Unchanged Scrapie Pathology in Brain Tissue of Tyrosine Kinase Fyn-Deficient Mice

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Key Words

Fyn-PrP signalling · Neurodegeneration · Prion diseases · Gliosis

Abstract

Fyn is a 59-kDa member of the Src family of tyrosine kinases synthesized on cytosolic polysomes and then targeted to the plasma membrane where it clusters in caveolae-like membrane microdomains. The cellular isoform of the prion protein (PrP) has also been identified to be a caveolar constituent and to participate in signal transduction events concerning cell survival and differentiation via recruitment of Fyn. We studied the scrapie infection of mice deficient for Fyn (Fyn^{-/-}) to clarify the role of Fyn in an in vivo model of transmissible spongiform encephalopathies. Fyn^{-/-} mice died on average 9 days earlier than wild-type control mice, but no differences were seen regarding activation of astrocytes, vacuolization of the neuropil, and accumulation of misfolded prion protein. The experimental model suggests that a deficiency for Fyn is detrimental in prion diseases, although it has no major effect on the clinical course of an experimental prion infection of the CNS.

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The biological functions of the Fyn kinase are manifold, and comprise signalling via T cell receptor and regulation of brain functions. A role for Fyn signaling has been proposed in neurodegenerative diseases, such as Alzheimer's disease and multiple sclerosis. In Alzheimer's disease, Fyn kinase is upregulated and important for tau phosphorylation [1]. Furthermore, Fyn^{-/-} mice display defects in myelination indicating a relevance of Fyn kinase in regulation of myelin-producing oligodendrocytes [2]. Fyn itself has been shown to regulate oligodendroglial cell development [3].

Previous studies pointed to a possible significance of Fyn signalling in prion diseases. It has been shown that the prion protein (PrP) triggers cell signalling in neurites through interaction with phosphorylated Fyn [4] and participates in signal transduction events important for cell survival and differentiation via recruitment of Fyn kinase [5, 6]. Furthermore, it has been shown in vitro that PrP is involved in neurite outgrowth and neuronal survival via Fyn signaling [7].

To assess the role of Fyn signaling and Fyn-PrP interactions in vivo, 5-week-old, female Fyn-deficient 129Sv × C57B/6 mice, age- and sex-matched wild-type 129Sv × C57B/6 (Jackson Laboratory, Bar Harbour, Me., USA) control mice were intracerebrally inoculated with 20 µl

Fig. 1. GFAP-positive astrocytes and vacuolisation of the neuropil in scrapie-infected 129Sv $\times$ C57B6Fyn^{-/-} and 129Sv $\times$ C57B/6 wild-type control mice. **a** 129Sv $\times$ C57B6Fyn^{-/-} mice. **b** 129Sv $\times$ C57B/6 wild-type control mice. Cerebellum 125 dpi. Scale bar = 50 μ m.

of diluted 10% brain homogenates prepared from a scrapie strain 139A (courtesy of R.H. Kimberlin, Edinburgh, UK) infected mouse. All animals were monitored twice weekly for clinical signs of disease development.

Mice were sacrificed at 125 days post infectionem (dpi) and at the terminal stage of the disease. The terminal stage was reached when the development of scrapie symptoms indicated that the animals would die, if not sacrificed, within the next 72 h. One brain hemisphere was formaldehyde-fixed for morphological evaluation, and the other hemisphere was frozen and used for characterization of misfolded proteinase K-resistant prion protein (PrP^{Sc}) by Western blot analysis.

For the Western blot procedure, 10% brain homogenates from 2 animals per group were subjected to proteinase K-digests as previously described [8]. The subsequent detection of PrP^{Sc} was performed using the anti-PrP antibody 6H4 (Prionics, Switzerland).

Immunohistochemical detection of glial fibrillary acid (GFAP)-expressing astrocytes was performed according to previously published procedures [9] using polyclonal anti-GFAP antiserum (Dako, Denmark).

In order to detect differences in the degree of spongiform lesions, PrP^{Sc} deposition, and astrogliosis, a quantitative scoring system was applied. The entire brain section in each specimen was examined and the degree of lesion was graded from 0 to 3+ according to the severity of damage. Examined areas were the cortex, hippocampus, brain stem, cerebellum, and the olfactory bulb. Differences between Fyn^{-/-} mice and the wild-type controls were evaluated by independent scoring of all tissue samples by three investigators without prior knowledge of the group to which the mice belonged.

The comparison of the Fyn^{-/-} mice with the wild-type controls concerning the gliosis, the accumulation of mis-

Fig. 2. Accumulation of proteinase K-resistant prion protein (PrP^{Sc}). Western blot analysis of proteinase K-resistant PrP^{Sc} in brain homogenates of Fyn^{-/-} mice at 125 dpi (lane 1) and the terminal stage (lane 3), and from wild-type controls at 125 dpi (lane 2) and the terminal stage (lane 4).

folded proteinase K-resistant prion protein (PrP^{Sc}) and the scrapie-associated vacuolization of the neuropil revealed no significant differences for the examined parameters.

The expression of activated, GFAP-expressing astrocytes (fig. 1) was unchanged in the Fyn^{-/-} mice compared to the wild-type controls. Thus, the Fyn signaling cascade is not involved in the development of the astrogliosis in our model of chronic neurodegeneration. However, a recent report by Chun et al. [10] described the overexpression of Fyn kinase by reactive astrocytes due to neural trauma, which points towards a possible participation of astrocytic Fyn activation in acute neurodegeneration.

The ablation of the Fyn gene did not affect the extent of PrP^{Res} deposition when analyzed by Western blotting (fig. 2) indicating that the Fyn kinase is not relevant for the conversion of the normal cellular prion protein into the disease-associated isoform PrP^{Sc}. Furthermore, no difference was seen concerning vacuolization of the neuropil, which is a hallmark of prion disease-associated neurodegeneration [11] (fig. 1).

Finally, Fyn^{-/-} mice succumbed to the disease on average at 175 $\pm$ 9 (dpi), whereas the wild-type controls died at 184 $\pm$ 9 dpi (table 1). The duration of the symptomatic phase and the clinical symptoms in the Fyn^{-/-} mice were identical to the controls. The survival time differences of on average 9 days are borderline significant ($p < 0.05$) and may suggest that intact Fyn signalling is to some extent neuroprotective. Interestingly, PrP expression has been suggested to be associated with neuroprotective anti-apoptotic functions [12]. Thus, PrP-triggered Fyn activation may be involved in signalling pathways, which promote neuronal survival. Conversely, cross-linking of cellular PrP by monoclonal antibodies, which was reported to induce Fyn kinase activation in vitro [13],

Table 1. Survival times (dpi) of scrapie-infected 129Sv × C57B6Fyn^{-/-} and 129Sv × C57B/6 wild-type control mice

Dilution of the 10% brain homogenate used as inoculum	129Sv × C57B/6Fyn ^{-/-}	129Sv × C57B/6 Control
10 ⁻⁴	175 ± 10, n = 8* [161, 164, 170, 174, 177, 185, 188, 188]	184 ± 9, n = 13 [170, 174, 177, 177, 181, 181, 181, 181, 188, 195, 197, 197, 198]
* p < 0.05 vs. control group. Values are mean ± SD (Student's t test).		

triggers neuronal apoptosis *in vivo*. Hence, Fyn could theoretically be also involved in signalling cascades leading to neuronal loss. However, it was recently shown that for example PrP-triggered activation of extracellular regulated kinases 1/2 (ERK 1/2) is not necessarily Fyn-dependent but can be controlled by NADPH oxidase in certain cell types [5] indicating that PrP-mediated signalling is complex and may vary for different tissues.

Taken together, we report here that a Fyn deficiency does not affect the development of the astrogliosis, nor the accumulation of misfolded PrP^{Sc} or the clinical symptoms in a murine scrapie model. The moderately shortened survival times of the Fyn^{-/-} mice suggest that Fyn kinase may be involved in mediating neuroprotective

functions of PrP and likewise argue against a prominent role for Fyn in the observed neurotoxicity following PrP cross-linking by monoclonal antibodies or possibly by multimeric aggregates of PrP^{Sc}.

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References

- Lee G, Newman ST, Gard DL, Band H, Panchamoorthy G: Tau interacts with src-family non-receptor tyrosine kinases. *J Cell Sci* 1998; 111:3167–3177.
- Osterhout DJ, Wolven A, Wolf RM, Resh MD, Chao MV: Morphological differentiation of oligodendrocytes requires activation of Fyn tyrosine kinase. *J Cell Biol* 1999;145:1209–1218.
- Sperber BR, McMorris FA: Fyn tyrosine kinase regulates oligodendroglial cell development but is not required for morphological differentiation of oligodendrocytes. *J Neurosci Res* 2001;63:303–312.
- Mouillet-Richard S, Ermonval M, Chebassier C, Laplanche JL, Lehmann S, Launay JM, Kellermann O: Signal transduction through prion protein. *Science* 2000;289:1925–1928.
- Schneider B, Mutel V, Pietri M, Ermonval M, Mouillet-Richard S, Kellermann O: NADPH oxidase and extracellular regulated kinases 1/2 are targets of prion protein signaling in neuronal and nonneuronal cells. *Proc Natl Acad Sci USA* 2003;100:13326–13331.
- Massimino ML, Griffoni C, Spisni E, Toni M, Tomasi V: Involvement of caveolae and caveolae-like domains in signalling, cell survival and angiogenesis. *Cell Signal* 2002;14:93–98.
- Chen S, Mange A, Dong L, Lehmann S, Schachner M: Prion protein as trans-interacting partner for neurons is involved in neurite outgrowth and neuronal survival. *Mol Cell Neurosci* 2003;22:227–233.
- Baier M, Norley S, Schultz J, Burwinkel M, Schwarz A, Riemer C: Prion diseases: Infectious and lethal doses following oral challenge. *J Gen Virol* 2003;84:1927–1929.
- Jeffrey M, Goodsir CM, Holliman A, Higgins RJ, Bruce ME, McBride PA, Fraser JR: Determination of the frequency and distribution of vascular and parenchymal amyloid with polyclonal and N-terminal-specific PrP antibodies in scrapie-affected sheep and mice. *Vet Rec* 1998;142:534–537.
- Chun JT, Crispino M, Tocco G: The dual response of protein kinase Fyn to neural trauma: Early induction in neurons and delayed induction in reactive astrocytes. *Exp Neurol* 2004; 185:109–119.
- Prusiner SB: Prions. *Proc Natl Acad Sci USA* 1995;95:13363–13383.
- Kim BH, Lee HG, Choi JK, Kim JI, Choi EK, Carp RI, Kim YS: The cellular prion protein (PrPC) prevents apoptotic neuronal cell death and mitochondrial dysfunction induced by serum deprivation. *Brain Res Mol Brain Res* 2004;124:40–50.
- Solforosi L, Criado JR, McGavern DB, Wirz S, Sanchez-Alavez M, Sugama S, DeGiorgio LA, Volpe BT, Wiseman E, Abalos G, Masliah E, Gilden D, Oldstone MB, Conti B, Williamson RA: Cross-linking cellular prion protein triggers neuronal apoptosis *in vivo*. *Science* 2004;303:1514–1516.