ABSTRACT

LEDGF/p75 protein is a human transcriptional co-activator and epigenetic reader associated with transcriptionally active chromatin. It is crucial for HIV integration and MLL1 fusion-driven leukemia development. Interactions of LEDGF/p75 with HIV integrase (HIV IN) and MLL1–menin complex are considered an attractive therapeutic target for drug development. LEDGF/p75 interacts with both HIV IN and MLL1–menin complex through its integrase binding domain (IBD).

While the pathophysiological interactions of LEDGF/p75 IBD were intensively studied, little was known about the physiological ones. In addition to HIV IN and MLL1, the LEDGF/p75 IBD also interacts with JPO2, PogZ, ASK and MLL2. In search for specific inhibitors of LEDGF/p75 IBD interaction with HIV IN and MLL1, it is essential to obtain detailed information about its interactions with all binding partners.

The IBD–MLL1–menin complex has been structurally characterized, but only partially. Using NMR spectroscopy, we identified and mapped a novel part of the IBD–MLL1 interface. This additional interface is able to maintain the interaction between LEDGF/p75 and MLL1 even without the presence of menin, which was considered necessary. Moreover, colony forming assays of primary leukemic blasts revealed that this additional interface is essential for leukemic transformation. Interestingly, the newly defined interface on IBD overlaps with the binding site of the HIV IN.

Our analyses revealed structural details of LEDGF/p75 interactions with other physiological binding partners. We found that interactions with the LEDGF/p75 IBD are maintained by an intrinsically disordered IBD-binding motif (IBM) common to all known cellular partners. This interaction interface and its importance have been thoroughly validated by mutation analyses and two solution structures of IBD–IBM complexes have been solved. Utilizing the structural information, we explained how HIV IN out-competes the cellular proteins.

Based on the knowledge of the IBM, we identified and validated IWS1 as a novel LEDGF/p75 interaction partner. IWS1 is a human transcription factor which plays a key role in defining the composition of the RNA polymerase II elongation complex. It also interacts with the H3K36 (histone 3, lysine 36) methyltransferase Hypb/Set2. Trimethylated H3K36 represents a signature chromatin mark of active transcription which is recognized by PWWP domain of LEDGF/p75.

Detailed characterization of physiological interaction interfaces on the IBD revealed a notable overlap with the region involved in interaction with HIV IN. Indeed, a large part of interaction interface on IBD is common for all the interaction partners and their interactions with LEDGF/p75 are mutually exclusive. The similar binding modes of LEDGF/p75 interaction partners represent a new challenge for the development of selective interaction inhibitors. However, in the case of MLL1, there is a unique additional menin-dependent interface engaged in its interaction with IBD. Such feature could allow for a specific inhibition.