·论著·

中国辽宁汉族群体 DNA 修复基因：*ERCC2/XPD* A35 931C 遗传多态性

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【摘要】 **目的　研究 DNA 修复基因 *ERCC2/XPD* A35 931C 及其遗传多态性在保护维持基因组整体性及抗癌发生过程中的重要作用。方法　应用 PCR－RFLP 方法调查了中国辽宁汉族群体 DNA 修复基因：*ERCC2/XPD* A35 931C 遗传多态性。结果　*ERCC2/XPD* A35 931C 基因型频率：AA = 0.98；AC = 0.05；CC = 0.00，基因型分布符合 Hardy-Weinberg 遗传平衡定律（$P = 0.77$）；*ERCC2/XPD* A35 931C 等位基因频率：A = 0.98；C = 0.02。结论　研究结果与前期所报道的其他群体的该等位基因频率分布进行比较，辽宁汉族群体该 SNP 频率分布与亚洲大多数群体的频率分布特点相似。**

【关键词】 DNA 修复基因；　*ERCC2/XPD* A35 931C；　遗传多态性；　中国汉族

Polymorphism of DNA Repair Genes: *ERCC2/XPD* A35 931C in Han Chinese from Liaoning Province

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【**Abstact**】 **Objective**　To study the important role of DNA repair gene *ERCC2/XPD* A35 931C and its genetic polymorphism in maintaining the integrity of the genome and protecting against mutations that can lead to cancer. **Methods**　We studied the genetic polymorphism of *ERCC2/XPD* A35 931C in Han Chinese from Liaoning Province by PCR－RFLP method. **Results**　The genotype frequencies were 0.98(AA), 0.05(AC), and 0.00(CC); and the allele frequencies were 0.98(A) and 0.02(C), respectively. **Conclusion**　The present study results were compared with ones of the previously reported control populations. Frequency distribution of *ERCC2/XPD* A35 931C among Han Chinese in Liaoning is similar to that of most population in Asia.

【**Key words**】 DNA repair genes;　*ERCC2/XPD* A35 931C;　Genetic polymorphisms;　Han Chinese

DNA 修复基因在保护维持基因组整体性及抗癌发生过程中起着重要作用，DNA 修复基因的产物：ERCC2/XPD（excision repair cross complementation group 2/xeroderma pigmentosum complementation group D 切除修复交叉互补 2 组/着色性干皮病互补 D 组）参与核苷酸切除修复过程。*ERCC2* A35 931C 是由于 A-C 颠换（GenBank accession No. L47234），导致氨基酸密码子 751 从赖氨酸变为谷氨酰胺。理论上推论，*ERCC2* A35 931C SNP 的颠换是影响 *ERCC2* 蛋白酶功能活性最重要的因素[1~3]。一些遗传流行病学研究已经调查了不同群体中这个 SNP 与不同癌症发生风险之间的关联[4~23]，但目前关联研究所报道的结果是十分矛盾的。*ERCC2* A35 931C 功能的意义还没有被阐明。单核苷酸多态性的群体遗传学信息对于遗传疾病的研究是非常重要的。我们报道了中国辽宁汉族群体 DNA 修复基因 *ERCC2* A35 931C 的遗传多态性。

1　材料与方法

1.1　研究群体：外周血样品（n = 150 人）来自于中国辽宁地区无亲缘关系的、健康的汉族人群。所有被研究个体均知情同意。

1.2　基因分型[4,5]：取 1.5mL 外周血提取基因组 DNA，使用 Puregene DNA Isolation kit（Gentra Systems，

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Minneapolis, MN, U S A)。

多态性的检测应用改进了 PCR-RFLP 的方法。*ERCC*2 A35 931C (rs # 13181) PCR 引物是:forward primer:5′-ATCCTGTCCCTACTGGCCATTC-3′和 reverse primer:5′-TGTGGACGTGACAGTGAGAAAT-3′. [TaKaRa Biotechnology(Dalian) Co., Ltd, China 合成]。

50μL PCR 反应液内含:10mmol/L Tris-HCl, 50mmol/L KCl (pH8.4), 1.5mmol/L $MgCl_2$, 每种 dNTP 各 0.2mmol/L, 引物各 1.0μmol/L, *Taq* DNA 聚合酶 2U[TaKaRa Biotechnology(Dalian) Co., Ltd, China]及基因组 DNA 50 ~ 200ng。PCR 循环条件:开始变性温度 96℃ 1min, 94℃ 30s, 60℃ 30s, 72℃ 1min, 30 circles, 最后延伸:72℃ 2min。

20μL 限制性内切酶反应液内含:10μL PCR 产物及 1U Pst Ⅰ限制性内切酶(New England Biolabs, Beverly, MA)。按说明要求操作:65℃ 孵育 1.5h。2%琼脂糖(Spainish 进口分装/上海生工)分离。UVP 凝胶成像仪(GDS-8000 UVP, U S A)成像。*ERCC*2 A35 931C:PCR 引物设计扩增目的片段为 324bp, Pst Ⅰ限制性内切酶切点在 A, 而不是在 C, 3 种基因型分别根据以下片段判断:AA:224bp, 100bp;AC:224bp, 158bp, 100bp, 66bp 及 CC:158bp, 100bp, 66bp。

1.3 统计学方法:群体基因频率分布的 Hardy-Weinberg 遗传平衡检验:HWE 软件(http://linkage.rockefeller.edu/ott/linkutil.htm)。中国辽宁汉族群体与前期所报道的其他群体之间的等位基因频率分布的比较:卡方检验(χ^2 test)。SPSS 软件(Version 11.5, U S A)完成。

2 结果

150 个 DNA 样品被用于基因分型。但 5 个 PCR 反应失败。

*ERCC*2 A35 931C 的基因型频率分布符合 Hardy-Weinberg 遗传平衡定律($P = 0.77$)(见表 1)。

中国辽宁汉族群体与前期所报道的其他群体之间等位基因频率分布的比较见表 2。

表 1 中国辽宁汉族群体基因型和基因频率分布

Table 1 Genotype and allele frequencies of *ERCC*2 A35 931C among Han Chinese in Liaoning, China

Genotypes	observed(%)	Allele frequencies	P for HWE[a]
AA	138(95.17%)	A = 0.98	$\chi^2_{(1)} = 0.089$
AC	7(4.83%)	C = 0.02	$P = 0.77$
CC	0(0.00%)		

a. Hardy-Weinberg equilibrium

表 2 中国辽宁汉族群体与前期所报道的其他群体之间等位基因频率分布比较

Table 2 Allele frequencies of *ERCC*2/*XPD* A35 931C in previously reported control populations

Reference	cancer	Ethnicity	Control(n)	MAF[a]	P[b]
Liang *et al*[10]	Lung cancer	Chinese(Beijing)	1 020	0.09	< 0.001
Yu *et al*[11]	Esophageal cancer	Chinese(Hubei)	152	0.07	0.01
Yeh *et al*[12]	Colorectal cancer	Chinese(Taiwan)	736	0.07	0.003
Chen *et al*[13]	Lung cancer	Chinese(Jiangsu)	109	0.40	< 0.001
Hamajima *et al*[24]	No	Japanese	240	0.05	0.077
Park *et al*[14]	Lung cancer	Korean(South Korea)	163	0.06	0.024
Shen *et al*[15]	Bladder cancer	Caucasian(Italy)	214	0.40	< 0.001
Winsey *et al*[16]	Skin cancer	Caucasian(UK)	211	0.40	< 0.001
Zhou *et al*[17]	Lung cancer	Caucasian(USA)	1 240	0.37	< 0.001
Vogel *et al*[18]	Skin cancer	Caucasian(USA)	117	0.36	< 0.001
Spitz *et al*[19]	Lung cancer	Caucasian(USA)	360	0.33	< 0.001
Caggana *et al*[20]	Gliomas	Caucasian(USA)	148	0.41	< 0.001
David-Beabes *et al*[21]	Lung cancer	Caucasian(USA)	453	0.35	< 0.001
Stern *et al*[22]	Bladder cancer	Caucasian(USA)	197	0.38	< 0.001
David-Beabes *et al*[21]	Lung cancer	African-American	234	0.25	< 0.001
Qiao *et al*[25]	No	Non-Hispanic White	102	0.36	< 0.001
Vogel *et al*[23]	Lung cancer	Caucasian(Denmark)	269	0.35	< 0.001
Qi *et al*	Lung cancer	Chinese(Liaoning)	145	0.02	present study

a. MAF: minor allele frequency

b. χ^2 test for comparison of allele frequencies of the previously reported control populations with present study result, respectively

3 讨论

前期研究结果表明,在欧洲及北美洲 *ERCC2* A35 931C C 变异等位基因频率是常见的:大约 50% 的个体携带有 AC 基因型;大约 10% ~ 15% 的个体是 CC 基因型。美国黑种人中:大约 5.6% 的个体是 CC 基因型。而在亚洲,携带有 CC 基因型的个体是很少见的[26]。

在本研究中,没有观察到 *ERCC2* A35 931C CC 基因型的个体(见表 1),辽宁汉族群体无该 SNP CC 基因型的个体;*ERCC2* A35 931C C 变异等位基因频率是 0.02,这是目前报道的所有群体中该 SNP 最低的次要等位基因频率,与欧洲及北美洲所报道的高加索群体(0.33 ~ 0.41)、非西班牙白人群体(0.36)及美国黑人群体(0.25)的研究结果有极显著的差异($P < 0.001$)(见表 2)。与日本群体的研究结果(0.05)一致($P = 0.077$),与中国北京(0.09)、中国湖北(0.07)汉族群体、中国台湾群体(0.07),以及韩国群体(0.06)的结果相似;但明显不同于中国江苏汉族群体(0.40),这个群体的该 SNP 基因频率分布符合于欧洲及北美洲群体的特点(见表 2),值得进一步研究探讨。

遗传流行病学研究已经调查了 *ERCC2* A35 931C多态性与癌症的关联并且揭示了 C 变异等位基因与不同癌症之间的关联[4,9~13,17,22,23]。前期已报道了丹麦及美国高加索群体中 *ERCC2* A35 931C SNP 与增加基底细胞癌的发生风险相关联[6~8]。在中国辽宁汉族群体中有统计学意义低的 *ERCC2* A35 931C C 变异等位基因频率提示不同种族间可能存在不同的与癌症发生风险相关的遗传学标记。在中国群体中进行更多的该方面研究,对于确定这个单核苷酸多态性的分布特点是十分有价值的。

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