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Letter to the Editor

Cell death in adult neural stem cells

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Dear Editor,

Neural stem cells (NSC) were discovered to exist in the adult brain. These cells have a capacity for self-renewal and can generate both neuronal and glial cells.[1] Stem cells undergo apoptosis as an essential component of neural development. Previous studies suggest that embryonic and perinatal progenitor cells undergo apoptosis via activation of caspases[2] and involvement of the Fas death receptor.[3] Nothing, however, is known about the mechanisms by which adult NSC undergo cell death. Here we show that adult NSC undergo apoptosis via the mitochondrial pathway with caspase-3 serving as the executioner caspase in the apoptotic machinery.

We utilized a primary culture method which promotes the growth of adult NSC.[4] Primary tissue was harvested from the ependymal layer and underlying subventricular zone from the adult rat. Procedures used in animal experimentation comply with the Karolinska Institute's regulations for care and use of laboratory animals. Cells were cultured for 6–8 days in a serum-free medium supplemented with epidermal growth factor in order to develop clonal spheres of cells, which were subsequently trypsinized and re-cultured for 4 days to generate secondary spheres before a final trypsinization procedure and cell attachment onto poly-L-lysine coated slides. After 45 minutes of cell attachment, cells were exposed for 18 h to staurosporine (STS) (0.25 μM), a known inducer of apoptosis. At the time of exposure, cells were found to express nestin (Figure 1a), an

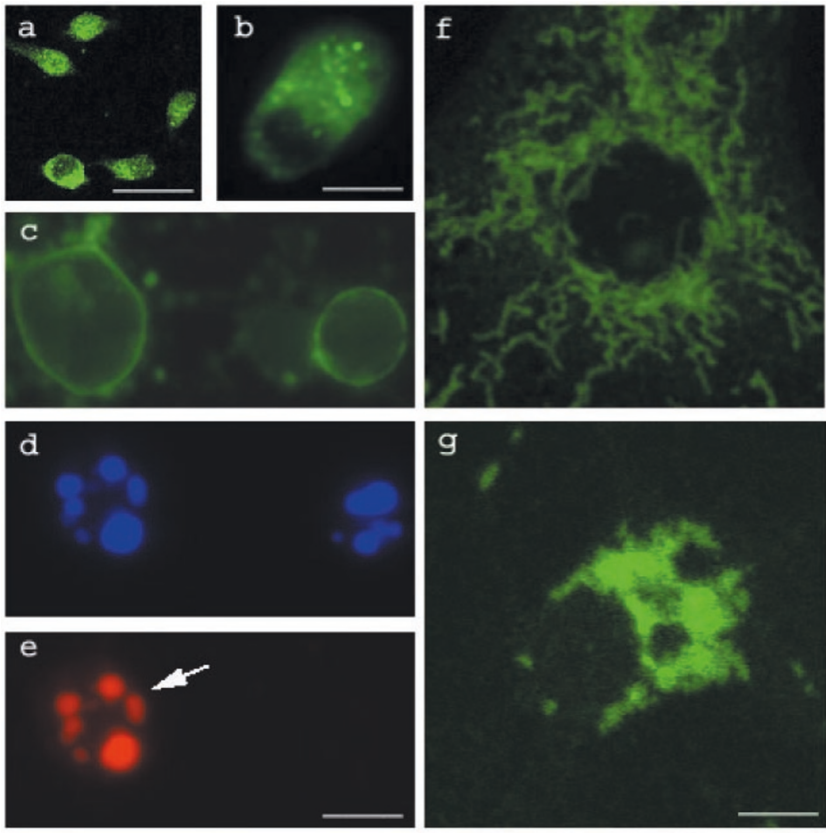

Figure 1 Control adult NSC expressed nestin immunoreactivity (**a**). After exposure to STS, cells underwent apoptosis and expressed active caspase-3 (**b**). Cells exposed to STS stained with Annexin V (**c**) and exhibited chromatin condensation and fragmentation as detected by the membrane permeable dye Hoechst 33342 (**d**). The membrane impermeable dye propidium iodide stained chromatin only in cells in a more advanced stage of apoptosis (arrow in **e**). Cytochrome *c* immunoreactivity was localized in mitochondria arranged in fine filamentous networks (**f**). After exposure to STS, cytochrome *c* was released from the mitochondria into the cytosol (**g**). Scale bars: **a**=7 μm; **b**–**e**=3.5 μm; **f**,**g**=1.75 μm

intermediate filament protein characteristic of CNS precursor cells.

Exposed cells demonstrated DNA breaks with 3′ hydroxyl ends visualized with TUNEL staining, phosphatidyl serine exposure as detected by Annexin V (Figure 1c), and an apoptotic morphology (Figure 1d,e). In addition to positive immunoreactivity against the active fragment (p17) of caspase-3 (Figure 1b), pre-incubation with the pan-caspase inhibitor, z-VAD-fmk (40 μM), prevented apoptosis ($P<0.05$).

Mitochondria are known to act as key regulators of apoptosis in several cell types.[5] Previous reports utilizing progenitor cell lines have found that apoptosis in these cells is inhibited by Bcl-2, an anti-apoptotic protein acting on the mitochondria.[6] Permeabilization of the outer mitochondrial membrane results in the release of several intermembrane space proteins, including cytochrome *c*, which is involved in subsequent activation of caspases, followed by the degradation phase of apoptosis.[7] Utilizing an antibody specific for cytochrome *c*, we observed that stained mitochondria in unexposed adult NSC were arranged in reticular networks (Figure 1f). Cells exposed to STS exhibited diffuse fluorescence throughout the cytosol (Figure 1g), implying a release from the mitochondria. Previous reports have suggested a functional integration of mitochondrial structures. The mitochondria may change shape during cell division and differentiation, likely due to the action of dynamin-related protein.[8] The reticular arrangement observed in adult NSC may not only help determine cell structure and mitochondrial location, but also allow functional linkages that enable intracellular Ca^{2+} regulation crucial for cellular energetics.[9]

These preliminary results indicate the importance of the mitochondria in mediating apoptosis in adult NSC, promoting a caspase-3-dependent mechanism. Thus it appears that the intrinsic cell death pathway is functional at this stage. Currently, we are investigating the role of the extrinsic cell death pathway. We have observed expression of the Fas receptor using immunocytochemistry, but exposure to an agonistic Fas mAb (250 ng/ml) failed to induce apoptosis. Further experiments are necessary to investigate the possibility that the Fas pathway may become operative only when NSC differentiate into glial cells as previously suggested.[10]

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