



Faculty of Resource Science and Technology

**ISOLATION AND RESTRICTION SITE CHARACTERIZATION OF  
THE TUMOUR SUPPRESSOR GENE, *p53*, FROM TOTAL RNA  
PROSTATE TISSUE**

Samsunnisha Begum Sultan Suhaibudeen

Bachelor of Science With Honours  
(Resource Biotechnology)  
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# ISOLATION AND RESTRICTION SITE CHARACTERIZATION OF THE TUMOUR SUPPRESSOR GENE, *p53* FROM TOTAL RNA PROSTATE TISSUE

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## ABSTRACT

*p53* is a tumour suppressor gene, located at the short arm of chromosome 17 at position 17p13.1. The ability of *p53* gene to induce apoptosis helps to maintain the genetic integrity of the genome. *p53* gene comprises five conserved domains, which would result in deficiency of function, when mutated. The present study was to isolate *p53* cDNA by Reverse Transcription - Polymerase Chain Reaction (RT-PCR) and restriction site characterization by using several types of restriction enzymes. Total RNA from normal prostate tissue (AMBION Cat: #7988) was used as template. Several trials and optimizations were made for PCR, with two specific primers on *p53* gene. However, the desired length of 1.322kb product failed to be amplified.

Key Words: *p53*, tumour suppressor gene, total RNA, normal prostate tissue, restriction enzymes.

## ABSTRAK

*p53* adalah satu gen ketumpasan tumor, terletak pada lengan pendek kromosom 17 pada lokasi 17p13.1. Kebolehan gen *p53* mencetus apoptosis membantu memelihara sifat semulajadi genetik genom. Gen *p53* terdiri daripada lima domain terpelihara, yang akan menyebabkan gangguan pada fungsinya, bila mengalami mutasi. Kajian melibatkan cDNA *p53* dengan menggunakan teknik Reverse Transcription - Polymerase Chain Reaction (RT-PCR) dan pengenalpastian tapak pemotongan dengan menggunakan beberapa jenis enzim penghad. RNA penuh daripada tisu prostat normal (AMBION Cat: #7988) digunakan sebagai templat. Beberapa percubaan dan strategi-strategi optimum telah dijalankan dalam teknik PCR, bersama sepasang primer pada gen *p53*. Walaupun begitu, produk dengan panjang 1.322kb yang dikehendaki gagal diampifikasikan.

Kata Kunci: *p53*, gen ketumpasan tumor, RNA penuh, tisu prostat normal, enzim penghad.

## INTRODUCTION

Cancer is a neoplasm characterized by the uncontrolled growth of anaplastic cells that tend to invade surrounding tissue and to metastasize to distant body sites (Anderson, 1994). According to Ross (1996), cancer is a disease of uncontrolled cell growth because of damage to their DNA, which garbles the genetic signals for normal growth. As cancer is distinguished by their nature, site or clinical course of the lesion, prostate cancer is called prostate adenocarcinoma. Anderson (1994) wrote that adenocarcinoma is any one of a large group of malignant, epithelial cell tumors of the glands, whereas prostate adenocarcinoma is a slowly progressive adenocarcinoma of the prostate.

Vassuer *et.al.* (2003) claimed that cancer is a disease caused by multiple genetic alterations that lead to uncontrolled cell proliferation, which often involves activation of cellular proto-oncogenes and inactivation of tumor-suppressor genes. Grossman (2001) stated that mutated p53 is uncommon in early prostate cancer. Navone (1993) reported that p53 mutation is on the late event of prostate carcinogenesis. In addition, Grossman (2001) showed that high expression levels of p53 were only detected in the third stage of prostate adenocarcinoma.

Cowell (1995) mentioned that *p53* gene fulfills all the four criteria for being analyzed as a tumor suppressor gene, has potentials as one of the strategies for gene therapy. The criteria are that *p53* is mutated in more than 50% of reported cancer cases (Jameson, 1998), rate of mutation in *p53* is high, 95% mutations in *p53* are point mutations (Cowell, 1995), and the size of *p53* gene is small

and easy to be handled in analysis. *p53* is used in this study as it is the most commonly mutated gene in human cancers, including on the late levels of prostate carcinogenesis.

This study aims to determine the origin of mutations occur in *p53*. As stated in many published journals, the central region of *p53* is the most frequently altered in cancers. According to Cowell (1995), this region was found to be the DNA binding region, and contains the four out of five most highly conserved regions II-V. Mutations in *p53* gene can be either inherited or as a response to carcinogenic agents.

Cowell (1995) wrote that cells expressing mutant *p53* are totally resistant to apoptosis, while cells expressing wild-type *p53* are very sensitive to some therapeutic agents that easily induce apoptosis in order to repair the DNA lesion. Ross (1996) stated that *p53* protein expresses antiproliferative effect on cells. Mutant *p53* gives rise to mutant protein, which destroys the capacity of the protein to induce damaged cells to apoptosis (Lemoine, 1994).

The development of cancer involves a multi-step process including mutation, failure of DNA repair, activation of oncogenes, and finally loss of tumor suppressor function (Ross, 1996). Cancer can only be detected when all these steps occurred. Researches in cancer are being carried out, hoping for earlier cancer diagnosis to save human lives.

Ross (1996) suggested that tumor suppressor gene and oncogene might have a strict correlation, where they alternatively suppress and encourage cell division during normal tissue growth and repair. Without any tumor suppressor gene, damage to DNA would results in tumor that cannot



be stopped. Many researchers suggest the reintroduction of wild-type *p53* to tumor cells for inducing apoptosis. Cowell (1995) suggests that the conversion of mutant *p53* to a wild-type conformation of *p53* may also be a treatment for cancer.

This research involved the isolation of *p53* cDNA region with the intended purpose of characterizing it using restriction site mapping.

## **LITERATURE REVIEW**

### **STRUCTURE OF *p53* GENE**

*p53* gene consists of 11 exons bearing 16-20 kb of DNA, with exons 2-11 coding for 2.2-2.5 kb mRNA and the remaining one exon codes for a 53 kDa protein (Knudson, 1990). According to Eeles (1996), there are five conserved domains in the gene; the most important domain for maintaining the overall structure of the protein is domain II-V, which is located within exons 4-9. Ponder (1995) wrote that *p53* is located mainly in the cell nucleus, at the short arm of chromosome 17 at position 17p13.1 (Figure 1). *p53* gene was discovered on 1979, when it was found to form complex with the large T antigen of the simian virus 40, which is a viral tumor (Lindahl, 1997).

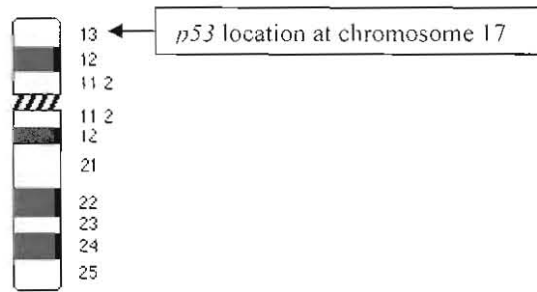


Figure 1: Location of *p53* gene in human chromosome. Retrieved from The World Wide Web: [http://www.infobiogen.fr/services/chromcancer/Indexbychrom/idx\\_17.html](http://www.infobiogen.fr/services/chromcancer/Indexbychrom/idx_17.html)

According to Lindahl (1997), at first, *p53* is considered as a tumor antigen since its accumulation takes place in the nucleus of tumor cells. Further researches bring *p53* to an oncogene, due to its potential of inducing neoplastic transformation (Lindahl, 1997). Only in late 1980's *p53* was regarded as a tumor suppressor gene because of its ability in suppressing transformation by other oncogene and mediate cell cycle arrest, or apoptosis.

TUMOUR SUPPRESSOR GENE, *p53*

*p53* has many functions in suppressing tumor growth and regulating cell cycle arrest. *p53* binds to specific sequences with single-strand or double-strand DNA, depending on the carboxyl terminal. When DNA strand is under stress or damaged by certain mutagenic agents, such as UV radiation the DNA strand breaks or faces lesions (Lindahl, 1996) that cause the overexpression of *p53* protein in the cell (Mihich & Hartwell, 1997). This accumulation creates a transient blockage in the G1 phase, just before DNA replication takes place (Cowell, 1995). Here the cells are arrested in the G1 phase to provide time for DNA repair machineries to do their work (Figure 2). One of the most common jobs of these machineries is that they induce apoptosis. This way the gene helps to maintain the genetic integrity of genome, thus called the “guardian” of genome



(Cowell, 1995). Cells with mutant or no *p53* gene could not experience cell growth arrest. This situation leads to abnormal cell growth, which creates tumors and malignancies. The cells with mutant or no *p53* gene may be reintroduced with wild-type *p53* for expressing the normal function of *p53*. This brought to the notion that *p53* act as negative regulator in cell growth.

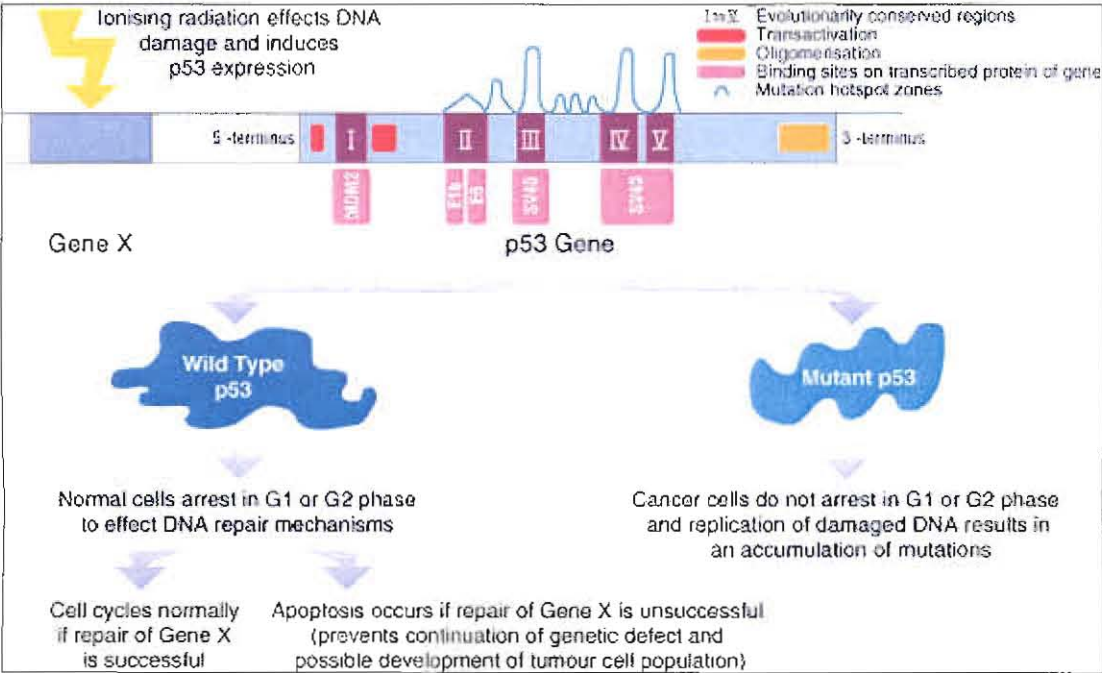


Figure 2: Regulation of cell growth and suppression of tumor cell proliferation. Retrieved from The World Wide Web: <http://www.novocastria.co.uk/oapdgl.htm>

When *p53* gene is mutated, absent, or bound to viral protein, this regulation cannot occur and lead to the genetic instability, which accumulate mutations and develops malignant clones (Cowell, 1995). Transformed cells that undergo somatic mutations express mutant proteins. Lemoine stated that these proteins from transformed cells have longer half-life and have the ability of triggering conformational change for the wild type protein, as mutant proteins have different conformation compared to wild type proteins.

## MUTATIONS IN *p53* GENE

According to Cowell (1995) 95% of *p53* alterations are point mutations that produce mutant proteins with no transactivational activity. Knudson (1990) claimed that 80% of cancers involving *p53* mutation lost one allele and the other allele shows a point mutation. Inactivation of both alleles leads to the total loss of *p53* function. Cowell (1995) wrote that mutations in *p53* were found in most human cancers (45-50%). *p53* gene is altered in 60% of human cancers (Ponder, 1995).

Roth (2003) claimed that most *p53* mutations in cancers are found within the central domain, which impairs the ability of protein to bind DNA. Cowell (1995) stated that 95% mutations in *p53* found in sporadic tumors found throughout the coding sequence of the gene, around the four highly conserved domains (II-V) that is identified as the DNA binding region.

Ponder (1995) stated that three of five prostate cancer cell lines showed mutations in the coding sequences of *p53* gene and can be suppressed by wild type *p53*. *p53* mutations in prostate cancer can only be diagnosed at the late stage of malignancy.