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液体活检对食管癌诊疗和预后的价值

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摘要 食管癌是一种全球性的恶性肿瘤, 由于其进展迅速并缺乏早期发现和有效预后的生物标志物, 造成了患者较高的死亡率和较差的预后情况。近些年来, 在精准肿瘤诊疗的大背景下, 液体活检作为一种新兴的非侵入性检测方法, 在食管癌疾病进展中可以实现动态监测, 逐渐在临床上引起关注。液体活检通过从体液中获取肿瘤相关的生物标志物, 如循环肿瘤DNA、循环肿瘤细胞、外泌体内容物等, 来评估肿瘤的存在、特征和预后等。对食管癌患者进行及时有效的预后评估有助于改善其临床结局, 故该文将对液体活检在食管癌的诊疗和预后中的科学研究及临床应用现状进行详细阐述, 并指出目前液体活检中存在的挑战及其未来发展方向, 期望能为食管癌的早期和超早期诊断、疗效动态监测、预后评估、个体化精准治疗决策的制定提供依据。

关键词 食管癌; 液体活检; 诊断; 预后

Diagnostic and Prognostic Values of Liquid Biopsy in Esophageal Cancer

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Abstract Esophageal cancer is a malignancy with high mortality and poor prognosis worldwide due to its rapid progression and lack of effective prognostic biomarkers for early detection. As a new non-invasive detection method, liquid biopsy can realize continuous monitoring during the progression of esophageal cancer, and has

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gradually attracted clinical attention. Liquid biopsy assesses the presence, characteristics, and prognosis of tumors by obtaining tumor-related biomarkers such as circulating tumor DNA, circulating tumor cells and exosomes from body fluids. Prompt and effective prognostic assessment of patients with esophageal cancer is beneficial in improving their clinical outcomes. Therefore, this paper will elaborate on the recent researches and clinical application of liquid biopsy in the diagnosis and prognosis of esophageal cancer, and point out the current challenges and future development, hoping to provide a basis for early diagnosis, continuous monitoring of therapeutic efficacy, prognosis evaluation, and development of individualized precision treatment decisions of esophageal cancer.

Keywords esophageal cancer; liquid biopsy; diagnosis; prognosis

食管癌(esophageal cancer, EC)是一种高度侵袭性的恶性肿瘤, 具有全球影响性。据统计, 它在2020年新增全球恶性肿瘤发病率中排名第十, 其死亡率位居第六[1]。EC有两种主要的组织学亚型: 食管鳞状细胞癌(esophageal squamous cell carcinoma, ESCC)和食管腺癌(esophageal adenocarcinoma, EAC)。EC的治疗方案根据疾病分期而异, 针对早期EC一般采用内镜治疗, 局部晚期EC在手术治疗前常规采用新辅助放化疗[2]。尽管EC的治疗选择多种多样, 但其早期症状隐匿, 大多患者确诊时已处于晚期或局部晚期[3], 并且由于其特征性的快速进展, 局部疾病也存在较高的复发率, 从而导致EC的存活率仍然很低[4]。因此, 为EC患者探索合适的治疗靶点和准确的预后生物标志物以监测和提高临床疗效是至关重要的。

目前, 任何生物标志物都不被推荐来补充或替代内镜检查和组织病理学检查, 内镜检查结合组织活检仍然是EC确诊的金标准[5]。但内镜检查对检测EC无症状局部复发的价值有限, 而传统的预后评估也主要依赖于临床病理学特征, 且存在侵入性、较高的医疗费用、患者依从性差等局限性[6-7]。计算机断层扫描(computed tomography, CT)等影像学检查也常用作诊断和监测EC的辅助检查, 但对微小病变的低敏感性一直是发现早期复发部位的一个难题[8]。来源于血液的常规生物标志物(如癌症自身抗体、肿瘤标志物、细胞因子等)在诊断和预后评估上被广泛应用, 它们虽然提供了简单、侵入性更小的替代方案, 但单一癌症相关自身抗体生物标志物的诊断价值有限, 而肿瘤标志物如糖类抗原19-9、癌胚抗原、鳞状细胞癌抗原等在早期诊断上也存在特异性和敏感性较低的问题[9-10]。

与上述病理学、影像学和血液学等常规检测方法相比, 液体活检(liquid biopsy)是一种非侵入性的检测方法, 近年来在EC的诊疗方面引起了广泛关注。本文将对液体活检在EC的早期诊断及预后评估中的应用现状作一阐述, 为实现EC患者早期干预、减少预后不良事件的发生提供理论支持。

1 液体活检概述

液体活检作为一种新兴的检测方法, 具有非侵入性、简单性、可在不同时间点重复进行和较高的成本效益比。它通过分析血液或其他体液(如唾液、胸腔积液、尿液等)中的与肿瘤相关的生物标志物, 简化了肿瘤取样过程, 并通过简单易得的重复取样实现了肿瘤进展的动态监测, 为肿瘤患者预后评估提供了前景[11]。近年来, 液体活检技术得到了迅速发展, 目前在临床诊疗中广泛应用的基于液体活检的生物标志物主要有循环肿瘤DNA(circulating tumor DNA, ctDNA)、循环肿瘤细胞(circulating tumor cells, CTCs)及基于外泌体(exosomes)的生物标志物[如微小核糖核酸(microRNA, miRNA)、非编码RNA(non-coding RNA, ncRNA)等], 这些肿瘤衍生或与肿瘤有关的成分可以提供关键的纵向信息和数据, 以便对患者治疗反应、耐药性以及肿瘤复发情况进行个体化的分析, 从而实现基于精准医学的治疗[12-13]。

2 液体活检在食管癌诊疗和预后评估中的应用

目前基于液体活检的生物标志物在EC早期诊断、复发预测、疗效监测、生存预后等多方面评估中均具有巨大潜力(表1)。

2.1 ctDNA

ctDNA是目前在液体活检样品中被广泛研究的分析物之一, 它是由特异来源的凋亡或坏死肿瘤细胞释放到外周血和体液中的较短DNA片段, 主要存在

表1 液体活检在食管癌诊断及预后评估中的应用

Table 1 Application of liquid biopsy in diagnosis and prognosis evaluation of esophageal cancer

标志物 Biomarker	样本来源 Sample source	病理类型 Pathological type	应用领域 Application	主要结果 Outcome
ctDNA	Plasma	EAC, ESCC	Relapse prediction	Find minimal residual disease, enabling earlier identification of recurrence than imaging [14]
	Plasma	ESCC	Relapse prediction	Enable earlier identification of recurrence than imaging [15]
	Plasma	EAC	Relapse prediction	Enable earlier identification of recurrence and progression than imaging [16]
	Plasma	EAC	Treatment response	Higher negative rate in the complete response group after treatment [16]
	Plasma	EAC, ESCC	Treatment response	Identification of responders to immunotherapy efficacy [17]
	Plasma	ESCC	Survival prediction	Initial positive results correlates with lower PFS and OS [15]
	Plasma	EAC, ESCC	Survival prediction	Positive results after chemoradiotherapy correlates with lower PFS and DSS [14]
CTCs	PB	ESCC	Treatment response	Higher positive rate in the progressive disease group than the partial response group after treatment [18]
	PB	GOA	Treatment response	Prediction of DCR after treatment [19]
	PB	EAC, ESCC	Relapse prediction	Higher risk of recurrence in positive groups than negative groups [20]
	PB	EAC, ESCC	Survival prediction	Positive results preoperative correlates with lower PFS and OS [20]
	PB	ESCC	Survival prediction	Positive results preoperative correlates with lower DFS and OS [21]
	PB	EAC, ESCC, GOA	Survival prediction	Positive results after treatment correlates with lower DFS and OS [22]
	PB	EAC, ESCC, GOA	Survival prediction	Positive results in nonmetastatic patients correlates with lower PFS and OS [23]
	PB	GOA	Survival prediction	Positive results before/after treatment correlates with lower PFS and OS [19]
miRNA	Plasma	EAC	Early diagnosis	Diagnosis through the changes of miRNA expression [24]
	Urine, saliva	ESCC	Early diagnosis	Similar diagnosis ability through miR-1246 with serum sample [25]
	Serum	ESCC	Survival prediction	Higher expression of miR-1246 correlates with lower OS [25]
	Serum	EAC	Survival prediction	miR-1238 and miR-5-4p correlates with risk of death [26]
	Serum	ESCC	Treatment response	Ientification of response to neoadjuvant chemotherapy based on miR-193b-5p-miR-873-3p-model [27]
	Saliva	ESCC	Treatment response	Reflection of tumor load based on G-NchiRNA [28]
circRNA	Plasma	ESCC	Early diagnosis	Identification of ESCC patients [29]
lncRNA	Serum	ESCC	Early diagnosis	Identification of tumor stage [30]
STMN-1	Serum	ESCC	Early diagnosis	Identification of ESCC patients [31]
tsRNA	Saliva	ESCC	Early diagnosis Survival prediction	Identification of patients based on two-tsRNA signature [32] Prediction of PFS and OS based on the bi-signature risk score for prognosis [32]
Metabolite	Plasma	ESCC	Relapse prediction	Prediction of recurrence based on four metabolome markers [33]
CD-14	Plasma	ESCC	Survival prediction	Higher gene expression levels correlates with lower OS [34]

ctDNA: 循环肿瘤DNA; CTCs: 循环肿瘤细胞; miRNA: 微小核糖核酸; circRNA: 环状RNA; lncRNA: 长链非编码RNA; tsRNA: 非编码小RNA; STMN-1: 微管解聚蛋白1; PB: 外周血; EAC: 食管腺癌; ESCC: 食管鳞状细胞癌; GOA: 胃食管腺癌; PFS: 无进展生存期; OS: 总生存期; DSS: 疾病特异性生存期; DCR: 疾病控制率; DFS: 无病生存期。

ctDNA: circulating tumor DNA; CTCs: circulating tumor cells; miRNA: microRNA; circRNA: circular RNA; lncRNA: long non-coding RNA; tsRNA: tRNA-derived small RNA; STMN-1: stathmin-1; PB: peripheral blood; EAC: esophageal adenocarcinoma; ESCC: esophageal squamous cell carcinoma; GOA: gastric and oesogastric junction adenocarcinoma; PFS: progression-free survival; OS: overall survival; DSS: disease-specific survival; DCR: disease control rate; DFS: disease-free survival.

于血浆中, 其样本易得性、较好的患者依从性、可重复性可以帮助实现肿瘤负荷的动态监测[35]。ctDNA的检测和定量技术的难点在于其在总血浆DNA中含量极低, 近年来其技术革新主要聚焦于突变基因的检测, 包括聚合酶链式反应(polymerase chain reaction, PCR)和二代测序等方法[36]。基于这些技术, ctDNA检测各种类型肿瘤(其中包括了原发性肿瘤和转移性肿瘤)的能力大幅提升[37-38]。在EC患者中, ctDNA在肿瘤早期诊断、残留和复发监测、预后等方面显示出了巨大的临床应用潜力, 给传统的EC诊疗带来了颠覆性的变化。

在EC中, 已经有研究发现由ctDNA检测确定的癌症相关基因及其驱动因素的异常高水平的甲基化可用于诊断和监测肿瘤复发, ctDNA甲基化已成为检测EC相关特征的一种高灵敏度方法[39-40]。通过对局部晚期ESCC患者放疗前后及放疗期间的血浆样本进行二代测序, 发现与基线ctDNA阴性患者相比, 基线阳性患者放疗后的无进展生存期(progression-free survival, PFS)(P=0.047)和总生存期(overall survival, OS)(P=0.005)显著降低, 并且初次放疗后血浆样本中ctDNA的存在是ESCC患者的独立预后因素(P=0.011)[15]。对于EAC患者, 一项研究纵向收集并检测了患者治疗过程中的血浆样本, 以评估ctDNA与疾病分期和治疗反应的关联性, 研究结果显示, 基线血浆中未检测到ctDNA的患者在接受新辅助化疗后呈现完全缓解的良好状态[16]。此外, 通过ctDNA检出的*TP53*、*ERBB4*肿瘤相关基因突变相比影像学检查更早地显示了疾病进展与复发[16]。ctDNA检测到的非同义突变表明存在微小残留灶, 比PETCT检测到的肿瘤复发平均提前期为114.9天[14]。ctDNA的检测还能有助于识别治疗后有肿瘤进展风险的EC患者, 研究表明接受术前新辅助放化疗的患者治疗后ctDNA的水平可以预测肿瘤进展、远处转移以及疾病特异性生存期[14]。由于仅存在较少的EC患者受益于基于免疫检查点抑制剂(immune checkpoint inhibitors, ICIs)联合化疗的治疗手段, 最近的研究也着力于探讨EC患者血浆ctDNA水平与ICIs治疗应答的关系, 该研究通过综合分析ctDNA中体细胞变化确定了一种分子肿瘤负荷指数(molecular tumor burden index, mTBI), 发现mTBI降低程度更高的患者拥有更长的PFS和持久的临床获益, 证明了ctDNA有助于识别免疫化学治疗的应答者[17]。可见在不同的治疗方案中, ctDNA均显示了其与治疗反应及疾病进展等预后情况的相关性。

2.2 CTCs

ASHWORTH等[41]在1869年证明了CTCs在癌症患者外周血中存在, 这种肿瘤细胞从原发肿瘤脱落并外渗进入血液循环。即使癌症处于早期阶段, CTCs也有可能存在于癌症患者的外周血中, 因此在肿瘤患者的早期诊断中可以借助CTCs的检测来判断是否存在无法被影像学技术发现的微小病变[42-44]。近年来, 富集和鉴定CTCs的检测方法非常多样, 包括基于免疫磁珠或逆转录聚合酶链式反应捕获叶酸受体阳性的CTCs等[45-46]。“CellSearch系统”已被开发并用于识别血液中的CTCs, 它是一种基于上皮细胞黏附分子免疫学测定的CTCs检测方法, 并且在一些转移性肿瘤中具有预测肿瘤进展和预后的临床效用[47-49]。

德国的一项针对123名接受手术的EC患者的前瞻性研究发现, 术前CTC阳性与肿瘤大小、转移、国际抗癌联盟(Union for International Cancer Control, UICC)分期等病理特征相关; 在预后方面, CTCs阳性患者的肿瘤复发风险是CTCs阴性患者的5.1倍, 并且CTCs可以作为OS的独立预后标志[20]。由此可见, 术前CTCs检测有望帮助实现EC的准确分期, 从而为制定治疗决策及改善预后提供机会[21]。对于较难在血液中检测到CTCs的非转移性EC, 有研究发现接受术前顺铂和5-氟尿嘧啶化疗联合放疗的新辅助治疗方案的患者, 治疗后任意随访期间(6个月、12个月、24个月) CTCs阳性都与更差的OS和无病生存期(disease-free survival, DFS)相关[22]。这表明了即使在未发生淋巴结转移或远处转移的情况下, CTCs的检测也存在显著的预后价值。在其他类似的研究中均提出了CTCs作为一种独立的预后标志物在疾病早期阶段预测预后状态的可能性[18,23]。在CTCs评估疗效方面, 有研究针对胃食管腺癌患者进行分析, 因胃食管腺癌是包含食管癌和胃食管交界处癌在内的恶性肿瘤, 其患者特征与EC具有一定可比性[19,22], 研究发现了接受两个周期的化疗后, CTCs计数与更差的PFS和OS相关, 并且揭示了动态评估CTCs计数对于需要通过影像学检查才能实现的目标病变的疗效评价的助益, 基线与治疗后的CTCs计数变化提示了适当改变治疗方案的潜在可能[19]。中国的一项研究分析了不同的CTCs临界值与ESCC患者临床结局的相关性, 证实了术前CTCs计数(≥5个/7.5 mL血液)可以预测肿瘤复发, 并

且CTCs计数升高与OS缩短有关[50]。

2.3 外泌体相关生物标志物

外泌体是一种直径为30~150 nm的细胞外囊泡，由包括肿瘤细胞在内的各种类型的细胞分泌，其内容物包括DNA、RNA、蛋白质等，这些来源于肿瘤细胞的囊泡通过促进局部和全身细胞通讯从而促进肿瘤进展，因此能够反映与肿瘤相关的生物学变化[51-53]。外泌体在体液中的大量存在增加了其检测的灵敏度，而近年来对于外泌体的分离与表征技术(包括超速离心等经典分离方法以及部分已经获批在临床上使用的商业试剂盒)已取得较大进展[54-56]。在临床应用方面，外泌体内容物(尤其是miRNA、环状RNA、其他非编码RNA等)在肿瘤的诊断与预后上均显示出巨大潜力[57-59]。

2.3.1 miRNA　miRNA是一类长度为20~24个核苷酸的ncRNA分子，其基因表达的改变已被发现与多种恶性肿瘤的发病机制有关，miRNA表达特征也被证明具有组织特异性和疾病特异性[60-61]。miRNA存在于一系列体液如血清、血浆、全血、尿液和唾液中，在癌症早期诊断、疾病进展监测和预后预测方面逐渐发挥出巨大潜力[62]。

已经有研究发现，EC患者组织样本中特定的miRNA与PFS和OS有关[63-64]。而最近的一些着眼于非侵入性手段获得miRNA的研究也揭示了其异常表达与EC相关[65-66]。对EAC患者的血浆样本进行miRNA表达谱分析发现，miR-382-5p显著上调而miR-133a-3p显著下调，这些变化说明了它们可能作为潜在的生物标志物[24]。同样地，血清miR-1246水平在ESCC患者中也具有诊断价值和预后价值[25,67]。除此之外，在非血清样本如尿液和唾液中，miR-1246水平也具备与前者相当的诊断能力，然而在预后方面，这些非血清样本的诊断能力可能由于收集时间不同而存在大幅波动，并没有发现miR-1246水平与OS的相关性[67]。在治疗反应监测方面，一项基于100例接受术前新辅助化疗的ESCC病例血清标本的研究发现，通过对miRNA微阵列的全面表达谱分析确定的血清miRNA生物标志物，能够在治疗前预测疗效，其中基于miR-193b-5p、miR-873-3p和有无淋巴结转移构建的模型在预测能力上优于其他治疗前临床特征如单一淋巴结转移、血清鳞状细胞癌抗原和临床T分期等[27]。在生存预后方面，美国的一项多中心研究发现miR-4253和miR-1238-5p水平每增加一倍(其对数每增加一个单位)，EAC患者任何原因导致的死亡风险就会分别增加4.85倍(P=0.04)和3.81倍(P=0.04)[26]。miRNA的珍贵价值不仅体现在其特异性改变能够预测预后，其作为潜在的癌基因或癌抑制基因与其靶标之间的相互作用更为新型疗法的产生提供了巨大前景[62]。

2.3.2 其他非编码RNA　除miRNA外，其他ncRNA[如长链非编码RNA(long non-coding RNA, lncRNA)、环状RNA(circular RNA, circRNA)和tRNA来源的小RNA(tRNA-derived small RNA, tsRNA)等]对于EC的早期诊断和预后评估同样具有潜在价值。这些外泌体相关ncRNA已被发现可参与肿瘤的多种进展过程，包括促进肿瘤增殖、转移和侵袭等[29,68-69]。有研究通过结合2种circRNA在血浆中的表达水平来建立基于circRNAs特征的诊断模型，发现其诊断性能优于单一的circRNA水平，且其中的circ_0001946被证实可以预测ESCC患者的OS和DFS[29]。来源于血清样本的4种lncRNA组合在区分健康人群与ESCC患者和鉴别不同肿瘤分期上都具有较优的诊断性能，其曲线下面积(area under the curve, AUC)分别达到了0.844和0.935[30]。除了血液中的外泌体之外，唾液中的外泌体也是近几年检测的热点，正逐渐展现出作为预测高风险临床不良结局的无创标志物的潜力[70]。有研究在多个前瞻性ESCC队列中评估唾液外泌体相关的高尔基体膜蛋白1(Golgi membrane protein 1, GOLM1)与*N*-α-乙酰转移酶35(*N*-α-acetyltransferase 35, NAA35)的嵌合RNA(G-NchiRNA)在治疗反应的纵向监测中的效用，发现G-NchiRNA水平能够反映肿瘤复发并预测放化疗反应，并且G-NchiRNA水平的降低先于对治疗反应的放射学证据[28]。最近的一项多中心前瞻性研究在唾液来源的外泌体中发现了与ESCC相关的两种转运RNA(tRNA-GlyGCC-5和一种新型非编码小RNA)，并且证实了这两种小RNA不仅可以检测早期和晚期的ESCC，还可以反映接受放化疗的患者的治疗反应，同时，基于这二者开发的双特征预后风险评分模型显示其评分与OS和PFS呈负相关[32]。

2.3.3 外泌体相关的其他内容物　外泌体的其他内容物(如蛋白、代谢物等)亦可作为EC进展的预测标志物。有研究发现血清来源的外泌体中存在的一种微管调节蛋白stathmin-1的浓度在ESCC患者中显著

表2 不同液体活检标志物的诊断性能及预后价值比较

Table 2 Comparison of diagnostic and prognostic values of different liquid biopsy biomarkers

标志物 Biomarkers	病理类型 Pathological type	应用领域 Application	ROC曲线下面积 AUC
ctDNA	ESCC	Treatment response	0.880 [71]
ctDNA	GOA	Survival prediction	0.946 [72]
miR-133a-3p, miR-382-5p, miR-451a	EAC	Early diagnosis	0.846 [24]
miR-193b-5p, miR-873-3p	ESCC	Early diagnosis	0.912 [27]
miR-193b-5p, miR-873-3p	ESCC	Treatment response	0.730 [27]
CD-14	ESCC	Early diagnosis	0.960 [34]
circ_0001946	ESCC	Early diagnosis	0.928 [29]
4-lncRNA	ESCC	Identification of stage	0.935 [30]
circ_0026611	ESCC	Identification of metastasis	0.724 [73]

ROC: 受试者工作特征; AUC: 曲线下面积; ctDNA: 循环肿瘤DNA; lncRNA: 长链非编码RNA; ESCC: 食管鳞状细胞癌; EAC: 食管腺癌; GOA: 胃食管腺癌。

ROC: receiver operating characteristic; AUC: area under the curve; ctDNA: circulating tumor DNA; lncRNA: long non-coding RNA; ESCC: esophageal squamous cell carcinoma; EAC: esophageal adenocarcinoma; GOA: gastric and oesogastric junction adenocarcinoma.

升高, 并由此证明了其浓度升高与淋巴结转移和分期有关[31]。血浆外泌体的靶向代谢组学分析有助于找到预测肿瘤复发的潜在代谢组特征, 一项针对ESCC患者的研究基于机器学习利用差异代谢物来区分复发和非复发患者, 并证实了基于血浆外泌体来源的4种代谢物组合的模型(3′-UMP、棕榈油酸、棕榈醛和癸酸异丁酯)可以预测肿瘤复发[33]。外泌体表面的CD-14蛋白也被发现在ESCC患者中具有潜在的诊断效能, 并且其基因表达水平高的患者OS更短[34]。

由此可见, 外泌体作为早期或晚期EC检测的一种非侵入性生物标志物, 在早期诊断、治疗反应和肿瘤复发监测, 以及其他临床结局预测上均具有巨大潜力。

2.4 液体活检在食管癌诊疗和预后评估中的应用小结

液体活检作为一种生物标志物检测手段在EC诊断和预后评估上具有重要的潜力。其主要通过收集和检测体液中的CTCs、ctDNA、外泌体(包括miRNA、ncRNA和其他内容物)等生物标志物, 实现对EC的早期诊断和动态预后评估。而不同的生物标志物侧重于不同的应用领域, 如ctDNA可以检测到肿瘤的存在、遗传变异和突变情况, 因此在监测疾病进展与疗效监测上更具优势[14-17,74]; CTCs则可以辅助确定肿瘤存在和转移情况, 其数量和特征可以预测患者的生存期和复发风险[19-20,22]; 某些特定的外泌体相关ncRNA被发现与肿瘤的发生、进展和预后相关, 从而可以辅助EC的诊断和预后评估[24,26-27,67]; 外泌体的其他内容物则可以提供多种肿瘤信息如突变情况、转移倾向和治疗反应等[31-33]。此外, 在诊断和预后价值的评价上, 不同液体活检生物标志物也各有侧重, 且其AUC存在一定差异, 但总体性能均较好(表2)。总之, 通过结合这些基于液体活检的生物标志物, 有望提高EC早期检测的准确性, 并帮助实现治疗决策制定和个性化患者管理。

3 液体活检在评估食管癌预后中的优势及可能存在的局限性

综上所述, 基于ctDNA、CTCs及外泌体内容物的液体活检在评估EC疾病进展、肿瘤负荷、治疗反应、临床结局等中已经取得较大进展。液体活检最大的优势在于其非侵入性和可重复性, 并与其他用于诊断和预后评估的临床检测方法相比存在较多优势(表3)。如前文所述, 液体活检在灵敏度和特异度上高于其他常规的血液学检查, 并且正因为液体活检相关的生物标志物来源于血浆、血清、唾液等容易获得的体液, 使得对肿瘤进行重复和纵向分析得以实现, 为监测肿瘤负荷和对治疗的反应提供了简单的方法[11]。与局部组织活检相比, 它在展示与肿瘤相关的特征方面更具整体性, 并且能更早地发现转移性肿瘤的存在[22]。与影像学检查相比, 液体活检可更早地提示微小残留灶和微小病变的存在, 更早地实行疗效评估, 从而能够帮助临床医生及时调整治疗策略及方案[14,19,28]。

然而, 目前在临床上普遍实施液体活检仍存在

表3 液体活检和其他临床检测方法特点比较

Table 3 Comparison between liquid biopsy and other clinical detection methods

特征 Characteristics	液体活检 Liquid biopsy	内镜下组织活检 Endoscopic biopsy	影像学检查 Imaging examination	其他血液生物标志物 Blood biomarkers
Invasive or not	Partly*	Yes	No	Yes
Quantitative/qualitative	Quantitative/qualitative	Qualitative	Qualitative	Quantitative/qualitative
Cost	Low	High	High	Low
Sensitivity, specificity	High	Depending on the experience of doctors	Depending on the experience of doctors	Low
Sampling	Simple and repeatable	Complex and low in acceptability	Simple	Simple

Partly*: 血液样本侵入性; 其他体液样本非侵入性。

Partly*: invasive in the blood sample; non-invasive in other body fluid samples.

一些挑战, 主要体现在以下两个方面。(1) 检测技术的局限性: 对于在血液中含量较低的CTCs来说, 其检测精度一直是需要克服的难点, 即使是获批于临床使用的CellSearch平台也无法满足高特异性和灵敏度的要求[75]。对于ctDNA也存在类似的问题, 尽管其在血液中的含量相比CTCs来说有所提高, 但由于来自其他有核细胞的DNA的存在导致ctDNA水平的稀释, 也需要极其灵敏和高效的分离技术[76]。(2) 评估标准难以统一: 于外泌体而言, 尽管其携带的许多分子已被证明可作为潜在的生物标志物, 但仍需要对不同体液的外泌体分类提取方法进行标准化, 并在更大的范围内验证[55]。由于CTCs、ctDNA、外泌体均存在一定的异质性, 且不同检测方法存在灵敏度和特异度差异, 较难在不同临床阶段的评估上设定统一标准[77-78], 仍需要更多的临床试验才能使液体活检成为临床常规工具。

4 液体活检在食管癌诊疗及预后评估中的应用前景

由此, 液体活检在EC诊疗和预后评估上所展现出的潜力需要未来更多的前瞻性研究及临床研究来验证。尽管液体活检的灵敏度、特异性目前可能受到限制, 但它仍能获取关于肿瘤特征、进展状态和预后的重要信息, 结合组织活检和其他临床检查, 可以为EC患者提供更有效的早期诊断以及更全面的癌症评估和监测, 从而更好地指导治疗和预后管理。目前, 随着各领域科学技术的不断发展, 液体活检也具有广泛的应用前景。

4.1 个体化精准药物治疗与液体活检

4.1.1 *治疗靶点筛查和指导用药* 液体活检能够提供有关肿瘤的分子特征和变异信息, 这些信息可以帮助识别患者中潜在的靶点突变和驱动基因, 从而指导治疗方案的制定和个体化用药。事实上, 在EC患者中已经发现了异常表达的miRNA通过调节细胞增殖、迁移和侵袭, 从而对肿瘤的发展和进展起重要作用[79-80]。一方面, 有些miRNA的过表达抑制肿瘤生长, 如miR-125b在靶向BCL-2修饰因子介导的ESCC中具有抗肿瘤作用[81]; 另一方面, 部分miRNA的高表达与预后不良有关, 这说明了它们具有促进肿瘤进展的作用, 抑制其靶基因的表达可能在ESCC调控中发挥重要作用, 因此可将这些miRNA靶标视为ESCC治疗的可能靶点[82]。而液体活检可以为靶点筛查提供一种快速、高通量的测试方法, 有望为携带相应靶点突变的患者制定治疗方案并指导用药。

4.1.2 *疗效实时动态监测和评估* 在多种EC治疗方案如手术治疗、放疗联合化疗、术前新辅助治疗、免疫治疗等中, 基于液体活检的生物标志物已经体现了其动态监测治疗反应的效用[14,16-17]。除此之外, 液体活检或可确定治疗目标, 鉴于手术过程中可能存在脱落的肿瘤细胞以及只有耐药细胞才能长时间存活并诱导转移的情况, 有研究将EC患者术前与术后13天的CTCs计数变化作为疗效评估的标准[83]。目前, 免疫治疗作为肿瘤治疗的热点, 对其疗效的及时监测十分重要。通过常规影像学检查进行初始反应评估通常无法确定患者能否获得持久的临床受益(durable clinical benefit, DCB), 然而, 能够监测疗效的液体活检却为此提供了可能。有研究证实了在非小细胞肺癌中治疗前ctDNA阳性水平与DCB的相关性, 这表明治疗前进行液体活检可能实现早期、无创、

准确的结果预测[84]。而外泌体中的程序性死亡受体配体1(programmed cell death ligand 1, PD-L1)因其在抗PD-1/PD-L1治疗反应预测中的潜力而受到研究人员的广泛关注[85]。虽然在EC中未进行验证, 但未来可对外泌体中PD-L1的临床应用进行研究。鉴于液体活检的非侵入性与可重复性, 应充分发挥其在疗效的动态监测与评估中的价值。

4.2 液体活检联合基于多组学的机器学习实现精准诊疗

人工智能(artificial intelligence, AI)是机器对人类智能过程(包括感知、合成和推断信息)的模拟, 机器学习也是AI的一种形式, 它通过不同的算法基于数据进行模式识别和预测。目前已经通过针对内镜成像的机器学习成功预测了EC黏膜下浸润并区分了T1a期与T1b期[86]。此外, 多组学(multi omic)作为一种综合性的研究方法, 整合了基因组学、转录组学、蛋白质组学、代谢组学等, 而液体活检能够提供获得其中多种数据的高通量测量方法, 因此将其与多组学及机器学习结合, 可能进一步揭示EC的生物学特征, 从而为开发早期诊断和预后评估的精准生物标志物提供前景[87]。

4.2.1 *食管癌不同阶段辅助诊断模型的建立* 基于液体活检生物标志物的机器学习可能有助于人群的大规模筛查, 并且能够避免在肿瘤病变的早期检测和巴雷特食管检测中不必要的侵入性操作, 同时对后者进行液体活检还有助于分析进展为肿瘤的风险[88]。目前, 已经有研究发现机器学习在多种早期诊断和超早期诊断领域有所建树, 揭示了它通过高维组学数据重建表型特征、区分原发性肿瘤和转移性肿瘤等的能力[89-90]。尽管在EC中没有进行大型队列的验证, 但液体活检提供的单一组学数据有望为EC多组学特征数据库的建立奠定基础, 更有效地实现肿瘤分期、病灶分级、危险分层等[91], 建立EC早期与超早期辅助诊断模型, 推进中晚期和局部晚期EC精准诊断, 从而为实现EC不同阶段的精准医疗提供强有力的技术支撑。

4.2.2 *治疗后肿瘤进展与不良预后早期预警模型构建* CTCs数量和ctDNA水平的相互关联性可能显示了液体活检的联合检测在评估EC预后上的潜力[92], 这也与多组学分析的思想类似, 但能解释这种相关性的生物学机制仍未被发现, 需要通过临床研究来提供理论依据。多组学技术的应用可能为EC的个体化治疗提供参考依据, 最近有研究综合分析了基因组、转录组、PD-L1表达和血清蛋白的变化, 并且揭示了这种多组学分析与新辅助化疗联合免疫治疗后的临床转归和疗效应答的相关性[93]。而AI也可用于识别诊断生物标志物, 预测EC的五年生存率[94]。基于这两种临床效用, 期望能通过二者的结合尽早发现肿瘤进展与预后不良的患者, 虽然还没有发现基于液体活检的机器学习成功预测预后的案例, 但未来这可作为发展方向, 通过液体活检技术实现高通量、快准稳的多组学分析, 结合完善而深度的机器学习, 确定EC的最佳预后生物标志物与早期预警模型。

4.3 类器官与液体活检结合促进食管癌临床前研究

近年来, 类器官作为一种模拟上皮细胞生物学和肿瘤研究的新工具, 是一种体外培养的组织模型, 可以更好地模拟人体内的疾病微环境, 为EC进展的分子机制研究、新型药物的筛选和新治疗决策的制定提供有力支持[95-96]。更重要的是, 类器官技术可以在样本量较小的情况下模拟EC进展, 这可能在很大程度上弥补基于液体活检的CTCs和ctDNA富集较为困难的不足。因此, 将液体活检与类器官技术结合具有较大的应用前景, 在结直肠癌和肺癌中已经发现CTCs衍生的类器官模型能够帮助监测肿瘤转移进展以及高通量药物筛选等[97], 而在EC中类器官也被证实能用于模拟患者的放化疗反应[98]。未来可着力于EC类器官与液体活检结合的临床前研究, 实现更全面、准确和高效的诊疗方案的制定。

展望未来, 有效的EC诊断、治疗和预后管理可能会更聚焦于上述这些基于液体活检的新兴技术与方法上, 而不是仅仅依靠基于单一生物标志物的分析, 期待日后基于液体活检的多参数诊断模型、预后模型的建立能为实现EC的精准治疗添砖加瓦。

5 结语

总而言之, 对于早期症状隐匿而预后不佳的EC而言, 目前的临床工具尚不能完全实现EC的早期诊断及精准的疗效和预后评估, 从而导致EC患者不良结局的发生。而液体活检在诊断、预后和治疗选择领域均展现出巨大的优势, 有望帮助EC目前的诊疗脱离上述这一恶性循环, 改善EC患者的预后。通过对ctDNA、CTCs、外泌体等基于液体活检的生物标志物的检测, 将其与其他临床指标或组学信息相结合, 加以新兴技术的辅助, 能够实现对

EC患者更加全面、准确而高效的诊疗和预后评估，并及时加以干预，从而为精准医学的进一步推广和应用提供更多的机会。未来需要更多的临床试验来证实液体活检广泛的预后价值，包括但不限于连续治疗反应监测、临床结果预测、风险评估和耐药机制研究等。相信随着科学和检测技术的不断进步与完善，液体活检在不久的将来能够成为肿瘤学的有前途的革命性武器。

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