**Treatment prospects for autosomal-dominant polycystic kidney disease**

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**Treatment prospects for autosomal-dominant polycystic kidney disease.** An increased understanding of the molecular genetic and cellular pathophysiologic mechanisms responsible for the development of autosomal-dominant polycystic kidney disease (ADPKD), made possible by the advances in molecular biology and genetics of the last three decades, has laid the foundation for the development of effective therapies. As the concept that a polycystic kidney is a neoplasm in disguise is becoming increasingly accepted, the development of therapies for ADPKD may benefit greatly from the expanding body of information on cancer chemoprevention and chemosuppression. This review summarizes the observations that already have been made and discusses therapies for PKD that deserve investigation.

Adult polycystic kidney disease (PKD) was once deemed to be a rare congenital malformation with a fate predetermined at birth. Recognition of its hereditary nature nearly a century ago (autosomal-dominant PKD or ADPKD) added to the burden of affected individuals. More recent studies have shown that the ADPKD kidney forms normally and that macroscopic cysts develop mainly after birth. It is the most common life-threatening monogenetic human disease and the third most common single cause of end-stage renal failure behind hypertension and diabetes mellitus. Advances in molecular biology and genetics in the last three decades have made possible a greater understanding of the molecular genetic and cellular pathophysiologic mechanisms responsible for the development of ADPKD, and have laid the foundation for the development of effective therapies [reviewed in 1–4]. As the concept that a polycystic kidney is a neoplasm in disguise is gaining acceptance [5], the development of therapies for ADPKD may benefit from the expanding body of information on cancer chemoprevention and chemosuppression [6]. The purpose of this review is to summarize the observations that already have been made and to discuss therapies for PKD that deserve investigation.

**GENETIC MECHANISMS**

Mechanisms by which mutations to one allele result in a characteristic phenotype include gain of function, haploinsufficiency, production of a toxic protein, a dominant negative effect, and somatic mutation of the allele not affected by the germline mutation. The analysis of X chromosome inactivation patterns in epithelial cells, isolated from individual cysts from women with ADPKD, has suggested that cysts develop from clonal expansions of single cells. Analysis of the PKD1 genes in these cells has revealed a loss of heterozygosity or intragenic mutations involving the nonaffected PKD1 allele in approximately 20% of renal and hepatic cysts [reviewed in 1] and similar changes involving the PKD2 gene in PKD2 cystic epithelium [7–9]. These observations support a two-hit model of cystogenesis, which is consistent with the inactivating nature of most germline PKD1 and PKD2 mutations [10, 11] and the focal development of renal and hepatic cysts. The mild cystic phenotype of Pkd1 or Pkd2 heterozygotes and the severe fetal cystic changes of homozygote mutant mice indicate that cystogenesis is associated with polycystin loss [2]. The more severe cystic disease in Pkd2<sup>WS25/WS25</sup> and Pkd2<sup>WS25/−</sup> mice, associated with the Pkd2 WS25 unstable allele (generated by tandem integration of a mutant exon 1 into the first intron of Pkd2), which results in an increased rate of somatic mutations by intragenic homologous recombination, further supports the two-hit mechanism. The mild cystic phenotype of Pkd1 or Pkd2 heterozygote mutant mice [12, 13] may point to different susceptibilities of murine Pkd and human PKD genes to somatic mutations. The human PKD1 gene might have a higher susceptibility due to polypyrimidine tracts, which may facilitate triple helixing, erroneous DNA repair, and mutagenesis during transcription [14], or to the existence of homologous PKD1 genes (HG), which may predispose to somatic...
mutations by gene conversion events [15]. The high rate of somatic mutations in the PKD2 gene remains unexplained. Of note is the variable development of the expected renal phenotype in other targeted mutant mouse models for diseases in which a two-hit mechanism is likely. For example, heterozygote mutant mice for Von Hippel-Lindau (VHL) disease remain phenotypically normal up to 15 months of age [16], while heterozygote tuberous sclerosis complex 2 (TSC2) mutant mice consistently develop renal cell carcinomas by 6 months of age [17]. Therefore, other mechanisms may need to be considered to account for the different phenotypic expression of PKD mutations in humans and mice. The different phenotypic expression of these diseases may simply reflect differences in life span, which determines whether somatic mutations develop in the kidney, an organ that may be particularly susceptible because of its high oxidative metabolism [18], as well as other factors that currently are not understood.

Whether somatic mutations of the PKD allele not affected by the germline mutation are the only, or even the most important, mechanism responsible for cyst formation in ADPKD has not been definitely established. The overexpression of polycystin 1 in the cystic epithelium detected immunohistochemically by C-terminal antibodies [reviewed in 4] can only be explained, within the context of the two-hit model, if the majority of second hits involve in-frame missense mutations rather than truncations or deletions. This may be particularly important in the TSC2/PKD1 contiguous gene syndrome and in early ADPKD, in which overexpression of polycystin 1 is more uniformly observed [19]. The extensive renal cystic disease observed in the PKD1/TSC2 contiguous gene syndrome and early onset ADPKD would require an extremely high rate of somatic mutations or somatic mutations occurring in stem cells at an early stage of nephrogenesis. If the latter was correct, many cysts in these conditions would share the same somatic mutations, a hypothesis that has not yet been tested. The increased rate of proliferation and apoptosis observed in many noncystic tubules cannot be explained by the two-hit hypothesis unless the rate of second hits is much higher than that suggested by the number of cysts [20–22]. Focal development of cysts has also been observed in autosomal recessive models [22, 23], as well as in transgenic models of PKD [24–26]. Overexpression of PKD1 as a transgene results in the development of a cystic phenotype, suggesting that the maintenance of a normal level of polycystin may be critical to maintain a differentiated tubular epithelial phenotype [27]. Transheterozygous mutations of PKD1 and PKD2 (for example, somatic mutations of PKD2 in cells carrying a germline PKD1 mutation) have been recently reported and proposed to explain the continued expression of polycystin 1 and polycystin 2 in ADPKD1 and ADPKD2 kidneys [28, 29]. If these transheterozygous mutations are pathogenetically important, it seems likely that mutations in multiple other genes encoding proteins that may be partners of the polycystins in the same signaling pathway may also occur.

The understanding of the molecular genetic mechanisms responsible for the cystic disease in ADPKD has treatment implications. If somatic mutations of either one or multiple genes were important, treatments directed at reducing the rate of mutations should be considered. Their effectiveness would depend on whether the somatic mutations occur mostly during the development, maturation, and growth of the kidney or at later stages in association with environmental exposures and cellular senescence. If the hypothesis that ADPKD is a recessive disease at the cellular level was confirmed, gene replacement therapy would be theoretically possible. The feasibility of gene replacement therapy has been demonstrated by the rescue of the renal phenotype in the Oak Ridge National Laboratory polycystic kidney (orpk) model by the expression of the wild-type orpk gene as a transgene [30]. The expression of PKD1 as a transgene has also been shown to rescue the embryonically lethal phenotype associated with the Pkd1del34/del34 knock-out mouse [27]. Technical considerations, such as the requirement for a highly selective, efficient, and durable gene transfer to somatic cells, safety issues, and the observation that the overexpression of PKD1 also results in a cystic phenotype cast doubt on the feasibility of gene therapy as a successful treatment for ADPKD.

### THE FUNCTION OF POLYCYSTINS

The predicted structures of the polycystins suggest that they are membrane-associated proteins capable of physical interaction, but their function remains uncertain [reviewed in 1–4]. Recent evidence supports the concept that they participate in the regulation of cell–matrix and cell–cell interactions and, by so doing, of cell differentiation, proliferation, and apoptosis. Polycystin 1 has been found to be a component of large multiprotein complexes associated with cell–matrix contacts at focal adhesions and intercellular contacts at adherens junctions and desmosomes [31, 32]. Polycystin 2 is expressed in the endoplasmic reticulum membrane of cells in tissue culture, and its C-terminus contains a 34 amino acid region essential to this localization [33]. Possibly the physical interaction between polycystin 1 and polycystin 2 is important for the trafficking of these proteins to the plasma membrane.

Cell–matrix interactions in focal adhesions are mediated by integrin receptors (Fig. 1). These are heterodimeric molecules composed of α and β subunits, of which there are multiple subtypes with specificity of binding to different matrix components [34]. Binding to specific
ligands induces clustering of integrin heterodimers and assembly of a multiprotein complex, which includes structural actin binding proteins such as tensin, vinculin, talin, and α-actinin, as well as the tyrosine kinases focal adhesion kinase (FAK) and c-src. This provides a mechanism for signal transduction leading to alterations in gene transcription. Cell–cell interactions at adherens junctions and desmosomes are mediated by classic and desmosomal cadherins (Fig. 1). These are single-pass transmembrane adhesion proteins, capable of forming lateral dimers in a calcium-dependent manner [35]. The cytoplasmic domains of these proteins connect directly to β-catenin or γ-catenin, which function as a bridge with α-catenin and cytoskeleton molecules such as α-actinin, vinculin, and F-actin. There is evidence for cross-talk between focal and adherens junctions. In many cellular systems, cadherin activity is under the dual control of signaling pathways activated by focal adhesions and growth factor receptors. Tyrosine phosphorylation of the NH2-terminus of β-catenin results in dissociation of the entire cadherin catenin complex from the cytoskeleton.

β-Catenin is a bifunctional protein that is not only involved in cell–cell adhesion by its link to E-cadherin, but also functions as a transcription factor (Fig. 1) [36, 37]. Cytosolic β-catenin levels are regulated by binding to the adherens junction and desmosomes and by ubiquitin-proteasome–mediated degradation following phosphorylation by glycogen synthase kinase-3β (Gsk3β) in a multiprotein complex that also includes the adenomatous polyposis coli (APC) protein, axin, and protein phosphatase-2A. The degradation of β-catenin can be inhibited by secreted Wnt glycoprotein acting upon its receptor Frizzled, which activates Disheveled (Dvl) and inhibits Gsk3β. Free cytosolic β-catenin can translocate to the nucleus, where it binds and activates the lymphoid-enhancing transcription factor (LEF-1) T-cell factor (TCF). One of the target genes of the β-catenin/LEF-1 transcription factor is c-myc, which is overexpressed in PKD.

The precise localization and the role of polycystins in cell–matrix and cell–cell contacts are not firmly established. Wilson et al have detected a direct physical association between polycystin 1 and α5 and β1 integrins, vinculin, paxillin, and FAK during the initial adherence of fetal collecting tubule epithelial cells to type 1 collagen matrix [31]. On the other hand, Huan and van Adelsberg have observed the colocalization of polycystin 1 with E-cadherin and α, β, and γ-catenins, but not with FAK or β-integrin, in human fetal kidney [32]. Scheffers et al have reported the colocalization of polycystin 1 with desmosomes (abstract; Scheffers M, Netherlands PKD Work-
Adelsberg also observed an increased PKD, that overexpression of c-myc as a transgene in epithelial cells. Wilson et al found a marked increase in the normal interaction between polycystin 1 and the E-cadherin/catenin complexes, and that it might represent an example of cross-talk between cadherins and integrins. They suggested that an alteration in polycystin 1 phosphorylation, resulting from an impaired association with FAK, could be responsible for a failure to down-regulate integrin-mediated epithelial cell adhesion [31]. Huan and van Adelsberg also observed an increased β1 integrin-mediated adhesion to extracellular matrix in cells from polycystic kidneys in the cpk mouse. These investigators, however, suggested that the up-regulation of β1 integrin-mediated adhesion was secondary to a disruption of the normal interaction between polycystin 1 and the E-cadherin/catenin complexes, and that it might represent an example of cross-talk between cadherins and integrins [32]. Daikha-Dahmane et al found a marked increase in α3 integrin staining in normal and cystic collecting duct cells and a severe disorganization of the expression and distribution of other integrins in the cyst lining cells from ADPKD and ARPKD kidneys [38]. The role of cell-matrix interactions in the pathogenesis of PKD is supported by the renal cystic phenotype of tensin knockout mice [39].

A number of recent studies suggest that polycystin 1 participates in the regulation of cell–cell interactions in renal tubular epithelial cells and that a disruption of these interactions may be an early event in the pathogenesis of ADPKD. Rocco et al first reported an attenuated expression of the epithelial cell adhesion molecules N-CAM and E-cadherin at mRNA and protein levels in the kidneys of cpk/cpk mice, and suggested that a defect in cell adhesion receptors in the cystic renal epithelium may play an important role in the pathogenesis of cyst formation and altered function of cystic epithelium [40]. More recently, Kim et al observed that the expression of the C-terminal domain of polycystin as a membrane-bound fusion protein stabilizes soluble endogenous β-catenin and stimulates LEF-1-dependent gene transcription in human embryonic kidney cells [41]. Charron et al have reported that the epithelial cells from polycystic kidneys not only have reduced levels of E-cadherin, but that this protein is improperly sequestered in an intracellular pool because of an impaired basolateral trafficking of proteins and a delayed exit from the Golgi apparatus [42]. The reduced levels of E-cadherin in the cystic epithelium may explain the increased rate of apoptosis in ADPKD kidneys since cell–cell adhesion inhibits apoptosis in the renal tubules by an E-cadherin–dependent mechanism [43]. In addition, the reduced sequestration of β-catenin by the E-cadherin complexes may result in higher cytoplasmic levels. Consistent with this is the observation by Huan and van Adelsberg of a prolonged half-life of β-catenin in immortalized cell lines isolated from cpk mice (abstract; Huan Y, 33rd ASN Meeting, 2000). An important role for β-catenin in the pathogenesis of ADPKD is also supported by the observations that transgenic mice expressing large amounts of a NH2-terminally truncated β-catenin develop severe PKD [44], that the expression of c-myc (a target of β-catenin/LEF-1) is up-regulated in the cystic epithelium from human ADPKD and rodent models of PKD, that overexpression of c-myc as a transgene induces the development of PKD [26], and that treatment with c-myc antisense oligonucleotides reduced the severity of the cystic disease in cpk/cpk mice (abstract; Ricker J, 32nd ASN Meeting, 1999). If the role of β-catenin in the pathogenesis of PKD is confirmed, treatment strategies to down-regulate its expression or activity could be considered.

**DOWNSTREAM PATHOGENETIC EVENTS**

While the exact molecular genetic and biochemical mechanisms that initiate the development of the cystic disease in ADPKD have not been firmly established, they result in a clonal expansion of partially differentiated epithelial cells characterized by disorganization of epithelial cell proliferation and apoptosis, expression of a secretory phenotype, and a disarray of cell matrix interactions that leads to interstitial inflammation and matrix accumulation (Fig. 2). In addition, the cysts produce a number of factors capable of affecting in an autocrine and paracrine fashion the development and enlargement of neighboring cysts. These include growth factors, chemokines, proinflammatory cytokines, nucleotides, bioactive lipids, matrix metalloproteinases, lysosomal enzymes, and vasoactive factors [reviewed in 45, 46]. Recent micropuncture and microdissection studies have demonstrated that tubular obstruction is a feature of PKD in some animal models and may contribute to the growth, if not the development, of the cysts [47]. Several investigators have drawn attention to the similarities between the pathogenesis of renal cystic disease and obstructive nephropathy. Nephroangiosclerosis develops early in ADPKD and probably contributes significantly to the progression of the renal disease and decline in renal function. Parallel with this downstream pathogenetic event, the analysis of the signal transduction pathways in cells derived
from cystic epithelium has demonstrated multiple abnormalities (Fig. 3). The increasing understanding of the abnormalities in cell–matrix and cell–cell communication, endocrine, paracrine and autocrine mechanisms, and signal transduction pathways in ADPKD has provided a framework for the delineation of possible therapies and opportunities for synergistic interventions in ADPKD.

ANTIMUTAGENS AND ANTIOXIDANTS

If somatic mutations are important for the development of the cystic lesions of ADPKD, which is an increasingly accepted hypothesis, then treatments capable of reducing the rate of somatic mutations may be able to slow down the progression of the disease. This could include drugs capable of preventing the activation of endogenous and exogenous mutagens (inhibitors of phase 1 enzymes) or of enhancing their detoxication (inducers of phase 2 enzymes). This approach has not yet been tried for ADPKD. One study has demonstrated an up-regulation of the cytochrome P450 1B1 in the Han:SPRD rat [48]. If a similar up-regulation of this enzyme could be demonstrated in other models of renal cystic disease, further studies to determine whether CYP1B1 can modulate the development of PKD and be a target for therapy would be warranted.

Oxidant species are generated during metabolic processes and contribute to the regulation of cell proliferation and differentiation under normal conditions and to tissue injury in response to pathologic stresses. Damage to DNA by oxidant species appears to occur naturally, and low steady-state levels of base-damaged products can be detected in nuclear DNA from human cells and tissues. It has been calculated that the total number of oxidative hits to the DNA per cell per day in humans may be more than $10^4$ [49]. A number of observations suggest that the severity of PKD can be modulated by oxidative stress, and depletion of glutathione by the administration of buthionine sulfoximine aggravates the development of PKD in Han:SPRD rats [reviewed in 50]. The evidence for a protective effect of antioxidants on the development of PKD is more limited. The administration of probucol to pcy mice fed a high-protein diet had a protective effect, possibly as a consequence of its antioxidant action [51]. On the other hand, the dietary content of α-tocopherol, a major lipophilic antioxidant of cell membranes, did not have a significant effect on the severity of the PKD in Han:SPRD rats [52]. Possibly

Fig. 2. Schematic representation of pathogenetic events and treatment strategies in PKD. Pathogenetic events are shown in shaded boxes, and treatment strategies are shown in open boxes.
other cytosolic antioxidants and antioxidant enzymes are more important in the pathogenesis of PKD.

**DIETARY AND METABOLIC INTERVENTIONS**

Studies in animal models of PKD have clearly demonstrated that its development can be markedly influenced by dietary interventions. While protein restriction consistently ameliorates it, the administration of a high-protein diet dependably aggravates renal cystogenesis in pcy mice and Han:SPRD rats [53, 54]. The responsible mechanisms are not clearly established. In the remnant kidney model, a high-protein diet has been associated with a lower arterial pH, higher intracellular pH and inorganic phosphate, higher oxygen consumption, higher generation of oxygen-free radicals, higher oxidized:reduced glutathione ratios, and increased ammoniogenesis, while the reverse occurs during protein restriction. The increased intracellular concentration of inorganic phosphate associated with a high-protein diet may result in an enhanced synthesis rate of adenosine 5'-triphosphate (ATP), but the steady-state tissue concentrations of ATP have not been found to be different [55]. Enhanced ammoniogenesis, oxidative stress, and extracellular release and paracrine effect of ATP have all been proposed as potential mechanisms of renal cystic disease progression in ADPKD. In addition, alterations in dietary protein have been shown to alter the activity of the renal renin-angiotensin system and the expression of transforming growth factor-β (TGF-β), which can also affect renal cystic disease progression [53, 54].

Not only the protein content, but the protein composition of the diet may influence the development of PKD. Soy protein-based diets have a beneficial effect on the development of PKD in the Han:SPRD rat and in the pcy mouse, as compared with casein-based diets [56–58]. The mechanism underlying this beneficial effect has not been established. Soy protein contains several phytoes-
trogens or soy isoflavones, including genistein, which is an inhibitor of tyrosine protein kinases. The addition of genistein in an amount comparable to that present in a soy protein-based diet, however, did not have a beneficial effect [56]. Since soy protein has a high l-arginine/l-lysine ratio compared with casein, the possibility exists that the beneficial effect of soy protein is related to an increased production of nitric oxide [56]. However, the administration of l-arginine has been found to have only a very modest beneficial effect on the development of renal cystic disease in the Han:SPRD rat [59]. An intriguing study relates the beneficial effect of a soy protein diet to its effect on polyunsaturated fatty acid metabolism [58]. In this study, soy feeding was associated with higher renal and hepatic linoleic acid content, higher hepatic a-linolenic acid, and lower hepatic arachidonic acid content, changes consistent with the reported effect of soy protein feeding to decrease the hepatic activity of Δ6-desaturase, the first step in the conversion of the essential a-6-polyunsaturated fatty acid (a-6-PUFA) to the eicosanoid precursor arachidonic acid (Fig. 4) [60]. It has been suggested that the beneficial effect of the soy protein may be due to the reduced production of arachidonic acid. Finally, the administration of soy protein results in lower levels of insulin-like growth factor-1 (IGF-1) in the kidneys of Han:SPRD cy/+ rats, suggesting a role for the IGF system in its beneficial effect [61].

The administration of flaxseed supplementation, a rich source of α-linolenic acid, has also been reported to ameliorate the interstitial nephritis associated with the renal cystic disease in Han:SPRD rats [62]. The mechanism responsible for this beneficial effect is thought to be through competition with linoleic acid for Δ6-desaturase and a reduction in arachidonic acid synthesis (Fig. 4) [58]. In addition, α-linolenic acid is converted into eicosapentaenoic acid (EPA), which displaces arachidonic acid from the cell membranes. EPA competes effectively for available cyclooxygenase and lipoxygenase activity, and the prostanoids and leukotrienes derived from EPA (3-series PGs and 5-series LTs) are less inflammatory and chemo-tactic than those derived from arachidonic acid [AA; 2-series prostaglandins (PGs) and 4-series leukotrienes (LTs)]. The beneficial effect of flaxseed may also be due to its high content of lignans, a main class of phytoestrogens.

In contrast to the observations with soy protein diet and flax oil supplementation, the administration of fish oil, a rich source of ω3-PUFA EPA and docosahexaenoic (DHA) acids, has a long-term detrimental effect on renal function and survival in pcy mice [63], despite a possible early beneficial effect in the short term. The administration of a fish oil diet reduced the renal content of tissue arachidonic acid, which was replaced by EPA and DHA. The authors suggested that the detrimental effect might have been due to a reduction in renal prostaglandin E2 production, but this seems inconsistent with the observations in the studies using a soy protein and flaxseed diet. Possibly, autoxidation of the ω3-PUFA or an oxidizing or nephrotoxic effect of the antioxidant ethoxyquin added to the fish oil preparation might have been responsible for the detrimental effect.

The possibility that renal ammoniagenesis or linked metabolic processes could have an effect on the progression of PKD was suggested by an association between chronic hypokalemia and acquired renal cysts [reviewed in 64]. Acquired renal cysts are also observed in chronic renal failure, both clinically and experimentally, and in patients with distal renal tubular acidosis. Increased renal ammoniagenesis, either in absolute terms or relative to the number of surviving nephrons, is common to these conditions. The link of an enhanced cortical production of ammonia associated with renal mass reduction, chronic hypokalemia, and dietary deficiency of antioxidants to the development of interstitial inflammation and fibrosis in these conditions suggested that a similar mechanism could be operative in PKD, as a result of a defective transfer of ammonia to the final urine analogous to that observed after subtotal nephrectomy [reviewed in 64]. Therefore, the demonstration that the administration of sodium bicarbonate or potassium bicarbonate or sodium/potassium citrate markedly attenuates the development of PKD in the Han:SPRD rat seemed to provide a strong rationale for the study of this safe and economical form of treatment in human ADPKD [64, 65]. Unfortunately, this beneficial effect has not been confirmed in other animal models of PKD. In fact, the administration of sodium bicarbonate or sodium/potassium citrate to pcy mice has no beneficial effect and can be detrimental [66, 67]. More recently, the administration of sodium bicarbonate to pck rats has been found to markedly aggravate the development of PKD (abstract; Qian Q, 33rd ASN Meeting, 2000). These opposing effects of base administration may be related to the origin of the cysts from different nephron segments in these animal models. In the Han:SPRD rats, the cysts develop from the proximal tubules. In the pcy mouse, the cysts originate from the distal tubules and collecting ducts. In the pck rats, the cysts derive from the thick ascending limb of Henle, distal tubules, and collecting ducts. The investigation of the mechanisms responsible for these different responses is likely to provide an insight into the role of metabolic factors in the modulation of renal cystogenesis.

ErbB RECEPTORS AND TYROSINE
KINASE INHIBITORS

A number of elegant studies during the last decade have provided evidence for a major role for the epidermal growth factor (EGF)-TGF-α-EGF receptor (EGFR) axis in promoting epithelial hyperplasia and cyst formation and enlargement, and for its potential as a target
Fig. 4. Schematic representation of the metabolism of omega-3 and omega-6 fatty acids. The carbon at the methyl group is referred to as the ω-carbon. ω-3 Fatty acids (FAs) have a double bond between the third and fourth carbon from the ω-carbon (ω-3 or n-3). ω-6 FAs have a double bond between the sixth and the seventh carbon from the ω-carbon (ω-6 or n-6). The ω-3 α-linolenic (LNA) and the ω-6 linoleic (LA) acids are essential FAs. Their metabolism involves increases in chain length and degree of unsaturation by the addition of extra double bonds. There is competition between the ω-3 and ω-6 FAs for the desaturases. LNA is converted to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). LA is converted to arachidonic acid (AA). The metabolites derived from EPA are less inflammatory and chemotactic than those derived from AA. Soy protein feeding decreases the hepatic activity of Δ-6 desaturase. Fish oil is a rich source of EPA and DHA. Rose and Connolly discuss more about the ω-3 fatty acids [60].

for treatment in PKD. EGF and TGF-α are the best known of a large family of EGF-related peptide ligands for a family of four structurally-related tyrosine kinase receptors known as ErbB receptors [68]. The EGFR, also known as ErbB-1, is the receptor for EGF and TGF-α. ErbB-2, which is expressed at low levels in normal epithelial cells, has no recognized high-affinity ligand. ErbB-3 and ErbB-4 and their ligands are shown in Figure 5. The binding of an EGF-like peptide to the extracellular domain of an ErbB receptor results in receptor dimerization, tyrosine kinase activation, and autophosphorylation. A large number of cytoplasmic proteins, containing phosphotyrosine binding motifs, such as Grb-2, phospholipase Cγ (PLCγ), Src, and many others, engage the activated ErbB receptors. The response triggered by specific growth factors includes diverse intracellular signaling cascades and the activation of particular transcription factors that lead to either cell proliferation or differentiation depending on cell–matrix and cell–cell interactions [69]. Internalization of the ligand receptor complex follows the binding of the ligand and activation of the receptor. Internalized receptors into clathrin-coated vesicles are still capable of eliciting mitogenic signals. The fate of the internalized receptors depends on the nature of the ligand. When the ligand is TGF-α, internalized receptors are recycled to the cell membrane, whereas EGF-bound receptors are targeted for degradation.

Although the expression of EGF mRNA and protein is markedly down-regulated in the kidneys of cpk and pcy mice and of Han:SPRD rats [70, 71], renal cyst fluids from ADPKD, autosomal-recessive polycystic kidney disease (ARPKD), and murine and rat PKD models con-
tain multiple EGF or EGF-like peptides in mitogenic concentrations [72, 73]. The expression of TGF-\(\alpha\) mRNA and protein is increased in ADPKD kidneys [73]. EGFR is overexpressed and mislocated to the apical surface of cystic epithelial cells in human ADPKD and ARPKD, as well as in the cpk, bpk, and orpk mouse models of PKD [74, 75]. Apically expressed EGFRs exhibit high-affinity binding for EGF, autophosphorylate in response to EGF, and transmit a mitogenic signal when stimulated by the appropriate ligand. Overexpression of ErbB-2 has also been detected in a small number of patients with ADPKD but not in those with ARPKD [76]. Transgenic mice that overexpress TGF-\(\alpha\) develop renal cystic disease and renal expression of TGF-\(\alpha\) as a transgene accelerates the progression of the PKD in pcy mice [24, 77]. The expression of ErbB-2 as a mouse transgene causes multifocal hyperplasia of renal tubular epithelial cells and cyst formation [25]. EGF and TGF-\(\alpha\) are cystogenic in a variety of in vitro systems [78, 79]. Treatment of murine metanephric organ cultures with the tyrosine kinase inhibitors tyrphostin or genistein inhibits the development of EGF and TGF-\(\alpha\)-induced proximal tubule cyst formation in explants from normal mice and induces proximal tubule cyst regression in explants from bpk mice [78, 80]. Treatment of postnatal kidney explants from bpk mice with the EGFR tyrosine kinase inhibitor EKI-785 markedly reduced the number of collecting tubule cysts [81]. The in vivo role for increased EGFR activity was demonstrated by breeding mice carrying the waved-2 mutation, a point mutation that decreases EGFR tyrosine kinase activity, and orpk mice [82]. Animals homozygous for both mutant genes (orpk and waved-2) had a marked binding for EGF, autophosphorylate in response to EGF, and transmit a mitogenic signal when stimulated by the appropriate ligand. Overexpression of ErbB-2 has also been detected in a small number of patients with ADPKD but not in those with ARPKD [76]. Treatment of murine metanephric organ cultures with the tyrosine kinase inhibitors tyrphostin or genistein inhibits the development of EGF and TGF-\(\alpha\)-induced proximal tubule cyst formation in explants from normal mice and induces proximal tubule cyst regression in explants from bpk mice [78, 80].

The interest on the inhibition of EGFR extends beyond the treatment of ADPKD and is an area of intense investigation for the prevention and treatment of neoplastic diseases in many academic centers and pharmaceutical companies [83, 84]. A link between activation of EGFR and ErbB-2 receptors and human cancer has been convincingly demonstrated. Several reversible and irreversible EGFR tyrosine kinase inhibitors, with excellent potency and selectivity and limited toxicity, are currently in phase 1 or phase 2 clinical trials.
Ras INHIBITORS

The Ras genes code for a family of guanosine triphosphate (GTP) binding proteins, with endogenous guanosine triphosphatase (GTPase) activity, which cycle between an active GTP-bound and an inactive guanosine diphosphate (GDP)-bound state and have a central role in the cellular signal transduction cascade [85]. The participation of Ras proteins in the pathogenesis of PKD is supported by the increased renal expression of H-ras and K-ras in cpk mice and human ADPKD, as well as by the renal cystic phenotype of Ras transgenic mice [86]. The activation of Ras requires attachment to the plasma membrane, which is dependent on a number of post-translational modifications (Fig. 6). The most crucial of these modifications is the attachment of a farnesyl group to a C-terminal cysteine by the enzyme farnesyltransferase, which recognizes a carboxyl terminal CAAX motif. This enzyme can be inhibited by molecules that resemble either the 15 carbon farnesyl donor or the Ras CAAX motif. Several of these drugs are currently in phase 1 and phase 2 clinical trials for neoplastic diseases and appear to have minimal toxicity [87, 88]. The possibility that these drugs may be effective to treat PKD is suggested by the observation that the administration of lovastatin, an HMG-CoA reductase inhibitor, had a beneficial effect on the development of renal cystic disease in Han:SPRD cy/+ rats, possibly by inhibiting farnesyl synthesis and Ras farnesylation [89].

CAMP ANTAGONISTS AND PROTEIN KINASE A TYPE 1 INHIBITORS

The activation of adenyl cyclase and generation of cAMP promote the secretion of solutes and fluid into the cysts [90, 91]. Recent studies have suggested that cAMP may also promote cell proliferation in polycystic kidneys and a variety of neoplastic tissues, including renal cell carcinoma [92–94]. Yamaguchi et al found that receptor-mediated agonists of adenyl cyclase, forskolin, and 8-Br-cAMP activate the extracellular signal regulated kinase (ERK) cascade and increase cellular proliferation in cells derived from polycystic kidneys, but that they had an inhibitory effect on cells derived from normal human kidney cortex [92]. The proliferative effect on ADPKD derived cells was complementary and additive to that caused by EGF. While the effect of EGF was blocked by genistein, a receptor tyrosine kinase inhibitor, the cAMP-dependent proliferation was not. The effect of cAMP, but not that of EGF, was blocked by a cAMP-dependent protein kinase [protein kinase A (PKA)] inhibitor. Both effects, those of EGF and of cAMP, were blocked by a mitogen extracellular kinase (MEK recep-
tor) inhibitor. From these observations, these authors concluded that cAMP stimulates the proliferation of ADPKD, but not normal kidney, epithelial cells by PKA activation of the ERK pathway at a place distal to the receptor tyrosine kinase and proximal to MEK (Fig. 3).

The role of cAMP and PKA on the regulation of cell proliferation in a variety of neoplastic tissues, including renal cell carcinoma, has received recent attention and may be relevant to ADPKD [94-96]. The effects of cAMP on mammalian cells are mediated by binding to either of two distinct isoforms of PKA, known as PKA1 and PKA2. PKA1 and PKA2 share identical catalytic (C) subunits, but differ in the regulatory (R) subunits termed R1 in PKA1 and R2 in PKA 2. The PKA holoenzyme is a tetramere formed of two identical regulatory and catalytic subunits. Upon cAMP binding to the R subunits, the active C subunits are released. The relative abundance of PKA1 and PKA2 isoforms is regulated during differentiation, cell growth, and neoplastic transformation. Increased levels of PKA1 are detected in neoplastic and proliferating cells, whereas a predominant expression of PKA2 is found in growth-arrested cells. There is evidence for functional cross-talk between ligand-induced EGFR activation and PKA1 expression and function. PKA1 is overexpressed and activated following TGF-α-induced transformation in several rodent and human cell lines. Ciardiello and Tortora have proposed that EGFR and PKA1 interact by direct binding of the R1 subunit to the Grb2 adaptor protein, downstream of EGFR, but upstream of the ERK pathway, which is consistent with the observations made in ADPKD-derived epithelial cells [94]. PKA1 may also activate MEK through the intermediacy of Rap-1 and B-Raf, as has been recently been described in PC-12 and COS cells, which are stimulated to proliferate by cAMP [reviewed in 92].

The observation that cAMP and PKA activation promotes the proliferation of neoplastic and ADPKD-derived epithelial cells has important therapeutic implications. A new class of cAMP analogues can discriminate between the two cAMP binding sites on R1 and R2, down-regulate R1 by facilitating the degradation of protein, and up-regulate R2 expression at the transcriptional level. One of these analogues (8-Cl-cAMP) has been shown to inhibit growth and induce differentiation in a variety of human cancer cell lines. Another approach in neoplastic disease has been to inhibit the synthesis and function of PKA1 by the use of R1 mRNA antisense oligonucleotides. The functional cross-talk between EGFR activation and PKA1 expression suggests that the combination of EGFR tyrosine kinase inhibitors and PKA1 inhibitors may have synergistic effects. In fact, anti-EGFR antibodies and PKA1 antisense oligonucleotides have a demonstrated cooperative inhibitory effect on the growth of renal cancer xenografts in athymic mice [95].

INHIBITORS OF PROTEIN KINASE EFFECTORS

The activation of Ras triggers a protein kinase cascade, which includes Raf and MEK. Kinase inhibitors of Raf and MEK are in early stages of development, while Raf antisense oligonucleotides are in phase 2 clinical trial for a number of carcinomas, including renal cell carcinoma [97]. One of the MEK inhibitors, PD-098059, has been shown to block the proliferative effect of EGF and cAMP on ADPKD-derived epithelial cells in vitro [92].

INHIBITORS OF LIPID-MEDIATED SIGNALING

The activation of hormone, growth factor, and cytokine receptors is also associated with changes in phospholipid and sphingolipid metabolism, and results in the generation of bioactive lipid signaling molecules. A number of abnormalities in the levels of these bioactive lipid molecules have been described in PKD in humans and in rodents.

The phosphorylated residues on the intracellular domain on the growth factor receptors bind and activate PLC-γ, which cleaves phosphatidylinositol (4,5)-bisphosphate (PIP₂) into diacylglycerol (DAG; Fig. 3). The levels of PIP₂ in the kidneys of pcy mice have been found to be markedly reduced, probably reflecting an increased PLC-γ activity [98]. DAG activates some forms of protein kinase C (PKC) such as PKC-α, which are involved in cell proliferative processes [99]. PKC-α antisense oligonucleotides and inhibitors of PKC kinase activity, which are in phase II clinical trials for neoplastic disease, may be of value to treat PKD.

The activation of Ras results in the activation of phosphatidylinositol-3 kinase (PI3K; Fig. 3). The product of this reaction, phosphatidylinositol-3 phosphate (P13P), is able to activate the protein kinase Akt (also known as protein kinase B or PKB), which is a suppressor of apoptosis [100]. An increased formation of P13P after intraperitoneal injection of 1H-myoinositol, which probably reflects the activation of PI3K, has been described in pcy mice [98]. In addition, an increased expression of Akt has been observed in PKD [101]. Inhibitors of PI3K, which would be predicted to inactivate Akt and promote apoptosis, are currently in a preclinical phase of development [102].

The binding of cytokines to their receptors can also trigger the activation of sphingomyelinases. A marked reduction in the renal concentration of ceramide and striking increases in the renal concentrations of glucosyl and lactosyl ceramides have been reported in the cpk mice [103]. These may reflect an increase in glucosyl ceramide synthase activity. The levels of glucosyl and lactosyl ceramide and the activities of glucosyl ceramide synthase and lactosyl ceramide synthase are markedly increased in human ADPKD kidneys and in epithelial cell cultures derived from human ADPKD and cpk kidneys [104].
Lactosyl ceramide has been found to stimulate the phosphorylation of mitogen-activated protein kinase (MAPK) in proximal tubular epithelial cells from normal human kidney [104]. The effect of drugs capable to inhibit the synthesis of lactosyl ceramide, such as 1-phenyl-2-decanoylamino-3-morpholino-1-propanol, on the development of PKD has not been ascertained [105].

RETINOIDS AND VITAMIN D

The retinoids are a class of biologically active derivatives of and synthetic compounds structurally related to vitamin A, which control normal cell growth, differentiation, and apoptosis during embryonic development and within epithelial tissues in later life [106]. They exert their effects by binding to at least six retinoid receptors, which are classified into two subfamilies: retinoid acid receptors (RARs) α, β, and γ, and retinoid X-receptors (RXRs) α, β, and γ (Fig. 3). The diversity of these receptors and their binding to DNA as dimers explains the complexity of the retinoid signaling mechanisms. The best studied retinoids, 13-cis-retinoid acid, 9-cis-retinoid acid, and all-trans-retinoid acid, occur endogenously at very low levels and have been synthesized and administered at pharmacological levels. Retinoids have been used as chemopreventive agents in experimental models of carcinogenesis, as well as in clinical trials, including chemoprevention of recurrent bladder carcinoma [107]. The focus of retinoid drug development is the synthesis of drugs with selectivity for specific retinoid acid receptors and less toxicity. One of these drugs, fenretinide, induces apoptosis in numerous neoplastic cells, has a favorable toxicity profile, has been found to be effective in rat and mouse models of bladder carcinogenesis, and is being tested in large placebo-control studies of chemoprevention for human bladder cancer. Whether fenretinide or other retinoids might be beneficial in the treatment of PKD has not been studied. However, the administration of N-(4-hydroxyphenyl) retinamide markedly inhibits cystogenesis in cultures of ADPKD-derived Madin-Darby canine kidney, and rat epithelial cells in a collagen matrix [108].

Like the retinoids, biologically active forms of vitamin D inhibit cellular proliferation and induce differentiation and apoptosis in a variety of cells by binding to a vitamin D receptor. Although the clinical use of vitamin D is limited by its calcemic effect, a number of analogues that inhibit cell growth without significant calcemic activity have been synthesized [109]. The effect of these drugs on cystogenesis has not been evaluated.

MODULATORS OF PROLIFERATION AND APOPTOSIS

Proliferation and apoptosis are tightly regulated during nephrogenesis. In normal mature kidneys and in the majority of kidneys with noncystic diseases, there is a very low rate of epithelial proliferation, and apoptotic DNA fragmentation is undetectable. On the other hand, it is easily detectable in rodent models of PKD and human ADPKD, involving both cystic and noncystic renal parenchyma [20, 21]. A marked increase in caspase (mainly caspase 4)-mediated apoptosis has been described in kidneys of cpk mice [110]. The exact role of apoptosis with respect to cystogenesis is uncertain. A recent study in the cpk mouse has demonstrated that nuclear expression of Pax 2, which is normally down-regulated in mature epithelial cells, is persistent in many epithelial cells. Reducing the Pax 2 gene dose led to an increase in apoptosis and less severe cystic disease [111]. On the other hand, the development of renal cysts and renal failure in knockouts of the antiapoptotic proto-oncogenes Bcl 2 and AP2-β suggests that accentuated apoptosis might exert a cystogenic effect. In addition, the high rate of apoptosis in polycystic kidneys may exert a certain degree of counterproliferative and counterprooenoctic effect, which may explain the low incidence of renal cell carcinoma in ADPKD. Although multiple strategies to increase or decrease the rate of apoptosis in tubular epithelial cells are possible [112, 113], further studies are needed to define unambiguously the role of apoptosis in cystic disease progression and the potential benefit of treatments acting at various levels of the apoptotic cascade.

TAXANES

A role for microtubules in cystogenesis was suggested by the demonstration that taxol, colchicine, and vinblastin reversibly inhibited the formation of cysts by epithelial cells from human and murine polycystic kidneys cultured in a collagen matrix. The administration of taxol to cpk mice markedly inhibited the formation of cysts and prolonged survival. Treatment with other microtubule active taxanes was also protective to an extent proportional to their ability to bind and promote spontaneous assembly of microtubules in vitro, whereas treatment with inhibitors of DNA synthesis was ineffective [114]. The mechanism by which stabilization of microtubules was protective in a rapidly progressive model of PKD is uncertain, but it might have been due to the promotion of apoptosis [115, 116]. The fact that the administration of taxol has no protective effect in Han:SPRD rats or pcy/pcy mice with slowly progressive renal cystic disease suggests that taxanes are not likely to have a beneficial effect in human ADPKD [117]. The administration of taxol also was not protective in orpk mice, a model of ARPKD less rapidly progressive than that in cpk mice [118].
HORMONAL MODULATORS

By the time the renal cysts reach a diameter of approximately 2 mm, they become disconnected from the tubular segment from which they derived. Further growth of the cysts depends on a process of transepithelial fluid secretion. The driving force is an active transport of chloride from the basolateral to the apical side. The potential energy for chloride secretion is developed by the sodium pump (Na\(^+\),K\(^+\)-ATPase) located in the basolateral membranes of cyst epithelial cells. The chloride entry mechanism into the basolateral membrane is a sodium-potassium-chloride, bumetanide-sensitive, cotransporter (NKCC1 or BSC2), which utilizes the gradient established by the sodium pump to bring K and Cl into the cells. Net chloride transport is accomplished by opening chloride channels within the apical membrane. Chloride ions flow down an electrochemical gradient into the cyst generating increased transepithelial electron activity that in turn drives sodium ions through ion selective paracellular pathways. An apical chloride channel that may play a role in cyst fluid accumulation is the cystic fibrosis transmembrane conductance regulator (CFTR). This is supported by the up-regulation of CFTR in some cysts, by whole cell patch clamp studies of ADPKD-derived epithelial cells, by the CFTR antisense oligonucleotide-induced inhibition of fluid secretion in cyst-derived cell monolayers, and by the observation of a milder cystic phenotype in two patients affected by both ADPKD and cystic fibrosis [119]. On the other hand, attenuation of the cystic phenotype was not observed in a third patient with ADPKD and cystic fibrosis [120]. On the other hand, attenuation of the cystic phenotype was not observed in a third patient with ADPKD and cystic fibrosis [120]. On the other hand, attenuation of the cystic phenotype was not observed in a third patient with ADPKD and cystic fibrosis [120]. On the other hand, attenuation of the cystic phenotype was not observed in a third patient with ADPKD and cystic fibrosis [120]. On the other hand, attenuation of the cystic phenotype was not observed in a third patient with ADPKD and cystic fibrosis [120].

The activation of the NKCC1/BSC2 cotransporter and of the chloride channels is regulated by PKA-mediated phosphorylation, which is under hormonal control. The secretion of chloride and fluid by cyst-derived epithelial cells is stimulated by a number of agonists that stimulate the production of cAMP, such as vasopressin, secretin, vasoactive intestinal peptide, prostaglandin E1 and E2, and cyst activating factor (CAF). Secretin has been shown to increase the rate of fluid secretion in vivo in renal and hepatic cysts of subjects with ADPKD [120]. In addition, testosterone has been found to activate adenylcyclase, increase cellular cAMP, and stimulate the secretion of chloride and fluid by monolayers of MDCK cells, possibly contributing to the gender dimorphism observed in human ADPKD and rat models of PKD [121].

The molecular mechanisms by which fluid is secreted by cyst-derived epithelial cells can potentially be targeted for treatment. Whether the inhibition of the basolateral NKCC1/BSC2 cotransporter by loop diuretics might be of therapeutic value has not been ascertained.

The administration of a vasopressin V2-receptor (AVP-V2R) antagonist reduced the severity of the cystic disease and renal insufficiency in cpk/cpk mice, a model characterized by an urinary concentration defect and up-regulation of AVP-V2R and aquaporins 2 and 3 [122]. This observation may be relevant to other experimental models of PKD, as well as to human ADPKD and ARPKD, in which there is also a urinary concentration defect. Anecdotal observations suggest that the inhibition of secretin release induced by the administration of H\(_2\) blockers or by octreotide might have a beneficial effect on polycystic kidney and liver disease. Nevertheless, a small prospective study with octreotide failed to demonstrate a benefit [123].

ANTI-INFLAMMATORY AGENTS

Interstitial inflammation is a consistent finding that develops early and contributes to the progression of the renal cystic disease in murine models of PKD and in human ADPKD. It is accompanied by local expression of chemokines, lymphokines, and other inflammatory mediators [reviewed in 45]. The chemokines are a large family of secreted, chemotactic, basic proteins of 8 to 10 kD molecular weight selectively targeting different leukocyte subpopulations. They have been divided according to their chemical structure into the C-X-C [for example, interleukin-8 (IL-8)], C-C [monocyte chemotactic protein 1 (MCP-1)] and C (for example, lymphotxin) chemokines. Osteopontin is a highly acidic, secreted glycoprotein that also exhibits chemotactic activity for macrophages. IL-8, MCP-1, and osteopontin are expressed at low levels in normal renal tubule epithelial cells. In Han:SPRD rats, MCP-1 and osteopontin are expressed at very high levels, primarily over the cystic epithelium. Elevated plasma concentrations of IL-6 have been reported in patients with ADPKD. High levels of the proinflammatory cytokines tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), IL-1, and IL-2 have been found in cyst fluids from patients with ADPKD [reviewed in 45]. TNF-\(\alpha\), a major mediator of inflammation and a strong inducer of other cytokines, is produced as a 26 kD membrane-associated precursor protein that requires proteolytic cleavage to the soluble 17 kD active protein. The TNF-\(\alpha\)-converting enzyme (TACE) responsible for this proteolytic cleavage belongs to the family of metalloproteinases. Most metalloproteinase inhibitors have limited potency against TACE, but more selective inhibitors currently under development and other strategies for inhibiting TNF-\(\alpha\) may be of interest in the treatment of ADPKD (discussed later in this article) [124, 125]. The importance of inflammation in the pathogenesis of ADPKD is highlighted by studies in which methylprednisolone, a steroid anti-inflammatory that inhibits phospholipase A\(_2\) and cyclooxygenase-2 (COX-2), atten-
uated renal enlargement and renal dysfunction in two rodent models of renal cystic disease [126]. Whether other anti-inflammatory drugs, such as COX-2 inhibitors or lipoxygenase inhibitors, might have protective effects on the development of PKD that outweigh their potential toxicity has not been ascertained.

INHIBITORS OF MATRIX METALLOPROTEINASES

Matrix metalloproteinases are a group of zinc dependent enzymes that include the interstitial collagenases (MMP-1, MMP-8, and MMP-13), stromelysins (MMP-3 and MMP-10), gelatinases (MMP-2 and MMP-9), and membrane-type metalloproteinases [127]. They are involved in the remodeling and turnover of the extracellular matrix and have been implicated in the pathogenesis of PKD by controlling matrix degradation and growth factor activation. While the levels of tissue inhibitors of MMPs (TIMPS) have been found to be uniformly elevated in tissues or cultured cells from human polycystic kidneys or livers and in renal tissues from Han:SPRD rats or cpk mice, the observations on MMPs have been less consistent [reviewed in 123]. A recent study has shown elevated serum or plasma levels of MMP-1, MMP-9, and TIMP-1 in patients with ADPKD [128]. MMPs are also thought to play an important role in tumor invasion, metastases, and angiogenesis. A number of selective inhibitors of specific MMPs are currently in phase 1, 2, or 3 clinical trials for a variety of neoplastic diseases [127]. Treatment of Han:SPRD rats with batimastat, a broad spectrum MMP inhibitor, has had a beneficial effect on cyst number and kidney weight (abstract; Obermuller N, Netherlands PKD Workshop, 2000). Recently, specific TACE inhibitors have been reported to inhibit TGF-α secretion from immortalized cystic collecting tubule cells in vitro and cystogenesis in bpk mice in vivo (abstract; Dell K, 33rd ASN Meeting, 2000). Therefore, the role of MMPs in the pathogenesis of ADPKD and their potential as a target for intervention deserve further study.

TREATMENT OF HYPERTENSION AND NEPHROANGIOSCLEROSIS

Hypertension often develops early in the course of ADPKD and is a predictor of renal functional decline. It is accompanied by an increase in renal vascular resistance and filtration fraction, resetting of the pressure-natriuresis relationship, salt sensitivity, and normal or increased extracellular fluid volume, plasma volume, and cardiac output. It is accompanied by severe sclerosis of the preglomerular vessels, even early in the course of the disease. Therefore, early detection and treatment of hypertension are of the utmost importance in management of these patients. The role of the different vasoactive factors and the degree to which they may contribute, not only to the development of hypertension and vascular lesions, but also to the proliferation of the cystic epithelium and interstitial inflammation and fibrosis, have been reviewed elsewhere [45] and are only briefly summarized here.

There is convincing evidence that the intrarenal renin-angiotensin system is activated in human ADPKD, as well as in experimental models of PKD, as least in the early stages. The administration of a converting enzyme inhibitor or an angiotensin II type 1 (AT-1) receptor antagonist to Han:SPRD cy/+ rats between 3 and 4 weeks and 10 to 20 weeks of age has had a protective effect on the severity of the renal cystic disease and renal function as compared with other antihypertensive regiments [129-131]. On the other hand, enalaprilat and hydralazine administered to Han:SPRD rats between 3 and 40 weeks of age were equally protective on the renal function as reflected by the level of serum creatinine, although only enalaprilat reduced proteinuria and kidney size [131].

Elevated plasma levels and the presence of endothelin in cyst fluid have been demonstrated in human ADPKD [132, 133] and cpk/cpk mice [134]. Elevated renal tissue levels of endothelin 1 have been found in Han:SPRD rats. On the other hand, the expression of endothelin type A and type B receptors has been found to be increased in the cpk/cpk mouse, but reduced in the Han:SPRD rat. Endothelin-1 (ET-1) transgenic mice have a cystic phenotype [135]. The acute administration of bosentan, a mixed endothelin receptor antagonist, significantly decreased the mean arterial blood pressure and glomerular filtration rate and increased the renal blood flow in Han:SPRD rats, but had no significant effect in the control rats [136]. Whether the chronic administration of endothelin antagonists might have a protective effect on the development of PKD has not been ascertained.

Little is known on the role of nitric oxide (NO) in the pathogenesis of ADPKD. Nitric oxide has potentially beneficial (preservation of renal blood flow and glomerular filtration, inhibition of platelet adherence to endothelial surfaces) and detrimental (cytotoxicity from oxidative damage) effects. Both beneficial and detrimental effects from NO inhibition have been described in a variety of experimental models of glomerular disease and acute renal failure [reviewed in 59]. The administration of l-arginine in the rat remnant kidney model, which is characterized by a reduced renal generation of NO, has produced variable results: protective at a low and detrimental at a high dose. The renal production of NO has been found to be reduced in Han:SPRD rats (abstract; Wang D, 33rd ASN Meeting, 2000) [58]. The administration of the NO synthase inhibitor Nω-nitro-l-arginine methyl ester (L-NAME) and l-arginine had only a modest, gender-dependent (observed only in the male animals) overall effect on the severity of the cystic disease [59]. On the other hand,
short-term administration of an HMG-CoA reductase inhibitor increased the glomerular filtration rate and renal blood flow in patients with ADPKD, presumably because of an increased endothelium-dependent, NO-mediated vascular relaxation (abstract; Van Dijk M, Netherlands PKD Workshop, 2000). The observation of a protective effect of an endothelial NO synthase DNA polymorphism associated with an increased NO production in male ADPKD patients also suggests a role for NO in the pathogenesis of ADPKD (abstract; Persu A, 33rd ASN Meeting, 2000).

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APPENDIX

Abbreviations used in this article are: AA, arachidonic acid; ADPKD, autosomal-dominant polycystic kidney disease; Ang, angiotensin; APC, adenomatous polyposis coli; ARPKD, autosomal-recessive polycystic kidney disease; AT1, angiotensin II type 1; ATP, adenosine 5'-triphosphate; AVP, arginine vasopressin; CAF, cyst activating factor; CAM, cell adhesion molecule; CAMP, cyclic adenosine 3'5'-monophosphate; CFTR, cystic fibrosis transmembrane conductance regulator; COX-2, cyclooxygenase-2; DAG, diacylglycerol; DHA, docosahexaenoic acid; DvI, Discheveled receptor; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; EPA, eicosapentaenoic acid; ERK, extracellular signal-regulated kinase; FA, fatty acid; FAK, focal adhesion kinase; GDP, guanosine diphosphate; GFR, glomerular filtration rate; GTP, guanosine triphosphate; GTPase, endogenous guanosine triphosphatase; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; IL, interleukin; LA, ω-6 linoleic acid; LFA-1, lymphoid-enhancing transcription factor; LNA, ω-3 linoleic acid; L-PHA, Nα,β-nitro-L-arginine methyl ester; LT, leukotriene; MAPK, mitogen activated protein kinase; MCP, monocyte chemotactic protein; MEK, mitogen extracellular kinase; MMP, matrix metalloproteinase; NO, nitric oxide; OPN, osteopontin; PG, prostaglandin; P13K, phosphatidylinositol-3 kinase; PI3P, phosphatidylinositol-3 phosphate; PIP2, phosphatidylinositol(4,5)-bisphosphate; PKA, protein kinase A; PKA-1, protein kinase A type 1; PKA-2, protein kinase A type 2; PKB, protein kinase B; PKD, polycystic kidney disease; PLCγ, phospholipase Cγ; RAR, retinoic acid receptor; RXR, retinoid X-receptor; TGF, tumor necrosis factor-α converting enzyme; TCF, T cell factor; TIMP, tissue inhibitor of matrix metalloproteinase; TNF-α, tumor necrosis factor-α.

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