New Roles of Filamins in Cell Signaling, Transcription and Organ Development

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin vid
Göteborgs universitet kommer att offentligen försvaras i
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av

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Avhandlingen baseras på följande delarbeten:

I. **Filamin A promotes VEGF-A activity through the HIF-1α-mediated hypoxic response**
Xiaowei Zheng*, Xianghua Zhou*, Meit Björndahl, Hidetaka Uramoto, Teresa Pereira, Lakshmanan Ganesh, Elizabeth G. Nabel, Yihai Cao, Jan Borén, Lorenz Poellinger, and Levent M. Akyürek
**Equal contribution to the paper*
Under revision

II. **Filamin A regulates c-MET signaling via SMAD2**
Xianghua Zhou, Aslı Toylu, Neşe Atabey, Carl-Henrik Heldin, Gisela Nilsson, Jan Borén, Martin O. Bergö, and Levent M. Akyürek
Submitted

III. **Filamin B deficiency in mice results in skeletal malformations and impaired microvascular development**
Xianghua Zhou, Fei Tian, Johan Sandzén, Renhai Cao, Emilie Flaberg, Laszlo Szekely, Yihai Cao, Claes Ohlsson, Martin O. Bergö, Jan Borén, and Levent M. Akyürek
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GÖTEBORGS UNIVERSITET

New Roles of Filamins in Cell Signaling, Transcription and Organ Development

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Abstract

Filamins are large actin-binding proteins that stabilize delicate three-dimensional actin networks and link them to cellular membranes. They integrate cell architectural and signaling functions and are essential for cell locomotion and development. This thesis includes studies of two abundantly expressed filamin members, filamin A (FLNA) and B (FLNB).

FLNA has recently been shown to bind to the proteins that are related to cell motility and are implicated in diseases. The number of known FLNA interacting proteins is increasing, thus a complete understanding of the role of FLNA in diseases still requires intensive study. We identified hypoxia-inducible factor-1α (HIF-1α), a transcription factor, as a novel interacting partner of FLNA and studied the influence of their interaction on HIF-1α signaling in *FLNA*-deficient and *FLNA*-expressing human tumor cells. At hypoxia, cleavage of FLNA by calpain was induced. The cleaved C-terminal fragment interacted with HIF-1α and facilitated nuclear translocation and transactivation activity of HIF-1α. As a consequence, *FLNA*-deficient tumor cells produced less VEGF-A and exhibited an impaired ability to induce proliferation and migration of endothelial cells. In addition, we discovered that the interaction between FLNA and another transcription factor SMAD2 partially regulates c-MET expression. *FLNA*-deficient tumor cells expressed less c-MET and displayed impairments in c-MET signaling and hepatocyte growth factor-induced cellular migration. These results suggest that FLNA is important for cellular motility and may influence tumor growth by regulating angiogenesis and tumor metastasis in response to chemoattractants.

FLNB mutations in humans are associated with devastating congenital malformations. However, the causal role of *FLNB* in these genetic disorders is unknown. Using a gene-trapping technique, we generated a mouse model of *Flnb*-deficiency, which led to a high embryonic lethality. A few *Flnb*-deficient mice that reached term displayed severe skeletal malformations and disorganized microvasculature. *Flnb*-deficiency impaired the cell motility of embryonic fibroblasts, which may partly explain the observed developmental consequences. Generation of *in vivo* and *in vitro* models of *Flnb*-deficiency will advance our understanding of the biological importance of FLNB in organ development and disease progression.

Keywords: filamins; F-actin-binding proteins; cell movement; integrins; GTP phosphohydrolases; genetic diseases, inborn; mice, knockout; hypoxia-inducible factor 1; vascular endothelial cell growth factor A; proto-oncogene proteins c-met; Smad2 protein; hepatocyte growth factor; cartilage; osteogenesis; neovascularization; neoplasm metastasis

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