758 BCL-2 INTRODUCED WITH RETROGRADE TRANSPORT OF ADENOVIRUS VECTORS RESCUES MOTONEURONS FROM CELL DEATH *IN VIVO*.

HIROYUKI YAGINUMA[1], MITSUHIRO HASHIMOTO[2], KEIKO OKABE[3], TAKAKO SHIMADA[1] & KATSUHIKO MIKOSHIBA[2]

[1]Dept. of Anat., Sch. of Med., Fukushima Med. Univ., Fukushima 960-1295, [2]Molecular Neurobiol. Lab. Tsukuba Life Sci. Center, RIKEN, Tsukuba 305-0074, [3]Dept. of Anat., Inst. Basic Med. Sci., Univ. of Tsukuba, Tsukuba 305-0006

To examine whether overexpression of Bcl-2 rescues motoneurons from programmed cell death in the developing chick spinal cord, we have generated adenovirus vectors capable of overexpressing Bcl-2. Following injection of Bcl-2 vectors into right leg buds of the chick embryos on embryonic day (E) 4.5-5 (st 25), many motoneurons in the right lateral motor column (LMC) in lumbosacral segments strongly expressed Bcl-2 by E7. On E7 colchicine was injected into the same leg buds to induce motoneuron cell death due to depletion of target-derived neurotrophic factors. Virtually all motoneurons in the LMC died by E10 in the control embryos that received injection of colchicine without preceding injection of Bcl-2 vectors. By contrast, remaining motoneurons were observed in the LMC of the embryos that received injection of Bcl-2 vectors followed by injection of colchicine. Most of the remaining motoneurons expressed Bcl-2. These results suggest that Bcl-2 adenovirus vectors can be retrogradely transported from the periphery to cell bodies, and that overexpression of Bcl-2 can rescue motoneurons from cell death *in vivo*.

759 TYROSINE PHOSPHATASE INHIBITOR PREVENTS PEROXYNITRITE-INDUCED CELL DEATH OF HUMAN NEUROBLASTOMA SH-SY5Y CELLS

MAKIO SAEKI, TOMOHIKO KANESAKI, TAKASHI YAMADA, YASUHIRO OOI, SADAAKI MAEDA

Dept. of Pharmacology, Fac. of Dentistry, Osaka Univ., Suita, Osaka 565-0871, Japan

Peroxynitrite ($ONOO^-$) is a potent oxidant and cytotoxic species produced by the reaction of superoxide with NO. The involvement of peroxynitrite-mediated cell death in neurodegenerative disorders such as Alzheimer's desease is suggested by the localization of nitrotyrosine-like immunoreactivity at sites of tissue damage. However, few specific cellular target proteins for peroxynitrite cytotoxicity containing nitrated tyrosine residues have been identified. In the present study, exposure of human neuroblastoma SH-SY5Y cells to the peroxynitrite donor 3-morpholinosyndnonimine (SIN-1) resulted in cell death characterized by retraction from the culture dish followed by detachment, and induced tyrosine nitration of a 130-kDa protein. Pretreatment of cells with tyrosine phosphatase inhibitor, vanadate, inhibited cell death induced by SIN-1, and stimulated the tyrosine phosphorylation of the 130-kDa protein. We propose that the 130-kDa protein is a potential signal molecule in cell death induced by peroxynitrite.

760 ANTI-APOPTOTIC FUNCTION OF BCL-2 REQUIRES LOCALIZATION IN THE INNER MITOCHONDRIAL MEMBRANE

SHIRO KANAMORI, TAKAHIRO GOTOW, MASAHIRO SHIBATA, KYOKO ISAHARA, YOSHIYUKI OHSAWA, NOBORU SATO, TSUYOSHI WATANABE, YASUO UCHIYAMA

Dept. of Cell Biol. & Anat. I, Osaka Univ. Med. Sch.; 2-2 Yamadaoka, Suita, Osaka 565-0871

Bcl-2, an anti-apoptotic protein, is believed to be localized in the outer mitochondrial membrane, endoplasmic reticulum, and nuclear envelope. However, Bcl-2 has been suggested to act on the maintenance of mitochondrial membrane potential, indicating its possible association with the inner mitochondrial membrane. We therefore further examined the exact localization of Bcl-2 in mitochondria purified from wild-type and *bcl-2*-transfected PC12 cells and pre- and postnatal rat brains. Double immunostaining demonstrated that Bcl-2 was co-localized with subunit β of F_0F_1ATPase in the inner mitochondrial membrane in *in vivo* and *in vitro* cells, while it was also associated with smooth endoplasmic reticulum in them. By subcellular fractionation of postnuclear supernatants from *bcl-2*-transfected cells, Bcl-2 appeared in mitochondrial and light membrane fractions. Using purified mitochondria from PC12 cells and postnatal rat brains, Bcl-2 and subunit β were neither dissolved into the outer membrane fraction by disruption with 1 mg digitonin/mg mitochondrial protein, nor digested with 250 μg/ml trypsin. The results suggest that Bcl-2 acts as an anti-apoptotic factor by localizing mainly to the inner mitochondrial membrane.