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The role of oxidants in ischemic limb vascularization – redox signaling by glutaredoxin-1

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Oxidants, or reactive oxygen/nitrogen species (RONS) are increased in ischemic tissues. Although antioxidants are widely used, clinical trials do not prove their effectiveness to protect people from cardiovascular disease. Genetic modulation of the enzymatic source of oxidants or the antioxidant system in mouse reveal that physiological levels of oxidants are essential to promote the revascularization after femoral artery occlusion. However, the mechanism remains unclear. Oxidants transduce cellular signaling through oxidative modifications of redox sensitive protein thiols. Of particular importance, the reversible modification with abundant glutathione, called S-glutathionylation (or GSH adducts), is relatively stable and alters protein function including signaling, transcription, and cytoskeletal arrangement.

Glutaredoxin-1 (Glrx) is an enzyme which catalyzes reversal of GSH adducts, and does not scavenge oxidants itself. Thus, Glrx may control redox signaling under fluctuation of

oxidants levels. We show that Glrx ablation stabilizes HIF-1□ by increasing GSH adducts on Cys^{520} (mouse Cys^{533}) and promotes *in vivo* HIF-1□ stabilization, VEGF-A production and revascularization in the ischemic muscles. As an endogenous Glrx is anti-angiogenic, we also show that Glrx overexpression inhibits angiogenic function in endothelial cells and in vivo ischemic vascularization. There are several Glrx targets including HIF-1□□which may contribute to inhibition of vascularization by reducing GSH adducts.

In conclusion, these animal studies provide a caution that excess antioxidants can be harmful for treatment of ischemic limbs, and Glrx is a potentially therapeutic target to improve ischemic limb vascularization.

Short Biography

Reiko Matsui

NAME: Reiko Matsui
POSITION TITLE: Assistant Professor of Medicine

Education and Qualifications

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Kanazawa University (Japan)	M.D.	04/1980	Medicine

A. Personal Statement

I was trained for basic medical research in pulmonary and cardiovascular fields at Boston University after working in clinical medicine in Japan. I have a broad view of medicine including gerontology since I worked at Tokyo Metropolitan Geriatric Hospital as an internist, while I engaged in medical research at Tokyo Metropolitan Institute of Gerontology in my early years. I gained various experiences in research techniques for basic science projects in Boston University Medical Campus. I have been focusing on vascular biology and oxidative stress in Vascular Biology Section, and working on NIH-funded projects as a co-investigator. I instruct students and post-doc fellows and publish independently. Recent data we obtained are in part published, and have been used to propose grant applications to continue my research work. I am studying the roles of glutaredoxin-1 (Glrx) in cardiovascular disease. Glrx regulates cellular signaling and transcription by reversing glutathione post-translational protein modification. This relatively unexplored enzyme and its targets and function are just now being uncovered. The role of Glrx on hypoxia-induced vascularization in the recently-funded proposal is a relevant to therapeutic approaches to vascular complications in diabetes. With my long-term interest in clinical medicine, I am enthusiastic to continue my studies and develop this in the translational research.

1. Bachschmid MM, Xu S, Maitland-Toolan KA, Ho YS, Cohen RA, Matsui R. Attenuated cardiovascular hypertrophy and oxidants generation in response to angiotensin II infusion in glutaredoxin-1 knockout mice. Free Radic Biol Med 49:1221, 2010. (PMID: 20638471)

2. Shao D, Fry JL, Han J, Hou X, Pimentel DR, Matsui R, Cohen RA, Bachschmid MM. A redox-resistant sirtuin-1 mutant protects against hepatic metabolic and oxidant stress. J Biol Chem. 289(11):7293-306, 2014. (PubMed PMID: 24451382)

3. Murdoch CE, Shuler M, Haeussler DJ, Kikuchi R, Bearelly P, Han J, Watanabe Y, Fuster JJ, Walsh K, Ho YS, Bachschmid MM, Cohen RA, Matsui R. Glutaredoxin-1 up-regulation induces soluble vascular endothelial growth factor receptor 1, attenuating post-ischemia limb revascularization. J Biol Chem. 289(12):8633-44, 2014. (PubMed PMID: 24482236)

4. Watanabe Y, Murdoch CE, Sano S, Ido Y, Bachschmid MM, Cohen RA, Matsui R. Glutathione adducts induced by ischemia and deletion of glutaredoxin-1 stabilize HIF-1¤ and improve limb revascularization. Proc Natl Acad Sci USA 113:6011-6, 2016. (PMID 27162359)

5. Shao D, Han J, Hou X, Fry J, Behring J, Seta F, Long MT, Roy HK, Cohen RA, Matsui R, Bachschmid MM. Glutaredoxin-1 deficiency causes fatty liver and dyslipidemia by inhibiting sirtuin-1. Antioxid Redox Signal 2016 Dec 13 (ePub ahead of print)

B. **Positions and Honors**

1980-1981	Residency in Orthopedics, Jichi Medical College
1981-1984	Residency in Internal Medicine, Tokyo Metropolitan Geriatric Hospital
1984-1987	Medical Staff, Pulmonary Division, Tokyo Metropolitan Geriatric Hospital
1985-1987	Research member, Experimental Pathology, Tokyo Metropolitan Institute of Gerontology
1984-1987	Research Associate Member, Internal Medicine, Department of Medicine, Kanazawa University
1987-1989	Postdoctoral Fellow, Department of Pathology, University of British Columbia, British Columbia, Canada
1989-1993	Postdoctoral Fellow, Pulmonary Center, Boston University School of Medicine, Boston, MA
1993-1997	Research Associate, Pulmonary Center, Boston University School of Medicine, Boston, MA
1997- 2000	Research Associate, Vascular Biology Unit, Boston University School of Medicine, Boston, MA
2001- 2011	Research Assistant Professor, Vascular Biology Unit, Boston University School of Medicine, Boston, MA
2012-	Assistant Professor, Vascular Biology Section, Boston University School of Medicine, Boston.

C. **Contribution to Science**

My early contributions were involved in lung development. There is a hypothesis that interference of environment during development of the lung may determine predisposing conditions for pulmonary emphysema which is a major pathology of chronic obstructive lung disease. These studies revealed that iso-caloric low protein diet during early age of rats decreased connective tissue proteins in the lung and resulted in structural and functional abnormalities resembling emphysema. I started these studies when I was at Tokyo Metropolitan Institute of Gerontology, and continued further to examine the effects of modulation of connective tissues in pre-natal lung development in the lab of Dr. William Thurlbeck who was the expert in the field. Also, research for growth factors which control lung development was continued after I moved to Boston University.

1. Matsui R, Thurlbeck WM, Yu SY, Fujita Y, Kida K. Connective tissue, mechanical and morphometric changes in the lungs of weanling rats fed a low protein diet. Pediatric Pulmonology 7:159,1989. (PMCID: 279730)
2. Ofulue AF, Matsui R, Thurlbeck WM. Role of calmodulin as an endogenous initiatory factor in compensatory lung growth after pneumonectomy. Pediatric Pulmonology 15:145,1993. (PMCID: 8327276)
3. Matsui R, Thurlbeck WM, Shehata EI, Sekhon HS. Two different patterns of airway branching regulated by different components of the extracellular matrix in vitro. Exp Lung Res 22:593, 1996. (PMCID: 8979045)
4. Matsui R, Brody JS, Yu Q. FGF-2 induces surfactant protein gene expression in foetal rat lung epithelial cells through a MAPK-independent pathway. Cell Signal 11:221, 1999. (PMCID: 10353697)

Furthermore, I worked in the study of connective tissue in the lung, specially type I collagen which is the major structural protein of the lung. Accumulation of collagen is characteristic of lung fibrosis. Alveolar type 2 cells normally produce type IV collagen and fibronectin (components of basement membrane) but I found that alveolar epithelial cells immortalized by viral gene synthesize type I collagen. This data suggest viral infection may stimulate synthesis of type I collagen from alveolar cells and cause fibrosis.

1. Fine A, Matsui R, Zhan X, Policks CF, Smith BD, Goldstein RH. Discordant regulation of human type I collagen genes by prostaglandin E2. Biochemica Biophysica Acta 1135:67, 1992. (PMCID: 1375511)
2. Matsui R, Goldstein RH, Brody JS, Steele M, Fine A. Type I collagen formation in rat type II alveolar cells immortalized by viral gene products. Thorax 49:201, 1994. (PMCID: 8202874)

I have been working on vascular oxidative stress since I joined Vascular Biology. Oxidative and nitrosative stress cause post-translational modifications and modulate protein function. Tyrosine nitration is one of these modifications. Nitric oxide (NO) relaxes aortic smooth muscle and this response involves the calcium pump in ER (SERCA). We found that NO-induced aortic smooth muscle relaxation was impaired and SERCA activity was decreased in hypercholesterolemic aorta. Feeding with anti-oxidants improved SERCA function and decreased nitro-tyrosine on SERCA. These studies indicate that oxidative stress associated with hypercholesterolemia causes tyrosine nitration of SERCA which impairs activity, and impaired vascular relaxation in response to NO. Also, we worked on the method to quantitatively assess nitro-tyrosine of tissue samples of angiotensin II-infused rats.

1. Adachi T., Matsui R., Weisbrod R., Najibi S., Cohen RA. Reduced Sarco/Endoplasmic reticulum Ca2+ uptake activity can account for the reduced response to NO, but not sodium nitroprusside, in hypercholesterolemic aorta. Circulation 104:1040, 2001. (PMCID: 11524399)
2. Adachi T, Matsui R, Xu S, Kirber M, Lazar HL, Sharov V, Schoneich C, Cohen RA. Antioxidant improves sarco/endoplasmic reticulum Ca^{2+}ATPase function and lowers tyrosine nitration in hypercholesterolemia and improves nitric oxide-induced relaxation. Cir Res 90:1114, 2002. (PMCID: 12039802)
3. Guo W, Adachi T, Matsui R, Xu S, Jiang B, Zou MH, Kirber M, Lieberthal W, Cohen RA. Quantitative assessment of tyrosine nitration of manganese superoxide dismutase in angiotensin II-infused rat kidney. Am J Physiol Heart Cir Physiol. 285:H1396, 2003. (PMCID: 12791589)

One of my major contributions in vascular biology was the study of glucose-6-phosphate dehydrogenase (G6PD) deficiency. G6PD is a key enzyme of the pentose phosphate pathway which contributes to NADPH generation. G6PD deficiency is the most prevalent enzymopathy in humans, and was thought to worsen cardiovascular disease due to oxidative stress and hemolytic anemia. However, we found that angiotensin II -induced cardiovascular hypertrophy was attenuated in G6PD-deficient mice. There are many enzymes that depend on NADPH including the oxidant source, NADPH oxidase, and enzymes in the cholesterol synthesis pathway. We also demonstrated higher blood pressure, less atherosclerosis, lower oxidants and plasma cholesterol in G6PD-deficient mice with apoE-/- background. These studies are actually consistent with clinical reports on G6PD-deficient people, and confirmed a contribution of NADPH oxidase to vascular hypertrophy and atherosclerosis.

1. Matsui R, Xu S, Maitland KA, Hayes A, Leopold JA, Handy DE, Loscalzo J, Cohen RA. Glucose-6-phosphate dehydrogenase deficiency decreases the vascular response to angiotensin II. Circulation 112:257, 2005 (PMCID: 15998684)
2. Matsui R, Xu S, Maitland KA, Mastroianni R, Leopold JA, Handy DE, Loscalzo J, Cohen RA. Glucose-6-phosphate dehydrogenase deficiency decreases vascular superoxide and atherosclerotic lesion formation in ApoE-/- mice. Arterioscler Thromb Vasc Biol 26:910, 2006. (PMCID: 16439706)

D. **Research Support**

Current Research Support
RO1 HL133013 Matsui R (PI) 7/1/2016 -6/30/2020
Regulation of ischemic limb vascularization by glutaredoxin-1

The major goal of this project is to elucidate mechanism of redox-regulation of angiogenesis and lead to improvement diabetic ischemic limb.
Role: Primary investigator

RO3 AG051857-01 Matsui R (PI) 8/1/2016 – 04/30/2018
Regulation of ischemic vascularization by glutaredoxin-1 in aging

The goal of this project is to study the role of glutaredoxin-1 on ischemic limb revascularization in aged animals.
Role: Primary investigator

The list of the publications:

Publications as corresponding author are marked as bold.

1. Gorelenkova Miller O, Behring JB, Siedlak SL, Jiang S, Matsui R, Bachschmid MM, Zhu X, Mieyal JJ. Upregulation of glutaredoxin-1 activates microglia and promotes neurodegeneration: Implication for Parkinson's disease. Antioxid Redox Signal. 25:967-82, 2016. (PubMed PMID: 27224303; PubMed Central PMCID: PMC5175443).

2. Shao D, Han J, Hou X, Fry J, Behring J, Seta F, Long MT, Roy HK, Cohen RA, Matsui R, Bachschmid MM. Glutaredoxin-1 deficiency causes fatty liver and dyslipidemia by inhibiting sirtuin-1. Antioxid Redox Signal 2016 Dec 13 (ePub ahead of print)

3. Han J, Weisbrod RM, Shao D, Watanabe Y, Yin X, Bachschmid MM, Seta F, Janssen-Heininger YM, Matsui R, Zang M, Hamburg NM, Cohen RA. The redox mechanism for vascular barrier dysfunction associated with metabolic disorders: Glutathionylation of Rac1 in endothelial cells. Redox Biol 9:306-19, 2016. (PMID: 27693992; PMCID: PMC5045950).

4. Watanabe Y, Murdoch CE, Sano S, Ido Y, Bachschmid MM, Cohen RA, **Matsui R.** Glutathione adducts induced by ischemia and deletion of glutaredoxin-1 stabilize HIF-1a and improve limb revascularization. Proc Natl Acad Sci USA 113:6011-6, 2016. (PMID 27162359, PMCID: PMC4889374).

5. Yao C, Behring JB, Shao D, Sverdlov AL, Whelan SA, Elezaby A, Yin X, Siwik DA, Seta F, Costello CE, Cohen RA, Matsui R, Colucci WS, McComb ME, Bachschmid MM.

Overexpression of Catalase Diminishes Oxidative Cysteine Modifications of Cardiac Proteins. PLoS One 10:e0144025, 2015. (PMID: 26642319; PMCID: PMC4671598).

6. Murdoch CE, Shuler M, Haeussler DJ, Kikuchi R, Bearelly P, Han J, Watanabe Y, Fuster JJ, Walsh K, Ho YS, Bachschmid MM, Cohen RA, **Matsui R.** Glutaredoxin-1 up-regulation induces soluble vascular endothelial growth factor receptor 1, attenuating post-ischemia limb revascularization. J Biol Chem. 289(12):8633-44, 2014. (PMID: 24482236, PMCID:PMC3961686)

7. Shao D, Fry JL, Han J, Hou X, Pimentel DR, Matsui R, Cohen RA, Bachschmid MM. A redox-resistant sirtuin-1 mutant protects against hepatic metabolic and oxidant stress. J Biol Chem. 289(11):7293-306, 2014. (PMID: 24451382, PMCID: PMC3953247)

8. Bachschmid MM, Xu S, Maitland-Toolan KA, Ho YS, Cohen RA, **Matsui R.** Attenuated cardiovascular hypertrophy and oxidants generation in response to angiotensin II infusion in glutaredoxin-1 knockout mice. Free Radic Biol Med 49:1221, 2010. (PMID: 20638471, PMCID: PMC2930025)
9. **Matsui R**, Xu S, Maitland KA, Mastroianni R, Leopold JA, Handy DE, Loscalzo J, Cohen RA. Glucose-6-phosphate dehydrogenase deficiency decreases vascular superoxide and atherosclerotic lesion formation in ApoE-/- mice. Arterioscler Thromb Vasc Biol 26:910, 2006. (PMCID: 16439706)

10. **Matsui R**, Xu S, Maitland KA, Hayes A, Leopold JA, Handy DE, Loscalzo J, Cohen RA. Glucose-6-phosphate dehydrogenase deficiency decreases the vascular response to angiotensin II. Circulation 112:257, 2005 (PMCID: 15998684)

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12. Adachi T, Matsui R, Xu S, Kirber M, Lazar HL, Sharov V, Schoneich C, Cohen RA. Antioxidant improves sarco/endoplasmic reticulum Ca^{2+}ATPase function and lowers tyrosine nitration in hypercholesterolemia and improves nitric oxide-induced relaxation. Cir Res 90:1114, 2002. (PMCID: 12039802)

13. Adachi T., Matsui R., Weisbrod R., Najibi S., Cohen RA. Reduced Sarco/Endoplasmic reticulum Ca2+ uptake activity can account for the reduced response to NO, but not sodium nitroprusside, in hypercholesterolemic aorta. Circulation 104:1040, 2001. (PMCID: 11524399)

14. Matsui R, Brody JS, Yu Q. FGF-2 induces surfactant protein gene expression in foetal rat lung epithelial cells through a MAPK-independent pathway. Cell Signal 11:221, 1999. (PMCID: 10353697)

15. Matsui R, Thurlbeck WM, Shehata EI, Sekhon HS. Two different patterns of airway branching regulated by different components of the extracellular matrix in vitro. Exp Lung Res 22:593, 1996. (PMCID: 8979045)

16. Nagai A, Katayama M, Thurlbeck WM, Matsui R, Yasui S, Konno K. Effect of indomethacin on lung development in postnatal rats: possible role of prostaglandin in alveolar formation. Am J Physiol 268:L56, 1995. (PMCID: 7840229)

17. Matsui R, Goldstein RH, Brody JS, Steele M, Fine A. Type I collagen formation in rat type II alveolar cells immortalized by viral gene products. Thorax 49:201, 1994. (PMCID: 8202874)

18. Ofulue AF, Matsui R, Thurlbeck WM. Role of calmodulin as an endogenous initiatory factor in compensatory lung growth after pneumonectomy. Pediatric Pulmonology 15:145, 1993. (PMCID: 8327276)
19. Massoud EAS, Sekhon HS, Puterman M, Rotschild A, Matsui R, Thurlbeck WM. In vitro branching morphogenesis of the fetal rat lung. Pediatric Pulmonology 15:89, 1993. (PMCID 7682683)

20. Fine A, Matsui R, Zhan X, Policks CF, Smith BD, Goldstein RH. Discordant regulation of human type I collagen genes by prostaglandin E2. Biochemica Biophysica Acta 1135:67, 1992. (PMCID: 1375511)

21. Matsui R, Thurlbeck WM, Yu SY, Fujita Y, Kida K. Connective tissue, mechanical and morphometric changes in the lungs of weanling rats fed a low protein diet. Pediatric Pulmonology 7:159, 1989. (PMCID: 279730)

22. Mizuuchi T, Karube S, Matsui R, Fujimori S, Ohta K, Kida K. Pulmonary complications subsequent to fractured neck of the femur in the elderly. Nihon Ronen Igakkai Zasshi. 26:174-8, 1989. (PMID: 2795969)

23. Yamanouchi H, Matsui R, Tomonaga M, Sakurai H, Yoshimura M, Shimada H. Unilateral hemispheric cerebral changes similar to Creutzfeldt-Jacob disease in case of hemiconvulsion. Acta Neuropathol 76:316, 1988. (PMCID: 3063050)

24. Matsuse T, Kida K, Matsui R, Mizuuchi T, Matsuka Y, Fukuchi Y, Orimo H. Pseudomonas aeruginosa bacteremia in the elderly. Nihon Ronen Igakkai Zasshi 25:153-9, 1988. (PMID 3418951)

25. Mizuuchi T, Yamaoka M, Kida K, Matsui R, Urayama K, Inamatsu T, Shimada K. A case of Legionella pneumophila serogroup III with pulmonary carcinoma: the first report in Japan. Nihon Naika Gakkai Zasshi 75:654-9, 1986. (PMID 3746079)

26. Kida K, Utsuyama M, Ohta T, Matsui R. Effects of repeated administration of elastase on lungs of rats with experimental diabetes mellitus. Nihon Kyobu Shikkan Gakkai Zasshi 24:56-62, 1986.

Editorials and Critical Reviews

1. Watanabe Y, Cohen RA, **Matsui R**. Redox regulation of ischemic angiogenesis - Another aspect of reactive oxygen species. (Review) Circ J. 80(6):1278-84, 2016 (PubMed PMID: 27151566).

2. Cohen RA, Murdoch CE, Watanabe Y, Bolotina VM, Evangelista AM, Haeussler DJ, Smith MD, Mei Y, Tong X, Han J, Behring JB, Bachschmid MM, **Matsui R**. Endothelial cell redox regulation of ischemic angiogenesis. (Review) J Cardiovasc Pharmacol. 67(6):458-64, 2016. (PubMed PMID: 26927696; PubMed Central PMCID: PMC4899292).

3. Murdoch CE, Bachschmid MM, Matsui R. Regulation of neovascularization by S-glutathionylation via the Wnt5a/sFlt-1 pathway. (Review) Biochem Soc Trans. 42:1665-70, 2014. (PubMed PMID: 25399587; PubMed Central PMCID: PMC4934611).

4. Pimentel D, Haeussler DJ, Matsui R, Burgoyne JR, Cohen RA, Bachschmid MM. Regulation of cell physiology and pathology by protein S-glutathionylation: lessons learned from the cardiovascular system. (Review) Antioxid Redox Signal 16(6):524, 2012. (PubMed PMID: 22010840; PubMed Central PMCID: PMC3270052)

5. Bachschmid MM, Schildknecht S, Matsui R, Zee R, Haeussler D, A Cohen R, Pimental D, Loo BV. Vascular aging: Chronic oxidative stress and impairment of redox signaling-consequences for vascular homeostasis and disease. (Review) Ann Med. 45:17-36, 2012. (PubMed PMID: 22380696; PubMed Central PMCID: PMC3717565)

Textbook Chapters:

1. Matsui R. Chapter 25 Growth factors. In Molecular Biology of Lung Disease, edited by Kawakami Y, Taniguchi N, Kida K. Igaku-Shoin Ltd, Tokyo 1998.