Proteomics of p53 in Diagnostics and Therapy of Acute Myeloid Leukemia

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Abstract: The anti-oncogene TP53 is frequently mutated in human cancer, but in hematological malignancies this is a rare feature. In acute myeloid leukemia (AML) more than 90% of the patients comprise wild type TP53 in their cancer cells, but if TP53 is mutated or deleted the disease is often found to be chemoresistant. In this review we define proteomics of the oncogene product p53 as the study of proteins in the p53 regulating signaling networks, as well as the protein study of members of the p53 family itself. Various messenger RNA splice forms as well as a multitude of post-translational modifications give a high number of protein isoforms in the p53 family. Some of the proteomic techniques allow detection of various isoforms, such as two-dimensional gel electrophoresis in combination with tandem mass spectrometry (MS/MS) and this methodology may therefore increasingly be used as a diagnostic tool in human disease. We introduce the p53 protein as an illustration of the complexity of post-translational modifications that may affect one highly connected protein and discuss the possible impact in AML diagnostics if the p53 profile is reflecting cell stress and status of signal transduction systems of the malignancy.

Key Words: p53, AML, diagnosis, therapy, signaling networks, two-dimensional electrophoresis.

INTRODUCTION

The p53 protein is the founding member of a family of proteins that regulate cell cycle progression, differentiation and apoptosis. The protein has been denominated "guardian of the genome" [1], and effectively senses DNA damage and various forms of cellular stress in most somatic cells. Most of its interest in biomedical research seems to be ignited by the fact that many human cancers comprise mutations in the p53 gene, and these mutations appear to limit the p53 protein's ability to respond to chemotherapy and irradiation.

The p53 protein is a 393 amino acid protein [2] composed of five main structural and functional domains (Fig. (**1**)). Several amino acids are available for modification (Table (**1**)) with the N- and C-termini as the main regulatory domains. Post-translational modifications are thought to change the p53 protein conformation and thus stabilize and activate the protein as a transcription factor.

Activation of p53 occurs by a number of stress signals (Fig. (**1**)). Stress such as ionizing and UV radiation, chemotherapeutics, hypoxia and other signals are known to influence p53 activity by affecting the activity of kinases, acetyl transferases and other modifying enzymes and following these stress signals, specific residues are rapidly modified [reviewed in 3]. Modification is accomplished through the addition of small proteins or chemical groups to the p53 protein. Already when p53 first was described in 1979 it was identified as a phosphoprotein [4]. After more than 25 years of research it has become clear that there are a number of different modifications (Table (**1**)) with unique functions that contribute to the sophisticated regulation of this complex protein.

Acute myeloid leukemia (AML) is a malignant disease of the myeloid lineage of hematopoietic cells, characterized by a differentiation block that results in accumulation of immature myeloblasts. The clinical signs of AML usually reflect general bone marrow suppression, and may include fatigue, hemorrhage, infections and fever [5].

During the last half-century the diversity of therapies has gone through a revolutionary development. However, even though anti-leukemic treatment is improving, the overall disease-free survival rate still does not exceed 50%. A major concern is the large number of older patients (>60 years) who cannot get the most intensive treatment because of therapy-related toxicity. It is therefore necessary to find new molecular targets to improve treatment specificity, thereby increasing efficiency and lowering toxicity with the ultimate goal of increased survival rates.

The World Health Organization (WHO) has proposed a classification of myeloid neoplasias that considers recurring genetic abnormalities and leukemic blast morphology upon AML diagnosis. Cytogenetic aberrations are further used, together with disease response after first course of chemotherapy, to determine the prognosis of disease outcome [8-10]. Recently it was suggested that gene expression profiling may refine risk stratification of AML and it has been shown that particular gene expression signatures can correlate to clinical outcome, also in patients without a particular chromosomal translocation [reviewed in 11].

TP53 mutations in AML are associated with cytogenetic aberrations involving chromosome 17p monosomy [12] as well as secondary leukemia [13] and have been known to correspond with resistance to chemotherapy and ultimately

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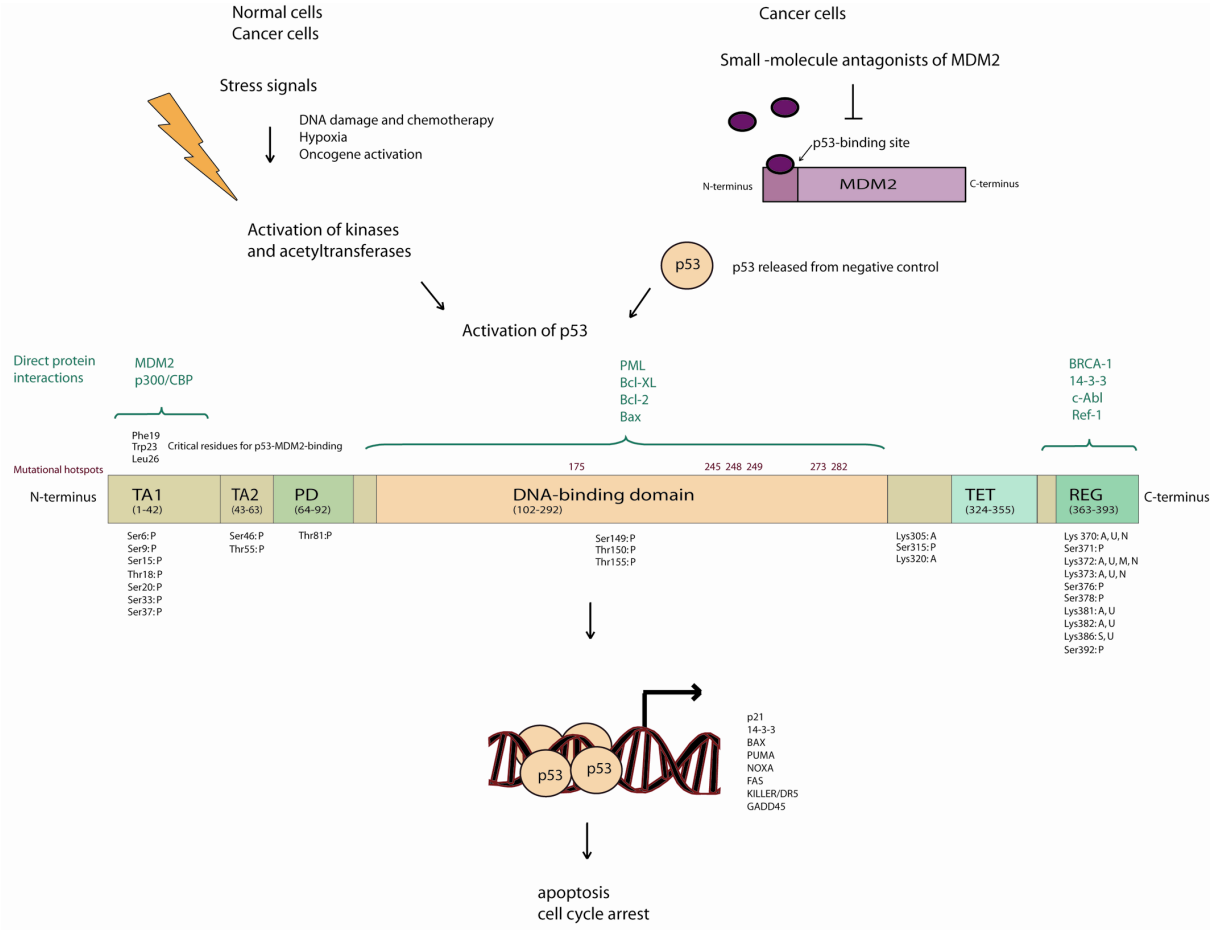

Fig. (1). Mechanisms of p53 activation in acute myeloid leukemia (AML). The full length p53 protein consists of 393 amino acids and is divided into five functional domains. The N-terminal transactivation domain (TA) is required for transcriptional activity, and is involved in regulating the stability and activity of p53 via interactions with proteins like MDM2. Through the proline rich domain (PD), p53 is influenced by diverse signaling molecules who can bind this region through their SH3 domains. Most of the interactions between p53 and its target genes take place at the central core DNA-binding domain. The C-terminal end contains the tetramerization domain (TET) and a regulatory domain (REG) that regulate the ability of p53 to bind to specific DNA sequences through the DNA-binding domain. As shown in the figure, the amino (N)- and carboxy (C)-termini are the main regulatory domains. Stress signals or treatment with small-molecule antagonists of MDM2 activate the p53 pathway by stabilization and accumulation of the p53 protein. p53 residues reported to be post-translationally modified by phosphorylation (P), acetylation (A), ubiquitination (U), neddylation (N), methylation(M) or sumoylation (S) are indicated below. p53 is also subject to direct interaction with a variety of proteins. Selected partner proteins and their approximate interaction domain are indicated (brackets). Further signaling involves activation of p53 target genes and induction of apoptosis or cell cycle arrest. A selection of the most prominent of the putative p53-inducable genes is indicated. The molecular switch that determines either expression of cell cycle arrest genes or apoptosis-related genes is incompletely understood, and a possible mechanism may include specific mRNA splice forms of p53 [6]. In addition to the p53 modulation of transcription, the p53 protein interacts directly with the Bcl-2 family of anti- and pro-apoptotic proteins mediating mitochondrial permeabilization [7].

lower complete remission rates [14]. Examination of the status of the p53 gene is not included routinely in AML diagnostics, partly because mutation is an infrequent feature. However, novel drugs that target wild type p53 networks, like the MDM2 targeting nutlin [15], may increase the relevance of p53 protein status evaluation in AML. We have described the use of p53 protein analysis in patients undergoing chemotherapy [16]. This work reported specific changes in protein distribution as a result of therapy, suggesting major changes in cell signaling. Based on the action of new therapeutics on p53-related proteins, and the fact that p53 is a crucial protein in key signaling networks, the question rises if protein analysis of p53 and its posttranslational modifications may provide important information for individualized therapy of AML.

THE p53 NETWORK

The p53 protein is a highly connected signaling node in the cell and is to a great extent controlled through post-translational modifications, so even though the protein itself generally is wild type in sequence in AML, other changes in the p53 network can change the specificity of its action. In a heterogeneous disease like AML which is characterized by a

myriad of genetic defects, it is highly likely that such alterations might take place. The p53 network is therefore a good candidate in the search of new targets for treatment.

It has been suggested that signaling pathways involving p53 can not be understood by looking at isolated components of the network and that it is essential to consider the entire network in order to comprehend the full potential of this highly connected protein [17]. This means that mutation of the TP53 gene is not the only marker that should be considered in prognostic evaluation of a patient. Aberrations in a p53 modifying enzyme, protein partner or any other member of the p53 network could potentially have dramatic effects on the control of cell fate.

One issue concerning the p53 network that could be of great importance is the production of several p53 isoforms. Major variants of the p53 protein may be formed through alternative splicing of mRNA or protein cleavage. To date, the human p53 gene has been reported to hypothetically encode ten isoforms, resulting from variously spliced mRNA [6, 18]. These are the full length protein, three N-terminally truncated isoforms translated from an alternative point of initiation at codon 40, or by alternative splicing of intron 2 [6, 19, 20], two C-terminally truncated isoforms produced by alternative splicing of intron 9 [6, 21], three isoforms produced from an internal promoter in intron 4 [6] and one isoform produced by alternative splicing in exons 7-9 [18]. In addition, truncated p53 protein products are known to be produced as a result of protease action, most likely through an autoproteolysis mechanism [22, 23]. In fact, one of the p53 cleavage products, p35, has protease activity and has the ability to cleave the full length p53 protein to generate an alternate protein product [23].

The different p53 isoforms have different functions. Most is known about the N-terminally truncated isoform _Np53, also termed p47. This isoform lacks the 40 most extreme N-terminal amino acids and thus has impaired transcriptional activation capacity. Also, it does not complex with the negative regulator of p53; MDM2 [19]. Furthermore, this isoform has been shown to have a dominant negative effect on full length p53 [19, 20] thus repressing transcriptional activity and growth suppression mediated by full length p53. p47 has also been shown to modify p53 localization and inhibit p53 degradation most likely due to deficient ubiquitination resulting from impaired ability to complex with MDM2 [20].

Bourdon *et al*. recently reported that all known p53 isoforms are expressed in normal human tissue in a tissue-dependent manner [6]. They also reported differential binding of these isoforms to p53-responsive promoters and alternate apoptotic responses. At least one additional isoform in addition to p47 was suggested to be dominant negative toward full length p53 (_133p53). Differential expression of isoforms can also lead to variable cellular responses because the isoforms have different potentials for transactivation. Rohaly *et al*. [18] reported that the isoform produced from alternative splicing of exons 7-9 can only transactivate genes associated with cell cycle arrest and not the apoptotic machinery. This may imply that expression of specific isoforms can contribute to decide the preferred mechanism of action of p53 in a given tissue.

Taken together these studies suggest a major role for p53 isoforms in the regulation of p53-mediated responses to cell stress and further studies are required to elucidate the complex organization of expression of p53 proteins.

POST-TRANSLATIONAL MODIFICATIONS OF p53

A second issue contributing to the importance of the p53 network is the high level of post-translational modifications associated with the p53 protein. Various modifications are, to a great extent, the key to regulation of p53 activity and differences in a cell's ability to apply these regulatory modifications could have great impact on protein signaling and transactivation of target genes.

1. Phosphorylation

Stress induced activation of p53 is to a great extent controlled through phosphorylation. Phosphorylation is the most common signal for protein activation in a cell and p53 has a number of serine and threonine residues available for accepting phosphate groups (Table **1**).

Phosphorylation is a highly controlled event with the N-terminus of p53 as the main target for initiation of activating signals. Serines 15, 20, 33 and 37 are the main initiation sites and phosphorylation of these residues is detected rapidly after stress induction [24] as a result of the activity of a number of kinases. Phosphorylation of serines 15, 20 and 37 will perturb the interaction between p53 and its negative regulator MDM2 [25, 26]. Phosphorylation thus inhibits MDM2 induced ubiquitination and subsequent degradation, thereby contributing to p53 stabilization. Inhibition of p53-MDM2 interaction is also followed by increased recruitment of transcriptional coactivators such as p300 and the p300/CBP-associated factor (PCAF) [27] and this results in increased acetylation of the C-terminus as discussed below.

Protein phosphatases counteract kinases and thus provide a switch mechanism through protein dephosphorylation. It has been shown that multiple phosphatases can dephosphorylate both the N- and C-terminus of p53 *in vitro*, including PP1, PP2A, PP5, Wip1 and Cdc14 [58-61].

2. The Ubiquitin Family of Proteins and their Conjugation to p53

The p53 protein is highly modified by a family of small polypeptides known as the ubiquitin protein family. In normal cells, p53 has a short half life with a turnover of about 20 minutes due to polyubiquitination, targeting p53 for degradation via the proteasome pathway. Covalent binding of ubiquitin to p53 is achieved through direct interaction of p53 with the ubiquitin E3 ligase MDM2 [62, 63]. MDM2 mediates monoubiquitination at several C-terminal lysine residues [64, 65], but monoubiquitination is not sufficient for degradation and the presence of a polyubiquitin chain is required. Polyubiquitination is achieved through interaction of p53 with the transcriptional co-activator p300 which has an intrinsic ubiquitin ligase activity as well as acetyl transferase activity [47]. However, this polyubiquitination is dependent on the previous monoubiquitination by MDM2 [47]. C-terminal ubiquitination of p53 ultimately leads to nuclear export and protein degradation [66].

Table 1. Overview of Post-Translationally Modified p53-Residuces. (A), Acetylation; (M), Methylation; (N), Neddylation; (P), Phosphorylation; (U), Ubiquitination

Residue	Modification	Modifying Enzyme	Reference
Ser6	Phosphorylation	Unknown kinase	[28]
Ser9	Phosphorylation	Casein Kinase 1 (P)	[28]
Ser15	Phosphorylation	ATM (P), ATR (P), Chk2 (P), DNAPK (P), ERK (P), p38 (P)	[25, 29-33]
Thr18	Phosphorylation	Casein Kinase 1 (P), Chk2 (P)	[30, 34]
Ser20	Phosphorylation	Chk1 (P), Chk2 (P), JNK (P)	[30, 35]
Ser33	Phosphorylation	CAK (P), p38 (P), GSK-3_	[36-38]
Ser37	Phosphorylation	ATR (P), Chk1 (P), Chk2 (P), DNAPK (P)	[25, 30, 31 33]
Ser46	Phosphorylation	ATM (P), p38 (P)	[29, 36]
Thr55	Phosphorylation	ERK (P)	[39]
Thr81	Phosphorylation	JNK (P)	[40]
Ser149	Phosphorylation	COP9 Signalosome (P)	[41]
Thr150	Phosphorylation	COP9 Signalosome (P)	[41]
Thr155	Phosphorylation	COP9 Signalosome (P)	[41]
Lys305	Acetylation	p300/CBP (A)	[42]
Ser315	Phosphorylation	CDK2 (P)	[43]
Lys320	Acetylation	PCAF (A)	[44]
Lys370	Acetylation, Ubiquitination, Neddylation	p300/CBP (A), Mdm2 (N, U), p300 (U)	[45-48]
Ser371	Phosphorylation	PKC (P)	[49]
Lys372	Acetylation, Ubiquitination, Methylation, Neddylation	p300/CBP (A), Set9 (M), Mdm2 (N, U), p300 (U)	[45-48, 50]
Lys373	Acetylation, Ubiquitination, Neddylation	p300/CBP (A), Mdm2 (N, U), p300 (U)	[45-48]
Ser376	Phosphorylation	PKC (P)	[51, 52]
Ser378	Phosphorylation	PKC (P)	[51, 53]
Lys381	Acetylation, Ubiquitination	p300/CBP (A), Mdm2 (U), p300 (U)	[46-48]
Lys382	Acetylation, Ubiquitination	p300/CBP (A), Mdm2 (U), p300 (U)	[46-48]
Lys386	Sumoylation, Ubiquitination	Ubc9 (S), p300 (U)	[46, 47, 54, 55]
Ser392	Phosphorylation	p38 (P), PKR (P)	[56, 57]

Two type I ubiquitin-like proteins, SUMO-1 and Nedd8, have also been found to modify p53. Conjugation of the small ubiquitin-like protein SUMO-1 was reported by two independent studies in 1999 [54, 55]. Unlike ubiquitin this protein does not appear to target p53 for destruction, but rather seems to change the ability of the modified protein to interact with other cellular proteins thereby increasing p53 transactivation ability [54]. It has also been shown that the conjugation of SUMO-1 to certain substrates can defend these molecules against modification by ubiquitin and thus prevent protein degradation [67] offering an additional mechanism for the regulation of p53 activity. Nedd8 is another ubiquitin-like molecule reported to be conjugated to p53 by MDM2 [45]. This modification appears to have the same negative regulatory effect as ubiquitin and has been reported to inhibit p53 transcriptional activities.

3. Acetylation

The C-terminal lysine residues functioning as acceptor sites for ubiquitin are also acceptor sites for acetyl groups (Table **1**). Acetylation by the acetyl transferases CBP, p300 and PCAF is, in contrast to ubiquitination, an event that stabilizes and activates the specific DNA binding activities of p53 [44, 48] and levels of acetylation are significantly raised in response to almost every type of stress [68]. Because ace-

tyl and ubiquitin compete for the same lysine residues, activation and inactivation of p53 function is tightly regulated through differences in the activity of modifying enzymes. Further it has been established that histone deacetylases HDAC1/2/3 interact with p53 and down-regulate its function by deacetylation [69] as also does the oncogenic transcription factor PML-RARα [70]. The PML-RARα fusion protein is the result of a translocation involving chromosomes 15 and 17 (t(15;17)(q22;q21)) in AML-M3 and it has been shown that PML-RARα mediated deacetylation is dependent on wild-type PML which acts as a bridge between p53 and PML-RARα. PML is also required for p53 acetylation by stabilizing the interaction between p53 and CBP/p300 [71].

In some cases, previous phosphorylation is required for subsequent acetylation of p53. A study by Sakaguchi *et al* [72] revealed that prior phosphorylation of serine 33 and/or serine 37 in response to DNA damage enhanced the interaction of p300 and PCAF with p53. A second report concluded with increased p300 binding to p53 as a response to phosphorylation of serine 15 [27]. Other reports have also provided evidence for a phosphorylation-acetylation cascade demonstrating a highly structured and cooperative activating process for p53 functions [73].

Detection of the level of p53 modifications is expected to give information about the activity of the protein and the application of proteomics techniques in the evaluation of p53 status may contribute to prognostic classification of the patients.

THE p53 FAMILY

The p53 protein is part of a family that includes p63 [74-76] and p73 [77]. These proteins are also transcription factors and share structural and functional similarities with p53. Sequence homology between p53 and its family members enables p63 and p73 to bind to p53 DNA-binding sites, thereby transactivating p53 target genes.

Many different isoforms of the p63 and p73 proteins are known. The p63 gene expresses six mRNA variants that encode six protein isoforms, and the p73 gene expresses at least 35 mRNA variants that theoretically could encode 28 protein isoforms. So far, 14 of the putative p73 protein isoforms have been described [6]. Both p63 and p73 have been shown to express N-terminally deleted isoforms that exert dominant negative effects on both themselves and p53 [76, 78] and via over-expression of these deleted isoforms it is therefore possible that the p53 protein can be functionally inactive, even in leukemia where the gene normally is wild type. It is becoming increasingly clear that most p63 and p73 isoforms have specific and distinct activities, many being able to induce apoptosis [79-81] or cell cycle arrest [81, 82] through specific transcriptional activation of target genes. It is also becoming clear that p63 and p73 may play a larger role in the development of malignant diseases than previously reported. It has been shown that tumors that express mutations in both p53 and a second p53 family member have a greater metastatic potential than other tumors [83]. Pathways involving p53 family members might therefore be a promising field for development of possible novel drugs in the treatment of acute leukemia.

PROTEOMICS OF P53 REGULATING PATHWAYS

Proteomic approaches, in particular mass spectrometry based ones, are the methods of choice to further address some of the challenges in the p53 research outlined above. Deciphering the roles of the various post-translational modifications and up-stream and down-stream effector proteins in p53 function is more feasible due to the mass spectrometers capacity for rapid and sensitive protein identification and quantification. Other more genomic methods have already been successfully used to investigate p53 function: Serial analysis of gene expression [84, 85], microarrys [86, 87], differential display [88], and subtractive hybridization [89] have revealed differential expression of many genes in response to DNA genotoxic stress and p53 –induced apoptosis. Often however, the analysis of mRNA expression correlates poorly with protein expression levels and in the case for p53 few of these genes have been characterized at the protein level. In combination with other proteomic techniques such as gel electrophoresis and ion exchange/reverse phase chromatography, mass spectrometry has been successful in the identification of a number of binding proteins and post-translational modifications. By mass spectrometry of affinity-purified p53-associated factors, Li *et al*. [90] identified the herpes virus-associated ubiquitin-specific protease (HAUSP) as a novel p53-interacting protein. HAUSP strongly stabilizes p53 even in the presence of excess MDM2, and also induces p53-dependent cell growth, repression and apoptosis. Furthermore two-dimensional gel electrophoresis in combination with stable isotope labeling for quantitative profiling has identified several distinct functional categories of proteins which display altered expression in p53-induced apoptosis [91]. Protein profiling exercises have been reported, identifying proteins which are differentially expressed either in response to stimuli or in the background of p53 knock-out cells. While these identify possibly relevant up-stream and down-stream effector proteins they do not elude to the precise regulation of p53 itself [92, 93]. More targeted approaches have been used to analyze p53 directly. Abraham *et al* [94] for example have used 2D gel electrophoresis to distinguish different, potentially phosphorylated isoforms of p53 and have successfully applied matrix assisted laser-desorption ionization time-of-flight (MALDI ToF) and nano-electrospray tandem MS on immuno-purified preparations of p53, to identify four sites of phosphorylation in response to radiation, in addition to a constitutively phosphorylated serine [95]. Similarly we have used Fourier Transform Ion Cyclotron Resonance (FTICR) MS to characterize the acetylation sites of p53 on immuno-purified endogenous protein. All published p53 acetylation sites were identified in addition to one novel site of acetylation (CD unpublished data). Data such as these together with the availability and use of anti-phospho and anti-acetyl specific antibodies demonstrate that profiling the post-translational pattern of p53 will point to signature patterns that regulate the protein in response to specific regulatory signals. These global and targeted analyses of p53 regulation have also been complimented by functional protein microarrays [96-98]. The interaction with key p53 regulatory proteins such as MDM2 have been analyzed on protein chips imprinted with wild type p53 or p53 variants with single amino acid substitutions. MDM2 directly binds to, and sub-

sequently inhibits p53 function by altering its stability, location and transcriptional activity [99]. Consequently the identification of key residues responsible for interactions with MDM2 and others will clearly improve understanding of how mutations disrupt protein function. Protein microarrays may thus provide an effective format for screening compounds across panels of proteins for the rescue of function leading to pharmaceutical rescue of the mutant activities [100-102]. Proteomic efforts such as these will together provide a coordinated view of how key cellular decision makers such as p53 regulate cellular function and how they contribute to the onset of disease such as AML.

THE POTENTIAL USE OF P53 PROTEOMICS IN AML DIAGNOSTICS

Gene arrays have previously made it possible to study the genetic signature of tumors and in breast cancer it has been shown that each tumor displays a distinct gene expression portrait [103]. However, the application of proteomics is, as described above, becoming increasingly important in terms of identifying proteins that are over-expressed or altered in cancer cells since the gene expression patterns do not always correlate with changes at the functional protein level [104]. Using proteomic techniques enables the detection of post-translational modifications attributed to proteins and thereby makes it possible to discover functional differences that can not be determined from genomic information. As such, the protein signature of a highly connected signaling molecule might provide an extensive insight into the regulation of the AML proteome based on protein partners and the activity of enzymes conferring post-translational modifications.

Expression and phosphorylation of p53 in AML primary blasts is highly heterogeneous, suggesting differences in cell signaling (N.A. and B.T.G., manuscript submitted). We have previously proposed that signaling pathways in AML have diverse signaling potential and this may reflect the clinical outcome for the patients [105]. Based on the complexity of the regulation of the p53 protein we hypothesized that signaling potential could be read from p53 expression and we recently suggested that the expression profile detected by two-dimensional electrophoresis may serve as a read-out for the entire p53 network in AML cells [106]. Using two-dimensional electrophoresis and immunoblots (2DI) is a good choice for visualization of the p53 protein in clinical material (Fig. (**2**)). The observed profile represents differences in isoform expression and in post-translational modifications, differences that may not entirely be distinguished by other methods. Using a novel gel analysis algorithm [106] we have been able to correlate the p53 signatures to clinical parameters such as leukemic cell morphology (Fig. (**3**)).

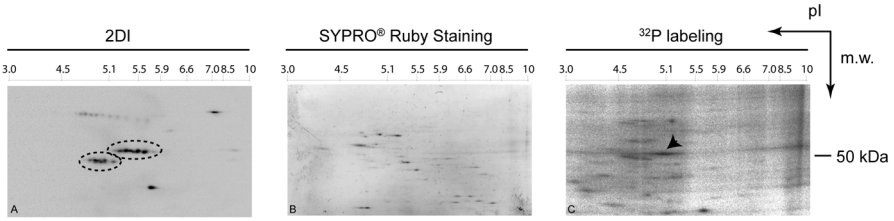

Fig. (2). Two-dimensional gel electrophoresis techniques for visualization of the p53 protein in AML material. For characterization of the p53 protein in primary material the optimal choice for visualization is two-dimensional electrophoresis and immunoblots (2DI) (A). The circled proteins are the main p53 isoforms found in AML material. The 2DI method allows acceptable separation of p53 isoforms and visualization with low background detection as compared to staining with SYPRO® Ruby (B) or ^{32}P labeling (C).

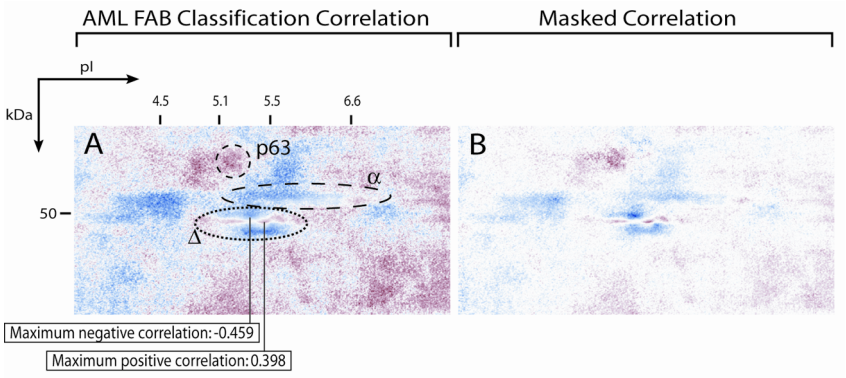

Fig. (3). Relations between p53 biosignature and the p53 FAB Classification (excluding M3). A novel gel analysis algorithm [106] was used to correlate expression of p53 protein to the differentiation stage of leukemic blasts (FAB). Image (A) shows which gel-areas correlate (red) and anti-correlate (blue) with increasing differentiation. Image (B) shows the significant correlations. p53-alfa, -delta and p63 have their own unique relation towards differentiation. The p53-delta region further contains many strong correlating and anti-correlating isoforms, suggesting that p53 expression patterns on 2DI portray the function of the protein (and its large variety of specific isoforms) as a central hub that integrates various pathways related to differentiation.

This correlation technique discloses protein patterns that are associated with specific clinical criteria. Distinct differences in patient protein profiles may imply that the p53 profile reflects cell stress and the status of signal transduction systems in AML and may indicate that the p53 protein signature could be used as a novel biomarker for clinical outcome in this particular disease. Evaluation of the p53 network may further aid individualization of treatment protocols and thereby ensure optimal treatment for all patient groups, also the older patients (>60 years) who currently can not receive optimal treatment due to related toxicity.

CONCLUDING REMARKS

The use of proteomic techniques in diagnostic procedures may in the future enable individual evaluation of signaling networks in the patients. This could contribute to the development of molecular targeted therapy. It has been suggested that biological networks have modular architecture and that highly connected clusters of proteins function in a network of protein interactions [107]. p53 has been proven to be a central node in an extensive protein signaling network and both a pre-therapy diagnostic characterization as well as monitoring p53 protein during therapy may represent a non-realized potential in future molecular medicine.

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ABBREVIATIONS

2DI	=	Two-dimensional electrophoresis and immunoblot
AML	=	Acute myeloid leukemia
FAB	=	French-American-British cooperative group
FTICR	=	Fourier Transform Ion Cyclotron Resonance
HAUSP	=	Herpes virus-associated ubiquitin-specific protease
HDAC	=	Histone Deacetylase
MALDI.ToF	=	matrix assisted laser-desorption ionization time-of-flight
MS	=	Mass Spectrometry
PCAF	=	p300/CBP-associated factor
UV	=	Ultra violet
WHO	=	World Health Organization

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