"P53 AND CANCER"

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BY

DEBASHREE DAS
ROLL NO. 409LS2037

UNDER THE SUPERVISION OF
ASSOCIATE PROF. SAMIR K. PATRA

DEPARTMENT OF LIFE SCIENCE
NATIONAL INSTITUTE OF TECHNOLOGY
ROURKELA-769 008, ODISHA, INDIA

DEPARTMENT OF LIFE SCIENCE
NATIONAL INSTITUTE OF TECHNOLOGY,
ROURKELA-769008

.....

Dr. SAMIR K. PATRA,
Associate Professor and Head.

Ref. No.

Date: .....

CERTIFICATE

This is to certify that the thesis entitled "p53 and Cancer" which is being submitted by Ms Debashree Das, Roll No. 409LS2037, for the award of the degree of Master of Science from National Institute of Technology, Rourkela, is a record of bonafide research work, carried out by her under my supervision. The results embodied in this thesis are new and have not been submitted to any other university or institution for the award of any degree or diploma.

Dr. SAMIR K. PATRA
ASSOCIATE PROFESSOR AND HEAD,
Department of Life Science
National Institute of Technology
Rourkela – 769008,Odisha, India.

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Phone no.: 0661-2462683. Email: skpatra_99@yahoo.com

DECLARATION

I, Debashree Das, hereby declare that this project report entitled **"**p53 and Cancer**"** is the original work carried out by me under the supervision of Dr. Samir K. Patra, Associate Professor and Head, Department of Life Science, National Institute of Technology Rourkela (NITR), Rourkela and to the best of my knowledge and belief the present work or any other part thereof has not been presented to any other University or Institution for the award of any other degree.

Debashree Das

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And to all mighty, who made all things possible……..

(DEBASHREE DAS)

CONTENTS

ABSTRACT

p53 acts as the single most important gene in cancer as a single mutation can lead to tumorigenesis. More recently p53 functions have been further diversified by the discovery of various isoforms of p53. These isoforms derived either by alternative splicing or by differential translational sites are expressed in a tissue specific manner and have been proposed to influence p53 activity. Here we are trying to confirm the influence of p53β isoform on p53 activity either by its transcriptional repression or by forming a complex with p53 thereby modulating its activity at p53 dependent promoters. Expression of both p53 and its isoform rules out the chances of transcriptional regulation. Therefore, it may be that p53β/p53 may be binding differentially to p53 dependent promoters producing aberrations.

INTRODUCTION

The body's system is composed of trillions of cells which continually grow and divide providing for the growth and maintenance of the body. Even though we stop growing when we become adults, many of our cells continue to grow and divide. Our bodies constantly replace the worn-out cells with new cells to stay healthy. To do this, cells must enter a "highway" called the cell cycle. The cell cycle has built-in controls for how fast, and for how long a cell will keep dividing. Such control mechanism is called the homeostatic mechanism otherwise called the “Traffic controllers” of the cell. Such a mechanism depends on signals to decide when to stay in or exit the cell cycle. The proto-oncogenes and tumor suppressor genes constitute the go and stop signals of this machinery, respectively. Tumor suppressor genes code for proteins that serve as the "stop" signals that tell a cell to leave the cell cycle and stop dividing. The proto-oncogenes code for the "go" signals that tell the cell to stay in the cell cycle and continue to divide.

Deregulation of these "stop" or "go" signals can lead the cells to escape from the tight controls that maintain homeostasis. Cells that accumulate DNA damage (called mutations) may lose their ability to respond to or make "stop" signals resulting in cancer developement.

The development of cancer is a complex process that requires the accumulation of damage to the cell's growth-controlling genes, including damage to tumor suppressor genes and proto-oncogenes. That is why cancer takes so long to develop.

A central player in protecting the integrity of the genome is p53. p53 protein is expressed at low levels under unperturbed conditions. However, the p53 pathway is activated by any cellular stresses that alter the normal cell-cycle progression or can induce mutations of the genome leading to the transformation of a normal cell into a cancerous cell. Depending on the tissue-type and the extend of the damage, activated p53 protein either stops the cell cycle to repair the lesions or switches ‘on’ the programmed cell death pathways (apoptosis), forcing the damaged cells to ‘commit suicide’. The p53 protein prevents the multiplication of damaged cells that are more likely to contain mutations and exhibit abnormal cellular growth than undamaged cells. Hence, p53 protein is the guardian of the genome preventing cancer formation. The mechanisms by which p53 accomplishes its tumour suppressor activity are still not completely understood. The best described mechanism is its ability to modulate gene expression. p53 is a transcription factor that binds directly and specifically as a tetramer to

target sequences of DNA (p53-responsive elements (p53RE). The ability of p53 to modulate gene expression is required for its tumour suppressor activity. Identification of the cyclin dependent kinase inhibitor Waf as a p53-responsive gene, helps to explain how p53 can induce cell-cycle arrest. Recently, several p53-inducible genes that encode for proteins with apoptotic potential have been identified. However, the tumour suppressor p53 can trigger cell death independently of its transcriptional activity through sub-cellular translocation and activation of proapoptotic Bcl-2 family members.

The importance of its role is exemplified by the facts that p53 activity is ubiquitously lost in human cancer either by p53 protein inactivation or by p53 gene mutation. A defective **p53** gene deprives the cells of crucial signals that normally put the "brakes" on inappropriate cell division and tumor development. Human p53 gene has indeed a dual gene structure. p53 gene transcription can be initiated in normal human tissue from two distinct sites upstream of exon1 and from an internal promoter located in intron 4. The alternative promoter leads to the expression of an N-terminally truncated p53 protein initiated at codon 133 (Δ133p53). The intron 9 can be alternatively spliced to produce three isoforms: p53, p53β (identical to p53i9) and p53g, where the p53b and p53g isoforms lack the oligomerisation domain. Therefore the human p53 gene can encode at least nine different p53 protein isoforms, p53, p53b, p53g, Δ133p53, Δ133p53b and Δ133p53g due to alternative splicing of the intron 9 and usage of the alternative promoter in intron 4, and also Δ40p53, Δ40p53b, Δ40p53g due to alternative splicing of the intron 9 and alternative initiation of translation or alternative splicing of the intron 2. p53 variant mRNA are expressed in several normal human tissues in a tissue-dependent manner, indicating the internal promoter and the alternative splicing of p53 can be regulated. Moreover, the tissue-specific expression of the p53 isoforms could explain the tissue-specific regulation of p53 transcriptional activity in responses to stresses such as ionising radiation, UV, pH and hypoxia. But still their expression patterns and functions remained a mystery.

REVIEW OF LITERATURE

The genetic basis of cancer development has only been established recently based on the evidence that familial, epidemiologic, and cytogenetic studies have provided over the last quarter century. The current understanding shows that cancers is a multistage process in which mutations (both inherited and somatic) of cellular genes lead to clonal selection of variant progeny with the most robust and aggressive growth properties.(1)

Two classes of genes, are targets for the mutations, i.e. Proto-oncogenes and Tumor Supressor Genes

Proto-oncogenes:

Proto-oncogenes have critical roles in a variety of growth regulatory pathways, and their protein products are distributed throughout many subcellular compartments. The oncogenic variant alleles present in cancers have sustained gain-of-function alterations resulting from point mutations, chromosomal rearrangements, or gene amplifications of the protooncogene sequences Whereas oncogenic alleles harbour activating mutations, tumor-suppressor genes are defined by their inactivation in human cancer [1].

Tumor-suppressor genes:

A tumor suppressor gene, called as an anti-oncogene, by Knudson, is a gene that protects a cell from developing cancerous properties. When this gene is mutated to cause a loss or reduction in its function, the cell can progress to cancer, usually in combination with other genetic changes.

Some of the epithets used for tumor suppressor genes are the ‘gatekeeper’, Caretaker’ and ‘Landscaper’ [2]. They are called the gatekeepers because, first, their loss of function is rate-limiting for a particular step in multi-stage tumorigenesis; second, they act directly to prevent tumor growth, and third, restoring ‘gatekeeper’ function to tumor cells suppresses neoplasia. Ex. Adenomatous Polyposis Coli (APC).

Kinzler and Vogelstein subsequently qualified the ‘gatekeeper’ definition of tumor suppressor genes to include all direct inhibitors of cell growth (suppressing proliferation, inducing apoptosis or promoting differentiation). This allows us to define genes such as p53 as a ‘gatekeeper’, albeit as a ‘progression gatekeeper’.

By contrast, 'caretaker' tumor suppressor genes act indirectly to suppress growth by ensuring the fidelity of the DNA code through effective repair of DNA damage or prevention of genomic instability (such as microsatellite or chromosome instability). As such, a large number of 'caretaker' tumor suppressor genes are DNA repair genes. Loss of 'caretaker' function predisposes to cancer by increasing the DNA mutation rate, thereby increasing the chances that 'gatekeeper' gene function will be lost. Mutation in both alleles of a 'caretaker' gene requires mutations in both alleles of gatekeeper genes to be functional whereas, gatekeeper muatation does not require caretaker mutation.

Mutations in tumor suppressor genes are recessive; that is, as long as the cell contains one normal allele, tumor suppression continues. (Oncogenes, by contrast, behave as dominants; one mutant, or overly-active, allele can predispose the cell to tumor formation) [2].

Tumor suppressor pathways:

Cells escape growth control by targeting key oncogenes/ tumor suppressor in molecular pathways. These pathways have evolved to integrate positive and negative growth signals according to cellular function and microenvironment during normal development and tissue repair. The RB pathway (RB/p16INK4a/cyclin D1) and the p53 pathway (p19ARF/mdm2/p53) are both frequently targeted in tumorigenesis and the mutation occurring in each pathway depends on the tumor type.

p53

TP53 gene and p53 protein:

The tumour suppressor protein p53 was first described in 1979 and ten years later identified as a tumour suppressor. In human, the TP53 gene that contains 11 exons is located in chromosome 17p13.1, the coded protein is approximately 53 kDa in size, containing 393 amino acids [3].

Structural and functional aspects of p53:

There are four conserved domains in p53:

1. The N-terminal domain is required for transcriptional transactivation
2. A hydrophobic central sequence-specific DNA binding domain, composed of two beta-

sheets and a zinc atom which stabilizes the structure

3. A tetramerization domain near the C-terminal end

4. The C-terminal domain interacts directly with single stranded DNA [4].

When normal mammalian cells are subjected to stress signals (e.g. hypoxia, radiation, DNA damage or chemotherapeutic drugs Δp53 is activated; additionally to its activation, ubiquitin-dependent degradation of the p53 protein is blocked. The resulting increase in p53-dependent gene transcription leads to the p53-mediated induction of programmed cell death and/or cell cycle arrest. Functional p53 is thought to provide a protective effect against tumorigenesis, and indeed, mutations of p53 have been found in nearly all tumor types and are estimated to contribute to around 50% of all cancers.

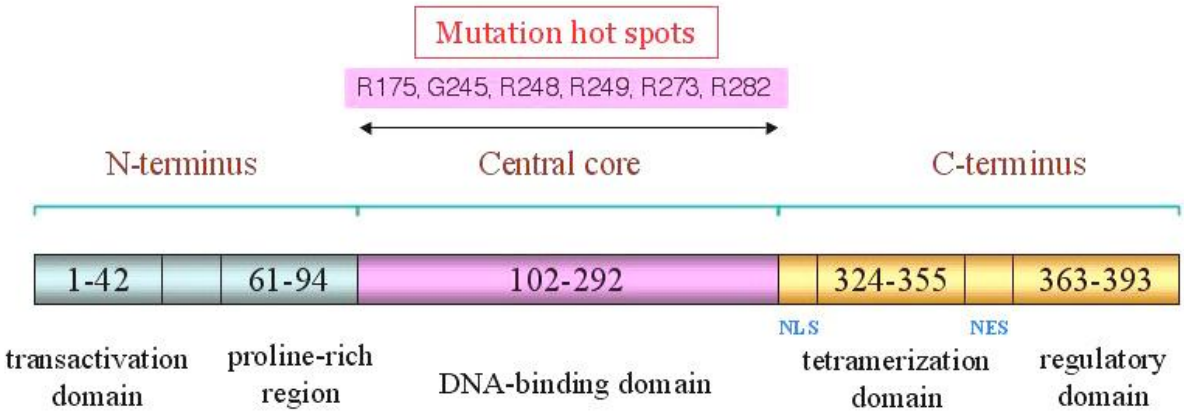


Figure 1: Schematic representation of the p53 structure (Ling Bai *et.al*, 2006).

Functions of the p53 protein:

Of the many functions of p53, the first ones identified were inhibition of abnormal growth of cells and triggering of programmed cell death. Because these processes ensure genomic integrity or destroy the damaged cell, p53 has been called the "guardian of the genome"[5]. Later on, other important functions, such as DNA repair and inhibition of angiogenesis, were discovered. p53 is a sequence-specific nuclear transcription factor that binds to defined consensus sites within DNA as a tetramer and affects the transcription of its target genes[6]. p53 regulates these genes either by transcriptional activation or by modulating other protein activities by direct binding. The p53-induced activation of target genes may result in the induction of growth arrest either before DNA replication in the G1 phase of the cell cycle or

before mitosis in the G2 phase. The growth arrest enables the repair of damaged DNA. By programmed cell death, which is often referred to as apoptosis according to its morphological appearance, the cells damaged beyond repair are eliminated thus preventing the fixation of DNA damage as mutations. The importance of p53 mutation in tumor cell biology is irrefutable. Wild-type p53 mediates imperative functions such as regulation of the cell cycle and programmed cell death. Deficiency of p53 function by mutation or inactivation abrogates normal cell cycle checkpoints and apoptosis, generating a favourable milieu for genomic instability and carcinogenesis.

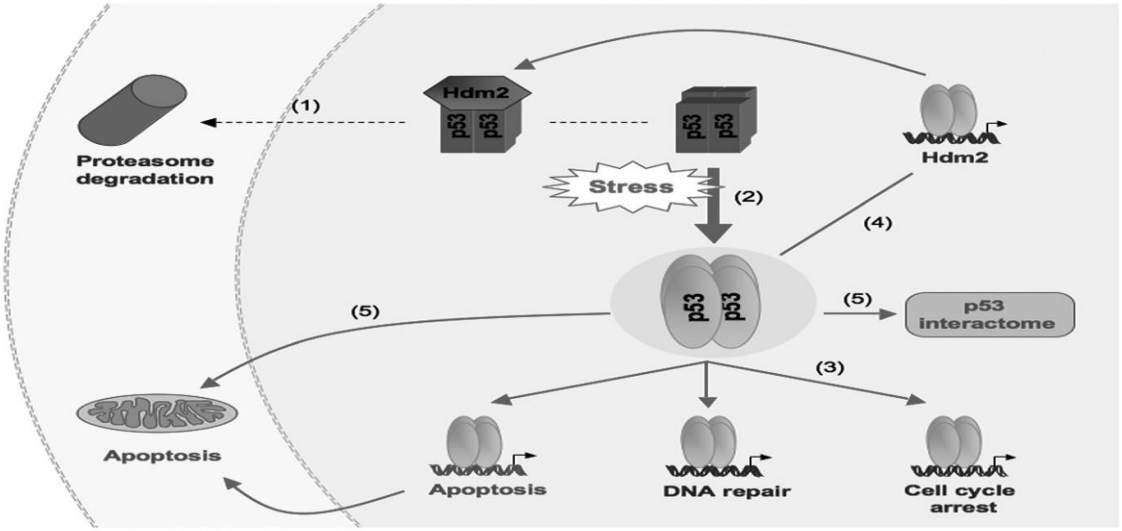


Figure.2: The classical p53 pathway[V. Marcel,et. al].

Activation of p53:

p53 is activated, among others, in response to DNA damage, and many factors interact to signal and modulate this response. There is still controversy over the pathways that lead to the activation of p53. Several mechanisms have been suggested:
One idea is that stress-activated protein kinases phosphorylate p53, protecting it from degradation and activating its function as a transcription factor. Indeed, many phosphorylated forms of p53 are found in cells, and by phosphorylation p53 can be released from a latent state, in which it cannot bind DNA. One attractive candidate for p53 activation by phosphorylation is the DNA-dependent protein kinase (DNA-PK). DNA-PK is activated by DNA damage, and one of its substrates is p53[7].

Instead of its phosphorylation, the dephosphorylation of p53 at serine 376 by the ATM-dependent activation of a specific phosphatase might enable DNA binding of p53 and its transcriptional activation. In this process, the so called 14-3-3 proteins bind to the C-terminus of the dephosphorylated p53, and by this possibly activate it.

Another pathway towards activation of p53 involves the mdm-2 gene product. MDM-2 can target p53 for nuclear export and degradation; nonfunctional MDM-2 results in accumulation of p53 and activation of p53-dependent transcription. The mdm-2 gene itself is activated for transcription by p53, so this model implies that p53 is constitutively active, driving transcription of the protein (MDM-2) that targets its own degradation. Blocking the p53 degradation pathway would result in the activation of the p53 response. Indeed, it was shown that the ARF tumor suppressor (also called $p14^{ARF}$) binds to the complex of p53 and MDM-2, by this stabilizing p53, possibly by inducing degradation of MDM-2 . ARF expression itself is regulated by the E2F-1 transcription factor! This connects the Rb pathway to p53: oncogenes like E1A or SV40 T block Rb function, thus activating E2F-1. E2F-1 transcriptional activity leads to the expression of a number of genes required for passage into and through S phase but also to the expression of ARF which stabilizes p53. This would result in either p53 dependent apoptosis or cell cycle arrest unless p53 itself is inhibited [8].

Regulation of p53:

The p53 protein is effectively able to inhibit cell growth, and its activity is therefore strictly regulated. There are several mechanisms for the regulation of p53. Although, in some models, chemical DNA damage, for example, seems to increase TP53 transcription, it is generally believed that the principal mechanisms governing the activity of p53 occur at the protein level. These include post-translational modifications, regulation of the stability of p53 protein, and control of its sub-cellular localization[9]. Post-translational modifications of the protein take place in response to stress, and different agents elicit diverse responses. The human p53 protein has been shown to be modified at least at 17 different sites. Of the post-translational modifications of p53, the most widely studied and best-known so far is phosphorylation. After DNA damage induced by ionizing radiation or UV light, phosphorylation takes place mostly at the N-terminal domain of p53[10]. Another important modification is acetylation, which has been shown to occur in response to chemically induced DNA damage and hypoxia. In response to DNA damage, the p53 protein is also modified by conjugation to SUMO-1, a

ubiquitin-like protein. Many proteins able to interact with p53 may also play a role in p53 regulation.

The murine double minute 2 (*mdm2, hdm2* in human) gene encodes a 90 kDa protein (97 kDa in human) that was originally identified as a dominant transforming oncogene. The *mdm2* gene has been found to be amplified in human cancers. The combination of overexpressed mdm2 and p53 gives a worse prognosis than either one of them alone. Deletion of the *mdm2* gene in mice is embryonically lethal, probably due to increased accumulation of p53, but this lethality can be counter-acted by deletion of the TP53 gene The p53-mdm2 relationship is vital in the regulation of cell growth and death.The mdm2 protein regulates the activity of the p53 protein with more than one mechanism. It can block the transcriptional activity of the p53 protein, export p53 from the nucleus to the cytoplasm and promote the degradation of p53 as The protein functions as a ubiquitin ligase and can ubiquitinate p53

Mdm2-mediated degradation regulates the stability of p53. For many of its functions, p53 needs to be localized in the nucleus. The p53 protein involves nuclear import and export sequences, and the activity of p53 is regulated by both nuclear import and nuclear export. The mdm2 protein is able to shuttle between the nucleus and the cytoplasm [11] and it is known to bind to the p53 protein in the N-terminal region. Through binding to p53, mdm2 shuttles p53 out of the nucleus to the cytoplasm for degradation [12].

Aberrations of p53 function:

There are many ways in which the p53 function may be altered in human cancers. p53 can be inactivated indirectly through binding to viral proteins, as a result of alterations in the *mdm2* or $p19^{ARF}$ genes or by localization of the p53 protein to the cytoplasm. The most common aberration of p53 in human cancers is, however, mutation of the TP53 gene. Most of the mutations in the TP53 gene occur in the exons 4-9, the coding region for the DNA-binding central domain of the protein. A large proportion of all mutations in TP53 are single base substitutions . Of all mutations, approximately 30% occur in six codons, which are called the hotspot codons. These residues are located in the DNA-binding part of the protein, and mutations in these codons influence the protein-DNA contacts and the conformation of the protein[13]. It also seems that, in cancer cells with normal TP53 alleles, the expression or regulation of the protein is often somehow altered. Other factors that prevent normal folding

of the p53 protein, such as cadmium, may influence its DNA-binding capacity. It has therefore been suggested that all cancer cells have some aberration of p53 [3].

p53 Target Genes:

Wild-type p53 binds to specific genomic sites with a consensus binding site 5'-PuPuPuC(A/T)(T/A)GPyPyPy-3'. p53 binds as a tetramer and stimulates expression of downstream genes that negatively control growth and/or invasion or are mediators of apoptosis. It was predicted that the expression of about 200-300 genes might be controlled by p53 transactivation.

1. p53 target genes involved in p53 control:
   mdm2
2. p53 target genes involved in cell cycle control:
   p21 WAF1/CIP1, GADD45, WIP1, mdm-2, EGFR, PCNA, CyclinD1, CyclinG, TGF alpha and 14-3-3sigma
3. p53 target genes involved in DNA repair:
   GADD45, PCNA, and p21 WAF1/CIP1
4. p53 target genes involved in apoptosis:
   BAX, Bcl-L, FAS1, FASL, IGF-BP3, PAG608 and DR5/KILLER, GML, P2XM
5. p53 target genes involved in angiogenesis:
   TSP-1, BAI1
6. p53 target genes involved in cellular stress response:
   TP53TG1, CSR, PIG3

Analysis of p53 binding sites throughout the human genome suggests the existence of many more p53 regulated genes.

Other p53 dependent proteins

Poly(ADP-ribose)polymerase:

Poly(ADP-ribose)polymerase (PARP) has long been known to play a role in the recognition of DNA damage and in DNA repair. PARP is known to be involved in the regulation of p53. It has been suggested that PARP plays a positive role in the activation and upregulation of p53[14] and have shown that, in human osteosarcoma cells, p53 is poly(ADP-ribosyl)ated by

PARP. PARP has also been shown to activate DNA-dependent protein kinase (DNA-PK) activity *in vitro* and thus to regulate the activity of p53 by phosphorylation[15]. Altogether, the relationship between PARP and p53 seems to differ in different models and still needs further studies to be thoroughly understood.

Oncogenic Ras:

Mammalian *ras* genes are considered crucial in the regulation of cell proliferation. In mammals, the *ras* family consists of three genes located in different chromosomes, encoding the homologous 21 kDa proteins H-Ras, N-Ras and K-Ras. It has been estimated that 30% of all human cancers express mutated forms of *ras*[16]. The signal of Ras can have either negative or positive effects on cell growth, differentiation and death[17]. The signal is subsequently transmitted by a cascade of kinases, which results in the activation of MAPK. The Ras-MAPK pathway is apparently involved in the regulation of basal and induced levels of p53 and $p19^{ARF}$ is required for oncogenic Ras-induced accumulation of p53.

$p21^{WAF1/Cip1/Sdi1}$:

The p21 protein was the first cyclin-dependent kinase (CDK) identified. The p21 protein has multiple functions. It codes a protein that mediates p53-induced growth arrest of the cell cycle and is also a regulator of CDK activity. Yet another group demonstrated its gene expression to be induced in relation to cellular senescence. p21 can inhibit CDK-cyclin activity[18] and directly inhibit DNA replication. The gene is transcriptionally upregulated by wild-type p53. The activation of p53 causes induction of p21, which in turn inhibits CDK-cyclin activity and arrests the cell cycle at the G1 or G2 cell cycle checkpoint. This gives time for DNA repair before replication or mitosis and thus links p21 directly to the tumour suppressor function of p53 [19].

"Family members" of p53: p63 and p73 proteins

Recently, two new genes notably similar to the TP53 gene have been found. One of these genes is called *p63*, *p51* or KET, and the other *p73*. They encode proteins that share high sequence similarity and conserved functional domains with p53 and can exert p53-like functions, such as transactivation of p53 target genes and induction of apoptosis. Both give rise to differentially spliced mRNAs and, respectively, to several different proteins

homologous to p53, There are at least three different forms of the p63 protein differing at the C-terminal end (α, β and γ) that may also differ at the transactivation domain (p63TA and p63ΔDN) and six different variants of the p73 protein, p73-. The p73 protein, like p53, accumulates in response to DNA damage, and it is noteworthy that different types of inducers of DNA damage seem to affect p73 in different ways. Both p63 and p73 take part in the regulation of normal cell development and apoptosis[20]. Different forms of p63 protein can act in a dominant-negative manner towards p53, but whether p63 dysregulation has a role in tumorigenesis remains to be seen. p73, on the other hand, has been suggested to be a tumour suppressor protein, although opposite opinions have also been presented [21]. The function of p63 or p73 as a tumour suppressor still remains unclear.

P53 isoforms

A protein isoform is any of several different forms of the same protein. Different forms of a protein may be produced from related genes, or may arise from the same gene by alternative splicing. Both family members TP63 and TP73 are expressed as multiple isoforms that share a common "p53-like" DNA binding domain with a different N- or C-terminus, generated by alternate promoter and alternative splicing. Such an observation led to the search for p53 isoforms.[22,23]

The human p53 gene flaunts a relatively simple architecture. According to previous studies p53 was supposed to comprise of only one promoter, and transcribe three mRNA splice variants encoding, respectively, full length p53, p53i9[24] and Δ40p53[25].

p53i9 encoded by alternative splicing of the intron 9 was re- ported as a p53 protein isoform truncated of the last 60 amino acids of p53, lacks the p53 tetramerization domain, devoid of DNA-binding activity, defective in transcriptional activity and even was not found expressed at protein level.

Δ40p53 (also named p47 or _Np53), is an N-terminally truncated p53 isoform deleted of the first 40 amino acids. It can be generated either by an alternative splicing of intron 2 or by alternative initiation of translation but the generated protein still contains a part of the p53 transactivation domain, and it can activate gene expression after transfection through a second transactivation domain located between amino acids 43 and 63. Δ40p53 can also act, after transfection, in a dominant-negative manner toward wild type p53, inhibiting both p53

transcriptional activity and p53-mediated apoptosis. Δ40p53 can modify p53 cell localization and inhibits p53 degradation by MDM2[26].

Previous observations also reported no alternative promoter for the p53 gene.

However recent studies have suggested that the p53 gene has a complex transcriptional expression pattern encoding different p53 mRNA variants, through both the use of alternative splicing and the existence of an internal promoter in intron 4[27]. The C terminus can be alternatively spliced to produce three isoforms—p53, p53β, and p53γ—where the p53β and p53γ isoforms lack the oligomerization domain. The alternative promoter leads to the expression of an N-terminally truncated p53 protein (Δ133p53), which is initiated at codon 133.

Therefore, the human p53 gene can encode at least nine different p53 protein isoforms—

- p53, p53β, p53γ,- due to alternative splicing of intron 9
- Δ133p53, Δ133p53β, and Δ133p53γ due to alternative splicing of intron 9 and usage of the alternative promoter in intron 4, and
- Δ40p53 due to alternative splicing of intron 2 and alternative initiation of translation.

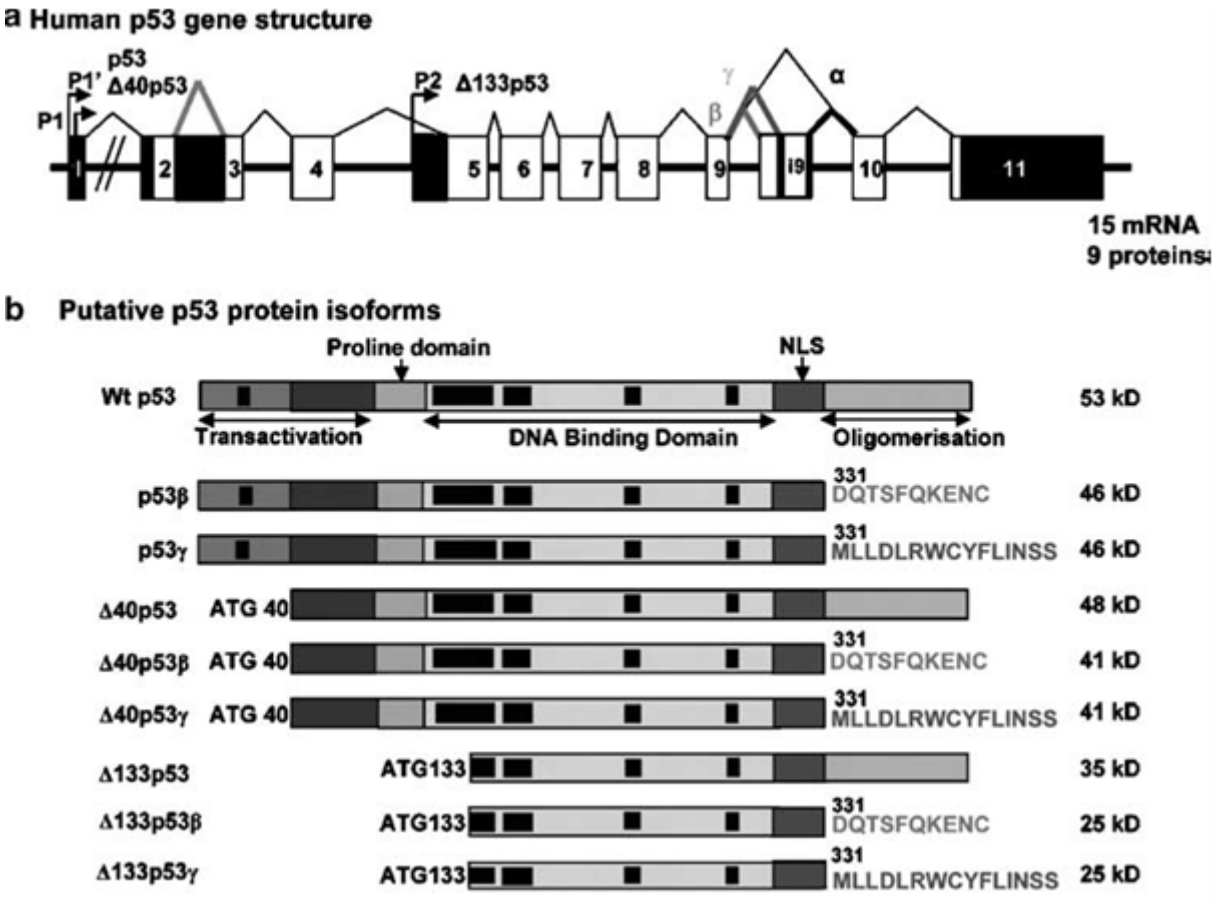


Figure.3: Human p53. (a) Schema of the human p53 gene structure: alternative splicing (a, b, g) and alternative promoters (P1, P10 and P2) are indicated. (b) p53 protein isoforms: p53, p53b and p53g proteins encoded from P1 or P10 promoters contain the conserved N-terminal domain (FxxcW) of transactivation (TA). D133p53 isoforms encoded from promoter P2 are amino-truncated proteins deleted of the entire transactivation domain and deleted of part of the DNA binding domain. Translation is initiated at ATG-133. D40p53 protein isoforms encoded from P1 or P10 promoters are amino-truncated proteins due to alternative splicing of exon 2 and/or alternative initiation of translation at ATG-40). D40p53 protein isoform have lost the conserved N-terminal domain of transactivation (FxxcW) but still contain part of the transactivation domain [F Murray-Zmijewski, et.al].

All p53 isoforms are expressed in normal tissue at the mRNA level. Moreover, the pattern of expression is tissue specific, indicating that their expression can be selectively regulated. The endogenous expression of p53 protein isoforms cannot be accessed by any existing p53 antibodies as they cannot distinguish isoforms from full-length p53 forms that have been subject to post-translational modification including phosphorylation, acetylation, methylation,

ubiquitination, sumoylation, and neddylation or protein degradation[28]. p53 protein isoforms are less abundant than full-length p53 protein. It is thus unclear what sub-fractions of total p53 are active in the different biochemical and biological functions of p53 (gene expression, cell cycle arrest, senescence, apoptosis, differentiation, replication, control of chromosome segregation, mitosis, meiosis, DNA repair, etc.). Moreover, p53 abundance is not necessarily associated with p53 transcriptional activity. Endogenous p53β and p53 proteins bind differentially to promoters. p53β binds preferentially to BAX and p21 promoters rather than the MDM2 promoter, while p53 binds preferentially (five times more) to p21 and MDM2 promoters than to the BAX promoter. This suggested that p53β could interfere with p53 transcriptional activity on the BAX but not the p21 promoter. p53β interferes with p53 transcriptional activity by forming a protein complex with p53. The p53/p53β complex may bind preferentially to specific p53-responsive elements, and therefore only a subset of p53-inducible promoters will be responsive to p53β. As p53 isoforms can bind differentially to promoters, can modulate p53 target gene expression and p53-mediated apoptosis, the differential expression of the p53 isoforms in human breast tumors could explain the difficulties in many clinical studies to link p53 status to the biological properties and drug sensitivity of human cancer. Furthermore, it suggests that failure of appropriate regulation of expression of p53 isoforms may have a role in tumor formation since attenuation of the wild-type p53 response would render the cells more susceptible to further genetic damage and therefore neoplastic transformation and tumor progression. It will be essential to extend our study to a larger series of tumors. In conclusion, the structure of the *p53* gene is similar to the p63 and p73 genes, revealing an unforeseen complex regulation. The interplay between p53 isoforms and p53 on specific targets may play a major role in controlling the activity of p53-related proteins.

Biochemical and biological functions of p53 isoforms

p53 isoforms differ by the absence of structural and functional domains that may alter biochemical properties known to be essential for p53 suppressive functions. Only one motif is common to all isoforms, the portion of the DNA-binding domain (residues 133–257), which is stabilized by a Zinc atom and provides the structural motif that binds in the minor groove of DNA[29]. In theory, p53 isoforms can exert their effects by two mechanisms: autonomous functional properties, different from those of p53, and/or modulation of p53 activity. All p53 isoforms discussed above appear to conserve some DNA-binding activity. The lack of

transcriptional activity is likely due to their poor capacity to recruit components of the transcriptional machinery onto promoters, either because of the lack of a suitable protein interaction domain (e.g. ΔNp53 and Δ133p53) or to the lack of domains essential for high DNAbinding affinity (e.g. Δp53: core domain; p53b: oligomerisation and/or regulation domains). How-ever, it cannot be ruled out that they may exert effects towards genes different from the known p53 target genes [30].

Δp53 has no detectable impact on p53 transcriptional activity. Δp53 is thus able to act as an inhibitor of p53, but only if Δp53 is forced to relocalize into the nucleus. Δp53 is not able to form oligomers with p53 but retains DNA-binding capacity, suggesting that the inhibitory effect results from competition between p53 and Δp53 for promoter binding ΔNp53 isoform can also inhibit p53 transcriptional activity. Transfected ΔNp53 may also affect p53 activity by altering its cellular localization, as exogenous ΔNp53 accumulated in the cytoplasm and induced the cytoplasmic re-localization of p53, thereby decreasing its transcriptional activity. ΔNp53 may work as an inhibitor of p53 suppression by two complementary mechanisms, depending upon stress context. In non-stressed cells, ΔNp53 may work in the nucleus as a competitive inhibitor of p53 to prevent transactivation. In stressed cells, accumulation of p53 may favor formation of hetero-oligomers with ΔNp53 that are exported from nucleus into cytoplasm, preventing p53 from regulating gene expression. In contrast with the above isoforms, p53b presents the capacity to increase p53 transcriptional capacity. Even if some intrinsic biochemical capacities have been attributed to p53 isoforms, most of the studies report their biochemical role towards p53 transcriptional activity either as inhibitory regulators (ΔNp53, Δ133p53 and Δp53) or as enhancer (p53b). In both instances, the biochemical mechanisms include DNA binding modulation, hetero-oligomers formation and/ or p53 sequestration in the cytoplasm. These three mechanisms may be dependent on the isoform, on the cellular context and also on the target gene considered, leading to a wide diversity of regulatory options, either positive or negative, by p53 isoforms. In this way, one of the most important factors is the relative expression level of the isoforms as compared to p53 itself, which may vary from one tissue or cell type to another.

Mutations affecting p53 splicing patterns.

Several studies reported that intronic or exonic mutations gave rise to particular truncated p53 proteins with strong similitude to p53 isoforms. A deletion of 8 bp was identified in

intron 9 leading to an insertion of 133 additional nucleotides derived from intron 9 this transcript corresponds to p53β RNAand we can speculate that the 8 bp deletion may favor the use of the alternative splice site producing p53β In one case of colorectal cancer, a substitution of A to G in the 3 part of intron 9 led to the expression of a p53 protein, which conserves residues 1 to 332 but presents new residues from 332 to 358[31]. This truncated protein presents some similitude with p53b but has a longer Cterminal domain. On the other hand, mutations sometimes occur at splice junctions, leading to the production of aberrant p53 truncated protein. In one study of chronic lymphocytic leukemia, 85% of patients expressed a Dex6 p53 transcript, which lacks the first 113 nucleotides of exon 6 leading to a premature stop codon at residue 189 that results in a C-truncated p53 product. This transcript was not found in healthy patients and no mutation on TP53 was detected, suggesting a deregulation of splicing giving rise to the production of a new p53 isoform. In Li-Fraumeni syndrome, defined as family carrying germline TP53 mutations, three atypical mutations have been described, all affecting acceptor or donor splicing sites [32] . For example, mutation of 5_ acceptor splice site in intron 3 resulted in the skipping of exon 4, suggesting that these cells were unable to produce p53 and ΔNp53.

Biological integration of the p53 isoforms Network

The diversity of processes leading to their production is a clue to the functionalsignificance of p53 isoforms, as cells have adopted number of strategies to produce them in different regulatory contexts. Second, most p53 isoforms are deficient for p53-like suppressive function but appear tomodulate p53 activity [33, 34, 35].Third, N-truncated p53 isoforms escapeHdm2-mediated degradation and may provide a regulatory system for controlling p53 activity independently of Hdm2 [36]. Fourth, patterns of p53 isoform appear to be deregulated in different cancer types and may provide an alternative mechanism to inactivate p53 suppressive function in wild-type TP53 tumors or to modulate mutation penetrance and phenotype in mutant TP53 tumors The complex effects of p53 isoforms on p53 response may have significant consequences for cancer development. Aside from a possible involvement in individual susceptibility, isoform expression may contribute to p53 inactivation in cells retaining wild-type TP53 alleles. However, based on the hypotheses detailed above, the biggest impact of isoforms may be on p53-dependent responses to cytotoxic therapies, as the capacity of cancer cells to undergo drug-induced apoptosis may be largely dependent upon p53 isoform expression patterns. ΔNp53, a “buffer” for regulating p53 response is more stable

than p53 as it escapes to Hdm2-degradation and is not activated by a stress as compared to p53. As DNp53 inhibits p53 transcriptional activity, a p53 response would be observed only when the quantity of stabilized p53 is higher than the quantity of ΔNp53. A change in p53 ratio would induce a decrease of p53 response. Epressing high levels of ΔNp53 or Δ133p53 may have poor apoptotic responses, whereas cells expressing high levels of p53b may be very good responders to treatment. Thus, studies on p53 isoforms will provide a rich field of new concepts and paradigms in the regulation of p53 activities.

p53 & Its Interaction with Other Genes

p53 and p300:

P300 are transcriptional co-factors and histone acetyl transferases that play a significant role in the p53 pathway. P300 has been found to both stabilize [37] and help in the degradation of p53[38]. p300 can forma complex withMDM2*in vitro* and *in vivo,* a negative regulator of p53, and this complex was shown to facilitate MDM2-mediated p53 degradation, on the other hand p300 was also shown to stabilize p53 after DNA damage.

Acetylation by p300 at lysine residues at C terminus occuring after DNA damage leads to activation of sequence-specific DNA binding of p53 and transcriptional activation, helps p53 to escape ubiquitination which share the same site, and thus escape degradation[39]. Inhibition of p300-mediated p53 acetylation by MDM2 would result in loss of sequence specific DNA binding and more efficient ubiquitination, consequently leading to the degradation of p53[40]. Phosphorylation of p53, at specific Lysine residues, stimulated by UV and γ irradiation, prevents MDM2 binding which prevents phosphorylation of p53 and recruits p300 which stabilizes p53.

P53 and DNMTs:

DNA methylation is the predominant epigenetic mechanism in regulation of gene expression. Epigenetic instability due to an imbalance of hypermethylation and global hypomethylation is a general feature of cancer cells and occurs early during cellular transformation[41]. The importance of DNA methylation in tumorigenesis has been demonstrated in cancer cells, which harbor global genomic DNA hypomethylation and regional hypermethylation at CpG islands of tumor suppressor genes. DNA methylation is mediated by a class of DNA

methyltransferases (Dnmts) involved in de novo methylation of genomic DNA and in the maintenance of DNA methylation patterns during replication. Global genomic DNA demethylation induced by 5-Aza-deoxycytidine activates the p53 signaling pathway and induces apoptosis, suggesting that DNA methylation mediated by Dnmts is associated with p53 signaling in maintaining genome stability. Although one mechanism for tumor promotion is the overexpression of Dnmts, which brings about hypermethylation of the transcriptional regulatory regions of tumor suppressor genes, Dnmts have also been shown to interact with transcriptional corepressors thereby suppressing transcription.

p53 and Dnmt1 cooperate to repress survivin gene expression. Activation of p53 leads to down-regulation of *survivin*, a member of the inhibitor of apoptosis (IAP) family .Induction of p53 leads to transcriptional and translational repression of *survivin in vivo* p53 recruits Dnmt1 and stimulates Dnmt1-mediated DNA methylation of the survivin promoter cooperation between DNMT1 and p53 is essential for *survivin* gene regulation, either through methylation-dependent or -independent pathways. A plausible model of this repressor complex would require DNA damage-mediated recruitment of HDAC1–DNMT1–p53 complexes to the CpG-rich *survivin* promoter, a preferred site for DNMT1 binding because of high density of CpGs. Presence of p53 may stabilize the HDAC1–DNMT1–p53 complex on the *survivin* promoter, either by direct or indirect contacts with DNA by means of methylated histones [42, 47].

Dnmt3a interacts with p53 directly and represses p53-mediated transactivation of the p21 gene. It was found that trans-repression by Dnmt3a does not require the methyltransferase activity implying that transcriptional repression does not involve promoter silencing through DNA methylation by Dnmt3a. Whether the interaction of Dnmt3a with p53 interferes with the oligomerization of p53 remains to be determined. The identification of p53 interaction with Dnmts may have some clinical relevance. Since Gadd45 is one of the downstream targets of p53, it will be of interest to determine whether the hypermethylation of Gadd45 promoter is due to recruitment of Dnmt3a or other Dnmts by p53 in HCC [43].

P53 and HDACs:

HDAC1 may also aid in survivin repression through interaction with p53 by means of the Sin3a repressor complex or by directly binding to DNMT1 Induction of p53 leads to transcriptional and translational repression of *survivin in vivo* [44].

P53 and GADD45:

The tumor suppressor p53 gene has been implicated in the control of cell cycle checkpoint in response to genotoxic stress. The role for p53 in G1-S arrest is clearly shown to be mediated though p21. However, the role of p53 in the control of the G2-M arrest is under debate and remains to be further elucidated. It is postulated that as one of the p53-targeted genes, *GADD45* might be a strong player in mediating p53-regulated cell cycle G2-M checkpoint. Previous studies have shown that Gadd45 protein interacts with Cdc2 and dissociates the Cdc2/cyclin B1 complex Subsequently, 'free' cyclin B1 protein dissociated from the Cdc2 complex is more likely pumped out from the nucleus, probably by the nuclear transport system. As a result of exclusion of cyclin B1 protein from the nucleus, Cdc2 kinase activity is inhibited and followed up by the cell cycle G2-M arrest. This goes along with the finding by that DNA damage causes increased nuclear export of cyclin B1 and in turn arrests cells at the G2-M transition. Our observations that inducible expression of *GADD45* protein alters cyclin B1 nuclear localization have suggested that exclusion of nuclear cyclin B1 protein by Gadd45 might be an essential step for the *GADD45*-induced G2-M arrest. Therefore, the findings in this work have further presented the precise evidence that the p53-*GADD45* pathway is well involved in the control of G2-M arrest.

Both p21 and MDM2 appear not to be the candidates to mediate the role for p53 in *GADD45*-induced G2-M arrest. Independence from p38 kinase activity suggest that Gadd45 acts at the late G2-M transition or early mitotic phase, instead of at the initiation of G2-M transition. Overexpression of cyclin B1 protein has been found in certain types of human tumors although the biological function of this overexpressed protein in tumorigenesis remains unclear [45].

OBJECTIVE

As it is suggested that failure of appropriate regulation of p53 isoforms may have a role in tumor formation, therefore the objective of our work is to check if any of the isoforms express in any cancer tissue which contain an intact, unmutated p53 gene.

MATERIALS AND METHODS

1. Collection of Samples:

Blood was collected as the normal tissue from the local CWS Hospital, Rourkela, Odisha, stored in ice and immediately processed for better RNA extraction. Cancer tissue (Lymph Node Carcinoma) was collected from National Medical College, Kolkata and stored in RNA later (Sigma) at -20^0C until the extraction of RNA.

2. Extraction of Total RNA:

Total RNA was extracted from both blood (normal) and cancer tissue using GeneJET^{TM} RNA Purification Kit (Fermentas).

2.1. For extraction from Blood:

The collected blood was centrifuged at 3000 rpm for 15 mins at 4° C. The supernatant containing the serum was separated from the pellet which contains the blood cells. The pellet was resuspended in 600 µl of Lysis Buffer (supplemented with 20 µl of 14.3 M β-mercaptoethanol for every 1ml of Lysis Buffer) and vortexed to mix thoroughly. 450 µl of ethanol (96-100%) was mixed with the solution. About 700 µl of the lysate was transferred to a GeneJET^{TM} RNA Purification Column inserted in a collection tube and centrifuged at 12000 rpm for 1 min at 4° C. The flow-through was discarded and the column was placed into a new 2 ml RNase-free microcentrifuge tube. 700 µl of Wash Buffer 1(supplemented with 250 µl of ethanol for every 1ml Wash buffer 1) was added to the column and centrifuged for 1 min at 12000 rpm. The flow-through was discarded and 600 µl of Wash Buffer 2 (supplemented with 850 µl of ethanol for every 0.5 µl Wash buffer 2) was added to the column. It was centrifuged at 12000 rpm for 1 min at 4° C. The flow-through was again discarded. Centrifugation was again done at 12000 rpm for 1 min at 4 ° C by adding 250 µl of Wash buffer 2. The flow-through was discarded and the column was transferred to a sterile 1.5 RNase-free microcentrifuge tube. 100 µl of nuclease-free water was added to the column and centrifuged for 1 min at 12000 rpm to elute RNA. The RNA was stored at - 20° C for further use or immediately processed for cDNA synthesis

2.2. For Extraction from Cancer tissue:

About 30 mg of frozen cancer tissue was taken and thoroughly homogenized using Lysis buffer. The grinded tissue was transferred into a sterile 2 ml microcentrifuge tube containing 300 µl of Lysis Buffer (supplemented with 20 µl of 14.3 M β-mercaptoethanol for every 1ml of Lysis Buffer). The mixture was vortexed for 10 secs for thorough mixing. The next steps of extraction were same as that followed in the previous protocol for blood RNA extraction.

3. Quantitative Estimation of RNA Concentration by Spectrophotometric Analysis:

The concentration of the extracted total RNA from both blood and cancer tissue was quantified by measuring the absorbance at 260 nm in a spectrophotometer (ELICO, BL 200 Bio Spectrophotometer, double beam) and calculated by using the formula as given below:

$$\text{Total RNA (µg /ml)} = OD_{260} \times 40 \times \text{Dilution factor.}$$

4. Quantitative Estimation of RNA Concentration by Denaturing Gel Electrophoresis:

The extracted RNA from both blood and cancer tissue was run on a denaturing agarose gel and the quantity of RNA estimated from the band intensity. For denaturation gel (40 ml), 0.6 g agarose (Sigma), 28.8 ml dH_2O (Sigma), 7.2 ml formaldehyde (Sigma), 4 ml 10X MOPS buffer were mixed properly. About 2 µl (2µg) of the total RNA was mixed with 18 µl 1X Reaction Buffer (2µl of 10X MOPS Buffer, 4 µl formaldehyde, 10 µl formamide (Sigma) ,2 µl 0.2 mg/ml Etbr (Sigma)) and incubated at 55 °C for 1 hr. It was then cooled on ice and loaded in the wells of the denaturing gel.

5. First strand cDNA synthesis:

Total RNA (4 µg) from both blood and cancer tissue were used for first strand cDNA synthesis by reverse transcription using RevertAid^{TM} First Strand cDNA Synthesis Kit (Fermentas) in a thermocycler (Biorad). The RNA were incubated with 1 µl of oligo$(dT)_{18}$ primers (100 µM, 0.2 µg/µl) and 12 µl of nuclease-free water at 65 °C for 5 min. The reaction

was cooled on ice to allow the primers to anneal to the RNA, then spin down and placed on ice again after which the following components were added to the reaction in order; 4 µl of 5X Reaction Buffer, 1 µl of Ribolock™ RNase inhibitor (20 U/µl), 2 µl of 10 mM dNTPs and 1.0 µL of RevertAid™ M-MuLV-Reverse Transcriptase (200 U/µl). The reagents were gently mixed and incubated for 1 hr at 42°C. Heating at 70°C for 5 min terminated the reaction and the synthesized cDNA was stored at –20 ^{0} C for further use.

6. Gene-specific PCR for amplification of the desired gene:

6.1. Primers selection:

A set of specific forward and reverse primers for the amplification of the desired gene under study was selected from published papers (Boldrup L.,Christophe J.B.et al 2007). The cDNA of both the blood and cancer tissue synthesized were used as the template for the specific primers. The constitutively expressed housekeeping gene, β-actin was used as a positive control to ensure high quality. The primer sequences used for the PCR reaction are shown in Table 1:

Table 1.Table showing the sequence of the forward and backward primers used.

PRIMER	TYPE	SEQUENCE
p53	***Forward***	5'-GTCACTGCCATGGAGGAGCCGCA-3'
	Reverse	5'-GTCACTGCCATGGAGGAGCCGCA-3'
p53β	***Forward***	5'-GTCACTGCCATGGAGGAGCCGCA-3'
	Reverse	5'-GTCACTGCCATGGAGGAGCCGCA-3'
β-ACTIN	***Forward***	5' TCTACAATGAGCTGCGTGTG 3'
	Reverse	5' TCTCCTTCTGCATCCTGTC 3'

Boldrup L.,Christophe J.B.et al 2007[48]

6.2. PCR conditions:

The PCR sample mixtures, in a 25 µl volume, contained 17 µl of dH_2O (Sigma), 2.5 µl of 1X PCR buffer (Sigma), 0.5 µl of dNTP (0.2 mM, Sigma), 1.5 µl of $MgCl_2$ (1.5 mM, Sigma), 0.5 µl each of the forward and reverse primers (0.2 µM, Sigma) p53 AND p53β and 0.5 µl Taq DNA-polymerase (1U/µl, Himedia). 2 µl of each cDNA sample was added. PCR amplifications of p53 and p53β were performed in a thermal cycler by initial denaturation at

94° C for 1 min, followed by 30 cycles of denaturation at 94° C for 1 min, annealing at 94 ° C for 30 secs, and extension at 60° C for 45 secs, followed by an final extension step at 72° C for 5 mins.

7. Agarose Gel Electrophoresis of the PCR products:

The generated PCR products were analyzed by electrophoresis on 1.5% agarose gel. Agarose gel was prepared with 1X TAE (Tris Acetate EDTA, Sigma) buffer. Before casting ethidium bromide(1µl of 10mg/ml stock in 30 ml) was added to the gel. 15 µl of sample (PCR product) was loaded to each well along with 3 µl 1 X loading dye. 5 µl of DNA marker (1 kb, Sigma). The gel was run in TAE buffer at 100 volt for 40 minutes.

8. Analysis of the Relative Expression level of the different genes:

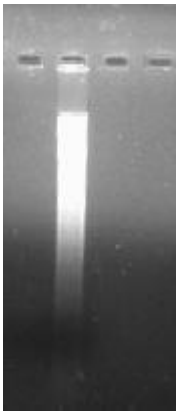
The relative levels of expression of each gene were analyzed by taking the absorbance through spectrophotometric readings. The ratios of desired genes/β-actin product were subsequently calculated after subtraction of the background pixel intensity for each gene of interest and used to assess the differences in expression levels between normal and cancer tissue.

RESULTS AND DISCUSSION

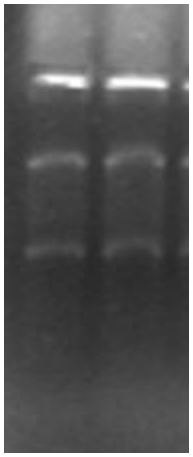
For Normal (Blood) Tissue:

Product	**Conc^{n}.** (µg/ml)	**Purity**	
		260/280	**260/230**
Total RNA	1570.32	1.34	0.82

Table.1: Spectrophotometer results of total RNA from blood tissue



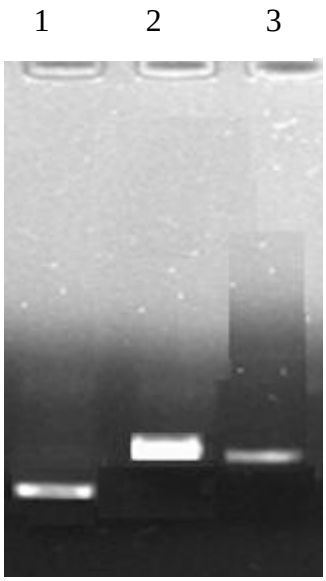
[Total RNA in 1% agarose gel]



[Total RNA in denaturation gel]

Gene	**Conc^{n}.** (µg/ml)	**Purity**	
		260/280	**260/230**
p53	2058.58	1.83	0.89
p53β	2608.23	2.01	0.86
B-Actin	1302.35	1.93	1.02

Table.3: Spectrophotometer results of gene specific amplification product from blood



Lane.1: β-Actin

Lane.2: p53

Lane.3-p53β

[Gene Specific PCR Amplification]

For Cancerous Tissue (Lymph Node):-

Tissue	**Conc^{n}.** (µg/ml)	**Purity**	
		260/280	**260/230**
Gall Bladder Cancer	234.67	1.03	0.65
Lymph Node Cancer	1478.51	1.61	1.02

Table.3: Spectrophotometer results of total RNA from cancerous tissue

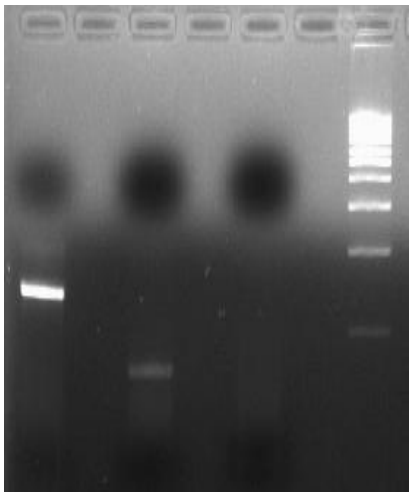
Lane.1: Lymph Node Cancer

Lane.2: Gall Bladder Cancer

[Total RNA in denaturation gel]

Gene	Conc^{n}. (µg/ml)	Purity	
		260/280	260/230
DNMT3A	168.23	1.74	1.28
HDAC	1746.0	0.93	0.19
GADD45	1764.0	0.96	0.21
p300	1864.0	1.00	1.24
p53	1474.0	1.05	1.36
p53β	2440.0	1.02	0.18
B-Actin	1282.0	0.93	0.71

Table.5: Spectrophotometer results of gene specific amplification product from lymph node cancer tissue



[Gene Specific PCR Amplification]

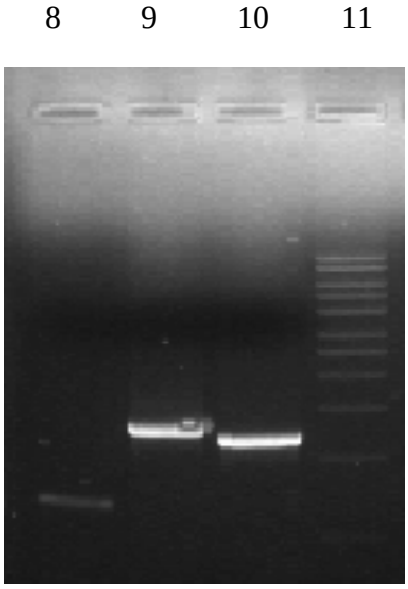
Lane.1 & 8: β-Actin

Lane3: HDAC

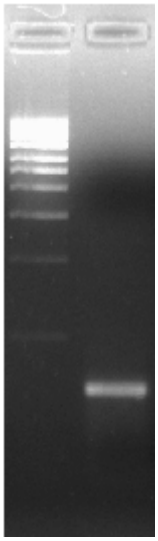
Lane.9: p53

Lane.10: p53β

Lane.7 & 11: Marker



[Gene Specific PCR Amplification]

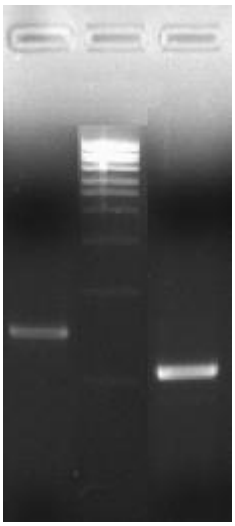


Lane.1 & 4: Marker

Lane.2: p300

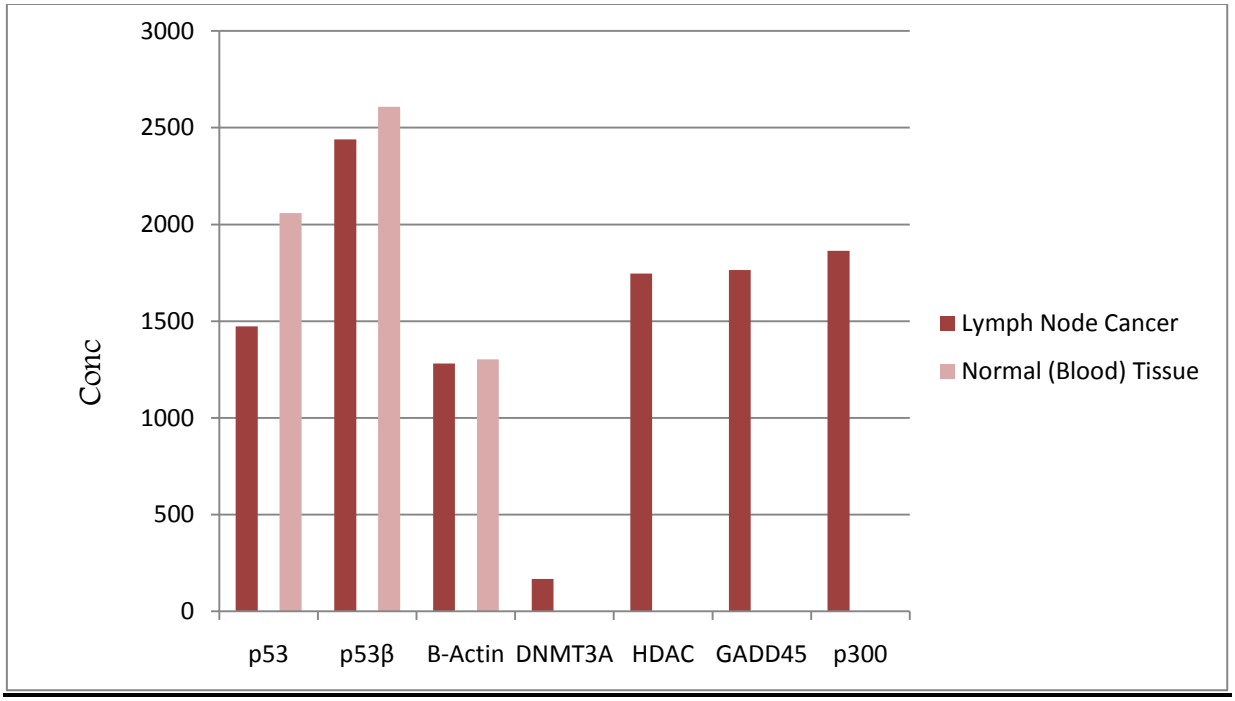
Lane.3: β-Actin

Lane.5: GADD45



[Gene Specific PCR Amplification]

[Gene Specific PCR Amplification]



[Comparative Study of Gene Amplification Product By Spectrophotometry Analysis]

P53 and p53i:

P53 being the frequently mutated gene event, leading to tumorigenesis is often found expressed at low or minimal levels in various cancers. In this study of lymp node carcinoma

both p53 and p53β are found to express fairly, indicating some other mechanism or mutation in some other pathway dependent upon p53 expression.

Or it may be that expression of p53β modulates the activity of p53. P53β has been seen to change the transcription activity of p53 and also forms complex with p53 and binds differentially to p53 responsive promoters. Since we have extracted the mRNA and then carried out cDNA synthesis, it is clear that p53 is active at the transcriptional level. So it may be that expression of p53β changes the activity of p53 by binding differentially to p53reponsive promoters.

P53 expression in cancer cells may be explained by interactions between p53 and p53β and other target genes. Some such interactions may be:

p53 and p300:-

P300 being a histone actyltransferase, has twin action on p53. It has been previously reported that it can either stabilize or help in the degradation of p53. Expression of both p300 and p53 in the lymph node carcinoma sample, suggests that p300 acts to stabilize the p53 by Acetylation. Stable p53 is expected to promote apoptosis and prevent tumor formation. But p53β in conjugation with p53 might be changing the activity of p53 at the respective promoters.

p53 and GADD45:-

As previously stated GADD45 is a p53 target protein which mediates p53 regulated G2-M checkpoint. Expression of GADD45 in lymph node carcinoma sample suggests active cell cycle regulation through the expressed p53. This anomalous behaviour of GADD45 and p53 may be due to the expression of p53β isoform which alters the interaction of p53 and GADD45.

P53 and DNMT 3a:-

DNMT3a represses the downstream activator p21 of p53 dependent cellcycle arrest pathway, expression of DNMT3a in lymph node carcinoma suggests down-regulation of p21 and hence unrestricted cell divivsions leading to tumorigenesis. This is in aggrement with the previous proposition that DNMT3a may lead to tumor formation by interfering with p53 pathway.

However, it is still not clear if it prevents p53 oligomerization.from this we can conclude that p53β does not change the DNMT3a mediated p53 target repression.

Moreover, expression of GADD45, a p53 target protein in the cancer sample suggests that, DNMT3a does not cause the hypemathylation of its promoter.

P53 and HDAC:-

As reported, p53 in combination with HDAC helps in the repression of survivin, an inhibitor of apoptosis gene either by a methylation dependent or independent mechanism. Expression of both p53 and HDAC in absence of DNMT1 may lead to repression of surviving in methylation independent mechanism. Such pro-apoptotic environment does not lead to tumor formation. Therefore it may be that p53β expression alters its interaction with HDAC, inactivating surviving repression and thus promoting tumor.

CONCLUSION

p53 being a tumor suppressor gene is expected to be not expressed by cancer cells. But the lymph node carcinoma cells show the expression of p53as well as its truncated isoform p53β. This indicates that some other mutation or aberration may be involved in cancer formation which may be dependent on p53 expression.

Moreover interactions of p53 with other cancer related genes show that, p53 isoform p53β, influences such interactions, often showing abnormal behaviour of tumor suppressor gene. As it has been stated previously that p53β can influence the p53 transcriptional activity or form a complex with p53, thereby modulating its interactions with other genes.

FUTURE PROSPECTS

The influence of p53 isoform p53β, on its transcription as well as its interactions with other target genes causing cancer is subject to further evaluation through RT-PCR studies, which give a quantitative estimation of the relative levels of expression, immunoblot analysis to confirm the nature of the isoforms as well as different knock out gene methods to ascertain the role of each p53 isoform.

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