Prx I  Prx II
Prx I  Prx II

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...
1. Prx I / Prx II mRNA

2. Prx I / Prx II mRNA

3. Prx I / Prx II mRNA

IV. ...
1. Prx I protein in adult rat lungs exposed to normoxia and hyperoxia ........................................ 9
2. Prx II protein in adult rat lungs exposed to normoxia and hyperoxia ........................................ 9
3. Prx I and Prx II protein in neonatal rat lungs exposed to normoxia and hyperoxia ......................... 10
4. Prx I protein in bronchoalveolar lavage fluid of adult rats exposed to normoxia and hyperoxia ........ 10
5. Prx II protein in bronchoalveolar lavage fluid of adult rats exposed to normoxia and hyperoxia ........ 11
6. Prx I mRNA in adult rat lungs exposed to normoxia and hyperoxia .............................................. 11
7. Prx II mRNA in adult rat lungs exposed to normoxia and hyperoxia .............................................. 12
8. Prx I and Prx II mRNA in neonatal rat lungs exposed to normoxia and hyperoxia ............................ 12
Peroxiredoxin (Prx) is a thiol-dependent peroxidase. It has multiple isoforms (Prx I, Prx II) that are regulated by antioxidant enzymes (CuZnSOD, Catalase, Glutathione Peroxidase) and anti-oxidative enzymes (antioxidant enzymes). Prx protects cells from oxidative stress (oxygen toxicity).

Peroxiredoxin (Prx) acts in a thioredoxin-dependent manner. Prx reduces thioredoxin (Trx), thioredoxin reductase (TR) is also involved in cell protection.

Isoforms Prx I, Prx II, Prx mRNA, Prx I mRNA, Prx II mRNA.
mRNA と細胞内から mRNA と遺伝子の形で遺伝を引き継ぎます。Prx と mRNA の関係を示す Prx が Prx の予測 mRNA の関係を示す。Prx mRNA と細胞内から mRNA と遺伝を引き継ぎます。Prx が Prx の予測 mRNA の関係を示す。

親子の関係 : antioxidant enzyme, peroxiredoxin, hyperoxia, neonate
(acute respiratory distress syndrome: ARDS) 1) O$_2^-$  2) superoxide dismutase(SOD)  3) spontaneous dismutation  4) H$_2$O$_2$  5) OH$^-$  6) O$_2^-$, H$_2$O$_2$  7) OH$^-$  8) catalase  9) superoxide dismutase  (recruitment)  10) (activation)  11) (necrosis)  12) (apoptosis)  13) (diffuse alveolar damage)  

1) Prx I  2) Prx II  

-3-
(multiorgan failure)\(^{8-10}\) MnSOD, Cu/ZnSOD \(^{12}\) glutathione peroxidase(GPx)\(^{8,10}\) (sublethal dose)\(^{11}\) (resistant to high oxygen)\(^{12}\) glutathione peroxidase(GPx)\(^{8-10}\) (resistant to high oxygen)\(^{12}\) Glutathione peroxidase(GPx)\(^{8-10}\)

Peroxiredoxin(Prx)\(^{14-16}\) 6-Prx \(^{14-16}\) Prx I - IV\(^{2-}:\) Cys Prx\(^{2}\) thioredoxin system(NADPH, thioredoxin reductase, thioredoxin)\(^{17-18}\) thioredoxin-dependent peroxidase(TPx)\(^{17-18}\) 1-Cys Prx\(^{14-16}\) catalase \(^{14-16}\) GPx\(^{14-16}\) catalase \(^{14-16}\) peroxisome \(^{14-16}\) H\(_2\)O\(_2\) \(^{14-16}\) Prx \(^{14-16}\) K\(_{cat}\) \(^{14-16}\) H\(_2\)O\(_2\) \(^{14-16}\) Prx \(^{14-16}\) H\(_2\)O\(_2\) \(^{14-16}\) Prx \(^{14-16}\) H\(_2\)O\(_2\) \(^{14-16}\) 0.1-0.4% \(^{14-16}\) Prx \(^{14-16}\) H\(_2\)O\(_2\) \(^{14-16}\) Prx \(^{14-16}\) H\(_2\)O\(_2\) \(^{14-16}\) 0.1-0.4% \(^{14-16}\) Prx \(^{14-16}\) H\(_2\)O\(_2\) \(^{14-16}\) Prx \(^{14-16}\) H\(_2\)O\(_2\) \(^{14-16}\) Prx \(^{14-16}\) H\(_2\)O\(_2\) \(^{14-16}\)

2- Cys Prx \(^{14-16}\) Prx I \(^{14-16}\) Prx II \(^{14-16}\) Prx I\(^{14-16}\) S- phase
DMSO, Prx II, Molt-4, serum deprivation, ceramide, etoposide, apoptosis, Prx II, DMSO, H2O2, cisplatin

Prx II, cisplatin, apoptosis, SNU638, H2O2, cisplatin

Prx II

Prx II, cisplatin, apoptosis

Prx II

Mouse monocyte-macrophage cells, menadione, Prx I, Prx II, Prx II

Prx I

Mouse monocyte-macrophage cells, menadione, Prx I

thioredoxin, thioredoxin reductase

thioredoxin system (Trx, TR)

thioredoxin (Trx, TR)
1. MATERIALS

Rat (Sprague-Dawley) & CO₂ & 99.9% & 1.2 cm² x 1.0 cm³ & acryl chamber & 90% & Beckman gas analyzer (Beckman Coulter Inc, Fullerton, CA, USA) & 6, 12, 24, 48, 72 & 3 & 24 & 3 & ether & -80°C & 12 & 2 & 3000 rpm & 5 & -80°C &

2. METHODS

phosphate-buffered saline (PBS) & 2 mM phenylmethylsulfonyl fluoride (PMSF), 5 µg/ml aprotinin & 1 µg/ml leupeptin & 20 mM Hepes- NaOH (pH 7.0) & 10,000 rpm & 30 & 10,000 rpm & 1 & BCA reagent (Pierce Inc, Rockford, IL, USA)
Westernblot:

- 12% SDS- polyacrylamide gel
- nitrocellulose membrane
- 2% BSA
- TTBS
- 15 min
- anti- Prx I, anti- Prx II
- 3 min
- TTBS
- alkaline phosphatase conjugated goat anti- rabbit IgG
- goat anti- actin antibody
- Fluor- STM Multi- Imager

Northernblot:

- TRI Reagent
- RNA
- 260
- 50 RNA
- 1% agarose- 0.66 M formaldehyde gel
- nylon membrane
- pCRPrx I, pCRPrx II
- Prx I, Prx II
- cDNA
- hybridization random labeling kit
- [32P] dCTP labeling
- actin cDNA
- Fluor- STM Multi- Imager
- Prx/actin mRNA
III. 図

図．図表

図．図表 1 2 の Prx I Prx II の比較を示す。図表 3 24 時間後の Prx I Prx II の比較を示す。

図．図表 4 5 の Prx II の mRNA の比較を示す。図表 6 7 の Prx I mRNA の比較を示す。図表 8 の Prx II mRNA の比較を示す。
1. **Prx I protein in adult rat lungs exposed to normoxia and hyperoxia.** Prx I protein and Actin protein in 3 separate adult rat lungs exposed to normoxia and hyperoxia were measured by Westernblot analysis, and the data are given as ratio. Hyperoxia did not induce obvious alteration of Prx I protein all the times in adult rat lungs.

2. **Prx II protein in adult rat lungs exposed to normoxia and hyperoxia.** Prx I protein and Actin protein in 3 separate adult rat lungs exposed to normoxia and hyperoxia were measured by Westernblot analysis, and the data are given as ratio. Hyperoxia did not induce obvious alteration of Prx II protein all the times in adult rat lungs.
3. Prx I and Prx II protein in neonatal rat lungs exposed to normoxia and hyperoxia. Prx I and Prx II protein and Actin protein in 3 separate neonatal rat lungs exposed to normoxia and hyperoxia were measured by Westernblot analysis, and the data are given as ratio. Hyperoxia did not induce obvious alteration of Prx I and Prx II protein at 24 hours in neonatal rat lungs.

4. Prx I protein in bronchoalveolar lavage fluid of adult rats exposed to normoxia and hyperoxia. Prx I protein and Actin protein in 3 separate adult rat lungs exposed to normoxia and hyperoxia were measured by Westernblot analysis, and the data are given as ratio. Hyperoxia did not induce obvious alteration of Prx I protein all the times in bronchoalveolar lavage fluid of adult rats.
5. Prx II protein in bronchoalveolar lavage fluid of adult rats exposed to normoxia and hyperoxia. Prx II protein and Actin protein in 3 separate adult rats exposed to normoxia and hyperoxia were measured by Westernblot analysis, and the data are given as ratio. Hyperoxia did not induce obvious alteration of Prx II protein all the times in bronchoalveolar lavage fluid of adult rats.

6. Prx I mRNA in adult rat lungs exposed to normoxia and hyperoxia. Prx I mRNA and Actin mRNA in 3 separate adult rat lungs exposed to normoxia and hyperoxia were measured by Northernblot analysis, and the data are given as ratio. Hyperoxia did not induced obvious increase of Prx I mRNA at 24 hour in adult rat lungs.
7. Prx II mRNA in adult rat lungs exposed to normoxia and hyperoxia. Prx II mRNA and Actin mRNA in 3 separate adult rat lungs exposed to normoxia and hyperoxia were measured by Northern blot analysis, and the data are given as ratio. Hyperoxia did not induce obvious alteration of Prx II mRNA all the time in adult rat lungs.

8. Prx I and Prx II mRNA in neonatal rat lungs exposed to normoxia and hyperoxia. Prx I and Prx II mRNA and Actin mRNA in 3 separate neonatal rat lungs exposed to normoxia and hyperoxia were measured by Northern blot analysis, and the data are given as ratio. Hyperoxia induce marked increase of Prx I mRNA at 24 hours in neonatal rat lungs (*P<0.05). But hyperoxia did not induce obvious alteration of Prx II mRNA at 24 hour in neonatal rat lungs.
Peroxiredoxin I & II mRNA levels were evaluated in ARDS patients. Peroxiredoxin I & II mRNA levels were significantly increased compared to control levels.

ARDS (acute respiratory distress syndrome) is a severe form of pulmonary injury that often leads to multi-organ failure. The peroxiredoxin transcripts were examined in ARDS patients. The peroxiredoxin I & II mRNA levels were significantly increased compared to control levels.

In ARDS patients, the peroxiredoxin I & II mRNA levels were significantly increased compared to control levels.
Prx I / Prx II mRNA ratio increases significantly by several-fold. This process is observed in vitro.

24 hours after exposure, Prx II mRNA levels are significantly increased compared to Prx I mRNA. This effect is not observed in isolated Prx I mRNA. After 24 hours, Prx I mRNA levels return to baseline. However, Prx II mRNA levels remain significantly increased.

Translation (translation) and posttranslational modification (posttranslational modification) of Prx I and Prx II also play a role in their expression and function.
isolectric point (pI) 

Prx I 

Prx II 

Prx I mRNA 

Prx II mRNA 

Prx I mRNA
V.

1. Peroxiredoxin (Prx) I or II mRNA
2. Peroxiredoxin I or II mRNA
3. mRNA of Prx I or II Prx I or II mRNA

1. 24-hour  Prx I or II Prx I or II mRNA
2. Prx I or II mRNA
3. Prx I or II mRNA

V. 1

1. Peroxiredoxin (Prx) I or II mRNA
2. Peroxiredoxin I or II mRNA
3. mRNA of Prx I or II Prx I or II mRNA
4. Prx I or II mRNA
5. Prx I or II mRNA

- 16 -

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Abstract

Expression of peroxiredoxin I and II in neonatal and adult rat lung exposed to hyperoxia

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(Directed by Professor Hyung Jung Kim)

In mammals, to adapt hyperoxia, there is gradual increase in the activities of multiple antioxidant enzymes in neonatal stage. And the relative tolerance of newborn animals to hyperoxia compared to adult animals is known to be closely associated with augmented induction of antioxidant enzymes.

Peroxiredoxin (Prx) in vivo reduces hydrogen peroxide with thioredoxin system, and Prx I and II as abundant cytosolic antioxidant proteins remove hydrogen peroxide generated during normal oxidative metabolism and stimulation of cell surface receptors.

The goal of this study was to examine expression of Prx I and II mRNA and protein in adult rat lungs in response to hyperoxia and compare with those of neonatal rat lungs in response to hyperoxia.

Adult Sprague-Dawley rats and neonates delivered from timed-pregnant Sprague-Dawley rats were randomly exposed to normoxia or hyperoxia (FiO₂ > 0.9).
For hyperoxia studies, the rats were exposed to hyperoxia in 3.4- ft² plastic chambers in which O₂ (>90%), CO₂ (<0.1%), room temperature, and humidity (40- 60%) were monitored. Air- breathing rats were exposed in identical chambers in which air (FiO₂=0.21) flowed from a compressed air generator. After exposure of high oxygen, animals were sacrificed by cutting the abdominal aorta and exsanguination, The broncholalveolar lavage was done in the right lungs with 3 times with 4 ml of normal saline and the left lungs were removed.

The expression of Prx I and Prx II protein in lung tissues and broncholalveolar lavage fluids were measured by western blot analysis using polyclonal rabbit anti- Prx I or anti- Prx II antibody. And the relative expression of Prx I and Prx II protein per actin protein as internal standard were obtained. The expression of Prx I and Prx II mRNA in lung tissues were measured by northern blot analysis using Prx I- and Prx II - specific cDNAs prepared from pCRPrx I and pCRPrx II. And the relative expression of Prx I and Prx II mRNA per actin mRNA as internal standard were obtained.

Hyperoxia did not induce obvious alteration of expression of Prx I and Prx II protein all the times in adult rat lungs(Fig 1, 2). And also did not induce obvious alteration of expression of Prx I and Prx II protein at 24 hours in neonatal rat lungs(Fig 3).

Hyperoxia did not induce obvious alteration of amount of Prx I and Prx II protein all the times in bronchoalveolar fluid of adult rats(Fig 4, 5).

Hyperoxia did not induce obvious alteration of expression of Prx I and Prx II mRNA all the times in adult rat lungs (Fig 6, 7). Interestingly, hyperoxia induced marked increase of Prx I mRNA at 24 hour in neonatal rats(Fig 8). But hyperoxia did not induce obvious alteration of Prx II mRNA at 24 hour in neonatal rats(Fig 8).
In conclusion, Prx I and II is differently regulated by hyperoxia in neonatal rat at transcriptional level. But the expression of Prx I and II protein by hyperoxia is not different. The upregulation of Prx I mRNA may be another mechanism of resistance to high oxygen and mechanism of posttranscriptional regulation of Prx I remained to be solved.

Key Words : antioxidant enzyme, peroxiredoxin, hyperoxia, neonate.