Identification of potential novel drug resistance mechanisms by genomic and transcriptomic profiling of colon cancer cells with p53 deletion

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Abstract
TP53 (p53) is a pivotal player in tumor suppression with fifty percent of all invasive tumors displaying mutations in the TP53 gene. In the present study, we characterized colon cancer cells (HCT116 p53−/−) with TP53 deletion, a sub-line derived from HCT116-p53+/+ cells. RNA sequencing and network analyses were performed to identify novel drug resistance mechanisms. Chromosomal aberrations were identified by multicolor fluorescence in situ hybridization (mFISH) and array comparative genomic hybridization (aCGH). Numerous genes were overexpressed in HCT116 p53−/− cells: RND3/RhoE (235.6-fold up-regulated), DCLK1 (60.2-fold up-regulated), LBH (31.9-fold up-regulated), MYB (28.9-fold up-regulated), TACSTD2 (110.1-fold down-regulated), NRIP1 (81.5-fold down-regulated) and HLA-DMB (69.7-fold down-regulated) are among the identified genes with potential influence on multidrug resistance (MDR) and they are associated with cancer progression and tumorigenesis, according to previously published studies. Probably due to TP53 deletion, disturbances in DNA repair and apoptosis are leading to aberrancies in cellular and organismal organization, ultimately increasing tumorigenesis and cancer progression potential. With NFκB, PI3K and HSP70, being at the center of merged protein network, and TH1-2 pathways, being among the influenced pathways, it can be speculated that the inflammatory pathway contributes to a resistance phenotype together with cell cycle regulation and heat-shock response. HCT116-p53−/− cells have more chromosomal aberrations, gains and losses in copy numbers than HCT116-p53+/+ cells. In conclusion, numerous genomic aberrations, which might be associated with yet unknown drug resistance mechanisms, were identified. This may have important implications for future treatment strategies.

Keywords Cancer · Chromosomal aberrations · Drug resistance · Genomic instability · Loss-of-function · Transcriptomics · Tumor suppressor

Abbreviations
aCGH Array comparative genomic hybridization
mFISH Multicolor fluorescence in situ hybridization

MDR Multidrug resistance
RNA-Seq RNA sequencing

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Introduction
TP53 has been described as the guardian of the genome (Lane 1992). Upon detrimental damage caused by xenobiotic and carcinogenic substances, p53 maintains cellular integrity. Aberrations and damage in DNA are recognized by p53, leading to cell cycle arrest and DNA repair. In case of persistent damage exceeding the limit of cellular repair capability, p53 can trigger apoptosis. The mechanisms of apoptosis induced by p53 consist of transcriptional activation of FAS, KILLER/DR5, and the mitochondrial pathways (Green and Kroemer 2009). Furthermore, genes promoting
cell survival such as BCL2, IGFR, MCL-1, survivin and PIK3CA are inhibited by p53 (Oren 2003; Riley et al. 2008). P53 plays role in various downstream processes, in addition to apoptosis and growth arrest, after activation by post-translational modifications such as phosphorylation, acetylation, and methylation (Bode and Dong 2004). It functions as a transcription factor responsible for maintaining the genomic integrity by regulating cell cycle arrest, DNA repair, and/or apoptosis-related pathways. In terms of cell cycle regulation, p53 activates p21/WAF1, an inhibitor for G2/M-specific cell division control protein 2 kinase and cyclin-dependent G1 kinase, subsequently leading to G2/M and G1 checkpoint control. Failure in arresting cells at both G1 and G2/M checkpoints due to mutated p53 can lead to drug resistance (Agarwal et al. 1995; Piovesan et al. 1998).

Both DNA repair and apoptosis mechanisms are essential to maintain a healthy condition in human cells. Under normal conditions, cells with excessive DNA damage or other aberrations are eliminated by apoptosis. If this control mechanism initiated by p53 is disrupted, abnormal cell proliferation with excessive DNA damage occurs. p53 mutations are among the main reason for disrupted DNA repair and apoptosis, which may initiate tumorigenesis due to increased population of abnormal cells which are more prone to mutations and chromosomally unstable. Abnormal proliferation of those cells with accumulated DNA damage because of nonfunctional p53 also affects the subsequent generations of cells with additional mutations. Ultimately, this leads to an increased risk of carcinogenesis.

P53 is mutated in more than 50% of all human carcinomas, and colorectal cancer is among the cancer types with frequent deleterious p53 mutations (Baker et al. 1989). Most of the mutations occur in the DNA-binding domain and lead either to protein-misfolding or disruption of the DNA-binding ability (Hainaut et al. 1997). The loss of its apoptotic function is an important reason for the development of radio- and drug-resistant cancer cells (Bertheau et al. 2008; Chen et al. 2012). Moreover, tumors with p53 mutations are commonly characterized by aggravated metastasis and genomic instability (Liu et al. 2010; Muller and Vousden 2013). Additional oncogenic functions of mutant p53 include promoting invasion, migration, angiogenesis and proliferation, which can lead to enhanced drug resistance and mitogenic defects (Muller and Vousden 2013). The above functions are just a few of the plethora of multiple pathways by which mutant p53 governs cancer progression (Muller and Vousden 2013). For instance, p53 has an impact also on drug metabolism (Krais et al. 2016; Wohak et al. 2018) and cell metabolism by limiting glycolysis and facilitating mitochondrial respiration (Gomes et al. 2018; Matsuura et al. 2016).

Resistance to multiple drugs has been well studied in ATP-binding cassette (ABC) transporters, which mediate the multidrug resistance (MDR) phenotype. Multiple drug resistance is, however not restricted to ABC transporters and other MDR phenomena have also been described, including, p53, Bcl-2, the proliferation rate of tumors and others (Efferth et al. 2008; Hientz et al. 2017; Reed 1995). Micro-array analyses were previously performed for HCT116 cell line (Bhattacharjee et al. 2005; Kabir et al. 2018; Khonthun et al. 2020; Ma et al. 2017), but the application of genomics and transcriptomics methods to isogenic knockout cells allows a superior and deeper comparison between cell lines to identify novel drug resistance mechanisms.

In this study, we applied RNA sequencing, array comparative genomic hybridization (aCGH) and multicolor fluorescence in situ hybridization (mFISH) to analyze HCT116 p53+/+ colon cancer cells and its drug-resistant subline with p53 deletion, HCT116 p53−/−, to characterize genes, pathways, protein networks and chromosomal aberrations responsible for drug resistance in the HCT116 p53−/− cell line. Overall, this study shall provide a better overview of the full complexity of mechanisms and genetic alterations in colon cancer cells and their contribution to drug resistance that occurred upon p53 deletion.

### Materials and methods

#### Cell culture

HCT116 p53+/+ and its drug-resistant HCT116 p53−/− subline, which were generously provided by Dr. B. Vogelstein and H. Hermeking (Howard Hughes Medical Institute, Baltimore, MD, USA) (Bunz et al. 1998) were grown as described previously (Saeed et al. 2015). HCT116 p53−/− cells possess a significant mitotic checkpoint deficit such that they cannot respond normally to DNA-damaging agents, enter mitosis and subsequently replicate their genomes in the presence of DNA damage (Bunz et al. 1998). The drug resistance profile of HCT116 p53−/− has been studied during the past years. Compared to wild-type cells, these knockout cells reveal resistance to established anticancer drugs of diverse pharmacological classes (doxorubicin, 5-fluorouracil and 5′-deoxy-5-fluorouridine, cisplatin and oxaliplatin, etoposide, and vincristine) as well as to investigational cytotoxic compounds with activity against cancer (arsenic trioxide as PML/RARA inhibitor, nutlin-3a as p53 activator, the fluoro-ropyrimidine F10, the HDAC inhibitor entinostat and the synthetic polyaniline DENSpm) and even cytotoxic but non-cancer drugs (the antimalarial quinacrine, the anticonvulsant valproic acid and the anti-inflammatory and COX1/2-inhibitory ibuprofen (Brachtendorf et al. 2018; Bunz et al. 1999; Coker-Gurkan et al. 2015; Dawood et al. 2018; Dominjanni and Gmeiner 2018; Gunasegaran et al. 2020; Hernlund et al. 2008; Janssen et al. 2008; Kralova et al. 2009; Lin...
et al. 2004; Mohapatra et al. 2012; Sonnemann et al. 2014; Terranova-Barberio et al. 2017).

RNA sequencing

The procedure was previously described (Kadioglu et al. 2016a). Gene expressions were quantified using the FPKM (fragments per kilobase of transcript per million mapped reads) measure (Choudhri et al. 2018; Wesolowski et al. 2013). The deregulation of genes in HCT116 p53−/− cells was calculated by dividing overall FPKM values of genes in HCT116 p53−/− cells by those in HCT116 p53+/+ cells.

Pathway and network analysis

Fold change in RNA expression of ± 7 were applied for filtering (Kadioglu et al. 2016a), and then the deregulated gene list was subjected to Ingenuity Pathway Analysis (IPA) (QIAGEN Redwood City, USA, www.qiagen.com/ingenuity) to identify specific networks and pathways in HCT116 p53−/− cells.

mFISH

HCT116 p53−/− and HCT116 p53+/+ cells were cytogenetically prepared to obtain metaphase spreads according to standard procedures and analyzed using molecular cytogenetics. mFISH was performed as previously reported using human whole chromosome paints as probes (Kadioglu et al. 2016a; Liehr et al. 2009a, b; Liehr and Pellestor 2009).

aCGH

Whole genomic DNA was extracted from HCT116 p53−/− and HCT116 p53+/+ cells with QIAmp DNA mini kit (QIAGEN GmbH, Hilden, Germany). aCGH was performed as previously reported (Aust et al. 2013).

Western blotting

The protein expression levels of selected genes (i.e., ANGPT2 and catalase) were evaluated in HCT116 p53−/− and HCT116 p53+/+ cells to validate their deregulation found by RNA sequencing analysis as previously described (Kadioglu et al. 2016a). Briefly, total proteins were extracted from cells using protein extraction buffer (M-PER™ mammalian protein extraction reagent mixed with 1% Halt™ protease inhibitor cocktail, Thermo Fisher Scientific). Samples equivalent to 30 µg were loaded to 10% SDS-PAGE to be separated and then transferred to Ruti®-PVDF membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 5% BSA (Carl Roth, Karlsruhe, Germany) for 1 h and probed with the selected primary antibodies at 4 °C against ANGPT2, catalase and β-actin (for all 1:1000, Cell Signaling Technology, Frankfurt, Germany). After 24 h, the membranes were incubated with secondary antibody conjugated to HRP (1:2000, Cell Signaling Technology) for 1 h and detected with Luminata™ Classicco Western HRP substrate (Merck Millipore Darmstadt, Germany). Images were analyzed using ImageJ software (NIH, Bethesda, MD, USA).

Results

Differential gene expression profile of HCT116 p53−/− cells, downstream pathways and network analysis

Ratios of RNA-seq-derived FPKM values for the expression of each gene in HCT116 p53−/− cells were considered as fold change of gene expression in comparison to that of HCT116 p53+/+ cells. For further analysis, differential gene expression with a threshold of ± 7 was taken into account (Kadioglu et al. 2016a), which yielded 300 differentially expressed genes (Supplementary Table 1). The top 10 up- and down-regulated genes in HCT116-p53−/− cells are listed in Table 1. RND3 (+ 235.6), MCPH1 (+ 85.7) and MYB

| Gene     | Differential expression (fold change) |
|----------|---------------------------------------|
| RND3     | 235.633                               |
| MCPH1    | 85.701                                |
| DCLK1    | 60.193                                |
| ZNF772   | 37.039                                |
| ANKRD31  | 37.028                                |
| ZNF419   | 32.420                                |
| LBH      | 31.954                                |
| MYB      | 28.855                                |
| MIR4477B | 26.768                                |
| MST1     | 25.177                                |
| DPEP1    | −431.858                              |
| ICAM1    | −166.463                              |
| EHF      | −163.282                              |
| TACSTD2  | −110.073                              |
| LAMA4    | −104.721                              |
| NPM2     | −85.415                               |
| NRIP1    | −81.519                               |
| HLA-DMB  | −69.678                               |
| HCG4B    | −61.255                               |
| MIR564, TMEM42 | −58.380                           |

Table 1 Top 10 up- and down-regulated genes in HCT116 p53−/− cells compared to HCT116 p53+/+ cells.
(+ 28.9) were among the most up-regulated genes, whereas DPEP1 (− 431.9), ICAM1 (− 166.5) and NPM2 (− 85.4) were the most down-regulated genes.

In network 1, histone H4, cyclin A and NFκB possessed the highest number of nodes, CD3 and Hsp70 had the highest number of nodes in the center of network 2. “Cancer”, “organisinal injury and abnormalities” in network 1, whereas “cellular assembly and organization” and “molecular transport” in network 2 were the affected biological functions. Erk1/2 showed the highest number of nodes in network 3. “Organisinal injury and abnormalities” and “carbohydrate metabolism” were the affected biological functions in network 3 (Fig. 1).

Several genes known to be involved in drug resistance were deregulated, implying that HCT116 p53−/− cells exerted a multi-factorial resistance phenotype. If a fold change threshold of ±7.0 was applied, one DNA repair gene, one oxidative stress gene, and one transcription factor gene were among the deregulated resistance genes implying that genes from those gene classes may have an important influence on the MDR phenotype of HCT116 p53−/−. These genes are depicted in Table 2 and a full list of all deregulated genes involved in resistance mechanisms is provided in Supplementary Table 2.

The top three networks were merged and the merged network was further analyzed. As can be seen from Fig. 2, NFκB resided in the center of the merged network together with PI3K and HSP70.

“Cancer”, “organisinal injury and abnormalities” and “cell to cell signaling and interaction” were among the most affected biological functions in HCT116 p53−/− cells (Fig. 3). Genes residing at the top 10 biological function list are shown in Supplementary Table 3.

“Th1 pathway” (p value: 0.000437), “IL4 signaling” (p value: 0.012589) were among the most significant signaling pathways in HCT116 p53−/− cells implying the possible immune response pathways influence on drug resistance (Fig. 4).

Among the 116 p53 target genes (Fischer 2017), 33 were down-regulated and 19 were up-regulated (fold changes were above a threshold of ±1.5) as can be seen in Table 3.

A validation of the selected genes was performed at the protein level for ANGPT2 and catalase. As shown in Fig. 5, ANGPT2 was up-regulated (+ 8.7-fold), whereas catalase was down-regulated (− 1.9-fold) in HCT116 p53−/− cells, correlating with the RNA sequencing output and validating the RNA expression data at the protein level.

**mFISH**

HCT116-p53+/+ cells showed the karyotype 45 < 2n >, X, dup(10)(q?q?), der(16)t(8;16)(p13;q?), der(18)t(17;18) (?p11.2), whereas HCT116-p53−/− cells had 45 < 2n > X, t(5;7)(q1?3;p22), dup(10)(q?q?), der(16)t(8;16)(p13;?), der(18)t(17;18) (?p11.2). The results of the mFISH analyses are depicted in Fig. 6. HCT116-p53−/− cells had a clonal de novo balanced translocation t(5;7)(q1?3;p22) compared to HCT116-p53+/+ cells.

**aCGH**

HCT116 p53+/+: Chromosomal amplification and deletions were well reflected in the deregulation of gene expression as observed in RNA Seq analysis. LVRN was 3.3-fold down-regulated and AP3S1 was 1.7-fold up-regulated. The corresponding chromosomal locus (5q23.1) was amplified. FLJ42393 was 1.7-fold down-regulated. There was a deletion at the corresponding chromosomal locus (3q27.3–q28).

The results are summarized in Table 4. HCT116 p53−/−: Compared to HCT116 p53+/+ cells, more amplifications and deletions were observed in HCT116-p53−/− cells. This implies that p53 deletion led to an accumulation of additional chromosomal aberrations, amplifications and deletions. NXPH2 was 1.7-fold up-regulated, and there was an amplification at the corresponding chromosomal locus. OR5K2 was 1.7-fold up-regulated, and there was an amplification at the corresponding chromosomal locus. PARK2 was 8.1-fold down-regulated, and there was a deletion at the corresponding chromosomal locus. Correlation of aCGH data with RNA-Seq results clearly showed a differential expression of genes at the corresponding chromosomal locus amplification/deletion, as can be seen in Table 4. Overall aCGH results are depicted in Table 5.

**Discussion**

In the present study, we aimed to identify novel drug resistance genes by using colon cancer cell line, HCT116 p53+/+ and the drug-resistant HCT116 p53−/− subline with TP53 deletion as a model. The gene expression profiles, affected signaling pathways, biological functions and chromosomal abnormalities were identified by RNA sequencing, mFISH and aCGH.

Several genes known to be involved in drug resistance were deregulated supporting a multi-factorial resistance phenotype in HCT116 p53−/− cells, those identified genes in various drug resistance clusters including apoptosis, DNA repair, ferroptosis, glutathione related, heat shock, oxidative stress, transcription factors as listed in Supplementary Table 2 may have an important influence on the MDR phenotype.

The most up-regulated gene RND3/RhoE (+ 235.6-fold) was previously associated with tumor invasion, metastasis and was reported as a potential marker of drug resistance of
Fig. 1 Affected protein networks in HCT116 p53−/− cells in comparison to HCT116 p53+/+ cells. Genes that are labelled in green were down-regulated, and genes that are labelled in red were up-regulated. The top three networks were depicted. a Network 1 b Network 2 c Network 3

**Affected functions:**
- Cancer
- Organismal injury and abnormalities

**Affected functions:**
- Cellular assembly and organization
- Molecular transport
gastric cancer as well as relapse and prognosis for colorectal cancer cases (Chang et al. 2014; Li et al. 2009; Zhou et al. 2013).

CARD11 mutations have been associated with ibrutinib (Bartlett et al. 2018).

CARD11 (−7.4-fold) appeared in the list of genes in the top five biological functions (Supplementary Table 3), indicating that apoptosis inhibition upon down-regulation of CARD11 might play an important role in the drug resistance phenotype of HCT116 p53−/− cells.

It was reported that CARD11 contributes to ibrutinib resistance in cancer (Grommes et al. 2017; Wu et al. 2016) supporting our observation that CARD11 could play role in drug resistance phenotype of HCT116 p53−/− cells. DCLK1 (+60.2-fold) has been reported to be associated with chemoresistance to cisplatin in non-small cell lung cancer cells and targeting DCLK1 by miR539 led to increased sensitivity to cisplatin (Deng et al. 2018). DCLK1 has also been associated with drug resistance in colorectal cancer, pancreatic cancer, and kidney cancer (Ge et al. 2018; Makino et al. 2020; Qu et al. 2019).

DCLK1 (+31.9-fold) has been reported as a potential marker for hepatocellular carcinoma, as its overexpression was associated with poor prognosis (Chen et al. 2018).

Myb (+28.9-fold expression in knockout cells) is an oncogenic transcription factor playing a role in the promotion of leukemic cell transformation (Introna and Golay 1999). Myb was linked to cisplatin resistance in colon cancer cells (Funato et al. 2001). It is also involved in the development and progression of several solid tumors, including melanoma (Ramsay and Gonda 2008; Schultz et al. 2009).

Loss of TACSTD2 promoted squamous cell carcinoma progression and resistance through attenuating chemotherapeutic reagent-induced apoptosis, implying that TACSTD2 could be used as a marker for pathological grading of SCC (Wang et al. 2013).
et al. 2014). Interestingly, it was 110.1-fold down-regulated in HCT116 p53−/− cells, pointing out that TACSTD2 down-regulation could be a mechanism contributing to aggressive growth and MDR of HCT116 p53−/− cells. Migration and invasion of esophageal squamous cell carcinoma cells were enhanced upon NRIP1 down-regulation (Ni et al. 2018). We observed that NRIP1 was 81.5-fold down-regulated in HCT116 p53−/− cells, implying that NRIP1 down-regulation could play a role in the MDR phenotype. HLA-DMB (− 69.7-fold) belongs to the major histocompatibility complex class II genes, and higher HLA-DMB expression was associated with higher survival rate via increased CD8 lymphocyte numbers in advanced-stage serous ovarian cancer (Callahan et al. 2008). Down-regulation of HLA-DMB may be linked with the MDR phenotype of HCT116 p53−/− cells by influencing tumor aggressiveness.

The NCF2 gene (+ 15.1-fold expression in knockout cells) encodes a subunit of NOX2. Depletion of NOX2 subunits reduced the formation of lung metastases following intravenous injection of murine tumor cells (Martner et al. 2019).
Up-regulation of NCF2 promoted gastric cancer metastasis by LINC1410-miR-532-5p-NCF2-NF-κB feedback loop activation (Zhang et al. 2018).

Overexpression of the MYB transcription factor (+28.8-fold expression in knockout cells) has been associated with poor prognosis and was frequently observed in colorectal cancer (CRC) (Cross et al. 2015). Another study pointed out that MYB expression in tumor cells due to its tumorigenic role modulated the host immune response, which has the potential to influence the use of immunotherapy in CRC patients (Millen et al. 2016). MYC (+5.1-fold) is another transcription factor with a critical role in tumorigenesis. It regulates the expression of cell cycle related genes, and overexpression was observed in various cancer types, including colon cancer (Kadioglu et al. 2016b; Pelengaris et al. 2002). We have identified IL4 signaling among the most affected signaling pathways in HCT116 p53−/− cells implying that immune response pathways possibly influence drug resistance. One study stated that IL-4 can augment BCR-signalling and reduce the effectiveness of BCR-kinase inhibitors such as ibrutinib in CLL cells (Blunt et al. 2017).

Another study reported that innate immune pathway activation via the interleukin-1 receptor-associated kinase 1 and 4 (IRAK1/4) complex contributes to adaptive resistance in FLT3-mutant AML cells (Melgar et al. 2019). This result supports our observation about the association of immune response pathways with drug resistance.

The validation of RNA-seq results was performed for ANGPT2 and catalase by Western blotting. ANGPT2 mRNA was up-regulated, whereas CAT mRNA was down-regulated in HCT116 p53−/− cells compared to HCT116 p53+/+ cells. This was confirmed for protein expression. CAT is frequently down-regulated in tumors (Glorieux et al. 2014), e.g. Breast cancer was characterized by down-regulation of catalase and concomitant overexpression of SOD (Wang et al. 2017). On the other hand, upregulation of ANGPT2 was associated with liver metastasis in colon cancer (Urosevic et al. 2020).

Network analysis pointed out “cancer”, “organismal injury and abnormalities” for network 1, “cellular assembly and organization”, “molecular transport” for network 2, “organismal injury and abnormalities”, “carbohydrate metabolism” for network 3 as major affected biological functions.
functions. Due to TP53 deletion, disruption in the DNA repair and apoptosis mechanisms were probably leading to aberrancies in cellular and organismal organization, ultimately increasing tumorigenic and cancer progressive potential. In network 1, the genes encoding histone H4, cyclin A and NFκB possessed the highest number of nodes. CD3 and HSP70 had the highest number of nodes in the center of network 2, implying an influence of cell cycle regulation,

| Gene    | Differential expression (fold change) |
|---------|---------------------------------------|
| SPATA18 | 4.207                                 |
| CSF1    | 4.139                                 |
| TSPAN11 | 3.365                                 |
| TP53I3  | 3.098                                 |
| PTP4A1  | 2.352                                 |
| ABCA12  | 2.058                                 |
| ZNF79   | 2.032                                 |
| DUSP14  | 2.026                                 |
| FAM210B | 1.975                                 |
| RPS27L  | 1.969                                 |
| GDF15   | 1.856                                 |
| NADSYN1 | 1.692                                 |
| FAS     | 1.639                                 |
| FAM212B | 1.611                                 |
| ATF3    | 1.599                                 |
| HSPA4L  | 1.579                                 |
| TRIAP1  | 1.549                                 |
| AEN     | 1.547                                 |
| MDM2    | 1.513                                 |

| Gene    | Differential expression (fold change) |
|---------|---------------------------------------|
| TNFRSF10D | – 2.205                        |
| TNFRSF10B | – 2.058                        |
| KITLG    | – 2.047                         |
| PGF      | – 2.047                         |
| BAX      | – 2.018                         |
| ACER2    | – 2.017                         |
| CCNG1    | – 1.874                         |
| GPR87    | – 1.873                         |
| XPC      | – 1.867                         |
| SERTAD1  | – 1.863                         |
| ARHGEF3  | – 1.834                         |
| SYTL1    | – 1.804                         |
| CD82     | – 1.789                         |
| FDXR     | – 1.756                         |
| EPS8L2   | – 1.718                         |
| SESN1    | – 1.706                         |
| SESN2    | – 1.654                         |
| APOBEC3C | – 1.648                         |
| TP53INP1 | – 1.609                         |
| Dyrk3    | – 1.586                         |
| ANKRA2   | – 1.581                         |
| ORAI3    | – 1.558                         |
| CES2     | – 1.535                         |

| Gene    | Differential expression (fold change) |
|---------|---------------------------------------|
| GRHL3   | – 7.153                              |
| PRDM1   | – 5.224                              |
| PADI4   | – 5.164                              |
| CPE     | – 4.718                              |
| FAM198B | – 3.568                              |
| WRN63   | – 3.419                              |
| FUC1    | – 2.999                              |
| CDIP1   | – 2.582                              |
| ASTN2   | – 2.441                              |
| SULF2   | – 2.315                              |
inflammation and heat-shock response for drug resistance. Importantly, the appearance of molecular transport genes in network 2 highlighted a possible cross-talk between p53 and cellular transporters to promote the MDR in cancer cells.

A member of ABC transporters, the \textit{ABCB1/MDR1} gene, is transcriptionally dependent on p53, where wild type p53 negatively affects \textit{ABCB1/MDR1} gene expression through sequence-specific binding to the downstream promoter (Strauss et al. 1995). On the contrary, mutant p53 activated \textit{ABCB1/MDR1} promoter in different cell lines (Nguyen et al. 1994; Sampath et al. 2001). The \textit{ERK1/2} gene had the highest number of nodes in network 3, pointing out a contribution of ERK-regulated cell proliferation pathway to drug resistance. NF\textit{k}B resided in the center of the merged network together with PI3K and HSP70, implying a contribution of inflammatory pathways together with cell cycle and heat shock response phenomena in the MDR phenotype. Th1, Th2 pathways and CD28 signaling were among the most affected signaling pathways in HCT116-p53 \textit{\textasciitilde\textasciitilde} cells supporting the hypothesis that inflammatory pathways play an important role in the MDR phenotype.

**Fig. 4** Affected signalling pathways (Top 10) in HCT116 \textit{p53 \textasciitilde\textasciitilde} cells in comparison to HCT116 \textit{p53 ++} cells. The orange line depicts the statistical significance threshold (\(p=0.05\)) and the orange chart depicts the ratio of deregulated genes in each pathway.

**Fig. 5** Protein expression of ANGPT2 and catalase in HCT116 \textit{p53 \textasciitilde\textasciitilde} and HCT116 \textit{p53 ++} cells as determined by Western blotting.
Fig. 6  mFISH analysis of HCT116 p53 \(^{+/+}\) (a) and HCT116 p53 \(^{-/-}\) (b) cells

| Chromosomal locus | Cytoband | # Probes | Amp/Del  | Gene       |
|-------------------|----------|----------|----------|------------|
| **HCT116 p53 \(^{+/+}\)** | chr3:187898258-188080406 | q27.3–q28 | 15 | − 1.088643 | FLJ42393 |
|                   | chr5:115220574-115433864 | q23.1 | 19 | 0.672502 | AP3S1     |
|                   | chr2:139060642-139586488 | q22.1 | 30 | 0.576345 | NXPH2     |
|                   | chr3:98146718-98600450 | q11.2–q12.1 | 33 | 0.480192 | OR5K2     |
|                   | chr6:162343673-162707662 | q26   | 32 | − 0.797749 | PARK2     |
| **HCT116 p53 \(^{-/-}\)** | chr2:139060642-139586488 | q22.1 | 30 | 0.576345 | NXPH2     |
|                   | chr3:98146718-98600450 | q11.2–q12.1 | 33 | 0.480192 | OR5K2     |
|                   | chr6:162343673-162707662 | q26   | 32 | − 0.797749 | PARK2     |

Table 4  Chromosomal aberrations and corresponding deregulated genes. Comparison between aCGH and RNA sequencing profiles.
Table 5 Overall aCGH results

| Chromosome | Cytoband | #Probes | Amp/Del | P value | Annotations |
|------------|----------|---------|---------|---------|-------------|
| HCT116 p53 | chr3:114336335-114441291 | q13.31 10 | 0.930778 | 1.43E-11 | **ZBTB20, CNV_98410** |
| HCT116 p53 | chr3:187898258-188080406 | q27.3–q28 15 | – 1.088643 | 7.50E-22 | **LPP, FLJ42393, CNV_8438...** |
| HCT116 p53 | chr4:169510103-169718417 | q32.3 18 | – 1.000585 | 3.99E-22 | **PALLD, CNV_68838, CNV_6350...** |
| HCT116 p53 | chr5:115220574-115433864 | q23.1 19 | 0.672502 | 1.72E-11 | **AP3S1, LVRN, COMMD10** |
| HCT116 p53 | chr6:76606357-67674714 | q12 7 | 1.187426 | 6.11E-13 | **OPHN1** |
| HCT116 p53 | chr7:6,689,115-6,823,761 | p11.2 12 | – 4.324700 | 1.07E-10 | **AMELY, TBL1Y** |
| chr2:139060642-139586488 | q22.1 30 | 0.576345 | 4.63E-14 | **SPOPL, NXPH2, CNV_6001...** |
| chr3:98146718-98600450 | q33 33 | 0.480192 | 3.77E-11 | **OR5K1, OR5K2, CLDND1...** |
| chr3:116781727-117480699 | q13.31–q13.32 18 | – 0.840402 | 1.93E-17 | **chrX:67606357-67674714** |
| chr3:1188,915,318–189,233,666 | q28 16 | 0.678746 | 7.43E-11 | **TPRG1, CNV_6224, CNV_36118...** |
| chr6:162343673-162707662 | q26 32 | – 0.797749 | 8.35E-27 | **PARK2, CNV_3649, CNV_8532...** |
| chr12:19212202-19371177 | p12.3 13 | – 0.764834 | 3.82E-11 | **PLEKH5, CNV_113275, CNV_5296...** |
| chr14:105957346-107258824 | q32.33 85 | 0.333574 | 2.22E-13 | **C1orf80, TMEM121, KIAA0125...** |
| chr15:94197624-94591102 | q26.1–q26.2 23 | 0.741784 | 2.28E-17 | **chrX:29398858-29528742** |
| chr16:78,328,380-78,670,327 | q23.1 24 | – 1.124667 | 1.46E-37 | **WWOX, CNV_4014, CNV_3128...** |
| chr20:14928568-15317311 | p12.1 33 | – 0.608444 | 7.87E-17 | **MACROD2, CNV_9315, CNV_30119...** |
| chrX:29398858-29528742 | p21.2 11 | 0.881627 | 2.58E-12 | **ILIRAPL1, CNV_3265** |
| chrX:96427803-96727959 | q21.33 25 | – 3.569660 | 3.65E-319 | **DIAPH2, CNV_68008** |

Observed in both cell lines

| Chromosome | Cytoband | Annotations | Chromosome | Cytoband | Annotations |
|------------|----------|-------------|------------|----------|-------------|
| chr1:192899886-193202164 | q31.2 | **UCHL5, TROVE2, GLRX2** | chr16:78949665-90163114 | q23.1—q24.3 | **WWOX, MAF, DYNLRB2** |
| chr2:89427365-90242018 | p11.2 | **CNV_34427, CNV_35873, CNV_107994** | chr17:34450405-34475514 | q12 | **CNV_4031, CNV_8842, CNV_34507** |
| chr2:141719777-142150170 | q22.1 | **LRP1B, CNV_98004, CNV_9962** | chr17:43457048-81093254 | q21.31—q25.3 | **ARHGAP27, SH3D20, PLEKHM1** |
| chr2:20547915-205794583 | q33.3 | **PARD3B, CNV_63286, CNV_3405** | chr19:20644642-20984793 | p12 | **ZNF737, ZNF626, CNV_4070...** |
| chr3:60193640-60230828 | p14.2 | **FHTI, CNV_8983, CNV_51130** | chr22:24347959-24390254 | q11.23 | **LOC391322, GSTT1, GSTTP2** |
| chr4:19271201-19814969 | p15.31 | **CNV_98645, CNV_91960, CNV_8998** | chrX:550458-2687250 | p22.33 | **SHOX, CRLF2, CSF2RA** |
Conclusions

In conclusion, the gene expression profiles of HCT116 p53−/− and HCT116 p53+/+ colon cancer cell lines were analyzed by RNA sequencing, mFISH and aCGH, to identify differentially expressed genes, affected protein networks, pathways, biological functions in addition to chromosomal aberrations in a comparative manner. Various genes, pathways and networks were identified that might be associated with drug resistance and aggressive behavior of colon cancer. This study clearly demonstrates that drug resistance in TP53-knockout cells is rather determined by multiple than by single factors. It is apparent that multi-factorial drug resistance complicates the development of novel treatment strategies. Nevertheless, our study may represent a starting point to design more specific and promising anti-cancer strategies bypassing drug resistance.

Author contributions OK: formal analysis; OK and MS: investigation; OK, MS, NM, SA, KM and TL: methodology; TE: supervision; OK: writing—original draft; MS, TL and TE: writing—review and editing.

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Data availability All data generated or analysed during this study are included in this published article.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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