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文献综述 REVIEW

循环肿瘤细胞分子鉴定与个体化肿瘤诊疗

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Molecular characterization of circulating tumor cells and individualized cancer diagnosis and therapy

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Abstract

The heterogeneity of cancer cells involved in oncogenesis and metastasis has always been the key factor that impedes tumor diagnosis and treatment (especially traditional chemotherapies). In recent years, molecular characterization of tumors and accordingly implementation of individualized treatment targeting specific molecular markers have become a hotspot for cancer research. As a link between the primary tumor and metastases, circulating tumor cells (CTCs) provide a window into tumor biology and the metastatic cascade. With their real-time, non-invasive and repeatable access, CTCs are excellent resources of tumor specimens. Molecular characterization of CTCs is of great significance for tumor molecular analysis and individualized treatment. Here we review the recent progress in molecular characterization of CTCs and individualized cancer diagnosis and therapy.

Key Words: Tumors; Circulating tumor cells; Molecular characterization; Individualized therapy

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摘要

肿瘤细胞表现的高度异质性严重困扰着肿瘤临床诊断和治疗. 因此, 肿瘤分子分型及其指导个体化治疗一直是肿瘤研究领域的热点. 循环肿瘤细胞(circulating tumor cells, CTCs)作为具有肿瘤代表性的"液体活检"样本, 允许多次、实时、非侵入性获取, 是指导个体化肿瘤诊疗的绝佳标本. 目前认为, 基于分子鉴定检测少数更具存活力和侵袭性的CTCs比单独CTCs计数更有价值. 而在肿瘤转移过程中CTCs表现的多种生物学特性及其分子机制都可被用于分型, 并且可能成为个体化肿瘤诊疗的靶点. 本文综述近年来关于CTCs分子鉴定的研究进展, 以及CTCs分子分型指导个体化诊疗的研究现状, 提出相关领域今后的研究方向.

关键词: 肿瘤; 循环肿瘤细胞; 分子鉴定; 个体化治疗

核心提示: 尽管关于循环肿瘤细胞(circulating tumor cells, CTCs)的研究报道越来越多, 但目前更多的是注重于CTCs富集、分离方法学的改进或升级以及CTCs计数临床意义的评价, 至于CTCs分子表型鉴定及其临床意义的研究尚处于起步阶段. CTCs具有特有的生物学特性和行为,

■背景资料
肿瘤异质性是困扰肿瘤临床诊断和治疗的一大问题, 分子分型是个体化治疗的基础. 脱离原发灶或转移灶进入血液的循环肿瘤细胞(circulating tumor cells, CTCs)是具有肿瘤代表性的绝佳标本, 以其代替肿瘤组织进行分子表型分析, 在临床上指导个体化诊疗已成为肿瘤学领域的一个研究热点.

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■研发前沿
随着生物技术的不断发展和进步, CTCs研究正在从单纯的分离计数逐渐发展为在分离计数基础上进一步进行分子鉴定, 尤其是单细胞测序技术的发展, 允许对单个CTCs进行分子鉴定和比较分析, 是未来的一个研究趋势.

有关分析对于进一步了解肿瘤异质性, 选择个体化治疗方案, 并在治疗过程中实时监测疗效具有广泛的应用前景.

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0 引言

恶性肿瘤通常经历一个由原发灶形成, 肿瘤细胞不断侵袭、迁移, 最终形成转移灶的过程. 在此过程中肿瘤细胞表现的高度异质性为临床诊断和治疗带来很大困扰, 严重挑战传统的诊疗方法[1]. 因此, 对肿瘤进行分子分型并指导个体化治疗一直是肿瘤研究领域的热点, 也是未来的发展方向[2]. 然而, 真正实现肿瘤分子分型并非易事, 一个最大局限是对肿瘤组织的获取, 对术后难以找到可测量病灶的肿瘤更是如此. 而且在漫长发展过程中肿瘤细胞不断发生基因型变化, 需要多次获取样本对肿瘤细胞表型进行实时监测[3,4]. 值得庆幸的是, 近来的研究表明, 脱离原发灶或转移灶后进入血液的循环肿瘤细胞(circulating tumor cells, CTCs)作为原发灶和转移灶之间的链接, 以及肿瘤生物学和转移的窗口, 不仅是研究肿瘤转移复发过程和机制的一个重要切入点, 而且可作为具有肿瘤(包括转移灶)代表性的“液体活检”(liquid biopsy)样本, 允许多次、实时、非侵入性获取, 是指导个体化肿瘤诊疗的绝佳标本[5]. 然而, 现有的研究表明, 固然CTCs检测具有评估疾病进程和预测肿瘤转移复发、生存预后的潜能, 但是CTCs也是具有高度异质性的细胞群体, 基于分子鉴定检测少数更具存活力和侵袭性的CTCs比单独CTCs计数可能更有价值[6]. 已知CTCs在历经侵袭、转移各个阶段的过程中, 需要获得上皮间质转化、干细胞特性、逃逸免疫监视、抵抗失巢凋亡、耐受治疗等生物学特性, 才能闯过层层难关生存下来并增殖, 最终形成远处转移灶[7]. 而上述每个生物学特性的分子机制都可被用来进行肿瘤分子分型, 并且可能成为个体化肿瘤诊疗的靶点. 本文综述近年来关于CTCs分子鉴定的研究进展以及CTCs分子分型促进个体化诊疗转化研究的现状, 提出相关领域今后的研究方向.

1 *CTCs*全基因表达谱检测

肿瘤全基因表达谱检测早已得到较多应用, 近来有人尝试检测CTCs全基因表达谱. Smirnov等[8]从3例转移性肿瘤患者外周血中富集CTCs, 比较富集前、后血样中基因表达谱变化, 筛选出35个差异表达基因, 然后在30例转移性结直肠癌患者CTCs中进行验证, 发现其中*KRT19*、*KRT20*、*AGR2(hAG-2)*、*CEACAM5*、*S100A14*、*S100A16*和*FABP1*等基因高表达. 如果以*AGR2*、*KRT19*、*S100A13*、*ASGR2*和*TST*联合表达区分转移病例和对照病例, 其准确率超过80%. Strati等[9]应用qRT-PCR检测66例早期乳腺癌患者CTCs基因表达谱, 发现*CK19*、*MAGE-A3*、*HER-2*、*TWIST1*、*hTERT*和乳腺珠蛋白转录本尽管表达率不同, 但均高于26例转移性乳腺癌, 提示这些基因与乳腺癌侵袭转移密切相关. Magbanua等[10]检测9例转移性去势抵抗性前列腺癌(castration resistant prostate cancer, CRPC)患者CTCs基因表达谱, 发现存在广泛的基因拷贝数异常(>50%). 值得注意的是, 其中7例(78%)X染色体上雄激素受体所在基因座拷贝数高水平扩增, 不仅提示这与CRPC疾病进展高度相关, 而且证明CTCs可用来检测CRPC患者雄激素受体扩增情况.

近年来的一个重要进展是单细胞基因测序技术和单细胞全基因表达谱检测在CTCs方面的应用[11,12]. Stoecklein等[13]应用比较基因组杂交技术对食管癌患者骨髓中获取的单个播散肿瘤细胞进行全基因组测序, 发现不同细胞之间的基因畸变存在偏差, 但在17q12-21染色体却均存在*HER-2*基因的获得, 并与生存预后相关. Powell等[14]应用qRT-PCR技术分析乳腺癌患者单个CTCs基因转录谱, 发现受检的87个肿瘤相关基因在不同CTCs中表达并不相同, 以其中31个基因高表达为标准, 可将所有CTCs分为两个亚群. 有趣的是, 培养的乳腺癌细胞系基因表达差异则与CTCs不一致. 因此认为, 以往通常利用肿瘤细胞系进行新药筛选及药敏研究的策略具有很大的局限性. 毫无疑问, CTCs单细胞基因测序将描绘更为完整的肿瘤全基因表达谱和差异表达谱, 更好地反映肿瘤细胞异质性, 为肿瘤分子分型及相应的个体化治疗提供更准确的依据.

2 CTCs转移潜能标志物检测

目前认为, 肿瘤转移效率非常低, 只有一小部分具有高转移潜能的CTCs可能存活下来并最终形成转移灶. 而反映肿瘤细胞转移潜能的分子标志物包括一系列癌基因、抑癌基因、肿瘤生存

基因以及microRNA[15]. Shen等[16]应用qRT-PCR检测结直肠癌患者CTCs中生存素mRNA表达, 发现阳性率(57.7%)显著高于良性疾病和健康对照组, 而且生存素阳性与Dukes分期和淋巴转移状态明显相关. Yie等[17]应用RT-PCR ELISA检测143例NSCLC患者CTCs生存素mRNA, 发现其表达与肿瘤侵犯程度、淋巴结转移状态以及疾病分期高度相关, 阳性患者转移和复发概率更高. 在乳腺癌、胃癌、结直肠癌中也有类似的发现, 且与血浆CEA相比, 生存素mRNA阳性CTCs能更准确地预测复发. Yoon等[18]应用巢式qRT-PCR检测79例NSCLC患者CTCs甲状腺转录因子-1(thyroid transcription factor 1, TTF-1)mRNA表达, 发现术后阳性患者的无进展生存时间(progression-free survival, PFS)较短, 提示CTCs表达TTF-1 mRNA与肿瘤转移潜能相关, 可以用于预测病情进展. 此外, 用于检测CTCs转移潜能的标志物还有AGR2(anterior gradient 2)[19]、M30(凋亡相关KRT18片段)[20]、端粒酶[21]等.

3 CTCs上皮–间质转化标志物检测

已知发生上皮-间质转化(epithelial-mesenchymal transition, EMT)的肿瘤细胞迁移和侵袭能力增强[22]. Lecharpentier等[23]应用免疫荧光染色检测6例转移性NSCLC患者CTCs间质标志物vimentin及上皮标志物Keratin表达情况, 结果在来自于6例的几乎所有CTCs中都可以观察到共同强表达这2个指标, 没有发现只表达keratin的CTCs, 但在3例患者中检测到很少几个只表达vimentin的CTCs. 此研究首次证实了具有上皮/间质混合性表型CTCs的存在. 而NSCLC原发性肿瘤的表型则是keratin阳性、vimentin阴性. Kasimir-Bauer等[24]应用AdnaTest方法检测乳腺癌患者CTCs中3种EMT标志物(TWIST1、Akt2、PI3Kα), 结果29%患者至少一种阳性. Powell等[14]分析乳腺癌患者CTCs单细胞转录谱后发现, 尽管CTCs之间存在基因表达差异, 但是普遍高表达EMT相关分子TGF-β1、vimentin和CXCR4. 最近的研究将CTCs的EMT表型与干细胞特性联系起来, 认为EMT可以赋予CTCs干细胞特性[25,26]. Armstrong等[27]证实肿瘤患者CTCs高频出现共表达上皮标志物(EpCAM、cytokeratins、E-cadherin)、间质标志物(vimentin、N-cadherin、O-cadherin)和干细胞标志物(CD133)的现象. 在另一项研究中, 研究者检测了乳腺癌CTCs中EMT相关转录因子(TWIST1、SNAIL1、SLUG、ZEB1、FOXC2)的表达情况, 并分析了接受或未接受新辅助化疗对CTCs表达这些转录因子的可能影响, 发现新辅助化疗并不能清除这些发生了EMT的CTCs[28]. 推测EMT很可能通过赋予CTCs干细胞特性使CTCs耐受化疗. 因此, 检测CTCs亚型对于指导分子靶向药物的研发及临床个体化治疗具有重要意义.

然而, 目前对于检测CTCs中EMT分子标志物存在一些争议. 一方面, 迄今对EMT现象仍缺乏共识, 尚没有被一致认可的EMT分子标志物, 以致于不同研究组选择的EMT标志物各不相同; 另一方面, 目前CTCs分离、检测大多基于上皮特异性分子(如EpCAM、CK等), 由于CTCs可能因EMT丢失或低表达这些分子, 导致具有EMT表型的CTCs无法被富集或漏检[29]. 因此, CTCs EMT分子表型检测的临床可行性依赖于更高敏感度和更高特异性的CTCs的富集方法.

4 CTCs干细胞标志物检测

有学者认为, CTCs就是来源于肿瘤干细胞的细胞亚群[30]. 比如有研究显示, 乳腺癌CTCs大多Ki67阴性(不增殖), 支持干细胞的“休眠”学说[31]. Balic等[32]最先研究了CTCs干细胞亚型, 发现转移性乳腺癌患者CTCs中72%具有$CD44^{+}/CD24^{-/low}$干细胞样表型, 而原发肿瘤灶中低于10%. 随后有多篇文献报道了类似的研究结果[33-35]. 乙醛脱氢酶1(acetaldehyde dehydrogenase 1, ALDH1)被认为是一个新的乳腺癌干细胞样标志物[36]. Aktas等[37]发现, 在转移性乳腺癌患者CTCs中ALDH1呈高表达, 且治疗无效组的表达阳性率(44%)显著高于治疗有效组(5%). Raimondi等[38]报道, 乳腺癌CTCs中ALDH1表达与间质指标vimentin和纤维连接蛋白表达相关, 并与肿瘤分期相关. 而Theodoropoulos等[33]则认为, 与只是ALDH1高表达的CTCs相比, ALDH1高表达且具有$CD44^{+}/CD24^{-/low}$表型的CTCs恶性程度更高. 然而, 目前有关CTCs干细胞标志物鉴定的研究报道较少, 一个主要的限速瓶颈可能是肿瘤干细胞标志物尚没有得到统一认定[39]. 因此, 肿瘤干细胞研究的进步将会推动CTCs干细胞标志物鉴定及其临床意义的研究.

5 CTCs分子靶向药物靶点检测

近来, CTCs中药物靶点鉴定替代原发组织标本指导靶向药物治疗和监测疗效、耐药的潜能受到重视. 已有多篇文献报道大部分乳腺癌患者(89%)CTCs表达HER-2与原发肿瘤组织一

■相关报道
目前国内外关于CTCs分离检测技术方法及其临床应用的综述较为多见. 国外关于CTCs分子鉴定及其临床意义的研究报道也越来越多.

■创新盘点

目前国内外鲜见关于CTCs分子鉴定及其临床意义的综述. 本文较为全面地介绍了CTCs分子分型指导肿瘤个体化诊疗的研究现状, 并展望了本领域今后的研究方向.

致[40-43], 这意味着依靠CTCs指导乳腺癌靶向药物Herceptin治疗成为可能, 从而可以摆脱目前单纯依靠肿瘤组织诊断的窘境. 有趣的是, 小部分患者(11%)原发肿瘤组织HER-2阴性, 却可以从相同患者CTCs中检测到HER-2阳性, 而在使用Herceptin治疗后, 其中部分患者有效. 说明检测CTCs表达HER-2甚至优于检测原发肿瘤组织. 另有报道, 检测CTCs表达雌激素受体和孕激素受体可以指导乳腺癌内分泌治疗[43-45]. 鉴于目前国际上对乳腺癌分子分型已形成共识[46], 可以预期, CTCs相关分子标志物检测在乳腺癌分子分型及个体化治疗方案制定方面将会大显身手.

其他类型肿瘤也有检测CTCs药物靶点的类似研究. Maheswaran等[47]应用CTC-chip检测接受吉非替尼治疗的NSCLC患者CTCs, 发现吉非替尼靶点EGFR突变与肿瘤组织具有高度一致性. Miyamoto等[48]检测前列腺癌患者CTCs中雄激素受体及其下游信号通路相关分子的表达情况, 可以指导前列腺癌内分泌治疗, 并通过治疗过程中雄激素受体变化情况评估疗效. 鉴于CRPC可以选择抗IGFR1治疗, 有报道应用CTCs检测IGFR1表达情况指导制定抗IGFR1治疗方案[49].

6 结论

尽管关于CTCs的研究报道越来越多, 但目前更多的是注重于CTCs富集、分离方法学的改进或升级, 以及CTCs计数临床意义的评价, 至于CTCs分子表型鉴定及其临床意义的研究尚处于起步阶段. CTCs具有特有的生物学特性和行为, 有关分析对于进一步了解肿瘤异质性, 选择个体化治疗方案, 并在治疗过程中实时监测疗效具有广泛的应用前景[50]. 然而, CTCs分子鉴定需要高敏感和高特异的CTCs分离方法以及针对单细胞或稀少细胞的分子分析技术. 现行的分离方法各有利弊, 尤其是分离发生EMT或干细胞样CTCs受到置疑和挑战[29]. 而现有的常用CTCs分子鉴定方法也存在一定的局限, 比如, 针对蛋白标志物的免疫学检测方法敏感性或特异性不够; 针对核酸标志物的PCR等检测技术需要破坏CTCs结构, 无法观察细胞形态学以及计数靶细胞, 更不能对靶细胞进行后续的细胞培养与药敏实验等. 另一个棘手的问题是CTCs具有高度异质性. 解决的策略是对单个CTCs进行分子鉴定和比较分析. 目前已有一些这样的尝试[51,52]. 相信这会成为未来的一个研究趋势.

总之, 实现CTCs分子鉴定在肿瘤个体化诊疗方面的临床转化在很大程度上取决于CTCs分离和鉴定技术的完善和发展. 尽管尚有很长的路要走, 相信通过全世界的共同努力, 在不久的将来CTCs分型检测作为一项新的实验技术一定会得到广泛的临床应用, 为临床医师提供有用信息, 指导正确诊疗方案的制订, 使广大肿瘤患者受益.

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■应用要点

目前认为, 基于分子鉴定检测少数更具存活力和侵袭性的CTCs比单独CTCs计数更有价值. 而在肿瘤转移过程中CTCs表现的多种生物学特性及其分子机制都可被用于分型, 并且可能成为个体化肿瘤诊疗的靶点.

■同行评价
本文较为全面地综述了近年来循环肿瘤细胞CTCs分子鉴定的研究进展, 介绍了CTCs分子分型促进个体化诊疗转化的研究现状和研究方向, 具有较高的学术参考价值.

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