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Abstract: Mutations in three of the genes encoding the XPB, XPD and TTDA components of transcription factor TFIIH can result in the clinical phenotype of trichothiodystrophy (TTD). Different mutations in XPB and XPD can instead cause xeroderma pigmentosum (XP). The completely different features of these disorders have been attributed to TTD being a transcription syndrome. In order to detect transcriptional differences between TTD and XP cells from the XP-D complementation group, we have compared gene expression profiles in cultured fibroblasts from normal, XP and TTD donors. Although we detected transcriptional differences between individual cell strains, using an algorithm of moderate stringency, we did not identify any genes whose e□pression was reproducibly different in proliferating fibroblasts from each type of donor. Following UV-irradiation, many genes were up- and down-regulated in all three cell types. The micro-array analysis indicated some apparent differences between the different donor types, but on more detailed inspection, these turned out to be false positives. We conclude that there are minimal differences in gene expression in proliferating fibroblasts from TTD, XP-D and normal donors.

Cover Letter

Dear Errol

I enclose a manuscript entitled “Transcriptional changes in trichothiodystrophy cells” that we would like to be considered for publication in DNAR. This paper reports on a very careful study that we have carried out, using micro-arrays, to look for specific transcriptional differences between TTD fibroblasts and XP-D or normal fibroblasts, both in unirradiated and irradiated cells. We were not able to detect any differences that could be attributed to a TTD-specific genotype. En route our study highlights a number of potential pitfalls that need to be avoided when using such an approach. In particular using a single defective cell line and its corrected derivative might generate data that can be easily misinterpreted.

We hope that the results in this paper will be of interest to readers of DNAR.

Best regards.

Alan

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Transcriptional changes in trichothiodystrophy cells

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Key words: Gene expression, Micro-arrays, UV-irradiation, xeroderma pigmentosum

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Abstract

Mutations in three of the genes encoding the XPB, XPD and TTDA components of transcription factor TFIIH can result in the clinical phenotype of trichothiodystrophy (TTD). Different mutations in *XPB* and *XPD* can instead cause xeroderma pigmentosum (XP). The completely different features of these disorders have been attributed to TTD being a transcription syndrome. In order to detect transcriptional differences between TTD and XP cells from the XP-D complementation group, we have compared gene expression profiles in cultured fibroblasts from normal, XP and TTD donors. Although we detected transcriptional differences between individual cell strains, using an algorithm of moderate stringency, we did not identify any genes whose expression was reproducibly different in proliferating fibroblasts from each type of donor. Following UV-irradiation, many genes were up- and down-regulated in all three cell types. The micro-array analysis indicated some apparent differences between the different donor types, but on more detailed inspection, these turned out to be false positives. We conclude that there are minimal differences in gene expression in proliferating fibroblasts from TTD, XP-D and normal donors.

1. Introduction

Defects in any of seven different genes (*XPA* to *G*) involved in nucleotide excision repair (NER) can result in the genetic disorder xeroderma pigmentosum (XP), the principal features of which are extreme sensitivity of the skin to sunlight-induced pigmentation changes and skin cancers. The incidence of skin cancer is about 2000-fold greater for XP patients compared to the normal population [1], and is entirely caused by exposure to sunlight. In addition, about one third of XP patients suffer from progressive neurological degeneration [2]. Cells isolated from XP patients are hypersensitive to UV-irradiation, which can be reversed by complementation with a wild type copy of the corresponding NER gene.

Mutations in the *XPD* gene can also result in several other clinical phenotypes, the most common of which is trichothiodystrophy (TTD) [3,4]. TTD shows none of the sunlight-induced skin pigmentation changes characteristic of XP, but is a multi-system disorder, the hallmark of which is sulphur-deficient brittle hair. This is caused by a reduced expression of cysteine-rich matrix proteins in the late stage of keratinocyte differentiation in the hair shaft [5]. Other symptoms include mental retardation, unusual facies, ichthyotic skin, and reduced stature. About half of all TTD patients are photosensitive with either moderate or severe cell sensitivity to UV [6].

A clue to understanding how defects in the same gene can result in such different clinical features came with the discovery that the XPD helicase was a component of the transcription factor TFIIH, which has dual roles in NER and transcription [7]. In NER, TFIIH opens up the site around the lesion permitting excision of the damaged base, whereas in transcription TFIIH opens up the DNA at the promoter site enabling access to the transcription machinery [8]. Both processes require helicase activity, but this is provided by the XPD protein only in the case of NER [9]. For transcription

initiation, the XPD helicase does not have to be active and XPD seems only to play a structural role in maintaining the stability of the TFIIH complex.

TFIIH consists of ten subunits, five of which (XPB, p62, p52, p44 and p34) make up the core complex [10-13]. The helicase activity of XPB, required for transcription, functions in the opposite direction to that of XPD (3' to 5' for XPB and 5' to 3' for XPD) [14]. XPD is associated both with the core complex via binding to p44 and with the CAK (cyclin activating kinase) complex (cyclin H, cdk7 and MAT1), bridging both sub-complexes together. The interaction with p44 stimulates the helicase activity of XPD by 10-fold [14]. The tenth subunit, TTD-A / p8, was identified recently and binds both p52 and XPD [12,13]. Mutations in *XPB* and *TTD-A* can also result in TTD [4].

Features of XP are thought to result from defective NER, whereas TTD has been considered a transcription syndrome [15]. Consistent with this hypothesis, it has been found that, with one or two exceptions, mutations generating the XP and TTD phenotypes are located at different, mutually exclusive sites in the *XPD* gene [16]. Even though each mutated site is only found in either XP or TTD patients, there are no disease specific domains and mutations causing changes in adjacent amino acids can result in different disease phenotypes.

It has been hypothesised that the disease phenotype of TTD is caused by defects in both basal [17] and activated transcription [18-21]. Transcriptional activation by different nuclear hormone receptors is reduced in TTD cells, for example the R722W mutation in mice results in the deregulation of thyroid hormone targets in the brain [21]. Although the transcription syndrome hypothesis for TTD is attractive and has gained wide acceptance, there is to date little direct or detailed evidence to indicate the precise nature of this transcriptional deficiency, nor has any satisfactory explanation been provided as to why, despite similar NER deficiencies in XP and

some TTD cell lines, TTD patients show none of the cutaneous abnormalities found in XP. To try and gain further insight into possible transcription anomalies in TTD, we have used expression profiling on microarrays to look for transcriptional differences between TTD, XP and normal primary fibroblasts, either without treatment or following exposure to UV-C-irradiation.

2. Materials and Methods

2.1. Cell strains

Cultured primary skin fibroblasts were used in all our experiments (Table 1). All cell strains were maintained in MEM supplemented with 15% fetal calf serum at 37°C. Details of the mutation analysis of these strains have been described previously [16,22,23].

2.2. RNA synthesis

The RNA synthesis levels after UV-C-irradiation were determined as described in [24].

2.3. UV-irradiation and RNA extraction

48 h after plating, when cells were semi-confluent, they were UV-C-irradiated with the doses described in Table 1. Total RNA was extracted 12 h after irradiation using the RNeasy kit (Qiagen). To ensure that a temperature shift to room temperature before or during cell lysis did not affect changes in gene expression, the cells were lysed at 37°C. The remainder of the extraction procedure was performed at room temperature following the manufacturer's protocol. A DNase treatment was included in the purification.

2.4. Preparation of cRNA samples and hybridisation to Affymetrix chips

The total RNA samples were converted to double stranded (ds) cDNA using the 'cDNA Synthesis System' (Roche) following the standard protocol with oligo[$(dT)_{24}$ T7 promoter]$_{65}$ primer. 5µg of total RNA was transcribed per sample. The ds cDNA was cleaned-up using the 'Affy clean-up' kit (Qiagen) following the manufacturer's instructions. All of the ds cDNA obtained was then amplified and biotinylated using the 'GeneChip Expression 3'-Amplification Reagents for IVT Labeling' kit (Affymetrix) following the standard protocol. The 'Affy clean-up' kit was used again to purify the

biotinylated cRNA. The quality of the starting total RNA and biotinylated cRNA was determined using the 2100 Bioanalyser (Agilent Technologies).

2.5. Analysis of gene expression with and without UV treatment

The biotinylated cRNA samples were fragmented and hybridized to Affymetrix HG U133 Plus 2.0 arrays following the standard protocol. These whole genome arrays contain more than 54,000 probesets, allowing analysis of the expression level of >38,500 human transcripts. The data obtained were analysed using Genespring 6.0, and also by LIMMA in the R environment for statistical computing. First, the data were normalized using the GCRMA model. This method performs within-chip and between-chip normalization in one step [25]. t-tests were then performed on the normalized data employing the False Discovery rate (Benjamini-Hochberg) multiple-testing correction [26].

LIMMA uses Bayesian statistics to compute the probability of a gene being differentially expressed in any defined contrast, for example TTD compared to normal [27]. This statistical test measures the probability of an outcome, for example a gene being differentially expressed, calculated from a ratio of the probabilities of the experimental outcome and the prior assumption of the experimental outcome. A log odds value of zero corresponds to a 50-50 chance that a gene is differentially expressed. Hence, the higher the log odds, the higher the probability that a gene is not a false positive. A log odds ratio around 5 was used as a cut off to identify genes which are most likely to be differentially expressed.

2.6. GSTT1 gene analysis by PCR amplification

A 480-bp fragment of the GSTT1 gene (primers described in [28]) was amplified in a multiplex PCR with a 704-bp control fragment (primer set DJg5/g6 [29]) from genomic DNA. The PCR products were analysed on a 1% agarose gel.

2.7. Identification of enriched biological themes

The software application EASE identifies gene categories containing an over-representatively high number of differentially expressed genes among the gene lists obtained from the LIMMA analysis. EASE uses the systems of Gene Ontology (GO) as categorization system and a variation of the one-tailed Fisher exact probability to measure over-representation referred to as EASE score. Gene ontology groups with an EASE score of less than 0.05 were considered to be statistically significant. If a number of associated GO groups were identified from the same gene list the group highest in the hierarchy without being too general was used.

2.8. Quantitative real-time PCR

All kits used for this analysis were obtained from Qiagen and standard protocols were followed unless otherwise stated. Real-time PCR assays were run on the Stratagene Mx3005P instrument. Samples for analysis by qPCR were prepared as follows. The UV treatment and RNA purification was performed as described above. 2µg of total RNA was converted to single stranded cDNA using the ‘QuantiTect Reverse Transcription’ kit using the RT primer mix provided, which contains a blend of oligo-dT and random primers. Real-time PCR reactions were performed using the ‘QuantiTect Primer Assays’ and the ‘QuantiTect SYBR Green PCR’ kit. First, standard curves were set up for each primer set. Three housekeeping genes were assayed to find the best gene to use as a normaliser for the comparative

quantification. TATA-binding protein (TBP) was chosen as the most suitable gene for normalisation as its expression level was the most similar to all the genes assayed and little variation was observed among the Ct values obtained from the UV treatment samples. For each gene of interest every sample was assayed in duplicate and the log_2(fold change) was calculated comparing the average of the treated with the untreated values for each cell strain.

3. Results

The goal of our study was to determine if specific genes were expressed at different levels in TTD cells mutated in the *XPD* gene compared to XP cells mutated in the same gene and to normal cells. By this means we hoped firstly to gain insights into any transcriptional deficiencies in TTD cells and also to understand why TTD patients do not get skin cancer whereas XP patients do.

3.1. Comparison of gene expression in corrected isogenic cell lines

Our first approach was to use an isogenic system of TTD primary fibroblasts transduced with a retroviral vector expressing either *XPD* cDNA to correct the defect or the *E. coli lacZ* gene as a negative control. In triplicate experiments using TTD1BEL as a recipient, we found 36 genes whose expression was changed reproducibly more than 2-fold ($p < 0.05$) in the corrected versus uncorrected cells. Encouraged by these findings, we carried out similar experiments using another TTD cell strain, TTD7BR, as well as the normal cell strains 1BR and XP1BR XP-D cells. We found about 50 genes whose regulation was changed ($p < 0.05$) on transduction of the TTD7BR cells but not in the other two cell strains. However, there was no overlap between the genes whose expression was changed by correction of TTD1BEL and of TTD7BR cells. Thus, although statistical analysis of the data showed that the expression changes observed for each cell line were significant and consistent, the changes that we observed appeared to be cell line-specific rather than genotype-specific.

3.2. Comparison of gene expression in untreated cultured fibroblasts

Since we were interested in identifying genes that might be fundamental to the TTD phenotype, we next adopted a different approach and compared the transcriptional profiles of three normal, three XP-D and three TTD fibroblast strains (Table 1) to look

for differences that were found in all three TTD strains compared with the three XP-D and the three normal cell strains. Data were analysed using the LIMMA package.

When we compared the transcription profiles for 54,673 probe sets of untreated cells in all three groups, we found only one gene, Glutathione S-transferase Theta (*GSTT1*) that appeared to be differentially expressed (down-regulated) in all TTD strains compared to normal and XP-D cells. *GSTT1*, however, is absent from 38% of the population [30]. PCR screening of the genome of the three strains used for the microarray experiment showed that the *GSTT1* gene was indeed absent (Fig. 1, lanes 5 - 7). However, analysis of an additional four TTD cell strains mutated in the *XPD* gene showed the presence of *GSTT1* in three of them (Fig. 1, lanes 1 - 4), thus discounting a correlation between the $GSTT1^-$ genotype and the TTD phenotype.

3.3. Comparison of UV-induced changes in gene expression

In subsequent experiments we compared the transcriptional changes in TTD, XP-D and normal cells irradiated with a UV-C dose that causes a reduction to approximately fifty percent of normal RNA synthesis (Fig. 2). The question we sought to address was whether there were genes up- or down-regulated by UV in all three TTD strains that were not altered in either group of XP-D or normal cells, or vice versa. Fig. 2 shows the responses of RNA synthesis to UV-irradiation in the cell strains used in the microarray experiments. Approximately 50% inhibition was achieved with 4 Jm^{-2} for all the XP-D strains, as well as for TTD1BEL and TTD9VI, 8 Jm^{-2} for TTD7BR and 12 Jm^{-2} for the normal cells. These doses were used in the microarray experiments (Table 1). We found that a total of 5283 probe-sets were differentially expressed after UV damage in one or other cell type (Tables 2 and S1, Fig. 4). 1172 genes were induced after UV in TTD cells compared to 1294 in XP-D and 1404 in normal cells, and 1488 genes were down-regulated in TTD cells compared to 2373 in XP-D and 1155 in normal cells.

To obtain a better understanding of the biological and molecular processes and pathways affected we analysed the lists of differentially expressed genes using GenMAPP (Gene MicroArray Pathway Profiler) and EASE (Expression Analysis Systematic Explorer). Both these software packages are used to identify groups of biologically related genes showing a large number of gene-expression changes [31,32]. Both programs classify large gene lists into functionally related gene groups allowing the analysis of gene expression data in the context of biological pathways. Using both software packages we identified similar functional groups and biological processes.

Fig. 3 shows the biological themes identified with EASE. The bars represent the percentage of genes in this gene list of all differentially expressed genes of a specific contrast. For example, 56 of a total number of 1172 genes up-regulated in TTD cells after UV were in the gene ontology group ‘RNA metabolism / processing’. In general, the biological processes identified from the genes differentially expressed in TTD, XP-D and normal cells were very similar. In addition, the number of genes found in the different Gene Ontology (GO) groups as a fraction of the genes differentially expressed after UV was similar for each cell type. This is especially the case for genes that were repressed after UV, where the differences observed were minimal (Fig. 3B). The only category, which differed, was ‘transcription’ where a slightly larger proportion of genes were down-regulated in XP-D cells (10.5% compared to 8.9% in TTD and 7.4% in normal cells). The up-regulated biological pathways differed more significantly (Fig. 3A). Specifically, in four categories more genes were induced in normal cells compared to TTD and XP-D cells. For example, 3.35% of genes in the protein targeting and transport group were induced in normal cells compared to 1.28% in TTD and 1.24% in XP-D cells. A similar pattern was observed for the ‘DNA metabolism/replication’ (4.91% in normal, 3.09% in XP-D and 2.9% in TTD cells), ‘protein biosynthesis / folding’ (6.91% in normal, 4.4% in XP-D and 4.61% in TTD

cells), and ‘RNA metabolism / processing’ (7.19% in normal, 4.33% in XP-D and 4.78% in TTD cells) GO gene groups. In addition, normal cells induced a larger no of cell cycle genes (5.3%) compared to TTD (4.1%) and XP-D (3.63%) cells.

The gene lists were compared to one another to identify any overlapping genes and genes uniquely changed in one of the contrasts (Fig. 4A and B). A large number of genes were differentially expressed in all three cell types after UV-irradiation; 630 genes were down-regulated and 515 genes up-regulated. This analysis also identified genes whose expression appeared to change after UV only in one specific cell type, for example TTD cells (Fig. 4A and B), where 225 probe sets were up- and 156 probe sets down-regulated only in this cell type. In addition we identified genes whose expression appeared to change in normal and XP-D but not in TTD cells. We analysed the raw data for all nine cell strains in more detail for all of these genes. It became evident that most of the apparent differences were false positives, due to either (1) very low gene expression levels such that the data were unreliable, or (2) increased scatter of the data from cells with one phenotype so that UV-induced changes with this phenotype did not reach statistical significance, whereas they were significant with the other phenotypes. Careful analysis of all the individual expression values enabled us to eliminate as false positives all but 11 of the probe-sets as candidates for differential expression between the different phenotypes. Nine of these eleven genes were chosen for further analysis by qPCR (Fig. S1). For eight of these, qPCR did not confirm the differences suggested by the microarrays. Only one of the genes analysed, *HOXC4*, was confirmed to be down-regulated after UV specifically in TTD cells (Fig. S2). We therefore analysed three additional TTD cell strains with different *XPD* mutations and the UV-induced change in *HOXC4* gene expression was measured by qPCR. However, the specific repression of this gene after UV-irradiation could not be confirmed in these cells (data not shown).

4. Discussion

We have used two different approaches to look for transcriptional differences between TTD cells on the one hand and XP and normal cells on the other hand. The first approach entailed comparing the transcriptional profiles of cells in which the phenotype has been corrected by introduction of the wild-type *XPD* gene. A similar approach was used by Costa et al. with XP-B cells [33]. They used a cell line from a patient with the combined features of XP and CS as recipient for *XPB* cDNA containing either the same mutation as found in the recipient cell or a mutation found in a TTD patient. This approach has the advantage that comparisons are made between isogenic cells, and both we and Costa et al. were able to identify differences in expression of several genes between the test and control samples. In our study however, we found that the changes in gene expression on correction of the phenotype differed between cell lines. In the study of Costa et al. only one recipient cell line was used, so we do not know if the changes that they observed would be confirmed using other recipient cells. In our experiments we found that the *XPD* gene was over-expressed up to 30-fold in the transduced cells, and this in itself could result in some distortion of the expression profiles. Selzer et al. also employed this approach to investigate the role of the CSB protein in basal transcription [34]. They used the Atlas human arrays (746 genes) and NIA cDNA arrays (15000 ESTs) to look for differential gene expression between a CS-B fibroblast line stably transfected either with functional *CSB* or empty vector. In this study however, the authors did not find any significant differences in the expression of the genes assayed between the two isogenic cell lines. Our results emphasize the importance of using several different cell lines from a particular disorder in this type of analysis.

Despite the attraction of the isogenic system, we decided that it was important to compare cohorts of unrelated TTD, XP and normal cells and to look for common differences between the cohorts. It has been suggested that the developmental

defects resulting in the features of TTD are caused by deficiencies in transcription [15]. When we analysed gene expression profiles in untreated TTD, XP-D and normal proliferating fibroblasts we could not identify any phenotype-specific differences. In a recent study, Dorioli et al found a deficiency in TTD fibroblasts in upregulation of the *COL6A1* gene when the cells entered confluence (personal communication). This difference in expression was, as in our study, not seen in proliferating cells. As the symptoms of TTD appear to be mainly caused by developmental defects, it is possible that significant differences in transcription only occur and/or can be measured in differentiating cells (as has been suggested by de Boer et al. [35]) and not in proliferating skin fibroblasts. Interestingly, transcriptional differences in differentiated tissue were observed in the TTD mouse (XPD^{R722W}) [17] and in erythrocytes from TTD patients [22]. The phenotype of the TTD mouse mimics the human condition extremely well, including the skin conditions observed in patients. The authors analysed the transcription levels of the *SPRR2* gene, a member of a family of small proline-rich proteins, which is expressed late in terminal differentiation of interfollicular keratinocytes [36]. They found that transcript levels were reduced by 2.5- to 3-fold in the dorsal skin of TTD mice compared to wt [17].

Many genes are known to be up- or down-regulated following exposure to DNA damaging agents and there are several reports on expression profile changes following UV-irradiation of human cells [33,37-40]. Likewise we observed many genes clearly up- or down-regulated following UV-C-irradiation of human fibroblasts. Several categories, DNA metabolism, replication and repair, RNA metabolism and processing, protein biosynthesis, folding and targeting, cell cycle, chromosome organisation and a number of other pathways contained both genes that were up- and down-regulated. Biological processes that seemed to only contain repressed genes include phosphate metabolism, proteolysis and peptidolysis, signal transduction, and telomere maintenance. GO groups specifically up-regulated

included nitrogen metabolism, nucleotide metabolism, and ribosome biogenesis and assembly. Similar GO groups affected by UV-irradiation were identified in other studies. Koch-Paiz et al. for example also found genes in DNA repair, cell cycle regulation, transcription, protein modification and chromatin related affected by UV-C [38]. Microarray analysis of rat cardiac fibroblasts identified similar groups as targets after UV [40]. They found up-regulation of a number of processes involved in regulation of the cell cycle, protein metabolism, transcription, translation and others. They also identified groups, which seemed to be unique to the cell system they were using like cholesterol biosynthesis and lipid metabolism.

Using the second approach, we identified a number of genes that appeared to be differentially expressed after UV only in TTD, but not in XP-D or normal cells. However, more careful analysis of the data and further tests using quantitative RT-PCR showed that these were all false positives. In conclusion, using the two experimental systems described above we were not able to identify, in proliferating fibroblasts, any transcriptional differences specific for TTD cells that could explain the symptoms of the disease or the lack of UV-induced skin cancer in TTD patients. Thus despite the clear evidence for a 40-60% decrease in the *in vitro* transcriptional activity of TFIIH containing TTD-specific mutant XPD protein [9] and the demonstration that TFIIH levels in TTD fibroblasts are only 40-60% of those in normal and XP fibroblasts [41], remarkably these deficiencies appear to have a minimal effect on the gene expression profile in untreated or UV-irradiated TTD fibroblasts. This further reinforces the hypothesis of [17] that the transcriptional deficiency associated with TTD-specific mutations is only manifest in specialised tissues dependent on high levels of active TFIIH.

Acknowledgements

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Table 1 - Primary fibroblast cultures and treatments

TTD		**XP**		**wt**	
Cell line[a]	UV treatment	Cell line	UV treatment	Cell line	UV treatment
TTD1BEL (R722W)	0, 4 J/m^2	XP1DU (R683W)	0, 4 J/m^2	1BR	0, 12 J/m^2
TTD7BR (R487G)	0, 8 J/m^2	XP1BR (R683W)	0, 4 J/m^2	48BR	0, 12 J/m^2
TTD9VI (R112H)	0, 4 J/m^2	XP30BR (R683Q)	0, 4 J/m^2	245BR	0, 12 J/m^2

[a]For each cell line the causative mutation is indicated

Table 2 - Genes differentially expressed after UV damage

Number of genes meeting significance criterion Benjamini-Hochberg p<0.05	TTD4-TTD0	XP4-XP0	WT12-WT0
Down-regulated	1488	2373	1155
No change	52015	51008	52116
Up-regulated	1172	1294	1404

Fig. 1 - *GSTT1* analysis in TTD cell strains.

The presence of the *GSTT1* gene was analysed by PCR in the three TTD cell lines used for the microarray analysis, and in addition in cell strains TTD11PV (XPD R112H), TTD2GL (XPD R112H), TTD1BR (TTD-A) and TTD1BI (XPD frameshift 730). Two individuals with wt *XPD* were used as negative and positive controls for *GSTT1*.

Fig. 2 - Recovery of RNA synthesis after UV-C-irradiation

Cells were UV-irradiated with the indicated doses and RNA synthesis measured by incorporation of ^{3}H uridine 16 h later.

Fig. 3 - Biological processes specifically induced (A.) or repressed (B.) after UV-C-irradiation.

The software application EASE was used to discover enriched biological themes by identifying gene categories containing an over-representatively high number of differentially expressed genes among the gene lists obtained from the LIMMA analysis. Black bar; normal cell strains. Grey bar; XP-D strains. White bar; TTD strains.

Fig. 4 - Analysis of UV treated compared to untreated cells.

Venn diagrams of genes identified to be differentially expressed after UV treatment: A. up-regulated after UV. B. down-regulated after UV.

Fig. S1 - Gene expression data repeated by qPCR.

To confirm the data obtained from the microarray analysis, RNA was isolated from a separate UV-irradiation experiment and gene expression was assayed using qPCR. Expression levels of the TATA binding protein (TBP) were used to normalise the expression data obtained for the different genes of interests. Expression levels of

eight genes were assayed. The same cell strains as used in the microarray were assayed. 1BR, dark blue. 48BR, turquoise. 245BR, light blue. XP1DU, organge. XP30BR, dark yellow. XP1BR, light yellow. TTD7BR, dark green. TTD1BEL, medium green. TTD9VI, light green. A $\log_2$(fold change) of x means that the gene was upregulated 2^x-fold.

Fig. S2 - Gene expression of HOXC4 repeated by qPCR.

To confirm the data obtained from the microarray analysis, RNA was isolated from a separate UV-irradiation experiment and gene expression was assayed using qPCR. Expression levels of the TATA binding protein (TBP) were used to normalise the expression data obtained for HoxC4. Expression levels were plotted as the log2 of the fold change.

Figure

TTD cells

TTD11PV TTD2GL TTD1BR TTD1BI TTD7BR TTD9VI TTD1BEL

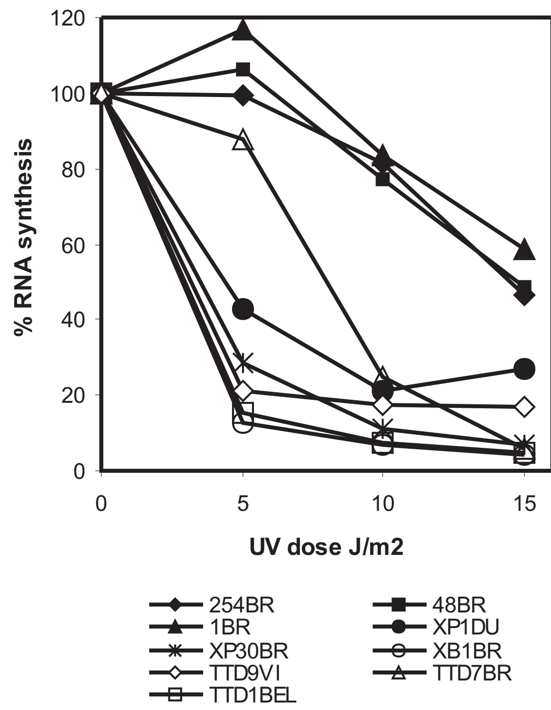
controls

$GSTT1^+$ $GSTT1^-$

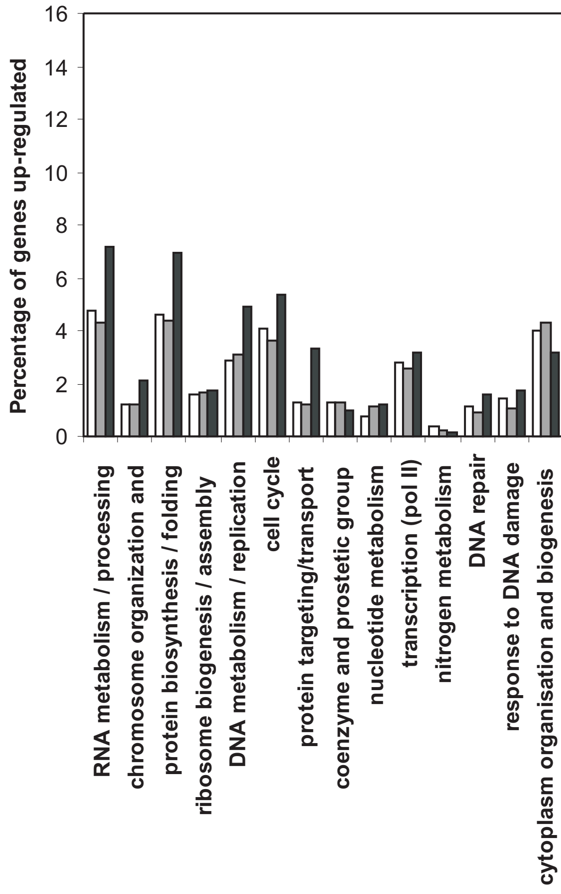
Control

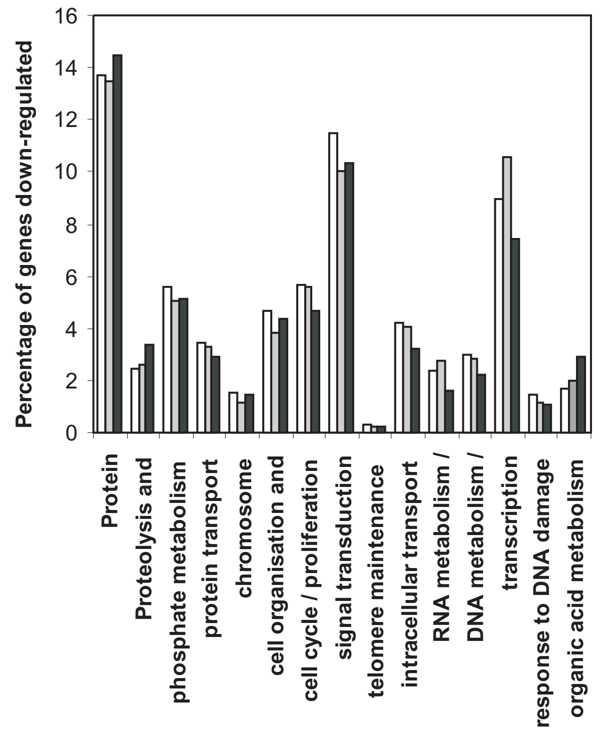
GSTT1

Figure



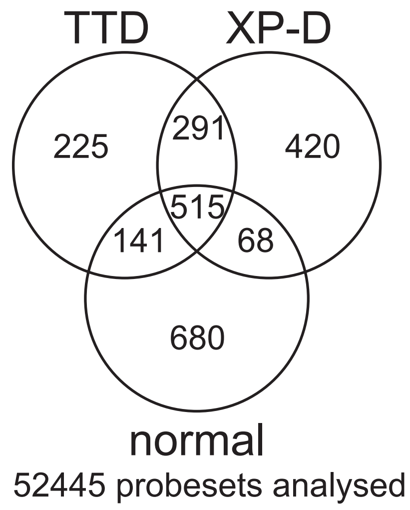
Figure





Figure

(A)



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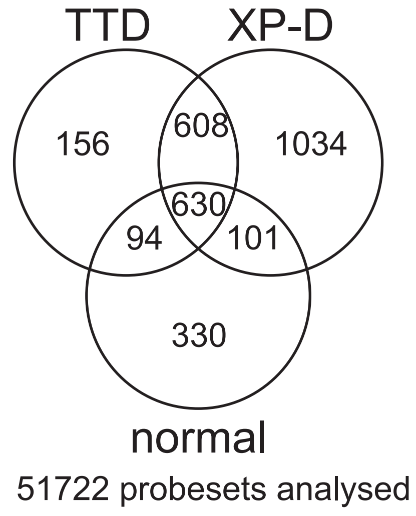

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