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帕金森病路易(小)体的蛋白质生物信息学数据分析 *

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摘要 原发性帕金森病(idiopathic Parkinson's disease，PD)的主要病理特征之一是出现于中脑特定脑区黑质致密部(substantia nigra pars compacta，SNpc)多巴胺能神经元的路易(小)体(Lewy bodies，LBs)，PD 病人 LBs 和 / 或路易轴突也出现于脑内其他脑区非多巴胺能神经元，比如蓝斑(locus coeruleus，LC)等脑干个别脑区去甲肾上腺素能神经元、额前叶皮层(prefrontal cortex，PFC)、颞叶皮层(temporal cortex，TC)等大脑多个脑区胆碱能神经元．为了明确 LBs 的蛋白质构成，本文通过蛋白质生物信息学数据分析，就 LBs 的蛋白质构成归纳了 5 个方面的要点：a．LBs 的组织结构单元是 α- 突触核蛋白(α-synuclein，α-SYN)表征的 2 类纤维状聚集物和 6 类非纤维状聚集物(通常被称为寡聚物)；b．病理性 α-SYN 在 LBs 内存在 5 种化学修饰形式；c．19 个 α-SYN 相关蛋白质分别与 α-SYN 共定位于 LBs；d．117 个 LBs 的已知蛋白质被划分为 10 组不同蛋白质功能群组；e．LBs 的蛋白质组学鉴定数据库包含了分别在 LC、SNpc 和 PFC 脑区组织水平鉴定的 84、124 和 120 个候选蛋白质，在 TC 脑区细胞水平鉴定的 108 个候选蛋白质，以及在 TC 脑区亚细胞水平鉴定的 29 个候选蛋白质．上述要点广泛、深入地概括了 LBs 的蛋白质构成．

关键词 原发性帕金森病，路易(小)体，蛋白质，生物信息学
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原发性帕金森病(idiopathic Parkinson's disease，PD)的主要病理特征之一是出现于中脑特定脑区黑质致密部(substantia nigra pars compacta，SNpc)多巴胺能神经元的路易(小)体(Lewy bodies，LBs)．PD 病人 LBs 和 / 或路易轴突(Lewy neurites，LNs)也出现于脑内其他脑区非多巴胺能神经元，比如蓝斑(locus coeruleus，LC)等脑干个别脑区去甲肾上腺素能神经元以及额前叶皮层(prefrontal cortex，PFC)、颞叶皮层(temporal cortex，TC)等大脑多个脑区胆碱能神经元．PD 流行病学研究显示，LBs 见于除常染色体隐性遗传的青春型帕金森综合征以外的 10%家族型 PD(familiar PD，fPD)病人和 90%散发型 PD(sporadic PD，sPD)病人[1]．临床诊断学将 LBs 确定为路易体痴呆(dementia with LB，DLB)等路易体病(LB diseases，LBD)的主要病理依据之一[2]．在神经退行性疾病(neurodegenerative diseases)范畴内，PD 患者 LBs、阿尔茨海默病(Alzheimer's disease，AD)患者淀粉样斑和亨廷顿病患者神经元包涵体分别代表了发生于细胞质、细胞外间质和细胞核基质的蛋白质包涵体，而且比之于后两者，前者更具有代表性[3]．基于疾病相关蛋白质与疾病病理机制之间的逻辑关系，PD 患者 LBs 的特征蛋白质成分 α- 突触核蛋白(α-synuclein，α-SYN)被认为是 LB 变异型 AD(LB variant of AD，LBVAD)等共核蛋白病(synucleinopathies)的标志性蛋白质[4]．鉴于 α-SYN 的分子构象在 PD 患者 LBs 内发生了改变[5]，一些学者根据化学(或药物)分子伴侣通过校正和稳定蛋白质构象以实现疾病治疗这一构想，提

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出了蛋白质构象脑病(protein-conformational brain diseases)等蛋白质构象病(protein conformational diseases)的概念[6-8]. 因此，在神经病学背景之下研究 PD 患者 LBs 具有指导意义(图 1).

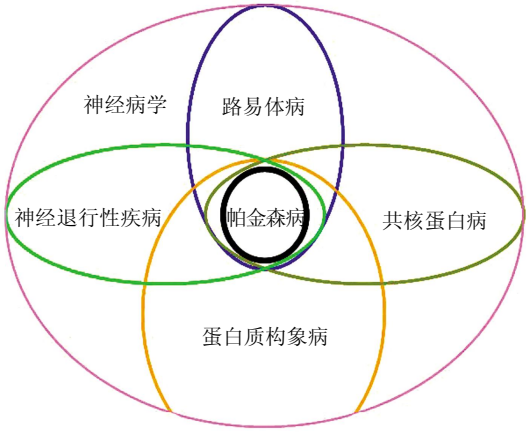

Fig. 1　Morphologic interrelations of Lewy body diseases, synucleinopathies, neurodegenerative diseases and protein conformational diseases, in which Parkinson's disease is a representative of the chronic progressive neurological disorders

图 1　帕金森病分别与路易(小)体病、神经退行性疾病、共核蛋白病和蛋白质构象病之间的相互关系

LBs 的蛋白质构成是“还原”PD 病理机制的“窗口”. 比如，α-SYN 基因突变生成突变体蛋白质，α-SYN 基因座扩增生成双、三体野生型蛋白质，它们通过阻断线粒体物质代谢和能量转换，抑制蛋白酶体酶活性，以及触发自身错误折叠，触及 fPD 病理机制[9-14]；再比如，多巴胺(dopamine, DA)代谢异常生成过剩氧化代谢物，它们通过激活线粒体膜电位去极化和解除呼吸链氧化磷酸化偶联，抑制蛋白酶体凝乳酶样活性、胰酶样活性和半胱天冬酶(“含半胱氨酸的天冬氨酸蛋白质水解酶”的简称)样活性，以及非特异性共价结合 α-SYN，触及 sPD 病理机制[15-18]. 一般认为，α-SYN 只是代表了 LBs 内的折叠蛋白质，它作为最早发现的一种 PD 相关蛋白质(PD related proteins，或 PD linked gene products)，为初步揭示 PD 病理机制积累了不可或缺的知识储备. 然而，越来越多的证据显示，在 PD 病变过程中，LBs 也富集了 PTEN 诱导基因表达的蛋白质激酶 1(PTEN induced kinase 1)、富含亮氨酸密码子序列重复基因表达的蛋白质激酶 2(leucine-rich repeat kinase 2)、癌基因 DJ-1 表达产物(oncogene DJ-1)等与线粒体功能相关的蛋白质，以及泛素(ubiquitin，Ub) C 端水解酶 L-1 (Ub C-terminal hydrolase L1，Uch-L1)、E2 依赖性 Ub-靶蛋白质连接酶 E3 异构体 parkin (E2-dependent ubiquitin-protein ligase E3 isoform parkin，Parkin)等与蛋白酶体功能相关的蛋白质[19]. 这些后续发现的 PD 相关蛋白质已经成为进一步揭示 PD 病理机制的热点领域.

实际上，在 LBs 所含有的生物大分子中，少部分来源于突触前神经递质囊泡、致密分泌囊泡以及溶酶体等生物膜系统的脂类物质，而其余绝大部分来源于细胞质等细胞单元、线粒体等细胞腔室以及蛋白酶体等蛋白质复合体的蛋白质[20]. 那么，LBs 的蛋白质构成具有几层涵义？本文通过蛋白质生物信息学数据分析解析了 LBs.

1　α-SYN 表征的聚集物

LBs 的组织结构单元是 α-SYN 表征的聚集物. 在 LBs 形成过程中，α-SYN 通过纤维化聚集方式形成 α-SYN 纤维状聚集物，通过非纤维化聚集或分子聚集(molecular crowding)或寡聚化(oligomerization)方式形成 α-SYN 非纤维状聚集物、共聚物(coformers)或寡聚物(oligomers)[21]. 一些报道显示，α-SYN 纤维状聚集物和 α-SYN 寡聚物是蛋白质聚集物在 LBs 内彼此独立存在的 2 种形态[5]. 另外一些报道显示，α-SYN 纤维状聚集物是蛋白质聚集物在 LBs 内的成熟形态，而 α-SYN 寡聚物仅是蛋白质聚集物在 LBs 内的过渡态[22]. 再有一些报道显示，在病变早期形成的 LBs 内，蛋白质聚集物以 α-SYN 寡聚物为主，而在病变晚期形成的 LBs 内，蛋白质聚集物以 α-SYN 纤维状聚集物为主[23].

α-SYN 在其表征的聚集物内具有 3 种不同的分子构象形式，即，新生 α-SYN、生理性 α-SYN 和病理性 α-SYN. 首先，新生 α-SYN 属于非折叠蛋白质(unfolding proteins)[5, 24]. 在新生 α-SYN 的 C 端区段内部、C 端区段与中间区段之间以及 C 端区段与 N 端区段之间，疏水性氨基酸残基之间均存在疏水作用. 同时，在它的 C 端区段与其他区段之间，酸、碱性氨基酸残基之间存在静电作用[5]. 这些氨基酸残基之间的相互作用阻止 α-SYN 形成 α 螺旋和 / 或 β 片层等典型二级结构，α-SYN 以自

由伸展状态呈现原始构象. 因此，新生 α-SYN 具有低疏水性和高负电荷性的基本特征，既不引发分子折叠又不发生分子间相互聚集，游离于神经元细胞质[25]. 其次，绝大多数新生 α-SYN 在生理状态下属于部分折叠蛋白质(partially folded proteins)[5, 24]. 在生理性 α-SYN 的 N 端区段内部，氨基酸残基之间通过氢键等相互作用形成 5 个 α 螺旋，然而，它的其他区段仍然保持了新生 α-SYN 的原始构象[25]. 因此，生理性 α-SYN(相对于新生 α-SYN)获得较高疏水性和较低负电荷性，虽然仅在小部分区段引发分子折叠，但是总体上不发生分子间相互聚集，借助于 N 端区段的 α 螺旋结合于神经元乃至神经递质囊泡的膜脂质[23]. 在中枢神经元内，新生 α-SYN 和生理性 α-SYN 共同构成了动态变化的 α-SYN 池，前者在池内具有相对高的含量而后者具有相对低的含量，它们在人脑的可溶性细胞提取物中占总蛋白质质量的 1%[25-28]. 再次，绝大多数新生 α-SYN 在病理状态下属于错误折叠蛋白质(misfolded proteins)[5, 24]. 在病理性 α-SYN 的中间区段及其两翼的部分区段范围内，氨基酸残基借助于非老年斑组分 Aβ 片段区(non-Aβ component of plague，NAC)形成了 5 个 β 片层，而它的其他区段仍然保持了新生 α-SYN 的原始构象[21]. 因此，病理性 α-SYN(相对于生理性 α-SYN)获得较高疏水性和较低负电荷性，不仅在绝大部分区段引发分子折叠而且容易发生分子间相互聚集，以蛋白质复合体等亚细胞结构物或暂时细胞器(intermediate organelles)的形式存在于细胞质[5].

为了明确 LBs 的组织结构单元，本文通过蛋白质生物信息学数据分析，(i)将 α-SYN 表征的纤维状聚集物归纳为直线带型和麻花样带型 2 个类别，(ii)将 α-SYN 表征的非纤维状聚集物归纳为点状、球状、链状、花环状、原(或前)纤维状以及纤维状 6 个类别(详见附录 1，http://www.pibb.ac.cn/cn/ch/common/view_abstract.aspx?file_no=20130065&flag=1).

2 病理性 α-SYN 的化学修饰形式

在 α-SYN 表征的聚集物形成过程中，蛋白质化学修饰通过改变病理性 α-SYN 的长度范围、电荷分布或分子构象等蛋白质结构特征，以及亲和性、疏水性或酶活力等蛋白质功能特性，影响 LBs 的结局[23]. 为了明确病理性 α-SYN 在 LBs 内的存在形式，本文通过蛋白质生物信息学数据分析，将 α-SYN 的化学修饰状态归纳为(i)磷酸化、(ii)氧化(包括硝基化、羟基氧化、巯基氧化和 DA 氧化)、(iii)泛素化和类泛素化等 3 种常见蛋白质修饰类别，以及(iv)蛋白质截断和(v)蛋白质交联等 2 种其他蛋白质修饰类别(详见附录 2，http://www.pibb.ac.cn/ cn/ch/common/view_abstract.aspx?file_ no=20130065&flag=1).

此外，α-SYN 在甲硫氨酸残基 1 位点被乙酰化修饰是新生 α-SYN 向生理性 α-SYN 转变过程中形成 α 螺旋的必要步骤之一[5]. 然而，α-SYN 的乙酰化修饰形式广泛地存在于 PD 病人 LBs 内，这可能是生理性 α-SYN 在 LBs 形成过程中被动地参与了蛋白质聚集物的形成[23]. 报道显示，乙酰化修饰在体外能够瞬间促进 α-SYN 形成 α 螺旋，并且降低 α-SYN 的聚集率[5].

3 α-SYN 相关蛋白质

α-SYN 在脑内的主要功能是调节 DA 的生理水平[22]. α-SYN 调控作用主要表现为通过作用于酪氨酸羟化酶(tyrosine hydroxylase，TH)调节 DA 合成，通过作用于囊泡单胺转运体 -2 (vesicular monoamine transporter 2，VMAT2)调节 DA 由神经递质囊泡外主动转运至神经递质囊泡内和 DA 囊泡从神经递质囊泡中分选出来，通过作用于磷脂酶 D2 调节轴突末端 DA 稳定性，通过作用于 DA 转运体(dopamine transporter，DAT)调节 DA 从突触间隙再摄取到细胞内，以及通过作用于抑癌基因 p53 产物(或 P53 蛋白质)抑制多巴胺能神经元凋亡[22-23]. 然而，许多 α-SYN 相关蛋白质与 α-SYN 之间通过共价、非共价结合和 / 或直接、间接作用协助 α-SYN 发挥功能. 尽管 α-SYN 相关蛋白质是一些来源广泛、功能各异的蛋白质，但是它们在相关领域已经成为研究人员认识 α-SYN 结构与功能的常用工具[29].

迄今为止，免疫组织化学分析显示，至少 30 个 α-SYN 相关蛋白质分别与 α-SYN 共定位于黑质多巴胺能神经元[5]. 为了明确 LBs 内的 α-SYN 相关蛋白质及其与 α-SYN 的共定位，本文通过蛋白质生物信息学数据分析归纳了 19 个 α-SYN 相关蛋白质. 它们包括(i)14-3-3 蛋白 5 (14-3-3 epsilon protein，14-3-3ε)、(ii)TH、(iii)丝裂原活化蛋白质激酶(mitogen-activated protein kinases，MAPKs)异构体细胞外信号调控激酶 2、(iv)MAPKs 异构体 p38、(v)DAT 、(vi)VMAT2、(vii)Aβ、(viii) α-SYN 亲和蛋白 1、(ix) 微管相关蛋白 1B

(microtubulin-associated protein-1B，MAP-1B)、(x)微管相关蛋白 τ(MAP tau)、(xi)微管蛋白 α(alpha tubulin，α-Tub)、(xii)β-Tub、(xiii)Ub、(xiv)Ub 结合蛋白 p62、(xv)Uch-L1、(xvi)Parkin、(xvii)G 蛋白偶联受体激酶 5、(xviii)E1A 结合蛋白 p300 以及(xix)聚集蛋白(详见附录 3，http://www.pibb.ac.cn/cn/ch/common/view_abstract.aspx?file_no=20130065&flag=1).

4 蛋白质功能群组

揭示 LBs 蛋白质构成的一种研究策略是对 LBs 进行免疫组织化学分析，被发现的新蛋白质因为具有生物学意义被认为是已知蛋白质(或确切蛋白质). 迄今为止，LBs 的已知蛋白质不少于 117 种(详见附录 4，第 1 部分，http://www.pibb.ac.cn/cn/ch/common/view_abstract.aspx?file_no=20130065&flag=1). 为了明确 LBs 的已知蛋白质的功能属性，Wakabayaashi 等[30](2013 年)通过蛋白质生物信息学数据分析，首次将 LBs 的 93 个 LBs 已知蛋白质初步归纳为细胞骨架网状结构、细胞内蛋白质降解通路和细胞周期机器等 13 组蛋白质功能群组. 本文在此基础之上，结合蛋白质在生理状态下组装蛋白质复合体、细胞器等亚细胞结构物中所扮演的角色[31-32]，以及蛋白质在 PD 细胞模型(如，线粒体功能抑制剂和蛋白酶体功能抑制剂分别作用于体外细胞诱导蛋白质聚集)和 PD 动物模型(如人 α-SYN 基因在模型动物中过表达导致蛋白质聚集)中形成蛋白质包涵体的报道[33-35]，将 LBs 的已知蛋白质进一步归纳为 10 组蛋白质功能群组. 它们既在生理状态下分别涉及(i)神经递质代谢、(ii)抗氧化防御系统、(iii)脑内局部免疫反应、(iv)细胞骨架网状结构、(v)线粒体有氧能量代谢和细胞凋亡线粒体通路、(vi)Ub 依赖性和非 Ub 依赖性蛋白酶体降解通路和溶酶体降解通路、(vii)蛋白质折叠、(viii)细胞内信号转导通路、(iv)细胞周　期机器以及(x)其他细胞功能[30, 34, 36-37]，又在病理状态下分别参与 PD 病理机制(详见附录 4 第 2 部分，http://www.pibb.ac.cn/cn/ch/common/view_abstract.aspx?file_no=20130065&flag=1).

当一个蛋白质具有多种功能并且有可能属于两组以上蛋白质功能群组时，多数学者认为应该将它划入多数文献倾向于描述的某一组蛋白质功能群组. 比如，α-SYN 与 14-3-3ε 之间存在同源氨基酸序列，涉及神经递质代谢[38-39]；α-SYN 也与 MAPs 异构体之间存在同源氨基酸序列，涉及细胞骨架网状结构[40]；α-SYN 还与分子伴侣蛋白质家族成员之间存在同源氨基酸序列，涉及蛋白质折叠等[41]. 鉴于多数文献提供了 α-SYN 具有分子伴侣蛋白质活性的证据[42]，甚至将 α-SYN 表述为分子伴侣样蛋白质[43]，本文将其划分为涉及蛋白质折叠的蛋白质.

5 蛋白质组学鉴定数据库

揭示 LBs 蛋白质构成的另外一种研究策略是对 LBs 进行蛋白质组学分析，获得鉴定的新蛋白质因为具有物理学意义而不具有生物学意义被认为是候选蛋白质(或可能蛋白质). 在 PD 发生、发展过程中，LBs 和 / 或 LNs 经历了从低位脑干向高位大脑逐步扩展的“旅程”，并且大致呈现了这样的规律性：始于 LC 等低位脑干个别脑区，途经中脑特定脑区 SNpc 以及终于 PFC、TC 等大脑多个脑区[44-45]. 这成为 LBs 应用于 PD 临床分型的主要病理学依据. PD 在临床上被划分为 6 个阶段：第 1、2 阶段 PD(特别是第 2 阶段 PD)呈现 LC 去甲肾上腺素能神经元丧失、LBs 和 / 或 LNs 形成等非特异性病理改变，并且在此病理基础之上表现睡眠、自主运动紊乱等非运动障碍症状，PD 病人在此期间处于疾病前期(或临床前期)；第 3、4 阶段 PD(特别是第 3 阶段 PD)呈现 SNpc 多巴胺能神经元丧失、LBs 形成等特异性病理改变(而 LNs形成属于非特异性病理改变)，并且在此病理基础之上表现静止性震颤、运动迟缓、肌肉僵硬和姿势步态障碍等运动障碍症状，PD 病人在此期间处于疾病发作期(或临床期)；第 5、6 阶段 PD(特别是第 6 阶段 PD)呈现 PFC、TC 胆碱能神经元丧失、LBs 和 / 或 LNs 形成等非特异性病理改变，并且在此病理基础之上表现日常行为异常、认知功能缺失等非运动障碍症状，PD 病人在此期间处于并发症状期(或临床后期)[46]. 为了明确 LBs 的候选蛋白质的蛋白质组学特征，本文通过蛋白质生物信息学数据分析，从三个研究层次归纳了 LBs 的蛋白质组学鉴定数据(详见附录 5 第 1 部分，http://www.pibb.ac.cn/cn/ch/common/view_abstract.aspx?file_no=20130065&flag=1)：(i)PD 病人 LC、SNpc 和 PFC 脑区的定性、定量蛋白质组学分析，分别在组织水平初步显示了 PD 发生前后的蛋白质差异表达谱[46-51]，鉴定数据分别涵盖了 84、124 和 120 个候选蛋白质；(ii)DLB 病人 TC 脑区定性蛋白质组学分析在细胞水平

初步显示了 DLB 的蛋白质表达谱[52]，鉴定数据涵盖了 108 个候选蛋白质；(iii)DVLB 病人 TC 脑区定量蛋白质组学分析在亚细胞水平初步显示了 DVLB 发生前、后的蛋白质差异表达谱[53]，鉴定数据涵盖了 29 个候选蛋白质．上述获得鉴定的候选蛋白质构成了 LBs 的蛋白质组学鉴定数据库，它们为揭示 LBs 的蛋白质构成成分提供了新线索(详见附录 5 第 2 部分，http://www.pibb.ac.cn/cn/ch/common/view_abstract.aspx?file_no=20130065&flag=1)．

通过上述分析，本文就 LBs 的蛋白质构成归纳了五个方面的要点：LBs 的组织结构单元是 α-SYN 表征的 2 类纤维状聚集物和 6 类非纤维状聚集物；病理性 α-SYN 在 LBs 内存在 5 类化学修饰形式；19 个 α-SYN 相关蛋白质分别与 α-SYN 共定位于 LBs；117 个 LBs 的已知蛋白质被划分为 10 组不同蛋白质功能群组；LBs 的蛋白质组学鉴定数据库包含了分别在 LC、SNpc 和 PFC 脑区组织水平鉴定的 84、124 和 120 个候选蛋白质，在 TC 脑区细胞水平鉴定的 108 个候选蛋白质，以及在 TC 脑区的亚细胞水平鉴定的 29 个候选蛋白质．上述要点广泛、深入地概括了 LBs 的蛋白质构成．

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An Analysis of Protein Bioinformatics Datasets Related to Lewy Bodies of Parkinson′s Disease*

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Abstract Lewy bodies (LBs) in dopaminergic neurons in substantia nigra pars compacta (SNpc) are the cardinal pathological hallmark of Parkinson's disease (PD), while LBs and/ or Lewy neurites are also seen in non-dopaminergic neurons in other brain regions of patients with PD, such as noradrenergic neurons in locus coeruleus (LC), and cholinergic neurons in prefrontal cortex (PFC) and temporal cortex (TC). To explicit protein contents of LBs in PD, here is an article that demonstrates five aspects of protein constitution of LBs by analyzing protein bioinformatics datasets related to LBs in PD: (1) LBs is filled with 2 types of alpha-synuclein (α-SYN) positive fibrilar aggregates and/or 6 types of α-SYN positive non-fibrilar aggregates (described frequently in literature as oligomers); (2) modification forms of pathological α-SYN in LBs are often associated with 5 species of chemical modifiers; (3) 19 α-SYN-associated proteins rich in LBs are respectively colocalized with α-SYN; (4) 117 previously reported proteins in LBs in immunochemistry study are at least classified into 10 different groups of functional proteins with close relationship; (5) proteomics database as protein candidate resource for LBs contains 84, 124 and 120 proteins identified respectively from raw tissue samples of LC, SNpc and PFC regions, and 108 proteins identified from a cellular separation of analyte captured from TC region, and 29 proteins identified from a sub-cellular fraction of analyte isolated from TC region. It is suggested that the aforementioned aspects comprehensively and fundamentally reflect protein composition of LBs in PD.

Key words Parkinson's disease, Lewy bodies, proteins, bioinformatics

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附录 1

α-SYN 表征的聚集物的生物信息学数据分析

i. 在 α-SYN 进行纤维化聚集过程中，病理性 α-SYN 之间形成了完整的四级结构，即，α-SYN 纤维状聚集物[1]. 在 α-SYN 纤维状聚集物内，单个 α-SYN 被称为基原纤维(proto-filament)，它的五个 β-片层依次平行排列并且形成一个被称为 β-交叉结构物(cross-β structure)或 β-三明治(β-sandwich)的结构域，组成 β-片层的每一个氨基酸残基将其侧链暴露于这个结构域的外表面[2]. 而在 α-SYN 纤维状聚集物内，相邻基原纤维像人左右手手指互补交叉似地叉合(interdigitate)，并且形成了一个被称为“立体拉链”(steric zipper)的结构[1, 3]. 在这个拉链样结构中，组成 β-片层的所有氨基酸残基将其侧链作为“拉链左右牙”，在相邻基原纤维交界面处形成一个高度保守的共有基序(a highly conserved common motif)，这个共有基序的外形呈纤维状或梭形状，纤维轴向就是 α-SYN 纤维状聚集物的形成方向[1-2]. 一个 α-SYN 纤维状聚集物通常含有至少两个或几个基原纤维[1]. 按照基原纤维在形成 α-SYN 四级结构过程中组合方式的微细差异，一些学者将 α-SYN 纤维状聚集物划分为直线带型(straight ribbon)和麻花样带型(twisted ribbon) 2 个形态类别[2-4].

ii. 在 α-SYN 进行非纤维化聚集过程中，病理性 α-SYN 之间没有形成完整的四级结构而仅仅通过共价、非共价结合随机性地聚拢；而且，非纤维化聚集诱导生理性 α-SYN 乃至新生 α-SYN 同病理性 α-SYN 发生相互作用[1]. 因此，病理性 α-SYN、生理性 α-SYN 和新生 α-SYN 共同形成了 α-SYN 寡聚物[1, 5]. 显然，α-SYN 寡聚物的形状会伴随蛋白质聚集时病理条件的不同而有所差异. 在不同来源的 LBs 中，α-SYN 寡聚物通常呈现点状寡聚物(annular oligomers，或 annular protofibrils)、球状寡聚物(spheroidical oligomers)、链状寡聚物(chain protofibrils)、花环状寡聚物(wreath-like oligomers)、原(或前)纤维状寡聚物(prefibrillar oligomers，或 protofibrils)以及纤维状寡聚物(fibrillar oligomers) 6 个形态类别[6-12]. 而且，α-SYN 寡聚物在从任意形状结构向具体形状结构转变过程中所呈现的上述构象类型与 α-SYN 的质和量存在一定的关联性[13]. 比如，比之于生理性 α-SYN 在球状寡聚物中占有相对较高的数量而在原(或前)纤维状寡聚物中占有相对较低的数量，病理性 α-SYN 在前者占有相对较低的数量而在后者占有相对较高的数量[12].

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附录 2

病理性 α-SYN 的化学修饰形式的生物信息学数据分析

i. 在 DLB 病人 LBs 内，α-SYN 的磷酸化修饰率达 90%(而在正常脑内，生理性 α-SYN 的磷酸化修饰率仅 4%)[1]. α-SYN 的丝氨酸残基 -87 (Ser-87)、Ser-125 和 Ser-129 是主要的磷酸化修饰位点，而且 Ser-129 位点的磷酸化修饰水平最高[2]. 蛋白质磷酸化修饰有助于稳定 α-SYN 的 β- 片层[3]. 蛋白质磷酸化修饰促进 α-SYN 进行纤维化聚集[3].

ii. 在 PD 病人 LBs 内，α-SYN 的氧化修饰水平不低于它的磷酸化修饰水平[3]. 首先，α-SYN 的酪氨酸残基 -39 (Tyr-39)、Tyr-125、Tyr-133 和 Tyr-136 是一组氧化修饰位点，氧化修饰方式是硝基化和羟基氧化[2]. 硝基化将弱极性的 Tyr 转变为强极性的 3- 硝基酪氨酸，α-SYN 由此不能形成稳定的 β- 片层；羟基氧化将两个 Tyr 转变为一个 Tyr 同二聚体，α-SYN 借此形成分子内、间交联物[4-5]. 其次，α-SYN 的蛋氨酸残基 -1 (Met-1)、Met-5、Met-116 和 Met-127 是另外一组氧化修饰位点，氧化方式是巯基氧化[2]. 巯基氧化将 Met 转变为刚性和极性均较强的亚砜和砜，从而弱化了 α-SYN 的 β- 片层[6-7]. 再次，DA 及其氧化代谢物(包括超氧阴离子、过氧化氢等过氧化物以及多巴醌、5, 6- 二羟基吲哚、5, 6- 吲哚醌等 DA 衍生物)广泛地共价结合于 α-SYN[2-3, 8]. 这种非特异性氧化修饰方式干扰 DA 及其氧化代谢物在生理状态下特异地非共价结合于 α-SYN，并且进一步干扰 α-SYN 形成 β- 片层[9-11]. 蛋白质氧化修饰促进 α-SYN 进行非纤维化聚集[2].

iii. 在 PD 病人 LBs 内，Ub 通过赖氨酸残基 -63 (Lys-63)共价结合于 α-SYN 的 2 个泛素化修饰位点，即，Lys-10 和 Lys-12[2]. Ub 与 α-SYN 一样也是 LBs 的特征蛋白质成分[8]. Ub 在 LBs 内的存在形式是 Ub 单体、2 分子 Ub 单位(一个 Ub 的 C 末端甘氨酸残基的羧基与另外一个 Ub 的 Lys-48 的 ε- 氨基通过异肽键形成的二肽)乃至多分子 Ub 单位(或 Ub 链). 蛋白质泛素化修饰促进 α-SYN 进行纤维化聚集[12]. 在 DLB 病人 LBs 内，Ub 样蛋白质修饰物(Ub-like protein modifiers，ULMs) 异构体 SUMO1 (small Ub-like modifier 1)以类泛素化(sumoylation)修饰方式结合于 α-SYN 的 N 端某个 Lys-(可能是 Lys-102)[13]；在 PD 病人 LBs 内，ULMs 异构体 NEDD8 (neural precursor cell-expressed, developmentally down-regulated protein)以同样方式结合于 α-SYN 的相同氨基酸残基位点[13]. 蛋白质类泛素化修饰促进 α-SYN 进行纤维化聚集[2-3].

iv. 在 DLB 病人 LBs 内，α-SYN 在天冬氨酸残基 -115 (Asp-115)、天冬酰胺残基 -122(Asn-122)、Asp-119、Tyr-133 或 Asp-135 位点被物质代谢产生的自由基有选择地截断[2-3]. 分子断裂使 α-SYN 失去了 C 端部分氨基酸序列，α-SYN 由此减弱了分子伴侣活性和蛋白质结合作用. 这种蛋白质化学修饰形式促进 α-SYN 进行纤维化聚集[2].

v. 在 PD 病人和 DLB 病人 LBs 内，α-SYN 通过酶促催化反应形成分子内、间交联物[14]. 可溶性组织型转谷氨酰胺酶(soluble tissue transglutaminase，tTGase)催化谷氨酰胺(Gln)与 Lys 之间的转酰胺基反应[2]. 在 α-SYN 内部和(或) α-SYN 分子之间，tTGase 催化 Gln-79 或 Gln-109 与 N 端区段一些 Lys-(Lys- 的具体位置不清楚)之间以及 Gln-79、Gln-99 或 Gln-109 与 Lys-60 之间的转酰胺基反应，α-SYN 借此形成分子内、间交联物[3]. 在 LBD 病人 LBs 内，α-SYN 通过非酶促催化反应形成分子内、间交联物[2, 8, 14]. 葡萄糖代谢和脂质过氧化产生的高反应性醛类物质通过自由基诱导的氧化还原反应广泛地在蛋白质 Lys- 位点形成高级糖基化终末产物(advanced glycation end-products，AGEs)，AGEs 之间相互作用使蛋白质形成交联物[2]. α-SYN 极有可能通过上述非酶促催化反应在它的 15 个 Lys- 位点形成分子内、间交联物. 蛋白质交联阻碍 α-SYN 形成完整的四级结构[2]. 这种蛋白质化学修饰形式促进 α-SYN 进行非纤维化聚集[15-17].

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附录 3

α-SYN 相关蛋白质的生物信息学数据分析

i. 14-3-3ε，或酪氨酸 3- 单加氧酶激活蛋白质(tyrosine 3-monooxygenase activation protein)，是 14-3-3 蛋白质家族成员在脑内的主要存在形式. 14-3-3ε 与 α-SYN 之间存在同源氨基酸序列的百分比是 40%，前者是 MAPKs 异构体细胞外信号调控激酶 -2 (extracellular signal-regulated kinase 2，ERK2)激活剂[1-2]，后者是 ERK2 抑制剂[3]，它们在酶磷酸化修饰及其活性调控过程中显示相互拮抗的生物学效应[1].

ii. TH，或酪氨酸 3- 单加氧酶 (tyrosine 3-monooxygenase)，是 DA 等儿茶酚胺神经递质合成的限速酶，在 Ser-19、Ser-31 和 Ser-40 等磷酸化修饰位点被 ERK2 等蛋白质激酶修饰后获得酶促催化功能[1]. 在 DA 合成过程中，α-SYN 负性调控 TH 酶活力.

iii. ERK2，或 MAPKs-1，是蛋白质磷酸化三级酶促级联反应第三级酶，在细胞膜穴样内陷小凹协同小窝蛋白质 (caveolin-1，Cav-1)共同组装信号分子[4]. ERK2 在 N 端 68～133 氨基酸序列形成蛋白质结合结构域，借此与 α-SYN 发生作用[5]. α-SYN 除直接地抑制 ERK 酶活力之外，尚通过调控 Cav-1 表达量间接地抑制 ERK 酶活力[4].

iv. MAPKs 异构体 p38 (P38)，或 MAPKs-11 或 MAPKs-12 或 MAPKs-13 或 MAPKs-14，在 N 端 68～134 氨基酸序列形成蛋白质结合结构域，借此与 α-SYN 发生作用[5]. P38 与 ERK2 互为 MAPKs 异构体.

v. DAT，或 DA 活化转运体(DA active transporter)，是囊泡单胺转运体(vesicle monoamine transporters，MATs)或突触前质膜单胺转运体 (presynaptic plasma membrane monoamine transporters，MATs)异构体，在 C 末端氨基酸序列(具体范围不清楚)形成蛋白质结合结构域，借此与 α-SYN 的 NAC 发生作用[6]. α-SYN 作为 DAT 抑制剂参与 DA 释放和再摄取[6].

vi. VMAT2，或突触囊泡胺转运体(synaptic vesicle amine transporter)，是 MATs 异构体. VMAT2 与 α-SYN 的作用方式类似于 DAT 与 α-SYN 的作用方式，但是 VMAT2 受 α-SYN 的影响程度弱于 DAT 受 α-SYN 的影响程度[6]. α-SYN 作为 VMAT2 抑制剂参与 DA 贮存.

vii. Aβ 是 I- 型跨膜蛋白质淀粉样前体蛋白质(beta amyloid precursor protein，APP)连续经过 β- 分泌酶和 γ- 分泌酶酶解后形成的肽片段[7]. α-SYN 通过 NAC 与 Aβ 形成分子间交联物[8]. 此外，α-SYN 在 N 端 1～56 氨基酸序列(特别是 N 端 25～35 氨基酸序列)形成蛋白质结合结构域，借此与 Aβ 发生作用.

viii. α-Syn 亲和蛋白质 -1 (synphilin-1，Sph1)，或 α-SYN 相互作用蛋白质(α-SYN interacting protein)，是锚定于突触前神经递质囊泡膜的突触前蛋白质. Sph1 含有 6 个锚蛋白样重复基序(ankyin-like repeats mortifs)和 1 个螺旋化螺旋结构域(coiled coil domain)，前者是神经递质囊泡膜的锚定区段，后者是蛋白质结合结构域[9]. α-SYN 在 N 端 1～39 氨基酸序列形成蛋白质结合结构域，借此与 Sph1 发生作用[10].

ix. MAP-1B 是由一个高分子量亚基(称为重链)和几个低分子量亚基(称为轻链)组成的复合蛋白质[11]. MAP-1B 重链和任一轻链均具有蛋白质结合结构域，借此与 α-SYN 的 C 端 109～140 氨基酸序列发生作用[11].

x. 微管相关蛋白质 -τ(MAP tau，Tau)是 MAP-2 基因转录物和(或)Tau 基因转录物被剪切之后产生的选择性异构体[12]. Tau 在 C 末端氨基酸序列(具体范围不清楚)形成蛋白质结合结构域，借此与 α-SYN 的 C 端 87～140 氨基酸序列发生作用[12].

xi. α-Tub 是微管蛋白质异二聚体亚基 -1，在 C 末端 10 个氨基酸序列形成蛋白质结合结构域[13-14]，借此与 α-SYN 的 C 端 60～100 氨基酸序列发生作用[15].

xii. β-Tub 是微管蛋白质异二聚体亚基 -2. 在 C 末端 18 个氨基酸序列形成蛋白质结合结构域[13-14]，借此与 α-SYN 的 C 端 60～100 氨基酸序列发生作用[15].

xiii. Ub，或热休克蛋白质 -8(8-kDa heat shock protein)，是介导 Ub 依赖性蛋白质与蛋白质相互作用的信号分子[16]. 蛋白质泛素化依次通过硫酯化反应(泛素化反应第一步)、转硫酯化反应(泛素化反应第二步)和非酶促缩聚反应(泛素化反应第三步)将 Ub 和 α-SYN 缩合为 Ub-α-SYN 加合物，Ub 与 α-SYN 之间最终以异肽键相连 接[17].

xiv. Ub 结合蛋白质 p62(Ub binding protein p62，P62)，或隔离体蛋白质 -1(sequestosome 1)或蛋白质毒性应激反应蛋白质(proteotoxic stress response protein)，是 Ub 结构域蛋白质(Ub domain proteins, UBPs)异构体[18]. P62 在 C 末端氨基酸序列(具体范围不清楚)形成 Ub 相关结构域，借此与 α-SYN 发生作用[19]. UBPs 作为 Ub 链靶导向因子(Ub chain-targeting factor)或 α-SYN 转运受体(cargo receptor)将 α-SYN 拖入蛋白酶体或溶酶体[18].

xv. Uch-L1，或神经元去泛素化酶 (neuronal

deubiquitinating enzyme)，是 Ub 羧基端水解酶异构体[20]. UchL1 将多泛素化的 Ub-α-SYN 加合物拆分为 α-SYN 和 Ub 链，并且进一步将 Ub 链拆分为 Ub 单体[21-22]. 另一方面，UchL1 同二聚体具有 E2 依赖性 Ub- 靶蛋白质连接酶 E3 活性，将单、双泛素化的 Ub-α-SYN 加合物分别聚合为含有不同 Ub 单位的 Ub-α-SYN 加合物[23].

xvi. Parkin，或 RING-between-RING 连接酶 E3 异构体，在 N 端氨基酸序列形成 Ub 样结构域(Ub-like domain)并且借此非共价结合于靶蛋白质，在 C 端氨基酸序列(具体范围不清楚)形成蛋白质结合结构域并且借此非共价结合于 Ub-E2 偶联物，通过非酶促缩聚反应将 Ub 从 E2 转移至 E3 并且进一步形成 Ub- 靶蛋白质加合物[24].O- 糖基化的 α-SYN 是 Parkin 蛋白质底物之一[25].

xvii. G 蛋白质偶联受体激酶 -5 (G-protein coupled receptorkinase 5，GRK5)是 GRK 异构体. 在 GRK 异构体中，GRK5 与 α-SYN 之间存在最适酶 - 底物构效关系[26]. 磷酸化的 α-SYN 成为磷脂酶 D2 抑制剂[26-27].

xviii. E1A 结合蛋白质 p300(E1A binding protein p300，P300)是乙酰基转移酶异构体，具有多个蛋白质相互作用结构域，借此与蛋白质发生作用. P300 可能通过与 α-SYN 发生作用负性调控 P53 蛋白质的表达量[28].

xix. agrin 是一种硫酸乙酰肝素蛋白质聚糖，可能通过氨基聚糖侧链与 α-SYN 发生作用[29].

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附录 4

1 LBs 的已知蛋白统计于表 1

Table 1 A List of Previously Reported Protein Components in LBs in Immunohistochemistry Study

表 1 LBs 的已知蛋白质统计表

No. Protein name, classification, and references
i Neurotransmitter metabolism
1. 14-3-3 protein epsilon (14-3-3 ε) #[1]
2. Choline acetyltransferase (ChAT) [2]
3. Chromogranin A (CgA) [3]
4. Dopamine transporter (DAT) #[4-5]
5. Synaptophysin (Syn) [3]
6. Synaptotagmin XI (Syt11) [6]
7. Tyrosine hydroxylase (TH) #[7]
8. Vesicular monoamine transporter 2 (VMAT2) #[8]
ii Antioxidant defense system
9. Cu/Zn superoxide dismutase (C,Z-SOD) [9]
10. Oncogene DJ-1 protein (DJ-1) § [10]
11. Mn superoxide dismutase (Mn-SOD) [9]
12. Nonselenium glutathione peroxidase (NSGP) [11]
iii Local immune reaction in brain
13. Complement component 3d (C3d) [12]
14. Complement component 4d (C4d) [12]
15. Complement protein component 7 (C7) [12]
16. Complement protein component 9 (C9) [12]
17. Immunoglobulin G (IgG) [13]
18. alpha Internexin (INA), or 66-kDa neurofilament protein (NF66) [14]
19. alpha Tubulin (α-Tub) #[15]
20. beta Tubulin (β-Tub) #[15]
21. TDP caspase cleavage product (TDPccp) [16]
22. gamma-Tubulin (γ-Tub) [17]
23. gamma-Aminobutyric acid type A receptor-associated protein (GABARAP) [18]
24. Gelsolin (GSN), or Gelsolin-related amyloid protein, Finnish type [19]
25. Histone deacetylase 6 (HDAC6) [20]
26. Microtubulin-associated protein-1B (MAP-1B) #[21]
27. Microtubulin-associated protein -1C (MAP-1C), or Dynein [22-23]
28. Microtubule-associated protein-l light 3 (MAP-LC3, or LC3) [18]
29. Microtubulin-associated protein-2 (MAP-2) [24]
30. Microtubulin-associated protein-5 (MAP-5) [25]
31. Microtubule-associated protein tau (Tau, or MAPT) #[26]
32. Neurofilament heay polypeptide (NF-H) [27]
33. Neurofilament light polypeptide (NF-L) [27]
34. Neurofilament medium polypeptide (NF-M) [27]
35. Pericentrin (Pent) [17]
36. Septin 4 (Sept4) [28]
37. Tropomyosine (Tm) [29]

Continued

No. Protein name, classification, and references
iv Cytoskeleton network
38. Tubulin polymerization promoting protein (TPPP, or P25) [30]
v Mitochondrial aerobic respiration and mitochondrial pathway of apoptosis
39. Cytochrome C (Cyt C) [31]
40. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [32]
41. High temperature requirement protein A2 (HtrA2) [33]
42. PTEN-induced kinase 1 (PINK1) § [34]
vi Ubiquitin-dependent and-independent proteasome and lysosome degradation pathways
43. beta Transducin repeats containing protein (β-TrCP) [35]
44. Cullin-1 (Cul-1) [36]
45. Dorfin [37]
46. Liposome associate proteins (LAPs) [38-39]
47. Lysosomal-associated membrane protein 2A (LAMP2A) [38-39]
48. Glucocerebrosidase (GCD) [40]
49. Neighbor of Brest cancer 1 gene protein (NBR1) [41]
50. Neural precursor cell-expressed, developmentally down-regulated protein (NEDD8) [42]
51. NEDD8 ultimate buster (NUB1) [43]
52. Multicatalytic proteinase (MCP) [44-46]
53. Proteasome subunit beta5 (PSMB5) [47]
54. Proteasome 26S subunit ATPase2 (PSMC2) [47]
55. Proteasome 26S subunit ATPase5 (PSMC5) [47]
56. Proteasome 26S subunit ATPase6 (PSMC6) [47]
57. Proteasome 26S subunit non-ATPase11 (PSMD11) [47]
58. Proteasome 26S subunit non-ATPase13 (PSMD13) [47]
59. Regulator of Cullin-1 (ROC1) [48]
60. Seven in absentia homologue1 (SIAH1) [49]
61. Sequestosome 1 (SQSTM1), or Ubiquitin-binding protein p62 (P62) #[50]
62. Small ubiquitin-like modifier1 (SUMO1) [51]
63. Ubiquitin (Ub) #[50]
64. Ubiquitin C-terminal hydrolase L1 (Uch-L1) #§ [52]
65. Ubiquitin activating enzyme E1 (Ube1) [17, 53]
66. Ubiquitin conjugating enzyme E2 H7 isoform (UbcH7) [17]
67. E2-dependent ubiquitin-protein ligase E3 parkin isoform(Parkin) #§ [54]
68. Tripartite motif-containing protein 9 (TRIM9) [55]
69. Tumor necrosis factor-receptor associated factor 6 (TRAF6) [56]
vii Protein folding
70. 14-3-3 protein zeta (14-3-3 ζ) [57]
71. alpha Synuclein (α-SYN) § [58]
72. C terminus of 70-kDa heat shock protein-interacting protein (CHIP) [59]

Continued

No.	Protein name, classification, and references
	73. DnaJ subfamily B, member 6 (DnaJB6), or HSP40 homologue [60]
	74. 22-kDa heat shock protein (HSP22), or alpha B-Crystallin (αB) [61]
	75. 27-kDa heat shock protein (HSP27) [62]
	76. 32-kDa heat shock protein (HSP32), or Heme oxygenase-1 (HO1) [63]
	77. 40-kDa heat shock protein (HSP40) [64-65]
	78. 60-kDa heat shock protein (HSP60) [64-65]
	79. 70-kDa heat shock protein (HSP70) [64-65]
	80. 90-kDa heat shock protein (HSP90) [64-65]
	81. 110-kDa heat shock protein (HSP110) [64-65]
	82. 70-kDa heat shock cognate (HSC70) [66]
	83. Synphilin-1 (Sph1) # [58]
	84. T-complex polypeptide 1 beta subunit (TCP-1β) [67]
	85. Torsin A [65]
	86. Valosin-containing protein (VCP) [68]
viii	**Cellular signaling pathways**
	87. Agrin #[69]
	88. Basic fibroblast growth factor (bFGF, or FGF-2, or FGF-β) [70]
	89. Calbindin D28K (Ca-28K) [71]
	90. Calcium/calmodulin-dependent protein kinase 2 (CaMK-II) [72]
	91. Casein kinase II (CKII β) [73]
	92. Clusterin (CLU), or Apolipoprotein J (ApoJ) [74]
	93. Chondroitin sulfate proteoglycans (CSPGs) [75]
	94. (phosphorelated) Extracellular signal-regulated kinase (pERK) [76]
	95.E1A binding protein p300 (P300, or EP300) #[77]
	96. Forkhead box subgroup O transcription factor 3A (FOXO3A) [78]
	97. G-protein-coupled receptor kinase 5 (GRK5) #[79]
	98. Glycogen synthase kinase-3beta (GSK-3β) [80]
	99. Hippocalcin (HPCA) [81]
	100. Histone deacetylase 4 (HDAC4) [82]
	101. Leucine-rich repeat kinase 2 (LRRK-2) § [83]
	102. MAPKs extracellular signal-regulated kinase 2 isoform (ERK-2) #[84]
	103. MAPKs p38 isoform (P38) #[76]
	104. Mixed lineage kinase 2 (MLK2) [81]
	105. Myxovirus resistence gene A (MxA), or Myxovirus resistence protein 1 [85]
	106. Nuclear factor κB (NFκB) [86]
	107. Parkin-associated endothelin receptor-like receptor (pael-R, or GRP37) [87]
	108. Phosphorylated nuclear factor IkBα (pIkBα) [48]
	109. Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (Pin1) [88]
	110. Phospholipase C delta (PLC-σ) [89]
	111. Tissue transglutaminase (tTGase, or TG2) [90]
ix	**Cell cycle machinery**
	112. Cyclin B (Cln B) [91]
	113. Cyclin-dependent kinase 5 (cdk5) [92]
	114. Cyclin-dependent kinase5, regulatory subunit 1 (CDK5R1, or P35nck5α, or P35) [92]
	113. Cyclin-dependent kinase 5 (cdk5) [92]
	114. Cyclin-dependent kinase5, regulatory subunit 1 (CDK5R1, or P35nck5α, or P35) [92]
	115. Retinoblastoma protein (Rb) [93]

Continued

No.	Protein name, classification, and references
x	**Others**
	116. Alzheimer's beta amyloid peptide (Aβ, or BAP) #[94]
	117. beta Amyloid precursor protein (APP, or BAPP) [95]

MAPKs: mitogen-activated protein kinases; TDP: 43 kDa transactivation response DNA-binding protein; PTEN: phosphatase and tensin homolog. #: α-SYN associated proteins co-localized with α-SYN in LBs. §: PD related proteins, or PD linked gene products.

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2 蛋白质功能群组的生物信息学数据分析

i. DA 合成、贮存、释放和再摄取是 DA 代谢的主要环节[1]. DA 代谢异常是 PD 病理机制的一个促进因素. 在涉及神经递质代谢的 6 个 LBs 的已知蛋白质中，14-3-3ε 和 TH 参与 DA 合成[1]；Syn、Syt11 和 VMAT2 参与 DA 贮存[1-2]；DAT 参与 DA 释放和再摄取[1]. 在这组蛋白质功能群组中，另外 2 个 LBs 的已知蛋白质分别是 CgA 和 ChAT，前者参与去甲肾上腺素合成而后者参与乙酰胆碱合成[3-4].

ii. 超氧化物歧化酶(superoxide dismutase, SOD)和谷胱甘肽过氧化物酶(glutathione peroxidase，GPx)是细胞内抗氧化防御体系的主要抗氧化酶，前者在氧化应激反应中暂时将超氧化物转化为氢 过氧化物，后者最终将氢过氧化物脱毒(即，转化为二氧化碳和 水)[5]. 细胞内抗氧化防御体系衰竭是 PD 病理机制的一个促进因素. 涉及细胞内抗氧化防御体系的 3 个 LBs 的已知蛋白质包括 2 个 SOD 和 1 个 GPx，前者是 C,Z-SOD 和 Mn-SOD，后者是 NSGP. 在这组蛋白质功能群组中，另外 1 个 LBs 的已知蛋白质是抗氧化酶 DJ-1，它实质是一种非典型性过氧化物酶，具有过氧化物还原酶样活力[6].

iii. 脑内局部免疫反应的一个主要途径是补体级联反应[7]. 脑内局部免疫反应异常导致的慢性神经性炎症是 PD 病理机制的一个促进因素. 涉及脑内局部免疫反应的 4 个 LBs 的已知蛋白质分别是补体蛋白质 C3d、C4d、C7 和 C9. 在这组蛋白质功能群组中，另外 1 个 LBs 的已知蛋白质是免疫球蛋白质 IgG，它是 B-免疫细胞在特异性系统免疫反应中产生的主要抗体.

iv. 细胞骨架网状结构具有多种生理功能. 然而，就狭义的细胞骨架而言，细胞骨架蛋白质通常以微管形式参与细胞内物质输送，以中间丝形式参与细胞形状维持，以及以微丝形式参与细胞形体改变和细胞位置迁移[8]. 在 PD 研究已近百年或 LBs 研究已逾百年的发展动态中，细胞骨架网状结构塌陷是首次反映 PD 病理机制的一个主要病理事

件[9]. 涉及细胞骨架网状结构的 17 个 LBs 的已知蛋白质分别属于除微丝蛋白质和中间丝相关蛋白质之外的其它四类细胞骨架蛋白质: GSN 和 Tm 属于微丝结合蛋白质[10]; INA、NF-H、NF-L 和 NF-M 属于中间丝蛋白蛋(或神经丝蛋白质)[11]; HDAC6、MAP-1B、MAP-1C(或 dynein)、MAP-2、MAP-5、MAP-LC3、Tau 和 TPPP 属于微管相关蛋白质[12-14]; 以及 α-Tub、β-Tub 和 γ-Tub 属于微管蛋白质. 在这组蛋白质功能群组中，另外 3 个 LBs 的已知蛋白质分别是 MAP-LC3 同源蛋白质 GABARAP、微管组织中心结构蛋白质 Pent 和细胞骨架结合蛋白质 Sept4[15-17]. 它们虽然不属于狭义的细胞骨架概念范围内的蛋白质，但是通过与细胞骨架蛋白质发生作用影响细胞骨架网状结构的稳定性和动态变化. 本文认为它们应该属于广义的细胞骨架蛋白质. 目前，揭示广义的细胞骨架蛋白质的结构与功能是分子细胞生物学研究的一个热点. 最近发现的 1 个 LBs 的已知蛋白质是 TDPccp，它是细胞核因子 TDP-43 经过半胱天冬酶酶解后形成的 C 末端产物(分子量为 25 kDa)[18]. 鉴于 TDP-43 具有调控微丝蛋白质基因转录和微丝蛋白质基因转录物选择性剪切的功能，本文认为 TDPccp 也应该属于广义的细胞骨架蛋白质.

v. 有氧能量代谢和细胞凋亡是线粒体的两个基本功能[19]. 线粒体功能障碍是 PD 病理机制的一个方面. 在涉及线粒体有氧能量代谢和细胞凋亡线粒体通路的 4 个 LBs 的已知蛋白质中，Cyt C 既是呼吸链电子传递复合体 -I 的组成型蛋白质，又是线粒体凋亡体(apoptosome)的激活蛋白质组分[20-21]; GAPDH 既是线粒体外膜膜 表面的糖酵解酶(glycolytic enzyme)，又是线粒体内的促凋亡蛋白质[22]; HtrA2 既是线粒体内、外膜间隙的组成型蛋白质，又是线粒体内的促凋亡蛋白质[23]; PINK1 既是线粒体内膜的组成型蛋白质，又是线粒体内的抗凋亡蛋白质[24].

vi. 真核细胞内蛋白质降解的两种主要途径是 Ub 依赖性和非 Ub 依赖性蛋白酶体降解通路(特别是 26S Ub 依赖性蛋白酶体降解通路形式)和溶酶体降解通路(特别是特异性溶酶体降解通路形式或分子伴侣蛋白质介导性自噬)，前者通过蛋白酶体清除可溶解的异常蛋白质和废弃的正常蛋白质，后者通过溶酶体清除不溶解的异常蛋白质、废弃的正常蛋白质和功能缺失的细胞器[25]. 在 26S Ub 依赖性蛋白酶体降解通路形式中，与蛋白质底物共价结合的 Ub 是蛋白酶体识别蛋白质底物的信号肽[26-27]; 在特异性溶酶体降解通路形式中，溶酶体通过膜表面 LAMP-2A 受体吞噬蛋白质底物[28]. 尽管蛋白酶体(和溶酶体)与蛋白质底物之间的蛋白质相互作用机制不完全清楚，但是 UBPs 异构体通过非共价结合于蛋白质底物(实质是通过分子间作用力形成受体 - 配体复合物)的方式促进蛋白质底物进入蛋白酶体(和溶酶体)[29]; 尽管溶酶体(和蛋白酶体)分解蛋白质底物的酶促反应机制不完全清楚，但是 ULMs 异构体通过共价结合于蛋白质底物(实质是通过异肽键形成蛋白质分子间交联物)的方式影响溶酶体(和蛋白酶体)酶活性[29-30]. 蛋白酶体功能抑制(和溶酶体功能障碍)是 PD 病理机制的另外一个方面. 涉及蛋白酶体降解通路的 18 个 LBs 的已知蛋白质是 26S Ub 依赖性蛋白酶体降解通路形式的组成型蛋白质: Ub 是泛素化酶的一个底物(另外一个底物是靶蛋白质); Ube1、Ub 结合酶 E2 异构体 UbcH7 和 8 个 Ub- 靶蛋白质连接酶 E3 异构体(包括 β-TrCP、Cul-1、Dorfin、Parkin、ROC1、SIAH1、TRAF6 或 TRIM9)分别是催化泛素化反应的激酶、载体蛋白质和接头蛋白质; PSMB5、PSMC2、PSMC5、PSMC6、PSMD11 和 PSMD13 是 26S 蛋白酶体亚基; Uch-L1 是 Ub- 蛋白酶体系统的 Ub 再生因子. 涉及溶酶体降解通路的 2 个 LBs 的已知蛋白质是特异性溶酶体降解通路形式的组成型蛋白质，即，LAMP-2A 和 GCD. 此外，在这个蛋白质功能群组中，NBR1、NUB1 和 P62 互为 UBPs 异构体，SUMO1 和 NEDD8 互为 ULMs 异构体.

vii. 蛋白质折叠是后基因组时代蛋白质代谢的一个基本内容(另外两个基本内容是蛋白质化学修饰和蛋白质分解)[31]. 热休克蛋白质家族(heat shock proteins，HSPs)或分子伴侣蛋白质家族(主要家族成员来自 HSPs)、分子伴侣素家族、共伴侣蛋白质以及分子伴侣样蛋白质在蛋白质代谢过程中扮演主要角色[31]. 蛋白质错误折叠是形成 PD 病理机制的第三个方面. 涉及蛋白质折叠的 8 个 LBs 的已知蛋白质是 HSPs 成员. 其中，HSP22、HSP27 和 HSP32 属于低分子量 HSPs，而 HSP60、HSP70、HSC70、HSP90 和 HSP110 属于高分子量 HSPs. 此外，在这组蛋白质功能群组中，TCP-1β 是 1 个分子伴侣素家族成员，CHIP、DnaJB6、HSP40(DnaJB6 的同系物)和 Sph1 是 4 个共伴侣蛋白质，以及 14-3-3ζ、α-SYN、Torsin A 和 VCP 是 4 个分子伴侣样蛋白质[32-33].

viii. MAPKs 信号转导通路是细胞信号依次通过下游蛋白质组件(包括刺激信号分子或配体蛋白质、受体蛋白质以及调节蛋白质)、中游蛋白质组件(包括特异性丝氨酸 / 苏氨酸蛋白质激酶或磷酸化三级酶促级联反应激酶及其调节蛋白质)以及上游蛋白质组件(包括非特异性丝氨酸 / 苏氨酸蛋白质激酶和转录因子)形成的细胞信号转导过程及其调控机制. 它的三条分支通路形式主要是 ERK1(或 P44)/ERK2(或 P42)-MAPKs、JNK-MAPKs 和 p38-MAPKs; 它的生理效应主要是对蛋白质进行化学修饰. MAPKs 信号转导通路异常是反映 PD 病理机制三个方面彼此重叠的共同点和相互联系的交叉点[34]. 在涉及 MAPKs 信号转导通路的 23 个 LBs 的已知蛋白质中，磷脂酶 PLC-σ、转谷氨酰胺酶 TG2、钙离子结合蛋白质 HPCA、细胞生长因子 bFGF、基质糖蛋白质 CLU 以及 2 个基质蛋白质聚糖(包括 CSPGs 和 Agrin)属于下游蛋白质组件[35-42]，去乙酰基酶 HDAC4、肽基－脯氨酰基顺反异构酶 Pin1、2 个蛋白质激酶(包括 MLK2 和 LRRK2)以及 3 个 MAPKs 异构体(包括 ERK2、pERK 和 p38)属于中游蛋白质组件[43-46]; GTP 酶 MxA、2 个钙离子结合蛋白质(包括 Ca-28K 和 CaMK-II)、3 个转录因子(包括 NFκB、pIκB 和 FOXO3A)、2 个蛋白质激酶(包括 CKII β 和 GSK-3βP)以及乙酰基转移酶 P300 属于上游蛋白质组件[47-48-54]. 此外，在这组蛋白质功能群组中，G- 蛋白质偶联受体异构

体 pael-R 及其蛋白质激酶 GRK5 属于 G- 蛋白质偶联受体信号转导通路的下游蛋白质组件[55].

ix.细胞周期机器(cell cycle machinery)的主要构成元件是细胞周期素 cyclins(Cln)、细胞周期素依赖性蛋白质激酶(cyclins-dependent kinases，CDKs)和 CDKs 抑制因子(CDKs inhibitors，INKs)[56]. 在细胞周期机器连续运转过程中，Cln 和 CDKs 以蛋白质复合体形式正性调控微管发生的成核过程和微管消长的动态变化，这意味着细胞进入下一个细胞周期时相[57]；在细胞周期机器暂时叫停过程中，INKs 通过抑制 CDKs 酶活力等机制负性调控微管发生的成核过程和微管消长的动态变化，这意味着细胞退出下一个细胞周期时相[58]. 细胞周期阻滞(cell cycle arrest)开创了认识多巴胺能神经元以凋亡形式丧失等 PD 病理机制的新思维[59-60]. 涉及细胞周期机器的 4 个 LBs 的已知蛋白质分别是细胞周期 M 时相决定因子 cyclin B、非典型性 CDK 异构体 CDK5 及其酶活性调节亚基 P35，以及细胞周期 G1～S 时相检测点调节蛋白质 Rb[61-62].

x．鉴于 Aβ 及其前体物 APP 是 AD 病人淀粉样斑的特征蛋白质成分，Wakabayaashi 等(2013)认为它们可能被动地参与了 LBs 的形成过程，不大可能反映 PD 病理机制[59].

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附录 5

1 蛋白质组学鉴定数据的生物信息学数据分析

i. 首先，在 PD 病人 LC 脑区的蛋白质组学分析中，获得鉴定的候选蛋白质为临床前期 PD 提供了 LBs 的蛋白质构成信息[1]. 分析结果显示，鉴定数据包括 NFs 和 TH 等不少于 2 个 LBs 的已知蛋白质和 84 个 LBs 的候选蛋白质. 其次，在 PD 病人 SNpc 脑区的蛋白质组学分析中，获得鉴定的候选蛋白质为临床期 PD 提供了 LBs 的蛋白质构成信息[2-4]. 鉴于 LBs 在多巴胺能神经元丧失之后被暂时释放于细胞外间质[5]，一种实验设计是以 PD 病人 SNpc 脑区为分析样品进行蛋白质组学分析[2-4]；鉴于 LBs 富集了功能缺失的线粒体和(或)线粒体蛋白质[6]，另外一种实验设计是以 PD 病人 SNpc 脑区的线粒体分级分离制备物为分析样品进行蛋白质组学分析[7]. 上述分析结果显示，鉴定数据包括 α-Tub、α-Syn、β-Tub、CaMK-II、HSP60、HSP70、INA、Mn-SOD、NF-L 和 NF-M 等不少于 10 个 LBs 的已知蛋白质和 124 个 LBs 的候选蛋白质. 此外，鉴于脑多巴胺能神经元除了密集地分布于 SNpc 之外尚散在分布于同属于中脑的相邻侧腹核(ventral tegmental area，VTA)脑区，还有一种实验设计是比较同一个 PD 病人 SNpc 脑区与 VTA 脑区(作为对照)之间的蛋白质差异表达谱[8]. 然而，考虑到 VTA 脑区不是 PD 病变所涉及的主要病变部位，而且自 VTA 发起的中脑 - 皮层神经通路和中脑 - 边缘系统神经通路(相对于自 SNpc 发起的黑质 - 纹状体神经通路)在 PD 发生、发展过程中不担任主要角色，本文未将这部分鉴定数据收录为 LBs 的蛋白质组学鉴定数据. 再次，在 PD 病人 PFC 脑区的蛋白质组学分析中，获得鉴定的候选蛋白质为临床后期 PD 提供了 LBs 的蛋白质构成信息[9]. 分析结果显示，鉴定数据包括 α-SYN、CLU、Cyt C、HSP70、SOD 和 Syn 等不少于 6 个 LBs 的已知蛋白质和 120 个 LBs 的候选蛋白质.

ii. 上述 PD 病人脑区的蛋白质组学分析的主要研究缺陷是复杂、不均一的脑组织导致一部分鉴定数据为假阳性，即，与 LBs 无任何关系的候选蛋白质获得鉴定[10]. 然而，蛋白质组学分析借助于激光捕获显微切割(laser capture microdissection，LCM)技术从复杂、不均一样品中获取同质性分析样品的技术优势，能够显著地提高分析样品与鉴定数据之间的相关性[10-12]. Leverenz 等(2007)先利用这项技术从 DLB 病人 TC 脑区冰冻切片中分离了靶细胞，后对靶细胞分离物进行了蛋白质组学分析[12]. DLB 是一种既表现 PD 一系列神经病学症状又表现诸多非运动障碍症状的复杂系统性疾病；约 30%PD 病人发展为 DLB[10]；DLB 涉及的脑区除了 LC、SNpc 和 PFC 脑区之外尚包括 TC 等脑区[13]. 分析结果显示，鉴定数据包括 14-3-3ε、αB、α-SYN、α-Tub、β-Tub、APP、CaMK-II、dynein、GAPDH、HSC70、INA、MAP-1B、MAP-2、NF-M、Tau、Ube1 和 Uch-L1 等不少于 17 个 LBs 的已知蛋白质和 108 个 LBs 的候选蛋白质. 显然，在细胞水平获得的鉴定数据中(相对在组织水平获得的鉴定数据中)，LBs 的已知蛋白质的数量百分比明显地增多，而 LBs 的候选蛋白质的数量百分比明显地减少.

iii. 鉴于以生物样品为研究对象的蛋白组学分析要求每个分析样品至少含有约 1～10 万个靶细胞[10]，上述 LCM 技术应用于蛋白质组学分析的主要技术瓶颈是耗时、繁重的分离靶细胞操作不能保证在操作期间一次性地或连续地从分析样品中收集足够数量的靶细胞. 然而，蛋白质组学分析借助于生物化学分级分离技术从组织匀浆中快速富集亚细胞结构物的技术优势，大大地缩短了分析样品制备与蛋白质组学技术操作之间的时间间隔[10]. Xia 等(2008)先利用这项技术从 DVLB 病人 TC 脑区的组织匀浆中富集了 LBs，后对 LBs 富集物进行了蛋白质组学分析[14]. DVLB 是一种既具有 AD 一系列精神病学症状又表现诸多 DLB 非运动障碍症状的复杂系统性疾病；约 25%AD 病人发展为 DVLB[15]；DVLB 涉及的主要脑区是 PFC 和 TC，等[14]. 分析结果显示，鉴定数据包括 α-SYN、Cul-1、dynein、GAPDH、GSN、HSP70、Ub、Ube1 和 Uch-L1 等不少于 9 个 LBs 的已知蛋白质和 29 个 LBs 的候选蛋白质. 显然，在亚细胞水平获得的鉴定数据中(相对在细胞水平获得的鉴定数据中)，LBs 的已知蛋白质的数量百分比明显地增多，而 LBs 的候选蛋白质的数量百分比明显地减少.

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2 蛋白质组学鉴定数据库见表 2

表 2 蛋白质组学鉴定数据库(源于 LBs 的候选蛋白质)统计表

Table 2 A list of Large Scale Proteomics Databases (as Protein Candidate Resource for LBs)

Analytic sample, protein name and reference
i Raw tissue samples of brain regions in patients[1−6]
Locus coeruleus[1]
1. (highly similar to) 4-Aminobutyrate aminotransferase
2. 7-dehydrocholesterol reductase
3. 28S ribosomal protein S6
4. 28S ribosomal protein S34
5. alpha Enolase
6. gamma Aminobutyric acid receptor-associated protein
7. gamma Butyrobetaine dioxygenase
8. gamma Synuclein
9. A kinase (PRKA) anchor protein 12
10. Acyl-coenzyme A thioesterase 9
11. Adenylate kinase isoenzyme 1
12. Aldehyde dehydrogenase X
13. Ankyrin-3
14. Arginine-rich, mutated in early stage tumors
15. Brefeldin A-inhibited guanine nucleotide-exchange protein 3
16. cAMP-dependent protein kinase type II alpha regulatory subunit
17. Carbonic anhydrase 2

Continued

Analytic sample, protein name and reference
18. Carbonyl reductase 3
19. Catenin alpha 1
20. c-Myc-responsive protein Rcl
21. Coatomer subunit beta
22. Cold shock domain-containing protein E1
23. Complement component 4B
24. Creatine kinase B
25. Cytosol aminopeptidase
26. Dopamine beta hydroxyls
27. ELAV-like protein 4
28. (highly similar to) Elongation factor 1 subunit gamma
29. Endoplasmic reticulum aminopeptidase 1
30. Ferritin heavy chain
31. FK506-binding protein 4
32. Flotillin-1
33. Gelectin-3
34. GrpE protein homolog 1
35. Hemopexin
36. HESB like domain containing 2
37. HLA class I histocompatibility antigen, B-59 alpha chain
38. HLA class II histocompatibility antigen, DRB1-1 beta chain
39. Inositol monophosphatase
40. Isoleucyl-tRNA synthetase
41. Kinectin
42. Leucine-rich PPR motif-containing protein
43. LYR motif-containing protein 4
44. Mammalian ependymin-related protein 1
45. Methylmalonyl-CoA epimerase
46. Neuroglobin
47. NHL repeat-containing protein 2
48. Nitric oxide synthase
49. Phenylalanyl-tRNA synthetase αlpha chain
50. Phenylalanyl-tRNA synthetase beta chain
51. Phospholipase D3
52. Podocalyxin-like protein 1 precursor
53. Probable E3 ubiquitin-protein ligase HERC1
54. Prolow-density lipoprotein receptor-related protein 1
55. Protein-arginine deiminase type 2
56. Protein FAM127A
57. Protein tweety homolog 1
58. Putative uncharacterized protein DKFZp686I04196 (Fragment)
59. Putative uncharacterized protein MRPL23
60. Pyruvate kinase isozymes M1/M2
61. Rab3 GTPase-activating protein non-catalytic subunit
62. Rap guanine nucleotide exchange factor 2
63. (highly similar to) Ras-related protein Ral-B
64. Regucalcin
65. Regulator of nonsense transcripts 1
66. Resistance to inhibitors of cholinesterase 8 homolog A69.
67. Ribophorin I

Continued

Analytic sample, protein name and reference
68. Ribosyldihydronicotinamide dehydrogenase
69. SAT2 protein
70. Serine/threonine-protein kinase Nek7
71. Seipin
72. Serine/threonine-protein kinase PAK 3
73. Small G protein signaling modulator 1
74. Synapsin-1
75. Thioredoxin domain-containing protein 4
76. Transgelin
77. Tumor protein D52
78. UDP-glucose :glycoprotein glucosyltransferase 1
79. Upregulated during skeletal muscle growth protein 5
80. Uncharacterized protein KIAA0513
81. V-crk sarcoma virus CT10 oncogene homolog
82. Versican core protein
83. Vimentin or neurofilament
84. Wolframin
Substantia nigra pars compacta[2-5]
1. 3',5'-Cyclic nucleotide phosphodiesterase 10A2
2. 6-Phosphofructokinase
3. 14-3-3 protein epsilon
4. 60 S ribosomal protein L3
5. alpha Actinin 4
6. alpha (or beta) Centractin
7. beta Adducing
8. beta Tubulin cofactor A
9. Acetolactate synthase
10. Acylphosphatase 2
11. (similar to) Adaptor-related protein complex 2 beta 1 subunit
12. Adenylate kinase 1
13. Adenylate kinase isoenzyme 1
14. Aldehyde dehydrogenase A1
15. Aldo-keto reductase family 1, member A
16. Annexin V
17. ARL-6-interacting protein-1
18. Astrocytic phosphoprotein PEA-15
19. ATP-dependent helicase DDX1
20. ATP synthase D chain
21. Brain link protein-1 precursor
22. Breast carcinoma amplified sequence 1
23. Calcium/calmodulin-dependent 3,5-cyclic nucleotide phosphodiesterase 1B
24. Carbonyl reductase 1
25. Cellular retinol-binding protein 1
26. Coactosin-like protein 1
27. Complexin 1
28. Creatine kinase
29. C-terminal binding protein 1
30. Cysteine desulfurase
31. Cytochrome C oxidase polypeptide I

Continued

Analytic sample, protein name and reference
32. Dihydropteridine reductase
33. Dihydropyrimidinase-related protein-1
34. Dynamin-like protein
35. Electron transfer flavoprotein-ubiquinone oxidoreductase
36. Elongation factor 1
37. Elongation factor 2
38. Endoribonuclease dicer
39. Excitatory amino acid transporter 1
40. Eukaryotic initiation factor 5A
41. Fatty acid-binding protein
42. Ferritin H
43. Ferrochelatase
44. Galectin 1
45. Gelsolin precursor
46. General vesicular transport factor p115
47. Glial fibrillary acidic protein
48. Glial maturation factor beta subunit
49. Glutamate decarboxylase
50. Glutamate dehydrogenase 1
51. Glutathione S-transferase M2
52. Glutathione-S-transferase M3
53. Glutathione-S-transferase O1
54. Glutathione-S-transferase P1
55. Glutathione transferase omega 1
56. (similar to) Glycolipid transfer protein
57. Guanine nucleotide-binding protein, alpha 13 subunit
58. Guanine nucleotide-binding protein Gi/Gs/Go, gamma 2 subunit
59. Guanine nucleotide-binding protein Gi/Gs/Go, gamma 4 subunit
60. Heat shock 20-kDa-like protein
61. Histidine triad nucleotide-binding protein 1
62. HSPC263
63. Ig chain C region membrane-bound segment
64. Integrin 8 precursor
65. L-type calcium channel delta subunit
66. Megakaryocyte-stimulating factor
67. Methylmalonate-semialdehyde dehydrogenase
68. Monoglyceride lipase
69. NADH-ubiquinone oxidoreductase, 42-kDa subunit
70. NADH-ubiquinone oxidoreductase, subunit B14.7
71. Neurocan core protein precursor
72. Nitrilase homolog 1
73. Nuclear transport factor 2
74. O00499 Myc box-dependent interacting protein 1
75. O14775 guanine nucleotide-binding protein subunit 5
76. O14983 sarcoplasmic reticulum calcium ATPase 1
77. OX-2 membrane glycoprotein precursor
78. P05556 integrin β-1 precursor, splice isoform beta 1D
79. P06905 myelin proteolipid protein
80. P09543 2',3'-cyclic nucleotide 3'- osphodiesterase
81. Peptidyl-prolyl cis-trans isomerase

Continued

Analytic sample, protein name and reference
82. Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1
83. Peroxiredoxin 1
84. Peroxiredoxin 2
85. PHR1 isoform 1/2
86. Placental ribonuclease inhibitor
87. Plexin-B1/SEP receptor precursor
88. Profilin I
89. Profilin II
90. Protein transport protein Sec23A
91. Protein-tyrosine phosphatase, non-receptor type 5
92. Pyruvate dehydrogenase protein X component
93. Pyruvate kinase 3
94. Q14168 MAGUK p55 subfamily member 2
95. Q8IXJ6 NAD-dependent deacetylase sirtuin 2
96. Q9UI40 sodium/potassium/calcium exchanger 2 precursor
97. Rab GDP dissociation inhibitor alpha
98. Rab GDP dissociation inhibitor beta
99. Radixin
100. Ras-related protein Rab-10
101. Ras-related protein Rab-14
102. Ras-related protein Rab-21
103. Ras-related protein Rab-3C
104. Ras-related protein Rap-1b
105. Rho-related GTP-binding protein Rho G
106.(similar to) Ribosomal protein L18 alpha
107. S-adenosyl homocysteine hydrolase 1
108. Secretogranin I precursor
109. Septin 7
110. Septin KIAA0202d
111. SH3-binding glutamic acid-rich like protein
112. SH3-containing protein SH3GLB2
113. Sodium/hydrogen exchanger 1
114. Sodium/potassium-transporting ATPase, beta 2 chain
115. Sorcin A
116. Succinate-semialdehyde dehydrogenase
117. Synaptoporin
118. Telomerase-binding protein p23
119. Transmembrane protein 10
120. Transforming protein RhoB
121. Tumor protein D53
122. Ubiquinol-cytochrome C reductase iron-sulfer subunit
123. Vesicular inhibitory amino acid transporter
124. V type ATPase A1
Prefrontal cortex[6]
1. 1-Phosphatidylinositol-4,5-bisphosphate phosphodiesterase beta 1
2. 10-formyltetrahydrofolate dehydrogenates
3. gamma Synuclein
4. Adducin 2
5. A-kinase anchor protein 12
6. Actin related protein 2/3 complex subunit 4

Continued

Analytic sample, protein name and reference
7. Amine oxidase B
8. Annexin A6
9. AP-2 complex subunit 1
10. Apolipoprotein E precursor
11. ATPase, alpha chain
12. ATPase, gamma chain
13. ATPase, epsilon chain
14. ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C1
15. ATPase, H+ transporting, mitochondrial F0 complex, subunit F2
16. ATP synthase, delta chain
17. Bassoon protein
18. Brain-specific polypeptide PEP-19
19. Breast carcinoma amplified sequence 1
20. Calcium-binding mitochondrial carrier protein Aralar 1
21. Calcium binding protein
22. Calnexin precursor
23. CDNA FLJ34946 fis, clone NT2RP7008714
24. CDNA FLJ90813 fis, clone Y79AA1000967
25. Cell division cycle 10
26. Cytochrome C oxidase polypeptide Va
27. Cytochrome C oxidase polypeptide Vb
28. Desmuslin
29. Dihydropteridine reductase
30. DPYSL3 protein
31. Drebrin 1
32. Drebrin-like protein
33. Dynactin 2
34. Enoyl-CoA hydratase
35. EPB41L1 protein
36. Epsin-1
37. Excitatory amino acid transporter 2
38. Fatty acid-binding protein
39. Fumarate hydratase
40. Glutaminase kidney isoform41. Glutamine synthetase
42. Glutathione S-transferase Mu 3
43. Glutathione transferase omega 1
44. Growth factor receptor-bound protein 2
45. GTP-binding nuclear protein Ran
46. Guanine nucleotide-binding protein alpha 13 subunit
47. Guanine nucleotide-binding proteins
48. Hepatocyte cell adhesion molecule
49. Heterogeneous nuclear ribonucleoproteins A2/B1
50. HU-K4
51. Heat shock protein beta 1
52. Huntingtin interacting protein 2
53. IGLV4-3 protein
54. Immunoglobulin superfamily member 8 precursor
55. Inositol monophosphatase
56. Isocitrate dehydrogenase
57. Kelch repeat and BTB domain-containing protein 11

Continued

Analytic sample, protein name and reference
58. Kinesin-like protein 2
59. Lambda-crystallin
60. Limbic system-associated membrane protein precursor
61. Methylglutaconyl-CoA hydratase
62. Microtubule-associated protein 6
63. Microtubule-associated protein RP/EB family member 3
64. Mitochondrial glutamate carrier 1
65. Myelin basic protein
66. NADH dehydrogenase 1beta subcomplex subunit 9
67. NADH dehydrogenase 1beta subcomplex subunit 10
68. NAD-dependent deacetylase sirtuin 2
69. NADH-ubiquinone oxidoreductase 18-kDa subunit
70. NADH-ubiquinone oxidoreductase 49-kDa subunit
71. NADH-ubiquinone oxidoreductase 75-kDa subunit
72. NAPB protein
73. Nuclear ubiquitous casein and cyclin-dependent kinases substrate
74. Neurofascin precursor
75. Neuronal protein NP25
76. Nicotinamide nucleotide transhydrogenase
77. OTTHUMP00000030828
78. Oxidation resistance protein 1
79. Paralemmin
80. Profilin 2
81. Prohibitin
82. Peroxiredoxin 6
83. Protein C2orf32
84. Protein disulfide-isomerase A6 precursor
85. Protein FAM10A5
86. Protein NDRG2
87. Protein pelota homolog
88. Pyridoxal kinase
89. Pyridoxal phosphate phosphatase
90. Pyruvate kinase isozymes M1/M2
91. Rab GDP dissociation inhibitor alpha
92. Ras-related protein Rab-2A
93. Ras-related protein Rab-11A
94. Ras-related protein Rab-6B
95. Ras-related protein Rab 7
96. Rho GDP-dissociation inhibitor 1
97. Ribonuclease inhibitor
98. Sarcoplasmic/endoplasmic reticulum calcium ATPase 2
99. Serine/threonine-protein kinase PAK 1
100. Septin 9
101. Septin 11
102. Serotransferrin precursor
103. SH3 domain binding glutamic acid-rich protein like 3
104. Sodium/potassium-transporting ATPase subunit beta 1
105. Succinate dehydrogenase flavoprotein subunit
106. Succinyl-CoA:3-ketoacid-coenzyme A transferase 1
107. Synaptosomal-associated protein 25

Continued

Analytic sample, protein name and reference
108. Tenascin-R precursor
109. Thioredoxin
110. Transforming protein Rho A precursor
111. Tripeptidyl-peptidase 1 precursor
112. Tropomodulin 2
113. Tu translation elongation factor
114. Ubiquinol-cytochrome C reductase complex core protein 2
115. Ubiquinol-cytochrome c reductase iron-sulfur subunit
116. Ubiquitin-activating enzyme E1
117. UV excision repair protein RAD23 homolog B
118. Vacuolar ATP synthase subunit B
119. Vacuolar proton translocating ATPase 116 kDa subunit
120. VGF nerve growth factor inducible precursor 162
ii A cellular separation of analyte captured from temporal cortex regions in patient [7]
1. 2',3'-cyclic-nucleotide 3'-phosphodiesterase
2. 86-kDa heat shock protein
3. 4F2 cell-surface antigen heavy chain
4. beta Globin gene from a thalassemia patient, complete cds
5. Actin, α-skeletal muscle
6. Actin, aortic smooth muscle
7. Actin, cytoplasmic 1
8. Ankyrin 2
9. Antigen KI-67
10. ATP5A1 protein
11. ATP synthase beta chain
12. Band 4.1-like protein 2
13. Band 4.1-like protein 3
14. B-cell lymphoma/leukemia 11B
15. BM88 antigen
16. Brain glycogen phosphorylase
17. Brain-specific Na-dependent inorganic phosphate cotransporter
18. Calnexin precursor
19. Carbonyl reductase 1
20. Carbonyl reductase 3
21. Clathrin coat assembly protein AP180
22. Clathrin heavy chain 1
23. Clathrin heavy chain 2
24. Claudin-11
25. Collagen alpha 1(I) chain precursor
26. Connexin 43
27. Contactin 1 precursor
28. Creatine kinase beta chain
29. Cytokeratin type II
30. Dermcidin precursor
31. Desmoglein 1 precursor
32. Desmoplakin
33. Dihydropyrimidinase-like 2
34. DJ68D18.1.2
35. DNM1 protein

Continued

Analytic sample, protein name and reference
36. Drebrin
37. Dynamin 2
38. Dynamin 3
39. Elongation factor 1 alpha 1
40. Elongation factor 1 alpha 2
41. Excitatory amino acid transporter 1
42. FSCN1 protein
43. Gelsolin precursor
44. Glucose phosphate isomerase
45. Glutathione S-transferase M3
46. GNAI2 protein
47. Guanine nucleotide-binding protein, αlpha-activating polypeptide O
48. Guanine nucleotide-binding protein gamma 2
49. Guanine nucleotide-binding protein G
50. H1 histone family, member 2
51. H2B histone family, member Q
52. HIST1H4F protein
53. Histone H2A
54. Histone H2A F/Z variant
55. HNRPA2B1 protein
56. Homo sapiens TUBB1 human beta tubulin 1, class VI
67. Hyaluronan and proteoglycan link protein 2 precursor
58. Isocitrate dehydrogenase alpha subunit
59. Isocitrate dehydrogenases
60. KIAA0607 protein
61. Laminin alpha 1 chain precursor
62. Laminin alpha 4 chain precursor
63. Mitochondrial inner membrane protein
64. Myelin basic protein
65. Myelin-oligodendrocyte glycoprotein precursor
66. Myelin proteolipid protein
67. NEFL protein
68. Neural cell adhesion molecule 1
69. N-ethylmaleimide-sensitive factor
70. Neuronal membrane glycoprotein M6-a
71. OTTHUMP00000040847
72. OTTHUMP00000062121
73. Peroxiredoxin 5
74. Plectin 1
75. Plectin 6
76. Phosphoglycerate dehydrogenase
77. Phosphoglycerate mutase 2
78. Prepro alpha 2 (I) collagen precursor
79. Protein FLJ34068
80. Protein FLJ37958
81. Ras-GTPase-activating protein binding protein 2
82. Ras-related protein Rab-1A
83. Reticulon protein 3
84. Retinoblastoma-associated factor 600
85. Rho-associated protein kinase 2
86. (similar to) RIKEN cDNA 4732495G21 gene
87. Rotatin

Continued

Analytic sample, protein name and reference
88. Secernin 1
89. Serotransferrin precursor
90. SLC25A3 protein
91. Sodium/potassium-transporting ATPase alpha 2 chain precursor
92. Sodium/potassium-transporting ATPase alpha 3 chain
93. Spectrin alpha chain, brain
94. Spectrin alpha non-erythrocytic 1
95. Synapsin 1
96. Synaptosomal-associated protein 25
97. Synaptotagmin 1
98. Syntaxin binding protein 1
99. Tenascin-R
100. Tetrodotoxin-resistant voltage-gated sodium channel
101. Titin
102. (similar to) Triosephposhate isomerase
103. Trypsin I precursor
104. TUBA6 protein
105. Ubiquitin and ribosomal protein S27a precursor
106. Vacuolar ATP synthase catalytic subunit A
107. Versican core protein precursor
108. Voltage-dependent anion channel 1
iii A sub–cellular fraction of analyte isolated from temporal cortex region in patient [8]
1.Aconitase 2
2. Adapter-related protein complex 2 alpha 1
3. Adapter-related protein complex 2 beta 1
4. ATPase, lysosomal 70 kDa, v1 alpha subunit
5. Calcium-dependent secretion activator
6. Calgranulin beta
7. Carbonyl reductase 1
8. Clathrin heavy chain 1
9. Coatomer-related protein complex alpha subunit
10. Collapsing response mediator protein HCRMP-2
11. Cytoskeleton components
12. Doublecortin-like kinase
13. Dynactin 1
14. Dynamin-like protein
15. E3 ubiquitin ligase kpc 1, ring finger protein 123
16. F-box protein 2
17. Glucose phosphate isomerase
18. Guanine nucleotide binding protein alpha
19. Imp cyclohydrolase
20. Mitogen-activated protein kinase kinase 1
21. Peroxiredoxin
22. Plectin 1
23. Proteasome 26S non-ATPase subunit 2
24. Protein disulfide isomerase-associated 3
25. Protein kinase C beta 1
26. Spectrin alpha
27. Spectrin beta
28. Vacuolar protein sorting 35
29. WD repeat-confaining protein 1

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