

Statins, Lipids, and Mutations: Consequences for the Heart and Immune System

Akademisk avhandling

Som för avläggande av medicine doktorexamen vid Sahlgrenska akademien,
Göteborgs universitet kommer att offentlig försvaras i Arvid Carlsson,
Medicinaregatan 3, den 6e september, klockan 09:00.

av Emil Ivarsson

Fakultetsopponent:
Professor Pontus Aspenström
Uppsala Universitet, Sverige

Avhandlingen baseras på följande delarbeten

- I. **Emil G. Ivarsson**, Zhiyuan Zou, Murali K. Akula, Margareta Scharin-Täng, Kristell Le Gal, Christin Karlsson, Johan Sternemalm, Azra Miljanovic, Malin Levin, Jan Borén, Martin G. Dalin, Martin O. Bergö. Long-term statin administration inhibits protein geranylgeranylation and causes cardiomyopathy in mice. *Manuscript*.
- II. Murali K. Akula, Mohamed X. Ibrahim*, **Emil G. Ivarsson***, Omar M. Khan*, Israiel T. Kumar, Malin Erlandsson, Christin Karlsson, Xiufeng Xu, Mikael Brisslert, Cord Brakebusch, Donghai Wang, Maria Bokarewa, Volkan I. Sayin, and Martin O. Bergo Protein prenylation restrains innate immunity by inhibiting RAC1 effector interactions. *Nat. Comm., In Press*.
- III. Martin G. Dalin, Pär G. Engström, **Emil G. Ivarsson**, Per Unneberg, Sara Light, Maria Schaufelberger, Thomas Gilljam, Bert Andersson, Martin O. Bergo. Massive parallel sequencing questions the pathogenic role of missense variants in dilated cardiomyopathy. *Int. J. Cardiol., Volume 228, 1 February 2017, Pages 742-748*.

**SAHLGRENKA AKADEMIN
INSTITUTIONEN FÖR MEDICIN**



Statins, Lipids, and Mutations: Consequences for the Heart and Immune System

Emil Ivarsson

Avdelningen för Molekylär och Klinisk Medicin, Institutionen för Medicin,
Sahlgrenska akademien, Göteborgs universitet, Sverige, 19.

Abstract

CAAX proteins are a group of proteins that undergo a three-step protein maturation process that renders the proteins carboxyl-terminus hydrophobic and prone to localize to cellular membranes, where they have their primary function. The first step in this process is called prenylation, which is the covalent attachment of a lipid, either a 15-carbon farnesyl or a 20-carbon geranylgeranyl lipid, to the carboxyl-terminal cysteine residue by the enzymes farnesyltransferase (FTase) and geranylgeranyltransferase type I (GGTase-I), respectively.

Statins are inhibitors of HMG-CoA reductase, the rate-limiting enzyme in the cholesterol biosynthesis pathway, and are widely used in the treatment of hypercholesterolemia. They are thought to improve myocardial function by inhibiting GGTase-I- and FTase-mediated prenylation of the CAAX proteins RHOA and RAC1, two known mediators of cardiomyopathy. In the first paper of this thesis, we show that, contrary to popular belief, long-term statin administration causes reduced heart function, hypertrophic cardiomyopathy, and hyperactive RHOA in the hearts of wild-type mice. Similarly, we show that inactivation of the prenylation enzymes GGTase-I, FTase, or both, in heart muscle cells causes severe dilated cardiomyopathy. These findings indicate that statins and prenylation inhibitors might have the capacity to cause heart problems.

In the second paper, we define the mechanism underlying a previous finding that inactivation of GGTase-I in mouse macrophages prevents prenylation of RHO family proteins, paradoxically causes them to become hyperactive, and that this leads to severe rheumatoid arthritis. We find that the RHO-protein RAC1 is responsible for the development of rheumatoid arthritis. We further show that non-prenylated RAC1 exhibit an increased interaction with the effector proteins TIAM1 and IQGAP1 which trigger GTP loading, activation, excessive inflammatory signaling, and arthritis. Inactivation of RAC1 or IQGAP1 reduces the inflammatory signaling and markedly improves rheumatoid arthritis in GGTase-I deficient mice. We conclude that inhibiting prenylation of RAC1 stimulates effector interactions and cause excessive inflammatory signaling. This finding suggests that prenylation normally restrains innate immunity by limiting RAC1 effector interactions and its activation.

Protein-altering germline mutations are a major cause of dilated cardiomyopathy (DCM). However, recent sequencing studies have shown that rare protein-altering variants are also present in individuals without reported DCM. This complicates the interpretation of genetic testing in the clinic, which is increasingly used for diagnosis. In the third paper, we analyzed genotype-phenotype correlations and variant prevalence in 41 DCM-associated genes in a cohort of 176 Swedish DCM patients, and compared the variants to those of healthy reference individuals. We found 102 rare protein-altering variants, many of which were not previously reported, and further analysis revealed that harboring any variant was correlated with earlier onset of disease and reduced transplant-free survival. Comparing the number of variants found in DCM patients to rare variants in a healthy population showed that, while frameshift and nonsense variant were more common in DCM patients, the prevalence, pathogenicity scores, and location of missense variants were similar in both groups. These findings question the role of many putatively disease-causing variants and suggest that results from genetic testing should be interpreted with caution.

Keywords: CAAX proteins, Statins, RHO proteins, Dilated Cardiomyopathy

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