PIPKIN REGULATES INTEGRIN ENDOCYTOSIS FOR FOCAL ADHESION DISASSEMBLY BY MODULATING THE RECRUITMENT OF THE ENDOCYTIC MACHINERY TO FOCAL ADHESIONS

S. Beisekenova[1], B. Ospanova[1], T. Kabdullayeva[1], W. T. Chao[2], J. Kunz[1,2*]

1) School of Science and Technology, Nazarbayev University, Astana, Kazakhstan; Jeanette.Kunz@nu.edu.kz; 2) Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, USA

Introduction. Cell migration and tissue invasion during tumor metastasis require the regulated disassembly of focal adhesions, but the underlying mechanisms remain poorly defined. We previously showed that the turnover of focal adhesions occurs through odpha5Peta1 integrin endocytosis from focal adhesions (1). We further discovered that only integrins in their active conformation are internalized during focal adhesion disassembly and we set out to define the molecular machinery responsible for integrin transport (1,2). These studies revealed that the pathway required for the trafficking of active integrins involves different components than the pathway required for the trafficking of inactive integrins (1,2). Significantly, only by inhibiting the endocytosis of activated integrins was cell migration inhibited (2). Hence, the further identification of key components of the focal adhesion disassembly pathway is likely to expose novel avenues to inhibit tumor cell invasion and metastasis.

Materials and methods. We used a focused RNA interference screen in combination with dominant negative approaches to systematically identify signaling and adaptor proteins necessary for integrin endocytosis during focal adhesion disassembly.

Results and discussion. We identified several new proteins that contribute to integrin endocytosis during focal adhesion disassembly, including proteins involved in cytoskeletal reorganization and/or regulation of membrane traffic. In particular, we found that focal adhesion disassembly occurs through a dynamin 2- and clathrin-dependent mechanism (1,2). We further identified the clathrin adaptor Disabled 2 (DAB2) and several tyrosine kinases as novel components of the focal adhesion disassembly pathway (1,2). Significantly, we showed that Type I phosphatidylinositol 4-phosphate 5-kinase beta PIPK1P, an enzyme that generates phosphatidylinositol-4,5-bisphosphate $PI4,5P_2$, is a master regulator of focal adhesion disassembly and we provide evidence that PIPK1P, via synthesis of $PI4,5P_2$, acts as a targeting hub for the recruitment of the endocytic machinery to focal adhesion sites (2).

Conclusions. Together, our findings identify new endocytic and signaling components specific for focal adhesion disassembly. Future goals are to determine how integrin endocytosis is regulated by these newly identified proteins, how the assembly of the endocytic and signaling network for focal adhesion disassembly is itself regulated, and how this affects the migration of normal and tumor cells. As many of the newly identified components have known roles as oncogenes, they may provide new therapeutic avenues to target cancer development and progression.

Acknowledgments. Funding support was provided by R01 grant from the National Institute of General Medicine of National Institutes of Health, USA.

References.

1. Chao WT & Kunz J (2009). *FEBS letters* 583(8):1337-1343.
2. Chao WT, *et al.* (2010). *Molecular and cellular biology* 30(18):4463-4479.