·论著·

恶性胸腔积液端粒酶活性与患者总生存期的相关性

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【摘要】　目的　探讨恶性胸腔积液患者的端粒酶活性与总生存期之间的相关性。方法　前瞻性收集2015年12月1日至2017年12月31日韶关市粤北人民医院呼吸内科收治的腺癌性胸腔积液(腺癌组)、鳞癌性胸腔积液(鳞癌组)、小细胞癌性胸腔积液(小细胞癌组)患者各20份的胸腔积液标本及其临床资料,采用ELISA法分别检测胸腔积液标本中的端粒酶活性,方差分析LSD法比较三组标本的端粒酶活性,采用生存分析KM法比较三组患者的总生存期(overall survival, OS),采用Pearson相关分析考察端粒酶活性与患者总生存期之间的相关性。OS定义为从诊断到患者死亡。结果　腺癌组、鳞癌组和小细胞癌组患者胸腔积液的端粒酶活性分别为198.8±23.9、196.9±7.9和210.5±6.9,小细胞癌组胸腔积液端粒酶活性明显高于腺癌组和鳞癌组,差异均有统计学意义($P<0.05$),而腺癌组和鳞癌组患者的胸腔积液端粒酶活性比较差异无统计学意义($P>0.05$);小细胞癌组患者的中位OS为(1.4±0.03)个月,明显短于腺癌组的(2.9±0.09)个月及鳞癌组的(3.0±0.12)个月,差异均有统计学意义($P<0.05$),而腺癌组及鳞癌组患者的中位OS比较差异无统计学意义($P>0.05$);胸腔积液端粒酶活性与患者总生存期呈中度负相关性($r=-0.54, P<0.05$)。结论　小细胞癌性胸腔积液患者总生存期短于鳞癌及腺癌患者,其端粒酶水平高于腺癌及鳞癌性胸腔积液端粒酶水平。恶性胸腔积液患者的不良预后可能与胸腔积液端粒酶活性水平相关,采取控制胸腔积液端粒酶水平的治疗方案可能改善恶性胸腔积液患者的预后。

【关键词】　恶性胸腔积液;端粒酶;腺癌;鳞癌;小细胞癌;生存期;相关性

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Correlation between telomerase activity and overall survival in patients with malignant pleural effusion. *CHENG Jiang-tao, LIU Qing-yi, YANG Yin-lou, ZHANG Hong-hua, XIE Bin, GONG Mei, MA Zhan-zhong. Department of Respiratory Medicine, Yuebei People's Hospital, Shaoguan 512000, Guangdong, CHINA*

【Abstract】　**Objective**　To investigate the correlation between the activity of telomerase and overall survival (OS) in patients with malignant pleural effusion. **Methods**　Pleural effusion samples were collected from 20 patients with adenocarcinoma, 20 patients with squamous cell carcinoma, and 20 patients with small cell carcinoma from Dec. 1, 2015 to Dec. 31, 2017 in Department of Respiratory Medicine, Yuebei People's Hospital. Telomerase activity in the pleural effusion samples of the three groups were detected with enzyme-linked immunosorbent assay (ELISA) and then compared with one-way analysis of variance (ANOVA) among the three groups. The OS of these groups were analyzed with

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Kaplan Meier method. The correlation between the activity of telomerase and OS were analyzed with Pearson correlation analysis. OS were defined as from the diagnosis to death. **Results**　Telomerase activity in pleural effusion of adenocarcinoma group, squamous cell carcinoma group, and small cell carcinoma group were 198.8±23.9, 196.9±7.9, and 210.5±6.9 respectively, which was significantly higher in small cell carcinoma group than adenocarcinoma group and squamous cell carcinoma group ($P<0.05$) but showed no significant difference between adenocarcinoma group and squamous cell carcinoma group ($P>0.05$). The median OS of small cell carcinoma group was (1.4±0.03) months, significantly shorter than (2.9±0.09) months of adenocarcinoma group and (3.0±0.12) months of squamous cell carcinoma group ($P<0.05$). There was no significant difference in OS between adenocarcinoma group and squamous cell group ($P>0.05$). Telomerase level in pleural effusion was moderately negatively correlated with OS ($r=-0.54$, $P<0.05$). **Conclusion**　The OS of patients with pleural effusion in small cell carcinoma is shorter than that of patients in squamous cell carcinoma and patients in adenocarcinoma pleural effusion, while telomerase level of patients with small cell carcinoma pleural effusion is higher. The poor prognosis of patients with malignant pleural effusion is correlated to the level of telomerase, and the treatment of controlling telomerase may improve the prognosis of patients with malignant pleural effusion.

【**Key words**】　Malignant pleural effusion; Telomerase; Adenocarcinoma; Squamous carcinoma; Small cell carcinoma; Overall survival; Correlation

恶性胸腔积液多为其他部位癌细胞转移或原发于胸膜的恶性肿瘤导致,其临床特点为胸腔积液增长迅速,常常为原发肿瘤如肺癌进展的临床表现之一,恶性胸腔积液患者的总生存期短,预后极差,虽然既往文献报道可采取诸如滑石粉胸膜固定[1]、顺铂[2]注射液胸腔内灌注及胸腔积液穿刺引流等方法处理恶性胸腔积液,但疗效均不理想。

端粒酶(telomerase)是核糖核酸蛋白酶,其利用自身的RNA为模板合成端粒DNA,可以延伸端粒并维持其稳定性[3],近90%的恶性肿瘤细胞端粒酶存在活性表达,是目前最为通用的肿瘤分子标志物之一。国内外已有多项临床研究证实,恶性胸腔积液患者端粒酶水平较良性胸腔积液增高,临床上可作为鉴别诊断的重要指标[4]。本研究旨在探讨不同种类的恶性胸腔积液端粒酶活性水平及其与患者总生存期(overall survival, OS)的相关性,为临床治疗提供一种新的思路。

1　资料与方法

1.1　一般资料　选取2015年12月1日至2017年12月31日韶关市粤北人民医院呼吸内科收治的腺癌性胸腔积液(腺癌组)、鳞癌性胸腔积液(鳞癌组)、小细胞癌性胸腔积液(小细胞癌组)患者各20份的胸腔积液标本及其临床资料。排除确诊恶性胸腔积液后采取放化疗、靶向治疗、免疫治疗及中医中药治疗患者。腺癌组男性16例,女性4例,平均年龄63.1岁;鳞癌组男性17例,女性3例,平均年龄59.5岁;小细胞癌组男性14例,女性6例,平均年龄66.9岁。三组患者年龄和性别比较差异均无统计学意义($P>0.05$),具有可比性。

1.2　肺癌诊断标准　通过查体及胸部影像学检查确诊胸腔积液,行胸腔积液穿刺引流取胸腔积液标本,进行脱落细胞学查见癌细胞,免疫组化检测确定癌细胞类型。

1.3　标本采集及检测方法　(1)标本收集:获取

患者书面知情同意,并经粤北人民医院伦理委员会批准后,取新鲜胸腔积液标本10 mL,在4℃低温离心机以1 500 r/min离心15 min,去除上清液,用磷酸盐缓冲液(PBS, pH 7.4, 4℃)清洗两次,然后将沉渣细胞移入1.5 mL Eppendorf管,置于-80℃冰箱冻存,集中检测。(2)样本检测:采用DLDEVELOP公司端粒酶ELISA试剂盒(批号:EDL2016092001)检测胸腔积液端粒酶活性。

1.4　患者生存期随访　患者确诊为胸腔积液后,因自身经济等原因未进行化疗或靶向治疗等抗肿瘤处理,而采用最佳支持治疗(best support care),严密追踪随访此类患者的临床症状及生存期,OS计算方法为自诊断恶性胸腔积液开始直至患者死亡。

1.5　统计学方法　应用SPSS 22.0进行统计分析,计量资料以均数±标准差($\bar{x}±s$)表示,组间比较采用方差分析LSD法;计数资料以频数表示,率的比较采用χ^2检验;OS比较采用生存分析Kaplan-Meier (KM)法,差异性检验采用Log rank检验。端粒酶活性与OS相关性采用Pearson相关分析。以$P<0.05$为差异有统计学意义。

2　结果

2.1　三组患者的端粒酶活性比较　腺癌组、鳞癌组和小细胞癌组的端粒酶活性分别为198.8±23.1、196.9±7.9、210.5±6.9,三组端粒酶活性比较差异有统计学意义($F=4.79$, $P<0.05$)。组间比较LSD法:小细胞癌性胸腔积液端粒酶活性明显高于腺癌性胸腔积液组($t=2.47$, $P<0.05$)及鳞癌性胸腔积液组($t=2.85$, $P<0.05$),差异有统计学意义。腺癌及鳞癌性胸腔积液端粒酶活性差异无统计学意义($t=0.39$, $P>0.05$)。

2.2　三组患者的OS比较　采用生存分析KM法比较三组OS,腺癌组患者中位OS:(2.9±0.9)个月,鳞癌组患者中位OS:(3.0±0.1)个月,小细胞癌性胸腔积液中位OS:(1.4±0.03)个月。小细胞癌性胸腔积液患

者中位总生存期明显短于腺癌性胸腔积液组及鳞癌胸腔积液患者中位总生存期,差异有统计学意义($P<0.05$)。腺癌及鳞癌胸腔积液患者中位总生存期比较差异无统计学意义($P>0.05$),见图1。
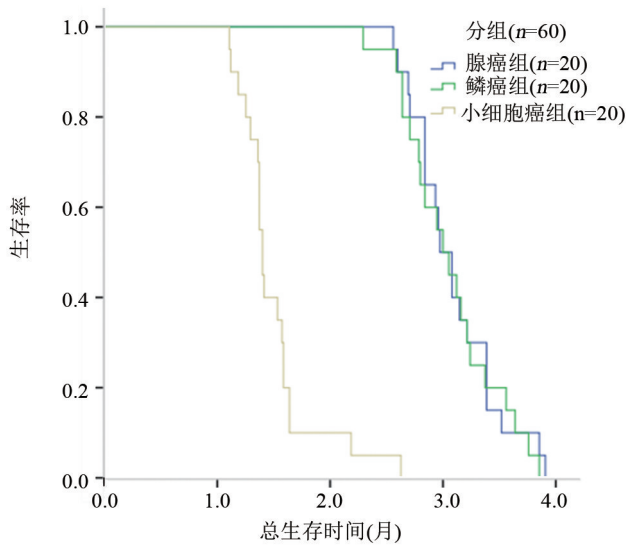

图1　三组OS生存分析KM曲线

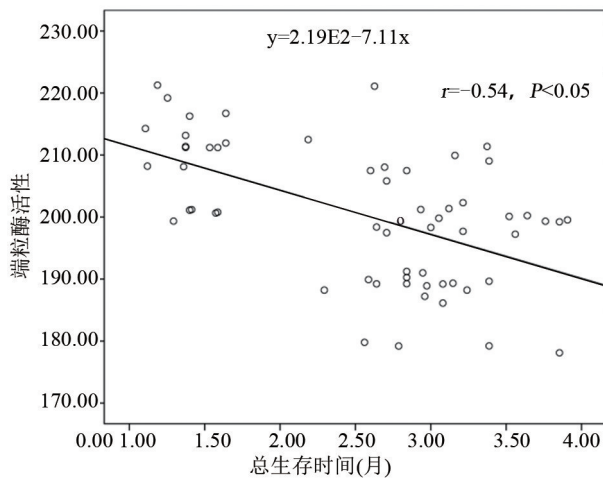2.3　端粒酶水平与患者总生存期的相关性　恶性胸腔积液端粒酶水平与患者总生存期呈中度负相关($r=-0.54$,$P<0.05$),见图2。

图2　恶性胸腔积液端粒酶水平与患者总生存期的相关性

3　讨论

人体胸膜腔是一个由脏层胸膜和壁层胸膜构成的潜在腔隙,两层胸膜间有少量液体,在胸腔内负压的作用下,脏层胸膜与壁层胸膜彼此紧贴并藉由液体相互滑动,起连接胸壁与肺脏的作用[5]。胸膜腔内液体的产生和吸收存在动态平衡,其产生过多或者吸收减少,都会导致胸腔积液[6]。根据其性质不同,可分为漏出液、渗出液两大类;渗出液进一步可分为炎症性、结核性及恶性胸腔积液。其中,恶性胸腔积液(malignant pleural effusion,MPE)是原发于胸膜,或其他部位的恶性肿瘤细胞转移至胸膜所导致的胸腔积液,是晚期恶性肿瘤的常见并发症之一。其产生机理为癌细

胞分泌蛋白因子引起炎症反应,进一步导致毛细血管通透性增加;癌细胞纵隔淋巴结转移所致的淋巴液和血液回流障碍,静水压增加等[7-8]。

胸腔积液为呼吸内科常见疾病,由于其病因多样造成诊断困难[9],在治疗方面,恶性胸腔积液的不良预后目前并未得到改善,因此造成了严重的管理挑战,欧洲和美国每年有大约75万人被诊断为恶性胸腔积液[10],因此带来巨大的财务负担[11]。恶性胸腔积液通常会导致呼吸困难、衰弱等临床表现[12],控制恶性胸腔积液的措施有滑石粉胸膜固定术[13]、胸腔内灌注顺铂注射液[14]、持续性置管引流[15-16]等。随着分子靶向治疗在胸部肿瘤治疗中的应用,已有新的治疗方式不断应用于胸腔积液的临床实践,但均不能有效地解决胸腔积液量快速增加以及复发等问题。

端粒(telomere)是存在于染色体末端的DNA序列,在细胞分裂时发挥维持染色体长度的作用,端粒通常会随着细胞的分裂而缩短,短端粒会限制细胞分裂,最终导致细胞发生复制性衰老[17],端粒缩短导致细胞衰老以防止物种在生命早期发生肿瘤[18],这可能是生物为防止在生命早期发生肿瘤而进化出来的一种抑制肿瘤的机制[19]。

端粒酶(telomerase)是一种具有逆转录酶活性的核糖核酶,由RNA和蛋白质构成,它可以在细胞分裂过程中延长端粒的长度[20-22]。端粒酶存在两个保守组分,即核心端粒酶蛋白质(TERT)组分和RNA组分,TERT包含端粒酶逆转录酶催化亚单位;RNA组可为端粒序列合成提供模板[23]。正常组织中几乎没有端粒酶活性,而在85%的肿瘤细胞中可以检测到端粒酶活性。因此,抑制肿瘤细胞中端粒酶的活性可能阻止端粒的延长,阻断癌细胞持续分裂的过程,从而导致癌细胞的衰老甚至死亡,可能对恶性肿瘤的发生发展产生抑制作用。

本研究发现,初诊的腺癌性胸腔积液端粒酶活性与鳞癌性端粒酶活性差异无统计学意义,小细胞癌性胸腔积液端粒酶活性显著高于腺癌和鳞癌胸腔积液组,差异具有统计学意义。小细胞癌性胸腔积液端粒酶活性较高,提示端粒酶活性存在较高水平的重新激活。

小细胞型肺癌(small cell lung cancer,SCLC)属于肺癌的一种尚未分化分型的类型,在肺癌患者中占20%~25%,其恶性程度较高,癌细胞生长和发展速度较快,更易较早的引起淋巴转移。在各类肺癌中,SCLC的预后相对较差,严重影响患者的生活质量与生命安全,而小细胞性胸腔积液患者的预后较腺癌性胸腔积液和鳞癌性胸腔积液差,与既往研究结论一致[24]。

未经抗肿瘤治疗的恶性胸腔积液患者中位生存期较正规治疗的患者差,提示对于癌性胸腔积液进行针对性治疗,如放化疗、靶向治疗等的治疗方案重要

性。小细胞癌性胸腔积液患者中位总生存期明显短于腺癌性胸腔积液组及鳞癌胸腔积液患者中位总生存期,结合前期研究成果,小细胞癌性胸腔积液端粒酶水平高于其他两种恶性胸腔积液,提示OS的差异可能与小细胞癌性胸腔积液的端粒酶活性增高有关。

进一步相关性分析显示,恶性胸腔积液端粒酶水平与患者总生存期有中度负相关($r=-0.54$)。提示端粒酶活性越高,患者总生存期越短,因此,可以通过检测端粒酶活性预测恶性胸腔积液患者生存期,为临床判断患者预后提供新的指标。另一方面,可以提出科学设想,使用端粒酶抑制剂进行治疗,如胸膜腔内灌注,可能控制患者胸腔积液端粒酶水平,延缓胸腔积液增加速度,以改善患者预后。

综上所述,恶性胸腔积液患者中,端粒酶活性水平与患者预后存在相关性,端粒酶活性水平越高,患者预后越差。抑制恶性胸腔积液端粒酶水平可能改善此类胸腔积液患者预后。但是本研究入组病例数较少,有一定局限性,尚需大规模前瞻性研究加以确认。

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