Göteborg, 2018

Defining the importance of protein geranylgeranylation in innate immunity

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin vid Göteborgs universitet kommer att offentligen försvaras i hörsal Åke Göransson, Medicinaregatan 11, Göteborg.

Fredagen den 14 december 2018, kl 13:00 av

Naga Venkata Muralikrishna Akula

Fakultetsopponent: **Professor Sharon Campbell**
Department of Biochemistry and Biophysics
University of North Carolina School of Medicine, USA

Avhandlingen baseras på följande delarbeten

1. **Control of the innate immune response by the mevalonate pathway.**
Akula MK, Shi M, Jiang Z, Foster CE, Miao D, Li AS, Zhang X, Gavin RM, Forde SD, Germain G, Carpenter S, Rosadini CV, Gritsman K, Chae JJ, Hampton R, Silverman N, Gravallese EM, Kagan JC, Fitzgerald KA, Kastner DL, Golenbock DT, Bergo MO, and Wang D. (2016). *Nature Immunol.* **17**: 922–929.

2. **Protein prenylation restrains innate immunity by limiting RAC1 effector interactions.**
Akula MK, Ibrahim MX, Khan OM, Kumar TI, Erlandsson MC, Karlsson C, Xu XF, Brisslert M, Brakebusch CH, Bokarewa M, Wang D. and Bergo MO.
Submitted

SAHLGRENSKA AKADEMIN
INSTITUTIONEN FÖR MEDICIN

GÖTEBORGS UNIVERSITET

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Defining the importance of protein geranylgeranylation in innate immunity

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ABSTRACT

RHO family proteins and other intracellular proteins are prenylated with a 20-carbon lipid—a product of the cholesterol synthesis pathway—by protein geranylgeranyltransferase type I (GGTase-I). Prenylation is widely believed to target proteins to membranes where they encounter effector molecules that stimulate GTP-binding and activation. However, my host group found that knockout of GGTase-I in mouse macrophages (*Pggt1b*$^{\Delta/\Delta}$) actually increases GTP-loading of RHO proteins such as RAC1, RHOA, and CDC42, and also increases pro-inflammatory signaling and cytokine production, and induces severe rheumatoid arthritis. These results suggest that prenylation may inhibit rather than stimulate RHO protein function. The mechanisms underlying increased GTP-loading and exaggerated innate immune responses in the absence of GGTase-I are not known. I have addressed these issues in two independent but interconnected projects.

In project 1, we found that there is an imbalance between inflammatory and anti-inflammatory cytokines produced by *Pggt1b*$^{\Delta/\Delta}$ macrophages. We also found that knockout of GGTase-I prevents the interaction between KRAS and PI3K catalytic subunit p110δ and that this reduces signalling through the PI3K-AKT-GSK3β pathway. Moreover, *Pggt1b*$^{\Delta/\Delta}$ macrophages exhibit increased caspase-1 activity that is directly responsible for the production of active interleukin IL-1β, and that this effect requires the *MEFV* (pyrin) inflammasome. Thus, we conclude that GGTase-I promotes an association between KRAS and p110δ and thereby controls major inflammatory pathways in macrophages.

In project 2, we tested the importance of RHO proteins in the development of arthritis in *Pggt1b*$^{\Delta/\Delta}$ mice. We found that knockout of *Rac1* (*i.e.*, in *Pggt1b*$^{\Delta/\Delta}$*Rac1*$^{\Delta/+}$ mice), but not *Rhoa* and *Cdc42*, markedly reduced inflammatory cytokine production and arthritis in *Pggt1b*$^{\Delta/\Delta}$ mice. We also found that non-prenylated RAC1 bound more strongly to the RAS GTPase-activating-like protein 1 (IQGAP1) – which facilitated RAC1 GTP-loading and activation. Knockout of *Iqgap1* in *Pggt1b*$^{\Delta/\Delta}$ mice abolished cellular phenotypes *in vitro* and inhibited arthritis *in vivo*. Thus, we conclude that blocking prenylation stimulates RAC1 effector interactions and activates wide-spread pro-inflammatory signaling. Thus, prenylation normally restrains innate immune responses by inhibiting RAC1 effector interactions.

Key words: *CAAX* proteins, GGTase-I, RAC1, innate immunity, statin, mevalonate

ISBN 978-91-7833-233-5 (PRINT) http://hdl.handle.net/2077/57427
ISBN 978-91-7833-234-2 (PDF