

The ESF Meeting on „The Proteomics, Epigenetics and Pharmacogenetics of Pendrin“

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Key Words

Pendrin • Inherited hearing loss • Functional test • Ion transport • Pendred syndrome • EVA • SLC26A4

Abstract

Human pendrin (SLC26A4, PDS) is a 780 amino acid integral membrane protein with transport function. It acts as an electroneutral, sodium-independent anion exchanger for a wide range of anions, such as iodide, chloride, formate, bicarbonate, hydroxide and thiocyanate. Pendrin expression was originally described in the thyroid gland, kidney and inner ear. Accordingly, pendrin mutations with reduction or loss of transport function result in thyroid and inner ear abnormalities, manifested as syndromic (Pendred syndrome) and non-syndromic hearing loss with an enlarged vestibular aqueduct (ns-EVA). Pendred syndrome, the most common form of syndromic deafness, is an autosomal recessive disease characterized by sensorineural deafness due to inner ear malformations and a partial iodide organification defect that may lead to thyroid goiter. Later, it became evident that not only pendrin loss of function,

but also up-regulation could participate in the pathogenesis of human diseases. Indeed, despite the absence of kidney dysfunction in Pendred syndrome patients, evidence exists that pendrin also plays a crucial role in this organ, with a potential involvement in the pathogenesis of hypertension. In addition, recent data underscore the role of pendrin in exacerbations of respiratory distresses including bronchial asthma and chronic obstructive pulmonary disease (COPD). Pendrin expression in other organs such as mammary gland, testis, placenta, endometrium and liver point to new, underscored pendrin functions that deserve to be further investigated.

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collaboration and explore new directions for research. It is an independent organization, owned by 78 Member Organizations, which are research funding and performing organizations, academies and learned societies from 30 countries. ESF promotes collaboration in research itself, in funding of research and in science policy activities at the European level.

The main objectives of ESF for the years 2006-2010 (extended to 2011), as defined by its current strategic plan, are to promote Science Strategy and Science Synergy, paving the way for initiatives across disciplinary and geographic boundaries in the European Research Area (ERA).

The Exploratory Workshops scheme is one of the key instruments of the Science Strategy “pillar”. Each year, ESF supports approximately 50 Exploratory Workshops across all scientific domains. The focus of the scheme is on workshops aiming to explore an emerging and/or innovative field of research or research infrastructure, also of interdisciplinary character. Workshops are expected to promote new directions in research or new domains. It is expected that a workshop will conclude with plans for specific follow-up research activities and/or collaborative actions or other specific outputs either within the frame of ESF or for submission to the EU 7th Framework Program or other European or international funding organizations.

Aim of the Meeting

The explorative Workshop on “Proteomics, Epigenetics and Pharmacogenetics of Pendrin” provided a foundation for significant extension of an existing collaboration (20 research groups - 15 in Europe, one in Israel, one in Japan and 4 in the USA) to a truly European Network focused on Pendred syndrome, i.e. the Pendrin Consortium. Pendred syndrome is the most common form of syndromic congenital hearing loss in which malfunction of the pendrin protein occurs. The common theme on which the scientific collaborations are based is “**P**roteomics, **E**pigenetics, and **P**harmacogenetics of **P**endrin” (PEPP). “Proteomics” within this collaboration refers to how the pendrin protein alone or in conjunction with other proteins impacts the observed function; “epigenetics” refers to how inherited genetic information and genetic information not encoded in the DNA sequence translates to variations in the overall function of pendrin; and finally, “pharmacogenetics” explores how genetic differences in the pendrin gene could be translated into

differences in the way that possible drugs affect pendrin function. Extension of the existing collaboration between the PEPP partner-laboratories into a truly European dimension will add significant momentum to the knowledge-transfer between the different groups.

The main sponsors of the meeting were the ESF, the County of Salzburg (Austria) and the Paracelsus Medical University in Salzburg, Austria. Opening remarks were given by the organizer (Prof. M. Paulmichl), as well as the responsible person for research in the County of Salzburg (Dr. G. Brandstetter). In addition, Prof. J. Syka, the official observer from the ESF, gave an overview of the ESF foundation. After this presentation, the meeting moved on to the scientific topics.

Scientific Content of the meeting

The Special Issue “Proteomics, Epigenetics, and Pharmacogenetics of Pendrin” consists of 20 papers, 11 of which are reviews and 9 are original contributions. Owing to their main topics, the papers have been divided in the following sections:

- Function of the Pendrin Anion Exchanger and its Pathogenic Allelic Variants
- Structure/Function Relation of Pendrin
- Tissue-Specific Expression and Function of Pendrin (further divided in five subsections: The Thyroid, The Inner Ear, The Kidney, The Airways, The Liver)
- Pendrin Pharmacology
- Genetic, Epigenetic and Transcriptional Regulation of Pendrin

Function of the Pendrin Anion Exchanger and its Pathogenic Allelic Variants

Human pendrin (SCL26A4, PDS) is a 780 amino acid integral membrane protein. After its initial cloning in 1997 [1], it was generally believed that the function of pendrin was to promote sulfate transport across the cellular membrane [1]. This hypothesis was corroborated by the high homology between pendrin and the other members of the multifunctional sulfate transporter Solute Carrier (SLC) 26 family [2]. However, it was later recognized that pendrin could not transport sulfate [3, 4], but could transport iodide [4], chloride [5], formate [6], bicarbonate [5], hydroxide [5] and thiocyanate [7], at least in heterologous overexpression systems. Moreover, these earlier studies established that pendrin acted as a sodium-independent anion exchanger. The coupling ratio of the

ion exchange was initially unclear; again, considering homology with other members of the same family, and owing to earlier reports, it was assumed that pendrin participated in electrogenic anion exchange [8]. Later, we and others showed that pendrin anion transport was indeed non rheogenic (electroneutral) [9-12].

Mutations of the gene encoding pendrin (*SLC26A4*) that lead to a reduction or loss of transport function can result in syndromic (Pendred syndrome) and non-syndromic hearing loss with an enlarged vestibular aqueduct (ns-EVA) [13]. Pendred syndrome (OMIM#274600), the most common form of syndromic deafness [14], was originally described more than one century ago by Vaughan Pendred, as a combination of deafness and goiter unrelated to environmental factors [15]. Pendred syndrome is an autosomal recessive disease characterized by sensorineural deafness (due to inner ear malformations, such as an EVA) and a partial iodide organification defect disclosed by a positive perchlorate discharge test, with or without goiter [16]. Ns-EVA is a condition where deafness, due to inner ear malformations, is not associated with thyroid dysfunction. In this Special Issue, four manuscripts (one review and three original papers) describe in detail the Function of the Pendrin Anion Exchanger and its Pathogenic Allelic Variants. The mechanism underlying anion transport by wild-type pendrin, as well as the impact of mutations in the *SLC26A4* open reading frame (ORF) on pendrin function, are explored. Specifically, Reimold et al. describe the function and regulation of pendrin in an heterologous overexpression system, with isotopic flux, electrophysiology, and protein localization methodologies. They confirm the electroneutrality and 4,4'-diisothiocyano-2,2'-stilbenedisulfonic acid (DIDS)-insensitivity of the transporter, show its pH insensitivity and anion selectivity sequence, and identify residues important for activity and regulation [12]. Two original papers in this Special Issue are devoted to assessment of the functionality of wild-type pendrin and several of its allelic variants found in the Palestinian, Israeli and Spanish populations [17, 18]. In addition, the findings concerning the functional characterization of wild type pendrin and all allelic variants for which a functional test is available as of now are summarized in a review [19]. The main outcome of the latter work is that clinical and radiological studies should be combined with genetic (i.e. the sequencing of *SLC26A4* ORF) and functional studies for a correct diagnosis of Pendred syndrome and ns-EVA due to pendrin mutations.

Structure/Function Relation of Pendrin

The structure of pendrin has not been experimentally determined up to now. For highly hydrophobic proteins, in silico predictions obtained with different programs lead to different results. Information from crystallography or nuclear magnetic resonance spectroscopy is currently not available. Besides the membrane topology and anion binding domains, much attention has been devoted to understanding the structure and function of the intracellular carboxy-terminal end of the protein, which contains a Sulfate Transporter and Anti-Sigma Factor Antagonist (STAS) domain, as well as a putative protein kinase A (PKA) phosphorylation site. Some aspects of the Structure/Function Relation of Pendrin are described in this Special Issue in two manuscripts (one review and one original contribution). The review by Sharma et al. specifically focuses on the STAS domain, which may play a role in regulation, nucleotide binding, membrane targeting of the protein and protein-protein interactions [20, 21]. Although widespread among bacteria, in higher organisms the STAS domain is largely restricted to SLC26 anion transporter polypeptides. The presence of disease-causing mutations in the STAS domains of SLC26A2/DTNST, SLC26A3/DRA, and SLC26A4/pendrin attests to their structural importance [22]. Accordingly, Bizhanova et al show, in their original contribution, that a partial or complete elimination of the pendrin STAS domain leads to reduced plasma membrane insertion of the protein and consequently to decreased iodide transport. Additionally, they suggest that the rapid plasma membrane insertion of pendrin observed in response to forskolin is no longer possible without a functional PKA site [23].

Tissue-Specific Expression and Function of Pendrin

Pendrin is expressed in tissues as diverse as the thyroid gland [24], kidney [25], inner ear ([26, 27], mouse ortholog), airways [28], mammary gland [29], testis [30], placenta [31], endometrium [32] and liver (mouse ortholog, [33]). Pendrin exerts different functions in these different tissues; in this Special Issue, nine manuscripts (three original contributions and six reviews) elucidate and further explore the Tissue-Specific Expression and Function of Pendrin.

The Thyroid. In the thyroid gland, pendrin is expressed exclusively at the apical membrane of a subset of thyroid follicular cells [24]. Several points of evidence indicate that pendrin is crucially involved in follicular iodide transport, i.e. (i) its localization, (ii) the

iodide organification defect presented by patients with Pendred syndrome [34] and (iii) the aforementioned functional studies in heterologous expression systems. Pendrin could sustain iodide flux into the follicular lumen by means of an iodide/chloride exchange activity. In contrast, pendrin might also secrete bicarbonate into the thyroidal follicle, since thyroid follicular transepithelial potential and pH are reduced in pendrin knock-out mice [35]. In this Special Issue, two reviews [36, 37] discuss supporting evidence as well as arguments questioning the role of pendrin in mediating iodide efflux in thyrocytes.

The Inner Ear. In the inner ear, pendrin is expressed in epithelial cells in the cochlea, the vestibular labyrinth and in the endolymphatic sac, where it sustains chloride/bicarbonate exchange, secreting bicarbonate into the endolymph [38, 39]. As already mentioned, pendrin mutations with loss or reduction of the transport activity induce deafness associated with inner ear malformations (EVA). The comparatively high prevalence of EVA provides a strong imperative to develop rational interventions that delay, ameliorate or prevent hearing loss associated with this phenotype. Mouse models have been invaluable in helping to determine the mechanisms underlying SLC26A4-associated deafness. In this Special Issue, the review by Wangemann is devoted to elucidating the function of pendrin in the inner ear. Studies in mouse models that have focused on delineating the role of pendrin in the physiology of the inner ear and the pathobiology that leads to hearing loss are summarized. The crucial role of pendrin in controlling endolymphatic pH and ion composition, as well as in maintaining the endocochlear potential and fluid absorption into the endolymphatic sac is highlighted [40].

The Kidney. Pendrin is expressed on the apical membrane of non-alpha intercalated cells of the distal nephron and functions mainly as a chloride/bicarbonate exchanger [25]. Studies using pendrin knock-out mice led to the conclusion that one of the functions of pendrin in the kidney is the secretion of bicarbonate [25, 41], with a crucial role in acid-base homeostasis. In this Special Issue, four manuscripts (two reviews and two original papers) summarize and expand on the role of pendrin in the kidney. Wagner et al. reviewed the recent findings on the role and regulation of pendrin in the context of acid-base homeostasis in health and disease. The review focuses on pendrin expression and activity in the kidney of adult mice, which is regulated by systemic acid-base status, dietary electrolyte intake (mostly chloride), and hormones such as angiotensin II and aldosterone [42].

Pendrin also plays a role in chloride reabsorption within the kidney, and could be responsible for the pathogenesis of hypertension [43]. Indeed, pendrin knock-out mice develop hypotension under NaCl restriction [44] and are resistant to aldosterone-induced hypertension [45]. Hadchouel et al. focus on the role of chloride in triggering hypertension [46]; this role is usually underestimated and the mechanisms by which chloride transport influences blood pressure have, until recently, not been studied in detail. Pendrin-mediated chloride reabsorption is coupled either directly (via the proton/bicarbonate-dependent sodium transporter NDCBE) or indirectly (by activating the membrane expression of the epithelial sodium channel ENaC) with sodium reabsorption. These findings indicate that pendrin plays a crucial role in extracellular fluid volume and blood pressure control, and may represent a new molecular target for antihypertensive drugs [47].

Interestingly, in controlling both acid-base homeostasis and blood pressure, pendrin establishes functional interactions with other proteins. Indeed, Wagner et al. showed that angiotensin II stimulated the activity and enhanced the membrane abundance of the H⁺-ATPase in beta intercalated cells, thereby providing the driving force for bicarbonate secretion and chloride reabsorption via pendrin [48]. Moreover, very recently, the water channel AQP5 was found to colocalize with pendrin at the apical side of beta intercalated cells. The membrane expression of both pendrin and AQP5 are inhibited by potassium depletion, suggesting a functional interplay between the two proteins [46].

Pendrin may also play a role in controlling cellular volume, intracellular pH and chloride concentration. In this context, Rodighiero et al. present an interesting original contribution exploring the control of transmembrane chloride fluxes by pendrin, with possible functional implications in cell volume recovery after hypotonicity-induced cell swelling in human kidney cells [49].

The Airways. Prominent expression of pendrin in the airways was not discovered until 2005, when Kuperman et al. described upregulation of the transporter mRNA in the lungs of 3 separate murine asthma models [28]. Since then, pendrin activity in the respiratory epithelium has been associated with increased antimicrobial activity within the airway surface liquid (ASL) [7], mucus production [50] and regulation of the ASL thickness [51]. Recent data underscoring the role of pendrin in respiratory distresses including bronchial asthma and chronic obstructive pulmonary disease (COPD) are reviewed by Nofziger et al. in this Special Issue [52].

The Liver. In an original contribution, Alesutan et al. show that pendrin is indeed expressed in the murine liver, and report that acidosis modified pendrin transcript levels, with opposite effects in the liver and kidney [33]. Accordingly, hepatic Slc26a4 transcript levels were upregulated by ammonium chloride and acetazolamide, which are known to cause acidosis. This group postulates that pendrin might participate in the regulation of volume, and consequently metabolism, of the hepatocyte. The functional role of pendrin in the liver is a new and interesting issue, and deserves further investigation.

Pendrin Pharmacology

As previously mentioned and reviewed by Hadchouel et al. in this Special Issue, chloride reabsorption via pendrin at the level of the kidney could be responsible for the pathogenesis of hypertension [43, 46]. Thus, pendrin may represent a potential target for blood pressure control [47]. As reviewed by Amlal et al. in this Special Issue, pharmacologic inhibition of pendrin and the thiazide-sensitive sodium-chloride cotransporter NCC may provide a novel and strong diuretic regimen for patients with fluid overload, including those with congestive heart failure, nephrotic syndrome and renal failure [53].

In the airways, pendrin has been associated with mucus production [50] and is upregulated following stimulation with pro-inflammatory cytokines [7, 50, 51, 54, 55]. As reviewed by Nofziger et al. in this Special Issue [52], pendrin-mediated chloride reabsorption could reduce the ASL thickness [51], exacerbating, together with mucus overproduction, the symptoms of asthma and COPD [50]. In such pathological conditions, blocking or reducing pendrin activity with the use of selective drugs might be beneficial. Moreover, pendrin allelic variants with a modest, but significant, gain of function have been identified [18]. These hyperfunctional mutants could be genetic modifiers contributing to the severity of the phenotype of hypertension, asthma and COPD. A considerable effort has been devoted in identifying drugs that could reduce pendrin activity. Pendrin shows an unusual inhibitor profile when compared to other anion exchangers, in that it seems to be scarcely sensitive [4] or even resistant [56] to classic inhibitors of chloride/bicarbonate exchangers. Interestingly, the most potent pendrin inhibitor available at present seems to be the nonsteroidal anti-inflammatory drug niflumic acid [56]. The identification of new pendrin inhibitors deserves further investigation.

Genetic, Epigenetic and Transcriptional Regulation of Pendrin

This section comprises four manuscripts, three of which are reviews and one is an original contribution. The field of genetics and its associated area of genomics have evolved tremendously over the past years. Since the discovery of *SLC26A4* mutations and association with disease, there has been remarkable progress in understanding pendrin function due to technological genomic advances that are reviewed in this Special Issue by Dror et al. While most of the mutations in patients with Pendred syndrome have thus far been identified in the *SLC26A4* gene, other genetic mutations may contribute to the phenotype as well. Furthermore, the work on the mouse models for deafness (Slc26a4^{-/-} [41, 57], Slc26a4loop [58], Slc26a4^{tm1.Dontuh/tm1.Dontuh} [59] mice) illuminated the physiological and functional role of pendrin in various systems, including the inner ear [60].

In the attempt of establishing a genotype-phenotype correlation, Ito et al., in a comprehensive review of the current knowledge on the topic, concluded that there is no correlation between the type of *SLC26A4* mutation and thyroid phenotype (i.e. Pendred syndrome versus ns-EVA), but the number of mutant alleles of *SLC26A4* is correlated with the thyroid and auditory phenotypes. Pendred syndrome correlated with the presence of two *SLC26A4* mutant alleles, while ns-EVA was associated with either one or zero *SLC26A4* mutant alleles. In families with one mutant allele of *SLC26A4*, EVA is likely caused by a second, undetected *SLC26A4* mutation, while in families with zero mutant alleles of *SLC26A4*, etiologic heterogeneity includes causes other than, or in addition to, monogenic inheritance [61].

Investigation of the transcriptional regulation of pendrin is crucial for understanding how the expression levels and activity of this transporter are modulated in health and disease. This knowledge could aid in the development of new pharmacological leads for asthma, COPD and/or hypertension therapy. The main findings regarding pendrin transcriptional regulation are reviewed by Rozenfeld et al. in this Special Issue [62]. Ambient pH and known modulators of electrolyte balance (aldosterone and the intestinal natriuretic hormone UGN) modulate human pendrin promoter activity by acting on distinct response elements. These findings provide the first direct evidence that pendrin-mediated chloride/bicarbonate exchange in the renal tubule, iodide accumulation in the thyroid, and endolymph ion balance in the inner ear may be differentially regulated at the transcriptional level by systemic pH and aldosterone [63].

In addition, we recently found that the human pendrin promoter contains a signal transducer and activator of transcription 6 (STAT6) binding site (the N₄, interferon-gamma activated site (GAS) motif) that renders the promoter responsive to the pro-inflammatory cytokines, interleukin (IL)-4 and IL-13. This finding could explain pendrin upregulation observed in murine asthma models, an event linked to asthma and COPD exacerbations [54, 62].

Although it is widely accepted that epigenetic factors could contribute to the inter- and intra-familial variability of the clinical manifestation of Pendred syndrome, very little is understood. Besides other epigenetic phenomena [64], DNA methylation plays an important role in determining gene expression patterns. In an original contribution, Lee et al. explored the methylation status of the human pendrin promoter in two separate cell models. They suggest that cell-specific differences in basal methylation may determine cell-specific responses, in terms of pendrin mRNA expression, to different stimuli [65].

Outlook and future perspectives

Since its cloning in 1997, tremendous advances have been made in understanding the role of pendrin in health and disease, owing especially to studies in mouse models for Pendred syndrome. Despite that, several aspects of pendrin physiology, molecular biology and genetics deserve further investigation. The molecular structure, membrane topology, and anion binding domains of the pendrin protein are not known, and the impact of mutations on its transport activity is difficult to predict. Correlation between the specific genotype (in terms of type of mutation) and phenotype of the patient is, at present, not

established. Unclear is the determinant of the phenotypic variability of Pendred syndrome, and how and which genetic, epigenetic and environmental factors contribute to the severity and progression of the clinical picture of the patient is only partially understood.

The precise mechanisms that, in case of pendrin hyperfunction or overexpression, would lead to pathological conditions such as asthma, COPD and hypertension are not fully elucidated, either. Inhibiting pendrin activity in such conditions could be beneficial, nevertheless the pharmacological profile of the transporter is poorly characterized, and no selective inhibitors have been identified so far.

Conclusions

The explorative Workshop on “Proteomics, Epigenetics and Pharmacogenetics of Pendrin” fortified the existing scientific collaboration among the members of the Pendrin Consortium, provided a concrete basis for future cooperation and prepared the ground for developing an application to the EU 7th framework program.

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