

c-Jun N-Terminal Kinase (JNK) and p38 Play Different Roles in Age-Related Purkinje Cell Death in Murine Organotypic Culture

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Abstract Several studies have shown that Purkinje cells die by apoptosis in organotypic slice cultures from postnatal 3-day-old (P3) mice. This cell death is age-dependent and has been proposed as indirect evidence for the programmed Purkinje cell death occurring in *in vivo* cerebellum. Here, we studied whether c-jun N-terminal kinase (JNK) and p38 kinase pathways contribute to the Purkinje cell death observed in cerebellar slice cultures obtained from P3 mice. Slice culture treatment with D-JNKI1 or SB203580, respectively inhibitors of JNK and p38 MAP kinases, results in a better survival of Purkinje cells. Interestingly, the combined treatment with the two inhibitors potentiated single treatment effects. These results suggest that p38 and JNK pathways might be differently implicated in this Purkinje cell death. Time course experiments found p38 activation immediately post-slicing, whereas JNK activation was detected only 2 h after the culture. We hypothesize that p38 activation might be due to the “sliced condition,” and JNK activation might be more specific to P3 age-dependent cell death. The study of JNK and p38 activation in cerebellar lysates from P0 slice

culture confirmed JNK activation being specific for the P3 explants, whereas p38 is activated both from P0 and P3 cerebellar slice culture lysates. These results suggest that p38 is activated by the slicing, whereas JNK activation is related to developmental Purkinje cell death.

Keywords Purkinje cell · Cell death · Organotypic culture · JNK · p38

Introduction

Developmental neuronal cell death is an essential process during the normal development of the nervous system. It is generally thought that during the period of developmental neuronal death, neurons are dependent on the presence of trophic factors for their survival [1]. However, the mechanisms triggering apoptosis in programmed developmental cell death are not completely understood. It has been proposed that mouse Purkinje cell death occurring in organotypic cultures from P3 explants may reflect the vulnerability of Purkinje cells during the postnatal period of programmed cell death. Indeed, this neuronal death is age-dependent since Purkinje cells massively die by apoptosis when explants are taken from postnatal day 1 (P1) to day 5 (P5), but they survive much better if explants are taken before or after these ages [2]. This model of age-dependent Purkinje cell death in organotypic culture at P3 has been successfully used to unravel the role of microglial cells in postnatal developmental Purkinje cell death [3]. However, the effect on Purkinje cell survival in P3 organotypic culture is less efficient after ablation of microglial cells than after treatments with an inhibitor of PKC [4] or with Mefispristone (RU486) [5]. These results prompted us to hypothesize that in this model of Purkinje cell death, two types of cell

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death occur, one intrinsic to the model and a second related to developmental cell death. Indeed, in this model, cutting and culturing slices also induces per se a general cell death. To validate this hypothesis, we analyzed candidate pathways that might be differently activated by these two types of cell death, focusing our attention on c-jun N-terminal kinase (JNK) and p38 pathways, since these two mitogen-activated protein kinase (MAPK) are required for different forms of cell death in cerebellar granule cell neurons [6].

JNK is critical for naturally occurring neuronal death during development [7, 8], although it also plays an important role in pathological death of adult brain following different insults [9–11]. p38 MAPK pathway plays a pivotal role in neuronal death due to a variety of harmful stimuli, such as oxidative stress, excitotoxicity, and inflammation [12].

We first analyzed whether JNK and p38 MAPK are involved in Purkinje cell death in murine P3 organotypic cultures and showed that inhibition of these two pathways promotes Purkinje cell survival. Interestingly, these two effects were additive. Furthermore, the time course of activation of the two kinases clearly showed that p38 is activated just after the slicing, whereas JNK activation is detected after 2 h in culture. Lastly, the study of JNK and p38 activation in P0 slice culture was not able to detect JNK activation at any time, whereas activation of p38 was observed after the slicing as for the P3 culture.

Altogether, these results clearly suggest that in our model, p38 activation is due to the slicing and JNK activation is due to the fact that P3 Purkinje cells are more vulnerable, underlining that the two MAP kinases respond to different stimuli. P3 organotypic culture is therefore a model of cell death that combines both traumatic and developmental cell death.

Materials and Methods

Cerebellar Slice Cultures

Organotypic cultures of mouse cerebellum were prepared from P0 (day of birth) or P3 (postnatal day 3) Swiss mice (Janvier, Le Genet St Isle, France) as described previously [13]. After decapitation, brains were dissected out into cold Gey's balanced salt solution (Invitrogen, Cergy Pontoise, France) containing 5 mg/ml glucose. Cerebellar parasagittal slices (350 μ m thick) were cut on a Macllwain tissue chopper and transferred onto membranes of 30-mm Millipore culture inserts with 0.4- μ m pore size (Millicell, Millipore, Molsheim, France). Slices were maintained in culture in six-well plates at the interface between the air and the culture media consisting of 50% basal medium Eagle, 25% Hank's buffered salt solution, 25% horse serum, 1 mM L-glutamine, and 5 mg/ml

D-glucose (all products were from Sigma, St Louis, MO, USA) in a humidified chamber with 5% CO₂ at 35°C [14].

The medium was changed every 2–3 days, and cultures were fixed at 5 days in vitro (DIV) for Purkinje cell survival evaluation or at different survival times (3–24 h) for immunohistochemical studies.

D-JNKI1 (Istituto di Ricerche Farmacologiche Mario Negri, Milano, Italy) or SB203580 (Calbiochem, France Biochem, Meudon, France) was added to the medium just before the culture to obtain a concentration of 20 and 10 μ M, respectively.

Immunostaining Procedure

Cerebellar slices were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 1 h at room temperature. For c-jun labeling, cerebellar slices were transferred to 0.12 M phosphate buffer containing 10% sucrose at 4°C for 48 h and then cut with a cryostat to obtain 20- μ m-thick sections. Immunohistochemistry was performed as described previously [2]. Mouse monoclonal antibody against calbindin (CaBP, dilution 1:5,000, Swant, Bellinzona, Switzerland) was used to label Purkinje cells and was revealed with Alexa488-conjugated goat anti-mouse antibody (dilution 1:400, Jackson Immuno-Research Laboratories, Inc, West Baltimore Pike, PA, USA). Rabbit polyclonal antibodies against P-c-jun (dilution 1:1,000, Cell Signalling Technology no. 9164, Beverly, MA, USA) were revealed with Cy3-conjugated goat anti rabbit (1:500 dilution, Jackson ImmunoResearch). After 2 h incubation in buffer containing the secondary antibody, the slices were washed several times and mounted in Mowiol (Calbiochem, VWR, Fontenay sous bois, France). Negative controls were performed for each immunohistochemistry reaction by omitting the primary antibodies.

Quantification of Purkinje Cell Survival

The number of Purkinje cells revealed by anti-CaBP immunostaining was determined using a fluorescence microscope (DMR Leica). Slices for this study were classified in one of the four of following groups (adapted from Ghomari et al. [2]): Group 1 included slices with no more than 50 Purkinje cells (Fig. 1a), group 2 slices containing between 50 and 100 Purkinje cells (Fig. 1b), group 3 slices with more than 100 Purkinje cells but with no group of more than 100 Purkinje cells (Fig. 1c), and finally, group 4 included slices with more than 100 Purkinje cells and at least one compact group of more than 100 cells (Fig. 1d, e).

The percentage of slices included in each group was calculated for each animal in each experiment. Six animals were used for control, DJNKI1 and DJNKI1+ SB203580 experimental conditions, and seven animals for the SB treatment.

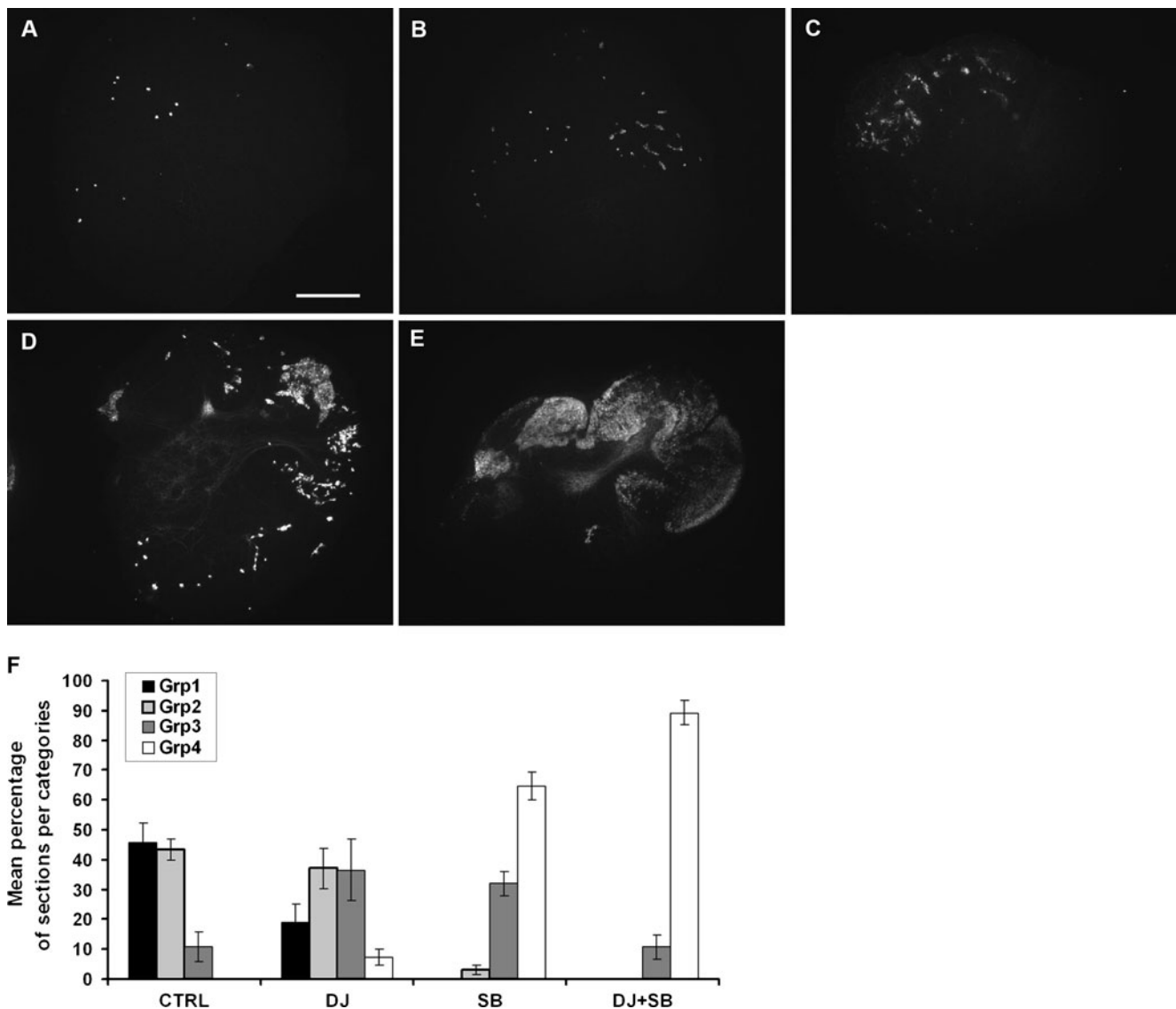


Fig. 1 Purkinje cell survival in organotypic cultures. **a–e** Photomicrographs of cerebellar slices maintained 5 days *in vitro* and immunostained with anti-CaBP antibodies. Four different slice culture conditions have been tested: control, D-JNK11 20 μ M, SB203580 10 μ M, and D-JNK11 20 μ M+SB203580 10 μ M. Slices for this study were classed according to the number of Purkinje cells as described in “Materials and Methods.” **a–d** Examples of slices belonging to the different groups. **a** Slice from a control condition, very few Purkinje cells are present (<50 cells), and it thus belongs to the group 1. **b** Slice from a control culture, it presents a number of Purkinje cells higher than 50 but smaller than 100, and it belongs to group 2. **c** Slice from a D-JNK11-treated culture, the number of Purkinje cells is higher than

100; it belongs to group 3. **d** Slice from the SB203580-treated condition, there is at least one cluster of >100 Purkinje cells, and it is classed in group 4. **e** Slice from the culture condition in which both inhibitors are used. The co-treatment clearly results in many clusters of >100 Purkinje cells. **f** Quantitative evaluation of Purkinje cells. Slices were classed in four groups according to the number of Purkinje cells as described in “Materials and Methods.” The absolute counts were expressed as category percentages in each animal and mean \pm SEM were calculated ($n=6$ animals for controls, DJNK11 and DJNK11+ SB203580 culture condition, and $n=7$ animals for the SB treatment). Scale bar in **a** is 350 μ m

Sample Preparation for Western Blot

At different culture times (from 0 to 24 h, $n=6/8$, $N=3$), slices from each animal were pooled and quickly homogenized in ice-cold lysis buffer [15] using a manual potter apparatus. Homogenates were sonicated for 30 s, amplitude

of 80, and then centrifuged at 13,000 rpm for 10 min at 4 $^{\circ}$ C. Supernatants were collected, and protein concentrations were determined by the Bradford method (protein assay; Bio-Rad, Munich, Germany). Samples were stored at -80° C until used. P3 or P0 cerebellum and slices at time 0 were used as earliest points for the time course studies.

Western Blot Analysis

Proteins were separated on 10–14% sodium dodecyl sulfate polyacrylamide gel and transferred to a polyvinylidene fluoride membrane. Incubation with primary antibodies was overnight at 4°C using: rabbit anti-JNK antibody (1:4,000; no. 9252, Cell Signaling Technology), rabbit anti-P-JNK (1:4,000; no. 4671, Cell Signaling Technology, Beverly, MA, USA), rabbit anti-p38 (1:1,000, no. 9212, Cell Signaling Technology), rabbit anti P-p38 (1:1,000; no. 9211, Cell Signaling Technology). P-antibodies are specific and recognize only the phosphorylated form of these proteins. Blots were developed using horseradish peroxidase-conjugated secondary antibodies (nos. 31430 and 31460, Thermo Fisher Scientific Inc., Rockford, IL, USA) and the ECL chemiluminescence system (no. 34075, Thermo Fisher Scientific Inc.). All blots were normalized against tubulin level (1:10,000, no. SC-8035 Santa Cruz, Biotechnology, CA, USA) and quantified by densitometry analysis (Scion Image, Scion Corporation, Frederick, MD, USA). For each sample, the P-JNK, JNK, P-p38, and p38 levels were normalized to tubulin, and subsequently, P-JNK/JNK and P-p38/p38 ratios were determined.

Statistical Methods

All experiments were repeated at least three times using independent slice culture preparations, using each time two or three animals.

For Fig. 1, the absolute counts were expressed as category percentages in each animal, and mean±SEM were calculated. The frequency distribution of sections in the four groups was compared between the different experimental conditions using the chi-square and Fisher's exact test. *P* values <0.05 were considered significant.

For Figs. 2, 3, and 4, data were calculated as mean±SEM. Differences between groups were compared using Student's *t* test (single comparisons) or one-way ANOVA (multiple comparisons) with Bonferroni post-test. *P* values <0.05 were considered significant.

Results

Specific JNK and p38 Inhibition Prevents Purkinje Cell Death in Organotypic Cultures of P3 Mouse Cerebellum

To investigate whether the two stress-activated MAPKs, JNK and p38, are involved in P3 Purkinje cell death in organotypic culture, we first evaluated if the specific kinase inhibition in this model could result in an increased Purkinje cell survival (Fig. 1). For this purpose, we performed P3 cerebellar slices, and we kept them in culture

for 5 DIV in the presence or absence of kinase inhibitors, D-JNKI1 20 μM for JNK and SB203580 10 μM for p38. Purkinje cell survival was evaluated after calbindin immunostaining as described in "Materials and Methods." Briefly, survival was scored as follows: group 1 contains <50 Purkinje cells, group 2 contains between 50 and 100, group 3 contains more than 100 but without a compact group of more than 100 Purkinje cells, and group 4 contains at least a compact group of more than 100 Purkinje cells. In the control condition, the vast majority of slices fell into group 1 (46%, Fig. 1a, f) or into group 2 (43%, Fig. 1b, f), with very few slices in group 3 (11%) and no slices in group 4. JNK inhibition resulted in an increased number of slices in group 3 (37%, Fig. 1c, f) as well as in group 4 (7%), whereas p38 inhibition strongly protected Purkinje cell from cell death, with 32% slices belonging to group 3 and 65% to group 4 (Fig. 1d, f). When we treated P3 cerebellar slices with both D-JNKI1 and SB203580, no slices were present in groups 1 and 2, 11% of slices belonged to group 3, and 89% belonged to group 4 (Fig. 1e, f).

The frequency distribution in the four groups was significantly different among the four culture conditions (control, D-JNKI1, SB203580, and D-JNKI1+SB203580; *P*<0.001 with chi-square test). Furthermore, the frequency distribution in groups 2–4 was significantly different (*P*=0.0005, Fisher's exact test) between SB and DJ+SB condition. No significant effect due to the experiment date was found. These experiments clearly demonstrate JNK and p38 involvement in Purkinje cell death from P3 cerebellar slices, and they suggest that p38 and JNK act as two separated pathways, differentially involved in Purkinje cell death.

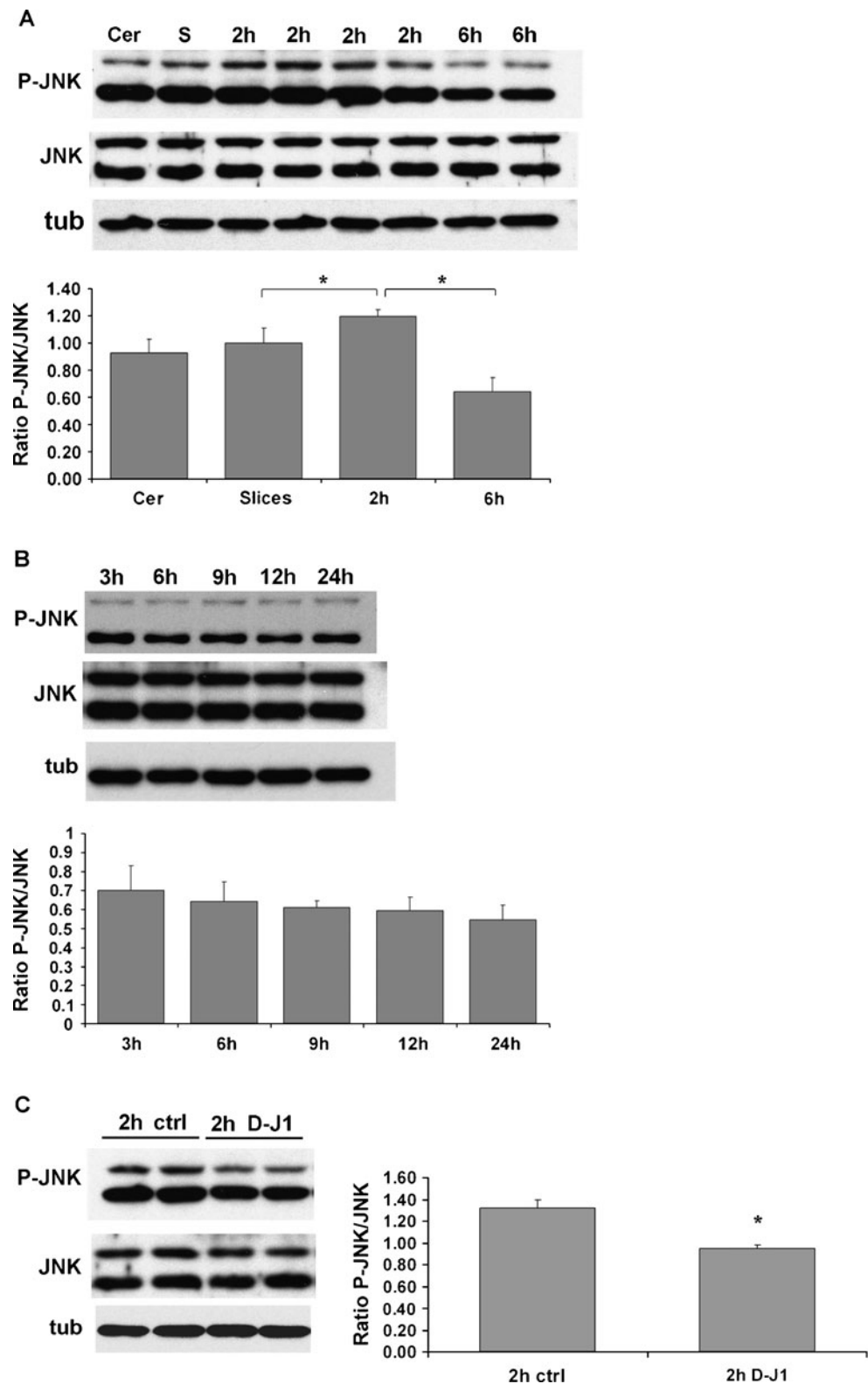
Time Course of JNK and p38 Activation in P3 Cerebellar Slices

To strengthen our results, we analyzed by Western blot the time course of activation of JNK and p38 in our model. For this purpose, cerebellar slice lysates were collected starting from 0 to 24 h after culture from P3 mouse. We included in our experiments a normal P3 cerebellum lysate, in order to test the difference between the in vivo (=not sliced) and the "ex vivo" (sliced) conditions.

The analysis of the P-JNK/JNK ratio showed JNK activation (20%) 2 h after the culture followed by a significant reduction (~50%) at 6 h (Fig. 2a), and no further activation was observed for the later time points studied (Fig. 2b). Importantly, a significant difference was never observed between the cerebellum lysates and the slices at time 0 in culture.

In addition, we investigated whether JNK inhibition by D-JNKI1 treatment was able to reduce the increase observed in P3 slices maintained 2 h in vitro: JNK

Fig. 2 Time course of JNK activation in lysates from P3 cerebellar slice cultures (**a** and **b**). Western blot analysis of JNK activation, evaluated as P-JNK/JNK ratio, from 0 to 6 h (**a**) and from 3 to 24 h (**b**) after the culture. Loading control=tubulin. JNK is activated at 2 h (20%), and no activation can be detected between the “sliced” and “not sliced” conditions. **c** D-JNKI1 treatment significantly reduces (30%) JNK activation at 2 h. Data for WB quantification are mean \pm SEM ($n=5$ animals per group). * $P<0.05$

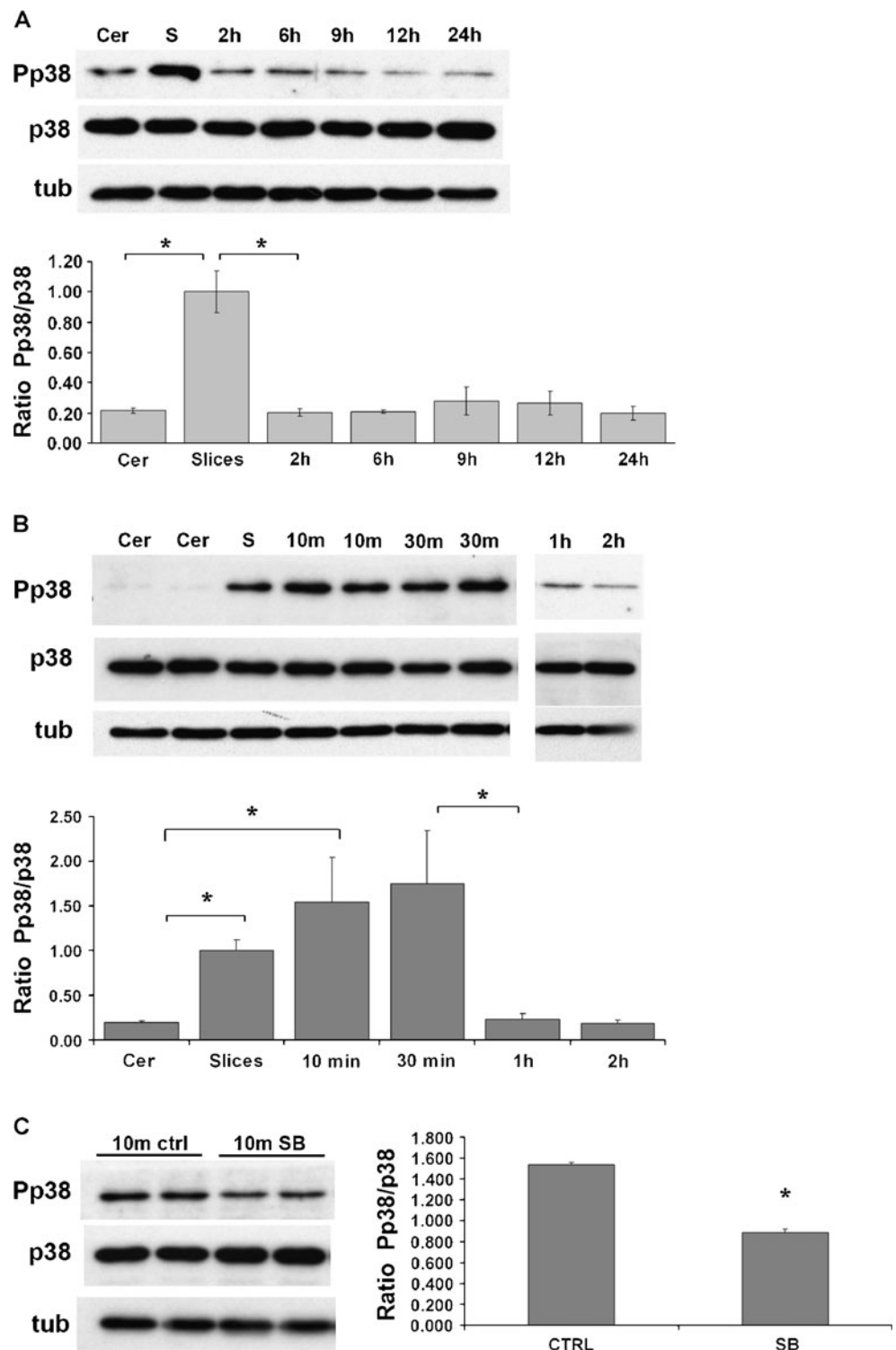


activation at 2 h after the culture was significantly reduced (30%) by D-JNKI1 treatment (Fig. 2c).

On the other hand, P-p38 level was dramatically high (80%) at 0 h in vitro (immediately after the culture) and then decreased to a lower level for all the time points

studied (Fig. 3a), clearly indicating an early strong p38 activation in this model. By analysing p38 activation in a smaller temporal window (Fig. 3b), we showed that this kinase is activated at 10 and 30 min in cerebellar slices and then declines so that at 1 and 2 h, it reaches levels

Fig. 3 Time course of p38 activation in lysates from P3 cerebellar slice cultures. **a** Western blot analysis of p38 activation, evaluated as P-p38/p38 ratio, from 0 to 24 h after the culture. The “sliced condition” induces a strong (80%) p38 activation, not present in the following time points. **b** Time course of p38 activation from 0 to 2 h after the culture. The ratio of P-p38 to p38 levels clearly indicates that p38 activation lasts for the first 30 min after the culture and then decreases to levels comparable to cerebellum lysates. Loading control=tubulin. **c** SB203580 treatment decreases p38 activation of 43% at 10 min of culture. Data for WB quantification are mean±SEM ($n=5$ animals per group). * $P<0.05$



comparable to cerebellum lysates (Fig. 3b). As for JNK, we then verified the effect of SB203580 on the p38 early activation. Indeed, Western blot analysis showed a 43% reduction in p38 activation at 10 min in cerebellar slices after SB203580 treatment (Fig. 3c).

The time course study demonstrates a different time course of activation for JNK and p38, thus suggesting a

different role for the two stress-activated kinases in Purkinje cell death from P3 cerebellar slice cultures.

JNK and p38 Activation in P0 Cerebellar Slice Cultures

We then decided to verify whether the difference in the timing of activation between JNK and p38 could reflect a

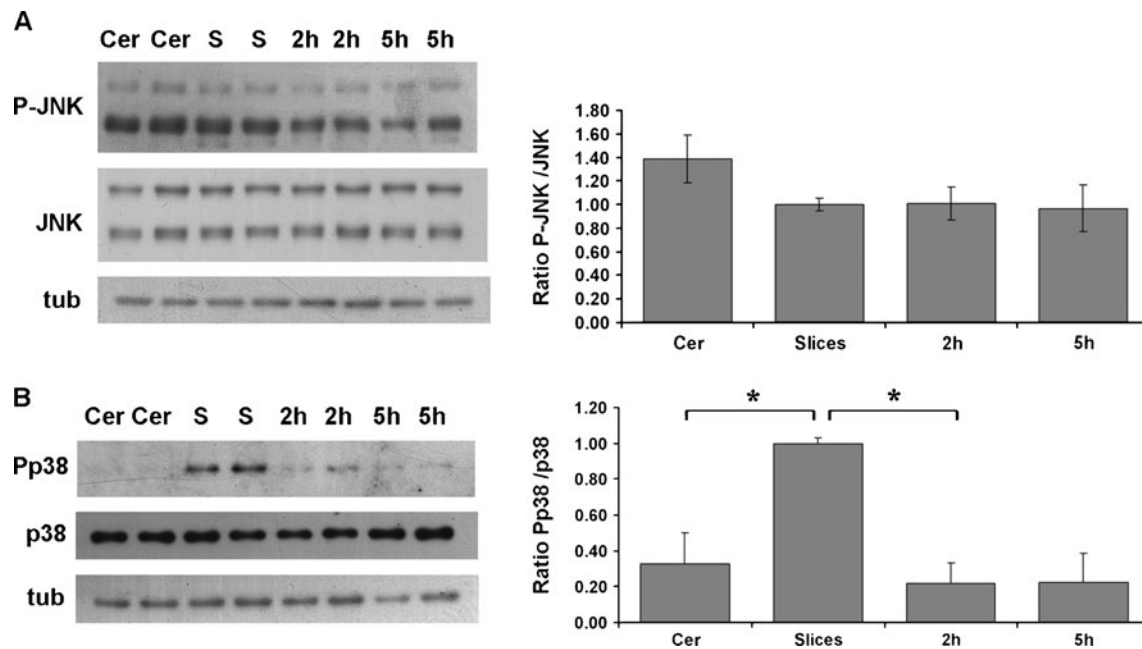


Fig. 4 Time course of JNK and p38 activation in lysates from P0 cerebellar slice cultures. **a** Western blot analysis of JNK activation from 0 to 5 h after the culture. Loading control=tubulin. No JNK activation is observed for the time points analysed. **b** Time course of p38 activation from 0 to 5 h after the culture. Loading control=tubulin.

The ratio of P-p38 to p38 levels shows a significant p38 activation (70%) at 0 h, as it was observed for the lysates from P3 cerebellar slice cultures. Data for WB quantification are mean \pm SEM ($n=5$ animals per group)

difference in the cell death stimuli. It is known that Purkinje cells undergo programmed cell death during a precise time window, P1–P5, whereas such cell death is not observed before or after these ages. A p38 or JNK activation before P1 or after P5 would thus result in a kinase activity not relevant to age-dependent Purkinje cell death. To test this hypothesis, we analyzed p38 and JNK activation in lysates from P0 cerebellar slice cultures.

The P-JNK/JNK ratio did not show any significant difference in unsliced samples and post-slicing (Fig. 4a). This result showed that JNK is activated neither after the slicing nor 2 h after the culture in slices from P0 cerebellum.

On the other hand, the analysis of the P-p38/p38 ratio for the same time points showed a strong p38 activation (70%) at 0 h in vitro (sliced condition) and a decrease in levels comparable to cerebellum lysates at 2 and 5 h, similar to what we observed for the lysates from P3 cerebellum (Fig. 4b).

These results demonstrated that although p38 is activated in slices from both P0 and P3 cerebellum, JNK is activated specifically in slices from P3 cerebellum.

c-Jun Activation in Purkinje Cells from P3 Cerebellar Slice Cultures

To verify that JNK activation occurs in Purkinje cells in cerebellar slices from P3 mice, we analyzed by immuno-

histochemistry the activation of c-jun, the main JNK target, at 2 h (Fig. 5) by double labeling Purkinje cell with CaBP and P-c-jun antibodies. The analysis was performed at 2 h because this time point corresponds to the peak of JNK activation in our Western blot experiments. We detected c-jun activation in many cell types, including Purkinje cells, supporting JNK activation in this cell type at 2 h. Indeed, numerous CaBP immunoreactive Purkinje cells (Fig. 5a, d) are also positive for activated c-jun (=P-c-jun), (Fig. 5b, e, c, f). No signal was detected in the absence of primary antibodies in cerebellar slices (Fig. 5g–i).

Discussion

Developmental cell death of Purkinje cells has long been ignored due to the difficulty in identifying and counting Purkinje cells during development [6]. In vivo, the number of Purkinje cells is increased by over 30% in Bax knockout mutants [17], suggesting that this protein may be involved in naturally occurring Purkinje cell death. Furthermore, the analysis of mouse lines with different temporal expression of the anti-apoptotic Bcl2 protein in Purkinje cells indicated that developmental death of Purkinje cell indeed occurs in two periods, one during embryonic development and the second one during the first postnatal week [18, 19]. At P3, there is a peak of the number of Purkinje cells expressing the active form of caspase 3 [3, 20, 21]. During the first

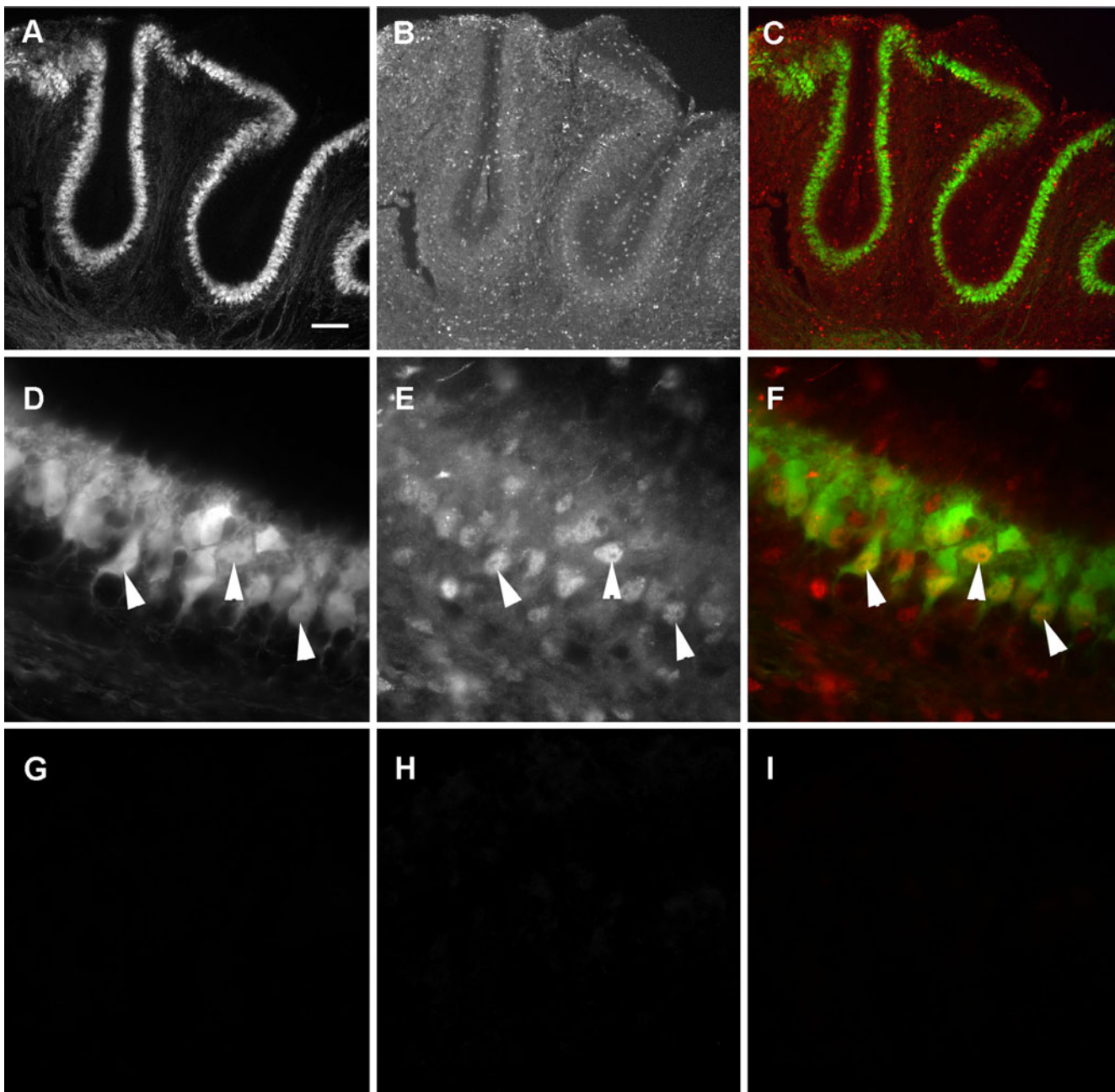


Fig. 5 c-Jun activation in PCs from P3 cerebellar slice cultures. P3 cerebellar slices were double labeled using CaBP immunostaining (**a**, **c**, **d**, **f**, **g**, **i**) and P-c-jun immunostaining (**b**, **c**, **e**, **f**, **h**, **i**). However, in **g**, **h**, and **i** the first antibodies were omitted. The pictures **d–g** and **e–i** have been taken exactly in the same conditions to be comparable.

Higher magnification clearly shows that numerous CaBP-positive PCs are also activated c-jun (=P-c-jun) immunoreactive (**d–f**). No signal was detected in the absence of the primary antibodies (**g–i**). Scale bar in **a** is 180 μm in **a–c** and 30 μm in **d–f**

postnatal week, Purkinje cells express caspase 3 [22]. These results suggest that during the developmental period of Purkinje cell death, these neurons are potentially more vulnerable. In organotypic culture, the age-related massive Purkinje cell death reflects the developmental Purkinje cell death occurring in the in vivo P1–P5 mouse cerebellum. Purkinje cells die by an apoptotic process characterized by caspase 3 activation in organotypic cerebellar slice cultures

from P3 mice, and a better Purkinje cell survival has been observed in P3 slices from hu-bcl-2 transgenic mice [2]. Thus, the model of P3 organotypic culture has been proposed as a model to study the mechanisms involved in developmental Purkinje cell death [16]. Here, we use this model to investigate JNK and p38 MAPK roles in Purkinje developmental cell death and to define more precisely the death occurring in P3 slices.

We first showed that the specific JNK or p38 inhibition could rescue Purkinje cell from death. Indeed, both D-JNKI1 and SB203580 were able to protect Purkinje cell, with a major effect of SB203580 compared to D-JNKI1 and with a stronger effect when used in combination. Furthermore, both p38 and JNK are activated in cerebellar lysates from P3 slice cultures with a different time course: JNK activation, evaluated as P-JNK/JNK ratio, increased 2 h after the culture and showed no differences between the “not sliced” and the “sliced” conditions, whereas p38 was strongly activated for the first 30 min after the culture.

Our data clearly indicated that the two stress-activated kinases were acting in a very different way.

The fact that p38 is similarly activated in both P0 and P3 lysates points out that p38 activation is unrelated to age-dependent cell death but is likely consequent to the slicing. In different experimental models, p38 inhibition protects cultured neurons against excitotoxic damage [23–25] and reduces brain injury after cerebral ischemia [26]. It is well known that p38 α - and β -specific inhibition blocks the production of the major inflammatory cytokines (TNF- α and IL-1 β) both in vitro and in vivo, and this seems to result from the effects at both transcriptional and translational level [27]. In addition, the p38 pathway is involved in the induction of several other inflammatory molecules, such as iNOS and COX2 [28, 29]. p38 activation is part of the mechanisms leading to apoptotic cell death induced by ROS and NO in primary mesencephalic dopaminergic neurons [30]. Overall, the p38 signaling pathway has been widely accepted as a cascade contributing to neuroinflammation. Thus, when analysing cell death in organotypic culture, it is important to consider the strong p38 activation intrinsically related to the model (slicing).

On the other hand, JNK activation is more specific since it is activated in P3 and not in P0 slices. It is thus correlated with the age-dependent Purkinje cell death observed in organotypic culture [2, 13]. Furthermore, we showed that c-jun, the main JNK target, was largely activated in Purkinje cells from P3 cerebellar slices, although other cell types can also present c-jun activation. The protection observed with the specific inhibition of JNK is less important than the one observed with SB203580. In the same way, by Western blot analysis, JNK activation appears less than p38 activation, though the effect of these two kinases is likely not Purkinje cells specific, as shown by c-jun activation in other cerebellar cells. Interestingly, the protective effect of the inhibition of both p38 and JNK is comparable to the one observed after inhibition of PKC [4] or addition of meflpristone [5], whereas the effect on Purkinje cell survival observed after D-JNKI1 treatment is comparable to the one observed after ablation of microglial cells or treatments with IGF1 [3, 4]. These two treatments have been involved in developmental cell death. Altogether,

these results suggest that in contrast to p38, JNK is involved in Purkinje cell death related to developmental cell death.

The activation of JNK pathway is critical for naturally occurring neuronal death during development as well as for pathological death of adult brain following different insults. Kuan et al. [7] were the first to show JNK1 and JNK2 role in the development of the nervous system and the overall relevance of JNK signaling in brain development has been broadly investigated [11]. In the context of neurodegeneration, the administration of the specific JNK inhibitor cell permeable peptide, D-JNKI1, was able to protect against excitotoxicity in vitro, cerebral ischemia in vivo [31, 32], and conferred neuroprotection and amelioration of neurobehavioral deficits after experimental TBI [33]. In a model of chronic excitotoxicity, the Lurcher mutant mouse, D-JNKI1 administration resulted in an increased number of Purkinje cells [34]. D-JNKI1 inhibitor peptide is thus a very powerful tool to study specific JNK involvement in neuronal death.

In conclusion, both JNK and p38, the two stress-activated MAPKs, contribute to Purkinje cell death occurring in P3 organotypic culture. However, our results clearly indicate that these two MAPKs are activated by two different causes, the culture model (slicing) and the developmental vulnerability of Purkinje cells, and the contribution of each MAPK is related to only one specific cause. p38 plays a role in Purkinje cell (PC) death consecutive to the slicing, whereas JNK activation is linked to the “age-related” Purkinje cell death.

Our data strengthen what was already known in the stress-activated protein kinases context in the central nervous system: Mechanistically different forms of cell death differently need p38 and JNK activation in neurons [6].

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Conflicts of Interest The authors certify that there is no conflict of interest concerning the work presented in this manuscript.

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