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Role of NADPH Oxidase and GSH in Cystic Fibrosis

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วัตถุประสงค์เชิงพฤติกรรม

1. เพื่อให้ได้ความรู้เกี่ยวกับโรค cystic fibrosis
2. เพื่อให้ทราบบทบาทของ NADPH oxidase และ GSH ที่เกี่ยวข้องกับพยาธิสภาพของ cystic fibrosis

Abstract

Cystic fibrosis (CF), an autosomal recessive disorder that is resulted from mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, leading to a reduction in apical membrane chloride transport. The mechanism of CF has been the subject of debate that it may be associated with nicotinamide adenine dinucleotide phosphate (NADPH) oxidases and depletion of glutathione (GSH). NADPH oxidases are proteins that transfer electrons across biological membrane, resulting in the reactive oxygen species (ROS) production and play a key role in host defenses in CF. Depletion of GSH in CF because of defect in CFTR may alter GSH availability by modulating epithelial GSH transport, and then contributes to an imbalance in the antioxidant defense and results in oxidative stress. These effects can make cells damage from ROS.

Keywords: Cystic fibrosis, NADPH oxidases, GSH

Cystic Fibrosis (CF)

CF is the most common lethal genetic disorder in Caucasians, which is caused by mutation in the cystic fibrosis transmembrane conductance regulator (CFTR), gene locates on chromosome 7. In CF patients, pulmonary disease is the major cause of morbidity and mortality, which resulting from respiratory complications, such as mucus plugging, frequent bacterial infections and declining lung function over time. The most common cause of death is respiratory failure secondary to *Pseudomonas aeruginosa* infections.[1,2,3]

The CFTR gene was first discovered in 1989 and currently, more than 1000 mutations within the CFTR gene have been identified. CFTR is known to be a temperature-sensitive, cAMP-mediated chloride channel that is found in the exocrine glands, secretory epithelia and airway epithelial cells. The CFTR also directly regulates other ion channels, such as the outwardly rectifying chloride channel (ORCC) and the amiloride-sensitive sodium channel (ENaC), thereby playing a major role in the control of ion and water balance in body tissues. Elevated sweat chlorides, pancreatic exocrine insufficiency, and chronic sinopulmonary inflammation and infection are common features of CF. This a multi-organ disease reflects the consequences of mutations in the CFTR gene. The most common mutation is the deletion of phenyalanine at position 508 (ΔF508), which is found on 70% of CF alleles and identified in 50% CF patients. This mutation results in an immature protein that is not fully glycosylated, and instead, is ubiquitinated and targeted in the ER for subsequent proteolytic degradation. Approximately 99% of the ΔF508 proteins are degraded in a pre-Golgi compartment, thus never reaching the cell surface. However, a small amount of ΔF508 CFTR has been shown to reach the plasma membrane of certain tissues *in vivo* and can caused abnormality in several organs, especially the lungs.[2,4,5,6,7]

The molecular mechanisms of pulmonary infection and lung damage have been the subject of debate. They may involve in excessive reactive oxygen species (ROS) which play a role in host defense. Furthermore, they may associate with decrease in glutathione (GSH) level that leading to an oxidative stress and resulted in cell damage. ROS are normally generated from phagocytic cells. Activating these cells undergo a respiratory burst discharging superoxide as a result of stimulation of the nicotinamide adenine dinucleotide phosphate oxidases (NADPH oxidases: NOX). Then, superoxide dismutase (SOD) will catalyze the conversion of superoxide into hydrogen peroxide and

then change to hypochlorus acid. Hypochlorus acid is the major oxidant produced by phagocytic cells, and is the potential cause of tissue injury.[8,9,10]

NADPH oxidases

It was found In 1933 that phagocytic cells, mainly the polymorphonuclear neutrophil (PMN), showed markedly increased oxygen consumption, or a respiratory burst, during phagocytosis which was postulated to be related to an increased energy demand for the phagocytic process. In inflammation, neutrophils appeared to be the principal phagocytic cells, which associated with host defense. Activated neutrophils utilize molecular oxygen to kill microbial pathogens. The immense rise in oxygen consumption and the associated production of oxygen-free radicals are designated "respiratory burst" that are catalyzed by NADPH oxidases.[11,12]

The NOX family NADPH oxidases are the proteins that transfer electrons across biological membranes. In general, the electron acceptor is oxygen and the product of the electron transfer reaction is superoxide. Therefore, the biological function of NOX enzymes is the ROS production. Thereby the NOX family consists of 7 members: NOX1, NOX2 ($gp91^{phox}$), NOX3, NOX4, NOX5, DUOX1, and DUOX2. All of them share a core structure consisting of 6 transmembrane domains with two heme binding regions, and a long cytoplasmic C-terminus containing falvin adenine dinucleotide (FAD) and NADPH-binding regions.[13,14]

NOX2 ($gp91^{phox}$)

The phagocyte NADPH oxidases (NOX) is a multicomponent enzyme that transfers electrons from cytoplasmic NADPH onto extracellular (or intraphagosomal) molecular oxygen, resulting in superoxide generation. The overall reaction catalyzed by this enzyme is:

$$NADPH + 2\ O_2 \longrightarrow NADP^+ + 2\ O_2^- + H^+$$

The electron transfer from NADPH to oxygen is a multistep process, during which the electrons are transported sequentially along several moieties of the oxidase:[15]

$$NADPH \longrightarrow FAD \longrightarrow 2\ Heme \longrightarrow 2\ O_2$$

This NOX2 consists of the flavocytochrome b_{558}, which is a heme-binding heterodimer composed of a large ($gp91^{phox}$) and a small ($p22^{phox}$) subunit. In 6 transmembrane of $gp91^{phox}$ subunit consists of N terminus and C-terminal portion, containing the FAD and NADPH binding domains that are essential for activity. The control of oxidase activation is the recruitment of regulatory proteins to the

flavocytochrome, including the $p40^{phox}$, $p47^{phox}$ and $p67^{phox}$, of which $p47^{phox}$ and $p67^{phox}$ are essential for activity. Thus, $gp91^{phox}$ contains the entire transmembrane redox machinery, in which electrons are transferred from NADPH on the cytoplasmic side via FAD and two hemes to molecular oxygen in the extracellular or intraphagosomal space.[16,17]

NADPH oxidase activity

The NOX core enzyme comprises five components: $p40^{phox}$ (*phox* for phagocyte oxidase), $p47^{phox}$, $p67^{phox}$, $p22^{phox}$ and $gp91^{phox}$. In the resting cell, $p40^{phox}$, $p47^{phox}$ and $p67^{phox}$ exist in the cytosol as a complex. On the other hand, $p22^{phox}$ and $gp91^{phox}$ are located in the membranes of secretory vesicles where they occur as a heterodimeric flavohemoprotein (cytochrome b_{558}). When the resting cell is exposed to any of a wide variety of stimuli, the cytosolic component $p47^{phox}$ becomes heavily phosphorylated and the entire cytosolic complex migrates to the membrane, where it associates with cytochrome b_{558} to assemble the active oxidase. It can transfer electrons from the NADPH to oxygen by means of its electron-carrying prosthetic groups, its flavin and then its heme group(s). Activation requires the participation of two low-molecular-weight guanine nucleotide-binding proteins: Rac2, which in the resting cell is located in the cytoplasm in a dimeric complex with Rho-GDI. During activation, Rac2 binds guanosine triphosphate (GTP) and migrates to the membrane along with the core cytosolic complex. When phagocytosis takes place, the plasma membrane is internalized as the wall of the phagocytic vesicle, with the enzyme pours $O_2^{\cdot}$ into the vesicle, and the rapid conversion of this $O_2^{\cdot}$ into its successor products bathes the internalized target in a lethal mixture of corrosive oxidants (Figure 1).[18]

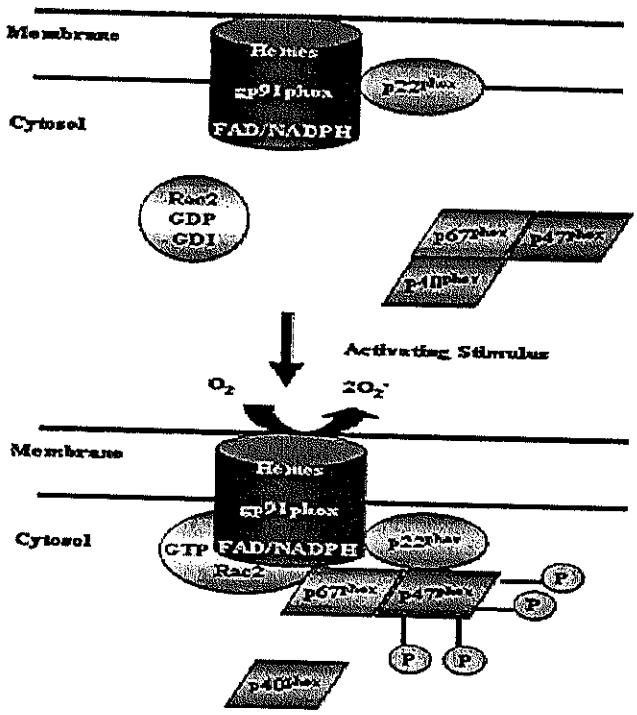

Figure 1 Activation and assembly of the phagocyte NADPH oxidase (Adapted from Bokoch and Dieboid, 2002).[19]

NADPH oxidase and host defense

The action of host defense apparatus is its ability to produce a group of highly reactive oxidizing agents, including oxidized halogens, oxidizing radicals, and singlet oxygen to kill the microbial. The phagocytes (neutrophils, eosinophils, and mononuclear phagocytes) and B lymphocytes generate superoxide, which is the precursor of reactive oxidants. Thereby, the phagocyte NADPH oxidases play a key role in host defense by generating large amounts of O_2^- and other ROS that call as the respiratory burst.[20,21,22]

The non phagocytic NOX1-5/DUOX1-2 enzymes participate in an NADPH oxidase activity in some non phagocytic cell (NOX1-5/DUOX1-2), generating ROS in intra and extra-cellular medium. New insights have been brought by the study of the role of NADPH oxidases in human airway epithelial cells. Investigations of a panel of tissues revealed the high expression of Dual oxidase 1-2 in whole lung as showed by Schwarzer and colleagues (2004) in human tracheal surface epithelium where both DUOX1-2 are expressed at levels 1000x fold superior to other NOX isoforms and thus represent the major NOX's isoforms in airway epithelial cells. Concerning their functions, Matt and coworkers (2005) have shown that Dual oxidase 1 plays a critical role in

mucin expression through Protein Kinase C (PKC) signalling pathway. In the same manner, Yan and colleagues (2008) have recently reported that the bacterium *P. aeruginosa* uses ROS to up-regulate mucin expression via a PKC-NADPHoxidase signalling pathway in human airway epithelial cells. In a recent paper, Moskwa and coworkers (2007) have presented a novel host defense system of normal airways that implies the Dual oxidases as H_2O_2 generating enzyme which permits the formation of the bactericidal hypothiocyanite ($OSCN^-$) from the red-ox system ($OSCN^-/SCN^-$). Moreover, in CF airway epithelium $OSCN^-$ is diminished due to a CFTR-dependent defect in SCN^- secretion, which leads to a collapse of the oxidative antimicrobial mechanism. They have demonstrated for the first time that CFTR is critical for this transepithelial SCN^- secretion process. As the oxidative antimicrobial system is inactive in CF cells, they suggest a new cellular and molecular basis for defective airway immunity in cystic fibrosis.[23,24,25,26]

Glutathione (GSH)

The tripeptide L-γ-glutamyl-L-cysteinyl-glycine (γ-L-Glu-L-Cys-Gly), or GSH plays a major role in maintaining intracellular reduction-oxidation (redox) balance and regulating signalling pathways augmented by oxidative stress. GSH is the most abundant low molecular weight thiol-containing substance in cells, is synthesized from glutamate, cysteine, and glycine.[27,28,29] GSH plays an important roles in proliferation of lymphocytes, spermatogenesis and cytokine production. It serves as an important antioxidant defenses for ROS and electrophilic compounds. GSH has been implicated in immune modulation and inflammatory responses. It has been shown to be critical to the lungs' antioxidant defenses. Alterations in reduced GSH levels in the lung have been displayed in various inflammatory conditions. A low GSH concentration in the lung may contribute to an imbalance between oxidants and antioxidants and may amplify inflammatory responses and potentiate lung damage.[30,31]

The mechanisms of GSH-related inflammation have shown by the ability of oxidative stresses or changes in the intracellular GSH redox status to trigger signal transduction and hence the transcription of specific genes via the activation of redox-sensitive transcription factors. Protective antioxidant genes are induced by modulation of cellular GSH/GSSG levels in response to various oxidative stresses, including inflammatory mediators in lung cells. Therefore, modulation of GSH redox status causes increased gene expression of both proinflammatory genes via activator protein-1 (AP-1) and nuclear factor-keppa B (NF-kB), and also activation of antioxidant-protective genes.

The balance may exist between pro- and anti-inflammatory gene expression related to GSH redox status in response to oxidative stress and during inflammation. Theses effects may lead to cell injury or protection against the injurious events of inflammation (Figure 2).[31]

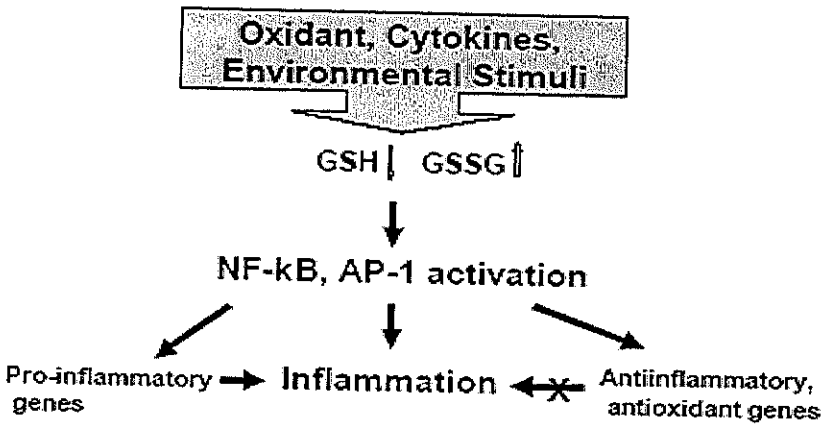

Figure 2 GSH/GSSG-related inflammation
(Adapted from Rahman and Macnee, 2000)[31]

Role of GSH in cystic fibrosis

CF is characterized by progressive GSH deficiency. The defect in CFTR may alter GSH availability in cells. The study of Henrion-Caude and colleagues (2002) showed that CFTR modulated epithelial GSH transport, and recent findings have shown that CFTR genetic defects induce a decrease in the concentrations of GSH, which contributes to an imbalance in the antioxidant defense.[32] Reduction of intracellular GSH may result in oxidative stress, making cells less able to resist cell damage from electrophiles. Reduction of GSH can alter the profile of important inflammatory mediators.[33] GSH is also an important antioxidant in the lung, and the GSH concentration is greatly reduced in airway surface fluid of CF patients. These defects can be attributed directly to missing or defective CFTR channels, because the GSH in the lung epithelial lining fluid of CFTR-deficient mice is decreased by half, compared with that in wild-type mice.[34] CFTR maintains a cellular homeostatic balance of ions, including sodium, chloride and GSH. The presence of GSH in the lung provides a sensor system for maintaining surfactant production, as well as a trigger for inflammation. Cellular GSH deficiency has been associated with an increased NF-kB transcription, which participates in the inflammatory cytokines regulation. Reduced GSH levels lead to inflammation, a hallmark of CF and oxidative stress that can result in

damage to cell membrane, cellular proteins and DNA.[35] Childer and colleagues (2007) have displayed apoptosis sensitivity could be correlated to GSH levels. While a reduced intracellular state is normal in epithelial cells, during the process of apoptosis, depletion of GSH is required and it has been repeatedly demonstrated that CF appear to have a defect in the process of normal cell death.[36]

Conclusion

In summary, the ROS and an oxidative stress in CF may associate with NADPH oxidases and depletion of GSH, which is related to apoptosis. The finding NOX in CF is correlated to host defense via inflammation and also ROS production. Moreover, the depletion of GSH in CF that may involve in defective CFTR can result in oxidative stress, making cells less able to resist cell damage. The defective CFTR may participate in an early phase of inflammation. With the discovery of NOX as a source of ROS generation and depletion of GSH, a very exciting area has begun, paving the way to more thorough understanding of ROS-production process, especially in CF. The exact function of non-phagocytic NOX proteins and molecular mechanism of reduced GSH remain to be explored.

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คำถาม

1. Cystic fibrosis เป็นโรคเกี่ยวกับอวัยวะใด
    1. Pulmonary disease
    2. Liver disease
    3. Heart disease
    4. Brain
    5. Blood

2. Cystic fibrosis เกิดจากการบกพร่องที่ gene ใด
    1. ORCC
    2. ENaC
    3. CFTR
    4. CFTE
    5. CFTY

3. ข้อใดไม่เกี่ยวข้องกับกลไกการเกิด Cystic fibrosis
    1. ROS production
    2. GSH depletion
    3. Apoptosis
    4. Inflammation
    5. Sodium channel

4. ข้อใดไม่เกี่ยวข้องกับ NADPH oxidases
    1. Respiratory burst
    2. ROS generation
    3. Inflammation
    4. GSH depletion
    5. Electron transfer

5. ข้อใดไม่เกี่ยวข้องกับ NADPH oxidase
    1. DUOX
    2. FAD
    3. $gp91^{phox}$
    4. NOX6
    5. NOX1

6. องค์ประกอบใดสำคัญในการทำงานของ NADPH oxidases
   1. $P67^{phox}$
   2. $p47^{phox}$
   3. $p40^{phox}$
   4. $p22^{phox}$
   5. $p21^{phox}$

7. ข้อใดคือบทบาทของ NADPH oxidases ใน cystic fibrosis
   1. Host defense
   2. Osteoarthritis
   3. Edema
   4. Trauma
   5. Autoimmune

8. ข้อใดไม่เกี่ยวข้องกับ GSH deficiency
   1. เพิ่ม NF-kB
   2. เพิ่ม oxidative stress
   3. ลด infection
   4. เพิ่ม apoptosis
   5. เพิ่ม inflammation

9. GSH คืออะไร
   1. ROS
   2. Antioxidant
   3. Oxidative stress
   4. NADPH oxidase
   5. FAD

10. ข้อใดเกี่ยวข้องกับ GSH ใน cystic fibrosis
   1. Decreased ROS
   2. Decreased infection
   3. Defective CFTR
   4. Synthesis NADPH oxidases
   5. ROS production