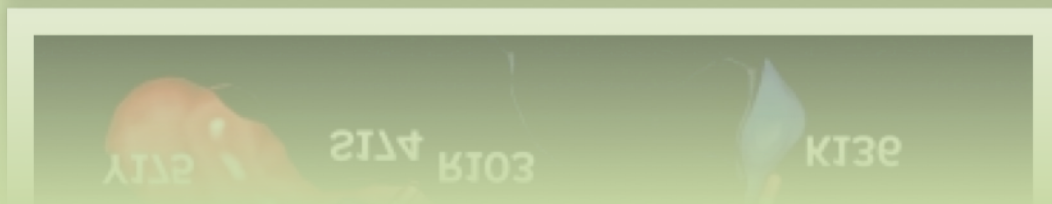
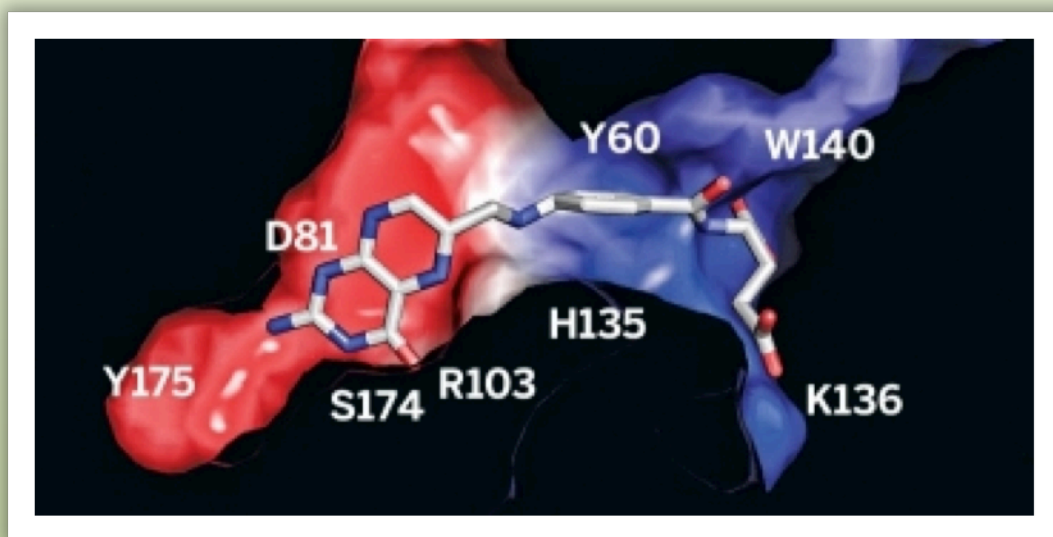


# Polycystic Kidney Disease

A look into folate conjugated drugs action on the kidney epithelial cells.

Angie Nato



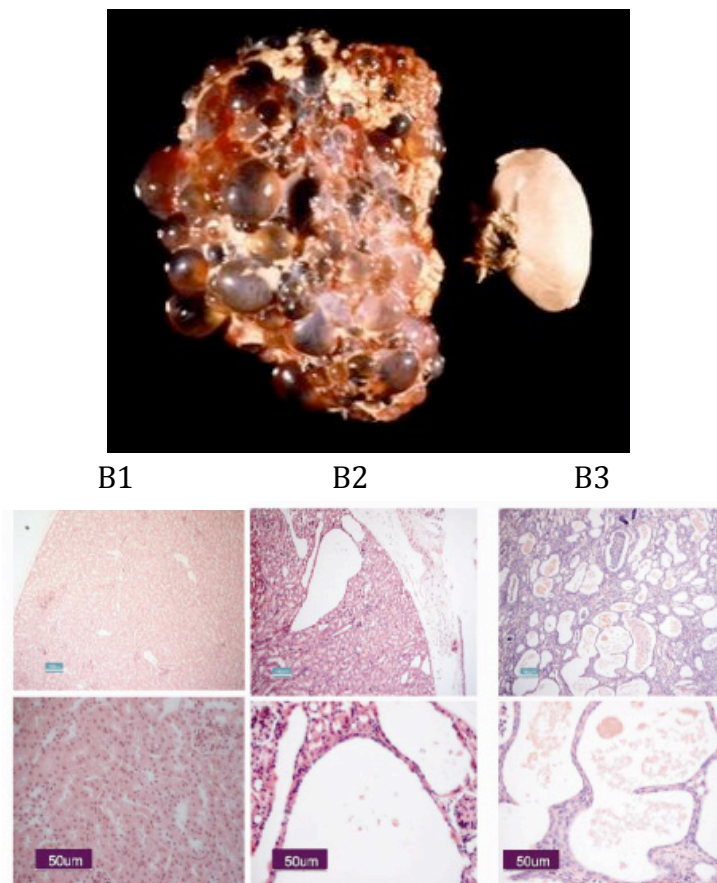
## Table of Contents

<b>Introduction.....</b>	<b>3</b>
<b>The role of the proximal tubule .....</b>	<b>7</b>
<b>The Folate Receptor .....</b>	<b>9</b>
<b>Renal Expression of Folate Receptors .....</b>	<b>11</b>
<b>Folate Receptor -Targeted Therapies .....</b>	<b>12</b>
<b>Designing FA-SMDCs .....</b>	<b>14</b>
<b>Assembly of FA-SMDC .....</b>	<b>15</b>
<b>Folate Receptor targeting in PKD .....</b>	<b>19</b>
<b>Other drugs used in Polycystic Kidney disease. ....</b>	<b>21</b>
<b>Somatostatin.....</b>	<b>21</b>
<b>Curcumin .....</b>	<b>21</b>
<b>Triptoliptide .....</b>	<b>22</b>
<b>Eternercept.....</b>	<b>22</b>
<b>Conclusion .....</b>	<b>22</b>
<b>References.....</b>	<b>23</b>

## Introduction

Polycystic kidney disease (PKD) is an inherited genetic disorder, characterized by the formation of clusters of cysts in the kidneys (1)(2). The cysts are multiple and are filled with fluid, and this leads to enlargement of the kidneys. Figure 1 shows a comparison of a polycystic kidney with a normal kidney, as well as a histopathological representation of cystic kidneys from *Pkhd1<sup>del3-4/del3-4</sup>* mice compared to a normal one. PKD usually affects both kidneys, however, in some instances it is present in one kidney but as the disease progresses, the other kidney is eventually affected (2). Other organs that can be damaged by the disease are the liver, pancreas the heart and the brain, indicating that it is a systemic disease (1). PKD is a prevalent life-threatening disease affecting approximately 12 million people worldwide (3).

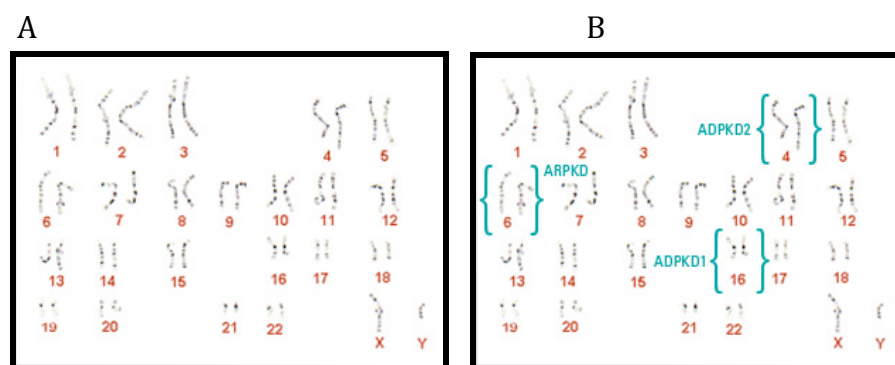
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**Figure 1:** (A) Polycystic Kidney compared to a normal kidney, (B1) Histological representation of a normal kidney having no cysts, (B2-B3) Histopathology representation of kidney from *Pkhd1<sup>del3-4/del3-4</sup>* mice that deteriorated with age. B2 at 6 months was categorized as cystic while B3 at 9 months as severely cystic (4).

Two types of PKD exist, Autosomal dominant polycystic kidney disease (ADPKD) and Autosomal recessive polycystic kidney disease (ARPKD). ADPKD occurs in both children and adults, although more commonly seen in adults. Being autosomal dominant, an individual has a 50% chance of getting the disease if one of the parents has the disease. ARPKD is less common and it is usually identified a few weeks after birth. It occurs when a child inherits the genetic mutation from both parents (1)(2).

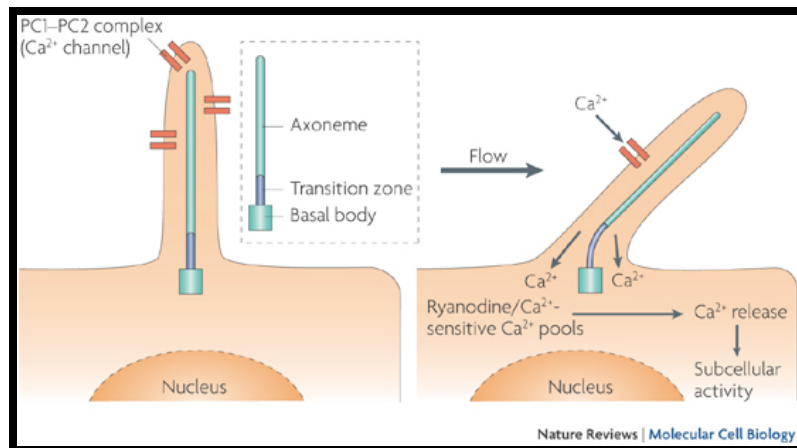
Approximately 85% of patients have ADPKD type 1, whereby there is a mutation on the short arm of chromosome 16, while 15% have ADPKD type 2 in which the mutation is found on the long arm of chromosome 4 (2). Figure 2 shows the chromosomes that undergo the mutations in PKD. There is a possibility of the existence of a third genotype, however no genomic locus has been assigned. PKD1 and PKD2, expressed by most organs in the body, encode the proteins polycystin-1 and polycystin-2 respectively, and together they regulate the morphologic configuration of epithelial cells.



**Figure 2:** (A) Karyotype of a normal male and (B) regions that undergo mutations in polycystic kidney disease. Approximately 85% of patients have ADPKD type 1, whereby there is a mutation on the short arm of chromosome 16, while 15% have ADPKD type 2 in which the mutation is found on the long arm of chromosome 4 (5)

Symptoms commonly seen are cyst formation in the renal epithelial cells as depicted in figure 1, excessive proliferation and fibrosis, which subsequently leads to a progressive destruction of the renal tissue and ultimately the kidney function is compromised (3)(6).

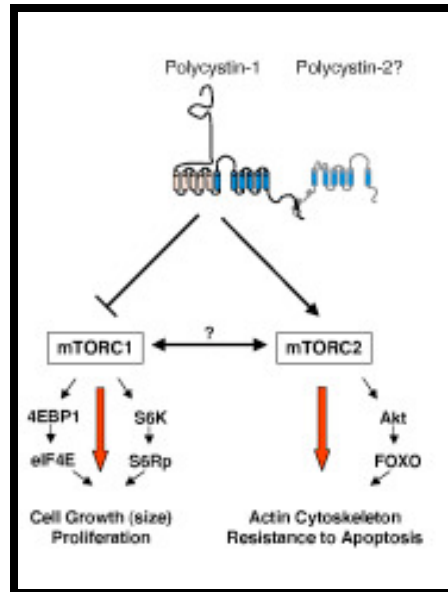
Polycystin-1 is an integral membrane protein with an extracellular domain, which contains a long N-terminal (1)(2). It is possibly involved in the interaction between cells and between the cells and the matrix. It is also implicated in calcium homeostasis when interacting physically with polycystin-2. In the renal epithelial cells, polycystin-1 is localized in the primary cilia, which are sensory organelles as shown in figure 3 below. Polycystin-2 is part of the voltage-activated calcium channels, and is also found on the primary cilia of renal epithelial cells. (1)(2)(7)



**Figure 3:** Polycystin-1 (PC1) –polycystin-2 (PC2) complex is normally found on the ciliary membrane and it responds to shear stress caused by the flow of urine. This causes the ciliary to bend thus activating  $Ca^{2+}$  channels leading to an influx of  $Ca^{2+}$  and subsequently subcellular activity. [8]

During cyst formation, several signaling molecules are involved, of which the main ones are mammalian target of rapamycin (mTOR), Calcium ( $Ca^{2+}$ ) and cyclic adenosine monophosphate (cAMP) (9). Rapamycin inhibits mTORC1 by binding to the endogenous protein FKBP12. This binding does not affect mTORC2 however long term exposure can lead to mild inhibition. These points in the pathway have been targeted therapeutically in order to impede the formation of cysts.

Shillingford et al (10) have previously demonstrated that the mammalian target of rapamycin (mTOR) pathway is upregulated in human patients with ADPKD as well as in PKD rodent models. mTOR, a serine/threonine kinase, is a catalytic subunit of two complexes. mTOR complex 1, is involved in protein synthesis, cell proliferation as well as autophagy while mTOR complex 2 regulates the cytoskeleton as well as cell survival as shown in figure 4 below(9). Rapamycin, an mTOR kinase inhibitor, preferentially inhibits mTOR complex 1 and only inhibits mTOR complex 2 upon prolonged exposure.



**Figure 4:** The mammalian target of rapamycin (mTOR) is known to exist in two complexes, mTORC1 implicated in cell growth and proliferation and mTORC2, which controls the actin cytoskeleton and apoptosis resistance. Polycystin-1 has been found to inhibit mTORC1 and upregulate mTORC2 (9).

Sirolimus and Everolimus are some of the mTOR inhibitors that have been used in clinical trials to determine their efficacy in patients with ADPKD (11-13). The results were however very disappointing, and this has been attributed to the use of sub therapeutic doses compared to what had been used in the animal studies. However, due to undesirable side effects experienced by many of the patients, higher doses could not be used (3).

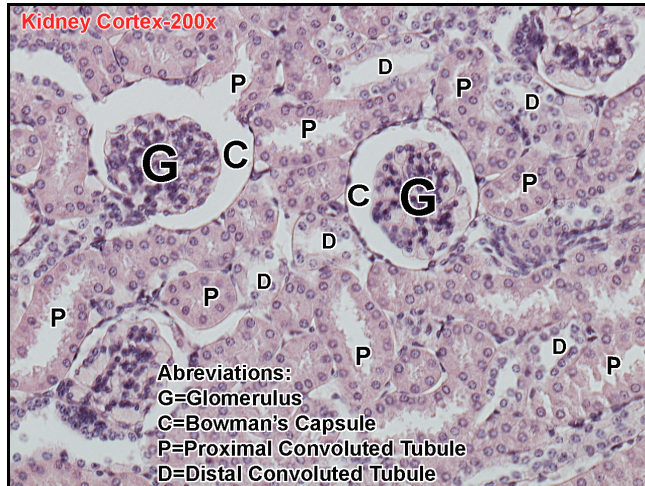
The concept of targeting mTOR inhibitors directly to the kidneys was the best approach in this circumstance and this was achieved by the use of folate receptor (FR) mediated endocytosis and this will be discussed in detail below (3). It has been possible to deliver folate-conjugated drugs in cancer therapy to cancer cells, as they are found to overexpress FR. Folate-conjugated rapamycin (FC-rapa) has been used in animal models of ADPKD with much success and with very few side effects compared to the use of rapamycin on its own. This has been possible since a specific drug was being targeted to a specific organ (3).

The aim of this review is to evaluate the literature on the possible targeting approaches that can be followed for PKD. This involves targeting the folate receptor to deliver folate conjugated drugs to the proximal tubular epithelial cells in the cysts.



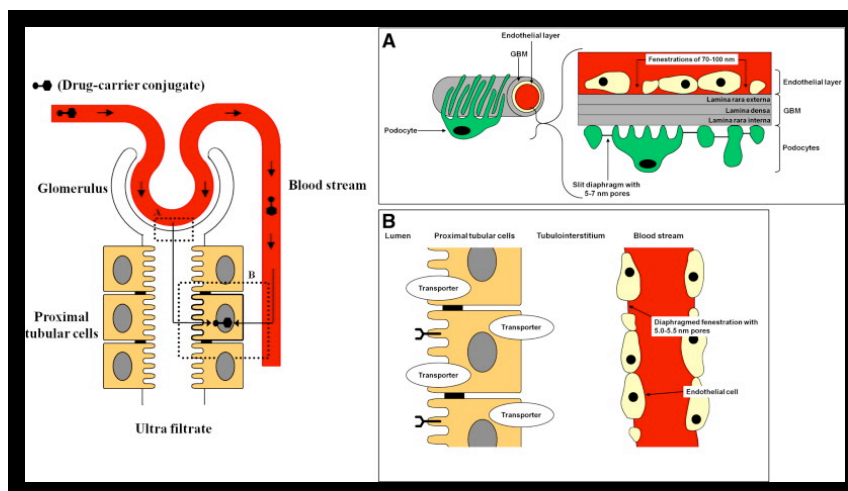
## The role of the proximal tubule

The functional unit of the kidney is the nephron, comprising of the glomerulus, which is the filtration unit, and the tubular part composed of the proximal tubule, the loop of Henle, a distal tubule and a collecting duct. Figure 5 is a microscopic representation of the different cells in the kidney.



**Figure 5:** microscopic representation of the different cells in the kidney. The sections labeled are: G- Glomerulus, C - Bowman's Capsule, P - Proximal convoluted tubule and D - Distal convoluted tubule (14).

The tubular part is responsible for the reabsorption of the filtered compounds both endogenous such as glucose and salts as well as exogenous such as drug molecules. The proximal tubule is the largest site where this occurs (15). Figure 6 illustrates the anatomical barrier between the proximal tubular cells and the circulation as well as ways in which a drug moves through the barrier.



**Figure 6.** Proximal tubular cells. A) Some Drug molecules can be filtered through the fenestrae in the glomerular endothelium. B) Drug molecules can pass through the diaphragmed fenestrae, through the tubulointerstitium to the basolateral membrane (16).

For a drug to reach the proximal tubule, it will either go through the basolateral side or the apical side. The endothelial cell layer of the renal peritubular capillaries is on the basolateral side, and it has fenestrations with a diameter of around 60-70 nm. A diaphragm, which is a filtration slit, of 3-5 nm thickness closes these fenestrations. Negatively charged heparin sulphate is found in these fenestrae, and this influences what can pass through since positively charged macromolecules and particles are easily transported through but not negatively charged drug carriers (17). The tubulointerstitium, which contains immune cells, is then encountered (16)

To get to the apical side of the proximal tubule cell, the drug is filtered through the glomerulus. To achieve this, it goes through the endothelial layer, the glomerular basement membrane, which allows positively charged molecules to pass through more easily than the negatively charged molecules, and subsequently the drug passes through the podocyte foot processes (16).



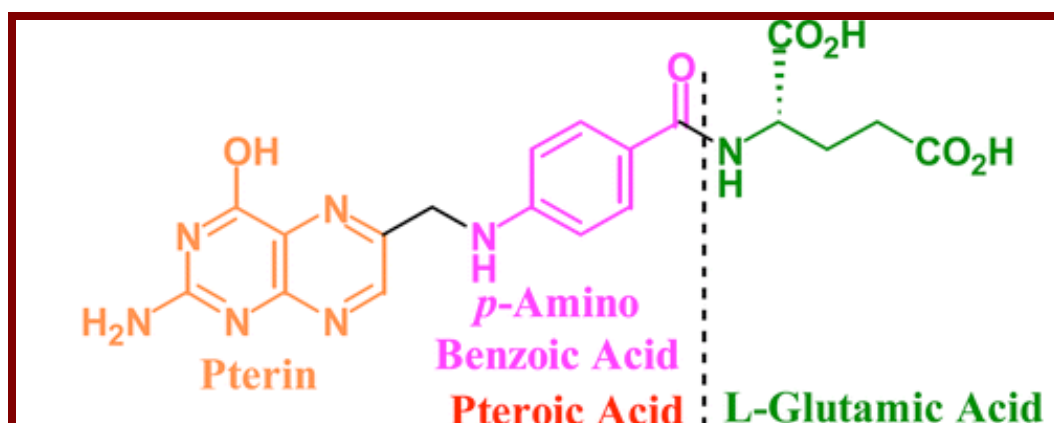
## The Folate Receptor

Folate is a vitamin required by all living cells, as it is essential for cell division and cell metabolism. It can enter the cell through two ways, the reduced folate carrier pathway (RFC) found on all cells, and is located on the baso-lateral surface of the proximal tubule in the kidney or by the folate receptor pathway (FR) found on the apical brush border of the proximal tubule. The FR is a glycosylphosphatidyinositol-linked membrane protein that transports folate or the ligand that binds to it, to the inside of the cell through an endosomal pathway. The FR pathway has a higher affinity compared to RFC (18). The RFC is the main route used by normally dividing cells to internalize the folate and since the folate is not in high demand, this route is the ideal one as the RFC transport protein has low affinity for folate. On the other hand, cancer cells over-express FR, which has a high affinity for folate and since they are rapidly dividing, the demand for folate is higher.

FR $\alpha$ , FR $\beta$ , FR $\gamma$  and FR $\delta$  are the different isoforms of the folate receptor, however FR $\alpha$  is the most widely expressed and also found in the proximal tubules (19)(20)(21). Once the FR captures the folate, it undergoes an internalization process, whereby an endosome is formed, thus enabling it to deliver the folate into the cell. Subsequently, the FR is taken back to the cell surface and the same process is repeated (18) (20). The binding of folate to the FR is optimal at a neutral pH since a low pH causes a rapid dissociation of the ligand receptor unit (21).

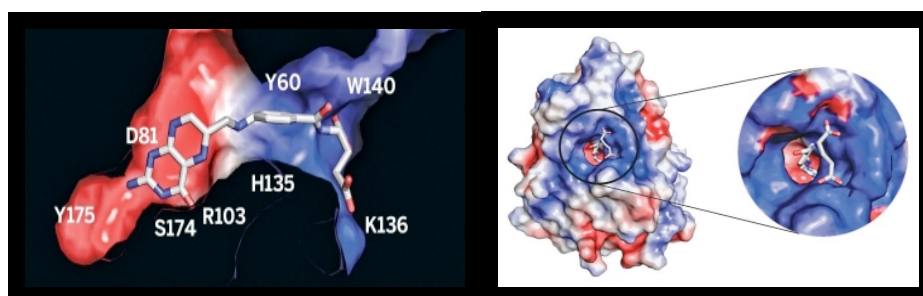
It is important to note that *Paulos et al* (19), demonstrated in cancer cell lines that the rate of folate transportation into the cell is independent on the number of FRs present but determined by the metabolic need of the cell. Therefore suggesting that the number of FRs are relevant for therapy that is targeted on the surface of a cell that is overexpressing the receptor, and not therapy that is directed into the cell (20).

*Chen et al* have recently been able to determine the structure of the FR $\alpha$  receptor. To get a better understanding of it, the structure of folate will be discussed briefly. Figure 7 depicts the structure of folic acid (folate), whereby it can be subdivided into two parts. The pteric acid part that has high affinity for the FR receptor, and the glutamic acid moiety, which can be modified or conjugated to a molecule for targeted delivery.



**Figure 7:** The structure of folic acid (folate), whereby it can be subdivided into two parts (dotted line). The pterinoic acid part that has high affinity for the FR receptor, and the glutamic acid moiety, which can be modified or conjugated to a molecule for targeted delivery (19)

The structure of the FR receptor as depicted in figure 8, shows how folic acid binds to it. The electrostatic charge distribution ranges from -3 (red) to +3 (blue) electron volts. The basic pterinoic group is deeply lodged inside the negatively charged pocket of the FR receptor, while glutamic acid moiety is sticking out of the binding pocket, since this group is negatively charged (12).



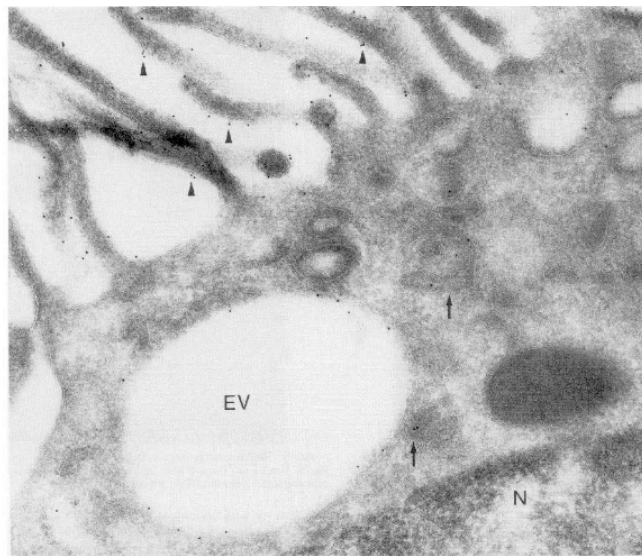
**Figure 8: (A)** Charge distribution surface of FR $\alpha$  with a close-up view of the ligand-binding pocket entrance. Folic acid carbon atoms are colored grey, nitrogen atoms blue, and oxygen atoms red. A color-code shows an electrostatic scale from -3 (red) to +3 eV (19).

**(B)** A side view of the folate receptor with folic acid in the binding pocket. The region in red is the negatively charged pocket, while the region in blue is the positively charged entrance (19).

When exploring folate-conjugated target delivery, the drug to be delivered is attached to the glutamate moiety as it does not influence the binding of the folic acid.

## Renal Expression of Folate Receptors

FRs are known to be expressed in the kidney. They are located in the apical brush boarder of the proximal tubule (21)(22)(23). In the mouse, immunocytochemical techniques have located the FRs in the glomerulus (24) (22). In humans, they have been found in the urine (25), and identification of the mRNA indicated that the isoforms FR $\alpha$  and FR $\beta$  are expressed in the human kidney (26) Figure 9 below is an immunohistochemical representation of the folate binding protein located in the microvilli of the proximal tubule, endocytic vacuole and the apical tubules.



**Figure 9:** Immunocytochemical representation of folate binding protein in the proximal tubule as shown by the arrowheads in the microvilli and in the endocytic vacuole (EV). The folate binding protein is also seen in the apical tubules as shown by the arrows (22)

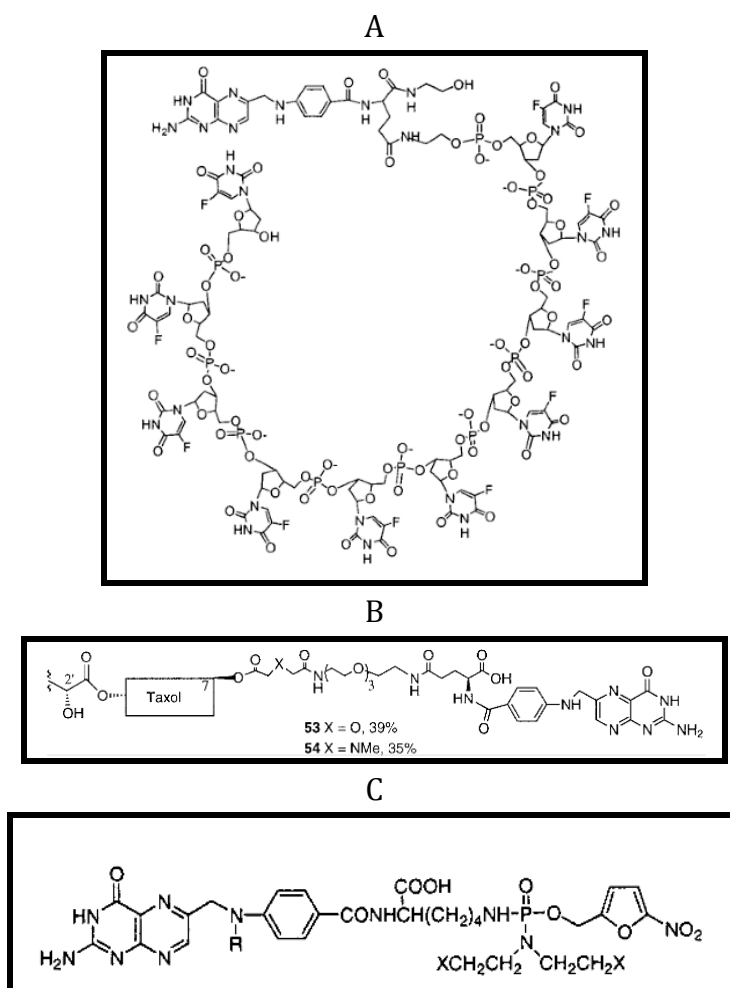
## Folate Receptor -Targeted Therapies

As mentioned previously, folate (FA) has a high affinity for the folate receptor FR. It is important to note that the folate receptor is also used as a tumor antigen/biomarker as it is largely expressed on cancer cells and almost absent in normal dividing cells (27)(28)(29).

Potent chemotherapeutic agents have also been delivered to cancer cells through the FR by attaching them to folic acid. This forms a small molecule drug conjugate SMDC (29). This receptor has been used to deliver molecules of various sizes into the cancer cells, for instance radionuclides, liposomal constructs as well as DNA (29).

Folate has been greatly studied as a carrier of conjugates into the cells as the conjugated drug does not change its affinity and uptake by the receptor into the cell. Therefore the design of the conjugates attached to folate (FA-SMDC), to be taken up by the cell and subsequently released from the folate while inside the cell is a very important area in folate-drug conjugate therapy, as subtle design changes impacts greatly on the pharmacology of the compound (30).

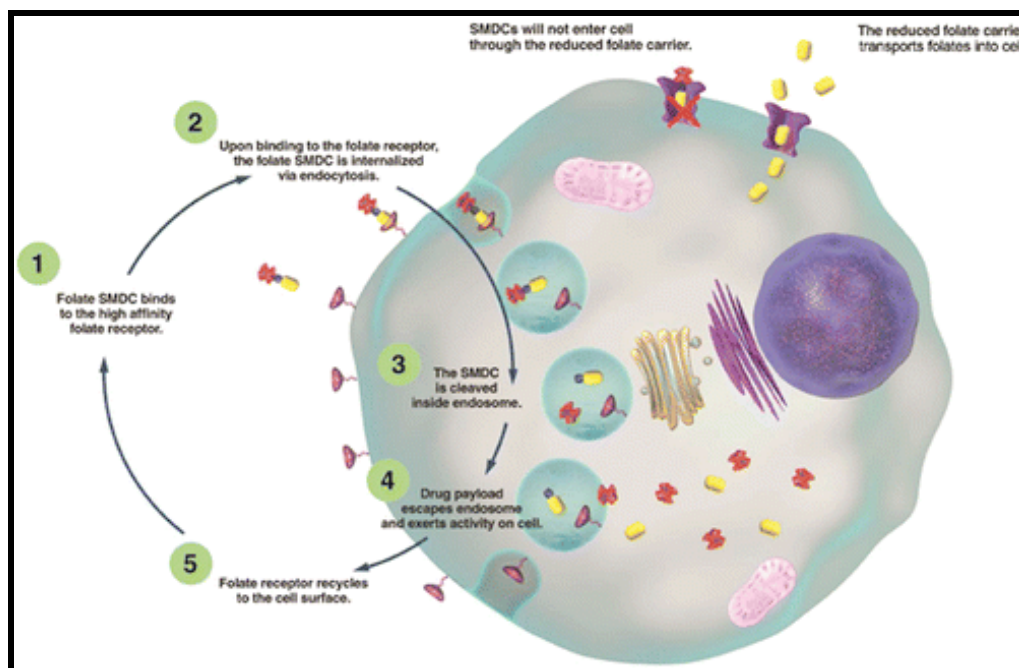
Previously there was limited success in the conjugates formed, as their designs were not adequately structured (30). For instance, some of the chemotherapeutic agents that had such a disadvantage as they were covalently attached to FA or pteronic acid are bis(haloethyl) phosphoramidites that had been used in IGROV human ovarian carcinoma cell lines (31), 5-fluoro-2'-deoxyuridine-5'-*O*-monophosphate (FdUMP) against human colorectal tumor cells (H630) (32), as well as paclitaxel (33) Their structures are shown in figure 10 below.



**Figure 10:** Chemotherapeutic agents that have been used as small molecular drug conjugates and covalently attached to FA or pteric acid. A: 5-fluoro-2'-deoxyuridine-5'-O-monophosphate (FdUMP), B: Paclitaxel derivative and C: bis(haloethyl) phosphoramidites (31)(32)(33)

Figure 11 below depicts how FR takes up a FA-SMDC. In Step 1, once the FA-SMDC leaves the blood circulation, it moves towards the folate receptor (FR) on the surface of a tumor cell. As the affinity of FA is still maintained even with a SMDC attached to it, it binds with ease to FR as seen in Step 2 and taken up by the cell, via endocytosis (29)(34). The endosome has a proton pump attached to it and this causes a slight drop in the pH level in the endosome. This drop in pH causes the FR to undergo a conformational change causing the FA of the SMDC to be released (35). In Step 3, the linking system in the SMDC is then cleaved inside the endosome, releasing the drug, which is able to diffuse out of the endosome and into the cell, and thus able to access its target in Step 4. The FR is then recycled back to the surface of the cell, a process that normally takes 8-12 hours (20).

The anion transporter, reduced folate receptor (RFC) also seen in figure 11 transports folate into the cell, however, the FA-SMDCs is not a substrate to it. This limits the FA-SMDCs to FR expressing cells, thereby reducing side effects



**Figure 11:** A depiction of how the folate receptor (FR) is taken up by a tumor cell by endocytosis of the FR. The yellow oval is the folic acid (FA) while the red is the small molecule drug conjugate (SMDC) (29).

### Designing FA-SMDCs

It is not enough for the SMDC to be transported into the cell, but it is vital that it is released from the endosome. To achieve this, a good linker system should be designed. Vlahov and Leamon came up with a list of characteristics of a successful FA-SMDC (29). This system was used for tumor targeting and these characteristics may be applied and be beneficial in PKD.

*High potency drugs.* It normally takes 8-12 hours for a folate receptor once endocytosed to be recycled back to the surface of the cell. Although the FR is heavily expressed in tumor cells, they can be saturable; therefore using highly potent drugs would circumvent this potential problem (29).

*Incorporating functional groups that are easy to modify.* For ease of attaching the drug to FA, functional groups such as  $-NH_2$ ,  $-NH-$ ,  $-OH$  or  $-COOH$  can be used (29).

*Enhanced hydrophilicity.* Addition of charges to lipophilic small molecules facilitated their movement through the FR and not to other cells that are not targeted (29).

*High Stability.* It is important that the SMDC detaches from the FA-SMDC while in the endosome and not earlier in order to ensure the drug is accumulated in the targeted cell and minimize the side effects (29).

*Releasable linkers.* The use of a disulfide linker system (which can easily be reduced in the endosome) (36) or a pH sensitive linker system (as there is a drop in pH in the endosome) facilitates cleaving of the linker thus releasing the drug.

*Low molecular weight.* The size of a typical FA-SMDC is normally <2000 Da. This makes it easy for the conjugate to be taken up by the folate receptor (29).

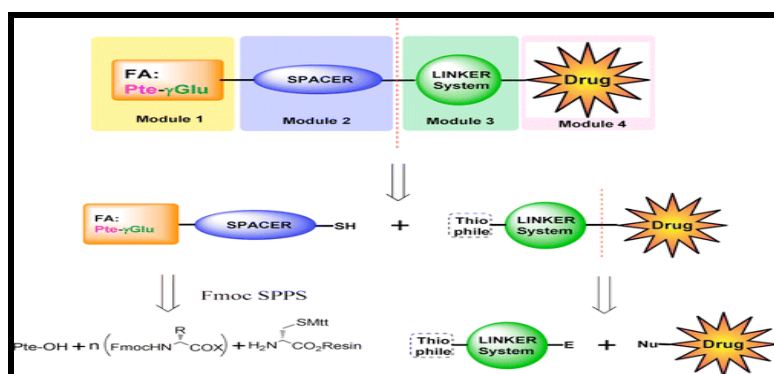
### Assembly of FA-SMDC

A good FA-SMDC conjugate, as seen in figure 12.A, has the FA (Module 1) attached to a spacer (module 2) which ensures that the binding of the folate to the FR is not affected and it also improves the hydrophilicity of the FA-SMDC structure by adding aspartic acid and arginine residues. Peptidic extension of FA that terminates in a cysteine residue is a commonly used spacer unit as shown in figure 12.B below. The synthesis techniques it normally undergoes are standard fluorenylmethyloxycarbonyl-based solid-phase peptide synthesis (Fmoc SPPS). The cys thiol group is the site that can be easily cleaved. Figure 13.A shows some of the structures with disulfide linker system (37).

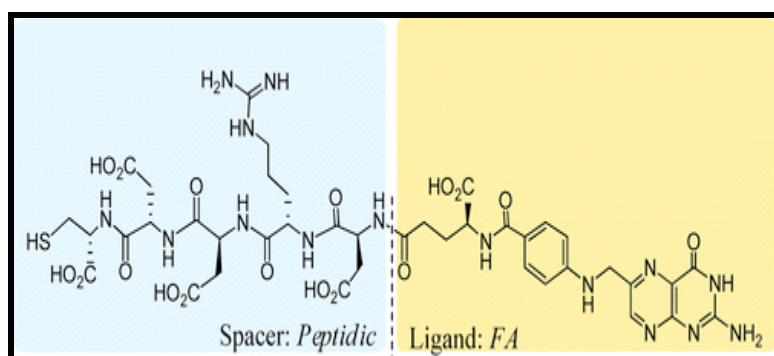
The drug (Module 4) is then attached to the spacer by a linker (Module 3), which can be cleaved to release the drug.



A

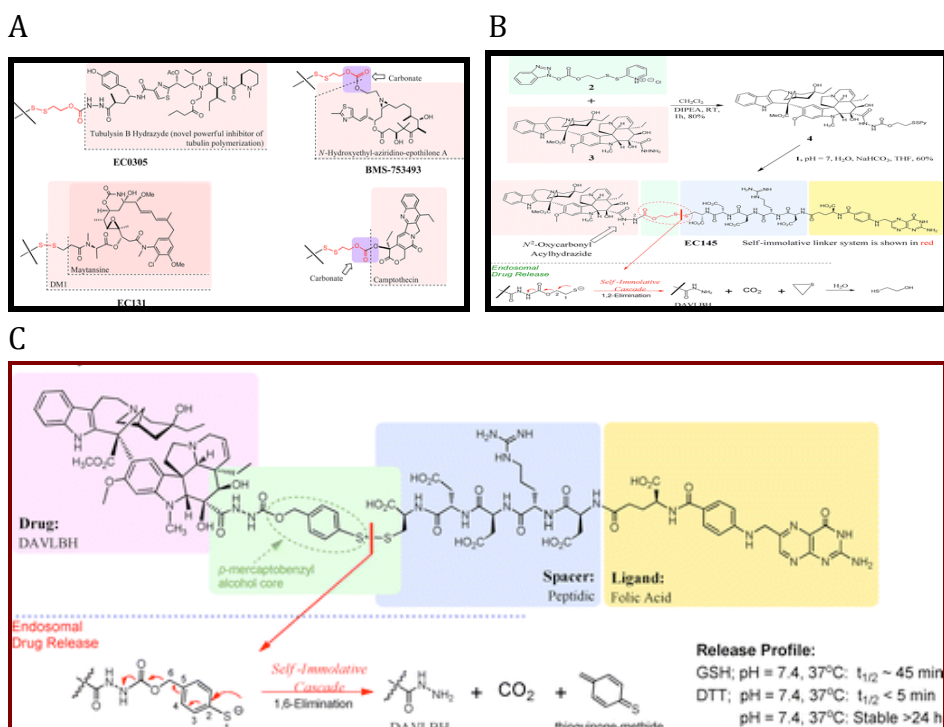


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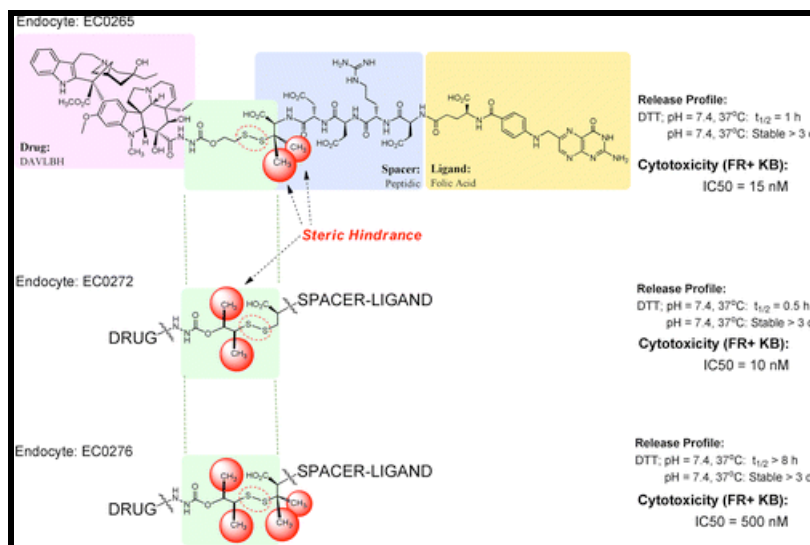
**Figure 12:** (A) a typical FA-SMDC (B) a peptidic-spacer unit (29)

Other techniques of ensuring the drug is released from the FA-SMDC are by the use of a self-Immolative Linker system. This utilizes the electronic influences for instance a compound with a mercaptoethanol core undergoes an episulfide reduction then a 1,2-elimination reaction, figure 13.B, while a compound that has a *para*-mercaptobenzylalcohol core in the disulfide trigger will undergo thioquinone-methide-based 1,6-elimination, as can be seen in figure 13.C.



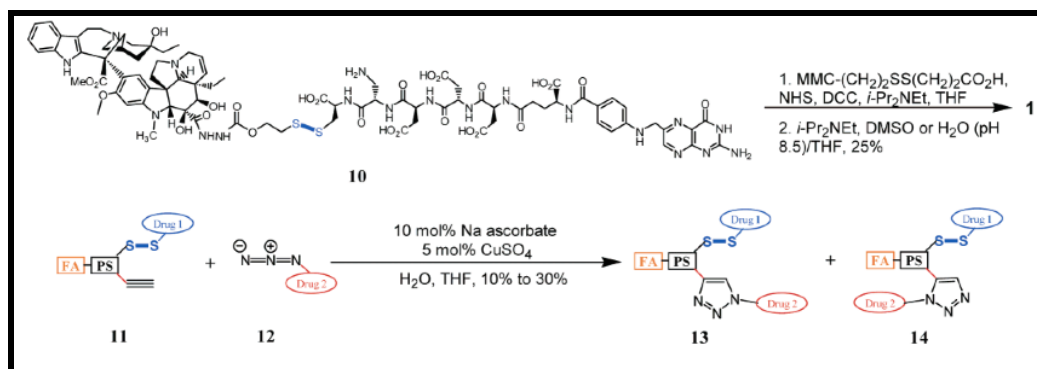
**Figure 13:** (A) Structures with linker systems that are disulfide-based (B) A scheme showing a 1,2-self Immolative linker system and (C) a 1,6- elimination of a self-immolative linker system (29).

Compounds such as glutathione, homocysteine cysteine and albumin are present in high numbers in the sera. These can easily attack the disulfide bond in the linker, releasing the drug into the blood stream and thus the drug does not reach its target. This especially happens when the FA-SMDC is present in the circulation for a long period of time. This problem can be circumvented by the use of sterical hindrance for instance by introducing methyl groups around the disulfide bond as depicted in figure 14 below (29). This can however decrease the potency of the drug conjugate.



**Figure 14:** The influence of Steric Hindrance on drug release from the conjugate and the cytotoxicity associated with it (29).

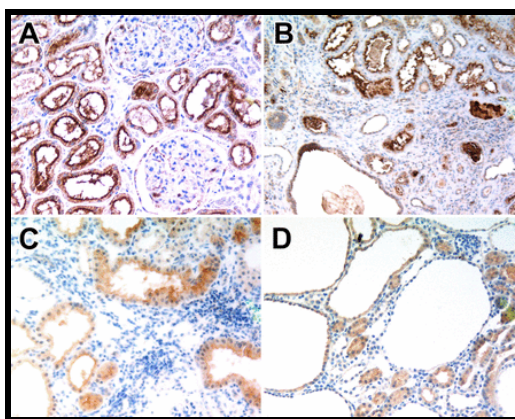
Multiple drugs can be attached to the same conjugate as shown below in figure 15, in the example below, the yields obtained of the dual drug conjugates were moderate (38). Unsymmetrical disulfide bonds can be assembled to the same conjugate of folic acid, and this is important in targeting multiple pathways that could be involved in a disease. Targeting multiple pathways can lead to lower doses of the drugs used therefore less side effects.



**Figure 15:** A scheme showing a multidrug conjugate of folic acid, where FA is folic acid, PS is peptidic spacer and drug1 and drug2 are the targeted drugs that have a self-immolative linker fragment (38).

## Folate Receptor targeting in PKD

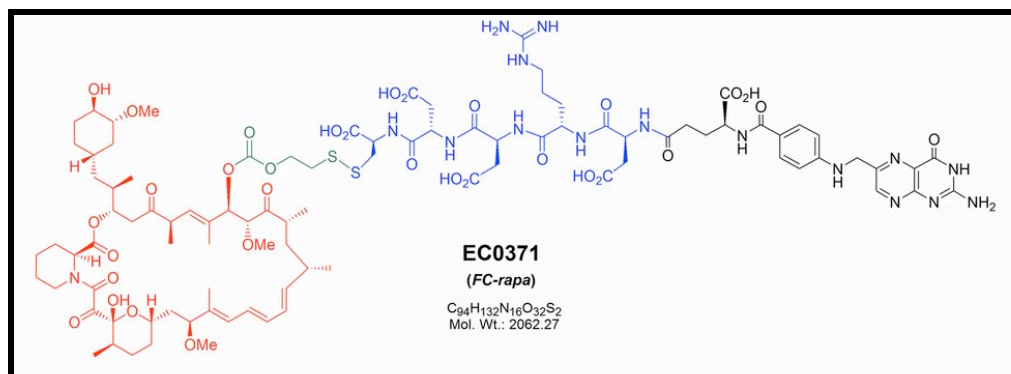
As previously mentioned, FR are present in the kidneys. However it is important to ensure that the expression of the FR is still maintained in the patients with ADPKD. Shillingford *et al* were able to establish this by doing an immunohistochemistry staining for FR on humans with ADPKD as well as on mouse models of ADPKD, the *orpk*-rescue and the *bpk* mouse. They found, as shown in figure 16, that the normal tubules and the cysts on the epithelial cells to be positive for FR, and as this had similar signal intensity as the FR found in human serous ovarian cancer, it showed that the FR was highly expressed in the renal cysts (39).



**Figure 16.** Immunohistochemistry staining of FR in PKD, counterstained with hematoxylin (A) Normal Kidney, (B) Human kidney with ADPKD, (C) *orpk*-rescue mouse kidney and (D) *bpk* mouse kidney. It is important to point out that both the epithelial cells in the tubule of the normal kidney and the cyst lining cells are FR positive (39).

As previously noted, the epithelial cells in the proximal tubule and as shown in figure 16 above, the cells lining the cysts in ADPKD express the folate receptor. Therefore folate targeted drug therapy can be used in the pharmacological management of the disease.

A folate-conjugated form of rapamycin FC-rapa (EC0371), shown in figure 17, was assembled with a hydrophilic pentapeptide spacer and a self-immolative disulfide linker system (39).



**Figure 17:** Chemical structure of folate-conjugated rapamycin (EC0371) depicting folate (black), hydrophilic spacer (blue), biologically cleavable linker (green), and rapamycin (red) (39).

To determine the effectiveness of FC-rapa in inhibiting the mTOR pathway *in-vitro*, folate adapted KB cells known to express high levels of FR (40) were used and the control was MDCK cells, as they do not express FR (41). The mTOR surrogate markers used were phosphorylated S6 kinase and S6, its downstream substrate. Both conjugated and unconjugated rapamycin were used, and it was found that the unconjugated rapamycin inhibited the phosphorylation of S6 kinase and S6 in both cell types. The conjugated rapamycin (FC-rapa) dose dependently, at a dose range of 0.125nM – 2nM, inhibited the mTOR pathway in the KB cells but not in the MDCK cells (39).

FC-rapa was also used *in-vivo* in the *bpk* mutant mice from postnatal days 7-21. There was a significant inhibition of renal enlargement and cyst growth, thus decreasing the kidney weight, the size of the cysts as well as a decrease in cell proliferation.

It is important to note that the normal cells in the renal tubule were not significantly affected by the FC-rapa treatment (10)(42). This is due to the low mTOR activity in these cells. This indicates that the treatment is mainly targeted to the cyst cells (39)(42). The conjugate is seen to accumulate in both normal tubular cells as well as in diseased PTEC, but the effects of the drug are only apparent in the diseased cell since the drug target mTOR is only active in PKD affected cells.

## Other drugs used in Polycystic Kidney disease.

There is currently no cure for PKD therefore symptomatic treatment and supportive measures are normally afforded to the patient. These can include pain management, antibiotic treatment when the cysts are infected and blood pressure control. Patients who progress to end stage renal disease can benefit from hemodialysis, peritoneal dialysis or renal transplantation.

There are some drugs under investigation that could lead to promising results in the management of PKD. These drugs target, for instance, cell signaling pathways and cell differentiations that are abnormal. Cell anaplasia and apoptosis are also targeted and since it is a cyst disease, fluid secretion is also addressed (7).

### Somatostatin

Somatostatin is a cyclic neuroendocrine hormone secreted by the D cells of the pancreatic islet, the nervous system and thyroid glands (43). It has a high affinity towards the 5 somatostatin receptors  $sst_1$  to  $sst_5$ . These receptors are overexpressed in various tumor cells, compared to normal cells, thus making them good candidates for targeted drug therapy (44).

The  $sst_2$  receptor is expressed in the kidney, and its ligands inhibit adenylate cyclase and stimulate phospholipase C (43). Somatostatin has been used in patients with ADPKD and the treatment prevented the growth of the parenchymal tissue in the kidneys (45).

Octreotide is an analogue of somatostatin that has a high affinity for the receptor  $sst_2$ . Its structure has d-amino acids, enhancing its resistance to enzymatic degradation and a cystin bridge to ensure the pharmacophore  $\beta$ -turn is stable (46). Various cytotoxic compounds have been conjugated to octreotide with some successful results.

### Curcumin

Curcumin is a spice from the rhizome plant *Curcuma Longa*. Due to its several beneficial properties like anti-inflammatory, anti-oxidant and anti-proliferative effects, it has been used in many studies to determine its therapeutic potential (47). It is known to inhibit several pathways, among them the mTOR signaling, Wnt/ $\beta$ -catenin signaling and the nuclear factor- $\kappa\beta$  (NF- $\kappa\beta$ ) (48). It also inhibits the signal transducer and activator of transcription 3 (STAT3), which is important in ADPKD, as demonstrated in the iKsp-Pkd1<sup>del</sup> mice (49).

## Triptoliptide

Triptoliptide is a natural product shown to be able to delay the growth of cysts in mice with a kidney-specific *Pkd1* mutation. Triptoliptide stimulates polycystin 2 dependent calcium release and this intracellular calcium leads to a decrease of cAMP by stimulating phosphodiesterase and inhibiting adenyl cyclase (50).

## Eternercept

Eternercept, a tumor necrosis factor- alpha (TNF- $\alpha$ ) inhibitor was used in a study for the pharmacological intervention of pkd in mice that had a *Pkd2* mutation. The mice were given the treatment for ten weeks and at the end of the study it was found that the mice that received the TNF- $\alpha$  did not develop any cysts while the ones that received a control treatment developed cysts (51).

## Conclusion

The elucidation of the molecular pathogenesis of PKD has shown that FR are highly expressed in the renal cyst therefore this makes folate conjugated drugs a brilliant approach in the delivery of drugs into the renal cysts. The success in the management of the disease now lies in the proper design of the pharmacological drugs that will enable easy delivery into the cell via the FR in order to reach the intended target, and since multiple drugs can be attached to the folic acid, several pathways in the disease pathogenesis can be targeted simultaneously.



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