

ABSTRACT

RHO family proteins control cell movement and intracellular signaling by cycling between active GTP-bound and inactive GDP-bound states. Aberrant signaling of RHO GTPases has been implicated in many diseases including cancer and inflammation. Geranylgeranyltransferase type I (GGTase-I) attaches a 20-carbon geranylgeranyl lipid to a carboxyl-terminal CAAX motif of most RHO family proteins. Geranylgeranylation is viewed as essential for membrane targeting and activation of RHO proteins. Consequently, inhibiting GGTase-I to interfere with RHO protein lipidation and activity has been proposed as a strategy to treat cancer and inflammatory disorders. Moreover, statins – widely prescribed cholesterol-lowering drugs – possess anti-inflammatory properties that are independent of their cholesterol-lowering effects. These pleiotropic statin effects are thought to be mediated by reduced synthesis of geranylgeranyl lipids and reduced geranylgeranylation and inhibition of RHO family proteins. Despite the therapeutic interest in GGTase-I, no studies have yet defined the impact of inactivating GGTase-I in mouse models of inflammation using genetic strategies.

Paper I of this thesis shows that mice lacking GGTase-I in macrophages develop joint inflammation and bone erosions similar to rheumatoid arthritis. The disease was initiated by GGTase-I-deficient macrophages which accumulated high levels of GTP-bound RAC1, CDC42, and RHOA, and RAC1 remained associated with the plasma membrane. Moreover, GGTase-I deficiency led to robust activation of p38 MAPK and NF- κ B, and increased production of proinflammatory cytokines. This effect was caused by non-geranylgeranylated GTP-bound RAC1. Thus, rather than being an anti-inflammatory drug target, GGTase-I protects mice from inflammation and arthritis development.

In Paper II, we tested if GGTase-I deficiency in macrophages would affect the development of atherosclerosis in LDL receptor-deficient mice. We hypothesized that aortic lesions would be enhanced due to local and systemic inflammation and the presence of rheumatoid arthritis – a disease that carries a high risk of atherosclerosis development in humans. Contrary to our expectations, GGTase-I deficiency markedly reduced atherosclerosis development. Cellular analyses revealed impaired foam cell formation due to high levels of cholesterol efflux. Molecular analyses revealed increased COX2 and PPAR γ activity and expression of the scavenger receptors CD36 and SR-B1. The pathway was triggered by RHOA which accumulated in the active GTP-bound state in the GGTase-I-deficient macrophages.

This thesis challenges the current dogma that geranylgeranylation is essential for RHO protein activation and suggest that this posttranslational modification may actually inhibit RHO protein function. The thesis also sheds new light on the role of RHO family proteins in macrophage inflammatory signaling and cholesterol homeostasis and mechanisms underlying pleiotropic statin effects.

Keywords: GGTase-I, RHO proteins, inflammation, rheumatoid arthritis, atherosclerosis

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Dissertation

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This thesis is based on the following articles:

- I. **GGTase-I deficiency hyperactivates macrophages and induces erosive arthritis in mice.**
Omar M. Khan, Mohamed X. Ibrahim, Ing-Marie Jonsson, Christin Karlsson, Meng Liu, Anna-Karin M. Sjogren, Frida J. Olofsson, Mikael Brisslert, Sofia Andersson, Claes Ohlsson, Lillemor Mattsson Hultén, Maria Bokarewa, and Martin O. Bergo. *J. Clin. Invest.* 2011; **121**:628–639.

- II. **Targeting GGTase-I activates RHOA, increases macrophage cholesterol efflux, and reduces atherosclerosis development in mice.**
Omar M. Khan, Murali K. Akula, Kristina Skålen, Christin Karlsson, Stephen G. Young, Jan Boren and Martin O. Bergo. *Submitted.*



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