DOI: 10.22055/IVJ.2021.253970.2323

Mutation of p53 as a tumor suppressor gene in lung fibroblast cells exposed to nano-alumina and zinc oxide nanoparticles

Zeinab Jalilian[1], Jamileh Salar-Amoli[2*], Fatemeh Jalousian[3], Tahereh Ali-Esfahani[4], Ali Bashiri Dezfouli[5] and Abbas Barin[6]

[1] MSc Graduated of Toxicology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[2] Professor, Department of Comparative Biosciences, Toxicology and Animal Poisoning Research Center, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[3] Assistant Professor, Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[4] PhD Student of Animal and Poultry Health and Nutrition, Toxicology and Animal Poisoning Research Center, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[5] PhD Graduated of Toxicology, Toxicology and Animal Poisoning Research Center, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[6] Assistant Professor, Department of Clinical Pathobiology, Toxicology and Animal Poisoning Research Center, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

Received: 21.11.2020 Accepted: 23.04.2021

Abstract

An increase in the broad usage of metal oxide nanoparticles in biological applications may have novel interactions with biological systems and result in emerging health problems. In this study, the effect of aluminum oxide (Al2O3, 35-45 nm) and zinc oxide (ZnO, 30 nm) nanoparticles (NPs) on the mutation of codon 248 of the p53 gene, a key gene in the tumor cell suppression, was conducted in the cellular growth medium. After 72 hours of exposure to the mentioned NPs (5, 10, 25, 50 µg/ml), lung fibroblast MRC-5 cells were evaluated through MTT assay for cytotoxicity and subsequent PCR and sequencing analysis for in vitro genotoxicity assessment. After zinc oxide nanoparticle (ZnO-NPs) treatment, cells underwent substantial cytotoxicity, and these toxicities were significant at doses of 25 and 50 μg/mL. Regarding aluminum oxide nanoparticles (nano-alumina, Al2O3-NPs), a concentration of 50 μg/mL affected the viability of MRC-5 cells. There was no significant difference in other treated groups compared to the control. Interestingly, the mutation in the 248 codons of the P53 gene was observed following 72 hours incubation of MRC-5 cells with 5 μg/mL of Al2O3-NPs. This mutation occurred in the form of the CGG to CCG, transforming the arginine codon into proline generators. The mutation in codon 248 p53 (replacement cytosine instead of guanine) will result in non-functional P53 protein production. Hence, following the modulation of p53 in lung cells, the possibility of cancer emerging will be increased. Moreover, determining the nanoparticles' accurate cytotoxic concentration is of great importance to reduce deleterious effects on the body's normal cells.

Key words: Aluminum oxide, Mutation, p53, Lung fibroblast MRC-5 cells, Zinc oxide

[*] **Corresponding Author**: Jamileh Salar-Amoli, Professor, Department of Basic Sciences, Toxicology and Animal Poisoning Research Center, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
E-mail: jsalar@ut.ac.ir

Refrences

Bai, D.-P., Zhang, X.-F., Zhang, G.-L., Huang, Y.-F., & Gurunathan, S. (2017). Zinc oxide nanoparticles induce apoptosis and autophagy in human ovarian cancer cells. *International journal of nanomedicine, 12*, 6521.

Bai, L., & Zhu, W.-G. (2006). p53: structure, function and therapeutic applications. *J Cancer Mol, 2*(4), 141-153.

Balasubramanyam, A., Sailaja, N., Mahboob, M., Rahman, M., Hussain, S. M., & Grover, P. (2009). In vivo genotoxicity assessment of aluminium oxide nanomaterials in rat peripheral blood cells using the comet assay and micronucleus test. *Mutagenesis, 24*(3), 245-251.

Barik, B. K., & Mishra, M. (2019). Nanoparticles as a potential teratogen: a lesson learnt from fruit fly. *Nanotoxicology, 13*(2), 258-284.

Bashiri Dezfouli, A., Salar-Amoli, J., Yazdi, M., Ali-Esfahani, T., & Barin, A (2017). Evaluation of the Antioxidant Activities and Cytotoxicities of Selected Medicinal Herbs Using Human Hepatoma Cell Line (Hepg2). *Iranian Journal of Toxicology, 11*(6), 13-20.

Bashiri Dezfouli, A., Salar-Amoli, J., Pourfathollah, A. A., Yazdi, M., Nikougoftar-Zarif, M., Khosravi, M., & Hassan, J. (2020). Doxorubicin-induced senescence through NF-κB affected by the age of mouse mesenchymal stem cells. *Journal of cellular physiology, 235*(3), 2336-2349.

Bergh, J. (1999). Clinical studies of p53 in treatment and benefit of breast cancer patients. *Endocrine-related cancer, 6*(1), 51-59.

Brown, C. J., Lain, S., Verma, C. S., Fersht, A. R., & Lane, D. P. (2009). Awakening guardian angels: drugging the p53 pathway. *Nature Reviews Cancer, 9*(12), 862-873.

Buhrmann, C., Yazdi, M., Dezfouli, A. B., Sahraneshin, F. S., Ebrahimi, S. M., Ghaffari, S. H., et al. (2020). Significant decrease in the viability and tumor stem cell marker expression in tumor cell lines treated with curcumin. *Journal of Herbal Medicine*, 100339.

Buzea, C., Pacheco, I. I., & Robbie, K. (2007). Nanomaterials and nanoparticles: sources and toxicity. *Biointerphases, 2*(4), MR17-MR71.

Demir, E., Burgucu, D., Turna, F., Aksakal, S., & Kaya, B. (2013). Determination of TiO2, ZrO2, and Al2O3 nanoparticles on genotoxic responses in human peripheral blood lymphocytes and cultured embyronic kidney cells. *Journal of Toxicology and Environmental Health, Part A, 76*(16), 990-1002.

Dey, S., Bakthavatchalu, V., Tseng, M. T., Wu, P., Florence, R. L., Grulke, E. A., et al. (2008). Interactions between SIRT1 and AP-1 reveal a mechanistic insight into the growth promoting properties of alumina (Al 2 O 3) nanoparticles in mouse skin epithelial cells. *Carcinogenesis, 29*(10), 1920-1929.

Dezfouli, A. B., Pourfathollah, A. A., Salar-Amoli, J., Khosravi, M., Nikogoftar-Zarif, M., Yazdi, M., & Ali-Esfahani, T. (2017). Evaluation of age effects on doxorubicin-induced toxicity in mesenchymal stem cells. *Medical journal of the Islamic Republic of Iran, 31*, 98.

Dixon, K., & Kopras, E. (2004). *Genetic alterations and DNA repair in human carcinogenesis.* Paper presented at the Seminars in cancer biology.

Friedler, A., Veprintsev, D. B., Freund, S. M., Karoly, I., & Fersht, A. R. (2005). Modulation of binding of DNA to the C-terminal domain of p53 by acetylation. *Structure, 13*(4), 629-636.

Ghosh, M., Sinha, S., Jothiramajayam, M., Jana, A., Nag, A., & Mukherjee, A. (2016). Cyto-genotoxicity and oxidative stress induced by zinc oxide nanoparticle in human lymphocyte cells in vitro and Swiss albino male mice in vivo. *Food and Chemical Toxicology, 97*, 286-296.

Hainaut, P., Hernandez, T., Robinson, A., Rodriguez-Tome, P., Flores, T., Hollstein, M., et al. (1998). IARC Database of p53 gene mutations in human tumors and cell lines: updated compilation, revised formats and new visualisation tools. *Nucleic acids research, 26*(1), 205-213.

Ickrath, P., Wagner, M., Scherzad, A., Gehrke, T., Burghartz, M., Hagen, R. et al. (2017). Time-dependent toxic and genotoxic effects of zinc oxide nanoparticles after long-term and repetitive exposure to human mesenchymal stem cells. *International journal of environmental research and public health, 14*(12), 1590.

Jeng, H. A., & Swanson, J. (2006). Toxicity of metal oxide nanoparticles in mammalian cells. *Journal of Environmental Science and Health Part A, 41*(12), 2699-2711.

Keller, A. A., McFerran, S., Lazareva, A., & Suh, S. (2013). Global life cycle releases of engineered nanomaterials. *Journal of nanoparticle research, 15*(6), 1692.

Lewis, P., & Parry, J. (2004). In silico p53 mutation hotspots in lung cancer. *Carcinogenesis, 25*(7), 1099-1107.

Li, Z., Guo, D., Yin, X., Ding, S., Shen, M., Zhang, R. et al. (2020). Zinc oxide nanoparticles induce human multiple myeloma cell death via reactive oxygen species and Cyt-C/Apaf-1/Caspase-9/Caspase-3 signaling pathway in vitro. *Biomedicine & Pharmacotherapy, 122*, 109712.

Madapura, H. S., Salamon, D., Wiman, K. G., Lain, S., Klein, G., Klein, E., & Nagy, N. (2012). p53 contributes to T cell homeostasis through the induction of pro-apoptotic SAP. *Cell cycle, 11*(24), 4563-4569.

Manke, A., Wang, L., & Rojanasakul, Y. (2013). Mechanisms of nanoparticle-induced oxidative stress and toxicity. *BioMed research international, 2013: 942916*.

McCormick, F. (2001). Cancer gene therapy: fringe or cutting edge? *Nature Reviews Cancer, 1*(2), 130-141.

Meulmeester, E., & Jochemsen, A. G. (2008). p53: a guide to apoptosis. *Current cancer drug targets, 8*(2), 87-97.

Nikolova, P. V., Wong, K. B., DeDecker, B., Henckel, J., & Fersht, A. R. (2000). Mechanism of rescue of common p53 cancer mutations by second-site suppressor mutations. *The EMBO Journal, 19*(3), 370-378.

Parveen, S., Misra, R., & Sahoo, S. K. (2012). Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging. *Nanomedicine: Nanotechnology, Biology and Medicine, 8*(2), 147-166.

Paskulin, D., Cunha-Filho, J., Souza, C., Bortolini, M., Hainaut, P., & Ashton-Prolla, P. (2012). TP53 PIN3 and PEX4 polymorphisms and infertility associated with endometriosis or with post-in vitro fertilization implantation failure. *Cell death & disease, 3*(9), e392-e392.

Petitjean, A., Mathe, E., Kato, S., Ishioka, C., Tavtigian, S. V., Hainaut, P., & Olivier, M. (2007). Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. *Human mutation, 28*(6), 622-629.

Powell, B., Soong, R., Iacopetta, B., Seshadri, R., & Smith, D. R. (2000). Prognostic significance of mutations to different structural and functional regions of the p53 gene in breast cancer. *Clinical cancer research, 6*(2), 443-451.

Radziun, E., Wilczyńska, J. D., Książek, I., Nowak, K., Anuszewska, E., Kunicki, A. et al. (2011). Assessment of the cytotoxicity of aluminium oxide nanoparticles on selected mammalian cells. *Toxicology in vitro, 25*(8), 1694-1700.

Sengul, A. B., & Asmatulu, E. (2020). Toxicity of metal and metal oxide nanoparticles: a review. *Environmental Chemistry Letters*, 1-25.

Sliwinska, A., Kwiatkowski, D., Czarny, P., Milczarek, J., Toma, M., Korycinska, A. et al. (2015). Genotoxicity and cytotoxicity of ZnO and Al2O3 nanoparticles. *Toxicology mechanisms and methods, 25*(3), 176-183.

Taheri, S., Banaee, M., Haghi, B. N., & Mohiseni, M. (2017). Effects of dietary supplementation of zinc oxide nanoparticles on some biochemical biomarkers in common carp (Cyprinus carpio). *International Journal of Aquatic Biology, 5*(5), 286-294.

Yang, D., Zhang, M., Gan, Y., Yang, S., Wang, J., Yu, M. et al. (2020). Involvement of oxidative stress in ZnO NPs-induced apoptosis and autophagy of mouse GC-1 spg cells. *Ecotoxicology and Environmental Safety*, *202*, 110960.

Zhang, L., Gu, F., Chan, J., Wang, A., Langer, R., & Farokhzad, O. (2008). Nanoparticles in medicine: therapeutic applications and developments. *Clinical pharmacology & therapeutics*, *83*(5), 761-769.