Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Journal of Chromatography B, 855 (2007) 28–34
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Review

Cardiovascular proteomic analysis[☆]

Toru Suzuki[a,b,*], Ryozo Nagai[a]

[a] *Department of Cardiovascular Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan*
[b] *Department of Clinical Bioinformatics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan*

Received 30 September 2006; accepted 16 February 2007
Available online 4 March 2007

Abstract

Here, we report on our proteomic studies in the field of cardiovascular medicine. Our research has been focused on understanding the role of proteins in cardiovascular disease with a particular focus on epigenetic regulation and biomarker discovery, with the objective of better understanding cardiovascular pathophysiology to lead to the development of new and better diagnostic and therapeutic methods. We have used mass spectrometry for over 5 years as a viable method to investigate protein–protein interactions and post-translational modifications in cellular proteins as well as a method to investigate the role of extra-cellular proteins. Use of mass spectrometry not only as a research tool but also as a potential diagnostic tool is a topic of interest. In addition to these functional proteomics studies, structural proteomic studies are also done with expectations to allow for pinpoint drug design and therapeutic intervention. Collectively, our proteomics studies are focused on understanding the functional role and potential therapeutically exploitable property of proteins in cardiovascular disease from both intra-cellular and extra-cellular aspects with both functional as well as structural proteomics approaches to allow for comprehensive analysis.
© 2007 Elsevier B.V. All rights reserved.

Keywords: Proteomic analysis; Cardiovascular

Contents

1. Introduction 28
2. Proteomic analysis of intra-cellular proteins 29
3. Proteomic analysis of extra-cellular proteins 31
4. Conclusions—implications and lessons learned 32
Acknowledgements 32
References 32

1. Introduction

The completed human genome project has shown that given the marginal increase in genes during evolution, diversity in humans is likely dictated by post-genomic regulation, namely proteins [1,2]. Aside from the recently addressed field of RNA regulation (e.g. micro-RNA, etc.), post-genomic regulation at the protein level is likely the major regulatory step. Splicing, processing, and post-translational modifications in addition to protein–protein interaction are but some of the notable regulatory pathways.

In contrast to genes/DNA which are quite stable as would be necessary for conserved preservation of the genetic information, proteins which are the products of genes are rather unstable and have short half-lives being produced and then degraded to be present to function only when and where their need is dictated as reflective of their physiologically active properties. Not only are different proteins produced at times through splicing,

☆ This paper was presented at the 31st Annual Meeting of the Japanese Society for Biomedical Mass Spectrometry, Nagoya, Japan, 28–29 September 2006.

* Corresponding author at: Department of Clinical Bioinformatics and Department of Cardiovascular Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. Tel.: +81 3 3815 5411x33117;
fax: +81 3 5800 8824.
E-mail address: torusuzu-tky@umin.ac.jp (T. Suzuki).

1570-0232/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2007.02.043

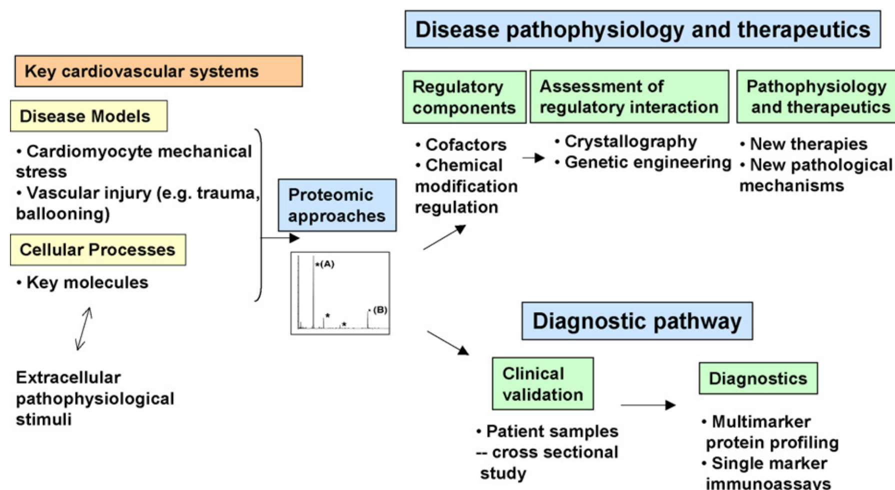

Fig. 1. Illustration describing study workflow and logic.

but once they are produced they are often processed/cleaved and/or modified (e.g. phosphorylation, glycosylation), and act in concert with other proteins as complexes before they are degraded. Indeed, understanding the complex regulation of proteins under a given condition (e.g. temporospatial context) is of utmost importance in understanding its/their function.

While proteomic analysis in general concerns the comprehensive understanding of proteins and their functions which often entails understanding their functions in a non-challenged or quiescent state, our interests are focused on the role of proteins as relevant in disease states and their regulation as manifested under disease conditions. Temporal changes in disease-associated factors are important in understanding pathophysiology. For instance, genetic disorders as represented by monogenic disorders often manifest phenotypes in early life. However, for acquired diseases such as oncogenic as well as lifestyle-oriented diseases, environmental factors play an important contributory role to the onset of disease besides their genetic underpinnings. Although regulation of gene expression undoubtedly plays an important role, temporal changes in later/adult life is regulated in main at the level of proteins, and therefore studies at the protein level are ideal for studies focused on disease onset and progression. We therefore strive to understand the molecular changes which affect proteins under disease conditions, not only to understand their role in the pathophysiological basis of disease, but further to exploit their possible roles in diagnostic as well as therapeutic applications.

The following discussion on our use of proteomic approaches will be separated into categories of analysis on intra-cellular proteins and extra-cellular proteins. A model of our study logic and workflow is illustrated in Fig. 1.

2. Proteomic analysis of intra-cellular proteins

For proteomic analysis of intra-cellular proteins, we have focused on epigenetic regulation, in particular on chromatin regulation of transcription in cardiovascular disease [3,4]. To briefly provide background on epigenetic regulation, in eukaryotes including mammals such as humans, genomic DNA is packaged into chromatin whose fundamental unit is the nucleosome in which DNA is twice wrapped around the histone octamer. This compaction of DNA is likely necessary to efficiently package the vast amount of genomic DNA as found in the eukaryotic cell. This adds an additional step necessary in activation of transcription as DNA is wound in the chromatin state under basal conditions. Only after the chromatin structure is relieved can transcription factors including both regulatory as well as general factors access the promoter to regulate transcription and thus gene expression.

Epigenetic regulation impacts a multitude of physiological as well as pathophysiological reactions ranging from cancer to cardiovascular disease. It had been poorly addressed in the field of cardiovascular disease likely due to more important focuses on physiology as well as membrane-oriented cellular biology. However, pioneering work in skeletal muscle, which is the prototype for cardiac as well as smooth muscle which are relevant in the cardiovasculature, suggested from early on that epigenetic regulation may be important in phenotypic regulation. Studies using the DNA methylation inhibitor, 5-azacytidine, showed that regulation of methylation state can induce myocyte differentiation [5]. This work led to the discovery of the transcription factor, MyoD, a master regulator transcription factor of myogenic differentiation [6]. Recent studies have shown that epigenetic regulation namely in the form of regulators of the post-transcriptional modification, acetylation, play a role in regulating cardiac growth and remodeling (e.g. cardiac hypertrophy and failure). The studies from the Olson lab have been instrumental in establishing that acetylation/deacetylation, in particular through the functions of deacetylases (HDACs, histone deacetylases) play a pivotal role in cardiovascular phenotypic modulation and disease [7,8].

We have also focused in parallel on the role of epigenetic regulation of cardiovascular disease with a focus on understanding the role of protein–protein interaction and the post-translational

modification, namely acetylation. For this, we have used proteomic approaches to understand the underlying mechanisms of functional regulation of the Sp- and Krüppel-like factor of zinc finger transcription factors which play an important role in cardiovascular regulation [3,9]. As protein–protein interaction and post-translational modifications are detectable by proteomic approaches, as a result, we were able to define novel mechanisms of epigenetic regulation of transcription and cardiovascular disease through our studies which will be discussed in detail below.

Zinc finger factors, especially those which contain a cysteine–histidine (Cys2–His2) zinc-finger motif, emerged through evolution and markedly increased in eukaryotes [10]. Although this common motif is widely found in cellular factors ranging from enzymes to transcription factors, often the motif is used as a DNA-binding domain in transcription factors. As these factors preferentially evolved in eukaryotes, they are thought to play functional roles in developmental and differentiation processes among others. The specificity protein- (Sp-) and Krüppel-like factors (KLFs) are a family of these factors which is centered on Sp1 (specificity protein-1), which was one of the first regulatory transcription factors identified as a factor which stimulates the simian virus 40 (SV40) early promoter by binding to its GC-rich promoter sites [11]. Subsequent categorization of the genome showed that Sp1 and its related factors show sequence similarity centered on the zinc-finger region with other zinc finger transcription factors related to the Krüppel gene as identified in Drosophila as being a gap segmentation gene [12–19]. We now understand that there are approximately 20 of these factors in mammals and through gene mutation studies that these factors often have individual biological functions. For instance, KLF1 (aka erythroid KLF, EKLF) is required for erythrocyte development. Interestingly, studies in zebrafish have shown that the KLFs have evolved to function mainly in the blood and circulatory organs [20]. Thus, the KLFs are an excellent target for our work which centers often on the cardiovascular role of proteins.

We set out to understand the role of transcription factors in chromatin regulation with a focus on the Sp/KLF factors in cardiovascular pathophysiology. Recent studies have shown that chromatin transcription is regulated by three classes of factors which include (1) modification enzymes such as methylases and acetylases, (2) ATP-independent nucleosome assembly factors which are also called histone chaperones, and (3) ATP-dependent nucleosome assembly factors. These factors through protein–protein interactions and post-translational modifications affect their target protein (e.g. histones, transcription factor) to regulate transcription.

Using the zinc finger DNA-binding domain region, which is often an interface for protein–protein interactions, of Sp1 and KLF5, the latter being a Krüppel-like factor important in mediating cardiovascular remodeling in response to external stress, we affinity purified proteins which interact with these peptides from nuclear extract and identified them by use of matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF-MS) with subsequent peptide mass fingerprinting/post-source decay (PMF/PSD) analysis. We identified an ATP-independent nucleosome assembly factor, template activating factor-I (TAF-I/SET), to interact with both Sp1 and KLF5 [21,22]. TAF-I/SET inhibited the DNA-binding and promoter activation activities of Sp1 and KLF5, and thus acts as a transcriptional repressor. We had previously shown through a focused approach that Sp/KLF factors are differently acetylated. We found that the coactivator/acetylase p300 interacts and acetylates KLF5 to coactivate transcription and interestingly that TAF-I/SET could mask KLF5 from being acetylated by p300. These showed that TAF-I/SET could inhibit acetylation of transcription factor. We also showed the regulation of these factors under pathophysiological conditions. Under phorbol ester stimulation (as a model agonist of pathophysiological stimulation), KLF5 was upregulated as was its downstream gene, platelet-derived growth factor A-chain (PDGF-A), but interestingly the repressor, TAF-I/SET, was downregulated under these conditions thus likely allowing for stimulation of transcription. As p300 has been shown to be induced by phorbol ester, it is likely that coordinated induction/repression of cofactor with transcription is important for transcriptional regulation. We further showed that protein–protein interaction (e.g. p300, TAF-I/SET) is coupled with post-translational modification (e.g. acetylation), and thus showed a novel mechanism of transcriptional regulation as defined by proteomic approaches. We further went on to show that the deacetylase, HDAC1, competes with p300 for interaction with KLF5, which showed that deacetylase regulates transcription at levels other than catalytic regulation [23]. Through our studies and those of others, we now know that the Sp/KLF factors, aside from histones, are the only family of factors which associates with all three classes of chromatin remodeling factors, with further regulation among these classes of chromatin remodeling factors [4]. Thus, proteomic approaches, which often excel in identifying protein–protein interactions as well as post-translational modifications, played an important role in identifying a novel pathophysiological transcriptional regulatory mechanism.

We have further carried out crystallographic studies to understand the biophysical mechanisms of the protein–protein interaction and post-translational modifications as stated with the ultimate goal to develop pinpoint drugs/compounds to specifically regulate chromatin transcription in the context of pathophysiological regulation. We have already solved the crystal structure of TAF-I/SET [24]. Co-crystal structure analysis should allow for designing specific compounds. Thus our functional and structural proteomic analysis is a model approach for comprehensive understanding of the role of proteins in cardiovascular pathophysiology.

In summary, proteomic approaches have been instrumental in the discovery of new pathophysiological mechanisms of cardiovascular disease involving protein–protein interaction and post-translational modification (acetylation). Given that our studies were done in a timely manner as concurrent with studies by others to study the role of epigenetic regulation with a focus on acetylation/deacetylation in the cardiovasculature, the field of cardiovascular research has much advanced in its comprehensive understanding of involved proteins in recent years. Importantly, in contrast to the work of others which have been mainly focused on use of animal models, our proteomic studies have allowed for understanding the underlying pathogenic mechanisms at the pro-

tein level which complement these works done using different approaches. These mechanistic insights will likely lead to new approaches for therapeutic intervention. Cardiovascular disease now sets a benchmark for understanding epigenetic regulation in disease and will likely impact other fields (e.g. metabolic disease, cancer) which are diseases with similar acquired changes in later life.

One concern which remains for proteomic analysis is that the abundant protein is the first to be identified, which may not necessarily physiologically relevant. More emphasis on combined functional assays with MS-based identification will be needed. We were fortunate that our selection of protein interaction domain and cell conditions resulted in favorable results. However, it is important to note that much trial-and-error was needed in establishing conditions which benefited much from our experience and training in classical cold-room techniques using chromatographic columns to separate proteins. Essentially, the MS-based techniques were more a technological advancement allowing for detection with less amounts of protein, but the questions and experimental logic for procedure used were more consistent with those of the past. Knowledge and experience in protein purification are thus likely prerequisites for effective use of MS-based techniques. Further studies will likely be aimed at the better understanding of the temporo-spatial regulation of protein–protein interaction and post-translational modification in the combinatorial context (e.g. complex) with further understanding of their roles *in situ* using imaging MS techniques which are being developed at present. The eventual development of an interaction and regulatory map of proteins in disease states, not only in cardiovascular medicine but in all medical fields, will surely allow for better understanding of the pathophysiology as well as allow for targeted therapeutic intervention.

3. Proteomic analysis of extra-cellular proteins

For extra-cellular proteins, we have focused on discovering new proteins and their functional roles for exploitation in diagnostic purposes (e.g. biomarkers) as well as for therapeutic purposes (e.g. bioactive molecules). Since the pioneering work of predecessors of the lab in which cardiac myosin light chain was purified and then applied to diagnosis of myocardial infarction [25], clinical application of protein chemistry has been a longstanding research topic. The authors have also recently shown that vascular smooth muscle proteins can be used in diagnosis of aortic dissection [26–29], which is the first diagnostic application of a blood test for this disease, as well as studying various oxidized LDL assays in the diagnosis of coronary artery disease [30–32], in addition to characterization of other cardiovascular diagnostic markers such as the natriuretic peptides among others (e.g. interleukin-6 in coronary artery disease) [33–35].

Thus, with this longstanding background in diagnostic applications of protein-based markers and assays, the authors were keen to introduce MS technology to the lab early on. We first used MALDI-TOF MS over 5 years ago not only to identify post-translational modifications (e.g. acetylation) in the above mentioned intra-cellular proteomic studies but also aimed at using this technology to identify new pathophysiological proteins in the cardiovasculature.

There are two critical determinants of using MS-technology for detection of proteins. First is the detector as exemplified by MALDI-based MS detection as well as electrospray ionization-type detection (ESI). Importantly, MS technology allows for detection of minute amounts of protein as would not have been possible by classical techniques. The other important determinant is fractionation or pre-MS separation procedures. This is the most important step for any protein identification or detection procedure, either being a classical technique or recent one. Finally, developing a workflow which best suits the specific needs of the lab must be developed based on these steps. For instance, if working with blood samples, a further workflow involving sample preparation prior to the separation procedure must be optimized.

Our studies have been focused on addressing the possibility of using MS-based technologies not only as a research tool but also as a diagnostic tool for clinical medicine with a particular focus on blood-based techniques. On the former point of the detector, while we also use ESI-based techniques as a research tool, we have focused in main on using MALDI-based detection. At present, four devices are used in the lab which include a PerSeptive Biosystems Voyager DE-STR, Ciphergen ProteinChip PBSII reader, and Shimadzu Biotech AXIMA-QIT and AXIMA-CFR plus instruments. Our findings and opinions are based primarily by use of such equipment and workflow as optimized for such. Clearly, the more sophisticated and sensitive instrumentation provides more information on the sample, but questions still remain on quantitative assessment, reproducibility (between-run), and standardization as would be necessary for diagnostic use. Further, the specifications as would be needed for diagnostic detection have yet to be determined. As our knowledge and experience with this technology advance and specific diagnostic content and protocols become established, the specifications as necessary for diagnostic use will become apparent. Only then will a MS device specific for clinical diagnostic use become available and most likely be refined for this purpose. One further important issue for clinical diagnostic use would be that the pricing of MS devices decrease to a reasonable level to allow wide use as well as ease-of-use, but this will likely be dictated by the necessary specifications needed.

The issue of protein fractionation has remained the most challenging aspect. In blood, approximately 20 proteins including albumin, IgG, and haptoglobin among others comprise the majority of the proteins available. At present, methods are available to facilitate specific removal of these abundant proteins from blood (e.g. Agilent, Beckman Coulter), but at times the protein of interest may be attached to these abundant proteins for which clearance may not necessarily be wise. Ion exchange and other protein purification procedures can be applied at micro-scale levels to separate proteins as well. One technology which warrants mentioning is the surface enhanced laser desorption ionization (SELDI) MS technology (Ciphergen/Bio-Rad) which is characterized by chemically modified surfaces similar to chromatography (e.g. ion-exchange, metal-affinity, etc.), but importantly manipulated on the MS plate thus allowing for

both separation of protein as well as subsequent detection. This seemed to be an advantageous solution to a single workflow for on-chip fractionation and high-throughput analysis, although a verdict has yet to be reached. Numerous diagnostic applications of this technology are presently under investigation mainly in the field of cancer (e.g. ovarian cancer, prostate cancer) [36,37], as are studies which address its reproducibility (e.g. different centers) [38] as would be necessary for a diagnostic platform. Although an approved diagnostic use has yet to become available, studies have shown that MS-based diagnosis may provide early and sensitive detection of ovarian cancer as compared to the classical tumor marker, CA125, for example [36].

Two-dimension electrophoresis (2D-PAGE) and immuno-based separation procedures remain major areas of research. 2D-PAGE has been classically used for protein separation as well as differential analysis, and presently labeling methods as well as computer-assisted identification procedures have much facilitated this technology. It is important to note that MS technology is most optimized for detection of peptides and small molecules (e.g. <5000 daltons) and when proteins and larger molecules are of interest, 2D-PAGE separation still remains a viable procedure despite its low-throughput. How to improve upon 2D separation procedures therefore remains an important topic. One recent technology which warrants mention is the PF 2D system (Beckman Coulter) which allows separation of proteins by two-dimensional liquid chromatography system based on separation by chromatofocusing with subsequent reversed-phase chromatography. We performed differential proteomic analysis using an animal model of diabetes mellitus and associated metabolic disorders (Otsuka Long-Evans Tokushima Fatty rat) using this system [39]. Differentially expressed proteins in serum were identified with MALDI-TOF mass spectrometry including apolipoproteins and alpha2-HS-glycoprotein. This was the first application of this approach to differential serum proteomics. While this technique still is in early stages and has poor throughput, it shows the potential of non-gel-based 2D techniques in identifying disease-associated proteins. A micro-scale 2D device for clinical use, if developed, would clearly be a welcomed tool not only for research but also for possible clinical diagnostic use.

Antibody-based immuno-separation is also an attractive procedure once the protein of interest is identified. Optimal procedures and platforms (e.g. beads, plates) for the use of immuno-based separation will also need to be addressed. Importantly, as protein fragments may be associated with disease as well as their modifications, immuno-based isolation will likely be pivotal for clinical application. For instance, fragmented and modified forms of the cardiac troponin protein are known to be associated with cardiac disease [40,41].

Whether MS instrumentation will find its way into mainstream diagnosis remains a question to be answered, but it is already used for diagnosis of inborn metabolic disorders and familial Amyloidosis among others. For this technology to become a viable addition to diagnostic testing, it will need to show that it is cost-effective and also to show important information which cannot be detected by any other technique. For instance, protein fragmentation and post-translational modification in disease are surely attractive targets in which detection by MS would excel, and for this to be a viable test would likely require that a panel of antibodies (e.g. protein chip) become available to allow for effective detection (e.g. cost, time). Quantization, reproducibility, and portability are also issues which remain to be addressed.

4. Conclusions—implications and lessons learned

In all, this review has summarized our approaches to proteomic medicine, and how we have used it in our lab to better understand cardiovascular pathophysiology with a focus on epigenetic regulation as well as biomarker discovery with further interest in clinical application of the technology. Although clinical application or exploitation of MS technology is still in young stages, further advancements will surely clarify the use of this biotechnology as a medical tool.

In a general context, the cardiovascular proteome is a current topic of interest and in the United States the National Heart, Lung, and Blood Institute (NHLBI) has started a clinical proteomics working group that is charged with identifying opportunities and challenges in clinical proteomics and using these as a basis for recommendations aimed at directly improving patient care [42]. The Human Proteome Organisation (HUPO) plasma proteome project also had a cardiovascular substudy which catalogued plasma proteins to be used as a resource [43]. Other studies have been ongoing on a global level to address the technologies and their results as would be relevant to cardiovascular disease. Although most studies have resulted in cataloging differential protein profiles in cells and disease states [44,45], this is an important initial step in determining the potential of the technology and cross-referencing of results. Given the various technologies and instrumentation available at present, these studies should be of value in determining which method meets the needs for specific aims. As we learn through our experiences in parallel with the advancements in technologies and instrumentation, this field will surely rapidly evolve within the coming years. Eventually, we aim to have a comprehensive understanding of protein dynamics (e.g. interaction, modification, degradation) in disease both at intra- as well as extra-cellular levels with ultimate goals of better understanding pathophysiological mechanisms which will lead to development of new diagnostic and therapeutic techniques.

Acknowledgements

This study was supported by grants from the Ministry of Education, Culture, Sports, Science, and Technology; New Energy and Industrial Technology Development Organization; and the Ministry of Health, Labor, and Welfare in addition to grants from the following foundations (Applied Enzymology, Takeda Science, Japan Heart).

References

[1] J.C. Venter, M.D. Adams, E.W. Myers, P.W. Li, R.J. Mural, G.G. Sutton, H.O. Smith, M. Yandell, C.A. Evans, R.A. Holt, J.D. Gocayne, P. Ama-

natides, R.M. Ballew, D.H. Huson, J.R. Wortman, Q. Zhang, C.D. Kodira, X.H. Zheng, L. Chen, M. Skupski, G. Subramanian, P.D. Thomas, J. Zhang, G.L. Gabor Miklos, C. Nelson, S. Broder, A.G. Clark, J. Nadeau, V.A. McKusick, N. Zinder, A.J. Levine, R.J. Roberts, M. Simon, C. Slayman, M. Hunkapiller, R. Bolanos, A. Delcher, I. Dew, D. Fasulo, M. Flanigan, L. Florea, A. Halpern, S. Hannenhalli, S. Kravitz, S. Levy, C. Mobarry, K. Reinert, K. Remington, J. Abu-Threideh, E. Beasley, K. Biddick, V. Bonazzi, R. Brandon, M. Cargill, I. Chandramouliswaran, R. Charlab, K. Chaturvedi, Z. Deng, V. Di Francesco, P. Dunn, K. Eilbeck, C. Evangelista, A.E. Gabrielian, W. Gan, W. Ge, F. Gong, Z. Gu, P. Guan, T.J. Heiman, M.E. Higgins, R.R. Ji, Z. Ke, K.A. Ketchum, Z. Lai, Y. Lei, Z. Li, J. Li, Y. Liang, X. Lin, F. Lu, G.V. Merkulov, N. Milshina, H.M. Moore, A.K. Naik, V.A. Narayan, B. Neelam, D. Nusskern, D.B. Rusch, S. Salzberg, W. Shao, B. Shue, J. Sun, Z. Wang, A. Wang, X. Wang, J. Wang, M. Wei, R. Wides, C. Xiao, C. Yan, A. Yao, J. Ye, M. Zhan, W. Zhang, H. Zhang, Q. Zhao, L. Zheng, F. Zhong, W. Zhong, S. Zhu, S. Zhao, D. Gilbert, S. Baumhueter, G. Spier, C. Carter, A. Cravchik, T. Woodage, F. Ali, H. An, A. Awe, D. Baldwin, H. Baden, M. Barnstead, I. Barrow, K. Beeson, D. Busam, A. Carver, A. Center, M.L. Cheng, L. Curry, S. Danaher, L. Davenport, R. Desilets, S. Dietz, K. Dodson, L. Doup, S. Ferriera, N. Garg, A. Gluecksmann, B. Hart, J. Haynes, C. Haynes, C. Heiner, S. Hladun, D. Hostin, J. Houck, T. Howland, C. Ibegwam, J. Johnson, F. Kalush, L. Kline, S. Koduru, A. Love, F. Mann, D. May, S. McCawley, T. McIntosh, I. McMullen, M. Moy, L. Moy, B. Murphy, K. Nelson, C. Pfannkoch, E. Pratts, V. Puri, H. Qureshi, M. Reardon, R. Rodriguez, Y.H. Rogers, D. Romblad, B. Ruhfel, R. Scott, C. Sitter, M. Smallwood, E. Stewart, R. Strong, E. Suh, R. Thomas, N.N. Tint, S. Tse, C. Vech, G. Wang, J. Wetter, S. Williams, M. Williams, S. Windsor, E. Winn-Deen, K. Wolfe, J. Zaveri, K. Zaveri, J.F. Abril, R. Guigo, M.J. Campbell, K.V. Sjolander, B. Karlak, A. Kejariwal, H. Mi, B. Lazareva, T. Hatton, A. Narechania, K. Diemer, A. Muruganujan, N. Guo, S. Sato, V. Bafna, S. Istrail, R. Lippert, R. Schwartz, B. Walenz, S. Yooseph, D. Allen, A. Basu, J. Baxendale, L. Blick, M. Caminha, J. Carnes-Stine, P. Caulk, Y.H. Chiang, M. Coyne, C. Dahlke, A. Mays, M. Dombroski, M. Donnelly, D. Ely, S. Esparham, C. Fosler, H. Gire, S. Glanowski, K. Glasser, A. Glodek, M. Gorokhov, K. Graham, B. Gropman, M. Harris, J. Heil, S. Henderson, J. Hoover, D. Jennings, C. Jordan, J. Jordan, J. Kasha, L. Kagan, C. Kraft, A. Levitsky, M. Lewis, X. Liu, J. Lopez, D. Ma, W. Majoros, J. McDaniel, S. Murphy, M. Newman, T. Nguyen, N. Nguyen, M. Nodell, S. Pan, J. Peck, M. Peterson, W. Rowe, R. Sanders, J. Scott, M. Simpson, T. Smith, A. Sprague, T. Stockwell, R. Turner, E. Venter, M. Wang, M. Wen, D. Wu, M. Wu, A. Xia, A. Zandieh, X. Zhu, Science 291 (2001) 1304.

[2] J.D. McPherson, M. Marra, L. Hillier, R.H. Waterston, A. Chinwalla, J. Wallis, M. Sekhon, K. Wylie, E.R. Mardis, R.K. Wilson, R. Fulton, T.A. Kucaba, C. Wagner-McPherson, W.B. Barbazuk, S.G. Gregory, S.J. Humphray, L. French, R.S. Evans, G. Bethel, A. Whittaker, J.L. Holden, O.T. McCann, A. Dunham, C. Soderlund, C.E. Scott, D.R. Bentley, G. Schuler, H.C. Chen, W. Jang, E.D. Green, J.R. Idol, V.V. Maduro, K.T. Montgomery, E. Lee, A. Miller, S. Emerling, Kucherlapati, R. Gibbs, S. Scherer, J.H. Gorrell, E. Sodergren, K. Clerc-Blankenburg, P. Tabor, S. Naylor, D. Garcia, P.J. de Jong, J.J. Catanese, N. Nowak, K. Osoegawa, S. Qin, L. Rowen, A. Madan, M. Dors, L. Hood, B. Trask, C. Friedman, H. Massa, V.G. Cheung, I.R. Kirsch, T. Reid, R. Yonescu, J. Weissenbach, T. Bruls, R. Heilig, E. Branscomb, A. Olsen, N. Doggett, J.F. Cheng, T. Hawkins, R.M. Myers, J. Shang, L. Ramirez, J. Schmutz, O. Velasquez, K. Dixon, N.E. Stone, D.R. Cox, D. Haussler, W.J. Kent, T. Furey, S. Rogic, S. Kennedy, S. Jones, A. Rosenthal, G. Wen, M. Schilhabel, G. Gloeckner, G. Nyakatura, R. Siebert, B. Schlegelberger, J. Korenberg, X.N. Chen, A. Fujiyama, M. Hattori, A. Toyoda, T. Yada, H.S. Park, Y. Sakaki, N. Shimizu, S. Asakawa, K. Kawasaki, T. Sasaki, A. Shintani, A. Shimizu, K. Shibuya, J. Kudoh, S. Minoshima, J. Ramser, P. Seranski, C. Hoff, A. Poustka, R. Reinhardt, H. Lehrach, Nature 409 (2001) 934.

[3] T. Suzuki, M. Horikoshi, R. Nagai, Gene Ther. Mol. Biol. 7 (2003) 91.

[4] T. Suzuki, T. Matsumura, R. Nagai, Trends Cardiovasc. Med. 15 (2005) 125.

[5] R.L. Davis, H. Weintraub, A.B. Lassar, Cell 51 (1987) 987.

[6] S.J. Tapscott, R.L. Davis, M.J. Thayer, P.F. Cheng, H. Weintraub, A.B. Lassar, Science 242 (1988) 405.

[7] E.N. Olson, Science 313 (2006) 1922.

[8] J. Backs, E.N. Olson, Circ. Res. 98 (2006) 15.

[9] T. Suzuki, K. Aizawa, T. Matsumura, R. Nagai, Arterioscler. Thromb. Vasc. Biol. 25 (2005) 1135.

[10] R. Tupler, G. Perini, M.R. Green, Nature 409 (2001) 832.

[11] W.S. Dynan, R. Tjian, Cell 35 (1983) 79.

[12] S. Philipsen, G. Suske, Nucleic Acids Res. 27 (1999) 2991.

[13] J. Turner, M. Crossley, Trends Biochem. Sci. 24 (1999) 236.

[14] D.T. Dang, J. Pevsner, V.W. Yang, Int. J. Biochem. Cell. Biol. 32 (2000) 1103.

[15] J.J. Bieker, J. Biol. Chem. 276 (2001) 34355.

[16] A.R. Black, J.D. Black, J. Azizkhan-Clifford, J. Cell. Physiol. 188 (2001) 143.

[17] P. Bouwman, S. Philipsen, Mol. Cell. Endocrinol. 195 (2002) 27.

[18] J. Kaczynski, T. Cook, R. Urrutia, Genome Biol. 4 (2003) 206.

[19] M.W. Feinberg, Z. Lin, S. Fisch, M.K. Jain, Trends Cardiovasc. Med. 14 (2004) 241.

[20] A.C. Oates, S.J. Pratt, B. Vail, Y.I. Yan, R.K. Ho, S.L. Johnson, J.H. Postlethwait, L.I. Zon, Blood 98 (2001) 1792.

[21] T. Suzuki, S. Muto, S. Miyamoto, K. Aizawa, M. Horikoshi, R. Nagai, J. Biol. Chem. 278 (2003) 28758.

[22] S. Miyamoto, T. Suzuki, S. Muto, K. Aizawa, A. Kimura, Y. Mizuno, T. Nagino, Y. Imai, N. Adachi, M. Horikoshi, R. Nagai, Mol. Cell. Biol. 23 (2003) 8528.

[23] T. Matsumura, T. Suzuki, K. Aizawa, Y. Munemasa, S. Muto, M. Horikoshi, R. Nagai, J. Biol. Chem. 280 (2005) 12123.

[24] S. Muto, M. Senda, N. Adachi, T. Suzuki, R. Nagai, M. Horikoshi, Acta Crystallogr. D Biol. Crystallogr. 60 (2004) 712.

[25] R. Nagai, S. Ueda, Y. Yazaki, Biochem. Biophys. Res. Commun. 86 (1979) 683.

[26] H. Katoh, T. Suzuki, Y. Hiroi, E. Ohtaki, S. Suzuki, Y. Yazaki, R. Nagai, Lancet 354 (1995) 191.

[27] T. Suzuki, H. Katoh, M. Watanabe, M. Kurabayashi, K. Hiramori, S. Hori, M. Nobuyoshi, H. Tanaka, K. Kodama, H. Sato, S. Suzuki, Y. Yazaki, R. Nagai, Circulation 93 (1996) 1244.

[28] T. Suzuki, H. Katoh, M. Kurabayashi, Y. Yazaki, R. Nagai, Lancet 350 (1997) 784.

[29] T. Suzuki, H. Katoh, Y. Tsuchio, A. Hasegawa, M. Kurabayashi, A. Ohira, K. Hiramori, Y. Sakomura, H. Kasanuki, S. Hori, N. Aikawa, S. Abe, C. Tei, Y. Nakagawa, M. Nobuyoshi, T. Misu, T. Sumiyoshi, R. Nagai, Ann. Int. Med. 133 (2000) 537.

[30] S.I. Toshima, A. Hasegawa, M. Kurabayashi, H. Itabe, T. Takano, J. Sugano, K. Shimamura, J. Kimura, I. Michishita, T. Suzuki, R. Nagai, Arterioscler. Thromb. Vasc. Biol. 20 (2000) 2243.

[31] T. Suzuki, H. Kohno, A. Hasegawa, S. Toshima, T. Amaki, M. Kurabayashi, R. Nagai, Clin. Biochem. 35 (2002) 347.

[32] T. Amaki, T. Suzuki, F. Nakamura, D. Hayashi, Y. Imai, H. Morita, K. Fukino, T. Nojiri, S. Kitano, N. Hibi, T. Yamazaki, R. Nagai, Heart 90 (2004) 1211.

[33] T. Suzuki, K. Yamaoki, O. Nakajima, T. Yamazaki, Y. Yamada, H. Akioka, Y. Yazaki, Jpn. Heart J. 41 (2000) 205.

[34] T. Suzuki, T. Yamazaki, Y. Yazaki, Cardiovasc. Res. 51 (2001) 489.

[35] T. Suzuki, S. Ishiwata, K. Hasegawa, K. Yamamoto, T. Yamazaki, Heart 83 (2000) 578.

[36] Z. Zhang, R.C. Bast Jr., Y. Yu, J. Li, L.J. Sokoll, A.J. Rai, H.M. Rosenzweig, B. Cameron, Y.Y. Wang, X.Y. Meng, A. Berchuck, C. Van Haaften-Day, N.F. Hacker, H.W. de Bruijn, A.G. van der Zee, I.J. Jacobs, E.T. Fung, D.W. Chan, Cancer Res. 64 (2004) 5882.

[37] B.L. Adam, Y. Qu, J.W. Davis, M.D. Ward, M.A. Clements, L.H. Cazares, O.J. Semmes, P.F. Schellhammer, Y. Yasui, Z. Feng, G.L. Wright Jr., Cancer Res. 62 (2002) 3609.

[38] O.J. Semmes, Z. Feng, B.L. Adam, L.L. Banez, W.L. Bigbee, D. Campos, L.H. Cazares, D.W. Chan, W.E. Grizzle, E. Izbicka, J. Kagan, G. Malik, D. McLerran, J.W. Moul, A. Partin, P. Prasanna, J. Rosenzweig, L.J. Sokoll, S. Srivastava, S. Srivastava, I. Thompson, M.J. Welsh, N. White, M. Winget, Y. Yasui, Z. Zhang, L. Zhu, Clin. Chem. 51 (2005) 102.

[39] T. Matsumura, T. Suzuki, N. Kada, K. Aizawa, Y. Munemasa, R. Nagai, Biochem. Biophys. Res. Commun. 351 (2006) 965.

[40] R. Labugger, L. Organ, C. Collier, D. Atar, J.E. Van Eyk, Circulation 102 (2000) 1221.
[41] J.L. McDonough, J.E. Van Eyk, Prog. Cardiovasc. Dis. 47 (2004) 207.
[42] C.B. Granger, J.E. Van Eyk, S.C. Mockrin, N.L. Anderson, National Heart, Lung, and Blood Institute Clinical Proteomics Working Group, Circulation 109 (2004) 1697.
[43] B.T. Berhane, C. Zong, D.A. Liem, A. Huang, S. Le, R.D. Edmondson, R.C. Jones, X. Qiao, J.P. Whitelegge, P. Ping, T.M. Vondriska, Proteomics 5 (2005) 3520.
[44] J.A. Westbrook, J.X. Wheeler, R. Wait, S.Y. Welson, M.J. Dunn, Electrophoresis 27 (2006) 1547.
[45] U. Mayr, M. Mayr, X. Yin, S. Begum, E. Tarelli, R. Wait, Q. Xu, Proteomics 5 (2005) 4546.