

## Circulating Nucleic Acids in Plasma and Serum V

*Editors*

PETER B. GAHAN AND R. SWAMINATHAN

This volume is the result of a meeting entitled **Fifth International Conference on Circulating Nucleic Acids in Plasma and Serum**, held on August 23–27, 2007 in the Cosmos Hotel, Moscow, Russia.

### CONTENTS

|                                                                                                                          |     |
|--------------------------------------------------------------------------------------------------------------------------|-----|
| Preface. <i>By</i> Peter B. Gahan and R. Swaminathan .....                                                               | xii |
| Circulating Nucleic Acids in Plasma and Serum: Recent Developments. <i>By</i><br>Peter B. Gahan and R. Swaminathan ..... | 1   |

### Part I. Basic Research

|                                                                                                                                                                                                                                                                         |    |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Metabolic DNA as the Origin of Spontaneously Released DNA? <i>By</i><br>Peter B. Gahan, Philippe Anker, and Maurice Stroun .....                                                                                                                                        | 7  |
| Circulating DNA: Its Origin and Fluctuation. <i>By</i> Maniesh van der Vaart and<br>Piet J. Pretorius .....                                                                                                                                                             | 18 |
| The Origin of Elevated Levels of Circulating DNA in Blood Plasma of Premature<br>Neonates. <i>By</i> N. O. Tuaeve, Z. I. Abramova, and V. V. Sofronov .....                                                                                                             | 27 |
| The Effect of Protein Transport Inhibitors on the Production of Extracellular<br>DNA. <i>By</i> Evgeniy S. Morozkin, Tatyana I. Babochkina, Valentin V. Vlassov,<br>and Pavel P. Laktionov .....                                                                        | 31 |
| Binding and Penetration of Methylated DNA into Primary and Transformed<br>Human Cells. <i>By</i> Tatyana E. Skvortsova, Valentin V. Vlassov, and<br>Pavel P. Laktionov .....                                                                                            | 36 |
| Extracellular DNA Fragments: Factors of Stress Signaling between X-Irradiated<br>and Nonirradiated Human Lymphocytes. <i>By</i> Aleksei V. Ermakov, Svetlana V.<br>Kostyuk, Marina S. Konkova, Natalya A. Egolina, Elena M. Malinovskaya,<br>and Natalya N. Veiko ..... | 41 |
| Isolation and Sequencing of Short Cell-Surface-Bound DNA. <i>By</i> Vera S.<br>Mal'shakova, Dmitry V. Pyshnyi, Alexander A. Bondar, Valentin V. Vlassov,<br>and Pavel P. Laktionov .....                                                                                | 47 |

|                                                                                                                                                                                                               |    |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Deamination of Adenosines in Extracellular RNA Spontaneously Internalized by Human Cells. <i>By</i> Elena V. Kuligina, Oksana V. Vratskih, Dmitry V. Semenov, Vera A. Matveeva, and Vladimir A. Richter ..... | 51 |
| Circulating Nucleic Acids in the Assessment of Endogenous Growth Hormone Production. <i>By</i> H. Thakkar, A.N. Butt, J. Powrie, R. Holt, and R. Swaminathan .....                                            | 58 |
| The Involvement of Different Mobile LINE Copies of Blood Plasma and Extrachromosomal DNA of Liver Cells in Systemic Adaptive Response. <i>By</i> Sophiya S. Sherlina and Viktor G. Vladimirov .....           | 66 |

## Part II. Methodology

|                                                                                                                                                                                                                                                                          |     |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Transrenal Nucleic Acids: From Proof of Principle to Clinical Tests: Problems and Solutions. <i>By</i> Hovsep S. Melkonyan, W. John Feaver, Erik Meyer, Vladimir Scheinker, Eugene M. Shekhtman, Zhenghan Xin, and Samuil R. Umansky .....                               | 73  |
| Removal of High-Molecular-Weight DNA by Carboxylated Magnetic Beads Enhances the Detection of Mutated <i>K-ras</i> DNA in Urine. <i>By</i> Ying-Hsiu Su, Janet Song, Zhili Wang, Xiao-He Wang, Mengjun Wang, Dean E. Brenner, and Timothy M. Block .....                 | 82  |
| A Method for Characterization of Total Circulating DNA. <i>By</i> Maniesh van der Vaart and Piet J. Pretorius .....                                                                                                                                                      | 92  |
| Isolation and Extraction of Circulating Tumor DNA from Patients with Small Cell Lung Cancer. <i>By</i> Ruth E. Board, Victoria S. Williams, Lucy Knight, Jacqueline Shaw, Alastair Greystoke, Malcolm Ranson, Caroline Dive, Fiona H. Blackhall, and Andrew Hughes ..... | 98  |
| Stability of $\beta$ -Actin mRNA in Plasma. <i>By</i> N.C. Holford, H.S. Sandhu, H. Thakkar, A.N. Butt, and R. Swaminathan .....                                                                                                                                         | 108 |
| Normalization of Circulating Nucleic Acid Results: Should We Use $\beta$ -Actin? <i>By</i> N.C. Holford, A. Ramoutar, A.N. Butt, and R. Swaminathan .....                                                                                                                | 112 |
| Splicing by Exon Exclusion Impaired by Artificial Box C/D RNA Targeted to Branch-Point Adenosine. <i>By</i> Dmitry V. Semenov, Oksana V. Vratskih, Elena V. Kuligina, and Vladimir A. Richter .....                                                                      | 119 |
| Optimization of Purification of Human Cell-Free mRNA from Plasma. <i>By</i> Tadeja Dovc-Drnovsek, Barbara Emersic, Primož Rozman, Darko Cerne, and Jana Lukac-Bajalo .....                                                                                               | 125 |
| Fragments of Noncoding RNA in Plasma of Human Blood. <i>By</i> Dmitry V. Semenov, Dmitry N. Baryakin, Tatyana P. Kamynina, Elena V. Kuligina, and Vladimir A. Richter .....                                                                                              | 130 |
| An Improved Method for the Isolation of Free-Circulating Plasma DNA and Cell-Free DNA from Other Body Fluids. <i>By</i> Christoph Kirsch, Sabine Weickmann, Bernd Schmidt, and Michael Fleischhacker .....                                                               | 135 |

## Part III. Fetal Nucleic Acids

|                                                                                                                                                                    |     |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Fetal Nucleic Acids in Maternal Plasma: Toward the Development of Noninvasive Prenatal Diagnosis of Fetal Chromosomal Aneuploidies. <i>By</i> Y.M. Dennis Lo ..... | 140 |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|

|                                                                                                                                                                                                                                                                                         |     |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Phenotype and mRNA Expression of Syncytiotrophoblast Microparticles Isolated from Human Placenta. <i>By</i> Belinda Kumpel, May-Jean King, Suren Sooranna, Dave Jackson, Jane Eastlake, Rong Cheng, and Mark Johnson . . .                                                              | 144 |
| Fetal Sex Identification in Maternal Plasma by Means of Short Tandem Repeats on Chromosome X. <i>By</i> Gennaro Vecchione, Michela Tomaiuolo, Michelina Sarno, Donatella Colaizzo, Rosella Petraroli, Maria Matteo, Pantaleo Greco, Elvira Grandone, and Maurizio Margaglione . . . . . | 148 |
| A New Y Chromosome Marker for Noninvasive Fetal Gender Determination. <i>By</i> Olga B. Vainer, Aleksey V. Katokhin, Sergey M. Kustov, Valentin V. Vlassov, and Pavel P. Laktionov . . . . .                                                                                            | 157 |

#### Part IV. Circulating Nucleic Acids and Cancer

|                                                                                                                                                                                                                                                                                                                                   |     |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| DNA Integrity in Plasma and Serum of Patients with Malignant and Benign Diseases. <i>By</i> Stefan Holdenrieder, Alexander Burges, Oliver Reich, Fritz W. Spelsberg, and Petra Stieber . . . . .                                                                                                                                  | 162 |
| Quantification of LINE1 in Circulating DNA as a Molecular Biomarker of Breast Cancer. <i>By</i> Eiji Sunami, Anh-Thu Vu, Sandy L. Nguyen, Armando E. Giuliano, and Dave S. B. Hoon . . . . .                                                                                                                                      | 171 |
| Effect of Adjuvant Chemotherapy on Integrity of Free Serum DNA in Patients with Breast Cancer. <i>By</i> Ugur Deligezer, Yesim Eralp, Elif Z. Akisik, Ebru E. Akisik, Pinar Saip, Erkan Topuz, and Nejat Dalay . . . . .                                                                                                          | 175 |
| Clinical Relevance of Circulating Nucleosomes in Cancer. <i>By</i> Stefan Holdenrieder, Dorothea Nagel, Andreas Schalhorn, Volker Heinemann, Ralf Wilkowski, Joachim von Pawel, Hannelore Raith, Knut Feldmann, Andreas E. Kremer, Susanne Müller, Sandra Geiger, Gerhard F. Hamann, Dietrich Seidel, and Petra Stieber . . . . . | 180 |
| Detection and Monitoring of Cell-Free DNA in Blood of Patients with Colorectal Cancer. <i>By</i> Heidi Schwarzenbach, Jan Stoecklacher, Klaus Pantel, and Eray Goekkurt . . . . .                                                                                                                                                 | 190 |
| Detection of Mutated <i>K-ras</i> DNA in Urine, Plasma, and Serum of Patients with Colorectal Carcinoma or Adenomatous Polyps. <i>By</i> Ying-Hsiu Su, Mengjun Wang, Dean E. Brenner, Pamela A. Norton, and Timothy M. Block . . . . .                                                                                            | 197 |
| Integrity of Cell-Free Plasma DNA in Patients with Lung Cancer and Nonmalignant Lung Disease. <i>By</i> Bernd Schmidt, Sabine Weickmann, Christian Witt, and Michael Fleischhacker . . . . .                                                                                                                                      | 207 |
| Cell-Surface-Bound Circulating DNA as a Prognostic Factor in Lung Cancer. <i>By</i> Svetlana N. Tamkovich, Nikolai V. Litviakov, Olga E. Bryzgunova, Aleksey Yu. Dobrodeev, Elena Yu. Rykova, Sergey A. Tuzikov, Aleksandr A. Zav'ialov, Valentin V. Vlassov, Nadezhda V. Cherdyntseva, and Pavel P. Laktionov . . . .            | 214 |
| Deoxyribonuclease Activity and Circulating DNA Concentration in Blood Plasma of Patients with Prostate Tumors. <i>By</i> Anna V. Cherepanova, Svetlana N. Tamkovich, Olga E. Bryzgunova, Valentin V. Vlassov, and Pavel P. Laktionov . . . . .                                                                                    | 218 |
| Methylation-Specific Sequencing of GSTP1 Gene Promoter in Circulating/Extracellular DNA from Blood and Urine of Healthy Donors and Prostate Cancer Patients. <i>By</i> Olga E. Bryzgunova, Evgeniy S. Morozkin, Sergey V. Yarmoschuk, Valentin V. Vlassov, and Pavel P. Laktionov . . . . .                                       | 222 |

|                                                                                                                                                                                                                                                                           |     |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Circulating DNA in the Blood of Gastric Cancer Patients. <i>By</i> Elena V. Kolesnikova, Svetlana N. Tamkovich, Olga E. Bryzgunova, Petr I. Shelestyuk, Valentina I. Permyakova, Valentin V. Vlassov, Aleksandr S. Tuzikov, Pavel P. Laktionov, and Elena Y. Rykova ..... | 226 |
| Methylation-Based Analysis of Circulating DNA for Breast Tumor Screening. <i>By</i> Elena Y. Rykova, Galina A. Tsvetovskaya, Galina I. Sergeeva, Valentin V. Vlassov, and Pavel P. Laktionov .....                                                                        | 232 |

## Part V. Circulating Nucleic Acids and Other Diseases

|                                                                                                                                                                                                                                                                                           |     |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Overview of Circulating Nucleic Acids in Plasma/Serum: Update on Potential Prognostic and Diagnostic Value in Diseases Excluding Fetal Medicine and Oncology. <i>By</i> Asif N. Butt and R. Swaminathan .....                                                                             | 236 |
| Effect of Hypoxia on Circulating Levels of Retina-Specific Messenger RNA in Type 2 Diabetes Mellitus. <i>By</i> Amy Wong, Simon Merritt, Asif N. Butt, Adrian Williams, and R. Swaminathan .....                                                                                          | 243 |
| Retina-Specific mRNA in the Assessment of Diabetic Retinopathy. <i>By</i> Z. Shalchi, H.S. Sandhu, A.N. Butt, S. Smith, J. Powrie, and R. Swaminathan .....                                                                                                                               | 253 |
| Measurement of Circulating Neuron-Specific Enolase mRNA in Diabetes Mellitus. <i>By</i> H. S. Sandhu, A. N. Butt, J. Powrie, and R. Swaminathan .....                                                                                                                                     | 258 |
| Hepatitis B Virus X-DNA: A Serum Marker for Early Detection of Resistance Development during Lamivudine Therapy. <i>By</i> Wei Zhang, Yan-Hong Li, Shao-Jun Zhu, Yan Zhang, Li Gong, Shu-Mei Wang, Hans Jörg Hacker, Claus Hobe Schröder, Dong-Sheng Cheng, and Ying-Ming Feng .....      | 264 |
| Effect of Cell-Free DNA of Patients with Cardiomyopathy and rDNA on the Frequency of Contraction of Electrically Paced Neonatal Rat Ventricular Myocytes in Culture. <i>By</i> N. Bulicheva, O. Fidelina, N. Mkrtumova, M. Neverova, A. Bogush, M. Bogush, O. Roginko, and N. Veiko ..... | 273 |
| Influence of Plasma DNA on Acid-Base Balance, Blood Gas Measurement, and Oxygen Transport in Health and Stroke. <i>By</i> Irina L. Konorova, Natalya N. Veiko, and Viktor E. Novikov .....                                                                                                | 278 |
| Circulating 11 $\beta$ -Hydroxysteroid Dehydrogenase Type 1 mRNA and Cardiovascular Risk Factors. <i>By</i> Maisa Al Bakir, Asif N. Butt, and R. Swaminathan .....                                                                                                                        | 283 |
| Hypertension and Circulating mRNA for 11 $\beta$ -Hydroxysteroid Dehydrogenase Type II. <i>By</i> S. Agalou, A. N. Butt, Phil Chowienzyk, and R. Swaminathan ..                                                                                                                           | 290 |
| Cell-Free Plasma DNA during Peritoneal Dialysis and Hemodialysis and in Patients with Chronic Kidney Disease. <i>By</i> Marie Korabecna, Sylvie Opatrna, Jan Wirth, Kamila Rulcova, Jaromir Eiselt, Frantisek Sefrna, and Ales Horinek .....                                              | 296 |
| Arrayed Primer Extension for the Noninvasive Prenatal Diagnosis of $\beta$ -Thalassemia Based on Detection of Single Nucleotide Polymorphisms. <i>By</i> Thessalia Papasavva, Ioannis Kalikas, Andreanni Kyrii, and Marina Kleanthous .....                                               | 302 |
| The Housekeeping Gene (GA3PDH) and the Long Interspersed Nuclear Element (LINE) in the Blood and Organs of Rats Treated with Cocaine. <i>By</i> Anatoliy M. Voskresenskiy and Lena S. Sun .....                                                                                           | 309 |

|                                                                                                                                                                                                                                                                                                        |     |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Extracellular Functional Noncoding Nucleic Acid Bioaptamers and Angiotropin RNP Ribokines in Vascularization and Self-Tolerance: 3D Episcritps on Domains Shared in Regulator Proteins of the Vascular and Cancer Epigenome. <i>By</i> Josef H. Wissler, Joerg E. Wissler, and Enno Logemann . . . . . | 316 |
| Index of Contributors . . . . .                                                                                                                                                                                                                                                                        | 343 |

### Financial support for the meeting

The general sponsor of the meeting was Roche Diagnostics (Russian branch)

The meeting was sponsored also by:

- BioChemMack (<http://www.biochemmack.ru>)
- Parallabs (Russian branch)
- Applera (Russian branch)
- Bio-Rad Laboratories (Russian branch)
- Dr. Fooke Laboratorien GmbH (<http://www.fooke-labs.de>)

The New York Academy of Sciences believes it has a responsibility to provide an open forum for discussion of scientific questions. The positions taken by the participants in the reported conferences are their own and not necessarily those of the Academy. The Academy has no intent to influence legislation by providing such forums.