Genomic Instability and Centrosomal Amplification Induced by Antiretrovirals

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ABSTRACT

•Exposure to the nucleoside reverse transcriptase inhibitor (NRTI) Zidovudine (AZT) induces genomic instability, causing: cell cycle arrest, micronuclei, sister chromatid exchanges and shortened telomeres. AZT, in addition, is a moderately strong transplacental carcinogen.

• In previous studies we demonstrated AZT-induced centrosomal amplification, by staining with an anti-pericentrin antibody and fluorescence microscopy, in Chinese Hamster Ovary (CHO) cells exposed for 24 hours to 1, 10, and 100 μM AZT; there were 0.27%, 0.66% and 1.13 % of cells with >2 centrosomes/cell.

• In this study AZT-induced centrosomal amplification was explored in CHO cells and in two normal human mammary epithelial cell (NHMEC) strains: M99005, which incorporates AZT into DNA, and M98040, which does not incorporate AZT into DNA.

• Dose response curves using 5 doses of each of 3 commonly-used NRTIs and thymidine, and time response curves at 24, 48 and 72 hours, were performed to determine cytotoxicity for each individual compound.

• The agents and doses chosen for pericentrin analysis of 1000 cells/group were: Lamivudine (3TC) 12 and 122 μM, Didanosine (ddI) 10 and 102 μM, and Stavudine (d4T) 8.9 and 89 μM and thymidine 9.9 and 99 μM.

• **In the CHO cells,** a dose-dependent, statistically significant ($p<0.05$) trend for increase in centrosomal amplification was observed for each of the NRTIs but not for thymidine. By t-test, the high dose for each compound was significantly ($p<0.05$) different compared to the control. The results are as follows: for 3TC, 0.29%, 0.98% and 1.26% of cells exposed to 0, 12, and 122 μM 3TC, respectively, showed amplification; for ddI, 0.42%, 0.97%, and 1.38% of cells exposed to 0, 10, and 102 μM ddI respectively, showed amplification; for d4T, 0.29%, 1.0%, and 1.11% of cells exposed to 0, 8.9, and 89 μM d4T, respectively, showed amplification; and for thymidine, 0.62%, 0.82% and 0.52% of cells exposed to 0, 9.9, and 99 μM thymidine, showed amplification.

• **In the NHMEC strain M99005,** an NRTI-induced increase in centrosomal amplification was observed. By t-test, the high dose for each NRTI and the low dose for 3TC and ddI were significantly different ($P < 0.005$) compared to the unexposed control. The results are as follows: for 3TC, 0.33%, 1.13%, and 1.27% of cells exposed to 0, 12, and 122 μM 3TC, respectively, showed amplification; for DDI, 0.36%, 1.46%, and 1.54% of cells exposed to 0,10, and 102 μM ddI, respectively, showed amplification; for d4T, 0.30%, 1.03%, and 1.33% of cells exposed to 0, 8.9, and 89 μM d4T, respectively, showed amplification; and for thymidine, 0.35%, 0.45%, and 0.50% of cells exposed to 0, 9.9, and 99 μM thymidine, respectively, showed amplification.

• **In the NHMEC strain M98040,** only the high doses of ddI and d4T showed significant ($P < 0.005$) increases in amplification, compared to the unexposed or thymidine-exposed controls. This suggests that incorporation of an NRTI into DNA is a necessary prerequisite for centrosomal amplification.

• **We conclude that centrosomal amplification is not just induced by AZT, but also occurs with 3TC, d4T and ddI in the following order of increasing toxicity: d4T<AZT<3TC<ddI.**

BACKGROUND

• AZT is a transplacental carcinogen in mice. The drug is a nucleoside analog that becomes incorporated into DNA where it then acts as a replication chain terminator, causing mutations. In cultured cells, exposure to AZT causes changes in the cell cycle and gene expression.

• Centrosomal amplification has been shown to occur in most cancers. The centrosome is an organelle comprised of a pair of centrioles surrounded by a pericentricular matrix (Figure 1). Centrioles are essential tubule organizing center, crucial to the formation of the spindle apparatus during mitosis. to the positioning of spindle fibers during cell division, and to morphogenesis of the cell during development. Centrosomes act as a microtubule organizing centers.

• In the cell cycle, the centrosome duplicates only once to form the 2 poles of a mitotic spindle, essential in chromosomal segregation and cell proliferation (Figure 2). Centrosomal activity producing extra spindle poles may be the cause of aneuploidy. Centrosomal amplification can be a primary cause of genomic instability.

• In previous studies we showed AZT-induced centrosomal amplification by staining with an anti-pericentrin antibody and fluorescence microscopy (Figure 3).

Figure 1: The Centrosome

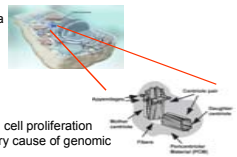
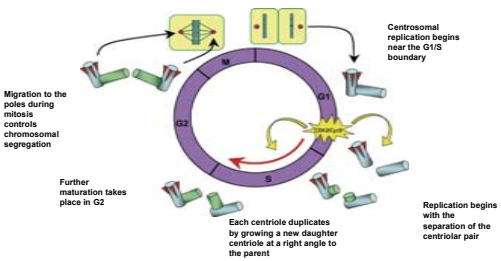


Figure 2: The Centrosome Replication Cycle

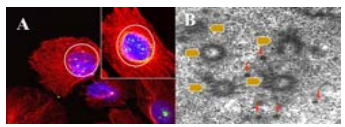


Fukasawa, K. Cancer Letters (2005) 230:6-19

Figure 3: Exposure of Chinese Hamster Ovary (CHO) cells to AZT revealed:

* Striking disorganization of centrosomal material in the form of amplification and fragmentation found by confocal microscopy (A) and confirmed by EM (B, brown arrows).

* Pericentriolar bodies associated with the centrosome, absent in untreated cells (B, red arrows).



CHO cells exposed for 24 hr to 200 μM AZT

OBJECTIVE

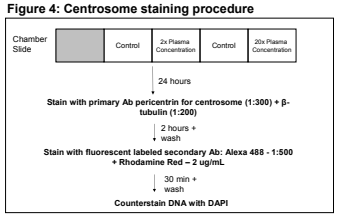
To determine if centrosomal amplification is a common phenomenon induced by nucleoside analogs.

•Cells were stained with anti-pericentrin antibody and an Alexa 488-conjugated (green color) secondary antibody for centrosome identification. For tubulin localization, the cells were stained with anti-β-tubulin and a rhodamine red-conjugated secondary antibody (red color). For DNA (nuclear) localization cells were stained with DAPI (blue color).

•Quantitation of positive centrosomal (pericentrin/Alexa 488 positive) signals was performed for 1000 cells per group by fluorescence microscopy. Each exposure was performed on 3 occasions; values shown are mean ± standard error.

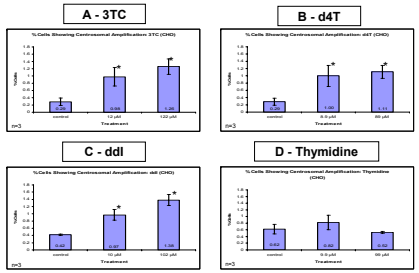
•Chinese Hamster Ovary cells (CHO) and Normal Human Mammary Epithelial cells (NHMEC, strains M98040 and M99005) were exposed to 3TC, d4T, ddI, and thymidine for 24 hours (Figure 4).

Figure 4: Centrosome staining procedure



RESULTS

Figure 5: Centrosomal amplification was induced in CHO cells by 3TC (A), d4T (B) and ddI (C) but not by thymidine (D) (* Statistically significant by t-test)



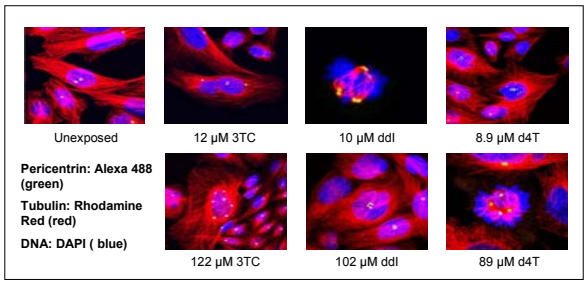
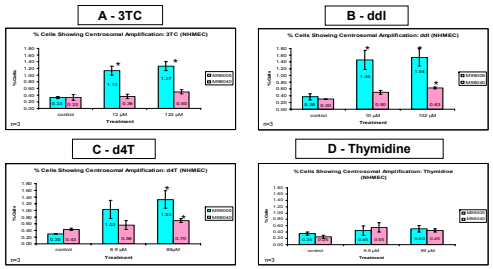
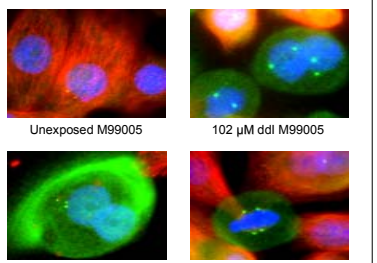


Figure 7: Centrosomal amplification was induced strongly in NHMEC strain M99005 , but not in strain M98040, by exposure to 3TC (A), ddI (B), d4T (C) but not by thymidine (D) (* Statistically significant by t-test)





SUMMARY

• NRTI exposure of CHO cells, and M99005 NHMECs, to 3TC, d4T and ddI showed dose-related, statistically significant, increases in centrosomal amplification.

• For each NRTI, the lower dose chosen was near plasma levels.

• No increase in centrosomal amplification was observed with thymidine alone in any strain or with low doses in the M98040 strain.

• Centrosomal amplification occurs in NHMECs that incorporate AZT (and presumably other NRTIs) into DNA, but not in NHMECs that do not show AZT-DNA incorporation.

CONCLUSIONS

•Centrosomal amplification is a novel mechanism of genomic instability observed in human and hamster cells exposed to 3TC, d4T and ddI, as well as AZT.

•This manifestation of genotoxicity appears at plasma levels, and is likely the result of NRTI incorporation into DNA.

•Centrosomal amplification occurs in the following order of toxicity: d4T<AZT<3TC<ddI.