Bioorganic Chemistry
- Molecular Clinical Chemistry -

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Mr ALIOTO, Tyler University of California, Berkeley, U.S.A., 2 June 2003 - 3 June 2003
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Scope of Research

This laboratory was founded in 1994 with the aim of linking (bio)chemical research and clinical medicine. Thus, the scope of our research encompasses the structure, function and pathophysiological significance of various biomolecules and bioreactions in relation to human diseases, and the application of molecular techniques to clinical diagnosis and therapy. Our current interest is focused on the role of poly(ADP-ribosyl)ation in protection of genome from apoptosis-inducing stresses, and the molecular etiology of neurodegenerative disorders including Alzheimer's disease and Parkinson's disease.

Research Activities (Year 2003)

Presentations

The Establishment of Evidence-Based Genetic Testing in Japan, Ueda K, 10th Annual Meeting of the Japanese Society for Gene Diagnosis and Therapy, Osaka, 24 - 25 July.

Role of Poly(ADP-ribose) Synthetase in Neuronal Cell Death, Tanaka S, Japanese-German Biochemistry Meeting, Marburg, 29 - 30 September.

Functional Characterization of Septin 3, Takehashi M, Japanese-German Biochemistry Meeting, Marburg, 29 - 30 September.

Poly(ADP-ribose) Synthetase Activation and Mitochondrial injury in Neuronal Cell Death, Tanaka S, and Ueda K, 76th Annual Meeting of Japanese Biochemical Society, Yokohama, 15 - 18 October.

Characterization of Novel Mammalian Septin, SEPT 3, Takehashi M, Tanaka S, Suzuki K, Nagai H, Maeda M, Kinoshita N, and Ueda K, 76th Annual Meeting of Japanese Biochemical Society, Yokohama, 15 - 18 October.

Development of Highly Sensitive *In Situ* Hybridization and RNA Amplification Methods, Iida S, Onaka S, Takehashi M, Hayashi T, TanakaS, and Ueda K, 76th Annual Meeting of Japanese Biochemical Society, Yokohama, 15 - 18 October.

Grant

Tanaka S, Suppression of neuronal cell death by poly(ADP-ribose) synthetase inhibitors, Japan Foundation for Applied Enzymology, 1 April 2003 - 31 March 2004.

Role of Poly(ADP-ribose) Synthetase in Neuronal Cell Death

Poly(ADP-ribose) synthetase (PARS) is a nuclear enzyme that, upon activation by DNA single-strand breaks, forms (ADP-ribose)n chains from β-nicotinamide adenine dinucleotide (NAD^+) on acceptor proteins, including histones and PARS itself. Poly(ADP-ribose) is known to be involved in various physiological and pathological events such as DNA repair, cell differentiation, cell cycle, and cell death. The excessive activation of PARS leads to depletion of cellular NAD^+. The depletion of NAD^+, a co-enzyme in energy metabolism, results in reduction of ATP generation, while ATP is used for replenishment of NAD^+. This "energy crisis" is considered to be causative of apoptotic and necrotic cell death.

We studied, using models derived from primary cell cultures of rat brain, role(s) of PARS in the neuronal cell death in Alzheimer's disease (AD) and cerebral infarction. In the AD model, cortical neurons became mostly apoptotic after exposure to Aβ amyloid fibrils. The Aβ fibrils treatment enhanced mitochondrial generation of reactive oxygen species (ROS). In the cerebral infarction model, cortical neurons became partly apoptotic and partly necrotic after oxygen-glucose deprivation. This treatment induced mitochondrial membrane depolarization and the opening of permeability transition pore (PTP), through which apoptotic factors such as cytochrome c and apoptosis-inducing factor (AIF) were released. In both AD and cerebral infarction models, PARS was activated in a dose- and time-dependent manner, resulting in the decrease of NAD^+ content. Addition of PARS inhibitors such as 1,5-dihydroxyisoquinoline and benzamide suppressed NAD^+ depletion, and also attenuated the mitochondrial injury. These *in vitro* models strongly suggest that PARS activation plays a critical role in the ROS-mediated neuronal cell death in the pathological conditions such as AD and cerebral infarction.

Functional Characterization of Septin 3

Septin 3 is a novel member of the septin subfamily with GTPase domain. The septin 3 gene was originally cloned with a phenotype of up-regulation during neuronal differentiation of the human teratocarcinoma cell line NT2. Septin 3 has, at least, two isoforms, A and B, that are produced by the alternative splicing of a single transcript. We studied the physiological and pathological roles of septin 3 isoforms. Using antibodies specific for the isoforms A and B, we found that both isoforms were abundantly expressed in normal human brain. We further investigated intracellular localization and interaction of the two isoforms in HeLa cells, transfected separately or together, by use of the antibodies in immunohistochemistry and immunoprecipitation. Both isoforms A and B were found to form fibrous structures in the cytoplasm, and the structures were to be composed of homologous as well as heterologous isoforms of septin 3. We also analyzed a genetic association of septin 3 polymorphisms with Alzheimer's disease (AD), Parkinson's disease (PD), and Lewy body variant (LBV) of AD. Genotyping of microsatellite polymorphisms in exon 11 indicated a significant difference in long/short allelic distribution between AD and control, suggesting a role played by septin 3 in pathogenesis of AD, but not PD or LBV.

Overexpressiom of CYP2D6 Attenuates the Toxicity of MPP^+ in Actevity Dividing and Differentiated PC12 Cells

Clonal pheochromocytoma cell lines overexpressing cytochrome P450 2D6 (CYP2D6) were established. CYP2D6 was localized in the endoplasmic reticulum, and its enzymatic activity in the microsomal fraction was confirmed by using high performance liquid chromatography analysis with [guanidine-^{14}C]debrisoquine as a substrate. Overexpression of CYP2D6 protected cells against the toxic effects of 1-methyl-4-phenylpyridinium ion (MPP^+) at the concentration range of 20~40 μM, as assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Further, reactive oxygen species generation was suppressed in the mitochondria. The cytotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was unchanged in cells overexpressing CYP2D6 versus mock-transfected controls at concentrations up to 500 μM. These results suggest that the lowered enzyme activity of CYP2D6 in individuals, termed "poor metabolizers" may represent a risk factor from exposure to select neurotoxicants.

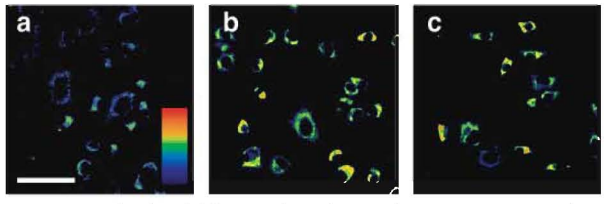

Figure 1. Mitochondrial generation of ROS after exposure to MPP^+ or MPTP.
Clonal cells were treated with a reduced form of chloromethyltetramethylrosamine before (a) after a 24-h incubation with 20 μM MPP^+ (b) or 500 μM MPTP (c). Mitochondrial ROS are visualized by oxidized chloromethyltetramethylrosamine (Scale bar = 40 μm).