径合成天冬氨酸。重要的是, 丙酮酸并不能促使GOT1缺失细胞增殖, 说明丙酮酸应该通过GOT1途径催化天冬氨酸合成, 进而促进ETC缺陷型细胞增殖[5]。当抑制ETC过程时, 丙酮酸也不能使MDH1缺陷型细胞合成天冬氨酸,

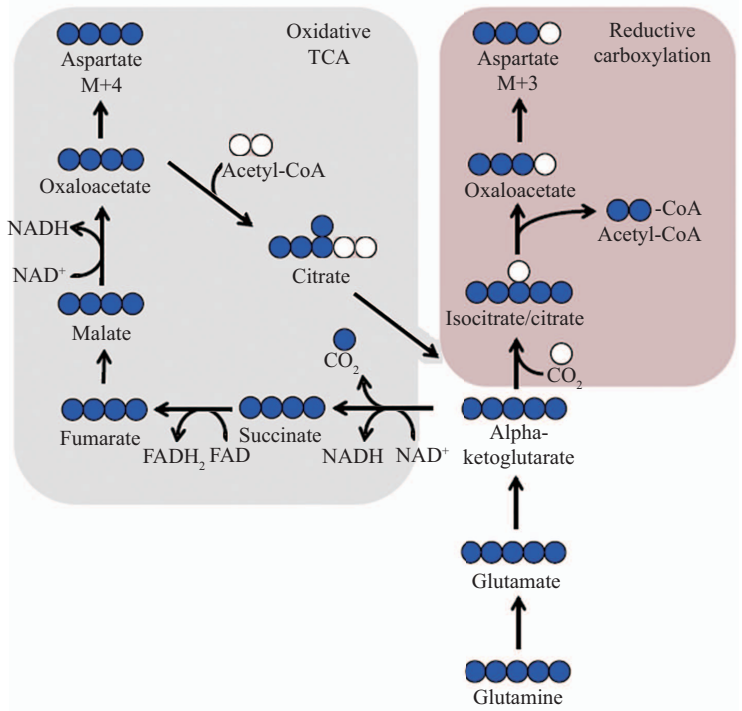


线粒体内膜上的电子传递链由复合体I、II、III、IV、V组成, 分别标为粉色、蓝色、黄色、绿色以及红色。各复合体的电子传递及质子转运分别以黑色及蓝色箭头标示。Cyt.c(ox.): 氧化型细胞色素c; Cyt.c(red.): 还原型细胞色素c。

The mitochondrial electron transport chain at the inner membrane is composed of five respiratory complexes. Complex I is colored in pink, complex II in blue, complex III in yellow, complex IV in green and complex V in red. The electron transfer pathways within each complex as well as the coupled proton translocations are indicated by black and blue arrows, respectively. Cyt.c(ox.): cytochrome c in oxidized state; Cyt.c(red.): cytochrome c in reduced state.

图1　线粒体电子传递链

Fig.1　The mitochondrial electron transport chain

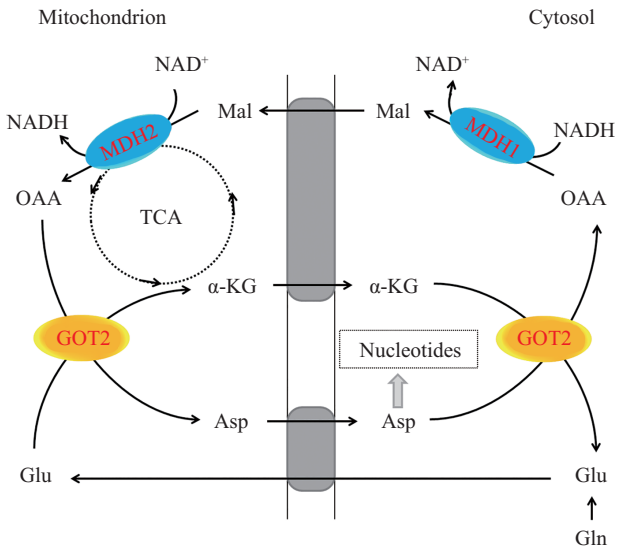


天冬氨酸合成有氧化和还原两种途径, 氧化途径主要通过TCA循环中的3个氧化反应完成; 还原途径主要通过还原性羧化作用完成。氧化途径以浅蓝色背景标示, 还原途径以浅紫色背景标示。蓝色圆形为同位素标记的碳原子, 白色圆形为非同位素标记的碳原子。

Aspartate synthesis includes oxidative TCA metabolism or reductive carboxylation from glutamine. The oxidative pathway is indicated in light blue background and the reductive pathway is shown in light purple background. Carbon atom labeled with isotope is in filled blue circle and carbon atom without labeling is in open white circle.

图2　由谷氨酰胺代谢合成天冬氨酸的途径(根据参考文献[4]修改)

Fig.2　Synthesis process of aspartate from glutamine (modified from reference [4])



胞质中草酰乙酸在MDH1作用下氧化生成苹果酸并消耗NADH, 苹果酸转运至线粒体内经MDH2作用生成草酰乙酸并间接将还原当量NADH转运至线粒体内。草酰乙酸和谷氨酸在GOT2作用下生成天冬氨酸和α-酮戊二酸, 二者分别转运至胞质后在GOT1的作用下再生草酰乙酸和谷氨酸。黄色和蓝色分别代表谷草转氨酶(GOT)和苹果酸脱氢酶(MDH)。

NADH is consumed in the cytosol by the oxidation of oxaloacetate (OAA) to malate (MAL). Then malate transfers into the mitochondrion where malate is reduced into oxaloacetate with accompany of regeneration of NADH. This transfer is also indirectly accomplished by transamination with glutamate (GLU) to form α-ketoglutarate (α-KG) and aspartate (ASP). These substances are then transferred to the cytosolic compartment where the reverse transamination regenerates oxaloacetate and glutamate. GOT is in yellow and MDH is in blue.

图3　天冬氨酸–苹果酸穿梭

Fig.3　The aspartate-malate shuttle

进而挽救细胞增殖; 而额外补充天冬氨酸却会挽救MDH1缺陷型细胞增殖, 说明丙酮酸诱导的NAD^+激活MDH1进而催化苹果酸转变为草酰乙酸, 并进一步驱动天冬氨酸的合成, 需要依赖于GOT1的存在[5]。总之, ETC受阻时, 天冬氨酸是维持细胞存活与增殖的限制因子。

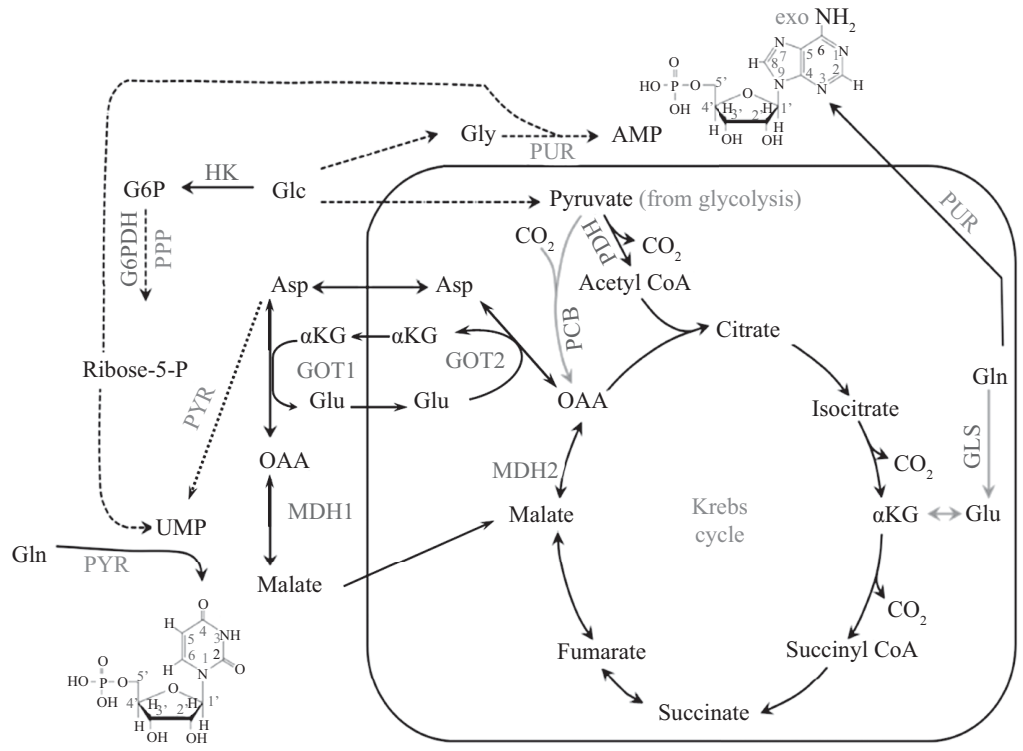
然而, 天冬氨酸并不改变线粒体耗氧量与细胞内NAD^+/NADH比例, 即天冬氨酸促进细胞增殖的作用方式与丙酮酸、α-酮丁酸作为外源电子受体而挽救ETC缺陷型细胞增殖的方式并不相同, 可能是其本身作为细胞增殖生物合成的必需物质而发挥作用[4]。天冬氨酸参与许多细胞合成途径, 如作为丝氨酸、苏氨酸、甲硫氨酸等氨基酸合成的前体物质, 也是嘌呤与嘧啶合成的前体物质[89], 天冬氨酸缺失会影响核苷的生物合成, 产生DNA损伤进而导致细胞S期阻滞[81]。

3　天冬氨酸与三羧酸循环、核苷酸代谢

细胞代谢调控是一个复杂的多元调控过程。天冬氨酸在细胞内参与多种代谢过程, 尤其与三羧酸循环、核苷酸代谢有着密切关系(图4)。

在核苷酸代谢过程中, 嘧啶合成首先是利用天冬氨酸等合成尿嘧啶[82]。嘧啶的4个碳原子中有3个碳原子来自于天冬氨酸, 并且主要源于谷氨酰氨而非葡萄糖[82,90]。这也从另一个侧面验证了前文所提到的, 天冬氨酸合成需要额外谷氨酰胺。嘧啶合成过程中的一个反应为二氢乳清酸脱氢酶反应, 与ETC相偶联, 因此ETC缺失时细胞不能合成尿嘧啶核苷酸[82]。天冬氨酸是一个非必需氨基酸, 可以通过Na^+依赖的阴离子转运蛋白进入细胞[91], 也可以通过草酰乙酸的转氨基作用从头合成, 是苹果酸–天冬氨酸穿梭的重要部分。草酰乙酸作为进入三羧酸循环的一个重要节点, 由丙酮酸羧化酶催化的回补反应产生[92]。除此之外, 另一个回补反应则是谷氨酰胺分解, 即谷氨酰胺水解为谷氨酸与氨, 而谷氨酸和草酰乙酸通过转氨基作用生成α-酮戊二酸和天冬氨酸[85], 或通过谷氨酸脱氢酶的作用生成α-酮戊二酸与氨离子, α-酮戊二酸可以通过三羧酸循环生成草酰乙酸等其他代谢中间物[70,93-94]。这两种回补反应的重要性主要依赖于细胞类型与生长条件, 以维持细胞能量代谢、氮平衡以及合成代谢[92,95-97]。

体外培养的细胞通过利用谷氨酰胺和葡萄糖在细胞内合成大量天冬氨酸用来合成嘧啶[90,98-101], 且并不依赖于天冬氨酸浓度[82]。用同位素标记的谷



葡萄糖经糖酵解产生丙酮酸, 进而通过丙酮酸脱氢酶或丙酮酸羧化酶产生的乙酰辅酶A或草酰乙酸进入三羧酸循环。核苷酸的核糖部分由葡萄糖经己糖激酶与6-磷酸葡萄糖脱氢酶催化产生的核糖构成。嘧啶核苷酸的碱基中的碳原子1个来自CO_2, 另外3个来自天冬氨酸, 经三羧酸循环及转氨基作用产生。谷氨酰胺也可以作为天冬氨酸产生的前体, 通过谷氨酰胺酶回补产生α酮戊二酸。

The glucose metabolism via glycolysis produces pyruvate, and the pyruvate enters the Krebs cycle via acetyl CoA (AcCoA) catalyzed by pyruvate dehydrogenase (PDH) or via oxalacetate (OAA) catalyzed by pyruvate carboxylase (PC). The ribose subunit of nucleotides is synthesized from glucose via hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6PDH) (the oxidative branch of the PPP). The pyrimidine nucleobases are synthesized using one carbon from CO_2 and three carbons from Asp, which is readily produced by transamination of oxalacetate (OAA) derived from the Krebs cycle by glutamate oxaloacetate transaminase (GOT) and malate dehydrogenase (MDH). Glutamine can also serve as the precursor for Asp and therefore pyrimidine bases by replenishing α-ketoglutarate (α-KG) via glutaminase (GLS) plus transaminase reactions, and the latter half of the Krebs cycle sequence.

图4　天冬氨酸与三羧酸循环、核苷酸代谢的关系(根据参考文献[82]修改)

Fig.4　The relationship between aspartate and the TCA cycle, nucleotide metabolism (modified from reference [82])

氨酰胺处理细胞发现, 尽管有高浓度外源天冬氨酸存在, 细胞内天冬氨酸库主要为利用谷氨酰胺合成的带有同位素标记的天冬氨酸[102]。谷氨酰胺代谢生成谷氨酸, 进而生成α-酮戊二酸, 后者进入三羧酸循环产生草酸乙酸, 再生成天冬氨酸。之后, 通过天冬氨酸–苹果酸穿梭将合成的天冬氨酸转运至胞质中进行嘧啶合成。

虽然对于核苷酸合成而言, 高浓度外源天冬氨酸存在时, 细胞依然合成内源的氨基酸。但对于三羧酸循环而言, 外源天冬氨酸可以缓解三羧酸循环中间物的缺失, 如草酰乙酸、苹果酸, 并且可以在局部缺血后促进三羧酸循环进而增加氨基转移过程中ATP的生成[103-104]。天冬氨酸氨基转移酶催化天冬氨酸与谷氨酸之间的α-氨基转移, 这一过程同三羧酸循环一样, 在细胞能量代谢过程中起着非常重要的作用[105]。

在三羧酸循环中, 丙酮酸分解为乙酰辅酶A, 最后氧化产生CO_2并为氧化磷酸化提供还原当量[106]。三羧酸循环由八个连续反应组成, 起始于乙酰辅酶A与草酰乙酸生成柠檬酸, 之后柠檬酸在不同的酶催化下生成异柠檬酸、α-酮戊二酸、琥珀酰辅酶A、琥珀酸、延胡索酸、苹果酸、草酰乙酸, 完成一轮循环。除丙酮酸外, 其他回补反应产物也可以进入三羧酸循环, 如谷氨酰胺的主要代谢产物α-酮戊二酸[107]。α-酮戊二酸不仅是三羧酸循环的代谢中间产物, 还有许多酶催反应可生成α-酮戊二酸。在缺氧或线粒体缺失情况下, 来自于谷氨酰胺的α-酮戊二酸通过还原性羧化反应生成柠檬酸以维持细胞合成代谢[73-75,108]。当ETC功能受阻时, 依赖于谷氨酰胺的还原途径是快速生长细胞的主要代谢方式[75], 该发

现说明了为何ETC受阻时天冬氨酸合成主要依赖于还原性途径的原因。谷氨酸除了通过谷氨酸脱氢酶生成α-酮戊二酸外, 还可以通过谷丙转氨酶或谷草转氨酶(GOT1、GOT2)发挥作用[106]。

显然, 三羧酸循环有两重生物学意义。它不仅可以介导分解反应产生能量, 还可以为细胞生长提供前体物质[106]。在非增殖细胞中, 三羧酸循环的主要功能是完全氧化底物, 最大化产生ATP; 增殖细胞中, 三羧酸循环则是生物合成前体的主要来源[2,109]。

4 天冬氨酸特异性促进细胞周期进程

Patel等[81]研究发现, 由谷氨酰胺缺失而引起的细胞S期阻滞是由于细胞内脱氧核苷酸缺失而无法满足DNA合成所致。谷草转氨酶(GOT)抑制剂处理细胞也会引起细胞周期阻滞。如前文所述, 抑制GOT活性导致α-酮戊二酸和天冬氨酸合成受阻。然而, 天冬氨酸可以挽救细胞S期阻滞, 但谷草转氨酶的另一产物α-酮戊二酸并无此作用[81]。这些研究说明, GOT催化的天冬氨酸合成受阻是谷氨酰胺缺失而引起细胞S期阻滞的关键原因。的确, 额外脱氧核苷酸可以挽救S期阻滞的细胞并使其增殖, 说明额外脱氧核苷酸不仅可以使细胞完成S期, 还可使细胞进行有丝分裂[81]。天冬氨酸是核苷酸合成的前体物质, 天冬氨酸缺失会导致细胞内核苷酸减少进而引起DNA合成受阻, 结果导致细胞S期阻滞[81]。Dornfeld等[89]也发现, 天冬氨酸转运至线粒体后转变为草酰乙酸足以驱动TCA循环产生能量及其他生物分子进而完成复制。

本实验室Liu等[6]发现, 大肠杆菌天冬氨酸氨基转移酶缺失导致细胞复制原点数减少、细胞变短、细胞生长速度显著减慢, 结果说明, 天冬氨酸的短缺导致复制起始和细胞分裂均延迟发生从而推迟细胞周期进程。过表达天冬氨酸氨基转移酶可以得到相反的作用, 即显著促进细胞周期进程。通过分别检测20种氨基酸对大肠杆菌细胞周期的影响, 发现额外天冬氨酸和谷氨酰胺显著促进细胞周期进程, 即促进染色体复制起始和细胞分裂的发生。其他氨基酸如甘氨酸、缬氨酸、亮氨酸、异亮氨酸、丙氨酸、精氨酸及其前体物质并不明显影响染色体复制与细胞分裂。

5 展望

本文阐述了天冬氨酸可挽救ETC缺陷型细胞的增殖, 且这种作用与丙酮酸挽救ETC缺陷细胞的增殖并不一样。丙酮酸是作为外源电子受体而发挥作用, 但天冬氨酸并不改变线粒体耗氧量与细胞内NAD^+/NADH的比值。此外, GOT抑制也会影响细胞增殖。因此, 天冬氨酸作为细胞周期限制因子可能直接参与细胞增殖调控。天冬氨酸涉及到多种物质的合成, 包括氨基酸、内消旋二甲基庚二酸(meso-diaminopimelate, mDAP)、核苷酸、NAD^+及泛酸[110-115]。来源于不同途径的天冬氨酸在不同细胞过程中起着非常关键的作用, 包括参与脂肪酸代谢[116]、鸟氨酸循环[117]、TCA循环[4-5]等。由于天冬氨酸与多种代谢过程相关, 那么天冬氨酸究竟通过何种途径参与细胞增殖调控呢？是通过调控天冬氨酸自身代谢产量来影响不同代谢过程, 继而简单影响细胞生长？还是作为信号分子, 通过特定途径来影响细胞增殖？这些问题的答案尚不清楚, 还有待于进一步深入研究。

谷氨酰胺作为一种重要的肿瘤营养物质, 几乎与增殖肿瘤细胞的每一个代谢功能都有关, 而Myc蛋白可调控谷氨酰胺的摄入与降解[107]。通过抑制谷氨酰胺代谢而控制癌细胞生长方面已有诸多研究, 天冬氨酸合成需要谷氨酰胺的存在, 那么研究天冬氨酸对细胞增殖和生长的调控是否会成为一个新的癌症治疗靶点？值得注意的是, 并非所有的癌细胞都需要额外的谷氨酰胺且对于谷氨酰胺的依赖程度并不相同, 如某些肺癌细胞和乳腺癌细胞[118-119]。这些细胞中, 天冬氨酸是否依然可以促进其增殖？与其他依赖于谷氨酰胺的细胞相比, 其作用机理是否相同？

有趣的是, mTOR(mammalian target of rapamycin)作为一个真核细胞信号通路, 可以调控细胞生长、代谢、增殖以及存活。线粒体代谢及生物合成也受mTOR调控, mTOR抑制时线粒体膜通透性、氧消耗与ATP水平均降低, 线粒体磷酸化蛋白组发生改变[120]。mTOR也是胞内ATP的感受器, 可以感知鱼藤酮、抗霉素A等线粒体抑制剂抑制mTOR通路中S6K1与4EBP1磷酸化导致的ATP损耗[121]。天冬氨酸可以挽救ETC缺陷型细胞的增殖, 那么天冬氨酸促进细胞增殖是否通过影响mTOR信号通路？已有研究证明, 氨基酸作为一类信号分子可以激活mTOR[122], 亮氨酸对于mTOR的激活是必需的, 以谷氨酰胺依赖的方式进入细胞[123], 并且是谷氨酸脱氢

酶的别构激活因子[124-125]。天冬氨酸的合成也需要谷氨酰胺的存在, 这二者之间是否存在某种联系? mTOR信号通路与癌症及2型糖尿病等疾病密切相关, 天冬氨酸是否也在其中扮演重要角色呢? 可以肯定的是, 用Rapamycin抑制mTOR可以延长寿命[126], 因为mTOR活性降低会阻滞细胞周期进程从而实现延长寿命。那么, 控制天冬氨酸摄入或降低天冬氨酸代谢活性是否可以用来延长寿命的新方法呢? 的确, 天冬氨酸本身是细胞周期进程的一个重要限制营养因子。

本实验室研究发现, 天冬氨酸直接促进细胞周期进程, 并使DNA复制和细胞分裂与其细胞生长进行协调; 还发现额外天冬氨酸提升单个细胞所含复制起始蛋白DnaA的量和细胞分裂信号UDP-葡萄糖的量。DnaA量的提升促进染色体复制起始, 而UDP-葡萄糖量的提升会延缓细胞分裂因为UDP-葡萄糖影响FtsZ隔膜的形成。但这些结果是否也适用于真核细胞还有待于进一步研究。

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