



· 论 著 ·

EGFR (rs884419) 基因多态性与前列腺癌临床病理学特征的相关性分析

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[摘要] 背景与目的: 表皮生长因子受体 (epidermal growth factor receptor, *EGFR*) 基因的高表达被证明在前列腺癌中可间接促进肿瘤细胞迁移, 并与较短的无转移生存期相关。但此前对于*EGFR*基因的研究主要集中于机制方面, 很少与临床指标建立联系。对比*EGFR*基因在西方国家和中国前列腺癌患者中基因型的差异, 同时探讨临床病理学特征与*EGFR*基因多态性的相关性。方法: 检测*EGFR* (rs884419) 基因在复旦大学附属肿瘤医院2014—2019年连续收治的182例前列腺癌患者外周血白细胞DNA中的多态性情况, 比较中国与西方人群基因型变异的差异, 并比较基因型与临床病理学特征及基线特征的相关性。结果: 与*EGFR* (rs884419) 基因型为AA和AG的变异组患者相比, 基因型为GG的正常组患者在初诊时为M₁期的占比更高 (65.3% vs 84.2%, $P < 0.05$)。 *EGFR* (rs884419) 基因多态性与确诊时年龄、确诊时前列腺特异性抗原、转移负荷及Gleason评分无相关性 ($P > 0.05$)。与美国前列腺癌患者相比, *EGFR* (rs884419) 基因的变异频率更高 (20.4% vs 79.1%, $P < 0.05$)。结论: *EGFR*基因型正常组患者初诊时M₁期占比较变异组高, 提示*EGFR*基因的表达差异可能参与前列腺癌转移的发生、发展过程, 根据基因型理论上可以预测患者初诊时的远处转移状态; 同时, 与西方人群相比, 中国前列腺癌患者*EGFR*基因的变异频率较高, 对确诊后治疗效果的预测效能会更高。

[关键词] 前列腺癌; 表皮生长因子受体; rs884419; 基因多态性; 临床病理学特征
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Correlation between *EGFR* (rs884419) gene polymorphism and clinicopathological characteristics of prostate cancer PAN Jian, WEI Yu, YE Dingwei, ZHU Yao (Department of Urology, Fudan University Shanghai Cancer Center; Department of Oncology, Shanghai Medical College, Fudan University, Shanghai 200032, China)

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[Abstract] **Background and purpose:** In prostate cancer, the high expression of epidermal growth factor receptor (*EGFR*) gene is confirmed to indirectly promote the migration of tumor cells and is associated with a shorter metastasis-free survival. However, majority of previous research on the *EGFR* gene focused on the mechanism and rarely established links with clinical indicators. This study aimed to compare the genotype differences of *EGFR* gene in the western countries and Chinese prostate cancer patients, to explore the correlation between clinicopathological characteristics and *EGFR* gene polymorphism. **Methods:** The polymorphism of *EGFR* (RS884419) gene in peripheral blood leukocyte DNA of 182 prostate cancer patients who were treated in Fudan University Shanghai Cancer Center from 2014 to 2019 was detected to compare genotype differences of *EGFR* gene in prostate cancer patients between Chinese and the western populations, and to explore the correlation between clinicopathological and basic characteristics and *EGFR* gene polymorphism. **Results:** Compared with patients in the variant group with *EGFR* (rs884419) genotypes AA and AG, patients in the normal group with genotype GG had a higher proportion of distant metastases (M₁ stage) at diagnosis (65.3% vs 84.2%, $P < 0.05$); *EGFR* (rs884419) gene polymorphism had no correlation with age at diagnosis, prostate-specific antigen at diagnosis, metastatic load and Gleason score ($P > 0.05$). Compared with American prostate cancer patients, the mutation frequency of *EGFR* (rs884419) gene was higher (20.4% vs 79.1%, $P < 0.05$). **Conclusion:** The M₁ stage

of patients at diagnosis is higher in the normal *EGFR* genotype group than in the variant group, suggesting that the difference in *EGFR* gene expression may be involved in the occurrence and development of prostate cancer metastasis. According to the genotype, the distant metastasis status of patients at the diagnosis can be predicted theoretically. And due to the high frequency of mutations in the Chinese patients, *EGFR* genotype has a stronger predictive value for treatment efficacy than it does in the western population.

[Key words] Prostate cancer; Epidermal growth factor receptor; rs884419; Gene polymorphism; Clinicopathological characteristics

前列腺癌是世界范围内男性第二大常见恶性肿瘤^[1]。虽然局限性前列腺癌患者的5年生存率接近100%，但转移性去势抵抗性前列腺癌目前仍然无法治愈^[2-3]。考虑到只有12%的前列腺癌患者死于该疾病，区分侵袭性和惰性前列腺癌仍然是一个重要的临床问题，因此寻找一种可以辅助判断肿瘤的早期侵袭情况、复发可能性并预测转移部位的标志物十分必要，有望为患者提供个性化的治疗。

对于预测前列腺癌的复发风险，最优决策是建立在包括临床分期、Gleason评分等临床变量的列线图指导评分和前列腺特异性抗原（prostate-specific antigen, PSA）值之上的。但随着高通量测序方法的出现，越来越多的研究将评估基因组生物标志物作为对前列腺癌风险进行分层的变量。人类基因组中最常见的遗传变异是单核苷酸多态性（single nucleotide polymorphism, SNP）。有研究^[4]发现，SNP与前列腺癌易感基因位点、雄激素生物合成之间存在关联。SNP也被发现是前列腺癌的预后标志物，Van Den Broeck等^[5]的综述指出，特定的SNP位点变异与前列腺癌的常规治疗效果、不良反应及复发概率之间存在关联。

上皮-间质转化（epithelial-mesenchymal transition, EMT）参与前列腺癌的转移进展^[6-8]。表皮生长因子受体（epidermal growth factor receptor, EGFR）在前列腺肿瘤的发生、发展中发挥着重要作用^[9-10]。EGFR信号转导通路是目前所知最主要和研究最深入的促进肿瘤细胞增殖、转化、凋亡、侵袭和转移的受体酪氨酸激酶通路^[11-12]，在生长因子调控细胞生长、发育和功能的多个过程中发挥着重要的生理作用。已有研究表明，EGFR表达与前列腺癌的高分级、晚

分期、复发^[13]和骨转移^[14]相关。此外，EGFR也被证明可以调控骨发育^[15]。作为EMT、分化、增殖和血管生成的调节因子之一，EGFR可能启动和（或）促进肿瘤的扩散和转移，因此被认为可能是预测高转移潜能的替代标志物^[14]。39%~47%的前列腺癌表达EGFR^[16]，并且在前列腺癌进展到晚期雄激素非依赖性阶段时也能观察到EGFR的表达增加^[17]。在包括前列腺肿瘤在内的临床前肿瘤模型中，EGFR的表达可能与不良表型增殖、血管生成和迁移直接相关^[18-20]，并与肿瘤的放射可治愈性负相关^[21-22]。

EGFR（rs884419）基因多态性与中国前列腺癌患者包括转移负荷在内的病理学特征的相关性研究尚未见报道。本研究旨在对比*EGFR*（rs884419）基因变异频率在中国与西方人群前列腺癌中的分布差异，并基于中国前列腺癌患者的突变特征，分析*EGFR*（rs884419）基因多态性与临床病理学特征的相关性并评估EGFR对转移情况的预测效能。

1 资料和方法

1.1 研究对象

选择复旦大学附属肿瘤医院2014—2019年连续收治的182例前列腺癌患者作为研究对象。纳入标准：经病理学或细胞学检查确诊为前列腺腺癌，无神经内分泌分化、无印戒细胞癌或小细胞癌分化；年龄≥18岁；患者自愿签署知情同意书，同意将血液样本及临床资料用于本研究。

1.2 基因测序

检测*EGFR*（rs884419）基因在纳入的182例患者外周血白细胞DNA中的多态性情况。提取患者血液中的DNA，采用DNA提取试剂盒对其进行

聚合酶链反应 (polymerase chain reaction, PCR) 扩增, *EGFR*引物的下游引物为5'-GACAGACTG GATAAAGAAAATTGTGGTACA-3', 上游引物 5'-CTCCATCCATGTTCTTGCAAAGTAC-3'。反应条件: 将DNA在98 °C下进行变性, 持续15 s; 在52 °C下进行退火, 持续20 s; 在68 °C下进行扩增, 持续20 s, 共38个循环。

1.3 文献纳入情况

采用关键词“prostate carcinoma” OR “prostate cancer” AND “polymorphism” AND “EGFR”在PubMed数据库中进行检索; 用“EGFR”、“前列腺癌”等关键词在中国知网和万方等数据库中检索。限定检索时间为2000年1月1日—2021年4月1日。文献纳入标准: ① 在2021年4月1日之前发表的与前列腺癌和*EGFR*基因相关的中英文研究; ② 研究对象为人类, 而不是机制研究; ③ 有具体的基因数据。文献排除标准: ① 同一人群基因数据的重复报道; ② 存在严重偏倚的数据。纳入文献的基本情况见表1。

表 1 美国和中国*EGFR* (rs884419) 基因的各基因型分布

Tab. 1 Distribution of genotypes of *EGFR* (rs884419) gene in the

America and China						
Country	Ethnicity	Patient <i>n</i>	GG	AA+ AG	Mutation frequency	<i>P</i> value
America	Caucasian	196	156	40	20.4%	<0.001
China	Asian	182	38	144	79.1%	

1.4 统计学处理

计量资料 (如年龄) 分析采用非配对 *t* 检验; 计数资料 (如基线PSA、基因型频率、转移负荷情况等) 分析则采用Fisher精确检验或 χ^2 检验。 $P < 0.05$ 为差异有统计学意义, 检验为双侧。所有的统计过程均采用R-Studio软件完成。

2 结 果

根据所设定的文献纳入和排除标准, 最后仅有一篇文献纳入分析 (表1) [23]。本研究将*EGFR* (rs884419) 基因型为AG和AA的患者命名为变异组, 基因型为GG的患者命名为正常组。所纳入文献包含196例美国前列腺癌患者, 其中至少1个*EGFR* (rs884419) 基因变异位点的患者占20.4% (40/196), 复旦大学附属肿瘤医院的182例前列腺癌患者中, 携带至少1个*EGFR* (rs884419) 基因变异位点 (数据符合Hardy-Weinberg平衡) 的患者占79.1% (144/182), 中国前列腺癌的*EGFR* (rs884419) 变异频率显著高于美国 ($P < 0.001$, 图1)。

在对*EGFR* (rs884419) 基因型与前列腺癌临床病理学特征进行分析时发现, 基因型与患者初诊时的远处转移状态相关, 正常组患者初诊时为M₁期的概率较变异组高 ($P = 0.028$); 而*EGFR* (rs884419) 基因与患者确诊时的年龄、确诊时的PSA水平、Gleason评分及转移负荷均无相关性 ($P > 0.05$, 表2, 图2)。

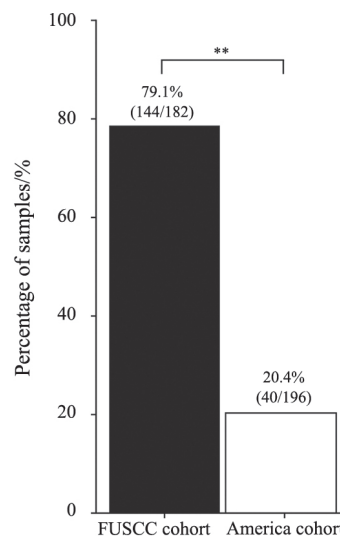


图 1 *EGFR* 基因突变频率

Fig. 1 Mutation frequency of *EGFR*

FUSCC: Fudan University Shanghai Cancer Center; **: $P < 0.001$

表 2 患者的临床病理学特征

Tab. 2 Clinicopathologic characteristics of patients

Characteristic	Genotype		P value
	AA/AG (n=144)	GG (n=38)	
Age at diagnosis/year $\bar{x}\pm s$	65.6±7.7	64.4±8.0	0.460
PSA level $\rho_B/(ng\cdot mL^{-1})$			0.903
≤20	44	12	
>20	100	26	
Gleason score*			0.523
≤7	29	9	
8-10	115	27	
Unknow	0	2	
Metastasis stage*			0.028
M ₀	49	6	
M ₁	94	32	
Unknow	1	0	
Metastases volume*			0.582
High	56	20	
Low	39	11	

*: The data in some patients were not available. High-volume disease was defined as the presence of visceral metastases or ≥4 bone lesions with at least one outside of the vertebral column and pelvis according to the CHAARTED trial

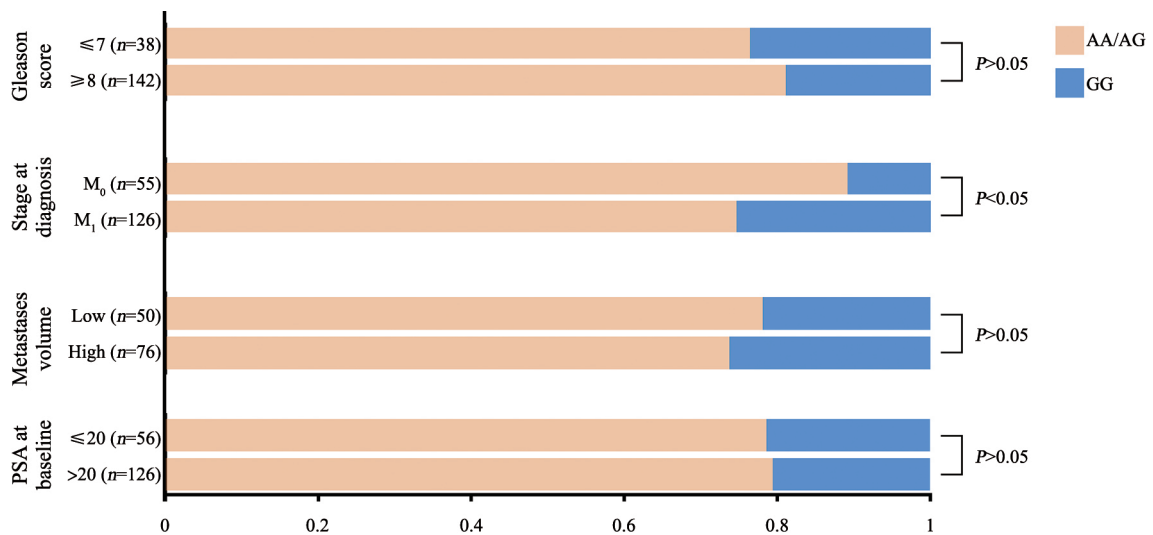


图 2 不同临床特征中基因型分布

Fig. 2 Distribution of genotype in different clinical characteristics

3 讨 论

*EGFR*基因表达的调控机制十分复杂, 受到基因多态性的影响。目前针对*EGFR*基因与临床病理学特征关系的研究罕见报道。本研究对中国人群前列腺癌患者*EGFR* (rs884419) 基因多态性进行了分析。一方面, 在与其他人种的对比中发现, 中国前列腺癌患者rs884419位点的变异频率较美国高, 提示与美国前列腺癌患者相比, 理论上*EGFR*基因型对于预测中国前列腺癌患者转移状态与治疗效果的效力更好。另一方面, 基因型为GG型的患者有着更高的远处转移发生率, 可能意味着在rs884419位点发生变异后, *EGFR*基因表达降低, 抑制了肿瘤细胞的远处侵袭能力, 最终使得患者的远处转移概率降低。

Xie等^[24]研究表明, *EGFR*基因状态与前列腺癌患者的死亡风险显著相关, *EGFR*基因的高表达与较短的无转移生存期相关。还有研究显示, *EGFR*表达与EMT相关^[25-26], 而EMT可促进肿瘤细胞迁移和存活^[6]。相比于*EGFR*正常或低表达的肿瘤组织, *EGFR*高表达的肿瘤组织内有大量的血管和(或)淋巴管, 可以促进癌细胞经血液和淋巴途径播散, 使其侵袭能力增强^[27]。Nastały等^[28]的研究则显示, *EGFR*高表达的前列腺癌细胞中, 刚性基质的产生显著增加, 由此推测*EGFR*也许可以促进细胞在刚性基质上的生长, 最终促进骨转移的发生。而Day等^[14]的研究也证实*EGFR*蛋白的高水平与前列腺癌进展及骨转移相关。

*EGFR*也可以在其他方面促进前列腺癌进展, 如促进肿瘤的自我更新等。有一些证据支持该观点, 即添加到培养基中的表皮生长因子(epidermal growth factor, EGF)可促进干细胞的生长^[29], 而*EGFR*的激活会增加前列腺微球体的形成^[30]。Perez等^[23]进行了一项回顾性分子流行病学研究, 提示*EGFR*基因的rs884419位点是前列腺癌根治术后3年内复发风险的遗传标记。尽管目前尚不清楚rs884419发挥作用的潜在机制, 但由于该SNP位于CREB、SREBF、ZNF

和KLF1的转录因子结合位点, rs884419位点的多态性差异可能会通过调节基因的表达影响转录, 最终引起蛋白功能的改变。

为探索不同*EGFR*基因型对前列腺癌恶性程度的影响, 本研究评估了*EGFR* (rs884419) 基因多态性与临床病理学特征之间的相关性。但本研究存在一些不足之处: 首先, 单中心数据不可避免地存在人群选择性偏倚, 但非选择性、连续性入组的方式降低了选择偏倚带来的误差; 其次, 有限的样本数在一定程度上会影响统计结果的准确性; 最后, 由于随访时间有限及部分患者治疗线数的不同, 本研究没有展示根据基因型区分的治疗数据。

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