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Poster presentation

HLA-C increases fusion efficiency between the HIV-1 envelope and the cell membrane and is part of the fusion complex

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Background

The HIV-1 viral envelope originates from the cell membrane and contains different cellular proteins such as MHC class I molecules, which play a role in modulating viral infectivity. HLA-C is known to enhance viral infectivity and reduce sensitivity to neutralizing antibodies by inducing gp120/41 conformational changes that may increase fusion efficiency and viral infectivity [1]. In this work we studied the effect of HLA-C on syncytia formation and of its association with the HIV-1 gp120/41 and with the CD4/CCR5 receptors in purified fusion complexes.

Materials and methods

Syncytia were formed by co-cultivating HeLa cells expressing the HIV-1 gp120/41 of different laboratory and primary isolates with target cells expressing HIV-1 receptors (3T3.T4.CCR5; 3T3.T4.CXCR4; HeLa-P4.2; TZM-bl; CHO-CD4-CCR5). HLA-C expression was silenced using the RNA interference technique (Dharmacon SMARTpool siRNAs) and verified by Western blot and cytofluorimetry. Fusion efficiency was determined by calculating the fusion index, and, with TZM-bl cells, by using the β-gal and the luciferase quantitative assays. Fusion complexes formed by co-cultivating CHO-gp120/41-HLA-C and CHO-CD4-CCR5 cells, and by co-cultivating CHO-gp120/41 and CHO-CD4-CCR5-HLA-C cells were purified and analyzed by Western blot and dot-blot as described [2].

Results

The level of syncytia formation between HLA-C silenced HeLa-ADA and 3T3.T4.CCR5 cells or HeLa-LAI and 3T3.T4.CXCR4 cells was significantly lower ($p < 0.01$) than between non silenced control and target receptor cells. The R5-tropic gp120/41 ADA was sensitive to HLA-C presence when fusing with 3T3.T4.CXCR4 cells ($p < 0.01$), whereas the X4-tropic gp120/41 LAI was not affected by HLA-C presence when fusing with 3T3.T4.CCR5 cells. Similarly, fusion efficiency was significantly lower ($p < 0.01$) using HLA-C silenced cells expressing HIV-1 gp120/41s with either HeLa-P4.2 or TZM-bl cells. No difference was evident using gp120/41 of the NDK isolate, confirming a previous report [1]. When HLA-C was expressed on target receptors cells, a similar positive effect on fusion efficiency was observed for all gp120/41s tested, including NDK. HLA-C molecules were detected in purified fusion complexes, either associated with the gp120/41 or with the receptors.

Conclusion

HLA-C silencing by RNA interference reduces fusion efficiency of X4 and R5-tropic gp120/41s, highlighting its role in increasing viral infectivity. The fusion efficiency of R5-tropic gp120/41 with CD4-CXCR4 cells is enhanced by HLA-C presence, whereas the X4-tropic gp120/41 LAI is not sensitive to HLA-C presence when fusing with CD4-CCR5 cells, suggesting that HLA-C might play a role in the transition from R5 to X4-tropism during the course of nat-

ural infection. The NDK env fusion capacity is not influenced by HLA-C presence when co-expressed with the gp120/41, but it is enhanced when HLA-C is co-expressed with the receptors, suggesting a different mechanism of action for HLA-C molecules in enhancing viral infectivity, whether they are expressed on the same membrane that contains the gp120/41 or expressed with the receptors. The comparison between HLA-C sensitive and insensitive gp120/41 sequences may help elucidating the role of HLA-C in the fusion process by indicating a possible interaction and/or association between these molecules. The co-purification of HLA-C with the fusion complex suggests its association either with gp120/41 or with HIV-1 cellular receptor/co-receptor, resulting in an increased efficiency of the fusion process and thus a greater infectivity of viral particles that include HLA-C in their envelope.

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