



Transmission electron microscopy analysis of Annexin 2-depleted MDM reveals inhibition of HIV assembly and maturation in internal vesicles

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BACKGROUND

HIV-1 infected monocyte-derived macrophages (MDM)

- MDM are important for HIV-1 transmission, replication, and pathogenesis.
- HIV buds into internal vesicles in MDM.
- HIV buds from T-cells via their plasma membrane.
- No cellular proteins have been discovered that govern assembly/budding in MDM.

Previously, we discovered that HIV-1 p55^{Gag} binds to Annexin 2 (Anx2)

- Anx2 is an endosome-associated protein involved in membrane organization/traffic.
- Anx2 cycles between the cytosol, the inner membrane of vesicles, and the plasma membrane.

PURPOSE AND HYPOTHESIS

Purpose of our study

To understand the role of Anx2 in HIV assembly/budding in MDM.

Our hypothesis

HIV assembly/budding in MDM is mediated by a p55^{Gag} - Anx2 interaction.

MATERIALS AND METHODS

siRNA knockdown of Anx2 expression

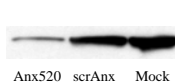
- MDM were transfected with Anx2-specific siRNA (or a neg. control) for 3 days.
- MDM were infected with 50 ng of HIV-1_{JAGO} for 16 hrs and cultured for 4 days.
- Cell culture supernatants were assayed for the presence of p24^{Gag}.
- Duplicate cell cultures were
 - lysed for ANX2 and GAPDH Western blotting or
 - prepared for transmission electron microscopy (TEM).

Transmission electron microscopy (TEM)

- Cultures were fixed for 1 hr in 2% glutaraldehyde.
- Cells were scraped off plate with 1% gelatin and pelleted.
- The resulting cell pellet was fixed in 2% osmium, washed, dehydrated in ethanol and propylene oxide, and embedded in plastic resin (EPON).
- 70 nm thick sections cut and stained with uranyl acetate.
- Observations were made using a JEOL JEM 1010 transmission electron microscopy.

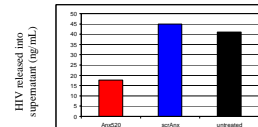
RESULTS

Reduction in Anx2 protein after siRNA treatment as determined by Western Blot for Anx2

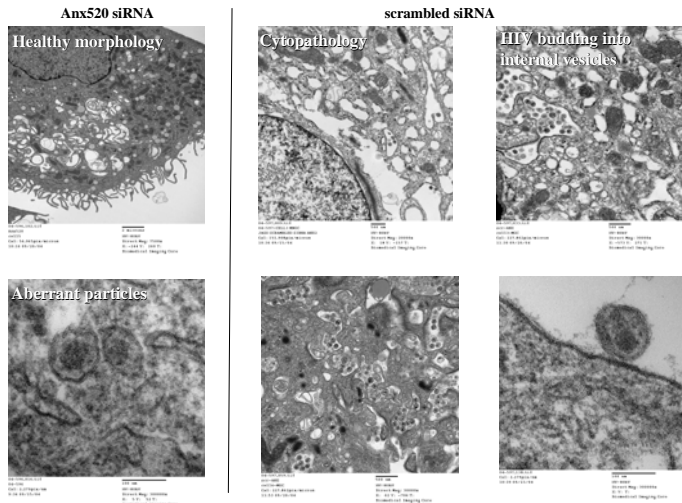


Anx520 siRNA resulted in a decrease of Anx2 protein in 16 out of 23 MDM treated with Anx2-specific siRNA.

Depleting Anx2 protein in MDM leads to a significant decrease in p24^{Gag} and infectious virus released



Transmission electron microscopy of HIV-1 infected MDM with and without Anx2 siRNA-treatment



MAIN RESULTS

- Depleting Annexin 2 protein with siRNA in MDM leads to a significant decrease in p24^{Gag} and infectious virus release.
- We observed no assembly/budding of HIV particles into internal vesicles (e.g. multivesicular bodies) in MDM in cultures depleted of Annexin 2.
- Instead we observed possible immature viral particles with membrane bilayer structures.
- Conversely, HIV infection of MDM expressing wild type levels of Annexin 2 (scrambled siRNA) resulted in HIV budding into internal vesicles and full particle maturation.

CONCLUSIONS

- The reduction of Annexin 2 protein inhibited HIV maturation in internal vesicles in MDM.
- These results support our hypothesis that binding of p55Gag to Annexin 2 is involved in directing viral assembly and budding to the endosomal membranes in MDM and that reduction of Anx2 leads to decreased production of mature HIV particles because this HIV maturation pathway has been disrupted.

