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由于目前国际对神经变性的本质和结局没有明确意义，更没有特异性分子标识，造成在阿尔茨海默病（AD）研究中常常用凋亡或坏死的指标评价变性．我们认为，神经变性是受独特机制有序调控的一种慢性细胞死亡，阐明相关调控机制对发现神经变性特异性分子标识、建立有效干预措施至关重要．

——王建枝

Tau 蛋白过度磷酸化机制及其在阿尔茨海默病神经元变性中的作用 *

王建枝 ** 田 青
(教育部神经系统重大疾病重点实验室，华中科技大学同济医学院病理生理系，武汉 430030)

摘要 Tau 蛋白是神经元中含量最高的微管相关蛋白，其经典生物学功能是促进微管组装和维持微管的稳定性．在阿尔茨海默病(Alzheimer's disease，AD)患者，异常过度磷酸化的 Tau 蛋白以配对螺旋丝结构形成神经原纤维缠结并在神经元内聚积．大量研究提示，Tau 蛋白异常在 AD 患者神经变性和学习记忆障碍的发生发展中起重要作用．本课题组对 Tau 蛋白异常磷酸化的机制及其对细胞的影响进行了系列研究，发现 Tau 蛋白表达和磷酸化具有调节细胞生存命运的新功能，并由此对 AD 神经细胞变性的本质提出了新见解．本文主要综述作者实验室有关 Tau 蛋白的部分研究结果．

关键词 阿尔茨海默病，Tau，磷酸化，蛋白激酶，磷酸酯酶，神经元，神经变性
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1 Tau 蛋白过度磷酸化的机制

Tau 蛋白是维系神经元骨架系统稳定的重要分子．在阿尔茨海默病(Alzheimer's disease，AD)患者，Tau 蛋白被异常过度磷酸化，并以配对螺旋丝结构形成神经原纤维缠结在细胞内聚积[1]．国外的研究报道，AD 患者脑内神经原纤维缠结的数量与其临床痴呆程度呈正相关；异常 Tau/ 缠结从内嗅皮质向海马和大脑皮层传播发展与患者临床表现吻合，是目前国际评估 AD 病程进展的金标准(即 Braak 分级)[2]；最近的研究显示，β- 淀粉样蛋白(Aβ，AD 的另一个致病分子)的毒性作用需要 Tau 蛋白介导[3]．这些研究结果提示，Tau 蛋白异常在 AD 患者神经细胞变性和学习记忆障碍的发生发展中起重要作用．

至今尚未在 AD 患者中发现 Tau 基因突变，因此，对 Tau 蛋白的研究主要集中在蛋白质翻译后修饰．在已经报道的 Tau 蛋白修饰中，对异常过度磷酸化的研究最为深入．蛋白激酶活性增高和 / 或磷酸酯酶活性降低是导致 Tau 蛋白过度磷酸化的直接原因．在众多的蛋白激酶和磷酸酯酶中，糖原合酶激酶 3β(GSK-3β)(图 1)和蛋白磷酸酯酶 2A(PP2A)(图 2)分别在 Tau 蛋白过度磷酸化中发挥重要作用[4-6]．

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** 通讯联系人.
Tel: 027-83693883, E-mail: wangjz@mails.tjmu.edu.cn
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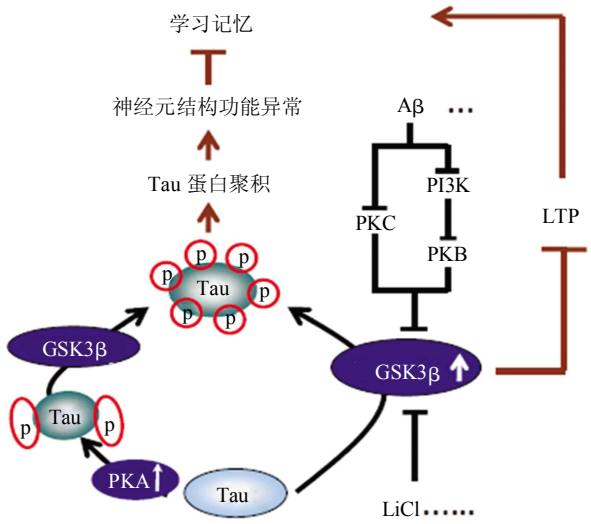

Fig. 1　Role of GSK-3β in Alzheimer-like Tau hyperphosphorylation and the upstream regulation

图 1　GSK-3β在 Tau 蛋白过度磷酸化中的作用和调节机制

Aβ 等可通过抑制 PI3K 和 PKC 途径而激活 GSK-3β 并使 Tau 蛋白发生过度磷酸化、聚积，从而导致神经元功能、结构改变，动物记忆障碍；同时抑制 PI3K 和 PKC 可持续激活 GSK-3β；Tau 蛋白经 PKA 预磷酸化后更易于被 GSK-3β 磷酸化；GSK-3β 激活可抑制 LTP 的形成．褪黑素等可下调 GSK-3β、抑制 Tau 蛋白过度磷酸化．

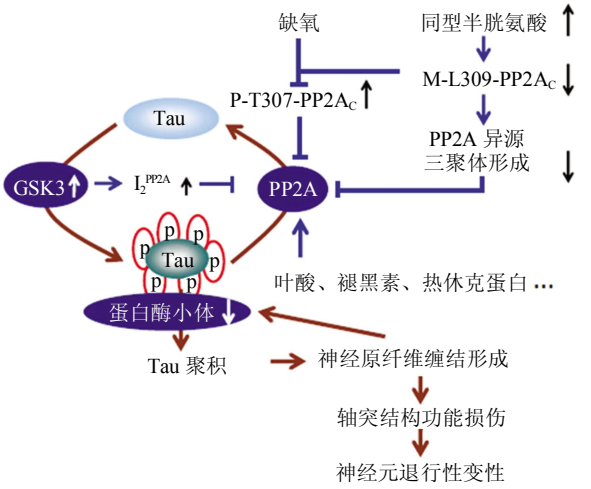

Fig. 2　Role of PP2A in dephosphorylating Tau and the regulation of the PP2A activity

图 2　PP2A 在 Tau 蛋白去磷酸化中的作用和调节机制

PP2A 使磷酸化的 Tau 蛋白去磷酸化；抑制 PP2A 使 Tau 蛋白发生过度磷酸化、聚积形成神经原纤维缠结(NFT)，神经元出现功能障碍，最终发生退行性变性；GSK-3β 激活可上调 PP2A 抑制因子 2 (I_2^{PP2A})的水平并抑制 PP2A 活性；同型半胱氨酸、急性缺氧等可通过升高 PP2A 催化亚基(PP-2Ac)磷酸化、降低其甲基化而升高 PP2A 的活性，降低 Tau 的磷酸化并改善神经元功能．

GSK-3β 可使 Tau 蛋白多个 AD 相关位点发生过度磷酸化[7]；GSK-3β 激活是过氧化亚硝酸盐、Aβ、高级糖化终末产物(AGEs)、持续光照、内质网应激、蛋白酶体功能障碍等因素诱导 Tau 蛋白发生过度磷酸化、聚积的共同机制[8-16]．Aβ 可通过抑制磷脂酰肌醇 -3 激酶(PI3K)和蛋白激酶 C(PKC)两条途径激活 GSK-3β[8]；在大鼠脑片和整体同时抑制 PI3K 和 PKC 可持续激活 GSK-3β，使 Tau 蛋白发生持续过度磷酸化和聚积、降低脑内乙酰胆碱水平、动物出现空间记忆障碍[8, 17-18]，而抑制 GSK-3β 可显著改善多种因素诱导的 Tau 过度磷酸化及动物认知障碍[19]．激活 GSK-3β 压抑长时程增强(LTP)的形成，而抑制 GSK-3β 则促进 LTP，其机制与突触前神经递质释放有关[20-21]，但 Tau 蛋白是否参与 GSK-3β 对突触功能的影响尚不清楚．

在对 Tau 进行磷酸化修饰时，GSK-3β 与蛋白激酶 A(PKA)具有正性协同作用，即 PKA 预磷酸化的 Tau 蛋白更容易被 GSK-3β 过度磷酸化，解释了短暂激活 PKA 导致 GSK-3β 位点持续过度磷酸化的机制[22-23]．海马齿状回神经元新生必须有 GSK-3β 相关的 Tau 蛋白过度磷酸化，但在脑室下区(SVZ)，虽然也可观察到磷酸化的 Tau 蛋白与新生神经元共定位，但激活 GSK-3β 并不能促进 SVZ 区的神经元新生[24-25]，提示不同脑区神经元中 Tau 蛋白的磷酸化可能发挥不同的功能．乙酰左旋肉碱、过表达脑红蛋白等可通过抑制 GSK-3 而降低 Tau 蛋白磷酸化[26-28]．此外，CDK5、PKA、MAPK、CAMK Ⅱ也参与 Tau 的过度磷酸化[29-34]；与 PKA 一样受 cAMP 调控的鸟苷酸交换因子(EPAC)，可通过调节微小 RNA 的转录而影响小鼠的学习记忆和社交活动[35]．

AD 患者脑中磷酸酯酶活性降低，因此，恢复磷酸酯酶活性可能成为一种潜在的干预策略．与磷酸酯酶 2B(PP2B)和 PP-1 相比，PP2A 使 AD 患者 Tau 蛋白去磷酸化作用活性最强[36-37]；在体外，PP2A 可使 AD 患者脑中分离的异常 Tau 蛋白多个位点去磷酸化，从而恢复其生物学功能[38]．通过抑制 PP2A 可诱导 Tau 蛋白过度磷酸化、导致轴突转运功能异常、动物学习记忆障碍[39-43]；热休克蛋白(HSP)、急性缺氧、褪黑素、叶酸 /VitB12、黄连素，苯基丁酸(PBA)等可通过升高 PP2A 活性而降低 Tau 蛋白磷酸化，改善神经元功能和学习记忆[44-52]；PP2A 活性增高还可促进神经元轴突生长[53]．PP2A 在 AD 转基因小鼠的星型胶质细胞中

活性升高，可能与 Aβ 吞噬有关[54]. 关于 PP2A 的上游调节因素目前尚不清楚，作者发现，GSK-3β 激活可通过上调 PP2A 的内源性抑制因子从而抑制 PP2A 的活性[55-56]，可见，GSK-3β 激活可通过多种机制参与 AD 的神经损伤. 此外，农用杀虫剂也可抑制 PP2A 和激活 GSK-3[57].

AD 患者脑内异常磷酸化的 Tau 蛋白被异常糖基化修饰. 糖基化包括 N- 糖基化和 O- 糖基化，AD 脑中 N- 糖基化 Tau 蛋白水平显著增高；异常 N- 糖基化在配对螺旋丝的螺旋结构形成和稳定性中起重要作用，促使 Tau 蛋白的磷酸化和聚积；在体外去 N- 糖基化使双配对螺旋丝螺旋结构消失，变成垂直的纤维丝[58]. 正常 Tau 蛋白的 O- 糖基化程度较高[59]，由于 O- 糖基化与磷酸化的位点相同(丝氨酸和苏氨酸)，故 O- 糖基化可抑制 Tau 蛋白的磷酸化. 饥饿时，随着 Tau 蛋白丝氨酸 / 苏氨酸位点的 O- 糖基化水平降低，Tau 蛋白的磷酸化水平显著升高[60]. 此外，硝基化修饰促进 Tau 蛋白聚积[61-62].

上述研究结果提示，适时适度干预导致 Tau 蛋白发生异常修饰的上游因素，如 GSK-3β 和 PP2A 等，可能达到阻止或延缓 AD 的神经变性. 然而，如何在临床上到达适时适度的干预尚待深入研究.

2　脑脊液中异常过度磷酸化 Tau 蛋白的检测方法及其诊断意义

脑脊液与脑组织毗邻，脑内的生化变化可直接反映在脑脊液中. 因此，定量检测人体脑脊液中 Tau 蛋白磷酸化的改变，对动态研究 Tau 蛋白在人脑神经元退行性变性中的作用和机制有重要意义. 国外研究报道，AD 患者脑脊液 Tau 的 181 位点磷酸化水平增高. PHF-1 是 AD 患者 Tau 蛋白的重要磷酸化表位，由于 PHF-1 阳性 Tau 蛋白在脑脊液中的含量低，用经典的酶联免疫吸附法(ELISA)不能检测. 我们巧妙地将具有高特异性的 ELISA 与高灵敏度的双酶底物循环方法联在一起，建立了高特异、高灵敏的 ELISA- 双酶底物循环技术. 将此技术用于检测脑脊液中 Tau 蛋白 PHF-1 位点的磷酸化水平，发现在 AD 患者显著增高；通过计算 p-Tau 蛋白与总 Tau 蛋白的比值，还可鉴别 AD 与血管性痴呆[63].此外，该方法还可检测脑脊液中磷酸化的神经细丝蛋白[64].

我们还利用脑功能影像学技术，在具有 AD 样骨架蛋白异常磷酸化特征的动物模型上，发现海马血氧水平依赖的(BOLD)信号与异常磷酸化 Tau 的分布高度一致[65]，发现 Tau 磷酸化水平与脑内乙酰胆碱降低的水平一致、与磁共振波谱采集的胆碱峰的变化一致[66]，AD 患者脑中特定区域铁聚积[67]. 这些研究对建立 AD 早期和无创性预警技术是一个初步尝试.

3　Tau 蛋白过度磷酸化对细胞生存命运的影响

由于至今尚无评估神经变性的分子指标，一些研究者将神经变性与凋亡的概念混为一谈，并用“凋亡”指标评价“变性”. 然而，国际著名神经病理学家 Braak 等的研究显示：AD 患者脑中凋亡细胞数与年龄匹配对照组比并无明显增强，更重要的是，他们发现，神经退行性变性的特征是神经元突起逐渐变短、变少或消失，而富含神经原纤维缠结的神经元胞体数量没有明显减少，这些形态学改变与凋亡的特征显然不同. 可见，神经变性不是凋亡，AD 脑萎缩的原因不是凋亡引起的. 随着老化，脑中促凋亡因素不断累积，神经元长期暴露在促凋亡环境，为什么 AD 患者神经元不发生凋亡(急性过程)而是选择慢性退行性变性呢？由于变性神经元细胞体中主要的成分是过度磷酸化的 Tau 蛋白，我们推测，Tau 蛋白磷酸化必定在神经元选择性变性中起作用. 因此，我们创建了过表达 Tau 和不表达 Tau 蛋白的细胞株，探讨 Tau 蛋白在调节细胞生存命运中的作用.

与预期结果相反，我们发现，过度表达 Tau 蛋白可使细胞抵抗由 GSK-3β 介导的细胞凋亡，其主要机制涉及 Tau 蛋白过度磷酸化通过底物竞争使抗凋亡因子 β- 连环素的磷酸化水平减低，从而保存 β- 连环素而发挥抗凋亡作用[68]. 此外，Tau 蛋白磷酸化还通过调节线粒体、p53 等凋亡途径，抵抗 Aβ 等因素引起的凋亡[69-71]，而 Tau 蛋白去磷酸化促进细胞凋亡[72].

同时，大量研究显示，Tau 蛋白过度磷酸化损伤神经细胞功能和结构，如过度磷酸化使 Tau 蛋白结合微管能力降低[37]，Tau 蛋白在神经元内聚积导致神经元轴突转运障碍[43]、乙酰胆碱释放减少[40]、蛋白酶体活性抑制[61-62,73-74]，这些功能和结构的改变可能导致神经元呈慢性进行性变性.

上述研究结果表明，Tau 蛋白磷酸化使神经元逃逸急性凋亡的同时，又启动了慢性退行性变性. 据此，作者提出了 AD 神经元变性是有别于凋亡的

一种受独特分子调控的细胞死亡形式，即“神经退行性变性死亡，neurodegenerasis”[4](图 3).

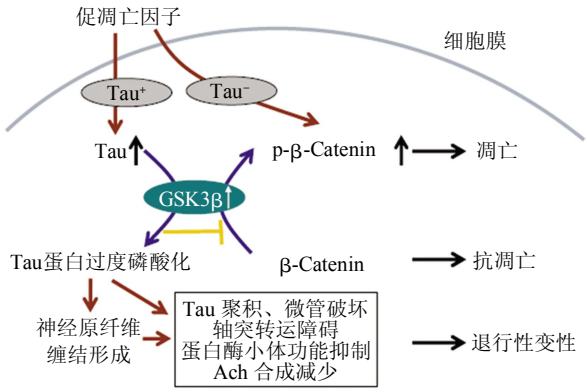

Fig. 3　Dual role of Tau hyperphosphorylation in neurons to abort apoptosis and to trigger neurodegeneration

图 3　Tau 蛋白过度磷酸化在神经元逃逸凋亡和发生退行性变性中起双重作用

当细胞缺失 Tau 蛋白时(Tau⁻)，促凋亡因子启动凋亡信号系统，如激活 GSK-3β 使生存因子 β-catenin 磷酸化并在细胞质中降解，细胞因生存信号不足而发生凋亡；当细胞中富含 Tau 蛋白时(Tau⁺)，Tau 蛋白通过底物竞争机制抑制 β-catenin 被激活的 GSK-3β 磷酸化，非磷酸化的 β-catenin 进入细胞核，促进生存因子表达而发挥抗凋亡作用，使神经元暂时存活．然而，过度磷酸化的 Tau 蛋白易于聚积、破坏微管和轴突运输功能等，导致神经元功能和结构异常而呈病态生存，最终发生神经退行性变性死亡(neurodegenerasis).

4　展　　望

AD 的发病因素和机制复杂，如基因变异[75-76]、表观遗传变化[77]、内外环境因素影响[78-79]、异常修饰与淀粉样蛋白在脑内的沉积[80-81]、神经营养及可塑性改变[82-83]、氧化应激[84-85]、离子通道沉默[86]、金属离子代谢[87]及能量代谢紊乱[88]等等．由于异常 Tau/ 缠结的脑区特异性发生和发展与 AD 神经变性和临床症状正相关，因此，阐明 Tau 蛋白毒性获得(gain of toxicity)及其在神经元变性死亡中的作用，对发展有效的 AD 防治策略至关重要．作者阐释的 GSK-3β 和 PP2A 在 Tau 蛋白过度磷酸化中的作用和调节机制，特别是原创性地提出 “Tau 蛋白过度磷酸化在神经元逃逸凋亡和发生退行性变性中起双重作用”学说，对诠释 AD 发生与发展的分子细胞机制、发现 AD 生物学标记物以及建立临床新干预方法，都具有重要的意义．

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Molecular Mechanisms Underlie Alzheimer-like Tau Hyperphosphorylation and Neurodegeneration*

WANG Jian-Zhi**, TIAN Qing

(*Key Laboratory of Neurological Diseases, Ministry of Education of China, Department of Pathophysiology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan* 430030, China)

Abstract Tau is the most abundant microtubule associated protein. The normal function of Tau is to promote microtubule assembly and stabilize microtubules. In Alzheimer's disease, Tau is abnormally hyperphosphorylated and the hyperphosphorylated Tau accumulates, in the form of paired helical filaments (PHF), in the neuron to form neurofibrillary tangles. Numerous studies indicate that the abnormal Tau modifications play a crucial role in AD neurodegeneration and the cognitive deficits. We have studied systemically the mechanisms underlie Tau hyperphosphorylation and the effects of Tau phosphorylation on cell viability. We found unexpectedly that expression of the hyperphosphorylated Tau, at certain point, renders the cells more resistant to the exogenously induced cell apoptosis, whereas dephosphorylation of Tau promotes cell apoptosis. We also found that persistent Tau hyperphosphorylation and the cellular accumulation damage the neural functions and thus decrease the viability. Based on these findings, we propose that Tau hyperphosphorylation may play a dual role in leading the neurons to abort from an acute apoptosis and at the same time triggering a chronic neurodegeneration, which may explain why the degenerated neurons observed in the postmortem Alzheimer's brain are enriched with the hyperphosphorylated Tau proteins/tangles. It is suggested that proper intervention of Tau hyperphosphorylation may serve as a promising strategy in rescuing cell apoptosis and arresting neurodegeneration in Alzheimer's disease.

Key words Alzheimer's disease, Tau, phosphorylation, protein kinase, protein phosphatase, neuron, neurodegeneration

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**Corresponding author.

Tel: 86-27-83693883, E-mail: wangjz@mails.tjmu.edu.cn

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