·论 著·

TP53 polymorphisms are involved in inverse colorectal cancer comorbidity in Chinese schizophrenia patients*

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Abstract: The inverse cancer comorbidity in schizophrenia patients may be related to the genetic factors, involving the regulation of apoptosis. The tumour suppressor gene TP53, involved in neural apoptosis, is one of the potential candidate genes associated with schizophrenia which might reduce colorectal cancer risk. We recruited 270 schizophrenia patients and 312 colorectal cancer patients without schizophrenia. To examine the genetic association between schizophrenia and colorectal cancer, we analysed eight SNPs (rs12951053, rs1625895, rs2909430, rs9895829, rs1042522, rs8079544, rs8064946, rs17806770) covering 14.35 kb in the region of TP53. We observed that one of the eight genetic polymorphisms showed statistically significant differences between the colorectal cancer subjects and the schizophrenia subjects (rs12951053, $P=0.000\ 1$, OR 1.70, 95%CI 1.30－2.23). In addition, the haplotype of A-G (rs12951053- rs8064946), giving a global $P=0.001\ 8$, was the most significant. Our data indicate that the polymorphisms of rs12951053 in TP53 confer reduced susceptibility to colorectal cancer and suggest a potential protective mechanism against colorectal cancer in the schizophrenia patients of Han Chinese origin.

Key words: colorectal cancer; schizophrenia; TP53; polymorphism; reduced susceptibility
DOI: 10.3969/j.issn.1673-4130.2015.15.046 **文献标识码**: A **文章编号**: 1673-4130(2015)15-2234-04

The relationship between cancer and psychiatric disorders has attracted interest among researchers for over a century. Lower than expected occurrence of cancer has been reported for psychiatric patients. Schizophrenia is still one of the most challenging psychiatric disorders, often resulting in negative changes in life styles and long periods of hospitalization, which may increase the health care costs.

Contradictory results in the correlation between schizophrenia and cancer have been observed[1-3]. Recent researches have observed reduced incidence of cancer in schizophrenia[4-7], and in their first degree relatives[8-9]. There was an evidence of colorectal cancer being reduced in schizophrenia patients from Catts's meta-analysis[9]. The hypotheses proposed to explain the decreased risk included genetic factors, neuroleptic medication, life differences and environmental aspects[9-10]. A lower than expected rates of cancer in schizophrenia patients has been ascribed to specific protective mechanism against cancer. The genetic factors are mostly related to the inverse relationship between cancer and schizophrenia[10]. To date, TP53 and XRCC4 genes have been reported to be involved in this protective mechanism[5,11-13].

The tumour suppressor gene TP53 is considered as a candidate susceptibility gene for schizophrenia because it was shown to control the neural stem/progenitor cells self-renewal and differentiation. Schizophrenia occurs as a consequence of abnormality in neurodevelopmental process[14-16]. Increased expression of TP53 at early stage of brain development results in neuronal damage[17], and also, increased neuronal apoptosis may be responsible for the high incidence of neuromotor anomalies found in schizophrenia[18-19]. Furthermore, the TP53 gene is located on 17p13.1 region and the region around 17p13.3 has been proved to have a significant linkage with schizophrenia[20]. These findings support that the TP53 gene may be related to the pathogenesis of schizophrenia.

Catts and Catts firstly proposed TP53 gene as a susceptibility gene in schizophrenia, was associated with a lower risk of cancer[13]. Yang et al. (2004) investigated genetic association between polymorphism of three SNPs and schizophrenia. With 701 cases and 695 controls, the study demonstrated that TP53 might play a role in the susceptibility to schizophrenia in the Chinese population[12]. Ni et al. (2005) performed case-control study on 286 Toronto schizophrenia cases and 264 controls, transmission disequilibrium test (TDT) on 163 Portuguese nuclear families to test the association between TP53 gene and schizophrenia. The results provided further evidence for the association[21]. Park et al. (2004) tested the genetic association between 179 schizophrenia patients and 104 lung cancer patients in Korean. The results suggested that p53 polymorphisms might be associated with reverse susceptibility to lung cancer in schizophrenia patients[11].

We therefore concluded that variants within the TP53 gene might confer genetically reduced susceptibility to colorectal cancer among patients with schizophrenia. To examine this assumption, we investigated eight genetic polymorphisms (rs12951053, rs1625895, rs2909430, rs9895829, rs1042522, rs8079544, rs8064946 and rs17806770) between Chinese colorectal cancer subjects without schizophrenia and schizophrenia subjects, a genetic association strategy similar to the one used

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by Park et al[11].

1 Subjects

For this study, 312 patients with colorectal cancer (178 male and 134 female, age 61.23±14.03 years) and 270 patients with schizophrenia (191 male and 79 female, age 57.25±11.55 years) were recruited. The CRC patients underwent curative resection through the year of 1999 to 2007 at the surgical department of Shanghai First People's Hospital or the Shanxi People's Hospital, China. The cancerous tissue and its adjoining normal control tissue (>10 cm) were promptly frozen in liquid nitrogen. The pathologic tumor staging was performed in accordance with Duke's criteria. All schizophrenia patients were diagnosed by senior psychiatrists according to the criteria DSM-III-R, and they all were from Shanghai and were Han Chinese in origin. At least two psychiatrists gave their assessments on the basis of interview data and hospital case records. Written informed consent for genetic analysis was obtained from all participants and was reviewed by the ethics committee of the Human Genetics Center in Shanghai. Genomic DNA was extracted using standard methods with phenol/chloroform purification. The extracted DNA was diluted to a final concentration of 100 ng/L and stored at −80 ℃ until further use.

2 Genotyping

Genotypes for eight SNPs in TP53 gene were determined (rs12951053, rs1625895, rs2909430, rs9895829, rs1042522, rs8079544, rs8064946, rs17806770). The markers were chosen from the HapMap project database (http://www.hapmap.org) and dbSNP (http://www.ncbi.nlm.nih.gov/SNP/) to cover a 14.35 kb region of TP53. All the eight markers are intronic SNPs except rs1042522(C/G) in exon 4. We genotyped the eight SNPs by the TaqMan® assay method using the ABI7900 DNA detection system (Applied Biosystems, Foster City, California). All probes and primers were designed with the Assay-on-Design service of Applied Biosystems. The standard PCR was performed using the Taqman® Universal PCR Master Mix (Applied Biosystems) reagent.

3 Statistical analysis

Hardy-Weinberg equilibrium, allelic and genotypic distributions were analysed on the SHEsis platform[22], which is a powerful platform for analysis of genetic association studies. The discrepancies of allele and genotype frequencies between CRC and schizophrenia patients were compared using Monte Carlo simulation strategy and 2 tests. As the standardized measure, Linkage disequilibrium (LD), using D′ to represent, was estimated for all SNP loci. *P* values of association analysis for multiplicity were corrected using a false discovery rate (FDR) controlling procedure. The statistic power was calculated using the G * Power program. All the *P* values in this study were two-tailed and the significance level was set at $P=0.05$. Results were also expressed in terms of *OR* and 95%*CI*, which were calculated on the website http://www.hutchon.net/ConfidOR.htm. The program UNPHASED was used to evaluate haplotype frequencies.

4 Results

For all our samples, distributions of genotype frequencies of all eight polymorphisms in either CRC or schizophrenia subjects were in Hardy-Weinberg equilibrium. The allele and genotype frequencies of the SNPs are listed in Table 1(See the "International Journal of Laboratory Medicine" website home page "paper attachment"). For genetic polymorphism rs12951053, there were statistically significant differences in allele frequencies between the 270 schizophrenia subjects and the 312 CRC subjects ($P=0.000\ 1$, $P=0.001$, after the FDR correction). The A allele and AA genotype of rs12951053 were significantly less common in the CRC group compared to the schizophrenia group (allele, 56.6% versus 68.9%, *OR* 1.70, 95%*CI* 1.30−2.23; genotype, 30.3% versus 49.2%).

We observed that many groups of the markers were in strong LD, by analysing linkage disequilibrium for each pair of the SNPs in the CRC and schizophrenia subjects (Table 2)(See the "International Journal of Laboratory Medicine" website home page "paper attachment").

We selected some of those haplotypes with significant frequency discrepancies between CRC and schizophrenia subjects (Table 3)(See the "International Journal of Laboratory Medicine" website home page "paper attachment") for presentation. Haplotype analysis of these polymorphisms revealed some significant global *P* values(Table4)(See the "International Journal of Laboratory Medicine" website home page "paper attachment").

A two-SNP-based haplotype, rs12951053-rs8064946, with a global p value of 0.0013, showed the most significant difference between the CRC and the schizophrenia groups. The haplotype A-G (rs12951053-rs8064946) was found to be correlated with an increased occurrence in the schizophrenia group compared to the CRC group ($P=0.001\ 8$, *OR* 0.633, 95%*CI* 0.481−0.832). The frequency of the haplotype A-G-C (rs12951053-rs1042522-rs8064946) was also greater in the schizophrenia group than in the CRC group ($P=0.002\ 9$, *OR* 0.641, 95%*CI* 0.486−0.845).

In the power calculations, G * Power 3 program was used. Our sample size had greater than 90% power to detect a significant ($P<0.05$) association for alleles, genotypes and haplotypes, with an effect size index of 0.1 (corresponding to a "weak" gene effect).

5 Discussion

Reports on the association between schizophrenia and cancer incidence are inconsistent. The schizophrenia patients may get higher, similar or lower cancer rates comparing against the general population, even though the schizophrenia patients may have multiple cancer-promoting factors including smoking behavior, alcohol consumption and other unhealthy life styles. The majority of recent studies suggest a protection mechanism in schizophrenia against cancer[4,6,10].

There are several reasons for schizophrenia patients getting less chance to develop cancers. The detection rate of cancers might be less in schizophrenia patients than that in the general population because patients with schizophrenia are less inclined to seek medical help. However, in the case of CRC, blood loss and obstructive symptoms often exist in CRC patients,

leading to the demand of medical attention. In addition, the neuroleptic medication and antidepressants may also lower the cancer occurrence rate. With respect to Levav's study, the reduced risk of morbidity for cancer in patients diagnosed with schizophrenia and in their blood relatives strongly points to genetic influences[8]. The medication cannot be persuasive enough to explain the lower risk in the blood relatives. More recent explanation for decreased cancer incidence in schizophrenia involves apoptotic mechanisms closely related to both the synaptic plasticity and cell cycle regulation[13].

The TP53 gene, a pivotal tumour suppressor gene, promotes cell-cycle arrest, DNA repair, senescence and apoptosis and restrains cancer development. Mutation of the TP53 is the most common genetic defect in human cancer. Catts and Catts[9,13] suggested that the reduced risk of cancer observed in schizophrenia patients might be associated with differences in apoptosis, and proposed TP53 as a candidate gene for the susceptibility. This hypothesis was attested progressively in the follow-up studies[11-12,21].

In the present study, our data, based on Han Chinese samples, provide further support on the assumption that TP53 may be involved in the cancer protective mechanism in schizophrenia patients. We conducted the genetic analysis by genotyping eight SNPs (rs12951053, rs1625895, rs2909430, rs9895829, rs1042522, rs8079544, rs8064946 and rs17806770) covering 14.35kb in the region of TP53. All these markers were selected from the HapMap project database http://www.hapmap.org and dbSNP http://www.ncbi.nlm.nih.gov/SNP/. At one of the eight markers (rs12951053), there were statistically significant discrepancies of allele or genotype frequencies between CRC and schizophrenia subjects. We observed that the A allele of rs12951053 was less frequent in CRC than in schizophrenia subjects (*OR* 1.70, 95% *CI* 1.30－2.23, $P=0.0001$, $P=0.001$, after the FDR correction), indicating that it might be a protective factor for schizophrenia patients against CRC.

Since haplotypes constructed from closely located markers will typically increase the statistical power for association with the disease, we performed haplotype analysis in SNPs with relatively strong linkage disequilibrium ($D'>0.7$). Our results indicated that one two-SNP-based haplotype, rs12951053-rs8064946 ($P=0.0013$), showed most significant global frequency difference between the CRC and schizophrenia groups (Table 4). In addition, we observed that the most significant haplotype A-G (rs12951053- rs8064946, *OR* 0.633, 95% *CI* 0.481－0.832, $P=0.0018$,) was much more common in the schizophrenia group (70.3%) than in the CRC group (60.0%), suggesting that A-G is a protective haplotype for CRC among schizophrenia patients. The haplotype A-G-G (rs12951053-rs1042522- rs8064946, *OR* 0.641, 95% *CI* 0.486－0.845, $P=0.0020$) also showed a protective role for CRC in schizophrenia patients.

In the genetic association analysis of study by Park et al. TP53, as a protective gene, induced a lower risk of lung cancer among Korean schizophrenia patients. We also observed that polymorphisms of TP53 might confer genetically reduced susceptibility to CRC in Chinese schizophrenia population, with the similar research strategy. Our sample was twice larger and included better age-matched subjects compared to the study by Park et al. Thus, the possibility of false-positive results was decreased. However, a series of susceptibility loci may contribute to this kind of reduced risk for CRC. Each of the loci influences but not determines the overall risk. Our sample size is relatively small. Thus additional replication studies using more SNPs in larger samples are needed.

There are confounding factors posing a major challenge for solving this epidemiological puzzle. Except for the firstly proposed candidate gene TP53, there are still some other genes involved, such as APC gene (adenomatous polyposis coli gene), which is significantly associated with schizophrenia and XRCC4 gene[5]. In addition, epigenetic mechanisms and microRNAs are implicated in schizophrenia vulnerability and cancer protection. Further understanding of the links between schizophrenia and cancer would be valuable to investigate related pathways and why certain people are protected from the malignancy. It will also reveal much about the etiology of schizophrenia and cast light on a variety of illnesses correlated with schizophrenia.

6 Conclusions

In summary, our results indicate that the polymorphisms of rs12951053 in TP53 confer reduced susceptibility to colorectal cancer in the Han Chinese schizophrenia population. Replicating studies with more markers and larger sample size in more ethnic groups will be necessary to provide insights into the correlation between schizophrenia and cancer.

(**Acknowledgements**: We appreciate the contribution of the members participating in this study, as well as the psychiatrists who helped us with diagnosis. This work was supported by the Open Project of Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders.)

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细胞聚集，堵塞肝血窦从而破坏肝组织[11]。经检测肝移植后再灌注 6 h 和 24 h 血浆 TNF-α、IL-1 水平；发现 GW3965 组两时间点其炎症因子均明显低于 OLT 组；说明在肝移植中 LXR 被激动后炎症因子表达也会受到抑制，也正是利用了 LXR 的负性炎症效应。NF-κB 在细胞中主要以 P65 异源聚体形式存在，并与其抑制剂 IκB 结合保留在细胞浆中[12]；当受到炎症因子 TNF-α、IL-1、ICAM-1 等的刺激，IκB 被泛素化降解，被释放的 NF-κB 进入细胞核与核酸结合诱导引起基因转录。LXR 被激动后可能会产生下游因子，阻止 IκB 被泛素化降解，因而 NF-κB 不能与 DNA 有效结合并启动相关基因转录。通过检测 NF-κB P65/P50 在肝组织中再灌注后 6 h 的表达，结果表明，通过 GW3965 预处理激动 LXR 后 NF-κB 表达受到明显抑制。本实验经过 GW3965 预处理激动供肝的 LXR，建立 SD 大鼠肝移植模型，检测 SD 大鼠肝移植手术结束后血清转氨酶、肝组织病理学改变、主要炎症因子 NF-κB 和其下游炎症因子 TNF-α、IL-1 的表达水平，发现 GW3965 预处理组肝移植后 IRI 的损伤轻于对比 OLT 组，表明 LXR 激动剂 GW3965 预处理减轻肝移植后 IRI 是有效的，本实验结果经 GW3965 预处理可以降低 SD 大鼠肝移植术后的血清转氨酶；减轻移植肝的病理损伤；降低内毒素信号通路的主要炎症因子 NF-κB 和其下游炎症因子 TNF-α、IL-1 的表达水平。通过以上几方面从而有效保护移植供肝缺血再灌注损伤。

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（收稿日期：2015-03-14）