International Journal of Pharmaceutical and Bio-Medical Science

ISSN(print): 2767-827X, ISSN(online): 2767-830X

Volume 05 Issue 03 March 2025

Page No : 195-201

DOI: https://doi.org/10.47191/ijpbms/v5-i3-08, Impact Factor: 8.163

Potential use of Alpha Klotho in Relation to Oxidative Stress in Chronic Obstructive Pulmonary Disease (COPD): A Systematic Review

Derallah A Lindra[1], Endang Purwaningsih[2], Ahmad Rusdan H Utomo[2], Faisal Yunus[3]

[1]Student of Biomedical Science Doctoral Program, University of Yarsi

[2]Lecturer of Biomedical Science Doctoral Program, University of Yarsi

[3]Lecturer of Pulmonology and Respiratory Medicine, University of Indonesia

ABSTRACT

Objectives: Chronic Obstructive Pulmonary Disease (COPD) is associated with ongoing inflammation, overuse injury, and accelerated aging of the lungs. Alpha Klotho is an anti-aging protein that protects cells from inflammation and damage. Alpha Klotho effectively reduces oxidative stress and maintains mitochondrial function by involving the reduction of ROS (reactive oxygen species) through the expression of antioxidant proteins as well as the suppression of ROS-related oxidative stress signaling pathways. However, there is still limited research on the potential use of alpha klotho in relation to oxidative stress in COPD. The aim of this study was to determine the potential use of alpha klotho in relation to oxidative stress in COPD.

Method: This research method uses the Systematic Review method by collecting secondary data from scientific research articles from 2014 - 2024. Data search used the databases PubMed, Google Scholar, Plos Medicine, Taylor and Francis, Nature. Conducted using the terms: alpha klotho, COPD, oxidative stress.

Results: 2 studies were selected for Systematic Review. Shows that alpha Klotho inhibits oxidative stress and the expression of inflammatory mediators so that it can protect lung cells from inflammation and further damage in COPD.

Conclusion: This review suggests that targeting the use of alpha klotho may be useful in suppressing oxidative stress that occurs in disease progression in COPD patients.

KEYWORDS: *alpha klotho, COPD, oxidative stress*.

ARTICLE DETAILS

Published On:
21 March 2025

Available on:
https://ijpbms.com/

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a disease characterized by airway limitation that is not completely reversible and can be prevented. This airway limitation is usually progressive and associated with an inflammatory response due to noxious substances or gases. Chronic obstructive pulmonary disease is not a single disease but is a general term used to describe chronic lung diseases that cause limitations in lung airflow. The terms chronic bronchitis and emphysema are no longer used, but are now included in the COPD phenotype.[1]

Based on data from the World Health Organization (WHO) from the Global Burden of Disease Study report, the global incidence of COPD was around 251 million cases in 2016. The mortality rate is estimated at around 3.17 million people died from COPD in 2015, where this figure constituted 5% of all global deaths in that year.[2] Basic Health Research (Riskesdas) in 2018 reported that people who smoke every day include 24.3% of the population aged over 10 years. In this Riskesdas, it is estimated that the prevalence of COPD in Indonesia is 3.7%.[3]

Oxidative stress results from several environmental factors and local airway abnormalities associated with bronchiolitis and chronic obstruction that are characteristic of COPD and play an important role in the pathomechanism of the disease.[4] Oxidative stress occurs when the oxidative load generated by exposure to exogenous and endogenous free radicals exceeds capacity. Antioxidant defense. This may occur due to excessive production of oxidants, fatigue, or impaired function of antioxidant mechanisms. Reactive oxygen species (ROS) such as hydroxyl radicals and superoxide anions, are produced by every cell in the body during mitochondrial respiration and cell signaling processes. ROS production by immune cells, especially phagocytic cells, is also important in immune defense against pathogens.[5]

In the development of COPD, exogenous radicals from cigarette smoke, biomass, air pollution and workplace exposure contribute greatly to small molecule oxidative stress. In addition, cigarette smoke can increase nitrogen oxide (NOX) activity in lung tissue and stimulate leukocyte migration. Nitrous oxide 4 (NOX4) is up regulated in airway smooth muscle cells of COPD patients which correlates with disease severity and is associated with pulmonary hypertension.[6,7] Long-term exposure to cigarette smoke and inflammation has been shown to reduce the activity of antioxidant enzymes such as catalase and superoxide dismutase which contribute to severe oxidative balance disorders in lung tissue.[8] Oxidative stress contributes to the development of COPD by weakening and disrupting certain anti-aging processes, such as sirtuin activity and the balance of the Klotho-fibroblast growth factor (FGF) protein system 23. Sirtuins (SIRTs) are enzymes of silent information regulator 2 (Sir2) class III deacetylase family. Because their activity is regulated by NAD+, they are very sensitive to redox. Sirtuins modulate transcription, cell growth, oxidative stress tolerance and metabolism thereby helping to alleviate aging-related mitochondrial dysfunction, genome instability and inflammation.[9]

The FGF23-Klotho (KL) system has emerged as an endocrine axis important for maintaining phosphate homeostasis and active vitamin D biosynthesis. Fibroblast growth factor 23 is a bone-derived hormone and its binding to the FGF receptor in the kidneys and parathyroid glands requires KL as an obligate co-receptor. Alpha klotho is a transmembrane protein, but also occurs in a blood-soluble form produced either through alternative splicing or proteolytic cleavage. Alpha klotho has been linked to anti-inflammatory and antiaging effects. The soluble form of alpha klotho can also be detected in blood, urine and cerebrospinal fluid.[10]

The alpha klotho protein was found to be associated with the emergence of oxidative stress. Thus, alpha klotho deficiency has been shown to increase the generation of endogenous ROS and accentuate oxidative stress, whereas administration of alpha klotho effectively reduces oxidative stress and maintains mitochondrial function and protects cells and tissues from oxidative stress. The mechanisms include activating the transcription factor forkhead box O (FoxO) and the nuclear factor kappa B (NF-κB) and nuclear factor-erythroid-2 related factor 2 (Nrf2) pathways. Klotho-deficient transgenic mice exhibit phosphate retention, accelerated aging, and pulmonary emphysema. Therefore, there is a suspicion that alpha klotho is protective against the development of COPD. The antioxidant effects of alpha klotho may involve the reduction of ROS through the expression of antioxidant proteins and the suppression of ROS-related oxidative stress signaling pathways. In this case, alpha klotho can increase renal transcription of Mn-SOD, catalase (CAT), heme oxygenase-1 (HO-1) and glutathione peroxidase (GPX) through the activation of FoxO protein and nuclear factor erythroid 2-related factor 2 (Nrf2).[11]

There is still limited research on the relationship between alpha klotho and oxidative stress in COPD patients. So researchers really want to explore alpha klotho, oxidative stress and COPD. The aim of this study was to determine the potential use of alpha klotho in relation to oxidative stress in COPD.

METHODOLOGY

The method in this literature review is a Systematic Review which is used to map the literature and identify gaps in the research areas carried out in the research. The framework used as a reference in preparing systematic reviews uses PRISMA for Systematic Reviews, which is a method for increasing quality assurance of the completeness of the systematic review structure and process. PRISMA for Systematic Reviews was chosen by researchers because of its preparation for creating detailed systematic reviews. This review includes several steps:

1. Identify article search results
2. Selection of articles based on title and abstract
3. Assessment of the suitability of the article based on the full text
4. Critical appraisal
5. Combine data, summarize and present results.

Identify article search results (Step 1)

In this literature review, the article search uses the PICO (Population, Intervention, Comparison and Outcomes) framework. This framework helps identify aspects of situations and populations that have specific conditions and

desired outcomes related to interventions. The description for the PICO framework is as follows:

Table 1. Framework PICO

Framework	Information
Patient / Population / Problem	COPD
Intervention / Prognostic factor / Exposure	Potential use of alpha klotho in relation to oxidative stress
Comparison / Control	-
Outcome	Alpha klotho in relation to oxidative stress

The eligibility criteria for articles are specified using inclusion and exclusion criteria. The inclusion criteria used are original articles, published in English, discussing the potential use of alpha klotho in relation to oxidative stress in COPD, journals published in 2014-2024, and free full text. While the exclusion criteria are review/comment articles, opinion articles, report documents/draft policies/guidelines from WHO/certain formal organizations/thesis reports. Article searches use the PubMed database, Google Scholar, Plos Medicine, Taylor and Francis, Nature. This review uses the keywords (alpha klotho) AND COPD AND oxidative stress.

Article Selection (Step 2 and 3)

At this stage, the articles obtained are then screened based on the number of duplicates, the suitability of the article related to the research objectives by paying attention to the title and abstract, analyzing the full text article according to the inclusion and exclusion criteria. The search results obtained 2 out of 562 articles that met the inclusion and exclusion criteria. The search results obtained amounted to 2 studies and were then entered into a data charting table with the criteria of researchers, research titles, research subjects, methodology and research results.

Critical Appraisal (Step 4)

Critical appraisal is the process of evaluating an article carefully, systematically and relevantly. The author evaluates the article using PICO by giving a score of 0 = NO, 1 = Not Applicable, 2 = Unclear, 3 = Yes.

Combine data, summarize and present results (Step 5)

Sources of data and information on literature studies were obtained from PubMed, Google Scholar, Plos Medicine, Taylor and Francis, Nature, which collected 562 literature studies using literature studies from 2014 - 2024. This literature study consists of articles or journals that were collected and have been carried out. Selection based on the title and related abstract information to see whether the article or journal meets the author's inclusion criteria to be used as literature in the literature review. 19 journals were analyzed, and 2 journals were selected with the number of literature studies released in 2015 being 2 articles or journals. With the large amount of material that has been collected and reviewed, there is a connection between the articles or journals obtained, so that by reviewing this literature study it can be used as information and recommendations to find out the role of alpha klotho on oxidative stress in COPD.

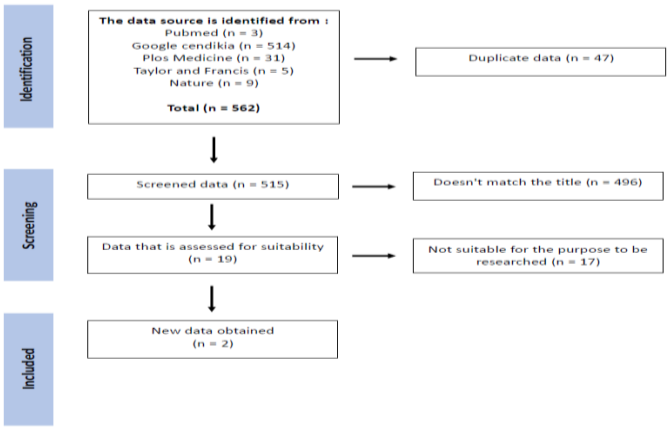

Figure 1. PRISMA for Systematic Review

RESULT

The number of literature obtained from various databases using the PICO method and the use of keywords, namely, 562 literature on PubMed, Google Scholar, Plos Medicine, Taylor and Francis, Nature. All literature will be selected using inclusion and exclusion criteria as well as screening for suitability of the research theme through reading the title and abstract. There are 2 journals that will be reviewed in this research. Details of the literature, namely, 2 pieces of literature obtained came from PubMed.

Table 1. Summary of journal contents

No	Researcher	Title	Subject	Methodology	Result
1	Gao W, Yuan C, Zhang J, Li L, Yu L, Wiegman CH, et al.	*Klotho expression is reduced in COPD airway epithelial cells: effects on inflammation and oxidant injury*	• The research subjects in this study consisted of lung tissue obtained from 59 subjects who underwent resection surgery to treat solitary peripheral carcinoma at the First Hospital of Nanjing Medical University. • Study subjects were classified as healthy non-smokers, smokers with normal lung function and smokers with COPD (according to GOLD guidelines) • Induction of Emphysema in Mice: Eight-week-old male C57BL/6 mice were exposed to ozone at a concentration of 3 ppm for 3 hours a day, twice a week for 1, 3, or 6 weeks. Lung tissue was then obtained for morphological and histological analysis.	This research methodology involves several techniques and procedures for collecting and analyzing data. Following are some of the main steps taken in this research: **Lung Tissue Collection:** Lung tissue was obtained from 59 subjects who underwent surgical resection to treat solitary peripheral carcinoma at the First Hospital of Nanjing Medical University. Subjects were classified as healthy non-smokers, smokers with normal lung function, and smokers with COPD according to GOLD guidelines. **RNA extraction and qPCR:** Total RNA was extracted from cells using TRIzol® (Invitrogen). qPCR was performed using SYBR Green to analyze gene expression. **Flow Cytometry:** The levels of apoptosis and intracellular ROS production by 16HBE cells were determined using flow cytometry with annexin V-FITC and propidium iodide and DCFH-DA. **ELISA:** The culture medium was centrifuged, and the supernatant stored at -80°C until analysis. KL, IL-1β, and TNFα were detected using an ELISA kit. **Western Blot:** Western blot analysis was performed to confirm the reduction of KL expression in healthy smokers compared with non-smokers, and further in patients with COPD. **Immunohistochemistry:** Immunohistochemistry results showed the distribution of KL expression along the airway epithelium and its reduction in healthy smokers and patients with COPD.	• Klotho is expressed along human airway epithelium and is decreased in the lungs of smokers compared with nonsmokers, but is reduced further in the lungs of COPD patients. • Klotho expression was also lower in the airway epithelium of ozone-induced emphysematous mice than in control mice. There is a decrease in klotho levels in human bronchial epithelial cells in response to exposure to cigarette smoke and inflammation. • NF-κB can bind directly to the klotho promoter to prevent klotho transcription and NF-κB acts indirectly by inhibiting PPARγ (peroxisome-proliferator-activated receptor γ) or Egr-1 (early growth response-1) which are known to activate the klotho promoter. • Intracellular klotho deficiency contributes to upregulation of pro-inflammatory cytokine expression (IL-8, IL-6 and MCP-1)

2	Li L, Wang Y, Gao W, Yuan C, Zhang S, Zhou H, et al.	*Klotho Reduction in Alveolar Macrophages Contributes to Cigarette Smoke Extract-induced Inflammation in Chronic Obstructive Pulmonary Disease*	• The research subjects in this study involved lung tissue and peripheral blood mononuclear cells obtained from three groups of participants: 22 non-smokers, 22 normal smokers, and 15 smokers with chronic obstructive pulmonary disease. • Basic characteristics of the patient, such as FEV1/FVC, DLCO and BMI. • A 10 ml blood sample was taken from each patient or volunteer to separate PBMCs using AccuSPIN and suspended in RPMI 1640 medium containing 10% heat-inactivated FBS and L-glutamine. • Murine alveolar macrophage cells from the MH-S cell line were also used in this study. MH-S cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% streptomycin/penicillin/glutamate solution.	The research methodology involved several key experimental techniques, including immunohistochemistry, Western blot, and ELISA. **Immunohistochemistry:** Tissue sections were incubated with peroxidase-conjugated secondary antibodies and developed using a 3,3'-diaminobenzidine substrate kit, with hematoxylin as a contrast dye. Each slide is evaluated under a microscope and scored based on the number of positively stained cells. **Western Blot:** Proteins are fractionated by SDS-PAGE and transferred to nitrocellulose or polyvinylidene difluoride membranes. The membrane was blocked with 5% BSA and incubated with primary antibody at 4°C overnight, then incubated with secondary antibody at 20°C for 1 h. **ELISA:** Culture medium is collected after treatment and centrifuged to separate cells. The supernatant was then analyzed to determine IL-6 and TNF-α levels using an ELISA kit. **Preparation of Cigarette Smoke Extract:** Cigarette smoke extract is prepared by burning one cigarette and passing the smoke into a culture medium. This extract is then filtered and used in experiments. **Cell Proliferation Analysis:** Cell proliferation was determined using the CCK-8 kit.	• There is a decrease in Klotho levels in alveolar macrophages in the lungs and PBMC (Lung Tissue and Peripheral Blood Mononuclear Cells) of COPD patients. • In vitro, cigarette smoke extract causes inhibition of Klotho expression and release in a time- and concentration-dependent manner in MH-S cells (Murine alveolar macrophage cell line), namely through the NF-κB pathway and coinciding with an increase in MMP-9 expression, TNF-α, and IL-6. • Klotho inhibits the expression of inflammatory mediators through the NF-κB pathway and through the insulin/IGF-1 pathway, Wnt and the activity of several ion channels.

DISCUSSION

Alpha klotho is expressed in human epithelial cells and is reduced in the lungs of smokers compared to non-smokers, but is significantly reduced in the lungs of COPD patients. Alpha klotho expression was also lower in the airway epithelium of mice exposed to ozone compared to control mice. There was a decrease in alpha klotho levels in the epithelium of human blood vessels due to exposure to cigarette smoke and burns. Conversely, a decrease in alpha klotho causes an increase in the production of proinflammatory cytokines, apoptosis, and a decrease in epithelial cell proliferation.[11]

Alpha klotho expression was reduced in ozone-induced COPD mouse models. Ozone exposure in mice, causes persistent airway inflammation and structural changes associated with increased oxidative damage to epithelial cells. The decrease in alpha klotho expression coincided with alveolar enlargement and damage after six weeks of ozone exposure. This suggests that decreased expression of the alpha klotho pathway may be associated with increased levels of inflammation and oxidative stress. A study by Li L et al,

showed that alpha klotho stimulates MH-S growth and inhibits MH-S senescence, thereby affecting tissue proliferation through inhibition at a concentration of 2000 pm. Alpha klotho is an important protein involved in the regulation of proinflammatory cytokine release, stress resistance, metabolism and aging, all of which are involved in the pathogenesis of COPD. Li L et al research found that NAC plays an important role in reducing the decrease in alpha klotho protein caused by cigarette smoke. This is possible because NAC can increase alpha klotho expression through suppressing ROS caused by tobacco smoke.[12]

The lung is the only organ that shows increased degenerative changes with age, including alveolar widening, indicating that the lung is more sensitive to alpha klotho activity. Intracellular alpha klotho production decreases with long-term exposure to passive smoking. In contrast, heavy exposure to cigarette smoke (24 hours) caused a decrease in alpha klotho protein and mRNA. Furthermore, alpha klotho expression is reduced by TNF in a concentration-dependent manner after acute and chronic exposure.[11]

A study by Li L et al, showed a decrease in alpha klotho in lung alveolar macrophages and PBMC (Lung Tissue and Peripheral Blood Cells) in COPD patients. This is because cigarette smoke causes inhibition of the expression and release of alpha klotho through the NF-κB pathway in MH-S cells (Murine alveolar macrophage cell line) depending on time and concentration, as well as increasing the expression of MMP-9, TNF and IL-6. The results show that smoking can increase airway inflammation through inhibiting alveolar macrophages in the development of COPD.[12]

Pharmacological approach, research by GAO W et al revealed an important role of the NF-κB pathway in the decrease in alpha klotho expression observed in epithelial cells. This is because NF-κB can directly bind to the alpha klotho promoter and act indirectly by inhibiting peroxisome-proliferator-activated receptor γ (PPARγ) or early growth response-1 (Egr-1) which are known to activate the alpha klotho promoter. Two mechanisms are involved in the release of alpha klotho first cigarette smoke and inflammatory cytokines cause degradation of alpha klotho by affecting ADAM tissue or through proteolytic activity that inhibits KL transmission. Increased regulation of the expression of pro-inflammatory cytokines (IL-8, IL-6 and MCP-1) and increased cell sensitivity to cigarette smoke-induced inflammatory processes due to the contribution of intracellular alpha klotho deficiency.[11]

The anti-inflammatory effect of alpha klotho is associated with reduced NF-κB activity in cells. In a study by GAO W et al, pharmacological inhibition of IL-8 expression by NF-κB and ERK inhibitors in KL cells was compared with control cells. Overall, loss of KL expression may exacerbate chronic inflammation, thereby accelerating COPD progression. Excessive reactive oxygen species induce inflammatory responses, regulate cell proliferation, induce apoptosis thereby directly affecting the lungs and contributing to COPD. Reduced alpha klotho levels will increase the vulnerability of airway cells to oxidative stress, as measured by ROS production, cell shrinkage and increased apoptosis. Phosphorylation of Akt/PKB, ERK1/2 and p38 MAPK, which play an important role in modulating inflammation and oxidative stress in COPD, was found in alpha klotho-deficient cells after exposure to hydrogen peroxide. A study by GAO W et al. also showed that nuclear factor erythroid 2-related factor 2 (Nrf2) plays an important role in protecting lungs from exposure to cigarette smoke as an antioxidant. Oxidative stress activates alpha klotho-induced Nrf2. Alpha klotho deficiency reduces Nrf2 expression and H2O2-induced Nrf2 translocation. Alpha klotho can act as an antioxidant to delay Nrf2 activation and suppress the expression of inflammatory cytokines.[11]

A study by Li L et al showed that alpha klotho protein inhibits the expression and release of MMP-9, TNF, and IL-6 due to cigarette smoke. These findings highlight the importance of klotho in the regulation of inflammation by alveolar macrophages.[12] Alpha klotho inhibits the expression of mediators (MMP-9, TNF, and IL-6) through the NF-κB pathway. The NF-κB transcription factor RelA is required to inhibit klotho gene transcription. Nuclear factor kappa B is an important transcription factor widely distributed in many cell types, consisting of a dimer of two subunits p50 and p65 (RelA). The p65 protein is a major transcription factor of NF-κB. In unstimulated cells, NF-κB is in the form of IκB. Release of the IκB subunit is followed by phosphorylation and translocation of the dimer to the nucleus, leading to NF-κB activation. In the nucleus, IκB regulates the transcription of various genes. NF-κB p65 phosphorylation, nuclear translocation and DNA binding activity will be significantly activated by cigarette smoke. Exogenous alpha klotho reduces the phosphorylation, nuclear translocation and DNA binding activity of NF-κB p65 by cigarette smoke. Research by Li L et al., also showed that treatment using dexamethasone increased the expression and release of alpha klotho in MH-S cells through the expression of alpha klotho transcription.[12]

CONCLUSION

This review suggests that targeting the use of alpha klotho may be useful in suppressing oxidative stress that occurs in disease progression in COPD.

REFERENCES

I. Global Initiative for Chronic Obstructive Lung Disease. Global Strategy for The Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease. 2023. Accessed on January 10, 2024.

II. WHO.The Top 10 Causes of Death. 2020. https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death

III. Kemenkes RI. Laporan Nasional RISKESDAS 2018. Lembaga Penerbit Badan Penelitian Dan Pengembangan Kesehatan 2019; 2019. p. 145–51.

IV. Barnes P.J. Oxidative Stress in Chronic Obstructive Pulmonary Disease. *Antioxidants.* 2022;11:965.

V. Antus B., Kardos Z. Oxidative Stress in COPD: Molecular Background and Clinical Monitoring. *Curr. Med. Chem.* 2015;22:627–50.

VI. Yang I.A., Jenkins C.R., Salvi S.S. Chronic obstructive pulmonary disease in never-smokers: Risk factors, pathogenesis, and implications for prevention and treatment. *Lancet Respir. Med.* 2022;10:497–511.

VII. Schiffers C., Reynaert N.L., Wouters E.F.M., van der Vliet A. Redox Dysregulation in Aging and COPD: Role of NOX Enzymes and Implications for Antioxidant Strategies. *Antioxidants.* 2021;10:1799.

VIII. Padmavathi P., Raghu P.S., Reddy V.D., Bulle S., Marthadu S.B., Maturu P., Varadacharyulu N. Chronic cigarette smoking-induced oxidative/nitrosative stress in human erythrocytes and platelets. *Mol. Cell. Toxicol.* 2018;14:27–34.

IX. Wu Q.-J., Zhang T.-N., Chen H.-H., Yu X.-F., Lv J.-L., Liu Y.-Y., Liu Y.-S., Zheng G., Zhao J.-Q., Wei Y.-F., et al. The sirtuin family in health and disease. *Signal Transduct. Target. Ther.* 2022;7:402.

X. Kuro-O M. The FGF23 and Klotho system beyond mineral metabolism. *Clin. Exp. Nephrol.* 2017;21:64–69.

XI. Gao W., Yuan C., Zhang J., Li L., Yu L., Wiegman C.H., Barnes P.J., Adcock I.M., Huang M., Yao X. Klotho expression is reduced in COPD airway epithelial cells: Effects on inflammation and oxidant injury. *Clin. Sci.* 2015;129:1011–1023.

XII. Li L, Wang Y, Gao W, Yuan C, Zhang S, Zhou H, et al. Klotho Reduction in Alveolar Macrophages Contributes to Cigarette Smoke Extract-induced Inflammation in Chronic Obstructive Pulmonary Disease. *J Biol Chem*. 2015; 290(46):27890-900.