News Release

Title

Cathepsin K-Mediated Notch1 Activation Contributes to Neovascularisation in Response to Hypoxia

Summary

Haiying Jiang (Visiting Researcher, Department of Community Healthcare & Geriatrics), Xian-Wu Cheng (Designated Associate Professor, Department of Community Healthcare & Geriatrics), Masafumi Kuzuya (Professor, Department of Community Healthcare & Geriatrics), collaborating with Toyoaki Murohara (Professor, Department of Cardiology), Yoshiharu Oshida (Professor, Department of Sport Medicine) and his team from Nagoya University Graduate School of medicine (Dean: Masahide Takahashi, MD, PhD) found that cathepsin K-mediated Notch1 activation contributes to inschemia-induced neovascularization in a mouse hindlimb model.

Here, we report an endothelial CatK-mediated Notch1 activation-based mechanism that plays an essential role in angiogenic actions in response to hypoxia injury. Cysteinyl CatK may be a potential therapeutic target for ischemic cardiovascular disease.

The paper on the above result was published online in an English journal *Nature Communication*s on June 6, 2014.

Key Points

- This study focused on a novel mechanism and molecular requirements for the CatK-mediated close association between Notch1 processing-related signaling transduction and EC/EPC angiogenic actions in ischemia-induced neovascularisation.
- In cultured ECs, hypoxia enhanced VEGF-induced CatK expression and activity; and CatK inhibition by genetic or pharmacological interventions impaired proangiogenic activities of mature ECs and EPCs, accompanied by reduced production of c-Notch1 associated with the reduction of Hes1, Hey1, Hey2, and VEGF protein expression as well as of Akt phosphorylation.
- *In vivo*, CatK deficiency resulted in decreased BM EPC mobilisation and homing in to support ischemic vasculogenesis associated with the reduction in Notch1-Hes1/Hey1/2 signaling transduction; and impaired vascular action was rescued by cell therapy with $CatK^{+/+}$ BM-derived c-Kit^{+} EPC-like cells in $CatK^{-/-}$ mice.

Research Background

Cats comprise the catalytic classes of serine, aspartate, and cysteine peptidases, and exhibit endo- or exopeptidase activities. There is growing evidence for specific intra- and extracellular functions for these lysosomal enzymes, which have been shown to participate in numerous cardiovascular pathogeneses and progressions. Recent findings of a few studies show that CatL plays crucial roles in the invasive and functional capacities of endothelial progenitor cells (EPC) in the EPC-mediated neovascularisation process. CatK is essential for extracellular type I collagen and elastin matrix metabolism. However, no studies thus far have reported on CatK's roles in tumor- or ischemia-related angiogenesis.

Notch receptors are important regulators of embryonic development and cardiovascular homeostasis. Among them, endothelial Notch1 has been shown to be critical for vasculogenesis and angiogenesis. Upon receptor activation, the Notch1 intracellular domain (NICD) is ultimately cleaved by the Presenilin protease (PS1/2) of the γ-secretase complex and translocates to the nucleus associated with RBP-J and Mastermind-like proteins to activate the transcription of downstream target genes such as hairy enhancer of split homolog-1 (Hes1), Hes-related repressor (also called Hey) family and angiogenic factors. Thus, γ-secretase strictly controls this terminal cleavage event and Notch1 signal activity. However, the mechanism underlying the proteolytic activation of Notch1 is largely unknown.

Research Results

1. Here we reveal that cathepsin K (CatK) has a role in ischemia-induced neovascularisation.
2. Femoral artery ligation-induced ischemia in mice increases CatK expression and activity, and CatK-deficient mice show impaired functional recovery following hindlimb ischemia.
3. CatK deficiency reduces the levels of cleaved Notch1 (c-Notch1), Hes1 Hey1, Hey2, vascular endothelial growth factor, Flt-1, and phospho-Akt proteins of the ischemic muscles.
4. In endothelial cells, silencing of CatK mimicked, whereas CatK overexpression enhanced, the levels of c-Notch1 and the expression of Notch downstream signalling molecules, suggesting CatK contributes to Notch1 processing and activates downstream signalling.
5. Moreover, CatK knockdown leads to defective endothelial cell invasion, proliferation, and tube formation, and CatK-deficiency is associated with decreased circulating EPC-like $CD31^{+}$/c-Kit^{+} cells in mice following hindlimb ischemia.
6. Transplantation of bone-marrow-derived mononuclear cells from $CatK^{+/+}$ mice restores the impairment of neovascularisation in $CatK^{-/-}$ mice.

Research Summary and Future Perspective

This study focused on a novel mechanism and molecular requirements for the CatK-mediated close association between Notch1 processing-related signaling transduction and EC/EPC angiogenic actions in ischemia-induced neovascularisation. Present study demonstrated a critical role for CatK in angiogenesis and vasculogenesis. This novel biological function of CatK may be exploited for the therapeutic control of pathophysiological neovascularisation.

The Title of the Paper

Jiang H, Cheng XW, Shi GP, Hu L, Inoue A, Yamamura Y, Wu H, Takeshita K, Li X, Huang Z, Song H, Asai M, Hao CN, Unno K, Koike T, Oshida Y, Okumura K, Murohara T, Kuzuya M. Cathepsin K-Mediated Notch1 Activation Contributes to Neovascularisation in Response to Hypoxia. *Nature Communications* (June 6, 2014)

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