

The two sides of Faim2 -- modulation of cell death and regeneration

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Most neurologic diseases are associated with neuronal cell death, and the surviving neurons consequently initiate regenerative processes. Activation of Fas/CD95, a so-called death receptor, can induce both apoptosis and alternative, non-apoptotic intracellular signaling¹. Consequently, modulators of the Fas/CD95 signaling pathways have gained importance. We have investigated the role of one such modulator, Fas inhibitory molecule 2 (Faim2), in neurologic diseases. We generated a Faim2 deficient mouse line and applied models of transient cerebral ischemia, bacterial meningitis, and Parkinson disease²⁻⁴. In brief, we found increased neuronal cell death in the acute phase of all disease models in Faim2 deficient mice in comparison to the Faim2 wildtype (WT). In addition, signs of increased regeneration in Faim2 deficient mice point towards an involvement of Faim2 and Fas/CD95 in regenerative processes. Finally, we found disease stage-dependent regulation of Faim2 expression, potentially enabling the switch between apoptotic and alternative Fas/CD95 signaling.

Thus, Faim2 appears to be an interesting protein for two reasons. First, it could be a target protein for neuroprotective strategies in the above mentioned diseases. Second, it may help us to understand the cellular signaling pathways involved in neuronal cell death and in regeneration. In spite of its name, Faim2 is not well known. We therefore start with nomenclature issues before describing the neurobiology of Faim2 in more detail.

What do we know about Faim2?

Faim2 is a member of two different protein families. The first family is defined functionally as Fas inhibitory molecules and comprises 3 members (Faim1, Faim2, Faim3) that are structurally unrelated and show different expression patterns throughout the organism^{1,5}. In addition, Faim2 is a member of a structurally defined family of proteins related to apoptosis named transmembrane BAX inhibitor-1 motif-containing (TMBIM) proteins, or life guard (Lfg) proteins. All family members (TMBIM1/Lfg3, TMBIM1b/Lfg5, TMBIM2/Lfg2, TMBIM3/Lfg1, TMBIM4/Lfg4) are highly conserved throughout evolution. Faim2 is TMBIM2. Another synonym for Faim2 is neuronal membrane protein 35 (NMP35) due to its molecular weight of 35 kD. TMBIM3 is also called Glutamate Receptor, Ionotropic, N-Methyl D-Aspartate-Associated Protein 1 (GRINA1). Among the TMBIM proteins, Faim2 and GRINA1 have been studied most. All TMBIM proteins have been associated with various intracellular processes, including apoptosis, calcium homeostasis, unfolded protein response, autophagy and lysosomal function, which is reflected in the confusing nomenclature⁶. Their precise functions have not been fully resolved.

Faim2 is primarily expressed in neurons^{7,8}. The constitutive expression of Faim2 in cultured cerebellar granule neurons prevents apoptotic cell death induced by Fas ligand⁹. Protective effects of Faim2 in cultured neurons have also been described for Purkinje cells and cortical neurons^{7,10}. In addition to these neuronal effects, Faim2 single nucleotide polymorphisms (SNPs) have been linked to an increased susceptibility for obesity, especially in childhood, in a genome wide association study (GWAS)¹¹. For certain subpopulations, i.e. type-2 diabetic patients, Faim2 SNPs have been linked to a higher risk of myocardial infarction¹². In accordance with its inhibition of apoptosis, Faim2 was found associated with the biology of certain types of tumors, including breast cancer¹³, neuroblastoma¹⁴ and small-cell lung cancer¹⁵, possibly with therapeutic implications¹⁶. This review focuses, however, on the role of Faim2 in non neoplastic neurological diseases.

Faim2 is protective in animal models of neurologic diseases

In an animal model of transient and focal cerebral ischemia, ischemia was induced by occlusion of the middle cerebral artery for 30 minutes followed by 72 hours of reperfusion. Cell death was associated with caspase-8 and caspase-3 activation, as expected for Fas dependent apoptosis. Faim2 deficient mice showed larger infarcts than Faim2 WT mice and a higher number of apoptotic neurons. This effect was rescued by lentiviral overexpression of Faim2 in the null mutant, in accordance with an inhibition of Fas dependent apoptosis by Faim2. Interestingly, lentiviral overexpression of Faim2 also reduced infarct size in WT mice, and Faim2 expression was transiently downregulated 18 h after artery occlusion².

In an animal model of bacterial meningitis, Faim2 deficient mice showed a higher number of apoptotic neurons in the hippocampus than WT littermates 24 hours after infection with *Streptococcus pneumoniae*. Animals were treated with antibiotics twice daily starting 18 hours after infection. Neuronal degeneration in this model occurs primarily in the hippocampus and is associated with a learning deficit after recovery. Interestingly, Faim2 expression was reduced to 50% 20 hours after infection, echoing the findings obtained in the ischemia model. As an indication of alternative Fas signaling we found increased ERK phosphorylation and a higher number of new (BrdU positive) neurons in the hippocampus of Faim2 deficient mice more than 8 weeks after infection. Accordingly, Faim2 deficient mice performed better than wildtype mice in a learning paradigm 8 weeks after bacterial meningitis. The relevance of these findings for human patients was supported by the fact that we found more Faim2 positive neurons in the hippocampus of patients with bacterial meningitis than in controls³.

Finally, in the classic animal model of Parkinson disease, Faim2 deficient mice displayed a more pronounced loss of TH positive dopaminergic neurons in the substantia nigra than WT. Mice were analyzed 14 days after injecting the dopaminergic neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), applied in 5 injections of 30 mg/kg spaced 24 hours apart. 14 days after MPTP we also found a more pronounced loss of dopaminergic axon terminals in the striatum of Faim2 deficient mice. Dopaminergic axon terminals recover by sprouting between 14 and 90 days after the last MPTP injection. This regeneration was more pronounced in Faim2 deficient mice, consistent with a potential role of Fas/CD95 in neuron regeneration. Faim2 expression was decreased by MPTP administration, again similar as in cerebral ischemia and bacterial meningitis⁴.

Towards a Faim2 based neuroprotective therapy

Taken together, we saw evidence of neuroprotection by Faim2 in three animal models of neurologic diseases. Common to these models is the occurrence of delayed and programmed cell death (see Figure 1). In our model of transient cerebral ischemia only the cells in the core of the infarct degenerate due to immediate glucose and oxygen deprivation. Most of the cells in the penumbra, however, degenerate through other mechanisms, including excitotoxicity, reactive oxygen species, and inflammation¹⁷. Similarly, cell death following bacterial meningitis is not primarily mediated by bacterial invasion, but by the inflammatory response that follows infection³. Finally, neurons in Parkinson disease and in the applied subacute MPTP model die by an apoptotic mechanism with contributions from excitotoxicity, oxidative stress and inflammation^{18,19}. This delayed and programmed cell death can potentially be modulated by Faim2.

Two findings indicate that there is a realistic therapeutic potential for neuroprotection by Faim2. First, in the study of transient cerebral ischemia, lentiviral overexpression of Faim2 decreased the infarct size in wildtype mice. This indicates that an increase in Faim2 may have protective effects even when Faim2 is constitutively expressed. Moreover, we have found in all three animal models a down regulation of Faim2. Therefore, increasing Faim2 or preventing Faim2 downregulation in early disease processes may attenuate cell death.

What do we need to develop this therapeutic option further? First, we need to know what regulates Faim2 expression in order to increase Faim2 expression in the disease state. Faim2 expression in cultured cells was regulated by the PI3-kinase / Akt pathway⁹. Since this pathway is downstream of several well-known growth factor receptors, it seems a plausible next step to test the therapeutic potential of agents stimulating these pathways.

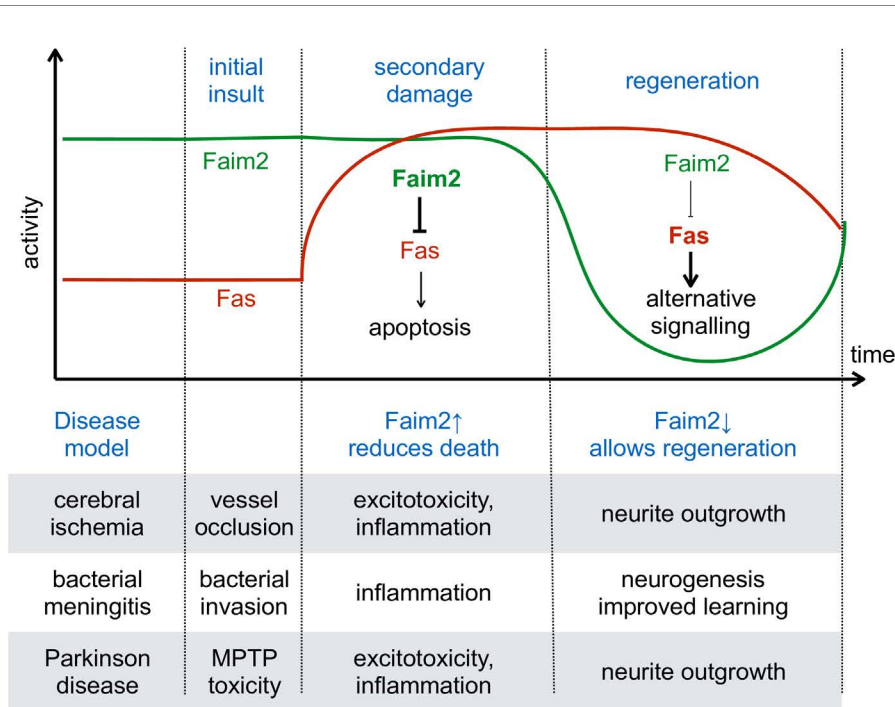


Figure 1: Schematic representation of the regulation of Fas and Faim during the different phases of the neurologic diseases studied. Faim2 is constitutively expressed, inhibiting Fas dependent apoptotic cell death. After the acute insult, cell delayed and programmed death occurs by excitotoxicity, oxidative stress and inflammation. In this phase, constitutive Faim2 can prevent Fas induced cell death, and increasing Faim2 can further reduce cell death. In the last phase, however, down regulation of Faim2 may allow alternative Fas signalling that underlies regenerative processes such as proliferation and differentiation of neuronal precursors and neurite outgrowth.

One of these pathways is erythropoietin, for which beneficial effects have been observed in animal models of cerebral ischemia^{20,21} and Parkinson disease^{22,23}.

Second, it will be beneficial to learn by which molecular mechanism Faim2 prevents cell death. Faim2 has been shown to physically interact with Fas and prevent downstream activation of caspases^{7,8,24}. Still, it is unclear whether the interaction of Faim2 with Fas prevents Fas trimerization, ligand binding, trafficking, or formation of the “death inducing signaling complex” with FADD and the initiator caspase 8. Moreover, some recent evidence indicates that Faim2 modulates cytosolic calcium as do other members of the TMBIM family^{25,26}. Interestingly, a regulation of calcium is also important for cell death in models of cerebral ischemia and Parkinson disease²⁷⁻²⁹.

Third, we have found evidence that deficiency of Faim2 or its downregulation can be associated with beneficial effects in the later phases of disease models. We found more neurogenesis (BrdU positive neurons) in the hippocampus 8 weeks after pneumococcal infection, improved learning after meningitis, and more pronounced sprouting 90 days after MPTP intoxication^{3,4}. These findings are consistent with the now established role of Fas/CD95 for regenerative processes, including proliferation and differentiation of neuronal precursors and neurite outgrowth^{30,24,31}. It is tempting to speculate that facilitating these regenerative

effects of Fas/CD95 signaling is the “purpose” of the Faim2 downregulation observed in disease states. In order to further develop a therapeutic strategy based on Faim2, it will therefore be crucial to determine when in the course of a disease Faim2 upregulation will be beneficial and when Faim2 inhibition would be more appropriate. One option to investigate this hypothesis will be to cross mice carrying the floxed Faim allele¹ with mice in which Cre recombinase can be induced by tamoxifen administration. It is likely though, that the time windows for beneficial and detrimental Faim2 activity differ between disease models and between mice and man.

Conclusion

Faim2 is constitutively expressed in neurons and inhibits Fas/CD95 signaling. It is therefore in an excellent position to prevent detrimental Fas/CD95 signaling and apoptotic cell death in response to a sublethal insult, and to allow Fas/CD95 signaling stimulating regenerative processes. However, it is exactly this dual function that makes it difficult to use Faim2 for neuroprotection. It is possible that further strategies tested for neuroprotection can similarly elicit beneficial and detrimental effects. Whether neuroprotection prevails may then depend on timing as outlined for Faim2, or may relate to dosing in other cases. In order to overcome this problem, we need to understand for each therapeutic strategy the signaling

pathway that mediates the beneficial and detrimental effects. We will then be able to develop biomarkers for both branches of signaling and determine in patients optimal dosing and timing for these therapeutic strategies.

Abbreviations used

Akt: protein kinase B

BrdU: Bromodeoxyuridine

Cre: causes recombination of two LoxP (locus of X-over P1) sites

ERK: Extracellular Signal-regulated Kinase

FADD: Fas associated protein with death domain

Faim2: Fas inhibitory molecule 2

floxed: flanked by LoxP (locus of X-over P1) sites

GWAS: genome wide association study

Lfg: life guard

MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

PI3-kinase: phosphatidylinositol 3-kinase

SNP: single-nucleotide polymorphism

TMBIM: transmembrane BAX inhibitor-1 motif-containing

WT: wild type

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