Laboratory of Signal Transduction

Plasma membrane signalosomes, mast cell, IgE receptor signalling

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The plasma membrane possesses a variety of molecules (mainly proteins and lipids) that are involved in such important cellular functions as cell migration, adhesion and signalling. Our recent studies have been focused on understanding the roles of selected plasma membrane-associated components at initial stages of mast cell activation. We analyzed functions of the transmembrane adaptor protein NTAL and the roles of protein tyrosine phosphatases (PTPs) at early stages of mast cell signalling induced via the high-affinity IgE receptor (FcεRI) or c-kit. We found that mast cells isolated from NTAL-deficient cells exhibited reduced spreading on fibronectin, enhanced filamentous actin depolymerization and enhanced migration towards antigen when compared to wild-type cells. To understand the molecular basis of these phenomena, we examined activities of two small GTPases, Rac and Rho, important regulators of actin polymerization. Stimulation of the cells via FcεRI enhanced activity of Rac[1,2,3] in both NTAL-deficient and wild-type cells. In contrast, the RhoA activity decreased and this trend was much faster and more extensive in NTAL-deficient cells, indicating a positive regulatory role of NTAL in the recovery of RhoA activity. After restoring NTAL into NTAL-deficient cells, both spreading and actin responses were rescued. Thus, our studies showed for the first time that NTAL has a crucial role in signalling, via RhoA, to the mast cell cytoskeleton.

The earliest known biochemical step in FcεRI-activated cells is tyrosine phosphorylation of the receptor subunits by Src family kinase Lyn. However, the exact molecular mechanism of this phosphorylation step is incompletely understood. We therefore tested our hypothesis that changes in the activity and/or topography of protein tyrosine phosphatases could play a major role in the FcεRI triggering. We found that exposure of mast cells to PTP inhibitors induced phosphorylation of the FcεRI subunits, similarly as FcεRI triggering. Interestingly, and in sharp contrast to antigen-induced activation, the inhibitors had no effect on association of FcεRI with detergent-resistant membranes and their topography in the plasma membrane. In cells stimulated with antigen or the inhibitors, enhanced oxidation of active site cysteine of several PTPs was detected. Unexpectedly, most of oxidized phosphatases bound to the plasma membrane were associated with the actin cytoskeleton. Based on these and other data we proposed that down-regulation of enzymatic activity of PTPs and/or changes in their accessibility to the substrates play a key role in initial tyrosine phosphorylation of the FcεRI and other multichain immune receptors.

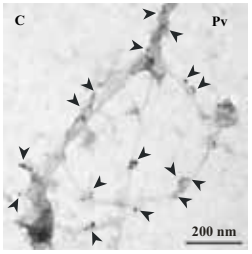

Fig. 1. Membrane topography of oxidized PTPs as detected by electron microscopy on isolated plasma membrane sheets (see Heneberg *et al.*, JBC 2010).

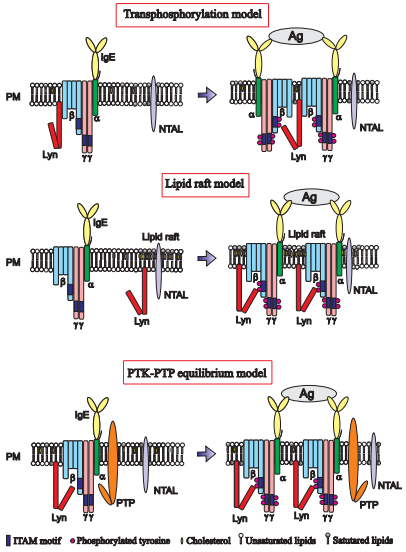

Fig. 2. Models of the high-affinity IgE receptor (FcεRI) phosphorylation by Lyn kinase (see Bugajev *et al.*, FEBS Lett 2010).

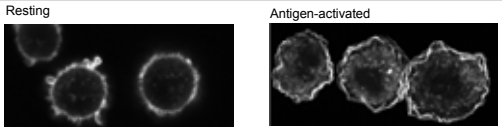

Fig. 3. Spreading of mast cells on fibronectin after antigen-induced activation (see Tůmová *et al.*, Eur J Immunol 2010).

- AS CR, KAN200520701 – Nano-PCR – ultrasensitive test for detection of specific proteins in body fluids, 2007-2011, Petr Dráber
- AS CR, M200520901 – Novel components of mast cell immune receptor signalling, 2009-2012, Petr Dráber
- GA CR, GA301/09/1826 – Topography and function of Csk-binding proteins of the plasma membrane in mast cells, 2009-2013, Petr Dráber
- GA CR, GP302/10/1759 – Function-structure relationships between transmembrane adaptor-based signalosomes in mast cells, 2010-2013, L. Dráberová

1. Heneberg P, Dráberová L, Bambousková M, Pompach P, Dráber P. Down-regulation of protein-tyrosine phosphatases activates an immune receptor in the absence of its translocation into lipid rafts. **J Biol Chem 2010** 285(17): 12787-12802.
2. Tůmová M, Koffer A, Šimíček M, Dráberová L, Dráber, P. The transmembrane adaptor protein NTAL signals to mast cell cytoskeleton via the small GTPase Rho. **Eur J Immunol 2010** 40(11): 3235-45.
3. Shaik GM, Dráberová L, Heneberg P, Dráber P. Vacuolin-1-modulated exocytosis and cell resealing in mast cells. **Cell Signal 2009** 21(8): 1337-1345.
4. Shaik GM, Dráberová L, Dráber P, Boubelík M, Dráber P. Tetraalkylammonium derivatives as real-time PCR enhancers and stabilizers of the qPCR mixtures containing SYBR Green I. **Nucleic Acids Res 2008** 36(15): e93.
5. Smrž D, Lebduška P, Dráberová L, Korb J, Dráber P. Engagement of phospholipid scramblase 1 in activated cells: Implication for phosphatidylserine externalization and exocytosis. **J Biol Chem 2008** 283(16): 10904-10918.

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