Oxidative Stress in Applied Basic Research and Clinical Practice

Toshio Miyata
Kai-Uwe Eckardt
Masaomi Nangaku *Editors*

Studies on Renal Disorders

Humana Press

Oxidative Stress in Applied Basic Research and Clinical Practice

Editor-in-Chief
Donald Armstrong

For other titles published in this series, go to
www.springer.com/series/8145

Note from the Editor-in-Chief

All books in this series illustrate point-of-care testing and critically evaluate the potential of antioxidant supplementation in various medical disorders associated with oxidative stress. Future volumes will be up-dated as warranted by emerging new technology, or from studies reporting clinical trials.

Donald Armstrong
Editor-in-Chief

Toshio Miyata • Kai-Uwe Eckardt
Masaomi Nangaku
Editors

Studies on Renal Disorders

Humana Press

Editors
Toshio Miyata
Division of Molecular Medicine
and Therapy
United Centers for Advanced Research
and Translational Medicine (ART)
Tohoku University Graduate School
of Medicine
2-1 Seiryo-Machi, Aoba-ku
Sendai, Miyagi 980-8575
Japan
miyata@med.tohoku.ac.jp

Kai-Uwe Eckardt
Department of Nephrology
and Hypertension
Friedrich-Alexander-University
Erlangen-Nuremberg
Krankenhausstrasse 10-12 D-91054
Erlangen, Germany
Kai-Uwe.Eckardt@med4.med.
uni-erlangen.de

Masaomi Nangaku
University of Tokyo School of Medicine
7-3-1 Hongo, Bunkyo-ku
Tokyo 113-8655
Japan
mnangaku-tky@umin.ac.jp

ISBN 978-1-60761-856-0 e-ISBN 978-1-60761-857-7
DOI 10.1007/978-1-60761-857-7
Springer New York Dordrecht Heidelberg London

Printed on acid-free paper

Humana Press is part of Springer Science+Business Media (www.springer.com)

Preface

Broad derangements of oxygen metabolism, such as oxidative stress and hypoxia, have been implicated in the genesis of kidney disease, independently of hemodynamic and metabolic abnormalities. They further impact various biological reactions linked to oxygen metabolism, such as nitrosative stress, advanced glycation, carbonyl stress, and endoplasmic reticulum stress. This causal role of impaired oxygen metabolism in kidney disease has implications for our understanding of current therapeutic benefits accruing from antihypertensive agents, the control of hyperglycemia/hyperinsulinemia or of hyperlipidemia, and the dietary correction of obesity. The defense mechanisms against oxidative stress (e.g., the Nrf2-Keap1 system) and hypoxia (e.g., the HIF-PHD system) have been recently explored in various cells, including kidney cells, and they include intracellular sensors for oxidative stress and hypoxia. Novel approaches targeting these sensors may offer clinical benefits in several disorders in which oxidative stress or hypoxia is a final, common pathway. Leading basic researchers and clinical scientists have contributed to this book and provide up-to-date, cutting-edge reviews on recent advances in the pathobiology of oxygen metabolism in kidney disease, especially oxidative stress and hypoxia.

Sendai — Toshio Miyata, MD, PhD
Erlangen — Kai-Uwe Eckardt, MD
Tokyo — Masaomi Nangaku, MD, PhD
February 8, 2010

Contents

Part I Oxidative Stress

1 Oxidative Stress Injury in Glomerular Mesangium 3
Josef Pfeilschifter and Karl-Friedrich Beck

2 Transition Metals and Other Forms of Oxidative Protein Damage in Renal Disease 25
Vincent M. Monnier, Ina Nemet, David R. Sell, and Miriam F. Weiss

3 Cyclooxygenase in the Kidney and Oxidative Stress 51
Raymond C. Harris

4 Renin-Angiotensin System in the Kidney and Oxidative Stress: Local Renin-Angiotensin-Aldosterone System and NADPH Oxidase-Dependent Oxidative Stress in the Kidney 71
Hideyasu Kiyomoto, Hiroyuki Kobori, and Akira Nishiyama

5 Thiamine in Diabetic Renal Disease: Dietary Insufficiency, Renal Washout, Antistress Gene Response, Therapeutic Supplements, Risk Predictor, and Link to Genetic Susceptibility 93
Paul J. Thornalley and Naila Rabbani

6 Novel Members of the Globin Family and Their Function Against Oxidative Stress 105
Hiroshi Nishi and Masaomi Nangaku

Part II Clinical Aspects of Oxidative Stress in the Kidney

7 Hypertension 121
Bernardo Rodriguez-Iturbe and Nosratola D. Vaziri

8 Uric Acid and Oxidative Stress 143
Yuri Y. Sautin, Witcha Imaram, Kyung Mee Kim, Alexander Angerhofer, George Henderson, and Richard Johnson

9 Reactive Oxygen and Nitrogen Species, Oxidative and Nitrosative Stress, and Their Role in the Pathogenesis of Acute Kidney Injury 161
Eisei Noiri, Francesco Addabbo, and Michael S. Goligorsky

10 Oxidative Stress in the Kidney: Proximal Tubule Disorders 179
Sara Terryn and Olivier Devuyst

11 Iron Metabolism and Oxidative Stress 205
Abolfazl Zarjou, József Balla, György Balla, and Anupam Agarwal

12 Hypoxia, Oxidative Stress, and the Pathophysiology of Contrast-Media-Induced Nephropathy 229
Samuel N. Heyman, Seymour Rosen, Mogher Khamaisi, Jean-Marc Odee, and Christian Rosenberger

13 Cardiovascular Complications in Renal Failure: Implications of Advanced Glycation End Products and Their Receptor RAGE 257
Hidenori Koyama and Yoshiki Nishizawa

14 Infection and the Kidney 293
Chih-Wei Yang

15 Oxidative/Carbonyl Stress in the Renal Circulation and Cardiovascular Renal Injury 305
Takefumi Mori, Toshio Miyata, and Sadayoshi Ito

Part III Current Therapy Targeting Oxidative tress

16 The Renin Angiotensin System 323
Josephine M. Forbes and Mark E. Cooper

17 Oxidative Stress in Kidney Injury: Peroxisome Proliferator-Activated Receptor-γ Agonists Are in Control 337
Li-Jun Ma and Agnes B. Fogo

18 Current Therapy Targeting Oxidative Stress: Statin 351
Ravi Nistala and Adam Whaley-Connell

19 *N*-Acetylcysteine in Kidney Disease 367
Giancarlo Marenzi, Erminio Sisillo, and Antonio L. Bartorelli

20 Advanced Glycation End Products Inhibitor 389
Takashi Dan, Charles van Ypersele de Strihou, and Toshio Miyata

Part IV Hypoxia

21 Involvement of Hypoxia-Inducible Factor 1 in Physiological and Pathological Responses to Continuous and Intermittent Hypoxia: Role of Reactive Oxygen Species 409
Gregg L. Semenza

22 Regulation of Oxygen Homeostasis by Prolyl Hydroxylase Domains 419
Kotaro Takeda and Guo-Hua Fong

23 Oxygen-Dependent Regulation of Erythropoiesis 437
Volker H. Haase

24 Intricate Link between Hypoxia and Oxidative Stress in Chronic Kidney Disease 465
Tetsuhiro Tanaka

25 RNA Interference and the Regulation of Renal Gene Expression in Hypoxia 479
Carsten C. Scholz, Colin R. Lenihan, Cormac T. Taylor, and Ulrike Bruning

Part V Hypoxia Pathology in Renal Disorders

26 Cardio-Renal Connection: The Role of Hypoxia and Oxidative Stress 499
Carsten Willam, Tilmann Ditting, Roland Veelken, and Johannes Jacobi

27 Hypoxia-Inducible Factors in Acute Kidney Injury: From Pathophysiology to a Novel Approach of Organ Protection 535
Wanja M. Bernhardt, Carsten Willam, and Kai-Uwe Eckardt

28 Hypoxia in Chronic Kidney Disease: The Final Common Pathway to End Stage Renal Disease 545
Masaomi Nangaku

29 Oxidative Stress and Hypoxia in the Pathogenesis of Diabetic Nephropathy 559
Fredrik Palm, Lina Nordquist, Christopher S. Wilcox, and Peter Hansell

30 Estimation of Kidney Oxygenation by Blood Oxygenation Level Dependent Magnetic Resonance Imaging 587
Lu-Ping Li and Pottumarthi V. Prasad

31 Anemia and Progression of Chronic Kidney Disease 611
Danilo Fliser and Ferdinand H. Bahlmann

Part VI Novel therapeutic approaches against oxidative stress and hypoxia

32 Novel Therapeutic Approaches Against Oxidative Stress and Hypoxia, Targeting Intracellular Sensor Molecules for Oxygen and Oxidative Stress 633
Toshio Miyata and Charles van Ypersele de Strihou

33 Endoplasmic Reticulum Stress as a Target of Therapy Against Oxidative Stress and Hypoxia 657
Reiko Inagi

34 Stem Cell Therapy Against Oxidative Stress and Hypoxia 673
Takashi Yokoo and Motoko Yanagita

Name Index 689

Subject Index 779

Contributors

Abolfazl Zarjou
Division of Nephrology, THT 647, University of Alabama at Birmingham, 1900 University Boulevard, Birmingham, Alabama 35294, USA

Adam Whaley-Connell
Division of Nephrology, Department of Internal Medicine, University of Missouri-Columbia School of Medicine, CE416, 5 Hospital Drive, Columbia, MO 65212, USA

Agnes B. Fogo
Division of Renal Pathology, Vanderbilt University School of Medicine, C-3310 Medical Center North Department of Pathology, Vanderbilt University Medical Center, Nashville, TN 37232-2561, USA
agnes.fogo@vanderbilt.edu

Akira Nishiyama
Department of Pharmacology and Hypertension and Kidney Disease Research Center, Kagawa University Medical School, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa Prefecture 761-0793, Japan
akira@kms.ac.jp

Alexander Angerhofer
Division of Renal Diseases and Hypertension, University of Colorado, Aurora, CO 80045 USA

Antonio L. Bartorelli
Centro Cardiologico Monzino, Via Parea 4, 20138 Milan, Italy

Anupam Agarwal
Division of Nephrology, THT 647, University of Alabama at Birmingham, 1900 University Boulevard, Birmingham, Alabama 35294, USA
agarwal@uab.edu

Bernardo Rodriguez-Iturbe
Servicio de Nefrología, Hospital Universitario, Universidad del Zulia and IVIC-Zulia, Maracaibo, Estado Zulia, Venezuela
bernardori@telcel.net.ve

Carsten C. Scholz
Conway Institute of Biomolecular and Biomedical Research,
School of Medicine and Medical Science, College of Life Sciences,
University College Dublin, Belfield, Dublin 4, Ireland

Carsten Willam
Department of Nephrology and Hypertension, Friedrich-Alexander-University
Erlangen, Krankenhausstrasse 12, D-91054 Erlangen, Germany
carsten.willam@uk-erlangen.de

Charles van Ypersele de Strihou
Division of Molecular Medicine and Therapy, United Centers for Advanced
Research and Translational Medicine (ART), Tohoku University Graduate
School of Medicine, 2-1 Seiryo-Machi, Aoba-ku, Sendai, Miyagi 980-8575, Japan

Chih-Wei Yang
Kidney Research Center, Department of Nephrology,
Chang Gung Memorial Hospital, 199, Tun-Hua North Road, Taipei, Taiwan
cwyang@ms1.hinet.net

Christian Rosenberger
Department of Medicine, Hadassah University Hospital,
Mount Scopus, 24035, Jerusalem 91240, Israel

Christopher S. Wilcox
Department of Medical Cell Biology, Uppsala University,
Box 571751 23 Uppsala, Sweden

Colin R. Lenihan
Conway Institute of Biomolecular and Biomedical Research,
School of Medicine and Medical Science, College of Life Sciences,
University College Dublin, Belfield, Dublin 4, Ireland

Cormac T. Taylor
Conway Institute of Biomolecular and Biomedical Research,
School of Medicine and Medical Science, College of Life Sciences,
University College Dublin, Belfield, Dublin 4, Ireland
cormac.taylor@ucd.ie

Danilo Fliser
Department of Internal Medicine – Renal and Hypertensive Diseases
Saarland University Medical Centre, Kirrberger Strasse,
66421 Homburg/Saar, Germany
indfli@uks.eu

David R. Sell
Department of Pathology and Biochemistry, Case Western Reserve University,
Wolstein Research Bldg., 2103 Cornell Road, Cleveland, OH 44106-7288, USA

Eisei Noiri
Department of Medicine, Tokyo University, Tokyo, Japan
noiri-tky@umin.ac.jp

Erminio Sisillo
Centro Cardiologico Monzino, Via Parea 4, 20138 Milan, Italy

Ferdinand H. Bahlmann
Department of Internal Medicine – Renal and Hypertensive Diseases
Saarland University Medical Centre, Kirrberger Strasse,
66421 Homburg/Saar, Germany

Francesco Addabbo
Department of Medicine, Tokyo University, Tokyo, Japan

Fredrik Palm
Department of Medical Cell Biology, Uppsala University,
Box 571751 23 Uppsala, Sweden
Fredrik.Palm@mcb.uu.se

George Henderson
Division of Renal Diseases and Hypertension, University of Colorado,
Aurora, CO 80045 USA

Giancarlo Marenzi
Centro Cardiologico Monzino, I.R.C.C.S. Department of Cardiovascular Sciences,
University of Milan, Milan, Italy
Giancarlo.marenzi@ccfm.it

Gregg L. Semenza
Vascular Program, Institute for Cell Engineering; McKusick-Nathans
Institute of Genetic Medicine; and Departments of Pediatrics, Medicine,
Oncology, Radiation Oncology, and Biological Chemistry,
The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
gsemenza@jhmi.edu

Guo-Hua Fong
Department of Cardiology, Kyushu University of Medicine,
3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

György Balla
Division of Nephrology, THT 647, University of Alabama at Birmingham,
1900 University Boulevard, Birmingham, Alabama 35294, USA

Hidenori Koyama
Department of Metabolism, Endocrinology and Molecular Medicine
Osaka City University School of Medicine, 1-4-3, Asahi-machi,
Abeno, Osaka 545-8585, Japan
hidekoyama@med.osaka-cu.ac.jp

Hideyasu Kiyomoto
Department of Pharmacology and Hypertension and Kidney Disease Research Center, Kagawa University, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa Prefecture 761-0793, Japan

Hiroshi Nishi
Division of Nephrology and Endocrinology, University of Tokyo School of Medicine, 7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan
mnangaku-tky@umin.ac.jp

Hiroyuki Kobori
Department of Pharmacology and Hypertension and Kidney Disease Research Center, Kagawa University, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa Prefecture 761-0793, Japan

Ina Nemet
Department of Pathology and Biochemistry, Case Western Reserve University, Wolstein Research Bldg., 2103 Cornell Road, Cleveland, OH 44106-7288, USA

Jean-Marc Odee
Department of Medicine, Hadassah University Hospital, Mountt Scopus, 24035, Jerusalem 91240, Israel

Johannes Jacobi
Department of Nephrology and Hypertension, Friedrich-Alexander-University Erlangen, Krankenhausstrasse 12, D-91054 Erlangen, Germany

Josef Pfeilschifter
Pharmazentrum frankfurt/ZAFES, University Hospital, Goethe University, Theodor-Stern-Kai 7, D-60590, Frankfurt am Main, Germany
pfeilschifter@em.uni-frankfurt.de

Josephine M. Forbes
Diabetes Complications Division, Baker IDI Heart and Diabetes Institute, Glycation and Diabetes, Baker IDI Heart and Diabetes Institute, 75 Commercial Road, Melbourne, VIC 3004, Australia
Josephine.Forbes@bakeridi.edu.au

József Balla
Division of Nephrology, THT 647, University of Alabama at Birmingham, 1900 University Boulevard, Birmingham, Alabama 35294, USA

Kai-Uwe Eckardt
Department of Nephrology and Hypertension, Friedrich-Alexander-University, Erlangen-Nuremberg, Krankenhausstrasse 10-12, D-91054 Erlangen, Germany

Karl-Friedrich Beck
Pharmazentrum frankfurt/ZAFES, University Hospital, Goethe University, Theodor-Stern-Kai 7, D-60590 Frankfurt am Main, Germany

Kotaro Takeda
Department of Cardiology, Kyushu University of Medicine,
3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
ktakeda@cardiol.med.kyushu-u.ac.jp

Kyung Mee Kim
Division of Renal Diseases and Hypertension, University of Colorado,
Aurora, CO 80045 USA

Li-Jun Ma
Division of Renal Pathology, Vanderbilt University School of Medicine,
C-3310 Medical Center North Department of Pathology,
Vanderbilt University Medical Center, Nashville, TN 37232-2561, USA

Lina Nordquist
Department of Medical Cell Biology, Uppsala University,
Box 571751 23 Uppsala, Sweden

Lu-Ping Li
Department of Radiology, Evanston Northwestern Healthcare, Suite G507,
Evanston Hospital, 2650 Ridge Avenue, Evanston, IL 60201, USA

Mark E. Cooper
Diabetes Complications Division, Baker IDI Heart and Diabetes Institute,
Glycation and Diabetes, Baker IDI Heart and Diabetes Institute,
75 Commercial Road, Melbourne, VIC 3004, Australia

Masaomi Nangaku
Division of Nephrology and Endocrinology, University of Tokyo School
of Medicine, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
mnangaku-tky@umin.ac.jp

Michael S. Goligorsky
Division of Nephrology and Endocrinology, University of Tokyo School
of Medicine, 7-3-1 Hongo, Bunkyo, Tokyo 113-8655, Japan

Miriam F. Weiss
Department of Pathology and Biochemistry, Case Western Reserve University,
Wolstein Research Bldg., 2103 Cornell Road, Cleveland, OH 44106-7288, USA

Mogher Khamaisi
Department of Medicine, Hadassah University Hospital, Mount Scopus,
24035, Jerusalem 91240, Israel

Motoko Yanagita
Division of Nephrology, Jikei Medical University, 3-25-8, Nishi-Shimbashi,
Minato-ku, Tokyo 105-8461, Japan

Naila Rabbani
Warwick Medical School, Clinical Sciences Research Institute, University of Warwick, University Hospital, Coventry CV2 2DX, UK

Nosratola D. Vaziri
Servicio de Nefrología, Hospital Universitario, Universidad del Zulia and IVIC-Zulia, Maracaibo, Estado Zulia, Venezuela

Olivier Devuyst
Laboratory of Nephrology, Université catholique, de Louvain Medical School, 54 Avenue Hippocrate, UCL 5450, 1200 Brussels, Belgium
olivier.devuyst@uclouvain.be

Paul J. Thornalley
Warwick Medical School, Clinical Sciences Research Institute, University of Warwick, University Hospital, Coventry CV2 2DX, UK
P.J.Thornalley@warwick.ac.uk

Peter Hansell
Department of Medical Cell Biology, Uppsala University, Box 571751 23 Uppsala, Sweden

Pottumarthi V. Prasad
Department of Radiology, Evanston Northwestern Healthcare, Suite G507, Evanston Hospital, 2650 Ridge Avenue, Evanston, IL 60201, USA
pprasad@enh.org

Ravi Nistala
Division of Nephrology, Department of Internal Medicine, University of Missouri-Columbia School of Medicine, CE416, 5 Hospital Drive, Columbia, MO 65212, USA
nistalar@health.missouri.edu

Raymond C. Harris
Division of Nephrology, Vanderbilt University, School of Medicine and Nashville Veterans Affairs Hospital, Nashville, TN 37232, USA
ray.harris@vanderbilt.edu

Reiko Inagi
Division of Nephrology and Endocrinology, University of Tokyo School of Medicine, 7-3-1, Hongo Bunkyo-ku, Tokyo 113-8655, Japan
inagi-npr@umin.ac.jp

Richard J. Johnson
Division of Renal Diseases and Hypertension, University of Colorado, Aurora, CO 80045, USA
richard.johnson@ucdenver.edu

Roland Veelken
Department of Nephrology and Hypertension, Friedrich-Alexander-University Erlangen, Krankenhausstrasse 12, D-91054, Germany

Sadayoshi Ito
Division of Nephrology, Endocrinology and Vascular Medicine, Tohoku University Graduate School of Medicine, 1-1 Seiryocho, Aoba-ku, Sendai 980-8574, Japan

Samuel N. Heyman
Department of Medicine, Hadassah University Hospital, Mount Scopus, 24035, Jerusalem 91240, Israel
heyman@cc.huji.ac.il

Sara Terryn
Laboratory of Nephrology, Université catholique, de Louvain Medical School, 54 Avenue Hippocrate, UCL 5450, B-1200, Brussels, Belgium

Seymour Rosen
Department of Medicine, Hadassah University Hospital, Mount Scopus, 24035, Jerusalem 91240, Israel

Takashi Dan
Division of Molecular Medicine and Therapy, United Centers for Advanced Research and Translational Medicine (ART), Tohoku University Graduate School of Medicine, 2-1 Seiryo-Machi, Aoba-ku, Sendai, Miyagi 980-8575, Japan
dantks@m.tains.tohoku.ac.jp

Takashi Yokoo
Division of Nephrology, Jikei Medical University, 3-25-8, Nishi-Shimbashi, Minato-ku, Tokyo 105-8461, Japan
tyokoo@jikei.ac.jp

Takefumi Mori
Division of Nephrology, Endocrinology and Vascular Medicine, Tohoku University Graduate School of Medicine, 1-1 Seiryocho, Aoba-ku, Sendai 980-8574, Japan
tmori@med.tohoku.ac.jp

Tetsuhiro Tanaka
Division of Nephrology and Endocrinology, University of Tokyo School of Medicine, 7-3-1 Hongo, Bunkyo-ku, 113-8655 Tokyo, Japan
tetsu-tky@umin.ac.jp

Tilmann Ditting
Department of Nephrology and Hypertension, Friedrich-Alexander-University Erlangen, Krankenhausstrasse 12, D-91054, Germany

Toshio Miyata
Division of Molecular Medicine and Therapy, United Centers for Advanced Research and Translational Medicine (ART), Tohoku University Graduate School of Medicine, 2-1 Seiryo-Machi, Aoba-ku, Sendai, Miyagi 980-8575, Japan
t-miyata@mail.tains.tohoku.ac.jp

Ulrike Bruning
Conway Institute of Biomolecular and Biomedical Research, School of Medicine and Medical Science, College of Life Sciences, University College Dublin, Belfield, Dublin 4, Ireland

Vincent M. Monnier
Department of Pathology and Biochemistry, Case Western Reserve University, Wolstein Research Bldg., 2103 Cornell Road, Cleveland, OH 44106-7288, USA
vmm3@cwru.edu

Volker H. Haase
Division of Nephrology and Hypertension, Vanderbilt University, C-3119A, MCN, 1161 21st Avenue, Nashville, TN 37232, USA
volker.haase@vanderbilt.edu

Wanja M. Bernhardt
Department of Nephrology and Hypertension, Friedrich-Alexander-University Erlangen, Krankenhausstrasse 10-12, D-91054 Erlangen, Germany
wanja.bernhardt@uk-erlangen.de

Witcha Imaram
Division of Renal Diseases and Hypertension, University of Colorado, Aurora, CO 80045, USA

Yoshiki Nishizawa
Department of Metabolism, Endocrinology and Molecular Medicine
Osaka City University School of Medicine, 1-4-3, Asahi-machi, Abeno, Osaka, 545-8585, Japan

Yuri Y. Sautin
Division of Renal Diseases and Hypertension, University of Colorado, Aurora, CO 80045, USA

Part I
Oxidative Stress

Chapter 1
Oxidative Stress Injury in Glomerular Mesangium

Josef Pfeilschifter and Karl-Friedrich Beck

Abstract The renal mesangium consists of glomerular mesangial cells and their surrounding extracellular matrix. This highly specialized pericapillary tissue constitutes the inner part of the glomerulus that supports the structure of the glomerular capillaries. In the healthy glomerulus, mesangial cells act by synthesis and control of extracellular matrix, by a well-organized production of a series of different growth factors, and by a fine-tuned cross-talk with the neighboring glomerular cells, namely the podocytes and the endothelial cells, as a regulatory device for the maintenance of glomerular structure and function. Besides invading immune cells, resident mesangial cells are considered the key players in modulating inflammatory signaling processes within the glomerulus. After exposure to cytokines, mesangial cells amplify the inflammatory process by synthesizing high amounts of cytokines, reactive oxygen species (ROS), and nitric oxide (NO). Oxidative and nitrosative stress affects surrounding glomerular cells that subsequently may collapse by apoptotic or necrotic mechanisms. We describe the sources and action of oxidative and nitrosative signaling processes in the mesangium and we discuss therapeutic strategies for the treatment of stress-induced cell damage in the course of glomerular diseases.

Keywords Reactive oxygen species · Nitric oxide · Oxidative stress · Nitrosative stress

J. Pfeilschifter (✉)
Pharmazentrum frankfurt/ZAFES, University Hospital, Goethe University,
Theodor-Stern-Kai 7, D-60590 Frankfurt am Main, Germany
e-mail: pfeilschifter@em.uni-frankfurt.de

T. Miyata et al. (eds.), *Studies on Renal Disorders*, Oxidative Stress in Applied Basic Research and Clinical Practice, DOI 10.1007/978-1-60761-857-7_1,

Abbreviations

AA	Arachidonic acid
ACE	Angiotensin converting enzyme
Ang II	Angiotensin II
AP-1	Activator protein 1
ELAV	Embryonal lethal abnormal vision
eNOS	Endothelial NO synthase
ET-1	Endothelin-1
FeTSPP	5,10,15,20-Tetrakis (4-sulfonatophenyl) porphyrinato iron III chloride
FP15	Fe(III)Tetrakis-2-(*N*-triethylene glycol monomethyl ether) pyridyl porphyrin
H_2O_2	Hydrogen peroxide
HIF	Hypoxia-inducible factor
IL-1β	Interleukin-1β
IL-8	Interleukin 8
iNOS	Inducible NO synthase
L-NIL	L-N^6-(L-iminoethyl) lysine dihydrochloride
L-NMMA	N^G-Monomethyl-L-arginine
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemoattractant protein1
MIP-2	Macrophage inflammatory protein
MMP-9	Matrix metalloproteinase 9
Mox1	Mitogenic oxidase 1
NADPH	Nicotinamide adenine dinucleotide phosphate
NFκB	Nuclear factor κB
NIK	NFκB-Inducing kinase
nNOS	Neuronal NO synthase
Nox	NADPH oxidase
O_2^-	Superoxide peroxynitrite
PDGF	Platelet-derived growth factor
PDGFRα	PDGF receptor α
PKB	Protein kinase B
PKC	Protein kinase C
PPAR	Peroxisome poliferator activated receptor
PTK	Protein tyrosine kinase
PTP	Protein tyrosine phosphatase
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RT-PCR	Reverse transcription polymerase chain reaction
sGC	Soluble guanylyl cyclase
SOD	Superoxide dismutase
TIMP-1	Tissue inhibitor or metalloproteinases 1

1 Introduction

The renal mesangium consists of glomerular mesangial cells and their surrounding extracellular matrix. This highly specialized pericapillary tissue constitutes the inner part of the glomerulus that supports the structure of the glomerular capillaries. Mesangial cells are able to contract, and this helps to maintain the structure of capillary loops and to regulate the capillary flow and ultrafiltration surface (for review see [1–4]). However, the major regulation of the single-nephron glomerular filtration rate (SNGFR) is conferred by the efferent and afferent arterioles. Therefore, mesangial cell contraction is currently considered to contribute to the fine tuning of SNGFR. In the healthy glomerulus, mesangial cells act by synthesis and control of extracellular matrix, by a well-organized production of a series of different growth factors and by a fine-tuned cross-talk with the neighboring glomerular cells, namely the podocytes and the endothelial cells, as a regulatory device for the maintenance of glomerular structure and function [4, 5]. However, disease states, caused by diabetes, autoimmune disorders, or inflammatory processes, dramatically disturb the glomerular homeostasis, and this processes may lead to necrosis, apoptosis, excessive production of extracellular matrix, fibrosis, and, subsequently, loss of glomerular function [4]. In an inflammatory setting, polymorphonuclear cells and macrophages, which produce large amounts of inflammatory mediators such as interleukin-1β (IL-1β) and tumor necrosis factor α (TNFα), but also reactive oxygen species and nitric oxide, invade the glomerulus and activate glomerular cells to participate in the production of these mediators. Besides invading immune cells, resident mesangial cells are considered the key players in modulating inflammatory signaling processes within the glomerulus [4, 5]. After exposure to cytokines, mesangial cells amplify the inflammatory process by synthesizing themselves into high amounts of cytokines, reactive oxygen species (ROS), and nitric oxide (NO). The primary ROS synthesized enzymatically is the radicalic superoxide anion O_2^-, which is decomposed to H_2O_2 or the highly reactive hydroxyl radical. NO can react with different ROS or even molecular oxygen to form the most prominent mediators of nitrosative stress, namely peroxynitrite or N_2O_3, respectively. Oxidative and nitrosative stress affects surrounding glomerular cells that subsequently may collapse by apoptotic or necrotic mechanisms. Here we describe the sources and action of oxidative and nitrosative stress in the mesangium and we discuss therapeutic strategies for the treatment of stress-induced cell damage in the course of inflammatory glomerular diseases.

2 Sources of Oxidative and Nitrosative Stress in Mesangial Cells

Generally, all enzymes that use molecular oxygen as a substrate are potentially generators of ROS. In most cases, ROS formation is undesirable and occurs when the respective enzymatic reaction runs under adverse conditions such as substrate

or cofactor deficiency. In contrast, nicotinamide adenine dinucleotide phosphate (NADPH) oxidases produce exclusively ROS to kill invading micro-organisms or to trigger mitogenic signaling cascades. Most of ROS-producing enzymatic reactions result first in the generation of the superoxide anion, which is rapidly further processed to more reactive compounds such as hydrogen peroxide, hydroxyl radical, or hypochlorite. The synthesis of NO occurs – with few exceptions in – in a more coordinated manner by the action of three different NO syntheses that are tightly regulated at expression and activity levels and that fulfill specific physiological tasks [6, 7]. Subsequently, reactive nitrogen species (RNS) are formed by the reaction of NO with ROS.

The first evidence that rat mesangial cells are capable of producing ROS was shown in 1983 [8]. Baud et al. demonstrated that phagocytosis of serum-treated zymosan particles by mesangial cells was accompanied with the production of superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2). Intriguingly, ROS production was associated with the activation of lipoxygenase. Inhibition of lipoxygenase activity by three different inhibitors attenuated ROS production, thus identifying lipoxygenase as a source of ROS in mesangial cells. Later, ROS production was also observed in human mesangial cells after stimulation with the cytokines IL-1β and TNFα [9], and an NADPH-dependent oxidase has been characterized to be the responsible enzyme for mesangial ROS production [10]. Importantly, cytokine-induced ROS production is responsible for TNFα-induced apoptosis in rat mesangial cells [11, 12]. Meanwhile, we know that a series of mediators such as high glucose, angiotensin II, and many others induce ROS formation in mesangial cells, indicating these glomerular pericytes as targets in diabetic nephropathy and hypertension (reviewed in [13, 14]). At the molecular level, in particular NADPH oxidases but also mediators derived from the arachidonic acid metabolism are the responsible devices in ROS generating processes. It is worth mentioning that even if NADPH oxidases have been known as the main source of ROS for a long time, the catalytic subunit of the classical phagocytic NADPH oxidase $p91^{phox}$ or Nox2 has never been detected immunologically in mesangial cells. This was amazing, as all accessory subunits such as $p22^{phox}$, $p47^{phox}$, and $p67^{phox}$, which are a prerequisite for a functional NADPH oxidase complex, are expressed in mesangial cells (for review see [15]). This enigma was deciphered in 2003 when Nox4 (formerly renox), a catalytic subunit that can replace gp91phox that was first characterized in 2000 in the renal cortex [16]. Nox4 has been demonstrated to confer angiotensin II-mediated activation of protein kinase B/Akt in mesangial cells [17]. Nox1 (formerly Mox1 for mitogenic oxidase), an NADPH oxidase subunit with high homology to Nox2 and Nox4, was first characterized in vascular smooth muscle cells and different tissues [18]. Nox1 was also found to trigger ROS formation in rat mesangial cells. Interestingly, Nox1 is expressionally controlled at the mRNA and protein level by nitric oxide (NO) via a cyclic GMP (cGMP)-dependent mechanism in rat mesangial cells [19].

The synthesis of NO by the inducible form of NO synthase (iNOS, also referred to as NOSII or macrophage type of NOS) in mesangial cells was first described indirectly by the measurement of cGMP, the main downstream effector of

NO-induced signaling in rat mesangial cells that were stimulated with IL-1β and TNFα [20, 21]. iNOS expression is also induced by cyclic AMP (cAMP) [22] and cytokine or cAMP-induced expression of iNOS is drastically inhibited by growth factors such as platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β), or endothelin-1 (ET-1) [23–26] and glucocorticoids [27, 28]. By contrast, cytokine-induced iNOS expression is upregulated in rat mesangial cells by basic fibroblast growth factor [29] and ROS donors such as the hypoxanthine/xanthine oxidase system or the redox-cycler DMNQ [30]. In human mesangial cells considerable iNOS expression occurs only when combinations of cytokines were administered as a cocktail [31]. Cloning and analysis of the 5'-flanking regions of murine, rat, and human iNOS revealed a high species and cell specificity. In mouse macrophages, induction of iNOS by interferon-γ or lipopolysaccharide (LPS) is mediated by γ-activated sites, IFN-γ-responsive elements, and nuclear factor κB (NFκB) [32–34]. In rat mesangial cells, binding of the NFκB at the appropriate site on the iNOS promoter is essential for the induction of iNOS by IL-1β [35, 36] in rat mesangial cells. In contrast, ET-1 inhibits cytokine-induced iNOS expression without affecting NFκB binding capacity, suggesting additional mechanisms that are essential for cytokine-induced iNOS expression [37]. For the induction of iNOS by cAMP, enhanced binding of the transcription factors CAAT/enhancer-binding protein (C/EBP) and cAMP-responsive element-binding protein (CREB) on the iNOS promoter has been reported [36]. The most important mechanism that triggers human iNOS expression is obviously the IFN-γ-JAK-2-STAT1α pathway [38]. The role of NFκB in the expression of iNOS is possibly restricted to warrant basal expression that is strongly modified by factors that confer posttranscriptional iNOS expression by regulating iNOS mRNA stability [39]. Probably the synthesis of NO by mesangial cells occurs exclusively via the iNOS. Conflicting results exist regarding the role of the so-called constitutive isoforms of NOS, namely the neuronal form (nNOS, also referred to as bNOS or NOSI) and the endothelial form (eNOS, also referred to as ecNOS or NOSIII) in mesangial cells. So far, there is only one report describing the activity of eNOS in mesangial cells [40]. Owada et al. [40] describe the formation of cGMP after activation of the endothelin B receptor. In contrast, another report demonstrates that ET-1 induces NO formation in the glomerulus but not in mesangial cells [41].

Interestingly, NO and ROS have a strong impact on the expression of their own sources in mesangial cells. As exemplified for iNOS, both ROS and NO are able to amplify cytokine-induced iNOS expression and NO synthesis in rat renal mesangial cells [30, 42]. Importantly, in the same cell system cytokine-induced NO inhibits the expression of Nox1 and the formation of ROS [19]. These results further corroborate the hypothesis that ROS and NO – by modulating the expression of their generating enzyme systems – shift the ROS/NO balance to a NO-dominated chemistry in the course of an inflammatory process in mesangial cells [43].

At this point, it is important to note that the description of the sources of mesangial ROS and NO production as mentioned above does not necessarily mean that mesangial cells react exclusively in an autocrine/paracrine manner to ROS or NO produced by their own synthesis machinery. The situation in the

glomerulus is much more complex. The main glomerular source of oxidative and nitrosative stress is invading immune cells that produce high amounts of ROS and NO predominantly by the activity of Nox2 and iNOS in the glomerulus. Moreover, the contribution of resident glomerular cells (e.g., endothelial cells by the activity of Nox4 or eNOS) or the macula densa by the activity of nNOS on glomerular ROS/NO production has to be considered.

3 Actions of Oxidative and Nitrosative Stress in Mesangial Cells

Both ROS and NO modulate a series of signaling pathways that finally may alter the susceptibility to cell death by necrosis and apoptosis or that affect other phenotypic changes as cell growth or migration. At the molecular level, ROS and NO determine the constitution of the phosphoproteome by affecting protein kinases or phosphatases, they modify protein activity by oxidation, nitration, or nitrosation, or they directly change the transcription by influencing the activity of several transcription factors [44–46]. Intriguingly, ROS and NO may counteract their specific functions by scavenging each other or they act together forming reactive metabolites such as peroxynitrite or other reactive compounds that exert toxic effects by nitrating proteins or lipids [43]. However, the synthesis of ROS and NO occurs timely and spatially separated within the glomerulus and, as mentioned above, the machineries of NO and ROS synthesis are potently influenced by their own products. Taking these phenomena into account, we developed the hypothesis that, for example, in the inflamed mesangium, the chemistry of oxidative and nitrosative stress is dominated in the early phases by the action of "classical" ROS such as O_2^- and H_2O_2 mainly via the activation of NADPH oxidases, followed by a phase of nitrosative stress after the induction of iNOS with subsequent formation of peroxynitrite and N_2O_3, and finally a resolution phase with suppressed ROS production and with the presence of high amounts of NO. We hypothesized that the activity of iNOS is eventually inhibited by substrate depletion or NO-mediated inhibition of the iNOS enzyme, leading to the resolution of the inflammatory process and retaining a balanced synthesis of small amounts ROS and NO within the glomerulus [43, 47].

3.1 Oxidative and Nitrosative Stress and Apoptosis

Removal of dispensable or injured cells by apoptosis is a prerequisite for the homeostasis in healthy tissue, but also for the resolution of many diseases. A role for apoptosis in removing mesangial cells in areas of mesangial hypercellularity was first demonstrated in 1994 by Baker et al. [48] in a rat model of anti-Thy1.1

mesangioproliferative nephritis. However, whether mesangial apoptosis has beneficial or detrimental effect depends on the biological context (e.g., the presence of growth factors, cytokines, ROS, NO, or the composition of the extracellular matrix) [49]. The participation of NO and ROS in apoptotic or necrotic cell death in cultured mesangial cells has been shown by several reports [11, 12, 50–52]. Remarkably, whereas ROS and NO donors dose-dependently increased apoptotic or necrotic cell death in rat mesangial cells, exposure of mesangial cells to equimolar amounts of NO and ROS had no toxic effects indicative for high susceptibility of mesangial cells to oxidative stress or NO. Otherwise, peroxynitrite, a most prominent anticipated mediator of nitrosative stress that is formed by the reaction of O_2^- with NO, does not obviously exert toxic effects. This reflects the potent intrinsic defense system against RNS in rat mesangial cells [51]. These results were later corroborated by a report that describes enhanced formation of ceramide after stimulation with NO or ROS, indicating a potential role for ceramide as a potential mediator of NO- and ROS-induced cell death [53]. Remarkably, SIN-1, a substance that produces equimolar amounts of NO and O_2^- and that is therefore accepted as a peroxynitrite generator, had no effect on ceramide formation and cell death, and this clearly indicates that peroxynitrite is not toxic to mesangial cells. In contrast, glomerular endothelial cells showed enhanced ceramide formation and cell death after treatment with SIN-1. Obviously, endothelial cells lack a protective system against nitrosative stress comparable to that of mesangial cells

3.2 Effects of ROS and NO on the Phosphoproteome

Phosphorylation and dephosphorylation of serine/threonine or tyrosine residues in target proteins by protein kinases or protein phosphatases determine the final outcome of most important cellular signaling processes. Consequently, the activity of the responsive kinases and phosphatases governs the constitution of the cellular phosphoproteome. Analysis of the human genome revealed the existence of about 500 protein kinases that represent the so-called kinome [54]. Moreover, alternative splicing of many of these genes may result in a high structural diversity of protein kinases [55]. Nearly 200 genes encode for protein phosphatases representing the phosphatome. Notably, around 700 genes control the phosphoproteome of a cell, and this indicates the high complexity of physiologic and pathophysiologic processes triggered by these important groups of enzymes.

Intriguingly, ROS and NO greatly affect the constitution of the cellular phosphoproteome. In a recent review, Chiarugi and Buricchi propose an elegant model for the effects of ROS on protein tyrosine phosphorylation [56]. Protein tyrosine phosphatases (PTPs) are commonly downregulated by oxidation of their sulfhydryl groups by hydrogen peroxide or other ROS [57]. In most cases this process is reversible by the action of antioxidants, and therefore, constitutes a well-defined regulatory mechanism [58]. In contrast, many protein tyrosine kinases (PTKs) are upregulated by oxidation. Mechanistically, as exemplified for the

insulin receptor kinase, activation by ROS is achieved by a conformational change of the enzyme after oxidation of several cysteine residues [59]. Since many PTKs regulate their activity by autophosphorylation, ROS-mediated downregulation of PTPs strongly contributes to a ROS-induced activity of PTKs. Importantly, this process is reversible, and a decrease of the intracellular redox potential by a reduced activity of ROS-producing enzymes or by an oxidant-mediated increase of protective mechanisms results in the reconstitution of the phosphoproteome. In this scenario PTPs become hyperactive due to their high phosphorylation state (as a result of high activity of PTKs in the presence of ROS) and the recovery of their activity by reduction of cysteine residues. Simultaneously, cysteine residues of PTKs were reduced and – due to the high activity of PTPs – PTKs become dephosphorylated, leading to an inhibition of PTK activity and subsequently to the abrogation of a series of signaling pathways induced by growth factors and cytokines. Taken together, a fine-tuned redox balance determines induction and resolution of PTK-mediated signaling cascades. As exemplified for PKCγ, a classical calcium- and diacylglycerol-dependent form of protein kinase C, ROS are also able to activate serine/threonine kinases [60].

Nitric oxide and peroxynitrite are also able to modulate kinase or phosphatase activity, leading to changes, for example, in the three main mitogen-activated protein kinase (MAPK) pathways [44, 61, 62]. Changes in the phosphorylation pattern by NO or peroxynitrite are triggered by different mechanisms, affecting, for example, sulfhydryl groups by nitrosation or tyrosine residues by nitration. However, in contrast to ROS, which exert their effects mainly by oxidative processes, NO bears additionally the ability to act via a well-defined physiological receptor, the soluble guanylyl cyclase (sGC). Cyclic GMP produced by the activity of sGC triggers phosphorylation cascades that may counteract the oxidative response. In rat mesangial cells, elevated cGMP levels as evoked by stimulation with atrial natriuretic peptide (ANP) augment the expression of MAP kinase phosphatase in glomerular mesangial cells, thereby affecting phorbol ester-induced mesangial cell proliferation [63] and inhibiting endothelin-1-induced activation of c-Jun, NH_2-terminal kinase (JNK), and p42/p44 (ERK-1/ERK-2) MAPK pathways [64, 65]. In contrast, both NO and ROS are able to activate p42/p44 (ERK-1/ERK-2) MAPK-mediated pathways in mesangial cells. Nitric oxide activates p42/p44 MAPK via cGMP-dependent and -independent mechanisms by inhibition of tyrosine phosphatases [66], whereas ROS formation induced by angiotensin II treatment stimulates phosphorylation of p42/p44 MAPK via the activity of Nox4 [67]. It is worth mentioning that angiotensin II also triggers PKB/Akt-dependent signaling [17], phosphorylation of 3-phosphoinositide-dependent protein kinase-1 (PDK-1) [68], and JNK [69] in a ROS-dependent manner, indicating that angiotensin II-evoked redox signaling triggers nearly all pathways required for mesangial cell matrix formation or proliferation. Augmented PDGF receptor phosphorylation by NO-dependent inhibition of PDGF receptor phosphotyrosine phosphatase by NO may counteract cGMP-mediated anti-proliferative effects in mesangial cells [70].

3.3 *Effects of Oxidative and Nitrosative Stress on the Transcription Pattern of Mesangial Cells*

Besides their ability to potently impact signaling processes that alter the gene expression pattern, both autacoids NO and ROS are able to directly affect gene expression at the transcriptional, posttranscriptional, and posttranslational levels [44–46, 71]. A series of so-called redox-sensitive transcription factors are responsive to nitrosative or oxidative stress, and such effects were best characterized for nuclear factor kappa B (NFκB), activator protein 1 (AP-1), and hypoxia-inducible factor 1 (HIF-1) [45, 71–74]. A series of reports describe the action of ROS or NO on the gene expression pattern in mesangial cells of murine, human, or rat origin. For these investigations, some authors administered ROS or NO exogenously using compounds such as the redox cycler 2,3-dimethoxy-1,4-naphthoquinone (DMNQ) or the xanthine oxidase/(hypo)xanthine system for the production of the superoxide anion, glucose oxidase for the production of H_2O_2, or different NO-producing agents such as diethylenetriamine/NO (DETA/NO) or S-nitrosoglutathione (GSNO). To assess the effects of endogenously produced ROS or NO, mesangial cells were prone to conditions mimicking (a) activation of the renin-angiotensin-aldosterone system (RAAS), (b) diabetic nephropathy by elevated glucose levels, or (c) inflammation by the cytokines IL-1β and TNF-α or aggregated immunoglobulin. The resulting expression patterns were then compared with that of mesangial cells that were additionally treated with suitable inhibitors for NADPH oxidases or inducible NO-synthase such as diphenylene iodonium (DPI) or N^G-monomethyl-L-arginine (L-NMMA), respectively, or with scavengers of ROS such as *N*-acetyl-cysteine.

To our knowledge, the first report that describes the action of endogenously produced ROS on gene expression in mesangial cells was published by Satriano et al. in 1993 [75]. The authors demonstrate that ROS, produced by murine mesangial cells after stimulation with TNF-α and aggregated immunoglobulin, enhanced the expression of monocyte chemoattractant protein 1 (MCP-1) and moncyte colony-stimulating factor 1 (CSF-1). The same group also observed later a ROS-dependent upregulation of the genes for RANTES (regulated upon activation, normal T cell expressed and secreted) and ICAM-1 (intercellular adhesion molecule-1), further corroborating a prominent role for ROS in mesangial chemokine signaling [76]. Intriguingly, originally noninflammatory pathways are able to induce the expression of proinflammatory chemokines. Ha et al. [77] demonstrated that high glucose induced the expression of MCP-1 via the activation of the transcription factor NFκB and the production of ROS, clearly indicating a proinflammatory effect of high glucose conditions. In fact, ROS-induced activation of MAP kinases, followed by activation of the transcription factors NFκB and AP-1, is a key signaling pathway induced by ROS in mesangial cells as exemplified for the expression of the matrix metalloprotease 9 gene [78]. Moreover, a further target that exerts chemokine-like functions, namely osteopontin, has been characterized to be upregulated via aldosterone/cytokine-induced ROS formation in rat mesangial cells [79].

High glucose potently induces generation of ROS via activation of protein kinase C (PKC) in mesangial cells [80]. Besides activation of the transcription factors AP-1 and NFκB by PKC-induced ROS, elevation of TGF-β levels is a main feature of ROS activity in mesangial cells. TGF-β-induced SMAD signaling cascades, in turn led by an upregulation of collagens 1, 3, and 4, fibronectin, and plasminogen activator inhibitor 1 (PAI-1) to an excessive formation of ECM, which caused glomerular fibrosis as a cardinal symptom of diabetic nephropathy not only by a enhanced expression of matrix components [81, 82] but also by a reduced expression of ECM-degrading factors [83]. Importantly, PKC is not only a prerequisite for high glucose-induced ROS formation. Activation of PKC is also regulated by a fine-tuned redox mechanism that may under certain circumstances constitute a positive regulatory loop, further amplifying the actions of ROS on cell death or gene regulation [84, 85].

It is worth mentioning that one of the first cell systems that was used to analyze NO-mediated gene expression were human renal mesangial cells. Brown et al. [86] found that protein and mRNA expression of the inflammatory chemokine IL-8 was drastically upregulated by NO. Meanwhile, a series of genes were found to be under expressional control by NO or ROS in mesangial cells. In our lab, we could demonstrate that certain genes are regulated in a coordinated fashion by NO and ROS [30, 42], whereas others are regulated in an opposite manner by the autacoids NO and ROS [78, 87].

The aim of most studies performed to analyze NO-dependent gene expression was to define a more or less unique and ubiquitous signaling machinery that triggers NO-mediated gene expression. This goal has so far not been attained. By contrast, the analysis of NO-modulated mRNA and protein expression revealed that a variety of signaling cascades are involved in NO-evoked cell responses.

Nevertheless, our current understanding of the biology of NO signaling focuses on three different pathways that are involved in triggering NO-driven responses directly to the transcriptional machinery of the cell or in mediating posttranscriptional or posttranslational mechanisms and, subsequently, to changes in the gene expression pattern. These pathways include (a) the cGMP signaling pathway [88], (b) interference with hypoxia-mediated signaling [89], and (c) interference with redox signaling (e.g., posttranslational changes of proteins by nitration of tyrosine residues or nitrosation of cysteine residues) [47].

During the past 15 years, several so-called differential display methods have been developed or improved to analyze the mRNA and protein expression patterns of cultured cells and tissues. We successfully used the RNA arbitrarily primed reverse transcription–polymerase chain reaction (RAP-PCR) to analyze NO-mediated gene expression on the mRNA level [90]. We also directly focused on NO-driven protein expression using a two-dimensional protein gel electrophoresis protocol. Besides these “arbitrary” approaches, we also investigated the NO-dependent regulation of gene products that are considered as key players in several forms of glomerulonephritis. These experiments revealed a series of genes that were expressionally controlled by NO. As exemplified for the NO-dependent regulation of SPARC (secreted protein acidic and rich in cysteine), SMOC-1