DOI：10.12287/j.issn.2096-8965.20230105

E3泛素连接酶RFWD家族成员在肿瘤中的作用研究进展

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【摘要】环指和WD重复结构域（RING Finger and WD Repeat Domain，RFWD）蛋白属于E3泛素连接酶，在细胞增殖、凋亡和DNA损伤修复的调控中发挥重要作用。越来越多的证据表明，RFWD蛋白通过靶向其底物泛素化降解参与肿瘤的发生发展。RFWD蛋白在不同类型的人类恶性肿瘤中具有抑癌和促癌的不同作用，可能与其底物蛋白的功能有关。本文分析了RFWD蛋白家族成员（RFWD1，RFWD2，RFWD3）的结构和功能，总结了RFWD蛋白家族成员的已知底物，并阐明其在不同肿瘤中的不同功能。此外，本文还讨论了RFWD家族成员作为癌症治疗潜在靶点的可能及策略。

【关键词】环指和WD重复结构域；E3泛素连接酶；肿瘤；靶向疗法

中图分类号：R730.2，R363.2+1　　**文献标识码**：A　　**文章编号**：2096-8965（2023）01-0030-09

Research progress on the E3 ubiquitin ligases RFWD family members in tumor

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【**Abstract**】RING finger and WD repeat domain (RFWD) proteins belong to E3 ubiquitin ligase and play an important role in the regulation of cell proliferation, apoptosis and DNA damage repair. Evidence indicates that RFWD proteins are involved in tumor development by targeting their substrates' ubiquitination and degradation. RFWD proteins have different inhibitory and carcinogenic effects in different types of human malignant tumors, which may be attributed to the functions of their substrate proteins. In this paper, the structure and function of RFWD protein family members (RFWD1, RFWD2, RFWD3) were analyzed, the known substrates of RFWD protein family members were summarized, and their different functions in different tumors were described. In addition, the possibility and the potential targeting strategies of RFWD family members for cancer therapy were discussed.

【**Keywords**】RING finger and WD repeat domain (RFWD); E3 ubiquitin ligase; Tumor; Targeted therapies

0　前言

泛素蛋白酶体系统（Ubiquitin Proteasome System，UPS）是真核细胞中具有进化保守性的翻译后修饰（Posttranslational Modification，PTM），控制着细胞增殖、细胞周期进展、转录和凋亡等多种生物学功能[1-3]。UPS对蛋白质的精确调控对于维持细胞活性和稳态至关重要，这始于多个泛素（一

收稿日期：2022-06-01；**修回日期**：2022-07-01

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种小的，高度保守的，由76个氨基酸组成的调节蛋白）分子与底物蛋白的附着（称为多聚泛素化）[4]。多聚泛素化是一个多步骤的过程，涉及三个连续的酶促反应。首先，泛素被E1泛素激活酶以ATP依赖的方式激活。随后，活化的泛素被瞬间转移到E2泛素结合酶。最后，E2反过来在E3泛素连接酶的帮助下将泛素标记到相应的底物上。这些多泛素化的蛋白随后被26S蛋白酶体复合物识别和降解[5]。UPS负责大约80%的细胞内蛋白质的水解，而该系统的失调则涉及到包括癌症在内的多种疾病[6-8]。至关重要的是，E3连接酶为泛素连接提供了底物特异性，而E3连接酶活性的改变是人类癌症发生发展的重要原因[9-11]。

迄今为止，在人类基因组中已经发现了超过600种推测的E3连接酶，它们可以根据不同的泛素化结构域分为三大类：环指（RING Finger）蛋白、U形盒（U-box）结构域蛋白和HECT结构域蛋白[12-14]。环指和WD重复结构域（RING Finger and WD Repeat Domain，RFWD）蛋白家族成员属于E3泛素连接酶环指亚型中的一员，参与调节细胞生长、凋亡和DNA损伤修复等多个生理过程[15, 16]。有研究表明，RFWD蛋白家族与多种肿瘤的发生发展密切相关，本文就RFWD蛋白家族的结构及在癌症中所起的作用进行综述。

1 RFWD家族

RFWD是一种环指蛋白，作为E3泛素连接酶，通过靶向底物蛋白进行泛素化降解，增加底物蛋白的更新。RFWD蛋白家族主要由RFWD1、RFWD2和RFWD3三个家族成员组成。这些E3泛素连接酶调节广泛的生物学功能，包括细胞生长凋亡、炎症反应、免疫系统调节和DNA损伤修复等[15, 16]。RFWD家族成员均包括一个RING环指结构域，构成泛素连接酶催化结构域的核心，最终导致底物蛋白的泛素化修饰。同时还包含一个卷曲螺旋结构域和WD40结构域，WD40结构域的基本功能是充当蛋白质-蛋白质和蛋白质-DNA相互作用的支架平台，其可以与一些功能相关的分子相互作用，因此可以发挥多种生物学功能[17]。E3泛素连接酶为泛素连接提供了底物特异性，因此RFWD家族参与了多种肿瘤的发生和发展。以往的研究表明，因为抑癌蛋白和促癌蛋白都是RFWD的底物蛋白，所以RFWD可以促进或抑制人类癌症的发生发展[18, 19]。鉴于RFWD家族成员之间的异同以及底物的特异性（见表1），在本文中我们将分别进行阐述。

表1 RFWD蛋白家族的底物蛋白

家族成员	底物	相互作用结构域	底物功能
RFWD1	MEKK3	WD40	蛋白激酶；促癌蛋白
	NEMO	CC	转录因子；促癌蛋白
	p65	CC	转录因子；促癌蛋白
	p53	未知	转录因子；抑癌蛋白
	p53	RING	转录因子；抑癌蛋白
	p27	RING	细胞周期抑制因子；抑癌蛋白
	14-3-3σ	RING	有丝分裂检查点因子；抑癌蛋白
	C/EBPα	未知	骨髓分化因子；抑癌蛋白
RFWD2	Jun	WD40	转录因子；促癌蛋白
	ETS	CC	转录因子；促癌蛋白
	β-catenin	未知	Wnt信号通路因子；促癌蛋白
	STAT3	RING	转录因子；促癌蛋白
	MTA1	RING	核小体重塑，DNA损伤反应；促癌蛋白
	MDM2	未知	E3泛素连接酶；促癌基因
RFWD3	RPA	WD40	DNA损伤反应；促癌蛋白
	RAD51	SSQ	DNA损伤反应；促癌蛋白
	E2F1	未知	促进细胞周期进程；促癌蛋白

MEKK3: Mitogen-Activated Protein Kinase Kinase Kinase 3; NEMO: Nuclear Factor-kappa B (NF-κB) Essential Modulator; C/EBPα: CCAAT/Enhancer-Binding Protein Alpha; STAT3: Signal Transducer and Activator of Transcription 3; MTA1: Metastasis-Associated Protein1; MDM2: Mouse Double Minute 2; RPA: Replication Protein A; E2F1: E2F Transcription Factor 1.

2 RFWD1的结构和功能

RFWD1又名肿瘤坏死因子受体相关因子7（Tumor Necrosis Factor Receptor-Associated Factor 7，TRAF7）或环指蛋白119（Ring Finger Protein 119，RNF119）。TRAF蛋白最初是作为肿瘤坏死因子受体（Tumor Necrosis Factor Receptor，TNFR）超家族成员的信号转导成分被发现的。在与它们各自的配体结合后，这些受体在细胞中传递各种不同的细胞外信号，这些信号调节重要的生物学过程，包括胚胎发育形成、先天性和获得性免疫反应、细胞存活和增殖、组织稳态和应激反应等[1, 20-22]。

在结构上，RFWD1具有典型的保守结构域，在其氨基末端包含一个环指结构域和一个卷曲螺旋结构域，然后是一个或多个锌指结构域。环指结构域和WD40重复序列的共存使TRAF7被归类为RFWD家族成员，并被命名为RFWD1。RFWD1蛋白的羧基末端包含七个重复的WD结构域[17]（见图1A）。RFWD1通过WD40重复序列与一些功能相关的分子相互作用，RFWD1可以与有丝分裂原活化蛋白激酶3（Mitogen-activated Protein Kinase Kinase Kinase 3，MEKK3）结合，二者的共表达会导致JNK和p38 MAP激酶途径的激活，它们构成了细胞因子和应激诱导的细胞凋亡的关键信号通路[23, 24]。RFWD1通过促进NF-κB基本调控子（NF-κB Essential Modulator，NEMO）和NF-κB p65亚基经泛素蛋白酶体途径降解，对NF-κB活性发挥负调控作用[25]。有研究表明RFWD1驱动突变与脑膜瘤发生有关[26]。通过对216个恶性胸膜间皮瘤样本的转录组和外显子组测序发现RFWD1存在显著复发性基因突变[27]。有趣的是，肿瘤抑制蛋白p53是RFWD1泛素化活性的另一个靶点，RFWD1的下调与乳腺癌队列中的不良预后相关，这可能与RFWD1介导的p53泛素化缺陷有关[28]。同样，RFWD1可以与p53结合并促进其在K48位点的泛素化促进蛋白降解，随后促进肝细胞癌（Hepatocellular Carcinoma，HCC）进展[29]，这表明RFWD1在癌症发生发展中发挥重要作用。

3 RFWD2的结构和功能

RFWD2又称为构成型光形态发生蛋白1（Constitutive Photomorphogenesis Protein 1 Homolog，COP1）或RNF200，最初被鉴定为拟南芥光形态建成的中央负调控因子[30]。RFWD2存在于非植物多细胞生物中，在哺乳动物、鸟类、两栖动物和鱼类等各种脊椎动物中高度保守[31]。人类RFWD2蛋白是由人类染色体1q24.1上的RFWD2基因编码的[32, 33]。与植物RFWD2相似，人类RFWD2具有三个高度保守的结构域：氨基末端环指结构域，内部卷曲螺旋区域和羧基末端7个WD40重复区域（见图1B）。核定位信号（Nuclear Localization Signal，NLS）和核输出信号（Nuclear Export Signal，NES）允许RFWD2在细胞核和细胞质之间穿梭[15, 34]。RFWD2环指基序，以锌结合半胱氨酸和组氨酸残基的保守模式为特征，通过募集E2泛素结合酶，将激活的泛素分子转移到其特定底物上[34, 35]。RFWD2的中心卷曲螺旋结构域负责RFWD2的同二聚化和与其他蛋白质的异二聚化[36, 37]。此外，RFWD2羧基末端的WD40重复序列由7个重复单元组成，其核心由大约40个氨基酸组成，介导RFWD2与其靶向底物的相互作用[32]。

RFWD2在人类癌症中具有促癌作用。RFWD2在许多人类癌症中过表达，包括白血病、淋巴瘤、黑色素瘤、胶质瘤、乳腺癌、肺癌、结直肠癌、肾细胞癌、肝细胞癌和卵巢癌等。Dornan等[38]首次发现RFWD2通过其环指结构域直接与p53相互作用，并在U2OS细胞中以泛素依赖的方式靶向降解p53。一项对人脑胶质瘤U251和U87细胞的体外研究表明，RFWD2泛素化并降解内源性p53，促进胶质瘤细胞增殖[39]。Kim等[40]发现，在HCT116细胞中，miR-944下调RFWD2会增加p53的表达，并诱导细胞周期阻滞和细胞凋亡，但在p53缺失的HCT116细胞中则不会诱导细胞凋亡。RFWD2通过其环指结构域直接与p27相互作用，加速U2OS细胞和乳腺癌MDA-MB-231细胞内源性p27的多聚泛素化和降解，从而促进细胞周期进展[41]。14-3-3σ在各种人类癌症中经常丢失或降低，RFWD2可以靶向14-3-3σ进行泛素依赖性蛋白水解[42]。Yoshida等[43]证明RFWD2是CCAAT增强子结合蛋白α（CCAAT/Enhancer-Binding Protein Alpha，C/EBPα）的E3泛素连接酶，并以蛋白酶体依赖的方式促进其降解，从而阻断小鼠32Dcl3未成熟髓系细胞的粒细胞分化，促进肿瘤发生。

RFWD2在人类癌症中也具有抑癌作用。Migliorini等[32]发现，小鼠RFWD2纯合缺失（RFWD2$^{-/-}$）导致了E9.5至E12.5之间的胚胎死亡，并伴有心脏畸

形，而RFWD2$^{+/-}$小鼠是可活和可育的，说明RFWD2对于小鼠存活至关重要。同样，一些遗传学研究表明，RFWD2在人类癌症中经常发生突变，Xie等[44]通过cBioPortal数据库分析证实了RFWD2在23 083例肿瘤中有20例（0.1%）纯合缺失，191例（0.8%）突变。研究表明RFWD2可特异性靶向c-Jun，促进其在人293T细胞[15]和侵袭性较小的乳腺癌细胞中泛素化降解[45]。Baert等[46]报道了在U2OS细胞中RFWD2可以作为多瘤病毒增强子激活物3（Polyomavirus Enhancer Activator，PEA3）基团蛋白的E3连接酶，促进其降解。Li等[47]发现RFWD2的过表达显著促进了U2OS细胞中转移相关蛋白1（Metastasis-Associated Protein1，MTA1）的泛素依赖性蛋白水解。RFWD2还可以靶向降解人前列腺上皮细胞和癌细胞中的信号转换和转录激活蛋白3（Signal Transducer and Activator of Transcription 3，STAT3）[48]。Xu等[49]指出，在人肝癌Bel-7402细胞中，RFWD2在Trib2蛋白的帮助下促进β-catenin降解。因此，这些数据共同表明，RFWD2可以促进或抑制人类肿瘤的发生，因为肿瘤抑制蛋白（如p53、p27、14-3-3σ和C/EBPα）和促癌蛋白（如Jun、ETS、β-catenin、STAT3和MTA1）都是RFWD2泛素连接酶的作用靶点。

4 RFWD3的结构和功能

RFWD3又名RNF201，在对响应DNA损伤而发生磷酸化的蛋白进行大规模蛋白质组学分析时，RFWD3首次被鉴定为共济失调-毛细血管扩张突变基因（Ataxia Telangiectasia-Mutated，ATM）和Rad-3相关蛋白（ATM-Rad3-Related，ATR）的底物[50]。ATM羧基端约100 kD功能片段与磷脂酰肌醇-3激酶（Phosphatidylinositol 3-Kinase，PI-3K）的催化功能域有高度的同源性，PI-3K蛋白家族均具有丝/苏氨酸蛋白激酶活性，作用下游靶蛋白相应丝氨酸（Ser）或苏氨酸（Thr）位点，在细胞周期调控、DNA损伤识别和修复中发挥重要作用[51]。ATR蛋白羧基末端的某一特定区域能够结合ATR相互作用蛋白（ATR-Interacting Protein，ATRIP），该区域在DNA复制过程中发挥重要作用[52]。ATM/ATR激酶可以感知DNA损伤，并将DNA损伤信号传导到下游靶蛋白，启动应激系统，产生细胞周期阻滞，从而完成DNA修复或启动细胞凋亡程序，因此ATM/ATR对维持细胞基因组的稳定和防止肿瘤的发生至关重要[53, 54]。RFWD3的关键功能结构域是具有E3泛素连接酶活性的环指结构域、卷曲螺旋结构域和包含三个WD40重复序列的羧基末端结构域。此外，N端的SSQ结构域有一簇SQ位点，由17个氨基酸残基的3个重复序列组成[55]（见图1C）。

在电离辐射的作用下，RFWD3可以与鼠双微基因（Mouse Double Minute 2，MDM2）相互作用，限制其在泛素化p53上延伸多泛素链，介导p53在DNA损伤晚期反应中的泛素化，从而作为p53稳定性的正调控因子，调控G1/S期DNA损伤检查点[56]。有研究报道[55]RFWD3与复制蛋白A（Replication Protein A，RPA）相关，RPA是一种单链DNA（single-stranded DNA，ssDNA）结合蛋白，在DNA复制、重组和修复中发挥重要作用。为了应对复制压力，RFWD3以RPA依赖的方式被招募到DNA损伤部位，促进RPA介导的DNA损伤信号传导和修复过程[55, 57, 58]。Elia等[59]通过定量蛋白质组学分析，发现RFWD3介导RPA泛素化也是复制叉重启、复制压力期间的正常修复动力学以及停滞复制叉处的同源重组（Homologous Recombination，HR）所必需的。除了同源重组，RFWD3被RPA募集到停滞的复制叉处对DNA链间交联（Intrastrand Cross-Linking，ICL）修复也很重要[60]，范可尼贫血（Fanconi Anemia，FA）患者表现为ICL修复缺陷与基因组不稳定性。Knies等[61, 62]发现RFWD3双等位基因失活突变的患者表现出范可尼贫血样症状，因此该基因也被命名为FANCW（FA Complementation Group W）。Inano等[63]进一步发现RFWD3在体外和体内多聚泛素化RPA和RAD51，促进其从DNA损伤位点及时去除以促进丝裂霉素C（Mitomycin C，MMC）诱导的同源重组。Dubois等[64]发现RPA磷酸化可以将Pre-MRNA加工因子19（Pre-MRNA-Processing Factor 19，PRP19）募集到RPA-ssDNA上并促进RPA-ssDNA上的RFWD3和PRP19的结合使其发生泛素化降解，从而促进ATR激活和同源重组。与这些观察结果一致，RFWD3可以以增殖细胞核抗原（Proliferating-Cell Nuclear Antigen，PCNA）依赖性方式稳定在复制叉处，使RPA泛素化并随后将其去除以促进DNA复制[65]。此外，RFWD3被ATR（可能还有ATM）磷酸化，这可能是其响应DNA损伤功能所必需的[56, 60]。起源识别复合体（Origin Recognition Complex，ORC）是DNA复制开始所需的复制前复合体的组成部分，ORC的

过表达导致RFWD3的稳定，同时，RFWD3以p53依赖性的方式稳定ORC[66]。RFWD3在多种肿瘤中发挥了重要作用，有研究表明RFWD3是多发性骨髓瘤和睾丸癌的易感位点[67, 68]。Zhang等[69]发现RFWD3可以促进非小细胞肺癌的增殖，并且在吸烟的肺癌患者中高表达。下调RFWD3的表达可以抑制胃癌的增殖和转移[70]。RFWD3可以通过与E2F转录因子1（E2F Transcription Factor 1，E2F1）相互作用来转录激活杆状病毒IAP重复5（Baculoviral IAP Repeat Containing 5，BIRC5），促进结直肠癌细胞的增殖和迁移[71]。

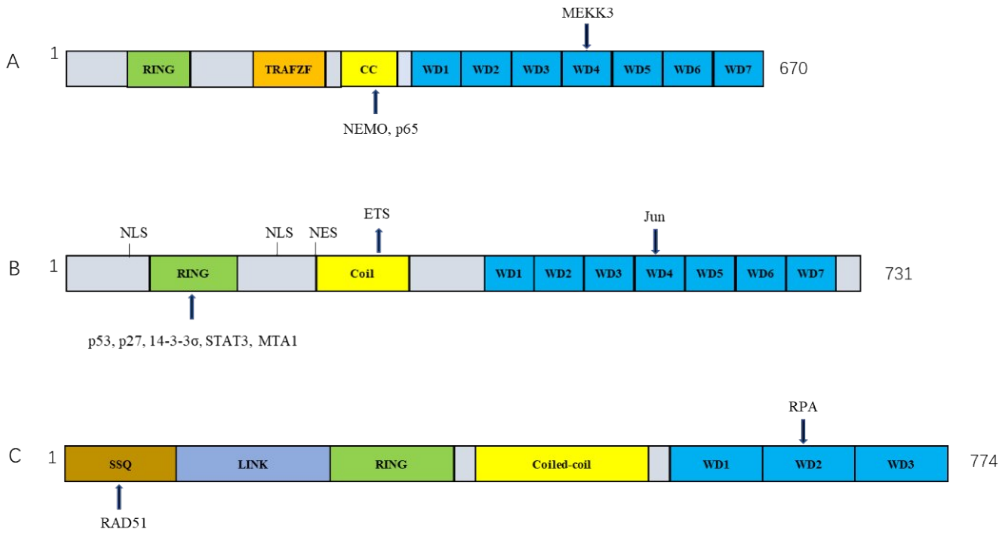


图1　RFWD蛋白结构域及与底物蛋白互作的结构域

5　RFWD蛋白作为治疗的靶点

E3泛素连接酶在许多生物学过程中发挥关键作用，一些E3泛素连接酶也被认为是有吸引力的治疗靶标。通过小分子或肽抑制致癌E3连接酶的可能途径包括抑制其表达、改变其亚细胞定位、抑制其与底物的相互作用、抑制其组装成多亚基复合物、抑制同源二聚化或异源二聚化以及抑制其催化结构域[72, 73]。基于蛋白结构的设计与先进的小分子筛选技术相结合，是目前用于开发E3泛素连接酶抑制剂的方法之一。许多E3泛素连接酶抑制剂的开发目前正在临床前和临床试验阶段进行评估，包括腺瘤性结肠息肉病毒基因产物APC[74, 75]、MDM2[76, 77]、S期激酶相关蛋白2（S-Phase Kinase-Associated Protein 2，SKP2）[78, 79]、斑点样POZ蛋白（Speckle-Type POZ Protein，SPOP）[80]和细胞凋亡抑制蛋白（cellular Inhibitor of Apoptosis Protein，cIAP）[72, 81]等。然而，开发蛋白质-蛋白质相互作用的调节剂并非易事，抑制E3泛素连接酶是目前药物开发中更具挑战性的领域之一。

在癌症治疗中靶向肿瘤抑制因子仍然具有挑战性。靶向肿瘤抑制性E3泛素连接酶的方法可能包括重新表达被抑制的E3（在没有失活突变或缺失的情况下）。通过利用协同致死性的原理来抑制下游致癌底物发挥作用（例如，在BRCA1缺陷或BRCA2缺陷型癌症[82]）。后者可以通过直接靶向单个致癌基因实现，例如，在F-box和WD重复包含结构域7（F-Box and WD Repeat Domain Containing 7，FBXW7）失活的癌症中靶向髓系细胞白血病1（Myeloid Cell Leukemia 1，MCL1）或更通用的蛋白水解靶向嵌合体技术方法，其中双功能分子用于引导其他E3泛素连接酶降解致癌底物[73, 83]。

越来越多的证据表明，RFWD蛋白在人类肿瘤的发生发展中起着重要作用，靶向RFWD可能成为一种新的癌症治疗方法。研究表明，p28是一种来自天青蛋白（azurin）的细胞穿透肽，它优先进入癌细胞，并与野生型p53或突变型p53蛋白的DNA结合域结合，这种特异性结合通过抑制RFWD2介导的泛素化和蛋白酶体降解，诱导细胞内p53水平在翻译后持续升高，从而导致细胞周期阻滞和癌细胞凋亡[84, 85]。重要的是，一项I期临床试验研究表明，在复发中枢神经系统肿瘤的儿童中，p28具有良好的耐受性，并在获得的12个癌症组织中的6个组织（50%）中引起肿瘤细胞核中p53的表达上调[86]。然而，直接靶向人类RFWD的小分子抑制剂尚未见报道。

值得注意的是，在将E3泛素连接酶作为治疗靶标时，必须考虑E3泛素连接酶的整体效应。首先，E3泛素连接酶可以作为肿瘤抑制因子或激活因子，并以一种依赖于基质和微环境的方式发挥作用；因此，靶向抑制这些因子需要在组织和肿瘤细胞的层面深入了解它们的作用。其次，特定的翻译后修饰可以将E3泛素连接酶从肿瘤抑制因子转化为肿瘤激活因子[87, 88]，反之亦然，突出了它们的功能和调控的复杂性。第三，由于E3泛素连接酶具有相似的催化结构域并可以同时靶向肿瘤促进蛋白和肿瘤抑制蛋白，理想的抑制剂是特异干扰E3泛素连接酶与抑癌蛋白的相互作用。了解E3泛素连接酶的结构以及调控E3的翻译后修饰将能更好地指导靶向这类蛋白质的药物的开发。

6 总结和展望

综上所述，RFWD蛋白家族通过促进下游底物的泛素化在癌症的发生发展中发挥双重作用。在某些RFWD过表达的癌症中，抑制RFWD可能是一种有效的治疗策略。然而，当RFWD在特定类型的肿瘤中表现出抑癌作用时，通过调节RFWD上游调控因子提高RFWD的表达水平可能有助于肿瘤治疗。

尽管一些研究已经确定了RFWD在某些类型人类癌症中的作用，但RFWD参与的致癌机制尚未完全阐明。为了解决这个问题，需要更先进的技术来发现促进肿瘤发生的RFWD的蛋白底物，同时需要合适的药物筛选方法来开发新的RFWD抑制剂或激动剂用于癌症治疗。

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