AGE-RELATED FUNCTION OF TUMOR SUPPRESSOR GENE TP53: CONTRIBUTION TO CANCER RISK AND PROGRESSION

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Aim: To examine the influence of combined genotypes of TP53 (exon 4, intron 3, intron 6) and XRCC1 (codon 10) on lung cancer age of onset. Methods: TP53 polymorphisms in codon 72 of exon 4 (Arg?Pro), in intron 3 (16 bp duplication), in intron 6 (G/A transition) and XRCC1 polymorphism in codon 10 (Arg399Gln) were analyzed in blood cells of 177 lung cancer patients and 196 healthy donors with Restriction Fragment Lenth Polymorphism PCR. Results: We showed that combination of TP53 variant genotypes and XRCC1 variant genotype is associated with the increased lung cancer risk in younger, but not elderly, smokers. In contrast, wild allele combination increases lung cancer risk for individuals over the age of 60. Conclusion: Our data confirm antagonistic pleiotropy hypothesis indicating that p53 protects the organism against cancer early in life, but promoting aging phenotype, including late life cancer in older persons.

Key Words: TP53 gene, lung cancer, aging.

Tumor suppressor TP53 gene is one of the most widely studied human genes. P53 protein plays a critical role in a cell cycle control, apoptosis initiation and DNA repair, controlling genome stability in the response to genotoxic factors. The choice of the p53 response is thought to depend on the strength of the stress signal and cellular damage extent. Mutations in TP53 gene are the most frequent genetic alterations in human cancer, including lung cancer. Functional inactivation of p53 owing to carcinogen-induced mutations in DNA-binding domains results in transcriptionally inactive p53, which is not capable to efficiently protect against cell damage.

Smoking is the main lung cancer risk factor, and several mutations in TP53 have been found to be specific lung cancer associated hotspot, induced by cancerous tobacco-derived metabolites [1]. Besides the common mechanisms that alter p53 function, TP53 gene polymorphisms are considered to be the risk factor for malignant disease, contributing to a person’s propensity to carcinogenic exposure as well as disease progression and/or modulation of therapeutic outcome. There are about 400 known polymorphisms for TP53 gene described in literature [2]. One of them, Arg72Pro (Ex4+119G>C, rs1042522) is the most well-known due to its ability to noticeably affect p53 function. A common polymorphism at codon 72 of TP53 results in either an arginine residue (TP53Arg) or proline residue (TP53Pro). Data on functional studies suggest that Arg and Pro allele variants are not biochemically equivalent because they affect p53 ability to bind DNA and interact with regulatory and target genes for activating repair or apoptosis processes in response to stress stimuli. TP53Pro variant has been shown to transcriptionally activate several p53 dependent genes involved in DNA repair better than the TP53Arg form, resulting in more effective DNA repair. TP53Arg in turn is capable of inducing apoptosis better than TP53Pro expressing variant [3]. Case-control studies suggested that codon 72 polymorphism of TP53 modulates the susceptibility of carriers to various malignancies including lung cancer (LC), but obtained data are controversial [2, 4–7]. Polymorphisms in TP53 gene introns 3 (rs17873862) and 6 (rs1625895) are associated with gene expression level by controlling mRNA stabilization [4, 8, 9]. X-ray repair cross-complementing group 1 (XRCC1) gene is required for the efficient repair of single-strand breaks and damaged bases of DNA. Base excision repair (BER) system is closely interrelated to p53 function [10]. Single nucleotide polymorphism in XRCC1 exon 10 (rs2548) results in the substitution Arg to Gln leading to the decrease of BER efficiency [11]. According to Human Genome Epidemiology Review the XRCC1 399Gln/399Gln genotype was associated with increased risk of tobacco-related cancers among light smokers but decreased risk among heavy smokers [12]. This unexpected result could be explained by enhanced apoptosis of cells with increased DNA damage from heavy tobacco smoking, which is the indirect suggestion to probable «crosstalk» between TP53 and XRCC1 polymorphisms.

Ageing is one of the most important risk factor of LC due to affecting the repair and immune system functions under the long deleterious exposures. P53 is able to induce cell cycle arrest and/or apoptosis in response to many stress stimuli that provides replicative senescence suppressing the development of cancer. Cell senescence is maintained by p53 and is able to be reversed following p53 inactivation [13]. Senescent cells have the phenotype entailed functional changes that can alter tissue structure and function that characterize aging. Thus tumor suppressor gene TP53 has both anticancer and pro-aging effects, depending on the age of the organism [14]. To our knowledge hitherto there are few studies devoted to the evaluation of the influence of TP53 polymorphism on age-related cancer [14–16]. To further define the role of the TP53 polymorphism in the view of «cross-talk» between TP53 and DNA-repair

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Abbreviations used: BER — base excision repair; LC — lung cancer; NSCLC — non small cell lung cancer; TP53 — tumor protein p53; XRCC1 — X-ray repair cross-complementing group 1; w/m — wild/mutated.
involving genes in LC risk we examined the influence of combined genotypes of TP53 (exon 4, intron 3, intron 6) and XRCC1 (codon 10) on LC age of onset.

MATERIALS AND METHODS

Patients. 136 primary non small cell lung cancer (NSCLC) patients collected from 2000 to 2004 at the Tomsk Cancer Research Institute Clinic were included in this study. Studies were performed according to the rules of Cancer Research Institute Ethical Committee. Patients mean age was 60.2 ± 8.6 (age range 42–79). All patients had a treatment including neoadjuvant chemotherapy and surgery. Tumors were staged according to the tumor node metastasis (TNM) classification of malignant tumors T1-4N0-2M0. 196 healthy individuals from the same region adjusted for age and sex were used as controls.

Samples. Peripheral blood was obtained from all LC patients and healthy donors. DNA was isolated from blood cells using chlorophorm/phenol extraction followed by ethanol precipitation according to the standard procedure [17].

Methods. TP53 polymorphisms in codon 72 of exon 4 (Arg72Pro), in intron 3 (16 bp duplication), in exon 6 (G/A transition, Mcp1) and XRCC1 polymorphism in codon 10 (Arg399Gln) were analyzed with Restriction Fragment Lenth Polymorphism PCR. Primer sequences for TP53 codon 72 exon 4 were 5′–CTGTAAGGACGAAGGTGTG–3′ and 5′–ACTGACGTTGCAAGTCAACAG–3′, for exon 3 were 5′–TGGGACTGACTTTCTGCTCTT–3′ and 5′–TCAAATCATCAGTGCTTG–3′, for exon 6 were 5′–TGGCCATCTACAAGCAGTCA–3′ and 5′–AGGGAGCTTTCTGCTTT–3′.

Statistics Deviation from Hardy Weinberg equilibrium of the polymorphisms under study were determined using Chi-square test. Cross tabulation and Chi-square test were performed when studying of association between different polymorphisms. Pearson Chi-square test or Fisher’s exact test was used when appropriate, and statistical significance level <0.05 was considered. Overall survival was computed and compared using Kaplan-Meier test. Computation was performed using STATISTICA 6.0.

RESULTS

Several main aspects, including polymorphism in TP53 gene exons and introns, «cross-talk» between the different genes polymorphisms, age dependence of lung cancer and TP53 antagonistic pleiotropism were taken into consideration in this study. The case and control groups were both genotyped for TP53 and XRCC1 polymorphisms, which were shown to be in Hardy-Weinberg equilibrium (data not shown).

First, we analyzed the distribution of each TP53 polymorphism among controls and LC patients. To assess LC risk-age relation, all LC patients were divided into 2 groups: 40-59 years old (54%, younger) and 60-79 years old (46%, elderly) according to WHO recommendations (1963).

The distribution of the three TP53 genotypes in the whole group of LC patients was similar to those of the controls. There was statistically significant difference in the prevalence of each polymorphism when stratified on smoking and age. We have revealed the higher frequency in w/m genotype at TP53 intron 6 (29% vs. 15% in controls, p<0.001) and Arg/Pro polymorphism at exon 4 (54% vs. 43% in control, p<0.000) in smoking younger LC patients in comparison with control. As to TP53 polymorphism at intron 3, only the trend to increasing frequency of heterozygote was shown in LC younger smokers as compared with control (data not shown).

To further define the role of the TP53 polymorphism in LC risk we examined the influence of combined genotypes of TP53 (intron 3, exon 4, intron 6) on LC age of onset. We analyzed the frequencies of combined either wild (i.e. w/w, Arg/Arg, w/w) or variant (i.e. w/m, Arg/Pro, w/m) TP53 genotype among cases and controls. Modifying effect of XRCC1 polymorphism in codon 10 towards association of TP53 genotype and LC was also examined under the condition of smoking. Among genotyped 196 controls the most frequent combinations of TP53 gene genotypes were w/w-Arg/Arg-w/w (wild genotype combination, 38%), w/w-Arg/Pro-w/w (4 exon heterozygote, 29%) and w/m-Arg/Pro-w/m (heterozygous genotype combination, 4%). Whereas in total LC group of patients these combinations occurred in 51% (p<0.05 vs. control), 21% and 13% (p<0.05 vs. control), respectively. Further we have evaluated the frequency of different genotypes combinations in LC patients stratified by smoking and age. No significant differences in the distribution of homozygous wild (major) genotype combination between younger LC patients and control were found (Table 1).

Table 1. Distribution of p53 exon 4-intron 3-intron 6 genotype combination among younger and elderly non small cell lung cancer patients

<table>
<thead>
<tr>
<th>Combination of p53 genotypes</th>
<th>Younger (40–59)</th>
<th>Elderly (60–79)</th>
</tr>
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<tbody>
<tr>
<td>w/w-Arg/Arg-w/w</td>
<td>38 (74/196)</td>
<td>38 (23/61)</td>
</tr>
<tr>
<td>w/w-Arg/Pro-w/w</td>
<td>29 (57/196)</td>
<td>23 (14/61)</td>
</tr>
<tr>
<td>w/m-Arg/Pro-w/m</td>
<td>4 (8/16)</td>
<td>5 (8/16)</td>
</tr>
</tbody>
</table>

Note: *significant differences compared with the control group; #significant differences compared with the elderly NSCLC patients group

We found the higher frequency in heterozygous w/m-Arg/Pro-w/m TP53 genotype combination in younger smokers (21% vs. 4% in controls, p<0.05) (Table 2). The same association was seen for TP53 genotype combination with Arg/Gln XRCC1 genotype (21% vs. 3% in control, p<0.05). So, heterozygous genotype combination was associated with NSCLC in younger smoking individuals. However, no increased LC risk was found for younger individuals carrying wild Arg/Arg XRCC1 genotype in combination with variant TP53 genotype (36% vs. 37% in control).

Table 2. Distribution of p53 exon 4-intron 3-intron-6 genotype combination among younger and elderly non small cell lung cancer patients stratified by smoking

<table>
<thead>
<tr>
<th>Group of examine patients</th>
<th>Frequency of genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy individuals</td>
<td>w/w-Arg/Arg-w/w</td>
</tr>
<tr>
<td>Younger NSCLC patients</td>
<td>34 (16/47)</td>
</tr>
<tr>
<td>Never smokers</td>
<td>80* (4/5)</td>
</tr>
<tr>
<td>Elderly NSCLC patients</td>
<td>34 (16/47)</td>
</tr>
<tr>
<td>Never smokers</td>
<td>80* (4/5)</td>
</tr>
</tbody>
</table>

Note: *significant differences compared with the control group

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We performed wild TP53 genotypic combination analyses in elderly individuals and found its significant elevation in comparison with control group (62% vs. 38% in controls, \(p < 0.05\)), but no modifying effect of smoking or XRCC1 genotype (4% in LC cases and controls) was found (Table 1, 2). In contrast to younger LC patients, no differences in the frequency of heterozygous TP53 genotype combination as well as its combination with XRCC1 heterozygotes was revealed in elderly LC individuals. Thus our findings point the association of major TP53 genotypes combination with LC in elderly individuals but smoking as well as XRCC1 gene polymorphism do not affect this risk factor significantly.

Next we determined whether the different TP53 genotype combination was associated with overall survival of LC patients who were under follow-up for 52 months after cancer diagnosis. The cumulative 3-year survival was reduced in carriers of wild TP53 genotype vs. those patients with heterozygous TP53 genotype (survival rate was 83% vs. 57%, respectively, \(p < 0.05\)) (Figure).

**Figure.** Kaplan-Meier estimated overall survival of LC patients with different combinations of TP53 genotypes (Arg72Pro ex4, dup16 in3, Mcp1 in6 polymorphism). Continuous blue curve — TP53 gene w/w-Arg/Arg-w/w genotype combination; broken red curve — TP53 gene w/m-Arg/Pro-w/m genotype combination; + — censored observation

**DISCUSSION**

Thus we found that TP53 polymorphisms in exon 4 and intron 3 and 6 are associated with LC in younger smoking individuals, suggesting the functional effect for intronic polymorphism in LC, reported by others [4, 8]. Despite the belief that introns are nonfunctional because they do not code the proteins, evidences exist indicating intron-regulated mRNA expression and coding-region mutations [4].

Although one variant single nucleotide polymorphism is found to have the influence on the disease, probably «cross-talk» between gene polymorphisms in the same pathway may provide a more accurate examination of LC risk [5]. In current study we tested the interactive effect of polymorphisms in TP53 exon 4, intron 3 and 6 and XRCC1 exon 10 on LC risk in individuals of different age. The main findings in this report are that combined analysis of TP53 and XRCC1 variant alleles showed the association of variant genotype combination with NSCLC in younger smoking individuals and, in contrast, that combination of wild genotypes increases LC risk in elderly patients independently of smoking factor.

The increase in heterozygous TP53 genotype combination frequency in younger smokers reflects the prevalence of variant alleles for each studied TP53 polymorphism, indicating their important effect on LC onset. Wu et al. [4] previously reported that patients with Pro/Pro genotype were diagnosed with LC at an earlier age and were heavy smokers in contrast to patients with Arg allele in TP53 genotype. We provided the evidence of interplay between TP53, XRCC1, age and NSCLC. Our data suggesting that variant Pro allele of TP53 (Arg72Pro polymorphism) increases LC risk in younger smokers whereas wild allele Arg promotes LC in elderly person, which is in accordance with tumor suppressors’ antagonistic pleiotropy hypothesis [14].

TP53 is known to be able to induce the senescence response to many DNA-damaging stimuli as well as oncogene expression. Activation of p53 in response to DNA damage usually leads to either proper repair of the injuries or elimination of the damaged cells from the proliferative cell pool [18]. There are several potential cellular outcomes, which are affected by cell type and DNA lesion severity. Transient arrest and proper repair occur when DNA lesions are not heavy and the repair is possible, and this outcome is the most favorable for cell and organism. Taking into account that TP53 Pro allele is more efficient than Arg allele in inducing G1 arrest, specifically activating p53-dependent target genes responsible for DNA repair resulting in the effective maintenance of genomic stability [3], we can suppose its benefit to reach favorable outcome in response to stress stimuli. Apoptosis is realized when the damage is very severe, due to p53-dependent transcriptional up-regulation of apoptotic effectors, such as PUMA, NOXA, BAX, and down-regulation of apoptotic repressors, such as BCL-2 and survivin. Preferential activation of PUMA and NOXA by TP53 Arg variant may contribute to more effective apoptosis induction [19]. Apoptosis prevents cancer by eliminating cells that are damaged or otherwise potentially oncogenic. Because apoptosis irreversibly removes cells from tissue, Arg allele can promote tissue depletion and organ degeneration. Senescent cells have been shown to accumulate with age in vivo and this is accompanied by several dramatic changes, best characterized for fibroblasts, which are as cells maintaining the stroma and regulating epithelial tissue function [18]. Senescent human fibroblasts acquire the secretory phenotype characterized by increased secretion of extracellular matrix remodeling enzymes, inflammatory cytokines and epithelial growth factors [14]. These functional changes in senescent cells may progressively promote aging related decline in structure and function, providing tumor stimulated microenvironment in aging organisms. Senescent stromal cells can stimulate the growth and promote the acquisition of invasive and migratory phenotypes by normal or premalignant epithelial cells possibly by epithelial-mesenchimal transition [20].

The age dependence of LC development could be explained by the accumulation of mutations, but evidences exist that cells with oncogenic mutations also require a permissive tissue microenvironment in which to progress into a malignant tumor. The age dependent accumulation of senescent stromal cells may synergize with the age-dependent accumulation of mutations, resulting in the rise of epithelial cancer.

In our case smoking carriers of TP53 gene Pro allele (Arg72Pro polymorphism), which codes p53 protein that
weakly induce apoptosis, are thought to have excessive mutation accumulation promoting neoplastic transformation in younger persons. Variant alleles of TP53 gene with intronic polymorphisms as well as polymorphism of XRCC1 gene in codon 10 contribute to tumor development because tumor cells are not efficient enough in TP53 gene expression and DNA repair. As for older individuals, Arg allele protecting from cancer in early period of life can provide accelerated ageing resulting in the establishment of senescent microenvironment that is able to promote tumor development and progression under the condition of age-related mutation accumulation. Wild genotypes of TP53 gene with intron 3 and intron 6 polymorphisms are expected to provide the high expression of Arg allele enhancing its deleterious effect.

The data presented in this study suggest that Arg/Pro genotype is beneficial for LC cancer patient survival in contrast to Arg/Arg genotype. Ørsted et al. also demonstrated that overall 12-year survival was higher for Pro/Pro carriers compared to Arg/Arg carriers in large scale study of Danish population [16]. It should be noted that disease outcome is strongly dependent on the therapeutic modality and tumor sensitivity to chemo- or radiotherapy. P53-dependent apoptosis is an important mechanism through which DNA-damaging anticancer agents exert their biological effect [19]. We have earlier reported that Arg/Pro TP53 gene genotype (Arg72Pro polymorphism) was associated with better tumor response to neo-adjuvant chemotherapy in LC patients [21]. There are few other studies on association of TP53 codon72 polymorphism with clinical tumor response to anticancer drugs, which are rather controversial [19, 22, 23].

CONCLUSION
Our findings provide evidence of interplay between TP53, XRCC1, age and cancer. We have shown the evidences for the interaction of exon and intron TP53 gene polymorphisms and LC risk. Obtained data indicate that combination of TP53 and XRCC1 variant genotypes is associated with the increased LC risk in smoking younger individuals. In contrast, major allele combination increases LC risk for individuals over the age of 60. Our data are in accordance with the antagonistic pleiotropy hypothesis indicating that p53 can protect organism from cancer early in life but promoting aging phenotypes, including late life cancer, in older organisms [10].

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