Isoform p53 Protein's Major Role in the Pathophysiology of Malignant Hematologic Diseases

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Aurelian Udristioiu,
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Cambridge
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This book first published 2024

Cambridge Scholars Publishing

Lady Stephenson Library, Newcastle upon Tyne, NE6 2PA, UK

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

ISBN (10): 1-5275-7243-9
ISBN (13): 978-1-5275-7243-0

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FOREWORD

The book "**Isoform p53 Protein's Major Role in the Pathophysiology of Malignant Hematologic Diseases**" is concerned with the identification of isoform p53 protein in malignant hematologic diseases and its impact on the clinical management of patients.

This research book presents an extensive study of malignant B cells, with presented isoform p53 protein, in Chronic Lymphocytic Leukemia (CLL). Cases of CLL were analyzed with the ELISA system to evaluate the relationship between isoform p53 protein and disease status, and the possible impact of this protein on responses to treatment and the survival of patients.

The book also provides important benchmarks in the field, studying the hematological and metabolic problems of the human body. The book details advances made over the past decade in cancer metabolism research that have increased the understanding of aerobic and anaerobic glycolysis and other metabolic changes associated with cell growth and proliferation by altering cell signaling and blocking their differentiation in malignancies.

The book, which has original content, deals not only with theoretical aspects, but also with practical aspects, highlighting the need for new practices in the biological sciences. It takes into account the progress in the pathophysiology and biochemistry of oncology over the years.

The new book, published by Cambridge Scholars Publishing, Great Britain, may be of interest to doctors of all specialties, medical students, as well as staff working in clinical laboratories, from different countries. It presents a large volume of data, experiments and laboratory research in a systematic and logical display.

Aurelian Udristioiu

ACKNOWLEDGEMENT

Dr Cristiana Tanase, Titu Maiorescu University of Bucharest, Faculty of Medicine, Professor Molecular Biology, Clinical Biochemistry and Research Management, PhD theses coordinator, MD, PhD.

CHAPTER ONE

INTRODUCTION TO THE FUNCTION OF THE P53 GENE AS A TUMOR SUPPRESSOR GENE

The p53 gene is a tumor suppressor gene and its activity stops the formation of tumors. If a person inherits only one functional copy of the p53 gene from their parents, they are predisposed to cancer and usually develop several independent tumors in a variety of tissues in early adulthood. Mutations in the p53 gene are found in most tumor types, and so contribute to the complex network of molecular events leading to tumor formation.

The International Cancer Genome Consortium has established that the p53 gene is the most frequently mutated (>50%) in human cancer, indicating that the p53 gene plays a crucial role in preventing cancer formation. The p53 gene encodes proteins that bind to DNA and regulate gene expression to prevent mutations in the genome.

Warren Maltzman, of the Waksman Institute of Rutgers University, first demonstrated that p53 was responsive to DNA damage in the form of ultraviolet radiation. In a series of publications in 1991–1992, Michael Kastan, Johns Hopkins University, reported that TP53 was a critical part of a signal transduction pathway that helped cells respond to DNA damage. In 1993, p53 was voted molecule of the year by Science magazine, USA.

The p53 gene has been mapped to chromosome 17. In the cell, its product, the p53 protein, binds DNA, which in turn stimulates another gene, CDKN, to produce a protein called p21 that interacts with a cell division-stimulating protein (cdk2).

When the p21 protein is complex with the cdk2 protein, the cell cannot pass through to the next stage of cell division. Therefore, the isoform p53 protein as a result of the p53 mutated gene can no longer effectively bind

DNA, and as a consequence, the p21 protein is not made available to act as the ‘stop signal’ for cell division.

Although the number of genes activated by the p53 gene is rather large, the outcome of p53 protein activation is either cell cycle arrest in G1 (by p21) or apoptosis (by BAX, PUMA or NOXA). The cell growth arrest activity of p53 protein allows the activation of the DNA repair system of the cell.

The amount of information that exists on all aspects of p53’s normal function and mutant expression in human cancers is now vast, reflecting its key role in the pathogenesis of human cancers. Mutant p53 genes are just one component of a network of events that culminate in tumor formation.

p53 gene alterations have been found in: 20-30% of chronic myeloid leukemia (CML) blast phases (or blast crises), often associated with 17q chromosome deletion; in 15% of Burkitt lymphomas; in 40% of aggressive chronic lymphocytic leukemia (CLL) cases, with transformation into Richter's syndrome; in 5-10% of multiple myelomas; in 5% of myelodysplastic syndrome (MDS) cases; in 60-80% of Hodgkin disease cases; and in 30% of high-grade B-cell non-Hodgkin lymphoma cases. p53 gene alterations in hematological malignancies are associated with a poor prognosis.

Inherited changes in the p53 gene greatly increase the risk of developing forms of cancer, as part of a rare cancer syndrome called Li-Fraumeni syndrome (LES). Most of these mutations change single protein building block amino acids in the p53 protein, which reduces or eliminates the protein's tumor suppressor function. Because the altered protein is less able to regulate cell growth and division, DNA damage can accumulate.

This damage may contribute to the development of a cancerous tumor by allowing cells to grow and divide. Although somatic mutations in the p53 gene are found in many types of cancer, LES appears to be the only cancer syndrome associated with inherited mutations in this gene.

This condition greatly increases the risk of developing several types of cancer, particularly in children and young adults. At least 140 different mutations of the p53 gene have been identified in individuals with LES.

Many of the mutations associated with LES change single amino acids in the part of the p53 protein that binds to DNA. Other mutations delete small amounts of DNA from the gene. Mutations in the p53 gene lead to a version of the p53 protein that cannot regulate cell growth and division effectively. Specifically, the altered protein is unable to trigger apoptosis in cells with mutated or damaged DNA. As a result, DNA damage can accumulate in cells. Such cells may continue to divide in an uncontrolled way, leading to the growth of tumors [1].

CHAPTER TWO

NORMAL FUNCTION OF THE P53 GENE

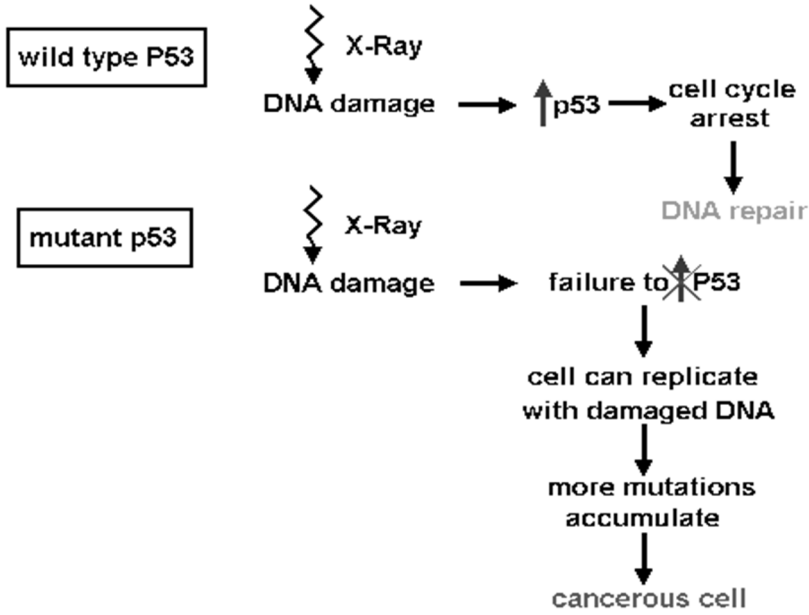
1.1. p53 protein discovery

The p53 protein (p53) was first identified in 1979 by Lionel Crawford, David P. Lane, Arnold Levine, and Lloyd Old, working at the Imperial Cancer Research Fund (UK), Princeton University, UMDNJ (Cancer Institute of New Jersey), and the Memorial Sloan-Kettering Cancer Center, respectively. Independently, it was also identified by Michel Kress, Pierre May, José A. Melero and Varda Rotter. It had been hypothesized to exist beforehand, as the target of the SV40 virus, a strain that induced the development of tumors. The human p53 gene was cloned in 1984.

The name p53 was given in 1979 to describe the apparent molecular mass; SDS-PAGE analysis indicated that it was a 53 kilodalton (k Da) protein. However, the actual mass of the full-length p53 protein (p53α), based on the sum of masses of the amino acid residues, is only 43.7 k Da. In addition to the full-length protein, the human p53 gene encodes at least 15 protein isoforms, ranging in size from 3.5 to 43.7 k Da. All these p53 proteins are called the p53 isoforms**.** In humans, the p53 gene is located on the short arm of chromosome 17 (17p13.11) [2].

Researchers have extensively studied factors known to damage DNA, such as UV light and tobacco smoke, along with the cellular mechanisms of DNA repair.

Cells also naturally accumulate a certain number of mutations with each division. If all mammalian cells were equally susceptible to mutations that could lead to cancer, then cancer risk should increase with the greater number of cells, species life span and greater number of cell divisions **[Scheme 1]**.



Scheme 1. *Potential path of mutant p53 genes leading to cancerous cells.*

However, researchers found no significant relationships between cancer risk and body size, life span, or basic metabolic rate among the species. For elephants, they estimated that the overall lifetime chance of dying from cancer was less than 5%. The lifetime cancer mortality rate for humans, by contrast, is about 20%. To gain insight into how elephants avoid cancer, researchers looked at elephant genomes, with a focus on the p53 gene.

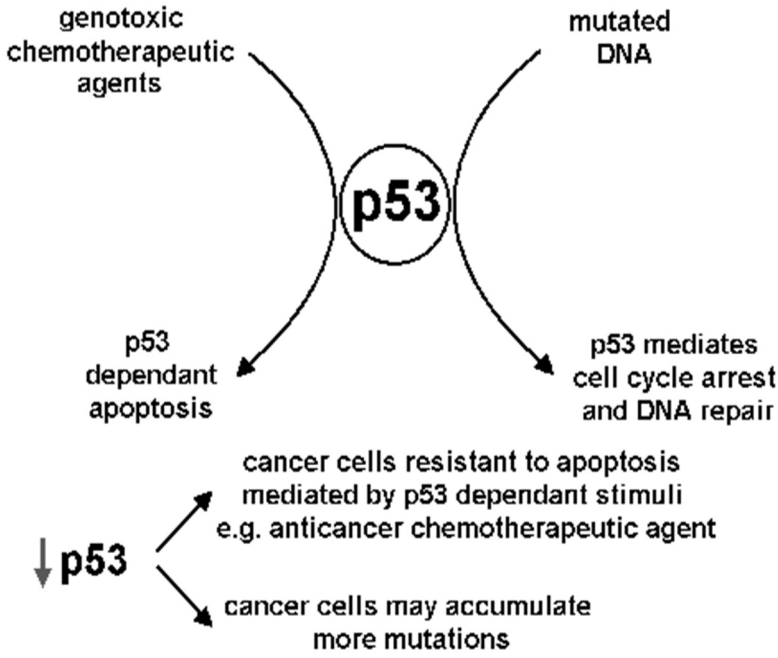
The team analyzed genomic sequences and found that African elephants have at least 40 p53 alleles. Functional assays comparing elephant and human cells showed that in response to DNA damage, cells from elephants had higher cell death (apoptosis) rates but didn't boost DNA repair mechanisms.

Both are known p53-mediated responses to DNA damage. Cells obtained from people with LES had significantly less apoptosis than the human controls and more than 5 times less than elephants. These results suggest that elephants may have evolved to resist cancer by triggering apoptosis

through the p53 protein to efficiently remove mutant cells (Harrison Wein, October 26, 2015. How Elephants Defend Against Cancer, NIH, USA).

The WHO defines a single category of myelodysplastic syndrome (MDS) with biallelic p53 gene inactivation, irrespective of the blast percentage, but excludes single-hit p53 mutant genes in MDS with bone marrow (BM) blasts <20%. Similarly, the recent International Prognostic Scoring System-Molecular acknowledged the poor outcome of the multi-hit p53 mutant genes but excluded the single-hit p53 mutant gene.

The ICC and ELN guidelines emphasize a p53 mutant variant allele frequency (VAF) of >10% regardless of the single- or multi-hit status of the genes for MDS/acute myeloid leukemia (AML) [3] **[Scheme 2]**.



Scheme 2*. A mutation of the p53 gene causing it to lose any of its functions will inevitably lead to carcinogenesis.*

1.2. Regulation of Isoform p53 Protein

As with 95% of human genes, the p53 gene encodes more than one protein. In 2005 several isoforms were discovered, and as of now, 12 human p53 isoforms have been identified (p53α, p53β, p53γ, Δ40p53α, Δ40p53β, Δ40p53γ, Δ133p53α, Δ133p53β, Δ133p53γ, Δ160p53α, Δ160p53β, and Δ160p53γ). Furthermore, p53 isoforms are expressed in a tissue-dependent manner and p53α is never expressed alone.

The full length isoform p53 proteins can be subdivided into different protein domains. Starting from the N-terminus, there are first the amino-terminal transactivation domains (TAD 1 and TAD 2), which are needed to induce a subset of p53 target genes. These domains are followed by the Proline rich domain (PXXP), whereby the motif PXXP is repeated (P is a Proline and X can be any amino acid). This is required, among other things, for p53 mediated apoptosis. Some isoforms lack the Proline rich domain, such as Δ133p53βγ and Δ160p53αβγ, hence some isoforms of p53 do not mediate apoptosis, which emphasizes the diverse roles of the p53 gene.

Next, there is the DNA binding domain (DBD), which enables the proteins to sequence specific bindings. The carboxyl terminal domain completes the protein. It includes the nuclear localization signal (NLS), the nuclear export signal (NES) and the oligo-merization domain (OD) [4].

Isoform p53 proteins often fail to induce MDM2 protein, which causes p53 protein to accumulate at very high levels. Moreover, the isoform p53 proteins themselves can inhibit normal p53 protein levels. The dynamics of p53 proteins, along with its antagonist, MDM2 protein, indicate that the levels of p53, in units of concentration, oscillate as a function of time.

This "dumped" oscillation is both clinically documented and mathematically modelled. Current models can also be useful for the modelling of p53 isoforms and their effects on p53 oscillation, thereby promoting *de novo* tissue-specific pharmacological drug discovery.

The protein kinases that are known to target the transcriptional activation domain of p53 protein can be roughly divided into two groups. The first group of protein kinases belongs to the MAPK family (JNK1-3, ERK1-2, and p38 MAPK), which is known to respond to several types of stress, such as membrane damage, oxidative stress, osmotic shock, heat shock, etc.

The second group of protein kinases (ATR, ATM, CHK1, CHK2, DNA-PK, CAK, and P53RK) is implicated in the genome integrity checkpoint, a molecular cascade that detects and responds to several forms of DNA damage caused by genotoxic stress. Oncogenes also stimulate p53 protein activation, mediated by the protein p14ARF. The critical event leading to the activation of p53 protein is the phosphorylation of its N-terminal domain.

The N-terminal transcriptional activation domain contains a large number of phosphorylation sites and can be considered as the primary target for protein kinases transducing stress signals [5].

Phosphorylation of the N-terminal end of the p53 protein by the above-mentioned protein kinases disrupts MDM2-binding. Other proteins, such as Pin1, are then recruited to p53 and induce a conformational change in p53, which prevents MDM2-binding even more. Phosphorylation also allows for the binding of transcriptional coactivators, like p300 and PCAF, which then acetylate the carboxy-terminal end of p53 protein, exposing the DNA binding domain of p53 protein, allowing it to activate or repress specific genes. Deacetylase enzymes, such as Sirt1 and Sirt7, can deacetylate p53 protein, leading to the inhibition of apoptosis. Some oncogenes can also stimulate the transcription of proteins that bind to MDM2 and inhibit its activity.

1.3. Function of p53 protein

Activated p53 protein binds to DNA and activates the expression of several genes, including microRNA miR-34a and WAF1/CIP1, thereby encoding p21 protein and hundreds of other downstream gene binds to the G1-S/CDK (CDK4, CDK6, CDK2, and CDK1) complexes (molecules important for the G1/S transition in the cell cycle), inhibiting their activity. When p21 protein (WAF1) is complexed with CDK2, the cell cannot continue to the next stage of cell division. An isoform p53 will no longer bind to DNA in an effective way, and, as a consequence, the p21 protein will not be available to act as the "stop signal" for cell division.

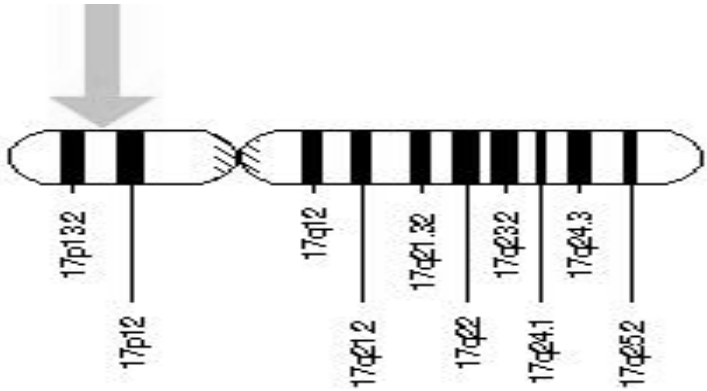
Studies of human embryonic stem cells (h ESCs) commonly describe the nonfunctional p53-p21 axis of the G1/S checkpoint pathway with subsequent relevance for cell cycle regulation and the DNA damage response (DDR).

Mathematical models also indicate that the p53 concentration oscillates much faster once teratogens, such as double-stranded breaks (DSB) or UV radiation, are introduced to the system. This supports and models the current understanding of p53 protein dynamics, whereby DNA damage induces p53 protein activation (see p53 regulation for more information). Current models can also be useful for modelling the mutations in p53 isoforms and their effects on p53 oscillation, thereby promoting de novo tissue-specific pharmacological drug discovery [6].

CHAPTER THREE

STRUCTURE OF THE P53 GENE

The p53 gene is located in the 17p13.1 chromosome and has: molecular location in chromosome 17; base pairs 7,668,402 to 7,687,550; and homo sapiens annotation release 107, GRCh38.p2. People have 2 alleles (copies) of the p53 gene. One is inherited from each parent, and both are crucial to prevent cancer. The p53 gene is mutated in most human cancers. Having only one functional allele causes LES, which is characterized by a more than 90% lifetime risk of cancer **[Scheme 3]**.



Scheme 3*. The TP53 gene is located on the short (p) arm of chromosome 17 at position 13.1. More precisely, the TP53 gene is located from base pair 7,668,402 to base pair 7,687,550 on chromosome 17.*

The p53 chip scans exons 2-11, containing more than 98% of all mutations described so far in the gene. This system has been designed to allow the detection of the most common mutations and all identified polymorphisms in the p53 coding sequence. It has been found that this system allows for the sequencing of an average of 97.5% of the arrayed p53 genes.

Altogether, 1329 bp of the sequence is analyzed from sense and anti-sense strands. The p53 chip detection limit for known alleles has been identified at 5%. The actual limit could sometimes be even less than 5%, but in real life, the possible alleles are mostly unknown and reliable controls and comparisons with results obtained with standard methods can be technically difficult because of their error rates.

The p53 chip has been tested in cooperation with partners from the IARC. In solid tumors, the p53 gene is mutated or deleted in about 50% of all tumors, but in leukemia, aberrations of the p53 gene are infrequent, ranging from 5-10% at diagnosis (Peller & Rotter, 2003). Mutations in the p53 gene occur mostly in the DNA binding domain (exons 5-8), as missense or nonsense mutations, deletions or inserts.

The p53 protein functions as a tetramer and the isoform p53 protein and the wildtype p53 protein form a tetrameric complex, while the mutant p53 acts as a dominant negative inhibitor of the wildtype p53 (loss-of-function) (Vousden & Lu, 2002). Patients with p53 gene mutations are not recognized at diagnosis by routine diagnostic screening with FISH.

Like patients with 17p del, patients with mutant p53 genes show poor survival rates and resistance to chemotherapy. Among patients with chemotherapy-refractory disease, about 40-50% have lost one 17p allele or have a mutated p53 gene, leading to reduced overall survival rates. More than 95% of the identified p53 mutations are found in exons 5-8, the DNA binding domain (Rossi et al., 2009).

In de novo acute myeloid leukemia (AML), the loss of the short arm of chromosome 17 is reported in 5-10% of cases (Stirewalt et al., 2001; Seifert et al., 2009). However, the deletion is mostly accompanied by complex aberrant karyotypes and is rare in other subgroups.

As in chronic lymphocytic leukemia (CLL), 17p del in one allele is most often associated with TP53 point mutations in the remaining allele, with adverse overall survival rates and low resistance to conventional chemotherapy. Haferlach et al. (2008) and Rűcker et al. (2012) have reported p53 mutation rates as high as 60-70%, respectively, in AML with complex karyotypes, most often associated with deletions of chromosomes 5, 7 and 17p.

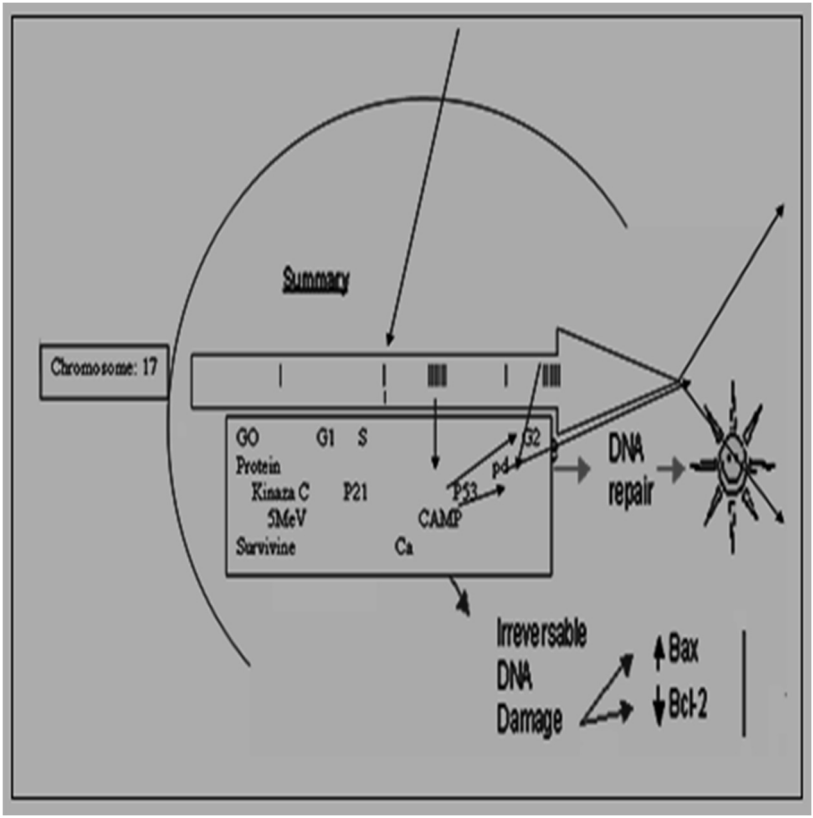
TP53 mutations in AML in any of the risk groups have been seen to represent a specific subset with the most un-favorable outcomes, displaying a very short OS (Grossmann et al., 2012).

A mutation of the p53 gene causing it to lose any of its functions will inevitably lead to carcinogenesis, which will allow the cell to grow indefinitely without any regulation. Another important factor in the process of carcinogenesis is the balance between the pro-apoptotic and anti-apoptotic members of the Bcl-2 family.

In a tumor cell, a mutation in the Bcl-2 gene results in increased expression and will suppress the normal function of the pro-apoptotic proteins BAX and BAK, leading to malignancy.

On the other hand, a mutation in the BAX or BAK genes can cause a down-regulation of expression, causing the cell to lose its ability to regulate apoptosis, once again leading to cancer cells. The inhibitor of apoptosis (IAP) family genes, which encodes negative regulatory proteins, can prevent apoptotic cell death. In a normal cell, the p53 protein binds to DNA, stimulating another gene to produce a protein called p21, which interacts with a cell division-stimulating protein (cdk2) [7].

The disruption of surviving microtubule interactions results in the loss of the anti-apoptotic function of the survivin protein and increased caspase-3 activity, a mechanism involved in cell death during mitosis. It has been suggested that the survivin protein may counteract the default induction of apoptosis in the G2/M phase **[Scheme 4]**.



Scheme 4. *In the normal cell, the p53 protein binds to DNA, stimulating another gene to produce a protein called p21, which interacts with a cell division-stimulating protein (cdk2*). *[Udristioiu A., Florescu C., Popescu MA., Cojocaru M. High Concentration of anaerobic ATP implicated in aborted apoptosis from CLL. Lab Medicine 2010; 41: 203-08].*

Activation of miR-34a and miR-145 can maintain pluripotency by inhibiting stem cell markers such as the Lin28a, Oct4, Klf4, and Sox2 genes. On the other hand, it has been observed that the homozygous deletion of the p53 gene can lead to a stemless phenotype in pancreatic acinar cells showing elevated expression of cancer stem cell (CSC) markers and stem cell regulators such as c-Myc gene, SOX9, and Klf4, along with several other genes. Therefore, the loss of the p53 gene can

also result in the development of stem cell-like characteristics that enhance tumor growth.

There are many p53 gene-induced mRNAs and encoded proteins involved in a variety of potentially anti-neoplastic processes, including inhibition angiogenesis, DNA repair, cell cycle checkpoints, senescence and apoptosis (Vogelstein et al., 2000; Riley et al., 2008; Brady and Attardi, 2010).

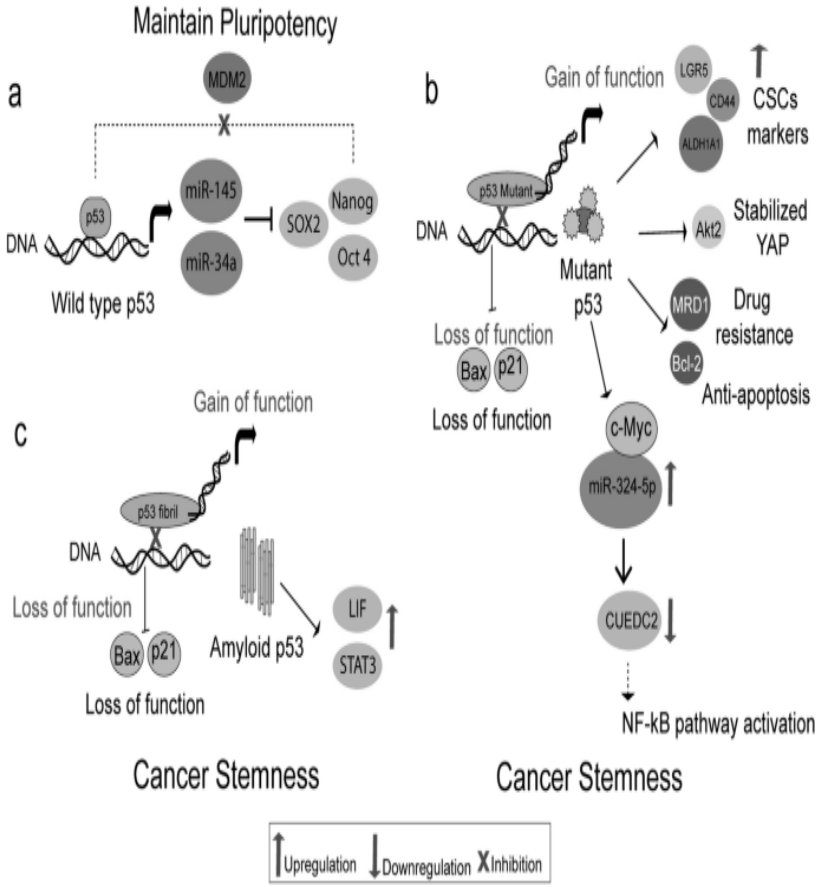
The activation of consecutive p53 gene responses, separated by a period of recovery, leads to the selective attenuation of a subset of p53-regulated mRNAs corresponding to those targeted by one or more of the p53-responsive miRNAs. Our results indicate that the long-term expression of p53-responsive miRNAs leads to an excess of miRNAs during the second response, and this likely prevents the induction of MDM2, BTG2 and CDKN1A mRNA and/or protein.

These observations are likely to have important implications for daily cancer therapies that activate p53 protein in normal tissues and/or tumor cells. The p53 protein regulates the expression of a subset of miRNAs, noncoding RNAs that contribute to post-transcriptional gene silencing, linking the p53 protein response to post-transcriptional regulation of gene expression in a separate way (Suzuki et al., 2009; He et al., 2007b; He et al., 2007c). Several recently identified miRNAs strongly induced in HT29-tsp53 cells are: miR-143-3p, miR-145-5p, miR-139-5p and miR-34a-5p (Cabrita et al., 2016).

These mRNAs were predominantly short-lived, permitting rapid recovery from p53 activation and rapid cell cycle re-entry. Here, we similarly detected the increased expression of p53-responsivness at miRNAs containing the miR-34, miR-139, miR-192 and miR-194 hairpins, and this was once again readily reversed at the restrictive temperature. The reversible expression of these mRNAs was not entirely surprising for two reasons. First, the p53-transcriptional mRNA response is reversed within 1 h at the restrictive temperature.

Ribonucleic acid miRNAs are similarly transcribed by RNA polymerase II, so their p53-dependent transcription is likely done through the same fundamental mechanism. It is suggested that this favors functional miRNA-mRNA interactions that selectively attenuate targeted gene expression. Although it remains to be tested, this type of delayed feedback may be a common feature of other transcriptional responses. One could

hypothesize that interacting miRNAs and mRNAs could be co-expressed in progenitor cell populations [8] **[Scheme 5]**.



Scheme 5. *Long-term expression of miRNAs in differentiating cell populations could contribute to the long-term silencing of specific mRNAs, preventing ectopic mRNA expression*.

A temperature-sensitive variant of p53 protein that permits rapid reversible activation of the p53 protein response was used to compare the induction and decay of mRNAs and miRNAs throughout two separately defined p53 responses. This resulted in evidence that p53-induced miRNAs provide delayed post-transcriptional feedback that inhibits the expression of

MDM2, BTG2 and p21WAF1 following an initial period of recovery. While the work presented here lacks functional assays, our analysis coupled with functional analysis from other laboratories builds a viable and novel working model for delayed post-transcriptional feedback that will be tested in future studies.

Based on all the above instances, it is evident that isoform p53 proteins and amyloid p53 proteins play a vital role in tumor initiation, progression, and metastasis. Several of the p53 isoforms are known to form p53 amyloids and display loss of function (LOF) and gain of function (GOF) phenotypes. The entire road of cancer progression is controlled by p53 protein via several interacting signaling molecules and pathways. Therefore, any therapeutic approach leading towards p53 protein reactivation could be of great importance to the field of precision medicine.

Specific germline mutations in hereditary breast and ovarian cancer susceptibility (HBC/HBOC) genes - the BRCA1, BRCA2 and PALB2 genes - have been shown to recur in French Canadians from Quebec, Canada, and this has been attributed to common ancestors. Germline p53 mutation carriers are known in Li-Fraumeni syndrome families, and feature the onset of breast cancer at a young age.

Reportedly, a rare p53 mutation was carried in French Canadian HBC families, though none recurred, possibly due to the limited number of cancer families investigated.

Information on p53 germline mutations found in French Canadian cancer families and provided by hereditary cancer clinics led to the investigation of 37 new BRCA1 and BRCA2 mutation-negative HBC/HBOC families for the p53 mutations, and the assessment of the frequency of p53 mutations in 1,235 French Canadian breast cancer cases not selected for family history of cancer.

p53 gene mutation-positive pedigrees from French Canadian cancer families were provided by local hereditary cancer clinics. Bi-directional Sanger sequencing of all the protein-encoding exons of p53 was performed using peripheral blood lymphocyte DNA from breast/ovarian cancer probands from 37 HBC/HBOC Canadian families. Targeted bi-directional Sanger sequencing assays of regions containing the identified p53 gene mutations was performed on 1,235 French Canadian breast cancer cases not selected for family history cancer.

Five new p53 mutations were identified in six pedigrees from hereditary cancer clinics. No deleterious mutations were identified in cancer probands from 37 HBC/HBOC families. A targeted mutation screen of the 1,235 breast cancer cases identified a c.844C>T [p. Arg282Trp] mutation carrier. This mutation was also found among the six mutation-positive cancer families provided by the local hereditary cancer clinics.

The targeted screen also uncovered a new p53 gene mutation - c.685T>C [p Cys229Arg] - in two breast cancer cases. p53 gene mutation carriers were found in all of the 656 women with breast cancer diagnosed at less than 50 years of age.

The p53 tumor suppressor protein has emerged as a universal sensor of genotoxic stress that regulates the transcription of numerous genes required for appropriate cellular response to DNA damage. Therefore, the transcriptional induction of p53 protein target genes can be considered a global and early indicator of genotoxic stress. By performing expression microarrays and RNA-Seq analysis on wild-type and mutant p53 genes in human lymphocytes respectively, derived from controls and Li-Fraumeni patients and exposed to different classes of genotoxic agents, we first determined a common p53-dependent transcriptional signature of DNA damage.

The specificity of the p53 genotoxicity assay can easily be demonstrated by performing the same experiment in control lymphocytes with heterozygous p53 mutations, which compromise responses to DNA damage. This assay allowed us to show that most of the drugs commonly used in cancer treatment, with the exception of the microtubule poisons, are highly genotoxic.

The p53 genotoxicity assay should facilitate the measurement of the genotoxic effects of chemical and physical agents and the identification of drugs that are not genotoxic and do not expose patients to the risk of secondary malignancies, especially those with a constitutional defect in response to DNA damage, such as patients with Li-Fraumeni syndrome.

Another strong limitation to routine analysis of p53 mutations resides in the fact that many tumors contain an excess of the wild-type p53 gene as compared with the mutant gene, resulting from the presence of intact

alleles in the tumor as well as in non-cancer cells (stromata, inflammatory cells and blood vessels).

CHAPTER FOUR

ISOFORM P53 PROTEIN AND CANCER

The function of the tumor suppressor of the p53 protein is well-highlighted in human cancer, where it is mostly present either in mutated or amyloid/aggregated form. Under normal conditions, p53 protein is inactivated by MDM2 protein (murine double minute 2), a negative regulator that causes proteasomal degradation of p53 protein.

Phosphorylation of p53 protein caused by diverse cellular stresses can reduce its binding affinity to MDM2 protein, which results in its activation. The p53 protein subsequently forms a homo-tetramer that binds to specific p53 response elements in the genomic DNA, where it acts as a transcriptional regulator of its downstream genes, regulating the cell cycle, apoptosis, DNA repair, and several other vital functions to maintain the genome's integrity.

Control of p53 protein activity is achieved by post-translational modifications, such as phosphorylation, acetylation, and ubiquitination, which influence p53 protein's binding to the DNA and also allow interaction with other proteins thereby affecting p53 protein transcriptional functions. On the other hand, isoform p53 protein exhibits a loss of its obligatory transcriptional activities, thereby gaining new functions.

Previous studies have suggested that in tumor cells, a non-functional p53 protein is often present in an aberrant, misfolded, and inactive conformation. Amyloid formation begins with the unfolding of p53 protein structural folds, followed by the formation of structures containing β-strands. Recent studies on the aggregation of p53 protein have shown its internalization into cells, its seeding capacity, and cell-to-cell transmission in a prion-like fashion. Interestingly, not only wild-type p53 but also mutant p53 proteins can also form amyloids, thereby leading to tumorigenesis.

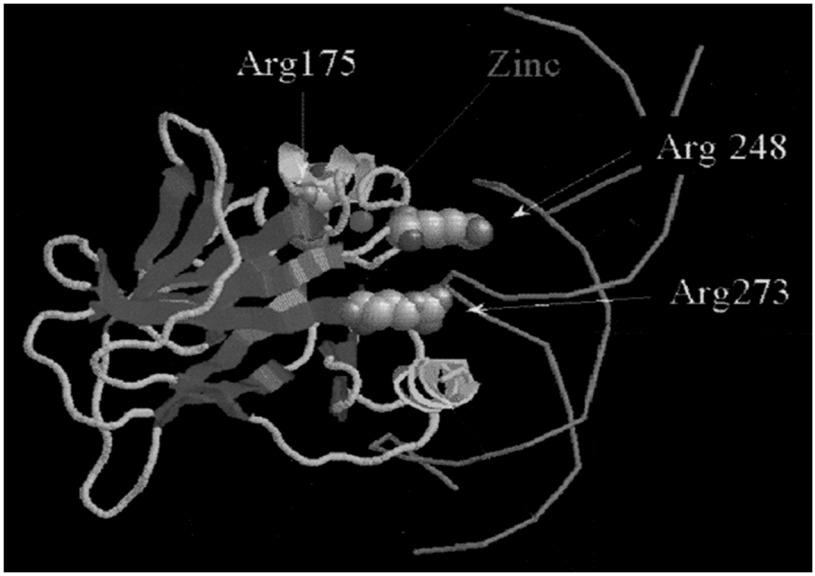
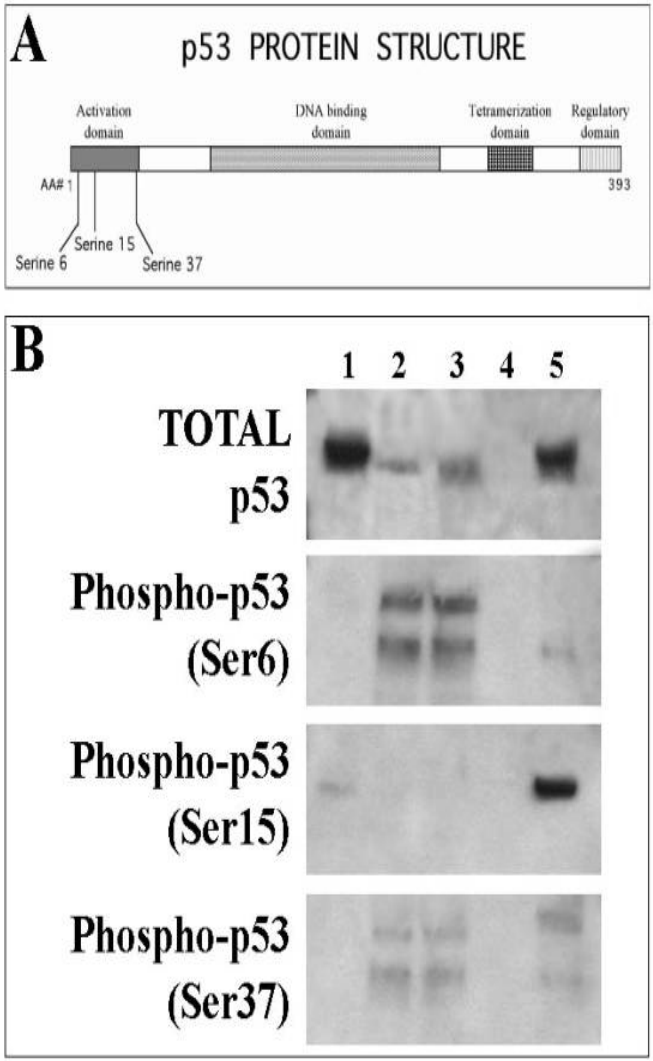


Figure 1. *Three-dimensional shapes of the x-rayed p53 protein structure in isomorphic form. Five p53 gene mutations were identified in hereditary cancer clinics. A targeted mutation screen of cancer cases identified an Arginin-282-Tryptophan mutation carrier.*

Cell lines investigated showed not only differences in the amount of total p53 protein but also displayed distinct differences in the phosphorylated forms of the protein. All tumor-derived cell lines showed p53 expression; as expected, the non-cancerous fibroblast cell line (lane #4) showed no p53 expression.

These results clearly show that p53 activity is affected in different ways in different cancers. For example, the ovarian cancer cell line (lane #3) showed increased expression of p53, activated through phosphorylation of serine 6, while the colon cancer cell line (lane #5) showed up-regulation of p53, phosphorylated at serine 15. The use of the Multi-Blot Kit greatly facilitated this analysis as all forms of p53 shown here are of virtually the same molecular weight [**Scheme 4**].



Scheme 4. *A schematic of the known protein domains in p53. Phosphorylation of residues within the activation domain at the N-terminus of the p53 protein results in the activation of the protein. Three serine residues are associated with this activation.*

The crystal structure of p53 protein DNA binding domains (as found in the bioactive homo-tetramer) has seven domains:

1. An acidic N-terminus transcription-activation domain (TAD), also known as activation domain 1 (AD1), activates transcription factors: residues 1-42. The N-terminus contains two complementary transcriptional activation domains - 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.

2. Activation domain 2 (AD2) is important for apoptotic activity: residues 43-63.

3. The proline-rich domain is important for the apoptotic activity of p53 protein by nuclear exportation via MAPK: residues 64-92.

4. Central DNA-binding core domain (DBD). Contains one zinc atom and several arginine amino acids: residues 102-292. This region is responsible for the binding of the p53 co-repressor LMO3 [22].

5. Nuclear localization signaling domain: residues 316-325.

6. Homo-oligo-dimerization domain (OD): residues 307-355. Tetramerization is essential for the activity of p53 in vivo.

7. C-terminal involved in downregulation of DNA binding of the central domain: residues 356-393. The International Cancer Genome Consortium has established that the p53 gene is the most frequently mutated (>50%) in human cancer, indicating that the p53 gene plays a crucial role in preventing cancer formation. The p53 gene encodes proteins that bind to DNA and regulate gene expression to prevent mutations in the genome.

The only way to study these proteins in the same sample using conventional Western blotting would be to strip and re-probe the blot multiple times. This is cumbersome, but more importantly, protein bound to the membrane is removed with each cycle of stripping. The Multi-Blot Kit allows confident comparisons of same-sized proteins within a sample. Characteristics of apoptosis are chromatin condensation, membrane blebbing, phosphatidyl-serine exposure on the cell's surface, cytoplasm shrinkage, the formation of apoptotic bodies, and DNA fragmentation **[Figure 2]**.