·综述·

液体活检在胶质瘤诊断中的意义

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摘　要：随着对胶质瘤发生发展机制的深入研究，越来越多的肿瘤标志物被用于胶质瘤的精准诊断，包括循环肿瘤细胞（CTCs）、循环肿瘤 DNA（ctDNA）、微小 RNA（miRNAs）、外泌体等。有别于病理组织活检，应运而生的液体活检技术，通过检测血液、脑脊液等体液中存在的特异性肿瘤标志物，不仅降低了侵入性，而且对于诊断胶质瘤，阐明肿瘤侵袭和播散的机制，揭示肿瘤异质性，设计个体化治疗和评估改善疗效意义重大。

关键词：胶质瘤；循环肿瘤细胞；循环肿瘤 DNA；微小 RNA；外泌体

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Diagnostic value of liquid biopsy in glioma

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Abstract: As research in the mechanism of glioma development and progression advances, more and more tumor markers are used for the precise diagnosis of glioma, including circulating tumor cells, circulating tumor DNA, microRNA, and exosome. The newly emerging technique, liquid biopsy, shows features differing from pathological tissue biopsy. Through detecting specific tumor markers in body fluids like blood and cerebrospinal fluid, liquid biopsy provides a less invasive but valuable way to diagnose glioma, clarify how it spreads, unravel its heterogeneity, design individualized treatment, and evaluate therapeutic effect.

Key words: glioma; circulating tumor cells; circulating tumor DNA; microRNA; exosome

胶质瘤是最常见的中枢神经系统原发性恶性肿瘤，每 100000 人中约有 7.3 例患者，其中高级别胶质瘤约占 85%，低级别胶质瘤约占 15%，多发于成年人[1]。胶质瘤细胞可以起源于不同的前体细胞，少突细胞前体细胞（oligodendrocyte precursor cells，OPCs）是重要的原始细胞之一，将 p53 和 NF1 基因敲除后，大部分时间处于稳定状态来源于成年人的 OPCs 将会被重新激活、增殖直至发生恶变。哺乳动物类雷帕霉素靶蛋白（mammalian target of rapamycin，mTOR）信号通路等参与了胶质瘤的发生发展[2]。由于胶质瘤的发生机制非常复杂，绝大部分胶质瘤早期不易发现，确诊胶质瘤仍然面临极大的挑战。传统的诊断技术主要包括病理组织活检和颅脑影像学检查，但两者均有一定的局限性：前者作为侵袭性手段，患者依从性不高；后者鉴别诊断仍有困难而可能延误最佳治疗时机[3-5]。目前，在肿瘤检测的临床研究中，科学家对无创诊断技术即液体活检技术表现出极大兴趣。该技术通过分离检测来自体液的循环肿瘤细胞（circulating tumor cells，CTCs）、循环肿瘤 DNA（circulating tumor DNA，

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ctDNA)、微小 RNA(miRNAs)、外泌体等为肿瘤学的临床决策提供丰富信息,它的应用能够较好地弥补传统诊断技术的不足。本文围绕胶质瘤的液体活检技术及新型肿瘤标志物进行综述,探讨其在临床中的巨大应用前景。

1 CTCs

通过激活信号通路,表达细胞外黏附分子等机制,来自原发肿瘤的胶质瘤细胞和胶质瘤干细胞(glioma stem cells,GSCs)可进入血管成为 CTCs[6],因 CTCs 具有干细胞的特性,对放化疗和循环应激诱导的细胞凋亡也具有一定的的耐受性[7]。在 GSCs 中,增殖细胞核抗原相关因子(proliferating cell nuclear antigen-associated factor,PAF)的过度表达影响了 DNA 的复制和嘧啶代谢通路,PAF 与增殖细胞核抗原(proliferating cell nuclear antigen,PCNA)相互作用并能够调节 DNA 的跨损伤修复(trans-lesion synthesis,TLS),因此 GSCs 拥有强大的自我更新能力,并导致胶质母细胞瘤(glioblastoma multiforme,GBM)患者对放疗不敏感,具有 PAF 类似作用的还有 Akt 家族基因以及磷脂酰肌醇 3-激酶(phosphoinositide 3-kinase,PI3K)/蛋白激酶 B(protein kinase B,Akt)信号通路[8-10]。Krol 等首次证实了胶质母细胞瘤患者 CTCs 簇的存在[11]。为了筛选分析 CTCs,Müller 等利用胶质细胞原纤维酸性蛋白(glial fibrillary acidic protein,GFAP)作为标志,通过比较基因组杂交技术(comparative genomic hybridization,CGH)、序列分析和荧光原位杂交(fluorescence in situ hybridization,FISH),进一步证明 CTCs 来源于 GBM,但是,在这一研究中,通过 GFAP 的免疫染色,仅有 20.6% 的 GBM 患者在外周血检测到了 CTCs[12]。利用原位杂交技术,同样可以检测 CTCs 的 miRNAs,Ortega 等利用 MishCTC 在上皮来源的循环肿瘤细胞中检测到 miRNA-21[13]。因此,利用这项技术有可能让胶质瘤得到早期诊断。由于 CTCs 获得率和纯度较低、获得的 CTCs 与原始肿瘤细胞间的异质性等有待研究,所以,该技术真正应用于临床还须进一步探索[14-16]。为了降低背景噪音,提高胶质瘤诊断技术的特异性,有科学家在尝试从脑脊液中分离 CTCs,期望能够从中获得胶质瘤细胞[17]。

2 ctDNA

循环血液中存在着一种 DNA,称之为血浆游离 DNA(cell-free DNA,cfDNA),其中来源于肿瘤细胞的称之为 ctDNA。ctDNA 的获取方法无创,利于 GBM 诊断和分型,可以协助制定治疗方案并检测患者对治疗的反应。对获取的 ctDNA 可以开展包括微滴式数字 PCR(droplet digital PCR,ddPCR)定量分析,突变基因检测,基因组、表观基因组和蛋白质组分析等[18-19]。在一项研究中,19 位脑脊液中分离检测到 ctDNA 的患者,仅有 3 位的 cfDNA 检测到 35 个突变位点,包括 1p/19q 共缺失、突变型 IDH1/IDH2 和生长因子受体信号通路的改变等,cfDNA 的这些突变位点的平均变异等位基因分数远低于脑脊液来源的 ctDNA(0.58% vs 23.96%),且大多数肿瘤组织中的克隆突变也存在于脑脊液中[20]。随着更多特异性突变位点被发现,这些位点的联合检测可以使肿瘤 DNA 诊断胶质瘤的特异性高达 100%[21]。尽管如此,不同体液来源的 ctDNA 方法学特征和提供的信息仍然具有差异,脑脊液来源的 ctDNA 提供的信息更加精确,更具有代表性,获得成本更低,相比 cfDNA,脑脊液来源的 ctDNA 突变位点的检测敏感性更高(100% vs 38%)[22],它的优势得益于检测分析手段的更新,即将体拷贝数改变(somatic copy number alteration,SCNAs)的分析与配对端测序确定的 DNA 片段模式相结合,借助 sWGS 数据检测 CSF 中的肿瘤细胞游离 DNA(cell-free tumor DNA,cftDNA)[23-25],同时将 DNA 分子片段长度测定联合特异性片段基因测序能够进一步提高 ctDNA 相关检测技术在胶质瘤诊断中的准确性,将受试者操作特征曲线下面积(area under curve,AUC)从小于 0.5 提高至大于0.91[26]。鉴于脑脊液中 ctDNA 的密度低于外周血液,Mair 等研究发现循环肿瘤线粒体 DNA(circulating tumor mitochondria DNA,tmtDNA)有助于提高胶质母细胞瘤的检测率,可以替代核 ctDNA(82% vs 24%),在脑脊液和尿液中也可以检测到,因此 tmtDNA 有更加广泛的应用前景[27-28]。

3 miRNA

miRNA 是长约 22nt 的非编码 RNA,广泛存在于从病毒到人类的各种生物中,通过与 mRNA 的 3'-UTRs 结合抑制 mRNA 表达或促进 mRNA 降解,防止蛋白合成[29-30]。许多研究表明 miRNA 在人类疾病的发生发展扮演重要的角色,如胶质瘤等肿瘤[31-37]。由于恶性肿瘤细胞具有异质性,ctDNA 并不会拥有完全相同的序列[38],相比之下,miRNAs 的一致性更高。Zhou 等通过荟萃分析得出,在胶质瘤的诊断中,miRNAs 的整体检测灵敏度达 85%

(95% *CI*:0.81 ~ 0.89),特异性为 90%(95% *CI*:0.85 ~ 0.93),AUC 是 93%(95% *CI*:0.91 ~ 0.95),其中来自血液标本的分别是 84%(95% *CI*:0.80 ~ 0.88),85%(95% *CI*:0.81 ~ 0.89)和 92%(95% *CI*:0.89 ~ 0.94),来自脑脊液标本的分别是 89%(95% *CI*:0.73 ~ 0.96),98%(95% *CI*:0.87 ~ 1.00),98%(95% *CI*:0.96 ~ 0.99);同时检测一组 miRNAs 可以提高灵敏度,如(miRNA-93, miRNA-590-3p, miRNA-454);(miRNA-15b,miRNA-21)等组合[39]。不仅如此,通过测序以及信号通路分析发现 miRNA 可以用于评估胶质瘤细胞的放化疗敏感性[40]。对患者临床资料的分析结果表明,低表达 miRNA-221 和 miRNA-222 的患者预后更好[34]。而且,miRNAs 与胶质瘤的侵袭性密切相关,通过慢病毒转导 miRNA-1270 的 LN-18 细胞系在雄性裸鼠体内的生长速度和体积得到了抑制;miRNA-605 的高表达可以抑制胶质瘤细胞系 U251 和 T98 细胞系增殖、迁移和侵袭的进展[32,35]。相比于肿瘤组织来源的 miRNA,基于脑脊液的 miRNA 因对核糖核酸酶和物理化学条件的耐受性更高,所以更加稳定、精确性也可能更高[41-43],并可以用于 CNS 其他恶性病变的鉴别诊断[44]。但是,由于 miRNA 序列较短,目前的探针不能很好分析 pri-,pre-以及成熟形式的区别,而且相关技术只能进行相对定量,因此,需要找到更加标准的内源性对照[41]。除了 miRNA,其他细胞外 RNA(extracellular RNA,exRNA)在胶质瘤检测中的标记作用也有待进一步挖掘,如 Y RNA 和 tRNA 等[43]。其中,tRNAs 的产生离不开 RNA 多聚酶Ⅲ(polymerase Ⅲ, Pol Ⅲ)的指导和转录因子ⅢB 和ⅢC(transcription factor ⅢB/C,TFⅢB/C)的控制,对于 mRNA 的表达至关重要,被证明与包括黑素瘤、GBM 在内的多种癌症密切相关,其关键在于癌基因和肿瘤抑制信号通路可以调节 Pol Ⅲ 和 tRNAs 的合成,进而影响细胞的生物学行为[45-47]。Yang 等发现 SOX4 通过结合特异的 tRNAs,如 tRNAiMet 等,阻碍 TATA box 结合蛋白和 Pol Ⅲ 对 tRNAs 基因的募集,从而抑制其表达以及 GBM 细胞的增殖[48]。

4　外泌体

外泌体是由多种细胞产生的脂双层分泌体,直径 30 ~ 100nm,包含 miRNAs 以及蛋白质等多种物质,参与细胞间信息传递和肿瘤微环境的形成[49-51]。在胶质瘤中,外泌体的产生与间充质干细胞和胶质瘤干细胞密切相关[52-54],如:来源于 GBM 的外泌体包含有凝血因子 TF/Ⅶa 复合体,进而诱导形成乏氧环境、促进血管生成、增强侵袭性[55]。Sun 等证明 GBM 干细胞通过分泌产生包含 Notch1 蛋白的外泌体促进 GBM 的侵袭转移[56]。外泌体凭借其提供的丰富信息可以用于胶质瘤的诊断,预后和治疗[57-58]。Santangelo 等发现血浆来源的外泌体包含的 mi-21/222/124-3p 与胶质瘤的分级和预后有关[59]。表皮生长因子受体(epidermal growth factor receptor,EGFR)与胶质母细胞瘤某一亚型密切相关,以突变型 EGFRvIII 为例,47% 左右的 GBM 患者 EGFRvIII 为阳性,它可以调控外泌体生物发生、细胞间转运和生物学效应[60-61]。大部分胶质瘤患者存在 EGFRvIII 突变体,利用脑脊液来源的外泌体检测 EGFRvIII 突变体,在诊断胶质瘤中特异性高达 98%,敏感性只有 61%[62]。当分离检测血浆来源的包含长链非编码 RNA-HOX 转录反义基因间 RNA(hox transcript antisense intergenic RNA,HOTAIR)的外泌体时,灵敏度为 86.1%,特异性为 87.5%,AUC 为 0.913(95% *CI*:0.845 ~ 0.982,$P < 0.0001$)[63]。然而由于分离检测方法步骤多耗时长,外泌体的纯度得不到保证,甚至有污染的风险[18]。为了提高临床应用价值,Lobbr 等报道了一种有效可重复,室温下能够维持外泌体足够稳定的方法[57]。同样的,经过人工设计的外泌体作为载体,可以顺利的通过血脑屏障,将纳米材料、化学药物和 miRNAs 靶向运输到病灶,甚至大脑小胶质细胞来源的外泌体也可以作为纳米治疗剂,用于诊断和治疗[53,64-65]。

通过筛选分析与肿瘤密切相关的细胞、分子与基因等物质,液体活检技术拓展了对肿瘤发生发展机制的认识,提高了诊断治疗的特异性,因其微侵袭性而更容易被患者接受。随着各项研究的开展,越来越多的循环肿瘤标志物被发现,但是由于灵敏度或特异性的限制,能够应用到临床的标记十分有限。更大的挑战在于如何找到合适的标记组合,收集分析不同信息,为胶质瘤的早期诊断分型、治疗方案的制定实施和疗效判定等提供科学依据;如何将基础研究和临床应用相结合,使丰富的生物标志更多地转化为胶质瘤的临床检测方法;如何降低高昂的检测成本,使液体活检技术能够在临床广泛开展。相信随着研究的深入进展,液体活检技术将在胶质瘤的临床应用中大放异彩。

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