Sirtuins, new potential targets for HIV-therapy

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Highly active antiretroviral therapy (HAART) has shown great efficacy in increasing the survival of Human Immunodeficiency Virus (HIV)-infected individuals. However, HAART, which is mainly based on targeting viral encoded molecules, cannot eradicate HIV.
Nevertheless, HIV critically relies not only on the action of viral proteins since many host genes have to interact with them in order to allow infection. In particular, after retrotranscription of the viral genomic RNA (enabled by the reverse transcriptase brought by this pathogen), integration of the proviral DNA into the host genome is allowed by the interaction between viral integrase with broken-DNA fixing proteins provided by the host cell, a process called 'post-integration repair'.
Based on these assumptions, we investigated the potential impact on HIV integration of those enzymes that catalyse the removal of acetyl groups from chromatin proteins. In particular we studied the sirtuin class of proteins, since this family of NAD+-dependent deacetylases is recruited to the sites of DNA damage to establish functional interactions with 'repairing factors', such as Ku70 or the ATM/Nsb1 complex.
To quantitatively assess the integration of viral DNA into the host genome, we challenged HeLa cells with an HIV-based, replication-defective, lentivirus that carries the 'green fluorescent protein' (GFP) reporter gene. Indeed, by infecting human cell lines in a range of 0,1-0,2 MOI (in order to avoid multiple infections in the same cell), we were able to measure 'integrational' events by discriminating, through flow cytometry, between population of cells endowed with low levels of the GFP protein (as consequent to 'episomal' expression of the viral gene), from cells characterized by a brighter green fluorescence enabled by the integration of the GFP gene into the host genome. In these experiments we identified, from a library of commercially available and newly synthesized sirtuins inhibitors, two molecules, B2 and compound-2, which were capable to inhibit HIV integration as efficiently as raltegravir (the 'reference' integrase inhibitor) with a good concentration/toxicity profile.
Importantly, the reliability of the data emerging from the flow cytometric assay was further confirmed, after purification of the genomic DNA of infected cells, via nested PCR of the Alu-gag sequences representing hybrid regions between host and viral DNA.
Moreover, as a proof-of-principle of the involvement of sirtuins in the molecular steps leading to viral integration, ablation of specific sirtuins sub-type by RNA interference reproduced the effect evoked by sirtuins inhibitors. Conversely, treatments with resveratrol, which activates the whole sirtuin family of proteins by increasing intracellular NAD+ levels, enhanced HIV integration, as determined by assessment of GFP-fluorescence intensity.
Taken together, our observations provide the first evidence that sirtuins represent a new potential class of targets for HIV-therapy, thus paving the way to the development of drugs able to inhibit HIV life-cycle on the cellular side, thus escaping the mechanism of resistance intrinsic to the viral machinery.