Available online at http://jbcp.shahed.ac.ir/

Journal of Basic and Clinical
PATHOPHYSIOLOGY

Volume 4, Number 1, Autumn-Winter 2015-2016

Effects of hydrogen peroxide-induced oxidative stress on the pattern of pro-apoptotic and anti-apoptotic genes expression during PC12 cells differentiation

Mehrnaz Mesbah-Ardakani, Farshad Homayouni-Moghadam, Mahin Izadi, Mohammad Ebrahim Rezvani*

Shahid Sadoughi University, Yazd, Iran.

Article info
Received: 16 Oct 2016
Revised: 17 Feb 2016
Accepted: 24 Feb 2016

p-ISSN:2322-1895
e-ISSN: 2345-4334

ABSTRACT

Background and Objective: In neurodegenerative disorders, oxidative stress mediated by reactive oxygen species is strongly associated with increased neuronal damages that lead to apoptosis. Pro-apoptotic and anti-apoptotic gene expressions were changed during cell differentiation that affect cell viability and differentiation. This study was conducted to determine the effects of hydrogen peroxide-induced oxidative stress on apoptotic cell death in the differentiated rat pheochromocytoma (PC12) cells.

Materials and Methods: Semi-differentiated PC12 cells were treated with 400 µM hydrogen peroxide. Characteristic morphological changes as anapoptotic index were evaluated by DAPI staining. MTT assay were used to evaluate cells survival and activity. Pro-apoptotic and anti-apoptotic gene expression were estimated by real time-PCR.

Results: The obtained data indicate that PC12 cell survival rate decreases in H2O2-treated condition during differentiation. Also, H2O2 increases apoptotic genes expressions including caspase 6 and PIN1 and decreases anti-apoptotic genes including sirt1 and sirt7.

Conclusion: H2O2-induced oxidative stress can retard the differentiation of PC12 cell to neural-like cells through the apoptotic gene expression. On the other hand, despite the PIN1 acts as an apoptotic gene, this study illustrated that the expression of this gene is increased during differentiation under oxidative stress conditions.

Key Words:
Differentiation
PC12
PIN 1
Caspase 6
Sirt 1
Sirt 7
Apoptosis

1. Introduction

Oxidative stress has a key role in apoptosis of neural cells (1) and leads to cell damage in a variety of neurodegenerative diseases including Alzheimer's and Parkinson's diseases (2). Various types of chemical and physiological oxidative stress inducers are available that can be apoptotic cell death (1-3). Reactive oxygen species (ROS) such as hydrogen peroxide, superoxide anion and hydroxyl radicals are causing oxidative stress (2, 4). These mediators are produced in the normal metabolic and inappropriate processes and consume molecular oxygen. ROS can attack proteins, membrane lipids and deoxynucleic acid and cause theirs malfunction and entirety. ROS through apoptosis leading to cell death (2). Apoptosis or programmed cell death is a regulated processes that involve molecular agents leading to cell death (5). Hydrogen peroxide (H_2O_2) is produced during the Redox process and involved cellular signaling cascades(3).

***Corresponding Author:** *Mohammad Ebrahim Rezvani*
Shahid Sadoughi University, Yazd, Iran.
Email: erezvani@yahoo.com

Apoptosis induced by H_2O_2 is associated with changes in apoptosis and anti-apoptosis proteins (4). Caspase-6 is a member of the cysteine-aspartic acid protease family. Continuous activity of caspases plays a central role in apoptosis induction. Caspase-6 can break down proteins that involve in the chromatin condensation and nuclear shrinkage, leads to start of the apoptosis (6). PIN1 (Peptidyl-prolyl cis -Trans isomerase NIMA-interacting 1) is a member of the peptidyl-prolyl isomerase enzyme family.PIN1 being disabled by its residues phosphorylation. Oxidative stress can reduce the level of PIN1 phosphorylation in the neurons and increase their activity (7). PIN 1 protein can induce apoptosis by strengthening the expression of proapoptotic proteins such as P53. PIN1 augments P53-induced mitochondrial damage and induces apoptosis by releasing of cytochrome C through the mitochondria (8).Sirt1 (silent mating-type information regulation 2 homolog 1) works as nicotinamide adenine dinucleotide-dependent histone deacetylase. Sirtuins are a group of enzymes that lead to the displacement of their substrates between the nucleus and cytoplasm by theirs acetylation. Sirtuins family proteins have a key role in the physiological regulation including regulation of gene transcription, metabolism, growth, cancer, circadian rhythms and aging (9). Sirt1 deacetylates both histone and Non-histone proteins that are involved in cell growth, apoptosis, cell senescence and tumor production (10).Increased levels of Sirt1can protect the cells against reactive oxygen species -induced DNA damage and reduce apoptotic death in in vitro (11). Sirt7 is another member of the sirtuins family that is less studied. Sirt7 can decrease P53 (proapoptotic protein) activity and DNA damage. So, it causes resistance to apoptosis and improves cell survival under genomic stress conditions. Sirt7 deficiency induces apoptosis (12).The apoptotic effects of caspase 6 and pin1 and anti-apoptotic effects of sirt1 and sirt7 were reported in the literature, but role of these genes in the differentiation rate ofPC12 cells under oxidative stress has not been studied. Since PC12 cells considered being as neural progenitor and these genes play a crucial role in their differentiation and growth, we decided to evaluate their expression under oxidative stress conditions during early stage of differentiation of PC12 cells to neural like cells.

2. Materials and Methods

2.1. Materials

Rat pheochromocytoma cells (PC12) were purchased from Pasteur Institute of Iran (Tehran, Iran). The tetrazolium salt 3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyltetrazolium bromide dye (MTT-M2128) and dimethyl sulfoxide (DMSO) were provided from SigmaAldrich. Dulbecco’s modified Eagle’s medium (DMEM) was purchased from GIBCO (Grand Island, NY, USA).

2.2. PC12 cell culture

PC12 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin(100 U/ml) and streptomycin (10 µg /ml) in a humidified atmosphere of 95% air and 5% CO2 at 37°C. Medium was changed every other day. For the experiments, the cells were seeded in 96-well plate ($1*10^4$ cells /well) and were allowed to grow.

PC12 cells were cultured and treated with retinoic acid (1µl/ml) and free serum medium to differentiate morphologically to neural cells. After 5 days, the PC12 cells were converted to the new differentiated cells that were morphologically similar to neurons with some neurites. For induction of apoptosis, the medium was treated by 400 µm H_2O_2 (13).

2.3. Measurement of cell viability

MTT assay was used to determine cell viability. Briefly, 24 h after treatment, the medium was removed and replaced with the medium (150 µl/well) containing 1% FBS. Then, 10 µl of a 5 mg/ml MTT solution in PBS was added to each well. After 3 h at 37°C, the cell supernatants were discarded; The MTT crystals (formazan) generated by the mitochondrial dehydrogenase activity of live cells were dissolved in 200 µl/well dimethyl sulfoxide. The absorbency of the specimens was evaluated at a wave-length of 560 nm with 690 nm as a reference wavelength. The range of MTT alternation in H2O2 treated cells is represented as a percent of the control values (100%).

2.4. DAPI staining assay

To detect the nuclear fragmentation, the fluorescent dye, DAPI, was used to distinguish the apoptotic cells from PC12 cells. The differentiated PC12 cells were incubated at 37°C with 400 µM H2O2 for 24 h and then stained with the DNA-specific fluorochrome dye, DAPI (1µg/ml) for 20 min. After staining, the cells were washed with PBS and fixed with paraformaldehyde (4%). The plates were observed under an inverted fluorescence microscope (Nikon eclipse Ti-u Japan) and some photos were taken from different fields of the cells(4) (Figure 1).

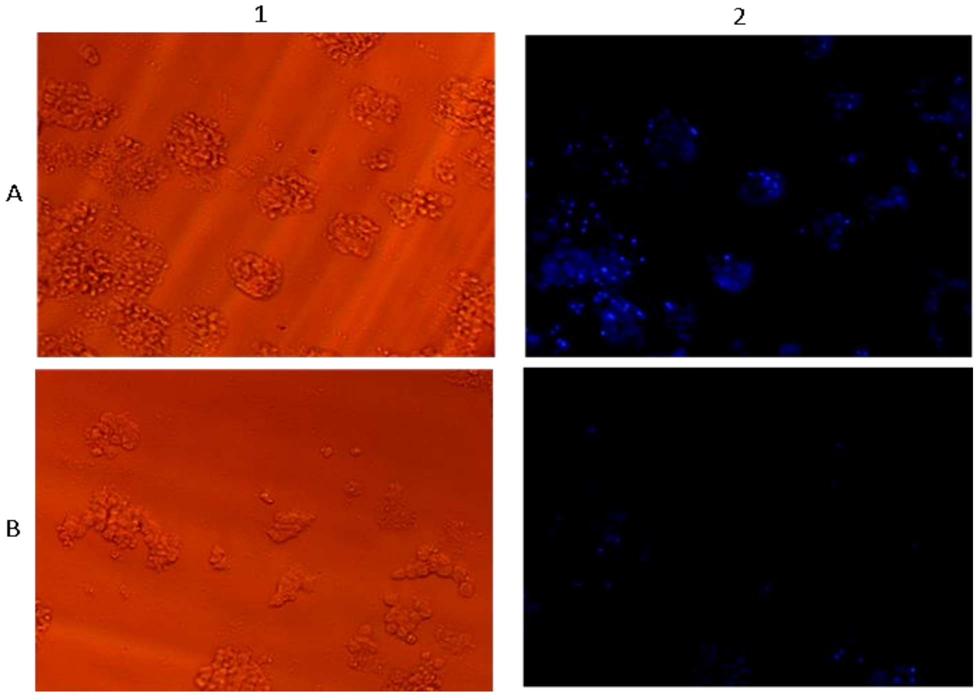

Figure 1. Apoptotic effect of H_2O_2 on morphology of differentiated PC12 cells. Chromatin condensation and nuclear fragmentation were analyzed by fluorescence microscopy utilizing the DNA binding fluorescent dye DAPI that illustrates apoptotic cells. (A-1) showed H_2O_2-treated PC12 cells by normal microscopy. (A-2) illustrated H_2O_2-treated PC12 cells by fluorescence microscopy. (B-1) indicated non-treated PC12 cells by normal microscopy. (B-2) showed non-treated PC12 cells by fluorescence microscopy

2.5. Real time PCR

The total RNA was extracted according to RNA purification kit (Jena Bioscience) directions. The extracted RNA was then reverse-transcribed into single stranded cDNA synthesis using MMLV Reverse Transcriptase and Oligo (dT)15 Primers according to the manufacturer's instructions (Vivantis) at 65 °C for 5 min, 25 °C for 10 min, 50 °C for 60 min, and 70 °C for 10 min and then samples were chilled on ice.

For gene expression analysis, relative quantitation PCR (qPCR) was performed using SYBR-Green-based protocols in Rotorgen (Qiagen) system and software (Qiagen, Australia). Primers were designed by using Allele ID software version 7.5 (Primer Digital Ltd). The studied genes were caspase 6, sirt1, sirt7, pin1 and β Actin, as a housekeeping gene). Toward the augmentation reactance, the qPCR SYBR Master Mix was used (Qiagen SYBR Green PCR Master Mix). The oligonucleotide primer sequences are provided in Table 1 (Primers were purchased from Takapo Zist Company, Tehran, Iran).

The qPCR conditions were set as follow: 10 min at 95 °C, followed by 35 cycles at 95 °C for 15 seconds, 52 °C for 1 min. The expression levels of the target genes in each sample were calculated by the comparative Ct method ($2^{-\Delta\Delta Ct}$ composition) since as in standardized to the Ct value of the βActin (housekeeping gene).

Table 1. The name of genes, their ID in Gen bank and their designed primer sequences

Gene	Primer sequences	size	Gene ID
cas6-F	5'- CACACATTTCCCTTCTACAC-3'	154	NM_001271984.1
cas6-R	5'- GATTTCTTTAGCCCTTTCCC-3'		
sir7-F	5'- GCAAAGCAGACACAATCC-3'	185	NM_001107073
sir7-R	5'- CCGCATTACATCATCACATT-3'		
sir1-F	5'- ATGAAGTATGACAAAGATGAAGT-3'	142	XM_006256146
sir1-R	5'- GTAGATGAGGCAGAGGTT-3'		
pin1-F	5'- CAGGAGAGGAGGACTTTG-3'	193	NM_001106701
pin1-R	5'- GTGCGTAGGATGATATGGA-3'		
Act b-F	5'- CTGTGCTATGTTGCCCTA-3'	103	NM_031144
Act b-R	5'- TAGTGATGACCTGACCGT-3'		

2.6. Statistical analysis

Data analysis among groups was performed using unpaired t student test using Prism 5 software. P-values less than 0.05 were statistically significant. Data were presented as mean± standard error of mean (SEM).

3. Results:

3.1. Cell viability

Our findings showed that the mean absorbance (560/690) in the H_2O_2-treated cells was significantly (17.9± 0.49) decreased as compared to non-treated cells (24.84±0.30). ($p<0.0001$) (figure 2-A).

3.2. Apoptosis in DAPI staining assay

DAPI staining was performed to estimate apoptotic rate. As shown in figure1, that nuclei from normal cells showed an approximately similar staining with no clues of chromatin condensation. In contrast, the H_2O_2-treated cells showed a staining pattern illustrating chromatin fragmentation. Our results demonstrated the mean percentage of apoptotic cells was remarkably increased in the H_2O_2-treated cells (70±3.077) as compared to non-treated cells (4±1.128) ($p<0.0001$) (Figure 2-B).

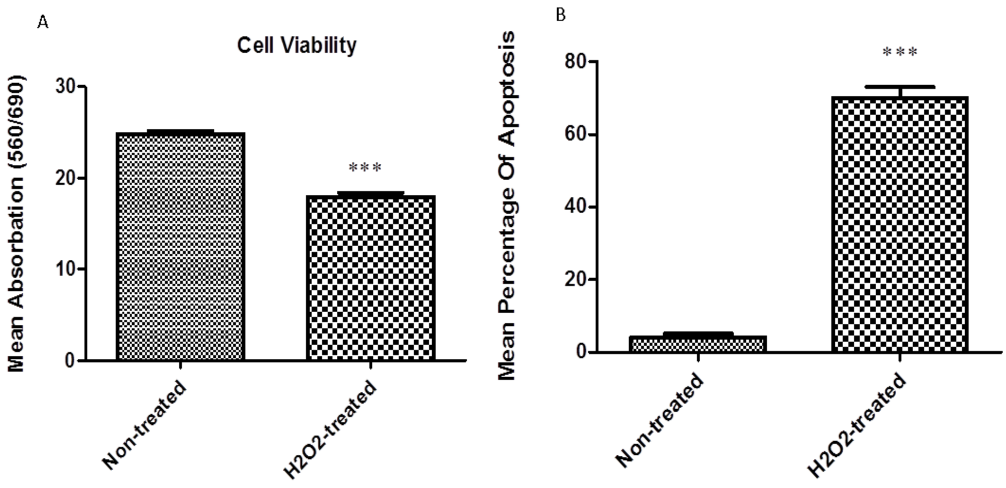

Figure 2. Quantification of MTT assay & DAPI staining: (A) MTT assay for cells viability after 24 h. (B) DAPI staining for indicating appoptotic cells rate after 24 h. *** $p<0.001$

3.3. Genes expression findings

Real time PCR assay showed that expression of sirt1 expression was significantly decreased in the H_2O_2-treated cells (0.0236±0.0011) compared with non-treated cells (1±0.057) ($p<0.001$) (Figure 3-A). Also, our data illustrated significant down expression of sirt7 in the H_2O_2-treated cells (0.00711±0.0007) compared with non-treated cells (1±0.057) ($p<0.001$) (Figure3-B). Figure 3-C showing that pin1 expression was exceptionally augmented in the H_2O_2-treated cells (9.694± 2.816) compared with non-treated cells (1±0.17) ($p<0.05$). It was also noted that caspase 6 was significantly increased in the H_2O_2-treated cells (1.806± 0.044) as compared with non-treated cells (1±0.057) ($p<0.001$) (Figure 3-D).

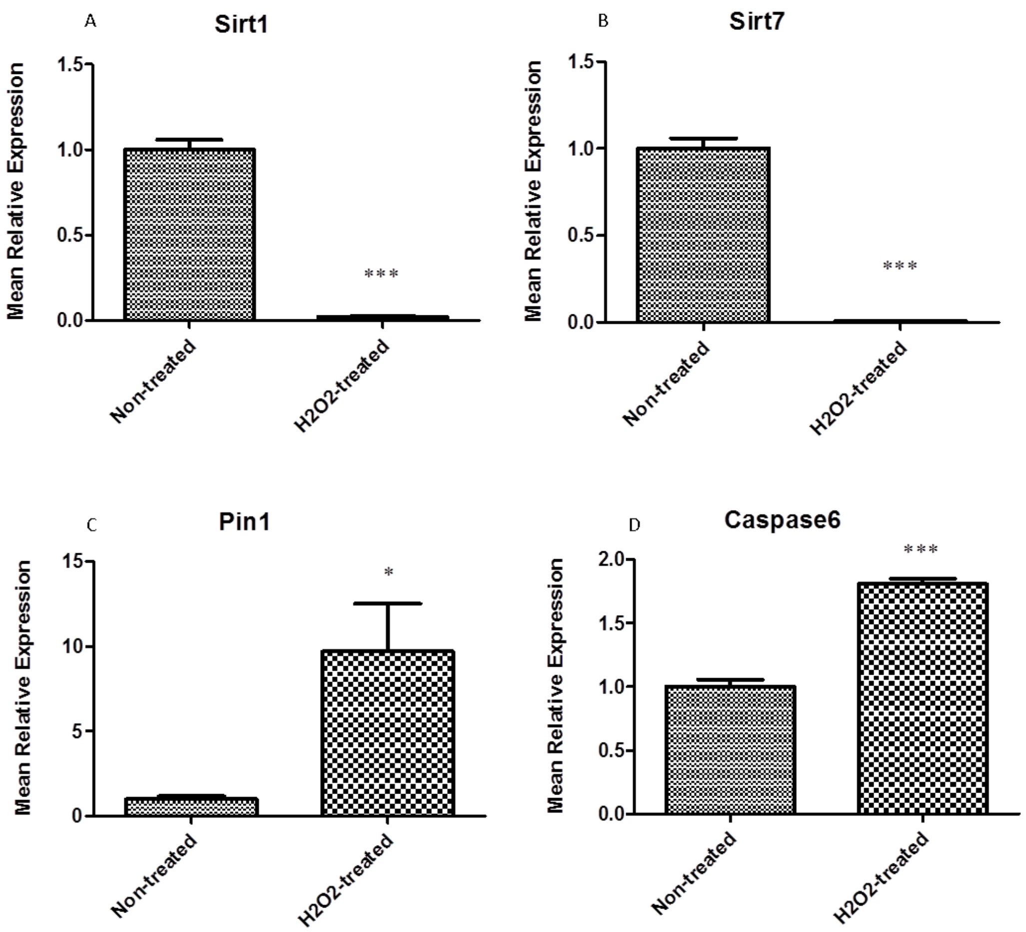

Figure 3. Gene expression in differentiated PC12 cells after 24 h. (A) Quantitative data for Sirt1 expression. (B) Quantitative data for Sirt7 expression. (C) Quantitative data for Pin1 expression. (D) Quantitative data for Caspase6 expression. * $p<0.05$, *** $p<0.001$

4. Discussion

In this study, the PC12 cells were cultured and underwent differentiation into neural-like cells in presence of RA. During ongoing differentiation, cells that were in the initial phases of transformation, received single oxidative stress by H_2O_2. Apoptosis induced changes included cell rounding, protrusions development (pseudopods), reduction of cell volume, concentration of chromatin (pyknosis), and nuclear fragmentation (karyorrhexis) (12). Previous studies have shown when PC12 cells exposed to H_2O_2-induced stress; some cells often show new morphology including plasma membrane changes. Also, DNA fragmentations have been reported following the stress of hydrogen peroxide in PC12 cells. At higher doses of H_2O_2, most cells showed necrotic cell death include signs of cell membrane perforation and the cell surface bubble (14). In our study, the results of DAPI staining confirmed whenPC12cells treated withH_2O_2 (400 μmol), the percentage of apoptotic cells was significantly increased in compared with the untreated cells. As well as the results of MTT assay showed that H_2O_2 can reduce the survival rate of the PC12 cells. These findings confirm the results of the related previous studies. Many studies on the effects of H_2O_2 on the expression of apoptotic and anti-apoptotic cell death pathway were conducted in PC12 cells and other cell lines. Shenglei Geandhis colleagues showed that H_2O_2 at concentrations ≥50 μm lead to increase in MnSOD (manganese superoxide dismutase) gene and apoptosis in the spiral ganglion cells (SGCs)(15). One study demonstrated that H_2O_2 at a dose of 120 μmol/L decrease sirt1 gene expression and protein, cell survival and increases expression of caspase3 in the pc12 cells (16).

Caspase 6 has been widely expressed in the brain and peripheral tissues. Apoptotic cell death is involved in neurodegenerative diseases including Huntington's, Alzheimer's, and Parkinson's diseases and stroke. The caspase 6 is emerged as a major player in the degeneration and death of nerve and is activated early in the disease process (17). Our results showed that H_2O_2 stress significantly increases caspase 6 gene expression in comparison with the untreated cells. PIN1 coordinates the activities of P53 family members and is involved to control the P53 accumulation and apoptotic function in the cells exposed to genotoxic stress (18). PIN1 is a key regulator of the P53-induced apoptotic in the neurodegenerative diseases. In neurons, PIN1 is located in the mitochondrial membrane and provides the possibility of its direct action on other metabolic and apoptotic regulators that are in the mitochondria (19). PIN1 strengthens P53-induced mitochondrial damage and initiates apoptosis by the release of cytochrome C from mitochondria (8). Our results showed that H_2O_2 significantly increased PIN 1 gene expression as compared to the untreated cells. Studies have shown that an increase in Sirt1 protects the cells against beta-amyloid-induced reactive oxygen species production and DNA damage and reduces apoptotic death in vitro conditions. One of the important proteins that are affected by Sirt1 is P53 that is proapoptotic protein. P53 deacetylation by Sirt1reduces P53 stability and inhibits apoptosis. So, it can improve cell survival (11).

Sirt1 in the stressful situations (such as metabolic and oxidative stress or hypoxia) is related with the pathology of many diseases including diabetes mellitus, cardiovascular diseases, neurodegenerative disorders and kidney disease. It inhibits the cells apoptosis and promotes the survival of cells in these diseases (20, 21). Our results showed that H_2O_2 significantly decreases Sirt1 gene expression. Sirt7 is a positive regulator of RNA polymerase I (pol1) and required for cell survival in mammals. Decreasing of Sirt7 or its catalytic activity inhibition leads to a reduction of pol1 and rDNA (Ribosomal DNA) relation and reducespol1 transcription. Reduction of Sirt7 stops cell proliferation and causes apoptosis (22). One study showed that lack of Sirt7 will be hyperacetylate P53invivo. The results demonstrated that Sirt7 causes resistance to doxorubicin-induced apoptosis. Finally, it was concluded that Sirt7 decreases DNA damage and P53 response and improves cell survival under genomic stress conditions (12). Our findings illustrated that H_2O_2 significantly decreases the expression of Sirt7. On the other hand, studies have shown that Sirt1 expresses in the cytoplasm in the cells PC12. Cytoplasmic Sirt1 improves NGF-induced neurite growth in the PC12 cells (23). Other studies have shown that Sirt1 is found in the cytoplasm of adult and fetus neural progenitor cells (NPC). Sirt1 as a factor enhancing growth could strengthen neuronal cell differentiation (24). Neural differentiation is reduced by inhibiting Sirt1 (25). A study showed that a decreased level of Pin1 suppresses neural differentiation in the neural progenitor cells (NPC) and overexpression of Pin1 has strengthened differentiation through catenin β (Inducer of NPC differentiation) activity.Pin1 levels increases in neuronal differentiation in vitro (26). NGF can lead to overexpression of Pin1 in the differentiated PC12 cells (27). Another study showed that after neuronal differentiation of the SY5Y cells with RA or NGF as well as the differentiation of NT2 cells into hNT nerve cells, has seen an increase in the levels of Pin1 (28). Our experimental results showed that under conditions of oxidative stress caused by hydrogen peroxide in PC12 cells that were in the first stage of differentiation, the Sirt1 gene expression has fallen sharply and almost is inhibited Moreover, the Pin1 gene expression is significantly increased. The results showed that Pin1 and Sirt1 both are involved in the differentiation and growth, but in terms of oxidative stress, Pin1 is more important to accelerate differentiation. So, it seems that Sirt1 normally acts as inducer of growth and differentiation, while Pin1 increases under the conditions of high oxidative stress in the cells and shows cell differentiation. Neural differentiation can be influenced by oxidative stress and hydrogen peroxide can generally reduce PC12 cell differentiation. In this study, we investigated apoptotic and anti-apoptotic genes that are involved at the early stages of PC12 cells differentiation into neuron-like cells. In the event that previous studies have shown that the role of genes in different stages of differentiation may vary in the apoptosis and growth.

Conclusion

The obtained data showed that H_2O_2 induces apoptosis in PC12 cells through increasing apoptotic genes such as PIN1 and caspase 6 and reducing anti-apoptotic genes such as Sirt1 and Sirt7. On the other hand, despite the PIN1 acts as an apoptotic gene, this study showed that the expression of this gene is increased during differentiation under oxidative stress conditions.

References

1. Cheng X-R, Zhang L, Hu J-J, Sun L, Du G-H. Neuroprotective effects of tetramethylpyrazine on hydrogen peroxide-induced apoptosis in PC12 cells. Cell biology international. 2007;31(5):438-43.

2. Hong H, Liu G-Q. Protection against hydrogen peroxide-induced cytotoxicity in PC12 cells by scutellarin. Life sciences. 2004;74(24):2959-73.

3. Jang J-H, Surh Y-J. Protective effects of resveratrol on hydrogen peroxide-induced apoptosis in rat pheochromocytoma (PC12) cells. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2001;496(1):181-90.

4. Cho ES, Lee KW, Lee HJ. Cocoa procyanidins protect PC12 cells from hydrogen-peroxide-induced apoptosis by inhibiting activation of p38 MAPK and JNK. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2008;640(1):123-30.

5. Jiang B, Liu J, Bao Y, An L. Catalpol inhibits apoptosis in hydrogen peroxide-induced PC12 cells by preventing cytochrome c release and inactivating of caspase cascade. Toxicon. 2004;43(1):53-9.

6. Zhang Y, Herman B. Ageing and apoptosis. Mechanisms of ageing and development. 2002;123(4):245-60.

7. Baik SH, Fane M, Park JH, Cheng YL, Yang-Wei Fann D, Yun UJ, et al. Pin1 promotes neuronal death in stroke by stabilizing Notch intracellular domain. Annals of neurology. 2015;77(3):504-16.

8. Polonio-Vallon T, Krüger D, Hofmann TG. ShaPINg cell fate upon DNA damage: role of Pin1 isomerase in DNA damage-induced cell death and repair. Frontiers in oncology. 2014;4.

9. Wang H, Li Q, Feng R, Wen T. Transcription levels of sirtuin family in neural stem cells and brain tissues of adult mice. Cell Mol Biol (Noisy-le-grand). 2012;58(Suppl.):OL1737-OL43.

10. Li C, Wang L, Zheng L, Zhan X, Xu B, Jiang J, et al. SIRT1 expression is associated with poor prognosis of lung adenocarcinoma. OncoTargets and therapy. 2015;8:977.

11. Gutierrez-Cuesta J, Tajes M, Jiménez A, Coto-Montes A, Camins A, Pallàs M. Evaluation of potential pro-survival pathways regulated by melatonin in a murine senescence model. Journal of pineal research. 2008;45(4):497-505.

12. Kiran S, Oddi V, Ramakrishna G. Sirtuin 7 promotes cellular survival following genomic stress by attenuation of DNA damage, SAPK activation and p53 response. Experimental cell research. 2015;331(1):123-41.

13. Zhang H, Mak S, Cui W, Li W, Han R, Hu S, et al. Tacrine (2)–ferulic acid, a novel multifunctional dimer, attenuates 6-hydroxydopamine-induced apoptosis in PC12 cells by activating Akt pathway. Neurochemistry international. 2011;59(7):981-8.

14. Kamata H, Tanaka C, Yagisawa H, Hirata H. Nerve growth factor and forskolin prevent H 2 O 2-induced apoptosis in PC12 cells by glutathione independent mechanism. Neuroscience letters. 1996;212(3):179-82.

15. Xie D, Liu G, Zhu G, Wu W, Ge S. (-)-Epigallocatechin-3-gallate protects cultured spiral ganglion cells from H2O2-induced oxidizing damage. Acta oto-laryngologica. 2004;124(4):464-70.

16. Li T, Li Z, Zhuang X, Fu Y. [Effects of caloric restriction on the oxidative stress injury and the expression of SIRT3 in PC12 cell]. Zhonghua yi xue za zhi. 2011;91(5):350-8.

17. Graham RK, Ehrnhoefer DE, Hayden MR. Caspase-6 and neurodegeneration. Trends in neurosciences. 2011;34(12):646-56.

18. Shen Z-J, Malter JS. Determinants of eosinophil survival and apoptotic cell death. Apoptosis. 2015;20(2):224-34.

19. Sorrentino G, Comel A, Mantovani F, Del Sal G. Regulation of mitochondrial apoptosis by Pin1 in cancer and neurodegeneration. Mitochondrion. 2014;19:88-96.

20. Kitada M, Kume S, Takeda-Watanabe A, Kanasaki K, Koya D. Sirtuins and renal diseases: relationship with aging and diabetic nephropathy. Clinical science. 2013;124(3):153-64.

21. Polak-Jonkisz D, Laszki-Szcząchor K, Rehan L, Pilecki W, Filipowski H, Sobieszczańska M. Nephroprotective action of sirtuin 1 (SIRT1). Journal of physiology and biochemistry. 2013;69(4):957-61.

22. Ford E, Voit R, Liszt G, Magin C, Grummt I, Guarente L. Mammalian Sir2 homolog SIRT7 is an activator of RNA polymerase I transcription. Genes & development. 2006;20(9):1075-80.

23. Sugino T, Maruyama M, Tanno M, Kuno A, Houkin K, Horio Y. Protein deacetylase SIRT1 in the cytoplasm promotes nerve growth factor-induced neurite outgrowth in PC12 cells. FEBS letters. 2010;584(13):2821-6.

24. Guo W, Qian L, Zhang J, Zhang W, Morrison A, Hayes P, et al. Sirt1 overexpression in neurons promotes neurite outgrowth and cell survival through inhibition of the mTOR signaling. Journal of neuroscience research. 2011;89(11):1723-36.

25. Hisahara S, Chiba S, Matsumoto H, Tanno M, Yagi H, Shimohama S, et al. Histone deacetylase SIRT1 modulates neuronal differentiation by its nuclear translocation. Proceedings of the National Academy of Sciences. 2008;105(40):15599-604.

26. Nakamura K, Kosugi I, Lee DY, Hafner A, Sinclair DA, Ryo A, et al. Prolyl isomerase Pin1 regulates neuronal differentiation via β-catenin. Molecular and cellular biology. 2012;32(15):2966-78.

27. Buschdorf JP, Chew LL, Soh U, Liou Y-C, Low BC. Nerve growth factor stimulates interaction of Cayman ataxia protein BNIP-H/Caytaxin with peptidyl-prolyl isomerase Pin1 in differentiating neurons. PloS one. 2008;3(7):e2686.

28. Hamdane M, Dourlen P, Bretteville A, Sambo A-V, Ferreira S, Ando K, et al. Pin1 allows for differential Tau dephosphorylation in neuronal cells. Molecular and Cellular Neuroscience. 2006;32(1):155-60.