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Association of TP53 Polymorphism in Lung Cancer Patients and Healthy People in Bangladesh

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Abstract: Lung cancer is one of the most common and lethal cancer. It's most important etiologic factor is tobacco smoking. The p53 tumor suppressor protein is important in cell-cycle control, apoptosis, and DNA repair. Polymorphisms in TP53 have been associates with inherited cancer susceptibility. The association of p5 codon 72 polymorphisms with lung cancer has been investigated by several scientific groups with controversial results. However, the genotype distribution of p53 codon 72 polymorphisms as well as the association of this polymorphism with lung cancer risk remains undefined in the Bangladesh population. It has been reported that the genotype distribution of p53 codon 72 polymorphism of lung cancer patients and non-cancer controls and association of demographic characteristics with lung cancer. In a case control study, we frequently matched (by age, sex, and smoking) lung cancer patients and control subjects. TP53 Polymorphisms were determined by restriction fragment length Polymorphisms (RFLP) analysis of PCR amplified exon 4 of p53 gene. Nearly one-third of total lung cancer incidence in Bangladesh was squamous cell carcinoma subtype and increased frequency of Arg/Arg and Pro/Pro genotypes were associated with non-small cell lung cancer (NSCLC). The absence of Pro/Pro genotypes in non-smoker and association with low pack years smoking status suggested that Pro/Pro genotypes was more frequently affected by tobacco carcinogens, p53 polymorphism may be associated with increased lung cancer risk and may affect the function of p53 gene. The aim of this present review is to Investigation of the effect of smoking and TP53 polymorphism in developing lung cancer and Study the association of TP53 polymorphism in lung cancer patient and healthy people in Bangladesh.

Keywords: p53 Tumor suppressor protein, Tobacco smoking, Restriction fragment length Polymorphisms, Lethal cancer, Lung cancer, Carcinoma

1. Introduction

Lung cancer involves malignant proliferation of the epithelial lining of the lower respiratory tract. It is one of the most common forms of malignancy leading to the major cause of cancer related deaths across the world (Parkin*et al.*,2001) and also in Bangladesh (CRR, 2009) Lung cancer has been the most common cancer in the world since 1985 and the leading cause of cancer death. Worldwide it is by far the most common cancer of men and increasingly being recognized in Bangladesh.An estimated 1.61 million people across the world were diagnosed with lung cancer in 2008, accounting for 13% of the total. More than half (55%) of the cases occurred in the developing world (Ferlay et al., 2008)**.** Lung cancer incidence is more than double in men than in women worldwide (rate ratio 2.5: 1.0) (Ferlay*et al.*, 2008)**.** Lung cancer was one of the common cancers among the patients who attended National Institute of Cancer Research and Hospital (NICRH), Dhaka, Bangladesh. A total of 3,209 lung cancer patients attended during 2005- 2007, of them 86% (2,763) were males. The number of lung cancer patients was increasing year by year; there were 902 lung cancer patients in 2005, 1,076 in 2006 and 1,231 in 2007(CRR. 2009). According to the latest who data

published in April 2011 lung cancers death reached 18,124 or 1.89% of total deaths. The age adjusted death rate is over 20.29per 100,000of population rank a #59 in the world.

Lung cancer is mainly two types. One type is small cell lung carcinoma, where the cancer cells contain dense neurosecretory granules (vesicles containing neuroendocrine hormones), which give this tumor an endocrine/paraneoplastic syndrome association. Most cases arise in the larger airways (primary and secondary bronchi) (Behzadi et al., 2009). This cancer grows quickly and spread early in the course of the disease. At presentation around 60–70% of this type of cancer has metastatic disease. This type of lung cancer is strongly associated with smoking (Hogan, 1999). The other type is non-small cell lung carcinoma (NSCLC), which is more common, accounting for approximately 80 percent of all lung cancer cases (Dayem, 2002). Further, non-small cell lung carcinoma (NSCLC) is divided into three subtypes, Adenocarcinoma, Squamous cell carcinoma and large cell carcinoma. Adenocarcinoma is more common in women and non-smokers; this type of NSCLC arises from the terminal bronchioles or alveolar walls (Dayem, 2002). Most cases of adenocarcinoma are associated with passive smoking or people who have smoked fewer than 100 cigarettes in their lifetimes ("never-smokers") (Hogan, 1999). It grows more slowly than the Squamous cell carcinoma. It spreads to other parts of the body at an early stage, and accounts for 40 percent of all cases (Dayem, 2002). Squamous cell carcinoma is associated with smoking and found mostly in men and older people of both sexes. It accounts for 30 to 35 percent of all NSCLC (Dayem, 2002). They typically occur close to large airways. A hollow cavity and associated necrosis are commonly found at the center of the tumor (Rahaman et al., 2011). Large cell carcinoma accounts for 5 to 15 percent of all case (Dayem, 2002). These are so named because the cancer cells are large, with a lot of cytoplasm, large nuclei and conspicuous nucleoli (Rahaman et al., 2011).

1.2 *TP53* Gene

The human *TP53* tumor suppressor gene, located on chromosome 17p13, is involved in several central cellular processes, including gene transcription, DNA repair, cell cycling, apoptosis and genomic stability. The *TP53* is one of the most frequently mutated genes in human cancers. It is reported that approximately half of all cancers have inactivated *TP53*(Robles and Harris ,2010).To date, several polymorphisms in the wild type *TP53* gene locus have been described. Among them the codon72 polymorphism on the exon 4 of the *TP53* gene, that have either arginine (CGC) or proline(CCC), has been reported to be associated with bladder and lung cancer (Carbone, 1991). The association of p53codon72 polymorphism has been studied in lung carcinoma by several authors. However, the results are contradictory. While some authors reported the frequency of Pro/Pro genotype to be higher in lung cancer (Fan *et al.*,2000; Kawajiri *et al.*, 1993; Wang *et al.*, 1999 ;Weston *et al.*, 1992b). Papadakis*et al.*, (2002)observed an increased frequency of Arg/Arg genotype in advanced lung cancer cases(Pandima*et al.*, 2010) indicated that p53Arg genotypes are more susceptible to lung cancer in India.(Pandima*et al.*, 2010) also showed that the frequency of Arg/Arg is higher in smokers. The association p53 codon72 polymorphism with lung cancer has not ever been studied in Bangladesh. Therefore, we are intending to study the association. Cancer caused about 13% of all human death worldwide (7.9 million). Rates are rising as more people live to an old age and as mass lifestyle changes occur in the developing world. (Jemal, A et al., 2011)

1.3 Lung Cancer in Bangladesh

Lung cancer is the most commonly diagnosed cancer (17% ofthe total new cancer cases) and leading cause of death in males (23 % of the total cancer deaths) whereas it is the fourth mostcommonly diagnosed cancer (8.5 %) and the second leading cause of cancer death (12.8 %) in female (Jemal*et al.*, 2011 and Ferlay*et al.*, 2008). In Bangladesh it is also the most prevalent cancer in male by considering incidence and mortality (24.9 % of incidence and 28.6 % ofmortality) where as it is the fourth most prevalent cancer by considering incidence and second leading cause of cancer related death in females (5.6 %) (Ferlay*et al.*, 2008) According to WHO (2007) total lung cancer cases in Bangladesh were 196,000 among those age 30 years and above (Haque, 2011and WHO, 2007). New lung cancer cases in Bangladesh were 20,904 and total mortality were 19, 393 in 2010 (Ferlay*et al.*, 2008) The annual number of new cases and mortality are estimated to rise to 43,048 and 40,252, respectively, by 2030 (Ferlay*et al.*, 2008) In Bangladesh, Lung cancer incidence was the highest among commonly occurred cancers (16.7% of the total cancer cases) (Cancer Registry Report of the NICRH, 2005-2007) (CRR,2009). In the similar fashion, lung cancer was the most common incident form of cancer in 2006 (16.4% of the total cases) and 2007(17.3% of the total cases) in Bangladesh (CRR, 2009).

1.4 Causes of Lung Cancer

Lung cancer is most often a "multifactorial" disease. There are many lung cancer causes, in addition to the well-known link with smoking. An overview of common lung cancer causes includes:

1.5 Population Characteristics

Lung cancer risk increases with age in both smokers and never smokers. The cancer is sufficiently rare in people under the age of 40, especially among never smokers (Samet*et al.*, 2009). Women who have never smoked are more

likely to develop lung cancer than men who have never smoked (Patel *et al.,* 2004)**.**

1.6 Lifestyle

Smoking, particularly of cigarettes, is by far the main contributor to lung cancer. The incidence of lung cancer is strongly correlated with cigarette smoking, with about 90% of lung cancers arising as a result of tobacco use (Biesalski *et al.,* 1998). Tobacco smoke contains over 60 known carcinogenic compounds, Tobacco smoke contains over 4,000 chemical compounds, many of which have been shown to be cancer-causing or carcinogenic. The carcinogens in tobacco smoke are chemicals known as radioisotopes from the radon decay sequence, nitrosamine, and benzopyrene(Sopori, 2002). Additionally, nicotine appears to depress the immune response to malignant growths in exposed tissue (Sopori, 2002). Across the developed world, 91% of lung cancer deaths in men during the year 2000 were attributed to smoking (71% for women) (Ezzatiand Lopez 2004).In the United States, smoking accounts for 80–90% of lung cancer cases (Hogan, 1999). The risk of lung cancer increases with the number of cigarettes smoked and the time over which smoking has occurred; doctors refer to this risk in terms of pack-years of smoking history (the number of packs of cigarettes smoked per day multiplied by the number of years smoked) (Hecht, 2003 and Sopori, 2002).

Passive smoking or the inhalation of tobacco smoke by nonsmokers, who share living or working quarters with smokers, also is an established risk factor for the development of lung cancer. Research has shown that nonsmokers who reside with a smoker have a 24% increase in risk for developing lung cancer when compared with nonsmokers who do not reside with a smoker (Taylor *et al.,* 2007). An estimated 3,000 lung cancer deaths that occur each year in the U.S. are attributable to passive smoking (Taylor *et al.,* 2007).

A higher intake of foods, such as salads, is associated with a lower risk of developing lung cancer. An excess intake of alcohol may raise the risk of lung cancer in smokers (Samet *et al.,* 2009).

1.7 Environmental Causes

Many environmental exposures can contribute to lung cancer risk in addition to smoking, and like smoking, many of these are avoidable if we are aware of them. You can reduce your risk by doing things as simple as testing your home for radon and using an appropriate mask when working with certain chemicals. Some of the most common environmental causes of lung cancer include:

1.8 Radon

Exposure to radon in the home is the second-leading cause of lung cancer and the leading cause in nonsmokers. Radon is a radioactive colorless and odorless gas that is produced by the natural decay of uranium, found in the Earth's crust. The radiation decay products ionize genetic material, causing mutations that sometimes turn cancerous (Alberg , 2007). It is estimated 12% of lung-cancer deaths attributable to radon gas, or about 20,000 lung-cancer-related deaths annually in the U.S., making radon the second leading cause of lung cancer in the U.S. The U.S. Environmental Protection Agency estimates that one out of every 15 homes in the U.S. contains dangerous levels of radon gas (Schmid *et al.,* 2010).

1.9 Asbestos

Asbestos fibers are silicate fibers that can persist for a lifetime in lung tissue following exposure to asbestos. Asbestos can cause a variety of lung diseases, including lung cancer. There is a synergistic effect between tobacco smoking and asbestos in the formation of lung cancer (Hubaux *et al.,* 2012). Exposure to asbestos is responsible for the majority of mesothelioma, a rare tumor involving the lining of the lung (which is different from lung cancer) (Davies*et al,*. 2010).

1.10 Air Pollution

Outdoor air pollution, especially in urban areas, appears to raise the risk of lung cancer. Outdoor air pollution is estimated to account for 1–2% of lung cancers (Alberg*et al.*,2010)**.**Atomic bomb survivors have an elevated risk of developing lung cancer, as do those who have undergone radiation therapy for other types of cancer, such as Hodgkin's disease. Fine particulates ($PM_{2.5}$) and sulfate aerosols, which may be released in traffic exhaust fumes, are associated with slightly increased risk (Chen *et al.*, 2008).

1.11 Occupational Causes

A number of occupations or occupational exposures are established or suspected risk factors for lung cancer. The International Agency for Research on Cancer has identified 12 occupational exposure factors as being carcinogenic to the human lung (aluminum production, arsenic, asbestos, bis-chloromethyl ether, beryllium, cadmium, hexavalent chromium, coke and coal gasification fumes, crystalline silica,nickel, radon, and soot). Diesel exhaust has been classifiedas probably carcinogenic to humans (Steenland*et al.*, 1996). Welding of stainless steel is possibly associated with anincreased risk of lung cancer, but the evidence for a carcinogenic effect of welding for other materials is weak (Sjogren et al.,1994 andMoulin*et al.*, 1997). However, the potential confounding effect from asbestos exposure may not have been fully controlled in many studies (Moulin*et al.*, 1997). The carcinogenic effect of certain polycyclic aromatic hydrocarbons (PAHs), especially benzo(*a*)pyrene, is well documented (International Agency for Research on Cancer ,1983). The lung cancer excess in occupations involving high exposure to combustion products is

often attributed to exposure to PAHs, possibly in combination with exposure to particles (Gustavsson*et al.,* 1998). While dermal exposure to lowgrade mineral oils is carcinogenic to humans .there is less evidence for a cancer hazard from inhalation of oil mist (Tolbert *et al.,* 1997).Occupational exposure to carcinogens is estimated to be responsible for 13 to 29% of lung cancers in men (Samet *et al.,* 2009).

1.12 Genetics

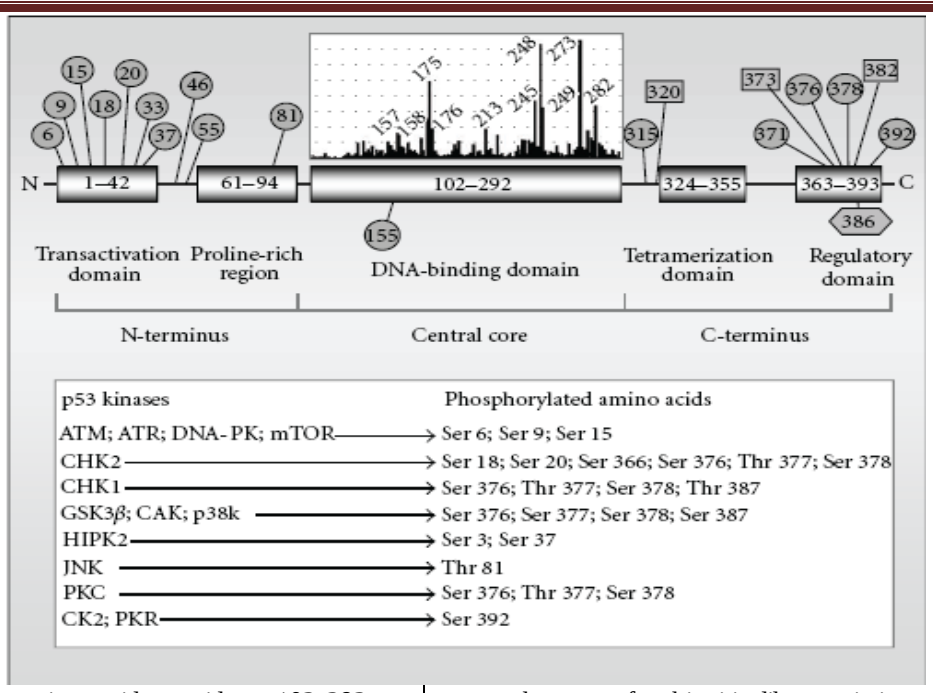
In general, a genetic predisposition to lung cancer does not mean someone will develop lung cancer. It means they are more likely to develop lung cancer, especially when combined with other risk factors. Having a first-degree family member (parent, sibling or child) with lung cancer roughly doubles the risk of developing lung cancer. This risk is more for women and less for men and stronger in nonsmokers than smokers. Having a second-degree relative (an aunt, uncle, niece or nephew) with lung cancer raises the risk by around 30% (Kern *et al.,* 2008)**.** In relatives of people with lung cancer, the risk is increased 2.4 times. This may be dueto genetic polymorphisms(Kern *et al.,* 2008)**.** Studies vary in the types of lung cancers that have the greatest hereditary component, but those with non- small cell lung cancers, especially lung adenocarcinoma are more likely to have a family history of lung cancer than those with small cell lung cancers(Kern *et al.,* 2008)**.**Current and former. Smokers are at greatest risk, but lung cancer does occur among non-smokers, with varying rates across countries (Jemal*et al.,* 2008). The association between cigarette smoking and increased risk of. Lung cancer is now undisputed. Despite this, less than 20% of smokers develop lung cancer, suggesting that the effect of tobacco smoke exposure is modified by other variables, including individual susceptibility (United States Department of Health and Human Services, 2004). The search for a gene or genes associated with susceptibility is still nascent. Genome wide association studies have independently reported chromosomal region 15q24-25.1, which contains nicotinic acetylcholine receptor sub-unit genes, to be associated with increased risk of lung cancer in ever smokers (Amos *et al.,* 2008; Hung *et al.,* 2008 and Thorgeirsson*et al.,* 2008). These findings have been replicated among individuals with a family history of lung cancer, and the relative risk of lung cancer associated with markers in this region are much higher for familial cases compared to the relative risk observed among sporadic cases (Liu*et al.,* 2008). Linkage analysis in families with aggregation of lung cancer also described a region on chromosome 6q23-25 associated with risk of lung cancer (Bailey*etal., 2004*). The clinical significance of these findings is still unclear. In the meantime, lung cancer risk models using epidemiologic data have been developed, and the most parsimonious models for both ever and never smokers include a family history of cancer variable (D'Amelio*et al.,* 2010).

1.13 p53 Protein

The *p53* protein was initially identified as an oncogene because of its high expression in tumours. Soon after its discovery, it was found that the sequence of tumour-derived *p53* differs from that originating from normal tissue. This led to the conclusion that the normal p53 protein acts as tumour suppressor rather than an oncogene. Meanwhile, *p53* has become one of the most studied tumour suppressors, because of its central role in counteracting cellular transformation. Interestingly, two *p53*- related genes, *p63* and *p73*, were identified in 1997**(**Aramayo *et al.*, 2011; B. P. *et al.*,2006). Both proteins share homology with p53 but differ in their biological functions from p53. In certain conditions, however, also *p63* and *p73* can function as tumour suppressors. The p53 protein is a transcription factor that specifically binds sequences as a tetramer to target (Bode and Dong 2004). The wt-p53 protein consists of 393 amino acids (aa) that constitute five functional domains, including two N-terminal transactivation domains (TAD1: aa 1–42, TAD2: aa 43–63) that are followed by a proline-rich domain (aa 64–92), the central DNA-binding domain (DBD: aa 102–292), the C-terminal tetramerization domain (aa 326–356) and basic domain (aa364–393)**(**Aramayo *et al.,* 2011 and B. P.*et al.,* 2006) **(**Figure 1)

Figure 1: Structure and Post-transcriptional Modification of the Human *p53* Protein. The insert vertical bars above the DNA-binding domain illustrate the distribution and the prevalence of point mutations found in *p53* in human cancers. The most frequently mutated codons ("hotspot" codons) are identified. Several proteins that phosphorylate *p53* and the target amino acids of *p53* are also shown in the box. ATM, ataxiatelangiectasia mutated; ATR, ataxia-telangiectasia and RAD3 related; DNA-PK, DNA-dependent protein kinase; mTOR; mammalian target of rapamycin; CHK1 and 2, checkpoint kinases 1 and 2; p38k, *p38* kinase; GSK3*β*, glycogen synthase kinase-3 beta; HIPK2, homeodomain interacting protein kinase 2; JNK, Jun-NH(2)-terminal kinase; PKC, protein kinase C; CK2, casein kinase 2; PKR, protein kinase R (Mirzayans *et al.,* 2012).

The N-terminal acidic transcriptional transactivation domain is required for activating *p53*-inducible genes. The N-terminus contains two complementary transcriptional activation domains, with a major one at residues 1–42 and a minor one at residues 55–75, specifically involved in the regulation of several pro-apoptotic genes (Venot *et al.,* 1998). Proline rich domain important for the apoptotic activity of *p53*: residues 64-92. The central DNA-binding domain facilitates sequence-specific binding of *p53* to *p53*- response elements in DNA. The domain contains one zinc atom and



several arginine amino acids: residues 102-292. This region is responsible for binding the *p53* co-repressor LMO3 (Larsen *et al.*, 2010)**.** The tetramerization domain facilitates the interaction of *p53* monomers to form dimers, and the interactions of dimers to form tetramers. Tetramerization is essential for the ability of *p53* to positively regulate gene expression.

Along with the function the proper regulation of *p53* abundance are necessary for the initiation of an accurate p53 response. The levels of p53 expression are key to its activity and are tightly controlled in the cell, mainly by covalent modifications (Lazar *et al.*, 1993). In unstressed cells, a constant proteasomal degradation of *p53* keeps low the expression level of *p53* (Figure 2). The mark for proteasomal degradation (i.e. polyubiquitylation) is attached to C-terminal lysine residues of *p53* by the E3 ligase MDM2 (Toledo and Wahl, 2006). Transfer of the ubiquitin moiety from an upstream E2 ligase depends on the RING (really interesting new gene) domain of MDM2 (Itahana *et al.*, 2007). Interestingly, although MDM2 is necessary and sufficient for ubiquitylation of p53, its homologue MDMX is required for efficient p53 regulation. MDMX also contains a RING (really interesting new gene) domain. However, it does not itself show significant ubiquitin ligase activity towards p53 (Linares *et al.*, 2003). Nevertheless, MDMX forms heteromers with MDM2 and enhances MDM2-mediated ubiquitylation of p53 (Linares *et al.*, 2003). The importance of both MDM2 and MDMX is highlighted by the fact that single knockout of each gene confers embryonic lethality (Toledo and Wahl, 2006). MDM2 and also MDMX are frequently over-expressed in tumours that possess wt-p53. The abundance, subcellular localization and activity of *p53* are regulated by post-translational modifications such as phosphorylation, acetylation, ubiquitylation and attachment of ubiquitin-like moieties such as SUMO and Nedd8 on certain residues mainly in the N- or C-terminal part of *p53* (Toledo and Wahl, 2006). Phosphorylation primarily occurs at N-terminal Ser and Thr residues and affects p53 stability as well as the decision of whether *p53* induces apoptosis or senescence. The phosphorylation at N-terminal residues influences binding of MDM2 and thereby modulates ubiquitylation of *p53* at C-terminal lysine residues (Toledo and Wahl, 2006). Acetylation of these C-terminal residues also prevents ubiquitylation(Bode and Dong , 2004). MDM2 binds to *TP53* mRNA, controlling the rate of translation (Mcevoy *et al.*, 2012), and MDM2 regulates the levels of itself, MDM4 (also known as MDMX) and p53 (Mcevoy*et al.*, 2012; Manfredi , 2010)

The pivotal role of MDM2 and MDM4 in the control of p53 function argues that polymorphisms at these loci should be scrutinized for potential modulation of p53 function. Polymorphisms at loci that alter the activity of any single upstream event that activates p53 should not entirely abrogate the p53 response, owing to the high level of redundancy in stress responses, but cellular responses could be attenuated by altering one or more of the triggers for p53 activation (Catherine*et al.*, 2009).

The range of post-translational modifications and p53 upregulation elicited by stress are well-studied features of the p53 network. Less is known about the physiological roles of p53 that are not necessarily linked to tumour suppression and that can be executed by low levels of p53 and in the absence of a severe insult or stress.

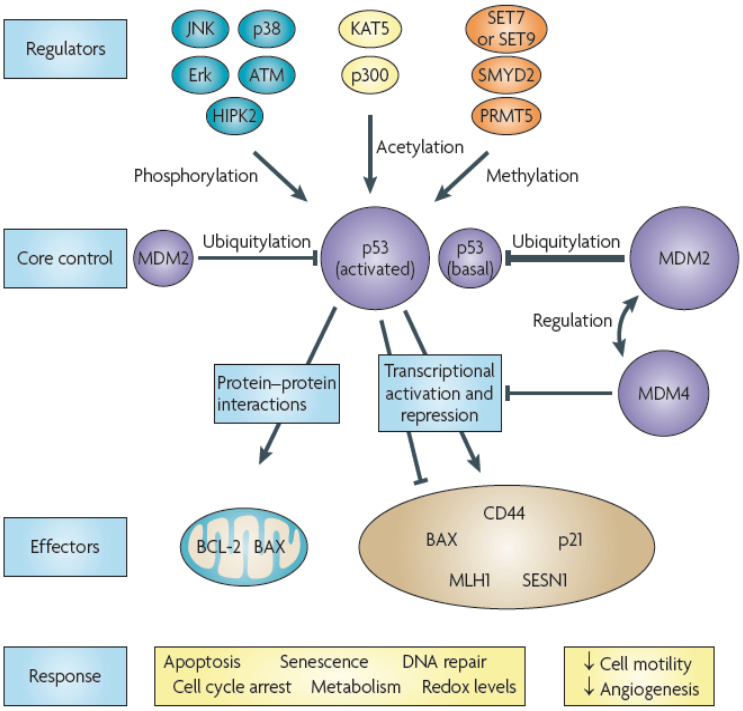


Figure 2.The p53 pathway (Catherine*et al.*, 2009)**.** The p53 pathway is complex. At least 50 different enzymes can covalently modify p53 to alter its stability, cellular location or activity17. Under normal cellular conditions, MDM2 represses p53 by binding and sequestering p53, and by ubiquitylating p53, targeting it for degradation. DNA damage, oxidative stress and oncogene activation are among the processes that can activate p53 by a range of regulators. Basal levels of p53 and p53 in cells that are undergoing low levels of stress can also affect cell physiology. Under high levels of stress, the interactions between MDM2, MDM4 and p53 are disrupted by post-translational modifications of these proteins. This allows activated p53 to act as a transcription factor, activating or repressing genes involved in apoptosis, cell cycle arrest and senescence. p53 can also move to the mitochondria, where it physically interacts with members of the Bcl-2 family to form pores in the mitochondrial membrane, leading to the release of cytochrome *c* and subsequent apoptosis. Some of the more intensively studied activators, regulators and effectors of p53 are shown in the figure. ATM, ataxia–telangiectasia mutated; BAX, BCL-2-associated X; HIPK2, homeodomain-interacting protein kinase 2; JNK, Jun N-terminal kinase; KAT5, K (lysine) acetyltransferase 5; MLH1, MutL protein homologu PRMT5,

Protein arginine methyltransferase 5; SESN1, sestrin 1; SMYD2, SET and MYND domain-containing 2.(Catherine *et al.*, 2009)

1.14 Polymorphism of *TP53* Gene

Mutation of *TP53* occurs in about 50% of human tumours, and most of these mutations are located in the DBD (Bode and Dong, 2004), influencing the specific binding of *TP53* to target sequences. The most common mutations concentrate on only a few nucleotides. Several of these hot spot mutations result in the exchange of amino acids that make contact with the DNA. Therefore, exchange of these amino acids weakens binding of p53 to DNA or alters sequence specificity of the mutant *TP53*. Altered sequence specificity in turn can even result in mutant *TP53* with oncogenic functions, thus turning the tumour suppressor to an oncogene. Mutations that cause structural destabilization of the DBD may result not only in its inactivation but also in deregulation of p53 by interfering with protein–protein interaction. Interestingly,mutations that interfere with *p53*oligomerization as well as deletion of *p53* are not common in human cancers. It is assumed that, once an inactivating mutation in one *TP53* allele has occurred, the resulting protein efficiently blocks the activity of the wt-*p53* expressed from the second allele (Essmann. and Schulze,2012). Thus, diverse mechanisms exist that impede a proper *TP53* response, and hence, also diverse strategies have been developed to overcome them.

Mutations in tumor suppressor *TP53* gene have been associated with inherited cancer susceptibility (Xifeng *et al.*, 2002)**.** Thirteen different polymorphisms have been detected in human *TP53* gene. Among them, five were found in exons and eight in introns. The five coding region polymorphisms include a C→T transition in codon 21 (Ahuja*et al.*, 1990)**,** a G→A transition in codon

36 (Felix*et al.,* 1999)**,** a C→T transition in codon 47 (Felley *et al.,* 1993)**,** a G→C transvertion in codon 72 (Weston*et al.,* 1992) and an A→G transition in codon (Carbone *et al.,* 1991)**.** However, among the five polymorphisms, those in codons 21, 36 and 213 are lack of significance- that means, they do not result in a change in the encoded amino acid sequence. The codon 47 polymorphism results in a proline to serine substitution and the polymorphism at codon 72 results in an arginine to proline substitution, which is in exon 4 (Xifeng*et al.,* 2002) **.** Whereas, the codon 72 polymorphism is common (Carbone *et al.,* 1991)**,** the polymorphism in codon 47 is rare (Felley *et al.,* 1993)**.**

More than 90% of the polymorphisms in *TP53* geneoccur are noncoding region polymorphism. A 16 base pair insertion in intron3 is the well-characterized intronic*TP53* polymorphism (Lazar*et al.,* 1993). This intronic polymorphism has been associated with an increase in the risk of several types of cancer. Several studies (Birgander*et al.,* 1995; Schneider *et al.,* 2004 and Walerych *et al.,* 2012)have estimated the haplotype frequencies for the polymorphisms in exon 4 and introns 3 and 6 and determined that the *TP53* haplotypes were associated with risk for lung (Birgander *etal.,*1995)**,**colorectal (Schneider *et al.,* 2004)**,**and breast cancers (Walerych *et al,*. 2012)**.**andWalerych*et al.,*2004)found a statistically significant difference in the distribution ofthe intron 6 polymorphism between healthy Caucasian controlsubjects and patients with ovarian cancer but found no differencebetween control subjects and patients with breast cancer.

Polymorphism at codon 47 (P47S) is rare located in the N-terminal transactivation domain of p53.This polymorphism results from a C>T base substitution at position 1 of codon 47. It has only been reported in populations of African origin. In which it is found at an allele frequency of approximately 5% (Weston and Godbold, 1997)**.** Dai and Gu (2010) showed that phosphorylation of the N-terminal domain of p53 regulate its transactivation properties. S46 is phosphorylate d by p38 and homeodomain-interacting protein kinase 2 (HIPK2), which enhances the transcription of apoptosis-related genes and hence promotes p53-mediated apoptosis (Kato *et al.,* 2003)**.** These two kinases are directed to phosphorylation sites by a proline residue adjacent to S46. For this reason, replacement of P47, as occurs with the P47S polymorphism, would be expected to decrease phosphorylation at S46 result in decrease transactivation of pro-apoptotic target genes and thus potentially increase cancer risk (Felley *et al.,* 1993)**.** Codon 217 polymorphism (V217M) (resulting from a G>A transition) is the only validated coding polymorphism located in the DNA binding domain (DBD) of p53. Thus, it could dramatically affect the activity of p53. Functional studies have been limited to transactivation assays in yeast, which indicate that this polymorphism results in little loss of activity (DAI and GU 2010)**.**The genes that show the most variation in activation are *CDKN1A*, *BAX* and *PMAIP1* (also known as NoXA). However, the p53-M217 variant leads to greater expression of these genes than the more common p53-V217 variant. Extrapolating from this result, one can speculate that the V217M polymorphism might be protective against cancer.

Codon 360 polynorphism (G360A) is located in the linker region adjacent to the tetramerization domain of p53. Again, the functional data for this polymorphic variant have been provided by transactivation studies in yeast, which showed a slight decrease in the transactivation of *BAX*, *MDM2* and *P53AIP*, and a more marked decrease in stratifin (*SFN*, also known as 14-3-3 sigma) and *GADD45* (growth arrest and DNA damage-inducible 45) (Kato *et al.,* 2003)**.**

Codon 72 polymorphism (R72P) is in exon 4 located in the segment of p53 gene that encodes the polyproline domain, which lies between the N-terminal transactvating domain and DNA binding domain (DBD). Most tumour associated mutations are found in this region (Venot *et al.,* 1998)**.** The polyproline domain is less well conserved across the species than the DBD. Deletion studies in cell and mice showed that the polyproline domain is essential for p53 to show a full apoptopic response to stress and inhibit tumoriogenesis(Rot *et al.,* 2000and Toledo *et al.,* 2007)**.**

From comparative sequence analyses in non-human primates, it was found that p53-P72 is the ancestral form, although p53-R72 occurs at a high frequency (>50%) in some populations (Puente *et al.,* 2006)**.** A latitude gradient in variant frequency (an increasing frequency of the p53–72 variant towards the equator (Beckman, 1994 ; Ozbey *et al.,* 2011 ; Ricksi *et al.,* 2010; Shi *et al.,* 2009) invited early speculation that p53-P72 might protect against adverse consequences of sunlight or other environmental cancer risk factors. Interestingly, p53 influences the tanning response to sunlight by inducing the expression of pro-opiomelanocortin (PoMC) (NAN *et al.,* 2008)**,** which leads to an idea that p53 polymorphisms influence *POMC* transactivation. The contribution of p53-mediated control of leukaemia-inhibitory factor expression, which is crucial for blastocyst implantation, has recently been considered in the discussion of evolutionary selection of specific alleles in the p53 pathway (Feng *et al.,* 2011; Hu 2009;Mathieu *et al.,*2012)**.**

1.15 Codon 72 polymorphism and lung cancer

p53 protein exists in two polymorphic forms, p53-Pro (p53-P72) or p53-Arg (p53-R72) in the general population due to single nucleotides change at codon 72 of exon 4 of the TP53 gene (MATLASHEWSKI et al. 1987) shows different structure and functional properties. The current

consensus is that p53-R72 is more effective at inducing apoptosis and protecting stressed cells from neoplastic development than p53-P72. However, to understand how universal these functional differences between p53-P72 and p53-R72 might be different cell type or whether they are relevant in vivo, the experimental data is insufficient (CHEN et al. 2011).NIH genetic association database, which is not comprehensive, has records on over 230 studies evaluating the effect of the codon 72 polymorphism on susceptibility to a wide variety of cancers (MATLASHEWSKI et al. 1987). Many of these studies have reported 'statistically significant' associations. The association of p53 codon 72 polymorphism with lung cancer risk has been studied by several groups, although with inconsistent result. Weston found the pro allele to be in excess in patients with adenocarcinoma in USA, but this could not be confirmed in its follow-up study (WESTON et al. 1994). In Japan, a study showed a significant association of the Pro allele with squamous cell carcinoma rather than lung adenocarcinoma (KAWAJIRI et al. 1993). Ying chuan Hu showed that the p53 Pro allele is associated with an increased frequency of p53 mutation in non-small cell lung cancer. They also stated that the codon 72 polymorphism did not influence patient's survival in either the entire patient group or among patients with p53 mutant tumor (Ying chuan Hu et al. 2005). Weston found that the frequency of polymorphic variants was similar in lung cancer cases and controls after adjustment for race (African-Americans and Caucasians in U.S. population). However, the proline variant at codon 72 was in excess in adenocarcinoma among lung cancer patient (AINSLEY WESTON et al. 1992). In Taiwan, a study showed that the Pro allele of the p53 codon 72 polymorphism increased the risk of lung cancer (especially adenocarcinoma) among female Taiwanese. Patients with lung cancer diagnosed at the early stage had an increased frequency of Pro/Pro genotype. Patients with the Pro/Pro genotype tended to have poorer prognosis than those with the Arg/Pro genotype (Ying chuan Hu et al. 1999). In India, Neeraj Jain found a strong association of p53 Arg homozygotes with advance lung cancer. They also expressed that the presence of Arg/Pro heterozygotes may associate with early progression of the disease possibly due to additional genetic alteration.

As to the correlation of p53 codon 72 polymorphism with smoking, there are conflicting results. Smoking is considered to be one of the principal causes of lung cancer. Murata found that lung cancer patients who did not smoke included a significantly larger proportion of Arg/Arg homozygotes and smaller proportion of Arg/Pro heterozygotes compared with the healthy controls (Murata et al. 1996). However, Jin reported that increased risks associated with the Pro/Pro genotype were noted in lighter smokers (Jin et al. 1995).

1.16 Reasons for studying p53 for Cancer Diagnostics and Prognosis

Many researchers focused on the study of *p53* structure and function for cancer diagnosis and prognosis. Mutations in the tumour suppressor TP53 gene are one of the most common genetic alterations, which present at high frequency in human cancers (vogelstein*et al.,* 2000;Hofseth, *et al.,* 2004; Levine and Oren , 2009). More than 26,000 somatic mutation data of *p53* appear in the international agency for research on cancer (IARC) *TP53* database version R14 (current version (November, 2009)(olivier *et al.,* 2010)**.**Up to 50% of all human cancers contain mutations in both alleles of the TP53 gene **(**Edlund *et al.,* 2012; Harris*et al.,* 2012)**.** Unlike most other tumor suppressor genes, such as RB, APC, and BRCA1 genes, they are inactivated by frameshift or nonsense mutations, which leads to disappearance or aberrant synthesis of the gene product, nearly 80% of TP53 gene mutations are missense mutations (Essmann and Schulze, 2012). Other mutations include frameshift insertions and deletions (9%), nonsense mutations (7%), silent mutations (5%), and other infrequent alterations (Cui *et al.,* 2007)**.** The frequency of *TP53* mutation varies from ~10% (hematopoietic malignancies) to 50–70% (ovarian, colorectal, and head and neck malignancies) (Rotter *et al.,* 2009)**.**Germline mutation of *TP53* causes Li-Fraumeni syndrome, which is a familial cancer syndrome including breast cancer, soft tissue sarcoma, and various other types of cancer (Malkin *et al.,*1990; Manfredi*et al.,* 2006)**.** Most *TP53* mutations in human cancers result in mutations within the DNA binding domain, thus preventing *p53* from transcribing its target genes. However, mutant *p53* has not only led to a loss of normal function of the wild-type protein but also led to new abilities to promote cancer (Rotter*et al.,* 2009)**.**

The key role of *p53* as a tumor suppressor is to block cell cycle progression and/or to induce apoptosis, in response to cellular stresses such as DNA damage. Numerous reports have described the mechanism by which *p53* induces apoptosis. As *p53* functions mainly as a transcription factor, it is important to explore the genes regulated by p53 that contribute to the regulation of apoptosis. Early studies showed that wild-type *p53* can bind the *bax*gene promoter region and regulate *bax* gene transcription (Reed*et al.,* 1995; Miyashita*et al.,* 1994)**.***bax*is a member of the Bcl-2 family, which forms heterodimers with Bcl-2, inhibiting its activity (Oltvai *et al.,* 1993)**.** The Bcl-2 protein family plays an important role in apoptosis and cancer (Plati *et al.,* 2011). For example, Bcl-2 controls the release of cytochrome c from the mitochondria, which activates the apoptotic pathway by activating caspase 9.

The p53 protein suppresses tumor formation not only by inducing apoptosis but also by causing cell cycle arrest. Depending on the type of cellular stress, p53 can induce G1 arrest through activation of transcription of the cyclin-dependent kinase inhibitor p21 (Chung and Bunz, 2010). *p53* also regulates the G2/M transition. For example, p53 can block cell entry into mitosis by inhibition of Cdc2. Cdc2 needs to bind to cyclin B1 in order to function. Repression of cyclin B1 by p53 also arrests of cells in G2 (Chung and Bunz, 2010)**.**

This is because most, but not all, human cancers harbor altered p53; the concept of restoration of p53 for cancer therapy is very attractive. An animal model showed the reactivation of wild type p53 to result in efficient tumor regression, including regression of lymphoma (WANG *et al.,* 2011) and liver carcinoma (Chien *et al.,* 2011)**.**p53 has been reported to induce apoptosis independent of its transcription of genes (Dixit *et al.,* 2012)**.** Surprisingly, activated p53can induce apoptosis in the cytoplasm by a *bax*-dependent mechanism (Stegh , 2012). Recent reports have showed that p53 regulates the process of self-renewal of neural stem cells (Hede *et al.,* 2011)and hematopoietic stem cells (Liu *et al.,* 2009).

It was reported that somatic *TP53* missense mutations were found in about 50% of human cancers, and inactivating mutations in the *TP53* gene were the most common genetic events in human cancers affecting a specific gene, with the vast majority arising from a single-point mutation in the DNA-binding domain encoding segment of *TP53*(Harris*et al.,* 2012; Leroy *et al.,* 2013). The mutant *TP53* protein rendered by the inactivating mutations unable to carry out its normal functions, that is, transcriptional transactivation of downstream target genes that regulate cell cycle and apoptosis (Hamzehloie *et al.,* 2012). Several recent studies indicated that in addition to abrogating the tumor suppressor functions of wild-type *TP53*, the common types of cancer-associated *TP53* mutations also furnish the mutant protein with new activities, so-called "gain-of-function" (GOF) activities. These new activities can contribute actively to various stages of tumor progression, including distant metastases, and to increased resistance to anticancer treatments. GOF activities of mutant *TP53* are exerted by aberrant protein interaction or gene regulation, such as MAPKK3, inhibitor of DNA-binding 4 (ID4), polo-like kinase 2 (Plk2), promyelocyticleukemia protein (PML), and prolylisomerase Pin1 (Hamzehloie *et al.,* 2012;Kim *et al.,* 2011). Although the occurrence of *TP53* mutations is not limited to a few particular sequences or codons along this gene, most mutations cluster in the *TP53* DNA binding domain, which encompasses exons five through eight and spans approximately 180 codons or 540 nucleotides (Pfeifer and Besaratinia, 2009). Most *TP53* missense mutations lead to the synthesis of a stable protein, which loses its specific DNA-binding and transactivation function and accumulates in the nucleus of cells. These mutant accumulated proteins play a vital role and are retained in distant metastasis. In moreover, the most frequent mutants have been shown to be capable of cooperating with oncogenes for cellular transformation (Markowitz *et al.,* 1994). About 30% of *TP53* missense mutations found in cancer correspond to nucleotide substitutions at highly mutable CpGdinucleotides and at codons encoding residues that play essential structural and chemical roles in the contact between the *TP53* protein and specific DNA sequences that constitute the *TP53* response elements (Olivier *et al.,* 2010). These mutations result in a significant loss of DNA-binding activity and transactivation capacity(Hamzehloie *et al.,* 2012).

Loss of heterozygosity (LOH) frequently detected in lung cancer cell lines and tumor samples at the location of the *TP53* gene on chromosome 17p13, which suggested that this gene was likely to be involved in the pathogenesis of lung cancer, and genetic abnormality of the *TP53* in lung cancers has been shown to be associated with a poorer survival prognosis and increased cellular resistance to therapy(Liu *et al.,* 2011**).** The highest frequency of *TP53* alterations is found in small cell lung cancer specimens (Lohmann *et al.,* 1993; Stewart, 2010). In contrast, the frequency of *TP53* mutations is the highest in squamous cell carcinomas and lowers in adenocarcinomas among non-small cell carcinoma samples **(**Lohmann *et al.,* 1993and Johnson *et al.,* 2012)**.** It has been reported that somatic mutations and increased expression of *TP53* were frequently found in 23% and 65% of non- small cell carcinoma respectively, (Baldi *et al.,* 2011; Bornachea *et al.,* 2012)**.** Moreover, *TP53* mutations are found in tumors both with and without allele loss at 17p13, which are mostly located within the DNA-binding domain of *TP53*(Hong *et al.,* 2008).Because of coding mutations of TP53 occur relatively early in the development of lung cancer and are potentially required for maintaining the malignant phenotype, the acquired *TP53* mutations are preserved during tumor progression and metastatic spread (D'Agostini *et al.,* 2008; Newnham *et al.,* 2011). It has been reported that the incidence of *TP53* mutations in primary tumors and metastatic lymph nodes was 23.2% and 21.4%, respectively. The *TP53* gene status in primary tumors and metastatic lymph nodes showed 92.9% harmony among 56 patients with non- small lung cancer who had undergone surgical resection, which explained the fact that *TP53* mutations usually precede lymph node metastasis(Rivlin *et al.,* 2011).Most *TP53* mutations occur before the tumor metastasizes. They are then preserved through subsequent stages of tumor development; as a result, no selection against *TP53* mutations occurs during metastasis.

2. Discussion and Conclusion

The population-base case-control study was carried out in Bangladesh population to examine the prevalence of p53 codon 72 polymorphism in lung cancer patients and to make relation of the polymorphisms with different demographic characteristics. We have found over half of the cancer patients (68.40%) have squamous cell carcinoma, which is slightly more than frequency (53%) estimated in the Cancer Registry Report (CRR) of the NICRH, 2005-2007 in Bangladesh(CRR, 2009). In an opposite fashion the frequency of adenocarcinoma in the present study is less than that of Cancer Registry Report (CRR, 2009) of the NICRH. The remarkable that there is no large cell carcinoma in lung cancer patients in Bangladesh. This outcome is similar to the Neeraj(52.50% of squamous cell carcinoma case and 2.50% of large cell lung cancer case) find in India. However, in Greek, 56.50% of lung cancer type was adenocarcinoma which is different from our study (PAPADAKIS et al. 2007). Nearly 95% of the lung cancer patients are more than 40 years old. This indicates, with the increase of age, the risk of lung cancer development increases. From our patient study, it is found that about half of the patients are in the age group of 51-60. That means lung cancer develop in the older people. This finding is supported by the CRR of the NICRH, Bangladesh. They showed that the highest number of lung cancer patients were 55-65 years old. According to present study and Cancer Registry Report (CRR) of the NICRH male are prone to lung cancer, which is supported by the WHO (WHO, 2008).

The relation of p53 codon 72 polymorphism with lung cancer is inconsistent as well as conflicting worldwide. Some studies found no correlation between p53 codon 72 polymorphism and lung cancer (BIRGANDER R et al.1995; WESTON and GODBOLD et al. 1997). On the other hand the worldwide scenario in the frequency of p53 polymorphism in lung cancer also indicate that Arg/Arg and Arg/Pro genotypes are more susceptible to lung cancer in Asian (JAIN N et al. 2007; TAGAWA M et al. 1998), European (PAPADAKIS et al. 2002), and North American population (JIN X et al.1995; MILLER DP et al. 2002). Similarly, in India, Neeraj Jain and Pandima Devi found association between Arg/Arg genotype with lung cancer (Neeraj Jain et al. 2005; Pandima Devi et al. 2010)

Other authors reported an increased frequency of Pro/Pro homozygotes in lung cancer (KAWAJIRI ET AL. 1993; WANG YC et al. 1999). Studies on the people of Asian and Mexican-Americans suggested at least at fivefold increase in lung cancer risk for the Pro/Pro homozygotes (KAWAJIRI ET AL. 1993; WANG YC et al. 1999). Also, an increased risk was observed for African-American (FAN et al. 2000) and white population with Pro/Pro genotypes(WANG YC et al. 1999). In agreement with the above results, we found increased frequency of Pro/Pro genotype in lung cancer patients compare to control and which was statistically more significant than Arg/Arg genotype. Our result also supported by majority of studies that associate p53 Pro/Pro genotype with lung cancer have been reported from Southeast Asia (China) (W. XUE et al. 2007), Taiwan or Japan (YI-CHING WANG et al. 1999). The discrepancies in the result may be attributed to various reasons such as selection of patients and control subjects, variation in laboratory protocols, and geographic and ethnic background. Fan, who demonstrated the association of p53 prolinehomozygosite with lung cancer, had studied only primary lung cancer stage I and II patients and excluded patients of advanced stage,(FAN et al. 2000) whereas Papadakis and Neeraj Jain studied advanced lung cancer patients (stage III and IV) and suggested association of p53 arginine homozygosity with lung cancer. Interestingly, we found increased frequency of both Arg/Arg and Pro/Pro homozygous genotype in lung cancer patient compare to control subject; although the results were not statistically significant (Pro/Pro genotype is more statistically significant) (PAPADAKISet al. 2002; NEERAJ JAIN et al. 2005)

The p53 plays important roles in apoptosis, cell cycle control and DNA repair, and these critical functions differ among p53 protein encoded by the Arg and Pro alleles. In the presences of Arg allele, conformational p53 mutants have been more potent in binding to p53 and neutralizing p53-induced apoptosis, which enhances tumariogenesis and provides a selective growth advantage to tumour cells (PANDIMA et al. 2010). Earlier studies reveal that people with Arg(Arg/Pro or Arg/Arg phenotype) in codon 72 of p53 gene are more susceptible for lung cancer at the later stage of life and longtime of smoking habit (PAPADAKIS et al. 2002; NEERAJ JAIN et al. 2005). The increased frequency Arg/Arg genotype was present in the patients who are more than 50 years old and smoke for a long time.

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