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BRAIN RESEARCH

Research Report

Apoptosis mediated by p53 in rat neural AF5 cells following treatment with hydrogen peroxide and staurosporine

Charlesene McNeill-Blue[a], *Barbara A. Wetmore*[a], *Joseph F. Sanchez*[b], *William J. Freed*[b], *B. Alex Merrick*[a,*]

[a]*Proteomics Group, National Center for Toxicogenomics, National Institute of Environmental Health Sciences, National Institute of Health, Department of Health and Human Services, D2-04, P.O. Box 12233, Research Triangle Park, NC 27709, USA*
[b]*Cellular Neurobiology Research Branch, National Institute on Drug Abuse, National Institute of Health, Department of Health and Human Services, 5500 Nathan Shock Drive, Baltimore, MD 21224, USA*

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ABSTRACT

AF5 neural cells derived from fetal rat mesencephalic tissue were immortalized with a truncated SV40 LT vector lacking the p53-inactivating domain to maintain long-term cultures with a p53-responsive phenotype. This study examined p53 function in producing programmed cell death in propagating AF5 neural cells after exposure to hydrogen peroxide (H_2O_2) and the kinase inhibitor staurosporine (STSP). Concentration-dependent exposure of AF5 cells to 0–800 mM H_2O_2 and STSP at 0–1000 nM revealed increasing cytotoxicity from MTS cell viability assays. Apoptosis occurred at 400 mM H_2O_2 as evidenced by subG_1 DNA and Annexin V flow cytometry analyses and cellular immunofluorescence staining with propidium iodide, anti-Annexin V and DAPI. DNA fragmentation, caspase-3/7 activity and cytochrome *c* release into cytosol also confirmed H_2O_2-mediated apoptotic events. p53 protein levels were increased over 24 h by H_2O_2 in a coordinated fashion with mdm2 expression. p53 activation by H_2O_2 was evidenced by elevated Ser15 phosphorylation, increased luciferase p53 reporter activity and upregulation of the downstream p53 targets $p21^{waf1}$ and apoptotic proteins, bax, Noxa and PUMA. STSP exposure produced apoptosis demonstrated by DNA fragmentation, caspase-3/7 activity, cytochrome *c* release and over 24 h was accompanied by sustained increase in p53 and Ser15 phosphorylation, rise in $p21^{waf1}$ and bax and a transient increase in p53 reporter activity but without Annexin V binding. These findings demonstrate that AF5 cells undergo apoptosis in response to H_2O_2-mediated oxidative stress and signal pathway disruption by STSP that therefore would be useful in studies related to p53-dependent neuronal cell death and neurodegeneration.

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* *Corresponding author*. Fax: +1 919 541 4704.
E-mail address: merrick@niehs.nih.gov (B.A. Merrick).

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Abbreviations:
bFGF, basic fibroblast growth factor
CNS, central nervous system
CO_2, carbon dioxide
DAPI, 4,6-diamidino-2-phenylindole
DAT, dopamine transporter
ddH_2O, distilled, deionized water
DMEM, Dulbecco's modified Eagle's medium
EDTA, ethylenediaminetetraacetic acid
G_1, gap1
G_2, gap2
GFAP, glial fibrillary acidic protein
H_2O_2, hydrogen peroxide
MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MTS, [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium
O^-_2, superoxide radical
OH^-, hydroxyl radical
PC12, pheochromocytoma cells
PD, Parkinson's disease
PMSF, phenylmethylsulfonylfluoride
P-Ser15-p53, phosphorylation of p53 at Ser15
PI, propidium iodide
PMSF, phenylmethylsulfonylfluoride
PUMA, p53 upregulated modulator of apoptosis
ROS, reactive oxygen species
SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis
STSP, staurosporine
TH, tyrosine hydroxylase

1. Introduction

Millions of people worldwide suffer from neurodegenerative disorders, but the pathogenic mechanisms leading to neuronal cell death are not well understood. Neural cell lines are valuable research tools for understanding the effectors and biochemical pathways underlying the neurodegenerative process. However, many oncogenically derived cell models often possess abnormal p53 function, altered cellular pathways and functions, and an inability to undergo apoptosis in a manner that limits their usefulness in the study of neuronal cell death. Tumor cell lines such as rat PC12 (Greene and Tischler, 1976), human SH-SY5Y (Pahlman et al., 1981) and mouse MN9D (a dopaminergic–neuroblastoma hybrid) (Choi et al., 1992) routinely used for neuronal studies face similar limitations in growth control, differentiation and apoptosis (Truckenmiller et al., 2002; Whittemore and Snyder, 1996).

The AF5 cell line was derived from the central nervous system (CNS) of 14-day-old rat mesencephalic tissue (Truckenmiller et al., 1998). Primary cultures were immortalized with an N-terminal fragment of SV40 large T antigen (T155 g) lacking the p53 inactivating domain while retaining wt p53 genotype. AF5 cells displayed genomic stability and growth factor responsiveness after months of continuous culture while retaining considerable plasticity in culture (Truckenmiller et al., 2002). These cells can be maintained in a propagating state in which nestin, a marker for immature neural precursors, is expressed or they can be differentiated upon confluency and exhibit differentiated morphologies, growth arrest and expression of mature neuronal cell markers such as βIII-tubulin. Approximately 1% of the cells in confluent cultures were immunopositive for tyrosine hydroxylase (TH), a marker of dopaminergic neurons and important for study of Parkinson's disease (Chen et al., 2005a,b,c). Microarray analysis showed gene expression or altered transcription consistent with neural specification when comparing undifferentiated (with and without bFGF) and differentiated AF5 cells (Truckenmiller et al., 2002). More recently, additional fetal rat mesencephalic and cerebral cortical lines have been derived using a similar construct T155c (Freed et al., 2005).

While both necrosis (Leist and Nicotera, 1998; Martin et al., 1998) and apoptosis (Anglade et al., 1997; Hirsch et al.,