Colostrinin-driven cell cycle arrest by p53 is required for cell differentiation in PC12 cells: Implications in Alzheimer's Disease

[1]Attila Bacsi, [1]Daniel Liebenthal, [1]T. Kley Hughes, [2]Marian L. Kruzel, [1]G. John Stanton, and [1]Istvan Boldogh

[1]Department of Microbiology and Immunology. The University of Texas Medical Branch, Galveston, Texas; [2]ReGen Therapeutics, Plc. London, England

ABSTRACT

Colostrinin (CLN), also known as proline-rich polypeptide complex, has been shown to be beneficial in the treatment of mild or moderate Alzheimer's disease. In our previous studies, we demonstrated that CLN has the ability to protect pheochromocytoma (PC12) cells from oxidative stress, particularly by reducing the 4-hydroxynonenal mediated cellular events. The goals of this study were to determine if p53 plays a direct role in mediating CLN driven G1 arrest and whether it influences the expression of cell cycle proteins during cell differentiation processes. Here we report that PC12 cells extend neurites and cease to proliferate upon treatment with CLN. The neuritogenesis and the block of the G1/S checkpoint correlate with the nuclear translocation and increase in the phosphorylation of p53 protein at serine[15] in CLN treated cells. Treatment of CLN-treated PC12 cells with specific antisense to p53, but not sense oligonucleotide, failed to arrest cell cycle or induce morphological changes. These cells also failed to over express p21^{WAF1}, as determined by immunoblotting analysis or exhibit growth arrest as measured by bromodeoxyuridine incorporation. CLN treatment of PC12 cells also resulted in transactivation of the p53 response element in a luciferase reporter construct, whereas this response was absent in mock-treated cells. PC12 cells treated with p53 sense oligonucleotides continued through the cell cycle, confirming the dependence of the CLN-mediated growth arrest signal on a p53 pathway. We have concluded that transient phosphorylation of wild-type p53 is indispensable in mediating the CLN antiproliferative signal required for differentiation in PC12 cells. These data may provide the molecular basis for explaining CLN's beneficial effect on Alzheimer's disease.

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INTRODUCTION

During the first few days post-parturition colostrums contain high levels of growth factors and antimicrobial compounds in addition to normal nutrients such as proteins, carbohydrates, fats, and minerals (1,2). Colostrinin was originally purified from ovine colostrum through various chromatographic steps, including ion exchange, affinity and molecular seiving, and ammonium sulfate precipitation (3). It has been shown that colostrinin and it's proline-rich polypeptides possess immunomodulatory effects and antioxidant activity and induce maturation and differentiation of murine thymocytes (1,3,4). They also promote peripheral blood leukocyte proliferation via induction of various cytokines (5).

The PC12 cells derived from a transplantable chromaffin tumor, provide a model system to study differentiation processes in a neuronal-cell phenotype (6). We have previously noted that CLN treatment mediates morphological changes (neurite-like outgrowth) (7). In general, neuronal-like morphological changes invokes two interrelated cellular processes: 1) progression through the stages of neurite outgrowth and 2) cell cycle arrest. In this study, we show that activation of p53 is an important event for CLN-induced cell cycle arrest and neurite outgrowth in PC12 cells. We also show that these coupled processes of cell cycle arrest and neuritogenesis share the overlapping regulators p53 and p21^{WAF1}, key coordinators of the G1/S phase cell cycle checkpoint.

CONCLUSION

We demonstrate here that colostrinin, a multi-component peptide, activates the p53 pathway, which is critical for the induction of morphological changes and neurite outgrowth. The data also indicate that activation of p21^{WAF1} may also be important in this differentiation response. Colostrinin's silencing effect on 4HNE-mediated activation of JNK, previously shown by us, suggests that this pathway could also be important in differentiation of PC12 cells by CLN. Together, these data identify possible therapeutic mechanisms that help explain it's therapeutic effects in Alzheimer's. Finally, the data suggest its potential use in other chronic neurological diseases.

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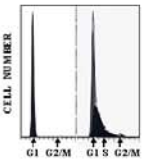
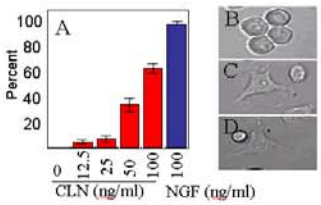
TABLE 1

	Cell Cycle Stages					
	G1		G1/S+S		G2/M	
Treatment	24h	72h	24h	72h	24h	72h
CLN	92%	97%	7.9%	2.9%	0.1%	0.1%
NGF	93%	99%	6.8%	0.9%	0.2%	<0.1%
EGF	59%	73%	34.5%	22.4%	6.5%	6.6%
Mock	73.6%	83%	23.5%	14.7%	2.9%	2.3%



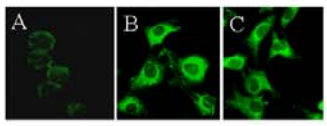
The cell cycle stage was determined by the cellular DNA content, which was evaluated by flow cytometry (FACScan flow cytometer, Becton Dickinson). Histograms were analyzed using ModFit LT cell cycle analysis software (Verity Software House, Inc) to determine the percent of cells in various stages of cell cycle. Instrument calibration was performed daily using Calibrate Beads according to the recommendation of the manufacturer (Becton Dickinson). 10,000 events were collected in all samples.

Fig. 1. Neurite Out-growth of PC12 Cells in Response to CLN.



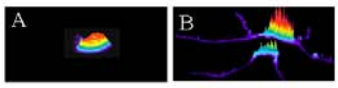
A. Percent of morphologically changed (neurite out-growth) cells vs. dose of CLN was determined on day 6 post-treatment. NGF (50ng/ml) was used as the positive control. B. Mock-treated, C CLN-treated, D. NGF-treated PC12 cells

Fig. 2. Morphological Changes of PC12 Cells Treated by CLN.



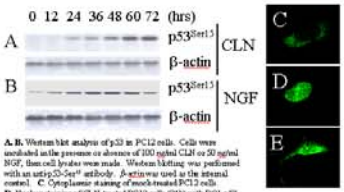
A. Mock-treated PC12 cells
B. Colostrinin-induced morphological change
C. Nerve Growth Factor-induced morphological change
*Fluorescent (485 nm) images of Eosin-stained cells

Fig. 3. Three-Dimensional Intensity Profile of Mock- and CLN-Treated PC12 Cells.



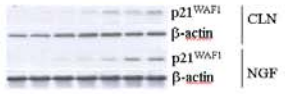
A. Mock-treated PC12 cells B. Colostrinin-induced morphological change.
*Fluorescent (495 nm) images of Hematoxylin-Eosin-stained PC12 cells

Fig. 4. Phosphorylation of p53 on Ser[15] in CLN-treated PC12 Cells Is Time-dependent.



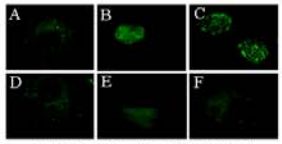
A, B. Western blot analysis of p53 in PC12 cells. Cells were incubated in the presence or absence of 100 ng/ml CLN or 50 ng/ml NGF, then cell lysates were made. Western blotting was performed with an anti-p53-Ser[15] antibody. β-actin was used as the internal control. C. Cytoplasmic staining of mock-treated PC12 cells. D. Nuclear staining of CLN-treated PC12 cells (24h) with DO1 p53 antibody. E. Staining pattern of p53 with DO1 antibody (96h) at a stage of cell differentiation in PC12 cells.

Fig. 5. Activation of p21^{WAF1} in CLN-treated PC12 Cells



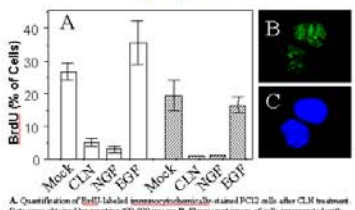
Western blot analysis of p21^{WAF1} in PC12 cells. Cells were incubated in the presence or absence of 100 ng/ml CLN or 50 ng/ml NGF, then cell lysates were made. Western blotting was performed with an anti-p21^{WAF1} antibody. β-actin was used as the internal control.

Fig. 6. Antisense, but Not Sense Oligonucleotides to p53 Inhibit Nuclear Accumulation of p53 in PC12 Cells.



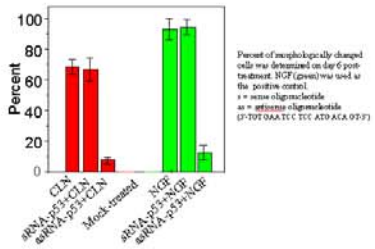
A. Mock-treated B. Sense-RNA and CLN-treated (100ng/ml)
C. Sense-RNA and NGF (50 ng/ml) D. Antisense-RNA treated only
E. Antisense-RNA treated plus CLN (100 ng/ml) F. Antisense-RNA treated plus EGF (50 ng/ml)-treated.

Fig. 7. Arrest of PC12 Cells in G1-Phase of the Cell Cycle by CLN.



A. Quantification of BrdU-labeled immunocytochemically-stained PC12 cells after CLN treatment. Data were obtained by counting 400-500 images B. Fluorescent image of cells incorporated with BrdU C. DAPI stained image of nuclei in B.

Fig. 8. Antisense, but not Sense Oligonucleotides to p53 Inhibit Neurite Outgrowth in PC12 Cells.



Percent of morphologically changed cells was determined on day 6 post-treatment. NGF (50ng/ml) was used as the positive control.
s = sense oligonucleotide
as = antisense oligonucleotide
(5'-TGT GAA TCC TCC ATG ACA GT-3')