

综述

Rab蛋白在神经退行性疾病中作用的研究进展

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摘要: Rab蛋白是Ras超家族成员, 是一类小分子GTP结合蛋白。在大分子物质进入细胞的内吞途径和细胞器间的物质转运过程中, Rab蛋白通过招募其效应蛋白可作用于囊泡形成、转运、黏附和融合, 是囊泡运输过程中的关键调节因素。Rab蛋白及其效应蛋白定位和功能的改变与多种疾病相关。本文就Rab蛋白的主要功能及其在帕金森病、阿尔茨海默病、亨廷顿病等神经退行性疾病发生与发展过程中的作用进行综述。

关键词: Rab蛋白; 神经退行性疾病; α -突触核蛋白; β -淀粉样蛋白; Tau蛋白; 亨廷顿蛋白

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Research progress of Rab proteins in neurodegenerative diseases

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Abstract: As a member of the Ras superfamily, Rab proteins are small GTP-binding proteins. In the process of endocytosis of macromolecules and substances delivery between organelles, Rab proteins act on vesicle formation, transport, tethering and fusion by recruiting their effectors, therefore being key regulatory factors in vesicle trafficking. Disturbance of localizations and functions of Rab proteins and their effectors are involved in the pathogenesis of several diseases. This review focuses on the main functions of Rab proteins and their possible roles in the onset and progression of neurodegenerative diseases including Parkinson's disease, Alzheimer's disease, and Huntington's disease.

Key words: Rab proteins; neurodegenerative diseases; α -synuclein; amyloid β -protein; Tau; Huntington protein

在真核生物中, 囊泡介导内吞膜蛋白及细胞器间的物质转运过程, 此过程受复杂的蛋白质机制调控, 囊泡运输障碍导致的细胞器间及细胞间信息传递异常是包括神经退行性疾病在内的疾病发生与发展的关键途径, 而 Rab 蛋白在囊泡运输过程中发挥关键调节作用, 主要调控囊泡形成、转运、黏附和融合的过程, 从而参与蛋白质等大分子物质进入内吞途径和在细胞器间的转运^[1]。Rab 蛋白是 Ras 超家族 (包括 Ras、Rho、Rab) 中最大的家族, 是一

类小分子 GTP 结合蛋白, 在酵母中发现了 11 种, 秀丽隐杆线虫中 29 种, 人类中大约有 70 种^[2]。1983 年, 第一个 Rab 基因 (*YPT1*) 在酿酒酵母中被发现^[3]; 1987 年, Touchot 等首次在哺乳动物中克隆出与 *YPT* 基因功能类似的同源基因, 并命名为 *Rab* (Ras-like in rat brain)^[4]。本文就 Rab 蛋白的主要功能及其在帕金森病 (Parkinson's disease, PD)、阿尔茨海默病 (Alzheimer's disease, AD)、亨廷顿病 (Huntington's disease, HD) 等神经退行性疾病发生与

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发展过程中的作用进行综述。

1 Rab蛋白的功能

Rab 蛋白与其它小分子 GTP 酶一样, 在激活与失活状态之间转换, 与 GTP 结合时处于激活状态, 与 GDP 结合时处于失活状态^[5]。将 GDP 交换为 GTP 可使 GDP 结合型 Rab 转化为 GTP 结合型 Rab, 这一转化过程由鸟嘌呤核苷酸交换因子 (guanine nucleotide exchange factors, GEFs) 催化; GTP 水解激活蛋白 (GTP hydrolysis activator proteins, GAPs) 使 GTP 水解为 GDP, 从而将 GTP 结合型 Rab 转化为 GDP 结合型 Rab^[6]。GDP 与 GTP 结合型 Rab 之间构象的差异主要在两个区域——开关 I 和开关 II, 与 GDP 结合时, 开关区域是未折叠的, 与 GTP 结合时, 开关区域有明确的构象, 能特异性地与效应蛋白 (外壳蛋白、马达蛋白、激酶、磷酸酶、黏附因子等) 结合^[5], 从而发挥其作用。

Rab 蛋白除了在激活与失活状态之间转换外, 还会在膜与胞质之间循环^[7]。Rab 蛋白半胱氨酸 C 端残基发生不可逆的异戊二烯化是可逆性膜附着的必要条件^[8]。Rab 蛋白在核糖体合成后, 先与 Rab 护送蛋白 (Rab escort protein, REP) 结合, 继而将 Rab 蛋白呈递给香叶烯基转移酶 (geranylgeranyl-transferase, GGase) 进行异戊二烯化, 解离后将异戊二烯化的 Rab 递送至靶膜^[9]。在一轮囊泡运输完成后, Rab 蛋白从膜上解离下来, 以可溶性的形式存在于细胞质中^[7], 最初认为 Rab3 互作蛋白负责此过程, 因其可抑制 GDP 的解离, 被称为 GDP 解离抑制剂 (GDP dissociation inhibitor, GDI)^[10]。GDI 只与异戊二烯化且无活性的 GDP 结合型 Rab 蛋白有高亲和力, 这确保了 Rab 蛋白在囊泡运输完成并已被 GAPs 失活后才从膜上被解离^[7]。

Rab 蛋白定位于质膜和膜性细胞器 (内质网、高尔基体、内体、溶酶体等), 特异性地控制真核生物细胞内的膜转运过程^[11], 参与内吞途径。Rab 蛋白通过这种方式, 赋予不同的膜身份, 以此来确保“货物”被正确地运输到细胞内特定的位置^[5, 12]。在大分子物质进入细胞的过程中, 可通过依赖网格蛋白内吞 (clathrin-dependent endocytosis, CDE) 和不依赖网格蛋白内吞 (clathrin-independent endocytosis, CIE) 两种形式形成内吞囊泡。内吞囊泡与内体融合, 进入内体腔^[13]。作为内吞途径的一个细胞器, 内体起源于反面高尔基网状结构 (trans Golgi network, TGN),

可分为早期内体、晚期内体、再循环内体^[14]。Rab5、Rab4A、转铁蛋白及其受体、以及早期内体抗原 1 (early endosome antigen 1, EEA1)^[15] 被认为是早期内体的标记物, 早期内体接受由上述大分子物质形成的内吞囊泡。内吞囊泡、早期内体表面都携带 Rab5, 内吞囊泡在 Rab 效应蛋白 EEA1 的帮助下与早期内体融合。一旦融合, “货物”进入内体腔, 膜受体可通过循环内体 (主要集中在微管组织中心, 由管状网组成, 标记物为 Rab11^[16]) 返回到质膜, 使其进入下一轮的内吞途径, 膜蛋白则被泛素连接酶标记, 并被分选到管腔内泡 (intraluminal vesicles, ILVs)^[17]。ILVs 在内体腔积累, 内体的组成和形态发生改变, 从早期内体时的管状小泡转变为多泡体 (multivesicular bodies, MVBs), 即晚期内体, 此时 Rab5 被 Rab7 代替^[17]。除了 Rab7, 还有 Rab9、甘露糖-6-磷酸受体 (mannose-6-phosphate receptor, MPR) 被认为是晚期内体的标记物^[18]。晚期内体最终与溶酶体融合, 将 ILVs 释放到溶酶体腔, 大分子物质被水解为氨基酸、脂质、碳水化合物, 被细胞所利用^[17]。由 CDE 形成的内吞囊泡有网格蛋白包被, 网格蛋白和外壳蛋白复合物 I、II 等被称为外壳蛋白, 外壳蛋白可识别要运输“货物”的特定元素, 决定哪些“货物”被分选到囊泡^[19]。内吞的“货物”通常在早期内体和晚期内体进行分选, 不同的货物可被分选到不同的路径, 除上述所说的晚期内体途径, 也可到达 TGN^[20]。晚期内体还可与质膜融合, 将 ILVs 向细胞外分泌, 即称为外泌体 (exosomes)^[21] (图 1)。

Rab 蛋白通过招募效应蛋白来启动下游膜转运过程^[22, 23]。囊泡转运是主动过程, Rab 蛋白通过招募马达衔接蛋白或直接与马达蛋白 (肌球蛋白、驱动蛋白、动力蛋白) 结合来驱动囊泡沿细胞骨架轨道 (肌动蛋白丝或微管) 转运^[24]。例如, Rab11A 的衔接蛋白 Rab11 家族互作蛋白 2 (Rab11 family interacting protein 2, Rab11-FIP2) 将囊泡与肌球蛋白 Vb 间接连接起来, 在肌球蛋白 Vb 的驱动下促进囊泡在质膜循环过程中的转运^[25]。Rab27A 招募马达衔接蛋白黑色素亲和素到黑色素小体 (melanosome) 膜上, 将黑色素小体与肌球蛋白 Va 连接起来, 在肌球蛋白 Va 的驱动下, 将黑色素小体转运到质膜附近^[26], 而另一 Rab27A 效应蛋白 SIP2 (synaptotagmin-like protein 2) 调节黑色素小体在黑色素细胞质膜附近的正确分布, 敲除 SIP2 可减少黑色素小体在质膜附近的分布, 黑色素小体集中在细胞中心, 细胞颜色会变浅, 集中

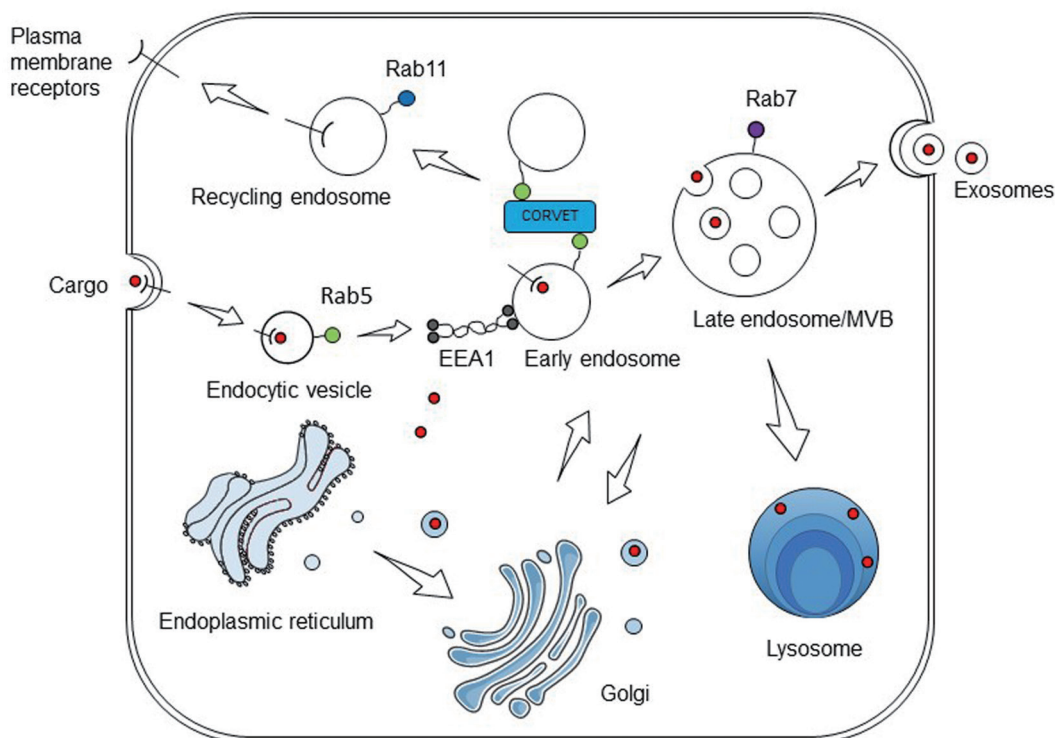


图 1. 内吞途径及细胞器间物质转运过程

Fig. 1. The process of endocytosis of macromolecules and substances delivery between organelles. Under physiological conditions, macromolecular substances are endocytosed via endocytic vesicles (Rab5 as a marker), and the latter is fused with the early endosome (Rab5 as a marker) in the presence of EEA1. CORVET can mediate the fusion of homotypic early endosome. Once plasma membrane receptors have released their cargo into the endosomal lumen, they are transported back to the plasma membrane by recycling endosome marked with Rab11, making them available for another round of endocytosis. Macromolecular substances are sorted at the endosome into intraluminal vesicles (ILVs), late endosome, and the late endosome eventually is fused with the lysosome, thereby releasing the intraluminal vesicles into the lumen of the lysosome. Macromolecular substances are also sorted to the trans-Golgi network. Late endosome can also be fused with the plasma membrane to release their ILVs into the extracellular space, termed “exosomes”. In addition, proteins synthesized by the endoplasmic reticulum can also be transported to the Golgi via vesicles and enter endosomes. EEA1: early endosome antigen 1; CORVET: class C core vacuole/endosome tethering; MVB: multivesicular body.

在质膜附近则会变深^[27]。此外,在将晚期内体的标记物——MPR从晚期内体转运到TGN的过程中,Rab9招募的效应蛋白TIP47可识别MPR,同时Rab9增加了TIP47对“货物”(MPR)的亲和力,有利于MPR分选到晚期内体出芽所形成的囊泡^[28]。

Rab蛋白及其效应蛋白还参与了囊泡的黏附和融合过程。Rab蛋白及其效应蛋白与可溶性N-乙基马来酰亚胺敏感性因子附着蛋白受体(soluble N-ethylmaleimide-sensitive factor attachment protein receptors, SNAREs)共同参与囊泡融合,囊泡膜上的SNARE(v-SNAREs)与靶膜上的SNARE(t-SNAREs)形成SNARE复合物,导致融合孔的形成,进而促进囊泡与靶标膜融合^[2]。内吞途径中的黏附有两个不同的机制:EEA1及其相关机制和黏附复合物

CORVET(class C core vacuole/endosome tethering)及其相关机制^[17]。Rab5效应蛋白EEA1是位于早期内体的长螺旋蛋白,其二聚体卷曲螺旋非常坚硬,一旦与内吞囊泡膜上的GTP结合型Rab5结合,就会失去其刚度,使内吞囊泡与早期内体靠近,Vps45(vacuolar protein sorting 45)蛋白(参与SNAREs装配的Sec1p/Munc-18家族成员)调控内吞囊泡v-SNAREs与内体t-SNAREs相互作用,继而促进内吞囊泡与早期内体的融合^[29]。Rab5另一效应蛋白CORVET,有Vps3、Vps8、Vps33等不同蛋白形成的亚基,Vps3、Vps8亚基与Rab5结合,Vps33亚基与v-SNAREs、t-SNAREs结合,继而促进同型早期内体的融合^[30]。在囊泡膜与靶标膜的融合过程中,为了确保运输的准确性,在膜性结构融合前由黏附因子将囊泡黏附

到靶膜上^[19]。黏附因子中的两大类——长螺旋蛋白(p115、Golgins、EEA等)和多蛋白复合物(TRAPP-I、TRAPP-II等)^[2]都是Rab效应蛋白。最近还有研究发现GEFs和GAPs除了参与GTP结合型Rab和GDP结合型Rab之间的互相转换外,还直接参与囊泡黏附。在囊泡黏附过程中GEFs直接启动黏附反应,再激活Rabs进一步促进黏附反应^[31];而GAPs中的TBC结构域蛋白家族成员TBC1D23一端与TGN上的长螺旋蛋白Golgin-97和Golgin-245末端保守残基结合,另一端与来源于内体的囊泡的WASH(Wiskott-Aldrich syndrome protein and Scar homologue)复合物结合,进而促进来源于内体的囊泡黏附到TGN^[32]。

Rab蛋白通过调控囊泡介导的细胞内物质转运过程,在癌症中可调节细胞分裂、迁移以及信号转导^[33–35],在免疫系统中可调节免疫受体的转运、趋化因子和细胞因子的分泌^[36, 37]。在神经元中,Rab蛋白定位于突触,在突触囊泡循环中调节轴突/树突中的顺行、逆行运输,其中Rab3、Rab27与突触囊泡及神经递质的释放密切相关^[38]。Rab3亚家族由Rab3A、Rab3B、Rab3C、Rab3D四个成员组成,Rab3A是突触活性区组成成分,与RIM1、MUNC13形成复合物,调节突触囊泡的锚定,进而释放神经递质^[39]。抑制乌贼突触前末梢Rab27活性,可导致突触囊泡的锚定减少^[40]。Rab蛋白可被不同的激酶磷酸化,比如CDK1(cyclin-dependent protein kinase 1)、LRRK1(leucine-rich repeat kinase 1)、LRRK2、PKC(protein kinase C)、TAK1(transforming growth factor- β -activated kinase 1)、TBK1(TANK-binding kinase 1)、Src(sarcoma)^[41]。早在1991年就有研究报告,在有丝分裂过程中,Rab1、Rab4被有丝分裂激酶CDK1磷酸化,阻止其与内体膜结合^[42]。最近又有证据表明TBK1可磷酸化Rab7A促进PINK-PARKIN途径的线粒体自噬,而对可被LRRK2磷酸化的Rab1A、Rab1B、Rab8A、Rab7L却没有作用(见后述)^[43]。PTEN诱导激酶1(PTEN-induced kinase 1, PINK1)的激活可诱导Rab8A、8B和13在第111位丝氨酸(Ser¹¹¹)的磷酸化^[44],并且PINK1依赖的Rab Ser¹¹¹位点的磷酸化可阻断LRRK2介导的Rab第72位丝氨酸(Ser⁷²)的磷酸化^[45]。

2 Rab蛋白与PD

PD是第二大最常见的神经退行性疾病,最典

型的病理学特征是黑质致密部多巴胺能神经元选择性丢失以及路易小体(Lewy bodies, LBs)的形成,其中LBs最主要的成分是异常聚集的 α -突触核蛋白^[46]。大多数PD患者是散发性的,家族性PD占所有PD患者的10%~15%^[47]。迄今为止,至少有18个基因的突变与PD病因相关,除了编码 α -突触核蛋白的*SNCA*(*PARK1/4*)、*UCHL1*(*PARK5*)、*LRRK2*(*PARK8*)、*GIGYF2*(*PARK11*)、*Omi/HTRA2*(*PARK13*)、*VPS35*(*PARK17*)、*EIF4G1*(*PARK18*)、*DNAJC13*、*CHCHD2*基因与常染色体显性遗传有关,*Parkin*(*PARK2*)、*PINK1*(*PARK6*)、*DJ-1*(*PARK7*)、*ATP13A2*(*PARK9*)、*PLA2G6*(*PARK14*)、*FBXO7*(*PARK15*)、*DNAJC6*、*SYNJ1*基因与常染色体隐性遗传有关,*RAB39B*与X-连锁遗传有关^[46]。其中一些突变与Rab功能和膜转运有关,如*LRRK2*、*SNCA*、*Rab39B*等。

2.1 Rab与LRRK2

诸多Rab蛋白是LRRK2的底物^[48, 49],*LRRK2*突变是导致PD最常见的原因,在家族性PD中约占4%,在散发性PD中约占1%^[50]。LRRK2是一个多结构域蛋白,包括激酶结构域、Ras复合物(Ras of complex, ROC)和ROC区C端组成的GTP酶结构域、蛋白质-蛋白质相互作用结构域^[51],定位于内质网、高尔基体、内体、溶酶体、自噬囊泡、突触囊泡等^[52],在自噬、突触囊泡运输、微管和线粒体动力学等过程中发挥着调节作用^[53–56]。*LRRK2*基因突变时会过度激活LRRK2,通常促进Rab在开关I区和开关II区被磷酸化,而磷酸化的Rab不能被GEFs激活、GAPs失活以及GDI回收,或不能与效应蛋白特异性结合^[49]。

LRRK2介导的Rab磷酸化影响内吞途径中囊泡运输。*LRRK2*突变促进高尔基体中Rab7L1 Ser⁷²位点的磷酸化,后者改变TGN形态结构^[57],使TGN片段化、MPR异常分布^[58],这可能影响MPR向晚期内体的逆行性转运;Rab7L1除了可作为LRRK2的底物外,还可作为上游调节蛋白招募LRRK2到TGN并激活LRRK2^[59, 60]。Rab7L1与核苷酸的结合较弱,异戊二烯化能力较弱,且不与细胞质中的GDI结合,但其与膜结合和GTP结合时可激活LRRK2^[61],由此导致恶性循环。整合组学分析显示含LRRK2-G2019S突变的诱导性多能干细胞(induced pluripotent stem cells, iPSC)来源的多巴胺能神经元内吞途径失调,内吞蛋白(endophilin I-III、各种Rab蛋白等)表达下调导致网格蛋白包被形成的囊泡功能受损。

尸检发现 LRRK2-G2019S 突变患者组织中 endophilin I-III 水平均高于对照组,表明 LRRK2 介导的 PD 可能与内吞途径失调有关^[62]。

LRRK2 及 Rab 蛋白的正常功能有助于维持溶酶体稳态。溶酶体应激诱导 Rab7L1、LRRK2 易位至溶酶体,并激活 LRRK2, LRRK2 可通过磷酸化下游效应蛋白 Rab8 减弱应激诱导的溶酶体体积的增大,磷酸化 Rab10 促进溶酶体内容物的释放,这些作用通过招募 Rab8/Rab10 的效应蛋白 EHBP1 和 EHBP1L1 介导^[63]。在腺癌人类肺泡基底上皮细胞(A549 细胞)中,敲除 Rab7L1 可减少 LRRK2 介导的 Rab10 磷酸化^[59]。果蝇在视网膜电图显示,多巴胺能神经元中 Rab10 与 LRRK2-G2019S 相互作用最强,而 Rab3 最弱, Rab10 可能是多巴胺能神经元中 LRRK2 的有效底物之一^[64]。磷酸化的 Rab8、Rab10 还可在中心体聚集,导致中心体内聚和纤毛形成缺陷,继而影响神经突生长、细胞极化和定向迁移,中心体内聚和纤毛形成缺陷可能会成为 PD 潜在的细胞生物标志物^[65],且 Rab10 也可能用作 LRRK2 抑制剂剂临床试验的生物标志物或患者富集生物标志物^[66]。

在原代皮层神经元中, LRRK2 突变使 Rab1A、Rab3C、Rab35 磷酸化诱导神经毒性,其中 Rab35 磷酸化诱导的神经毒性最为严重,向小鼠黑质区注射 Rab35-T72A 腺病毒导致多巴胺能神经元变性,表现为黑质致密部 TH 神经元和 Nissl 神经元的丢失,但其在神经元中的功能有待进一步确定^[51]。LRRK2 还可通过介导 Rab35 磷酸化调节 α -突触核蛋白传播(详见后述)^[67]。更有趣的是,PD 的另一突变基因 *VPS35/D620N* 突变可增强小鼠胚胎纤维细胞中 LRRK2 介导的 Rab8A、Rab10、Rab12 的磷酸化以及小鼠组织(肺、肾、脾、脑)中 Rab10 的磷酸化;在 *VPS35/D620N* 突变的患者中 LRRK2 介导的 Rab10 的磷酸化也增强,并且 *VPS35/D620N* 突变比 LRRK2 突变更能促进 Rab10 磷酸化^[68]。因此, LRRK2 激酶活性和 Rab 磷酸化过程可能是延缓 PD 发展进程的治疗靶点^[67]。

2.2 Rab与 α -突触核蛋白

SCNA 编码 α -突触核蛋白,是第一个被发现的 PD 致病基因^[69]。在神经元中, Rab 蛋白功能的缺失使囊泡运输障碍,导致 α -突触核蛋白代谢异常。在正常生理条件下, α -突触核蛋白在神经元的突触前末梢高度富集,对突触可塑性、囊泡包装和运输

至关重要^[70],并在调节膜转运过程中与 Rab 蛋白共定位且相互作用^[71-73]。在健康的神经末梢, α -突触核蛋白需要经历膜结合和解离的循环过程, α -突触核蛋白与 GTP 结合型 Rab3A 相互作用,后者能够稳定突触囊泡上的 α -突触核蛋白。Rab3A 突变时,与其结合的 GTPase 功能缺陷导致 α -突触核蛋白无法从膜上解离,使 α -突触核蛋白在膜上积累,而 α -突触核蛋白在膜上的积累可能会诱导 PD,但其确切机制仍不清楚^[71]。在酵母细胞中, α -突触核蛋白的表达可抑制囊泡从内质网转运到高尔基体,导致毒性的增加以及多巴胺能神经元的丢失,在果蝇、秀丽隐杆线虫和大鼠模型中, Rab1 均可挽救 α -突触核蛋白诱导的多巴胺能神经元的丢失^[74]。对弥漫性路易小体疾病(Lewy body disease, LBD)患者尸检结果显示,脑组织中 α -突触核蛋白与 Rab3A 及其效应蛋白 rabphilin 发生异常相互作用,由于 Rab3A 参与神经末梢突触囊泡的运输,因此推测在 LBD 中 α -突触核蛋白代谢可能受损^[73]。Dalfo 等在 *A30P* 转基因小鼠中发现 α -突触核蛋白聚集体,并且这种突变型 α -突触核蛋白与 Rab3A、Rab5、Rab8 相互作用。突变型 α -突触核蛋白与 Rab3A 结合可导致突触功能异常;与 Rab5 结合可导致神经递质的异常内吞,并可增加黑质致密部神经元的多巴胺毒性;与 Rab8 结合可影响胞质中 α -突触核蛋白的转运,进而可能导致 α -突触核蛋白在胞质中的沉积^[72]。

Rab 蛋白可调控 α -突触核蛋白的聚集和清除。 α -突触核蛋白中心结构域,或被称为非淀粉样 β 成分(non-amyloid-beta component, NAC)易受突变和环境的影响导致蛋白错误折叠和聚集,继而影响其生理功能^[75],而在人 H4 神经胶质细胞中,沉默 Rab8B 促进 α -突触核蛋白寡聚或聚集,并且对细胞具有毒性,过表达 Rab11A、Rab13 可促进 α -突触核蛋白包涵体的清除并挽救 α -突触核蛋白诱导的毒性^[76]。在果蝇中, Rab11 降低 α -突触核蛋白的聚集并改善了 α -突触核蛋白依赖性表型异常,如运动能力下降、多巴胺能神经元变性和寿命缩短^[77];在人 H4 神经胶质细胞中,过表达 Rab11A 降低 α -突触核蛋白的聚集,而敲低 Rab11 增加细胞内 α -突触核蛋白的聚集^[78]。作为晚期内体的标记物, Rab7 调节晚期内体和自噬体的运输,已有研究报道其能够诱导 α -突触核蛋白聚集体的清除^[79],后期的研究发现, Rab7 效应蛋白——含 FYVE 和卷曲螺旋结构域蛋白 1 (FYVE and coiled-coil domain-containing protein 1,

FYCO1) 过表达降低了含有 α -突触核蛋白聚集体的细胞数量, 敲除 FYCO1 使 Rab7 诱导的 α -突触核蛋白聚集体的清除减少, 这表明 Rab7-FYCO1 通路促进了 α -突触核蛋白的降解^[80]。另有研究显示, 在小鼠原代皮层神经元和 HeLa 细胞中, 干扰素 β (interferon- β , IFN β) 诱导 microRNA1 (Mir1) 的转录, 后者可与 TBC 结构域蛋白家族 (属 GAPs) 的 TB-C1D15 3' 未翻译区结合, 抑制其翻译表达, 使 Rab7 活性增强, 促进自噬, 从而降低蛋白聚集体 (α -突触核蛋白) 的积累^[81], 据此推测 IFN β -Mir1-TBC1D15-Rab7-FYCO1 可能是促进 α -突触核蛋白聚集体清除的一个完整通路。磁共振波谱显示, α -突触核蛋白 C 末端与 Rab8A 开关区域结合, Rab8A 促进 α -突触核蛋白的清除并降低 α -突触核蛋白诱导的细胞毒性^[82]。Rab11 可减少 Hook 同型二聚体并抑制内体成熟的负调控作用来促进自噬通量^[83]。最近有研究表明, 在多西环素诱导神经母细胞瘤细胞旁分泌 α -突触核蛋白的模型中, 敲低 Rab27B 减少 α -突触核蛋白经非分泌途径的释放, 增加细胞内不溶性 α -突触核蛋白的表达, 并导致 LC3 的表达增加, 其机制与敲低 Rab27B 导致自噬通量缺陷有关。与对照组相比, PD 患者尸检脑组织中 Rab27B 表达升高, 提示其可能是一种代偿性的增加^[84]。

近年来, 大量研究证实 α -突触核蛋白可作为病理性“种子”在细胞间传播和扩增, α -突触核蛋白聚集体沿神经网络扩散, 可能是其在脑中特征性分布的主要机制^[85]。游离型和囊泡型 α -突触核蛋白进入细胞, 膜融合特性可促进 α -突触核蛋白的内化, 内化后的 α -突触核蛋白在 Rab4A 标记的囊泡中聚集, 在后期, 它到达自噬溶酶体途径并被降解^[86]。 α -突触核蛋白还可以作用于邻近的星形胶质细胞, 并且在星形胶质细胞中内化后可激活星形胶质细胞进而诱导神经炎症^[87]。最近有研究显示, 细胞外 α -突触核蛋白通过网格蛋白介导的内吞途径进入细胞, 在此过程中肌球蛋白-7B 通过维持质膜相关的肌动蛋白网络来调控细胞表面膜动力学, 进而调节 α -突触核蛋白进入细胞^[88], 进入细胞的 α -突触核蛋白可通过 TLR4-NF- κ B-p62 途径介导的选择性自噬被清除^[89], 还可通过胞吐作用或以分泌体形式被分泌到细胞外^[90, 91]。体外研究显示, Rab 可调节 α -突触核蛋白向细胞外的分泌, 例如: Rab11、Rab11A^[78, 90]。最近有研究显示, 在 SH-SY5Y 细胞中, 细胞外的单体和聚集体形式的 α -突触核蛋白被细

胞内化后, LRRK2 G2019S 的表达可增加 α -突触核蛋白在细胞内的积累, 同时, *LRRK2 G2019S* 突变导致 LRRK2 激酶活性增加, 促进 α -突触核蛋白聚集体向细胞外的分泌, 即在增加细胞内毒性作用的同时促进细胞间传播^[67]。在神经母细胞瘤细胞中, 过表达 Rab35 能促进野生型 α -突触核蛋白和 A53T α -突触核蛋白的表达, 并且导致 A53T α -突触核蛋白的聚集和分泌增加。PD 小鼠模型 (MPTP、鱼藤酮小鼠, *LRRK2 G2019S* 转基因小鼠) 黑质区和 PD 患者血清中 Rab35 蛋白表达均升高, 且血清 Rab35 水平与 PD 发病年龄显著相关^[92]。Rab 蛋白在 α -突触核蛋白加工、清除和传播中的调节作用表明其在 PD 中可能具有重要的治疗价值。

2.3 Rab39B

在 18 个 PD 的明确致病基因中, *Rab39B* 基因编码的 Rab39B 是 Rab 家族成员, 在神经元内特异性表达, 在突触的形成和维持中起作用^[93]。*Rab39B* 突变导致早发性 PD, 出现路易小体等典型神经病理改变。最先在澳大利亚的一个家庭中发现早发性 PD 与 *Rab39B* 基因突变有关, 随后又在美国的一个家庭中发现 *Rab39B* 基因错义突变 (p.Thr168Lys)^[94]; 此后又发现几例 *Rab39B* 基因错义突变 (p.G192R/p.W186stop/p.E179fsX48) 导致的 PD 病例^[95-97]。*Rab39B* 基因突变被认为与 PD 认知障碍有关^[94-97], 但也有研究认为 *Rab39B* 基因突变不是引起 PD 和路易小体痴呆的常见原因^[98]。在小鼠大脑中, Rab39B 在大脑皮层、海马、黑质致密区广泛表达, 且主要表达在神经元中^[99]。神经元中的 Rab39B 定位于高尔基体, 在正常情况下可将 AMPA 受体 (alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor) 亚基 GluA2 转运到海马神经元突触后膜, 从而与谷氨酸发挥正常生理功能相关, *Rab39B* 突变的神经元中未成熟 GluA2 的水平升高, 导致缺乏 GluA2 亚基的 AMPA 受体形成, 这与未成熟突触的形成和智力障碍有关^[100, 101], 但这一机制还并未证实与 PD 患者认知障碍有关。这些证据表明, 有缺陷的 Rab 可能是导致家族性 PD 病人神经退行性变的基础, 但 Rab39B 功能丧失如何导致多巴胺能神经元选择性丢失的确切机制仍不清楚^[101]。

3 Rab蛋白与AD

AD 是痴呆症最常见的诱因, 65 岁以上人群中的发病率为 1%~3%^[102, 103]。AD 的典型病理学特征

是错误折叠的 β -淀粉样蛋白(amyloid β -protein, A β)异常聚集与过度磷酸化 Tau 蛋白的神经原纤维缠结(neurofibrillary tangles, NFTs)^[104, 105]。

在 A β 的形成过程中, 正常情况下, Rab5 标记的早期内体是加工 β 淀粉样前体蛋白的主要场所, β -分泌酶(β -secretase, BACE1)在 β 位点切割 β 淀粉样前体蛋白, 在晚期内体或 TGN 中进一步加工产生 A β ^[106]。在小鼠 AD 模型和 AD 患者大脑尸检中发现 Rab5 的过度激活^[107]和 β 切割的羧基末端片段(β -cleaved carboxy-terminal fragment of APP, β CTF)的表达水平升高^[108]。 β CTF 招募 Rab5 的效应蛋白 APPL1 (adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1)到 Rab5 标记的内体, 激活 GTP 结合型 Rab5, 导致 Rab5 的过度激活, A β 形成增加, 导致内体膨胀、轴突运输受损^[108], β CTF-APPL1 相互作用所介导的 Rab5 过度激活为研究 AD 病理机制提供了一个新的途径。

Tau 蛋白的积累是 AD 的重要病理特征, 与神经元功能和认知功能障碍密切相关, 主要由降解途径功能障碍引起^[109, 110]。Tau 蛋白的降解有三种不同的机制: 泛素蛋白酶体系统、自噬溶酶体途径^[111, 112]、内溶酶体途径。最近有研究表明, 之前未被关注的内溶酶体分选途径也可参与 Tau 蛋白清除, 该途径需要 Rab35 和转运所需的内体分选复合物(endosomal sorting complex required for transport, ESCRT)。Tau 蛋白位于 Hrs (hepatocyte growth factor regulated tyrosine kinase substrate, 定位于早期内体, 负责招募网格蛋白^[113])、EEA1、Rab5 阳性的早期内体、晚期内体和溶酶体, 表明 Tau 蛋白通过内溶酶体途径转运; 进一步的研究发现糖皮质激素通过抑制 Rab35 转录降低 Rab35 的表达, 进而抑制 Tau 蛋白降解; 在大鼠海马神经元注射腺相关病毒使其过表达 Rab35 可以挽救糖皮质激素诱导的 Tau 蛋白的积累。因此, Rab35 和内溶酶体途径对 Tau 蛋白的清除至关重要, 对 Tau 蛋白积累所导致的神经元功能障碍的治疗也值得进一步研究^[114]。

Rab10 作为囊泡运输蛋白, 最近被确定作为一种与 AD 相关的蛋白。对 AD 患者脑组织进行尸检发现, AD 患者神经元中 Rab10 表达水平较正常人高^[115]; 在 AD 患者海马组织 NFTs 中, 第 73 位苏氨酸磷酸化的 Rab10 (Rab10 phosphorylation at the amino acid residue threonine 73, pRab10-T73) 表达水平显著高于对照组, 用双重免疫荧光染色发现 pRab10-T73 与高度

磷酸化 Tau 蛋白共定位^[116]。在神经母细胞瘤细胞中, 过表达 Rab10 导致 A β 42、A β 42/A β 40 比率显著升高, 敲除 Rab10 导致 A β 42、A β 42/A β 40 比率下降, 提示 Rab10 可能是预防和治疗 AD 的有效靶点^[115]。还有研究利用生物信息学的方法鉴定出几个可能与 AD 相关的潜在生物标记物, 其中就包括 Rab10^[117]。

4 Rab蛋白与HD

HD 是一种常染色体显性遗传的神经退行性疾病, 患病率为 0.5‰^[118]。HD 的主要临床特征是运动、行为和认知能力进行性下降, 舞蹈症是最易识别的症状^[119]。HD 是由编码亨廷顿蛋白(huntington protein, Htt)的基因发生突变导致的, 胞嘧啶-腺嘌呤-鸟嘌呤(CAG)序列重复扩增, 使得编码的 Htt 氨基末端至少含有 35 个延长的谷氨酰胺, 这种突变使 Htt 具有毒性和聚集性^[118]。

Rab11 功能缺失与 HD 相关。在发育中的大脑皮层中, Htt 通过调控 Rab11 依赖性 N-钙黏蛋白(Rab11-dependent N-Cadherin)的转运来调节投射神经元多极-双极过渡、定向迁移和成熟, 突变的 Htt 在投射神经元内富集, 并改变了 Rab11 依赖的神经元迁移^[120]。在 HD 小鼠的纹状体和小脑中, GDP 结合型 Rab11 向 GTP 结合型 Rab11 的转换受损导致 Rab11 活性降低, 即 GEFs 不能介导对 Rab11 的激活, 这可能随着时间的推移对神经元功能产生累积影响, 与 HD 缓慢进行性发展的特征相一致。在 HD 皮层神经元中, 提高 Rab11 的活性可降低神经元对谷氨酸诱导的细胞死亡的敏感性^[121]。因此, 这对 HD 的治疗提供了一个新的靶点。

Rab 蛋白与 Htt 相互影响。例如在细胞和果蝇模型中, 抑制 Rab5 的表达使突变 Htt 的毒性增加, 过表达 Rab5 使突变 Htt 的毒性降低。研究表明, Rab5 和 / 或其激活的下游分子 Vps34 活性的降低会抑制 Atg5-Atg12 的结合, 影响早期自噬体的伸长, 进而影响自噬体的形成^[122]。自噬体的形成减少使突变 Htt 的降解减少, 从而导致突变 Htt 的聚集和毒性增加。此外, 亨廷顿相关蛋白 40 (Htt-associated protein 40, HAP40) 是 Rab5 效应蛋白, 介导 Rab5 将 Htt 招募到早期内体, 在 HD 患者成纤维细胞和脑组织中以及 HD 小鼠模型纹状体细胞中 HAP40 表达上调, 通过影响 Rab5 在调控早期内体与肌动蛋白和微管蛋白细胞骨架之间的作用, 导致内体的运动性和内吞活性受损^[123]。除了 Rab 蛋白影响 Htt

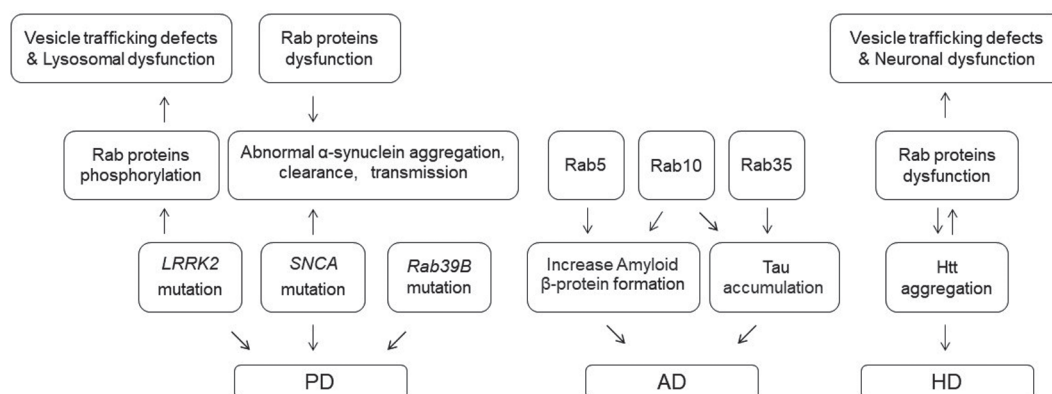


图 2. Rab蛋白在神经退行性疾病中的可能作用

Fig. 2. Possible roles of Rab proteins in neurodegenerative diseases. *LRRK2*, *SNCA*, and *Rab39B* mutations are genetic risk factors in Parkinson's disease (PD). *LRRK2* mutations promote Rab proteins phosphorylation, leading to vesicle transport defects and lysosomal dysfunction in the endocytic pathway; *SNCA* mutations and Rab proteins dysfunctions cause abnormal α -synuclein aggregation, clearance and transmission. The typical pathological features of Alzheimer's disease (AD) are the abnormal aggregation of misfolded A β and the neurofibrillary tangles of hyperphosphorylated Tau protein. Rab5 and Rab10 regulate the formation of A β , Rab35 regulates the clearance of Tau protein, and Rab10 was observed colocalized with hyperphosphorylated Tau. Huntington's disease (HD) is caused by mutations in the gene encoding Htt. Htt mutations lead to the loss of several Rab proteins function, whereas the latter regulates Htt aggregation and toxicity. This eventually results in vesicle trafficking defects and neuronal dysfunction.

的聚集和毒性，也有证据表明 Htt 反过来影响含不同 Rab 的囊泡的轴突运输。在果蝇幼虫中，Htt 表达水平降低时，含 Rab3、Rab9 的囊泡的双向运输、含 Rab7 的囊泡的逆行运输受到干扰，却同时刺激了含 Rab2 的囊泡的顺行运输，同时双视图成像显示，Htt 和 Rab2、Rab7、Rab19 在轴突运输过程中一起移动^[124]，这表明 Htt 可能影响含不同 Rab 的囊泡的运输，进而影响神经元的功能。

5 小结与展望

Rab 蛋白作为囊泡运输的重要调节因素，在大分子物质通过内吞作用进入细胞内以及细胞内物质转运的过程中发挥重要的作用。在此过程中 Rab 蛋白及其效应蛋白功能的失调将会导致大分子物质的转运受损，这与大多数神经退行性疾病的蛋白质聚集病理学特征相关。虽然 Rab 蛋白在囊泡形成、转运、黏附、融合等过程中的作用已被证实，并且越来越多的证据表明 Rab 蛋白与神经退行性疾病之间存在密切关系（图 2），但是 Rab 蛋白、囊泡运输受损、蛋白质聚集之间的具体机制尚未完全明确，还需进一步加深对 Rab 蛋白在神经退行性疾病中的分子作用机制的研究，我们相信 Rab 蛋白有可能成为神经退行性疾病早期诊断的生物标志物，并为疾病的治疗提供新的靶点。

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