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结核性腹膜炎与恶性腹水端粒酶活性

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Telomerase activity in tuberculous peritonitis and malignant ascites

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Abstract

AIM: To determine telomerase activity of exfoliated cells in tuberculous peritonitis and malignant ascites, and study the diagnostic value of telomerase activity in differentiating tuberculous peritonitis from malignant ascites.

METHODS: TRAP-PCR-ELISA and TRAP-PCR-silver staining were employed to determine telomerase activity in 18 specimens of malignant ascites and 13 specimens of tuberculous peritonitis. Telomerase activities in tuberculous peritonitis and malignant ascites were analysed.

RESULTS: Telomerase activity in malignant ascites (0.387±0.023) was significantly higher than that in tuberculous peritonitis (0.023±0.004, P <0.01). The positive rate of telomerase activity in malignant ascites was significantly higher than that in tuberculous peritonitis, 88.9 % (16/18) *vs* 7.7 % (1/13), P <0.01. The sensitivity, specificity and accuracy of determination of telomerase activity in diagnosis of malignant ascites were 88.9 %, 92.3 %, and 90.3 %, respectively.

CONCLUSION: Telomerase activity is positive in malignant ascites and may serve as a useful indicator for differentiating between tuberculous peritonitis and malignant ascites.

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摘要

目的: 研究端粒酶在结核性腹膜炎腹水与恶性腹水的活性水平,探讨腹水端粒酶检测鉴别结核性腹水与恶性腹水的诊断价值.

方法: 应用TRAP-PCR-ELISA方法和TRAP-PCR-银染法分别对13例结核性腹膜炎和18例恶性腹水标本(肝癌7例, 胃癌6例, 结肠癌2例, 胰腺癌1例, 卵巢癌2例)进行定性、定量端粒酶活性检测. 对比腹水细胞学检查,分析腹水端粒酶活性鉴别结核性腹膜炎与恶性腹水的诊断价值.

结果: 恶性腹水组端粒酶活性(0.387±0.023)高于结核性腹膜炎腹水组(0.023±0.004), 统计差异显著(P<0.01); 恶性腹水端粒酶阳性率88.9 % (16/18)明显高于结核性腹膜炎腹水组7.7 % (1/13), P<0.01. 仅1例结核性腹膜炎腹水端粒酶阳性.

结论: 恶性腹水端粒酶阳性,结核性腹水端粒酶阴性. 腹水端粒酶活性检测可能是鉴别结核性与恶性腹水的重要依据.

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0 引言

良恶性腹水的鉴别诊断是临床常见的难题, 尤其棘手的是结核性腹膜炎与恶性腹水的鉴别诊断. 为探索良恶性腹水的鉴别诊断, 近年有很多检测指标被尝试应用. 如腹水AFP、CEA、铁蛋白、腺苷脱氨酶、LDH等, 但由于这些指标受器官组织特异性或良恶性腹水有重叠的限制, 而使其实际应用价值大打折扣. 端粒酶的研究发现, 人类90 %的肿瘤组织存在异常端粒酶活性, 而正常组织细胞端粒酶活性低, 甚至无端粒酶活性. 端粒酶是一个不受组织器官限制的肿瘤标志物, 故腹水端粒酶活性的研究受到国内外关注[1,2]. 我们探讨腹水端粒酶活性检测鉴别结核性腹膜炎与恶性腹水的价值.

1 材料和方法

1.1 材料 2001-05/2002-12 腹水患者31例, 男15例, 女16例, 年龄22-67(平均48.6岁). 其中恶性腹水18例(肝癌7例, 胃癌6例, 结肠癌2例, 胰腺癌1例, 卵巢癌2例), 结核性腹膜炎腹水13例. 所有病例均未经放疗或化疗. Telomerase PCR-ELISA试剂盒(boehringer manheim公司). PCR扩增仪(PTC-100-60, MJ RESEARCH, 美

国)，酶标仪(Elx800，BIO-TEX，美国)，低温离心机(RC-SC， DUPONT/SORVALL，美国).

1.2 方法 常规腹腔穿刺抽腹水10 mL，3 000 r/min离心10 min，弃上清，PBS洗2次. 加裂解液200 μL，混匀，冰浴30 min; 4 ℃ 16 000 g低温离心20 min，上清 -80 ℃保存待用. 取细胞抽提液2 μL加TRAP-PCR扩增反应液，DEPC水补足至50 μL，按下列顺序在PCR仪上进行扩增反应: 25 ℃ 30 min，95 ℃ 5 min循环1周期; 继以94 ℃ 30 s变性、50 ℃ 30 s退火、72 ℃ 90 s延伸共循环30个周期; 最后72 ℃延伸10 min. 取PCR扩增产物5 μL，加变性液20 μL，室温10 min，加入杂交液后，取出100 μL，加入已包被的微孔板上，37 ℃水浴2 h，洗板，每孔加入酶标抗体100 μL，室温20 min，洗板，经TMB底物显色，室温20 min，终止反应，在酶标仪上测吸光度值(*A* 460 nm-*A* 690 nm). 取上述PCR扩增产物25 μL，加入125 g/L非变性聚丙烯酰胺凝胶上进行垂直电泳，电泳完成后，取下凝胶进行银染. 显示端粒酶特异性6-碱基阶梯带为阳性. 阴性标本不出现6-碱基阶梯带. 腹水细胞学检测为本院检验科常规检查.

统计学处理 均数比较显著性差异采用 *t* 检验，率的比较采用 χ^2 检验.

2 结果

在18例恶性腹水中端粒酶活性阳性检出率88.9 %(16/18)，而在13例良性腹水中其阳性检出率仅7.7 %(1/13)，两组间差异显著(*P* <0.01). 恶性腹水组脱落细胞端粒酶活性吸光度值(0.387 ± 0.023)明显高于结核性腹膜炎组(0.023 ± 0.004)，差异显著(*P* <0.01). 腹水癌细胞阳性者，端粒酶活性均阳性. 腹水端粒酶活性阴性者，癌细胞均阴性. 细胞学诊断准确率77.4 %，端粒酶诊断准确率达90.3 %(表1).

表1 端粒酶与细胞学诊断恶性腹水的敏感性及特异性(%)

	端粒酶	癌细胞
敏感性	88.9	61.1
特异性	92.3	100
阳性预测值	94.1	100
阴性预测值	85.7	65

3 讨论

目前良恶性腹水腹水鉴别诊断问题，尤其是结核性腹膜炎与恶性腹水的鉴别一直未能得到满意解决. 由于缺乏诊断结核性腹膜炎的敏感、特异性指标，同时也缺乏诊断恶性腹水的广谱、特异性标志物，所以给结核性腹膜炎与恶性腹水的鉴别诊断带来困难. 已有的腹水癌细胞检查，虽有满意的特异性，但其敏感性不足; 肿瘤标志物AFP，CEA明显受器官肿瘤来源限制; 铁蛋白、腺苷脱氨酶浓度在良恶性腹水之间存在一定重叠. 学者们一直在寻找一项特异性的广谱肿瘤标志物，以供临床需要，用于良恶性腹水鉴别诊断，但尚未取得突破性进展. 因此在临床实际工作中往往需要多项指标联合检测，以提高诊断正确率.

端粒(telomere)是真核细胞染色体末端的一种特殊结构，由串联排列的重复DNA序列和端粒结合蛋白构成，是维持染色体完整性的保护结构. 人类端粒DNA序列一般5-20 kb，细胞分裂一次，端粒丢失一段，缩短到一定程度时，出现细胞衰老或死亡. 端粒酶是一种能延长端粒末端的核酸蛋白酶，由RNA和蛋白质组成，能以其RNA为模板，反转录合成端粒DNA，并加到染色体末端，以补偿细胞分裂时端粒DNA的缩短，维持端粒长度. 所以，端粒酶的激活可导致异常分化的肿瘤细胞不断分裂增生. 近年来，对各种恶性肿瘤端粒酶活性的研究日渐广泛、深入，如胃癌[3-7]、肝癌[8-14]、结肠癌[15-19]、胰腺癌[20]、卵巢癌等[21,22]. 端粒酶与肿瘤细胞永生关系的确立，端粒酶被认为是目前已知最广谱的肿瘤特异性的肿瘤标志物之一[23]. 并且对判断抗肿瘤疗效、预后均有重要意义[24-28]. 国内外学者已将脱落细胞端粒酶活性检测用于恶性肿瘤的诊断研究，如检测肠灌洗液端粒酶活性诊断结肠癌[29]、检测胰液端粒酶活性诊断胰腺癌[30-32]、检测腹水端粒酶活性诊断恶性腹水等[2,33-35]. 但将端粒酶活性检测用于结核性腹膜炎与恶性腹水鉴别诊断的研究目前文献中尚少有报道.

我们对腹水标本PCR产物分别采用杂交ELISA和银染法检测了端粒酶活性，检测结果一致，定量法检测为阴性的标本定性法检测亦为阴性. 研究发现恶性腹水组中端粒酶活性为0.387 ± 0.023，阳性率88.9 %，与文献[1,35]报道相近. 恶性腹水组端粒酶活性明显高于结核性腹膜炎腹水组(0.023 ± 0.004，阳性率7.7 %，*P* <0.01). 在恶性腹水中仅2例端粒酶阴性，而结核性腹膜炎中仅1例端粒酶阳性. 另外，本实验中发现1例结核性腹膜炎出现端粒酶活性增高，这种假阳性结果曾有报道，其原因能是由于腹水中大量淋巴细胞所致[36]. TRAP-ELISA是一高度敏感性检测方法，只要反应体系中有约10个端粒酶活性细胞存在，即可检测出来. 与腹水癌细胞检查比较，腹水癌细胞阳性者，端粒酶活性均为阳性. 在5例腹水癌细胞阴性的恶性腹水中，端粒酶活性也呈阳性. 端粒酶诊断的准确率(90.3 %)明显高于癌细胞检查的准确率(77.4 %). 说明腹水端粒酶活性检测对结核性腹水与恶性腹水鉴别优于细胞学检查，二者联合应用更有价值.

本研究结果表明，腹水端粒酶活性检测鉴别结核性腹膜炎腹水与恶性腹水，具有诊断特异性强，准确率高. 我们认为腹水端粒酶活性检测是鉴别结核性腹膜炎腹水与恶性腹水一项新的重要指标，腹水端粒酶活性阳性时是提示恶性腹水的有力证据. 如能进一步克服假阳性的影响，并改进实验方法，使其操作简单、方便、经济，这一指标更具有临床实用价值.

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