論文 / 著書情報
Article / Book Information

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Title(English)	Placental Mammals Acquired Functional Sequences in NRK for Regulating Cell Proliferation and Apoptosis in Placenta
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論 文 要 旨

THESIS SUMMARY

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学籍番号： Student ID Number	20D40262	指導教員（主）： Academic Supervisor(main)	加藤 明
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

The title of this thesis is "**Placental Mammals Acquired Functional Sequences in NRK for Regulating Cell Proliferation and Apoptosis in Placenta**". The contents of this thesis cover the unique evolutionary history of the *Nrk* gene and its new functions in regulating placental growth through suppressing cell proliferation and promoting cell apoptosis. The details are described as below:

Introduction

The eutherian placenta is an evolutionarily novel organ whose acquisition must be associated with the molecular evolution of many genes regulating placental development[1]. NIK-related kinase (NRK) is one of the germinal center kinase (GCK) IV family members with the *N*-terminal kinase domain, an unstructured middle region, and the *C*-terminal citron homology (CNH) domain. NRK is highly expressed in the mouse placenta, and *Nrk* knock-out (KO) mice experiments suggested its role in preventing placental hyperplasia[2]. Previous studies also reported that NRK could inhibit the proliferative AKT signaling and enhance cell apoptosis[3,4]. However, the evolutionary history of the *Nrk* gene and molecular mechanisms by which NRK regulates placental development remain unclear. This study aimed to investigate these issues.

Results

Evolutionary history of the *Nrk* gene. Synteny and gene loci analysis revealed that the *Nrk* gene was widely present in vertebrates, and the neighboring genes were reorganized in the eutherian ancestor and conserved among eutherians. All eutherian *Nrk* genes were located on the X chromosome. Tissue expression analysis suggested that eutherian *Nrk* genes were restrictedly expressed in the placenta. Phylogenetic analysis of the amino acid sequences of vertebrate NRK demonstrated an accelerated evolutionary rate in the eutherian ancestor; during this period, the NRK sequence underwent many amino acid insertions/substitutions in several regions, including the middle region and the CNH domain. After this period, these regions were under strong purifying selection in eutherians.

The anti-proliferative effects of NRK. Immunoblotting analysis on AKT phosphorylation in

HEK293 cells showed that mouse NRK (mNRK) expression decreased AKT signaling, but chicken NRK (cNRK) and the other GCK IV family members did not. Our group previously identified casein kinase-2 (CK2) as an mNRK-interacting protein. CK2 is known to phosphorylate and inactivate PTEN, a phosphatidylinositol 3,4,5-triphosphate (PIP_3) phosphatase, thereby increasing PIP_3 and downstream AKT signaling. I found that amino acids 565-868 in the middle region of mNRK interact with and inactivate CK2, and I named it the CK2-inhibitory region (CIR). I also found that the CNH domain of mNRK can recruit mNRK to the plasma membrane (PM). This ability was required for mNRK to inhibit AKT signaling. Overall, I modeled that mNRK localizes at the PM and inhibits CK2, leading to decreased CK2-dependent phosphorylation of PTEN. This activated PTEN downregulates AKT signaling to inhibit cell proliferation. mNRK expression in cells decreased PTEN phosphorylation and PIP_3 levels, and *Nrk* knock-out mice placenta showed increased phosphorylation levels of PTEN and AKT, confirming my model. In contrast, cNRK and the other GCK IV family members had no homologous sequences to the CIR, no binding ability to CK2, and no PM-localizing CNH domain.

The pro-apoptotic effects of NRK. Our group previously suggested that mNRK is cleaved by caspase-3 upon the induction of apoptosis, and the C-terminal fragment containing the CNH domain promotes apoptotic cell death[3]. In this study, I found that the CNH domain of mNRK alone was sufficient to promote cell death and remained localized at the PM during apoptosis progression. I mapped the responsible region in the CNH domain for the PM localization and identified polybasic clusters, which were conserved only in eutherian NRK. I found that the CNH domain of mNRK can bind to phosphatidylserine, and the clusters were required for the phospholipid-binding ability and pro-apoptotic effects. To identify the molecular target of the CNH domain, I performed proteomic analysis of the CNH domain-interacting proteins and identified several PM-localizing cell death regulators.

Discussions

My study revealed the unique evolutionary history of the *Nrk* gene; it evolved rapidly during early eutherian evolution and was co-opted for regulating placental development. Especially the acquisition of two functional sequences, the CIR and the phospholipid-binding region in the CNH domain, enabled eutherian NRK to inhibit AKT signaling and promote apoptosis. In late pregnancy, the maternal nutrient must be distributed preferentially to the fetus than the placenta, for which placental development stops. NRK may be involved in this process by controlling proliferative AKT signaling and apoptosis machinery. My study highlights NRK and its downstream pathway as a putative molecular target for developing diagnostic and therapeutic methods for placenta abnormality and pregnancy complications.