泛素特异性蛋白酶调控乳腺癌的研究进展

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摘要：乳腺癌作为女性最常见的恶性肿瘤，受到多种因素共同调节。泛素-蛋白酶体系统失调与乳腺癌发病和进展密切相关。其中，作为去泛素化酶家族的主要成员，泛素特异性蛋白酶(ubiquitin-specific proteases，USPs)大多在乳腺癌中过度表达，已成为乳腺癌疾病研究的潜在治疗靶点。目前，已有很多学者将USPs分子的靶向抑制剂研发作为乳腺癌抗癌药物研究的重要方向。本文总结了USPs不同成员在乳腺癌增殖、凋亡、迁移、药物疗效等进程中的进展，同时汇总了USPs靶向抑制剂的研发情况，以及抑制剂对乳腺癌的作用效果及机制，为开发疗效更好、选择性更佳的临床候选药物提供参考。

关键词：去泛素化酶；去泛素化酶抑制剂；乳腺癌；USPs

Research progress on ubiquitin-specific proteases in regulation of breast cancer

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Abstract: Breast cancer, as the most common malignancy in women, is regulated by multiple factors. Dysregulation of the ubiquitin-proteasome system has been reported to be closely associated with breast cancer pathogenesis and progression. Among them, ubiquitin-specific proteases, as the main members of the deubiquitinating enzyme family, are mostly overexpressed in breast cancer and have become potential therapeutic targets for breast cancer disease research. At present, many scholars have taken targeted inhibitors of USPs molecules as an important direction for exploring anticancer drugs for breast cancer. In this paper, we summarized the research progress of different members of USPs in breast cancer proliferation, apoptosis, migration, and drug efficacy. Besides, the research and development of USPs targeted inhibitors are summarized, as well as the effect and mechanism of inhibitors on breast cancer, providing a reference for the discovery of clinical candidates with better efficacy and selectivity.

Key Words: ubiquitin-specific proteases; USPs inhibitors; breast cancer; USPs

乳腺癌是女性最常见的恶性肿瘤，目前已成为世界第一大癌种[1]。证据表明，泛素-蛋白酶体系统的失调与乳腺癌发病和进展密切相关，在乳腺癌发生的各种细胞蛋白中发挥关键作用[2]。乳腺癌研究中常见的蛋白质，如S期激酶相关蛋白2、BRCA1、BARD1、Efp等，均为泛素化途径的主要

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参与者[3]。目前，已发现的去泛素化酶约有99种，根据去泛素化酶活性部位不同可分为7大家族，分别是泛素特异性蛋白酶家族(ubiquitin-specific proteases，USPs)、泛素羧基末端水解酶家族(ubiquitin C-terminal hydrolases，UCHs)、卵巢肿瘤相关蛋白酶家族(ovarian tumor-related proteases，OTUs)、MJD结构域蛋白酶家族(Machado-Joseph disease protein domainproteases，MJDs/Josephins)、含JAB1/PAB1/MPN结构域的金属蛋白酶家族(JAB1/PAB1/MPN domain-con-taining metallo-enzymes，JAMMs)和单核细胞趋化蛋白诱导的蛋白酶家族(mococyte chemotactic protein-induced protein，MCPIPs)，以及最近发现的锌指含泛素肽酶1。USP家族是目前已知数量最多、结构最多样的去泛素化酶，已成为众多疾病研究的潜在靶点。

1 USPs家族

USPs家族都具有高度保守的USPs结构域，由Finger、Thumb和Palm三个子域形成[4]。Finger结构域负责与远端泛素的相互作用，催化位点位于Thumb和Palm结构域之间。大多数USPs在泛素结合后具有典型构象变化的共同特征，驱动了从无活性形式向催化活性状态的转变[5]。2002年，Hu等[6]用HAUSP/USP7的催化核心解析了USPs蛋白的第一个X射线结构。2005年，USP14的45 kDa催化结构域的晶体结构被报道[7]。2018年，Ward等[8]报道了去泛素化酶USP15的结构，揭示了一个错位的催化三联体和一个开放的泛素结合通道。

2 USPs与乳腺癌

大多数USPs在乳腺癌中过表达并参与乳腺癌发生发展的进程，如USP1、USP4、USP7、USP9X、USP14、USP18、USP20、USP22、USP25、USP37、USP39、USP48等(表1)[9]。

2.1 USPs与乳腺癌疾病特征的相关性

多种泛素特异性蛋白酶被证明与乳腺癌疾病特征及预后相关。USP7的过表达与乳腺癌的组织学分级呈正相关[10]。金堃等[11]发现，USP9X的表达与乳腺癌的预后呈负相关。USP11被发现与ERα阳性乳腺癌患者的低生存率相关，同时与患者新辅助治疗的预后相关[12,13]。USP20高表达与ER阴性乳腺癌患者不良预后有关[14]。周方方等[15]发现，乳腺癌组织中USP20的阳性表达与乳腺癌分化程度、临床分期有密切关系。USP22是乳腺癌总生存期和无病生存期的独立预后因素[16]。USP28的过表达与浸润性导管乳腺癌患者更好的生存率相关[17]。USP37高表达与乳腺癌淋巴结分期、分子分型和增殖标志物Ki67正相关，并可以作为乳腺癌总生存、无复发生存时间和无转移生存时间的独立预后因子[18,19]。2022年，葛永利等[20]发现，USP48 mRNA水平和蛋白质水平在乳腺癌中下调，可能在乳腺癌中发挥抑癌基因作用，预后相关性仍需进一步研究。

表1 USPs在乳腺癌的表达情况及功能

USPs	表达情况	功能
USP1	高表达	促癌基因
USP2-69	高表达	促癌基因
USP4	高表达	抑癌基因
USP7	高表达	促癌基因
USP9X	高表达	促癌基因 抑癌基因
USP11	高表达	促癌基因
USP14	高表达	促癌基因
USP15	高表达	促癌基因
USP18	高表达	促癌基因
USP20	高表达	促癌基因
USP22	高表达	促癌基因
USP28	高表达	抑癌基因
USP32	高表达	促癌基因
USP33	高表达	抑癌基因
USP37	高表达	促癌基因
USP39	高表达	促癌基因
USP48	低表达	抑癌基因
USP51	高表达	促癌基因

2.2 USPs与乳腺癌增殖

USPs可通过多种机制影响乳腺癌细胞增殖。USP1被证明是一种新的调节因子，可通过提高TAZ蛋白稳定性增加乳腺癌细胞的增殖[21]。USP1的非基因组机制也可以通过稳定ERα蛋白促进乳腺癌进展[22]。刘君樱等[23]在乳腺浸润性导管癌组织中发现USP2-69表达升高，并与促进肿瘤细胞增殖能力密切有关。USP4被确定为PAK5-DNPEP通路

的下游靶点，支配乳腺癌细胞生长[24]。USP9X对乳腺癌发生的促进作用已在许多研究中得到证实，可能的信号通路包括Hippo通路[25]、Notch信号[26]、细胞周期蛋白依赖性通路[27]、Wnt信号[28]。USP11可通过调节XIAP促进乳腺肿瘤恶性表型[29]。USP14被证明参与乳腺癌的进展，促进乳腺癌细胞增殖，此外USP14的AR去泛素化对于乳腺癌生长同样至关重要[30]。作为阻止ERα降解的新型保护剂，USP15是乳腺癌进展的关键驱动因子[31]。USP18可通过上调EGFR和激活AKT/Skp2通路促进乳腺癌生长[32]。USP39表达下调显著降低了三阴性乳腺癌细胞的增殖和集落形成能力[33]。

2.3 USPs调控乳腺癌细胞周期和细胞凋亡

乳腺癌细胞周期和细胞凋亡也受多种USPs调节。Hayal等[34]发现，抑制USP7活性增强了乳腺癌细胞系的凋亡基因表达。此外，ERα的过表达挽救了USP7沉默诱导的细胞周期停滞和凋亡，提示ERα状态对USP7在乳腺癌发生中的功能至关重要[35]。USP14可以通过调控CyclinB1的泛素化来调控乳腺癌细胞的细胞周期[36]。2022年，杨红星等[37]发现，USP22在乳腺癌组织中高表达，并通过调节BMI1表达调控乳腺癌恶性细胞的生物学行为。荣欣欣等[38]发现，在乳腺癌泛素蛋白酶体系统中，USP25功能作用明显增强，敲减*USP25*可显著提高乳腺癌细胞的凋亡水平。

2.4 USPs与乳腺癌侵袭、迁移

乳腺癌是一种侵袭性强、转移性强的恶性肿瘤，能够通过多种途径实现乳腺癌细胞向其他组织的转移。在乳腺癌中，敲减*USP1*可通过KPNA2抑制乳腺癌转移[39]。对于三阴性乳腺癌，USP1可通过稳定TAK1促进TGF-β诱导的迁移[40]。盛智梅等[41]证明，miR-4319通过靶向结合USP2抑制NF-κB信号通路，从而抑制乳腺癌细胞的侵袭。USP4是TGF-β和AKT信号通路的重要调节因子，Relaxin/TGF-β1/Smad2/MMP-9信号可能是USP4促进乳腺癌侵袭的机制[42]。曾响等[43]发现，USP4可通过其去泛素化酶的活性解除TGF-βRI耦联的泛素链，稳定TGF-βRI的表达，从而增强乳腺癌细胞的迁移能力。Garcia等[44]证明，USP11可改变TGF-β下游信号，促进人乳腺癌转移。荣欣欣等[45]发现，USP25与乳腺癌细胞的侵袭与转移能力有关，其在乳腺癌发展中可能扮演着重要角色。USP33被发现过表达并抑制乳腺转移[46]。USP51被发现是CDK4/6的真实靶点，CDK4/6-USP51-ZEB1轴在乳腺癌转移中起关键作用[47]。

2.5 USPs与乳腺癌耐药

乳腺癌化疗耐药是影响患者化疗效果的重要原因，寻找有效化疗药物敏感性的独立预测因子十分关键。据报道，USP4可参与乳腺癌进展并与他莫昔芬耐药相关[48]。USP7与紫杉类药物反应相关，USP7蛋白是紫杉药物敏感性的潜在预测因素[49]。受USP7影响的Aurora-A激酶的稳定性可能是调节有丝分裂进程和紫杉药物敏感性的潜在机制[50]。此外，USP9X还参与乳腺癌耐药，去泛素化酶USP9X的功能丧失可阻止他莫昔芬的作用[51]。USP9X抑制可增加雌激素受体阴性乳腺癌细胞对顺铂的敏感性[52]。*USP9X*敲低显著增强了对PARP抑制剂奥拉帕尼和甲磺酸甲酯的敏感性[53]。USP9X可以作为TRAIL耐药乳腺癌的治疗靶点，USP9X-YAP1轴可能是提高细胞对化疗敏感性的重要调节机制[54]。USP14抑制可通过AR相关信号通路增强乳腺癌对恩扎卢胺的敏感性，包括Wnt/β-catenin和PI3K/AKT通路[55]。癌症相关*USP15*突变可降低USP15-BARD1相互作用，增加癌细胞对PARP抑制剂的敏感性[56]。USP22的去泛素化活性是其维持ERα的稳定性功能所必需的，从而增强ERα的作用，赋予乳腺癌内分泌抵抗[57]。USP37可降低乳腺癌细胞对顺铂的敏感性[58]。敲除*USP37*基因表达可逆转乳腺癌细胞对阿霉素的耐药性，下调USP37可能是乳腺癌治疗中对抗多药耐药性的一种有价值的策略[59]。沈俐萍等[60]研究发现，曲妥珠单抗治疗后乳腺癌中USP39的mRNA表达量显著低于治疗前，提示USP39具有预测曲妥珠单抗治疗的潜在价值。

3 USPs抑制剂在乳腺癌中的研究进展

泛素特异性蛋白酶抑制剂可通过阻断USPs的去泛素化调控靶蛋白，进而影响机体的抗炎、抗病毒、抗肿瘤作用。目前众多研究者致力于开发新型USPs抑制剂，然而，2014年前报道的USP抑制剂的发现主要依靠高通量筛选。近来，基于USPs-抑制剂复合物的共晶结构进行结构引导的药

物设计成为主流。USPs抑制剂开始逐渐出现，已有超过60种USPs抑制剂被报道，其中b-AP15和VLX1570正在进行多发性骨髓瘤治疗的临床试验[5]。USPs抑制剂在乳腺癌中的研究亦有报道，但尚无USP抑制剂获批用于临床(表2)。

3.1 USP1抑制剂

USP1抑制剂用于乳腺癌研究的主要有Pimozide、N-苄基-2-苯基嘧啶-4-胺的衍生物ML323、Trifluoperazin和Rottlerin。Pimozide作为一种潜在的抗癌治疗已被广泛研究，在黑色素瘤、中枢神经系统肿瘤、骨肉瘤、神经母细胞瘤、骨髓增殖性肿瘤、乳腺、肺、前列腺、卵巢、结直肠、胰腺和肝细胞癌中显示了令人满意的结果[61]。早在1992年，Pimozide被认为是他莫昔芬耐药后的替代品用于乳腺癌治疗[62]。在三阴性乳腺癌中，Pimozide可通过磷酸化STAT3显著降低乳腺癌细胞侵袭和迁移[63]。ML323是目前已知性能最优的USP1抑制剂，对非小细胞肺癌和骨肉瘤效果良好。但是关于ML323在乳腺癌中的研究报道有限，KPNA2可能是ML323抑制乳腺癌转移的靶点[39]。Trifluoperazin可通过诱导G_0/G_1期阻滞和细胞凋亡抑制三阴性乳腺癌肿瘤生长和脑转移[64]。Rottlerin可以在乳腺癌细胞中表现出抗血管生成作用[65,66]。对于乳腺癌干细胞，Rottlerin诱导的自噬会导致细胞凋亡[67,68]。

3.2 USP2抑制剂

目前，只有2个USP2抑制剂被报道用于乳腺癌研究。6-硫鸟嘌呤可选择性杀死BRCA2缺陷肿瘤，克服PARP抑制剂耐药性[69]。BRCA1缺陷型乳腺癌细胞系对6-TG具有不同的敏感性[70]。6-TG在三阴性乳腺癌中的功能与lncRNA有关[71]。竞争性内源性RNA分子和差异表达基因可能参与了6-TG抑制MCF-7生长的机制[72,73]。另一种USP2抑制剂ML364可使HER2阳性乳腺癌细胞对HSP90抑制敏感[74]。

3.3 USP7、USP7/47抑制剂

USP7抑制剂costunolide可显著抑制乳腺癌生长和转移，可能是十分有前景的抗癌药物，尤其是用于转移性乳腺癌[75]。通过靶向细胞周期调控，costunolide能有效诱导乳腺癌细胞凋亡[76]。Costunolide和脱氢木香内酯联合治疗可通过诱导细胞周期阻滞和凋亡抑制乳腺癌[77]。USP7/47抑制剂P5091可降低MCF-10A细胞EMT标志物的表达，逆转EMT表型[78]。对于MCF7和T47D乳腺癌细胞

表2　USPs抑制剂

USPs	化合物	乳腺癌分子分型	研究进展	信号通路
USP1	Pimozide	ER阴性乳腺癌 三阴性乳腺癌	体内、体外	细胞周期、AKT、EMT、MMP-9、vimentin、STAT3
	Trifluoperazin	三阴性乳腺癌	体内、体外	G_0/G_1阻滞、cyclinD1/CDK4，cyclinE/CDK2
	Rottlerin	ER阴性乳腺癌 三阴性乳腺癌	体外	NF-κB、cyclinD-1、p38MAPK、AMPK、Skp2、
	ML323	乳腺癌	体内、体外	KPNA2
USP2	6-TG	BRCA2缺失PARP抑制剂耐药乳腺癌 BRCA1突变乳腺癌 三阴性乳腺癌	体内、体外	DNA修复、PI3K-AKT、细胞凋亡、lncRNA-miRNA-mRNA、ceRNA、DNMT1
	ML364	ER阳性乳腺癌	体外	酶促降解
USP7	Costunolide	转移三阴性乳腺癌 乳腺癌	体外	NF-κB、细胞周期、c-Myc/p53、AKT/14-3-3、p38MAPK
USP7/47	P5091	乳腺癌	体外	EMT
USP14	b-AP15	ER阳性乳腺癌 三阴性乳腺癌	体内、体外	自噬、ERα
	IU1	AR阳性乳腺癌	体内、体外	Wnt/β-catenin、PI3K/AKT
	Auranofin	ER阳性乳腺癌 三阴性乳腺癌	体内、体外	PTGR1、ERK1/2-MYC、p38MAPK、线粒体凋亡
USP9x	WP1130	ER阴性乳腺癌	体外	Mcl-1

系，P5091阻断去泛素化可以减少细胞增殖、细胞迁移、克隆形成和球体播散[34]。

3.4 USP9x抑制剂

USP9x抑制剂很少被报道。WP1130被发现可增强ER阴性乳腺癌细胞中顺铂的细胞毒性。同时，WP1130联合治疗可增加雌激素受体阴性乳腺癌细胞对顺铂的敏感性，呈USP9x依赖性[52]。

3.5 USP14抑制剂

USP14抑制剂b-AP15可以抑制MCF-7乳腺癌细胞系的肿瘤进展[79]。2015年，有研究证明了b-AP15和RA-9对三阴性乳腺癌细胞系的作用[80]。此外，b-AP15和PtPT可能具有治疗雌激素受体阳性乳腺癌的潜力[81]。USP14抑制剂Auranofin对乳腺癌抑制表现出协同作用。Auranofin和维生素C联合用药可以有效地对抗三阴性乳腺癌，表现出协同和过氧化氢介导的细胞毒性[82]。Auranofin和抗PD-L1抗体联合治疗三阴性乳腺癌有效，Auranofin和曲美替尼的协同作用可作为乳腺癌的一种新的治疗策略[83]。此外，另一种USP14抑制剂IU1在体外和体内乳腺癌细胞系中具有增强恩杂鲁胺抑制细胞生长和诱导细胞凋亡的能力[55]。

4 结论

USPs是乳腺癌研究中具有潜力的新型靶点，参与许多重要的信号通路，包括ERα信号通路、Hippo信号通路、TGF-β信号通路、PI3K/AKT信号通路、Notch信号通路等。USPs作为乳腺癌的潜在治疗靶点越来越受到关注，其抑制剂开始逐渐出现。尽管目前还没有USP抑制剂被批准用于临床，但生物学疗效表明了它们在乳腺癌治疗中的潜力。

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