Programming of Cell Resistance to Genotoxic and Oxidative Stress

Ilya O. Velegzhaninov 1,2*, Vitaly A. Ievlev 3, Yana I. Pylina 1, Dmitry M. Shadrin 1 and Olesya M. Vakhrusheva 2

1 Institute of Biology of Komi Science Centre of Ural Branch of RAS, Syktyvkar 167982, Russia; yanapylina@yandex.ru (Y.I.P); shdimas@yandex.ru (D.M.S);
2 Vyatka State University, Kirov 610000 Russia; nimf04ka@mail.ru
3 St. Olaf College, Northfield, MN 55057, USA; ielev1@stolaf.edu

* Correspondence: vellio@yandex.ru; Tel.: +7-909-121-9693

Abstract: Different organisms, cell types, and even similar cell lines can dramatically differ in resistance to genotoxic stress. This testifies to the wide opportunities for genetic and epigenetic regulation of stress resistance. These opportunities could be used to increase the effectiveness of cancer therapy, develop new varieties of plants and animals, and search for new pharmacological targets to enhance human radioresistance, for example, for manned deep space expeditions. Based on the comparison of transcriptomic studies in cancer cells, in this review we propose that there is a high diversity of genetic mechanisms of development of genotoxic stress resistance. This review focused on possibilities and limitations of the proposed regulation of the resistance of normal cells whole organisms to genotoxic and oxidative stress by overexpressing of stress-response genes. Moreover, the existing experimental data on the effect of such overexpression on the resistance of cells and organisms to various genotoxic agents has been analyzed and systematized. We suggest that the recent advances in the development of multiplex and highly customizable gene overexpression technology that utilizes the mutant Cas9 protein and the wealth of available data on gene functions and their signal networks open new opportunities for research in this field.

Keywords: cell programming; stress resistance; gene overexpression; radiation; oxidative stress; chemical genotoxins; malignant transformation; diversity of mechanisms

1. Introduction

Genotoxic stress, including oxidative stress, causes DNA damage. The evolutionary conservative cellular mechanisms of DNA-damage prevention and response (DNA repair, defense against reactive oxygen species, cell cycle checkpoints and apoptosis) protect cells from mutations and tissues from marination [1,2] On the one hand genotoxic stress can induce carcinogenesis, on the other hand it is used to treat cancer. The advancement of knowledge on regulation of stress-resistance in cells and organisms is extremely important for increasing the effectiveness of cancer treatment. In particular, the creation of new in vitro models of upregulated cell resistance to genotoxic and oxidative stresses allows to expand the spectrum of in vivo models for studies of genetic regulation of carcinogenesis. In addition, it was suggested multiple times that gene therapy of normal tissues surrounding tumor can be used for increasing their resistance to genotoxins. This can help to minimize the negative side effects of cancer treatment by chemotherapy and radiation therapy [3–5]. This technology can also be used for gene therapy and gene prophylaxis of diseases associated with increased sensitivity to DNA-damaging agents [6]. Understanding the mechanisms of cellular stress resistance, and especially resistance to oxidative stress is one of the most important tasks in studies of lifespan extension [7,8]. Knowledge of stress-resistance is also important when creating new genetically modified varieties of plants and breeds of animals [9]. Additionally, the problem of prolonged exposure of astronauts to cosmic ionizing radiation is a great challenge that needs to be addressed in order to make deep space expeditions possible [10,11]. One of the possible solutions is
a pharmacological or geno-therapeutic enhancement of human radioresistance. Lastly, cell cultures with multiple enhanced stress resistance can find application in recombinant therapeutic protein production [12]. To achieve all the objectives listed above, excluding the last one, it is necessary to ensure that tissue function and cells’ ability to elicit apoptotic and cell cycle responses are both not affected as a result of genetic engineering interventions. Ideally, an increase in resistance to genotoxic stress should lead to a decrease in the frequency of somatic mutations and neotransformations at the organismal level.

The functions of many stress-response genes have been well studied. Signal-cascade networks of gene activation in response to various damaging agents have also been elucidated. Such knowledge can help identifying potential gene targets and their combinations for transcriptional activation to increase resistance to genotoxic and oxidative stress. However, without an array of experimental data, it is not possible to accurately predict the results of such activations. Moreover, it is difficult to predict the biological consequences of overexpression of the same gene to varying degrees. The discovery of the CRISPR/Cas adaptive immunity and the development of methods for its application for genome [13] and epigenome editing [14–18] significantly expands the possibilities for further studies of stress resistance programming. In particular, relatively simple and adjustable multiplex overexpression of genes by nuclease-null Cas9 (dCas9) can successfully activate multi-subunit molecular complexes or entire signal cascades. Moreover, this technology provides activation of genes in endogenous context, covering splice variants [16]. These advantages distinguish it from the previously dominant gene overexpression technology which relies on introduction of cDNA into the cell under a constantly active or inducible promoter. To date, there are very few works in the literature that used CRISP/dCac9 for gene overexpression. Most of the articles are devoted to optimization of the technology and its application in various fields of biological science. However, this technology has already begun to prove its high potential. For example, it was shown the possibilities of reactivation of silenced tumor suppressors in vitro [19] and regulation of tumor phenotypes in vivo [20]. In this regard, this review discusses the current state of knowledge about modulation of resistance of normal and cancer cells as well as whole organisms to genotoxic and oxidative stress by genes overexpression. To assess the potential of genetic regulation of stress-resistance the review also discusses transcriptomic studies in cancer cells with different levels of radioresistance.

2. The diversity of mechanisms of stress resistance in cancer cells

Mechanisms for development of genotoxic and oxidative stress resistance in tumor cells are well described in a variety of reviews [21–24]. Clearly, cells lacking the capacity for apoptosis or irreversible cell cycle arrest will exhibit a resistant phenotype due to their continued ability to proliferate even under severe genotoxic stress conditions. Continued exposure to genotoxins in a combination with abnormal response to DNA damage can lead to further loss of control mechanisms and can increase resistance to stress in tumor cells. For example, this can happen through the missense mutations in tumor suppressors. It can also be induced by a shift in a balance between homologous (HR) and non-homologous end-joining (NHEJ) in double strand break DNA repair [22]. In addition, resistance to genotoxic stress is associated with the activation of oncogenes N-ras, K-ras [25,26], MET [27], YAP [28]. Radioreistance is also associated with the activity of the Sox2 and Oct3/4 genes that induce pluripotency and stem cell-like properties in cancer cells [29].

Due to the risk of carcinogenesis the mechanisms described above cannot be used as practical targets for induction of cellular stress-resistance. However, stress resistance of tumor cells is often formed by the mechanisms that are not associated with initiation of malignant transformation. As mentioned above, when components of genome stability machinery are altered it could lead to an increase in mutation rate in tumors, and result in an increased genetic heterogeneity of cells. This heterogeneity facilitates rapid selection of subpopulations of cells that are resistant to stress [23]. The possibility of this selection-based mechanism of resistance has been repeatedly confirmed in direct selection experiments [30–32]. However, there is also evidence that stress-resistance can be induced at the epigenetic level, independently from the selection process [33]. The resistance developed by
selection or independently of it often results from overexpression of the genes encoding transporter proteins which support enhanced drug efflux [24]. In many cases, overactivation of DNA damage recognition and repair as well as detoxification of free radicals are also observed. For example, Rad51 gene which is involved in homologous recombination is overexpressed in a variety of human cancer types. This often leads to chemo-resistance of these tumors [34]. An inverse correlation was observed between the expression of the excision repair gene ERCC1 and the sensitivity to platinum treatment of various types of tumors [35]. An enhancement of excision repair activity in lung cancer cells can also be associated with a SIRT1 dependent increase in XPA sensitivity to DNA damage [36]. Expression of the antioxidant defense gene - MnSOD - correlates with resistance to doxorubicin and mitomycin C in gastric carcinoma cells [37]. RPA1 gene which is involved in DNA replication and repair is overexpressed as a result of selection of a radioresistant clone in esophageal carcinoma cell line TE-1. Inhibition of RPA1 in that radioresistant clone restored the normal sensitivity to ionizing radiation [38].

There are many other examples of a established link between genotoxic stress resistance and overexpression of genes involved in DNA repair, xenobiotic detoxification or efflux. However, the diversity of possible mechanisms of resistance seems to be evenlarger. This is supported by the studies comparing transcriptomes of similar cell lines that differ in sensitivity to genotoxic agents. For example, a comparison of ten microarray studies performed on cancer cells with different degrees of resistance to ionizing radiation did not identify any commonly overexpressed genes [39–48]. We could not find a gene that would be significantly overexpressed in three or more comparison pairs. Approximately 95 percent of the total number of overexpressed genes were observed in only one study and were absent in others (Figure 1).

Thus, the diversity of pathways leading to resistance in cancer cells, allows us to suggest a wide range of possibilities for increasing resistance of normal cells to genotoxic and oxidizing agents. We suppose, that if we exclude all targets that affect cell cycle control, apoptosis, proliferation and differentiation, we can enhance stress-resistance without the risk of increasing malignancy. Moreover, the increased efficiency of cellular defense systems should in theory lead to a decrease in carcinogenesis. This assumption is supported by the fact that the activity of DNA repair systems inversely correlates with the risk of neotransformation[49]. In addition, a decrease in alkylating agent-induced carcinogenesis has been repeatedly demonstrated upon overexpression of the gene 06-methylguanine-DNA methyltransferase (MGMT), which is responsible for DNA damage recognition and repair [50–55].

3. Genotoxic stress resistance in experimental models with gene overexpression

Change in gene transcription is only one of the existing ways of readjusting the mechanisms of stress resistance. Another way of establishing stress resistance is pharmacological targeting of proteins and signaling cascades which seem more acceptable for clinical applications. However, accumulation of experimental data on the effects of overexpression of individual genes and their combinations is required to develop pathways of stress-resistance regulation which might help finding new pharmacological targets. The literature on the effects of overexpression of stress-responsive genes on the resistance of cells and organisms to genotoxins is overwhelmingly broad. However, we attempted to systematically analyze such published experimental studies to reveal any patterns and/or commonalities. Being mindful of the scale and the variety of the published studies, in our analysis we chose a simple algorithm of grouping the target genes by their function. The resulting lists of reviewed published reports are presented in Tables 1 and 2 for in vitro and in vivo studies, respectively. One interesting, but not totally surprising, finding of our analysis was that most studies driven by a targeted hypothesis (about involvement of a particular gene in stress resistance based on previous experimental evidence) found that overexpression of the gene did increase stress resistance. On the other hand it seems that in case of randomly selected targets, the predominant outcome would be sensitization to stress, likely due to a disruption of normal gene activity regulation.

As suggested above, the two most promising gene categories for inferring resistance by overexpression are the genes involved in DNA damage recognition and repair, as well as the genes...
responsible for efflux and detoxification of xenobiotics. Overexpression of these genes tends to be the most successful strategy to enhance resistance to genotoxic stresses without the risk of increasing the frequency of neoplastic transformations. However, overexpression of these targets does not always lead to an expected/desired outcome. Firstly, an increase in survival can mask the decrease in DNA repair quality. For example, overexpression of the gene encoding DNA polymerase β in CHO cells lead to an increase in survival after treatment with cisplatin, melphalan or mechlorethamine. However, it also dramatically increased the frequency of mutations in surviving cells. It has been repeatedly shown that this is due to the fact that DNA polymerase β is the most error prone eukaryotic DNA polymerase [56–59]. Therefore, the required outcome and endpoints to be used should be carefully selected. Secondly, the effect of overexpression of various single elements of a repair or detoxification system/pathway can sometimes produce an effect that is opposite of the expected one. At the cellular level, the two main groups of reasons for this are a) the imbalance between the elements of the protective systems, and b) the absence of the expected relationship between the level of gene transcription and the activity of gene product. The latter primarily applies to all proteins whose activity depends on post-translational modifications. The mismatch between the mRNA levels and the protein function may also arise when a gene encodes only one subunit of a multisubunit protein complexes. Foreexample, stability of the DNA repair protein XPC depends on the levels of HR23A and HR23B proteins [60], therefore overexpression of XPC gene may not be sufficient to enhance nucleotide excision repair. Consistent with this, an averaged quantitative relationship between the levels of mRNA and corresponding protein tends be weak [61]. However, estimations of this correlation are still the subject of discussion and differ widely in the range from 0.21 to 0.9 [62]. In exceptional cases, for example in the case of ribosomal proteins, mRNA can be a repressor of translation of its own product. This phenomenon is known to occur for the RpS3 protein which is involved in stress responses [63].

**Figure 1.** Genes that are overexpressed in radioresistant cancer cells in comparison with parental or similar but radiosensitive cells. The results of ten studies performed with microarrays were used. Only 15 of the 337 overexpressed genes are repeated twice in different studies: * - C-JUN; † - CXCL10, IFI44, IFIH1, IFITM1, STAT1, DDX60, HERC6, IFI27, PLSCR1, IFIT1, IFIT3; ‡ - ISG15; †† - ERP70.

Numbers in parenthesis is the quantity of transcripts analyzed. * - Genes that are involved in apoptosis, DNA repair, cell cycle control, cell proliferation and other mechanisms of stress response. ** - only tumor-related genes.
The imbalance of protective systems resulting from overexpression of individual genes may be caused by several different mechanisms. First, it can be driven by the imbalance in productivity of successive stages of a single cascade. For example, a wide range of modified bases in S. cerevisiae is excised using MAG1 (3-methyladenine DNA glycosylase). The abasic sites generated by MAG1 are processed normally by the major yeast APN1-encoded AP endonuclease. Disproportionately high expression of MAG1 compared to the AP endonuclease increases spontaneous mutation by up to 600-fold in S. cerevisiae and by 200-fold in Escherichia coli [64]. CHO cells with overexpressed MPG gene are more sensitive to alkylating agent N-methyl-N'-nitro-N-nitroso-guanidine (MNNG) which is also associated with excessive accumulation of abasic sites [65].

Secondly, there are situations when an increase in resistance to one agent is accompanied by sensitization to others. For example, overexpression of APE1 increases the resistance of CHO cells to dioxolane cytidine [66], but it sensitizes cells to agents which are activated by reduction reactions. The latter takes place because the product of APE1 gene has a RedOx function in addition to AP endonuclease activity [67]. Another mechanism is a shift in balance between the two competing processes. For example, overexpression of XRCC1 required for base excision repair (BER) slows gap-filling, because of the competion of BER with nucleotide excision repair for the PCNA protein [68].

The listed nuances of regulation of resistance to genotoxic stress explain the opposite outcomes observed during the overexpression of the same genes in different experiments (Tables 1 and 2). The same opposite outcomes are observed on the level of functional groups of gene, which obtained using PANTHER classification system [69,70]. The classification shows that researchers mainly chose the genes encoding nucleic acid binding proteins and proteins that catalyzes a redox reactions. This is expected, since the many proteins of these groups are involved in DNA repair and oxidative stress defence, respectively. At the same time, if we divide the experiments based on the direction of the effect on stress-resistance, the ratio of the functional groups does not change significantly (Fig. 2). This means that we can not say that in fact overexpression of the genes of one of these functional groups more effectively increases the stress resistance than the overexpression of the genes of the other group. At the level of the whole organism, potential disruptions of functional interactions between cells, tissues, organs and organ systems are added to the intra-cellular mechanisms of imbalance listed above. But improvements in survival, decrease in frequency of mutations, fewer incidence of cancer and some others desirable outcomes are still observed as a result of overexpression of stress-responsive genes in a number of studies, which holds promise (Table 2).

In addition to the above, there are, apparently, many other factors that can radically change the influence of overexpression of certain genes on cellular stress-resistance. This is supported by the cell line specific effect of overexpression of the proto-oncogene HER2/neu in human breast and ovarian cancer cells. In six different cell lines, overexpression led to either a decrease, or an increase in sensitivity to chemotherapeutic agents of different classes [71]. These experimental data provide additional evidence in favor of the need for further studies of genetic regulation of stress resistance in normal and cancerous cells as well as stress-resistance of an organism as a whole.
**Figure 2.** The functional classification of overexpressed genes using PANTHER protein classes.

Human orthologues of genes listed in table 1 were divided into two groups, depending on the effect of their overexpression on the resistance of cells ("In vitro"). The same division was performed for orthologues of genes listed in Table 2 ("In vivo"). Each groups was classified using PANTHER classification system using Protein class ontology [69,70]. * - total number of hits of analyzed genes to "PANTHER protein class" classification.

4. Prospects

The decrease in stress-resistance of cells in the variety of experiments described above is largely due to the multicomponent nature of stress response mechanisms that the studied genes participate in. Numerous experimental data that support the high efficiency of overexpression of the MGMT gene support this assumption (Tables 1 and 2). Product of this gene solely performs recognition and repair of damaged DNA bases, in contrast to most other elements of cell protective systems that operate in cooperation with many other gene products [72]. Considering the accumulated detailed knowledge of such interactions, development of multiplex gene activation systems with mutant RNA-guided Cas9 protein opens up the widest opportunities for studying regulation of stress resistance. Multiplex activation using one large [73] or a number of small [16] plasmids, using activators with different degrees of efficiencies, allows selecting the appropriate range of activation. To some extent, the level of superactivation of individual genes can be adjusted by selecting sgRNA for sequences located at different distances from the transcription start site.

<table>
<thead>
<tr>
<th>Gene (Gene ID*; Origin if different)</th>
<th>Cells</th>
<th>Agents</th>
<th>R*</th>
<th>References</th>
</tr>
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<tr>
<td>Genes involved in DNA damage recognition and repair</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPA3 (6119)</td>
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<td>X-ray</td>
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<td>[74]</td>
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<td>XPA (7507)</td>
<td>SV-40 transformed primary human cells</td>
<td>UV</td>
<td>↑</td>
<td>[75]</td>
</tr>
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<td>APN1 (853746; yeast) coding homolog of mammalian APE1</td>
<td>Chinese hamster (CHO-9)</td>
<td>MMS</td>
<td>↑</td>
<td>[76]</td>
</tr>
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<td>APE1 (328)</td>
<td>Chinese hamster (CHO)</td>
<td>dioxolane cytidine</td>
<td>↑</td>
<td>[66]</td>
</tr>
<tr>
<td>Mammalian cells</td>
<td>γ-ray</td>
<td>0</td>
<td>[66,77]</td>
<td></td>
</tr>
<tr>
<td>Alkylating agents</td>
<td>0</td>
<td>[66,77]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chinese hamster (CHO)</td>
<td>H2O2</td>
<td>0</td>
<td>[66]</td>
<td></td>
</tr>
<tr>
<td>Mitomycin C, porfiromycin, daunorubicin and aziridinyl benzoquinone (drugs that are activated by reduction)</td>
<td>↓</td>
<td>[67]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chinese hamster XRCC1-deficient (CHO)</td>
<td>Alkylating agents</td>
<td>↓</td>
<td>[78]</td>
<td></td>
</tr>
<tr>
<td>Chimeric MGMT (4255)+APE1 (328)</td>
<td>Human cervix adenocarcinoma (HeLa)</td>
<td>Alkylating agents</td>
<td>↑</td>
<td>[79]</td>
</tr>
<tr>
<td>Ku70 (2547)</td>
<td>Human renal carcinoma 786-O</td>
<td>γ-ray</td>
<td>↑</td>
<td>[80]</td>
</tr>
<tr>
<td>Ku70 (2547; human) + Ku80 (34930; human)</td>
<td>Rat cell lines Rat-1 and R708</td>
<td>X-ray</td>
<td>↓</td>
<td>[81]</td>
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<td>DNA-PK (5591)</td>
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<td>Adriamycin</td>
<td>↑</td>
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<td>[83,84]</td>
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<td>↑</td>
<td>[85]</td>
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<tr>
<td>Mouse hybridoma cells</td>
<td>Mitomycin C</td>
<td>↑</td>
<td>[84]</td>
<td></td>
</tr>
<tr>
<td>Gene</td>
<td>Function</td>
<td>Cell Type</td>
<td>Treatment</td>
<td>Effect</td>
</tr>
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<td>------</td>
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<tr>
<td>ALC1 (9557)</td>
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<td>phleomycin</td>
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<td>Ligg III (3980)</td>
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<td>MNNG</td>
<td>↑</td>
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<td>[56]</td>
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<td>Tag (947137; E.coli)</td>
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<td>MMS, MNU, EMS</td>
<td>↑</td>
<td>[88,89]</td>
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<td>AlkA (947371; E.coli)</td>
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<td>DMS, EMS, MMS</td>
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<td>[91]</td>
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<td>MMS</td>
<td>↓</td>
<td>[92]</td>
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<tr>
<td>dOGG1 (31806)</td>
<td>Drosophila S2 cells</td>
<td>paraquat, H₂O₂</td>
<td>↓</td>
<td>[99]</td>
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<tr>
<td>OGG1 (4968; human)</td>
<td>Chinese hamster (AA8 and AS52)</td>
<td>potassium bromate or [R]-1-[[10-chloro-4-oxo-3-phenyl-4H-benzo[a]quinolizine-1-yl]-carbonyl]-2-pyrididinemethanol plus light</td>
<td>↑</td>
<td>[100]</td>
</tr>
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<td>Chinese hamster (AA8)</td>
<td>melphalan, cisplatin, UV</td>
<td>↓</td>
<td>[101]</td>
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<td>NTH (947122; E.coli)</td>
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<td>H₂O₂</td>
<td>↑</td>
<td>[102]</td>
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<td>alkylating agents</td>
<td>↑</td>
<td>[103–105]</td>
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<td>alkylating agents</td>
<td>↑</td>
<td>[103–116]</td>
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<td>aklB (946708; E.coli)</td>
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<td>MMS, EMS</td>
<td>↑</td>
<td>[125]</td>
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</table>

**Genes involved in detoxification and efflux of free radicals and xenobiotics**

<table>
<thead>
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<th>Cell Type</th>
<th>Treatment</th>
<th>Effect</th>
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<td>SOD1 (6647)</td>
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<td>γ-ray</td>
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</tr>
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<td></td>
<td>Human primary lung fibroblasts (HPLF)</td>
<td>γ-ray</td>
<td>↑</td>
<td>[127]</td>
</tr>
<tr>
<td></td>
<td>Astrocytes of mice</td>
<td>xanthine oxidase with hypoxanthine, menadione</td>
<td>↑</td>
<td>[128]</td>
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<tr>
<td></td>
<td>Brain neurons of mice</td>
<td>S-nitroso-N-acetylpenicillamine, spermine NONOate, diethylamine-NONOate</td>
<td>↑</td>
<td>[129]</td>
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<tr>
<td></td>
<td>Normal human keratinocytes</td>
<td>UV</td>
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<td>[130]</td>
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<tr>
<td>Gene</td>
<td>Cell Type</td>
<td>Treatment/Condition</td>
<td>Regulation</td>
<td></td>
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<tr>
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<tr>
<td>SOD2 (6648)</td>
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<td>cisplatin</td>
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<td>↑ [126, 127, 133, 134]</td>
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<td>paraquat</td>
<td>↑ [126]</td>
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<td>X-ray</td>
<td>↑ [135]</td>
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<td>doxorubicin</td>
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<td>ALDH3A1 (218)</td>
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<td>↑ [130]</td>
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<td>CAT (847)</td>
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<td>TRX (41737)</td>
<td>Drosophila S2 Cells</td>
<td>H2O2</td>
<td>↑ [138]</td>
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<td>MTII (17750)</td>
<td>Chinese hamster ovary cells (K1-2)</td>
<td>Cadmium chloride, MNU, MNNG</td>
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<td>Mouse C127</td>
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<td>MTII (17748)</td>
<td>Mouse embryo fibroblasts (NIH/3T3)</td>
<td>tert-butyl hydroperoxide</td>
<td>↑ [141]</td>
<td></td>
</tr>
<tr>
<td>MTII (17748)</td>
<td>Chinese hamster (V79)</td>
<td>Amscarine, menadione, arsenite, TPA</td>
<td>↑ [142]</td>
<td></td>
</tr>
<tr>
<td>MTII (17748)</td>
<td>Human gastric carcinoma cells</td>
<td>doxorubicin</td>
<td>↑ [143]</td>
<td></td>
</tr>
<tr>
<td>MTII (17748)</td>
<td>Human breast cancer cells (MDA-MB-231)</td>
<td>γ-ray</td>
<td>↑ [149]</td>
<td></td>
</tr>
<tr>
<td>MTII (17748)</td>
<td>Human non-small cell lung carcinoma (H1299)</td>
<td>Ionizing radiation (not specified)</td>
<td>↓ [150]</td>
<td></td>
</tr>
<tr>
<td>MTII (17748)</td>
<td>Normal human foreskin fibroblasts (HCA2)</td>
<td>Endonuclease induced DBS</td>
<td>↑ [161]</td>
<td></td>
</tr>
</tbody>
</table>

**Genes involved in control of proliferation and cell cycle**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cell Type</th>
<th>Treatment/Condition</th>
<th>Regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCND1 (595)</td>
<td>Human adenocarcinoma cells (MCF7)</td>
<td>γ-ray</td>
<td>↓ [144]</td>
</tr>
<tr>
<td>p21 (1026)</td>
<td>Glioma cells (T-98G, U-251MG with mutant p53 allele and U-87MG with wild-type p53), Medulloblastoma cells MED-3.</td>
<td>γ-ray</td>
<td>↑ [145]</td>
</tr>
</tbody>
</table>

**Genes involved in regulation of apoptosis**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cell Type</th>
<th>Treatment/Condition</th>
<th>Regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCL2 (596)</td>
<td>Mouse thymocytes</td>
<td>Ionizing radiation (not specified)</td>
<td>↑ [146]</td>
</tr>
<tr>
<td>BCL2 (596)</td>
<td>Rat 6 fibroblast (R6)</td>
<td>UV</td>
<td>↑ [147]</td>
</tr>
<tr>
<td>BCL2 (596)</td>
<td>Human bladder cancer cells BIU87</td>
<td>adriamycin</td>
<td>↑ [148]</td>
</tr>
<tr>
<td>BCL2 (596)</td>
<td>Mouse embryo fibroblasts (NIH/3T3)</td>
<td>γ-ray</td>
<td>↑ [149]</td>
</tr>
<tr>
<td>BCL2 (596)</td>
<td>Human breast cancer cells (MDA-MB-231)</td>
<td>γ-ray</td>
<td>↑ [149]</td>
</tr>
<tr>
<td>BCL2 (596)</td>
<td>Human non-small cell lung carcinoma (H1299)</td>
<td>Ionizing radiation (not specified)</td>
<td>↓ [150]</td>
</tr>
</tbody>
</table>

**Genes with other function**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cell Type</th>
<th>Treatment/Condition</th>
<th>Regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>USP22 (23326)</td>
<td>Human lung carcinoma cells (A549)</td>
<td>cisplatin</td>
<td>↑ [151]</td>
</tr>
<tr>
<td>IGF1R (3480)</td>
<td>Mammalian cells</td>
<td>γ-ray</td>
<td>↑ [152–156]</td>
</tr>
<tr>
<td>SirT1 (23411)</td>
<td>Hepatocellular carcinoma cells (SK-Hep1)</td>
<td>doxorubicin</td>
<td>↑ [157]</td>
</tr>
<tr>
<td>SirT1 (23411)</td>
<td>Human skin fibroblasts (HS27)</td>
<td>UV</td>
<td>↑ [158]</td>
</tr>
<tr>
<td>SirT1 (23411)</td>
<td>Human endometrial carcinoma cells (HHL/A)</td>
<td>cisplatin</td>
<td>↑ [159]</td>
</tr>
<tr>
<td>SirT1 (23411)</td>
<td>Human gastric cancer cells (SGC7901)</td>
<td>adriamycin, cisplatin, fluorouracil</td>
<td>↑ [160]</td>
</tr>
<tr>
<td>SirT1 (23411)</td>
<td>Normal human foreskin fibroblasts (HCA2)</td>
<td>Endonuclease induced DBS</td>
<td>0 [161]</td>
</tr>
<tr>
<td>BRCC3 (79184)</td>
<td>Nasopharyngeal carcinoma cells (CNE2)</td>
<td>X-ray</td>
<td>↑ [165]</td>
</tr>
<tr>
<td>Bmi1 (12151)</td>
<td>Mice hematopoietic stem cells</td>
<td>γ-ray</td>
<td>0 [166]</td>
</tr>
</tbody>
</table>
### Table 2. Effect of overexpression of stress responsive genes on resistance to genotoxic agents in vivo

<table>
<thead>
<tr>
<th>Gene (Gene ID*; Origin, if different)</th>
<th>Object</th>
<th>Overexpression specificity</th>
<th>Agents</th>
<th>R*</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genes involved in DNA damage recognition and repair</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>mus210</strong> (36697)</td>
<td>D. melanogaster</td>
<td>ubiquitous</td>
<td>γ-ray</td>
<td>0</td>
<td>[189]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>paraquat</td>
<td>↓</td>
<td>[190]</td>
</tr>
<tr>
<td><strong>mei9</strong> (31373)</td>
<td>D. melanogaster</td>
<td>ubiquitous</td>
<td>γ-ray</td>
<td>↓</td>
<td>[189]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>paraquat</td>
<td>σ↑; Ψ↑</td>
<td>[190]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>neurospecific</td>
<td>paraquat</td>
<td>↓</td>
</tr>
<tr>
<td><strong>Rrp1</strong> (33500)</td>
<td>D. melanogaster</td>
<td>ubiquitous</td>
<td>paraquat</td>
<td>σ↑; Ψ↑</td>
<td>[190]</td>
</tr>
<tr>
<td><strong>Ku80</strong> (34930)</td>
<td>D. melanogaster</td>
<td>ubiquitous</td>
<td>γ-ray</td>
<td>0</td>
<td>[189]</td>
</tr>
<tr>
<td>Gene ID</td>
<td>Organism</td>
<td>Role</td>
<td>Treatment</td>
<td>Resistant</td>
<td>ID</td>
</tr>
<tr>
<td>---------</td>
<td>--------------</td>
<td>--------------------</td>
<td>-----------</td>
<td>-----------</td>
<td>----</td>
</tr>
<tr>
<td>paraquat</td>
<td>D. melanogaster</td>
<td>ubiquitous</td>
<td>γ-ray</td>
<td>0</td>
<td>189</td>
</tr>
<tr>
<td>spinB</td>
<td>D. melanogaster</td>
<td>ubiquitous</td>
<td>γ-ray</td>
<td>0</td>
<td>189</td>
</tr>
<tr>
<td>dPrp19</td>
<td>D. melanogaster</td>
<td>ubiquitous</td>
<td>paraquat, cisplatin</td>
<td>191</td>
<td></td>
</tr>
<tr>
<td>Ada (946710; E.coli) and its truncated and modified versions</td>
<td>Mice</td>
<td>ubiquitous</td>
<td>dimethylnitrosamine, diethylnitrosamine</td>
<td>↑</td>
<td>192</td>
</tr>
<tr>
<td>mammalian</td>
<td>ubiquitous</td>
<td>hepatic</td>
<td>MNU, nitrosodimethylamine</td>
<td>↑</td>
<td>193</td>
</tr>
<tr>
<td>MGMT (4255) and its modified versions</td>
<td>Mice</td>
<td>bone marrow</td>
<td>alkylating agents</td>
<td>↑</td>
<td>118,119,123,194</td>
</tr>
<tr>
<td>Mice</td>
<td>ubiquitous</td>
<td>bone marrow</td>
<td>alkylating agents</td>
<td>↑</td>
<td>52,54,55,195–197</td>
</tr>
<tr>
<td>Mice</td>
<td>bone marrow</td>
<td>alkylating agents</td>
<td>↑</td>
<td>50,198</td>
<td></td>
</tr>
<tr>
<td>D. melanogaster</td>
<td>ubiquitous</td>
<td>paraquat</td>
<td>0</td>
<td>201</td>
<td></td>
</tr>
<tr>
<td>Mice</td>
<td>ubiquitous</td>
<td>benzo(a)pyrene</td>
<td>↑</td>
<td>202,203</td>
<td></td>
</tr>
<tr>
<td>D. melanogaster</td>
<td>ubiquitous</td>
<td>100% O2</td>
<td>0</td>
<td>204</td>
<td></td>
</tr>
<tr>
<td>EC-SOD (6649)</td>
<td>Mice</td>
<td>alveolar type II and nonciliated distal bronchial epithelial cells</td>
<td>4-MV photons</td>
<td>↑</td>
<td>205</td>
</tr>
<tr>
<td>D. melanogaster</td>
<td>ubiquitous</td>
<td>H2O2</td>
<td>↑</td>
<td>206</td>
<td></td>
</tr>
<tr>
<td>Mice</td>
<td>heart-specific</td>
<td>doxorubicin</td>
<td>↑</td>
<td>207</td>
<td></td>
</tr>
<tr>
<td>Mice</td>
<td>ubiquitous</td>
<td>benzo(a)pyrene</td>
<td>↑</td>
<td>202,203</td>
<td></td>
</tr>
<tr>
<td>Mice</td>
<td>ubiquitous</td>
<td>streptozotocin</td>
<td>↑</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>Mek (35288)</td>
<td>D. melanogaster</td>
<td>neurospecific</td>
<td>paraquat</td>
<td>↓</td>
<td>190</td>
</tr>
<tr>
<td>dGADD45 (35646)</td>
<td>D. melanogaster</td>
<td>ubiquitious</td>
<td>γ-ray</td>
<td>↓</td>
<td>189</td>
</tr>
<tr>
<td>D. melanogaster</td>
<td>neurospecific</td>
<td>paraquat</td>
<td>↑</td>
<td>210</td>
<td></td>
</tr>
<tr>
<td>D. melanogaster</td>
<td>neurospecific</td>
<td>γ-ray</td>
<td>0</td>
<td>210</td>
<td></td>
</tr>
<tr>
<td>BCL2 (596; human)</td>
<td>Mice</td>
<td>ubiquitious</td>
<td>X-ray</td>
<td>↑</td>
<td>211</td>
</tr>
<tr>
<td>WRNexo (42208)</td>
<td>D. melanogaster</td>
<td>neurospecific</td>
<td>paraquat</td>
<td>↓</td>
<td>190</td>
</tr>
<tr>
<td>Per (31251)</td>
<td>D. melanogaster</td>
<td>neurospecific</td>
<td>paraquat</td>
<td>↑</td>
<td>212</td>
</tr>
<tr>
<td>D. melanogaster</td>
<td>neurospecific</td>
<td>paraquat</td>
<td>↑</td>
<td>212</td>
<td></td>
</tr>
<tr>
<td>Cyd (40162)</td>
<td>D. melanogaster</td>
<td>neurospecific</td>
<td>paraquat</td>
<td>↓</td>
<td>212</td>
</tr>
<tr>
<td>IGFIR (13480; human)</td>
<td>KSN nude mice</td>
<td>tumor generated by transgenic HeLa cells</td>
<td>X-ray</td>
<td>↑</td>
<td>152</td>
</tr>
<tr>
<td>Sirt1 (93759)</td>
<td>Mice</td>
<td>heart-specific</td>
<td>paraquat</td>
<td>↑</td>
<td>213</td>
</tr>
<tr>
<td>VASH1 (22846; human)</td>
<td>Mice</td>
<td>intratracheally infected with adenovirus vector encoding human VASH1</td>
<td>paraquat</td>
<td>↑</td>
<td>163</td>
</tr>
<tr>
<td>D. melanogaster</td>
<td>pericerebral fat body</td>
<td>paraquat</td>
<td>↑</td>
<td>214</td>
<td></td>
</tr>
</tbody>
</table>

**Genes involved in detoxification and efflux of free radicals and xenobiotics**

- **Gclc (53581)**
- **SOD1 (6647)**
- **SOD2 (36878)**
- **EC-SOD (6649)**
- **CAT (847)**
- **MTII (17750)**

**Genes involved in control of proliferation and cell cycle**

- **Mnk (35288)**
- **dGADD45 (35646)**

**Genes involved in regulation of apoptosis**

- **BCL2 (596; human)**

**Genes with other function**

- **WRNexo (42208)**
- **Per (31251)**
- **CLOCK (38872)**
- **Cyd (40162)**
- **IGFIR (13480; human)**
- **Sirt1 (93759)**
- **VASH1 (22846; human)**
- **dFOXO (41709)**

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**Gene ID* - EntrezGene ID for the organism from which the cDNA originated. When listed experiments performed in different species the human EntrezGene ID are specified. R* - resistance estimated based on survival, growth inhibition, DNA damage, mutagenesis or neoplastic transformation and points.**
Acknowledgments: We thank Dmitry Klokov for critically reading and correcting the manuscript. The work was supported by a Grant of The President of The Russian Federation (МК-2929.2017.4)

Conflicts of Interest: The authors declare no conflict of interest.

References


34. Klein, H. L. The consequences of Rad51 overexpression for normal and tumor cells. DNA Repair 2008, 7,
Manganese superoxide dismutase expression correlates with chemosensitivity in human gastric cancer cell
J. N.; Gu, Y. M. RPA1 expression in esophageal carcinoma and its influence on radiosensitivity of
of cervical cancers to ionizing radiation according to expression profiles of 62 genes selected by cDNA
D.; Bae, D.-S. Increased expression of ICAM-3 is associated with radiation resistance in cervical cancer. Int.
Y.; Hayashizaki, Y.; Yamagishi, H. Differential gene expression profiles of radioresistant oesophageal cancer
cell lines established by continuous fractionated irradiation. Br. J. Cancer 2004, 91, 1543–1550,
doi:10.1038/sj.bjc.6602187.
radioresistance of nasopharyngeal carcinoma by integrating gene ontology and protein-protein interaction
45. Khodarev, N. N.; Beckett, M.; Labay, E.; Darga, T.; Roizman, B.; Weichselbaum, R. R. STAT1 is
overexpressed in tumors selected for radioresistance and confers protection from radiation in transduced


Mollenhauer, M.; Slotta-Huspenina, J.; Boese, J.-H.; Banitscheff, M.; Gerstmair, A.; Faerber, F.; Kuster, B.


504  Calléja, F.; Jansen, J. G.; Vrieling, H.; Laval, F.; van Zeeland, A. A. Modulation of the toxic and mutagenic
effects induced by methyl methanesulfonate in Chinese hamster ovary cells by overexpression of the rat N-
505  Bramson, J.; O’Connor, T.; Panasci, L. Effect of alkyl-N-purine DNA glycosylase overexpression on cellular
Overexpression of human DNA repair protein N-methylpurine-DNA glycosylase results in the increased
removal of N-methylpurines in DNA without a concomitant increase in resistance to alkylating agents in
507  Trivedi, R. N.; Almeida, K. H.; Fornsaglio, J. L.; Schamus, S.; Sobol, R. W. The role of base excision repair in
the sensitivity and resistance to temozolomide-mediated cell death. Cancer Res. 2005, 65, 6394–6400,
doi:10.1158/0008-5472.CAN-05-0715.
DNA Glycosylase and DNA Polymerase Expression Collectively Predict Sensitivity to Temozolomide. Mol.
509  Laval, F. Expression of the E. coli fpg gene in mammalian cells reduces the mutagenicity of gamma-rays.
510  Radyuk, S. N.; Michalak, K.; Rebrin, I.; Sohal, R. S.; Orr, W. C. Effects of ectopic expression of Drosophila
doi:10.1016/j.freeradbiomed.2006.05.021.
511  Hollenbach, S.; Dhéault, A.; Eckert, I.; Radicella, J. P.; Epe, B. Overexpression of Ogg1 in mammalian cells:
effects on induced and spontaneous oxidative DNA damage and mutagenesis. Carcinogenesis 1999, 20, 1863–
1868.
512  Harris, L. C.; Margison, G. P. Expression in mammalian cells of the Escherichia coli O6 alkylguanine-DNA-
513  Abrams, N.; Margison, G. P. Mammalian Cells Expressing Escherichia coli O 6-Alkylguanine-DNA
Alkyltransferases Are Hypersensitive to Dibromoalkanes. Chem. Res. Toxicol. 1999, 12, 544–551,
doi:10.1021/tr980250h.
514  von Hoef, E.; Fairbairn, L.; Margison, G. P. Relationship between O6-alkylguanine-DNA alkyltransferase
activity and N-methyl-N’-nitro-N-nitrosoguanidine-induced mutation, transformation, and cytotoxicity in
11203.
E. coli O6-methyltransferase gene into repair-deficient human cells and restoration of cellular resistance to


radiation-induced cell death in the human hepatocellular carcinoma cell line HLE. Cancer Res. 2001, 61, 5382–5388.


190. Davis, B. M.; Reese, J. S.; Koç, O. N.; Lee, K.; Schupp, J. E.; Gerson, S. L. Selection for G156A O6-methylguanine DNA methyltransferase gene-transduced hematopoietic progenitors and protection from...


