NADPH oxidase mediated oxidative stress in hepatic fibrogenesis
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NADPH oxidase (NOX) is a multicomponent enzyme complex that generates reactive oxygen species (ROS) in response to a wide range of stimuli. ROS is involved as key secondary messengers in numerous signaling pathways, and NADPH oxidases complex has been increasingly recognized as key elements of intracellular signaling of hepatic fibrogenesis. In the liver, NADPH oxidase is functionally expressed both in the phagocytic form and in the non-phagocytic form. The non-phagocytic NADPH oxidase complex is structurally and functionally similar to the phagocytic NADPH, resulting in reduction of molecular oxygen to generate superoxide. There are six homologous NOX proteins in the non-phagocytic forms of NADPH oxidase. An emerging concept is that both phagocytic and nonphagocytic NADPH oxidase components in hepatic stellate cells (HSCs) mediate hepatic fibrosis, suggesting its potential role as a pharmacological target for anti-fibrotic therapy. The molecular components and signaling pathways of various NADPH oxidase homologues that are critical for the fibrotic activity in HSCs need to be more clearly identified. (Korean J Hepatol 2011;17:251-257)

Keywords: NADPH oxidase; Reactive oxygen species; Oxidative stress; Hepatic fibrosis; Hepatic stellate cell

INTRODUCTION

Chronic hepatic inflammation which is caused by excessive alcoholic consumption, hepatitis B or C virus, and non-alcoholic steatohepatitis results in hepatic fibrosis.¹ The terminal outcome of liver fibrosis is liver cirrhosis, characterized by excessive deposition of extracellular matrix proteins and the appearance of regenerative nodules, accompanied by hepatic failure, portal hypertension and hepatocellular carcinoma. Portal hypertension may cause serious complications such as esophageal variceal bleeding, ascites, and hepatic encephalopathy that are the major causes of death in cirrhotic patients. However, there is no effective anti-fibrotic therapy to treat or reverse hepatic fibrosis and liver cirrhosis. Therefore there is an urgent need to develop anti-fibrotic agent through the research for the molecular pathogenesis of hepatic fibrogenesis.

The hepatic stellate cell (HSC) is the main fibrogenic cell type in the injured liver and it also has been identified as an important effector in liver inflammation.¹ HSCs are located in the space of Disse in close proximity to hepatocytes on one side and endothelial and Kupffer cells on the other side. Quiescent HSCs are desmin-positive, perisinusoidal cells that are the primary cell in the body responsible for vitamin A storage.¹ Upon activation by liver injury, quiescent HSCs transdifferentiate into myofibroblast which produce inflammatory cytokines and several extracellular matrix proteins and glycoproteins including at least five collagen types, fibronectin, laminin, entactin, tenascin, and several proteoglycans.²

Reactive oxygen species (ROS) are involved as key secondary messengers in numerous signaling pathways including...
transcriptional regulation, differentiation, proliferation to oncogenic transformation, and activation of programmed cell death. It has previously been demonstrated that ROS significantly contributes hepatic fibrogenesis from various liver injuries including alcohol abuse, hepatitis C virus infection, iron overload and chronic cholestasis. ROS can stimulate the production of the type I collagen and may act as an intracellular signaling mediator of the fibrogenic action of TGF-β. ROS may be generated by multiple sources including mitochondrial respiratory chain, cytochrome p450E1, peroxisomes, and NADPH oxidases (NOXs) in the liver. Cumulating evidences indicate the critical role of NOX-mediated ROS in hepatic fibrogenesis which will be described in this review.

### NADPH oxidase homologues and components

NOX is a multicomponent enzyme complex that generates ROS in response to a wide range of stimuli including TNF-α, IL-1β, leptin and angiotensin II (Ang II). NOX generates superoxide (O2·⁻) from molecular oxygen using NADPH as an electron donor, and superoxide converts to hydrogen peroxide (H2O2) by superoxide dismutase (SOD). Classic NOX is the phagocytic NOX found in neutrophils. The phagocytic NOX complex consists of the catalytic subunit gp91phox (renamed NOX2) together with the regulatory subunit p22phox located in the membrane. The other regulatory components p47phox, p40phox, p67phox and the small GTPase Rac are normally located in the cytoplasm. Upon stimulation with agonists, the cytosolic subunits translocate to the membrane-bound cytochrome complex leading to enzymatic activity. Humans have 4 additional homologous NOX proteins (NOX1, NOX3, NOX4, NOX5) and 2 related enzymes (DUOX1, DUOX2) that may function in non-phagocytes. Among the seven members of the NOX family, NOX1 is structurally and functionally similar to NOX2. While NOX1 is also p22phox-dependent, NOX1 may use NOXO1 (homologue of p47phox) to organize the enzyme assembly and NOXA1 (homologue of p67phox) for enzyme activation. In contrast, NOX4 requires only p22phox without recruitment of cytosolic regulatory components to exert its enzymatic activity. All NOX enzymes are able to catalyze the reduction of molecular oxygen to superoxide, but there are key differences in their activation, subunit composition, localization, and expression (Table 1, Fig. 1).

### Table 1. The components of NADPH oxidase homologues

<table>
<thead>
<tr>
<th>Classification</th>
<th>Membrane bound components</th>
<th>Cytoplasmic components</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOX2</td>
<td>NOX2, P22phox</td>
<td>p40phox, p47phox, p67phox, Rac</td>
</tr>
<tr>
<td>NOX1</td>
<td>NOX1, p22phox</td>
<td>p40phox, p47phox or NOXO1, P67phox or NOXA1, Rac</td>
</tr>
<tr>
<td>NOX3</td>
<td>NOX3, p22phox</td>
<td>p40phox, NOXO1, NOXA1, Rac</td>
</tr>
<tr>
<td>NOX4</td>
<td>NOX4, p22phox</td>
<td>p40phox, p47phox, p67phox, Rac</td>
</tr>
</tbody>
</table>

NOX, NADPH oxidase; NOXO, NADPH oxidase organizer; NOXA, NADPH oxidase activator; Rac, Ras-related C3 botulinum toxin substrate.

### NADPH oxidase-mediated ROS generation and human diseases

Chronic granulomatous disease (CGD) has been described for several decades as a human disease resulting from defects in NOX complex. The genetic defect of phagocytic NOX activity results in an inability to produce the superoxide anion necessary for killing bacteria and fungi by neutrophils and phagocytes. The patients with CGD predispose to serious bacterial and fungal infections as well as granulomatous complications. It has been reported that at least 5 different genes involved in NOX activity may cause CGD. The gene mutation of NOX2 on the X chromosome account for about two thirds of the cases and present as severe form with earlier symptom manifestation. Meanwhile, mutations in p47phox, p67phox, and p22phox account for about one thirds of the cases as less severe form characterized by autosomal recessive inheritance.

In contrast with CGD that are defective in NOX function, several human diseases are associated with excessive ROS production by an overactive NOX. NOX mediated oxidative stress may cause endothelial dysfunction, vascular smooth muscle contraction and hypertrophy that results in various vascular diseases such as hypertension, hypercholesterolemia,