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Physiological significance of Rag1 in optic nerve neuropathy

Takuma Hayashi[1*], Takao Hirano[2] and Toshinori Murata[2]

[1]Department of Immunology and Infectious Disease, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan
[2]Department of Ophthalmology, Shinshu University Graduate School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan
Corresponding author

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A B S T R A C T

Although the transcription factor, nuclear factor-κB (NF-κB) is known to regulate 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-κBp50* spontaneously developed optic neuropathy. We studied the expression and activation of pro-apoptotic factor(s), which mediate optic nerve neuropathy in *p50*-null mice. Recombination activating gene 1 (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 apoptosis of Brn3a-positive retinal ganglion cells (RGCs), and also showed the specific effects of a *p50*-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, Bax, and cleaved caspase 3, 8, and 9 were detected in HEK293 cells expressing the external molecule of Rag1, and a human histopathological examination revealed the expression of Rag1 in RGCs. Recent studies indicated that Rag1 played a role in optic nerve neuropathy as a pro-apoptotic candidate in *p50*-null mice. These results may lead to new therapeutic targets in optic nerve neuropathy.

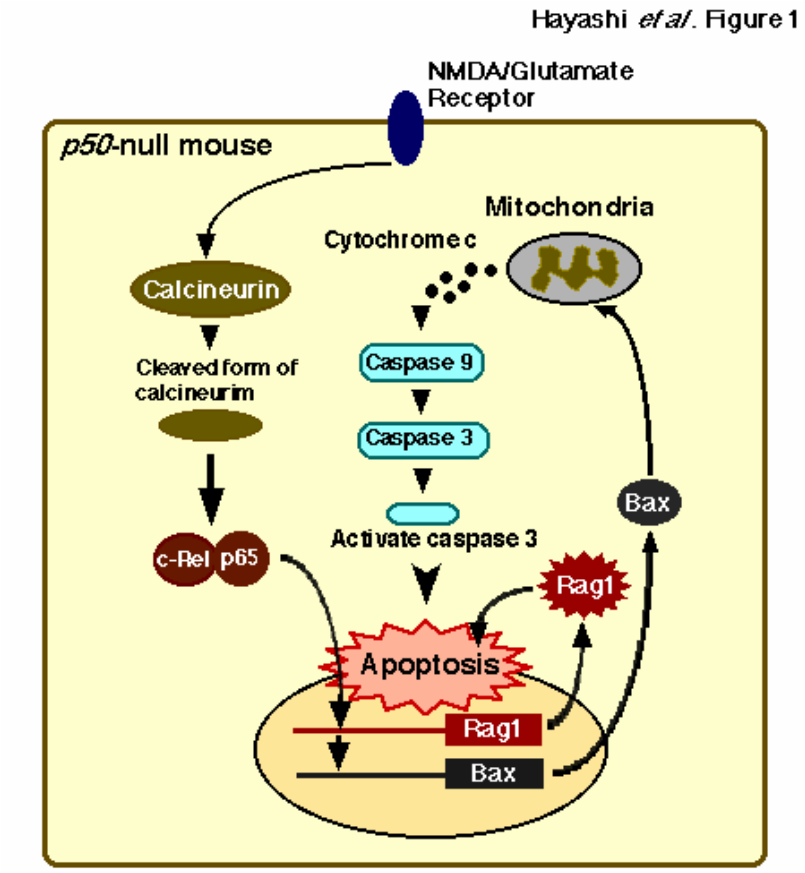
Introduction

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 -κB (NF-κB) signaling pathway was discovered in 1986 as a transcription modulator of the κ-light chain of B lymphocyte immunoglobulins (1). Subsequent studies have shown that NF-κB is a ubiquitously expressed dimeric transcription factor involved in numerous cellular processes, such as inflammation, differentiation, apoptosis, and tumorigenesis. NF-κB is a dimer composed

of members of the Rel family, which includes RelA (p65), RelB, and c-Rel (2). The classical NF-κB family, which is primarily composed of p50/RelA(p65) heterodimers, has been detected in almost animal cell types and is involved in cellular responses to stimuli such as stress and cytokines (3). NF-κB is sequestered in the cytoplasm of unstimulated cells by a class of inhibitors called 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 (4). NF-κB family also plays important roles in nervous system development and pathology by influencing neuronal apoptosis, neurite outgrowth, and synaptic plasticity (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 signaling pathway. For instance, *p50*-null mice, which are mice with a homozygous deficiency for *NF-κBp50*, exhibited the age-related degeneration of neuronal and non-neuronal cells, and defective NF-κB activation resulted in apoptosis in the striatal neurons of a Huntington disease model (7-9). 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 central nervous system (CNS) (10-13). We previously reported that the number of retinal ganglion cells (RGCs) in *p50*-null mice was significantly lower than that in its parental mice, *p50*-wild type mice, suggesting that these animals exhibited features resembling those of human non tension glaucoma (NTG) (14). 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 signaling pathways in optical neurons.

NF-κB was reportedly relevant in the B-cell receptor-mediated regulation of recombination activating gene (*Rag*) locus transcription (15). Recent studies suggested that immediately activated NF-κB signaling pathways may facilitate quick antigen receptor-regulated changes in Rag expression, which is important for editing (15). *Rag* genes encode two enzymes that play key roles in the adaptive immune system: both Rag1 and 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 (16,17). Rags have been detected not only in the immune network systems of mammals and amphibians, but also in their nervous systems; *Rag1* transcripts have been found in the murine CNS, particularly in areas of high neural density, such as the cerebellum and hippocampal formation (18-21). 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 (22). Based on the above-described findings, we focused on Rag1 as a novel candidate target related to NF-κB signaling pathways in neurons using *p50*-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.

Fig.1 Signal cascade of cell death mediated by Rag1 in p50-null mouse. A recent study demonstrated that the binding site of the hetero dimer p50/RelA(p65) could also be occupied by the homo dimer p50/p50, and may function as a repressor to regulate the role of p50/RelA(p65) as a transcription factor essential for neuronal responses. In p50-null neuronal cells, the c-Rel/RelA(p65) hetero dimer markedly induced Rag1 gene activation as a transcription factor. Rag1 may play a role in neuronal cell death signaling as a nuclear mediator. The cell death factors, Bax and cleaved caspase-3, and 9, were also clearly detected in Rag1-expressing cells



The absence of *Rag1* in *p50*-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 (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 (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 *p50*-null mice, which exhibit age-dependent decreases in RGCs. A genetically lack of Rag1 in *p50*-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 *p50*-null mice. We found that Rag1 was also localized in the nucleus of RGCs in 15-month-old *p50*-null mice whose RGC number had already markedly decreased, therefore we proposed that Rag1 may specifically influence apoptotic signaling pathway in the nucleus (26) (Fig. 1).

Many questions still 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 similar to the active site of several transposases and integrases (28). Kelch motifs, which mediate the interactions of Rag2 and Rag1, 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 (26). A protein homology studies revealed that the human Rag1 molecule was 90% homologous with its mouse ortholog, 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 may 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.

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