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Epstein-Barr Virus encoded deconjugases and AmotL2 in control of cell topology

AKADEMISK AVHANDLING
Som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i CCK lecture hall R8:00

Friday the 24th of January, 2014, 9.00 a.m.

av Sebastian Hildebrand

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ABSTRACT - *EPSTEIN-BARR VIRUS ENCODED DECONJUGASES*

The post-translational conjugation or deconjugation of proteins by ubiquitin (Ub) or ubiquitin-like molecules (UbLs: e.g. SUMO, NEDD8, ISG15) has emerged as a major regulatory mechanism of various cellular activities. Epstein-Barr virus (EBV) is a large double-stranded DNA tumor virus encoding ~100 open reading frames (ORFs). The overall aim of this study was to identify and functional characterize EBV encoded Ub- or UbL-deconjugases. We screened an EBV-ORFeome library for their activity against Ub-, NEDD8-, SUMO-1,-2,-3 and ISG15-GFP reporter. As a result we discovered that the BSLF1- and BXLF1-ORF comprised deubiquitinating activity. We could also detect that the large tegumental protein BPLF1-N cleaves the NEDD8-GFP reporter with similar efficiency as the Ub-GFP reporter. Following this observation we could show that BPLF1-N was able to process Ub- and NEDD8-linked functional probes with similar efficiency suggesting equal affinities towards ubiquitinated and neddylated substrates. We could show that BPLF1-N binds to and deneddylates cullins, which are assembled in cullin-RING ligases (CRLs). This CRL deneddylation facilitated the stabilization of their substrates involved in cell cycle regulation. Those accumulated BPLF1-N controlled CRL substrates were essential for an S-phase like cellular environment and endoreduplication in BPLF1-N expressing cells. We further demonstrated that the impact of BPLF1-N expression on viral genome replication was dependent on stabilization of the DNA licensing factor CDT1.

ABSTRACT - *AMOTL2 IN CONTROL OF CELL TOPOLOGY*

During developmental morphogenesis, cells migrate, differentiate and organize into multicellular structures. As a distinct step in organ formation, epithelial cells join together via cell-cell junctions to form sheets of cells that separate cellular compartments from each other. Endogenous forces, generated by contractile actin, are transmitted over cell-layers in part by the connection to adhesion junctions and E-cadherin. These forces affect cellular geometry (cell size and shape) and topology (connectivity among cells in a tissue). Exactly how E-cadherin connects to the actomyosin network has been less clear.

In our work, we show that the scaffold protein AmotL2 p100 binds to the adherens junction components E-cadherin and MAGI1 and associates to contractile actin fibers which connect cells over multiple layers. Silencing of AmotL2 in epithelial cells *in vitro* and in zebrafish keratinocytes *in vivo* resulted in loss of actin filaments perpendicular to cellular junctions and dramatic changes in cellular geometry. As a consequence, the packing of epithelial cells in the typical hexagonal patterns was severely perturbed and the ability to form 3-D structures was lost. Cells depleted of AmotL2 also showed increased fluidity and elasticity when subjected to mechanical force. We propose that AmotL2 is a critical component in the adhesion junctions that controls intracellular contractility as well as relaying forces between cells.