以循环肿瘤细胞为主的液体活检在鼻咽癌的研究进展

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【摘要】鼻咽癌是最常见的头颈部恶性肿瘤之一。近年来，随着治疗技术和治疗模式的不断发展，早期鼻咽癌患者 5 年生存率可达 80%以上，但多数患者确诊时已是中晚期，因此，提高疗效、预测预后是降低局部复发和远处转移的重要手段。液体活检领域新起点—循环肿瘤细胞，是从原发肿瘤脱落并渗入到血液中循环的肿瘤细胞，作为一个提供实时关键分子信息的临床工具，可以根据个体患者肿瘤特征确定新治疗的方法、避免无效治疗的继续进行和防止非必要不良反应的发生，进一步加强癌症的管理和治疗。虽然液体活检在肿瘤领域有着令人瞩目的成果，但在国内鼻咽癌相关研究数据并不丰富。本文将集中于以循环肿瘤细胞为主的液体活检在鼻咽癌应用的最新进展和临床证据进行综述，并对未来的发展方向予以展望。

【关键词】循环肿瘤细胞；液体活检；鼻咽癌；疗效监测；预后预测
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Research progress on liquid biopsy mainly based on circulating tumor cells in nasopharyngeal carcinoma

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【**Abstract**】Nasopharyal carcinoma is one of the most common head and neck tumors in China. In recent years, with the continuous development and progress of the treatment technology and treatment model, Early nasopharyngeal carcinoma patients can survive by more than 80%in 5 years. However, most patients are in the middle and late stages when diagnosis. Therefore, improving efficacy is an important means to reduce local recurrence and distant metastasis. Circulating tumor cells are tumor cells that detach from the primary tumor and infiltrate into the bloodstream. As a clinical tool that provides real-time key molecular information, they can determine new treatment methods based on individual patient tumor characteristics, avoid the continuation of ineffective treatment, and prevent unnecessary adverse reactions, further strengthening cancer management and treatment. Although liquid biopsy has achieved remarkable results in the field of tumors, there is not abundant research data related to nasopharyngeal carcinoma in China. This article will focus on the latest progress and clinical evidence of liquid biopsy mainly using circulating tumor cells in nasopharyngeal carcinoma, and provide prospects for future development.

【**Keywords**】Circular tumor cells; Liquid biopsy; Nasopharyal carcinoma; Efficacy monitoring; Prognostic prediction

鼻咽癌（Nasopharyngeal carcinoma，NPC）是一种与潜在致瘤性 EB 病毒（EBV）感染有关的上皮性癌，在我国最头颈部恶性肿瘤发病率居于首位，主要流行于我国华南两广地区。据国际癌症研究机构统计，2020 年鼻咽癌新发病例约 13.3 万例，死亡病例数约 8 万例，其中，我国每年新发病例占世界的 47%[1]。60%的患者在确诊时已是中晚期，失去最佳治疗时机，局部复发（15%）或远处转移（20%）是导致治疗失败甚至死亡

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的主要原因[2]。因此，提高疗效是降低局部复发和远处转移的有效手段。液体活检越来越多地用于肿瘤细胞的分子分析，与传统的金标准组织病理活检相比[3]，该方法无创、可重复性高、能实时精准反映肿瘤随时间发生的动态负荷变化和表观遗传变化，进一步探究肿瘤发展过程中的异质性以及原发和转移性病变之间的差异性[4]。另外，先进的成像技术，如磁共振成像（MRI）和正电子发射计算机断层显像（PET-CT）只能检测到已经确定的原发肿瘤和转移瘤（包括>10^9细胞）[5]，存在一定滞后性。基于上述，虽液体活检已广泛用于多项实体恶性肿瘤的诊断、疗效监测以及预后预测。但国内，液体活检在鼻咽癌的应用数据并不丰富，本文主要介绍液体活检在鼻咽癌的早期诊断、疗效监测和预后预测中的最新进展和发展潜力。

1 液体活检在鼻咽癌的应用

液体活检是指通过对血液、尿液等非实性生物组织进行分子审问来获得源自于组织的生物标志物，进一步分析相关信息，可反复多次、非侵入性地监测疾病的演变、发展和实时的治疗反应,提供了一个更全面的异质性肿瘤遗传特征并可能识别播散性侵袭性克隆[6]。目前主要检测对象包括但不限于循环肿瘤细胞（Circulating Tumor Cells，CTC）、循环肿瘤 DNA（Circulating Tumor DNA，ctDNA）、外泌体（exosomes）及微小循环 RNA 等。

1.1 循环肿瘤细胞

循环肿瘤细胞是指从原发肿瘤脱落并渗入到血液中循环的肿瘤细胞，通过沉降和播散形成继发病灶，与转移直接相关，可检测 DNA 基因组、RNA、蛋白、代谢组等信息[7]，被认为是液体活检领域的新起点。CellSearch 是首个也是唯一一个获得美国食品药品监督管理局（FDA）临床验证批准用于检测 CTC 计数的血液检测系统，已广泛应用于多种实体瘤患者的疗效监测和预测生存结果[8–11]。CTC 已被多个重要临床指南收录如表 1。

表 1　CTC 已被收录临床指南

Time	Guideline	Significance
2010	AJCC Cancer Staging Guidelines,7th edition	For the first time, CTC was included in the TNM staging system as a new M staging (distal metastasis) standard, listed as cM0 (i+), appearing between M0 and M1
2017	Breast Cancer NCCN Guidelines 2017.V3	Formal introduction of cM0 (i+) staging
2018	AJCC 8th Edition Cancer Staging Guidelines	In addition to retaining cM0 (i+) staging, CTC is listed as a prognostic assessment tool for breast cancer
2018	Expert consensus on the application of liquid biopsy in clinical tumor diagnosis and treatment and medical laboratory practice	For routine clinical testing of CTC, it is recommended that each laboratory choose a detection technology suitable for their own conditions, and after fully evaluating its detection performance, apply it to daily work
2019	Breast cancer CSCO Guidelines	Guidelines for the First Official Entry into China: CTC Clinical Application Moving towards the Era of Genotyping and Cell Sequencing
2019	Expert consensus on the use of circulating tumor cell detection in colorectal cancer; 2019 Expert consensus on early diagnosis and treatment of colorectal cancer	It can provide real-time information about the disease status of colorectal cancer patients, which is helpful for prognosis evaluation and treatment response monitoring of colorectal cancer
2019	Prostate Cancer NCCN Guidelines	The expression status of AR-V7 (androgen receptor splicing variant) in CTC can guide the selection of treatment options
2019	Primary liver cancer diagnosis and treatment standards	Detection of CTC has predictive value for liver cancer recurrence and progression after transcatheter arterial chemoembolization and radiotherapy
2020	Chinese Expert Consensus on Prevention and Treatment of Recurrence after hepatocellular carcinoma Resection (2020 edition)	Peripheral blood CTC is an independent risk factor for recurrence of HCC after hepatectomy
2020	Expert consensus on biomarker detection and application of hepatocellular carcinoma	Peripheral blood CTC count is an independent risk factor for postoperative recurrence in HCC patients
2020	Chinese Medical Association Oncology branch of pancreatic cancer early diagnosis and early treatment expert consensus	CTC can be used as a marker for early diagnosis and differential diagnosis of pancreatic cancer
2020	Prostate Cancer NCCN Guidelines	CTC can be used in imaging to detect tumor micrometastasis or residual lesions in the body, and early predict prostate cancer patients with high risk of recurrence and metastasis
2021	Expert consensus on clinical application and laboratory detection of circulating tumor cells	The detection of circulating tumor cells has the advantages of non-invasive sampling, dynamic reflection of the full spectrum of tumor genes, and providing real-time information related to the disease status of tumor patients

CTC 的数量及变化与 T、N 分期、治疗疗效和预后有明显相关性[8,11]。无论使用何种方法检测，检出 CTC 即提示更差的 DFS 预后， 并且 CTC 与更短的 DFS 预后密切相关[12]，另外，CTC 还可以对放化疗反应和治疗前后 PFS 和 OS 进行评估[13]。值得注意的是，Li 等人发现 CTC 总数在 T 分期、N 分期以及 EBV DNA 阳性和阴性病例之间没有差异[14]，我们考虑这可能与 CTC 的分子特性有关。从肿瘤的发展来看，肿瘤在发生转移前，原发灶可发生上皮间质转化（Epithelial-Mesenchymal Transition，EMT），在 EMT 中，细胞基因表达谱如上皮、间质分子标志物及转录因子表达也会发生改变，从而影响 CTCs 检出率[15]。

既往的检测技术存在一定局限性，依靠单一标志物（如 EpCAM），无法全面检出更多类型 CTC，便不能用于 EpCAM 阴性或低表达的肿瘤。以往方法的检测阳性率为 30%-40%（cellsearch®免疫磁珠法）、60%（RT-PCR 法）[16]。Canpatrol 平台是 CTC 的 EMT 与干性分析应用最广泛的应用型平台，其检测阳性率显著提升至 86%，这归因于其不依赖标志物分离 CTC，并运用多组核酸探针实现原位杂交，即 mRNA-ISH，更全面捕获、更灵敏特异检测 CTC 的检测方法。除外 CTC 计数和上皮间质转化（EMT）分型，Canpatrol 可同步原位检测其他目标基因的，如 LGR5[17]和 COX-2[18]，实现了更创新的液体活检新型技术研究与应用。通过该项技术，有研究发现 CTCs 的核型与化疗敏感性及耐药性有关，可用于预测鼻咽癌的疗效及评价耐药情况[19]；原发性肿瘤与 CTCs 之间特异性标志物的表达存在不一致，其克隆选择或克隆获得的遗传不稳定[20]。一项前瞻性研究发现鼻咽癌患者循环肿瘤细胞上 COX-2 的表达与不良的治疗反应、较高的复发和转移风险密切相关 [21]，这提示动态监测 CTC 特异性标志物的表达，能为鼻咽癌的疗效监测提供动态反应和进展评估，指导后续是否需要进一步加强治疗。此外，其他的生物标志物，如人表皮生长因子受体-2（HER2）[22–24]、雌激素受体[25–27]、前列腺特异性膜抗原[28,29]和叶酸受体[30]等具有不同的临床意义，已被描述为不同癌症的 CTC 标志物，期待未来有更多的研究进行临床验证。

近年来，免疫治疗也是肿瘤领域的一大转折点，有研究发现，循环肿瘤细胞的 PD- L1/PD-1 检测同样能够在免疫治疗上获益。一项 II 期研究表明将 CTC 的 PD-L1 表达比例作为预测生物标志物，可以发现 ORR 和 OS 与 PD-L1 的表达比例相关。另一项国际、随机、开放标签的 III 期确认了 PD-L1 表达的 CPS 是该人群预测疗效和生存的生物标志物且肿瘤表达 PD-L1 的患者可以从这一类型的免疫药物中获益。CTC 阳性及 CTC PD-L1 阳性患者比阴性患者有更短的 PFS[31]。另一项前瞻性研究纳入了 113 名在基线、两个周期的诱导化疗（第 6 周）和同期放化疗（第 15 周）结束时接受治疗的局部晚期患者。这项研究的结果表明，治疗结束时过度表达 PD-L1 的 CTC 患者的预后较差，而 CTC PD-L1 阴性与完全缓解密切相关[32]。

CTC 在指导个体化用药、监测早期 NPC 患者常规治疗疗效及晚期患者免疫治疗疗效方面都有相当大的贡献。现阶段已有研究人员发现 CTC 存在不同亚型，并研发检测 CTC 及其细胞表面表达的目标基因同步检测技术，但国内外将其应用于监测鼻咽癌根治性放疗疗效的临床验证较少，还需要进行更多的研究，以便更好地了解该疾病及其治疗方法。

1.2 循环肿瘤 DNA（ctDNA）

ctDNA 通常是由原发性肿瘤、转移性肿瘤和循环肿瘤细胞主动分泌或在肿瘤细胞凋亡或坏死过程中释放入循环系统中的 DNA 片段，会携带来源于肿瘤细胞相关的遗传学特征[33]，能够进一步评估分子异质性、监测肿瘤动态、识别治疗的遗传决定因素、跟踪基因组进化和获得性耐药的发展。ctDNA 作为一种肿瘤特异性标志物，可通过疗效监测指导化疗及靶向用药[34–38]。

鼻咽癌患者血浆中 EBV 特定区域 ctDNA 可完善 TNM 分期系统，个体化选择最优治疗方案[39–43] 。一项荟萃分析显示不论是在血浆样本还是血清样本，EBV DNA 的高敏感性和高特异性极大的提高了鼻咽癌的早期诊断。ctDNA 治疗期间的变化可用于近期疗效的监测和识别有复发风险患者的生存预后预测[44][45]。个案中发现 ctDNA 可以用于检测鼻咽癌微小残留病灶（MRD），证明了在临床复发前使用个性化 ctDNA 检测疾病的可行性[46]。但与 CTC 相比，ctDNA 在预后预测方面的特异性较低[47]。另外，近年来针对复发或转移性鼻咽癌患者的免疫治疗取得了重大突破，在一项正在进行的 II 期临床试验中，对于检测 ctDNA 的放化疗治疗后的患者将给予 Pembrolizumab 治疗，并测量 ctDNA 的 OR 率和清除效率；在接受抗 PD-1 治疗的患者中，观察到的耐药性约高达 60%。以上进一步揭示了 ctDNA 在鼻咽癌中的潜在应用。

通过以上研究结果，我们可以进一步理解 ctDNA 在鼻咽癌的诊断、治疗和预后评估提供新的线索和方法。但针对 ctDNA 用于监测鼻咽癌靶向治疗和免疫治

疗疗效及耐药性的研究十分有限，仍然需要更多的研究进一步探索。

1.3 外泌体

外泌体是细胞释放到周围生物液体中的小囊泡。常用的无细胞 DNA（cfDNA）来源于垂死的细胞，而外泌体核酸（exonas）来源于活细胞，能更好地反映肿瘤的生物学基础[48]。Skog 等人发现从患者血浆中分离出来的外泌体中可以检测到肿瘤源性突变，这为肿瘤的诊断提供了信息[49]。外泌体 RNA 与 ctDNA 结合时，提高了检测血液样本中突变的几率[50]，显著提高了生物标志物与治疗结果的相关性和液体活组织检查成功的可能性[48]。

外泌体可以操纵肿瘤微环境，参与化疗和抗辐射，诱导免疫抑制，促进病理性血管生成，支持转移，也是一种很有前途的生物标志物，鼻咽癌循环外泌体 Circmyc 明显增多[51]，它与细胞增殖呈正相关，与放射敏感性呈负相关[52]，可以作为 NPC 疗效监测和预后的独立预测指标。 低血浆 miR-9 水平与鼻咽癌淋巴浸润恶化和 TNM 分期进展密切相关，与鼻咽癌局部和转移性鉴别诊断也有较高的敏感性和特异性[53]。Yang 等人研究发现，血清外泌体水平上调与鼻咽癌的进展和总生存率下降有关。外泌体/miR-205-5p/EGFR/ERK 轴可能是鼻咽癌转移治疗的新靶点[54]。外泌体在 PD-L1 介导的免疫逃避中发挥作用的最显著数据集中于外泌体 PD-L1 与治疗反应之间的相关性，监测循环外泌体 PD-L1 可能是预测肿瘤对免疫治疗反应的有效方法，研究发现缺乏外泌体 PD-L1 增加的患者被认为缺乏 IFN-迟钝 T 细胞反应的充分恢复，这可能是治疗失败的原因。由于外泌体在 NPC 的早期诊断、疗效监测和预后预测方法等临床验证甚少，样本量均很小，需要未来更多、规模更大的临床研究进一步验证。

1.4 微小循环 RNA

微小循环 RNA 即 MicroRNAs （miRNAs）是由 19-24 个核苷酸组成的非编码单链进化保守的 RNA 分子，在细胞外环境中以稳定的形式存在，称为循环 miRNAs（cir-miRNAs），可调控基因表达，如早期胚胎发育、细胞增殖、细胞凋亡、细胞周期调控及免疫调节等[55,56]。早期有研究发现 miRNA-93 和 miRNA-221 可能在局部和局部晚期前列腺癌的疾病监测中发挥作用[57]。近年越来越多的研究结果表明，miRNA 在肿瘤细胞的增殖、迁移及肿瘤血管生成等方面也发挥重要作用，与包括乳腺癌、结肠癌、食管癌在内的诸多恶性肿瘤关系密切[58–60]。

随着液体活检在肿瘤学中的快速发展，存在于血浆、血清、唾液等体液中的 miRNA，又称为循环 miRNA（cir-miRNA）或细胞外 miRNA（EC-miRNA），引起了许多研究者对鼻咽癌生物标志物研究的兴趣。相关研究结果表示 microRNAs 对鼻咽癌具有很强的诊断能力[61–64]。Wu 等人首次将唾液 miRNAs 作为鼻咽癌的潜在指标进行了研究，他们基于对来自 22 名鼻咽癌患者的唾液样本和 25 名健康对照者使用微阵列 miRNA 表达的评估，他们发现唾液 miRNA 的差异表达可能通过靶向其靶基因在鼻咽癌中发挥关键作用[65]。有研究成功识别并验证了 16 个 miRNA 特征，用于与位于头颈部的其他肿瘤和非癌样本进行比较，以区分鼻咽癌，且该诊断模型具有 100%的准确率、100%的特异性和 100%的敏感性[61]。一些循环 miRNA 如 miR-20a、miR-21、miR-34a 等可以调节 PD-L1 的表达，同时也调可以节肿瘤免疫微环境，从而影响免疫治疗的效果。以上，微小循环 RNA 在鼻咽癌的发生和发展中发挥重要调控作用，可能为鼻咽癌的预防和治疗提供新的治疗方法。

2 讨论与总结

液体活检在肿瘤领域已经取得了相当大的进步，临床医生可用的检测数量正在增长。它们在解决与常规活检相关的问题方面提供了许多优势，越来越多地被用于肿瘤的分子分析和促进精确的药物治疗方法。液体活检也有一定的局限性和挑战，如液体活检评估和分析物验证的标准化，以及在临床试验中使用它们作为生物标志物的监管考虑。

晚期鼻咽癌通常表现出向颈部淋巴结和次级器官转移的强烈倾向，预后较差，局部复发和远处转移仍然是鼻咽癌患者治疗失败甚至死亡的主要原因，因此寻找可实时、精准预测鼻咽癌疗效和预后预测的生物标志物对于鼻咽癌治疗至关重要。CTC 对非转移性鼻咽癌长期生存的负面影响已得到证实，多项研究发现，处在 EMT 途径的循环肿瘤细胞具有更强的侵袭性，更能在远处形成新的肿瘤病灶，发生 EMT 的 CTC 与肿瘤进展或长期生存相关，因此分析循环肿瘤细胞 EMT 分子特性，能更好地了解肿瘤患者对治疗的反应效果，早期肿瘤复发的风险和预后情况。

基于上述，CTC 的分型、基因型和功能特征为研究与疗效相关的放化疗敏感性提供了机会，可以进一步揭示潜在的药物靶点，将是未来精准医学的重要组成部分。然而，但现阶段将相关生物标志物联合 CTC 分型监测鼻咽癌根治性放疗疗效的临床验证较少，现

有研究基本存在病例数少、没有大量临床研究验证等问题，期待将来有更多更有力的临床研究证据进行一个突破性的进展。

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