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CDK4/6抑制剂在HR+/HER-2−乳腺癌中耐药机制及生物标志物研究进展

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摘 要

CDK4/6抑制剂(CDK4/6i)联合内分泌治疗是激素受体阳性、HER-2阴性(HR+/HER-2−)晚期乳腺癌的主要治疗手段。然而，无论是原发性还是获得性的耐药性总是会发生，导致治疗失败和癌症进展。随着CDK4/6抑制剂应用的推广，研究者发现其并非对所有ER+乳腺癌患者有效，缺乏可靠的预测疗效或筛选患者群体的生物标志物是限制其临床应用的主要挑战。本文将对CDK4/6抑制剂在乳腺癌治疗中的耐药机制和对CDK4/6抑制剂生物标志物进行综述。

关键词

乳腺癌，CDK4/6抑制剂，耐药，生物标志物

Research Progress on Resistance Mechanism and Biomarker of CDK4/6 Inhibitor in HR+/HER-2− Breast Cancer

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Abstract

CDK4/6 inhibitor (CDK4/6i) combined with endocrine therapy is the primary treatment for hormone receptor-positive, HER-2-negative (HR+/HER-2−) advanced breast cancer. However, drug

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resistance, whether primary or acquired, always occurs, leading to treatment failure and cancer progression. As the use of CDK4/6 inhibitors has expanded, researchers have found that they are not effective in all patients with ER+ breast cancer, and the lack of reliable biomarkers to predict efficacy or to screen patient populations is a major challenge limiting their clinical use. In this paper, we will review the latest research progress of the mechanism of CDK4/6 inhibitor resistance in breast cancer treatment and CDK4/6 inhibitor biomarkers.

Keywords

Breast Cancer, CDK4/6 Inhibitor, Resistance, Biomarkers

1. 引言

乳腺癌是全世界女性中最常见的恶性肿瘤之一，也是大多数癌症相关死亡的主要原因[1]。在所有乳腺癌中，雌激素受体阳性(ER+)和 HER-2 阴性肿瘤所占比例最大，约为 65%~70% [2]，因此，内分泌治疗是乳腺癌有效的治疗方式。细胞周期蛋白依赖性激酶(Cyclin dependent kinase, CDK) 4/6 抑制剂联合内分泌治疗能显著提高 ER+/HER-2−乳腺癌患者的无进展生存期(PFS) [3]。因此，CDK4/6 抑制剂成为乳腺癌治疗的重要靶点之一。CDK4/6 抑制剂(Palbociclib、Ribocilib 和 Abemaciclib)用于 ER+/HER-2−乳腺癌患者的一线治疗现已被美国食品药品监督管理局批准[4] [5] [6]。

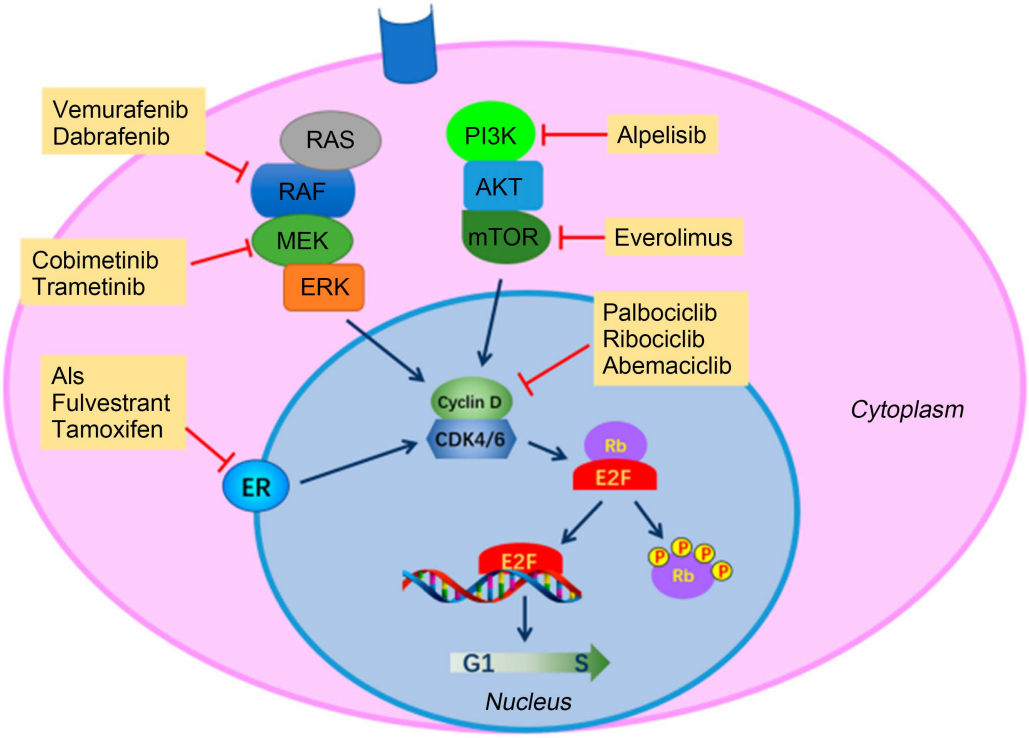
然而由于原发或获得性耐药的产生，CDK4/6 抑制剂在临床中的应用受到了很大限制，且目前尚缺乏能够预测其治疗效果的可靠生物标志物。精准医学时代中，基于生物信息学、组织芯片和二代测序等技术手段筛选预测标志物，有助于准确预测疗效，对实现个体化治疗和提示临床治疗效果具有重要意义。本文主要综述了 CDK4/6 抑制剂在乳腺癌治疗中的耐药机制和 CDK4/6 抑制剂的疗效预测生物标志物。

2. CDK4/6 抑制剂在乳腺癌中的作用及机制

细胞分裂是正常细胞在严格控制下的常见细胞过程，以防意外增殖，这通常是癌症的原因。参与细胞周期调控的途径很多，CDK 家族是细胞分裂调控中最重要的蛋白家族之一[7] (见图 1)。在细胞周期的 G1 期，CDK4/6 与细胞周期素 D (Cyclin D)相互作用形成 Cyclin D-CDK4/6 复合物，使 Rb 蛋白磷酸化[8] [9]。失活的 Rb 与转录因子 E2F 紧密结合，Rb 的磷酸化从 Rb-E2F 复合物中释放 E2F，随后诱导 E2F 靶基因上调，启动 DNA 合成，导致细胞周期进入 S 期[9] [10] [11]。

在乳腺癌和其他恶性肿瘤中，观察到 Cyclin D1-CDK4/6-Rb 信号级联的异常调节，并促进了不受控制的细胞增殖[12] [13]。近 15%的乳腺癌被检测到 *Cyclin D* 基因 *CCND1* (*Cyclin D1*)的扩增，并且研究发现在高达 50%的原发性 ER+乳腺癌和高分化肿瘤中 *Cyclin D1* 在 mRNA 和蛋白水平上的表达上调[14] [15]。在乳腺癌细胞系中，*Cyclin D* 的诱导启动了细胞周期过程，并增加了从 G1 期到 S 期的细胞数量[16]，体内研究表明，*Cyclin D* 过表达使得转基因小鼠乳腺细胞发生异常增殖，促进了乳腺癌的发生发展[17]。在许多癌症中，*Cyclin D1* 的过度表达与预后不良有关，并且常常与转移增加有关[14] [18] [19]。同样，散发性乳腺癌中 *CDK4* 的过度表达与肿瘤细胞的高增殖能力呈正相关[20]。*CDK6* 在 5 个鳞癌细胞系中活性升高[21]，抑制 Cyclin D3-CDK6 导致肿瘤细胞凋亡[22]。所有这些证据表明，CDK4/6 和 Cyclin D 可能

是肿瘤治疗的潜在靶点。



图注：*MAPK*、*PI3K* 和 ER 等上游信号通路的激活促进了细胞周期蛋白 D-CDK4/6 复合体的形成，使 Rb 蛋白磷酸化。随着 Rb 的磷酸化，E2F 从 Rb-E2F 复合物中解离出来。作为一种转录因子，释放的 E2F 启动 DNA 合成，导致细胞周期从 G1 期进入 S 期。CDK4/6 抑制剂(Palbociclib、ribociclib 和 abemaciclib)可阻止 CDK4/6 的激活，导致细胞周期阻滞于 G1 期[7]。

Figure 1. Mechanism of CDK4/6 inhibitors and possible combined therapy with CDK4/6 inhibitors
图 1. CDK4/6 抑制剂的作用机制及可能与 CDK4/6 抑制剂的联合治疗

3. CDK4/6 抑制剂的耐药机制

CDK4/6 抑制剂的发现改善了 ER+/HER-2−乳腺癌的预后，也可能使 HER-2+乳腺癌和其他实体肿瘤受益。然而，并非所有患者对 CDK4/6 抑制剂都有反应，甚至对 CDK4/6 抑制剂敏感的患者也可能产生耐药[23]。目前 CDK4/6 抑制剂的耐药机制尚不清楚。

3.1. *Rb* 的缺失

成视网膜细胞瘤基因(*Retinoblastoma gene*, *Rb*)的缺失已经成为一种内在和获得性耐药的机制。*Rb* 作为 CDK4/6 的靶点，被认为是 CDK4/6 靶向治疗敏感性的最重要的生物标志物之一[24]。临床前研究证明，在接受帕博西尼(Palbociclib)或瑞博西尼(Ribociclib)治疗的晚期乳腺癌患者中，*Rb* 功能丧失[25]。随着疾病的进展，检测到 *Rb1* 体细胞突变[26]，提示 *Rb* 突变可能与对 CDK4/6 抑制剂的获得性耐药有关。然而，在 Paloma-3 试验中，研究者并未发现 CDK4/6 抑制剂与 *Rb1* 表达之间的相互作用，因此需要对 *Rb* 通路进行更广泛深入的分析。

3.2. *Cyclin E1*、*CDK2*、*CDK4*、*CDK6* 的过表达

除 Cyclin D-CDK4/6 复合物外，Cyclin E-CDK2 复合物也可通过磷酸化 *Rb* 释放 E2F [27]，从而使肿

瘤细胞产生耐药性。*Cyclin E1* 是 CDK2 的一个调节亚单位，在 G1/S 检查点启动 DNA 复制的过程中起着中心作用。由于 Cyclin E-CDK2 磷酸化事件位于 Cyclin D-CDK4/6 介导的磷酸化事件的下游，*Cyclin E1* 的过表达使得 CDK4/6 的抑制在诱导 G1 期阻滞或随后的生长抑制方面无效。因此，*Cyclin E1* 水平高的肿瘤很可能对 CDK4/6 的抑制具有内在的抵抗力[28]。

作为促进细胞周期从 G1 期进入 S 期的三种间期 CDK 之一，*CDK4* 是一个公认的原癌基因[29]。在有丝分裂刺激下，*CDK4* 和 *CDK6* 与 Cyclin D 形成活性复合物，通过直接磷酸化启动 *Rb* 和相关蛋白的失活[30]。*Rb* 蛋白的磷酸化导致它们从转录抑制复合物中解离，从而激活依赖于 E2F 的基因表达，从而促进细胞周期的 G1-S 期转变，并最终推动增殖[29] [30]。

3.3. *p16* 扩增

p16INK4A 是一种固有的肿瘤抑制因子，可以与 CDK4/6 结合，破坏 Cyclin D-CDK4/6 复合物的形成[31] [32] [33]。在致癌应激过程中观察到 *p16* 的过度表达。当 *p16* 过表达与 *Rb* 缺失同时发生时，由于 *Rb* 功能障碍，获得了对 CDK4/6 抑制剂的耐药性[34]。在 *Rb* 存在的情况下，*p16* 的过表达由于 CDK4 的降低而表现出对 CDK4/6 抑制剂的耐药性[35]。

3.4. 血清 TK1 水平

胸苷激酶-1 (Thymidine kinase 1, TK1)是细胞周期的关键调节因子，在 S/G2 期高表达，催化 DNA 前体合成[36]。血清 TK1 水平和活性在实体肿瘤中升高，包括乳腺癌、肺癌和结直肠癌[37]。在乳腺癌患者中，高 TK1 水平和活性与肿瘤大小和预后不良有关[38] [39]。在 HR+/HER-2−转移性乳腺癌(HR+/HER-2−MBC)患者中，基线 TK1 活性降低与 PFS 延长相关，治疗 1 个月后 TK1 活性降低与 PFS 明显改善相关[40]，提示 TK1 是 HR+/HER-2− MBC 中有意义的潜在治疗靶点。ECLIPS 是一项前瞻性的药物遗传学研究，旨在确定对 Palbociclib 加 ET (来曲唑或氟维司群)敏感/耐药的预测性生物标志物。结果表明，进展期患者治疗前 TK1mRNA 拷贝数/mL 明显高于治疗 3 个月后(1200 vs 3350 拷贝/mL，P = 0.01)，提示 TK1mRNA 拷贝数/mL 与 CDK4/6 抑制剂获得性耐药有关[41]。

3.5. *FAT1* 的缺失

FAT1 是一种潜在的肿瘤抑制因子，是与河马信号通路相互作用的钙粘蛋白超家族成员之一，最近发现它可以调节 *CDK6* 的表达，其缺失可能介导了对 CDK4/6 抑制剂的耐药性。*FAT1* 基因敲除导致河马信号通路下调和 *CDK6* 过度表达。对 348 例接受基于 CDK4/6 抑制剂治疗的患者的活检组织进行基因测序显示，在这些患者中，大约有 6%的患者 *FAT1* 发生了突变。因此，*FAT1* 缺失可能是 CDK4/6 抑制剂耐药的有效预测因子[42]。

3.6. *FGFR* 的扩增

成纤维细胞生长因子受体(fibroblast growth factor receptor, FGFRs)家族包括四个高度保守的跨膜受体酪氨酸激酶(FGFR1-4)和一个能够结合成纤维细胞生长因子配体(FGFs)但缺乏细胞内激酶结构域的受体(FGFR5，也称为 FGFRL1)。FGF 介导重要的生理机制，如组织和新陈代谢的动态平衡、内分泌功能和伤口修复。在许多肿瘤类型中，FGF 信号轴的失控与肿瘤发生、肿瘤进展和抗癌治疗耐药有关。尽管已有多项研究提出异常的 FGFR 信号通路可作为各种肿瘤的潜在治疗靶点，但临床上抗 *FGFR* 治疗的疗效各不相同[43]。

据报道，在激素受体阳性的乳腺癌中有近 15%发生 *FGFR1* 扩增，并且与不良预后有关。*FGFR1* 与 CDK4/6 抑制剂的耐药性以及内分泌抵抗有关。*FGFR1* 扩增通过持续的 MAPK 活化诱导内分泌抗性，通

过激活 PI3K-AKT 和 RAS/MEK/ERK 信号通路，并以 ER 依赖性和独立的方式促进 *CCND1* 表达导致对 CDK4/6 抑制剂产生耐药性[43]。

3.7. *PTEN* 的缺失

PTEN 是指人第 10 号染色体缺失的磷酸酶及张力蛋白同源的基因，其缺失可导致对 CDK4/6 抑制剂的原发和获得性耐药。从机制上讲，*PTEN* 的缺失使 p27 (细胞周期素依赖性激酶抑制物)被排除在细胞核外，进而导致 CDK4 和 CDK2 的激活增加，最终通过维持 Rb 的磷酸化来降低对 CDK4/6 抑制剂的敏感性，从而减轻细胞周期停滞[44]。

3.8. PI3K/AKT/mTOR 通路

在 ER+乳腺癌中，mTOR 通路经常被过度激活，丝氨酸/苏氨酸激酶 mTOR 整合了多种细胞信号，包括有丝分裂原和营养信号，以控制细胞增殖、细胞周期和细胞大小。mTOR 激酶形成两个不同的多蛋白复合物，称为 mTORC1 和 mTORC2。调节 mTOR 的输入信号之一是 PI3K/AKT 通路，它可以激活 mTORC1 复合体。CDK4/6 抑制剂通过磷酸肌醇依赖性蛋白激酶 1 (PDPK1)使 AKT 磷酸化，并激活 Ribociclib 耐药乳腺癌细胞中 PI3K/AKT 通路，*PI3K* 能使 *Cyclin D1* 的表达增加，从而使乳腺癌细胞对 CDK4/6 抑制剂产生耐药性[45]。

此外，研究还发现 CDK4/6 抑制剂耐药细胞株重新激活了 CDK-Rb-E2F 通路，但对 mTORC1/2 抑制仍然敏感，这表明 mTORC1/2 抑制剂可能是 CDK4/6 抑制剂耐药患者的一种选择[45]。这些证据表明 PI3K/AKT/mTOR 通路与 CDK4/6 抑制剂耐药有关，针对这两个通路的联合策略可能是有效的[46]。

4. CDK4/6 抑制剂的疗效预测生物标志物

由于晚期乳腺癌患者转移病灶活检的困难、潜在风险和患者不适，循环生物标记物在非侵入性识别耐药患者、监测治疗效果和潜在指导后续治疗方面具有巨大潜力。HR 阳性和 HER-2 阴性是目前临床上用于选择 CDK4/6 抑制剂治疗患者的唯一肿瘤生物标志物。

Rb 是 CDK4/6 的主要磷酸化靶点，磷酸化 Rb (Prb)的存在是一个重要的生物标志物。对于 Paloma-2 和 Paloma-3 研究的分析表明，Cyclin E/CDK2 复合体也可能是有用的生物标志物，在接受 CDK4/6 抑制剂治疗的患者(来自 Paloma-3 的队列)中，Cyclin E1 mRNA 的高表达与较短的 PFS 相关，而在之前未治疗的患者(来自 Paloma-2 的队列)中，Cyclin E1 mRNA 的高表达与较短的 PFS 无关。p16 扩增作为生物标志物的作用仍然存在争议，因为对 Paloma-1、Paloma-2 和 Paloma-3 的生物标志物分析结果显示，*p16/CCND1* 队列中的 PFS 与未选择的队列相比没有显著差异[47] [48] [49]。

正在进行的 IIIb 期试验 Bioitalee (NCT03439046)正在研究 ctDNA 的改变及其在使用 CDK4/6 抑制剂和来曲唑作为第一线治疗期间的演变：在参与研究的 287 名绝经后患者中，有 271 名患者的样本适合进行初步的生物标志物分析。研究发现，改变最频繁改变的基因是 *PIK3CA* (22.14%)、*TP53* (15.50%)、*FGFR1* (6.64%)、*CDK4* (3.69%)、*AKT1* (3.32%)、*PTEN* (3.32%)、*ERBB2* (2.58%)、*CCND3* (2.58%)、*APC* (2.21%)和 *MAP2K4* (2.21%)。28%的患者出现一种以上基因改变。提示这些生物标志物可能是对 CDK4/6 抑制剂和来曲唑一线治疗产生内在耐药性的潜在标志。然而，最终的生物标记物动力学和药物基因组学分析仍在进行中。

5. 总结与展望

CDK4/6 抑制剂的临床应用代表着 HR+/HER-2−乳腺癌治疗的重大进展。这些药物在改善临床结果方面是有效的，但固有或获得性耐药的发展可能会限制这些治疗的疗效。目前，临床研究的重点是研究

CDK4/6 抑制剂的敏感性或耐药性机制，以及旨在改善临床结果的新的治疗策略。尽管在鉴定 CDK4/6 抑制剂耐药的潜在基因组驱动因素方面做出了显著而集中的努力，但到目前为止，这些标记都没有显示出临床实用价值。生物标记物前景看好，但需要进一步验证。

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