Interactions Between Mammalian DNA Repair Proteins

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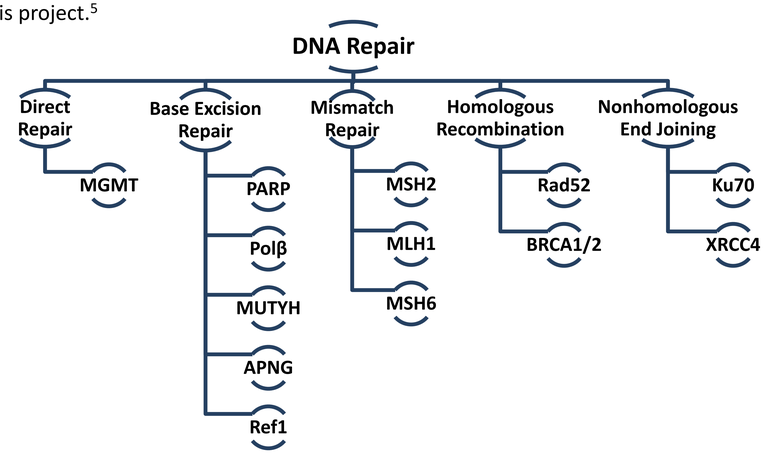
INTRODUCTION

Genomic DNA of all living organisms undergoes physicochemical damage as a result of various physiological, pharmacological and environmental insults. Methylation of purines, especially guanines is one of the most commonly observed chemical modification in damaged DNA. Under normal conditions the body attempts to maintain homeostasis by repairing DNA damage using various enzymes found in multiple DNA repair pathways. Methylated guanines are repaired in a single step using an enzyme called O6-methyl- guanine-DNA methyltransferase (MGMT). The objective of this study was to assess relationship between MGMT and expression of other DNA repair proteins in human mammary cancer cells as a model. Here we present a comparative analysis of possible interactions between MGMT and poly (ADP-ribose) polymerase (PARP), alkylpurine-DNA-N-glycosylase (APNG) and mutS homologues (MSH and MLH) – representative proteins involved in DNA strand break repair, base excision repair and mismatch repair pathways, respectively.

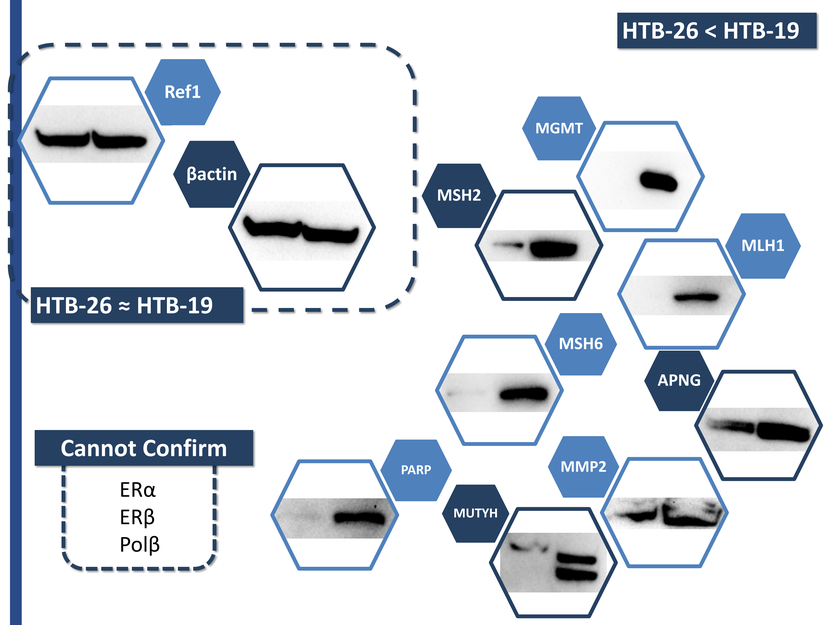
High expression of MGMT in certain cancer cells is known to induce resistance to alkylating chemotherapy agents e.g. Carboplatin and Cisplatin.[1-4] By determining the relationship between MGMT and other DNA repair enzymes targeted chemotherapy treatment can become more individualized to the patient's cell protein expression. HTB-26 is a breast adenocarcinoma cell line available for scientific research that is known to have very low to no expression of MGMT that is utilized in this project.[5]



PARP is one of the DNA repair proteins that has been identified and is already being utilized in the treatment of cancer.[6] While PARP inhibitors are used in the treatment of some cancer types, it is also theorized that they may be useful to prevent heart failure in post-myocardial infarction patients and possibly other cardiac indications[7], therefore exploring the interactions PARP plays with other DNA repair enzymes is necessary.

The interactions between other DNA repair proteins and MGMT are less well understood in comparison to PARP, but they appear to play an important role in development and treatment of cancer due to DNA damage and alterations in DNA repair.

RESULTS



METHODS

The interactions between different DNA repair proteins were assessed utilizing whole cell extracts (WCE) of HTB-26 and HTB-19 breast adenocarcinoma cell lines available for scientific research. WCE used for analysis were created with tissue cell cultures maintained within our laboratory. HTB-26 was used as the MGMT negative control and HTB-19 was used as the standard control.

The presence or absence of individual proteins were compared using western blot and Coomassie stain SDS gel techniques. The method of analysis was qualitative (present, not present, present in greater or lesser quantity).

Antibodies utilized for protein comparisons on western blots were from either Cell Signaling Technologies (CST) or Santa Cruz Biotechnology. Protein concentrations between the two different cell line WCE were equalized using both Coomassie blue staining on SDS gels and CST βactin antibody on western blot.

Equalized protein concentrations were analyzed using western blot analysis. Each protein antibody of interest was probed on individual blots, and in many cases, confirmation of protein equivalence was completed using the βactin antibody after the blot was probed for the protein of interest.

All images were obtained using a Bio-Rad Imager and Image Lab program.

When equalizing protein concentrations, it was found that the total protein concentration between HTB-19 and HTB-26 was approximately a 1:1 ratio. It was then decided to process each blot using 10µl of WCE.

DISCUSSION

There appears to be a correlation between MGMT presence or absence and expression of a variety of other DNA repair proteins. It was observed that multiple repair proteins demonstrate lower expression when MGMT is not present or present in such low quantities that it cannot be probed using western blot analysis.

Even though the different proteins probed do not all act on the same type of DNA damage, they appear to have a link to MGMT expression. We theorize that these proteins may work together within a protein complex while the cell infrastructure is performing DNA repair.

It must be emphasized that our studies are conducted at the protein level, which represents the closest correlation to the function as compared to the respective mRNA or genes, making our observations clinically significant as potential biomarkers.

Another strength of this study is that the same sample of each WCE was used for all protein assays, which allows us to assume equivalent protein concentration in all samples. Moreover, all results are highly reproducible as confirmed by multiple western blot analyses with at least two independent WCE preparations. Protein equivalency between preparations was confirmed using βactin as internal control.

One limitation of our study is that these experiments were carried out using tissue culture cell lines as an experimental model. It would be interesting to see if these observations are reproducible in clinical samples. We are also eager to explore if our results could be extended to other types of cancers in humans. Further studies are in progress.

CONCLUSIONS AND FUTURE STUDIES

This study demonstrates a potential relationship between MGMT expression and other DNA repair proteins. The specific relationships and how this relationship will apply to clinical use is yet to be determined. Further testing is needed to confirm differences in expression utilizing a quantitative method, along with structure-function relationship studies.

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References

1. Ding J, Miao ZH, Meng LH, Geng MY. Emerging cancer therapeutic opportunities target DNA-repair systems. Trends Pharmacol Sci. 2006;27(6):338-344. doi:10.1016/j.tips.2006.04.007
2. Madhusudan S, Hickson ID. DNA repair inhibition: a selective tumour targeting strategy. Trends Mol Med. 2005;11(11):503-511. doi:10.1016/j.molmed.2005.09.004
3. Martin NM. DNA repair inhibition and cancer therapy. J Photochem Photobiol B. 2001;63(1-3):162-170. doi:10.1016/s1011-1344(01)00213-5
4. Costello, J. F. "Methylation Matters." Journal of Medical Genetics, vol. 38, no. 5, 1 May 2001, pp. 285–303, doi: 10.1136/jmg.38.5.285.
5. Chen S, Zhang Y, Zhang D. Endoplasmic reticulum protein 29 (ERp29) confers radioresistance through the DNA repair gene, O(6)-methylguanine DNA-methyltransferase, in breast cancer cells. Sci Rep. 2015;5:14723. Published 2015 Sep 30. doi:10.1038/srep14723
6. Chen A. PARP inhibitors: its role in treatment of cancer. Chin J Cancer. 2011;30(7):463-471. doi:10.5732/cjc.011.10111
7. Henning RJ, Bourgeois M, Harbison RD. Poly(ADP-ribose) Polymerase (PARP) and PARP Inhibitors: Mechanisms of Action and Role in Cardiovascular Disorders. Cardiovasc Toxicol. 2018;18(6):493-506. doi:10.1007/s12012-018-9462-2