Aquaporins: translating bench research to human disease

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Summary

There is considerable potential for translating knowledge of aquaporin structure, function and physiology to the clinic. One area is in aquaporin-based diagnostics. The discovery of AQP4 autoantibodies as a marker of the neuromyelitis optica form of multiple sclerosis has allowed precise diagnosis of this disease. Other aquaporin-based diagnostics are possible. Another area is in aquaporin-based genetics. Genetic diseases caused by loss-of-function mutations in aquaporins include nephrogenic diabetes insipidus and cataracts, and functionally significant aquaporin polymorphisms are beginning to be explored. Perhaps the greatest translational potential is aquaporin-based therapeutics. Information largely from aquaporin knockout mice has implicated key roles of aquaporin-facilitated water transport in transepithelial fluid transport (urinary concentrating, gland fluid secretion), water movement into and out of the brain, cell migration (angiogenesis, tumor metastasis, wound healing) and neural function (sensory signaling, seizures). A subset of aquaporins that transport both water and glycerol, the ‘aquaglyceroporins’, regulate glycerol content in epidermal, fat and other tissues, and are involved in skin hydration, cell proliferation, carcinogenesis and fat metabolism. Aquaporin-based modulator drugs are predicted to be of broad potential utility in the treatment of edematous states, cancer, obesity, wound healing, epilepsy and glaucoma. These exciting possibilities and their associated challenges are reviewed.

Key words: aquaporin, AQP, water transport, cell migration, angiogenesis, cancer, diuretic epidermis, brain swelling.

Introduction

There are 13 mammalian aquaporins (AQPs), constituting a family of small, hydrophobic, membrane proteins. There is a considerable body of information about AQP structure from electron and x-ray crystallography (reviewed by Fujiyoshi et al., 2002), showing AQP monomers (~30 kDa) containing six membrane-spanning helical domains surrounding a narrow aqueous pore. AQP monomers are super-assembled in membranes as tetramers. While the primary function of most AQPs is in facilitating water movement across cell membranes in response to osmotic gradients, a subset of AQPs, called aquaglyceroporins, also transport glycerol and possibly other small polar molecules. There is controversial evidence that some AQPs may transport gases and ions across membranes. The mammalian AQPs are expressed in various epithelia and endothelia involved in fluid transport, such as kidney tubules and glandular epithelia, as well as in other cell types such as brain glial cells, epidermis and adipocytes. Much of our understanding of AQP functions in mammalian physiology has come from phenotype analysis of mice lacking each of the AQPs (reviewed by Verkman, 2005). Mouse phenotype studies have confirmed the anticipated involvement of AQPs in the urinary-concentrating mechanism and glandular fluid secretion, and led to the discovery of unanticipated roles of AQPs in brain water balance, cell migration, cell proliferation, neural activity, epidermal hydration and ocular function.

This review focuses on translational aspects of AQP research. What AQPs do and don’t do in mammalian physiology is reviewed, followed by consideration of AQP-based diagnostics, genetics and therapeutics.

Functions of aquaporins in cell and organ physiology

Anticipated roles of AQPs in urinary-concentrating function and gland fluid secretion

Water transport across kidney tubules and microvessels is important for reabsorption of water filtered by the glomerulus and for the formation of a concentrated urine, which involves countercurrent multiplication and exchange mechanisms and vasopressin-regulated water permeability in the collecting duct. AQP1 is expressed at cell plasma membranes in proximal tubule and thin descending limb of Henle epithelia, and in descending vasa recta endothelia (reviewed by Verkman, 2008). AQP2, the vasopressin-regulated water channel, is expressed in collecting duct apical membrane and intracellular vesicles, and AQPs 3 and 4 are expressed constitutively at the basolateral membrane of collecting duct epithelia. As anticipated, defective urinary-concentrating function was found in mice lacking AQPs 1–4 (Ma et al., 1997; Ma et al., 1998; Ma et al., 2000b; Yang et al., 2001) and in humans with mutations in AQP1 (King et al., 2001) or AQP2 (Deen et al., 1994). Transepithelial water permeability and near-isosmolar fluid absorption in proximal tubule are impaired in mice lacking AQP1 (Schnerrmann et al., 1998). AQP1 deletion also reduces water permeability in thin descending limb of Henle (Chou et al., 1999) and vasa recta microvessels (Fallone et al., 2000), impairing the generation of a hyperosmolar medullary interstitium. Deletion or mutation of AQPs 2–4 reduces collecting duct water permeability, impairing osmotic equilibration between urinary fluid in the collecting duct lumen and the renal interstitium, as illustrated in Fig. 1A. AQP inhibitors are thus predicted to have ‘aquaretic’ activity, producing a water>salt diuresis.
The other anticipated role of AQPs is in active fluid transport across epithelia. AQP5 deletion in mice impairs fluid secretion by salivary (Ma et al., 1999) and airway submucosal (Song and Verkman, 2001) glands, resulting in reduced secretion of a relatively hyperosmolar fluid. Impaired fluid secretion has also been found in AQP1 knockout mice in choroid plexus (Oshio et al., 2005), where cerebrospinal fluid is produced, and in ciliary epithelium (Zhang et al., 2002), where ocular aqueous fluid is produced. AQP1 is involved in the regulation of intracranial and intraocular pressure in these closed fluid compartments. As shown in Fig 1B, active, near-isosmolar fluid transport involves water movement across a highly water permeable epithelium in response to osmotic gradients produced by salt transport. Reduced epithelial cell water permeability results in the secretion of a relatively low volume of a hyperosmolar fluid. In the various epithelia mentioned above the rates of transepithelial fluid secretion normalized to epithelial surface area are very high, such that the reduced water permeability in AQP deficiency impairs transepithelial osmotic equilibration. In other epithelia having much lower rates of fluid absorption or secretion, including lacrimal gland (Moore et al., 2000), sweat gland (Song et al., 2002), alveolus (Bai et al., 1999; Ma et al., 2000a), and airways (Song et al., 2001), AQP deletion does not impair transepithelial fluid transport. Transepithelial water transport is not rate limiting for fluid secretion when rates of fluid secretion are low.

Unanticipated roles of AQP-facilitated water transport in brain function and cell migration
The main water channel in brain is AQP4, which is expressed in glial cells at fluid–parenchymal interfaces at the blood–brain and ependymal–CSF barriers. In cytotoxic brain edema, water moves into the brain through an intact blood–brain barrier in response to osmotic driving forces. Mice lacking AQP4 showed improved outcome and reduced brain water accumulation compared with wild-type mice in models of cytotoxic brain edema, including water intoxication and ischemic stroke (Manley et al., 2000) and bacterial meningitis (Papadopoulos et al., 2005). Recent results show a remarkably improved outcome in AQP4 null mice following spinal cord injury (Saadoun et al., 2008), which was attributed to reduced spinal cord edema early after injury. AQP deletion also influences tissue water accumulation in stress-induced corneal (Thiagarajah and Verkman, 2002) and retinal (Da and Verkman, 2004) edema, as well as cataract formation (Ruiz-Ederra and Verkman, 2006).

In vasogenic, or ‘leaky-vessel’ brain edema, excess water moves into the brain by a bulk fluid flow mechanism through a leaky blood–brain barrier, and exits the brain by movement into the CSF through the AQP4-rich glia limitans lining brain ventricles and the brain surface (Fig 1C). When these water exit routes are blocked in obstructive hydrocephalus, water also moves out of the brain back into microvessels through the blood–brain barrier. Mice lacking AQP4 have a worse clinical outcome and greater brain water accumulation in models of vasogenic brain edema, including cortical-freeze injury and brain tumor (Papadopoulos et al., 2004) and brain abscesses (Bloch et al., 2005). AQP4 null mice also manifest an accelerated course of brain swelling in obstructive hydrocephalus (Bloch et al., 2006). We concluded that AQP4 facilitates removal of excess brain water in vasogenic brain edema and hydrocephalus. AQP4 inhibitors are thus predicted to reduce brain swelling in cytotoxic edema, whereas AQP4 enhancers
Aquaporins: bench-to-bedside

Aquaporins (AQPs) are integral membrane proteins that serve as water channels in biological membranes. They are involved in various physiological processes, including water transport, ion regulation, and cellular function. AQPs are found in epithelial, endothelial, and glial cells, and their expression levels can vary significantly across different tissues and cell types.

Relevance in Neural Function:

AQPs are involved in neural function, particularly in the brain. Studies have shown that AQPs are present in glial cells and neurons, and they play a role in regulating extracellular space and K+ homeostasis. AQP4, in particular, is highly expressed in astrocytes, and altered expression or function of AQP4 can affect brain function.

Roles of AQPs in Neuronal Excitation and Seizure Disorder:

AQP4 is an important marker for astrocytic dysfunction, and its expression is decreased in the brains of patients with epilepsy. In animal models, AQP4 deficiency can lead to altered neuronal excitability, which may contribute to seizure disorders.

Roles of AQPs in Tumor Biology:

AQPs are also important in tumor biology. AQPs are expressed in various tumor cell types, and they play a role in tumor angiogenesis, cell proliferation, and metastasis. AQPs facilitate the movement of cells through extracellular matrices, which is crucial for tumor invasion and spread.

Roles of AQPs in Skin Physiology:

In the skin, AQPs are involved in water homeostasis, cell proliferation, and differentiation. AQPs like AQP3 and AQP7 are crucial for maintaining skin barrier function, and their expression levels can be altered in various skin diseases.

Impact on Epidermal Function:

Epidermal differentiation and barrier function are critical for maintaining skin health. AQPs are involved in these processes, and their dysregulation can lead to alterations in skin barrier integrity, which is seen in various skin disorders.

Consequences of AQPs Dysfunction:

Disruption of AQPs function can have significant consequences on various physiological processes. Understanding the role of AQPs in different tissues and cell types is crucial for developing targeted therapies for diseases associated with AQPs dysfunction.

Further Research:

Future research should focus on the role of AQPs in specific diseases and conditions, as well as the development of novel therapeutic strategies targeting AQPs for the treatment of various disorders.

Fig. 2. Roles of AQPs in mammalian physiology based on their glycerol transport function. (A) Reduced glycerol content in epidermis and stratum corneum in skin in AQP3 deficiency, accounting for reduced skin hydration. (B) Proposed mechanism of AQP3-facilitated cell proliferation involving reduced cellular glycerol and consequent reduced ATP energy and biosynthesis. (C) Proposed mechanism for adipocyte hypertrophy in AQP7 deficiency, showing impaired AQP7-dependent glycerol escape from adipocytes resulting in cellular glycerol and triglyceride accumulation. Glycerol 3-P, glycerol 3-phosphate; TG, triacylglycerol; FFA, free fatty acid.
inhibitors may thus have utility in skin tumor prevention and therapy. Recognizing the relationship between AQP3 expression and skin moisturization, several companies have marketed cosmetics containing ingredients claimed to increase AQP3 expression. However, given the relationship between AQP3 expression and skin tumorigenesis, caution seems warranted in the use of AQP3-upregulating cosmetics.

The aquaglyceroporin AQP7 is expressed in the plasma membrane of adipocytes. AQP7 null mice manifest progressive increases in fat mass and adipocyte hypertrophy as they age, with accumulation of glycerol and triglycerides in adipocytes (Hara-Chikuma et al., 2005; Hibuse et al., 2005). Biochemical studies suggested that adipocyte hypertrophy in AQP7 deficiency is the consequence of reduced plasma membrane glycerol permeability, with cellular glycerol accumulation and triglyceride biosynthesis (Fig. 2C). We proposed that increasing adipocyte glycerol permeability, perhaps by enhancers of AQP7 expression, might reverse this process and thus provide a novel therapy for obesity. AQP roles unrelated to their water and glycerol transport functions

The diverse group of physiological functions described above can be attributed to the plasma membrane water- and/or glycerol-transport functions of AQPs. Various other roles of AQPs have been proposed. There is controversial evidence that AQPs can transport certain gases, including CO2, NO and O2 (reviewed by Wu and Beitz, 2007). Because the permeability of lipid bilayers to these gases is very high, their permeability across cell membranes is predicted to be unimpaired. However, AQP independent, as has been found experimentally for CO2 (Yang et al., 2000; Fang et al., 2002; Missner et al., 2008). The less membrane-permeable gas NH3 has been found to pass through AQP8 (Holm et al., 2005), though a study utilizing knockout mice concluded that AQP8-facilitated NH3 is not of physiological importance (Yang et al., 2006d). There is evidence for transport of small ions, urea and arsenite by some AQPs, though in some cases the findings are controversial and so far no evidence has been reported to support the physiological importance of AQP-facilitated transport of these substances. Evidence for AQP functioning in mitochondria in liver and brain has been proposed (Calamita et al., 2005; Amiry-Moghaddam et al., 2005), though subsequently refuted by direct permeability measurements (Yang et al., 2006b). It is unlikely that AQP-facilitated water or glycerol transport in organellar membranes is of importance to cell functioning because of the high surface-to-volume ratio of organelles and consequent rapid water/solute equilibration even in the absence of AQPs. Finally, as mentioned with regard to neuroexcitation phenomena, various AQP protein–protein interactions have been proposed, such as AQP4–Kir4.1 interaction, though subsequently refuted (Ruiz-Eduera et al., 2007). Stoichiometric interactions between AQPs and ion channels seem unlikely because the membrane density of AQPs is 100- to 1000-fold greater than that of ion channels. Another recently proposed non-transporting role of AQPs is in cell–cell adhesion, including AQP4-facilitated glial cell adhesion (Hiroake et al., 2006). However, direct measurements have refuted the initial findings (Zhang and Verkman, 2008a). Together, the present evidence supports the conclusion that AQPs are involved primarily in plasma membrane water and/or glycerol permeability.

Aquaporin-targeted therapies

Potential clinical indications of AQP modulators

Notwithstanding some differences in human vs mouse physiology, the phenotype findings in AQP-deficient mice suggest various clinical indications of AQP modulators. The requirement of AQPs for the formation of a concentrated urine suggests that AQP inhibitors, or ‘AQP-aquaretics’, would reduce urine concentration, producing a water-salt diuresis. Though inhibitors of AQP2 would have similar aquaretic activity to existing vasopressin receptor-2 antagonists for therapy of hyponatremia associated with high vasopressin, AQP1 inhibitors are predicted to have utility in diuretic-refractory edematous states, such as severe congestive heart failure, where conventional salt-blocking diuretics are of limited efficacy. Inhibitors of AQP4 are predicted to reduce brain swelling in cytotoxic edema, potentially offering neuroprotection following brain and spinal cord injury, and ischemic stroke, and potentially reducing mortality in infectious meningitis and various encephalitides. Inhibitors of AQPs in tumor cells and microvessels are predicted to reduce tumor spread and angiogenesis, offering adjunctive tumor chemotherapy. Inhibition of AQP4-facilitated glial cell migration is predicted to inhibit glial scar formation following brain and spinal cord injury, promoting axonal regeneration and improving long-term neurological outcome. Topical inhibitors of AQP1 in the eye may reduce intraocular pressure in glaucoma, and inhibitors of AQP3 in the skin may reduce skin cancer. Compounds that increase AQP function, acting by increasing AQP expression, are predicted to have potential efficacy in reducing fat mass in obesity, in accelerating brain water clearance in vasogenic edema, in promoting wound healing and tissue regeneration following injury, and in inhibiting cataractogenesis. Validation of these predictions in humans will require the development of AQP-specific modulators. Challenges will include the identification of potent, AQP subtype-selective inhibitors, and, in the case of AQP4 inhibitors, inhibitors that penetrate the blood–brain barrier. Identification of AQP enhancers presents an even great challenge as AQPs probably already have maximal per-channel function that cannot be further increased, and identification of selective transcriptional upregulators is without precedent in drug discovery.

Aquaporin inhibitors

There are at present no reported AQP inhibitors that are suitable candidates for clinical development. Though multiple AQPs are inhibited by sulphydryl-reactive mercurials such as mercury and gold (Niemietz and Tyerman, 2002), these metal ions are non-selective in their action and very toxic. Various candidate blockers of AQP1 have been reported, including tetroethylammonium (Brooks et al., 2000), acetazolamide (Ma et al., 2004) and DMSO (van Hoek et al., 1990); however, a careful evaluation of their inhibition efficacy using sensitive measurement methods indicates little or no AQP1 inhibition by tetroethylammonium or acetazolamide, and apparent inhibition by DMSO resulting from an osmotic clamp effect rather than true inhibition (Yang et al., 2006a). A careful analysis in Xenopus oocytes also showed no AQP1 inhibition by acetazolamide or tetroethylammonium (Sogard and Zeuthen, 2008). Recently, several papers from one group reported AQP4 inhibition by a series of arylsulfonamides, antiepileptic drugs, and related molecules, with strong inhibition at low micromolar concentrations (Huber et al., 2007; Huber et al., 2008a; Huber et al., 2008b); however, these results could not be confirmed, with no inhibition activity found even at high concentrations of any of the putative AQP4 inhibitors (Yang et al., 2008). The identification of bona fide AQP inhibitors will likely require high-throughput screening of diverse small-molecule collections, utilizing sensitive assays of water transport function.
Screening methods for identification of AQP modulators

There are a number of possible strategies for identification of AQP modulators by screening of large compound collections. Many methods have been developed to measure water permeability across cell membranes, based largely on changes in cell volume in response to osmotic gradients. Cell volume has been measured in unlabeled cells by light scattering, phase-contrast microscopy and interferometry, and in fluorescently labeled cells by total internal reflection microscopy and confocal microscopy (reviewed by Verkman, 2000). Some of these methods are amenable to platereader or imaging (high-content screening) platforms. Calcein fluorescence quenching provides a simple approach to quantify cell membrane water permeability (Solenov et al., 2004), in which osmotically induced cell shrinking reduces cytoplasmic calcein fluorescence by cytoplasmic protein-mediated quenching. A similar strategy, following the development of second generation green fluorescent protein-based chloride sensors (Galiotta et al., 2001), involves measurement of cell membrane water permeability from the time course of fluorescence in labeled cells following osmotic challenge. Cell shrinking produces an instantaneous increase in cytoplasmic chloride concentration and consequent reduction in sensor fluorescence. Challenges in compound screening include the generation of stable cell lines with appropriate AQP expression to allow accurate measurement of water transport rates, and rapid imposition of osmotic gradients to drive cell volume changes.

We recently devised a simple screening method to identify inhibitors of AQP1 water permeability and UT-B urea permeability using human erythrocytes (Levin et al., 2007), which was successful in identifying nanomolar potency UT-B inhibitors of phenylsulfoxoxyoxozole and benzenesulfonanilide classes. Prior urea analog-based inhibitors have millimolar potency. As shown diagrammatically in Fig.3, the method involves measurement of erythrocyte lysis after imposing a large, outwardly directed gradient of acetamide, a urea analog that is transported efficiently by UT-B. The acetamide gradient causes cell swelling, which is limited by UT-B-facilitated acetamide efflux. Under appropriate conditions, UT-B inhibition slows acetamide efflux and increases cell lysis, as assayed by near-infrared light scattering. Minor assay modification has allowed identification of AQP1 inhibitors, in which AQP1 inhibition slows water influx and protects against osmotic lysis. Similar lysis-based assays are potentially suitable for studying other AQPs.

**Aquaporin-based diagnostics**

**Antibody-based diagnostics**

There is one prominent example of an AQP antibody-based diagnostic test. AQP4 has been implicated as a marker of the central inflammatory demyelinating disease neuromyelitis optica (NMO), or Devic’s disease (Wingerchuk et al., 2007). NMO is a unique form of multiple sclerosis (MS) in which inflammatory lesions are restricted to the optic nerve and spinal cord, causing acute ocular pain with loss of vision, and myelitis with symmetric paraplegia, sensory loss and bladder dysfunction. A serum immunoglobulin was discovered in NMO subjects, but not in MS or normal subjects, which was found to target external epitope(s) on AQP4 (Lennon et al., 2005). Seropositivity for NMO-IgG is reasonably sensitive (74%) and specific (>90%) for NMO (Jarius et al., 2008), enabling early diagnostic distinction of NMO from MS. The characteristic vasculocentric deposition of immunoglobulins and complement activation products in NMO has suggested the possibility that the AQP4 autoantibody is involved in NMO disease pathogenesis. However, various observations have challenged the proposed role of AQP4 antibody in disease pathogenesis, such as the lack of correlation of NMO-IgG antibody titer with disease severity, and the restricted sites of NMO lesions compared with the wide distribution of AQP4. Notwithstanding the incomplete understanding of the origin and importance of NMO antibodies in disease pathogenesis, the detection of NMO-IgG has opened a new area in the diagnosis of a neurological disease where alternative tests were not available. There are various other disease states in which AQP antibodies may be of utility for diagnosis and possibly involved in disease pathogenesis. As yet untested possibilities include AQP3 autoantibodies in autoimmune skin diseases and AQP5 autoantibodies in Sjogren’s syndrome.

**Protein-based diagnostics**

Assay of AQP protein content in bodily fluids and tissue specimens may have diagnostic value. The one established example is assay of AQP2 immunoreactive protein in urine for distinguishing among various etiologies of nephrogenic diabetes insipidus (NDI) (Rai et al., 1997; Ishikawa, 2000). The rationale for urinary AQP2 assay is the shedding, by an exosomal mechanism, of a small amount of AQP2 protein when present at the luminal membrane of kidney collecting duct. With suitable caveats, urinary AQP2 protein is a marker of apical membrane AQP2 expression, being absent in NDI caused by AQP2 deficiency or defective cellular processing. However, diagnostic assay of urinary AQP2 has not been widely used because alternative, reliable methods are available to evaluate NDI. The possibility of ‘shedding’ of other AQPs in urine, or in other bodily fluids, such as aqueous humor or CSF, has not been explored. Another potential role for AQP protein-based diagnostics is in evaluating AQP expression in tissue specimens. Several studies have attempted to correlate AQP expression in tumor cells with tumor grade (reviewed by Verkman et al., 2008a), and AQP expression with human epilepsy (Lee et al., 2004), and oculcar (reviewed by Verkman et al., 2008b) and skin (Olsson et al., 2006) diseases. Whether diagnostically useful or unique information can be obtained by such measurements remains to be seen.
Aquaporin genetics and human disease

‘Aquaporinopathies’

Though exceedingly rare, there exist loss-of-function mutations in human AQPs. Mutations in AQP2 produce non-X-linked NDI by a recessive mechanism, which involves defective mutant AQP2 protein folding/function, and by a dominant mechanism, which results from endoplasmic reticulum (ER)/Golgi interactions between wild-type and mutant AQP2 that prevent plasma membrane targeting of wild-type AQP2 (reviewed by Bichet, 2006). The incidence of NDI caused by AQP2 mutations is less than one in 20 million births. For other AQPs only a handful of subjects have been identified with loss-of-function mutations. The few subjects that lack functional AQP1, which were identified by blood group screening, are phenotypically normal but manifest defective urinary-concentrating function when deprived of water (King et al., 2001), similar to findings in AQP1 null mice. Because of the rarity of AQP1-deficient individuals, as well as a few subjects that apparently lack functional AQP3 or AQP7 (Roudier et al., 2002; Kondo et al., 2002), and because of wide phenotype variations in humans, little useful information is available about the roles of these AQPs in humans. Mutations in the major intrinsic protein (MIP) of the lens cause congenital cataracts (Berry et al., 2000). MIP (also called AQP0) is homologous to the AQPs, though its function in lens and the relationship between loss-of-function mutations and cataract formation are unclear (reviewed by Verkman et al., 2008b). Disease-causing mutations of other AQPs in humans have not been described.

The rarity of NDI caused by AQP2 mutation precludes clinical development of ‘new chemical entities’ for therapy because of the large costs involved. NDI patients are treated primarily by water replacement and salt restriction, and in some cases by drugs such as thiazides that impair urinary diluting ability. We have been interested in the possibility of using existing drugs for therapy of recessive NDI caused by defective cellular processing of AQP2 mutants. An emerging paradigm in molecular medicine is the therapy of protein folding diseases by chemical or molecular chaperones, which facilitate folding of the mutant protein by direct binding and/or modulation of components of the molecular quality control machinery. We have focused attention on the T126M mutation in AQP2, one of the mutations causing recessive NDI in humans. Studies in mammalian cell culture models indicated ER retention and protein misfolding (Tamarappoo and Verkman, 1998). We also found that incubation of cell cultures with the ‘chemical chaperones’ glycerol or trimethylamine-N-oxide rescued...
defective AQP2-T126M cellular processing, resulting in its plasma membrane expression and restoration of cell membrane water permeability (Tamarappoo et al., 1999). However, chemical chaperones are not suitable for use in vivo because of the high concentrations required.

For in vivo analysis, we created a mouse model of human NDI caused by the T126M AQP2 mutation. Initially, an AQP2-T126M knock-in mouse model was generated by targeted gene replacement using a Cre-loxP strategy in which the targeted gene locus contained an engineered T126M mutation (Fig. 4A) (Yang et al., 2001). Unfortunately, the homozygous mutant mice appeared normal just after birth, they generally died in the first week of life because of polyuria-induced renal failure (Fig. 4B, left). Immunoblot analysis of kidneys of the mutant mice showed endoglycosidase H-sensitive, core glycosylated AQP2-T126M, indicating ER retention (Fig. 4B, right). As a first step in developing an AQP2-T126M ‘conditional knock-in’ model of NDI, we generated an inducible mouse model of AQP2 gene deletion (‘conditional knock-out’ mouse) manifesting severe polyuria in adult mice (Yang et al., 2006c). LoxP sequences were inserted into introns 1 and 2 in the mouse AQP2 gene. Mating of germ-line AQP2-loxP mice with tamoxifen-inducible Cre-expressing mice produced offspring with inducible homozygous Cre-AQP2-loxP. Tamoxifen administration led to Cre recombinase expression and AQP2 gene excision, resulting in severe polyuria and an inability to concentrate their urine in response to water deprivation (Fig. 4C,D). The adult polyuric mice survived well. To create ‘conditional AQP2-T126M knock-in’ mouse model, mice heterozygous separately for floxed wild-type AQP2 and AQP2-T126M were bred to produce hemizygous mice containing a floxed wild-type AQP2 allele and a mutant AQP2-T126M allele (Yang et al., 2009). Conditional deletion of the wild-type AQP2 gene in adult mice by tamoxifen administration produced mice expressing only the mutant AQP2-T126M protein. The conditional knock-in adult mice showed polyuria, urinary hypo-osmolality and ER retention of AQP2-T126M in collecting duct. Screening of candidate protein folding ‘correctors’ in AQP2-T126M-transfected kidney cells showed increased AQP2-T126M plasma membrane expression with the Hsp90 inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG), a compound currently in clinical trials for tumor therapy. 17-AAG increased urine osmolality in the AQP2-T126M mice (without effect in AQP2 null mice) and partially rescued defective AQP2-T126M cellular processing. These proof-of-concept findings suggest the possibility of using existing drugs for therapy of some forms of NDI.

Aquaporin polymorphisms as disease markers

The possibility of functionally significant AQP polymorphisms has received little attention, though recent data support further research into this area. Two recent studies have investigated possible AQP4 polymorphisms. Kleffner and colleagues (Kleffner et al., 2008) studied 10 AQP4 polymorphisms in 41 stroke patients with middle cerebral arterial occlusion, tentatively identifying one polymorphism associated with increased severity of brain edema. Sorani and colleagues (Sorani et al., 2008) identified 24 AQP4 variants in an ethnically diverse cohort of 188 normal subjects, some of which altered AQP4 water permeability when expressed in cell cultures, though the results were inconclusive because water permeability was not normalized for plasma membrane AQP4 protein expression. In other studies, associations were reported for single nucleotide polymorphisms in AQP1 with priapism in sickle cell disease (Elliott et al., 2007) and with diabetic nephropathy (Ewens et al., 2005). The significance of these observations is unclear. Single nucleotide polymorphisms in AQP7 have also been associated with obesity and type II diabetes (Prudente et al., 2007). Research in disease-related AQP polymorphisms is at a very early stage, with its real impact to be determined. It may be worthwhile, for example, to investigate polymorphisms in AQP4 in brain diseases such as obstructive hydrocephalus, in AQP3 in skin diseases, and in various AQPs in cancer.

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