Identification of oxidative stress-regulated genes during nigral dopaminergic cell death

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The search for the molecular basis of DA cell death has been the recent focus in Parkinson's disease (PD) research. Although the initial causes appear to be heterogeneous, there is substantial evidence indicating excessive oxidative damage in the SN of affected PD brains.[1] One major postulated pathogenic mechanism of the SN-DA cell death in PD is a vicious cycle of oxidative stress. The oxidative stress triggered by various pathogenic mechanisms generates reactive oxygen species (ROS) and reactive nitrogen species including hydrogen peroxide (H_2O_2), superoxide ($O_2^{\cdot-}$), hydroxy radical ($^{\cdot}OH$), nitric oxide ($^{\cdot}NO$) and peroxynitrite ($ONOO^-$).[2] In particular the ROS not only inflict direct cellular damage, but also act as important intracellular messengers, targeting transcription factors, G-protein, ion channel, protein kinases and NOS.[3,4]

Molecular dissection of apoptotic cell death of substantia nigra (SN) dopaminergic (DA) neurons in both primary embryonic mesencephalic cultures and *in vivo* animal models has been technically difficult because DA neurons in SN are relatively rare and present with many heterogeneous cell populations in midbrain. Thus, a clonal SN-DA neuronal cell line, SN4741, was established from transgenic mouse embryos.[5]

Several common proapoptotic molecules have been implicated in the degenerating DA neurons of both MPP^+-induced model and idiopathic PD brain.[6] One proapoptotic pathway involves the activation of JNK MAP kinase. Subsequently, CEP1347 (an inhibitor of JNK activation) and JNK interacting protein-1 (JIP-1) was found to partially attenuate the MPP^+-induced DA cell loss.[7,8] Accordingly, our previous studies have identified a role for the ROS-dependent pathway during MPP^+- and other PD-related neurotoxicants-induced DA cell death.[6] The experimental data revealed that treatment with MPP^+ and related neurotoxicants induced apoptotic cell death in SN4741 cells. Following initial increases of

H_2O_2-related ROS activity and subsequent activation of JNK1/2 MAP kinases, activation of caspase-1 and caspase-3 activities resulted in apoptotic cell death. Thus, employing this biochemical model, further cDNA microarray analyses were performed in an attempt to identify potential downstream response genes during the oxidative stress-induced DA cell death. cDNA microarray analysis was performed as described before.[6] Briefly, Poly(A)$^+$ mRNA samples were prepared from the nontreated control cultures and from SN4741 cells treated with H_2O_2 for 6h, after the peak of JNK1/2 activation. Then, two fluorescence-labeled cDNA probes (Cy3 and Cy5) were prepared from the mRNA samples and hybridized with the Mouse GEM1 cDNA microarray (Incyte-Genomics), which contains about 8,700 sequence-verified clones of both known genes and EST clones. To confirm the cNDA expression level, RNA slot blot was carried out as described before.[9] The signals were quantified by PhosphoImage Analyzer (Fujifilms BAS-2500) and ImageGage 3.12 program.

The SN4741 cell is appropriate for the high throughput analysis of DA cell death because it maintains many essential features of SN-DA specific phenotype. A group of differentially regulated genes (over 150 clones) was identified, of which expressions were either increased or decreased (>1.8 fold). The high throughput cDNA microarray screening using the current model identified downstream response genes, such as Heme Oxygenase 1 HO-1),[10] Adenosine triphosphate-binding cassette transporter 1 (ABC1),[11] Apolipoprotein E (ApoE),[12,13] and GTP binding protein,[14] that can be the useful biomarkers to monitor the pathological conditions of DA neurons under oxidative stress. The results obtained by cDNA microarray analysis were further confirmed using the corresponding cDNA clone probes for RNA slot blot analysis. Parallel studies of these characterized cDNA probes by *in situ* hybridization and bioinformatic analysis revealed some novel genes regulated by oxidative stress.

Further characterization of the downstream EST clones identified in the current model may facilitate the development of novel surrogates for diagnosis of the complex pathological status of the degenerating DA neurons in PD.

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