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Author(s)	TAKAHASHI, Kenji
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regulation of the MAPK signaling pathway and the induction of a tolerant state by up-regulating HSPs in the brain.

In summary, we demonstrated that PBN induced neurite outgrowth via ERK and PKC pathways which was inhibited by thiol-based antioxidant in the *in vitro* studies using PC12 cells, suggesting that this PBN-induced formation of neurite in PC12 cells was associated with intracellular redox regulation. Besides this, in the *in vivo* studies using a model for

ischemia-reperfusion injury in gerbil hippocampus, it was also shown that PBN attenuated neuronal cell death via the up-regulation of ERK and HSPs and down-regulation of SAPK and p38. These results suggested that the neuroprotective effects of PBN *in vitro* and *in vivo* were involved in not only scavenging activity against oxygen radical but also its redox-regulation of signal transduction

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Fas-mediated signal transduction pathway in apoptosis induced by ionizing radiation

Kenji Takahashi

*Laboratory of Radiation Biology, Department of Environmental Veterinary Sciences,
Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan*

The studies of radiation-induced apoptosis are very important to understand the basic mechanisms of not only cell death but also the protection against it. To clarify signaling pathway concerning caspase family in radiation-induced apoptosis in lymphoblast cells, the effects of a protein synthesis inhibitor, cycloheximide, on the apoptotic signaling pathway including the activation of caspases and stress-activated protein kinase/ c-Jun N-terminal kinase (SAPK/JNK), the expression of Fas/CD 95 /APO-1 (Fas), the reduction of mitochondria membrane potential ($\Delta\Psi_m$) and the release of cytochrome c were examined in X-irradiated MOLT-4 cells.

MOLT-4 cells pretreated with 0.5 $\mu\text{g/ml}$ cycloheximide (CHX) for 1 h were exposed to 7.5 Gy of X-rays. The appearance of apoptosis,

expression of Fas, activation of caspases-3, -8, -9, SAPK/JNK and AP-1, the release of mitochondrial cytochrome c and the formation of death-induced signaling complex (DISC) between Fas and Fas-associated protein with death domain (FADD) were observed by fluorescence microscopy, Western blotting, flow cytometry, gel shift assay and immunoprecipitation methods, respectively. The ligation of Fas and Fas ligand was also examined.

When cells were exposed to 7.5 Gy of X-rays, the typical morphological alterations, characteristic of apoptosis, including nuclear fragmentation and chromatin condensation were observed 6 h after X irradiation and gradually increased up to 12 h. Apoptosis induction was significantly attenuated by CHX. Moreover, the activation of caspases-3 and -8,

-9 which was observed 6 h after X irradiation was significantly attenuated by CHX. SAPK/JNK was activated within 30 min after X irradiation. Interestingly, CHX did not inhibit the activation of SAPK/JNK but inhibited the expression of c-Jun and the activation of AP-1 existing downstream of SAPK/JNK. On the other hand, the expression of Fas, a tumor necrosis factor (TNF) receptor existing upstream of caspase-8, increased 2 h after X irradiation. However, neither a neutralizing antibody against Fas (ZB4) nor an agonistic antibody against Fas (CH-11) influenced X-ray-induced apoptosis, suggesting that caspase-8 activation was independent of Fas/ Fas-

ligand ligation. The DISC formation without the expression of Fas ligand was observed after X irradiation and CHX considerably inhibited it. Moreover, the reduction of $\Delta\Psi_m$ and the release of cytochrome c from mitochondria to cytoplasm, which were known to be upstream of caspase-9 activation, occurred after X irradiation. These phenomena were also inhibitable with CHX.

The present results indicated that apoptosis induction was dependent on the activation of caspases regulated by *de novo* protein synthesis through SAPK/JNK activation and Fas was an important apoptosis factor in X-irradiated MOLT-4 cells.

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Studies on population ecology of the spotted seal, *Phoca largha*, in the coastal waters of Hokkaido, Japan

Ayako W. Mizuno

*Laboratory of Wildlife Biology, Department of Environmental Veterinary Sciences,
Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan*

The spotted seal is a common sight in Japan. However, the conservation and management of this species has been inadequate. One of the principal reasons is lack of basic biological information. Therefore, the present study examined the spotted seal distribution patterns and abundance, cranial morphology and genetic features in the Hokkaido waters.

Seasonal/regional variations in spotted seal distribution were confirmed along the entire coastline of Hokkaido. Incidental harvests, damage control kills, and strandings were most common during salmon harvest season (fall), sea ice season, and the weaning period, respectively. Sub-adults and adults were

found in areas with seasonal sea ice, while immature seals were recovered from areas with little or no sea ice. The fewest seal sightings and no haul-out sites were reported from southern Hokkaido, probably because warm sea currents discourage seals from using this area.

Based on aerial surveys, the distribution and abundance of spotted seals were assessed in the breeding season off the Okhotsk coast of Hokkaido. The total abundance was estimated to be 13,653 seals (95%CI=6,167-30,252) in March, and 6,545 seals (95%CI=3,284-815,644) in April. The pack ice area off Hokkaido had higher densities (0.54 and 0.58