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ctDNA在结直肠癌检测中的应用

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摘　要

循环肿瘤DNA (Circulating tumor DNA, ctDNA)半衰期很短，可实时和精确地对疾病进行动态监测，作为一种新的诊断生物标志物，其在临床诊断上有广泛的应用前景。结直肠癌(Colorectal cancer, CRC)发病率高，多数CRC在确诊时进入局部进展期，寻求针对CRC的早期发现、早期诊断的标志物尤为必要。ctDNA在CRC的诊断中已有一定的应用，本文就ctDNA在CRC诊断中的应用进展作一综述。

关键词

ctDNA，结直肠癌，标志物

Application of ctDNA in Colorectal Cancer Detection

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Abstract

Circulating tumor DNA (ctDNA) has a short half-life and can be used for real-time and accurate dynamic monitoring of disease. As a new diagnostic biomarker, ctDNA has a wide range of clinical applications. Colorectal cancer (CRC) has a high incidence, and most CRC enter the local advanced stage when diagnosed, so it is particularly necessary to seek markers for the early detection and diagnosis of CRC. ctDNA has been used in the diagnosis of CRC. This article reviews the progress of ctDNA in the diagnosis of CRC.

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Keywords

ctDNA, CRC (Colorectal Cancer), Marker

1. 引言

ctDNA 被认为是一种新的诊断生物标志物[1]。ctDNA 的半衰期很短，从几分钟到几个小时不等，因此可以对疾病进行更准确和实时的动态监测[2]。在临床试验中，ctDNA 可用于基于检测是否符合临床试验条件的患者选择，以确定高风险和低风险的研究人群，并评估对抗癌治疗的反应[3]，在过去的几年里，通过血浆 ctDNA 分析监测肿瘤基因组学在不同的临床检测中得到较为广泛的应用[4] [5]。由于半衰期较长，敏感性和特异性有限，肿瘤标志物作为反应预测的生物标志物的效果有限，除常规使用肿瘤标志物和成像之外，ctDNA 正在成为预测反应的辅助工具[6]-[8]。ctDNA 与肿瘤负荷的变化相关，并预测治疗反应，一些研究显示优于标准肿瘤标志物[6]-[8]。

2. ctDNA

循环游离 DNA (cyclic cell-free DNA, cfDNA)是循环的双链 DNA 的细胞外片段，其长度在 120~220 bp 之间，以 167 bp 为中心，与细胞凋亡中 cfDNA 的核小体模式有关[9]。cfDNA 可在健康个体、癌症患者或其它疾病患者中检测到的碎片化血液循环分子，除血液外，在其它多种体液，如尿液或脑脊液中也可以检测到。cfDNA 的半衰期很短，从 4 分钟到 2 小时不等，可作为生物标志物应用于监测[10]-[13]。正常情况下，cfDNA 可以来自细胞凋亡、中性粒细胞胞外诱捕网(neutrophil extracellular traps, NETs)和红细胞成熟过程中的脱核[14] [15]。在血浆中，cfDNA 来源于粒细胞(32%)、红细胞祖细胞(30%)、淋巴细胞(12%)、单核细胞(11%)、血管内皮细胞(9%)和肝细胞(1%) [16]。cfDNA 在炎症、败血症或心肌梗死病理过程中可出现增加[17] [18]。ctDNA 属于 cfDNA 一种形式，其源于凋亡或坏死的肿瘤细胞，或由巨噬细胞吞噬坏死的肿瘤细胞产生[19]，虽然凋亡细胞可以产生 ctDNA，其片段长度与健康患者相似，而 ctDNA 比 cfDNA 更碎片化或更短[20]。在接受化疗和/或靶向治疗的患者中，ctDNA 4 周的变化可以预测临床疗效和无进展生存期(progression-free survival, PFS)改善的预后[8]。有研究在 durvalumab 单独或联合 tremelimumab 的 16 种不同实体瘤类型患者的 VII 期试验中描述了 ctDNA 对预后和预测的影响。研究显示治疗后 ctDNA 可预测预后。而使用 ctDNA 监测对早期诊断无效的，可以转向化疗或考虑添加抗细胞毒性 T 淋巴细胞相关分子 4 [21]。一项前瞻性 II 期试验中，随访了 94 例 25 种晚期实体肿瘤类型的患者，使用 pembrolizumab 治疗，并进行了系列 ctDNA 评估，利用 ctDNA 预测免疫治疗的反应在 INSPIRE 研究中显现出一定的应用前景[22]。

3. ctDNA 在 CRC 检测中的应用

CRC 是发生于结肠上皮和腺体的消化系统恶性肿瘤，根据 2022 年中国癌症统计报告的结果：CRC 发病率在所有肿瘤发病中排第 2 位，死亡率已居第 4 位。近年来 CRC 发病率和死亡率呈现上升趋势，多数 CRC 患者确诊时已属于局部进展期，这使得 CRC 的治疗变得更加困难。故针对 CRC 的早期发现、早期诊断显得极为重要。考虑到 ctDNA 检测的灵敏度很高，ctDNA 可以预测哪些 CRC 患者可能在疾病隐匿状态下的复发[2]。这已经引起了 CRC 中 ctDNA 作为各种临床应用的生物标志物的越来越多的兴趣。

最近的研究评估了 ctDNA 动态与辅助化疗疗效的关联，首次表明 ctDNA 是一种预测性生物标志物，ctDNA 具有识别新出现的基因突变和追踪肿瘤的克隆进化的潜力，而这些因素可导致原发性耐药性[23] [24]，ctDNA 更便宜、更安全、更方便，与重复的肿瘤活检相比，它提供了更准确的耐药机制，这与接受抗 EGFR 治疗或其他靶向治疗的 CRC 患者尤其相关[25]。根据 VAF 百分比和/或 ctDNA 清除率变化的 ctDNA 动力学，在包括 CRC 在内的多种肿瘤类型的临床试验中，是一种不可或缺的生物标志物[26]。有研究发现，在近 1000 名结直肠癌患者的大队列中，188 名患者微小残留病变(minimal residual disease, MRD)阳性，95 名患者接受了辅助化疗。24 周时，接受辅助化疗的患者 ctDNA 清除率明显高于未接受辅助化疗的患者[27]。

3.1. ctDNA 在 CRC 检测中的优势

在 CRC 的许多研究中，有可检测到的 ctDNA 的患者如果不给予任何治疗，几乎普遍会复发。ctDNA 不仅是一种高风险标志，而且是一种持续性疾病的指标，95%~100%持续检测到 ctDNA 的患者术后复发，如果没有接受全身治疗，通常在 2 年内随访[28]-[30]。目前 II 期结肠癌切除后的辅助治疗是基于临床和病理预后因素的风险分析，相比之下，尽管近 50%的 III 期患者仅通过手术治愈，但在可耐受的情况下，推荐对每个 III 期患者进行辅助化疗[31]。大约 15%和 30%的 II 型和 III 型患者尽管分别完成了适当的治疗，但仍出现复发[32] [33]。目前，局部结直肠癌(CRC)患者的标准治疗方法是手术切除，然后根据临床病理特征进行辅助化疗。这些患者的复发风险分层对于指导临床医生避免治疗不足和过度治疗至关重要。近来提出了 MRD 概念，即在术后患者血液中检测携带肿瘤特异性基因组或表观基因组改变的 ctDNA。MRD 的检测有可能成为一种有效的预后生物标志物，用于识别复发风险较高的患者，以及哪些患者可能从全身辅助治疗中获益最多。基于这一前所未有的发现，一些 II 期和 III 期 CRC 患者的临床试验正在进行，通过基于 ctDNA MRD 检测的升级或降级辅助化疗来评估 ctDNA 指导治疗的影响[34]。早期的规范化治疗后的 ctDNA 检测可以有益于那些需要强化治疗的患者。它可以为没有 ctDNA 的低风险患者提供了降级护理的机会，这些患者可能不需要副作用较强的全身治疗[4] [35] [36]。使用规范化治疗后的 ctDN 作为结直肠癌患者的整体生物标志物有望在临床中得以实际应用。虽然免疫治疗延长了微卫星高度不稳定 CRC 的 PFS，但在 KEYNOTE-177 中，近 30%的患者对 pembrolizumab 耐药[37]。从临床角度来看，ctDNA 检测可能因分期、转移负荷和转移部位而异。比如，与肺和腹膜转移患者相比，肝转移的 CRC 患者 ctDNA 检出率升高[38]-[40]。

由于循环肿瘤碎片(细胞、DNA 和甲基化标记物)脱落率高，故 CRC 适合液体活检方法[41]。有些标本中缺乏肿瘤组织，可能会导致第二代测序技术的失败，这可能是由于采样位置和/或肿瘤组织学的特点，如粘液性肿瘤所导致。ctDNA 可以补充或可能取代 CRC 中基于组织的基因分型，用于识别关键生物标志物，如 RAS/BRAF/HER2，以及检测微卫星不稳定性(MSI) [27]。液体活检的优势在于所需时间短(7~10 天，而基于组织的测序则由于获取肿瘤标本的时间，需要数周时间)，ctDNA 有望成为临床试验匹配和推进精准医疗的有效工具[42]。样本处理时间和周转时间的减少可以使加入临床试验的患者比例显著增加[43]。通过 ctDNA 分析，评估了 3 个月和 6 个月的化疗，支持对术后可检测到 ctDNA 的 IIICRC 患者加强治疗。术后检测到 ctDNA 并接受更长时间(6 个月)辅助化疗的患者的无病生存期(DFS)也有显著改善[44]。

3.2. ctDNA 检测的改进

在过去的几十年里，结直肠癌的分子图谱已经得到了很好的描述，包括染色体畸变，如拷贝数改变(CNAs)、倒位、易位、插入和缺失，以及单核苷酸点突变[45]。随着表观基因组学应用到了结直肠癌研究中，这些分子变化对癌症具有高度特异性，因此，在个体血液中检测到它们可能表明癌症的存在。特别

是在疾病的早期阶段，ctDNA 的浓度极低(约占总 cfDNA 的 0.01%)使其检测具有挑战性[46]-[48]。通过核小体定位或转录因子结合位点的表观基因组改变来分析甲基化肿瘤谱，当一个基因被甲基化时，它会在启动子的不同位置发生甲基化，从而增加了检测这些区域的机会，从而提高了技术的灵敏度。该方法最近在 CRC 患者中通过 ddPCR 使用两种甲基化标记物(WIFI 和 NPY)检测 ctDNA 进行了探索[49]。以前结果显示，仅检测 ctDNA 血浆的方法敏感性较差。通过使用附加标记，如甲基化或表观基因组学标记，可提高这些方法的敏感性[50]。仅使用血浆的方法也可以缩短对患者 MRD 评估的时间，因为它们不需要在进行 MRD 状态评估之前获取组织、测序和设计的 ctDNA 模板[FF]。最近的研究发现，除了预后标志物外，ctDNA 也可能是对辅助治疗反应的预测性生物标志物。Henriksen [51]等 35 研究了 160 例 III 期结直肠癌患者辅助化疗后 ctDNA 状态对复发风险的影响，只有 23% (3/13)的患者在辅助治疗下 ctDNA 永久清除，在 36 个月的随访中没有复发的迹象。相比之下，100% (10/10)有短暂清除或无清除的患者复发。术后检测到 ctDNA 的患者中，约 25%的患者通过标准输注氟尿嘧啶、亚叶酸钙和奥沙利铂辅助化疗获得清除。这一高危人群可能受益于延长治疗时间(超过规定时间)。

组织的二代测序技术不能反映肿瘤内和/或时间异质性[52]，而肿瘤异质性已引起越来越多的关注[53]，目前 ctDNA 检测已经有了越来越多的应用，但组织活检仍然是实体瘤分析的金标准。首先，从技术角度来看，组织活检在检测基因融合时更为敏感，因为这种基因重排的片段较大[42]。尽管 ctDNA 可以用于拷贝数变异的检测，但这一应用仅限于具有较大拷贝数扩增的患者，在约 15%的转移性肿瘤患者中依然无法检出足够的 ctDNA [54]。ctDNA 在肿瘤检测中的应用，还需要进一步研究。

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