Clinical and Prognostic Significance of R282W p53 Gene Mutation in North India Patients with Non Small Cell Lung Cancer

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Abstract

Background: p53 plays a central role in protecting the integrity of the genome. Its activity is ubiquitously lost in cancers, either by inactivation of its protein (p53 pathway) or by mutation in the p53 gene, thereby indicating its importance in understanding cancer and as a therapeutic target. Given the high frequency of the hotspot R282W p53 gene mutation in our NSCLC patients, we have evaluated the association of R282W mutation with the progression of the malignancy.

Methods: Blood DNA was extracted from cases. The R282W hotspot p53 gene mutation was detected by using ASO-PCR. Most of the NSCLC patient’s submitted samples for EGFR gene mutation analysis.

Results: The clinical significance of R282W hotspot p53 gene mutation in exon 8, codon 268 (C>T) was studied in hundred clinically confirmed NSCLC patients samples. Sixty two of hundred (62%) cases were reported positive for R282W p53 mutation. The clinically significant difference was reported between early and the advanced stages (72% vs. 43%) (p<0.007). Similarly higher frequency of this mutation was reported in adenocarcinoma (76.08%) than squamous cell carcinoma 27 (50%) (p<0.0134). Significantly higher frequency of R282W p53 mutation was reported in distant metastasis 23 (85.18%) than the metastasis (4.0075), current smokers than the ex smokers (p=0.02).

These findings suggest that stage, smoking, histological type, metastasis is strongly associated with the incidence of R282W mutation. Other variables as gender, age, smoking level, and family history of any cancer does not showing any significant association with p53, R282W mutation. Slightly lower overall survival was reported in NSCLC patients with R282W mutation than wild p53 cases (p<0.049).

Conclusion: Our results suggest that the hotspot R282W p53 mutation may influence the susceptibility, progression of NSCLC patients in Indian population. Large population-based prospective studies are required to validate our findings.

Keywords: NSCLC: Non Small Cell Lung Cancer; ADC: Adeno-Carcinoma; SCC: Squamous Cell Carcinoma; ASO: Allele Specific Oligonucleotide

Introduction

Non Small Cell Lung Cancer (NSCLC) is the major cancer killer worldwide in both sexes, accounting for >1.2 million deaths each year [1]. As of 2002, the one year prevalence of lung cancer in India for males was 11,511, and the 5 year prevalence was 27,477 accounting for approximately 3% of global prevalence, and 55% of total prevalence in South Central Asia [2]. The p53 gene, located on the short arm of human chromosome 17, encodes for a nuclear phosphoprotein involved in the regulation of cell proliferation [3]. The mutant gene product, which tends to accumulate to high levels in cancer cells, is believed to exert a dominant negative effect over coexpressed normal p53. Alterations of either the gene or protein product have turned out to be one of the most common changes identified in human malignancies. In resected lung cancers, point mutations of the p53 gene have been found in all histologic types, including approximately 45% of resected NSCLC [4,5]. In most studies, it has found that the risk factors of getting lung cancer are related to high percentage of passive smoking [6], cooking oil vapours [7,8] and occupational exposures [9].

The genetic mistake of p53 in NSCLC, as a result of either p53 protein over-expression [10] or p53 gene mutation [11,12], is found to be strongly correlated with tumor grade and can predict a poor prognosis [13,14]. The p53 gene is a large gene composed of 11 exons and 10 introns that are made up of 420,000 bp [15,16]. However, 90% of the known mutations exist in exons 4–9, 70% of research studies focusing on exons 5–8. This focus is because p53 exons 5–8 code for the DNA-binding domain of the p53 protein, an area where the structure can be highly affected by sequence changes [17]. In solid tumours, p53 gene mutations are generally considered to be a late event in carcinogenesis because they pre- dominate in advanced stages of the disease and have been correlated with short survival in carcinoma of the breast, prostate, lung, and stomach [18,19].

The main reason why the p53 gene is so frequently mutated in cancers (overall in over 50% of invasive cancers) is that the p53 protein plays multiple, coordinated anti-proliferative roles in response to many different types of stress stimuli [4,20]. The p53 tumor-suppressor gene is commonly mutated in human cancer [21], and 30%–80% of human
carcinomas contain sectors with a mutation in this gene, depending on
the type and stage of the tumor investigated [22,23]. Mutation in p53
has been found to be a prognostic factor in several cancers, but whether
it is an independent prognostic factor, is not yet known [24-26]. The
p53 plays a central role in protecting the integrity of the genome.
Its activity is ubiquitously lost in cancers, either by inactivation
of its protein (p53 pathway) or by mutation in the p53 gene, thereby
indicating its importance in understanding cancer and as a therapeutic
target. Given the high frequency of the p53 gene mutation in NSCLC
patients, we investigated whether the R282W p53 gene mutation
influences progression of Non small cell lung cancer in north Indian
populations.

Materials and Methods

Study population

Non Small Cell lung cancer patients were assessed on the basis of
clinical and pathological examinations. This study is a Hospital-based
study conducted on North Indian population. All incidents of NSCLC
cases are newly diagnosed during the study period, Ethical committee
approved the study. The procedures followed according to the ethical
standards of responsible committee of the Institutes/Hospitals, to
participate in a face-to-face interview using a structured questionnaire.

Selection criteria

Senior pathologists confirmed all diagnoses. We interviewed and
collected the demographic factors data from the patient. We
collected the information on age, smoking, chewing, usual alcohol
intake, and previous cancer diagnoses. Participant’s family history of
cancer and the clinical information for these cases are obtained from
medical records, tumor size, stage, and chemotherapy drugs. Patients
were recruited based on inclusion and exclusion criteria, which were
determined before the beginning of the study.

Sample collection

A total of 100 Non Small Cell lung cancer patients are enrolled in
the study. Sampling was done from two major hospital, MAMC and
Associated hospitals New Delhi, All India Institute of Medical Sciences,
New Delhi and Department of Radiation Oncology, SKIMS, Srinagar,
India between the periods June 2009 to September 2012. Most of the
NSCLC patients were referred to our molecular oncology lab for
EGFR gene mutation screening. From each patient 3 to 5 ml peripheral
blood sample was collected in EDTA collection vials in the Molecular
oncology lab.

DNA extraction from blood

Genomic DNA was extracted from blood samples using DNA
sure blood mini kit (Nucleo-pore Genetix brand) according to the
manufacturer’s protocol.

Mutation Analysis by ASO-PCR

The mutation was detected by allele specific oligonucleotide PCR
in which allele specific primers were used, one was specific for C allele
and other primer was for T allele. The R282W p53 mutation in exon
8(C>T) was evaluated with reaction mixture of 25 μl containing 2.5
μl of 400 μg/μl DNA, 0.25 μl of 25 pmol of each primer (Table 1) and
previously used by Stoehr et al. [27] 2.5 μl of 10X PCR buffer, 2.5 μl of
10 mM dNTP mixture, 0.3 μl of 3U/L Taq polymerase and 15.75 μl of
nuclease free water.

The thermal cycler conditions used consists of 35 cycles of
denaturation for 40 sec at 94°C, annealing for 40 sec at 58°C, and
extension for 40 sec at 70°C.

The amplification was performed under the following conditions:
95°C for 5 minutes for initial denaturation followed by 40 cycles of
denaturation at 95°C for 30 seconds, annealing at 58°C for 40 seconds,
extension at 72°C for 1 minute 30 seconds and completed with a
final elongation step at 72°C for 5 minutes. The PCR products were
visualized with ethidium bromide on a 2% agarose gel under a UV
transilluminator. The amplimers obtained had a band-size of 207 bp
(Figure 1).

Statistical Analysis

The variables measured in the study were investigated for association
by using the Hardy Weinberg equilibrium equation. The differences in
the incidence of p53 mutations among dependent variable like tumor
type, stage, histological type, cytological type, sex, smoking history,
smoking level, metastasis and family history with of any cancer were
calculated by the Chi square test. The Kaplan-Meier method was used to
calculate the overall survival in all 100 patients with p53 R282W
mutations or without p53 R282W mutations. Statistical difference was
considered significant for P values <0.05. SPSS version17 was used for
analysis.

Result

Demographic characteristics of study population

The study population consists of clinically confirmed NSCLC
patients. All demographic features of the subjects are depicted in the
table 2. A total of hundred Non Small Cell lung cancer patients were
analyzed among which 47 were adenocarcinoma and 53 squamous
cell carcinoma and 35 patients were in early Stage (I & II) and 65 in
advanced Stage (III & IV). Seventy smoked cigarettes, bidi and hukka
(pipe) and 30 cases were of non-smokers were defined as subjects who
had not smoked ever while in smokers there were 28 cases of current
smokers and 42 were ex-smokers. In hundred cohorts of NSCLC
patients, 27 were metastatised and 63 were none metastatised as

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depicted in table 2. Out of hundred NSCLC patients, 62(62%) were positive for p53 R282W mutation and 38 were negative. The difference was statistically significant (p<0.0011). The p53 mutation status in relation to clinicopathological features is shown in the table 3.

Frequency of R282W p53 mutation with respect to histological type, gender and age

The significant association was seen between R282W mutation and histological types of lung cancer patients. The higher frequency was seen in ADC (76%) than SCC (50%). The difference was statistically significant (p<0.0134). There was not much difference in R282W mutation in NSCLC patients with respect to gender however the higher frequency of this mutation was reported in lower age group ≤ 45(84.61%) than higher age group >45(58%).

Frequency of R282W mutation with respect to stage

Among the different stages, the higher frequency of R282W mutation was reported in NSCLC patients in advanced stage (72%) than the early stages (42.85%). The difference was statistically significant (p<0.0007). Also the progression was reported to be faster among the NSCLC patients in advanced stage with R282W mutation.

Frequency of R282W p53 mutation with respect to smoking type

It has been indicated that there is a strong coincidence of G to T transversion hotspots in lung cancers and sites of preferential formation of PAH adducts along the p53 gene. A significant association was seen between the frequency of R282W p53 mutation in current smokers (82.14%) and exsmokers (52.38%), (p<0.02). There was a strong coincidence of C to T transversion hotspots in our lung cancers patients.

Frequency of p53 R282W mutation with respect to metastasis

A significant association was seen between R282W p53 gene mutation and metastasis. The significantly higher frequency of R282W p53 mutation was reported in NSCLC patients with metastasis (85%) (p<0.0075).

Frequency of p53 R282W mutation with respect other clinicopathological feature

There was no significant association between level of smoking (mild, moderate and heavy), cytological type, family history and p53 R282W gene mutation.

Survival Analysis

Overall survival of patients with stage I, II, or IIIA NSCLC was statistically significantly lower in those with p53 mutant tumours than in those with p53 wild-type tumors (p<0.049). The survival analysis between the NSCLC cases with and without R282W p53 mutation show some good correlation (Figure 2).

Recent studies have indicated that there is a strong coincidence of G to T transversion hotspots in lung cancers and sites of preferential formation of PAH adducts along the p53 gene. The identification of R282W p53 mutation acting as modifiers of p53 protein may assist in the assessment of individual cancer progression and risk in NSCLC families. It may also play an important role in the delineation of cancer screening and intervention guidelines in these patients.

It may also help in appropriate management protocols, taking into account the risk of developing cancer either earlier or later in life.

Discussion

To the best of our knowledge, the present study is the first report of prevalence of p53 R282W (exon-8, C>T) mutation in NSCLC patients from India. NSCLC is characterized by multiple genetic alterations in proto-oncogenes and in the tumour suppressor genes. p53 is the most common molecular events in the NSCLC, suggesting a key role in lung tumour carcinogenesis. Mutations in codons 175,
245, 248, 273, and 282 are the most common in sporadic tumours [28]. In present study the frequency of p53 R282W mutations 62%, patient with adenocarcinoma have high frequency of p53 R282W mutation (76.08%) than the squamous cell carcinoma (50%).

Present study revealed a significant association of p53 R282W (exon-8, C>T) with increased risk of NSCLC if patients in advanced Stage (III & IV) found more risk for the p53 R282W gene mutation 72.30% than the early Stage (I & II) 42.85%. In addition current smokers have high frequency of p53 R282W (82.14%) mutation than ex smokers (52.38%) and non smokers (56.66%). Patients with distant metastasis also have high frequency for p53 R282W mutation in comparison to case without any distant metastasis, where the metastasis have 85.18% positive cases for mutation while no metastasis cases is with 53.42%. Other group like gender, age, smoking level, cytological type and patients with family history with any type of cancer does not show any significant association. Frequency of p53 R282W mutation in poorly differentiated cytological type in ADC (80%) is high and moderately differentiated cytological type is high in SCC (66.66%). As we analysed the level of smoking those who smoked more than 10 pack year have high frequency of p53 R282W mutation.

Rozenblum et al. [29], who analyzed p53 in exons 2–11 in first passage xenografts from 47 resected pancreatic cancer, recently reported p53 mutation in 76% of cases. Investigators analyzed p53 chiefly in exons 5–8, which is highly conserved through evolution and presumably of functional importance, 95% of the reported mutations have been found in exons 5–8 [30]. However, of 560 mutations in entire coding region of p53 was sequenced, 87% were in exons 5–8, and most of the others were in exons 4(8%) and 10(4%) [4].

Information on p53 database has indicated that 80% are GC to AT transitions occurring predominantly at CpG islands. Mutations in five hotspots codons (175, 245, 248, 273 and 282) accounted for approximately 43% of all p53 mutations in colorectal cancer [31]. All mutations were present in exons 5–8, which encode the DNA binding domain. Based on the updated p53 gene mutation database containing 5961 mutations, codons 175, 245, 248, 249, 273 and 282 were identified as hotspots mutation in human cancers [32].

**Conclusion**

The incidence of hotspot R282W p53 mutation in our NSCLC patients showed a tendency toward association with progressive disease status. Although the associations appeared to be statistically significant in our population, these initial findings should be independently

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**Table 3:** Clinicopathological feature of NSCLC patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>+ve case</th>
<th>- ve case</th>
<th>Chi-Square</th>
<th>df</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>50(62.5%)</td>
<td>30(37.5%)</td>
<td>0.0027</td>
<td>1</td>
<td>0.95</td>
</tr>
<tr>
<td>Females</td>
<td>12(60%)</td>
<td>8(40%)</td>
<td></td>
<td></td>
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<tr>
<td>Age group</td>
<td></td>
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</tr>
<tr>
<td>≤45</td>
<td>11(84.61%)</td>
<td>2(15.38%)</td>
<td>2.2343</td>
<td>1</td>
<td>0.134</td>
</tr>
<tr>
<td>&gt;45</td>
<td>51(58.62%)</td>
<td>36(41.37%)</td>
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<td></td>
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<tr>
<td>Stage of the disease</td>
<td></td>
<td></td>
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<tr>
<td>Early Stage (I &amp; II )</td>
<td>15(42.85%)</td>
<td>20(57.14%)</td>
<td>7.1718</td>
<td>1</td>
<td>0.007</td>
</tr>
<tr>
<td>Advanced Stage (III &amp; IV )</td>
<td>47(72.30%)</td>
<td>18(27.69%)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
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</tr>
<tr>
<td>Non Smoker</td>
<td>17(56.66%)</td>
<td>13(43.33%)</td>
<td>0.24</td>
<td>1</td>
<td>0.6242</td>
</tr>
<tr>
<td>Smokers</td>
<td>45(64.28%)</td>
<td>25(35.72)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Current Smoker</td>
<td>23(82.14%)</td>
<td>5(17.85%)</td>
<td>5.25</td>
<td>1</td>
<td>0.02</td>
</tr>
<tr>
<td>Ex Smoker</td>
<td>22(52.38%)</td>
<td>20(47.61%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Smoking level ( pack year )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild (≤10)</td>
<td>6(85.71%)</td>
<td>1(14.28%)</td>
<td>1.61</td>
<td>2</td>
<td>0.4471</td>
</tr>
<tr>
<td>Moderate (≤ 40)</td>
<td>20(60.60%)</td>
<td>13(39.39%)</td>
<td></td>
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<tr>
<td>Heavy (&gt; 40)</td>
<td>19(63.33%)</td>
<td>11(36.66%)</td>
<td></td>
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<tr>
<td>Histological type</td>
<td></td>
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</tr>
<tr>
<td>ADC</td>
<td>35(76.08%)</td>
<td>11(23.91%)</td>
<td>6.1105</td>
<td>1</td>
<td>0.0134</td>
</tr>
<tr>
<td>SCC</td>
<td>27(50%)</td>
<td>27(50%)</td>
<td></td>
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<tr>
<td>Cytological type (ADC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Well Differentiated</td>
<td>6(77.77%)</td>
<td>3(22.22%)</td>
<td>0.66</td>
<td>2</td>
<td>0.7189</td>
</tr>
<tr>
<td>Moderately Differentated</td>
<td>9(75%)</td>
<td>3(25%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poorly Differentiated</td>
<td>20(80%)</td>
<td>5(20%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytological type (SCC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well Differentiated</td>
<td>14(46.66%)</td>
<td>16(53.33%)</td>
<td>2.8</td>
<td>2</td>
<td>0.2466</td>
</tr>
<tr>
<td>Moderately Differentated</td>
<td>10(66.66%)</td>
<td>5(33.33%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poorly Differentiated</td>
<td>3(33.33%)</td>
<td>6(66.66%)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Metastasis</td>
<td></td>
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</tr>
<tr>
<td>Positive</td>
<td>23(85.18%)</td>
<td>4(14.81%)</td>
<td>7.1447</td>
<td>1</td>
<td>0.0075</td>
</tr>
<tr>
<td>Negative</td>
<td>39(53.42%)</td>
<td>34(46.57%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history of any cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significant (Positive)</td>
<td>11(61.11%)</td>
<td>7(38.88%)</td>
<td>0.0332</td>
<td>1</td>
<td>0.8553</td>
</tr>
<tr>
<td>Non Significant (Negative)</td>
<td>51(62.19%)</td>
<td>31(37.80%)</td>
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</table>

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verified by other large independent population-base studies to validate our findings.

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