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TITLE: DNA Base Excision Repair (BER) and Cancer Gene Therapy:
Use of the Human N-mythlpurine DNA Glycosylase (MPG) to Sensitize
Breast Cancer Cells to Low Dose Chemotherapy

PRINCIPAL INVESTIGATOR: Tia Harvey
Mark R. Kelly, Ph.D.

CONTRACTING ORGANIZATION: Indiana University
Indianapolis, Indiana 46202-5167

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Indiana University Indianapolis, Indiana 46202-5167 E-Mail: tmharvey@iupui.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
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13. ABSTRACT (Maximum 200 Words) The DNA Base Excision Repair (BER) pathway is responsible for the repair of alkylation and oxidative DNA damage resulting in protection against the deleterious effects of endogenous and exogenous agents encountered on a daily basis. The first enzyme in the human DNA BER pathway, N-methylpurine DNA glycosylase (MPG), is the focus of this proposal. This enzyme is responsible for the removal of damaged bases from the DNA resulting in an abasic site. Our laboratory has found that the overexpression of this DNA repair protein is cytotoxic to tumor cells in response to the classic alkylating agent, methyl methanesulfonate (MMS). It will be interesting to further investigate the use of MPG constructs to kill breast cancer cells in response to clinically relevant drugs used in breast cancer treatment protocols, such as thiotepa and cytoxan (cyclophosphamide). Gene transfer of MPG could result in increased kill of breast cancer cells using lower doses of chemotherapy, therefore minimizing peripheral damage and eliminating the need for bone marrow rescue or transplant. This is particularly important in advanced stage (IV) treatments currently using high dose chemotherapy and bone marrow transplants.				
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DNA Base Excision Repair (BER) and Cancer Gene Therapy: Use of the Human N-Methylpurine DNA Glycosylase (MPG) to Sensitize Breast Cancer Cells to Low Dose Chemotherapy

Task 1: To overexpress MPG in three breast cancer cell lines above endogenous levels.

This was accomplished in the preceeding period of grant funding.

Task 2: To investigate whether the breast cancer cell lines that overexpress MPG are more sensitive to MMS and chemotherapeutic agents such as mafosfamide and thiotepa.

MMS data was published this year in Cancer Research {Fishel, 2003 #12791} and a brief summary is presented below. This is the work of my predecessor on this grant. Current studies with mafosfamide and thiotepa are on-going. An addition to the original tasks is the targeting of MPG to the mitochondria to enhance tumor cell killing.

Initial studies using nuclear and mitochondrial targeted overexpression of MPG in breast cancer cell lines have been successful and recently published. In these studies, we were able to show that overexpressing MPG in the mitochondria enhanced tumor cell line sensitivity to an alkylating agent more than the nuclear targeting of MPG (Fig 1). The 231+nMPG cells and the 231+ mito-MPG cells had a p value < 0.05 (*) at all doses compared with 231+pcDNA cells using the one-way ANOVA test. The p value was < 0.05 (**) comparing the 231+nMPG cells and the 231+mito-MPG cells at 0 dose, 0.05, 0.1, and 0.2 mM MMS using the one-way ANOVA test ($n \geq 6$). The number of cells undergoing apoptosis in the mito-MPG-overexpressing cells and the nMPG-overexpressing cells after MMS treatment was significantly higher than the number of vector control cells undergoing apoptosis (data not shown). In Figure 2, a graphical representation of the percentage of cells undergoing apoptosis in the 231+mito-MPG and the 231+nMPG cells after treatment with 0.1 and 0.2 mM MMS continuously for 36-48 hours. The average of three independent experiments is represented. The 231+mito-MPG cells had a significant difference ($p < 0.05$) at the zero dose, 0.1, and 0.2 mM MMS compared to vector control cells using the one-way ANOVA test. The 231+nMPG cells had a significant difference ($p < 0.05$) at the 0.1 and 0.2 mM MMS compared to 231+pcDNA cells using the one-way ANOVA test. Additionally, the cells were shown to be dying via

Figure 1. Targeting MPG to the mitochondria further sensitizes the cells to MMS.

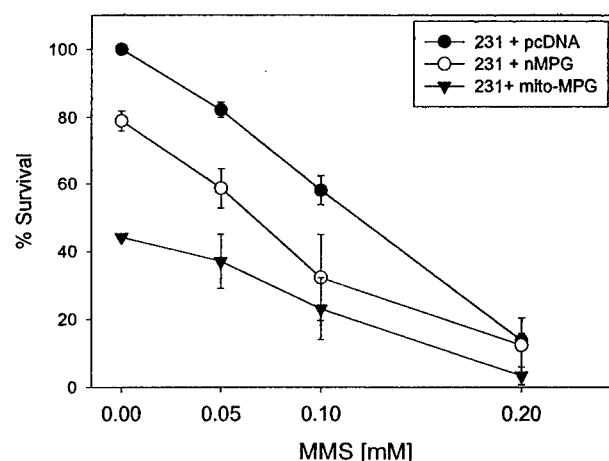
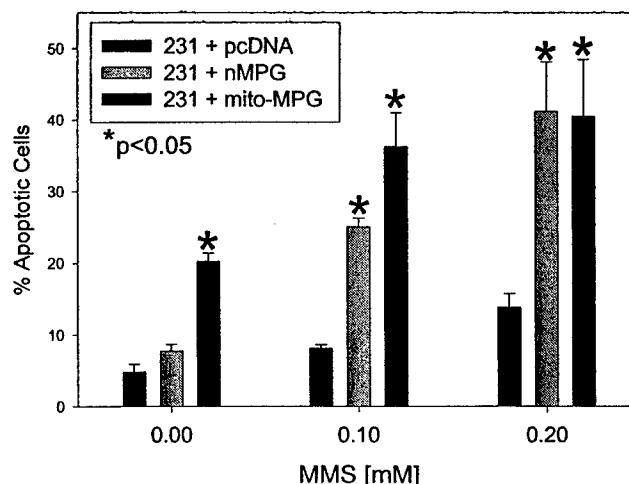


Figure 2.



apoptosis (Fig 2). However, these studies used a CMV-plasmid based expression system and stable cell lines. We feel this, and other expression systems should be evaluated, to confirm our initial findings of this effect of nuclear and mitochondrial MPG overexpression.

Task 3: *To construct the adenoviral transfer vector containing the breast cancer-specific promoter and human MPG.*

This has not been accomplished. An alternative promoter, hTERT (human telomerase reverse transcriptase) has been chosen as a more appropriate promoter as it is elevated in a number of cancers vs. normal human cells.

Task 4: *Use adenovirus-mediated transfer of MPG with a breast cancer-specific promoter in the three cell lines from Task 1.*

Adenoviral infection with generic CMV promoter has been accomplished, but not with a tumor specific promoter. This is dependent on the final construction of the promoter described in Task 3.

Progress this past year has been hampered due to my having to complete course work for my minor, which is now completed, as well as preparing and taking the Departmental Comprehensive exam to enter into candidacy. These tasks have been completed and full time can now be devoted to the completion of the proposed tasks.

Reportable Outcomes:

Fishel, M., Seo, Y.R., Smith, M.L. and **Kelley**, M.R. (2003) Imbalancing the DNA base excision repair pathway in the mitochondria; Targeting and overexpressing n-methylpurine DNA glycosylase in mitochondria leads to enhanced cell killing. *Cancer Research* 63: 608-615.