GENOMIC INSTABILITY AND CENTROsomAL AMPLIFICATION
INDUCED BY THE ANTIRETROVIRAL AGENT AZT.

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Abstract

Materials & Methods

Results
Patients infected with the human immunodeficiency virus-1 (HIV-1) undergo therapy with antiretroviral nucleoside reverse-transcriptase inhibitors, among which AZT is used frequently.

In cultured cells, exposure to the carcinogenic agent AZT induces cell cycle arrest, micronuclei, sister chromatid exchanges, shortened telomeres, and alterations in gene expression. A predicted consequence of these events is genomic instability.

In this study two normal human mammary epithelial cell (NHMEC) strains were exposed to AZT for 24 hours and analyzed for abnormality by immunofluorescence and confocal microscopy. Centrosomal amplification, identified by a pericentrin antibody and evidenced by the percentage of cells containing ≥2 centrosomes, was evaluated. The NHMEC strain 05, previously identified as a strain that incorporates high levels of AZT into DNA, showed centrosomal amplification in 31.67% of cells exposed to 200 µM AZT for 24 hrs and 5.83% centrosomal amplification in unexposed cells. The AZT strain NHMEC 40, which did not incorporate AZT into DNA, showed centrosomal amplification in 20.8% of cells exposed for 24 hrs to 200µM AZT and 7.83% centrosomal amplification in unexposed cells.

Because aberrations in centrosome morphology are associated with chromosomal mis-segregation, micronuclei bearing intact chromosomes, stained using a CREST antibody, were scored revealing increased positive signals for centromeric kinetochores within micronuclei of cells exposed to AZT. The NHMEC 05 strain exposed to 200 µM AZT for 24 hrs showed 36.4% of micronuclei containing centromeric signals compared to 25.9% of the control, respectively.

Immunohistochemistry with an Aurora A antibody revealed abnormal polarity in the mitotic spindle. Western blot studies confirmed an up-regulation of the protein in NHMEC 05. Flow cytometric studies revealed that cells bearing multiple centrosomes are able to replicate and exhibit a cell cycle similar to cells bearing a normal number of centrosomes, thus producing daughter cells bearing an unbalanced number of chromosomes.

Since centromeric positive micronuclei contribute to unbalanced chromosomal segregation or aneuploidy, the evidence presented here suggests that AZT induced genomic instability could be underlying the mechanism of AZT carcinogenicity.

Exposure of Chinese Hamster Ovary (CHO) cells to AZT revealed:

- Striking disorganization of centrosomal material in the form of nuclear amplification and fragmentation in found by confocal microscopy (Photograph 1, circle) and confirmed by EM (Photograph 2, brown arrows)
- Pericentriolar bodies associated with the centrosome, absent in untreated cells (Photograph 2 red arrows)
-kinetochore-positive micronuclei with centromeric portions of chromosomes lost from the nucleus and present in micronuclei (Photograph 3, arrows)

CHO cells exposed 24 hr to 200 and 400 µM AZT

**Objective**

To explore the ability of AZT to induce centrosomal damage in normal human mammary epithelial cells (NHMECs).

**Background**

Exposure of Chinese Hamster Ovary (CHO) cells to AZT revealed:

- Striking disorganization of centrosomal material in the form of nuclear amplification and fragmentation in found by confocal microscopy (Photograph 1, circle) and confirmed by EM (Photograph 2, brown arrows)
- Pericentriolar bodies associated with the centrosome, absent in untreated cells (Photograph 2 red arrows)
- Kinetochore-positive micronuclei with centromeric portions of chromosomes lost from the nucleus and present in micronuclei (Photograph 3, arrows)

**Results**

Two strains of Normal Human Mammary Epithelia Cells (NHMEC) were exposed to 0, 10 or 200 µM AZT for 24 hours.

The NHMEC strains were chosen based upon their AZT-DNA incorporation profile:

- NHMEC 05 cells incorporate AZT into DNA, and
- NHMEC 40 cells do not incorporate AZT into DNA

- Both, human cell strains, were analyzed by confocal microscopy, following staining with pericentrin antibody and a secondary antibody Alexa 488-conjugated (green), to identify centrosomes, and DAPI to localize nuclei.
- Cells were also stained with anti αtubulin and a secondary antibody rhodamine-conjugated (red) to localize tubulin (Peloponesse Jr., J. et al, PNAS 2005). Quantitation of positive pericentrin-Alexa 488 (centrosomal) signals was performed for 500-1000 cells.
- Identification of micronuclei containing positive kinetochore (centromeral) signals was achieved utilizing a CREST antibody and a secondary antibody Alexa 488-conjugated (green), and counted in 25 micronuclei (50-1000 cells) from each treatment group.

- Aurora A identification was carried out with specific antibodies and a secondary antibody Alexa 488-conjugated (green).
- Aurora A was also stained with anti -tubulin and a secondary antibody rhodamine-conjugated (red) to localize tubulin.

**Summary**

Exposure of NHMECs to AZT induced multiple manifestations of chromosomal instability including:

- Centrosomal amplification
- Lack of tubulin polymerization in cells bearing centrosomal amplification
- CREST positive micronuclei containing centromeric proteins
- Increase in centrosomal kinase activators Aurora A and B
- Multipolar metaphases

**Conclusion**

Genomic instability may be a major mechanism contributing to the carcinogenic potency of AZT.