ABSTRACT

A NOVEL TIP30 COMPLEX REGULATES ENDOCYTIC TRAFFICKING OF SIGNALING RECEPTORS BY FACILITATING THE TRANSPORT OF VACULOLAR (H+)-ATPASES TO EARLY ENDOSOMES

By

Chengliang Zhang

TIP30, a 30-kDa HIV-1 Tat-interacting protein, is a tumor suppressor whose expression is altered in a variety of human cancers. Tip30-deficient mice spontaneously develop hepatocellular carcinomas and other tumors as well as mammary preneoplasic lesions. However, the molecular mechanism of TIP30 function remains largely unraveled.

In this study, we show that a novel protein complex consisting of TIP30, Endophilin B1 (Endo B1), acyl-CoA synthetase long-chain family member 4 (ACSL4) interacts with Rab5a, and facilitates Rab5a recruitment to early endosomes by promoting efficient fusion between Rab5a vesicles and endocytic vesicles. Rab5a vesicles are EEA1-negative vesicles carrying vacuolar (H+

)-ATPases (V-ATPases), an endosome acidification enzyme that causes ligandreceptor dissociation. Inhibition of TIP30, ACSL4 or Endo B1 impairs Rab5a vesicles loading on early endosomes and causes the mislocalization of V-ATPases, leading to delayed EGF-EGFR dissociation and prolonged EGFR signaling. Furthermore, we show that both arachidonic acid and coenzyme A are essential for the fusion of Rab5a vesicles with endocytic vesicles in vitro. TIP30, ACSL4 and Endo B1can promote vesicle fusion in the presence of arachidonic acid and coenzyme A and can transfer the arachidonyl group to endosomal phosphatidic acid to produce triacylglycerol, which induces membrane tethering and stacking. Together, these results identify

a novel function for Rab5a in endocytic trafficking and suggest a mechanism, in which addition of the hydrophobic arachidonyl group to phosphatidic acid by the TIP30 protein complex may bring membranes into close contact to allow for membrane fusion.

Supporting the above findings is the observation that Tip30 deletion dramatically accelerated the onset of mammary tumors in the MMTV-Neu transgenic mouse model of breast cancer, which overexpresses another EGFR family member, HER2/Neu. Similar to liver cells, deletion of Tip30 in mouse mammary cells also caused the trapping of EGF-EGFR complexes in early endosomes, thereby leading to delayed EGFR destruction and sustained EGFR signaling in response to EGF treatment.

We further found that unlike tumors developing in MMTV-Neu mice, almost all of which are estrogen receptor-negative and progesterone receptor-negative (ER-/PR-), tumors arising in Tip30-/-/MMTV-Neu mice are almost exclusively ER+/PR- mammary tumors.

Immunofluorescence studies showed that Tip30 is predominantly expressed in ER+ mammary epithelial cells (MECs) and its deletion leads to an increase in the number of phospho-ER positive cells in mammary glands and accelerated activation of Akt in MMTV-Neu mice.