

血清糖蛋白非转移性黑色素瘤蛋白B对表皮生长因子受体扩增伴随突变的非小细胞肺癌预后的预测作用

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摘要 **目的** 评估游离的糖蛋白非转移性黑色素瘤蛋白B (GPNMB) 对表皮生长因子受体 (EGFR) 扩增伴随突变的非小细胞肺癌 (NSCLC) 患者的耐药和预后的预测价值。**方法** 选取2018年3月至2019年9月在唐山市人民医院接受治疗的55例EGFR扩增伴随突变的NSCLC患者作为观察组, 患者均应用EGFR酪氨酸激酶抑制剂 (EGFR-TKI) 作为一线治疗方案; 随机选取同期体检中心67例健康人群血液样本作为对照组。比较2组游离GPNMB表达水平; 采用t检验或 χ^2 检验分析GPNMB表达和患者临床病理特征的相关性; 结合临床疗效, 评估其作为耐药标志物的价值。随访患者的无进展生存时间 (PFS), 并采用多因素Cox回归分析影响EGFR扩增伴随突变NSCLC患者生存的独立危险因素。**结果** 与对照组相比, 观察组游离GPNMB表达水平显著升高。观察组游离GPNMB水平和EGFR-TKI的临床疗效显著相关 ($P = 0.016$), GPNMB高表达患者的耐药程度更强, PFS也更短 ($P = 0.032$)。游离GPNMB高水平 ($HR = 4.029, 95\%CI: 1.942-8.358, P < 0.001$) 是影响患者生存的独立危险因素。**结论** EGFR扩增伴随突变的NSCLC患者游离GPNMB表达水平显著上调; 且其高表达与患者耐药程度的增强及不良预后显著相关, 是影响患者生存的独立危险因素。

关键词 非小细胞肺癌; 糖蛋白非转移性黑色素瘤蛋白B; 表皮生长因子受体扩增伴随突变; 耐药; 预后

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Predictive value of serum glycoprotein non-metastatic melanoma protein B in patients with non-small cell lung cancer accompanied by epidermal growth factor receptor amplification with mutation

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Abstract Objective To evaluate the clinical value of free glycoprotein non-metastatic melanoma protein B (GPNMB) as a drug resistance and prognostic marker for non-small cell lung cancer (NSCLC) patients with epidermal growth factor receptor (EGFR) amplification accompanied by mutations. **Methods** Fifty-five cases of NSCLC patients with EGFR amplification associated with mutations who received treatment from March 2018 to September 2019 were included as the observation group. All patients received an EGFR-tyrosine kinase inhibitor (EGFR-TKI) as the first-line treatment; 67 blood samples from the physical examination center during the same period were randomly included as healthy control. We compared the expression levels of free GPNMB between the two groups, explored the correlation between GPNMB expression and the clinicopathological information in the observation group; and combined the clinical efficacy to evaluate its value as a drug resistance marker. Through follow-up, the progress free survival (PFS) of patients was statistically analyzed, and through multivariate Cox regression analysis, independent risk factors affecting the survival in the observation group were explored. **Results** Compared with that in the control group, the expression level of free GPNMB in the observation group was significantly up-regulated. The expression level of free GPNMB in the observation group is significantly related to the clinical efficacy of EGFR-TKI ($P = 0.016$). Patients with high GPNMB expression have significantly stronger drug resistance, and patients with high GPNMB expression have significantly shorter PFS duration ($P = 0.032$). A high free GPNMB expression ($HR = 4.029, 95\%CI: 1.942-8.358, P < 0.001$) is also an independent risk factor affecting patient survival. **Conclusion** The expression level of free GPNMB in patients with EGFR amplification accompanied by mutant NSCLC is significantly up-regulated, and its high expression is significantly related to the enhancement of the patient's drug resistance. High GPNMB expression is significantly related to the

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poor prognosis of patients and is an independent risk factor affecting patient survival.

Keywords non-small cell lung cancer; glycoprotein non-metastatic melanoma protein B; epidermal growth factor receptor amplification accompanied by mutations; drug resistance; prognosis

非小细胞肺癌(non-small cell lung cancer, NSCLC)是一种高发的恶性肿瘤^[1]。表皮生长因子受体(epidermal growth factor receptor, EGFR)是NSCLC临床研究中最受关注且最重要的靶点^[2]。近年来,临床相关资料表明EGFR在多种肿瘤中过表达,EGFR的扩增伴随突变是主要的突变类型之一^[3],激活EGFR能够引起肿瘤细胞内的酪氨酸活化及其受体磷酸化,促进肿瘤细胞增殖、分化以及血管生成等过程,加速肿瘤细胞的恶性进展^[4]。

研究^[5-6]表明,酪氨酸激酶抑制剂(EGFR-tyrosine kinase inhibitor, EGFR-TKI)在EGFR基因突变的NSCLC治疗中效果显著。此外,越来越多的研究^[7]表明NSCLC患者的EGFR基因突变类型和患者对EGFR-TKI的应答情况显著相关。随着肿瘤患者临床治疗中EGFR-TKI的利用率日益增高,耐药问题成了亟需解决的难题。挖掘EGFR-TKI耐药标志物和筛选适用EGFR-TKI治疗的NSCLC患者,实现个体化治疗,是改善临床治疗效果、延长患者生存期的关键。

糖蛋白非转移性黑色素瘤蛋白B(glycoprotein non-metastatic melanoma protein B, GPNMB)是一种高度糖基化的I型跨膜糖蛋白,作为肿瘤细胞生长的调节基因而备受关注^[8]。在不同的肿瘤中,GPNMB高表达都被证实能够促进肿瘤的恶性进展,例如,GPNMB的表达水平越高,乳腺癌细胞的增殖迁移侵袭能力越强,神经胶质瘤和小细胞肺癌患者的生存时间越短,生存状况越差^[9-11]。最新研究^[12]指出,GPNMB的表达水平可能和胃肠道恶性肿瘤患者EGFR-TKI治疗后的耐药有关。也有研究^[13]表明,GPNMB在EGFR突变NSCLC细胞中表达水平的变化与NSCLC的进展相关,但系统性的研究尚未见报道。

本研究选择将EGFR-TKI作为一线治疗的EGFR基因扩增伴随突变的NSCLC患者作为研究对象,检测其血液中游离GPNMB的表达水平,探讨其与患者耐药及预后的相关性。

1 材料与方法

1.1 研究对象

选取2018年3月至2019年9月唐山市人民医院收治的143例病理确诊为NSCLC患者的临床资料。利用ctDNA检测和FISH法筛选EGFR扩增伴随突变的NSCLC患者,排除资料不全和合并其他肿瘤者,最终纳入55例患者作为观察组。患者均以EGFR-TKI(奥希替尼)作为一线治疗,且未接受过局部治疗。收集患者血液样本和临床资料。此外,随机收集同期本院体检中心的健康人群血液样本作为对照组。所有研究对象均签署了知情同意书,本研究获得医院伦理委员会批准。

1.2 血液中游离GPNMB检测

2组血液样本均置于EDTA管并于-80℃保存。使用人GPNMB的ELISA商品化试剂盒(美国ELH-Osteoactivin, RayBiotech公司)对血液样本中游离的GPNMB进行定量分析,操作均按照试剂盒说明书进行。

1.3 随访

对观察组患者进行随访。记录患者应用EGFR-TKI的疗效。本研究通过疗效评价患者的耐药情况,根据实体瘤疗效评价标准^[14],EGFR-TKI临床疗效分为部分缓解(partial response, PR)、疾病稳定(stable disease, SD)和疾病进展(progressive disease, PD)。收集患者出院后的随访资料,记录患者的无进展生存时间(progression free survival, PFS),将患者的PFS作为本研究的主要终点事件。

1.4 统计学分析

采用SPSS 26.0和Graphpad Prism 8.4.2软件进行统计分析。计数资料以例(率)表示,组间比较采用Fisher精确概率法或 χ^2 检验。计量资料首先进行正态和方差齐性检验,所有正态分布的计量资料以 $\bar{x} \pm s$ 表示,组间比较采用双侧 t 检验。患者PFS的生存分析采

用Kaplan-Meier法和log-rank检验,采用多因素Cox回归分析筛选影响患者生存的独立危险因素。 $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 EGFR扩增伴随突变NSCLC患者血液中游离的GPNMB的表达水平

血液中游离的GPNMB表达水平对照组为 (1.49 ± 0.61) ng/mL,观察组为 (3.09 ± 0.88) ng/mL。根据观察组中GPNMB表达水平的中位值(3.13 ng/mL),将EGFR

扩增伴随突变的NSCLC患者分为高表达组($n = 27$)和低表达组($n = 28$)。随后,对GPNMB表达和EGFR扩增伴随突变NSCLC患者临床病理信息的相关性进行探究。结果显示,在所有EGFR扩增伴随突变NSCLC患者中,女性(32例)居多,33例患者存在吸烟史,临床分期均为Ⅲ期~Ⅳ期,组织分型以肺腺癌(37例)为主。GPNMB高表达组和低表达组年龄、性别、体质量指数(body mass index, BMI)、ECOG评分、临床分期、组织分型以及吸烟史比较均无统计学差异(均 $P > 0.05$),见表1。

表1 观察组GPNMB表达与各项临床指标的相关性

Tab.1 Correlation between GPNMB expression and the clinical data in the observation group

Item	High expression group ($n = 27$)	Low expression group ($n = 28$)	P
Age (year)	57 (41-86)	57 (37-87)	0.356
Sex [n (%)]			0.587
Male	10 (37.04)	13 (46.43)	
Female	17 (62.96)	15 (53.57)	
Body mass index (kg/m^2)	24.66 ± 2.85	24.14 ± 4.53	0.612
History of smoking [n (%)]			0.787
Never	11 (40.74)	13 (46.43)	
Ever	16 (59.26)	15 (53.57)	
ECOG score [n (%)]			0.964
0	5 (18.52)	6 (21.43)	
1	17 (62.96)	17 (60.71)	
2	5 (18.52)	5 (17.86)	
Clinical staging [n (%)]			0.768
Ⅲ	7 (25.93)	9 (32.14)	
Ⅳ	20 (74.07)	19 (67.86)	
Tumor classification [n (%)]			0.775
Adenocarcinoma	19 (70.37)	18 (63.29)	
Non-adenocarcinoma	8 (29.63)	10 (35.71)	
EGFR-TKI therapeutic efficacy [n (%)]			0.016
PR	4 (14.81)	7 (25.00)	
SD	7 (25.93)	15 (53.57)	
PD	16 (59.26)	6 (21.43)	

2.2 GPNMB表达水平和EGFR扩增伴随突变NSCLC患者耐药的相关性

高表达组中4例为PR,7例为SD,16例为PD;低表达组中7例为PR,15例为SD,6例为PD。2组疗效比较有统计学差异($P = 0.016$),低表达组患者使用EGFR-TKI治疗疗效更优,见表1。2组患者的客观反应率(objective response rate, ORR)比较,差异有统

计学意义(4/27 vs. 7/28, $P < 0.01$)。患者血液中游离GPNMB高表达可能提示耐药程度更强,对于EGFR-TKI治疗的敏感性更差。

2.3 GPNMB表达水平和EGFR扩增伴随突变NSCLC患者预后的相关性

观察组随访时间为1.2~18.7个月,平均11.58个月。随访结果显示,GPNMB高表达组患者的中位

PFS为7.38个月(95%CI:0.9~12.5),GPNMB低表达组患者的中位PFS为9.92个月(95%CI:5.3~14.7)。Kaplan-Meier生存曲线结果显示,GPNMB高表达组患者的PFS显著短于低表达组($P = 0.032$),见图1。加入GPNMB表达对患者进行分层生存分析,结果显示,女性($P = 0.009$)、非腺癌($P < 0.001$)、从未吸烟($P = 0.006$)、ECOG评分为1($P < 0.001$)、临床分期为Ⅳ期($P = 0.009$)患者的PFS和GPNMB表达水平显著相关。

2.4 GPNMB表达水平是EGFR扩增伴随突变NSCLC患者无进展生存的独立危险因素

通过Kaplan-Meier法进行患者生存分析,采用log-rank检验所有自变量与因变量的关系,结果显示,性别($P = 0.008$)、吸烟史($P = 0.009$)、ECOG评分($P = 0.002$)、临床分期($P = 0.009$)、组织类型($P = 0.002$)是患者生存可能的影响因素。随后,将GPNMB表达分组和性别、组织类型、吸烟史、ECOG评分以及临

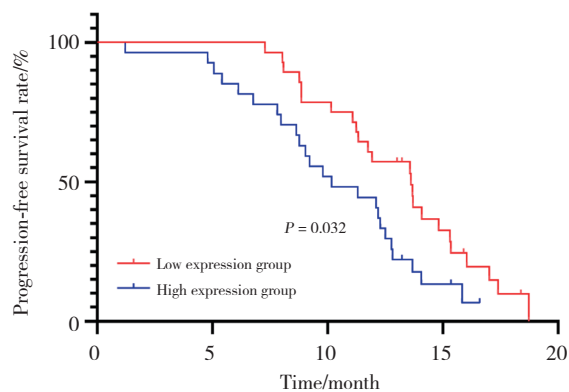


图1 不同GPNMB表达NSCLC患者的PFS曲线

Fig.1 PFS curve of NSCLC patients with different GPNMB expression

床分期等因素纳入多因素Cox回归分析。结果显示,血液中游离的GPNMB高表达($HR = 4.029, 95\%CI: 1.942\sim 8.358, P < 0.001$)是影响EGFR扩增伴随突变NSCLC患者无进展生存的独立危险因素。见表2。

表2 NSCLC患者无进展生存影响因素的多因素Cox回归分析

Tab.2 Multi-factor Cox regression analysis of progression-free survival in NSCLC patients

Factor	HR	95%CI	P
ECOG score (0)	1	-	-
ECOG score (1)	11.770	3.820-36.262	<0.001
ECOG score (2)	106.394	25.117-450.687	<0.001
Sex (female)	1	-	-
Sex (male)	1.614	0.798-3.263	0.183
Clinical staging (Ⅲ)	1	-	-
Clinical staging (Ⅳ)	4.194	1.933-9.099	<0.001
History of smoking (never)	1	-	-
History of smoking (ever)	1.160	0.608-2.212	0.652
Tumor classification (adenocarcinoma)	1	-	-
Tumor classification (non-adenocarcinoma)	0.978	0.455-2.098	0.954
GPNMB expression (low)	1	-	-
GPNMB expression (high)	4.029	1.942-8.358	<0.001

3 讨论

NSCLC患者的血液样本含有多种游离蛋白^[15-16]。NAKASHIMA-NAKASUGA等^[17]使用ELISA方法检测结直肠癌患者血清样本中LOX-1的蛋白表达,发现LOX-1与结直肠癌的预后显著相关。本研究应用ELISA方法检测了EGFR扩增伴随突变NSCLC患者血清中的GPNMB蛋白表达,发现GPNMB表达与患者预后以及耐药性显著相关。

HAN等^[13]研究发现GPNMB与肿瘤的EGFR突变呈正相关,且在EGFR L858R突变的NSCLC细胞中过表达。GPNMB糖基化可以激活突变的EGFR,开启下游STAT3信号通路,促进NSCLC细胞转移。本研究也发现EGFR扩增伴随突变NSCLC患者血清GPNMB显著高表达。根据GPNMB表达水平对EGFR扩增伴随突变NSCLC患者进行分组,并评价GPNMB与耐药性的关系。发现GPNMB高表达与EGFR-TKI的疗效显著相关。以上研究表明,GPNMB与肿瘤

EGFR突变有关,且在EGFR扩增伴随突变NSCLC患者中高表达,并增强其耐药性。

GPNMB与肿瘤患者的预后密切相关^[18]。FENG等^[10]发现GPNMB与胶质瘤不良预后密切相关。MA等^[16]研究证实GPNMB的过表达预示着上皮性卵巢癌的不良预后。本研究对EGFR扩增伴随突变NSCLC患者进行生存分析,发现血液中游离GPNMB高表达患者的PFS显著缩短。进一步分层生存分析和多因素Cox回归分析发现,GPNMB高表达不利于患者无进展生存,且是影响EGFR扩增伴随突变NSCLC患者无进展生存的独立危险因素。以上结果表明,血液中游离GPNMB表达水平可作为EGFR扩增伴随突变NSCLC患者预后标志物;GPNMB高表达与患者预后不良显著相关,并对患者的生存状况产生显著影响。

综上所述,EGFR扩增伴随突变NSCLC患者血液中游离GPNMB高表达,其高表达与患者的耐药程度增强密切相关,是潜在的耐药标志物。与此同时,本研究也证实GPNMB高表达和患者的预后不良显著相关,是影响患者生存的独立危险因素。本研究样本量较少,患者仅来自同一医院,未来应纳入更多样本量进行多中心深入研究。

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