Nucleotide Excision Repair Gene Expression after Cisplatin Treatment in Melanoma

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Abstract

Two of the hallmark features of melanoma are development as a result of chronic ultraviolet (UV) radiation exposure and the limited efficacy of cisplatin in treatment. Both of these DNA damaging agents result in large helix distorting DNA damage that is recognised and repaired by nucleotide excision repair (NER). The aim of this study was to examine the expression of NER gene transcripts, p53 and p21 in melanoma cell lines treated with cisplatin compared to melanocytes. Basal expression of all genes was greater in the melanoma cell lines compared to melanocytes. Global genome repair (GGR) transcripts showed significantly decreased relative expression (RE) in melanoma cell lines 24 hours after cisplatin treatment. The basal RE of p53 was significantly higher in the melanoma cell lines compared to the melanocytes. However, induction of p53 was only significant in the melanocytes at 6 and 24 hours after cisplatin treatment. Inhibition of p53 expression significantly decreased the expression of all the GGR transcripts in melanocytes at 6 and 24 hours post cisplatin treatment. Although the RE levels were lower with p53 inhibition, the induction of the GGR genes was very similar to the control melanocytes and increased significantly across the time-points. The findings from this study revealed reduced GGR transcript levels in melanoma cells 24 hours after cisplatin treatment. Our findings suggest a possible mechanistic explanation for the limited efficacy of cisplatin treatment and the possible role of UV light in melanoma.
Introduction

Two of the hallmark features of melanoma are the development of disease as a result of chronic repeated exposure to ultraviolet (UV) radiation (1, 2); and the limited efficacy of the DNA-damaging agent, cisplatin in disease treatment (3). Both of these agents result in large helix distorting DNA damage that is recognised and repaired by the DNA repair pathway, nucleotide excision repair (NER). NER is a versatile DNA repair system that eradicates UV-light induced lesions such as cyclobutane pyrimidine dimers (CPD) and pyrimidine (6-4) pyrimidine photoproducts (6-4PP) as well as lesions induced by many chemical compounds including cisplatin (4-6). The importance of the NER pathway is very evident in the rare autosomal recessive disease xeroderma pigmentosum (XP). Patients with XP have a drastically diminished NER capacity, therefore DNA damage accumulates rapidly after UV-light exposure, resulting in up to a 1000 fold increase in development of skin cancers on sunlight exposed areas of skin (7). Despite this, the role of NER in the development of melanoma in the general population as a result of UV radiation has not been thoroughly investigated. To date, only weak evidence of a genetic association of NER related genes with melanoma have been reported (8-11).

Another example of DNA damage recognised and repaired by NER is intra- and inter-strand crosslinks induced by DNA damaging or cross-linking agents. The most commonly used example of a DNA damaging agent is cisplatin. Despite the high level of efficacy of cisplatin treatment for many types of malignancies, several factors such as cell resistance and adverse drug reactions have undermined its therapeutic potential. In melanoma, the efficacy of cisplatin is limited (12) which is in contrast to most germ-cell tumours with cure rates of over 90% (13). This contrast in efficacy is yet to be explained and in recent years many studies have focused on elucidating the anti-cancer mechanism of cisplatin. NER is now known to be a vital component of this process however,
studies investigating the role of NER in cisplatin efficacy and resistance are yet to be conducted in melanoma.

NER consists of approximately 30 proteins that remove helix-distorting lesions via four steps: 1) recognition of the DNA lesion, 2) opening of a bubble around the lesion, 3) incision of the DNA upstream and downstream of the lesion by endonucleases and 4) DNA resynthesis and ligation (14). There are two damage recognition arms of the NER pathway, global genome repair (GGR) and transcription coupled repair (TCR). GGR encompasses the non-coding parts of the genome, silent genes, and the non-transcribed strand of active genes (15). TCR ensures that the transcribed strand of active genes is repaired with higher priority than the rest of the genome, by using RNA polymerase II (RNAPII) as a lesion sensor (15). Once the damage is recognised via one of these processes the remainder of the repair process follows a convergent pathway (7).

The tumour suppressor protein p53 has recently been implicated in transactivation of the GGR components of the NER pathway following DNA damaging events (16, 17). p53 plays an important role in many cellular responses to DNA damage, including cell cycle arrest and apoptosis. Recent evidence suggests that p53 is essential for both basal and induced GGR response to certain types of UV and chemical induced DNA damage (18-21). The role of p53 in regulating GGR seems to be mediated in part by its ability to transactivate the gene transcripts encoding the GGR proteins, DDB2 and XPC.

In contrast with other tumour types, melanoma has a relatively infrequent p53 mutation rate of only around 9% (reviewed in(22, 23). In addition, p53 protein and mRNA expression have been reported in melanoma, with reports showing increases in p53 with tumour progression (24-27). The presence of wild-type p53 expression in melanoma indicates other events that bypass p53 must be occurring.
for tumour development and/or progression to occur. One candidate event could be the lack of transactivation of GGR in response to DNA damage. Given the limited knowledge available detailing the role of NER in melanoma, the aim of this study was to determine the level of NER activity and its relationship with p53 in response to cisplatin induced DNA damage in melanocytes and melanoma cell lines.
Methods

Cell Lines and Cisplatin Treatment:

One melanocyte, three primary melanoma (MM200, IgR3, Me4405) and two metastatic melanoma (Mel-RM and Sk-mel-28) cell lines were used for this study. The derivation of MM200, IgR3, Me4405, Mel-RM and Sk-Mel-28 melanoma cell lines has been described previously (28-31). Melanocytes were purchased from Cascade Biologics at the commencement of this study. DNA for cell line authentication was extracted from all the cell lines whilst cultured for this study. Individual cell line authentication was confirmed using the AmpFISTR Identifiler PCR Amplification kit from Applied Biosystems and GeneMarker V1.91 software. A panel of 16 markers were tested and each cell line had a distinct individual set of markers present.

All of the melanoma cell lines were cultured in DMEM (5% FCS) and the melanocytes were cultured in Medium 154 (Cascade Biologics). All cell lines were maintained in exponential growth at 37°C and 5% CO₂. Cells were treated with 10μg/mL cisplatin (Pharmacia Upjohn) as previously described (28) and were harvested before treatment and 6 and 24 hours after treatment for gene expression analysis.

Inhibition of p53 by shRNA:

Constructs

Short hairpin RNA (shRNA) sequences to p53 or a control were expressed in the pSIHI-HI-copGFP (Copepod green fluorescent protein) shRNA expression vector (Systems Biosciences, Mountain View, CA, USA) The p53-directed shRNA sequence corresponds to nucleotides 1026-1044 (Genebank accession number NM_000546) (32). The control shRNA sequence 5’-TTAGAGGCGAGCAAGACTA-3’ showed no homology to any known human transcript.

Stable transduction of melanoma cell lines
Lentiviruses were produced in HEK293T cells using the pSIH1-H1-copGFP (Copepod green fluorescent protein) shRNA expression vector (Systems Biosciences, Mountain View, CA) encased in viral capsid encoded by three packaging plasmids as described previously (33). Viruses were concentrated as described previously (34). Viral titres were determined using $1 \times 10^5$ U2OS cells/well in 6-well plates, transduced with serial dilutions of the concentrated viral stocks in the presence of Polybrene ($8\mu$g/ml; Sigma, Castle Hill, NSW, Australia). Cells were harvested 48 hours post-transduction, analysed by flow cytometry for copGFP expression and viral titre calculated.

To generate a p53 silenced stable melanocyte cell line, melanocytes were transduced at an MOI of 10 with either a virus encoding p53 shRNA or a control shRNA that has no homology to any human gene. Cells were transduced twice with three days in between each transduction. The efficiency of transduction was monitored with co-expression of copGFP and was consistently over 95%. All cell lines tested negative for the presence of replicative competent virus using the Retrotek HIV-1 p24 antigen ELISA kit (ZeptoMetrix Corporation; Buffalo, NY, USA).

Sample Preparation and Real-time PCR:
Total RNA was extracted from all of the cell lines after cisplatin treatment using the SV Total RNA Isolation System Kit (Promega). The total RNA was quantified using the Quant-iT RiboGreen RNA Assay Kit (Invitrogen) and a fluorometer (BMG Labtech). To ensure consistency across all the samples being tested, a standardised amount (500ng) of total RNA was reverse transcribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) and a 1/20 dilution of the resultant cDNA was used in triplicate for each sample. Relative gene expression was measured in triplicate and normalised to $\beta$-Actin ($\Delta$Ct) using TaqMan® gene expression assays (Applied Biosystems) and a 7500 real-time PCR system (Applied Biosystems) for the following gene transcripts: DDB2 (XPE) (Hs00172068_m1), DDB1 (Hs_00172410_m1), XPC (Hs01104206_m1),
ERCC8 (CSA) (Hs01122123_m1), ERCC6 (CSB) (Hs00972920_m1), XPA (Hs00166045_m1), RPA1 (Hs00161419_m1), RPA2 (Hs00358315_m1), ERCC3 (XPB) (Hs01554450_m1), ERCC2 (XPD) (Hs00361161), ERCC5 (XPG) (Hs00164482), ERCC4 (XPF) (Hs00193342), ERCC1 (Hs01012161_m1), p53 (Hs00153340_m1) and p21(Hs00355782_m1). To ensure β-Actin itself did not change between cell lines and/or treatments the ratio of β-Actin to a second reference gene, GAPDH, was measured. The average β-Actin/GAPDH ratio was 1.02 ± 0.04 across all the individual cell lines and treatment timepoints.

**Statistical Analysis:**

Relative gene expression was calculated using $2^{-\Delta Ct}$ and unpaired, 2-tailed t-tests (p<0.05) were used to identify significantly altered expression in melanoma compared to melanocytes as described previously (35).

**Western Blot Analysis:**

Protein extraction, separation by SDS-PAGE, and Western blot analysis of cell lines was done as described previously (28). The mouse monoclonal antibody, Bp53-12, used for the detection of p53 was purchased from Upstate. The mouse monoclonal antibody for the housekeeping gene GAPDH was purchased from Ambion.
Results

Global Genome Repair

The relative expression (RE) of the Global Genome Repair (GGR) gene transcripts, XPC, DDB1 and DDB2 (XPE), was measured at 0, 6 and 24 hours after cisplatin treatment in melanoma and melanocyte cell lines. The RE of all 3 GGR transcripts was higher at the basal level and 6 hours post-treatment in the melanoma cell lines, although not significantly different when compared to melanocytes (Figure 1). The expression of these transcripts significantly increased in a time-dependent manner following cisplatin treatment in melanocytes. No cisplatin-dependent increase of these transcripts was observed in melanoma cell lines (Figure 1). The induction of XPC, DDB1 and DDB2 (XPE) relative to the basal expression level was measured at 6 and 24 hours post-treatment. At 24 hours after cisplatin treatment, there was a 38.77 (p=0.0003), 12.27 (p=0.0005) and 81.46 (p=0.0002) fold increase in RE in the melanocytes, respectively (Figure 2). In comparison, a 1.33 fold decrease in expression was observed for DDB1 in the melanoma cell lines and only a small fold increase in RE was observed for XPC (3.68) and DDB2 (1.84), all of which were not significant (Figure 2).

Clearly, a significantly lower RE level of the GGR genes was observed in the melanoma cell lines, 24 hours after cisplatin treatment (Figure 1). In addition, a significant increase in induction of these transcripts was observed in the melanocytes but was not observed in the melanoma cell lines (Figure 2).

Transcription Coupled Repair

The RE of the Transcription Coupled Repair (TCR) gene transcripts, ERCC6 (CSB) and ERCC8 (CSA), was very low in all of the melanoma and melanocyte cell lines at all time points. However, the RE for ERCC 6 (CSB) was significantly lower (p=0.005) in the melanoma cell lines (1.34) at
the 24 hour time point compared to the melanocyte cell line (6.52). For ERCC8 (CSA) RE was significantly higher in the melanoma cell lines (0.99) than the melanocyte cell line (0.14) at the 6 hour time point (p=0.04). No significant differences in RE between the melanoma and melanocyte cell lines were observed for ERCC6 (CSB) or ERCC8 (CSA) at all other time points. The relatively low level of expression of all the TCR transcripts at all time points suggests limited involvement of TCR in response to cisplatin induced DNA damage.

**Nucleotide Excision Repair**

The Nucleotide Excision Repair (NER) related genes, XPA, RPA1, RPA2, ERCC1, ERCC2 (XPD), ERCC3 (XPB), ERCC4 (XPF), ERCC5 (XPG), showed an overall trend of higher basal expression in melanoma cell lines compared to the melanocyte cell line (Table 1). Specifically, the basal RE of RPA1, RPA2, ERCC2 (XPD), ERCC3 (XPB) and ERCC4 (XPF) was significantly greater in the melanoma cell lines. In addition, RPA1 and RPA2 RE levels were significantly greater in the melanoma cell lines compared to the melanocytes cell line 6 hours after cisplatin treatment, but the expression levels were similar at 24 hours. In contrast to this, ERCC3 (XPB), ERCC4 (XPF) and ERCC5 (XPG) RE levels were significantly lower in the melanoma cell lines than the melanocyte cell line 24 hours after cisplatin treatment. In the melanoma cell lines, ERCC2 (XPD) and XPA were slightly increased in RE from 0 to 24 hours after cisplatin treatment however this was not significantly different to the RE observed in the melanocytes at the 24 hour time point (Table 1).

**p53**

p53 is currently thought to transactivate expression of the GGR genes DDB2 and XPC in response to DNA damage. The RE of p53 was measured in all the cell lines after 0, 6 and 24 hours cisplatin treatment. The RE of p53 was significantly higher in the melanoma cell lines compared to the melanocytes at 0 and 6 hours after cisplatin treatment but not at 24 hours (Figure 3). Although
transcript levels of p53 were higher in the melanoma cell lines at 0 and 6 hours, the induction of p53, was of borderline significance at 24 hours (3.91 fold increase, p=0.05). Conversely, induction of p53 was significant in the melanocytes at 6 hours (1.6 fold increase, p=0.0001) and 24 hours (19.57 fold increase, p=0.007) after cisplatin treatment (Figure 3).

p21

Increased expression of p21 is indicative of p53 activation in response to DNA damage. The RE of p21 was measured in all cell lines after 0, 6 and 24 hours of cisplatin treatment. The RE of p21 appeared to be higher in melanoma cell lines at the 0 and 6 hour time points, but it was not statistically significant. Conversely, the RE of p21 was significantly lower in the melanoma cell lines compared to the melanocytes cell line after 24 hours cisplatin treatment (Figure 3). Although transcript levels of p21 were higher in the melanoma cell lines at 0 and 6 hours, the induction of p21 was not significantly elevated by 24 hours compared to the melanocytes cell line. Induction of p21 was observed in the melanocyte cell line at 6 hours (9.85 fold increase, p=0.00004) and 24 hours (142.44 fold increase, p=0.002) after cisplatin treatment (Figure 3).

GGR transcript expression with shRNA inhibition of p53.

To determine if GGR transcript level changes in the melanoma cell lines were related to the absence of p53 knock-down experiments were undertaken using the melanocyte cell line. The transcript level of all the GGR genes, XPC, DDB1 and DDB2 was significantly lower in the p53 shRNA melanocyte cell line compared to the control melanocyte cell line at 6 and 24 hours post cisplatin treatment. The basal transcript levels however, were not significantly different (Figure 4). Although the RE levels were lower in the p53 shRNA melanocyte cell line, the induction of the GGR genes was very similar to the control melanocyte cell line and increased significantly across the time-points (Figure 5).
shRNA inhibition of p53 and its effect on p21 transcript expression.

shRNA was used to inhibit the transcript expression of p53 in the melanocyte cell line. To confirm inhibition of p53 transcription, p53 and p21 transcript expression was measured in the control shRNA melanocyte cell line and the p53 shRNA melanocyte cell line. The RE of p53 was 8.12 fold lower (p=0.01) in the p53 shRNA melanocyte cell line than the control melanocyte cell line. Similarly, The RE of p21 was 2.33 fold lower (p=0.07) in the p53 shRNA melanocyte cell line than the control melanocyte cell line. In addition, western blots were used to confirm inhibition of p53 protein levels in the p53 shRNA melanocyte cell line (Supplementary data).
Discussion

Despite overwhelming evidence that one of the primary causal agents of melanoma is chronic repeated exposure to ultraviolet (UV) radiation, the exact relationship between it and disease remains to be fully elucidated. Cisplatin is a common DNA-damaging agent that is used in the treatment of many types of cancer, the efficacy of cisplatin in the treatment of melanoma, however is very limited (12). Cisplatin binds to DNA forming helix distorting intra- and inter-strand cross-links (36, 37) which must be removed prior to either transcription or replication. The removal and repair of large helix distorting DNA damage induced by both UV-light and DNA-damaging agents such as cisplatin is orchestrated by NER. The aim of the present study was to examine changes in gene expression of key constituents of the NER pathway in melanoma cell lines treated with cisplatin compared to a control melanocyte cell line and to examine the relationship to the cell cycle checkpoint control gene, p53.

GGR operates throughout the entire genome and is a crucial step in the initial recognition of DNA damage (38). The intra and interstrand crosslinks caused by cisplatin are recognised by the GGR component XPC then the DDB1/DDB2 complex is recruited to bind specifically to the large helix distorting DNA adducts (38). Thereafter, the repair process proceeds through the rest of the NER pathway. Previous studies have identified a strong correlation between reduced XPC mRNA and protein levels and increased resistance of cancer cells to cisplatin treatment (39-41). In this study, the genes involved in the GGR pathway, XPC, DDB1 and DDB2 (XPE), were shown to have no increase in RE in the melanoma cell lines after cisplatin treatment. In contrast, a significant increase in RE 24 hours after cisplatin treatment was seen in the melanocyte cell line. These results demonstrate that following cisplatin treatment, the GGR genes are poorly induced in melanoma cell lines compared to melanocytes.
The most important mechanism for cisplatin’s anti-cancer action is recognition of cisplatin induced DNA damage which triggers cell death. This process is known as DNA damage-mediated apoptotic cell death (40). The cisplatin treatment-mediated p53 response and activation of caspase-3, both key mechanisms involved in triggering apoptosis, are significantly reduced in XPC-defective cell lines, which suggests XPC plays a critical role in initiating cisplatin DNA damage-mediated apoptosis (40, 41).

In addition to the key role XPC plays in DNA damage-mediated apoptosis after cisplatin treatment, DDB2 also has a role in this process. DDB2-deficient cells exhibit enhanced resistance to cell growth inhibition and apoptosis induced by cisplatin(42, 43) and DDB2 expression in cisplatin-resistant ovarian cancer cell lines is lower than their cisplatin-sensitive parental cells (42). Overexpression of DDB2 sensitizes cells to cisplatin-induced cytotoxicity and apoptosis via activation of the caspase pathway and downregulation of antiapoptotic Bcl-2 protein (42).

What we have observed in the melanoma cell lines is a failure to up-regulate key damage recognition genes including XPC and DDB2, in response to cisplatin induced DNA damage. The failure to illicit the appropriate response of the GGR pathway after cisplatin treatment suggests that the normal relationship between DNA damage recognition and subsequent apoptosis is no longer functionally accomplishable in these melanoma cell lines. The low expression of XPC and DDB2 may therefore, play a key role in cisplatin resistance in melanoma.

Basal expression levels of the GGR genes, XPC, DDB1 and DDB2 (XPE) appeared to be greater in the melanoma cell lines compared to the melanocytes. In addition, the NER genes, RPA1, RPA2, ERCC3 (XPB), ERCC2 (XPD) and ERCC4 (XPF), showed significantly higher basal level expression in the melanoma cell lines compared to the melanocytes. Increased basal expression of
several NER genes has been previously related to cisplatin resistance (44). Specifically, ovarian tumours resistant to cisplatin treatment have shown a high level of expression of XPA, ERCC1, XPF and ERCC3 (XPB). Conversely, testicular cancers display low expression of XPA, ERCC1 and XPF and are highly responsive to cisplatin (reviewed by (44). Even though only a subset of the NER genes previously reported to have higher basal expression in relation to cisplatin resistance were identified in this study, overall, increased basal level expression of NER genes appears to be associated with cisplatin resistance. Taken together with the results of this study, this could provide some explanation as to why cisplatin is ineffective in melanoma treatment.

p53 is a critical regulator of NER both at the basal level and in the induction of GGR following UV-light and chemically induced DNA damage (18-21, 45). The role of p53 in regulating GGR appears to be mediated in part by its ability to transactivate gene transcripts encoding the GGR proteins, DDB2 and XPC. In this study a key downstream p53 target gene, p21, showed a similar trend to p53, indicating that altered expression of p53 was indeed affecting downstream signalling. We confirmed that induced p53 and GGR gene transcript levels occur in concert in melanocytes 24 hours after cisplatin treatment. In melanoma cell lines studied herein the relationship is not as clear. Basal levels of p53 are significantly higher in melanoma cell lines relative to the basal levels of the majority of the GGR genes. Furthermore, the RE of p53 after 24 hours cisplatin treatment is remarkably similar in melanoma and melanocyte cell lines, but the RE of the GGR genes in the melanoma cell lines is very low. The most likely explanation for this occurrence is the difference in the induction of p53 after cisplatin treatment in the melanoma cell lines compared to the melanocytes. There is a highly significant increase in p53 induction at the 6 and 24 hour time points for the melanocytes but the melanoma cell lines fail to respond as rapidly and p53 is only differentially expressed after 24 hours (p=0.05). This suggests that it is the induction of p53 that is required for GGR activation rather than just a high constitutive level of p53 expression.
The current understanding of the relationship between p53 and GGR relies on the hypothesis that an increase in p53 results in transactivation of GGR transcript expression (16, 17). To investigate this notion further, shRNA was used to inhibit p53 expression in the melanocyte cell line used for this study. Once reduced p53 expression was confirmed by Western blot and direct measurement of p53 transcript levels and the consequent reduction in p21 mRNA, the expression of the 3 GGR transcripts was investigated. The RE levels of the 3 GGR genes, XPC, DDB1 and DDB2 (XPE) was significantly lower in the p53 shRNA melanocytes at all time points after cisplatin treatment. However, the induction of these transcripts was not altered by the inhibition of p53, as all 3 GGR genes showed significant fold change increases in expression at 24 hours after cisplatin treatment, thereby demonstrating their similarity to the control melanocytes. This result confirms that a high level of p53 expression is not the only requirement for GGR activation. In addition, the induction of GGR in the melanocytes despite p53 inhibition suggests that p53 may not be responsible for the lack of GGR induction in the melanoma cell lines.

In 2006, a study by Yang et al. identified a number of p53 target genes that were significantly down-regulated in melanomas compared to melanocytes (46). One of their major findings was the association of reduced DDB2 activity and melanoma development (Yang et al. 2006). In addition, the expression of other p53 target genes was also altered, including the cell cycle regulator CDKN1A (p21CIP), which was confirmed by Kaufmann et al. (2008) (47). Taken together, the findings of these studies and those of the current study, reveal that reduced DDB2 activity is potentially implicated in melanoma development and or progression and that a number of other p53 targets may have altered expression in melanoma.
In summary, this study has revealed that melanoma cell lines treated with cisplatin have lower expression of the GGR genes, XPC, DDB1 and DDB2 (XPE) when compared to melanocytes after 24 hours. Interestingly, the basal expression levels of p53 and several NER repair genes in melanoma cell lines was greater than that observed in the melanocyte cell line yet upon stimulation by the DNA damaging agent cisplatin, only the melanocyte cell line was capable of response. This was confirmed by studies aimed at inhibiting p53 expression in the melanocytes that resulted in reduced expression of the GGR transcripts but not their induction.

Low mRNA (40, 41) and protein (39) expression of GGR genes in relation to cisplatin resistance have been previously reported. Although the present study has only reported mRNA expression of NER genes, the results are in accordance with the previous studies and suggest that GGR deficiency may play a role in cisplatin resistance in melanoma. Further studies confirming the differences in NER expression after cisplatin treatment at the protein level would further support this finding.

These findings provide evidence of the possible biological mechanisms involved in the reduced efficacy of cisplatin in melanoma. Notwithstanding, the DNA damage caused by cisplatin is similar to ultraviolet (UV) radiation as both result in large helix distorting DNA damage that is recognised by the NER pathway. Therefore, the findings of this study point towards a biological mechanism involved in melanoma resistance to cisplatin and in melanoma development as a result of UV exposure.
References


Table 1: Relative Expression (RE) of Nucleotide Excision Repair (NER) gene transcripts in melanoma and melanocyte cell lines after 0, 6 and 24 hours after cisplatin treatment.

<table>
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<tr>
<th>Time (hrs)</th>
<th>Cell Type</th>
<th>XPA</th>
<th>RPA1</th>
<th>RPA2</th>
<th>ERCC3 (XPB)</th>
<th>ERCC2 (XPD)</th>
<th>ERCC5 (XPG)</th>
<th>ERCC4 (XPF)</th>
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<td>0</td>
<td>Melanocytes</td>
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<td>Melanoma</td>
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<td>10.91*</td>
<td>7.54*</td>
<td>4.34*</td>
<td>2.89</td>
<td>1.68*</td>
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<td>3.42****</td>
<td>2.07**</td>
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</tr>
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*p<0.05, **p<0.001, ****p<0.0001
Figure 1: Relative Expression (RE) of GGR genes, XPC, DDB1 and DDB2 (XPE), 0, 6 and 24 hours after Cisplatin Treatment in melanoma and melanocyte cell lines. The RE of all 3 GGR transcripts was higher at the basal level and 6 hours post-treatment in the melanoma cell lines, although not significant. 24 hours after cisplatin treatment the RE of all 3 GGR transcripts was significantly lower in the melanoma cell lines compared to the melanocyte cell line. Data points are the mean of triplicates of independent experiments done in duplicate; bars = SE. **p<0.005.

Figure 2: The effect of cisplatin treatment on the induction of GGR genes, XPC, DDB1 and DDB2 (XPE), in melanoma and melanocyte cell lines at 6 and 24 hours after cisplatin treatment.

After 6 and 24 hours, induction of the GGR genes was significant in the melanocyte cell line, but not significant in the melanoma cell lines. Results are expressed as the normalized fold induction of mRNA RE relative to the RE at 0 hours (which has been set to 1). Data points are the mean of triplicates of independent experiments done in duplicate; bars = SE. *p<0.05, **p<0.005, ***p<0.0005.

Figure 3: Relative Expression (RE) and induction of p53 and p21, 0, 6 and 24 hours after Cisplatin Treatment in melanoma and melanocyte cell lines. A) The RE of p53 transcript was significantly higher at the basal level and 6 hours post-treatment in the melanoma cell lines. 24 hours after cisplatin treatment the RE of p21 transcript was significantly lower in the melanoma cell lines compared to the melanocyte cell line. B) After 6 and 24 hours, induction of p53 was highly significant (p<0.005) in the melanocyte cell line, but only significant in the melanoma cell lines at 24 hours. Similarly, after 6 and 24 hours, induction of p21 was highly significant (p<0.0005) in the melanocyte cell line, but not significant in the melanoma cell lines. Results are expressed as the normalized fold induction of mRNA RE relative to the RE at 0 hours (which has been set to 1).
Data points are the mean of triplicates of independent experiments done in duplicate; bars = SE.*p<0.05, **p<0.005, ***p<0.0005, ****p <0.00005

**Figure 4.** Relative expression (RE) of GGR gene transcripts in melanocytes and p53 shRNA melanocyte cell lines after 0, 6 and 24 hours cisplatin treatment. The RE of all 3 GGR transcripts was significantly lower at 6 and 24 hours after cisplatin treatment in the p53 shRNA melanocyte cell line compared to the melanocyte cell line. Data points are the mean of triplicates of independent experiments done in duplicate; bars = SE. *p<0.05, **p<0.005, ***p<0.0005, ****p <0.00005

**Figure 5:** The effect of cisplatin treatment on the induction of GGR gene transcripts in melanocytes with p53 inhibition. The induction of XPC, DDB1 and DDB2 (XPE), represented as fold change, increased significantly (p<0.0005) from 6 to 24 hours after cisplatin treatment in the control and p53 shRNA melanocytes. Data points are the mean of triplicates of independent experiments done in duplicate; bars = SE. *p<0.05, ***p<0.0005, ****p <0.00005
Nucleotide excision repair gene expression after cisplatin treatment in melanoma.

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