液体肿瘤生物指标检测对乳腺癌患者的临床价值

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［摘要］ 循环肿瘤标志物在乳腺癌中的应用受到越来越多的关注，研究水平已从蛋白质水平深入到基因水平，相应的检测指标则从传统的肿瘤标志物扩展到相对特异的HER-2蛋白质细胞外段、循环肿瘤细胞、循环肿瘤DNA及循环RNA等。作为“液体检测”，循环肿瘤标志物的检测因其实时动态、操作简便、可重复性好等优势，被广泛应用于协助早期诊断、判断预后、预测疗效、监测疾病复发及病情变化等方面。对循环肿瘤标志物的深入研究，有助于实现患者的个体化治疗。

［关键词］ 乳腺癌；CA15-3；CEA；蛋白质细胞外段；循环肿瘤细胞；循环肿瘤DNA；循环肿瘤RNA

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［**Abstract**］ Circulating tumor markers have been paid more attention in the application of the treatment for breast cancer, the level of which has extended from protein to gene, including traditional tumor markers, HER-2 extracellular domain, circulating tumor cells, circulating tumor DNA (ctDNA), circulating RNA (ctRNA) and so on. As “liquid detection”, the detection of circulating tumor markers with real-time dynamic, easy operation, good reproducibility and other advantages are widely used in aiding early diagnosis, determining prognosis, prospectively predicting response or resistance to specific therapies, surveillance after primary surgery, and monitoring therapy in patients with advanced disease, The further study of circulating tumor markers may contribute to patient’s individual treatment.

［**Key words**］ Breast cancer; CA15-3; Carcino-embryonic antigen; Extracellular domain; Circulating tumor cells; Circulating tumor DNA; Circulating tumor RNA

目前乳腺癌是女性最常见的恶性肿瘤，致死率高，因此其早期诊断、有效随访及疗效的动态监测一直是临床科研工作者的研究重点。同时乳腺癌是一种高度异质性的恶性肿瘤，随着蛋白质组学、分子病理学及基因组学的发展进步，乳腺癌的诊疗已进入分子分型指导下的个体化诊疗时代。循环血中的肿瘤细胞及其释放的蛋白、基因片段等，作为循环肿瘤标志物可实时动态反映肿瘤负荷，同时携带大量肿瘤生物学行为相关信息，具有预后、预测价值。鉴于其重要的临床价值及操作简便、可重复等优势，目前液体肿瘤生物指标检测在各阶段乳腺癌诊疗中的应用日益广泛。其检测指标主要包括传统的血清标志物CA15-3、CEA，相对特异的蛋白质细胞外段(extracellular domain，ECD)、循环肿瘤细胞(circulating tumor cells，CTCs)、循环肿瘤DNA(circulating tumor DNA，ctDNA)及循环肿瘤RNA(circulating tumor RNA，ctRNA)等，从蛋白水平的检测发展到基因水平的研究，液体肿瘤生物指标检测的临床价值受到越来越多的关注，而不同检测指标的应用价值因自身的特异性及灵敏性而各不相同。

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1 CA15–3、CEA的临床价值

CA15-3、CEA作为乳腺癌最传统的血清标志物，其检测简便易行，是目前临床上应用最广泛的动态检测指标。Lee等[1]的研究指出CA15-3、CEA是乳腺癌患者的独立预后因素。Bahrami等[2]的研究显示，患者术后血清CA15-3、CEA水平的动态监测能预测复发转移，且有研究[3]表明，给予仅有癌标升高、无影像学复发证据的患者干预治疗，能显著改善预后。但由于二者检测的敏感性和特异性较差，限制了其在乳腺癌早期诊疗阶段的应用[4]。CA15-3、CEA在晚期乳腺癌患者的阳性检出率较高，大量研究表明二者检测水平的动态变化与治疗疗效具有相关性，在无目标病灶者中的临床价值更大，且CEA的临床价值在CA15-3检测值正常者中更为显著，但由于二者的检测值存在“闪烁”现象，目前多数专家共识建议其与其他临床指标联合应用，综合判定患者的病情变化[4]。

2 HER–2 ECD临床价值

HER-2阳性乳腺癌是乳腺癌的一种特殊亚型，其肿瘤侵袭性强、易复发，多对化疗及内分泌治疗耐药，但可从靶向治疗中获益。目前临床上一般依据组织学HER-2状态选择靶向治疗的适用患者群，但由于原发或继发耐药的存在，几乎所有患者均不能从靶向治疗中持续获益，因此探索动态预测靶向治疗疗效的指标成为研究热点。HER-2 ECD在金属蛋白酶的作用下脱落到外周血中，能被ELISA方法定量检测，是乳腺癌相对特异性的指标，能动态反映乳腺癌患者的病情变化，因此其临床价值受到越来越多的关注。

Pedersen等[5]的研究表明，对组织学检测HER-2阳性患者而言，ECD预测复发的敏感性优于CA153、CEA，但尚无基于ECD检测值升高而给予预先治疗致临床获益的相关研究报道。就曲妥珠单抗靶向治疗而言，目前相对一致的观点认为治疗过程中ECD下降者疗效较好，但ECD下降的定义尚不明确；而关于ECD基线检测值对靶向治疗疗效预测的价值尚无定论[6-8]。就ECD对拉帕替尼治疗疗效的预测作用而言，Lipton等[9]的研究表明ECD基线检测值高者客观有效率(objective response rate，ORR)高，治疗早期ECD检测值下降超过20%者ORR升高、无进展生存时间(progression free survival，PFS)延长。同时部分学者探讨了ECD检测值高水平对组织学HER-2阴性患者靶向治疗疗效的预测作用。Ardavanis等[10]的研究表明多重解救治疗后、组织学检测HER-2阴性而ECD检测值呈高水平的患者可能从曲妥珠单抗治疗中获益。Finn等[11]的研究显示组织学检测HER-2阴性、ECD高表达的患者未能从拉帕替尼治疗中获益。此外ECD检测对解救化疗、内分泌治疗同样具有预测价值。绝大多数研究认为，接受解救化疗且ECD基线检测值高的患者PFS、缓解持续时间(duration of response，DOR)较短；同时大部分研究结果显示，ECD基线检测值高的患者ORR低[12-13]。研究关于ECD检测对解救内分泌治疗疗效预测作用的结论相对一致：ECD基线检测值高的患者ORR低，DOR、疾病进展时间(time to progression，TTP)、OS较短[14]。

3 CTCs的临床价值

CTCs是指从癌症原发部位脱落通过血管或淋巴系统进入血液循环的肿瘤细胞。国内外研究先后证实了CTCs计数、CTCs HER-2对乳腺癌患者的预后、预测价值。相较于既往传统指标，CTCs能更好地反映肿瘤的生物学特性，近年来其分子、基因特性成为研究重点[15-19]。

有研究表明辅助治疗前后表达细胞角蛋白19信使RNA(cytokeratin-19 mRNA，CK-19 mRNA)的CTCs计数高者DFS、OS较短；在三苯氧胺辅助内分泌治疗过程中，若其持续存在则预示疾病的复发[20-21]。Xenidis等[22]的后续研究表明加用紫杉类辅助化疗能显著降低表达CK-19 mRNA的CTCs的阳性检出率，提高患者的DFS与OS。此外Georgoulias等[23]的研究表明，对标准辅助化疗结束后表达CK-19 mRNA的CTCs检测结果仍阳性的患者给予后续曲妥珠

单抗治疗能显著降低表达CK-19 mRNA的CTCs的阳性检出率及个数，降低复发转移的概率。

就解救阶段而言，Liu等[24]的个案报道显示了表达EGFR的CTCs计数的动态变化与拉帕替尼治疗疗效的相关性。Gradilone等[25]的研究表明外周血中检出表达多重耐药相关蛋白(multidrug-resistance-related proteins，MRPs)的CTCs者PFS较短。Yu等[26]的研究表明发生上皮间质细胞转化的CTCs计数与晚期乳腺癌患者的病情变化具有相关性。另有研究表明CTCs可检测到过甲基化的抑癌基因的启动序列，但其临床价值亟需大样本、前瞻性研究进一步探索[27]。

4 ctDNA的临床价值

基因改变普遍存在于乳腺癌的发生、发展过程中，主要包括点突变、过甲基化等形式。ctDNA是指由肿瘤组织释放到外周血中的DNA片段，所携带的肿瘤相关信息与肿瘤组织具有良好的一致性[28-29]，其对乳腺癌的临床价值成为近年研究的热点，主要包括早期诊断、判断预后、风险评估、疗效监测等方面。

Radpour等[30]的研究表明细胞周期调控及DNA损伤修复相关基因(*p16*、*p21*、*BRCA1*)、信号转导相关基因(*APC*、*BIN1*)、侵袭转移相关基因(*CST6*、*TIMP3*)、细胞解毒相关基因(*GSTP1*)、细胞增殖相关基因(*ESR-b*)联合检测早期乳腺癌的敏感性及特异性均超过90%。Silva等[31]的研究表明，ctDNA是独立的预后因素，ctDNA (同时在肿瘤组织和血清水平检测到的改变/突变基因)检测水平高者DFS较短。另有研究先后证实ctDNA的检测对辅助、新辅助、解救治疗的疗效有监测作用[32-35]。其中Murtaza等[34]的研究结果指出患者在解救治疗至疾病进展的过程中会获得与治疗药物相关的耐药基因的突变。Dawson等[35]通过标记扩增深度测序法(tagged-amplicon deep sequencing，Tam-Seq)检测乳腺癌最常见的突变基因*PIK3CA*、*TP53*或通过全基因组末端配对测序法检测肿瘤组织的其他突变基因或结构变异，后续通过PCR等技术动态检测相应的ctDNA水平，结果表明ctDNA的敏感性及与病情变化的相关性优于CTCs及CA15-3，且ctDNA水平高者OS较短，再次展示了其潜在的临床价值。

然而由于乳腺癌发生、发展过程中基因改变位点及形式的多样性，目前尚无明确的敏感性及特异性均能达到临床应用要求的靶基因，而这也将成为今后研究的重点。

5 RNA的临床价值

鉴于并非所有DNA的改变均可导致细胞表观遗传学的改变，mRNA的改变或许能更好地反映肿瘤的生物学特性，具有一定的预后价值[36]。

相较于mRNA来说，微小RNA(microRNA，miRNA，miR)稳定性强，通过调控多种基因的表达参与细胞的生长、凋亡、癌变、侵袭转移等过程。近年来众多研究证实了多种miRNA如miR-200b、210、128等对乳腺癌患者的临床价值[37-41]。Cuk等[37]的研究表明miR-148b等微小RNA的检测有助于乳腺癌的早期诊断。Buffa等[38]的研究证实了miR-210、128、27b的独立预后价值，其高表达者DFS较短，且与mRNA联合检测时预后价值更强。Volinia等[39]建立了包含30种mRNA、7种miRNA的联合检测模型，能较目前常用的MammaPrint and Oncotype DX更好地预测患者的复发风险。Madhavan等[40]的研究首次证实了miRNA的检测水平与CTCs计数的相关性，并显示了其对晚期乳腺癌患者的预后价值。同时有研究结果显示，通过调整miRNA-29b的水平能改变抑癌基因的过甲基化状态，从而抑制肿瘤细胞的生长[41]。

6 小结与展望

“液体肿瘤学检测”因其简便易行、侵害性小、实时动态佳等优势受到越来越多的关注，从蛋白检测到基因探索，多层面的循环肿瘤标志物更全面、深入、动态地反映着肿瘤的演变过程，且ctDNA/RNA的敏感性和特异性均较既往指标有所提升，显示预后、预测价值的同时展现了早期诊断乳腺癌的临床价值。此外，关于CTCs HER-2、CK-19 mRNA、miRNA

的研究结果更是初步展示了循环肿瘤标志物检测对于传统治疗理念的冲击，即通过检测携带肿瘤细胞生物学信息的循环肿瘤标志物来实时、动态指导个体化治疗，而非仅依靠组织学及影像学检查制定治疗策略。但证据力度、检测技术等多方因素在一定程度上限制了其在临床的推广应用，因此亟需大样本、前瞻性临床研究进一步证实液体肿瘤学检测对不同类型、不同阶段乳腺癌患者的临床价值，探索灵敏实用的靶向标志物，从而真正实现乳腺癌的分类、个体化治疗。

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