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Significant Value of p53 Accumulated in Invasive Ductal Breast Carcinoma

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Abstract

Background: The presence of a functional p53 protein is a key factor for the appropriate suppression of cancer development. The tumor suppressor p53 accumulates under stressful conditions, such as DNA damage, heat shock, hypoxia and/or proto-oncogene activation, although conflicting reports exist on its transcriptional activity. A loss of p53 activity, by mutations or inhibition, is often associated with human malignancies. This work investigated the significant value of p53 accumulated in IDBC (Invasive Ductal Breast Carcinoma) and at the same time tries to arise different supports of this value.

Results: To ensure this objective, we referred to two types of statistical analysis, the *chi-square* and logistic regression analysis. They confirmed the poor prognosis of p53 accumulated in IDBC (β^*=-0.456 with p=0.00001) and showed that the independent variables (MDM2, BCL2, BAX and ER) formed an interesting model to explain the significant value of p53 accumulated in the IDBC. The predictive value of the model including the four biomarkers is AUC=93.5%, showing that if we take the expression status of the four biomarkers, we can deduce the status of p53 with a reliability of 93.5%.

The residual term, representing 6.5% and involved in this significant value, corresponds to intrinsic modifications of p53: Alterations of the *TP53* gene, p53-oncoprotein interaction or cytoplasmic sequestration. In fact, following the IHC results of three different antibodies that recognize wild type or mutant p53, we examined the status of polymorphism 72, which may inform LOH (loss of heterozygozity). We found LOH associated with *TP53* mutations in the context of down-regulated p53 target genes revealed by IHC. Although wild type in some cases, p53 loses its transcriptional activity; this may be due to oxidation of cysteine residues in the core domain, either iSAPP interaction or its cytoplasmic sequestration.

Conclusion: p53 accumulated in IDBC had a significant value and the etiological factors of this value should be target for effective therapy.

Keywords: p53; MDM2; BAX; BCL2; Oestrogen receptor; Invasive ductal breast Carcinoma; Bad prognostic

Abbreviations: ER: Oestrogen Receptor; IDBC: Invasive Ductal Breast Carcinoma

Introduction

Invasive Ductal Breast Carcinoma (IDBC) is the most common form of invasive or expanding breast cancer. IDBC accounts for approximately 83.3% of invasive breast cancer cases in Tunisia [1]. It is also called invasive ductal carcinoma. The cancer begins in the milk ducts, which are responsible for transporting milk from the breast to the nipples for breastfeeding. Unlike Ductal Carcinoma *In Situ* (DCIS), where cancer cells are only found in the lining of the ducts, invasive ductal carcinoma means that cancer cells have spread outside the milk ducts to other parts of the breast. Depending on when it is detected and how aggressive the tumor is, breast cancer can spread to the lymph nodes and throughout the body.

Inactivation of the tumor suppressor protein p53 is considered to be a major risk of acquiring multiple gene lesions, thus promoting tumour development [2]. p53 is a transcription factor. In response to various types of genotoxic stresses, p53 trans-activates a number of genes by binding to specific DNA sequences, thereby targeting cell cycle arrest, repair of damaged DNA, differentiation or apoptosis as cell fate [3].

p53 has been shown to accumulate under stressful conditions, although conflicting reports exist on its transcriptional activity. Traditionally, p53 controls cellular homeostasis by affecting cell cycle progression and apoptosis. This function, however, is lost in many tumours, which contributes to dysfunction.

Normally, p53 has an extremely short half-life and protein levels are kept very low, often undetectable. Upon exposure to stress such as DNA damage, oncogene activation or hypoxia, p53 is stabilized, primarily by post-translational modification. Consequently, p53 becomes active as a transcription factor and promotes the transcription of cell cycle regulatory genes such as *p21WAF1/CIP1* or *mdm2* but also of genes involved in apoptotic events such as BAX or Fas [4]. BAX suppresses the ability of BCL2 to block apoptosis. It has been shown that the expression of BCL2 is essential in the regulation of apoptosis in breast carcinoma [5].

Since p53 is a nuclear sequence-specific transcription factor that transactivates a set of its target genes involved in cell cycle arrest and/or apoptotic cell death, mutant forms of p53 lose their critical function in maintaining genomic integrity. Moreover, the mutant forms of p53 acquire a much longer half-life compared to that of wild type p53 and some show dominant-negative behaviour towards wild-type p53 [6]. This dominant-negative effect of mutant p53 on wild-type p53 might be mediated by hetero-oligomerization through their oligomerization domains [7]. In this regard, the TP53 mutation confers resistance of tumor cells to anti-cancer drugs by inhibiting the p53-dependent pro-apoptotic pathway [8].

Understanding apoptotic signaling pathways helps in the development of particular inhibitors for anti-apoptotic proteins and activators of pro-apoptotic proteins. In both apoptosis pathways (extrinsic and intrinsic), pro-apoptotic and anti-apoptotic proteins act as potential regulators of cell division and cell growth. BAX pro-apoptotic proteins trigger the activation of the intrinsic pathway, an excellent target for the development of therapeutics and are currently in clinical trials. Similarly, the anti-apoptotic protein inhibitor is also well on the way in the drug development process. The considerable importance of apoptosis-based anti-cancer drugs is also due to improving drug sensitivity by reversing resistance mechanisms in cancer cells. The dysregulated or inactivated mechanism of apoptosis involves BCL-2 family proteins that include both pro-apoptotic member downregulation and anti-apoptotic upregulation and tumour suppressor (p53) regulation [9].

The first mechanistic connection between p53 and BCL-2 came from the identification of the multi-domain pro-apoptotic BCL-2 family member BAX, initially identified by the Korsmeyer laboratory [10]. Moreover, stress-activated BAX induction by p53 can overcome the anti-apoptotic effects of BCL-2, indeed, BAX-deficient cells are resistant to certain stimuli known to promote p53-dependent apoptosis [11]. For example, dysregulated expression of an oncogene induces p53-dependent apoptosis which is attenuated in the absence of BAX [11]. Thus, p53-mediated regulation of the ratio of BAX protein level to BCL-2 may influence a cells fate in response to stress.

Besides its ability to act on the transcription of BCL2 antagonists, p53 may act in other way to regulate BCL2. For example, it can repress BCL2 transcription under certain conditions. In this context, it has been shown that the introduction of p53 into some null cell lines generates a repressed expression of BCL2. Gamma irradiation leads to the induction of p53 and consequently to a reduced expression of BCL2 in leukemic cells. Although the mechanism is not fully understood, the BCL2 promotor contains a negative p53 responsive element, suggesting that BCL2 could be a potential target for repressions by the p53 transcription factor [12].

Materials and Methods

Tissue samples

Samples of 70 primary breast carcinomas, diagnosed with Invasive Ductal Carcinoma of the Breast (IDBC), were obtained from the antomic cytological pathology department, CHU Habib Bourguiba Sfax, Tunisia. Tumours were graded using the Scarf-Bloom Richardson scale (well differentiated=SBR-I-, moderately differentiated=SBR-II- and poorly differentiated=SBR-III-). The clinicopathologic information of the patients was obtained from patient files and anatomo-pathologic reports from the radiotherapy and clinical oncology department of the CHU Habib Bourguiba Sfax, Tunisia.

Immunohistochemistry

Tumours, fresh-frozen sections 4 µm thick, were treated with 0.3% hydrogen methanol to remove endogenous peroxidase activity. After blocking with 2% skimmed milk, sections were incubated with an appropriate monoclonal antibody. The antibodies produced by the mouse were: p53:DO7 from DAKO, which recognizes wild type and mutant; 1801 (DAKO) which only recognizes the wild type and 240 (DAKO) which only recognizes the mutant form; MDM2 (from Calbiochem); BCL2 (DAKO) and ER (DAKO). For the immuno-detection of BAX, a polyclonal antibody (Calbiochem) was used. Goat anti-mouse antibody (DAKO) was used as a secondary antibody and revealed by streptavidin-linked peroxidase (DAKO).

The slides were incubated in the presence of diaminobenzidine or the chromogenic substrate of peroxidase and hydrogen peroxide, to form a coloured precipitate.

We considered p53 and ER nuclear immunoreactivity to be positive when present in 20% or more of tumour cells nuclei. Cytoplasmic immunostaining for BCL2 and BAX were declared positive when present in 20% and 30% of cells, respectively. For MDM2, cytoplasmic or nuclear immunostaining with more than 20% of cells was considered positive.

Statistical analyses

Univariate and bivariate analysis: Positivity rates for the biomarkers and SBR grade were estimated as frequencies and age distribution was summarized as mean ± standard deviation and media (Q1-Q3). Age was then recoded as a binary variable according to the median.

The *chi-square* χ^2 test was used to statistically evaluate the relationships between the p53 status and the other biomarkers (MDM2, BCL2, BAX and ER), histological grade (SBR) and age. The association of each of the biomarkers with p53 was further evaluated using logistic regression with age and SBR grade as confounding factor and adjusted Odds Ratio (OR) and their confidence interval were obtained.

The OR is thus a measure of the strength and direction of the association between each biomarker and p53, adjusted for age and SBR grade (Figure 1).

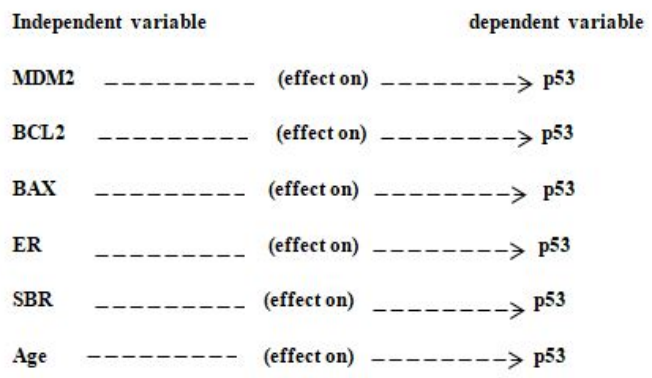

Figure 1: Brute association measure, univariate analysis; the effect of one independent variable, on p53 accumulated (dependent variable).

Multivariate association analyses: Multivariate binary logistic regression analysis was used to study the relationship between p53 status as dependent variable (Y) and the other biomarker as explanatory variables, adjusting for age and SBR grade. It allows estimating the effect size of each explanatory variable through an adjusted Odds Ratio (OR) and its 95% confidence interval as well as the accuracy with which the status of the four biomarkers considered is predictive of p53 status (Figure 2).

Logistic regression fits a generalized linear model that links the probability p of p53 being positive with the positivity status of the other biomarkers taken together, while adjusting for age and SBR grade, through the following equation:

$$Log\left(\frac{p}{1-p}\right) = \beta 0 + \beta 1ER + \beta 2BCL2 + \beta 3MDM + \beta 4BAX + \beta 5SBR + \beta 6AGE + e$$

Where β are regression coefficients estimated from data and e is the residual of the model. OR are calculated by exp(β) (exp is the exponential mathematical function). Naive Bayesian classifier was also used to estimated conditional probabilities of p53 positivity given positivity of other biomarkers.

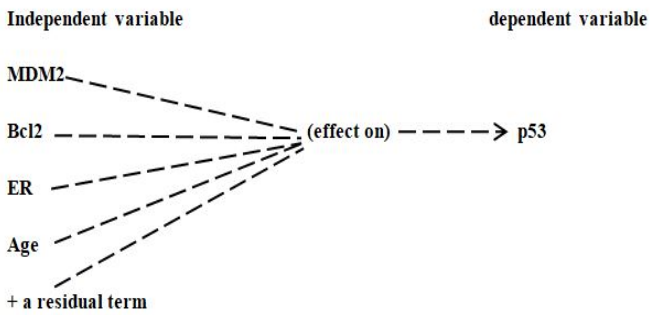

Figure 2: Principe of logistic regression analysis, the resultant effect study of three molecular markers (MDM2, BCL2 and ER) on p53 accumulated (dependent variable).

The goodness of fit of the model was evaluated using several metrics: AIC (Akaike Information Criterion), Area under the Curve (AUC) of the ROC curve (representing sensitivity versus 1-specificity for different probability cutoffs and sensitivity, specificity and accuracy for the best probability cutoff.

All statistical analyses were performed using R language with appropriate functions. Package e1710 was used for Naive Bayes classifier and package pROC was used to generate ROC curves, AUC and sensitivity/specificity for the best cutoff.

Molecular analyses

Identification of the polymorphism at codon 72 was used as a method for detecting Loss of Heterozygosity (LOH), according to our method: Exon 4-6 of the *TP53* gene is amplified by PCR on the extracted DNA corresponding tissues and blood, then digested with the restriction enzyme AccII.

Genomic DNA extraction: Genomic DNA was extracted from tumour tissues by proteinase K digestion and phenol/ chlorophore treatment according to the method of Bos, et al. The corresponding blood DNA was extracted according to the method of Sambrook, et al. The PCR and AccII digestion conditions were carried out [13-15].

Sequencing: The PCR fragments were sequenced according to the Sanger method with the thermo-sequenase cycle sequencing kit (Amersham).

Ethics: Informed consent was obtained from all patients and the study obtained the agreement of the ethics committee of South Tunisia acting under the Ministry of health (CPP Sud).

Results

Description of the sample

The age of patients varied between 25 and 83 years with a median of 53 years (IQR: 42-63). p53 was accumulated in 58.6%of cases. ER, BCL2, BAX and MDM2 were positive in 50.7%, 37.7%, 11.6% and 63.7% of tumours, respectively. Most tumours were in SBR grade II and III with only 5 cases over 96 in grade I. For multivariate analyses, SBR and age were recoded as binary variables with grade I and II tumours merged and age below and over 53 years, in order to avoid inflation of the estimated of regression coefficients.

p53 accumulated in IDBC is a bad prognostic

Figure 3 shows a sample of p53 immunostaining detection with nuclear and cytoplasmic localization. P53 was accumulated in 58.6% of cases.

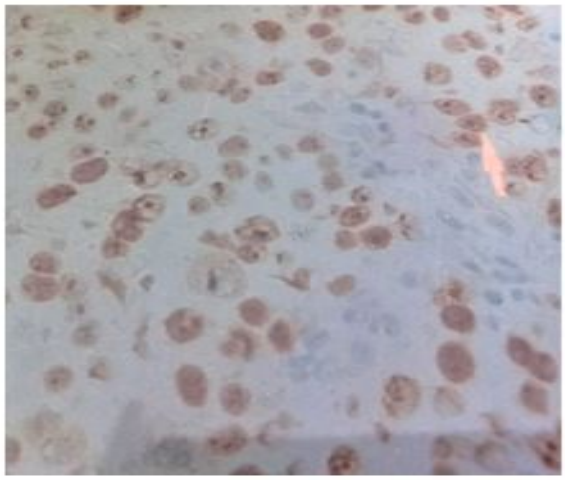

Figure 3: Immunostaing photos of p53.

Bivariate analysis: p53 overexpression was found to be significantly associated with overexpressed MDM2, downregulated BCL2 and repressed ER and in advanced SBR grade (III). No significant association was found with neither BAX nor age (Table 1). Highly significant negative associations were found with ER and BCL2, showing that most overexpressing these two proteins have low expression of p53. However, a significant positive association was found with MDM2, with 78%of tumours overexpressing p53 also having MDM overexpression. Tumours of SBR grade III accumulate more p53 than lower grade tumours.

Table1: Association of p53 expression status with biomarkers, age and SBR grade.

Variable	% Positive in p53 classes	p-value	Adjusted OR	95% CI
ER	85.3% *vs.* 26.8%	5.10^{-6}	0.58	0.47-0.71
BCL2	64.3% *vs.* 19.5%	0.0004	0.66	0.53-0.82
BAX	3.6% *vs.* 17.1%	0.18	1.26	0.87-1.83
MDM2	42.8% *vs.* 78.1%	0.006	1.47	1.18-1.84
Age (>53)	53.5% *vs.* 51.2%	0.85	1	0.79-1.26
SBR grade I+II *vs.* III	7.1% *vs.* 29.3%	0.025	1.39	1.04-1.89

Multivariate analyses: Multivariate analysis using logistic regression shows a significant association between p53 expression and all four biomarkers, including BAX which was not significant in bivariate analysis. A negative association with ER and BCL2 is reported indicating that tumours expressing high levels of p53 are less likely to express ER and BCL2 and vice versa. On the other side a positive association with BAX and MDM2 was reported, indicating that tumours with high p53 expression are more likely to express MDM2; and BAX detected in IDBC in context of p53 overexpression. The predictive value of the model including the four biomarkers is AUC=93.5% showing that if we take the expression status of the four biomarkers, we can deduce the status of p53 with a reliability of 93.5%. The overall correct (accuracy) of the model was 87% (for a probability cutoff of 0.54) indicating that the model correctly predicts the p53 status of 87% of the tumours, while the sensitivity is 90.2% indicating that 90% of tumours over expressing p53 are correctly classified as p53-positive with the model (Table 2).

The Naive Bayes model gives similar performance with AUC=91.3%, 85.5% accuracy and 92.7% sensitivity (for a probability cutoff of 0.4).

Table 2: Multivariate analysis using logistic regression.

Independent variable	β*	SE*	OR (95% CI)	p value
ER	-0.456	0.096	0.63 (0.52-0.76)	0.00001
BCL2	-0.288	0.099	0.75 (0.62-0.91)	0.0049
BAX	0.378	0.144	1.46 (1.10-1.94)	0.011
MDM2	0.303	0.089	1.35 (1.14-1.61)	0.0011

Thus, the statistical analysis showed a significant value of p53 accumulated in ICBC as bad prognostic.

What can be the residual term, which involved in 6.5% of p53 accumulated value in IDBC?

This residual term involves the intrinsic p53 modi fications: As *TP53* is tumor suppressor gene, it responds to The Knudson "two-hit" hypothesis: One copy of the gene is harbouring mutation, while the other paternal copy is targeted for chromosomal aberration. We adopted for looking for such *TP53* alterations a following strategy [16]:

Firstly, we study by IHC the state of p53 using three antibodies: DO7 that recognize wild type and mutant p53, 1801 that recognize only wild type and 240 that recognize only mutant p53, in fact, the conformational change of wild-type p53 exposes the Pab240 epitope, which is a feature of mutant p53. Then for the cases, revealed by IHC mutant p53 (DO7+, 1801- and 240+), we looked for LOH by exanimating the polymorphism 72 profile of *TP53* from blood; whose showing an heterozygotic profile we passed to examinate from corresponding tissue (Figure 4). Which presented LOH, we passed then to examinate the *TP53* sequence looking for eventual mutation.

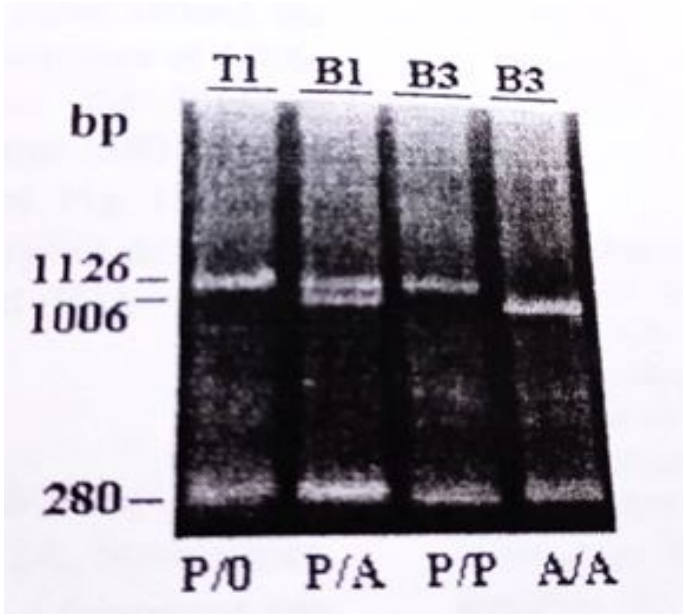

Figure 4: In the case of a heterozygote, the comparison of tumor and blood polymorphism profile allowed the identification of a potential LOH in the TP53 gene.

The tumor (T1) shows an example of LOH total (loss of allele Arg) compared to the corresponding blood (B1).

- 17% of cases are DO7+/1801-/240+ some of which, MDM2- BAX- BCL2+ ER-, presented LOH total and mutations in codns175, 259 and 268 and developed metastasis.
- 76% DO7+/1801+/240+ these cases presented the hallmark of heterogeneity tumor some of which presents partial LOH and mutation in codons 151 and 74 and developed metastasis (Figure 5).

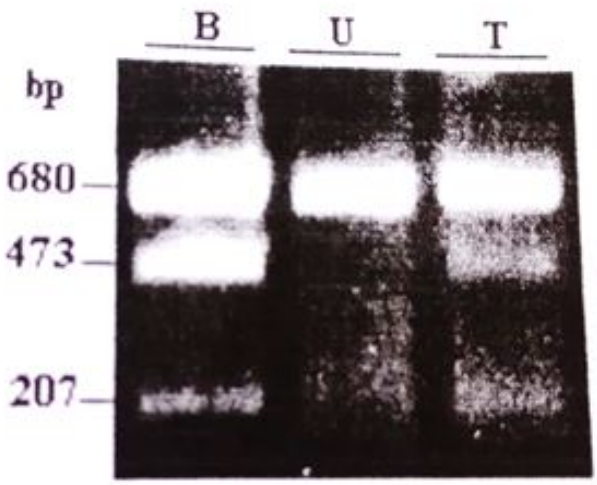

Figure 5: Partial LOH.

When comparing between polymorphism blood profile with tumor profile, we found a difference in band intensity; revealing a difference in alleles representation in the case of tumor tissue. This according to the heterogeneity of the tumors. This case showing a tumoral population lost the Arg allele.

- 7% of cases are DO7+/1801+/240- with MDM2- BAX- BCL2- ER- phenotype: Even though these cases corresponding to p53wt, they are transcriptionally inactive related to downregulated p53 target genes revealed by IHC.

Discussion

MDM2 accumulation is one of etiologic factor of p53 significant value

Mdm2 gene is expressed in 77% of cases. MDM2 is involved significant value of accumulated p53 in IDBC. In fact, *Mdm2* is known as p53 target gene; *Mdm2* contains two promotors P1 and P2; the promotor P1 is a target of PTEN/PI3K/AKT signaling pathway that can modulates the transcription of *Mdm2* independently of p53; whereas, the function of the promotor P2 is dependent of p53. The oncogenic potential of MDM2 has been rapidly associated to its ability to bind and inhibit the tumor suppressor p53 [17]. In normal conditions, MDM2 is expressed in nucleus, but it is translocated to the cytoplasm for inducing the degradation of certain its targets by the proteasomes (Figure 6).

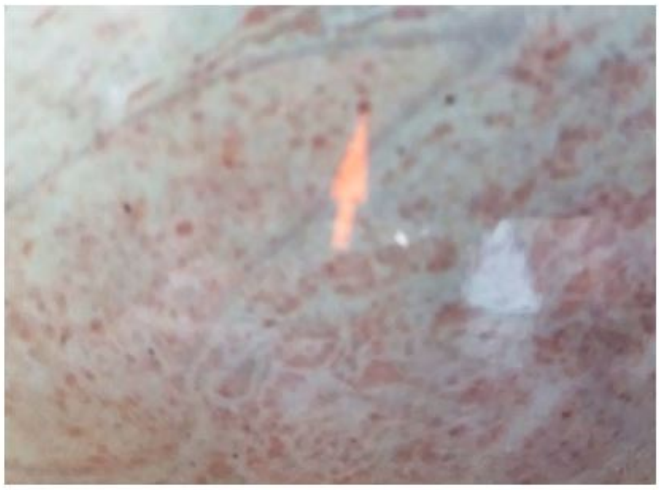

Figure 6: Immunostaining of MDM2 *in situ* and in invasive ductal carcinoma.

The statistical analysis showed that MDM2 is involved in the significant value of p53 accumulated in IDBC according to *chi-square* and justified by logistic regression analysis. MDM2 is a component of a p53-dependent negative feedback loop. The N-terminal hydrophobic pocket of MDM2 binds to p53 and thereby inhibits the transcription of p53 target genes. Additionally, the C-terminus of MDM2 contains a RING domain with intrinsic ubiquitin E3 ligase activity. By recruiting E2 ubiquitin-conjugating enzyme(s), MDM2 acts as a molecular scaffold to facilitate p53 ubiquitination and proteasome-dependent degradation.

MDM2 efficiently degrades wild-type p53 but fails to degrade mutant p53 in tumor cells. Mutant p53 interferes with the intramolecular autoactivation mechanism of MDM2, contributing to reduced ubiquitination and increased accumulation in tumor cells [18].

MDM2 can complex with p53 wild type associated with other proteins sustaining p53 stabilised but inactive, since the activation domain of p53 is engaged in MDM2 interaction. Such protein, HAUSP, the deubiquitinase HAUSP was first identified as a Herpes virus-associated cellular factor and subsequently shown to deubiquitinate and stabilize p53. HAUSP deubiquitinates p53 through an indirect interaction mediated by MDM2. The close proximity of HAUSP to p53, through this indirect interaction, is sufficient for the deubiquitination of p53 [19].

p53 and BAX relationship: M DM2 interferes with BAX p53 induction

BAX was detected in only 15% of cases comparing to MDM2. The results of statistical binary analysis showed no significant association between p53 and BAX. This state can be interpreted, in part:

In referring to two genetic facts: In fact, we distinguish among p53 target genes, early target genes, as *p21/Waf1* and *mdm2* and lates ones, as BAX. In fact, in response to DNA damage, stressed activated p53 triggers, in the first step, the cycle arrest by induction of the *p21/Waf1* expression; in order to allow DNAreparation.

To the extent where the reparation is failed, the stressed activated p53 triggers the expression of apoptotic protein, such as, BAX.

These differential times of target gene expression can be related to two genetic facts:

- The availability of strength or weakness of p53 target gene promotor. The canonical p53 response element (p53RE), which contains two repeats of a decamer motif "RRRCWWGYYY" separated by a spacer of 0 to 13 base-pairs, has been characterized as the regulatory region on the target genes that p53 binds for transcriptional activation. Thus, the late p53 target genes lack this canonical p53RE triggering p53 to require other stressed transcriptional factors to induce these late genes which explains the absence of direct statistical link [20].
- The different access to the canonical p53RE which related to epigenetic landscape, whose is regulated by stressful conditions in time and in space.

MDM2 p53 inactivation impacts on BAX expression but not on BCL2 repression, in second part: Although there is no significant association between the accumulated p53 and BAX expression, the rate of p53+/BAX- is 78% and in multivariate analysis, we found a significant association of p53+/BAX-/ER-, p=0.000004. The looking for the role of MDM2, in interfering with p53 in mediating BAX expression, showed the significant association of MDM2+ with p53+/BAX-, p=0.04. But MDM2 didn't interfere with p53 in mediating BCL2 repression. In fact, the BCL2 down regulation is not affected in IDBC; the multivariate analysis showed the MDM2+/P53+/BCL2- with p=0.00005. BCL2 protein expression analysis provides a better prognostic value [21]. That means, that the two activities of p53, transactivation and repression, are contexts independents (Figure 7).

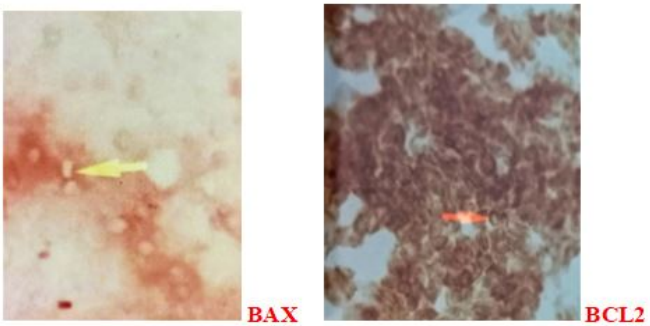

Figure 7: Immunostaining photos of BAX and BCL2 showing cyotplasmic localisation of either BAX and BCL2.

TP53 gene mutation another hit of p53 inactivation

In fact, mutations in *TP53* tumour suppressor gene were identified in most human cancers [22]. More than 70% of these mutations are missense. Inactivating missense mutations of TP53 are advantageous during cancer development due to their action as trans-dominant inhibitors of wild-type p53. Moreover, accumulation of point-mutated p53 protein in the cancer cell contributes to transformation and metastasis [23].

In this case, mutated p53 protein gains new pro-oncogenic functions. Molecular mechanisms underlying the gain-of-function phenotype, leading to increased cell migration and invasion, are still not clear. Several laboratories presented evidence that mutant p53 can be a transcription factor in its own right and that it can interfere with or modify functions of other proteins, with these scenarios not being mutually exclusive [24,25]. Mutated forms of p53 interact with their paralogs-p63 and p73, negatively regulating their function [26].

Inactivated p53 wild type: DO7+/1801+/240- with MDM2- BAX- BCL2- ER- phenotype

This phenotype can be the results of three processes:

Oxidation of cysteines residues of p53 DNA binding domain: The structure of the p53 core DNA-binding domain (residues 94-312) that binds directly to the DNA sequence has been resolved by x-ray crystallography and both x-ray crystallography and NMR analysis have been used to deduce the structure of the tetramerization domain (residues 323-356), which is needed for optimum function [27]. p53 functions primarily as a transcription factor and is biologically active as a homotetramer. It has a modular domain structure, consisting of folded DNA-binding and tetramerization domains, flanked by intrinsically disordered regions at both the amino- and carboxy-termini. The structure of the DNA-binding core domain (residues 94-292) consists of a central immunoglobulin-like β-sandwich scaffold and additional structural elements that form the DNA-binding surface; which include a loop-sheet-helix motif and two large loops (L2 and L3). The architecture of the L2/L3 region is stabilized by a zinc ion, which is tetrahedrally coordinated by Cys176, His179, Cys238 and Cys242 [28].

The p53 tetramer cooperatively binds to its target duplex DNA in a sequence-specific manner. The fundamental active unit of p53 appears to be the tetramer. P53 itself is redox active due to the presence of cysteines (Cys) that contain redox sensitive thiol groups (-SH) (Figure 8). In fact, in human p53, there are two clusters of cysteines in the DNA-binding domain, which are essential to the specific binding of p53 to its consensus sequence. Cys 176, 238 and 242, along with histidine 179, consist of a binding site for Zn^{2+} [29]. Mutations of these Zn^{2+}-ligands diminish the sequence-specific DNA binding of p53. Cys 124, 135, 141, 182 and 277 are located in the loop-sheet-helix region of the proximal DNA-binding domain of p53. They constitute a structural platform for redox modulation. Theoretically, there are multiple possible structures of oxidized thiol groups in proteins, including sulphenic acid (-SOH), disulfide (-S-S-), sulphenamide ($-SNR_1R_2$), sulphinic acid ($-SO_2H$) and sulphonic acid ($-SO_3H$) [30]. p53 accumulated under O2 deficiency it remained transcriptionally inactive [31,32]. It has been observed that treating p53 with oxidizing reagents abolishes its DNA-binding activity. Two recent studies identified the sites and structural details of p53 oxidation, GSH was found to be attached to either Cys124 or 141 and to 182 of p53 *via* disulfide bond after oxidant treatment, decreasing the DNA-binding activity of p53, which could be reversed by antioxidants [33,34]. Similar effects of S-glutathionylation of the conserved cysteines, which were observed in the study of p53, were also observed for AP-1, NF-κB and Pax-8 [35,36]. S-glutathionylation of p53 occurs both *in vitro* and *in vivo* and is regulated by the ratio of GSH/GSSH [37].

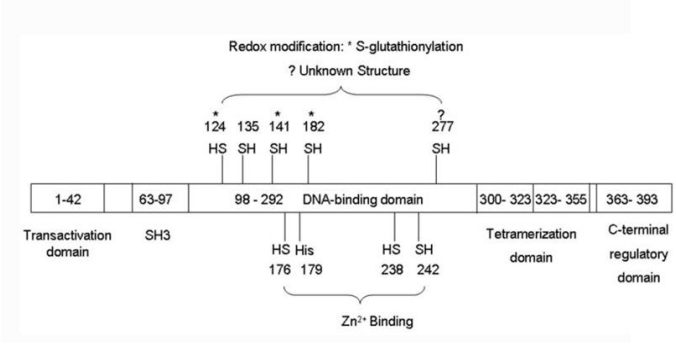

Figure 8: plan structure of p53 protein revealing cysteine residues in core domain involved in DNA binding and subject of redox modification.

Interestingly, both Cys182 and Cys277 have previously been implicated in redox-regulation of p53. Cys277 is a DNA-binding residue and oxidation of this residue has been implicated in differential gene recognition [38,39]. Bezek et al, previously reported that oxidation of Cys 277 decreases p53 binding to GADD45 but not to $p21^{CIP1}$. Although the structural nature of the redox modification on Cys 277 in p53 is still unknown and GADD45 and $p21^{CIP1}$ are both involved in DNA repair and cell growth arrest, Cys277 has also been identified as a possible substrate for selenomethionine (SeMet)-dependent redox-regulation of p53 [40]. Furthermore, an electrophilic cyclopentenone prostaglandin, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 ($15d\text{-}PGJ_2$), has been shown to bind to Cys277 *in vivo*, leading to an increase in p53 stability and reduction of its transcriptional activity. Cys182 has recently been shown to be particularly susceptible to diamide oxidation *in vivo*. Both Cys229 and Cys275 located at the N-terminal and C-terminal ends, respectively, of strands of the anti-parallel β-sheet sandwich. Jenna et al. postulated that alkylation of either of these residues disrupts this stabilizing structural feature and thus allows access of NEM to the cluster of buried cysteines 124, 135 and 141, which are located in between the two sides of the β-sandwich [41].

Interaction p53-oncoprotein, example with iSAPP: iSAPP, inhibitory member of the ASPP (Apoptosis-Stimulating Protein of p53) family is also known as the Rela-associated inhibitor, RAI and NF-kappa-B-interacting protein-1, NKIP1. It is one of the conserved inhibitors of p53. The discovery of the ASPP family of proteins as specific regulators of p53 identifies a new mechanism by which the apoptotic function of p53 is regulated; iASPP regulates the proliferation and motility of lung cancer cells [42]. This effect is intimately associated with the p53 pathway; iASPP has also been found to be over-expressed in breast carcinomas [43,44]. Classically, their carboxyl (C)-terminal conserved regions, each comprising ankyrin repeats and a Src homology 3 (SH3) domain, directly bind to p53 Domain Binding Domain (DBD) and iASPP has been shown to interact additionally with p53 regions flanking its DBD that interfere with transcriptional activity of p53 [45,46].

Cytoplasmic sequestration of p53: Accumulation of p53 can also occur in conjunction with altered subcellular localization. A significant number of IDBC accumulated wild-type p53 in the cytoplasm (Figure 9).

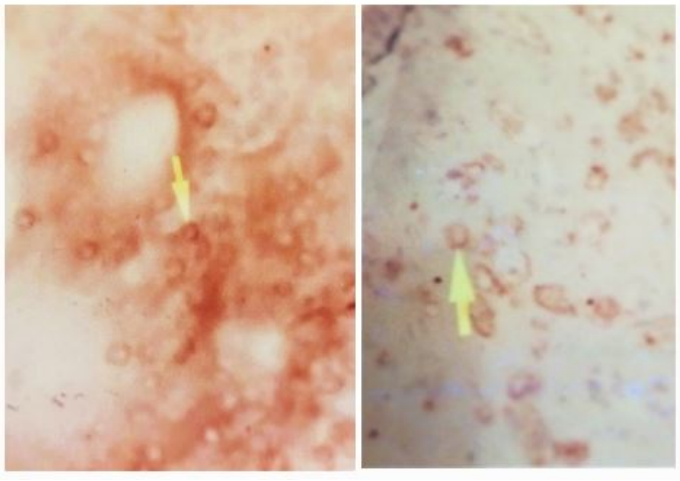

Figure 9: Cytoplasmic localisation of wild type p53: Immunostaing with antibody 1801 of cases with phenotype: DO7+ and 240-.

Cytoplasmic accumulation of wild-type p53 in tumor cells indicates that the tumor suppressor is inactive with regard to growth suppressive functions. Thus, cytoplasmic p53 localisation is an alternative mechanism of inactivation and interferes with downstream mediators of p53 function [47]. Despite intensive study of p53, the regulation of p53 subcellular localization although important for its function is still poorly understood. The regulation of p53 localization depends on factors that influence its nuclear import and export, subnuclear localization and cytoplasmic tethering and sequestration.

A cytoplasmic accumulation of wildtype p53 has also been found in undifferentiated neuroblastomas and in glioblastomas. An immunocytochemical study of frozen neuroblastomas reported cytoplasmic sequestration of p53 in undifferentiated neuroblastomas [48].

In some neuroblastoma cell line, MYCN amplified cell lines, an irradiation induced G(1) arrest does not occur, despite the presence of normal p53. MYCN amplification may alter downstream mediators of p53 function in neuroblastoma.

Conclusion

p53 accumulated in IDBC had a significant value and it can be used as prognostic marker for tumors. Nevertheless, overall, a network of multiple factors affects p53 transcriptional response, our understanding of p53 life or death decisions constitutes only the tip of the iceberg. More systematic studies are required to address the questions of p53 biology and provide new ideas for combination therapies to direct p53 response to the desired outcome.

Ethics Approval and Consent to Participate

Informed consent was obtained from all patients and the study obtained the agreement of the ethics committee of South Tunisia acting under the Ministry of Health (CPP Sud).

Competing Interests

The authors have declared that no competing interests exist.

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