Biochem. Cell. Arch. Vol. 22, No. 1, pp. 2415-2422, 2022	www.connectjournals.com/bca	*ISSN 0972-5075*
DocID: https://connectjournals.com/03896.2022.22.2415		*eISSN 0976-1772*

DEREGULATION OF AUTOPHAGY FLUX AND GENE EXPRESSION INDUCED BY TOBACCO SMOKE AMONG IRAQI SMOKERS

Miyada Kh Hassan[1,*], Adel M. Rabee[1] and Haider F. Ghazi[2]

[1]Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq
[2]Immunology, Department of Microbiology, Collage of Medicine, Al-Nahrain University, Baghdad, Iraq.
*e-mail : maiadaalaloosh@gmail.com

(Received 4 April 2021, Revised 5 June 2021, Accepted 12 June 2021)

ABSTRACT : Cigarette smoking is a lifestyle behavior that causes significant adverse health effects. Cigarette smoke contains chemicals, many of which are lead to the production of reactive oxygen species (ROS), which can lead to apoptosis and autophagy. To estimate the association of Cigarette smoking with the autophagy and immunity, technology of real time polymerase chain reaction (RTPCR) for gene expression of (LC3A, LC3B, LC3C, myd88) was used. Enzyme-linked immunosorbent assay (ELISA) technique was utilized to measurement the amount of TNF-α protein. The ratios of LC3A/LC3B and LC3B/LC3C were calculated to estimate the autophagy flux. The results indicate the expression of LC3B, LC3C and Myd88 genes in smokers is increased significantly ($p \leq 0.001$) compared with non-smokers. While the expression of LC3A gene is decreased significantly. Findings show that the serum mean concentration of (TNF-α) for smokers group is increased significantly ($p \leq 0.001$). The ratio of LC3A/Lc3B is increased highly significant ($p \leq 0.001$) in smokers individuals. While there is no significant differences in the ratio of LC3B/ LC3C between smokers and non-smokers.

Key words : Smokers, free radical production, autophagy flux, LC3 gene, gene expression.

How to cite : Miyada Kh Hassan, Adel M. Rabee and Haider F. Ghazi (2022) Deregulation of autophagy flux and gene expression induced by tobacco smoke among Iraqi smokers. *Biochem. Cell. Arch.* **22**, 2415-2422. DocID: https://connectjournals.com/03896.2022.22.2415

INTRODUCTION

Autophagy is a process that removes damaged organelles and proteins by lysosomal degradation which greatly contributes to cell homeostasis and the prevention of various diseases. Autophagic flux is a process that used to measure autophagic degradation activity (Xiaojuan *et al*, 2018).

Autophagy is not only an adaptive response to the stress, but it is also involved in a many of other physiological mechanisms, such as intracellular quality control, inhibition of cellular ageing, differentiation and development of the cells, apoptosis, and both innate and adaptive immunity (Yin *et al*, 2016). Therefore, weaken or deficiency in this pathway result in severe pathologies, like specific types of cancer, neurodegeneration, and muscular dystrophies (Jiang and Mizushima, 2014). As a result, it is very important to quantify autophagic process in specific, accurate, and reliable way to be able to assess the changes of autophagic flux process and eventually the blocked step when this pathway is defect (Orhon and Reggiori, 2017).

One of the main steps in the autophagy regulation is the binding of microtubule-associated protein 1 light chain 3 (LC3) with phosphatidylethanolamine and forming microtubule-associated protein 1 light chain 3 lipidated form (LC3-II). The Lipidated LC3 bind to the elongated phagophore after that the phagosome fuse with lysosomes. Then, LC3-II can be either delipidated and recycled or hydrolyze by lysosomal enzymes. This pathway is called LC3-associated phagocytosis (LAP). The gold standards for measuring autophagy activity are monitoring LC3-II conversion, LC3 distribution or its flux through the pathway of autophagy. Therefore, measuring the formation of cellular autophagosome puncta that contain LC3, and quantifying the ratio of LC3-II to LC3-I enable monitor autophagy flux activity (Kono *et al*, 2021).

Asare *et al* (2020) found that the materials contained in cigarette smoke weaken LC3-associated phagocytosis (LAP) pathway, leading to chronic inflammatory response which is initiate the lung injury and dysfunction of airway that is associated with chronic obstructive pulmonary disease COPD. While, many Previous studies have shown

expression of myd88 gene and the level of TNF-α. In particular, cigarette smoke acts as a double-edged sword that either exacerbates pathological immune responses or attenuates the normal defensive function of the immune system, possibly owing to the complexities and functional diversities of cigarette smoke components and individuals' medical condition. Nevertheless, smoking plays a harmful rather than beneficial role in either case. The results illustrated that changes in gene expression may has an important role in susceptibility of smokers to various diseases.

REFERENCES

Akata K and van Eeden S F (2020) Lung Macrophage Functional Properties in Chronic Obstructive Pulmonary Disease. *Int. J. Mol. Sci*. **21**, 853.

AlQasrawi D and Naser S (2020) Nicotine Modulates MyD88-Dependent Signaling Pathway in Macrophages during Mycobacterial infection. *Microorganisms* **8**, 1804; doi:10.3390/microorganisms8111804.

Aryanpur M, Mortaz E, Masjedi M R, Tabarsi P, Garssen J, Adcock I M, Mozafarian A and Sharifi H (2016) Reduced Phagocytic Capacity of Blood Monocyte/Macrophages in Tuberculosis Patients Is Further Reduced by Smoking. *Iran J Allergy Asthma Immunol*. **15**(3), 174-182.

Asare Patrick Roscioli, Eugene Hurtado, Plinio Tran, Hai Maiolo S and Hodge S (2020) THP-1 Macrophages Exposed to the Factors in Cigarette Smoke Exhibit Dysregulation of LC3-Associated Phagocytosis (LAP). *ajrccmconference* **201**(1).

Bai H, Inoue J, Kawano T and Inazawa J (2012) A transcriptional variant of the LC3A gene is involved in autophagy and frequently inactivated in human cancers. *Oncogene* **31**, 4397–4408. https://doi.org/10.1038/onc.2011.613.

Barbieri S, Zacchi E, Amadio P, Gianellini S, Mussoni L, Weksler B and Tremoli E (2011) Cytokines present in smokers' serum interact with smoke components to enhance endothelial dysfunction. *Cardiovascular Research* **90**(3), 475–483.

Bodas M, Van Westphal C, Carpenter-Thompson R, KMohanty D and Vij N (2016) Nicotine exposure induces bronchial epithelial cell apoptosis and senescence via ROS mediated autophagy-impairment. *Free Radic Biol Med*. **97**, 441-453.

Bjørkøy G, Lamark T, Pankiv S, Øvervatn A, Brech A and Johansen T (2009) Monitoring autophagic degradation of p62/SQSTM1. *Methods Enzymol*. **452**, 181-197. doi: 10.1016/S0076-6879(08)03612-4. PMID: 19200883.

Chen P, Xiao Z, Wu H, Wang Y, Su W and Li P (2020) The Effects of Naringin on Cigarette Smoke-Induced Dynamic changes in Oxidation/Antioxidant system in lung of mice. *Natural Product Communications* **15**(8), 1–9.

Chen Z H, Kim H P, Sciurba F C, Lee S J, Feghali-Bostwick C, Stolz D B, Dhir R, Landreneau R J, Schuchert M J, Yousem S A, Nakahira K, Pilewski J M, Lee JS, Zhang Y, Ryter S W and Choi A M (2008) Egr-1 regulates autophagy in cigarette smoke-induced chronic obstructive pulmonary disease. *PLoS One* **3**(10), e3316.

Chen Z H, Lam H C, Jin Y, Kim H P, Cao J, Lee S J, Ifedigbo E, Parameswaran H, Ryter S W and Choi A M (2010) Autophagy protein microtubule-associated protein 1 light chain-3B (LC3B) activates extrinsic apoptosis during cigarette smoke-induced emphysema. *Proc Natl Acad Sci. USA* **107**, 18880 –18885.

Cheng Y, Wang D, Wang B, Li H, Xiong J, Xu Sh, Chen Q, Tao K, Yang Z, Zhu Y and He S (2017) HMGB1 translocation and release mediate cigarette smoke-induced pulmonary inflammation in mice through a TLR4/MyD88-dependent signaling pathway. *Mol Biol Cell*. **28**(1), 201-209. doi: 10.1091/mbc.E16-02-0126. Epub 2016 Nov 2.

Deguine J and Barton G M (2014) MyD88: a central player in innate immune signaling. *F1000Prime Rep*. **6**, 97.

Ding S, Hou X, Wang G, Qiu H, Liu Y, Zhou Y, Du M, Tan X, Song J, Wei Y, Shu L, Li Z, Feng L and Jia X (2018) Autophagy Flux Contributes to Regulation of components of *Eclipta prostrata* L. on Cigarette smoking-Induced Injury of bronchial epithelial cells. *Frontiers in Pharmacology* **9**, 107. https://doi.org/10.3389/fphar.2018.00107.

Doz E, Noulin N, Boichot E, Guénon I, Fick L, Le Bert M, Lagente V, Ryffel B, Schnyder B, Quesniaux V and Couillin I (2008) Cigarette smoke-induced pulmonary inflammation is TLR4/MyD88 and IL-1R1/MyD88 signaling dependent. *J. Immunol*. **180**, 1169-1178. 10.4049/jimmunol.180.2.1169.

Farhang A A and Fikry A Q (2013) Effects of cigarette smoking on some immunological and hematological parameters in male smokers in Erbil city. *Jordan J Biol Sci*. **6**, 159-166.

Geraghty P, Dabo A J and D'Armiento J (2011) TLR4 protein contributes to cigarette smoke-induced matrix metalloproteinase-1 (MMP-1) expression in chronic obstructive pulmonary disease. *The J. Biol. Chem.* **286**(34), 30211–30218. https://doi.org/10.1074/jbc.M111.238824.

Huang H Q, Li N, Li D Y, Jing D, Liu Z Y, Xu X C, Chen H P, Dong L L, Zhang M, Ying S M, Li W, Shen H H, Li Z Y and Chen Z H (2021) Autophagy Promotes Cigarette Smoke-Initiated and Elastin-driven Bronchitis-Like Airway Inflammation in mice. *Front Immunol*. **22;12**, 594330.

Hwang J, Lee H, Lee W and Suk K (2010) NF-κB as a common signaling pathway in ganglioside-induced autophagic cell death and activation of astrocytes. *J. Neuroimmunol.* **226**(1–2), 66-72.

Jiang P and Mizushima N (2014) Autophagy and Human Diseases. *Cell Res*. **24**, 69–79.

Khachigian L M (2006) Early growth response-1 in cardiovascular pathobiology. *Circ Res.* **98**, 186–191.

Kiffin R, Bandyopadhyay U and Cuervo A M (2006) Oxidative stress and autophagy. *Antioxid Redox Signal*. **8**, 152–162.

Kim H P, Wang X and Chen Z-H (2008) Autophagic proteins regulate cigarette smoke induced apoptosis: protective role of heme oxygenase-1. *Autophagy* **4**(7), 887–895.

Kono Y, Colley T, To M, Papaioannou A, Mercado N, Baker J, To Y, Abe Sh, Haruki K, Ito K and Barnes P (2021) Cigarette smoke-induced impairment of autophagy in macrophages increases galectin-8 and inflammation. *Sci Rep.* **11**, 335. https://doi.org/10.1038/s41598-020-79848-0.

Koukourakis M I, Kalamida D, Giatromanolaki A, Zois C E, Sivridis E, Pouliliou S, Mitrakas A, Gatter K C and Harris A L (2015) Autophagosome Proteins LC3A, LC3B and LC3C Have Distinct

Subcellular Distribution Kinetics and Expression in Cancer Cell Lines. *PLoS ONE* **10**(9): e0137675. doi:10.1371/journal.pone.0137675

Kumar and Singh (2020) Study of TLC, DLC and SpO2 of hemoglobin changes in healthy smokers & non-smokers. *Int. J. Hlth Clin. Res.* **3**(5), 222-226.

Lee S E, Park H R, Park C S, Ahn H J, Cho J J, Lee J and Park Y S (2019) Autophagy in Crotonaldehyde-Induced Endothelial Toxicity. *Molecules* (Basel, Switzerland) **24**(6), 1137. https://doi.org/10.3390/molecules24061137.

Levine B and Kroemer G (2008) Autophagy in the pathogenesis of disease. *Cell.* **132**, 27–42. doi: 10.1016/j.cell.2007.12.018.

Li X, Yan Z, Wu Q, Sun X, Li F, Zhang S, Li K, Li L, Wu J, Xu L, Feng J, Ning W, Liu Z and Chen H (2016) Glucocorticoid receptor contributes to the altered expression of hepatic cytochrome P450 upon cigarette smoking. *Mol Med Rep*. **14**(6), 5271-5280.

Li X, Zhong CH, Yin Z, Qi H, Xu F, He Q and Shuai J (2020) Data-Driven Modeling identifies TIRAP-Independent MyD88 activation complex and Myddosome assembly strategy in LPS/TLR4 signaling. *Int. J. Mol. Sci*. **21**, 3061.

Liu G, Arimilli S, Savage E and Prasad G L (2019) Cigarette smoke preparations, not moist snuf, impair expression of genes involved in immune signaling and cytolytic functions. *Scientific Reports* **9**, 13390.

Liu S, Sun Y and Li Z (2018) Resveratrol protects Leydig cells from nicotine-induced oxidative damage through enhanced autophagy. *Clin Exp Pharmacol Physiol*. **45**, 573-580.

Lv Z Q, Han J J, Liu Y Q, Wang L L, Tang Q L and Sun Q (2015) Expression of beclin 1 in non-small cell lung cancer: an immunohistochemical study. *Clin. Respir. J.* **9**, 359–365.

Mercado N *et al* (2019) Bicaudal D1 impairs autophagosome maturation in chronic obstructive pulmonary disease. *FASEB BioAdv.* **1**, 688–705.

Mizumura K, Cloonan S M, Nakahira K, Bhashyam A R, Cervo M, Kitada T, Glass K, Owen C A, Mahmood A, Washko G R, Hashimoto S, Ryter S W and Choi A M (2014) Mitophagy-dependent necroptosis contributes to the pathogenesis of COPD. *J Clin Invest.* **124**, 3987–4003.

Monick M M, Powers L S, Walters K, Lovan N, Zhang M, Gerke A, Hansdottir S and Hunninghake G W (2010) Identification of an autophagy defect in smokers' alveolar macrophages. *J. Immunol.* **185**(9), 5425–5435. https://doi.org/10.4049/jimmunol.1001603

Nikota J K and Stampfli M R (2012) Cigarette smoke-induced inflammation and respiratory host defense: Insights from animal models. *Pulm Pharmacol Ther*. **25**, 257-262.

Orhon I and Reggiori F (2017) Assays to Monitor Autophagy Progression in Cell Cultures. *Cells* **6** (20), 1-13.

Pan M, Zheng Z, Chen Y, Sun N, Zheng B, Yang Q, Zhang Y, Li X and Meng Y (2018) Angiotensin-(1-7) Attenuated Cigarette Smoking–related Pulmonary Fibrosis via Improving the Impaired Autophagy Caused by Nicotinamide Adenine Dinucleotide Phosphate Reduced Oxidase 4–Dependent Reactive Oxygen Species. *Am J Respir Cell Mol Biol*. **59**(3), 306–319.

Petrescu F, Voican S C and Silosi I (2010) Tumor necrosis factor-alpha serum levels in healthy smokers and nonsmokers. *Int. J. Chronic Obstructive Pulmonary Dis.* **5**, 217–222.

Ryter S W, Lee S and Choi A (2010) Autophagy in cigarette smoke-induced chronic obstructive pulmonary disease. *Expert Rev Respir Med*. **4**(5), 573–584.

Shallal A F (2020) The changes in some inflammatory markers and biochemical aspects during smoking in males. *Biomed. Res.* **31**(1), 1-4.

Shi Ji Yin, Ning Xuan, Ling-ling Yao, Chun-Suo Meng and Aimin Hou Qi (2012) Vam3, a derivative of resveratrol, attenuates cigarette smoke-induced autophagy. *Acta pharmacologica Sinica* **33**, 888-896. 10.1038/aps.2012.73.

Soldin O P, Makambi K H, Soldin S J and OMara D M (2011) Steroid hormone levels associated with passive and active smoking. *Steroids* **76**, 653-659.

Song I, Sohn H S, Kim H, Lim E, Kwon, Ha M, Kwon J and Jin W (2014) Impact of smoking on the effectiveness of TNF-á inhibitors in patients with rheumatoid arthritis or Crohn's disease. *Trans. Clin. Pharmacol*. **22**(2), 92-101.

van der Toorn M, Rezayat D and Kauffman H F (2009) Lipid-soluble components in cigarette smoke induce mitochondrial production of reactive oxygen species in lung epithelial cells. *Am J Physiol Lung Cell Mol Physiol*. **297**, L109–L114.

Vij N, Chandramani-Shivalingappa P, Van Westphal C, Hole R and Bodas M (2018) Cigarette smoke-induced autophagy impairment accelerates lung aging, COPD-emphysema exacerbations and pathogenesis. *Am J Physiol Cell Physiol*. **314**, C73-C87.

Wang X, Du Z, Li L, Shi M and Yu Y (2015) Beclin 1 and p62 expression in non-small cell lung cancer: relation with malignant behaviors and clinical outcome. *Int. J. Clin. Exp. Pathol.* **8**, 10644–10652

Wang Z, Liang W, Ma C, Wang J, Gao X and Wei L (2021) Macrophages Inhibit Ciliary Protein Levels by Secreting BMP-2 Leading to Airway Epithelial Remodeling Under Cigarette Smoke Exposure. *Front. Mole. Biosci.* **8**, 663987. https://doi.org/10.3389/fmolb.2021.663987

Vij N, Chandramani-Shivalingappa P, Van Westphal C, Hole R and Bodas M (2018) Cigarette smoke-induced autophagy impairment accelerates lung aging, COPD-emphysema exacerbations and pathogenesis. *Am J Physiol Cell Physiol*. **314**, C73-C87.

Xiaojuan Chao, Hong-Min Ni and Wen-Xing Ding (2018) Insufficient autophagy: a novel autophagic flux scenario uncovered by impaired liver TFEB-mediated lysosomal biogenesis from chronic alcohol-drinking mice. *Autophagy* **14:9**, 1646-1648.

Yi L, Yong-cheng D, Jian-ying X and Xiao-yun H (2012) Expression and significance of myeloid differentiation factor 88 in marrow dendritic cells in asthmatic rats with cigarette smoke exposure. *Chin Med J*. **125**(14), 2556-2561.

Yin Z, Pascual C and Klionsky D J (2016) Autophagy: Machinery and Regulation. *Microb. Cell* **3**, 588–596.

Zhou J S, Zhao Y, Zhou H B, Wang Y, Wu Y F, Li Z Y, Xuan N X, Zhang C, Hua W, Ying S M, Li W, Shen H H and Chen Z H (2016) Autophagy plays an essential role in cigarette smoke-induced expression of MUC5AC in airway epithelium. *Am J Physiol Lung Cell Mol Physiol.* **310**, 1042–1052.