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Alpha-defensins increase HIV transcytosis: role of STI-mediated enhancement of HIV transmission

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Background: Although mucosal epithelial cells provide the first-line of defense against HIV infection, HIV can cross the genital or intestinal epithelium by transcytosis. The risk of HIV transmission is increased in individuals with sexually transmitted infections (STIs). Defensins are antimicrobial peptides important for mucosal innate immunity. Alpha-defensins, including human defensin 5 (HD5) and human neutrophil defensins 1-3 (HNPs1-3), are elevated at the genital mucosa in individuals with STIs. HD5 promotes HIV infectivity and contributes to enhanced HIV infection by STIs in vitro. Unlike HD5, HNPs exhibit anti-HIV activity in vitro. However, enhanced HIV acquisition is associated with increased levels of HNPs in cervicovaginal fluid. In this study, we examined the effect of alpha-defensins on epithelial integrity and the subsequent impact on HIV transcytosis in polarized epithelial cells.

Methods: HNP1 and HD5 and their linear analogs were chemically synthesized. Cysteine residues were replaced by alanine for linear HNP1 synthesis; whereas, isosteric a-aminobutyric acid (Abu) replaced cysteine residues for linear HD5 synthesis. Defensins were added to the apical side of polarized intestinal epithelial (Caco-2) cells. Transepithelial electrical resistance was measured by cellZscope to monitor epithelial permeability. Distribution of tight junction markers was observed by immunofluorescent staining of ZO-1 or occludin. To examine HIV transcytosis, HIV-1_{BAL} was added to the apical side of polarized epithelial cells, and HIV that crossed to the basolateral side was measured by HIV p24 ELISA. Statistical analysis was performed using a two-tailed paired *t*-test; $p<0.05$ was considered significant.

Conclusions: We found that HNP1, but not HD5, significantly disrupted epithelial integrity and promoted HIV traversal of epithelial barriers. Linear HNP1 with the same charges did not affect epithelial permeability, indicating that the observed effect of HNP1 on the epithelial barrier was structure dependent. These results suggest a role for HNP1 in STI-mediated enhancement of HIV transmission.

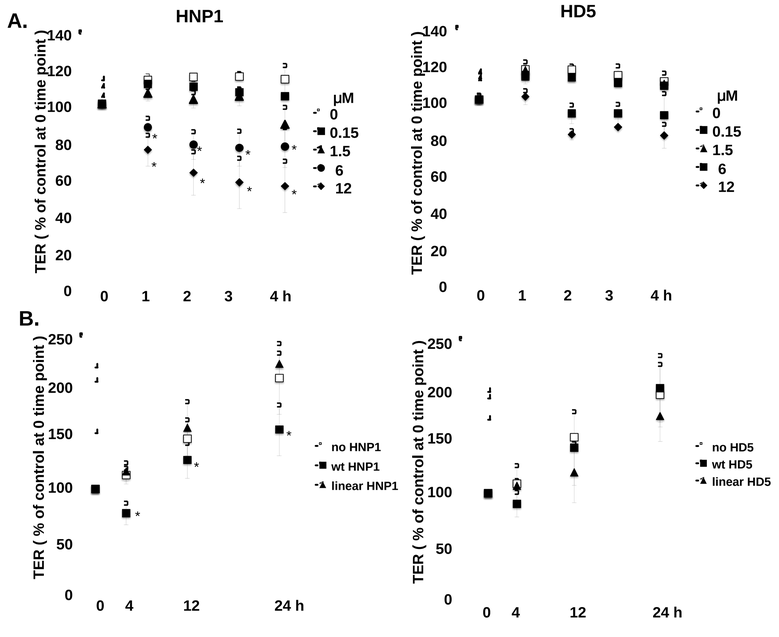


Fig.1. HNP1, but not HD5 or linear analogs, increases epithelial permeability. (A) The effect of HNP1 and HD5 on short-term dynamics of epithelial integrity in polarized Caco-2 cells were determined by measuring transepithelial electrical resistance (TER) using a CellZscope. **(B)** Effect of defensins and their linear analogs on epithelial integrity. TER values were normalized by the value at time 0 for each treatment. Data are mean±SD of averages of several experiments indicated in each panel; *p<0.05, defensin treated versus non-treated control at the same time point.

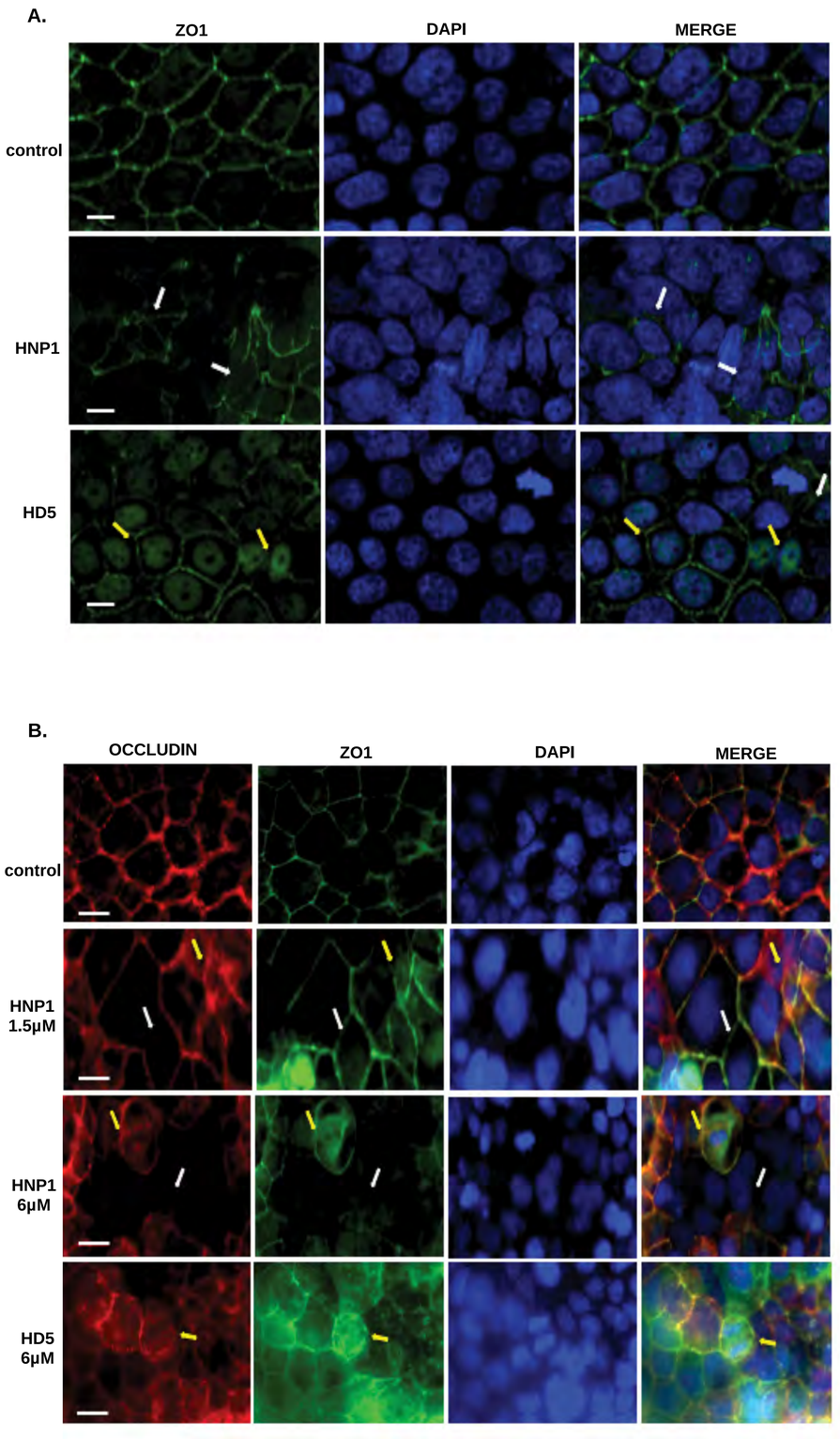


Fig. 2. Defensins alter distribution of tight junction markers. Monolayers of Caco-2 cells grown on MatTek plates (Panel A) or polarized Caco-2 cells grown in transwell inserts (panel B) were treated with defensins (6 mM) for 4 h followed by immunostaining with antibodies against tight junction markers ZO-1 and occludin. White arrows indicate loss intercellular tight junction borders; yellow arrows indicate translocation of markers. Images in Panel A were acquired on a Nikon A1Rsi confocal microscope; images in Panel B were acquired on a Zeiss Axio Observer Z1 microscope. Scale bar = 10 mm (panel A) or 20 mm (panel B).

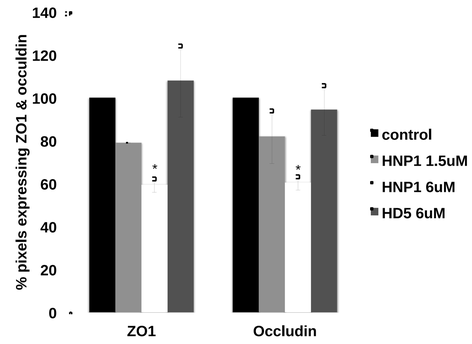


Fig. 3. HNP1 reduces tight junction expression in polarized intestinal epithelial cells. Quantification of ZO1 and occludin expression in microscope images of untreated and defensin-treated polarized Caco cells. Intensity between samples were normalized with DAPI intensity by dividing the number of pixels expressing ZO1 or occludin by the number of DAPI pixels. The values for immunofluorescence intensity represent means of a minimum of 3 representative fields for each experiment and averages of 2 independent experiments.

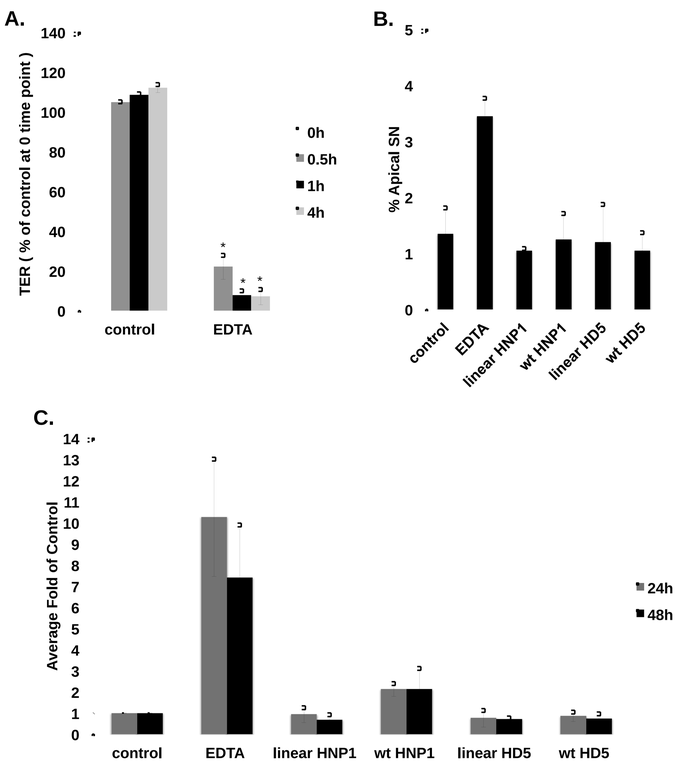


Fig. 4. HNP1, but not HD5, promotes HIV traversal of polarized intestinal epithelial cells. (A) The effect of 2.5mM EDTA treatment on epithelial integrity was determined by the Cellzscope. **(B)** FITC-Dextran was used to examine permeability after 4hr treatment with WT and linear analogs of defensins, or EDTA. Data are mean±SD of averages of 2 independent experiments. **(C)** Polarized Caco-2 cells were treated with 2.5mM EDTA, or 6 μM of WT and linear analogs of HNP1 and HD5 for 4 h before addition of HIV-1_{BAL} to the upper chamber. Basolateral supernatant was collected 24 and 48h after the addition of HIV to the apical chamber. The amount of HIV in the bottom chamber at the indicated times were determined by HIV p24 ELISA. The fold of untreated control for HIV transversal was determined for samples treated samples. Data are mean±SD of two to three independent experiments. The gray line represents no change in level of HIV transversal between untreated and treated samples.

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