靶向mTOR的抗肿瘤与抗衰老药物研究进展

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摘要 哺乳动物雷帕霉素靶蛋白(mammalian target of rapamycin, mTOR)是一种丝/苏氨酸蛋白激酶, 在调节细胞生长、增殖、存活、血管生成、蛋白质合成、细胞周期中发挥着重要的作用。mTOR信号通路异常与肿瘤及衰老密切相关, 已成为相关疾病治疗的靶点。该文综述了mTOR对肿瘤和衰老调控的研究进展, 对于揭示肿瘤及衰老相关疾病的发生机制具有重要意义, 并为研发以mTOR信号通路为靶点的抗肿瘤、抗衰老的治疗药物提供了新的思路和方法。

关键词 mTOR; 抗肿瘤; 抗衰老; 抑制剂

Advances in Anti-Tumor and Anti-Aging Drugs Targeting mTOR

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Abstract Mammalian target of rapamycin (mTOR), a serine/threonine protein kinase, plays important roles in regulating cell growth, proliferation, survival, angiogenesis, protein synthesis and cell cycle. The abnormality of mTOR signaling pathway is closely related to tumor and aging, and has become a target for the treatment of related diseases. This review summarizes the progress of mTOR in the regulation of tumor and senescence, which is of great significance to the mechanism of tumor and aging-related diseases, and to provide new ideas and methods for the development of anti-tumor and anti-aging therapeutic drugs targeting mTOR signaling pathway.

Keywords mTOR; anti-tumor; anti-aging; inhibitors

衰老和肿瘤是人类研究中的两个非常重要的课题[1]。各种肿瘤及衰老相关重大疾病严重影响着人们的生活质量, 给家庭和社会带来了沉重的经济负担。近年来, 随着现代生物学技术的不断进步及在细胞分子水平上研究的不断深入, 人们发现多种影响细胞衰老和肿瘤的分子及其参与的信号通路, 并且发现肿瘤与衰老的发展过程中有很多分子机制是共享的, 其中mTOR及mTOR相关信号通路在衰老和肿瘤中所起的作用越来越受到人们的关注。

1 mTOR简介

哺乳动物雷帕霉素靶蛋白(mammalian target of rapamycin, mTOR)是细胞内一种丝氨酸(Ser)/苏氨酸(Thr)蛋白激酶, 其主要包括两种不同类型的蛋白质复合物, 即mTORC1和mTORC2[2]。mTORC1包含mTOR蛋白、Raptor(mTOR调节相关蛋白、PRAS40(富含脯氨酸的Akt底物40)、mLST8(mammalian lethal with Secl3 protein 8, 也称为G蛋白β亚基样蛋白, GβL, LST8的酵母同源物)、Deptor(含有DEP结构域的mTOR

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相互作用蛋白)[3]。mTORC1信号主要通过Ras/Raf/MEK/ERK通路和PI3K/Akt/mTOR这两条通路激活, 并通过其两个主要底物S6K(S6激酶)和4E-BP1(4E-binding protein 1)控制细胞生长、存活、血管生成和蛋白质翻译[4]。mTORC2包含mTOR蛋白、Rictor、PROTOR、mLST8、Deptor、mSIN1[5]。研究发现, 生长因子如胰岛素信号可通过PI3K活化mTORC2, mTORC2的过度活化会通过磷酸化Akt的Ser473位点反馈激活Akt, 导致PI3K/Akt/mTOR信号通路激活[6]。mTORC2在细胞存活, 肌动蛋白细胞骨架形成, 降解新合成的多肽、糖类、脂质等方面发挥重要作用[7-8]。

mTOR作为一个重要的信号转导分子, 对整个信号通路的作用及功能的显示起极其重要的作用。研究发现, mTORC1的功能在高达70%的人类肿瘤中被过度活化, 在衰老相关疾病中也出现过表达的情况, 这些特征使得mTOR成为肿瘤和衰老中研究的潜在药物靶点。

2 mTOR对肿瘤的调控

近年来的多项研究指出, mTOR在多种肿瘤中被过度活化, 与肿瘤的发生和发展密不可分。过度活化主要表现为PI3K/Akt/mTOR、Ras/MEK/ERK/MNKs和MAPK/MNKs信号通路的过度激活、PTEN功能的缺失和自噬途径受阻。

研究表明, 乳腺癌、子宫内膜癌、卵巢癌、前列腺癌、肝癌、肺癌、白血病、胃肠道间质肿瘤、甲状腺癌和胰腺癌等均存在PI3K/Akt/mTOR信号通路的失调[9-10]。mTOR主要通过PI3K/Akt/mTOR和Ras/Raf/MEK/ERK这两条通路激活。促有丝分裂信号生长因子、激素、细胞活素类(cytokines)通过激活Akt, 进而激活mTOR, mTOR活化后会促使4E-BP1、PDCD4磷酸化[11-12]。其中, 4E-BP1在低磷酸化状态时, 与真核起始因子4E(eukaryotic initiation factor 4E, eIF4E)紧密结合, 阻止eIF4F复合物(由三个亚基eIF4E、eIF4A和eIF4G组成)的形成并抑制帽依赖的翻译起始[13]。当4E-BP1被mTORC1磷酸化时,会诱导4E-BP1释放出eIF4E; 同理, PDCD4在低磷酸化状态时, 与eIF4A紧密结合, 当PDCD4被mTOR磷酸化时, 会诱导它们释放出eIF4A, 从而促进eIF4F复合体的形成, 缩短蛋白质翻译过程, 导致肿瘤的生长。另外, Ras/MEK/ERK/MNKs和MAPK/MNKs通路能通过磷酸化eIF4E的Ser209位点调节eIF4E的活性, 从而影响翻译进程, eIF4E的过度活化可以促进细胞增殖和恶性转化, 因此, 也被认为是一种重要的致癌基因[14]。

研究发现, PTEN也可以对PI3K/Akt/mTOR信号通路进行负反馈调节。PTEN(phosphatase and tensin homolog deleted onchromosome 10, 10号染色体上缺失与张力蛋白同源的磷酸酶基因)是一种抑癌基因, 是PI3K/Akt/mTOR通路的一个重要的负反馈调节机制, PTEN的功能缺失使其解除了对PI3K/Akt/mTOR信号通路的抑制作用, 使PI3K及Akt产物增加、活性增强, 促使肿瘤发生[15]; AMPK可以直接使Raptor磷酸化[16], 抑制mTORC1的活性, 从而促进自噬的发生, 减少炎症因子的产生[17], 并且PTEN可抑制AMPK细胞信号转导途径, 从而抑制细胞生长和分化[18]。

当mTORC1被活化时, 它也可以通过抑制自噬而间接地促进肿瘤的发生[19]。自噬在肿瘤的抑制中也起重要作用, 支持自噬的抗癌作用的最直接的证据来源于自噬蛋白Beclin和ATG4C缺陷的小鼠, 这两种小鼠都容易发生肿瘤[20-21]。

mTOR介导的蛋白质翻译调控、PTEN功能紊乱和自噬抑制对肿瘤的发生发展起到重要的调控作用, 那么在对衰老的调控中它是否也扮演着如此重要的角色呢?

3 mTOR对衰老的调控

衰老是随着时间推移, 生物体的结构和机能逐渐老化、衰退的复杂生物学过程, 伴随着分子、细胞和组织器官的损伤, 患病的危险性增高并最终导致生物体的死亡。近年来, 延缓衰老以及延长人类健康寿命已成为生物学研究的热点, 随着现代生物学技术的不断进步及在细胞分子水平上研究的不断深入, 人们发现了多种影响细胞衰老的分子及其所参与的信号通路, 其中mTOR信号通路在衰老中所起的作用越来越受到人们的关注。研究发现, mRNA翻译、异常生长信号、PTEN缺失和自噬都与衰老的发生有关[22]。

2003年, Vellai等[23]发现, 降低mTORC1活性能明显延长秀丽隐杆线虫的寿命和功能, 自此揭示了mTOR在细胞衰老过程中的作用。近年来, 研究表明, 通过遗传方法减少mRNA翻译可以延长酵母、蠕虫

和苍蝇的寿命[22]。mTORC1通过S6K1和4E-BP1在翻译的调节中起关键作用。酵母、秀丽隐杆线虫和黑腹果蝇的实验证明, S6K1缺失延长了雌性小鼠的寿命, 而4E-BP1的缺失阻止了苍蝇的寿命延长[24]。

异常生长信号或应激信号可加速干细胞衰老和组织衰老。在最近的报告中, Wnt信号分子在小鼠表皮中的持续表达导致上皮干细胞的过度增殖, 最终导致它们衰老[25], 用雷帕霉素治疗可防止过表达Wnt的表皮干细胞的过度增殖和过早衰老[25]。在小鼠造血干细胞(hepatic stellate cells, HSCs)中, 检测到mTORC1活性呈年龄依赖性增加, 通过缺失TsC1, 激活mTORC1, 导致p16、p19和p21的表达增加, 导致HsCs的消耗, 延长雷帕霉素治疗将HSCs保持在与年轻动物相似的水平[26]。

PTEN的缺失过度激活了PI3K途径也导致了HSCs的过度增殖。事实上, 用雷帕霉素治疗恢复了PTEN-HSCs的重建能力, 以重建小鼠血液谱系。总之, 这些发现提示, mTORC1是促进造血干细胞从静止状态退出的生长信号的关键调节剂。

诱导自噬也是与长寿相关的mTOR抑制的一个因素。自噬基因的失活减少了酵母的寿命[27]。自噬是允许细胞在营养受限条件下生存并且去除受损组分的中心机制。在营养充足的条件下, mTOR磷酸化并抑制自噬起始激酶ULK1从而促进衰老[28]。

因此, mTOR作为一个重要的信号转导因子, 在抗肿瘤和抗衰老的研究中起着关键作用, 随着围绕mTOR研究的日益深入, 靶向mTOR的治疗药物被不断地开发和完善, 也就此产生了由雷帕霉素为开端的几代mTOR靶向治疗药物。

4　mTOR信号通路调节剂

4.1　mTOR抑制剂

雷帕霉素是人们发现的第一个mTOR抑制剂[29]。它已被FDA批准用于在移植手术后用作免疫抑制剂和用于治疗肾细胞癌, 并且已经用作冠状动脉支架的涂层和用于诸如淋巴管平滑肌瘤病和自身免疫病症的许多临床试验[29-30]。随着研究的深入, 研究人员发现, 雷帕霉素能有效抑制横纹肌肉瘤细胞系、神经细胞瘤、胶质细胞瘤、鼠黑素瘤、B细胞淋巴瘤、小细胞肺癌、胰腺癌、前列腺癌、乳腺癌和骨肉瘤等[31]。研究发现, 雷帕霉素通过结合细胞内受体FKBP12, 形成抑制性复合物, 然后一起结合FRB结构域的TOR蛋白的C-端区域, 通过抑制mTOR与raptor的相互作用来抑制mTOR功能, 破坏mTORC1与其底物的偶联, 从而抑制mTOR信号对下游靶标的功能发挥其细胞生长抑制和细胞毒性作用[32]。同时, Kaeberlein等[7]发现, mTOR通路下游的S6K可能与延长寿命有关, 它能通过抑制mTORC1, 进而抑制mTORC1下游效应因子S6K, 从而起到延长寿命的作用。这表明, mTORC1的抑制对于肿瘤细胞和正常细胞有着不同的生物学影响, S6K被抑制后, 细胞代谢被减缓, 肿瘤细胞因其恶性增殖的特性而导致生长的抑制, 最终达到抗肿瘤的目的, 而正常细胞被证明适当的能量抑制会达到抗衰老的目的, 所以抗衰老与抗肿瘤这两个截然不同的过程在mTORC1的调控下可以达到平衡, 并不矛盾[33]。

虽然雷帕霉素具有上述的临床效用, 但也存在一些副作用。首先, 其最大的副作用是抑制免疫系统的功能。研究发现, 肾移植患者雷帕霉素使用过程中, 34%患者遭受过病毒感染, 16%患者患有真菌感染[34]。其次, 雷帕霉素也经常与皮肤病学事件相关, 在肾移植患者中发现, 雷帕霉素导致60%的患者的水肿和55%的患者发生口疮性溃疡[34]。再者, 雷帕霉素治疗也与头发和指甲病症相关, 90%的患者出现秃发[34], 并且在人和小鼠中出现睾丸功能丧失和雄性生育力降低的情况[35-36]。同时, 雷帕霉素治疗导致代谢变化, 例如高脂血症导致胰岛素敏感性降低、葡萄糖耐受不良和新发糖尿病的发病率增加[30,37]。雷帕霉素治疗也与会引发一些胃肠道疾病, 包括腹泻等[38]。

基于雷帕霉素的副作用, 开发和研究具有优异特性的雷帕霉素衍生物成为研究热点。目前开发并研究的雷帕霉素抗肿瘤衍生物包括RAD001(Everolimus)、替西罗莫司(Temsirolimus, CCL-779)、AP23573(Deforolimus)、SAR943(32deoxy-rapamycin)、AB7-578(Zotarolimus)和Ridaforolimus。其中, 依维莫司RAD001(Everolimus)经FDA批准用于治疗先天性神经内分泌肿瘤[39]、乳腺癌[40]和肾细胞癌以及在TSC1和TSC2携带种系突变的患者中的肾下巨细胞星形细胞瘤[41]。Temsirolimus被FDA批准用于治疗晚期肾细胞癌[42]。替西罗莫司作为复发性或难治性细胞淋巴瘤的单一药物的III期临床试验已经完成, 显著提高了无进展生存与研究者的选择治疗[43]。Ridaforolimus在III期临

床试验中用于肉瘤[43]。AP23573(Deforolimus)单独或与几种化学治疗剂组合使用已经显示出对体外不同肿瘤细胞系的增殖有强效抑制作用, 并在携带异种移植物的小鼠中诱导部分肿瘤消退, 与雷帕霉素相比, Deforolimus具有更好的药物和药理学性质[44]SAR943(32deoxy-rapamycin)和AB7-578(Zotarolimus)是具有更好的药物特性的两种雷帕霉素类似物, 用于恶性肿瘤[45]、心血管支架植入[46]或慢性过敏性炎症的治疗[47]。然而, 这些衍生物是否也具有抗衰老作用还在进一步的研究中。

近几年的研究发现, 雷帕霉素效果不理想大部分是由于S6K/IRS-1反馈环的抑制, 这些治疗失败部分归因于雷帕霉素诱导Akt Ser473位点磷酸化, 从而激活Akt[48-49]。雷帕霉素介导的Akt激活可能是雷帕霉素及其类似物在患者中观察到的抗肿瘤效果减弱的原因。针对这一问题, mTOR的双靶点抑制剂和ATP竞争性抑制剂成为药物开发的思路。例如, NVP-BEZ235(NCT00620594)是mTOR的双靶点抑制剂。越来越多的证据表明, NVP-BEZ235通过结合PI3K和mTOR激酶的ATP结合裂口, 从而抑制其活性, 它能够阻断mTORC1/2的下游效应物S6k和4E-BP1的激活, 有效逆转PI3K/mTOR通路的过度活化, 从而在广泛的癌细胞系和实验肿瘤模型中产生有效的抗增殖和抗肿瘤活性[50-51], 具体的研究进展详见表1。

同时, 针对雷帕霉素诱导的Akt反馈激活mTORC2问题, ATP竞争性抑制剂的开发很大程度上解决了这个缺陷。与雷帕霉素相比, PP30、PP242和Torin1在较大程度上抑制了原代细胞的增殖, PP242在白血病小鼠模型中比雷帕霉素更有效[52], ATP竞争性抑制剂具体的研究进展详见表1。

4.2 其他调节剂

二甲双胍作为降糖药物已经被使用60多年, 但近年来流行病学调查结果提示二甲双胍有抗肿瘤的功效后, 二甲双胍抗肿瘤的研究便成为研究热点。随后陆续发现, 二甲双胍对乳腺癌、子宫内膜癌、卵巢癌、前列腺癌、胰腺癌、甲状腺肿瘤、肺癌、喉癌等多种肿瘤均能有效降低疾病患病风险[53]。研究发现, 二甲双胍可以通过激活AMPK信号转导通路和抑制胰岛素样生长因子-1(insulin-like growth factor-1, IGF-1)等途径调控肿瘤细胞的增殖[54]。

研究发现, 二甲双胍也可以通过激活AMPK信号转导通路, 抑制哺乳动物雷帕霉素靶蛋白(mTOR)及其下游相关分子延缓衰老并延长寿命。大量证据表明, 二甲双胍具有促进蠕虫、线虫、啮齿动物和人类长寿的功能[55]。这些影响独立于胰岛素信号通路, 但依赖于AMPK以及氧化应激转录因子SKN-1/NRF2[55]。二甲双胍延长了短寿命、肿瘤易发性的HER2/neu小鼠和雌性SHR小鼠的寿命[56-57]。这些结果都显示了二甲双胍有作为抗癌药物或抗衰老药物的前景。至于二甲双胍是否能成为抗肿瘤和抗衰老的双重特效药, 还需要进一步的研究。

越来越多的研究表明, 一些食物衍生的天然产物(diet-derived natural products), 包括姜黄素(curcumin)、白藜芦醇(resveratrol)、表没食子儿茶素没食子酸酯(epigallocatechin gallate, EGCG)、染料木素、3,3-二吲哚甲烷(3,3-diindolylmethane, DIM)和咖啡因(caffeine), 它们能通过直接或间接抑制mTOR信号来抑制肿瘤细胞增殖。其中, 姜黄素能打破mTOR-Raptor复合体, 白藜芦醇、EGCG和染料木素能抑制PI3K/Akt/mTOR信号通路[58]。食物衍生的天然产物通常对人类毒性较小, 为了在体内实现治疗效果, 有必要开发一些更有效的天然产物的衍生物或具有改善的药物性质的有效mTOR制剂。

5 展望

mTOR是细胞生长、增殖、代谢和血管生成等的中枢控制剂。研究表明, mTOR信号通路从许多方面参与肿瘤和衰老及年龄相关疾病的发生发展。了解mTORC1和mTORC2调控的直接分子机制, 并确定将多种mTOR调节信号转导给mTORCs的信号中间体是未来研究的重要领域。虽然目前已经开发出多种mTOR抑制剂对各类肿瘤有显著的抑制作用, 但存在很多副作用。雷帕霉素在动物模型中显示出作为用于治疗衰老相关疾病的药剂有着显著前景, 但是需要进一步研究, 从而确定是否有可用的靶向mTOR的策略最终针对肿瘤和衰老相关疾病都有一定的治疗效果。最近的研究表明, 在动物实验中, 通过组织特异性的基因敲除等功能获得或缺失研究方法, 能够深入了解mTOR通路的组织特异性功能, 为开发低毒、高效的抗肿瘤和抗衰老药物提供靶点[24]。总而言之, 深入对mTOR调控网络的研究将增强我们对肿瘤、衰老及衰老相关疾病发病机制的了解,

表1　mTOR通路调节剂

Table 1　mTOR pathway modulators

mTOR抑制剂 mTOR inhibitor	作用机制 Mechanism	发展现状 Development status
Rapamycin and rapalogs		
Rapamycin	Complex with FKBP12 and then combined with the FRB domain of mTOR to inhibit mTOR function	Rapamycin is approved by the FDA for use as an immunosuppressant after transplantation surgery and for the treatment of renal cell carcinoma, And has been used as a coating for coronary stents and for many clinical trials such as lymphangioleiomyomatosis and autoimmune disorders
Temsirolimus (CCI-779)	mTORC1 inhibitor	Tesirolimus has been approved by the FDA for the treatment of advanced renal cell carcinoma, and its phase III clinical trial as a single agent for relapsed or refractory cell lymphomas has been completed, significantly improving survival
Everolimus (RAD001)	Cell-type specific mTORC2 inhibitor	Everolimus approved by the FDA for the treatment of congenital neuroendocrine tumors, breast cancer, and subrenal giant cell astrocytoma
Deforolimus (AP23573)	Cell-type specific mTORC2 inhibitor	The phase II combination study is underway
Ridaforolimus	Allosteric inhibition of mTORC1	Ridaforolimus is conducting phase III clinical trials in sarcoma
32 deoxy-rapamycin (SAR943)	Complex with FKBP12 and then combined with the FRB domain of mTOR to inhibit mTOR function	Has been developed to prevent chronic allergic inflammation
Zotarolimus (ABT-578)	Complex with FKBP12 and then combined with the FRB domain of mTOR to inhibit mTOR function	Has been developed for cardiovascular stent implantation
Dual PI3K-mTOR inhibitors		
NVP-BEZ235	mTOR and PI3K dual specificity inhibitor	NVP-BEZ235 is in phase I/II of renal cell carcinoma, stage I/II of breast cancer, stage I of prostate cancer, and stage II clinical trial of pancreatic neuroendocrine tumors
PI-103	mTOR and PI3K dual specificity inhibitor	PI-103 did not enter clinical trials due to problems associated with rapid metabolism in the body
XL765	mTOR and PI3K dual specificity inhibitor	XL765 is in phase I clinical trial of breast cancer and glioblastoma
NVP-BGT226	mTOR and PI3K dual specificity inhibitor	NVP-BGT226 in phase I/II breast cancer clinical trial
GNE477	mTOR and PI3K dual specificity inhibitor	GNE477 shows stagnation in studies of tumor growth inhibition in MCF7 and PC3
WJD008	mTOR and PI3K dual specificity inhibitor	WJD008 significantly prevents cell proliferation in prostate cancer PC-3 cell phase I trials
GSK2126458	mTOR and PI3K dual specificity inhibitor	GSK2126458 is undergoing human breast ductal tumor I/II clinical trial
mTORC1/2 inhibitors		
MLN0128	Active-site mTOR inhibition	INK128 has anti-metastatic properties, selectively targeting cancer cells while retaining normal bone marrow cells in animal models
AZD8055	Active-site mTOR inhibition	AZD8055 is effective in xenograft models
Torin1	mTOR kinase inhibitor	Torin1 is effective in killing tumor cells and controlling mouse tumors in tissue culture and preclinical animal models
PP242	mTOR kinase inhibitor	PP242 is more effective than rapamycin in a mouse model of leukemia
PP30	mTOR kinase inhibitor	PP30 can inhibit PIKK family related kinase activity, but also inhibit mTOR and PKC, RET, JAK2 and other protein kinases
WYE-354	ATP competitive inhibitor of mTOR	WYE-354 can inhibit the proliferation of MDA361 and U87MG cells and induce cell death. It can stagnate tumor growth in the mouse model of U87MG xenograft tumors.
WAY-600	ATP competitive inhibitor of mTOR	WAY-600 inhibits cell proliferation and induces G_1 cell cycle arrest in different cancer cell lines
WYE-687	ATP competitive inhibitor of mTOR	WYE-687 inhibits cell proliferation and induces G_1 cell cycle arrest in different cancer cell lines

(续表1)

mTOR抑制剂 mTOR inhibitor	作用机制 Mechanism	发展现状 Development status
Ku-0063794	Specific mTORC1 and mTORC2 inhibitor	Ku-0063794 inhibits cell proliferation and induces G_1 cell cycle arrest in different cancer cell lines
Indirect mTOR inhibitors		
Metformin	Indirect mTOR inhibition	Metformin reduces cancer incidence in patients with type 2 diabetes and inhibits tumor growth in mouse and hamster models
Phenformin	Indirect mTOR inhibition	Phenformin has been used to treat type 2 diabetes and inhibits tumor growth in mouse and hamster models
Diet-derived natural products		
Curcumin	Disrupts the mTOR Raptor Complex	Curcumin has entered early clinical trials as a novel anticancer agent and can inhibit the proliferation of rhabdomyosarcoma cells.
Resveratrol	Inhibits PI3K/Akt/mTOR signaling pathway	Resveratrol has anti-inflammatory, antioxidant, neuroprotective and anti-cancer properties and inhibits cell proliferation in human glioma cells and breast cancer cells
epigallocatechin gallate (EGCG)	Inhibits PI3K/Akt/mTOR signaling pathway	Epigallocatechin gallate (EGCG) inhibits proliferation of hepatoma cells by inhibiting protein translation
Genistein	Inhibits PI3K/Akt/mTOR signaling pathway	Long-term low-dose genistein treatment with genistein inhibits estradiol-stimulated MCF-7 cell growth by down-regulating PI3K/Akt signaling pathway
3,3-diindolylmethane (DIM)	Inhibits both mTOR and Akt activity	3,3-diindolylmethane (DIM) inhibits cell proliferation by inducing autophagy in prostate, breast cancer, and xenograft mouse models
Caffeine	Inhibits TORC1	It has been shown that theophylline and related compounds theophylline inhibit the phosphorylation of mTOR-dependent substrates *in vitro* as well as *in vivo*

为研发治疗肿瘤、衰老及衰老相关疾病新药提供有用信息。

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