
In their recent papers in Cancer Research (1, 2) Bronstein et al. propose that in human cells AGT is not efficient in the removal of O6-alkylguanine from the chromosomal DNA unless it cooperates with nucleotide excision repair. This conclusion is based on a comparative study of three human B-cell lines. GM2250C (referred to as X-cell line) is a xeroderma pigmentosum (complementation group A) cell line, which has a deficiency in nucleotide excision repair (3) but normal expression of AGT (2). The TK6 (A-cell) line appears to have a normal nucleotide excision repair capacity (2) and does not express AGT (2). The GM0130B (N-cell) line appears to be efficient in both types of DNA repair (2).

These three lines responded differently when exposed to N-ethyl-N-nitrosourea, a potent alkylating agent of the SN1 type which produces large amounts of O6-ethylguanine adducts in the cell DNA. The rate of removal of O6-alkylguanine adducts from the DNA of X-cells was 5-fold slower than that in N-cells. A-cells, as expected, were totally incapable of removing this adduct, a behavior consistent with previous observations (3, 4). In accord, both A- and X-cells were approximately 3.4-fold more sensitive to the cytotoxic action of N-ethyl-N-nitrosourea than N-cells (2).

My interpretation of these results differs from that offered by the authors.

It seems to me that the link between a xeroderma pigmentosum phenotype and reduced ability to remove O6-alkylguanine adduct is a special case rather than a general rule. Although some correlation between a deficiency in nucleotide excision repair and the sensitivity of some types of xeroderma pigmentosum cells to a SN1 alkylating agent has been reported previously (5, 6), this correlation is weak (on the average 1.35-fold difference in sensitivity to the cytotoxic effect of N-methyl-N-nitrosourea between normal and xeroderma pigmentosum-derived cell strains), and, importantly, this correlation does not extend to all xeroderma pigmentosum cell lines, some of which are being as resistant to the cytotoxic effect of this alkylating agent as the strains derived from normal individuals (5, 6). These data have not been discussed by Bronstein et al. (1, 2). Another study reports that several xeroderma pigmentosum cell lines are as efficient in the removal of O-methylguanine DNA adducts as normal cell lines (3). Bronstein et al. speculate that because AGT removes O6-ethylguanine adducts more slowly than O6-methylguanine adducts, cell lines that are equally efficient in the removal of the latter may differ in the ability to remove the former (1). This hypothesis is essential in the authors' argument and it should have been tested experimentally on the same cells in the same experiment. In fact, there is very little if any experimental evidence in the literature favoring this hypothesis and it may well be incorrect.

Secondly, while not stating it directly, the authors imply (or at least that was my understanding) that the low levels of UV-induced unscheduled DNA synthesis and the absence of alkaline elution DNA strand breaks following UV exposure indicate a deficiency in the nucleotide excision repair of the DNA alkylation damage in the X-cell line (2). However, a number of cell strains of the xeroderma pigmentosum of the same complementation group (group A) exhibited levels of methyl-N-nitrosourea-induced unscheduled DNA synthesis similar to that in cells derived from normal donors. Thus, as far as this indirect test goes, the deficiency of cells in repairing UV-induced DNA damage may not correlate with the ability of the cells to repair DNA lesions produced by S,N-l alkylating agents (5, 6).

Therefore, the conclusion that human cells require both AGT and nucleotide excision repair pathways to efficiently repair O6-ethylguanine adducts in genomic DNA (1) has not been proven by the authors and, moreover, seems to be incorrect.

The authors give two possible explanations for the nature of the poor ability of the X-cell line to remove the O6-ethylguanine adduct from the DNA (1): (a) the excision repair pathway is needed to improve the access of AGT to regions of chromatin containing the alkylated DNA adducts; or (b) the nucleotide excision pathway is directly involved in the repair of the alkylating damage.

There is another interesting possibility. One could speculate that due to a DNA repair deficiency, the O6-alkylguanine-containing areas of DNA in the X-cells and some other xeroderma pigmentosum strains of the A-complementation group may be single-stranded for a considerable length of time making them poor substrates for AGT. Indeed, there is some evidence that double-stranded DNA that has O6-alkylguanine adducts is a better substrate for AGT than O6-alkylguanine-containing single-stranded DNA (7, 8).

In summary, more work is needed to understand the interaction of AGT and nucleotide excision repair.

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References
Reply

In his Letter to the Editor, Dr. Goldmacher questioned the model we proposed for the interaction between AGT and NER in the removal of O\textsubscript{6}etGua. He is correct in stating that this model is untested and unproven. What we have done is to measure biological and biochemical endpoints in human lymphoblasts exposed to ENU, present the results, and propose a model which might explain these results and provoke further scientific dialogue.

Currently accepted models have attributed the repair of O\textsubscript{6}etGua in mammalian cells to the independent action of AGT (1) and would predict that the XPA cell line GM2250, which expresses high levels of AGT, should be fully capable of O\textsubscript{6}etGua repair. What we found, however, through independent measurements of toxicity, mutagenesis, and DNA adducts, was that these XPA cells do not efficiently repair O\textsubscript{6}etGua (2, 3). Since this result is not accounted for by most currently accepted models concerning the actions of human AGT, we have proposed a modification of these models which can explain our data by borrowing concepts from more established pathways of DNA repair in bacteria, yeast, and human cells.

To address specific points of the Letter, some of the limitations of our published experiments should be underscored; we examined three cell lines, but only a single XPA cell line GM2250, which expresses high levels of AGT, should be fully capable of O\textsubscript{6}etGua repair. What we found, however, through independent measurements of toxicity, mutagenesis, and DNA adducts, was that these XPA cells do not efficiently repair O\textsubscript{6}etGua (2, 3). Since this result is not accounted for by most currently accepted models concerning the actions of human AGT, we have proposed a modification of these models which can explain our data by borrowing concepts from more established pathways of DNA repair in bacterial, yeast, and human cells.

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The abbreviations used are: AGT, O\textsubscript{6}-alkylguanine DNA alkyltransferase; O\textsubscript{6}etGua, O\textsubscript{6}-ethylguanine; NER, nucleotide excision repair; ENU, N-ethyl-N-nitrosourea; XPA, xeroderma pigmentosum complementation group A.

Additional support for our model has recently been published (6), where the effect of the AGT inhibitor, O\textsubscript{6}-benzylguanine, was examined in the same cell lines exposed to ENU. The results indicated that the lack of either AGT or nucleotide excision repair significantly impairs the ability of human cells to withstand DNA ethylation damage. Furthermore, the inhibition of AGT in xeroderma pigmentosum group A cells did not increase toxicity or mutagenicity, suggesting that AGT and nucleoside excision repair cooperate in the removal of DNA ethyl adducts.

Finally, Dr. Goldmacher has questioned whether this XPA cell line shows decreased rates of single strand breaks following alkylation. We presented alkaline elution data only as measured following UV exposure and did this to document the NER-deficient phenotype of the XPA cell line (2). If the presentation of these results caused confusion, we apologize. Dr. Goldmacher is correct in pointing out that the proposed model invoking the cooperation of AGT and NER in the removal of O\textsubscript{6}etGua has as one of its corollaries the production of single strand DNA breaks following ENU exposure. Although we did not present these results, we did indeed examine alkaline elution in these cells following ethylation damage, as has been done by other groups with both Chinese hamster ovary cells (7) and human cells (8, 9). Our findings indicate that large numbers of strand breaks occur following ENU exposure in all three cell lines and that no significant correlation was seen between strand break accumulation and DNA repair phenotype. Our interpretation is that a large number of strand breaks could be the result of lesions other than O\textsubscript{6}etGua, such as N\textsuperscript{7}-ethyguanine and N\textsuperscript{3}-ethyladenine. Further investigation in this direction may be informative, but may need to be done through a more controlled approach, perhaps by investigating the repair of O\textsubscript{6}etGua in an in vitro excision repair system, as has been done with other adducts (10).

We have presented the results of a defined set of experiments, and although these results are all internally consistent, they are in some disagreement with the conventional understanding of how AGT works in human cells. Therefore, we have presented a modification of a popular model of AGT function to account for our results. This new model, while consistent with much of the existing literature on alkylation, is unproven and as yet untested, and indeed is not the only possible model which might explain our results. Certainly further investigation is necessary if the repair of O\textsubscript{6}etGua in human cells is to be understood, and we hope that the proposed model will provide a heuristic framework to aid in the logical approach to this question. We thank Dr. Goldmacher for his thoughtful input and for opening this forum for scientific discussion.

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References


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