International Journal of Life Sciences Vol. 3 No.3. 2014. Pp.75-91

SJIF Impact Factor (2013): 4.402

Full Length Research Paper

Immunological Studies on P^{53} Expression by Using Different Mono Clonal Antibodies and Various Carcinoma Cell Lines

Zakaria Teleb[b1], Gerhard Unteregger[a], Thomas Zwergel[a], and Mathias Montenarh[b]

[a]*Department of Urology; University of the Saarland, Hamburg/ Saarland Germany.*
[b]*Department of Medical Biochemistry and Molecular Biology, University of the Saarland, Hamburg/ Saarland, Germany.*
[b1]*National Organization for Drug Control and Research, 6, Abou-Hazem St., Pyramids, Cairo, Egypt.*

**Corresponding Author: Zakaria Teleb*

Abstract

Immunological studies with different P^{53} antibodies and expression of P^{53} was investigated in the following cell lines: HT 29" Human colon carcinoma"; Ha Cat "Human keratinocytes"; SAOS "Human Osteosarcoma", MCF-7 "Human mammary carcinoma and PC-3 "prostate carcinoma". Antibodies "mouse monoclonal" against wild type P^{53} (PAb1620), mutant type P^{53} (PAb 240), antibodies (PAb, 1801) and PAb 421 which recognize both, wild type and mutant P^{53} proteins were performed. Expression of P^{53} was monitored by using indirect immune-fluorescence technique "FITC & TRITC". The specific staining pattern in these experiments was analyzed with the aid of a confocal laser scanning microscope (LSM). A clear nuclear staining was achieved in the control cell lines HT29 which bears a mutant form of P^{53} with the antibody 240. SAOS cells which serve as a negative control fails to show any P^{53} signal independent from the type of antibody.In contrast, MCF-7 cells exhibited a weak cytoplasmic staining pattern only by Ab240. More pronounced pattern by this antibody occurs in the human keratinocyte cell lines (Ha Cat), which showed a strong nuclear staining with Ab240. Additionally, this cell line was negative for all other antibodies. It was obtained from the prostatic carcinoma cell line "PC-3", this androgene-independent cell line provides quite different patterns of P^{53} expression depending on the particular type of antibody. Thus the wild type of P^{53} exhibits a prominent staining within the cell nucleus. By using image overlay with phase contrast, this staining pattern could be assigned to the nucleoli. A somewhat sparkled pattern occurs in this cell line with the antibody 1801, whereas Ab 240 demonstrated only a week staining of the nuclei. The antibody 421 shows a pattern like the wild type P^{53} against Ab 1620. We looked on P^{53} expression of individual cell lines obtained by radical prostatectomy. All the cells were negative for the mutant form recognized by Ab240. PC-3, exerted a pronounced immune fluorescence in the cell nucleoli, sometimes, this pattern is accompanied by a weak staining of the nucleoplasm.

Key words:*Different carcinoma cell lines: ("Ha Cat"; HT 29; SAOS;; MCF-7 and PC-3"), P^{53} gene expression.*

Introduction

P^{53} plays a key role in mediating cell response to various stresses, mainly by increasing or repressing a number of genes involved in cell cycle arrest, senescence, apoptosis, DNA repair, and angiogenesis. P^{53} mutations are observed in a significant minority of breast tumors, it was provide the updated information on P^{53} regulation and function with specific interest on its role in breast cancer by (Lacroix *et al.,* 2004).

Among the growth suppressor genes, there is also P^{53} which has been extensively studied and mutations in this gene have been documented in numerous human malignancies (Harris, 1996). Mutations in the P^{53} gene have been reported in a significant proportion of prostate (Effert *et al*., 1993). P^{53} plays an important role in regulatory cell fate in response to various stresses, either genotoxic (DNA alteration induced by: irradiation, UV, carcinogens, cytotoxic drugs). As a consequence of the mutations in the P^{53} gene, the half-life of the P^{53} protein increases, this event leads to an over expression of the P^{53} protein. Over expression in tumor tissue is correlated with a poor rate of patients, survival (Vousden and Lu, 2002 & Haupt *et al*., 2003 and Yu and Zhang, 2005).

Moreover, over expression of P^{53} was used to indicate aggressiveness of the tumor (Sarkis *et al*., 1995). P^{53} mutations are late events in prostate cancer and occur while the cancer cell undergoes transition from an androgen-dependent to an androgen-independent phenotype. Besides mutation interaction of P^{53} with viral and cellular proteins and an altered subcellular localization (Navone *et al*., 1993).

The analysis of various genes in human cancer cells contributed valuable information to the understanding of the mechanism of carcinogenesis. The most common genetic changes in colorectal tumors are deletions of the short arm of chromosomes 17 which is the site where the tumor-suppressor gene P^{53} maps. Wild type P^{53} which is mainly found in normal non-trans formed cells exerts a negative control on cellular proliferation (Levine *et al.,* 1991) and is able to suppress the transformed phenotype when transfected into neoplastic cells (Selter and Montenarh, 1994). The tumor suppressor gene P^{53} is isolated on chromosomes17. Its inactivation, usually caused by mutation of a single copy followed by allelic loss of the remaining chromosomes (Strano *et al*., 2007). Wild-type P^{53} which is mainly found in normal non-trans formed cells exerts a negative control on cellular proliferation and is able to suppress the transformed phenotype when transfected into neoplastic cells (Yu and Zhang, 2005). Following genotoxic stress, transcriptional activation of target genes by P^{53} tumor suppressor. It was reported that the restoration of P^{53} function in human cancer cell lines the deficient in P^{53} function up regulated the expression. The expression of wild-type P^{53} in human prostate and breast cancer cell lines correlated well increased the expression-furthermore, knockdown of P^{53} expression in cancer cells that express wild-type P^{53} result in the expression of gene. Importantly, genotoxic stress to cancer cells that resulted in activation of P^{53} unregulated the expression of gene.

P^{53} is one of the most intensively studied tumor suppressor proteins, with mutation that lead to loss of wild-type P^{53} activity frequently detected in many different tumor types. More recent studies are identifying further activities of mutant P^{53}, such as a role in cell reprogramming and expansion or in the maintenance and interaction with tumor stroma. Wild-type P^{53} was characterized as a suppressor of somatic stem cell reprogramming (Kawamura *et al*., 2009 & Marion *et al*., 2009). P^{53} is a key tumor suppressor that is mutated in more than 50% of human cancers (Vogelsteins *et al.,* 2000). P^{53} is a transcriptional that activities the transcription of its target by binding to a P^{53} DNA-binding consensus sequences. Importantly, proteins encoded by P^{53} target genes contribute to the determination of cell fate after stress (Vogelsteins and Lu, 2002 & Ljungman, 2000). P^{53} mediated its tumor-suppressive functions by inducing cell growth arrest apoptosis, senescence (Pucci *et al*., 2000).

P^{53} phosphorylation has been widely investigated. In most cases, it is associated with protein stabilization (Meek, 2004 and Mayo *et al*., 2005). Besides mutation interaction of P^{53} with viral and cellular proteins and an altered subcellular localization result in an inactivation of the growth suppressor function of P^{53}. The analysis of various genes in human cancer cells contributed valuable information to the understanding of the mechanism of carcinogenesis. The most common genetic changes in colorectal tumors are deletions of the short arm of chromosome 17 which is the site where the tumor-suppressor gene P^{53} maps (Dorman *et al*., 2003 and Weinberg *et al*., 2004)). Three functionally distinct regions have been identified in P^{53} (Savkur and Burris, 2004; Dorman *et al*., 2003 and Weinberg *et al*., 2004).

A number of monoclonal antibodies have been raised again P^{53}, the growth suppressor form of human P^{53} which is represented mainly by wild-type P^{53} is recognized by monoclonal antibody PAb 1620 whereas the mutant P^{53} is mainly reactive with PAb 240 (Gannon *et al.,* 1990). This finding would suggest that the different point mutations found for P^{53} in human tumors would exert a common conformational change. Most P^{53} mutations observed in breast cancer are of somatic origin (Blandino and Dobbelstein, 2004), P^{53} mutations are the most frequent genetic events in human cancer. Mutations of $P^{53}$are observed at a high frequency in human tumors, and recognized in about half of all malignant tumors in human. In the both systems of a human cell culture and their transplanted tumor, the sensitivities to radiation, heat and anti-cancer agents were observed in wild-type P^{53} cells, but not in mutated or deleted P^{53} cells (Takeo, 2005). WT P^{53} exists in mutant conformation in hypoxic core of MCF-7 solid tumors, and its conformation is oxygen-dependent.

The introduction of wt P^{53} into tumor cells results in tumor regression (Gogna *et al*., 2012) through enhancement of apoptosis (Vazquez *et al.,* 2008) although the molecular mechanism of P^{53}-induced effect is unknown. Structural P^{53}mutants are shown to inactivate the wild-type allele, and their dominant negative capacity and gain of function effect result from their aggregation propensity that causes co-aggregation of wt P^{53} (Xu *et al.,* 2011).

Mutations the P53 gene was shown to occur at different phases of the multistep process of malignant transformation, thus contributing differentially to tumor initiation promotion, aggressiveness, and metastasis (Brosh and Rotter, 2009; Levine and Oren, 2009; ; Oren and Rotter, 2010; Petitjean *et al.,* 2007; Schlomm *et al*., 2008, and Soussi, 2005).

Various lines of evidences indicate that, in addition to abrogating the tumor suppressor functions of wild-type P^{53}, the common types of cancer-associated P^{53} mutations also endow the mutant protein with new activities that can contribute activity to various stages of tumor progression and to increased resistance to anticancer treatments(Moshe and Rotter, 2009).
P^{53} induction of expression was also noted in prostate tumor cell lines PC-3 and in the breast tumor cell line MCF-7, it was observed on the up regulation of expression by functional P^{53} in human SAOS, it's important to determine whether the functional status of P^{53} in human cancer cell lines correlates expression levels. However, the expression of P^{53} wasn't detectable in PC-3 cells, the expression of P^{53} was detectable in MCF-7 (wild-type P^{53}) and (mutant P^{53}). Moreover, consistent, with the functional status of P^{53} in these cell lines, expression level were relatively high in extracts from MCF-7 cells (Shou et al., 2001 & Van Bokhoven *et al.,* 2003). Several recent reviews address in detail the various aspects of mutant P53 (Brosh and Rotter, 2009; Donzelli *et al*., 2008; Lozano, 2007; Olivier *et al*., 2009).

Immuno-fluorescence analysis of cell cultures or immuno-staining of histological sections from several cellular origins revealed that P^{53} is localized predominantly in the nucleus. Furthermore, P^{53} has lost its growth suppressor activity. The P^{53} protein was band to the major oncogenic protein of SV40, strongly suggesting that it was downstream effectors of the large T-antigen pathway (Vogelstein *et al.,* 2010).

In the present study, we have analyzed the subcellular localization of P^{53} in cancer cells of the human colon carcinoma "HT29"; human keratinocytes "Ha Cat" human osteosarcoma "SAOS" and human mammary carcinoma "MCF-7" and prostate carcinoma cell lines "PC-3". Using different monoclonal antibodies directed against P^{53} and by means of laser scanning microscopy.

Materials and Methods

Cell Culture

Colon cancer cell line "HT29", human Keratinocytes "Ha Cat", human Osteosarcoma "SAOS", human mammay carcinoma "MCF-7" and PC-3 "prostate carcinoma cell lines" were obtained from the tumor bank of the German Cancer Center in Heidelberg. Each of the cell lines was propagated as a monolayer culture in the appropriate medium supplemented with heat-inactivated fetal calf serum "FCS".

HT29 was cultured in Dulbecco's minimal essential medium "DMEM"/F_{12} supplemented with 10% FCS and 1% 200mM glutamine, the cells were propagated at 37ºC in 5% Co_2. SAOS cell lines were propagated as monolayer culture in appropriate medium McCoy's 5a medium, 85%; fetal bovine serum, 15%. MCF-7 and PC-3 cells was obtained from ATCC and cultured in MEM/F-12 medium containing 10% fetal calf serum and antibiotics (Gibco). All cells were routinely tested for the absence of mycoplasma DAPI staining and ELISA (Boehringer, Mannheim, Germany). For immunological staining cells were grown close to confluence on glass microscope slides within plastic dishes (Quadriperm, Heraeus, Germany) (Benninghoff *et al*., 1999).

Antibodies

Affinity purified monoclonal antibody PAb 1801 recognizes a denaturation stable determinant of P^{53}. This antibody was used in a 1:30 dilution for immune-histochemistry. Also, PAb 240 recognizes P^{53} location; it was used in a 1:30 dilution. This antibody has been shown to preferentially recognize mutant forms of the protein. PAb 1620 reacts preferentially with wild type P^{53}, this antibody was used in a 1:10 dilution. Results of immune-histochemistry analysis were reported as either positive or negative.

Immunostaining of P^{53} analysis in cell cultures

Culture cells on microscopic slides/inserts/ membranes or cover slips, remove and add medium for various periods to stimulate cells and proliferation. Cold buffer PBS was used two times for 10min and cold paraformaldehyde 4% (PBS) was freshly prepared. Cells were fixed on ice for 10min, then add methanol for 30min at room temperature, cells were washed in P135/0.02% tween 20 for 3 times. After fixation cells were incubated for 2hrs with monoclonal antibodies (35-60µl) of PAb 1620 (Milner *et al*., 1987) or PAb 240 (Gannon *et al*., 1990 and Benninghoff *et al.,* 1999), PAb 1801 and PAb 421 at a dilution of 1:10. Slides were washed with PBS/0.1% BSA and incubated for 45min with secondary antibodies (goat anti- mouse IgG labeled with FITC/TRITC (flourescein iso thiocyanate), at a dilution of 1:100, dianova for deletion. The remaining primary antibodies were blocked by incubation with goat-anti-mouse IgG containing only one F (ab)-fragment (Dianova, Hamburg, Germany). After washing, the second monoclonal antibody against P^{53} was applied and detected with sheep anti-mouse IgG. TRITC, immune-flurescence was detected by a confocal laser scanning microscope (Zeiss, Oberkochen, Germany) to define the exact sub-cellular distribution of P^{53}. Photographs were taken from a high resolution screen.

Experimental Procedures:

1) Types of Carcinoma cell lines:

- HT 29 (Colon carcinoma cell line).

- SAOS (Osteosarcoma).
- Ha Cat (Keratinocytes of Human)
- MCF-7 (Mammals carcinoma)
- F_{277} (Human Fibroblast)
- Prostatic carcinoma cell line:
  PC-3 p78; p1/ 289 p8; p1/289 p10; p1/290 p8; p2/224 p6;
  P288 p8; pc-3 p80; p1/290 p67; PC-3 p79.

All these cell lines were treated by different mono clonal antibodies,
These are:

- Ab 240 : against mutant type conformation.
- Ab 1620: against wild-type conformation.
- Ab 421 : against both (W.T & M.T): pan.
- Ab 1801: against both (W.T & M.T): pan.

2) Protocol for sub culturing different cell lines:

- Remove old medium by using a long Pasteur-pipette and vacuum.
- Add trypsin/EDTA to the cell culture vessel, use 4 ml. for 25 cm and 8 ml . for 75cm.
- Incubate at 37º C for 2-10 min. depending on the specific cell line.
- Control removal of cells from the vessel surface by phase contrast microscopy.
- Suspend the cell in a 10 ml. sterile centrifuge tube; then centrifuge at 1.200-1.500 rpm/ 3-5 min.
- Remove the supernatant carefully by using a short Pasteur pipette; suspend the cell pellet in an appropriate volume of fresh medium; for example: if a cell line has to be splitted from 75 cm vessel to another one, and additionally to a quadriperm with 4 slides, the dilution factor will be 2 with respect to the original growth area. In this case, add 4ml. medium, resuspend the cells carefully and transfer only 2ml. in the 75 cm vessel and 0.5ml. on each slide in the quadriperm dish, because the growth area of 4 slides is near 75cm., too.
- Prior suspending the cells in fresh medium, add 9ml. to a 75 cm. bottle, 4.5ml. to one slide and 3ml. to a 25 cm. bottle.
- Close the bottle and incubate at 37ºC until the cells has become confluent. Wash, fix and stain the cells as indicated in the immune-staining protocol.

3) Protocol for immunostaining of P^{53} antigen in cell cultures:

- Culture cells on microscope slides/inserts/membranes or cover slips.
- Remove, and add new medium for various periods to stimulate cells to proliferate.
- Remove slides and wash cells in a buffer tank 100ml. containing cold PBS.
- Change PBS two times and wash / 10min (total time).
- Transfer slides to a second tank containing cold freshly prepared paraformaldehyde 4% (PBS).
- Fix cells on ice/10 min.
- Transfer slides to another tank containing 100ml. methanol and fix for 30min. /RT.
- Wash cells for 10 min. in PBS/0.02% tween 20 (change 3 times).
- Remove slides and allow the buffer to evaporate until the slides are only wet.
- Add 35- 60µl of P^{53} antibody diluted 1:50 in PBS/ 0.1% BSA.
- Cover slides with cover slips and incubate at 4ºC overnight.
- Remove cover slips very carefully in 200ml. tank.
- Wash three times in PBS/ 0.02% tween 20 (10min.).
- Dry the slides as above until they are wet (semi dry).
- Add 35-60 µl the second antibody onto the slides.

The following antibodies can be used:

a) Goat-anti mouse labeled with FITC/TRITC/Peroxidase/Alk. Phosphatase.
b) Goat-anti mouse biotin labeled.
c) Any other labeled antibody recognizing mouse immunoglobuline, we perform staining by using (b).

- Incubate cells for 45 min. /RT in a humidified chamber without covering them with cover slips.
- Wash as above.
- Add 35-60µl of striptavidine/FITC-LRSC-TRITC or another label for 15 min./RT.
- Wash as above.

- Add one drop of embedding medium (PBS: Glycerol, 1:9) or anti-fade medium onto the slides, cover by coverslips.
- Follow immunostaining by fluorescence microscopy.

In other experiment, we used directly Goat-anti mouse labeled with FITC/TRITC/Peroxidase., without using striptavidin and in dark condition.

4) Laser Scanning Microscopy:

To follow the co-localization and cellular distribution of both mutant P^{53} (PAb 240 reactive) and wild type P^{53} (PAb 1620 reactive) within the same cell the samples were analyzed with a confocal laser scanning microscope (LS 410 invert, ZEISS). Samples were monitored using a standard objective (40x1.3 oil). Fluorescent dyes were excited with an external agon laser using the wavelength 488nm (FITC) and an internal He Ne-laser with 543 nm (Lissamin rhodamin, LRSC). Using the He Ne laser at 543nm, no cross-reaction with the FITC signals occurred. The samples were analyzed using the overlay function (three frams) and a pinhole-setting of 20. No additional zoom-factor was used. The pictures were scanned using the extended depth of focus mode with four scans at distance of 0.75µm and scanning time of 4s without additional filters.

Results

Expression of P^{53}gene was investigated in the following cell lines:

Human colon carcinoma "HT 29"; human keratinocytes "Ha Cat"; human Osteosarcoma "SAOS" and human mammary carcinoma "MCF-7" cell lines were cultured according to the instructions of the ATCC. Different antibodies as: "mouse monoclonal" against the wild type P^{53} (PAb 1620); mutant type P^{53} (PAb 240) and the antibodies 1801 and 421 which recognize both, wild type and mutant P^{53} proteins were used. Expresssion of P^{53} was monitored by using indirect immunofluorescence technique "FITC and TRITC". The specific staining pattern was analyzed with the aid of a confocal laser scanning microscope (LSM).

We analyzed the paint mutation in the P^{53} gene which correlates with immunocytochemical staining for P^{53} protein. In colon carcinoma cell lines "HT 29", the analysis of the tumor samples revealed some cases with negative P^{53} nuclear membrane staining patterns against PAb 1620 as indicated in Figure 1A. The other cases stained well positive nucleoli with the monoclonal antibody directed against P^{53} PAb 240 was indicated in Figure 1B. On the other hand, a positive nucleoli reactive with PAb 240 was seen in "Ha Cat" cell lines in P^{53} gene in Figure 2A, the mutant P^{53} was recognized by a monoclonal antibody 1620 (Figure 2B). As can be seen in Table (1), staining of P^{53} protein with PAb 240 was appeared as slightly positive or negative nuclei in MCF-7 carcinoma cell lines as indicated in Figure 3A. As well as, a negative reactivity with few nuclei and cytoplasmic staining recognized by PAb 1620 and PAb 1801 directed against P^{53} in MCF-7 cell lines in Figure 3B and 3C. Also, it has been found in Table (1) that SAOS cell lines appear to be negative nuclei staining patterns. This cell lines doesn't contain P^{53} protein by using PAb 1801 in Figure 4.

As revealed from Figure (5A), some of prostate carcinoma cell lines "PC-3 p79 starv" a signal from anti-wild type p53 antibody 421 localized exactly with the structure of the nucleus of the cells were seen. We analyzed the p53-staining pattern on prostatic carcinoma cell lines with PAb 1801. The staining of cultured leads to negative results as indicated in Fig. 5B. In addition to, different types of prostate carcinoma cell lines pl 290 which recognized p53 against PAb 1801 & PAb 1620, a weak positive nuclei staining was appeared (Fig. 6A and 6B). On the other hand, other cell types "pl 289" exerted weak positive nuclei against p53 gene with PAb 240, PAb 1620 and PAb 421 for recognizing the wild type protein and the mutant type (as shown in Fig. 7A, 7B, and 7C).

Table 1: Analysis of P^{53} in Human Cancer Cell Lines by Different Monoclonal Antibodies.

	Immunohistochemistry			
Cell Line	**PAb 1801**	**PAb 240**	**PAb 1620**	**PAb 421**
HT29	N.D	(+) nucleoli "Figure B"	(-) and some tissues with few nuclear membrane staining "Figure A"	N.D
Ha Cat	N.D	(+) nucleoli	(+) nucleoli	N.D
MCF-7	(-) nucleoli	(-) or slightly (+)	(-) nucleoli with few nuclei and cytoplasmic staining	N.D

SAOS	(-) Nucleoli It doesn't contain P^{53}	N.D	N.D	N.D
PC-3 p79 strav.	(-) Nucleus	N.D	N.D	N.D
P1/290	(+) nucleoli	(+) nucleoli	Weak (+) nucleoli	(+) Nucleoli
P1/289	N.D		(+) Nucleoli	(+) nucleoli

(-) nucleoli: it doesn't contain P^{53}
N.D: Not Determined

Table 2: The results of experiment of different carcinoma cell lines were collected in the following data.

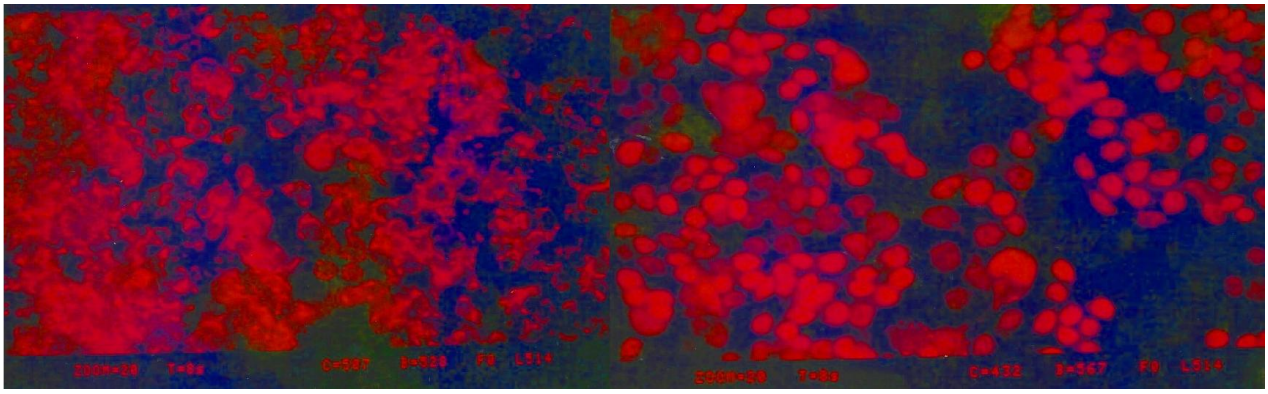
Cell type	Monoclonal antibody type	Type of treatment	Results
Ha Cat	Ab 240	Biotin system	(+) nucleoli
Ha Cat	Ab 1620	Biotin system	(-)
HT 29	Ab 240	Biotin system	(+) nucleoli
HT 29	Ab 1620	Biotin system	(-); few nuclear
PC – 3	Ab 240	Biotin system	(-)
PC – 3	Ab 1620	Biotin system	(+) nucleoli
MCF – 7	Ab 240	FITC system	(-); slightly ++
MCF – 7	Ab 1801	FITC system	(-); with few nuclei
MCF – 7	Ab 1620	FITC system	(-)
SOAS	Ab 1801	FITC system	(-): it doesn't contain p53 protein
PC - 3 p79 starv.	Ab 1801	Biotin system	(-)
PC – 3 starv.	Ab 421	Biotin system	(+) nucleus
PC - 3	Ab 240	Biotin system	(-)
P1 / 290	Ab 1801	Biotin system	(+) nucleoli
P1 / 288 P_8	Ab 1801	Biotin system	Very weak (+)
P1 / 288 P_8	Ab 1801	Biotin system	One cell (+)/ 50 in field
P1 / 224 P_6	Ab 1801	Biotin system	one nuclei + from, 3 nuclei in the cell, and 3+ nuclei staining in the same cell.
P1 / 289 P_{10}	Ab 1801	Biotin system	Completely (-)
P1 / 289 P_{10}	Ab 421	Biotin system	Completely (-)
P1 / 289	Ab 1620	Biotin system	Completely (-)
P1 / 290 P_{67}	Ab 1620	FITC system	One cell nuclear staining
P1 / 290 P_8	Ab 1620	FITC system	(-)
P1 / 289	Ab 240	FITC system	(-), but some cytoplasmic staining
P1 / 290 P_8	Ab 240	FITC system	(-) staining
P1 / 290	Ab 421	FITC system	(-)staining
P1 / 289	Ab 240	FITC system	Weak, but (+) nuclei

P1 / 289	Ab 1620	FITC system	Weak (+) nuclei staining
P1 / 289	Ab 421	FITC system	Weak (+) nuclei staining
P1 / 289	Ab 1801	FITC system	Weak (+) nuclei staining
P1 / 290	Ab 240	FITC system	Weak (+) nuclei staining
P1 / 290	Ab 1620	FITC system	Weak (+) nuclei staining, other slide has clear field positive
P1 / 290	Ab 421	FITC system	Weak (+)staining
P1 / 289	Ab 1801	FITC system	Weak expression
PC - 3 p82	Ab 240	FITC system	Very Weak (+)
PC – 3 p82	Ab 1620	FITC system	(+) nuclei staining, 5 cells clear (+)
PC – 3 p82	Ab 421	FITC system	Weak (+)
PC - 3 p82	Ab 1801	FITC system	Weak (+)
P1 / 277	Ab 240	FITC system	(+) nuclei staining
P1 / 277	Ab 1620	FITC system	(+) nuclei staining as expecting
P1 / 277	Ab 421	FITC system	Many cells are (+), nuclei
P1 / 277	Ab 1801	FITC system	Near (+) nuclei staining
F277 P4	Ab 1801	Biotin-Rhodamin	Few cytoplasmic staining; weak pere nuclei (+)
F277 P4	Ab 421	Biotin-Rhodamin	Strong cytoplasmic staining, but few cells

Key of Immunofourescence Pictures and Slides of Different Carcinoma Cell Lines

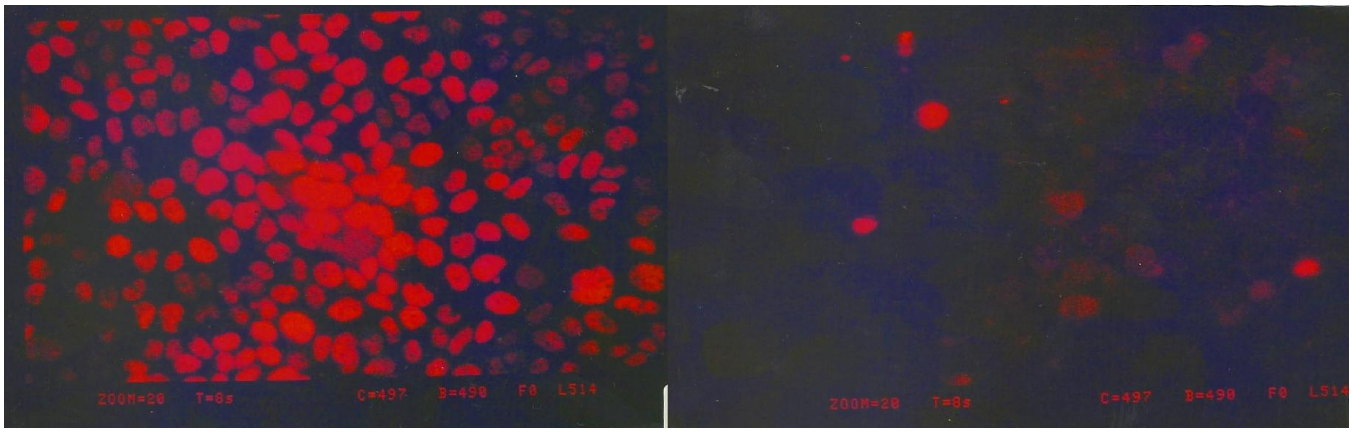
Number of slides	Cell type	Antibody type	Staining system	Results
1	Ha Cat	Ab 240	Biotin system	Positive
2	Ha Cat	Ab 1620	Biotin system	Weak (+)
3	HT 29	Ab 240	Biotin system	Positive
4	HT 29	Ab 1620	Biotin system	(-), nuclear membrane staining.
5	PC - 3	Ab 240	Biotin system	Negative
6	PC - 3	Ab 1620	Biotin system	Positive
7	PC - 3	Ab 1620	Biotin system	Positive
8	PC - 3	Ab 1620	Biotin system	Positive
9	MCF - 7	Ab 240	FITC system	Negative
10	SAOS	Ab 1801	FITC system	Negative, no p53 gene
11	PC-3 P_{79}(starv.)	Ab 1801	FITC system	Negative
12	PC-3 P_{79}(starv.)	Ab 1801	FITC system	Negative
13	PC-3 P_{79}(starv.)	Ab 1801	FITC system	Negative
14	PC-3 P_{79}(starv.)	Ab 421	Biotin-Rhodamin	Good positive nucleus staining, specific
15	PC - 3	Ab 240	Biotin-Rhodamin	Negative
16	P1/290	Ab 1801	Biotin-Rhodamin	Clear (+) nuclei staining.
17	P288 P8	Ab 1801	Biotin-Rhodamin	Weak staining (+)
18	P288 P8	Ab 1801	Biotin-Rhodamin	One cell (+)/ 50 cells/ field

19	P2/224 P6	Ab 1801	Biotin system	One nuclei (+) from three nuclei in the same cell.
20	P2/224 P6	Ab 1801	Biotin system	three nuclei are (+) in the same cell.
21	P1/290 P8	Ab 1620	FITC system	One cell (+) nuclei/field
22	P1/290 P8	Ab 1620	FITC system	One cell (+) nuclei/field
23	P1/289	Ab 240	FITC system	Weak nuclei staining most of cells (+)
24	P1/289	Ab 1620	FITC system	Weak (+) staining
25	P1/289	Ab 421	FITC system	Weak (+) staining
26	P1/289	Ab 1801	FITC system	Negative
27	P / 290	Ab 240	FITC system	Weak staining
28	P / 290	Ab 1620	FITC system	Weak (+) nuclei
29	P / 290	Ab 1620	FITC system	Clear positive nuclei
30	P / 290	Ab 1620	FITC system	It doesn't clear
31	P / 290	Ab 421	FITC system	Weak (+) staining
32	PC-3 P82	Ab 240	FITC system	(+) staining
33	PC-3 P82	Ab 1620	FITC system	(+) staining
34	PC-3 P82	Ab 1620	FITC system	(+) staining
35	PC-3 P82	Ab 421	FITC system	(+) staining
36	PC-3 P82	Ab 1801	FITC system	Two cells have (+) nuclei staining.
37	P1 / 277	Ab 240	FITC system	Clear (+) nuclei



(A) **(B)**

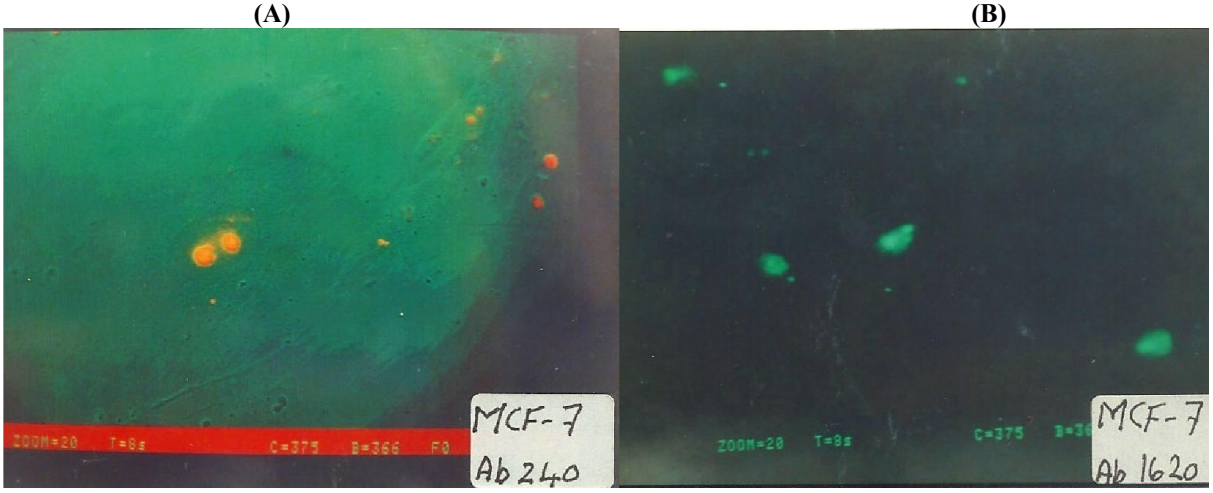
Figure (1): Human Colon Carcinoma Cell Line "HT29" was stained & labeled either with PAb 1620 to detect the wild type P^{53} (A) or with PAb 240 to detect the mutant type P^{53} (B), by using indirect immunoflourescence technique "FITC & TRITC". The staining pattern was analyzed with a confocal Laser Scanning Microscope (LSM). A clear nuclear staining was appeared.



(A) (B)

Figure 2: Human Keratinocyte Carcinoma Cell Line "Ha Cat" was stained & labeled either with PAb 240 (A) or with PAb 1620 (B) by using the same Protocol as in Figure (1). Positive nucleoli reactivity was seen with PAb 240.

(A) (B)



(C)

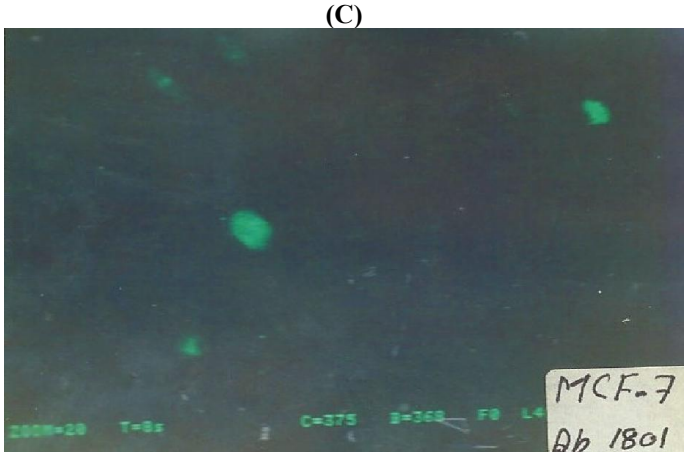


Figure 3: Human Mammary Carcinoma Cell Line "MCF-7" was stained & labeled either with PAb 240 (A) or with PAb 1620 (B) & with PAb 1801 to detect the both wild type and the mutant type (C), by using the same Protocol as in Figure (1). These cells exhibited a weak cytoplasmic staining pattern only by PAb 240.

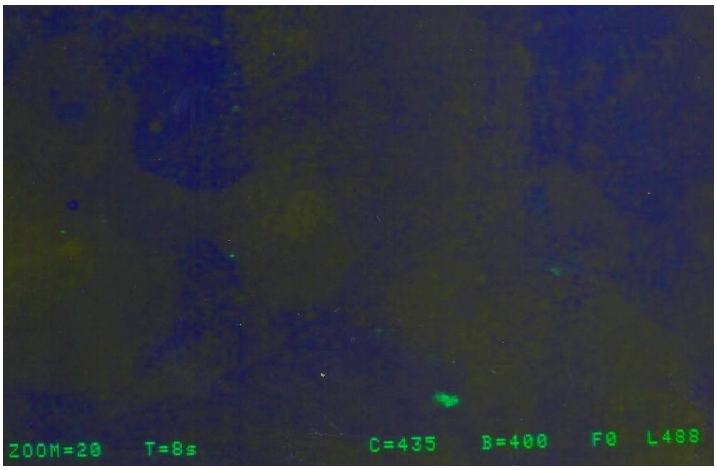
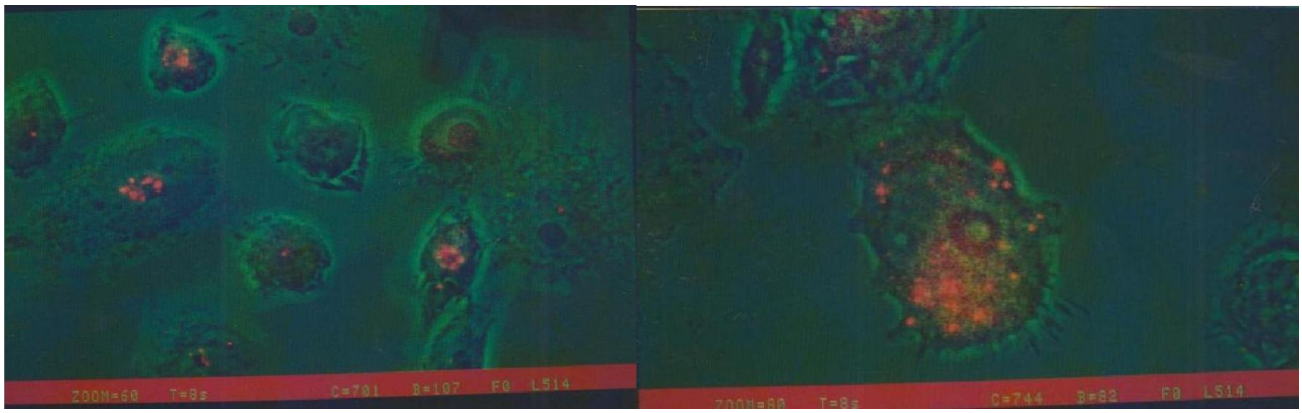
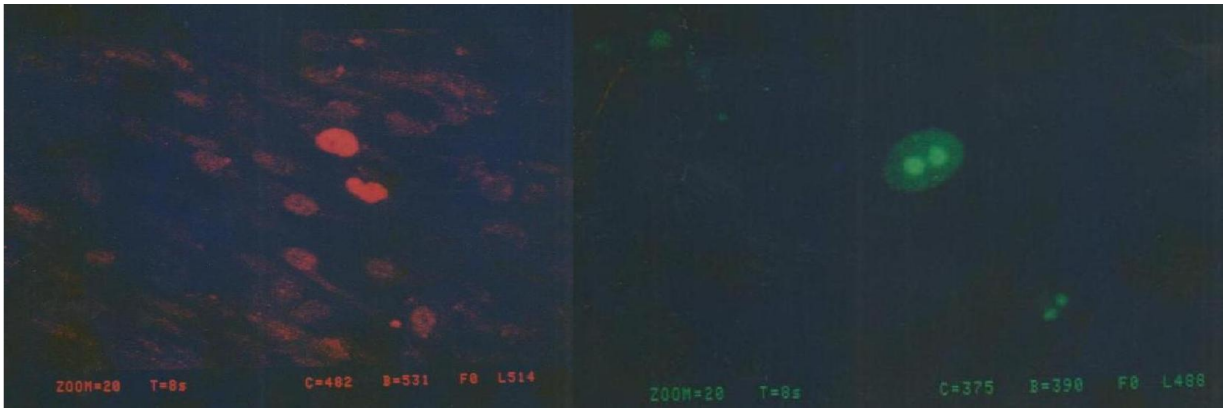


Figure 4: Human Osteosarcoma Carcinoma Cell Line "SAOS" was stained & labeled with PAb 1801 to detect the both conformation of P^{53} the wild type and the mutant type, by using the same Protocol as in Figure (1). These cells serve as a negative control fails to show any P^{53} signal independent from the type of antibody.



(A) **(B)**

Figure 5: Serum starvation of PC-3 cell line was stained and labeled either with PAb 421 (a) or PAb 1801 (b) to react with both conformation of P^{53}, by using the same protocol in the experiment. All cells were negative for the mutant form recognized by PAb 240. Immuno reactivity occurs only with the antibodies 1801.



(A) **(B)**

Figure 6: Prostatic carcinoma cell line P1/290 was stained and labeled either with PAb 1801 (a) or PAb 1620 (b) to react with the conformation of P^{53}, by using the same protocol in the experiment. The wild type of P^{53} exhibits a prominent staining within the cell nucleus, a somewhat sparkeled pattern occurs in this cell with the PAb 1801.

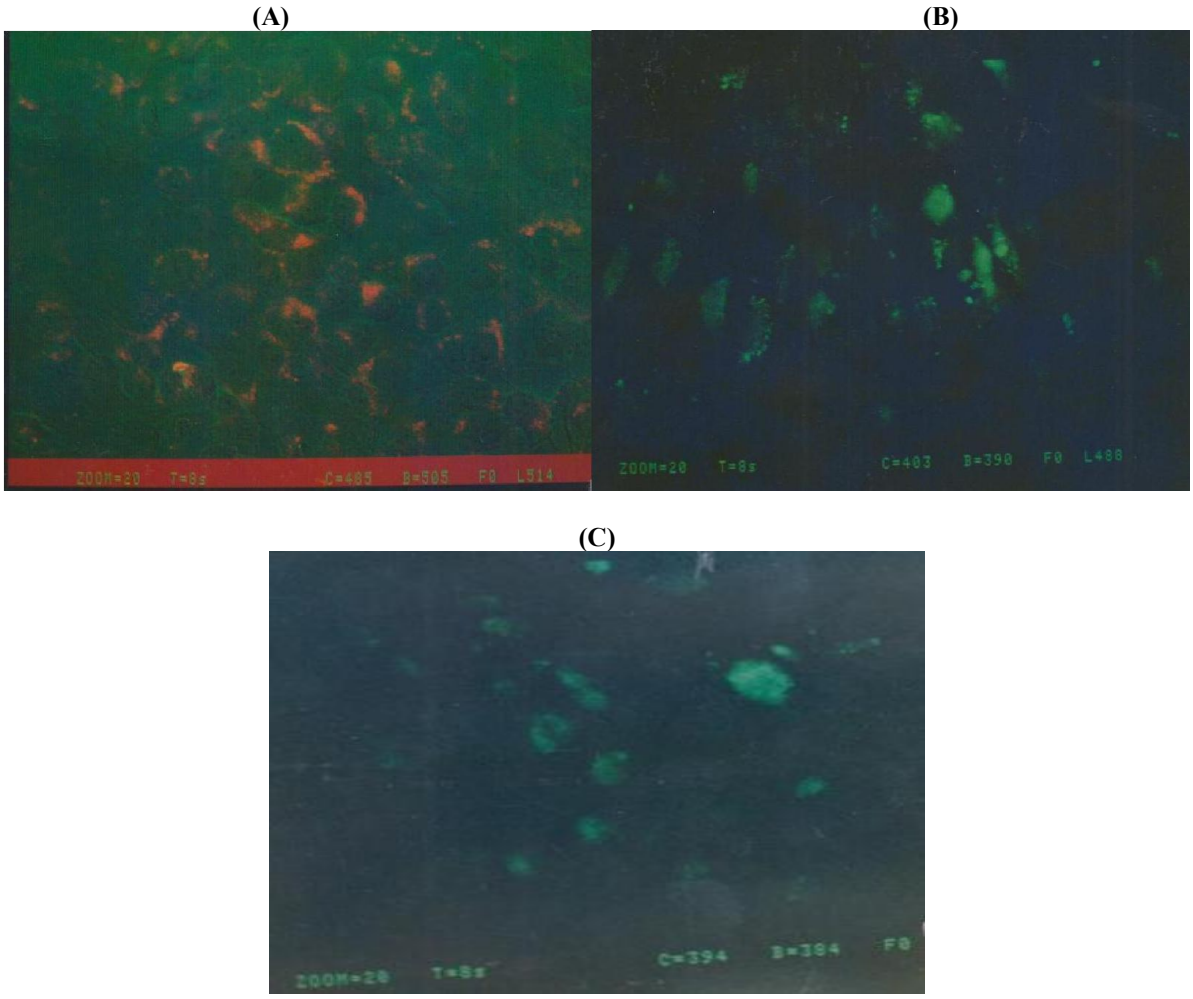


Figure 7: Prostatic carcinoma cell line P1/289 was stained and labeled either with PAb 240 (a) or PAb 1620 (b) or with PAb 421 (c) for detecting the conformation of P^{53}, by using the same protocol in the experiment. The reactivity of PAb 240 demonstrated only a week staining of the nuclei, the PAb 421 shows a pattern like the wild type P^{53} against PAb 1620.

The Final Conclusion of Our Results:

- HT 29 exerted a positive nuclei staining against Ab 240.
- SAOS revealed a negative staining for all antibodies, because it has not contain P53 protein.
- MCF-7 exerted a slightly positive nuclei against Ab 240.
- Ha Cat cells shows a positively nuclear staining against Ab 240.
- PC -3, also exerted a positive staining against Ab 1620; negative staining against Ab 240, and sparkle against Ab 421.
- P2/224 shows a positive staining against Ab 1801.
- P1/289 shows a negative staining by all antibodies.
- P 288 shows a positive staining against Ab 1801 (one cell only).
- P1/290, also exerted a positive staining against Ab 1620 & Ab 1801.
- F 277 revealed a weak cytoplasmic staining and weak pere nuclei staining against Ab 1801, but stronger cytoplasmic staining for few cells against Ab 421 was observed.

Discussion

The P^{53} gene is altered in a variety of different human tumors. Mutations in the gene coding for the phosphoprotein P^{53} are among the most common genetic alterations in human cancers (Hainaut et al., 1997). In normal cells, P^{53} is virtually undetectable by immunohistochemical methods due to the short half-life of the wild-type protein and the low amount of P^{53} present since mutation of P^{53} results in stabilization of the protein (Rotter, 1983), levelsof P^{53} which are detectable by immunohistochemistry in tissues

may thus indicate the expression of a mutant form of P^{53}. In all tumors with awild-type P53 gene no expression could be detected immunohistochemically with monoclonal antibodies PAb 1801, PAb 240 or PAb 1620. Furthermore, immunological evidence for a P^{53} over expression was found in most but not all colorectal tumors with a mutant P^{53} gene by immunohistochemical staining with the same monoclonal antibodies. PAb 1801 recognizes a human specific epitope and both the wild-type and the mutant P^{53}.

The P^{53} transcription factor is a critical element in the cells ability to regulate the cell cycle and its response to DNA damage. Mutations within the DNA binding domain of p53 are common and allow the formation of tetramers; however, these alterations prevent this protein complex from associating with target gene promoters. In the present study, it was examined the effects of P^{53} functionally in prostate cancer cells that harbored wild-type (wt) or mutant forms of the protein in response to commonly used chemotherapeutic drugs (Chappell *et al*., 2012).

A clear nuclear staining was achieved in the control cell lines HT 29 which bears a mutant form of P^{53} with the antibody PAb 240 (Lang *et al*., 1993). He examined the colorectal carcinomas cells for over expression of the P^{53} protein using different monoclonal antibodies directed against P^{53} "PAb 1801, PAb 240, PAb 421, Pab 1620" by immunohistochemical analysis and immune-blotting.

Subsequent studies identified many genes that were transcriptionally activated by P53 through P53 binding sites in either regulatory region. These included the cell cycle inhibitor, and the pro-apoptotic proteins (Nakano and Vousden, 2001 & Yu and Zhang, 2001). The P^{53} gene is altered in a variety of different human tumors. Mutations in the gene coding for the phosphoprotein P^{53} are among the most common genetic alterations in human cancers. In normal cells, P^{53} is virtually undetectable by immunohistochemical methods due to the short half-life of the wild-type protein and the low amount of P^{53} present, since mutation of P^{53} results in stabilization of the protein.

The study of P^{53} has revealed many of the principles underlying human tumorigensis. These include the critical differences between an oncogene and a tumor suppressor gene, the relationship between environmental exposures and cancer. The mechanisms through which cancer genes stimulate cell birth or inhibit cell death, and the striking networks that control the transcription, translation and function of key cellular proteins. The many facets of these studies, coupled with the fact that P^{53} inactivation is essential for the formation of the majority of human tumors, has, made P^{53} a uniquely valuable target for basic as well as applied research (Vogelstein *et al*., 2010).

The difficulties in linking P^{53} status to the biological properties and drug sensitivity of cancer cells could be partly explained by the recently discovered differential expression of the P^{53} isoforms in cancer. However, the pattern of isoform expression may vary from tumor to tumor (Bourdon *et al*., 2005), level of P^{53} which are detectable by immunohistochemistry in tissues may thus indicate the expression of a mutant form of P^{53}. Several studies based on immunohistochemistry have been reported that an increased level of P^{53} in some present cell lines such as HT 29 cell lines and Ha Cat cells (Hsueh *et al*., 2002), while other cell lines as SAOS exhibited no signals against any monoclonal antibodies. Also, MCF-7 cell lines exerted a weakly cytoplasmic staining pattern.

At the present study, we used four different carcinoma cell lines "HT 29"; Ha Cat; MCF-7 and SAOS against P^{53} localization to investigate the immune-reactivity of P^{53} protein. These cell lines grows as a monolayer exhibiting a typical epithelial cell shape with distinct nucleoli as shown in Figure (1) for HT 29 cell line phase contrast microscopy. The monoclonal antibody "PAb 240" was chosen for its specific affinity to the mutant conformation of P^{53} (Brosh and Rotter, 2009). As can be seen in Figure 1B staining of P^{53} protein with monoclonal antibody PAb 240 occurs predominantly within the nuclei of HT29 cell line. A clear nuclear staining was achieved in the control cell lines HT29 which bears a mutant form of P^{53} with the antibody PAb 240 (Lang *et al.,* 1993). It was examined the colorectal carcinomas cells for over expression of the P^{53} protein using different monoclonal antibodies directed against P^{53} "PAb 1801, PAb 240, PAb 421, PAb 1620" by immunohistochemical analysis and immune blotting system. A quite different localization could be observed with P^{53} antibody PAb 1620 recognizing wild type protein (Robinson *et al*., 2003). In order to follow exactly the topology of the P^{53} expression we examined simultaneously phase contrast laser scanning microscopy "CLSM" in an overlay modus. Thus as clearly visible signals, in figure 2 "Ha Cat" cell lines derived from anti-wild type P^{53} PAb 1620 co-localize exactly with the phase contrast structure of the nucleoli.

When the cell line Ha Cat cells were simultaneously labeled with both antibodies "PAb 240 in Figure 2A" and" PAb 1620 in Figure 2B", the subcellular structure again was positive nucleoli reactivity. It was confirmed with PAb 240by phase-contrast microscopy "Figure 2A". These data demonstrated the presence of at least two different subsets of P^{53} within the same cell. Since it was investigated whether this phenomenon was restricted only to the carcinoma cell lines, we analyzed the P^{53}- staining pattern on carcinoma cells using specific cell lines which exhibits hormone-insensitive growth behavior (Petitjean *et al.,* 2007). Staining of cultured carcinoma MCF-7 cells with PAb 240 leads to a uniform staining of all nuclei of cell lines (Figure 3A). In contrast to

these findings PAb 1620 reacted with the wild type confirmation of P^{53} in the nucleoli of MCF-7 cells (Figure 3A). Double immunofluorescence staining and simultaneous phase-contrast revealed that both subsets of P^{53} are present within the same cells (Figure 3C). Thus, the presence of wild type and mutant forms of P^{53} within the same cells seems not to be restricted to carcinoma cells but also present in MCF-7 cells.

SAOS cells which serve as a negative control fails to show any P^{53} signal independent from the type of antibody. This cell lines doesn't contain P^{53} protein (Ehrhart *et al*., 1988). In contrast, MCF-7 cells exhibited a weak cytoplasmic staining pattern only by PAb 240. A more pronounced pattern by this antibody occurs in the human keratinocyte cell line "Ha Cat", which show a strong nuclear staining with PAb 240 (Dippold *et al.,* 1981). Additionally, this cell line was negative for all other antibodies too.

The importance of P^{53} in cell death and the high frequency of mutations affecting this protein have generated a significant interest in exploiting the P^{53} pathway for novel cancer therapies. Small compounds have been used for the restoration of P^{53} function to lesions that carry full-length P^{53} protein with one amino acid change in the DNA binding core domain. In theory, such compounds should only have an effect on cancer cells, because the core domain of wild-type P^{53} in normal cells is already structurally intact (Bykov *et al*., 2002 and Peng *et al*., 2003). Most P^{53}- based therapeutic approaches aim to restore P^{53} function. However, in some tumors, P^{53} could have lost its apoptotic function but not its ability to direct prolonged cell growth arrest and DNA repair. In such cases P^{53}, could favour the recovery of cells damaged by therapy and prevent them inducing a mitotic catastrophe. Thus, P^{53} inhibitory therapies could be of interest in such cases (Gudkov and Komarova, 2003, 2005).

In addition to, we investigated the reactivity of P^{53} expression by using the antibodies (PAb 240, PAb 1620, and PAb 1801) as indicated in Figure 4, these cell lines serve as a negative control fails to show any P^{53} signals from the type of antibody. It is generally assumed that wild-type P^{53} cannot be detected by immunehistochemistry due to its short half-life. However, besides mutation complex formation of wild-type P^{53} with viral and cellular proteins may also stabilize the protein which allows the P53 protein to be detected by immunohistochemistry (Benninghoff *et al*., 1999). In addition to P^{53} gene mutations, binding to viral and cellular proteins is an alternate mechanism of inactivating in wild-type suppressor function of P^{53} (Georger *et al*., 2004).

In a subset of primary human tumors like inflammatory breast cancer, neuroblastoma (Moll *et al*., 1995), colon carcinoma (Bosari *et al.,* 1995) and malignant melanoma (Weiss *et al.,* 1995) P^{53} was also found in the cytoplasmic with nuclear exclusion. In colon carcinoma, cytoplasmic accumulation of P^{53} correlates well with un favorable prognosis (Bosari *et al*., 1995). In neuroblastoma cells, cytoplasmic P^{53} shows multiple dots representing large protein aggregates (Moll *et al.,* 1996). The precise mechanism of the cytoplasmic localization isn't yet known. The functional consequence of this localization is a marked impairment of the P^{53} mediated G1 cell cycle arrest in response to DNA damaging agents (Knippschild *et al.,* 1996 and Faria *et al.,* 2007).

Mutations in the gene coding for the phosphoprotein P^{53} are among the most common genetic alterations in human cancers (Hainaut *et al*., 1997). These alterations are more commonly found in high-stage tumors. Expression of wild type P^{53} in cancer cell lines with mutant P^{53} alleles will suppress their growth. Mutation in the P^{53} gene commonly leads to a prolonged half-life of the P^{53} protein which allows the detection of P^{53} in tumor tissue by immunohistochemistry. It is generally assumed that wild type P^{53} cannot be detected by immunohistochemistry due to its short half-life. However, besides mutation complex formation of wild type P^{53} with viral and cellular proteins may also stabilize the protein which allows the P^{53} protein to be detected by immunohistochemistry (Stattin *et al.,* 1996). In addition to P^{53} gene mutations, binding to viral and cellular proteins is an alternate mechanism of inactivating the wild type suppressor functions of P^{53} (Benninghoff *et al*., 1999).

In most cases, P^{53} is detected by immunofluorescence or immunohistochemistry in the nucleus of the cells (Montenarh, 1992). Nuclear sub fractionation revealed that P^{53} in normal and transformed cells is found in the chromatin, nuclear matrix fraction and nucleoplasmic fraction (Deppert and Haug, 1986). In addition to, a nuclear localization signal was identified on the polypeptide chain of P^{53}. Moreover, nuclear location of P^{53} was shown to be essential for its proper functioning (Shaulsky *et al.,* 1991). An exclusive cytoplasmic localization has been reported for P^{53} in some non-trans formed cell lines although this protein also harbors the nuclear location signal (Rotter, 1983). In a subset of primary human tumors like inflammatory breast cancer (Moll *et al*., 1992), neuroblastoma (Moll *et al*., 1995), colon carcinoma and malignant melanoma (Weiss *et al.,* 1995), P^{53} was also found in the cytoplasm with nuclear exclusion. In colon carcinoma, cytoplasmic accumulation of P^{53} correlates well with unfavorable prognosis (Bosari *et al*., 1995). In neuroblastoma cells, cytoplasmic P^{53} shows multiple dots representing large protein aggregates (Moll *et al*., 1996). The precise mechanism of the cytoplasmic localization isn't yet known. The functional consequence of this localization is a marked impairment of the P^{53} - mediated G cell cycle arrest in response to DNA damaging agents (Knippschild *et al*., 1996).

Thus a cytoplasmic localization of P^{53} has emerged as an alternate mechanism of inhibiting P^{53} function. In the present work, we demonstrated a new sub-cellular localization of P^{53} using monoclonal antibody PAb 1620, which is known to recognize the wild type confirmation of P^{53} (Milner *et al.,* 1987) and laser scanning microscopy, we found this particular subset of P^{53} localized in the nucleoli. In addition to, P^{53} in the wild type confirmation, P^{53} in a mutant confirmation was detected by the monoclonal antibody PAb 240 in the nucleus of prostate and bladder carcinoma cell (Gannon *et al.,* 1990). Thus, this is the first time to demonstrate two different conformational states of P^{53} within the same cells. Immunostaining was proven by immunoprecipitation using the same monoclonal antibodies. Also, P^{53} was detected in the nucleoli by the use of monoclonal antibody PAb 1620. The mechanism how P^{53} might be targeted to the nucleoli remains to be elucidated. It might be that this is achieved by binding to other cellular proteins. Indeed it was shown that P^{53} binds to the β-subunit of protein kinase CK2 (Appel *et al*., 1995) and CK2 was also found in the nuclei of the prostate carcinoma cells (Yenice *et al*., 1994). Furthermore, p^{53} is known to bind to RNA (Oberosler *et al*., 1993). Thus, our results clearly demonstrated another sub-cellular localization for P^{53} namely in the nucleoli and in addition provide evidence for the presence of at least two different forms of P^{53} within the same cells.

SAOS cells which serve as a negative control fails to show any P^{53} signal independent from the type of antibody. The cell lines don't contain P^{53} protein (Ehrhart *et al.,* 1988). In contrast, MCF-7 cells exhibited a weak cytoplasmic staining pattern only by PAb 240. A more pronounced pattern by this antibody occurs in the human keratinocyte cell line "Ha Cat", which show a strong nuclear staining with PAb 240. Additionally, this cell line was negative for all other antibodies too. The most surprising results were obtained from the prostatic carcinoma cell line; this androgene-independent cell line provides quite different patterns of P^{53} expression depending on the particular type of antibody. Thus, the wild-type of P^{53} exhibits a prominent staining within the cell nucleus. Following serum starvation of PC-3 cell lines no major changes of P^{53} expression was observed. In contrast to a wide variety of human tumors only a few mutations of P^{53} were found in prostatic carcinomas (Effert *et al.,* 1993).

Transfection of P^{53} into PC-3 cells resulted in a decreased cell growth rate. It was provided the first evidence on a correlation between loss of P^{53} function and centrosome amplification in prostate cancer cells. Our results indicate that P^{53} may play a role in the regulation of centrosome amplification and loss of P^{53} may be one of the mechanisms involving in prostate cancer cells (Ouyang *et al.,* 2001).

Prostate cancer is one of the most common cancers in men, and is one of the common features of prostate cancer in the mutation of the P^{53} gene, which occurs in over 50% prostate cancer cases. Abnormal P^{53} expression is also associated with increased stage and grade of this cancer. In addition, chromosomes instability and centrosome abnormalities are common events in prostate cancer and abnormal mitosis and chromosomal abnormalities are frequently found in prostate cancer tissues and established cell lines.

It is generally assumed that wild-type P^{53} cannot be detected by immunohistochemistry due to its short half-life. However, besides mutation complex formation of wild-type P^{53} with viral and cellular proteins may also stabilize the protein which allows the P^{53} protein to be detected by immunohistochemistry (Moll *et al*., 1996 and Benninghoff *et al*., 1999).

In addition to P^{53} gene mutations, binding to viral and cellular proteins is an alternate mechanism of inactivating in wild-type suppressor function of $P^{53.}$ In most cases P^{53} is detected by immunofluorescence or immunohistochemistry in the nucleus of the cells (Montenarh, 1992). Nuclear subfractionation revealed that P^{53} in normal and transformed cells is found in the chromatin, nuclear matrix fraction and nucleoplasmic fraction. In addition, a nuclear localization signal was identified on the poly peptide chain of P^{53}, Moreover, nuclear location of P^{53} was shown to be essential for its proper functioning (Shaulsky et al., 1991). An exclusive cytoplasmic localization has been reported for P^{53} in some non-transformed cell lines although this protein also harbors the nuclear location signal. In a subset of primary human tumors like inflammatory breast cancer, neuroblastoma (Moll *et al*., 1995), colon carcinoma (Bosari *et al*., 1995) and malignant melanoma (Weiss *et al.,* 1995) P^{53} was also found in the cytoplasmic with nuclear exclusion. In colon carcinoma, cytoplasmic accumulation of P^{53} correlates well with un favorable prognosis (Bosari *et al.,* 1995). In neuroblastoma cells, cytoplasmic P^{53} shows multiple dots representing large protein aggregates (Moll *et al.,* 1996). The precise mechanism of the cytoplasmic localization is a marked impairment of the P^{53} mediated G1 cell cycle arrest in response to DNA damaging agents (Moll *et al*., 1996). Thus a cytoplasmic localization of P^{53} has emerged as an alternate mechanism of inhibiting P^{53} function.

In our work, we demonstrated a new subcellular localization of P^{53} by using monoclonal antibody PAb 1620, which is known to recognize the wild type conformation of P^{53} (Milner et al., 1987) and laser scanning microscopy we found this particular subset of P^{53} localized in the nucleoli in different type of prostate carcinoma cell lines. In contrast to, we found negative results in other different carcinoma cell lines such as Ha Cat cells, MCF-7 cells and HT 29 cell lines. While in colon carcinomas "HT 29" it appears to be negative with few nuclear membranes staining against PAb 1620. In addition to, P^{53} in the wild-type conformation, P^{53} in a mutant type conformation was detected by the monoclonal antibody PAb 240 in the nucleoli of HT 29 cell lines and Ha Cat

cell lines and in other prostatic carcinoma cells. In contrast, MCF-7 cells exerted negative or slightly positive nuclei against PAb 240. The mechanism how P^{53} might be targeted to the nucleoli remains to be elucidated. It might be that this is achieved by binding to other cellular proteins. Indeed it was shown that P^{53} binds to the β-subunit of protein kinase CK2 (Appel *et al*., 1995) and CK2 was also found in the nuclei of prostate carcinoma cells (Yenice *et al., 1994).*

Recommendation

Our results indicated that P^{53} expression can occur at different sites within the cell. Especially in those malignancies were no mutations of P^{53} was observed. Whether this mechanism is involved in prostatic carcinomas has to be elucidated. From our observations one can conclude that only by using immunofluorescence technique in combination with image analysis, we can get detailed information about the exact localization of P^{53} within the cell.

Conclusion

It was conclude that there are two different subsets of P^{53} simultaneously present within the same cells. One subset of P^{53} is localized in the nucleus, while another entity is found to be associated with the nucleoli.

Acknowledgment

This work was supported by grants from Deutsche Krebshilfe W77/93/M02, and Funding from Fonds der chemische Industrie to MM.

References

Appel, K.; Wagner, P.; Boldyreff, B.; Issinger, O.G., and Montenarh, M. (1995): Mapping of the interaction sites of the growth suppressor protein P^{53} with the regulatory β-subunit of protein kinase CK2. *Oncogene*, 11: 1971-1978.

Benninghoff, J.; Kartarius, S.; Teleb, Z.; Selter, H.; Untergger, G.; Zwergel, T.; Wullich, B., and Montenarh, M. (1999): Two different forms of P^{53} localized differently within cells of urogenital tumors. *Cancer Letters,* 144: 55-64.

Blandino, G and Dobbelstein, M. (2004): P^{73} and P^{63}: Why do we still need them? *Cell Cycle.* 3:886-894.

Bosari, S.; Viale, G.; Roncalli, M.; Graziani, D.; Borsani, G.; Lee, A.K.C., and Coggi, G. (1995): P^{53} gene mutations, P^{53} protein accumulation and compartmentalization in colorectal adenocarcinoma. *Am. J. Pathol.,* 147:790-798.

Bourdon, J.C.; Fernandes, K.; Murray-Zmijewski, F.; Liu, G.; Diot, A.; Xirodinas, D.P.; saville, M.K., and Lane, D.P. (2005): P^{53} isoforms can regulate P^{53} transcriptional activity. *Genes and Development* 19: 2122-2137.

Brosh, R., and Rotter, V. (2009): When mutants gain new powers: news from the mutant P^{53} field. *Nat. rev. Can*. 9: 701-13..

Bykov, V.J.; Issaeva, N.; Selivanova, G., and Wiman, K.G. (2002): Mutant P^{53}-dependent growth suppression distinguishes PRIMA-1 from known anticancer drugs: a statistical analysis of information in the National Cancer Institute Database. *Carcinogenesis* 23: 2011-2018.

Chappell, W.H.; Lehmann, B.D.; Terrian, D.M.; Abrans, S.L.; Steelman, L.S.; Mc Cubrey, J.A. (2012): P^{53} expression controls prostate cancer sensitivity to chemotherapy and the MDM2 inhibitor Nutlin-3. *Cell Cycle*, 11(24): 4579-4588.

Deppert, W., and Haug, M. (1986): Evidence for free and metabolically stable P^{53} protein in nuclear sub fractions of simian virus 40-transformed cells. *Mol. Cell Biol.,* 2233-2240.

Dippold, W.G.; Jay, G.; Deleo, A.; Khoury, G., and Old, L.J. (1981): P^{53} transformation related protein, detection by monoclonal antibody in mouse and human cells. *Proc. Natt. Acad. Sci. USA,* 78:1695-1699.

Donzelli, S.; Biagioni, F.; Fausti, F.; Strano, S.; Fontemaggi, G., and Blandino, G. (2008): Oncogenomic Approaches in Exploring Gain of function of mutant P^{53}. *Curr. Genomics*, 9: 200-207.

Dorman, D.; Shimizu, H.; Burch, L.; Smith, A.J., and Hupp, T.R. (2003): The proline repeat domain of P^{53} binds directly to the transcriptional coactivator P300 and allosterically controls DNA-dependent acetylation of P^{53}. *Mol. And Cell. Biol*., 23: 8846-8861.

Effert, P.J.; Mccoy, R.H.; Walther, P.J.; Lin, E.T. (1993): P^{53} gene alterations in human prostate carcinoma. *J. Urol.,* 150:257-261.

Ehrhart, J.C.; Duthu, A.; Ullrich, S.; Appella, E., and May, P. (1988): Specific interaction between a subset of the P^{53} protein family and heat shock proteins hsp 72/hsc 73 in a human osteosarcoma cell lines. *Oncogene*, 3: 595-603.

Faria, M.H.G.; do Patrocinio, R.M. do S.V.; de Moraes Filho, M.O., and Rabenhorst, S.H.B. (2007): Immunoexpression of tumor suppressor genes P^{53}, P^{21} WAF1/CIP1 and P^{27} KIP1 in human astrocytic tumors. *Arq. Neuro-Psiquiatr*., 65:46. http:// ax.doi.org./ 10.1590/s004-2822007000700004.

Gannon, J.V.; Greaves, R.; Iggo, R., and Lane, D.P. (1990): Activating mutations in P^{53} produce a common conformational effect, a monoclonal antibody specific for the mutant form. *EMBO J*., 9: 1595-1602.

Georger, B.; Vassal, G., and Opolon, P. (2004): Oncodytic activity of P53-expressing conditionally replicative adeno-virus Ad Delta 24-P53 against human malignant glioma. *Cancer Res*., 64: 5753-5759.

Gogna, R.; Madan, E.; Kuppusamy, P., and Pati, U. (2012): Chaperoning of mutant P53 protein by Wild-type P53 protein causes hypoxic tumor regression. J. of Biolog. Chem., 287: 2907-2914.

GudKov, A.V., and Komarova, E.A. (2003): The role of P^{53} in determining sensitivity to radiotherapy. *Nat. rev. Cancer* 3: 117-129.

GudKov, A.V., and Komarova, E.A. (2005): Prospective therapeutic applications of P^{53} inhibitors. *Biochem. And Biophys. Res. Communi*., 331: 726-736.

Hainaut, p.; Soussi, T.; Shomer, B.; Hollstein, M.; Greenblatt, M.; Hoving, E.; Harvis, C.C., and Montesano, R. (1997): Database of P^{53} gene somatic mutations in human tumors and cell lines: updated compilation and future prospects. *Nucleic Acids Res*., 25: 151-157.

Harris, C.C. (1996): P^{53} tumor suppressor gene: from the basic research laboratory to the clinic-an abridged historical perspective. *Carcinogenesis*, 17: 1187-1198.

Haupt, S.; Berger, M.; Goldberg, Z., and Haupt, Y. (2003): Apoptosis the P^{53} network. *J. of Cell Sci*. 116: 4077-4085.

Hsueh, C.; Wang, H.; Gonzalez-Crussi, F.; Lin, J.N.; Hung, N.; Yang, C.P., and Jaing, T.H. (2002): Infrequent P^{53} gene mutations and lack of P^{53} protein expression in clear cell sarcoma of the Kidney: Immunohistochemistry study and mutation analysis of P^{53} in renal tumors of unfavorable prognosis. *Mod. Pathol*., 15: 606-610.

Knippschild, U.; Oren, M., and Deppert, W. (1996): Abrogation of Wild type P^{53} mediated growth-inhibition by nuclear exclusion. *Oncogene*, 12: 1755-1765.

Lacroix, M.; Toillon, R.A., and Leclercq, G. (2004): Stable "Portrait" of breast tumors during progression: data from biology, Pathology and genetics, *Endocrine-Related Cancer*. 11: 497-522.

Lang, P.; Maurer, J.; Hoher, D.; Bornhoft, G.; Montenarh, M.; Berdel, W.; Thiel, E., and Kreuser, E.D. (1993): Mutation and expression of the P^{53} tumor suppressor gene in tumor samples and cancer cell lines: Comparison of non-isotopic direct DNA-sequencing, immunoblotting and immunohistochemistry. *Int. J. Onc.,* 2: 347-355.

Levine, A.J.; Momand, J., and Finlay, C.A. (1991): The P^{53} tumor suppressor gene. Nature, 251:453-456.

Levine, A.J., and Oren, M. (2009): The first 30 years of P^{53}: growing ever more complex. *Nat. Rev. Cancer*. 9: 749-58.

Ljungman, M. (2000): Dial 9-1-1 for P^{53}: mechanisms of P^{53} activation by cellular stress. Neoplasia

Lozano, G. (2007): The oncogenic roles of P^{53} mutants in mouse models. *Curr. Opinion in gen. and Devel.,* 17: 66-70.

Mayo, L.D.; Rok, Seo, Y.; Jakson, M.W.; Smith, M.L.; Rivera, Guzman, J.R.; korgaonkar, C.K., and Donner, D.B. (2005): Phosphorylation of human P^{53} at serine 46 determines promoter selection and whether apoptosis is attenuated or amplified. *J. of Biol. Chem*., 280: 25953-25959.

Meek, D.W. (2004): The P^{53} response to DNA damage. *DNA repair* 3: 1049-1056.

Milner, J.A.; Cook, A., and Sheldon, M. (1987): A new anti-P53 monoclonal antibody, previously reported to be directed against the large T antigen of simian virus 40. *Oncogene*, 1:453-455.

Moll, U.M.; La Qugglia, M.; Benard, J., and Rion, G. (1995): Wild type P^{53} protein undergoes cytoplasmic sequestration in undifferentiated neuroblastomas but not in differentiated tumors. *Proc. Natl. Acad. Sci. USA*. 92:4407-4411.

Moll, U.M.; Ostermeyer, A.G.; Haladay, R.; Winkfield, B.; Frazier, M., and Zambetti, G. (1996): Cytoplasmic sequestration of wild type P^{53} protein impairs the G1 check-point after DNA damage. *Mol. Cell Biol*., 16:1126-1137.

Moll, U.M.; Riou, G., and Levine, A.J. (1992): Two distinct mechanisms alter P^{53} in breast cancer: mutation and nuclear exclusion. *Proc. Natl. Acad. Sci. USA*. 89:7262-7266.

Montenarh, M. (1992): Biochemical, immunological and functional aspects of the growth suppressor/oncoprotein P^{53}, Critical revieus in *Oncogenesis*, CRC Press, Boca Raton, pp. 233-256. Moul. J. W., Bettencourt, M.C., Sesterhenn, I.A.; Mostof; F.K.

Moshe, O and Rotter, V. (2009): Mutant P^{53} Gain of function in cancer. Cold spring Harbor Perspectives in Biology. *Published in Advance*. Dec. 16, copyright 2010 Cold spring Harbor Laboratory Press.

Nakano, K., and Vousden, K.H. (2001): PUMA, a novel proapoptotic gene, is induced by P^{53}. *Mol. Cell* 7:683-694.

Navone, N.M.; Troncoso, P.; Pisters, L.L.; Goodron, T.L.; Palmer, J.L.; Nichols, W.W.; Von Eschenbach, A.C., and Couti, C.J. (1993): P^{53} protein accumulation and gene mutation in the progression of human prostate carcinoma. J. Nat. Cancer Inst., 85: 1657-1669.

Oberosler, P.; Hloch, P.; Ramsperger, U., and Stahl, H. (1993): P^{53} catalyzed annealing of complementary single-stranded nucleic acids. *EMBO J.* 12:2389-2396.

Olivier, M.; Petitjean, A.; Marcel, V.; Petre, A.; Mounawar, M.; Plymoth, A.; de Fromental, C.C., and Hainaut, P. (2009): Recent advances in P^{53} research an interdisciplinary perspective. *Cancer Gene Thera*. 16: 1-12.

Oren M., Rotter, V. (2010): Mutant P53 gain of function in cancer. *Cold spring Harb Respect Biol*., 2: a001107.

Ouyang, X.; Wang, X.; Xu, K.; Jin, D.Y.; Cheung, A.L.M.; Tsao, S.W., and Wang, Y.C. (2001): Effect of P^{53} on centrosome amplification in prostate cancer cells. *Biochem. And Biophys. Acta., (BBA).* Molecular Cell Research. 1541(3): 212-220.

Peng, Y.; Li, C.; Chen, L.; Sebti., and Chen, J. (2003): Rescue of mutant P^{53} transcriptional function by ellipticine. *Oncogene* 22: 4478-4487.

Petitjean, A.; Mathe, E., and Kato, S. (2007): Impact of mutant P^{53} functional properties on TP^{53} recent developments in the IARC TP^{53} database. *Hum. Mutant*. 28: 622-629.

Pucci, B.; Kasten, M., and Giordano, A. (2000): Cell cycle and apoptosis. Neoplasia, 2: 291-299.

Robinson, M.; Jaing, P.; Cui, J.; Li, J.; Wang, Y.; Swaroop, M.; Madore, S.; Lawrence, T.S., and Sun, Y. (2003): Global gene chip profiling to identify genes responsive to P^{53}-induced growth arrest and apoptosis in human lung carcinoma cells. *Cancer Biol. Ther.,* 2: 406-415.

Rotter, V. (1983): P53, a transformation-related cellular encoded protein, can be used as a biochemical marker for the detection of primary mouse tumor cells *Proc. Natl. Acad. Sci. USA*. 80: 2613-2617.

Sarkis, A.S.; Bajorin, D.F.; Reuter, V.E.; Herr, H.W.; BNetto, G.; Zhang, Z.F.; Schultz, P.k.; Cordon-Cardo, C., and Sicher, H.I. (1995): Frognostic value of P53 nuclear over expression in patients with invasive bladder cancer treated with neoadjuvant MVAC. J. Cli. Oncol., 13: 1384-1390.

Savkur, R.S., and Burris, T.P. (2004): The co activator LXXLL nuclear receptor recognition motif. *J. of Peptide Res*., 63: 207-212.

Schlomm, T.; Iwers, L.; Kirstein, P. (2008): Clinical significance of P^{53} alterations in surgically treated prostate cancers. *Mod. Pathol*., 21: 1371-8.

Selter, H., and Montenarh, M. (1994): The emerging picture of P^{53}. Int. J. Biochem., 26: 145-154.

Shaulsky, G.; Gold finger, N.; Tosky, M.S.; Levine, A.T., and Rotter, V. (1991): Nuclear location is essential for the activity of P^{53} protein. *Oncogene*. 6:2055-2065.

Shou, J.; Ross, S.; Koeppen, H.; de Sauvage, F.J., and Gao, W.Q. (2001): Dynamics of notch expression during development and tumorigenesis. Cancer Res., 61: 7291-7297.

Soussi, T. (2005): Analysis of P^{53} gene alterations in cancer: a critical view. In: Pierre Hainaut KGW, edit. 25years of P^{53} research. *New York Springer*. P: 255-92.

Stattin, P.; Bergh, A.; Karlberg, L.; Nordgren, H., and Damberm, J.E. (1996): p^{53} immuno reactivity as prognostic marker for cancer specific survival in prostate Cancer. *Eur. Urol*., 30:65-72.

Strano, S.; Dell'orso, S.; Di Agostino, S.; Fontemaggi, G.; Sacchi, A., and Blandino, G. (2007): Mutant P^{53}: An oncogenic transcription factor. *Oncogene*. 26: 2212-2219.

Takeo, O. (2005): The role of the P^{53} molecule in cancer therapies with radiation and hyperthermia. *J. of Cancer Res. And Thera. JCRT*, 1(3): 147-150.

Van Bokhoven, A.; Varella-Garcia, M.; Korch, C.; Johannes, W.U.; Smith, S.S.; Miller, H.L.; Nordeen, S.K., and Lucia, M.S. (2003): Molecular characterization of human prostate carcinoma cell lines. Prostate, 57: 20.

Vazquez, A.; Bond, E.E.; Levine, A.J., and Bond, G.L. (2008): The genetics of the P^{53} and pathway, apoptosis and cancer therapy. *Nat. Rev. Drug Discov*., 7: 979-987.

Vogelstein, B.; Lane, D., and Levine, A.J. (2000): Surfing the P^{53} network. Nature, 408: 307-310.

Vogelstein, B.; Sur., S., and Prives, C. (2010): P^{53}: The most frequently altered gene in human cancers. *Nature Education* 3(9):6.

Vousden, K.H., and Lu, X. (2002): Live or let die: The cell's response to P^{53}. *Nat. Rev. Can*., 2:594-604.

Weinberg, R.L.; Freund, S.M.; Veprintsev, D.B.; Bycroft, M., and Fersht, A.R. (2004): Regulation of DNA binding of P^{53} by its C-terminal domain. *J. of Mol. Biol*., 342: 801-811.

Weiss, J.; Heine, M.; Korner, B.; Pilch, H., and Jung, E.G. (1995): Expression of P^{53} protein in malignant melanoma: Clinicopathological and prognostic implication. *Br. J. Dermatol*., 133: 23-31.

Xu, J.; Reumers, J.; Couceiro, J.R.; De Smet, F.; Gallarado, R.; Rudyak, S.; Comelis, A.; Rozenski, J.; Zwolinska, A.; Marine, J.C.; Lambrechts, D.; Suh, Y.A.; Roussean, F., and Schymkowitz, J. (2011): Gain of function of mutant P53 by coaggregation with multiple tumor suppressors. *Nat. Chem. Biol*., 7: 285-295.

Yenice, S.; Davis, A.T.; Goneli, S.A.; Akdas, A.; Limas, C., and Ahmed, K. (1994): Nuclear casein kinase 2 (CK-2) activities in human normal, benign hyper plastic, and cancerons prostate. *Prostate* 24:11-16.

Yu, J., and Zhang, L. (2005): The transcriptional targets of P^{53} in apoptosis control. *Biochem. And Biophys. Res. Comm*unications. 331: 851-858.

Zou, Z.; Gao, C.; Nagoich, A.K.; Connell, T.; Saito, S.; Moul, J.W.; Seth, P.; Appella, E., and Srivastava, S. (2000): P^{53} regulates the expression of the tumor suppressor gene maspin. The J.of Biologo. Chem., 275: 6051-6054.