Review

Transcriptional modulation induced by ionizing radiation: p53 remains a central player

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c Sheba Cancer Research Center, The Chaim Sheba Medical Center, Tel Hashomer, Israel

Abstract

The cellular response to DNA damage is vital for maintaining genomic stability and preventing undue cell death or cancer formation. The DNA damage response (DDR), most robustly mobilized by double-strand breaks (DSBs), rapidly activates an extensive signaling network that affects numerous cellular systems, leading to cell survival or programmed cell death. A major component of the DDR is the widespread modulation of gene expression. We analyzed together six datasets that probed transcriptional responses to ionizing radiation (IR) — our novel experimental data and 5 published datasets — to elucidate the scope of this response and identify its gene targets. According to the mRNA expression profiles we recorded from 5 cancerous and non-cancerous human cell lines after exposure to 5 Gy of IR, most of the responses were cell line-specific. Computational analysis identified significant enrichment for p53 target genes and cell cycle-related pathways among groups of up-regulated and down-regulated genes, respectively. Computational promoter analysis of the six datasets disclosed that a statistically significant number of the induced genes contained p53 binding site signatures. p53-mediated regulation had previously been documented for subsets of these gene groups, making our lists a source of novel potential p53 targets. Real-time qPCR and chromatin immunoprecipitation (ChIP) assays validated the IR-induced p53-dependent induction and p53 binding to the respective promoters of 11 selected genes. Our results demonstrate the power of a combined computational and experimental approach to identify new transcriptional targets in the DNA damage response network.

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1. **Introduction: the DNA damage response and transcriptional dynamics**

Maintenance of genome stability and integrity is essential for cellular homeostasis and prevention of undue cell death or neoplasia (Negrini et al., 2010). DNA damage caused by internal or external damaging agents is a major threat to the integrity of the cellular genome. Failure to repair DNA lesions may result in obstruction of replication and transcription or mutagenesis, leading to cellular malfunctions, undue cell death or cancer (Halazonetis et al., 2008; Jackson and Bartek, 2009).

The cellular defense system against this threat is the DNA damage response (DDR) – an elaborate signaling network that repairs the damage while swiftly modulating many physiological processes (Ciccia and Elledge, 2010). One of the most powerful triggers of the DDR is the DNA double-strand break (DSB) (Bassing and Alt, 2004; Hartlerode and Scully, 2009; Hiom, 2010). DSBs are induced by ionizing radiation (IR), radiomimetic chemicals, or reactive oxygen species formed during the course of normal metabolism, and may also result from replication fork stalling. DSBs also accompany normal genomic transactions such as meiotic recombination and the rearrangement of the antigen receptor genes in lymphocytes via V(D)J recombination.

One of the most potent exogenous inducers of DSBs is IR. As a major tool in cancer therapy, the success of IR relies on its ability to selectively kill tumor cells while minimizing the detrimental effect to normal surrounding tissues (Jen and Cheung, 2003; Snyder, 2004). It is therefore essential to understand the IR-mediated responses at the molecular, cellular and tissue levels (Jen and Cheung, 2003).

The major DSB repair pathways in eukaryotic cells are error-prone non-homologous end-joining (NHEJ) and a high-fidelity process based on homologous recombination (HR) between sister chromatids (Hartlerode and Scully, 2009; Hiom, 2010). However, the global cellular response to DSBs goes far beyond repair. This broad, powerful signaling network works swiftly and vigorously to coordinate a large number of cellular systems simultaneously (Ciccia and Elledge, 2010) in a hierarchical process executed through a series of highly controlled steps. It is based on a signal transduction mechanism that begins with **sensor** proteins, which rapidly accumulate at the damaged sites (Bekker-Jensen and Mailand, 2010). These proteins are thought to sense the damage and/or chromatin alterations following damage induction, initiate damage processing, and transmit a signal to **transducers**, which in turn relay the signal to numerous downstream **effectors** involved in specific pathways. The initial DSB response involves extensive dynamics of a host of protein post-translational modifications (Al-Hakim et al., 2010; Bensimon et al., 2010; Ciccia and Elledge, 2010; Polo and Jackson, 2011).

The primary transducer of the DSB alarm is the protein kinase **ATM**, which is rapidly activated in response to DSBs and proceeds to phosphorylate a plethora of key players in various damage response pathways (Derheimer and Kastan, 2010). ATM belongs to a conserved family of **PI3K-like** protein kinases (PIKKs) (Lempiainen and Halazonetis, 2009) that includes, among others, two additional major DDR transducers: the DNA-dependent protein kinase (DNA-PK) (Neal and Meek, 2011), and the ataxia–telangiectasia and Rad3-related (ATR) kinase (Lopez-Contreras and Fernandez-Capetillo, 2010). ATM, DNA-PK and ATR share substrates in the DSB response but exhibit selective substrate specificities in response to different genotoxic stresses and different DSB inducers. These three kinases maintain close functional relationships (Lempiainen and Halazonetis, 2009).

In humans, germ-line mutations in genes encoding DNA damage response proteins can lead to inherited genomic instability syndromes that involve various degrees of tissue degeneration (most notably in the nervous and immune systems), sensitivity to specific genotoxic stresses, cancer predisposition, and occasionally premature aging (Eysford and Bodvarsdottir, 2005; O’Driscoll and Jeggo, 2006). Null alleles in the human ATM locus lead to the genomic instability syndrome ataxia–telangiectasia (A–T) characterized by progressive cerebellar atrophy, immunodeficiency, cancer predisposition, and acute radiation sensitivity (Chun and Gatti, 2004; Lavin, 2008).

The DSB response involves a marked modulation of the cellular transcriptome (Begley and Samson, 2004; Elkon et al., 2005; Rashi-Elkeles et al., 2006). One of the major transcription factors (TFs) that mobilizes this response is the tumor suppressor protein **p53**, known to regulate genomic integrity by stabilizing the genome and preventing polyplidization (Aylon & Oren; p53: Guardian of ploidy). p53 plays a major role in determining the cell’s fate – whether it will survive DNA damage and growth arrest, or render such growth arrest irreversible and activate programmed cell death (Fei and El-Deiry, 2003; Braithwaite et al., 2005; Zamzami and Kroemer, 2005; Helton and Chen, 2007; Riley et al., 2008; Jackson et al., 2011). p53 exerts its transcriptional regulatory activity mostly through direct binding to the regulatory sequences of its target genes. The list of p53 targets, which is constantly growing, currently exceeds 100 genes whose biological role in the p53-mediated network has been studied in detail (Elkon et al., 2008; Riley et al., 2008; Paz et al., 2011). These genes are involved in a variety of pathways, including DNA repair (e.g., MSH2, MLH1, PCNA, XPC, DDB2 and RAD51), cell cycle progression (e.g., CDKN1A, GADD45A, BTG2, CCNG1 and 14-3-3ε), cell death (e.g., BAX, BCL, FAS/APO and the death receptors TNFRSF10B and TNFRSF6), transcription regulation (e.g., JUND, FOS and ATF3), and other signaling pathways (e.g., RRAD, SNX5 and HNRPK) (el-Deiry, 1998; Wei et al., 2006; Zschenker et al., 2006; Helton and Chen, 2007). Dozens more

### Table 1 – The studied cell lines.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Nature of Origin</th>
<th>Establishment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bj-hTert-1</td>
<td>Normal human foreskin fibroblast</td>
<td>(Rodnar et al., 1998)</td>
</tr>
<tr>
<td>G361</td>
<td>Human Skin melanoma</td>
<td>(Peebles et al., 1978)</td>
</tr>
<tr>
<td>HepG2</td>
<td>Human hepatocellular carcinoma</td>
<td>(Knowles et al., 1980)</td>
</tr>
<tr>
<td>Tk6</td>
<td>Normal human B-lymphoblastoid</td>
<td>(Skopek et al., 1978)</td>
</tr>
<tr>
<td>U2OS</td>
<td>Human Osteosarcoma</td>
<td>(Ponten and Saksel, 1967)</td>
</tr>
</tbody>
</table>
potential p53 targets have been identified by genome-wide profiling of p53 binding sites (Wei et al., 2006; Bandele et al., 2010), though the mere physical binding to the DNA does not automatically mean functional significance (Hiom, 2009). Numerous wide-scale expression studies collectively profiled cellular responses to a multitude of DNA-damaging agents (Heinloth et al., 2003a,b). They demonstrated that the transcriptional response to genotoxic stress goes beyond the core

![Hierarchical clustering of the conditions in our dataset](image1)

**Figure 1** — Hierarchical clustering of the conditions in our dataset. Samples were ordered in a hierarchical tree (dendrogram) according to similarity in their expression profiles: samples with similar profiles are connected by a short tree edges while samples with very different expression profiles have longer tree distance. The main divider of the samples in the dendrogram is cell line, demonstrating that cell type rather than irradiation is the major determinant of expression profile in our dataset. The name of each condition (shown below its location along the dendrogram) contains the initial of the corresponding cell line (B = Bj-1; G = G361; T = TK6; H = HepG2 and U = U2OS); C stands for control and IR represents the irradiated sample. The scale at the side of the dendrogram indicates the relative distance between the different conditions.

![IR-responsive gene clusters in TK6 cells](image2)

**Figure 2** — IR-responsive gene clusters in TK6 cells. We used the clustering algorithm CLICK to divide the set of responding genes in each cell line into clusters, each of which represents a set of genes with similar expression patterns. The total number of genes in each cluster is indicated. For each cluster, the graph shows the mean expression pattern of all its genes. Error bars represent one S.D. Prior to clustering, gene expression levels were standardized to mean = 0 and SD = 1; the y-axis corresponds to the standardized levels. The horizontal blue line represents the expression level in the untreated t0 sample (‘basal expression level’). The x-axis corresponds to the examined conditions: C: untreated control. IR: irradiated cells. Post-irradiation time points are indicated (0, 3 or 6 h).
Table 2 — Enriched biological pathways in the two major clusters obtained in each cell line.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cluster (size)</th>
<th>Enriched pathway</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BJ-1</td>
<td>Up (344)</td>
<td>p53 signaling</td>
<td>1.3 x 10^{-6}</td>
</tr>
<tr>
<td></td>
<td>Down (617)</td>
<td>Cell cycle</td>
<td>5.7 x 10^{-5}</td>
</tr>
<tr>
<td>G361</td>
<td>Up (229)</td>
<td>p53 signaling</td>
<td>3.1 x 10^{-9}</td>
</tr>
<tr>
<td></td>
<td>Down (173)</td>
<td>Cell cycle</td>
<td>8.4 x 10^{-6}</td>
</tr>
<tr>
<td>HepG2</td>
<td>Up (495)</td>
<td>p53 signaling</td>
<td>2.0 x 10^{-10}</td>
</tr>
<tr>
<td></td>
<td>Down (373)</td>
<td>Cell cycle</td>
<td>2.3 x 10^{-4}</td>
</tr>
<tr>
<td>Tk6</td>
<td>Up (842)</td>
<td>p53 signaling</td>
<td>4.3 x 10^{-7}</td>
</tr>
<tr>
<td></td>
<td>Down (1007)</td>
<td>Cell cycle</td>
<td>7.1 x 10^{-7}</td>
</tr>
<tr>
<td>U2OS</td>
<td>Up (295)</td>
<td>p53 signaling</td>
<td>1.7 x 10^{-16}</td>
</tr>
<tr>
<td></td>
<td>Down (330)</td>
<td>Cell cycle</td>
<td>1.4 x 10^{-7}</td>
</tr>
</tbody>
</table>

Table 3 — Biological functions of the genes that responded in at least 3 of the 5 cell lines tested.

<table>
<thead>
<tr>
<th>Biological function</th>
<th>Induced genes</th>
<th>Repressed genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell cycle regulation</td>
<td>CDKN1A, MDM2, PPM1D, PSMB4, PTP4A1, SESN1, SESN2</td>
<td>ASPM, AURKA, AURKB, BUB1, C13orf4 (BORA), MIS18BP1, C15orf23 (SKAP), C9orf14 (p42.3), CCNB1, CCNF, CDC20, CDC25C, CDC3, CENPA, CENPE, CKS2, FAM83D, GSPT1, GTE1, HJURP, KIF18A, KIF20A, KIF23, NEK2, PLK1, PSRC1, SGO2, WEE1, XPO</td>
</tr>
<tr>
<td>DNA repair</td>
<td>BTG2, DDB2, GADD45A, POLH, REV3L, BM25, XPC</td>
<td></td>
</tr>
<tr>
<td>Apoptosis</td>
<td>ARHGEF3, CYFIP2, DRAM, FAS, PHLDA3, RPS27L, TP53INP1, ZMAT3</td>
<td>G2E3</td>
</tr>
<tr>
<td>Transcriptional regulation</td>
<td>ATF3, C5orf41, TRIM22</td>
<td>CDCA7, DEPDC1</td>
</tr>
<tr>
<td>Other/uncharacterized</td>
<td>ANKRA2, ASCC3, C12orf5, CCD90B, DCP1B, DGKA, ENC1, F2R, FBXW7, FXR, FEZ1, FST, GDF15, GREB1, KLHL24, C2orf10, LOC643401, LOC727770, LOC727916, ORAI3, PANK1, PGF, PLCD2, PLK3, PRKX, RNF198, RPS11, SERTAD1, TMEM68, T0B1</td>
<td>CKAP2L, EIF4A1, FAM72A, GPSM2, KIF14, LRR8C1, PIF1, SLCO2A1, TNK1, TRIB1, TROAP</td>
</tr>
</tbody>
</table>

Most experimental investigations of the DDR are carried out in commonly used cell lines, most of them derived from human tumors. Such cell lines are expected to exhibit a widespread variation in their response to DNA damage, most notably to IR. This variation stems primarily from genetic variation among the donors, which is known to markedly affect their radiation response (Turesson, 1990; Bentzen, 2006; Chistiakov et al., 2008; Andreassen and Alsner, 2009; Popanda et al., 2009; Pugh et al., 2009; Niu et al., 2010). Variations in response to IR also come from the multiple genetic changes that take place in the course of prolonged culturing, particularly in tumor cell lines.

We carried out large-scale expression analysis to examine the transcriptional response induced by 5 Gy of IR in 5 malignant and non-malignant cell lines (Table 1). Expression profiles were recorded in mock-irradiated and irradiated cells 3 and 6 h after irradiation. Other than irradiation, all cultures underwent similar handling. The majority of the transcriptional response of these cell lines to IR was cell line-specific, with substantial differences from one line to another. There was, however, a core of induced or repressed genes common to the different cell lines, with the induced ones consisting almost exclusively of validated p53 targets.

Hierarchical clustering clearly demonstrated that cell line was the principal determinant of the expression profiles (Figure 1). After identifying the set of genes in each cell line
Figure 3 — Core response to IR depicted using the SPIKE knowledge base of signaling pathways (Paz et al., 2011). Violet nodes are proteins, green nodes are protein complexes, and yellow nodes are protein families. Blue edges represent regulations: arrows correspond to activation; T-shaped edges to inhibition, and open circles denote regulations whose effect is still not clear. Green edges represent association between nodes (e.g., association between a protein complex and its components). Red and green dots within a node indicate that not all the regulations and associations stored in SPIKE database for the node are displayed in the map. A. The core set of IR-induced genes is significantly enriched for genes in the p53-regulated network. Red bars denote genes that were induced in at least 3 of the 5 cell lines. B. The core set of IR-repressed genes is significantly enriched for genes involved in the G2/M transition in the cell cycle. Red bars denote genes that were repressed in at least 3 of the 5 cell lines.
whose level was changed at least 1.5-fold following irradiation, and subjecting these sets to cluster analysis, we found two major programs in each cell line: IR-induced gene induction or gene repression (Figure 2 and Figure S1). The vast majority of the responding genes were cell line-specific, with more than 90% of the responding genes responding to IR in only one cell line. A search for enriched biological processes in the various gene sets pointed to a motif shared by all cell lines: the clusters of induced genes were significantly enriched for p53 targets while clusters of down-regulated genes were enriched for cell cycle-related genes (Table 2). In some of the cell lines (e.g., G361; Figure S1), this core program was activated also in mock-treated samples compared to the zero time point control, albeit to a lesser extent compared to the matched irradiated cultures, indicating that this response may represent a general stress program. This result highlights the importance of using matched mock-treated controls while measuring transcriptional profiles.

To further characterize the core response to IR, we selected the genes that responded in at least three of the 5 cell lines: 53 induced genes and 41 repressed genes met this criterion (Table 3), and examined their enrichments in SPIKE signaling maps (Paz et al., 2011). The IR-induced core was significantly enriched in the p53 signaling map, and included key players in cell cycle regulation (e.g., CDKN1A), DNA repair (e.g., DDB2, REV3L and XPC), and apoptosis (e.g., FAS and DRAM) (Table 3, Figure 3A). The repressed core was significantly enriched in the G2/M map (e.g., AURKA, AURKB, CDC25C, PLK1 and WEE1; Table 3, Figure 3B).

3. Meta-analysis of transcription profiles data and experimental validation

To further characterize the transcriptional networks induced by IR, we carried out a meta-analysis of our own dataset and 5 publicly available ones in which transcriptional responses of human cells to IR had been examined (Supplementary Table A). The meta-data set included information on 7190 genes under 21 independent conditions (Supplementary Table B). IR-induced or repressed genes were defined as those that were responsive ($Z > 2.5$) in at least 4 of the 21 conditions (detailed information can be found in the Supplementary Methods section). Of the 248 genes that met this criterion, 199 were up-regulated and 49 were down-regulated (Supplementary Table C). The set of induced genes was enriched for genes that function in apoptosis, cell cycle regulation, stress responses, and metabolic processes (Table 4A), while the set of repressed genes was enriched mainly for genes that function in mitosis (Table 4B).

Novel putative targets of p53 in the IR-induced core were identified by computational analysis of cis-regulatory elements embedded in the promoter regions of these genes, using the PRIMA algorithm (Elkon et al., 2003). The p53 binding signature was highly enriched in the promoters of the up-regulated group, appearing in 61 genes ($p = 4.72 \times 10^{-15}$). p53 regulation had previously been documented for only 28 of these genes, making the remaining 33 a source of potential new p53 targets in response to IR. The set of down-regulated genes was significantly enriched for the binding site signature of NF-Y (22 out of 49 genes, $p = 1.0 \times 10^{-7}$), suggesting that this TF plays a major role in regulating the response of these genes to DNA damage.

Quantitative real-time RT-PCR (real-time qPCR) was used to experimentally validate the response of selected known and potential p53 target genes. We used the TK6 cell line with functional p53 and its p53-deficient derivative, NH32 (Chuang et al., 1999). TK6 cells have been extensively characterized and found to exhibit a robust IR response (Amundson et al., 2005). Fifteen genes that had been identified by PRIMA to contain p53-binding sites were selected: 4 previously known p53 targets and 11 potential p53 targets. The CD164 gene served as a negative control. Good agreement was found between the RT-PCR results, microarray data and PRIMA predictions: 14 of the 15 genes were indeed induced upon IR in a p53-dependent manner, while the negative control showed no induction in either TK6 or NH32 cells (Figure 4A).

The novel candidate p53 targets were further examined by chromatin immunoprecipitation (ChIP) to measure the IR-induced binding of p53 to the regulatory elements identified by PRIMA, 90 min after irradiation with 10 Gy of IR. We selected for this analysis 9 new p53 targets that we previously

<table>
<thead>
<tr>
<th>GO category</th>
<th>Number of genes</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Induced genes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regulation of apoptosis (GO:0042981)</td>
<td>20</td>
<td>$1.35 \times 10^{-09}$</td>
</tr>
<tr>
<td>Intracellular signaling cascade (GO:0007242)</td>
<td>28</td>
<td>$2.27 \times 10^{-09}$</td>
</tr>
<tr>
<td>Regulation of cell cycle (GO:0051726)</td>
<td>15</td>
<td>$6.91 \times 10^{-09}$</td>
</tr>
<tr>
<td>Response to stress (GO:0006950)</td>
<td>28</td>
<td>$4.70 \times 10^{-08}$</td>
</tr>
<tr>
<td>DNA damage response, signal transduction (GO:0042770)</td>
<td>6</td>
<td>$1.08 \times 10^{-06}$</td>
</tr>
<tr>
<td>Regulation of cellular metabolic process (GO:0031323)</td>
<td>43</td>
<td>$2.07 \times 10^{-06}$</td>
</tr>
<tr>
<td>Protein amino acid dephosphorylation (GO:0006470)</td>
<td>8</td>
<td>$2.42 \times 10^{-06}$</td>
</tr>
<tr>
<td>Cell cycle checkpoint (GO:0000075)</td>
<td>6</td>
<td>$3.07 \times 10^{-06}$</td>
</tr>
<tr>
<td>B: Repressed genes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuclear division (GO:0000280)</td>
<td>14</td>
<td>$2.31 \times 10^{-20}$</td>
</tr>
<tr>
<td>Cell division (GO:0051301)</td>
<td>13</td>
<td>$9.20 \times 10^{-17}$</td>
</tr>
<tr>
<td>Organelle organization (GO:0006996)</td>
<td>19</td>
<td>$1.15 \times 10^{-15}$</td>
</tr>
<tr>
<td>Microtubule-based process (GO:007017)</td>
<td>10</td>
<td>$8.62 \times 10^{-13}$</td>
</tr>
<tr>
<td>Regulation of mitotic cell cycle (GO:0007346)</td>
<td>6</td>
<td>$2.36 \times 10^{-08}$</td>
</tr>
</tbody>
</table>
Figure 4 – A. Validation of selected p53 novel targets using real-time qPCR. The figure represents the fold-induction of various genes 4 h after irradiation with 5 Gy. Blue bars: p53-proficient TK6 cells; Red bars: p53-deficient NH32 cells. The fold of induction represented is averaged over three independent measurements. B. Binding of p53 to selected novel p53 target promoters demonstrated using a ChIP assay. TK6 cells were harvested prior to and 1.5 h after irradiation with 10 Gy of IR, and ChIP was carried out as described in the supplementary methods section using a monoclonal antibody (DO-1) against human p53. PCR was performed using the immune complexes as templates and 13 pairs of primers designed to identify p53 binding sites within the promoters of 12 selected genes (sequences are available in Supplementary Table D). GAPDH served as negative control. Input samples corresponding to eluted DNA before immunoprecipitation, as well as IgG-immunoprecipitated chromatin served as positive and negative controls, respectively, for the ChIP assay.
<table>
<thead>
<tr>
<th>Gene name</th>
<th>Gene ID</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGR2</td>
<td>1959</td>
<td>EGR2 encodes for the human early growth response protein 2 (EGR2), a transcription factor with three tandem C2H2-type zinc fingers. Mutations in this gene are associated with the autosomal dominant Charcot-Marie-Tooth disease type 1D (CMT1D), Charcot-Marie-Tooth disease type 4E (CMT4E), and with Dejerine–Sottas syndrome (DSS). (Joseph et al., 1988; Warner et al., 1998; Bellone et al., 1999).</td>
</tr>
<tr>
<td>FUCA1</td>
<td>2517</td>
<td>FUCA1 (fucosidase, alpha-L-1) encodes an enzyme called &quot;tissue alpha-L-fucosidase&quot;. This enzyme is found in the lysosomes, where it plays a role in the breakdown of complexes of sugar molecules (oligosaccharides) attached to glycoproteins and glycolipids. Its main target is the sugar molecule called fucose. Mutations in the FUCA1 gene cause Fucosidosis, an autosomal recessive lysosomal storage disease with accumulation of fucose in various tissues (e.g., brain, liver, spleen, skin, heart, pancreas, and kidneys) (Occhiodoro et al., 1989; Willems et al., 1991).</td>
</tr>
<tr>
<td>GCH1</td>
<td>2643</td>
<td>GCH1 encodes a member of the GTP cyclohydrolase family. The encoded protein is the first and rate-limiting enzyme in tetrahydrobiopterin (BH4) biosynthesis, catalyzing the conversion of GTP into 7,8-dihydronicotinamide ribotide triphosphate. BH4 is an essential co-factor required by aromatic amino acid hydroxylases as well as nitric oxide synthases. Mutations in this gene are associated with malignant hyperphenylalaninemia and dopa-responsive dystonia (Thony et al., 2000).</td>
</tr>
<tr>
<td>HRAS</td>
<td>3265</td>
<td>HRAS is a proto-oncogene that belongs to the Ras oncogene family, whose members have intrinsic GTPase activity and they function in signal transduction pathways. HRas is involved in regulating cell division in response to growth factor stimulation. Mutations in HRAS cause Costello syndrome, a disease characterized by increased growth at the prenatal stage, growth deficiency at the postnatal stage, predisposition to tumor formation, mental retardation, skin and musculoskeletal abnormalities, distinctive facial appearance, and cardiovascular abnormalities. Defects in this gene are implicated in a variety of cancers, including bladder cancer, follicular thyroid cancer, and oral squamous cell carcinoma (Wong-Staal et al., 1981; Aoki et al., 2005).</td>
</tr>
<tr>
<td>LMNA</td>
<td>4000</td>
<td>LMNA encodes for the human protein lamin A/C, also known as LMNA. Lamin A/C belongs to the lamin family of proteins. The nuclear lamina consists of a two-dimensional matrix of proteins located next to the inner nuclear membrane. The proteins in the lamin family make up the matrix and are highly conserved in evolution. During mitosis, the lamina matrix is reversibly disassembled as the lamin proteins are phosphorylated. Lamin proteins are thought to be involved in nuclear stability, chromatin structure and gene expression. Mutations in the LMNA gene are associated with several diseases, including Emery–Dreifuss muscular dystrophy, familial partial lipodystrophy, limb girdle muscular dystrophy, dilated cardiomyopathy, Charcot-Marie-Tooth disease, and Hutchinson–Gilford progeria syndrome (Kamat et al., 1993; Rankin and Ellard, 2006).</td>
</tr>
<tr>
<td>NINJ1</td>
<td>4814</td>
<td>NINJ1 encodes for the ninjurin 1 protein, which is up-regulated after nerve injury both in dorsal root ganglion neurons and in Schwann cells. It demonstrates properties of a homophilic adhesion molecule and promotes neurite outgrowth from primary cultured dorsal root ganglion neurons. It was suggested to play a role in nerve regeneration and in the formation and function of other tissues (Araki and Milbrandt, 1996).</td>
</tr>
<tr>
<td>PGF</td>
<td>5228</td>
<td>PGF encodes for the human placental growth factor (PGF). This protein is a member of the VEGF (vascular endothelial growth factor) sub-family – a key molecule in angiogenesis and vasculogenesis, in particular during embryogenesis. The main source of PGF during pregnancy is the placental trophoblast (Maglione et al., 1993; Fischer et al., 2007).</td>
</tr>
<tr>
<td>TNFSF9</td>
<td>8744</td>
<td>The protein encoded by TNFSF9 is a cytokine that belongs to the tumor necrosis factor (TNF) ligand family. This transmembrane cytokine is a bi-directional signal transducer that acts as a ligand for TNFRSF9/4-1BB, which is a co-stimulatory receptor molecule in T-lymphocytes. This cytokine and its receptor are involved in the antigen presentation process and in the generation of cytotoxic T cells. The receptor, TNFRSF9/4-1BB, is absent from resting T-lymphocytes but rapidly expressed upon antigenic stimulation. The ligand, TNFSF9/4-1BBL, has been shown to reactivate anergic T-lymphocytes as well as promote T-lymphocyte proliferation. It has also been shown to be required for the optimal CD8 responses in CD8 T cells. This cytokine is expressed in carcinoma cell lines, and is thought to be involved in T-cell-tumor cell interaction (Goodwin et al., 1993; Alderson et al., 1994).</td>
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</tbody>
</table>

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Figure 5 – Integration of novel p53 targets in the IR-induced, p53-regulated network. The map was created using the SPIKE tool. New p53 targets are marked with red bars. Violet nodes are proteins, green nodes are protein complexes, and yellow nodes are protein families. Blue edges represent regulations: arrows correspond to activation; T-shaped edges to inhibition, and open circles denote regulations whose effect is still not clear. Green edges represent association between nodes (e.g., association between a protein complex and its components). Red and green dots within a node indicate that not all the regulations and associations stored in SPIKE database for the node are displayed in the map.

Table 5 – (continued)

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Gene ID</th>
<th>Summary</th>
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<tbody>
<tr>
<td>ZNF79</td>
<td>7633</td>
<td>ZNF79 encodes for the human zinc finger protein 79. cDNA clones encoding zinc finger motifs were isolated by screening human placenta and T cell (Peer) cDNA libraries with zinc finger (ZNF) consensus sequences. Unique cDNA clones were mapped in the human genome by rodent-human somatic cell hybrid analysis, and in some cases in situ chromosomal hybridization. ZNF79 mapped to 9q34 centromeric to the ABL gene, and between a constitutional chromosomal translocation on the centromeric side and the CML-specific ABL translocation on the telomeric side (Huebner et al., 1993).</td>
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</table>
validated using real-time qPCR and one bona fide p53 target (CDKN1A); GAPDH served as negative control. p53 physically bound all 9 targets and IR treatment enhanced its binding (Figure 4B). Thus, unequivocally identified 9 new p53 targets in the core transcriptional response to IR (Table 5). Finally, we used the SPIKE database developed in our labs (Paz et al., 2011) to create an interaction map integrating the old and new p53 targets in the IR response (Figure 5).

4. Conclusions: the transcriptional response to IR

The cellular response to DNA damage consists of an integrated signaling network mobilized by protein modifications and gene regulation (Bekker-Jensen et al. 2010; Ciccia and Elledge, 2010; Polo and Jackson, 2011). Transcriptional profiling is a major tool to monitor the DDR and identify new players in its web. We and others have characterized alterations in gene expression profiles following exposure to IR (Amundson et al., 1999, 2000, 2003; Jung and Cheung, 2003, 2005; Akerman et al., 2005; Elkon et al., 2005; Mori et al., 2005; Rashi-Elkeles et al., 2006; Wei et al., 2006; Zschenker et al., 2006; Landmark et al., 2007). The results of our comparison of the responses of different cell lines, described above, highlight the susceptibility of this analysis to substantial variations that reflect differences in genetic backgrounds and possibly inherent genomic instability of commonly used cell lines, and call for prudence in interpreting such data. In view of this caveat, the emergence of p53 as a major determinant of the gene expression response to IR even in this dataset is remarkable, and emphasizes the pivotal and universal role of p53 in the induction of the transcriptional response to IR. Thus, it is not surprising that p53 emerged from the meta-analysis of additional datasets as a key player in this response.

NF-Y appeared as a central player among IR-repressed genes. NF-Y was reported to control the transcription of several key regulators of the cell cycle (Yun et al., 1999, Jung et al., 2001; Manni et al., 2001), and three bioinformatics studies tied it specifically to promoters regulated during the G2/M transition of the cell cycle (Elkon et al., 2003; Linhart et al., 2005; Zhu et al., 2006). NF-Y was found to be involved in the modulation of cell cycle-associated promoters in response to DNA damage, through wild-type p53-dependent transcriptional inhibition (Yun et al., 1999; Adachi et al., 2000; Ceribelli et al., 2006), and to be required for p53-mediated transcriptional repression of several targets in the process of cellular transformation (Tabach et al., 2005). Our profiling results support the notion that upon DNA damage, NF-Y represses the transcription of genes related to the G2/M transition. Recently, Benatti et al. (2008) suggested that a delicate balance between NF-Y and p53 governs the pro- and anti-apoptotic transcriptional response and enables fine control of cell cycle progression upon DNA damage.

Our meta-analysis identified a core of 374 genes that respond to IR in several cell lines, pointing to a large group of genes that together control a significant volume of biological activity. It is important to note that this core is significantly enriched for genes involved in the apoptotic pathway. The combined use of microarrays and computational analysis, based on the PRIMA algorithm followed by real-time qPCR and ChIP, and the subsequently use of the SPIKE database provides a robust experimental paradigm for the dissection of the IR response and identification of functional nodes and novel players in this network.

The ongoing process of identifying p53-regulated genes, which began in the early 1990s with the discovery of p53’s first documented transcription targets (CDKN1A, GADD45A, Cyclin G, Bax and others (Ko and Prives, 1996)), is resulting in a constantly growing list. p53 targets were identified either individually or using screening techniques such as DNA microarrays (Laptenko and Prives, 2006). Wei et al. (2006) recently coupled ChIP assay with PET sequencing in search of global localization of transcription factors binding sites and discovered p53-binding sites throughout the human genome and identified 98 new p53 targets. Interestingly, our novel p53 targets were not identified in that study. Moreover, except for 6 known p53 targets (BAX, CDKN1A, TNFRSF10B, PCNA, XPC and DDB2) which were identified in both studies, there was no overlap between the two lists. Here too, our results attest to the power of using a combined computational and experimental approach to identify new transcriptional targets in the DNA damage response network.

p53 appears to activate conflicting response arms: DNA repair and cell cycle arrest, which help the cell cope with and survive genotoxic stress, and apoptosis, which eliminates the afflicted cell. Which arm prevails seems to be tissue-specific. Indeed in p53-null mice, some tissues show enhanced radiosensitivity (intestinal) under genotoxic stress while others are turned more radio-resistant (spleen, thymus, embryonic CNS) (Gudkov and Komarova, 2003; Komarova et al., 2004; Jackson et al., 2011). These conflicting effects of p53 activation also complicate the role of p53 in predicting cancer response to IR and chemotherapy (Bertheau et al., 2008). The dual functionality of p53 is also a major factor when considering p53 as a potential therapeutic target in cancer: its reactivation in p53-deficient tumors can improve outcome by enhancing cell death or cellular senescence (Ventura et al., 2007), but in some circumstances its inhibition (for instance, in p53-wt tumors) reduces tumor cell recovery (Gudkov and Komarova, 2003, 2005). Clearly, more research is needed in order to delineate the factors that affect the balance between p53’s life and death choices.

Studies of gene expression profiles naturally draw functional conclusions based on the protein products of the identified transcripts. It is important to use the current proteomic technology to examine the correlation between the dynamics of gene expression and the corresponding proteins. Is there a direct correlation? Furthermore, next-generation deep-seqencing methods should be used to decipher alternative splicing on a genomic scale following DNA damage induction. The integration of these technologies may yield the long-sought systems level, multi-layered understanding of the DNA damage response.

Acknowledgments

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molonc.2011.06.004.

REFERENCES


