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REVIEW

More targets, more pathways and more clues for mutant p53

S Garritano[1], A Inga[2], F Gemignani[1] and S Landi[1]

Mutations in the transcription factor p53 are among the most common genetic alterations in human cancer, and missense p53 mutations in cancer cells can lead to aggressive phenotypes. So far, only few studies investigated transcriptional reprogramming under mutant p53 expression as a means to identify deregulated targets and pathways. A review of the literature was carried out focusing on mutant p53-dependent transcriptome changes with the aims of (i) verifying whether different p53 mutations can be equivalent for their effects, or whether there is a mutation-specific transcriptional reprogramming of target genes, (ii) understanding what is the main mechanism at the basis of upregulation or downregulation of gene expression under the p53 mutant background, (iii) identifying novel candidate target genes of WT and/or mutant p53 and (iv) defining cellular pathways affected by the mutant p53-dependent gene expression reprogramming. Nearly 600 genes were consistently found upregulated or downregulated upon ectopic expression of mutant p53, regardless of the specific p53 mutation studied. Promoter analysis and the use of ChIP-seq data indicate that, for most genes, the expression changes could be ascribed to a loss both of WT p53 transcriptional activation and repressor functions. Pathway analysis indicated changes in the metabolism/catabolism of amino acids such as aspartate, glutamate, arginine and proline. Novel p53 candidate target genes were also identified, including *ARID3B*, *ARNT2*, *CLMN*, *FADS1*, *FTH1*, *KPNA2*, *LPHN2*, *PARD6B*, *PDE4C*, *PIAS2*, *PRPF40A*, *PYGL* and *RHOBTB2*, involved in the metabolism, xenobiotic responses and cell differentiation.

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INTRODUCTION

The tumor suppressor p53 is a 393-amino-acid nuclear phosphoprotein that responds to numerous stress stimuli, including DNA damage[1] and hypoxia.[2] Following homotetramerization, it acts as a transcription factor[3] and modulates the expression of a variety of genes, leading to enhanced DNA repair, control of cell cycle and apoptosis, and maintaining cellular homeostasis.[4–6] The p53 targets are only partially known, with assessments suggesting their number to be nearly 2000 genes.[7]

CDKN1A, *MDM2*, *BAX*, *GADD45* and *BBC3* are paradigmatic examples of upregulated target genes where p53 exerts its activity via evolutionarily conserved *cis*-response elements (p53RE).[8] The importance of p53 for the biology of cancer is evident by the fact that colorectal, breast and most other human solid tumors show a high frequency of somatic mutations within the *TP53* gene (www.iarc.fr/p53). Moreover, germline mutations within *TP53* cause the Li–Fraumeni syndrome, a dominantly inherited cancer proneness syndrome with an elevated risk of developing adrenocortical carcinoma, choroid plexus carcinomas, sarcomas and other types of cancer in multiple sites at a young age.[9] So far, nearly 2000 different single amino-acid changes in p53 have been reported in tumors,[10] and their frequencies vary markedly: next to exceedingly rare mutations, strong hotspots are evident.[11,12] This latter group of mutations affects, in particular, codons 175, 248, 249, 273 or 282. The impact of mutations on p53 functions can vary from a wild-type-like activity, for example, the R337H mutations associated with predisposition to adrenocortical carcinoma,[13] to a partial function or to a suspected complete loss of function (LOF).[12,14]

According to Resnick *et al.*, different mutant p53s retaining a partial activity (for example, T123A or S215C) show specific effects on the transactivation of target promoters, leading to mutation-specific altered regulation of hundreds of genes (the 'piano model'), resulting in a variety of biological consequences.[15,16] Cells can show lack of control of their cell cycle and weakened apoptosis and DNA repair. However, in selected examples, separation of p53 functions was observed, with defective apoptotic control, but wild-type function in cell cycle arrest.[17] Moreover, knockin mouse models showed varied phenotypes, suggesting the occurrence of mutation-specific gene expression reprogramming also *in vivo*.[18,19] However, most studies related to mutant p53 activity were performed on hotspot mutations, using reconstituted assays[12,14,20] or other *in vitro* models. Following these experimentations, it was observed that hotspot mutations have the least transactivating activity of common targets and therefore they were suggested to cause a p53 LOF. Hotspot p53 mutations were reported to be associated with more aggressive malignancies and could confer novel phenotypes *in vivo*, including an increased metastatic capacity and resistance to chemotherapies.[21–27] The acquired phenotypes of specific mutant p53s are generally referred to as gain-of-function properties,[28] but it is unclear if these features are restricted to or distinct among specific p53 hotspot mutations. Examining the impact of hotspot p53 mutations at a transcriptome level, a large number of genes are downregulated. However, there are also a restricted number of WTp53 targets whose transactivation seems not to be hampered by p53 mutations.[7] Moreover, there are also genes that are upregulated under mutant but not WT p53 expression. It is not clear whether different mutants can lead to similar transcriptional changes or have

[1]Department of Biology, University of Pisa, Pisa, Italy and [2]Laboratory of Transcriptional Networks, Centre for Integrative Biology, CIBIO, University of Trento, Trento, Italy. Correspondence: Professor S Landi, Department of Biology, University of Pisa, via Derna, 1, Pisa 56100, Italy.
E-mail: slandi@biologia.unipi.it
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different impact on it (like an extension of the 'piano model') and whether the gained phenotypes can be related to specific genes upregulated in the p53 mutant background. Thus, in this work, the focus was placed on cancer-associated p53 hotspot mutations that exhibit a loss of transactivation function in reconstituted assays,[12,14] and a review of the literature was performed, with the following aims: (1) to verify whether different p53 mutations can be equivalent for their effects, or whether there is a mutation-specific transcriptional reprogramming of target genes, (2) to understand what is the main mechanism at the basis of upregulation or downregulation of gene expression under the p53 mutant background, (3) to identify the novel candidate target genes of WT and/or mutant p53 and (4) to define cellular pathways affected by the mutant p53-dependent gene expression reprogramming.

SELECTION OF THE PUBLISHED LITERATURE

In order to identify genes differentially modulated upon the expression of mutant p53, only potentially unbiased transcriptome studies published in the literature were collected. In fact, in transcriptome studies, target genes are analyzed without formulating any *a priori* hypothesis and, virtually, all the genes are evaluated with the same relevance. An extensive literature search was carried out using PubMed (http://www.ncbi.nlm.nih.gov/pubmed) to collect original papers. Articles were selected by screening title, abstract and full text, and only those reporting the effects of ectopic expression of p53 mutants on the transcriptome were considered further.

Out of over 2000 known p53 mutations reported by the IARC (www.iarc.fr/p53) or UMD TP53 databases,[10,29] only 12, falling in 11 different codon sites (Figure 1), were studied through global gene expression changes. Those 11 mutated codons lie within the sequence-specific DNA-binding domain, correspond to hotspot mutations in tumors and result in LOF in functional assays. Studies on p53-dependent transcriptomes were few and heterogeneous in their experimental design, with a variable p53 status of the cell lines used, thus limiting the strength of the comparisons. Therefore, conservatively, conclusions on mutant p53-dependent gene deregulation were drawn only when at least three independent p53 mutations showed a coherent effect on the same target. Now on, genes upregulated under the ectopic expression of at least three different mutant p53 genetic backgrounds are defined as UMB, whereas DMB are the genes downregulated under at least three different mutant p53s. The results were obtained relying on the statistical analyses imbedded within each study, and a list of differentially expressed genes was compiled for further analysis (Supplementary Data S1). For each chosen article, in Table 1, the p53 missense mutation studied, and the cell lines used to perform the experiments were reported.

IN SILICO ANALYSES OF PROMOTERS AND PATHWAYS

COMPASSS (COMplex PAttern of Sequence Search Software),[30] a software that allows to perform custom pattern searches in entire genomes, was used to analyze the promoters. Given that most of the deregulated genes are not well-established p53 target genes, the focus was placed on the identification of non-canonical p53REs,[31,32] particularly a half-site RE motif. In the exploratory search, a conservative approach was used by limiting inspection to 2-kb upstream of annotated transcriptional start sites, not allowing mismatches in the half-site decameric motif, and requiring the presence of a cluster of at least two half-sites within one nucleosome.[32] Hence, the following input were used: RRRCWWGYYY($N_{0\text{-}50}$)RRRCWWGYYY and NRRCWWGYYN($N_{0\text{-}50}$)NRRCWWGYYN. Two closely spaced p53 half-sites either in a direct orientation (RRRCWRRRCW) or lacking the CWWG core (WGYYYRRRCW), or having a relaxed motif definition (RRNCNNGNYN) (all sequence features that have been associated with genes repressed by WTp53),[8] were also queried. Thus, COMPASSS was used to analyze the promoters of *UMB* and *DMB* genes and to measure the 'baseline' number of p53REs found in the whole human genome. Then, a binomial distribution-based statistics (approximated as normal distribution) was used, in order to verify whether the promoters were enriched for the input motifs, as compared with the baseline level.

The complex pattern of gene transcription changes was further analyzed with the tool Database for Annotation, Visualization and Integrated Discovery, in order to detect whether mutations within p53 could affect specific biological pathways.[33] Database for Annotation, Visualization and Integrated Discovery uses all the human genes as background to perform the comparisons, and if a group of genes is enriched within a specific biological process or pathway, the *P*-value of the modified Fisher's exact test will be lower than the cutoff (0.05). First, the short lists of *UMB* and *DMB* genes, either separated or combined, were used as input, but the total number of genes was not large enough to obtain statistically significant results. Thereafter, the analyses were repeated with a broadened input list, that is, the list of genes changing their expression under at least one p53 mutant background (that is, those reported in Supplementary Table S1).

RESULTS AND DISCUSSION

Similar deregulation profile of genes by distinct p53 mutation hotspots

By observing *UMB* and *DMB* genes, consistent trends emerged (Table 2, see also Supplementary Table S1). A total of 401 genes were found downregulated under the ectopic expression of at least three different mutant p53s, whereas 260 genes were found upregulated (reported in Tables 3 and 4, respectively). Given the

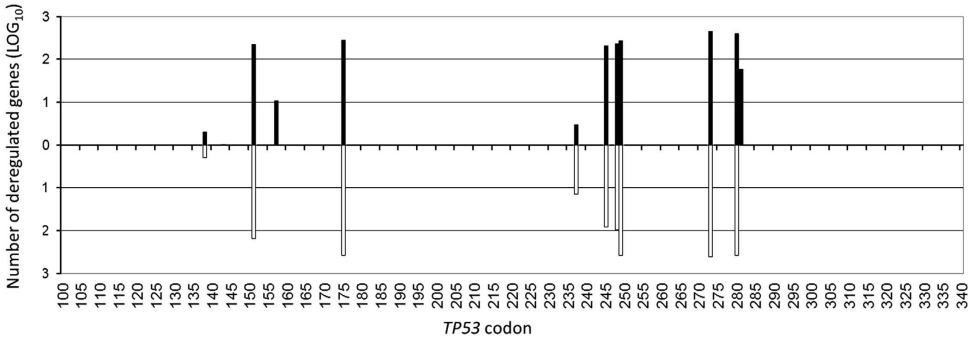

Figure 1. Number of genes (Log10) upregulated (black bars) and downregulated (white bars) following *in vitro* studies where a mutant form of p53 is overexpressed. Only few codons were assayed and for each mutation it is shown that the number of genes going overexpressed is approximately similar to those downregulated. The missense mutations falling within the codon 248 (R248Q and R248W) were considered as a unique one, in order to empower the study.

Table 1. List of selected article

Article	*Cells used*	*Mutation tested*
88	TKS, WTK1	M237I
89	HCT 116	A138P; R175H
90	H1299	R175H
91	HME1	R175H; R273H; R280K; R249S
65	H1299	R175H; R273H; D281G
92	U2OS	R175H; V157F; R248Q
93	LNCaP	G245S; R248W; R273H; R273C
94	H1299	R175H; R248W; R273H; D281G
95	H1299	D281G
96	H1299	R175H; R273H; D281G

For each article, the investigated p53 missense mutations and the cell lines used to perform the experiments are reported.

Table 2. Number of genes upregulated and downregulated following *in vitro* studies where a mutant form of p53 is overexpressed

Number of genes	*% Of the total number of genes*	*Number of mutations on different p53 codons, leading to upregulation*	*Number of mutations on different p53 codons, leading to downregulation*
151	8.2%	0	1
698	37.8%	1	0
446	24.2%	0	>1
503	27.2%	>1	0
401	22%	0	$\geqslant 3$
260	14%	$\geqslant 3$	0
Inconsistent			
48	2.6%	$\geqslant 1$	$\geqslant 1$

Only 48 out of 1846 were described as behaving differently according to the p53 mutated codon assayed. The greatest majority of genes (949) showed a reproducible upregulation or downregulation when various p53 mutations were assayed.

heterogeneity among studies, it is likely that consistent findings reveal true p53 target genes. Similar, although less confident, results could be obtained when the expression of genes was evaluated comparing at least two p53 mutants: 446 genes were found to be downregulated, whereas 503 genes were found to be upregulated. Thus, overall, the gene expression reprogramming did not seem to differ in relation to the mutant p53 hotspot analyzed. However, it should be also noticed that a small share of genes (48 out of 1846, 2.6%) was described as behaving discordantly, in relation to the p53 mutant assayed. It should be acknowledged that a systematic comparison of all mutant p53s under the same experimental conditions was not found in the literature, and thus subtle mutation-specific differences cannot be ruled out.

Hypothesized mechanisms at the basis of DMB and UMB phenotypes

In order to better understand the possible mechanisms related to the changes of expression caused by mutations within p53, *DMB* genes were first compared with the information from Riley's list,[3] who reported 126 experimentally validated p53 target genes. These genes were crossed with those reported in Table 3 and 26 in common were found (bolded in Table 3). Almost all of them, 25, were genes normally activated by the WTp53. Then, COMPASSS was used (the detailed statistics are reported for each chromosome and for each p53RE motif in Supplementary Table 2) and it was observed that *DMB* genes were enriched for p53RE motifs typically found in genes transactivated by WTp53. This was expected and was consistent with the comparison made with Riley's data. Thus, it is conceivable that, for most of the *DMB* genes, the lack of expression is related to the LOF of p53.

When Riley's list was compared with the *UMB* genes (Table 4), only three were in common (bolded), preventing to draw any conclusion. According to COMPASSS, *UMB* genes were specifically enriched for a pair of the p53RE variant motif (RRNCNNGNYN) that was previously related to WTp53-dependent gene repression.[8] Out of 260 *UMB* genes, 242 contained a putative repressor element. It is, however, important to note that the p53RE variant pattern search may retrieve false-positive results. This motif was also enriched over the baseline for the *DMB* genes (this because it represents a more degenerated version of the canonical p53RE) confirming the difficulty in separating p53-upregulated and p53-downregulated genes purely on the basis of the *cis*-regulatory elements.[3,8,32] The fact that WTp53 could bind p53RE within specific *UMB* genes is reinforced by studies of chromatin immunoprecipitation followed by DNA sequencing (ChiP-seq) coupled to transcriptome analysis.[34] In fact, high-confidence p53 occupancy sites have been mapped not only for 57 *DMB* genes but also for 23 *UMB* genes (underlined in Tables 3 and 4). In summary, also *UMB* genes could be explained with the loss of activity, that is, a loss of transcriptional repression, toward specific targets. Actually, it was shown recently that p53 bears a repressor activity for genes such as *CHEK1*, *BCL2*, *ARF* and *FOS*,[8] MDR1 and Lasp1.[8,35] Moreover, experiments in the yeast assays showed that mutant p53s lose the transactivating capability towards several target REs from target genes.[12,14,20]

Three generally accepted mechanisms of direct p53-mediated transcriptional repression are known: (1) steric interference by masking overlapping transcription factor binding sites,[36,37] (2) sequestration of transcription activators[38] and (3) recruitment of histone deacetylases.[3,39] Moreover, other indirect mechanisms were suggested, such as the transcriptional activation of microRNAs, known inhibitors of mRNA translation and stability.[39,40] Thus, a 'full-loss-of-function hypothesis' could explain both the *UMB* and the *DMB* genes. However, alternatives are discussed further in final remarks section.

Novel targets for WTp53

Previous analyses were also useful to detect novel putative direct p53 targets. In fact, a short list of highly likely candidate p53 targets was obtained applying the *in silico* analysis of p53REs within the *DMB* and *UMB* genes, crossed with the results from a ChIP-seq study.[34] In Supplementary Table S3, all the *UMB* and *DMB* genes positive for a p53REs within the promoter (through COMPASSS) were listed. Following the cross with the ChIP-seq study, known p53 targets were found (including *ATF3, BTG2, BTG3, MYC, CDKN1A, ENC1, TP53I3* and *TP53INP1*). However, interestingly, a restricted number of novel potential p53 targets were also suggested. These are: *ARID3B*, *ARNT2*, *CLMN*, *FADS1*, *FTH1*, *KPNA2*, *LPHN2*, *PARD6B*, *PDE4C*, *PIAS2*, *PRPF40A*, *PYGL* and *RHOBTB2*. Intriguingly, some of them, belonging to the UMB category, were shown to be in causal relationship with features of the malignant phenotype and their upregulation in tumor correlates with a worsening of the prognosis. For example, an overexpression of *ARID3B* in human neuroblastoma cell lines is more common in stage IV neuroblastoma than in stages I–III, indicating its role in the progression of malignant neuroblastoma.[41] The upregulation of *ARNT2* is also common in neuronal-derived tumors. *ARNT2* forms complexes with HIF-1a (Hypoxia-inducible factor 1-alpha), and it allows for initiating hypoxia/nutrient deprivation-induced vascular endothelial growth factor expression, therefore permitting tumor angiogenesis.[42] *FTH1* was found to be overexpressed in tumorspheres, and its upregulation has an important anti-apoptotic role.[43,44] Moreover, *FHIT* overexpression was shown to have a role in increasing the multidrug resistance of cancer cells,[45] whereas its silencing caused an increased sensitivity.[46] *KPNA2* was found to be highly expressed in different types of cancer,

Table 3. List of genes consistently downregulated where at least three independent mutations were assayed

ABAT	*C15orf41*	*COBL*	*EDNRB*	*GABARAPL1*	*GTPBP2*	*KIAA1211*	*MARVELD2*	*P9*	*PSA*	*SHC4*	*TBXA2R*	***VCAN***
ABCA12	*C16orf5*	***COL18A1***	*EFS*	*GAD1*	*H19*	*KIAA1324*	*MCC*	*PADI3*	*PSEN2*	*SHROOM2*	*TEAD3*	*VSNL1*
ABCG1	*C17orf103*	*COL2A1*	*EGR2*	***GADD45A***	*HAGH*	*KIAA1751*	*MDFIC*	*PAK6*	*PSTPIP2*	*SI*	*TFAP2E*	*WDR8*
ABHD15	*C19orf59*	*CPE*	*ENC1*	*GAGEB1*	*HES2*	*KITLG*	***MDM2***	*PALMD*	*PTGES*	*SLC2A13*	*TGFBR1*	*WFDC5*
ABHD4	*C1orf187*	*CPN2*	*EPB49*	*GALE*	*HHATL*	*KLRK1*	*MEF2A*	*PARD6G*	*PTPN22*	*SLC2A8*	*TINAGL1*	*WNT5A*
ABHD6	*C2orf3*	*CRISPLD2*	*EPHB4*	*GAMT*	*HIC2*	*KREMEN2*	*MGC16703*	*PARP10*	*PVRL4*	*SLC2A9*	*TINP1*	*WWP1*
ABTB2	*C4A*	*CRYAB*	*EPPK1*	*GATA3*	*HIST1H2AE*	*KRT78*	*MGC4248*	*PARP14*	*PYHIN1*	*SLC35D1*	*TLR3*	*XLalphas*
ACPP	*C4orf18*	*CSNK1G1*	*ETNK1*	*GCH1*	*HLA-DMB*	*KRTAP2-1*	*MIB2*	***PCBP4***	*RALGDS*	*SLC35E4*	*TM7SF3*	*YPEL3*
AHSA2	*C5orf4*	*CST1*	*ETV7*	*GCLC*	*HOXC13*	*KSR*	*MICALL2*	*PCLO*	*RASAL1*	*SLC39A8*	*TMEM144*	*ZBTB1*
AIFM2	*C6orf204*	*CST11*	*F2RL2*	*GDAP1L1*	*HOXD10*	*LAMP3*	*MIR*	*PDE3A*	*RASGEF1B*	*SLC4A11*	*TMEM27*	*ZMAT3*
AK1	*C7orf10*	*CTSH*	*FAM105A*	***GDF15***	*HSDL2*	*LCE1B*	*MLF2*	*PDE4C*	*RASSF6*	*SLC4A4*	*TMEM63B*	*ZNF197*
AKR1B10	*C7orf57*	*CYFIP2*	*FAM134B*	*GDF9*	*HSPA5BP1*	*LDLRAP1*	*MME*	*PDE4D*	*RDH10*	*SLC9A1*	*TMG4*	*ZNF236*
ALDH7A1	*C9orf100*	*CYLC1*	*FAM13C*	*GGT1*	*HTR2A*	*LHX3*	*MPZL2*	*PENK*	*RET*	*SLC9A3R1*	*TMTC3*	*ZNF385A*
AMACR	*C9orf98*	*CYP2C9*	*FAM43A*	*GGT6*	*IDUA*	*LIN54*	*MRAS*	***PERP***	*retII*	*SLCO2B1*	***TNFRSF10B***	*ZNF441*
ANK1	*CABC1*	*CYP2S1*	*FAM46C*	*GH2*	*IFIT3*	*LOC132671*	*MRPL44*	*PF4V1*	*RGS12*	*SMARCD3*	*TNFRSF14*	*ZNF492*
ANO4	*CABYR*	*CYP3A7*	*FAM69B*	*GHR*	*IFITM1*	*LOC203274*	*MSX1*	*PGF*	*RHOD*	*SOM*	*TNP2*	*ZNF746*
APAF1	*CACNA2D2*	*D4S234E*	*FAM82A1*	*GJC1*	*IL24*	*LOC283585*	*MT1G*	*PLA2G2A*	*RNASE7*	*SORBS1*	*TNRC6C*	*ZNF786*
APOBEC3C	*CALML3*	***DDB2***	*FAM87A*	*GLS*	*IL2RB*	*LOC284837*	*MTMR6*	*PLAC8*	*RNF128*	*SORL1*	*TP53I11*	
ARAP1	*CAPN1*	*DDC*	*FBXO2*	*GLS2*	*INPP1*	*LOC286434*	*MYO6*	*PLAT*	***RNF144B***	*SPAG1*	***TP53I3***	
ARG1	*CARD18*	*DFNB31*	*FBXO22*	*GM2A*	*INPP5D*	*LOC348938*	*MYO7A*	*PLEK*	*RNF4*	*SPN*	***TP53INP1***	
ARHGAP6	*CASP6*	*DGKZ*	*FGF1*	*GNA11*	*ISG15*	*LOC80154*	*NADSYN1*	*PLXNB3*	*RPL36*	*SSB2*	*TP53TG1*	
ARHGEF3	*CBFA2T3*	*DHRS2*	*FITM2*	*GNA14*	*ISYNA1*	***LRDD***	*NCRNA00085*	*PMAIP1*	***RPS27L***	*STAR*	***TP73***	
ASPA	*CCNA1*	*DISP1*	*FLJ14312*	*GNG2*	*ITFG1*	*LRP10*	*NEFL*	*PODXL*	*RPS6KA1*	*STARD5*	*TRAF4*	
ATP11B	*CCT6B*	*DKK2*	*FLJ32065*	*GPC1*	*KAT2B*	*LTB4R*	*NHLH2*	*POLH*	***RRM2B***	*STAT4*	*TREM2*	
BCHE	*CDH10*	*DMRTC1*	*FLJ36336*	*GPR126*	*KCNB1*	*LUM*	*NIFU*	*POMZP3*	*RTN1*	*STOX2*	*TRIM11*	
BCL11B	***CDKN1A***	*DNAJC18*	*FLJ40773*	*GPR137B*	*KCNC4*	*MAB21L1*	*NLRP1*	*POU3F1*	*SAA2*	*SULF2*	*TRIM2*	
BDKRB1	*CDKN1C*	*DNAJC21*	*FLNC*	*GPR155*	*KCNJ12*	*MAD1L1*	*NOTCH1*	*PPM1F*	*SAC3D1*	*SYK*	***TRIM22***	
BDNF	*CEACAM1*	*DPYSL4*	*FMN1*	*GPR56*	*KIAA0247*	*MAEL*	*NOTCH3*	*PPP2R2B*	*SCN3B*	*SYNC*	*TRIM3*	
BLNK	*CES2*	*DSG3*	*FREQ*	*GPR87*	*KIAA0284*	*MAFB*	*NRCAM*	*PPP2R2C*	*SCNN1G*	*SYTL2*	*TSGA10*	
BTF3L3	*CLCA2*	*DUOX1*	*FRMD8*	*GRAMD2*	*KIAA1026*	*MAGEA4*	*NRP2*	*PRKX*	*SEC14L5*	*TAF3*	*TSPAN14*	
BTG2	*CLDN19*	*DUSP13*	*FXYD2*	*GREB1*	*KIAA1052*	*MAP2K3*	*OIP106*	*PRKY*	***SERPINB5***	*TAGLN*	*TSPY1*	
C13orf31	*CNNM4*	*EDAR*	*G6PC*	*GRN*	*KIAA1199*	*MAPK13*	*OTP*	*PRRX1*	***SESN1***	***TAP1***	*ULBP2*	

The listed genes are reproducibly deregulated irrespectively on the mutated codon. Note that all the assayed mutations fall within the p53 DNA-binding domain. Bolded genes are in common with the functional assay proposed by Riley *et al.*[3] Underlined are genes for which a high-confidence p53 occupancy sites had been mapped.

Table 4. List of genes consistently upregulated where at least three independent mutations were assayed

ABLIM1	*CA9*	*DRG1*	*HIST1H4C*	*LPP*	*MYO5B*	*ProSAPiP1*	*SLCO4A1*	*XRCC5*
ACTA2	*CARS*	*E2F3*	*HMGB2*	*LRRFIP1*	*NAP1L1*	*PRPF40A*	*SLPI*	*YARS*
ADAMTSL4	*CBR4*	*E2F5*	*HOMER1*	*LRRK1*	*NARS*	*PRSS7*	*SMA4*	*ZBTB45*
ADPRTL2	*CCNB1IP1*	*EBAG9*	*HOMER2*	*LYN*	*NBEA*	*PRSS8*	*SMTN*	*ZNF217*
AGGF1	*CCNB2*	*EFHA1*	***HSP90AB1***	*MAD2L1*	*NDC80*	*PVRL3*	*SNTB2*	*ZNF238*
AKT2	*CCNH*	*EXPH5*	*HYAL3*	*MAL2*	*NDUFA4L2*	*PYGL*	*SPAG5*	*ZNF24*
ALDH1A3	*CCNL1*	*F2R*	*ID1*	*MAP2K5*	*NFATC2IP*	*QARS*	*SQSTM1*	*ZNF273*
ALDH2	*CD14*	*FADS1*	*ID3*	*MAP4*	*NFKBIA*	*RAD51C*	*SRM*	*ZNF415*
ALDH3A1	*CD44*	*FAM169A*	*IL1RL1*	*MAPKAPK2*	*NKTR*	*RBBP6*	*SS18*	*ZNF44*
ANGPT1	*CDC2*	*FBXO31*	*IMPDH2*	*MAPKAPK3*	*NLRX1*	*RBBP8*	*STAMBP*	*ZNF579*
APS	*CDC6*	*FDFT1*	*INADL*	*MAPKAPK4*	*NMI*	*RHOBTB2*	*STAT3*	*ZNF580*
AR	*CDH1*	*FECH*	*INF2*	*MARCH_6*	*NPC1*	*RHOG*	*STATH*	*ZNF652*
ARHGEF2	*CDKL3*	*FTH1*	*INPPL1*	*MARCKS*	*NUP153*	*RLN1*	*TAF1A*	
ARID3B	*CDS1*	*FZD3*	*ITGA6*	*MAZ*	*PAQR3*	*RNF44*	*TARS*	
ARNT2	*CEBPB*	*GAGE3*	*ITPR3*	*MCL1*	*PARD6B*	*RNF6*	*TFAP2A*	
ASB13	*CKS1B*	*GAPVD1*	*JUNB*	*MCM3*	*PARP1*	*RP3-402G11.5*	*TMC6*	
ATF3	*CLEC18C*	*GARS*	*JUP*	*MCM6*	*PCGF2*	*RPGRIP1*	*TNFRSF9*	
ATIC	*CLMN*	*GHDC*	*KIAA0516*	*ME1*	*PDE3B*	*RPS6KA3*	*TRAF3IP2*	
BAG2	*CPT1B*	*GINS1*	*KIF13B*	*MED13*	*PDLIM5*	*RRM1*	*TREM1*	
BARD1	*CPVL*	*GLI2*	*KIF24*	*MEF2D*	*PGAP3*	*SARNP*	*TRIM29*	
BCAN	*CREB1*	*GMIP*	*KIF2C*	*MELK*	*PGM1*	*SEC31A*	*TROVE2*	
BMP6	*CTPS*	*GNB2L1*	*KLF16*	*MEST*	*PHKB*	*SEZ6L2*	*TUBB*	
BPTF	*CTSF*	*GOSR1*	*KPNA2*	*MFGE8*	*PI3*	*SF3A2*	*TUSC3*	
BTG3	*CUL5*	*GPR153*	*KRAS*	*MINK*	*PIAS2*	*SFPQ*	*TXNIP*	
BTN3A3	*CUL7*	*GTF3A*	*KRT16*	*MMP28*	*PIK3CA*	*SGPP2*	*TXNL4A*	
C10orf116	*DAAM1*	*GUCY1A3*	*LARP*	*MNX1*	*PLEKHH1*	*SIRT6*	*UCN*	
C13orf1	*DCAF4*	*HAS3*	*LOC115871*	*MOCOS*	*POLA2*	*SLC16A4*	*UGT1A10*	
C16orf45	*DHFRL1*	*HAX1*	*LOC120450*	*MRPL46*	*POLD2*	*SLC26A2*	*UGT2B28*	
C1orf63	*DHRS9*	*HBA2*	*LOC139376*	*MRPS6*	*POLR2E*	*SLC29A2*	*UGT2B7*	
C21orf63	*DICER1*	*HHL*	*LOC81691*	*MTAP*	*PRDM15*	*SLC4A7*	*UPF1*	
CA2	*DMXL1*	*HIBCH*	*LPHN2*	*MYC*	*PRKCI*	*SLC6A8*	*VIM*	

The listed genes are reproducibly deregulated irrespectively on the mutated codon. Note that all the assayed mutations fall within the p53 DNA-binding domain. Bolded genes are in common with the functional assay proposed by Riley *et al.*[3] Underlined are genes for which a high-confidence p53 occupancy sites had been mapped.

Table 5. The tool DAVID groups cluster of genes into biological pathways

Category	*Term*	*Count*	*%*	P-*value*	*Benjamini*
KEGG_PATHWAY	p53 signaling pathway	26	1.9	8.6×10^{-8}	1.6×10^{-5}
KEGG_PATHWAY	Metabolism of xenobiotics by cytochrome P450	19	1.4	1.3×10^{-4}	7.9×10^{-3}
KEGG_PATHWAY	Progesterone-mediated oocyte maturation	23	1.7	3.2×10^{-4}	0.015
KEGG_PATHWAY	Ascorbate and aldarate metabolism	9	0.7	3.5×10^{-4}	0.013
KEGG_PATHWAY	Alanine, aspartate and glutamate metabolism	12	0.9	5.5×10^{-4}	0.017
KEGG_PATHWAY	Cell cycle	28	2	1.3×10^{-3}	0.029
KEGG_PATHWAY	Beta-alanine metabolism	9	0.7	2.7×10^{-3}	0.052
KEGG_PATHWAY	Arginine and proline metabolism	15	1.1	2.8×10^{-3}	0.05

The p53 signaling pathway and pathways related to the control of the cell cycle are deregulated, as expected (analysis carried out including all 1846 genes collected among all the published studies).

and its aberrant expression is often linked to a poor prognosis.[47] Finally, *PARD6B* was found amplified and overexpressed in a high number of breast cancer cell lines. The encoded protein, PAR6B, has a central role in tight junction assembly, maintenance of cell polarity, all features important for tumor progression and invasion.[48] Although the precise mechanism at the basis of the upregulation is not established (a loss of transcriptional repression is likely, as stated before), the increase in gene expression could, at least in part, explain some of the novel phenotypes gained by cancer cells, including angiogenesis, drug resistance and altered cell–matrix and cell–cell interactions.

Pathway analysis of deregulated genes using Database for Annotation, Visualization and Integrated Discovery

The possible pathways and biological functions modulated by mutant p53s were evaluated *in silico* using the tool Database for Annotation, Visualization and Integrated Discovery and in Table 5 the main results (with a KEGG-pathways based analysis) are reported. As expected, the p53 signaling pathway ($P = 8.6 \times 10^{-8}$), and pathways related to the control of the cell cycle ($P = 1.3 \times 10^{-3}$), is among the most significant semantic terms. Moreover, an over-representation of genes encoding for enzymes in the metabolism of xenobiotics ($P = 1.3 \times 10^{-4}$) was found, where, in general, the cytochrome p450 genes are overexpressed. This might be related to the known resistance to chemotherapeutic drugs associated with p53 mutation status of patients' cancer cells.[49] Intriguingly, an enrichment of deregulated genes in pathways devoted to the catabolism of amino acids was also found (for example, *ARG1*, arginase; *PRODH*, proline oxidase; *GLS2*, glutaminase; *GAD1*, glutamate decarboxylase 1, all downregulated). The amino-acid catabolism leads to the formation of α-ketoglutarate, one of the key substrate for the tricarboxylic acid cycle, which in turn results in enhanced mitochondrial respiration and ATP generation. It is worth to stress here that p53 was shown to have a role not only in the regulation of cell cycle, apoptosis, differentiation, senescence, angiogenesis,[50] antioxidant response[51] and glutaminolysis[52,53] but also in the modulation of both glycolysis[54–56] and mitochondrial respiration.[57–60] Metabolic enzymes including glucose transporters (such as *GLUT1* and *GLUT4*), glycolytic enzymes (such as *PGM5* and *HK2*) and tricarboxylic acid cycle enzymes are downstream targets of p53 (ref. 61), and WTp53 was shown to slow the glycolysis. The inhibition of glycolysis can also be achieved by p53-dependent transcriptional activation of synthesis of cytochrome C oxidative 2, resulting in enhanced mitochondrial respiration.[62] Thus, mutations within p53 could lead to an increase of the glycolysis, characteristic of cancer cells.[63] One of the most important genes linking the energy metabolism with p53 was proposed to be *GLS2*, encoding for glutaminase. It was shown that *GLS2* is transactivated by WTp53, and it regulates the cellular energy metabolism by increasing the production of α-ketoglutarate, the mithochondrial respiration and the ATP generation.[52] It is noteworthy that *GLS2* expression was shown to be decreased in hepatocellular carcinomas, whereas its overexpression reduced tumor cell colony formation in an *in vitro* assay. *GLS2* downregulation in cancer could be obtained by LOF mutations within p53, and this is consistent with the fact that *GLS2* was found among the *DMB* genes. In addition, *PRODH* (proline dehydrogenase) was found downregulated in various transcriptome studies (Supplementary Table S1). Interestingly, *PRODH* functions as a tumor suppressor, and it suppresses hypoxia-inducible factor signaling by increasing α-ketoglutarate.[64] Thus, overall, p53 mutations could lead to increasing levels of glycolysis and, in parallel, to reduced mitochondrial respiration. This suggests a role of mutant p53s in the Warburg effect. The modulation of α-ketoglutarate production, through the alteration of amino-acid catabolism, could be one possible mechanism to be considered in a putative p53-dependent metabolic shift.

FINAL REMARKS

As stated before, collected data seem to be in favor of a general lack of activity at the basis of *UMB* (LOF) and *DMB* (loss of transcriptional repression) genes. Given that all the p53 mutants taken into consideration here were classified as LOF in *in vitro* reporter assays, their expected impact corresponds to the lack of the hand in the 'piano model' analogy[15] and, therefore, the consequence of this gene expression reprogramming could result as a 'sound of silence'. However, several other aspects deserve discussion and open to the possibility of other mechanisms at play. In fact, the majority of p53 mutations encountered within tumors are of missense type. Moreover, tumors commonly retain and overexpress the full-length mutant p53 (ref. 65) and mutations whose effect is a true ablation of the gene sequence, such as large deletions, nonsense substitutions or in/del frame-shifts, account for only about 16% of the cases (www.iarc.fr/p53). This is in striking contrast to the majority of tumor suppressors (for example, *RB1*, *APC*, *NF1*, *NF2* and *VHL*), where the primary mutations are deletion or nonsense, leading to little or no expression of the respective proteins. Dominance or dominant-negative potential of mutant p53s when heterozygous with the WT allele has been considered as an underlying reason for the high preponderance of p53 missense mutations in cancer. However, one should wonder whether the classification of the hotspot mutations as 'inactivating' based on *in vitro* assays (commonly performed on yeast systems) is completely correct. The fact that missense mutations are preferred to the abrogation of the locus suggests that these two possibilities are not equivalent. p53 knockin mutant mouse models produced an altered tumor spectrum as compared with the knockout models, with more metastatic tumors.[66,67] Similarly, in an analysis of Li–Fraumeni patients, germline missense mutations in *TP53* have been shown to be associated with an earlier age of onset (9 years) when compared with germline deletions, suggesting a gain-of-function effect of missense p53 mutants in human tumors.[68] In addition, tumors with mutant p53 proteins may be more

aggressive (for example, conferring a poor prognosis) as compared with tumors where p53 is lost.[18,69–71] Thus, it could be hypothesized that these mutations alter profoundly, but not completely abrogate, some of the p53 functions. Various authors suggested a gain-of-function at molecular level for mutant p53s.[65,72–76] Mutant p53s can directly bind promoters of various targets such as it is for miR-130b,[77] miR-128-2,[78] Axl[79] or NF-kB2.[80] It was also shown that they form aberrant protein complexes with interacting partners, such as NF-Y, Sp1, Ets-1 or VDR, perturbing their activity.[81] More interactions of this type were reported in a recent review.[82] Among them, it is noteworthy to underline that, although WTp53 does not form heterotetramers with p63,[83] mutant p53s have been shown to bind and sequester TAp63 away from its target genes, hampering its anti-metastatic capacity.[84–87] Furthermore, other studies showed that mutant p53s bind to p63 and use it as chaperone for the transactivation of novel targets.[7] In this regard, it should be considered that the enrichment of the degenerated motif detected with COMPASSS within the promoters of *UMB* genes could be also a marker of the presence of p63-responsive elements, given the sequence similarities with the p53REs. Thus, we cannot rule out that some of the *UMB* genes are directly/indirectly transactivated by mutant p53s.

In summary, the elaboration of data already present in the literature allowed to gain novel insights in the biology of p53 and to define novel targets. Further studies are warranted in order to better define the extent of differences in the transactivating activities of different mutant p53s and to validate the novel targets suggested here.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies this paper on the Oncogenesis website (http://www.nature.com/oncsis).