Wahiba Aouadi, Virol Mycol 2017, 6:1(Suppl)
http://dx.doi.org/10.4172/2161-0517.C1.018

Binding of the methyl donor SAM to MERS-CoV 2'-O-methyltransferase nsp16 promotes the recruitment of the allosteric activator nsp10

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The Middle East respiratory syndrome coronavirus (MERS-CoV), causing severe pneumonia and renal failure in humans, appeared in the Middle East with secondary spread to Europe, Africa, Asia and North America since 2012. The World Health Organization reported 1618 cases with high case-fatality rate around 36%. The fatal outcome of MERS-CoV is caused by a poorly coordinated innate immune response with ineffective activation of the adaptive immune response that failed to clear MERS-CoV viremia from the host. The 5' end of coronaviruses positive stranded genome is decorated by a cap-1 structure (^{7m}GpppN2'$_{Om}$). The MERS-CoV encodes for two putative SAM-dependent methyltransferases, non-structural proteins (nsp) nsp14 and nsp16, supposed to methylate cap structure at the N7 position of the guanosine and at the 2-oxygen of the first nucleotide (N1) ribose, respectively. These cap modifications are essential for virus replication. The N7-methylation promotes RNA translation into viral proteins and the 2'-O methylation prevents virus detection by cell innate immunity mechanisms and viral translation inhibition by the interferon-stimulated IFIT-1 protein. In this work, we demonstrate that nsp14 is an N7-methyltransferase (MTase) converting GpppG-RNA into a cap-0 structure (^{7m}GpppG-RNA). These cap-0 RNAs are next methylated at the 2'O position of the first RNA nucleotide by nsp16 forming 7mGpppG2'Om-RNA.This 2'-O MTase activity is regulated by nsp10. To unravel the regulation of nsp10/nsp16 2'-O-MTase activity, we used purified MERS-CoV nsp16 and nsp10. First, we showed that nsp16 recruited N7-methylated capped RNA and SAM. The SAM binding promotes then the assembly of the enzymatically active nsp10/nsp16 complex that converted ^{7m}GpppG (cap-0) into ^{7m}GpppG2'$_{Om}$ (cap-1) RNA by 2'-OH methylation of N1 in a SAM-dependent manner. The subsequent release of SAH speeds up nsp10/nsp16 dissociation that stimulates the reaction turnover. Alanine mutagenesis and RNA binding assays allowed the identification of the nsp16 residues involved in RNA recognition forming the RNA binding groove (K46, K170, E203, D133, R38, Y47 and Y181) and the cap-0 binding site (Y30, Y132 and H174). In order to develop specific inhibitors blocking both enzymatic activities, we set up a robust enzymatic assay to screen chemical library. We first screened a chemical library containing 1200 compounds having the AMM (marketing authorization) and 320 natural compounds on both nsp14 N7 MTase and nsp16 2'-O-MTase activity, we have obtained 11 and 5 primary hits, respectively with inhibition percentage superior to 80%. In a second set of experiments we tried to identify compounds blocking the nsp10/nsp16 interaction by using the 2P2I3D library enriched in compounds (2000) that targeted the protein-protein interaction surface. We have obtained 68 with 50% inhibition and 122 with 40% inhibition. The IC_{50} of these compounds will be determined soon in order to select the best compounds for the hit to lead determination.

Biography

Wahiba Aouadi has done her Diploma of Higher Studies (DES) in Biochemistry in the year 2009 from University Mohamed Boudiaf - M'sila, Algeria. She obtained her Master's in Development and Immunology from Aix-Marseille University (France) in the year 2013. Currently, she is doing her PhD from the AFMB Laboratory of Aix-Marseille University with Infectiopole Grant on the Characterization of the Middle East respiratory syndrome coronavirus (MERS-CoV) capping enzymes, a prerequisite for drug design. Recently, her work has been accepted for publication in *Journal of Virology.*

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