The *FoxF2* Gene in Development and Disease

Akademisk avhandling

för avläggande av filosofie doktorexamen i Naturvetenskap, inriktning genetik, som kommer att offentligt försvaras i föreläsningssal Ragnar Sandberg, medicinaregatan 7A Göteborg, torsdagen den 26 november, 2015, kl. 10:00

av

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Prof. Vladimir Kalinichenko,
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This thesis is based on the following publications, referred to by roman numerals in the text:

I. Foxf2 is required for brain pericyte differentiation and development and maintenance of the blood-brain barrier
Reyahi A, Nik AM, Ghimai M, Gritli-Linde A, Pontén F, Johansson BR, Carlsson P.
Developmental Cell (2015) 34, 19-23.

II. *FOXF2*, a novel risk locus for stroke and small artery disease
Ganesh Chauhan, Corey R Arnold, Audrey Y Chu, Myriam Fornage, Azadeh Reyahi, Joshua C Bis, Aki S Havulinna (equal contribution first authors) ... *additional co-authors* excluded for brevity... (joint senior authors:) Lenore J Launer, M Arfan Ikram, Peter Carlsson, Daniel I Chasman, Sarah J Childs, William T Longstreth, Jr, Sudha Seshadri, Stéphanie Debette.
Submitted

III. Foxf2 in intestinal fibroblasts reduces numbers of Lgr5(+) stem cells and adenoma formation by inhibiting Wnt signaling
Nik AM, Reyahi A, Pontén F, Carlsson P.
Gastroenterology (2013) 144(5), 1001-11.

IV. Foxf2 enhances Tgfβ signaling in secondary palate development
Ali M.Nik, Jeanette Astroga-Johansson, Azadeh Reyahi, Mozhgan Ghiami, Fredrik Pontén and Peter Carlsson.
Manuscript

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The *Foxf2* gene in development and disease

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Abstract

In this thesis I present our recent data on the involvement and the mechanism of action of the forkhead transcription factor Foxf2 in development of the brain microvasculature, formation of the blood-brain barrier, control of the intestinal stem cell niche, and fusion of the secondary palate. The potential clinical significance of these findings is strengthened by a correlation between *Foxf2* expression and intestinal adenoma formation, and by association between genetic variants in human *FOXF2* and incident stroke.

We showed that *Foxf2* is expressed in brain pericytes, but not in mural cells of other organs. *Foxf2* null mutants have a defective brain vasculature and suffer from intracranial hemorrhage and a leaky blood-brain barrier with increased endothelial vesicular trans-cytosis. *Foxf2*$^{-/-}$ brain pericytes have diminished Pdgfrβ expression, and the cerebral vasculature a reduced activity of the Tgfβ –Alk5–Smad2/3 signaling pathway, associated with decreased expression of integrins, *Tgfb2*, *Tgfbr2*, *Alk5* and other pathway components.

In a large GWAS performed by an international consortium, we identified a genome-wide significant association of common variants near *FOXF2* with risk of stroke. Conditional knockout mice, in which *Foxf2* was deleted in healthy adults, developed clinical signs of stroke and exhibited cerebral ischemia, reactive gliosis and microhemorrhage. The animal model results thus corroborate the human genetic association and identifies *FOXF2* as a novel risk locus for stroke.

In the intestine we showed that *Foxf2* is expressed by subepithelial fibroblasts and restricts the size of the stem cell niche, and thereby the number and proliferation of Lgr5^{+} stem cells. *Foxf2* is a target of epithelial hedegehog signaling and inhibits the Wnt pathway by increasing the expression of the extracellular Wnt inhibitor Sfrp1. As a consequence, reduced *Foxf2* expression significantly increases both initiation and growth of intestinal tumors.

Reduced proliferation and decreased extracellular matrix production in the neural crest-derived mesenchyme of the palatal shelves was found to be responsible for the cleft palate phenotype in *Foxf2* null mutants. Mechanistically, the defect is associated with reduced canonical Tgfβ signaling and integrin expression. The *Tgfb2* mRNA level was not affected, but the amount of Tgfβ2 protein was significantly decreased in mutant palatal shelf mesenchyme.

Keywords: *Foxf2*, Pericyte, Pdgfrβ, Blood-brain barrier, Stroke, Wnt signaling, *sFRP-1*, Intestinal stem cell niche, Lgr5, Palatogenesis, Cleft palate, Tgfβ signaling

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