Physiological Significance of Recombination-Activating Gene, RAG1 in Optic Nerve Neuropathy

Takao Hirano, MD, PhD¹, Tomoko Nakamura-Yanagidaira, MD, PhD¹,², Toshinori Murata, MD, PhD¹, Takuma Hayashi, MD, PhD³

¹Department of Ophthalmology, Shinshu University Graduate School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan
²Department of Ophthalmology, Japanese Red Cross Society, Azumino Hospital, 5685 Toyoshina, Azumino, Nagano 399-8292, Japan
³Department of Obstetrics and Gynecology, Shinshu University Graduate School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan

*Corresponding Author: Takuma Hayashi, Department of Obstetrics and Gynecology, Shinshu University Graduate School of Medicine. 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan, Email: yoyoyo224@hotmail.com

Abstract: Although the transcription factor, nuclear factor-kappa-B (NF-κB) is known to regulate programmed-cell death and survival, its precise role in cell death within the central nervous system (CNS) remains unknown. We previously reported that mice with a homozygous deficiency for Nf-κB1p50 spontaneously developed optic neuropathy. We investigated the expression and activation of pro-apoptotic factor(s), which mediate optic nerve neuropathy in Nf-κB1p50-null mice. Recombination activating gene1 (RAG1) is known to control the recombination of immunoglobulin V(D)J. Experiments with genetically engineered mice revealed the involvement of RAG1 expression in the programmed-cell death of POU domain protein (POU4f2)/brain-specific homeobox3A (BRN3a)-positive retinal ganglion cells (RGCs), and also showed the specific effects of a Nf-κB1p50-null on the activation of RAG1 gene transcription. Furthermore, a genetic analysis of murine neuronal stem-like cells clarified the biological significance of RAG1 in N-methyl-D-aspartate (NMDA)-induced neuronal cell death. The apoptotic inducing factors, Bcl-2-associated X protein (BAX), and cleaved cysteine-dependent aspartate-directed protease (CASPASE) 3, 8, and 9 were detected in Human Embryonic Kidney (HEK) 293 cells expressing the external molecule of RAG1, and human histopathological examinations with retina tissues revealed the expression of RAG1 in RGCs. Recent studies indicated that RAG1 played a key role in optic nerve neuropathy as a pro-apoptotic candidate in Nf-κB1p50-null mice. These results may lead to new therapeutic targets in optic nerve neuropathy.

Keywords: RAG1, NF-κB1p50, programmed cell death, RGCs, optic neuropathy

1. INTRODUCTION

The intracellular pathways related to cell survival regulate neuronal physiology during embryonic development as well as the pathogenesis of various neurodegenerative disorders. The nuclear factor-kappa-B (NF-κB) signal pathway was discovered in 1986 as a transcription modulator of the κ-light chain of B lymphocyte immunoglobulins. Subsequent studies have shown that NF-κB is a ubiquitously expressed dimeric transcription factor involved in numerous cellular processes, such as inflammation, differentiation, programmed cell death, and tumorigenesis. NF-κB is a dimer composed of members of the REL transcriptional factor family, which includes RELA (p65), RELB, and proto-oncogene c REL. The classical NF-κB family, which is primarily composed of NF-κB1p50/RELA (p65) heterodimers, has been detected in almost animal cell types and is involved in cellular responses to stimuli such as stress and cytokines. NF-κB is sequestered in the cytoplasm of unstimulated cells by a class of inhibitors called nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor (IκB). The physiological degradation of IκB allows NF-κB to enter the nucleus, in which it specifically initiates the expression of target genes. Accordingly, the impaired regulation of NF-κB has been linked to various diseases, including oncogenesis, inflammatory disorders, and autoimmune diseases, as well as deficiencies in the processes of synaptic
plasticity and memory\textsuperscript{[4].} NF-κB family also plays important roles in central nervous system (CNS) development and pathology by influencing neuronal apoptosis, neurite outgrowth, and synaptic plasticity\textsuperscript{[5,6].} However, the range of cellular signals and transduction mechanisms that regulate activation of NF-κB family in neurons is broad and complex. Genetically modified mice have been extensively used to assess different gene components in the NF-κB signal pathway. For instance, \textit{Nf-κb1p50}\textsuperscript{-null} mice, which are mice with a homozygous deficiency for \textit{Nf-κb1p50}, exhibited the age-related degeneration of neuronal and non-neuronal cells, and defective activation of NF-κB resulted in apoptosis in the striatal neurons of a Huntington disease model\textsuperscript{[7,8,9].} Activated RELA (p65) has been suggested to glutamate-induced neurotoxicity, N-methyl-D-aspartate (NMDA)-induced retinal neuronal cell death, retinal ischemia, and reperfusion injury in the CNS\textsuperscript{[10,11,12,13].} We previously reported that the number of retinal ganglion cells (RGCs) in \textit{Nf-κb1p50}\textsuperscript{-null} mice was significantly lower than that in its parental mice, \textit{Nf-κb1p50}\textsuperscript{-wild type} mice, suggesting that these animals exhibited features resembling those of human non-tensioning glaucoma (NTG)\textsuperscript{[14,15].} However, the precise role of NF-κB in cell death within the CNS remains controversial. Therefore, we searched for a new target related to NF-κB signal pathways in optical neurons.

\section*{2. Biological Significance of RAG1 in Neuropathy}

NF-κB was reportedly relevant in the B-cell receptor-mediated regulation of \textit{recombination activating gene} (\textit{Rag}) locus transcription\textsuperscript{[16].} Recent studies suggested that immediately activated NF-κB signal pathways may facilitate quick antigen receptor-regulated changes in RAG expression, which is important for editing\textsuperscript{[16].} \textit{Rag} genes encode two enzymes that play key roles in the adaptive immune system: both \textit{Rag1} and \textit{Rag2} mediate the recombination of V(D)J, a process that is essential for the maturation of B and T lymphocytes in the development and maturation of lymphocytes\textsuperscript{[17,18].} RAGs have been detected not only in the immune network systems of mammals and amphibians, but also in their nervous systems; \textit{Rag1} transcripts have been found in the murine CNS, particularly in areas of high neural density, such as the cerebellum and hippocampal formation\textsuperscript{[19,20,21].} RAG1 may function in neurons to site-specifically recombine elements of the neuronal genome or prevent detrimental alternations in the genomes of long-lived cells. Although the role of the RAG1 locus in the CNS is currently unclear, RAGs are known to be directly regulated by NF-κB\textsuperscript{[16].} Activation of IKK-β and NF-κB in the hypothalamus of mice is shown to accelerate the ageing process, leading to shortened lifespan; inhibition of hypothalamic or brain IKK-β and NF-κB delays ageing and increases lifespan\textsuperscript{[22].} Based on the above-described findings, we focused on RAG1 as a novel candidate target related to NF-κB signal pathways in neurons using \textit{Nf-κb1p50}\textsuperscript{-null} mice as a model of optic nerve neuropathy. Since no studies have been published on the expression of RAG1 in the visual system, we first confirmed the presence of RAG1, but not the RAG2 transcript in RGCs. The absence of RAG1 in \textit{Nf-κb1p50}\textsuperscript{-null} mice resulted in a decrease in optic nerve neuropathy. In vertebrate embryonic development, the retina and optic nerve originate as outgrowths of the developing brain, and, thus, the retina is considered to be part of the CNS. In further experiments, three-dimensional cultures of mouse embryonic stem cell aggregates have demonstrated the autonomous formation of the optic cup, which develops into the outer and inner layers of the retina structure from brain balls\textsuperscript{[23]} Glutamate is a major excitatory neurotransmitter in vertical pathways through the retina, wherein RGCs first express the NMDA/glutamate receptors that are typical in the brain\textsuperscript{[24,25].} Since the brain and retina have a close relationship in genesis and neurotransmission, it is plausible that RAG1, which has been detected in the hippocampus, is also expressed in the retina.

We assessed the precise role of RAG1 in the retina using experiments with \textit{Nf-κb1p50}\textsuperscript{-null} mice, which exhibit age-dependent decreases in RGCs. A genetically lack of RAG1 in \textit{Nf-κb1p50}\textsuperscript{-null} mice diminished the loss of RGC, which was confirmed by several lines of evidence in the present studies. These results promoted us to speculate that RAG1 may play a role in the programmed cell death of RGCs, which was accelerated by RELA (p65)/c-REL, in \textit{Nf-κb1p50}\textsuperscript{-null} mice. We found that RAG1 was also localized in the nucleus of RGCs in 15-month-old \textit{Nf-κb1p50}\textsuperscript{-null} mice, whose RGC number had already markedly decreased, therefore we proposed that RAG1 specifically influence apoptotic signal pathway in the nucleus\textsuperscript{[26]} (Figure 1).
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Figure1. Induction of RAG1 by NF-κB activation in nerve systemic ageing.

NF-κB in the hypothalamus of mice is shown to accelerate the ageing process[22]. NF-κB induced RAG1 may mediate optic neuropathy.

Many questions remain regarding the molecular mechanisms involved in RAG1 functions in the retina. However, RAG1 may play a role in RGCs that is entirely distinct from somatic recombination. The evidence for this lies in studies of the molecular structure of the recombinase enzymes themselves; although both RAG1 and RAG2 share several roles, i.e., DNA cleavage and rearrangement of V(D)J recombination, only RAG1 molecule contained the catalytic DNA-binding core of the recombinase[27]. These domains are known to be like the active site of several transposases and integrases[28]. Kelch motifs, which mediate the interactions of RAG1 and RAG2, have been observed in numerous proteins, and RAG1 may interact with an identified protein via a kelch motif in the retina[29]. As in the mouse retina, we confirmed the intracellular localization of RAG1, but not RAG2, in RGCs in the human retina and other types of neuron[26,30]. A protein homology studies revealed that the human RAG1 molecule was 90% homologous with its mouse orthologue, the catalytic domains, zinc-finger, recombinase, and RING-finger in RAG1 molecule appeared to be conserved between species. These results indicated the physiological significance of RAG1 observed in mice may extend to the regulation of human RGC survival. We concluded that RAG1 might also be involved in the programmed cell death of RGCs in the human glaucomatous retina. Further studies on the physiological role of RAG1 in RGCs are expected to contribute to the development of preventive and therapeutic treatments for human NTG and optic nerve neuropathy.

3. CONCLUSION

As in the mouse retina, we confirmed the localization of RAG1, but not RAG2, in human RGCs. The catalytic domains, zinc-finger, recombinase and RING-finger in the RAG1 molecule appear to be conserved between species. These results indicated the physiological significance of RAG1 in human RGC survival, similarly to that in murine RGCs. We conclude that RAG1 may also be involved in the programmed cell death of RGCs in the human glaucomatous retina. Further studies on the role of RAG1 in RGCs are expected to contribute to the development of preventive and therapeutic treatments for human glaucoma.

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