Megsin Gene: Its Genomic Analysis, Pathobiological Functions, and Therapeutic Perspectives

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Abstract: It is critical to uncover genes specifically expressed in individual cell types for further understanding of cell biology and pathology. In order to elucidate pathogenesis of renal disease, we performed functional quantitative analysis of the genome in human kidney cells and compared the expression levels of a variety of kidney transcripts with those in other non-kidney cells. As a result, we identified a novel human gene, megsin, which is a new serine protease inhibitor (serpin) predominantly expressed in the kidney. Megsin is up-regulated in kidney disease. Genomic analysis revealed an association of the polymorphisms of megsin gene with susceptibility and/or progression of kidney disease. Its overexpression in rodents has led to the recognition of two different kidney abnormalities. The first disorder is linked to megsin biological effect itself and the other to its conformational abnormality recently called the serpinopathy. In the latter model, the cellular and tissue damage is induced by the endoplasmic reticulum (ER) stress due to conformational disorder resulting from megsin tertiary structure. In both types, the inhibition of megsin's activity or abnormal conformational change should open new therapeutic perspectives. The desire to prevent these abnormalities with the hope to offer new therapeutic strategies has stimulated the development of megsin inhibitors by a structure based drug design approach relying on a precisely known three dimensional megsin structure.

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ISOLATION OF A KIDNEY-SPECIFIC GENE, MEGSIN

Sixty trillion (6×10^{13}) cells in the human body share essentially identical genomic DNA. Nonetheless, for normal physiological function distinct for each cell type, expression of genes is tightly regulated by the cell lineage. It is therefore critical to uncover which genes are specifically expressed in individual cell types for further understanding of cell biology and pathology of diseases.

Some molecular biological approaches such as subtraction cloning and differential hybridization allowed us to compare libraries from two different sources and detect cell- or organ-specific genes. However, no quantitative information about expression levels of specific and non-specific genes can be obtained. No more than two libraries can be compared at one time using these methods, either.

In order to elucidate pathogenesis of kidney disease, we performed functional quantitative analysis of the genome in human kidney cells and compared the expression levels of a variety of kidney transcripts with those in other non-kidney cells. For this purpose, we employed a unique strategy utilizing a rapid large scale sequencing of a 3'-directed cDNA library and computerized data processing [1]. As kidney

cells, we focused on mesangial cells, as they play a central role in maintaining a structure and function of the kidney glomerulus. In the pathophysiology of kidney disease, the proliferation of mesangial cells and the accumulation of extracellular mesangial matrix are primary events leading to the progression of a variety of kidney diseases such as chronic glomerulonephritis and diabetic nephropathy, two major causes of end-stage renal failure.

Sequencing of a 3'-directed cDNA library of cultured human mesangial cells was performed to determine partial sequences of 1836 randomly picked clones [2]. The sequence similarities of the clones were compared with each other and with the DNA databank GenBank utilizing the FASTA program [3]. We compared their transcripts with those in various other cells and organs [2], and finally selected six clones [4, 5], which had not been recorded in any other databases and were thought to be predominantly expressed in mesangial cells. Dot blot analysis of mRNAs from various organs and cells supported mesangium-predominant expression of these clones. One clone was obtained which was most abundant among those detected only in our human mesangial cell cDNA library (0.3 % of all the mRNA population). Utilizing PCR techniques, a full length cDNA of this gene was obtained [4].

MEGSIN GENE AND PROTEIN

Amino acid homology search by the FASTA program throughout the SwissProt database revealed that this gene

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was highly homologous to members of the serpin superfamily [6], which is a group of structurally related proteins and generally serves as extracellular, irreversible serine protease inhibitors. We therefore termed this gene 'megsin' after mesangial cell-predominant gene with a homology to serpin.

The amino acid sequence in the reactive loop site of megin exhibits the characteristic features of functional serpins [4]. Our *in vitro* assays utilizing recombinant megin indeed confirmed that megin serves as a functional serpin [7].

EXPRESSIONS OF MEGSIN GENE AND PROTEIN IN THE KIDNEY

Northern blot and reverse-transcribed polymerase chain reaction analyses of various tissues and cells demonstrated that megin was predominantly expressed in human mesangial cells [4]. These findings were further confirmed by *in situ* hybridization and by immunohistochemistry (Fig. 1) using megin-specific antibodies [4, 8, 9]. In IgA nephropathy and diabetic nephropathy, megin mRNA expression in glomeruli was up-regulated. A similar up-regulation of megin was observed in the experimental anti-Thy1 nephritis model of rats [10]. The increased expression of megin gene is thus associated with renal disorders with mesangial proliferation and its matrix accumulation.

Fig. (1). Megin protein expression in the kidney glomerulus. Immunofluorescence study utilizing anti-human megin demonstrates that megin is predominantly localized in the glomerulus, especially in the mesangial area (x 200).

GENOMIC ASSOCIATION OF MEGSIN WITH KIDNEY DISEASE

Recent studies have demonstrated the interesting association of the polymorphisms of megin gene with susceptibility and/or progression of kidney disease in Chinese patients [11-13].

The correlation between polymorphisms of megin gene and IgA nephropathy were investigated by using the family-based association study. Polymorphisms C2093T and

C2180T within the 3' untranslated region of megin were first examined. Transmission disequilibrium test (TDT) and haplotype relative risk (HRR) analyses revealed that megin 2093C and 2180T alleles were significantly more transmitted from heterozygous parents to patients, which suggested that the genetic variation in megin conferred susceptibility to IgA nephropathy [11].

To further examine the relationships of these genetic variants with clinical manifestations and renal histological lesions, haplotypes were constructed by using the C2093T and C2180T alleles. The genotype-phenotype relationship study found that the 2093C-2180T haplotype is associated with more severe forms of IgA nephropathy and more rapid disease progression [12]. It raised the question that whether these two variants confer the effect or just in linkage disequilibrium with other variants nearby. To answer this question, 12 known SNPs from different functional regions of megin were selected from GenBank. The genotypes were determined by PCR-RFLP and direct sequencing and the heterozygosity rates were calculated if the genotypes were heterozygote. When the rate exceeded 10 %, the TDT and HRR analysis were performed. We found two novel SNPs which hadn't been reported before (23179 9T/10T and 23103 7A/6A), and six heterozygous SNPs, among which five SNPs with the rate more than 10 % were analyzed. TDT and HRR analyses showed that 23167G alleles were transmitted more frequently from parents to patients than expected. The scores of glomerular index and glomerular sclerosis index were higher in GG genotype patients than those in other genotypes and the distribution frequency of GG genotype in the progressive group was higher than that of the stable group. The polymorphism of megin A23167G is thus associated with susceptibility and progression of IgA nephropathy in Chinese population. GG genotype is associated with severe histological lesions and progression of the disease [13]. The analysis of other four SNPs found no statistical significance. These data suggest the possible involvement of genetic variations of megin in the susceptibility and progression of IgA nephropathy.

PATHOBIOLOGICAL FUNCTION OF MEGSIN

To further understand a pathobiological role of megin, we overexpressed the human megin cDNA in mouse genome [7]. Two lines of megin transgenic mice have been obtained. They developed progressive mesangial matrix accumulation, an increase in the number of mesangial cells (proliferation), and an augmented immune complex deposition (Figs. 2A and B). The transgenic model is characterized by the expression of megin in all tissues due to the ubiquitous promoter for the transgene. Although immunohistochemical studies revealed the presence of megin in a host of tissues as well as in non-mesangial areas of the kidney, pathogenic effects of megin overexpression were restricted within glomeruli. The mechanism of glomerular abnormalities still remains unknown. We speculate that overexpression of megin, a novel serpin expressed in the glomerulus, may lead to mesangial dysfunction, impair the disposal of immune complexes, and increase mesangial matrix by tipping the balance towards lower matrix degradation. By contrast, histological abnormalities were not evident in knock-out mice for megin (our unpublished observation).

We subsequently cross-bred megin transgenic mice with RAGE (the receptor for advanced glycation end products)/iNOS double transgenic diabetic mice [14], and produced a severe diabetic nephropathy model [15]. These triple transgenic mice were the first to exhibit diabetic nodular lesions, the end result of mesangial matrix accumulation, similar to those observed in humans (Fig. 2C). A genetic manipulation of megin thus engenders kidney diseases, suggesting its biopathological involvements.

Fig. (2). Histopathological analysis of megin transgenic mouse kidneys (PAS staining). As compared to 40-week-old wild-type mice (A, x 200), F₁ megin transgenic mouse of the same age developed mesangial matrix expansion and an increase in the number of mesangial cells (B, x 200). This megin transgenic mice were cross-bred with RAGE/iNOS transgenic mice to generate the triple transgenic mice, which developed more severe glomerular lesion such as glomerular hypertrophy, global mesangial expansion, adhesion of parietal epithelial cells to the tuft, nodular-like lesions (C, x 200).

SERPINOLOGY: A NOVEL PATHOLOGY INDUCED BY MEGIN GENE OVEREXPRESSION

As an extension of our search, we generated megin transgenic rats utilizing the same ubiquitous promoter for the transgene [16]. Homozygotes had an impaired growth. They failed to gain weight and all died within 10 weeks. Western blot analysis revealed that megin expression is ubiquitous in all tissues with different degrees: high in heart, kidney and pancreas, and moderate in lung. Homozygotes had clear evidence of renal and pancreatic dysfunction, as shown by a nephrotic syndrome (proteinuria, hypoproteinemia, and elevated cholesterol levels) with deterioration of renal function (creatinine and BUN) and hyperglycemia with insulin deficiency. Compared to size-matched wild-type rats, they have a markedly lower creatinine clearance, a marker of renal function.

By histology, numerous large Periodic Acid Schiff (PAS)-positive droplets were observed within the cytoplasm of kidney and pancreatic cells (Fig. 3). In heterozygotes, a significantly lower number of smaller PAS-positive droplets were observed with essentially the same distribution pattern.

Many questions beg for an answer. Why is kidney pathology so different between mice and rats overexpressing megin while we utilize the same promoter? Why is diabetes associated only with megin transgenic rats while it is absent in mice? What is the molecular identity of the PAS-positive droplets and electron-dense inclusion bodies within the ER?

Fig. (3). PAS staining of a megin transgenic rat (homozygote) at the age of 8 weeks. Numerous, large, PAS-positive droplets were observed within the cytoplasm of glomerular epithelial cells, distal tubular cells, and collecting ducts. x 200.

Fortunately, the clinical literature provided us a stimulating model [17]. Patients deficient in α_1 -antitrypsin (AT), another serpin, suffer from pulmonary emphysema. Some suffer from additional liver cirrhosis. In the latter patients, the mutated α_1 AT molecule is retained within the liver endoplasmic reticulum (ER) as PAS-positive but diastase-resistance droplets, meaning that they are not glycogen [18]. Recent advances in molecular analysis unraveled this pathology. A mutation of α_1 AT enlarges the strand 4 pocket, thereby facilitating an aberrant intermolecular linkage between the loop of one α_1 AT molecule and the strand 4 pocket of another α_1 AT molecule. The resulting dimer eventually polymerizes within the ER. The polymers cannot be exported into other organelles such as Golgi, accumulate within cells as PAS-droplets, and trigger a series of hepatotoxic events [19].

The pathology underlying pulmonary emphysema and liver cirrhosis in α_1 AT deficiency is thus quite different. A loss-of-function of α_1 AT reduces the inactivation of elastase, the target protease, with attendant pulmonary emphysema. By contrast, a gain-of-function due to the aggregation of mutated α_1 AT within the ER induces liver dysfunction. The former is a biological disorder and the latter a conformational disorder.

This pathology has been recently referred to as the serpinopathy [17]. Two serpinopathies have been documented to date in humans: liver cirrhosis due to the mutation of α_1 AT and familial encephalopathy due to neuroserpin [20]. Conformational alterations of protein tertiary structure involving beta sheet are already well known as they are at the root of prions, amyloidosis and serpinopathy [21].

The disorders observed in our megin transgenic rats are caused by megin polymerization and may be called a serpinopathy. On electron microscopy, indeed the huge electron-dense inclusions positive for megin accumulate in the dilated rough ER [16]. Such a histological abnormality of ER is absent in megin transgenic mice. Our model of megin serpinopathy is the first to involve the kidney and pancreas.

The phenotypic difference between megsin transgenic mice and rats might reflect different pathogenic mechanisms depending upon the degrees of megsin gene expression. In mice, overexpression of megsin only enhances the inactivation of its target serine protease. Histological abnormalities develop late at the age of 40 weeks. Megsin polymerization is absent in this model. In rats, by contrast, the marked overexpression of megsin is one order magnitude higher than in mice. It induces early megsin polymerization and cellular toxicity within 10 weeks.

ENDOPLASMIC RETICULUM (ER) STRESS

Why is megsin overexpression toxic only for the kidney and pancreas while megsin is also overexpressed in other organs? What are the mechanism(s) underlying the cytotoxicity and tissue damage associated with the serpinopathy?

We took advantage of our model to elucidate the mechanism of cellular toxicity in serpinopathies. We demonstrated that an ER stress induced by megsin polymerization initiates cellular toxicity. ER is an intracellular compartment that plays a critical role in the processing, folding and transport of newly synthesized proteins [22]. All cells regulate the capacity of their ER to process synthesized proteins and adapt to an imbalance between protein load and folding capacity, recently referred to as the ER stress [23, 24]. ER stress is triggered by various stimuli and pathophysiological conditions [25, 26]. As a defensive system against the ER stress, an unfolded protein response (UPR) develops [27]. UPR involves transient attenuation of new protein synthesis, degradation of misfolded proteins, expression of a variety of ER stress proteins such as oxygen-regulated protein (ORP) 150 [28] and glucose-regulated proteins (GRPs). Under normal conditions, these ER stress inducible proteins serve as protein chaperones, complex with defective proteins and target them for degradation. During stress, UPR may limit accumulation of abnormal proteins within the ER, allowing cells to tolerate the ER stress. When the ER stress exceeds the balance beyond the limit of cellular UPR, the cell undergoes the apoptosis by activating caspase 12 and CHOP. ER is thus a very interesting organella because it is a crucial center for the maintenance of life by protein synthesis as well as for cell death [29].

On immunohistochemistry, ER stress inducible chaperons, ORP150 and GRP 78, were markedly up-regulated in glomeruli of megsin transgenic rats as compared to wild-type [30]. By double immunostaining, OPR150-expressed podocytes showed accumulation of megsin inclusion bodies. Increased expression of ORP150 was confirmed by Western blot analysis. Up-regulation was markedly in homozygotes, mildly in heterozygotes, but absent in wild-type kidney. Interestingly, megsin expression was equally abundant in the heart in the absence of PAS droplets and electron dense inclusions. Indeed, ER stress inducible chaperone were not detectable in the heart [30].

Altogether, in transgenic rats, the aggregation of megsin as a consequence of aberrant intermolecular linkage within the ER perturbs the function of the ER and an unfolded protein response ensues. Despite an increased expression of ER stress inducible chaperons, some cells are damaged and undergo apoptosis.

MECHANISM OF MEGSIN'S ACTION AND ITS THERAPEUTIC PERSPECTIVE

The analysis of genes expressed in kidney mesangial cells has thus allowed the identification of a new kidney specific serpin, megsin. Its overexpression in rodents has led to the recognition of two different kidney abnormalities. The first disorder is linked to megsin biological effect itself and the other to its conformational abnormality recently called the serpinopathy. In both types, the inhibition of megsin's activity or abnormal conformational change should open new therapeutic perspectives. As yet, however, only very few serpin inhibitors have been reported and none is in clinical use.

In the last part of this article, I discuss strategies designed to inhibit megsin's activity or abnormal conformational change leading to the serpinopathy.

The mechanism of megsin's activity is fortunately deduced and might provide a clue to develop such an inhibitor. All serpins share a common structure [31]: a beta sheet-rich body and an exposed mobile reactive loop which functions as a pseudo-substrate for the target protease (Fig. 4). The target protease first cleaves the loop which traps the protease. The cleaved loop then penetrates into a pocket, named the strand 4 position in the A beta sheets. This step is crucial for the serpin's anti-protease activity, because, without insertion of the cleaved loop into this pocket, the trapped protease is released from the loop. Indeed, pre-incubation of a megsin with a 14 amino acid peptide of the loop abolishes the megsin's activity [our unpublished observation].

Identification of low molecule compounds able to enter into this pocket as a mock molecule may therefore be a key to develop a serpin inhibitor. Of note, the loop amino acid content and hence the pocket size and charge differs in each serpin, potentially determining its specificity.

We also have a clue to prevent the progression of the megsin serpinopathy, because identification of such a low molecule compound able to enter into the strand 4 pocket may be also a key to prevent aberrant intermolecular linkage between the loop and the pocket.

STRUCTURE BASED DRUG DESIGN FOR DEVELOPMENT OF MEGSIN INHIBITORS

Up to now, very few serpin inhibitors have been reported. Most have been discovered by high-throughput random screening of a large chemical library [32-34], a rather inefficient strategy. We tried a new approach, the structure based drug design (SBDD). This approach requires a precise knowledge of the three dimensional structure of the serpin. As the megsin crystal structure was not available, I chose another clinically important serpin, plasminogen activator inhibitor (PAI-1), whose crystal structure has been described [35], and tested our strategy with SBDD.

We virtually screened a library, encompassing more than two millions chemicals by SBDD (Fig. 5). Two different filters reduced the number of compounds to about 3,000. The first filter assesses the drug-likeness, calculated from the specific distributions of the molecular descriptors for drug molecules clinically used. The second filter assesses the spe-

Fig. (4). Mechanism of serpin's action and conformational change.

Serpins consist of a beta sheet-rich body. It contains an exposed mobile reactive loop. The target protease first cleaves the loop. The cleaved loop then traps the protease and inserts its *N*-terminus part into the strand 4 position (s4A) of the A beta sheet, triggering its anti-protease activity. Occasionally, an aberrant intermolecular linkage induced by the insertion of serpin's loop into the s4A position of another serpin molecule results in intracellular aggregation and subsequent cellular damage, called the serpinopathy. Therefore, small loop peptide or compounds able to enter into the s4A position of the A beta sheet as a mock molecule may prevent the serpin biological activity or an aberrant intermolecular linkage of serpins leading to the serpinopathy.

cific lead-likeness of the inhibitory molecules, calculated from distributions of the molecular descriptors common to known reference inhibitors and the inhibitory 14 amino acid peptide to PAI-1. Docking simulation was then undertaken

Their PAI-1 inhibitory activity appeared specific as they fail to modify other serpin/serine protease systems. On SDS-PAGE, PAI-1 formed a covalent complex with tPA whereas no PAI-1/tPA complex formation was observed when PAI-1 was preincubated with our PAI-1 inhibitory compounds. This, we have succeeded in isolation of a low molecular inhibitory compound for PAI-1, a model serpin.

Based upon these results, we are trying to elucidate the megsin crystal structure in order to analyze its strand 4 pocket to identify a compound potentially able to enter into this pocket. Such a compound will hopefully prevent the consequences of experimental kidney diseases as well as the serpinopathy phenotype of our megsin transgenic rat model.

SUMMARY

The analysis of genes expressed in human kidney cells has allowed the identification of a new kidney specific serpin, megsin. It is up-regulated in human kidney diseases. Furthermore, recent genomic analysis revealed an association of the polymorphisms of megsin gene with susceptibility and/or progression of kidney disease. Interestingly, its over-expression in rodents has led to the recognition of two different kidney abnormalities. One of which (megsin transgenic rat) has provided a major clue to understand a new family of clinical diseases, called the serpinopathy. The desire to prevent these abnormalities with the hope to offer new therapeutic strategies has stimulated the development of new megsin inhibitors by a structure based drug design approach relying on a precisely known dimensional megsin structure. Preliminary results obtained for PAI-1, another serpin, are very promising.

Fig. (5). Structure based drug design (SBDD) approach.

by a program Ph4Dock [36] to evaluate whether the compound fits within the PAI-1 pocket. Eventually, we identified 95 candidate compounds theoretically able to bind this pocket and purchased or synthesized 28 of them to test their biological activities *in vitro*. Two of the 28 candidate compounds were highly effective [our unpublished observation].

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REFERENCES

- [1] Okubo, K., Hori, N., Matoba, R., Niiyama, T., Fukushima, A., Kojima, Y., Matsubara, K. *Nature Genet.* **1992**, 2: 173.
- [2] Yasuda, Y., Miyata, T., Nangaku, M., Iida, Y., Maeda, K., Kurokawa, K., Okubo, K. *Kidney Int.* **1998**, 53: 154.
- [3] Pearson, W.R., Lipman, D.J. *Proc. Natl. Acad. Sci.* **1988**, 85: 2444.
- [4] Miyata, T., Nangaku, M., Suzuki, D., Inagi, R., Uragami, K., Sakai, H., Okubo, K., Kurokawa, K. *J. Clin. Invest.* **1998**, 102: 828.
- [5] Wada, T., Miyata, T., Inagi, R., Nangaku, M., Wagatsuma, M., Suzuki, D., Wadinski, B.E., Okubo, K., Kurokawa, K. *J. Am. Soc. Nephrol.* **2001**, 12: 2601.
- [6] Carrell, R.W., Boswell, D.R. *Elsevier Science.* **1986**, 403.
- [7] Miyata, T., Inagi, R., Nangaku, M., Imasawa, T., Sato, M., Izuhara, Y., Suzuki, D., Yoshino, A., Onogi, H., Kimura, M., Sugiyama, S., Kurokawa, K. *J. Clin. Invest.* **2002**, 109: 585.
- [8] Suzuki, D., M, T., Nangaku, M., Takano, H., Saotome, N., Toyoda, M., Mori, Y., Zhang, S., Inagi, R., Endoh, M., Kurokawa, K., Sakai, H. *J. Am. Soc. Nephrol.* **1999**, 10: 2606.
- [9] Inagi, R., Miyata, T., Suzuki, D., Toyoda, M., Wada, T., Ueda, Y., Izuhara, Y., Sakai, H., Nangaku, M., Kurokawa, K. *Biochem. Biophys. Res. Commun.* **2001**, 286: 1098.
- [10] Nangaku, M., Miyat, T., Suzuki, D., Umezono, T., Hashimoto, T., Wada, T., Yagi, M., Nagano, N., Inagi, R., Kurokawa, K. *Kidney Int.* **2001**, 60: 641.
- [11] Li, Y.J., Du, Y., Li, C.X., Guo, H., Leung, J.C., Lam, M.F., Yang, N., Huang, F., Chen, Y., Fang, J.Q., Maxwell, P.H., Lai, K.N., Wang, Y. *J. Am. Soc. Nephrol.* **2004**, 15: 1739.
- [12] Xia, Y.F., Li, Y.J., Du, Y., Yang, N.S., Li, C.X., Leung, J.C., Lam, M.F., Huang, W.J., Chen, S.Q., Maxwell, P.H., Lai, K.N., Wang, Y.M. *Nephrol. Dial. Transplant.* **2006**, 21: 1570.
- [13] Xia, Y.F., Huang, S., Li, X., Yang, N., Huang, J., Xue, C., Zhang, M., Leung, J.C., Lam, M.F., Li, J. *Clin. Nephrol.* **2006**, 65: 153.
- [14] Yamamoto, Y., Kato, I., Doi, T., Yonekura, H., Ohashi, S., Takeuchi, M., Watanabe, T., Yamagishi, S., Sakurai, S., Takasawa, S., Okamoto, H., Yamamoto, H. *J. Clin. Invest.* **2001**, 108: 261.
- [15] Inagi, R., Yamamoto, Y., Nangaku, M., Usuda, N., Okamoto, H., Kurokawa, K., van, Ypersele, de, Strihou, C., Yamamoto, H., Miyata, T. *Diabetes* **2006**, 55: 356.
- [16] Inagi, R., Nangaku, M., Usuda, N., Shimizu, A., Onogi, H., Izuhara, Y., Nakazato, K., Ueda, Y., Oishi, H., Takahashi, S., Yamamoto, M., Suzuki, D., Kurokawa, K., van, Ypersele, de, Strihou, C., Miyata, T. *J. Am. Soc. Nephrol.* **2005**, 16: 1339.
- [17] Lomas, D.A., Carrell, R.W. *Nat. Genet. Rev.* **2002**, 3: 759.
- [18] Lomas, D. A., Evans, D.L., Finch, J.T., Carrell, R. W. *Nature* **1992**, 357: 605.
- [19] Lomas, D.A., Mahadeva, R. *J. Clin. Invest.* **2002**, 110: 1585.
- [20] Davis, R.L., Shrimpton, A.E., Holohan, P.D., Bradshaw, C., Feiglin, D., Collins, G.H., Sonderegger, P., Kinter, J., Becker, L.M., Lacbawan, F., Krasnewich, D., Muenke, M., Lawewnce, D.A., Yerby, M.S., Shaw, C.M., Gooptu, B., Elliott, P.R., Finch, J.T., Carrell, R.W., Lomas, D.A. *Nature* **1999**, 401: 376.
- [21] Carrell, R.W., Lomas, D.A. *Lancet* **1997**, 350: 134.
- [22] Ellgaard, L., Helenius, A. *Curr. Opin. Cell. Biol.* **2001**, 13: 431.
- [23] Lee, A.S. *Trends Biochem. Sci.* **2001**, 26: 504.
- [24] Lee, A.S. *Curr. Opin. Cell Biol.* **1992**, 4: 267.
- [25] Aridor, M., Balch, W.E. *Nat. Med.* **1999**, 5: 745.
- [26] Ron, D. *J. Clin. Invest.* **2002**, 109: 443.
- [27] Kaufmann, R.J. *Genes Dev.* **1999**, 13: 1211.
- [28] Kitao, Y., Ozawa, K., Miyazaki, M., Tamatani, M., Kobayashi, T., Yanagi, H., Okabe, M., Ikawa, M., Yamashima, T., Stern, D.M.; Hori, O., Ogawa, S. *J. Clin. Inves.* **2001**, 108: 1439.
- [29] Pahl, H.L. *Physiol. Rev.* **1999**, 79: 683.
- [30] Inagi, R., Nangaku, M., Onogi, H., Ueyama, H., Kitao, Y., Nakazato, K., Ogawa, S., Kurokawa, K., Couser, W.G., Miyata, T. *Kidney Int.* **2005**, 68: 2639.
- [31] Gils, A., Declerck, P. *J. Thromb. Haemost.* **2004**, 91: 425.
- [32] Elokda, H., Abou-Gharbia, M., Hennan, J.K., McFarlane, G., Mugford, C.P., Krishnamurthy, G., Crandall, D.L. *J. Med. Chem.* **2004**, 47: 3491.
- [33] Crandall, D.L., Elokda, H., Di, L., Hennan, J.K., Gorlatova, N.V., Lawrence, D.A. *J. Thromb. Haemost.* **2004**, 2: 1422.
- [34] Liang, A., Wu, F., Tran, K., Jones, S.W., Deng, G., Ye, B., Zhao, Z., Snider, R.M., Dole, W.P., Morser, J., Wu, O. *Thromb. Res.* **2005**, 115: 341.
- [35] Bernstein, F.C., Koetzle, T.F., Williams, G.J., Meyer, E.F. Jr., Brice, M.D., Rodgers, J.R., Kennard, O., Shimanouchi, T., Tasumi, M. *J. Mol. Biol.* **1997**, 112: 535.
- [36] Goto, J., Kataoka, R., Hirayama, N. *J. Med. Chem.* **2004**, 47: 6804.