Relationship Between Oxidatively-induced DNA Damage and DNA Repair Enzyme APE1 in Cancer and Normal Cell Lines

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Abstract

Exploitation of DNA damage and repair mechanisms offers new avenues for cancer treatment and screening of environmental contaminants. It is therefore necessary to quantitatively measure DNA lesions and DNA repair enzymes in cancerous and normal cells to better understand the basic relationships between DNA damage and DNA repair. In this work, we used gas chromatography-tandem mass spectrometry (GC-MS/MS) with isotope dilution to measure the level of six oxidatively-induced DNA lesions (5-OH-5MeHyd, 5-OH-Cyt, ThyGly, FapyAde, FapyGua and 8-OH-Gua) in four human cells lines (HeLa, HepG2, MCF7 and MCRF10a). We also used liquid chromatography-tandem mass spectrometry (LC-MS/MS) with isotope dilution to measure the levels of APE1 (apurinic/apyrimidinic endonuclease) DNA repair enzyme in total protein extracts from cell culture samples paired to those used for DNA extraction. We further used GC-MS/MS to measure levels of cyclo lesion *S*-cdA. We found all seven lesions to be present at different levels in all four cells lines. For example, MCF7 had significantly higher levels of FapyAde, FapyGua and ThyGly than MCF10A. The levels of *S*-cdA detected with GC-MS/MS and LC-MS/MS were in agreement and were to those found previously in a rodent model. APE1 was detected in total extracts from all four cells lines, and was highest in MCF7 cells. We show for the first time that APE1 expression is surprisingly low in HeLa cells. Further work is currently underway to quantify levels of endogenous DNA repair enzyme MTH1 and to investigate the effects of environmental contaminant exposure on DNA damage and DNA repair.

Human Cell Cultures

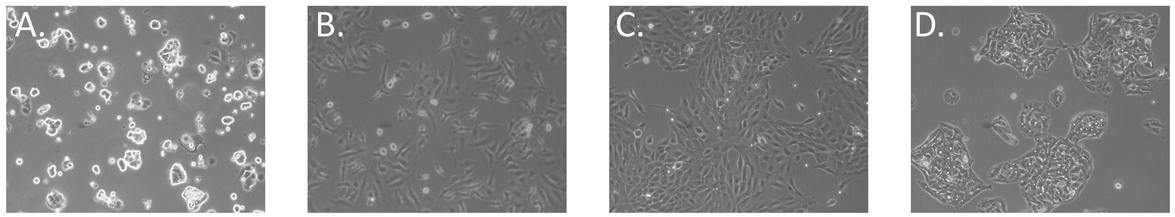

Photo credit: Alex Tona

Human Cell Models

A. MCF7 mammary gland epithelial metastatic adenocarcinoma.
B. MCF10A mammary gland epithelial non-cancer (fibrocystic tissue).
C. HeLa cervical epithelial adenocarcinoma.
D. HepG2 liver epithelia, hepatocellular carcinoma.

Methods

Cell Culture and Collection

Each cell line was grown as per ATCC guidelines and split into large flasks to obtain millions of cells per passage. Cells were counted microscopically and aliquoted into cryovials of 6 x 10^6 cells per sample. During this process, cells were "paired", so that cells for the same culture could be used for both DNA analyses and for protein analyses. See Sample Flow, right.

DNA Extraction

DNA was extracted using digestion with SDS and Proteinase K and a high-salt phase separation followed by clean-up with RNAse A and 70% ethanol. DNA levels were quantified via spectrophotometry and RNA levels were measured with Qubit high-sensitivity RNA assay.

Protein Extraction

Total protein was extracted from cells with the M-PER extraction buffer from Thermo Fisher. Protein concentration was measured with Coomassie blue assay.

Measurement of DNA Lesions

Each 50 µg DNA sample was spiked with known amounts of isoptically-labeled DNA lesion standards and incubated with *E.coli* formamidopyrimideine DNA glycosylase (Fpg) and Endonuclease (Nth) to remove DNA lesions from the genomic DNA. Samples were derivatized with BSTFA. Lesion levels were detected and quantified using GC-MS/MS.

Measurement of (5'*R*)- and (5'*S*)-8,5'-cyclo-2'-deoxyadenosines

50 µg DNA samples extracted from cells paired to DNA samples were spiked with isotopically-labeled *(5'R)*- and *(5'S)*-8,5'-cyclo-2'-deoxyadenosine standards and treated with Nuclease P1, Phosphodiesterase I and Alkaline Phosphatase to digest DNA into single nucleosides. Samples were analyzed on both LC-MS/MS and GC-MS/MS-derivatized with BSTFA.

Measurement of APE1

^{15}N-labeled APE1 standard was added to each protein extract and APE1-containing protein fractions were collected from protein extracts using LC. Proteins were digested with trypsin and APE1 levels were determined by integrating LC-MS/MS analysis of the APE-1 tryptic peptides.

Statistical Analysis

Two-tailed t-test was performed in Prism by GraphPad.

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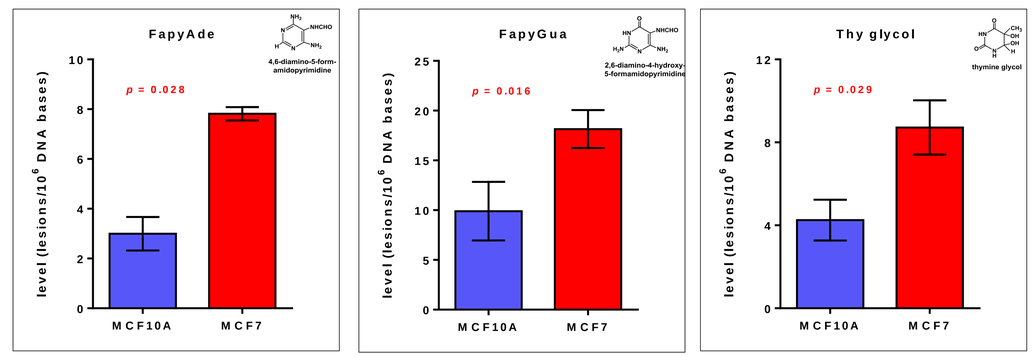
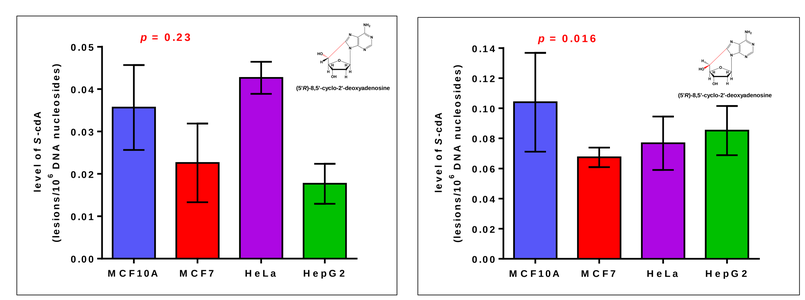
DNA Lesions



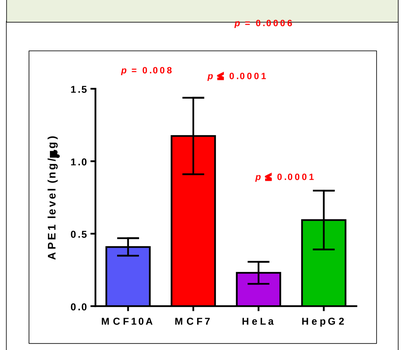
DNA lesion levels were higher in cancer cell line MCF7 than in the corresponding normal cell line MCF10A. n = 5

(5'*R*)- and (5'*S*)-8,5'-cyclo-2'-deoxyadenosines



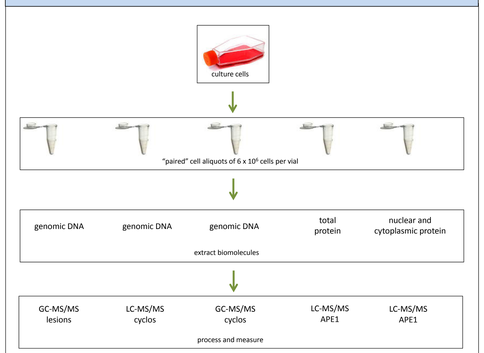
MCF10A had significantly higher levels of cdA than MCF7. Levels of *R*-cdA and *S*-cdA measured by GC-MS/MS agreed with those measured by LC-MS/MS (n = 5).

APE1



APE1 levels were significantly higher in MCF7 cells than in MC10A cells (n = 5).

Sample Flow



Conclusions and Discussion

- Preliminary analysis shows that lesions are detected at different levels in mammary cancer and normal cells.
- Lesions repaired by base excision repair are found at higher levels in cancer cells.
- Lesions repaired by nucleotide excision repair (cyclos) are found at higher levels in normal cells.
- The non-cancerous cell line (MCF10A) has a lower level of DNA repair enzyme APE1 than the corresponding cancerous cell (MCF7).
- Further work is underway to quantify effects of contaminants (BPS, smoke) on DNA damage and repair.