We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

12

Portrait of Transcriptional Expression Profiles Displayed by Different Glioblastoma Cell Lines

Paulo R. D. V. Godoy et al.[1]
University of São Paulo, Brazil

1. Introduction

Glioblastoma multiforme (GBM) is among the most lethal of all human tumors, with the average survival of approximately 1 year from diagnosis (Avgeropoulos and Batchelor, 1999). In glioblastomas, LOH 10q is the most frequent genetic alteration (69%), followed by *EGFR* amplification (34%), *TP53* mutations (31%), *p16INK4a* deletion (31%), and *PTEN* mutations (24%)(Ohgaki and Kleihues, 2005).

Treatments of patients with GBM include surgery, radiotherapy and parallel adjuvant chemotherapy (Stupp et al., 2005). While radiotherapy has been found to significantly prolong survival rates for GBM patients, a poor prognosis and radioresistance are typical characteristics of this disease (Stupp et al., 2007).

The antitumoral drug Temozolomide (TMZ) constitutes, in combination with radiotherapy, the current standard of care for glioblastoma (Stupp et al., 2005). However, the action of TMZ may be counteracted in tumors by the expression of the DNA repair enzyme MGMT, which repairs TMZ-induced DNA lesions (Hegi et al., 2005). MGMT activity and resistance to TMZ were highly correlated, indicating that MGMT is a major predictor of response to TMZ in glioma cells (Hermisson et al., 2006). According to the authors, collectively, MGMT expression and *TP53* status may become valuable parameters to predict cell responses to TMZ treatment in patients with GBM. Modulation of *MSH6*, *PARP1* and *NTL1*, DNA repair genes involved in mismatch repair and base excision repair (BER), were found at transcription and protein levels in GBM cells resistant to TMZ (Zhang et al., 2010).

Other genotoxic agents exert different effects in GBM cells with different *TP53* status. UV light (UV-C) (Batista et al., 2009) and chloroethylating agents (ACNU and BCNU) (Batista et al., 2007), significantly induce apoptosis in *TP53*-mutated glioma cells, while WT *TP53* cells are more sensitive to methylating agents, including TMZ (Hermisson et al., 2006; Roos et al., 2007).

Efforts have been made to overcome drug and radio-resistance of GBM cells, but the heterogeneity of these tumors seems to be critical, and molecular analysis is an important tool to elucidate the mechanisms underlying cellular responses to antitumoral agents. Recently, application of genome-scale methodologies has opened the opportunity to study

[1] Stephano S. Mello, Daniele A. Magalhães, Flavia S. Donaires, Ana P. Lima-Montaldi, Patricia Nicolucci, Eduardo A. Donadi, Geraldo A. S. Passos and Elza T. Sakamoto-Hojo

transcript profiles for thousands of genes simultaneously, thus providing a picture on how different biological processes can be modulated under irradiation, drug treatment or even between different cell types, on the basis of the lists of differentially expressed genes (either induced or repressed genes) provided by the microarray experiments. This approach allows comparisons between different biological situations (Tusher et al., 2001; Sakamoto-Hojo et al., 2003; Fachin et al., 2007; Fachin et al., 2009). In a previous work, several stress response/DNA repair genes, such as *HSPA9B*, *INPP5A*, *PIP5K1A*, *FANCG*, and *TPP2* were found up-regulated in U343MG-a GBM cells analyzed at 6 h following irradiation with 1 Gy, reflecting the radio-resistance of these cells; at this condition, the survival rate was 61%, and a broad spectrum of other biological processes was found associated to the list of differentially expressed genes in irradiated cells (Bassi et al., 2008).

TP53 gene plays a role in drug and radioresistance mechanisms, but the complex network of signaling pathways involving this gene is not well elucidated. TP53 is a multifunctional protein that acts in cell cycle blockage and signaling pathways towards DNA repair, contributing to the maintenance of genome integrity in response to a variety of genotoxic stresses (Bartussek et al., 1999). Alternatively, TP53 protein triggers a cascade of signaling pathways culminating in apoptosis, depending on the extent of DNA damage (Prise et al., 2005). The TP53 protein is also a transcription factor that regulates the expression of a large number of target genes (Vogelstein et al., 2000). Many TP53 target genes have been described (el-Deiry, 1998; Horn and Vousden, 2007; Laptenko and Prives, 2006; Sbisa et al., 2007), and some other targets have been computationally predicted by the analysis of their binding sites (Hoh et al., 2002; Smeenk et al., 2008; Veprintsev and Fersht, 2008).

According to the literature data, there are controversial findings about the outcome of patients in relation to the *TP53* status of tumors; this gene may positive or negatively influences the cell radioresistance, as well as it can exert no influence in cellular responses to therapies (McIlwrath et al., 1994; Slichenmyer et al., 1993; Smith et al., 1995).

2. Objective

In the present work, we aim to compare gene expression profiles displayed by four GBM cell lines in the absence of any kind of treatment, using the microarray method, looking for molecular signatures that can provide new clues towards the understanding of GBM biology and radioresistance mechanisms. The results on the transcriptional profiles presented by a number of genes with different biological functions are discussed on the light of literature data regarding GBM cell responses to ionizing radiation and antitumor drugs, generally provided by survival assays.

3. Materials and methods

3.1 GBM cell lines

Human GBM U343 MG-a cell line was kindly donated by Dr. James T. Rutka (The Arthur and Sonia Labatt Brain Tumour Research Center, Canada), while T98G, U251MG, and U87MG were supplied by the American Type Culture Collection (ATCC) (Rockville, Maryland, USA). T98G and U251MG cell lines harbor three mutations (*TP53, CDKN2A*, and *PTEN*) while U343MG-a and U87MG cells are wild-type (WT) for *TP53*, and mutant (MT) for *CDKN2A* and *PTEN* genes (Ishii et al., 1999).

3.2 Cell culture and total RNA extraction

For gene expression analysis, cells were thawed from the nitrogen and sub-cultured for four passages in the presence of HAM F10 + DEM medium plus 15% fetal bovine serum and kept at 37°C and 5% CO_2. After the fourth sub-culturing, they were incubated for two days, and total RNA extraction was performed in replicate at 48 h, by using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The quality of RNA samples was evaluated by denaturing agarose gel electrophoresis under standard conditions. To remove the contaminating DNA, RNA samples used in cDNA microarrays were treated with the Deoxyribonuclease I (Amplification Grade kit, Invitrogen), according to manufacturer's instructions.

3.3 cDNA microarray method

Four experiments in duplicate using GBM cells were carried out using a glass slide microarray containing 4500 clones of cDNA probe (in duplicates) from the human IMAGE Consortium cDNA library [http://image.llnl.gov/image/; kindly provided by Dr. Catherine Nguyen (INSERM-CNRS, Marseille, France)], and prepared according to the protocol described by Hegde et al (2000).

Microarrays were spotted onto glass slides (Corning) using a Generation III Array Spotter Amersham-Molecular Dynamics according to the manufacturer's instructions. Each cDNA sample was spotted twice on the slide (duplicate spots). The cDNA complex probes were prepared using the CyScribe Post Labelling Kit (Amersham Biosciences, England) (Fachin et al., 2009). Hybridizations were carried out using an automatic system (Automatic Slide Processor, Amersham Biosciences, England) and signals were immediately captured after the final wash procedure, using a Generation III laser scanner (Amersham Biosciences, England).

In an attempt to characterize the clones present in the array slide, the gene set was submitted to the NIH-DAVID bioinformatic tool, in order to obtain biological functions associated with the gene sequences present in the arrays, as well as the number of genes associated with each biological process. Among all clones, 2334 were identified by official gene symbol (HUGO), distributed in classes according to biological processes (Table 1).

3.3.1 Data acquisition and gene expression analysis

The image quantification was performed using the Spot software, (http://spot.cmis.csiro.au/spot/, CSIRO, Australia). Filtering, normalization and data analysis were done using the R statistical environment (Ihaka and Gentleman, 1996), in addition to Limma (Smyth et al., 2005), Bioconductor (Ihaka and Gentleman, 1996), Aroma (Bengtsson, 2004) and KTH (Wirta, 2004). The background to each feature was subtracted from the foreground value. Furthermore, the spots were evaluated by their circularity and calculations on the median versus mean deviation, so that those presenting irregular circularity, or with large differences between mean and median values, were considered unreliable. The raw data (red – R and green – G) was transformed into MA format before normalization, where $M = \log2(R/G)$ and $A = 1/2 \times \log2(R \times G)$. These procedures were followed by the application of the Print-tip Lowess normalization for each slide. Following the normalization procedure, microarray data were exported to tab-delimited tables in MEV format and analyzed in MEV (v. 3.1) software (Saeed et al., 2003). The microarrays data analysis involved the application of the statistical method SAM - Significance Analysis of

GOTERM-BIOLOGICAL PROCESS - FAT	%	PValue
GO:0007242~intracellular signaling cascade	10.28	3.90E-09
GO:0006793~phosphorus metabolic process	9.43	8.50E-16
GO:0006468~protein amino acid phosphorylation	6.73	6.81E-13
GO:0008104~protein localization	6.68	2.00E-04
GO:0042981~regulation of apoptosis	6.47	1.20E-05
GO:0007049~cell cycle	5.96	2.63E-04
GO:0033554~cellular response to stress	5.78	1.27E-11
GO:0006259~DNA metabolic process	4.50	4.42E-06
GO:0006974~Response to DNA damage stimulus	4.07	5.32E-10
GO:0006281~DNA repair	3.43	8.95E-11
GO:0007243~protein kinase cascade	3.43	1.53E-05
GO:0007167~enzyme linked receptor protein signaling pathway	3.08	1.04E-04
GO:0009314~response to radiation	1.93	5.82E-04
GO:0000165~MAPKKK cascade	1.89	1.69E-04
GO:0022604~regulation of cell morphogenesis	1.41	4.85E-04
GO:0006310~DNA recombination	1.29	9.19E-05
GO:0031344~regulation of cell projection organization	1.11	2.05E-04
GO:0006302~double-strand break repair	0.94	3.35E-05
GO:0006289~nucleotide-excision repair	0.86	5.76E-05
GO:0050770~regulation of axonogenesis	0.81	3.26E-04
GO:0051291~protein heterooligomerization	0.77	2.99E-04
GO:0050772~positive regulation of axonogenesis	0.51	1.85E-04
GO:0000723~telomere maintenance	0.51	6.05E-04
GO:0032925~regulation of activin receptor signaling pathway	0.30	7.62E-04

Table 1. Percentage of genes for each biological process analyzed for a total of 2334 genes present in a glass slide microarray. The array gene set (containing 4300 image clones) was submitted to NIH-DAVID (Dennis et al. 2003).

Microarray (Tusher et al., 2001), with the objective to compare MT versus WT *TP53* cell lines in terms of expression profiles. We used FDR< 0.68% to select only highly significant differentially expressed genes.

Information regarding biological functions were obtained at S.O.U.R.C.E. (http://genome-www5.stanford.edu/cgi-bin/SMD/source/source), and NCBI (http://www.ncbi.nlm.nih.gov/). Gene functional groups were given by DAVID tool (Dunne et al., 2003),

choosing *Homo sapiens* as the current background, Gene Ontology: GOTERM_BP_FAT as parameters, and Functional Annotation Chart as the analyzing tool. The main biological functions associated to the list of differentially expressed genes were selected.
Every modulated gene was compared to a list of previously identified genes with TP53 binding sites using genome-wide tiling Chromatin immunoprecipitation (ChIP)-on-chip approach (Smeenk et al., 2008) or (ChIP) with the paired-end ditag (PET) (Wei et al., 2006) in order to point out genes that could be modulated by TP53 transactivation.

4. Results

4.1 Transcript profiles displayed by GBM cell lines

4.1.1 Hierarchical cluster analysis

Gene expression profiles studied by the cDNA microarray method generated interesting results about the transcriptional profiles exhibited by each cell line. Analysis of gene cluster uses standard statistical algorithms in order to arrange genes according to similarity of expression patterns, and the results can be graphically represented (Eisen et al., 1998). The analysis of hierarchical clustering was performed to compare MT and WT *TP53* cells regarding transcript profiles by using a set of genes previously selected by the SAM analysis. The results of the hierarchical clustering showed that MT *TP53* cells were grouped apart from the WT *TP53* cells. The mutant cell lines (T98G and U251) were not separated within a gene cluster, indicating more similarity in the basal transcription levels between cell lines; in contrast, the proficient cell lines (U87 and U343) were clustered apart from each other (**Fig.1**).

4.1.2 Differentially expressed genes indicated by Significant Analysis of Microarrays (SAM)

The statistical analysis performed by SAM indicated that MT *TP53* cell lines showed 29 down-regulated and 68 up-regulated genes, compared with WT *TP53* cells, for FDR ≤ 0.68%. For this small list of highly significant differentially expressed genes, the magnitude of fold-changes ranged from –1.68 to +1.93 by comparing MT *versus* WT *TP53 (*Supp. Table 1*)*.
For the list of differentially expressed genes, biological gene functions were studied by the DAVID-NIH bioinformatic tool (Dennis et al., 2003; Huang da et al., 2009). Out of 97 modulated genes, 73 were suitable for functional grouping procedure, since 10 genes were not grouped and 14 I.D. clones were still unknown. The most relevant categories (represented by a variable number of genes) were related to neurological system process (11%), regulation of apoptosis (10%), cellular response to stress (8%), regulation of cell proliferation (8%), cell-cell adhesion (5%), DNA repair (5%), response to ionizing radiation (4%), histone modification (4%), cell division (4%), etc. (**Fig.2.)**. For discussion, we selected 36 genes on the basis of biological functions that can be possibly related to responses to genotoxic agents (**Table-2**), in order to find out clues for understanding the mechanisms underlying the sensitivity or resistance to anticancer therapies. Some of candidate genes that may participate in chemo- and/or radioresistance are involved in stress responses (*RUVBL2*, *ASNS*, *RNF8*, *LIG4* and *CAV1*) and cell adhesion (*CDH8*, *CDH13*, *CD93* and *ITGA5*), and other important cathegories, such as regulation of cell proliferation (*IGF1R*, *CDH13*, *CAV1, DUSP22, ADAMTS1, LIG4*) and apoptosis (*IGF1R, CDH13, IFT57, CRH*, *HSPB1, ASNS* and *LIG4*.

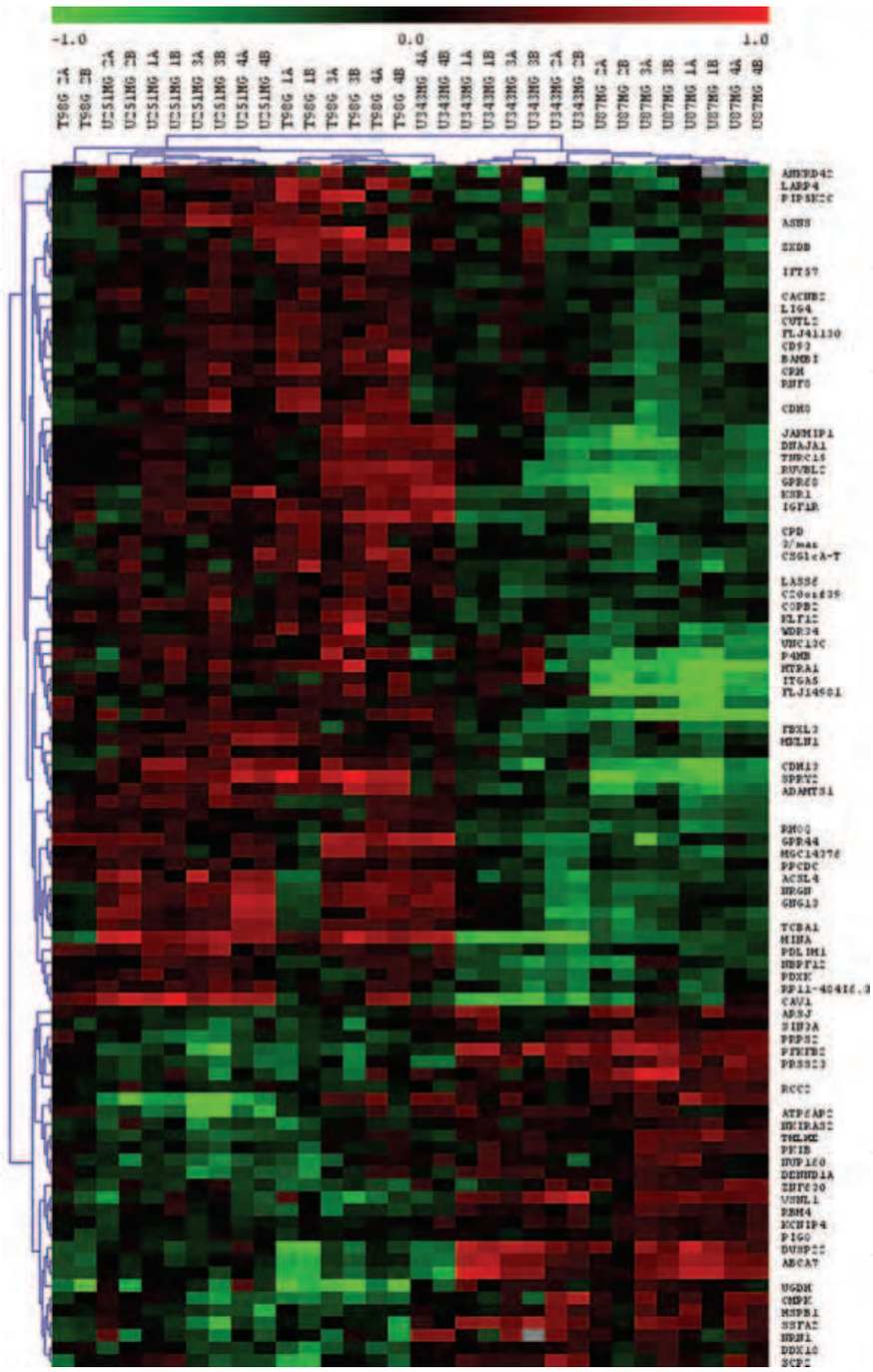


Fig. 1. Hierarchical clustering obtained for a gene set previously selected by the SAM method (FDR≤0.68%) for the results displayed by four GBM cell lines (T98G, U251MG, U343MG-a e U87MG), under normal proliferation in culture. The data set was provided by 4 experiments in duplicate. Experiments are represented by numbers (1-4) and the replicates are represented by letters (A and B). The expression level of each gene is represented according to the scale at the top (red indicates induction; green means repression; grey color represents data loss).

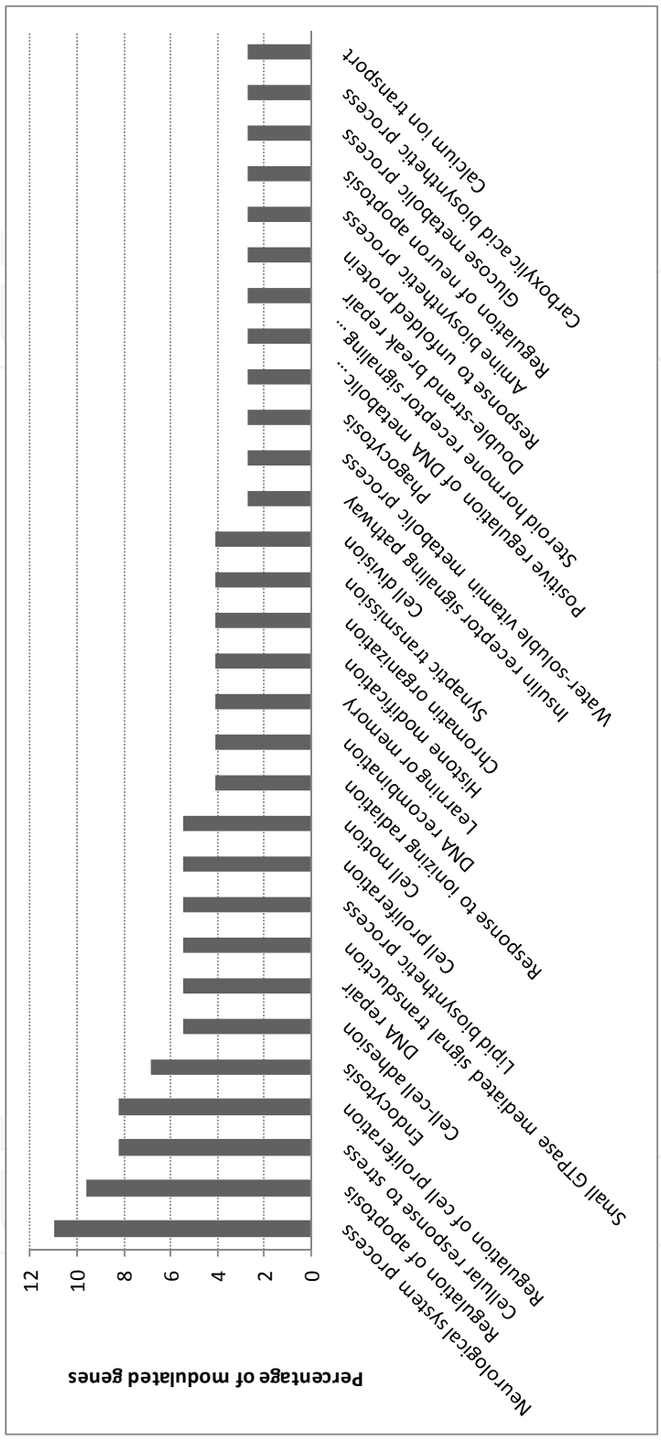


Fig. 2. Main Biological functions associated to the list of differentially expressed genes (out of 97 genes, 73 genes were recognized by the system) when comparing MT and WT *TP53* GBM cell lines. The results were analyzed by DAVID-NIH (Dennis et al., 2003; Huang da et al., 2009) as a bioinformatic tool to group differentially expressed genes according to their functions.

A comparative analysis of differentially expressed genes (obtained by SAM) was carried out with previously identified genes with *TP53* binding sites using ChIP-PET and ChIP-on-chip analysis (Smeenk et al., 2008; Wei et al., 2006). When comparing with ChIP- PET gene lists, we observed only one common gene (*LASS6*), while the comparison involving the gene list of our work with the ChIP-on-chip list, we found five common genes (*ARSJ*, *RCC2*, *CDH8*, *CDH13* and *HTRA1*).

5. Discussion

Expression profiles displayed by GBM cells provided results to compare these cells with other tumor types (Castells et al., 2010; Castells et al., 2009; Dreyfuss et al., 2009; Marucci et al., 2008; Reddy et al., 2008), normal tissues (Sallinen et al., 2000), or even regions in the same tumor (Mehrian Shai et al., 2005). However, there is no data in the literature comparing transcript profiles of GBM cell lines presenting similar genetic background. While T98G and U251MG harbor mutations for *TP53*, *CDKN2A*, and *PTEN*, U343MG-a and U87MG cell lines differ from others by the presence of a known WT *TP53* (Ishii et al., 1999). Expression profiles displayed by GBM cell lines were studied in the absence of any treatment. The hierarchical clustering analysis compared WT and MT *TP53* GBM cells regarding transcript profiles using a set of highly significant differentially expressed genes previously selected by SAM (FDR ≤ 0.68%). The results showed distinct expression profiles for WT *TP53* and MT *TP53* cells, whose patterns separate the mutant from WT cells. The mutant cell lines (T98G and U251MG) were not completely separated from each other within the cluster, indicating similarity of transcript patterns between the two cell lines. In contrast, *TP53* proficient cell lines (U87MG and U343MG-a) were clustered apart from each other. These results indicate the potential of the DNA microarray analysis to discriminate molecular profiles displayed by GBM cell lines presenting similar mutational background for *CDKN2A* and *PTEN* genes, but with different *TP53* status.

The statistical analysis performed by SAM (FDR ≤ 0.68%) indicated that MT *TP53* cell lines showed 28 down-regulated and 66 up-regulated genes, when compared to WT *TP53* cells (Table. 2). For the list of differentially expressed genes, biological gene functions were studied by the DAVID-NIH bioinformatic tool (Dennis et al., 2003; Huang da et al., 2009), and several biological processes were associated to the list of significant differentially expressed genes, such as: metabolism, response to ionizing radiation, cell adhesion, cell motion, apoptosis, DNA repair and transcription.

In addition, metabolic process was directly related with several biological functions as follow: insulin receptor signalling, water soluble vitamin metabolic process, steroid hormone receptor signalling, cellular response to nutrient levels, amine biosynthetic process, lipid biosynthetic process, calcium ion transport, lipid transport, glucose metabolic process, carboxylic acid biosynthetic process, and cellular amino acid derivative metabolic process. Other gene functions related to learning/memory, regulation of neuron apoptosis, synaptic transmission, and neurological system processes are intrinsic to the neural nature of GBM cells. DNA and RNA metabolism/regulation were also associated to several differentially expressed genes (Table. 2).

Three genes (*RNF8*, *CAV1* and *LIG4*) playing roles in ionizing radiation responses were found up-regulated in MT *TP53* cells, and this feature may influence the responsiveness to radiotherapy. It is already known that ionizing radiation causes DNA double-strand breaks (DSBs) that are highly cytotoxic lesions. Cells have a complex DNA-damage response that

Symbol	CloneID	Biological Process	Fold change	q-value (%)
SPRY2	40262	cell-cell signaling, development, organogenesis, regulation of signal transduction	1.93	0.00
CAV1	24651	cellular calcium ion homeostasis, cholesterol efflux, response to gamma radiation, regulation of apoptosis,	1.86	0.00
CDH13	31093	cell adhesion, homophilic cell adhesion	1.61	0.00
HTRA1	132044	proteolysis and peptidolysis, regulation of cell growth	1.55	0.00
ADAMTS1	34684	integrin-mediated signaling pathway, negative regulation of cell proliferation, heart trabecula formation, kidney development, ovulation from ovarian follicle, proteolysis	1.54	0.00
ITGA5	135671	cell-matrix adhesion, integrin-mediated signaling pathway	1.35	0.75
GPR68	22652	G-protein coupled receptor protein signaling pathway, inflammatory response, signal transduction	1.34	0.00
GPR44	25625	G-protein coupled receptor protein signaling pathway, immune response, calcium-mediated signaling, chemotaxis	1.34	0.75
RUVBL2	22267	chromatin modification, DNA recombination, DNA repair, regulation of growth, regulation of transcription	1.33	0.00
MKLN1	33715	cell motility, cell-matrix adhesion, signal transduction	1.31	0.00
KSR1	220655	Ras protein signal transduction, intracellular signaling cascade, protein amino acid phosphorylation	1.31	0.75
GNG13	178213	G-protein coupled receptor protein signaling pathway, signal transduction	1.30	0.75
CDH8	38939	cell adhesion, homophilic cell adhesion	1.30	0.00
DNAJA1	24473	protein folding, response to unfolded protein	1.28	0.75
RHOQ	131061	small GTPase mediated signal transduction	1.26	0.00
FBXL3	25778	protein ubiquitination	1.26	0.00
CRH	34671	immune response, learning and/or memory, pregnancy, signal transduction, synaptic transmission	1.24	0.00
IGF1R	21519	axonogenesis, brain development, exocrine pancreas development, male sex determination, regulation of apoptosis	1.24	0.00
ASNS	27208	amino acid biosynthesis	1.23	0.90
CD93	35503	cell-cell adhesion, macrophage activation, phagocytosis	1.19	0.00
COPB2	24627	intracellular protein transport, vesicle-mediated transport	1.18	0.00
CACNB2	34651	Synaptic transmission, ion transport	1.16	0.71
LASS6	35147	lipid biosynthetic process, regulation of transcription, DNA-dependent	1.15	0.75
LIG4	39274	single strand break repair	1.14	0.75
RNF8	39161	protein ubiquitination	1.14	0.00

Symbol	CloneID	Biological Process	Fold change	q-value (%)
KCNIP4	21478	ion transport	-1.13	0.00
RCC2	136887	cell cycle, cell division, mitosis	-1.14	0.90
RBM4	141446	DNA recombination, DNA repair, DNA replication, estrogen receptor signaling pathway, glucocorticoid receptor signaling pathway, mRNA splicing	-1.19	0.00
DENND 1A	21467	synaptic vesicle endocytosis	-1.21	0.74
HSPB1	23827	cell death, response to heat	-1.23	0.00
ARSJ	32854	Unknown	-1.24	0.00
ATP6AP 2	131821	angiotensin maturation, positive regulation of transforming growth factor-beta1 production, regulation of MAPKKK cascade	-1.29	0.00
UGDH	139835	UDP-glucose metabolism, electron transport	-1.41	0.00
DUSP22	182999	apoptosis, cell proliferation, development, inactivation of MAPK, protein amino acid dephosphorylation	-1.55	0.00
PFKFB2	53158	glucose catabolic process, positive regulation of insulin secretion, pyruvate metabolic process	-1.68	0.00

Table 2. List of highly significant differentially expressed genes provided by SAM (FDR ≤0.68%), comparing MT and WT TP53 GBM cell lines. Positive fold-change value means up-regulation, while negative fold-change value means down-regulation in MT *TP53* cells, compared to WT TP53 cells.

includes the spatial reorganization of DSB repair and signalling proteins into subnuclear structures surrounding DSB sites (Bartek and Lukas, 2007; Maser et al., 1997). In this context, RNF8 protein is an important component of the DNA damage response; it can be recruited to the DNA damage sites, thus triggering the formation of ubiquitin conjugates, promoting the recruitment of important proteins to DSB sites, thereby enhancing DNA-damage checkpoint events (G2/M) and guaranting cell survival (Kolas et al., 2007). There is also evidence that RNF8 participates in histone ubiquitylation and protects the genome integrity by licensing the DSB-flanking chromatin to concentrate repair factors near the DNA lesions (Mailand et al., 2007). LIG4 is also a key protein playing role in DSB repair by non-homologous end joining (NHEJ) pathway (Helleday et al., 2007), while *CAV1* presents a tumor suppressor function in non-neoplastic tissue, being down-regulated upon transformation, but re-expressed upon progression in metastatic and multidrug-resistant tumors (Burgermeister et al., 2008). *CAV1* was also associated with multidrug resistance (Belanger et al., 2004; Belanger et al., 2003), and radioresistance (Barzan et al., 2010; Cordes et al., 2007; Li et al., 2005).

In MT *TP53* cells, other stress response genes (*RUVBL2* and *ASNS*) were up-regulated, while *RBM4* was down-regulated. *RUVBL2* participates in chromatin-remodelling (Lee et al., 2010), which could also let the lesion available to DNA repair genes. *ASNS* is a gene whose response elements function as an enhancer to mediate the transcriptional activation of the gene, either by the amino acid response (AAR) or the unfolded protein response (UPR pathway), triggered by amino acid limitation or endoplasmic reticulum stress (Siu et al.,

2002), respectively. These pathways initiate a wide array of adaptive mechanisms and ultimately, if necessary, programmed cell death (Harding et al., 2003; Zinszner et al., 1998); The role of *RBM4* in response to stress stimulus is the activation of internal ribosome entry site (IRES)-mediated translation, promoting the expression of stress-response genes; therefore, the down-regulation of *RBM4* in MT *TP53* cells might compromise the translational regulation of stress-associated mRNAs (Markus and Morris, 2009).

Several genes playing roles in cell adhesion were also differentially expressed in GBM cell lines, and this result is in accordance with the association of these genes in acquired chemo- and radioresistance (Kraus et al., 2002). In the present work, three cadherins (*CDH8*, *CDH13* and *CD93*), and one integrin *ITGA5* were found up-regulated in MT *TP53* cells, relatively to WT counterparts. Cadherins are integral membrane proteins that mediate calcium-dependent cell-cell adhesion. They may play an important role in the development and maintenance of tissues, and possibly are involved in the invasion and metastasis of malignant tumors. While *CDH8* participates in neural circuitry, *CD93* is involved in phagocytosis (Bohlson et al., 2005), *CDH13* play a role in apoptosis (Chan et al., 2008), and *ITGA5* in cell spreading (Fang et al., 2010).

A comparative analysis of differentially expressed genes (provided by SAM) was performed with previously identified genes with TP53 binding sites; this comparison was possible by using ChIP-PET and ChIP-on-chip analyses (Smeenk et al., 2008; Wei et al., 2006). For the ChIP- PET gene lists, we observed only one common gene (*LASS6*), which was up-regulated in MT *TP53* cells. *LASS6* is a ceramide sintase, producing ceramides that have antiproliferative and pro-apoptotic effects (Ruvolo, 2003). This gene plays a role in apoptosis induction in colon cancer cells (Schiffmann et al., 2010), and enhanced tumor development and growth *in vivo* in human head and neck squamous cell carcinomas (HNSCCs) (Senkal et al., 2010).

In addition, the comparison involving our modulated gene list, comparing MT and WT *TP53* GBM cells, using the ChIP-on-chip list, provided five common genes: *ARSJ*, *RCC2*, *CDH8*, *CDH13* and *HTRA1*). While *CDH8*, *CDH13* and *HTRA1* were up-regulated, *ARSJ* and *RCC2* were down-regulated in MT *TP53* cells. *CDH8* and *CDH13* are cadherins and were already discussed as possible targets to chemo- and radioresistance (Kraus et al., 2002). *HTRA1* is a tumor suppressor-like factor when overexpressed in cancer cell lines. (Baldi et al., 2002; Chien et al., 2004). This gene was also associated to cisplatin therapy responses in various cancer types (Catalano et al., 2011; Chien et al., 2006; Komatsu et al., 2006). These genes cannot be transactivated by TP53, according to the *TP53* status in T98G and U251MG cells. However, the majority of the genome-wide TP53 target sites can also be bounded by overexpressed p63 and p73 in vivo, suggesting that they may possibly play an important role at TP53 binding sites (Smeenk et al., 2008). *ARSJ* and *RCC2* genes were not described to be related with stress responses or cancer, and were up-regulated in WT *TP53* GBM cells, compared to MT, and probably, they can be associated to transactivation properties of TP53.

Thus, in the present work, most genes involved in stress responses (*RUVBL2, ASNS, RNF8, LIG4* and *CAV1*), cell adhesion (*CDH8, CDH13, CD93* and *ITGA5*), and genes associated to p63 and p73 binding sites (*LASS6* and *HTRA1*) were up-regulated in MT *TP53* cells. At least some of them may have the potential to be directly involved in radio- or chemoresistance. Therefore, the loss of *TP53* function may compromise cellular responses to anticancer agents.

It is already known that mechanisms underlying the cellular radiosensitivity seem to vary among different cell lineages, but at what extent the radiosensitivity depends on *TP53* function is a matter of investigation since long time ago. Several authors demonstrated that the loss of *TP53* function decreases the sensitivity of GBM cells to irradiation (Bartussek et al., 1999; McIlwrath et al., 1994; Roy et al., 2006; Yount et al., 1996), and the same was observed for other tumor types (Fan et al., 1994; Komarova et al., 1997; Merritt et al., 1994). However, in primary gliomas, the *TP53* mutation confers an improved prognosis in adult glioma patients due to a better response to radiation therapy (Tada et al., 1998). This is consistent with the results obtained in *in vitro* studies, in which glioma cells lacking WT *TP53* function were more susceptible to radiation-induced apoptosis than their isogenic counterparts expressing WT *TP53* (Hara et al., 2004).

It is important to emphasize that all these articles in the literature took into account the *TP53* status, but not other mutations inherent to GBM. In the present work, we studied four GBM cell lines with different *TP53* status; T98G and U251MG are mutated and U343MG-a and U87MG are wild-type for *TP53* gene, but they are similar regarding the fact that they harbor mutations for *CDKN2A* and *PTEN*, according to Ishi et al. (1999). In experiments on cell survival performed by the clonogenic survival assay, the results provided by several authors indicated that MT *TP53* cells were, in general, more radioresistant than WT *TP53* cells (Bassi et al., 2008; Chautard et al., 2010; de la Pena et al., 2006; Lee et al., 2006; Roy et al., 2006).

As described above, several differentially expressed genes provided by the comparison between MT *versus* WT *TP53* GBM cells are involved in the mechanism of resistance to ionizing radiation and/or multidrug resistance. In general, cytotoxicity of DNA damaging agents correlates with the induction of DSB, which can be produced directly or indirectly into the DNA molecule. Whereas ionizing radiation induces DSB directly, the drug TMZ generates DSB only after two or more cycles of DNA replication, as a secondary effect (Caporali et al., 2004). Therefore, *TP53* mutation has the potential to change the sensitivity of GBM cells to anticancer agents currently used in therapy, and probably, the changes in the expression profiles exhibited by several genes acting down-stream in the signalling cascade of damage responses may compromise the outcome of drug- and radio- therapies. In order to understand these alterations in terms of transcript profiles, we are currently studying at what extent *TP53* status influences gene expression profiles in irradiated GBM cells (*manuscript in preparation*); we found several differentially expressed genes in irradiated MT *TP53* cells that are probably implicated in tumor resistance; among them, we can mention *CLSTN2*, *ROBO2*, and *BMPR1B* (with role in cell-cell adhesion), *BTRC*, *CYP26B1*, and *ANLN* (cell cycle regulators).

However, the data in the literature regarding the role of *TP53* in mediating sensitivity to anticancer agents still present controversies. It has been reported that the absence of a functional *TP53* increases TMZ sensitivity in glioma cell lines, an effect that is independent of MGMT status (Blough et al., 2011). Glioma cell lines that did not express a functional *TP53* were significantly more sensitive to TMZ than cell lines that were functionally intact for *TP53* expression (Blough et al., 2011). An example of this inconsistency comes from the results obtained with T98G cell line (MT *TP53*), in with the role of MGMT in mediating TMZ resistance was confirmed by the co-exposure to the MGMT inhibitor O6-BG, causing a reduction in the EC50, as evaluated by the clonogenic survival assay (Hermisson et al.,

2006). Nevertheless, the roles of *TP53* in terms of its influence on drug sensitivity are difficult to elucidate, since the dependence on the cell type, and the kind of antitumor agent used(Fukushima et al., 2009). In addition, the genetic background is also critical, since GBM cells often harbor other mutations for cell cycle regulator genes, and other tumor suppressor genes, as already mentioned for the four GBM cell lines studied in the present work, which carry two other mutations (*CDKN2A* and *PTEN*).

The control of cell proliferation is very critical in the tumor development, and may also be influenced by the *TP53* status (Facoetti et al., 2008). Several genes playing roles in the regulation of cell proliferation were found differentially expressed in MT *TP53* cells, relatively to WT: *IGFR1, CDH13, DUSP22, ADAMTS1, CAV1*, and *LIG4*. These last two genes also play a role in stress responses, as mentioned before.

Tumor growth also depends on the rate of apoptosis (Amirlak and Couldwell, 2003), and apoptosis is directly involved in gliomagenesis and resistance towards classical genotoxic approaches in cancer therapy. It has been reported that GBM cells are virtually resistant to different apoptotic stimuli (Adamson et al., 2009; Eisele and Weller, 2011), but the mechanisms underlying these responses are still unclear, and may depend on several treatment conditions and type of genotoxic agent. In the present work, the list of differentially expressed genes also indicated several genes implicated in apoptosis. Those genes, such as *IGF1R*, *CDH13*, *IFT57, CRH*, *HSPB1* and *ASNS*, were up-regulated in MT *TP53* GBM cells, relatively to WT cells. *IGF1R* participates in cell proliferation and protection of cell death (Trojan et al., 2007), while *CDH13*, *ITF57*, *CRH* and *ASNS* participates in apoptosis induction (Andreeva and Kutuzov, 2010; Chan et al., 2008; Gdynia et al., 2008; Gervais et al., 2002; Minas et al., 2007; Siu et al., 2002). Considering that the ability of cells to undergo apoptosis is dependent of a complex signaling cascade involving pro- and anti-apoptotic genes, among other gene classes, the up-regulation of several apoptosis related genes in MT *TP53* cells might influence responses of GBM cells submitted to anticancer agents.

In this work, we showed some distinct transcript profiles for MT and WT *TP53* GBM cells, pointing out several genes that might influence cell sensitivity to chemo- and/or radiotherapy.

6. Conclusions

The present data comparing transcript profiles displayed by GBM cell lines with different *TP53* status showed that several biological processes were associated to the list of highly significant differentially expressed genes. Gene classes associated with those genes (stress response, DNA repair, proliferation, cell division, cell adhesion, apoptosis, etc) provided a picture on transcript profiles under normal conditions of cell proliferation in cultured GBM cell lines. However, these gene classes reflect wide amplitude of cellular processes that might be involved in cellular defense mechanisms under conditions of cell injury provoked by irradiation or drug treatment. These results support the hypothesis that MT *TP53* cells might be more resistant than WT *TP53* cells to some genotoxic stresses, such as ionizing radiation and TMZ. While this hypothesis still should be tested, altogether, the information obtained in this work provides a relevant basic contribution towards the understanding of GBM responses to therapies, and for designing novel therapeutic strategies for patients with GBM, based on their *TP53* status, but also considering other gene mutations.

7. Supplementary data

Symbol	Gene Name	CloneID	Fold change	q-value (%)
SPRY2	Sprouty homolog 2 (Drosophila)	40262	1.93	0.00
CAV1	Caveolin 1, caveolae protein, 22kDa	24651	1.86	0.00
MINA	MYC induced nuclear antigen	139217	1.79	0.00
	Clone 25220 mRNA sequence	25220	1.63	0.00
CDH13	Cadherin 13, H-cadherin (heart)	31093	1.61	0.00
HTRA1	HtrA serine peptidase 1	132044	1.55	0.00
ADAMTS1	ADAM metallopeptidase with thrombospondin type 1 motif, 1	34684	1.54	0.00
MPND	MPN domain containing	24532	1.39	0.00
ARRB1	Arrestin, beta 1	21814	1.38	0.00
ACSL4	Acyl-CoA synthetase long-chain family member 4	133988	1.36	0.00
ITGA5	Integrin, alpha 5 (fibronectin receptor, alpha polypeptide)	135671	1.35	0.75
P4HB	Prolyl 4-hydroxylase, beta polypeptide	132702	1.34	0.00
GPR68	G protein-coupled receptor 68	22652	1.34	0.00
GPR44	G protein-coupled receptor 44	25625	1.34	0.75
PDLIM1	PDZ and LIM domain 1	135689	1.33	0.00
RUVBL2	RuvB-like 2 (E. coli)	22267	1.33	0.00
	In multiple ClusterIDs	261714	1.32	0.00
	Unknown	134004	1.32	0.00
ZXDB	Zinc finger, X-linked, duplicated B	38972	1.31	0.90
MKLN1	Muskelin 1, intracellular mediator containing kelch motifs	33715	1.31	0.00
LARP4	La ribonucleoprotein domain family, member 4	41347	1.31	0.71
KSR1	Kinase suppressor of ras 1	220655	1.31	0.75
JAKMIP1	Janus kinase and microtubule interacting protein 1	32109	1.30	0.00

GNG13	Guanine nucleotide binding protein (G protein), gamma 13	178213	1.30	0.75
ANKRD42	MRNA; cDNA DKFZp761C0524 (from clone DKFZp761C0524)	30094	1.30	0.00
CDH8	cadherin 8, type 2	38939	1.30	0.00
NRGN	Neurogranin (protein kinase C substrate, RC3)	178825	1.30	0.75
USP53	Ubiquitin specific peptidase 53	142468	1.30	0.69
PIP5K2C	Phosphatidylinositol-5-phosphate 4-kinase, type II, gamma	133173	1.30	0.75
WDR34	WD repeat domain 34	133474	1.29	0.00
TCBA1	Na+/K+ transporting ATPase interacting 2	41427	1.29	0.00
MGC14376	Chromosome 17 open reading frame 91	24659	1.28	0.00
DNAJA1	DnaJ (Hsp40) homolog, subfamily A, member 1	24473	1.28	0.75
KLF12	Kruppel-like factor 12	34367	1.28	0.00
UNC13C	unc-13 homolog C (C. elegans)	22137	1.27	0.00
RHOQ	Ras homolog gene family, member Q	131061	1.26	0.00
FBXL3	F-box and leucine-rich repeat protein 3	25778	1.26	0.00
RP11-484I6.3	Chromosome 13 open reading frame 27	24463	1.25	0.00
	Unknown	132487	1.25	0.75
CRH	Corticotropin releasing hormone	34671	1.24	0.00
IGF1R	insulin-like growth factor 1 receptor	21519	1.24	0.00
ASNS	Asparagine synthetase (glutamine-hydrolyzing)	27208	1.23	0.90
CSGlcA-T	Chondroitin polymerizing factor 2	39955	1.23	0.75
NBPF12	Neuroblastoma breakpoint family, member 1	24976	1.23	0.90
BAMBI	BMP and activin membrane-bound inhibitor homolog (Xenopus laevis)	41406	1.22	0.00
PDXK	Pyridoxal (pyridoxine, vitamin B6) kinase	25360	1.22	0.00
	Transcribed locus	136399	1.21	0.75
TNRC15	Transcribed locus	37482	1.21	0.75

	Calneuron 1	39092	1.20	0.75
3-Mar	Membrane-associated ring finger (C3HC4) 3	24707	1.20	0.00
	Unknown	39306	1.19	0.69
CD93	CD93 molecule	35503	1.19	0.00
C20orf39	Transmembrane protein 90B	35704	1.19	0.00
	In multiple ClusterIDs	41186	1.19	0.71
	CXXC finger protein 5	136782	1.18	0.00
PPCDC	Phosphopantothenoylcysteine decarboxylase	135984	1.18	0.00
COPB2	Clone 24627 mRNA sequence	24627	1.18	0.00
	CDNA FLJ34038 fis, clone FCBBF2005645	38618	1.18	0.00
FLJ41130	hypothetical LOC401113	40009	1.18	0.00
CPD	Carboxypeptidase D	40521	1.18	0.75
	Unknown	34967	1.16	0.90
CACNB2	calcium channel, voltage-dependent, beta 2 subunit	34651	1.16	0.71
CUTL2	Cut-like homeobox 2	41354	1.16	0.90
	Unknown	34966	1.15	0.00
LASS6	LAG1 homolog, ceramide synthase 6	35147	1.15	0.75
LIG4	Ligase IV, DNA, ATP-dependent	39274	1.14	0.75
RNF8	Ring finger protein 8	39161	1.14	0.00
IFT57	Intraflagellar transport 57 homolog (Chlamydomonas)	34942	1.12	0.00
PIGO	Phosphatidylinositol glycan anchor biosynthesis, class O	21678	-1.12	0.71
KCNIP4	Kv channel interacting protein 4	21478	-1.13	0.00
RCC2	Regulator of chromosome condensation 2	136887	-1.16	0.90
PKIB	Protein kinase (cAMP-dependent, catalytic) inhibitor beta	152289	-1.17	0.00
DDX18	DEAD (Asp-Glu-Ala-Asp) box polypeptide 18	149416	-1.17	0.00
SIN3A	SIN3 homolog A, transcription regulator (yeast)	26455	-1.18	0.00

RBM4	RNA binding motif protein 4	141446	-1.19	0.00
NKIRAS2	NFKB inhibitor interacting Ras-like 2	137971	-1.20	0.00
DENND1A	DENN/MADD domain containing 1A	21467	-1.21	0.74
TMLHE	Trimethyllysine hydroxylase, epsilon	21457	-1.22	0.00
HSPB1	heat shock 27kDa protein 1	23827	-1.23	0.00
ZNF630	Zinc finger protein 630	141069	-1.24	0.00
ARSJ	Arylsulfatase family, member J	32854	-1.24	0.00
NUP160	Nucleoporin 160kDa	33299	-1.25	0.00
SCP2	Sterol carrier protein 2	137004	-1.26	0.68
NRN1	Neuritin 1	140197	-1.28	0.00
ATP6AP2	ATPase, H+ transporting, lysosomal accessory protein 2	131821	-1.29	0.00
PRPS2	Phosphoribosyl pyrophosphate synthetase 2	146194	-1.32	0.00
	Transcribed locus, weakly similar to XP_933787.2 PREDICTED: hypothetical protein [Homo sapiens]	53331	-1.34	0.00
PRSS23	Protease, serine, 23	143887	-1.35	0.00
CMPK	Cytidine monophosphate (UMP-CMP) kinase 1, cytosolic	140570	-1.36	0.00
UGDH	UDP-glucose 6-dehydrogenase	139835	-1.41	0.00
VSNL1	Visinin-like 1	26570	-1.42	0.00
	Transcribed locus	139645	-1.44	0.00
SSFA2	Sperm specific antigen 2	140589	-1.45	0.00
ABCA7	ATP-binding cassette, sub-family A (ABC1), member 7	182933	-1.45	0.00
	Unknown	181796	-1.54	0.00
DUSP22	Dual specificity phosphatase 22	182999	-1.55	0.00
PFKFB2	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2	53158	-1.68	0.00

Table 1. List of significantly modulated genes provided by the statistical analysis by SAM (FDR ≤0.68%), comparing MT versus WT *TP53* GBM cell lines.

8. Acknowledgment

This study was supported by FAPESP (Fundação de Amparo à pesquisa de São Paulo), proc. nº: 04/15611-6, 06/01947-8 and 01953-8/06; CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico); CAPES (Coordenação de Aperfeiçoamento de Pessoal de nível Superior); and FAEPA - HC FMRP/USP (Fundação de Apoio ao Ensino, Pesquisa e Assistência).

9. References

Adamson, C., Kanu, O. O., Mehta, A. I., Di, C., Lin, N., Mattox, A. K., and Bigner, D. D. (2009). Glioblastoma multiforme: a review of where we have been and where we are going. Expert Opin Investig Drugs *18*, 1061-1083.

Amirlak, B., and Couldwell, W. T. (2003). Apoptosis in glioma cells: review and analysis of techniques used for study with focus on the laser scanning cytometer. J Neurooncol *63*, 129-145.

Andreeva, A. V., and Kutuzov, M. A. (2010). Cadherin 13 in cancer. Genes Chromosomes Cancer *49*, 775-790.

Avgeropoulos, N. G., and Batchelor, T. T. (1999). New treatment strategies for malignant gliomas. Oncologist *4*, 209-224.

Baldi, A., De Luca, A., Morini, M., Battista, T., Felsani, A., Baldi, F., Catricala, C., Amantea, A., Noonan, D. M., Albini, A., *et al.* (2002). The HtrA1 serine protease is down-regulated during human melanoma progression and represses growth of metastatic melanoma cells. Oncogene *21*, 6684-6688.

Bartek, J., and Lukas, J. (2007). DNA damage checkpoints: from initiation to recovery or adaptation. Curr Opin Cell Biol *19*, 238-245.

Bartussek, C., Naumann, U., and Weller, M. (1999). Accumulation of mutant p53(V143A) modulates the growth, clonogenicity, and radiochemosensitivity of malignant glioma cells independent of endogenous p53 status. Exp Cell Res *253*, 432-439.

Barzan, D., Maier, P., Zeller, W. J., Wenz, F., and Herskind, C. (2010). Overexpression of caveolin-1 in lymphoblastoid TK6 cells enhances proliferation after irradiation with clinically relevant doses. Strahlenther Onkol *186*, 99-106.

Bassi, C., Mello, S., Cardoso, R., Godoy, P., Fachin, A., Junta, C., Sandrin-Garcia, P., Carlotti, C., Falcao, R., Donadi, E., *et al.* (2008). Transcriptional changes in U343 MG-a glioblastoma cell line exposed to ionizing radiation. Hum Exp Toxicol *27*, 919-929.

Batista, L. F., Roos, W. P., Christmann, M., Menck, C. F., and Kaina, B. (2007). Differential sensitivity of malignant glioma cells to methylating and chloroethylating anticancer drugs: p53 determines the switch by regulating xpc, ddb2, and DNA double-strand breaks. Cancer Res *67*, 11886-11895.

Batista, L. F., Roos, W. P., Kaina, B., and Menck, C. F. (2009). p53 mutant human glioma cells are sensitive to UV-C-induced apoptosis due to impaired cyclobutane pyrimidine dimer removal. Mol Cancer Res *7*, 237-246.

Belanger, M. M., Gaudreau, M., Roussel, E., and Couet, J. (2004). Role of caveolin-1 in etoposide resistance development in A549 lung cancer cells. Cancer Biol Ther *3*, 954-959.

Belanger, M. M., Roussel, E., and Couet, J. (2003). Up-regulation of caveolin expression by cytotoxic agents in drug-sensitive cancer cells. Anticancer Drugs *14*, 281-287.

Blough, M. D., Beauchamp, D. C., Westgate, M. R., Kelly, J. J., and Cairncross, J. G. (2011). Effect of aberrant p53 function on temozolomide sensitivity of glioma cell lines and brain tumor initiating cells from glioblastoma. J Neurooncol *102*, 1-7.

Bohlson, S. S., Zhang, M., Ortiz, C. E., and Tenner, A. J. (2005). CD93 interacts with the PDZ domain-containing adaptor protein GIPC: implications in the modulation of phagocytosis. J Leukoc Biol *77*, 80-89.

Burgermeister, E., Liscovitch, M., Rocken, C., Schmid, R. M., and Ebert, M. P. (2008). Caveats of caveolin-1 in cancer progression. Cancer Lett *268*, 187-201.

Caporali, S., Falcinelli, S., Starace, G., Russo, M. T., Bonmassar, E., Jiricny, J., and D'Atri, S. (2004). DNA damage induced by temozolomide signals to both ATM and ATR: role of the mismatch repair system. Mol Pharmacol *66*, 478-491.

Castells, X., Acebes, J. J., Boluda, S., Moreno-Torres, A., Pujol, J., Julia-Sape, M., Candiota, A. P., Arino, J., Barcelo, A., and Arus, C. (2010). Development of a predictor for human brain tumors based on gene expression values obtained from two types of microarray technologies. OMICS *14*, 157-164.

Castells, X., Garcia-Gomez, J. M., Navarro, A., Acebes, J. J., Godino, O., Boluda, S., Barcelo, A., Robles, M., Arino, J., and Arus, C. (2009). Automated brain tumor biopsy prediction using single-labeling cDNA microarrays-based gene expression profiling. Diagn Mol Pathol *18*, 206-218.

Catalano, V., Mellone, P., d'Avino, A., Shridhar, V., Staccioli, M. P., Graziano, F., Giordani, P., Rossi, D., Baldelli, A. M., Alessandroni, P., *et al*. (2011). HtrA1, a potential predictor of response to cisplatin-based combination chemotherapy in gastric cancer. Histopathology.

Chan, D. W., Lee, J. M., Chan, P. C., and Ng, I. O. (2008). Genetic and epigenetic inactivation of T-cadherin in human hepatocellular carcinoma cells. Int J Cancer *123*, 1043-1052.

Chautard, E., Loubeau, G., Tchirkov, A., Chassagne, J., Vermot-Desroches, C., Morel, L., and Verrelle, P. (2010). Akt signaling pathway: a target for radiosensitizing human malignant glioma. Neuro Oncol *12*, 434-443.

Chien, J., Aletti, G., Baldi, A., Catalano, V., Muretto, P., Keeney, G. L., Kalli, K. R., Staub, J., Ehrmann, M., Cliby, W. A., *et al.* (2006). Serine protease HtrA1 modulates chemotherapy-induced cytotoxicity. J Clin Invest *116*, 1994-2004.

Chien, J., Staub, J., Hu, S. I., Erickson-Johnson, M. R., Couch, F. J., Smith, D. I., Crowl, R. M., Kaufmann, S. H., and Shridhar, V. (2004). A candidate tumor suppressor HtrA1 is downregulated in ovarian cancer. Oncogene *23*, 1636-1644.

Cordes, N., Frick, S., Brunner, T. B., Pilarsky, C., Grutzmann, R., Sipos, B., Kloppel, G., McKenna, W. G., and Bernhard, E. J. (2007). Human pancreatic tumor cells are sensitized to ionizing radiation by knockdown of caveolin-1. Oncogene *26*, 6851-6862.

de la Pena, L., Burgan, W. E., Carter, D. J., Hollingshead, M. G., Satyamitra, M., Camphausen, K., and Tofilon, P. J. (2006). Inhibition of Akt by the alkylphospholipid perifosine does not enhance the radiosensitivity of human glioma cells. Mol Cancer Ther *5*, 1504-1510.

Dreyfuss, J. M., Johnson, M. D., and Park, P. J. (2009). Meta-analysis of glioblastoma multiforme versus anaplastic astrocytoma identifies robust gene markers. Mol Cancer *8*, 71.

Eisele, G., and Weller, M. (2011). Targeting apoptosis pathways in glioblastoma. Cancer Lett.

Eisen, M. B., Spellman, P. T., Brown, P. O., and Botstein, D. (1998). Cluster analysis and display of genome wide expression patterns. Proc Natl Acad Sci U S A *95*, 14863-14868.

el-Deiry, W. S. (1998). Regulation of p53 downstream genes. Semin Cancer Biol *8*, 345-357.

Facoetti, A., Ranza, E., and Nano, R. (2008). Proliferation and programmed cell death: role of p53 protein in high and low grade astrocytoma. Anticancer Res *28*, 15-19.

Fan, S., el-Deiry, W. S., Bae, I., Freeman, J., Jondle, D., Bhatia, K., Fornace, A. J., Jr., Magrath, I., Kohn, K. W., and O'Connor, P. M. (1994). p53 gene mutations are associated with decreased sensitivity of human lymphoma cells to DNA damaging agents. Cancer Res *54*, 5824-5830.

Fang, Z., Yao, W., Xiong, Y., Zhang, J., Liu, L., Li, J., Zhang, C., and Wan, J. (2010). Functional elucidation and methylation-mediated downregulation of ITGA5 gene in breast cancer cell line MDA-MB-468. J Cell Biochem *110*, 1130-1141.

Fukushima, T., Takeshima, H., and Kataoka, H. (2009). Anti-glioma therapy with temozolomide and status of the DNA-repair gene MGMT. Anticancer Res *29*, 4845-4854.

Gdynia, G., Lehmann-Koch, J., Sieber, S., Tagscherer, K. E., Fassl, A., Zentgraf, H., Matsuzawa, S., Reed, J. C., and Roth, W. (2008). BLOC1S2 interacts with the HIPPI protein and sensitizes NCH89 glioblastoma cells to apoptosis. Apoptosis *13*, 437-447.

Gervais, F. G., Singaraja, R., Xanthoudakis, S., Gutekunst, C. A., Leavitt, B. R., Metzler, M., Hackam, A. S., Tam, J., Vaillancourt, J. P., Houtzager, V., *et al.* (2002). Recruitment and activation of caspase-8 by the Huntingtin-interacting protein Hip-1 and a novel partner Hippi. Nat Cell Biol *4*, 95-105.

Hara, S., Nakashima, S., Kiyono, T., Sawada, M., Yoshimura, S., Iwama, T., Banno, Y., Shinoda, J., and Sakai, N. (2004). p53-Independent ceramide formation in human glioma cells during gamma-radiation-induced apoptosis. Cell Death Differ *11*, 853-861.

Harding, H. P., Zhang, Y., Zeng, H., Novoa, I., Lu, P. D., Calfon, M., Sadri, N., Yun, C., Popko, B., Paules, R., *et al.* (2003). An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. Mol Cell *11*, 619-633.

Hegi, M. E., Diserens, A. C., Gorlia, T., Hamou, M. F., de Tribolet, N., Weller, M., Kros, J. M., Hainfellner, J. A., Mason, W., Mariani, L., *et al.* (2005). MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med *352*, 997-1003.

Helleday, T., Lo, J., van Gent, D. C., and Engelward, B. P. (2007). DNA double-strand break repair: from mechanistic understanding to cancer treatment. DNA Repair (Amst) *6*, 923-935.

Hermisson, M., Klumpp, A., Wick, W., Wischhusen, J., Nagel, G., Roos, W., Kaina, B., and Weller, M. (2006). O6-methylguanine DNA methyltransferase and p53 status

predict temozolomide sensitivity in human malignant glioma cells. J Neurochem *96*, 766-776.

Hoh, J., Jin, S., Parrado, T., Edington, J., Levine, A. J., and Ott, J. (2002). The p53MH algorithm and its application in detecting p53-responsive genes. Proc Natl Acad Sci U S A *99*, 8467-8472.

Horn, H. F., and Vousden, K. H. (2007). Coping with stress: multiple ways to activate p53. Oncogene *26*, 1306-1316.

Kolas, N. K., Chapman, J. R., Nakada, S., Ylanko, J., Chahwan, R., Sweeney, F. D., Panier, S., Mendez, M., Wildenhain, J., Thomson, T. M., *et al.* (2007). Orchestration of the DNA-damage response by the RNF8 ubiquitin ligase. Science *318*, 1637-1640.

Komarova, E. A., Chernov, M. V., Franks, R., Wang, K., Armin, G., Zelnick, C. R., Chin, D. M., Bacus, S. S., Stark, G. R., and Gudkov, A. V. (1997). Transgenic mice with p53-responsive lacZ: p53 activity varies dramatically during normal development and determines radiation and drug sensitivity in vivo. Embo J *16*, 1391-1400.

Komatsu, M., Hiyama, K., Tanimoto, K., Yunokawa, M., Otani, K., Ohtaki, M., Hiyama, E., Kigawa, J., Ohwada, M., Suzuki, M., *et al.* (2006). Prediction of individual response to platinum/paclitaxel combination using novel marker genes in ovarian cancers. Mol Cancer Ther *5*, 767-775.

Kraus, A. C., Ferber, I., Bachmann, S. O., Specht, H., Wimmel, A., Gross, M. W., Schlegel, J., Suske, G., and Schuermann, M. (2002). In vitro chemo- and radio-resistance in small cell lung cancer correlates with cell adhesion and constitutive activation of AKT and MAP kinase pathways. Oncogene *21*, 8683-8695.

Laptenko, O., and Prives, C. (2006). Transcriptional regulation by p53: one protein, many possibilities. Cell Death Differ *13*, 951-961.

Lee, J. S., Kim, Y., Kim, I. S., Kim, B., Choi, H. J., Lee, J. M., Shin, H. J., Kim, J. H., Kim, J. Y., Seo, S. B., *et al.* (2010). Negative regulation of hypoxic responses via induced Reptin methylation. Mol Cell *39*, 71-85.

Lee, Y. J., Chung, D. Y., Lee, S. J., Ja Jhon, G., and Lee, Y. S. (2006). Enhanced radiosensitization of p53 mutant cells by oleamide. Int J Radiat Oncol Biol Phys *64*, 1466-1474.

Li, J., Hassan, G. S., Williams, T. M., Minetti, C., Pestell, R. G., Tanowitz, H. B., Frank, P. G., Sotgia, F., and Lisanti, M. P. (2005). Loss of caveolin-1 causes the hyper-proliferation of intestinal crypt stem cells, with increased sensitivity to whole body gamma-radiation. Cell Cycle *4*, 1817-1825.

Mailand, N., Bekker-Jensen, S., Faustrup, H., Melander, F., Bartek, J., Lukas, C., and Lukas, J. (2007). RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. Cell *131*, 887-900.

Markus, M. A., and Morris, B. J. (2009). RBM4: a multifunctional RNA-binding protein. Int J Biochem Cell Biol *41*, 740-743.

Marucci, G., Morandi, L., Magrini, E., Farnedi, A., Franceschi, E., Miglio, R., Calo, D., Pession, A., Foschini, M. P., and Eusebi, V. (2008). Gene expression profiling in glioblastoma and immunohistochemical evaluation of IGFBP-2 and CDC20. Virchows Arch *453*, 599-609.

Maser, R. S., Monsen, K. J., Nelms, B. E., and Petrini, J. H. (1997). hMre11 and hRad50 nuclear foci are induced during the normal cellular response to DNA double strand breaks. Mol Cell Biol *17*, 6087-6096.

McIlwrath, A. J., Vasey, P. A., Ross, G. M., and Brown, R. (1994). Cell cycle arrests and radiosensitivity of human tumor cell lines: dependence on wild-type p53 for radiosensitivity. Cancer Res *54*, 3718-3722.

Mehrian Shai, R., Reichardt, J. K., Ya-Hsuan, H., Kremen, T. J., Liau, L. M., Cloughesy, T. F., Mischel, P. S., and Nelson, S. F. (2005). Robustness of gene expression profiling in glioma specimen samplings and derived cell lines. Brain Res Mol Brain Res *136*, 99-103.

Merritt, A. J., Potten, C. S., Kemp, C. J., Hickman, J. A., Balmain, A., Lane, D. P., and Hall, P. A. (1994). The role of p53 in spontaneous and radiation-induced apoptosis in the gastrointestinal tract of normal and p53-deficient mice. Cancer Res *54*, 614-617.

Minas, V., Rolaki, A., Kalantaridou, S. N., Sidiropoulos, J., Mitrou, S., Petsas, G., Jeschke, U., Paraskevaidis, E. A., Fountzilas, G., Chrousos, G. P., *et al.* (2007). Intratumoral CRH modulates immuno-escape of ovarian cancer cells through FasL regulation. Br J Cancer *97*, 637-645.

Ohgaki, H., and Kleihues, P. (2005). Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas. J Neuropathol Exp Neurol *64*, 479-489.

Prise, K. M., Schettino, G., Folkard, M., and Held, K. D. (2005). New insights on cell death from radiation exposure. Lancet Oncol *6*, 520-528.

Reddy, S. P., Britto, R., Vinnakota, K., Aparna, H., Sreepathi, H. K., Thota, B., Kumari, A., Shilpa, B. M., Vrinda, M., Umesh, S., *et al.* (2008). Novel glioblastoma markers with diagnostic and prognostic value identified through transcriptome analysis. Clin Cancer Res *14*, 2978-2987.

Roos, W. P., Batista, L. F., Naumann, S. C., Wick, W., Weller, M., Menck, C. F., and Kaina, B. (2007). Apoptosis in malignant glioma cells triggered by the temozolomide-induced DNA lesion O6-methylguanine. Oncogene *26*, 186-197.

Roy, K., Wang, L., Makrigiorgos, G. M., and Price, B. D. (2006). Methylation of the ATM promoter in glioma cells alters ionizing radiation sensitivity. Biochem Biophys Res Commun *344*, 821-826.

Ruvolo, P. P. (2003). Intracellular signal transduction pathways activated by ceramide and its metabolites. Pharmacol Res *47*, 383-392.

Sallinen, S. L., Sallinen, P. K., Haapasalo, H. K., Helin, H. J., Helen, P. T., Schraml, P., Kallioniemi, O. P., and Kononen, J. (2000). Identification of differentially expressed genes in human gliomas by DNA microarray and tissue chip techniques. Cancer Res *60*, 6617-6622.

Sbisa, E., Catalano, D., Grillo, G., Licciulli, F., Turi, A., Liuni, S., Pesole, G., De Grassi, A., Caratozzolo, M. F., D'Erchia, A. M., *et al.* (2007). p53FamTaG: a database resource of human p53, p63 and p73 direct target genes combining in silico prediction and microarray data. BMC Bioinformatics *8 Suppl 1*, S20.

Schiffmann, S., Ziebell, S., Sandner, J., Birod, K., Deckmann, K., Hartmann, D., Rode, S., Schmidt, H., Angioni, C., Geisslinger, G., and Grosch, S. (2010). Activation of

ceramide synthase 6 by celecoxib leads to a selective induction of C16:0-ceramide. Biochem Pharmacol *80*, 1632-1640.

Senkal, C. E., Ponnusamy, S., Bielawski, J., Hannun, Y. A., and Ogretmen, B. (2010). Antiapoptotic roles of ceramide-synthase-6-generated C16-ceramide via selective regulation of the ATF6/CHOP arm of ER-stress-response pathways. FASEB J *24*, 296-308.

Siu, F., Bain, P. J., LeBlanc-Chaffin, R., Chen, H., and Kilberg, M. S. (2002). ATF4 is a mediator of the nutrient-sensing response pathway that activates the human asparagine synthetase gene. J Biol Chem *277*, 24120-24127.

Slichenmyer, W. J., Nelson, W. G., Slebos, R. J., and Kastan, M. B. (1993). Loss of a p53-associated G1 checkpoint does not decrease cell survival following DNA damage. Cancer Res *53*, 4164-4168.

Smeenk, L., van Heeringen, S. J., Koeppel, M., van Driel, M. A., Bartels, S. J., Akkers, R. C., Denissov, S., Stunnenberg, H. G., and Lohrum, M. (2008). Characterization of genome-wide p53-binding sites upon stress response. Nucleic Acids Res *36*, 3639-3654.

Smith, M. L., Chen, I. T., Zhan, Q., O'Connor, P. M., and Fornace, A. J., Jr. (1995). Involvement of the p53 tumor suppressor in repair of u.v.-type DNA damage. Oncogene *10*, 1053-1059.

Stupp, R., Hegi, M. E., Gilbert, M. R., and Chakravarti, A. (2007). Chemoradiotherapy in malignant glioma: standard of care and future directions. J Clin Oncol *25*, 4127-4136.

Stupp, R., Mason, W. P., van den Bent, M. J., Weller, M., Fisher, B., Taphoorn, M. J., Belanger, K., Brandes, A. A., Marosi, C., Bogdahn, U., *et al.* (2005). Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med *352*, 987-996.

Tada, M., Matsumoto, R., Iggo, R. D., Onimaru, R., Shirato, H., Sawamura, Y., and Shinohe, Y. (1998). Selective sensitivity to radiation of cerebral glioblastomas harboring p53 mutations. Cancer Res *58*, 1793-1797.

Trojan, J., Cloix, J. F., Ardourel, M. Y., Chatel, M., and Anthony, D. D. (2007). Insulin-like growth factor type I biology and targeting in malignant gliomas. Neuroscience *145*, 795-811.

Veprintsev, D. B., and Fersht, A. R. (2008). Algorithm for prediction of tumour suppressor p53 affinity for binding sites in DNA. Nucleic Acids Res *36*, 1589-1598.

Vogelstein, B., Lane, D., and Levine, A. J. (2000). Surfing the p53 network. Nature *408*, 307-310.

Wei, C. L., Wu, Q., Vega, V. B., Chiu, K. P., Ng, P., Zhang, T., Shahab, A., Yong, H. C., Fu, Y., Weng, Z., *et al.* (2006). A global map of p53 transcription-factor binding sites in the human genome. Cell *124*, 207-219.

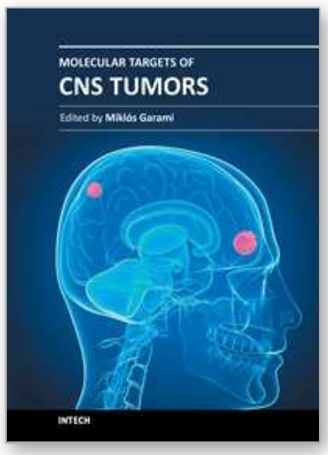
Yount, G. L., Haas-Kogan, D. A., Vidair, C. A., Haas, M., Dewey, W. C., and Israel, M. A. (1996). Cell cycle synchrony unmasks the influence of p53 function on radiosensitivity of human glioblastoma cells. Cancer Res *56*, 500-506.

Zhang, J., Stevens, M. F., Laughton, C. A., Madhusudan, S., and Bradshaw, T. D. (2010). Acquired resistance to temozolomide in glioma cell lines: molecular mechanisms and potential translational applications. Oncology *78*, 103-114.

Zinszner, H., Kuroda, M., Wang, X., Batchvarova, N., Lightfoot, R. T., Remotti, H., Stevens, J. L., and Ron, D. (1998). CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. Genes Dev *12*, 982-995.

IntechOpen

IntechOpen



Molecular Targets of CNS Tumors

Edited by Dr. Miklos Garami

ISBN 978-953-307-736-9
Hard cover, 674 pages
Publisher InTech
Published online 22, September, 2011
Published in print edition September, 2011

Molecular Targets of CNS Tumors is a selected review of Central Nervous System (CNS) tumors with particular emphasis on signaling pathway of the most common CNS tumor types. To develop drugs which specifically attack the cancer cells requires an understanding of the distinct characteristics of those cells. Additional detailed information is provided on selected signal pathways in CNS tumors.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Paulo R. D. V. Godoy, Stephano S. Mello, Daniele A. Magalhães, Flavia S. Donaires, Ana P. Lima-Montaldi, Patricia Nicolucci, Eduardo A. Donadi, Geraldo A. S. Passos and Elza T. Sakamoto-Hojo (2011). Portrait of Transcriptional Expression Profiles Displayed by Different Glioblastoma Cell Lines, Molecular Targets of CNS Tumors, Dr. Miklos Garami (Ed.), ISBN: 978-953-307-736-9, InTech, Available from: http://www.intechopen.com/books/molecular-targets-of-cns-tumors/portrait-of-transcriptional-expression-profiles-displayed-by-different-glioblastoma-cell-lines

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike-3.0 License, which permits use, distribution and reproduction for non-commercial purposes, provided the original is properly cited and derivative works building on this content are distributed under the same license.

IntechOpen

IntechOpen