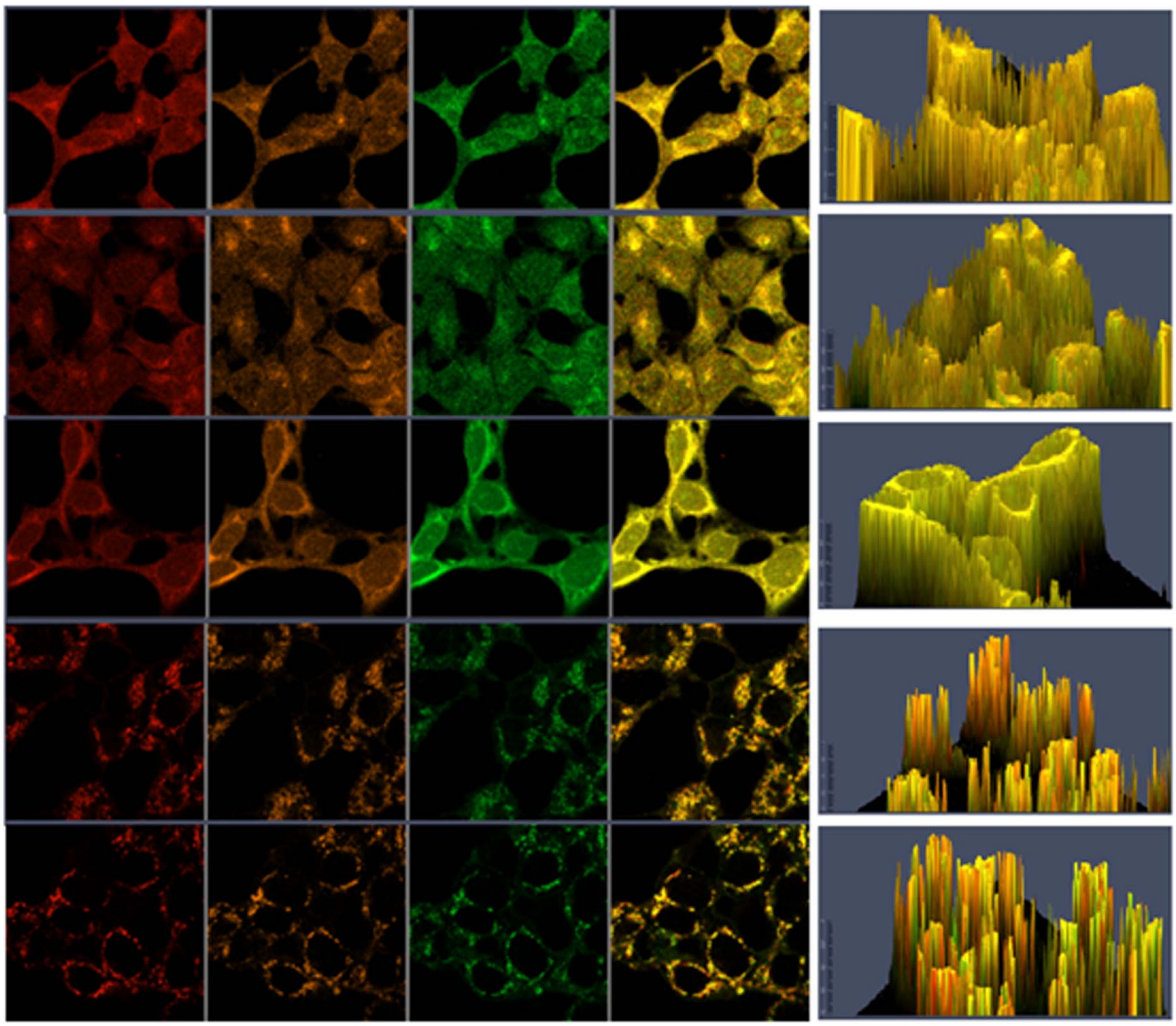
E-ISSN: 2149-7222 (Online)

Journal Cellular Neuroscience and Oxidative Stress



OPEN ACCESS and NO PUBLICATION FEE

http://dergipark.org.tr/jcnos

Former name; Cell Membranes and Free Radical Research

Volume 15, Number 3, 2023

Journal of Cellular Neuroscience and Oxidative Stress

http://dergipark.gov.tr/jcnos

BSN Health Analyses, Innovation, Consultancy, Organization, Industry and Trade Limited Company

http://www.bsnsaglik.com.tr/

info@bsnsaglik.com.tr

Formerly known as:

Cell Membranes and Free Radical Research (2008 - 2014)

Volume 15, Number 3, 2023

[CONTENTS]

Volume 15, Number 3, 2023
E-ISSN Number: 2149-7222 (Online)
Indexing: Scopus (Elsevier), CAS (Chemical Abstracts Service), Citation Index Database, EBSCOhost Research Database, Google Scholar, Index Copernicus,

AIM AND SCOPES
Journal of Cellular Neuroscience and Oxidative Stress is an online journal that publishes original research articles, reviews and short reviews on the molecular basis of biophysical, physiological and pharmacological processes that regulate cellular function, and the control or alteration of these processes by the action of receptors, neurotransmitters, second messengers, cation, anions, drugs or disease.

Areas of particular interest are four topics. They are;

A- Ion Channels (Na^+- K^+ Channels, Cl^- channels, Ca^{2+} channels, ADP-Ribose and metabolism of NAD^+, Patch-Clamp applications)

B- Oxidative Stress (Antioxidant vitamins, antioxidant enzymes, metabolism of nitric oxide, oxidative stress, biophysics, biochemistry and physiology of free oxygen radicals)

C- Interaction Between Oxidative Stress and Ion Channels in Neuroscience
(Effects of the oxidative stress on the activation of the voltage sensitive cation channels, effect of ADP-Ribose and NAD^+ on activation of the cation channels which are sensitive to voltage, effect of the oxidative stress on activation of the TRP channels in neurodegenerative diseases such Parkinson's and Alzheimer's diseases)

D- Gene and Oxidative Stress
(Gene abnormalities. Interaction between gene and free radicals. Gene anomalies and iron. Role of radiation and cancer on gene polymorphism)

READERSHIP

Biophysics	Biochemistry
Biology	Biomedical Engineering
Pharmacology	PhysiologyGenetics
Cardiology	Neurology
Oncology	Psychiatry
Neuroscience	Neuropharmacology

Keywords
Ion channels, cell biochemistry, biophysics, calcium signaling, cellular function, cellular physiology, metabolism, apoptosis, lipid peroxidation, nitric oxide, ageing, antioxidants, neuropathy, traumatic brain injury, pain, spinal cord injury, Alzheimer's Disease, Parkinson's Disease.

SHORT COMMUNICATION

J Cell Neurosci Oxid Stress 2023;15(3): 1157-1161

miR-140-5p regulates the hypoxia-mediated oxidative stress through Nrf2

Megharani MAHAJAN[1,2*] and Sandhya SITASAWAD[1*]

[1]*Redox Biology Laboratory, National Centre for Cell Science (NCCS), Pune 411007, India*
[2]*Department of Medicine, Hematology & Oncology, UT Health Science Center San Antonio, San Antonio, TX, USA*

Received: 22 August 2023; **Accepted:** 2 November 2023

***Address for correspondence:**

Dr. Megharani Mahajan
Department of Medicine, Hematology & Oncology,
UT Health Science Center San Antonio,
San Antonio, TX, USA – 78229
E-mail: *mahajanm@uthscsa.edu*
ORCID ID*: 0000-0002-6259-5754*

Dr. Sandhya Sitasawad
National Centre for Cell Science,
NCCS Complex, Savitribai Phule Pune University Campus,
Ganeshkhind Road, Pune - 411 007,
Maharashtra, India
E-mail: *ssitaswad@nccs.res.in*

List of Abbreviations;

ARE, *Antioxidant response element;* ***BC,*** *Breast cancer;* ***DCFH-DA,*** *Dichloro-dihydro-fluorescein diacetate;* ***DHE,*** *Dihydroethidium;* ***Keap1,*** *Kelch-like ECH-associated protein 1;* ***miRNA,*** *microRNA;* ***Nrf2,*** *Nuclear factor-erythroid 2-related factor 2;* ***ROS,*** *Reactive oxygen species;* ***SOD,*** *Superoxide dismutase.*

Abstract

Rapid and uncontrollable cell proliferation, altered metabolism, and abnormal vasculature of cancer cells make them hypoxic and result in the generation of reactive oxygen species (ROS), causing oxidative stress. Hypoxia-mediated oxidative stress represents a significant barrier to effective cancer treatment. miRNAs are emerging as a potential regulator of hypoxia-responsive genes and hypoxia-mediated oxidative stress. Based on the role of miR-140-5p in regulating a hypoxia-responsive gene, this study is aimed at understanding the miR-140-5p role in regulating hypoxia-mediated oxidative stress under breast tumor hypoxia. We found that the miR-140-5p might control the hypoxia-mediated ROS generation by regulating the Nrf2 expression. Knowing the significance of miR-140-5p in regulating hypoxia-mediated oxidative stress and breast tumor progression, targeting miR-140-5p might represent a promising strategy for anti-breast cancer therapy.

Keywords: miR-140-5p, Nrf2, Hypoxia, Breast Cancer, Reactive Oxygen Species (ROS)

Introduction

Oxidative stress is an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defense system in the body. Superoxide (O_2•−), hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and singlet oxygen ($^1O_2$) are known as ROS (Sato et al. 2013; Navarro-Yepes et al. 2014). ROS is a byproduct of aerobic metabolism, which possesses a strong oxidizing ability. Every cell in the human body is exposed to ~1.5×10^5 oxidative hits per day (Perillo et al. 2020). At low concentrations, ROS functions as a secondary messenger maintaining cellular homeostasis while they are deleterious to cells at a higher concentration by causing damage to DNA, lipids, and proteins (Thannickal et al. 2000). Cancer cells are metabolically active and hypoxic, and thus, tend to produce more ROS (Vera-Ramirez et al. 2012). These ROS can activate the prosurvival pathway, including the PI3K/MAPK pathway, thus contributing to tumor progression (Brahimi-Horn et al. 2007). Therefore, regulating oxidative stress has been an important strategy for cancer prevention (Arfin et al. 2021). Cells have a special defense system called 'antioxidants' controlled by Nuclear factor-erythroid 2-related factor 2 (Nrf2), a master antioxidant regulator of the cellular response to oxidative stress.

Nrf2 is an antioxidant transcription factor that binds to and mediates the expression of antioxidant response element (ARE) containing genes. Nrf2 is complexed with Kelch-like ECH-associated protein 1 (Keap1) in the cytoplasm under normal cellular conditions, maintaining Nrf2 at a low level. However, in response to various stresses, Nrf2 is de-repressed and induces the expression of ARE-containing genes (e.g., HO1, NQO1, γ-GCS, and GST). The expression of Nrf2-dependent antioxidant genes is essential for maintaining cellular redox homeostasis by decreasing oxidative stress and protecting from many diseases. However, Nrf2 and its downstream genes are overexpressed in various cancers, including breast cancer (BC), giving cancer cells survival and growth advantage (Homma et al. 2009). Activated Nrf2 in cancer cells has been shown to promote angiogenesis (Kim et al. 2011; Ji et al. 2014), metastasis (Shen et al. 2014; Arfmann-Knübel et al. 2015), radioresistance (Singh et al. 2010), chemoresistance (Shibata et al. 2008), and thereby contribute to tumor progression. Given the importance of Nrf2 in tumor cell response to low oxygen levels by regulating the coordinated expression of antioxidant genes and tumor-promoting genes involved in angiogenesis, invasion and metastasis, and therapeutic resistance; a better understanding of the regulation of Nrf2 is necessary to inhibit tumor progression.

Apart from the traditional transcriptional genes, non-transcriptional RNAs, especially microRNAs (miRNAs), have increasingly been shown to play an important role in different cancers, including BC. Because of their short size, stability, ability to respond rapidly to a variety of stresses, and their ability to target multiple genes and pathways simultaneously, miRNAs are emerging as promising players in cancer therapeutics.

Our previous study showed the miRNA-mediated regulation of Nrf2 in BC cells under hypoxia and confirmed miR-140-5p as a potential regulator of Nrf2. miR-140-5p is significantly downregulated in BC cells under hypoxia and thereby contributes to Nrf2-mediated breast tumor angiogenesis and metastasis (Mahajan et al. 2021). Given the importance of miR-140-5p in regulating the Nrf2, a master antioxidant regulator in a cell, raised an important consideration, in particular the role of miR-140-5p in regulating hypoxia-mediated oxidative stress. Therefore, the current study is aimed at understanding the role of miR-140-5p in regulating hypoxia-mediated oxidative stress.

Materials and Methods

Cell lines used and generation of stable Cell line

Maintenance of cell lines (MCF-7 and MDA-MB-231) used in the study including the generation of miR-140-5p overexpressing and knockout stable cell lines was performed as described previously (Mahajan et al. 2021).

Measurement of ROS

The generation of superoxide and hydrogen peroxide was measured using Dihydroethidium (DHE) and Dichloro-dihydro-fluorescein diacetate (DCFH-DA) by flow cytometry. Briefly, cells were trypsinized and washed with chilled PBS (2000 rpm for 5min at 4°C). Cells were then stained with DHE (10μM) and DCFH-DA (5μmol/l) for 30 min at 37 °C in the dark. Cells were then washed twice with 1xPBS. The fluorescent signals were detected by BD FACS CantoTM flow cytometer and analyzed using DIVA software.

Statistical Analysis

Data were expressed as the mean ± standard deviation (SD). The results were from at least three independent experiments. Statistical comparisons were made between two groups with the t-test and between multiple groups by ANOVA. Prism software (GraphPad, San Diego, CA, USA), was used to analyze statistical significance. A value of $p<0.05$ was considered statistically significant.

Results and Discussion

Tumor hypoxia induces the generation of ROS

To address this, we measured the generation of superoxide and hydrogen peroxide in BC cell lines under hypoxic conditions by flow cytometry using specific fluorescent dyes. As shown in Fig.1, exposure to a hypoxic condition significantly increases the generation of superoxide and hydrogen peroxide species in both MCF-7 (Fig.1A-B) and MDA-MB-231 (Fig.1C-D) cells as determined by increased oxidation of DHE and DCFH-DA. Our results are in agreement with the previous study in different cancers including BC showing the hypoxia-mediated increase in oxidative stress (Joshi et al. 2016; Williams et al. 2001). Collectively, these results confirm the role of hypoxia in increased oxidative stress in BC cell lines.

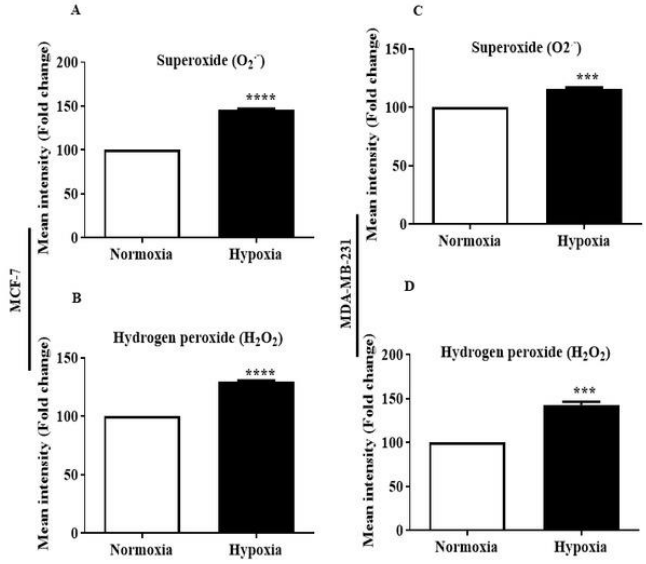


Fig. 1. Tumor hypoxia induces the generation of ROS. *MCF-7 and MDA-MB-231 cells were exposed to either normoxia or hypoxia and then stained with DHE and DCFH-DA. The generation of superoxide (A) and hydrogen peroxide (B) in MCF-7 cells. The generation of superoxide (C) and hydrogen peroxide (D) in MDA-MB-231 cells. Results were represented as mean fluorescence intensity. Error bars represent mean ± SEM (n = 3). *** p < 0.001, and ****p<0.0001 compared to EV.*

miR-140-5p regulates the generation of hypoxia-mediated ROS through Nrf2

To address the role of miR-140-5p in regulating the hypoxia-mediated oxidative stress we used the MDA-MB-231 cell line and miR-140-5p expression was inhibited (miR-140-5p-KD) under normoxia or overexpressed (miR-140-5p-OE) under hypoxia (Mahajan et al. 2021) and measured the generation of superoxide and hydrogen peroxide in these cells through flow cytometry. As shown in Fig.2, the knockdown of miR-140-5p under normoxia significantly reduced the generation of both superoxide and hydrogen peroxide levels (Fig.2A-D). In contrast, overexpression of miR-140-5p under hypoxia reduced superoxide levels while increasing the generation of hydrogen peroxide (Fig. 2E-H). The decrease in superoxide generation in miR-140-5p-OE cells under hypoxia might be because of the conversion of superoxide to hydrogen peroxide and molecular oxygen by superoxide dismutase (SOD) (Wang et al. 2018). These results together with our previous study (Mahajan et al. 2021) indicate that miR-140-5p might control the hypoxia-mediated ROS generation by regulating the Nrf2 expression. In line with our results, a study by Liu et al. showed that increased miR-140-5p elevated ROS levels, causing oxidative stress by Nrf2/Sirt2/Keap1/HO-1 pathway in mice with atherosclerosis (Liu et al. 2019). As miRNAs regulate oxidative stress and various genes involved in it, oxidative stress also affects expression levels of various miRNAs (Konovalova et al. 2019). However, the role of oxidative stress in the regulation of miR-140-5p expression in breast tumor hypoxia needs further investigation.

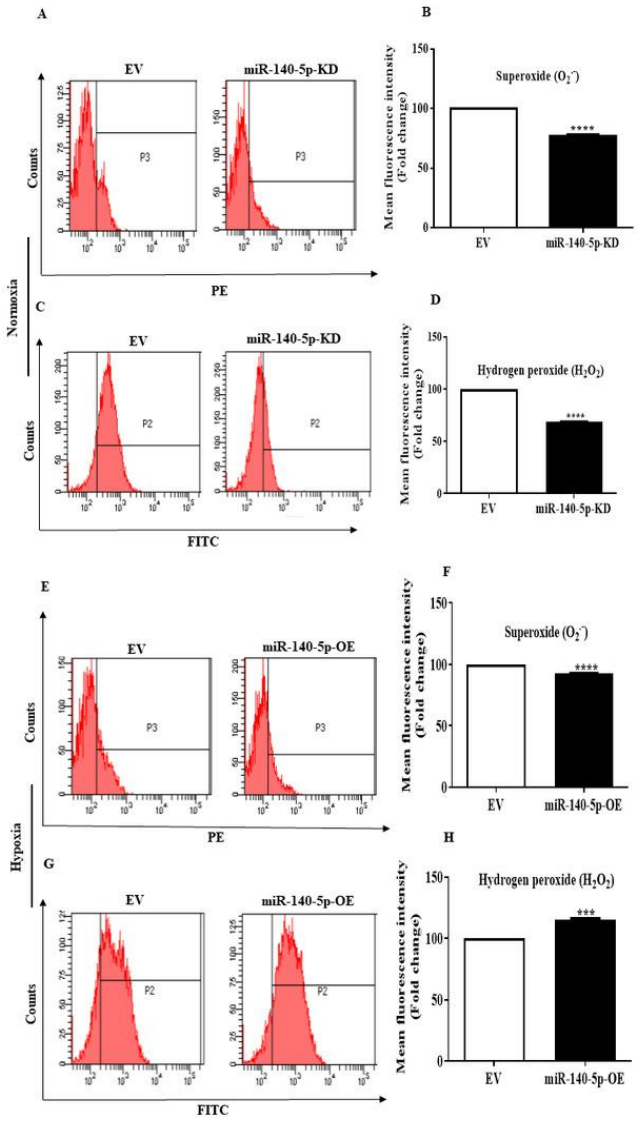


Fig. 2. miR-140-5p regulates the generation of ROS through Nrf2. *MDA-MB-231 cells with miR-140-5p knockdown under normoxia or overexpression under hypoxia were stained with DHE and DCFH-DA. The generation of superoxide (A, B) and hydrogen peroxide (C, D) in miR-140-5p-KD cells under normoxia. The generation of superoxide (E, F) and hydrogen peroxide (G, H) in miR-140-5p-OE cells under hypoxia. Results were represented as mean fluorescence intensity. Error bars represent mean ± SEM (n = 3). *** p < 0.001, and ****p<0.0001 compared to EV.*

Conclusion

In conclusion, in the current study, we inspected the role of miR-140-5p in regulating hypoxia-mediated oxidative stress. Our data indicate that miR-140-5p might control the hypoxia-mediated ROS generation by regulating the Nrf2 expression. Given the importance of miR-140-5p in regulating the hypoxia-mediated oxidative stress, angiogenesis, and metastasis through Nrf2, targeting miR-140-5p might represent a promising strategy for anti-BC therapy.

Significance of the study

Chemo-resistance is a significant problem in successful cancer treatment strategies. Resistance can develop after prolonged exposure to chemotherapeutic drugs or can be existing inherently in the patient. Tumors rapidly develop resistance after exposure to drugs, leading to most cancer-related deaths. Also, traditional drugs work by inhibiting one or two protein targets. Therefore, to develop novel therapeutic strategies to fight drug resistance and improve patient survival, miRNA-based therapy may provide a novel approach for the future of cancer therapy. The advantage of miRNA-based therapeutics over protein therapeutics is that they are short-sized, stable, respond rapidly to various stresses, and simultaneously regulate multiple genes and pathways. They can simultaneously target several effectors of pathways involved in tumor progression. Also, it is possible to supplement tumor-suppressive miRNAs with synthetic oligonucleotides and alleviate effects caused by oncogenic miRNAs through artificial antagonists. Additionally, miRNAs hold the power to inhibit all targets, including non-druggable targets. Therefore, miRNAs are emerging as promising candidates in successful cancer treatment strategies.

Author Contributions

M.M.: conceptualization, methodology, resources, formal analysis, validation, and original draft preparation; S.S.: investigation, conceptualization, formal analysis, visualization, validation, writing—original draft preparation and writing, review and editing, supervision. All authors have read and agreed to the published version of the manuscript.

Funding

This work was supported by intramural funds of NCCS.

Ethics Approval and Informed Consent Statement

Not applicable.

Patient consent for publication

Not applicable.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Acknowledgments

We express thanks to Sumit Das for helping in the generation of miR-140-5p overexpressing stable cell lines.

Conflicts of Interest

The authors declare no conflict of interest.

References

Arfin S, Jha NK, Jha SK, Kesari KK, Ruokolainen J, Roychoudhury S. et al. (2021). Oxidative stress in cancer cell metabolism. Antioxidants 10(5): 642. https://doi.org/10.3390/antiox10050642.

Arfmann-Knübel S, Struck B, Genrich G, Helm O, Sipos B, Sebens S. et al. (2015). The crosstalk between Nrf2 and TGF-β1 in the epithelial-mesenchymal transition of pancreatic duct epithelial cells. PLoS One 10(7): e0132978. https://doi.org/10.1371/journal.pone.0132978

Brahimi-Horn MC, Chiche J, Pouysségur J. (2007). Hypoxia and cancer. J Mol Med. 85: 1301-1307. 10.1007/s00109-007-0281-3

Homma S, Ishii Y, Morishima Y, Yamadori T, Matsuno Y, Haraguchi N. et al. (2009). Nrf2 enhances cell proliferation and resistance to anticancer drugs in human lung cancer. Clin Cancer Res. 15(10): 3423-3432. 10.1158/1078-0432.CCR-08-2822

Ji X, Wang H, Zhu J, Zhu L, Pan H, Li W. et al. (2014). Knockdown of Nrf2 suppresses glioblastoma angiogenesis by inhibiting hypoxia-induced activation of HIF-1α. IJC. 135(3): 574-584. 10.1002/ijc.28699

Joshi S, Kumar S, Ponnusamy, MP, Batra S. K. (2016). Hypoxia-induced oxidative stress promotes MUC4 degradation via autophagy to enhance pancreatic cancer cells survival. Oncogene. 35(45): 5882-5892. 10.1038/onc.2016.119

Kim TH, Hur EG, Kang SJ, Kim JA, Thapa D, Lee, YM. et al. (2011). NRF2 blockade suppresses colon tumor angiogenesis by inhibiting hypoxia-induced activation of HIF-1α. Cancer Res. 71(6): 2260-2275. 10.1158/0008-5472.CAN-10-3007

Konovalova J, Gerasymchuk D, Parkkinen I, Chmielarz P, Domanskyi, A. (2019). Interplay between MicroRNAs and oxidative stress in neurodegenerative diseases. Int J Mol Sci. 20(23): 6055. 10.3390/ijms20236055

Liu QQ, Ren K, Liu SH, Li WM, Huang CJ, Yang XH. (2019). MicroRNA-140-5p aggravates hypertension and oxidative stress of atherosclerosis via targeting Nrf2 and Sirt2. Int J Mol Med. 43(2): 839-849. 10.3892/ijmm.2018.3996

Mahajan M, Sitasawad S. (2021). Mir-140-5p attenuates hypoxia-induced breast cancer progression by targeting nrf2/ho-1 axis in a keap1-independent mechanism. Cells. 11(1): 12. 10.3390/cells11010012

Navarro-Yepes J, Zavala-Flores L, Anandhan A, Wang F, Skotak M, Chandra N. et al. (2014). Antioxidant gene therapy against neuronal cell death. Pharmacology & therapeutics. 142(2): 206-230. 10.1016/j.pharmthera.2013.12.007

Perillo B, Di Donato M, Pezone A, Di Zazzo E, Giovannelli P, Galasso G. et al. (2020). ROS in cancer therapy: The bright side of the moon. Exp Mol Med. 52(2): 192-203. 10.1038/s12276-020-0384-2

Sato H, Shibata M, Shimizu T, Shibata S, Toriumi H, Ebine T. et al. (2013). Differential cellular localization of antioxidant enzymes in the trigeminal ganglion. Neurosci. 248: 345-358. 10.1016/j.neuroscience.2013.06.010

Shen H, Yang Y, Xia S, Rao B, Zhang J, Wang J. (2014). Blockage of Nrf2 suppresses the migration and invasion of esophageal squamous cell carcinoma cells in hypoxic microenvironment. Dis Esophagus. 27(7): 685-692. 10.1111/dote.12124

Shibata T, Kokubu A, Gotoh M, Ojima H, Ohta T, Yamamoto M, Hirohashi, S. (2008). Genetic alteration of Keap1 confers constitutive Nrf2 activation and resistance to chemotherapy in gallbladder cancer. Gastroenterology 135(4): 1358-1368. https://doi.org/10.1053/j.gastro.2008.06.082

Singh A, Bodas M, Wakabayashi N, Bunz F, Biswal S. (2010). Gain of Nrf2 function in non-small-cell lung cancer cells confers radioresistance. ARS. 13(11): 1627-1637. https://doi.org/10.1089/ars.2010.3219

Thannickal V J, Fanburg BL. (2000). Reactive oxygen species in cell signaling. Am J Physiol Lung Cell Mol Physiol. 279(6): L1005-L1028. https://doi.org/10.1152/ajplung.2000.279.6.L1005

Vera-Ramirez L, Ramirez-Tortosa M, Perez-Lopez P, Granados-Principal S, Battino M, Quiles JL. (2012). Long-term effects of systemic cancer treatment on DNA oxidative damage: The potential for targeted therapies. Cancer Lett. 327(1-2): 134-141. https://doi.org/10.1016/j.canlet.2011.12.029

Wang Y, Branicky R, Noë A, Hekimi S. (2018). Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. JCB. 217(6): 1915-1928. https://doi.org/10.1083/jcb.201708007

Williams KJ, Cowen RL, Stratford IJ. (2001). Hypoxia and oxidative stress in breast cancer Tumour hypoxia–therapeutic considerations. BCR. 3(5): 1-4. https://doi.org/10.1186/bcr316