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基因检测在结肠癌预后中的临床应用

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[摘要] 准确预测II期结肠癌患者的术后复发风险对制定适合患者的治疗方案十分重要。然而, 传统临床病理中的风险因素并不足以准确地评估患者的复发风险进而指导临床决定。随着基因组学技术的发展, 越来越多的研究证实基因检测能提供更个体化更准确的预后信息。这些方法主要包括检测基因突变、基因表达及术后循环肿瘤DNA等3个方向。

[关键词] 结肠癌; 预后; 基因突变; 基因表达; 循环肿瘤DNA

Clinical applications of gene test in colon cancer prognosis

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Abstract Accurate prediction of recurrence risk is of vital importance for formulating suitable therapeutic regimen for patients who have had surgery for stage II colon cancer. However, conventional clinicopathological criteria are not accurate enough to evaluate the individual recurrence risk of patient. With the development of genomics, several gene assays have been confirmed to accurately assess the individual recurrence risk for colon cancer prognosis. These assays include detection of gene mutation, gene expression, and circulating tumor DNA (ctDNA).

Keywords colon cancer; prognosis; gene mutation; gene expression; circulating tumor DNA

世界范围而言, 结直肠癌是男性中第三普遍、女性中第二普遍的癌症^[1]。而在中国, 结直肠癌在男性癌症发病率中高居第五, 在女性中高居第四^[2]。约30%的结肠癌患者确诊时处于II期, 其中约80%的II期患者仅通过手术治疗即可治愈^[3]。辅助化疗的价值仍争论不止, 额外的辅助化疗几乎不能提供绝对的受益^[4]。对于整体II期结肠癌患者而言, 单药5-氟尿嘧啶(5-FU)的辅助化疗

只能额外提供2%~5%的绝对生存获益^[3]。因此准确找到高复发风险的患者, 避免过度治疗, 是临床医师尤为关心的课题。

目前指南里普遍建议对具有临床病理高危因素的II期结肠癌患者进行术后辅助化疗^[5-7]。然而, 这些临床病理的风险因素并不能充分地评估患者的复发风险以指导临床决定。首先, 传统临床病理学指标不够标准化, 不同指南里定义的高风险

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因素并不完全一样(表1)。其次,即使是这些指南里共同列出的危险因素,比如T4、组织分化程度、穿孔等,除T4是一个明确的不良预后因素之外,其它因素和临床复发相关性都存在比较大的争议^[8]。比如最近5项研究、3 000多名患者的数据表明肿瘤低分化并不表示复发高风险^[9-13]。而II期结肠癌患者中T4患者比例约为15%。再次,该标准与临床实际并不十分吻合。Erin等^[14]研究24 847名II期结肠癌患者的数据,发现75%的患者至少具有

一种高危因素,而事实上只有20%的患者会复发。根据临床病理标准区分的高/低危人群均不能从辅助化疗中明显获益。

随着基因组学技术的发展,越来越多的研究证实基因检测能提供更个体化更准确的预后信息,比如指南^[7]已经推荐检测MSI对II结肠癌手术患者进行预后预测,而根据肿瘤组织一些基因表达水平进行预后预测的临床试验^[9,15-17]也正在进行。本文主要回顾基因检测在结肠癌预后中的临床应用。

表1 三大指南里复发相关的高危因素

Table 1 High-risk features for recurrence in three guidelines

高危因素	美国临床肿瘤医学会(2004)	美国国家癌症资讯网(2017)	欧洲肿瘤学学会(2012)
T4	+	+	+
组织学分化差	+	+	+
淋巴结数量不足	<13	<12	<12
肠穿孔	+	+	+
肠梗阻		+	+
脉管/淋巴/神经浸润	+	+	+
切缘太近、阳性或不确定		+	

1 基因突变

1.1 微卫星不稳定

微卫星序列是遍布于全基因组上的短串联重复序列。微卫星不稳定(microsatellite instability, MSI)指DNA甲基化或基因突变导致微卫星重复序列长度的改变。大量的研究表明II期结肠癌中MSI是明确的预后良好因素^[18-19]。MSI的患者复发风险较低,不但不能从5-FU的辅助化疗中获益,甚至会降低总生存期(overall survival, OS)^[20]。然而II期结肠癌患者中,MSI患者的比例大约只有15%。另外,MSI在III期结肠癌中并没有明确的预后价值^[21]。有趣的是,最近一项研究^[22]发现在III期结肠癌中,如果肿瘤发生在右侧,MSI是一个预后良好因素。

1.2 BRAF 突变

BRAF^{V600E}突变是早期结肠癌中独立的预后因子^[23]。BRAF^{V600E}突变与复发风险关系不大,但预示OS差,部分原因在于其和复发后较短的生存期显著相关^[24]。有意思的是,在微卫星稳定(microsatellite stability, MSS)的左侧结肠癌患者中,BRAF^{V600E}突变是非常明显的不良预后因素^[23]。对于同时具有MSI和BRAF^{V600E}突变的患者而言,MSI的

良性预后效应更明显,患者整体预后良好^[23]。

1.3 KRAS 突变

在BRAF野生型的III期结肠癌患者中,KRAS突变是一个比较不好的预后因素,预示较低的无疾病生存期和OS。然而整体而言,KRAS突变的影响是比较弱的^[25]。但如果肿瘤位于左侧,并且KRAS的12号密码子发生突变,这种影响则比较显著^[26]。在II期结肠癌中,KRAS突变的预后价值还不清楚^[27]。

2 基因表达

近年来一些技术通过检测结肠癌组织基因mRNA表达水平来评估患者个体化复发风险,有些已经实现商业化服务,比如Oncotype DX Colon, ColoPrint, GeneFx Colon和OncoDefender等(表2)。其中Oncotype DX Colon和ColoPrint的临床验证较多。除通过mRNA表达进行预后之外,Zhang等^[28]还发现可以通过miRNA的表达对结肠癌患者进行预后预测。其建立一个基于6个miRNA表达水平的分类模型,把II期结直肠癌患者的复发风险分为高低两组,两组5年无疾病生存期分别为60%(高)和89%(低)^[28]。

表2 检测多基因表达评估结肠癌术后复发风险的方法

Table 2 Multi-gene expression assays proposed for predicting outcome in colon cancer postoperative patients

检测技术	发表时间	检测基因数	样本类型	方法	风险分组
Oncotype DX ^[9]	2010	12	FFPE	RT-PCR	3组
ColoPrint ^[15]	2011	18	FF	Microarray	2组
GeneFx Colon ^[16]	2011	634	FFPE	Microarray	2组
OncoDefender ^[17]	2012	5	FFPE	RT-PCR	2组

2.1 Oncotype DX Colon

Oncotype DX Colon通过检测II期、IIIA/B期结肠癌患者癌组织中12基因mRNA的表达水平来预测肿瘤患者个体化的复发得分(recurrence score, RS)。这些信息有助于医生决定II期患者术后是否需要联合辅助化疗, 以及III期患者术后化疗中是否需要联合奥沙利铂。这12个基因包括7个结肠癌相关基因及5个参考基因。根据评分复发风险可分为高($RS \geq 41$), 中($31 \leq RS < 41$), 低($RS \leq 30$)三组, 三组10年远端复发比例分别为25%(高)、11%(中)及8%(低)^[9]。

QUASAR试验^[29]中1 436名II期结肠癌患者的数据表明RS和复发风险明显相关, 能够提供与T分期或MMR状态相当的预后价值, 这三组3年复发风险分别为22%(高)、18%(中)及12%(低)。高复发风险组中辅助化疗的绝对受益率是6%, 而低复发风险组中辅助化疗的绝对受益率是3%^[29]。后续CALGB 9581和NSABP^[12,30]的研究结果也进一步证实Oncotype DX的预后价值。临床实际应用中Oncotype DX Colon的价值如何? Brenner等^[31]的结果显示有近38%的患者会根据Oncotype DX Colon的结果改变他们的治疗方案, 很大一部分患者会减少不必要的辅助化疗。辅助化疗的减少相应会降低潜在的医疗费用。Hornberger等^[32]的结果显示参考Oncotype DX Colon结果进行治疗, 可以使平均质量生命年增加0.035, 平均减少花费2 971美元。Alberts等^[33]的研究表明平均质量生命年增加0.114, 平均减少花费991美元。

2.2 ColoPrint

ColoPrint是通过微阵列芯片检测结肠癌组织18个基因的表达水平, 评估II/III期结肠癌患者术后复发风险。该检测把患者分为术后复发高风险组和低风险组, 低风险组术后5年复发率约为9%, 而高风险组术后5年复发率约为26%^[15]。相较于ASCO的临床高危标准, ColoPrint检测可以使高

危人群的绝对值减少20%^[34]。一些回顾性研究已经证明ColoPrint的临床价值, 而前瞻性的临床试验正在进行, 比如PARSC(Prospective Analysis of Risk Stratification by ColoPrint)^[35]。这是一个多国家多中心的前瞻性临床试验, 用ColoPrint评估II/III期结肠癌患者的复发风险。从2008年开始, 预计2019年结束, 计划招募575名II期结肠癌患者。目前初步结果同样证实ColoPrint的临床价值。

除以上两种方法之外, 结肠癌中还有很多其它根据基因表达进行预后评估的方法。然而, 这些方法真的能明显提高预后分层的效果吗? Di等^[36]比较4种不同基于基因表达评估复发风险的临床效用, 其中包括Oncotype DX, 发现在现有临床病理的预后模型基础上, 这4种方法额外提供的临床价值有限。以Oncotype DX为例, 表明无论是无复发生存期, 还是复发后生存期和OS, 相对于现有的临床病理预后模型, RS提供的额外价值都很少。此外, 4种方法之间的一致性较差。

3 循环肿瘤 DNA

除了根据基因表达来进行预后分层之外, 循环肿瘤DNA(circulating tumor DNA, ctDNA)检测技术的发展和运用提供了新的角度去评估个体化的术后复发风险。ctDNA是血液循环中来自肿瘤细胞的DNA片段, 携带有肿瘤基因组的突变信息, 可以用于肿瘤的预后评估和复发监测^[37-38]。Tie等^[39]的研究对230名II期结肠癌患者术后的ctDNA进行监测, 根据ctDNA有无进行复发风险分层, 风险比(hazard ratio, HR)高达18, 并且可以对临床病理指标分层的结果进行进一步的细分。考虑根据传统临床病理指标分层的HR大约只有1.5, 根据基因表达水平分层的HR基本在2~4^[9,15-17], 而术后ctDNA分层的HR高达18, 不得不说这是一个非常巨大的提升。最近, Diehn等^[40]研究了145名II或III期结肠癌患者术后ctDNA与预

后的关系,发现术后ctDNA阳性患者的无复发生存率远远低于术后ctDNA阴性患者(17% vs 88%)。

除了结直肠癌,也有研究表明ctDNA可以用于乳腺癌^[41]、非小细胞肺癌的预后评估^[42-43]。其中乳腺癌中的数据表明术后ctDNA可以有效的进行复发风险分层,连续监测可以显著提高分层的准确性,整体达到80%灵敏性,96%特异性^[41]。24名肺癌患者的研究^[42]表明术后ctDNA预后评估达到90%灵敏性,90%特异性。

尽管目前还需要更多的临床试验来验证ctDNA在结肠癌术后预后分层中的临床价值、标准化ctDNA的检测分析方法与阈值,但明显提升的HR表明这一方法在术后复发风险评估中有着非常远大前景。尤其是对约占II期结肠癌患者70%的T3且MSS的患者而言。

4 高通量测序技术的应用

结直肠预后相关的基因检测中,高通量测序技术的应用越来越多。比如MSI的检测,传统通过免疫组化的或多重PCR的方法来判断,最近研究^[44]表明也可以通过二代测序的方法进行检测。对于BRAF和KRAS等基因的突变检测而言,二代测序除以更低的成本检测全部编码序列之外,其检测灵敏性更是可以达到2%,经过一些实验和生信分析方法处理之后,甚至可以达到0.01%或更低^[45]。这对以低丰度为特征的ctDNA检测而言尤为重要。

尽管新一代的PacBio测序或纳米孔测序拥有测序读长更长、单分子测序、测序速度快等特点,然而目前仍面临样本需求量大、测序错误率较高、成本较高等问题的挑战^[46-47],因此相对于二代测序技术而言,其并不适合临床上肿瘤这种需要高测序深度的体细胞突变检测。在一段时间之内,肿瘤临床基因检测中的高通量测序仍将以二代测序为主,现在的读长足以满足大部分临床检测需求。

5 结语

从生物学特性出发揭示肿瘤本质,是实现肿瘤个体化精准治疗的方向。无论是基于基因突变,还是根据基因表达或者术后是否存在ctDNA都需进一步研究。然而,考虑到术后ctDNA显著的分层效果,以及其用于疗效评估与复发监测中的价值,在未来的临床实践中,ctDNA检测结合每一个患者的临床背景进行分析会具有巨大的临床应用前景。

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