**Reviewer’s report**

**Title:** Sequential decitabine and carboplatin treatment increases the DNA repair protein XPC, increases apoptosis and decreases proliferation in melanoma.

**Version:** 0  **Date:** 09 Aug 2017

**Reviewer:** David Melton

**Reviewer's report:**

I have two fundamental problems with this manuscript: the first is the assertion in the Introduction and Abstract that chemoresistance in melanoma is as a result of a defect in a key DNA repair pathway- Nucleotide Excision Repair (NER); the second is that the data presented in the paper support the suggestion that the increased apoptosis and reduced proliferation observed in the melanoma cell lines studied following combined treatment with carboplatin and a demethylating agent (Decitabine) are due to increased expression of the NER gene XPC.

Chemoresistance in melanoma is as a result of an NER defect

Whilst I accept the authors' previous findings of low levels of XPC expression in melanoma, I do not accept that these are representative of the levels of NER itself in melanoma. High expression levels of genes in multiple DNA repair pathways, including NER, have been found in melanoma metastases (1) and in melanoma relapses following chemotherapy (2). High levels of NER were seen in melanoma cells used to assay NER inhibitors (3).

High, rather than low, levels of the NER protein ERCC1 are associated with poor responses to chemotherapy in numerous cancer types, including non-small cell lung cancer, squamous cell carcinoma and ovarian cancer (4-11). Testicular cancers have very low levels of ERCC1 and are effectively treated by cisplatin (12). In a melanoma xenograft model loss of NER gene ERCC1 resulted in extreme sensitivity to cisplatin (13). NER inhibitors resulted in increased sensitivity of melanoma (3) and other cancer cells to cisplatin (14).

The high mutational load in melanoma (and non-melanoma skin cancers) can be explained by continuous skin exposure to the DNA-damaging UV component of sunlight rather than by an NER defect.

Data in the paper

Figure 1: the 4 melanoma lines studied all showed decreased methylation following exposure to both high and low concentration of Decitabine, but at the lower clinically relevant dose, only 1 line Mel-276 RM showed both a substantial (3-fold) and significant increase in XPC mRNA.
Another line Me4405 showed a smaller 1.5-fold and significant increase. No supporting data for a corresponding increase in XPC protein levels are provided here.

Figure 2: very clear data showing a low level of methylation across the XPC CpG islands for all 4 cell lines that is unaffected by Decitabine treatment. Significance of some variable and local demethylation of island shores is unclear and, as the authors conclude, "no remarkable pattern of methylation in XPC is able to explain why 0.26 μM decitabine increases expression of XPC in Me4405 and Mel-276 RM but not MM200 or Sk-mel-28."

Figure 3: Carboplatin alone led to an increase in XPC mRNA in 4 melanoma cell lines (range 1.52-3.86 fold increase). The combination with decitabine resulted in a further small significant increase in 3 out of 4 lines (1.49-7.55 fold). There is confirmation for an increase in XPC at the protein level, although there are considerable variations in the level of the loading control TATA BP. The action of carboplatin, rather than in combination with demethylation, resulting in increased levels of XPC seems to be the dominant effect here. Increased levels of other NER proteins in response to platinating agents have been reported previously and found to be linked to increased, rather than decreased, chemoresistance (15).

Figure 4: The levels of apoptosis (5-7%) seen in all 4 untreated melanoma lines are surprisingly high. Separate carboplatin and decitabine treatments both lead to increased apoptosis in all 4 lines. The combination treatment produces a bigger effect for 3 out of 4 lines, but not for SK-mel-28 which did not show a detectable increase in XPC mRNA. While this does provide some support for the authors' argument, it is hardly "remarkable" as they suggest. No significance data are provided for any of these results.

Figure 5: Each treatment alone, or in combination slowed proliferation of all 4 lines. Pooled data for all 4 cell lines are shown so there is nothing here to indicate that this response is due to increased XPC expression as a result of demethylation, which is seen in only some of the lines.

Figure 6: convincing data are shown that siRNA knockdown of XPC prevented the XPC mRNA and protein increase after decitabine and carboplatin treatment in the mel-RM and Me 4405 lines. In both lines there was a very small but significant decrease in apoptosis and a very small but significant increase in viability compared to the siRNA control. While two toxic agents in combination would be expected to cause increased apoptosis and decreased proliferation in melanoma, these effects of XPC knockdown are not large enough to prove involvement of a decitabine-induced increase in XPC expression.
Major changes required

1. Provide a more balanced consideration in the abstract, introduction and discussion of the relationship in melanoma and other cancers between levels of NER activity, levels of NER proteins and sensitivity/resistance to chemotherapy with platinating agents.

2. Many of the effects reported here are small and are not seen in all the melanoma lines studied. Significance values for some of the differences observed are not always provided (Figure 4). Some of the data (Fig 5A) are not displayed in a way that allows the link suggested by the authors between decitabine-induced increases in XPC expression and chemosensitivity to be tested (pooled growth data for all 4 lines is shown, while only two lines show a significant increase in decitabine-induced XPC expression). The discussion of causal changes between demethylation, increased XPC expression and chemosensitivity should be moderated to properly reflect these limitations. The results part of the abstract should also be rewritten to reflect this.

Minor changes required

3. Change the title so that the reader does not presume that a causal link between demethylation, increased XPC expression and chemosensitivity is established in this paper.

4. Comment on the high level of apoptosis observed in all untreated melanoma lines.

4. Line 310 delete "Remarkable"

5. Line 378 change "probable" to possible

References supporting review


6. Wang L et al. (2008) ERCC1 and BRCA1 mRNA expression levels in metastatic malignant effusions is associated with chemosensitivity to cisplatin and/or docetaxel. BMC Cancer, 8, 97.


15. Li W et al. Cisplatin regulates the MAPK kinase pathway to induce increased expression of DNA repair gene ERCC1 and increase melanoma chemoresistance. Oncogene 2012; 31; 2412-2422.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes
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