# The role of tumor specific DNA/gene dose in the development of papillary rencel cell tumors

Doctoral (Ph.D.) thesis

Author: Donát Péter Sarlós M.D.

Department of Urology

University of Pécs, Medical School

Pécs, 2019

Head of the Doctoral school: Prof. Lajos Bogár M.D., Ph.D., D.Sc.

Program leader: Prof. László Pajor M.D., Ph.D., D.Sc.

Mentors: Prof. Gyula Kovács M.D., Ph.D., D.Sc.

Árpád Szántó M.D., Ph.D.

# 1. Introduction

The classification of renal cell carcinoma (RCC) is traditionally based of the microscopis evalutation of HE stained slides. After classification systems based on cytological and architectural alterations, a change in the paradigm happened in the late 80' and early 90's: the new classification was based on specific chromosomal changes in tumors (Kovacs, 1993 a,b). The Heidelberg classification notices tumor-specific genetical alterations that identify the type of the tumor, even in cases, when histological analysis is controversial (Kovacs et al., 1997). Papaillary renal cell carcinoma can show high histological variability, but it shows well defined choromosomal and genetical changes. During tumor development, first the trisomy of tetrasomy of choromosomes 7 and 17 develop. This is may be followed by the loss of chromosome Y. The later chromosomal trisomies of 3q, 8, 12, 16, 20 might also develop, which merks the progression into a more aggressive tumor (Kovacs 1993a, Szponar et al., 2009). These data have high significance knowing the currently used WHO classification, where the difference between the papillary adenoma and carcinoma is made only by the size of the tumor. Thus a tumor under 15mm-s is benign, and above 15mms is malignant (Moch et al., 2016). This might lead to a false prediction of the prognosis. There are two theories about the development of papillary renal cell carcinoma. According to the opinion of the WHO and ISUP (International Society of Urological Pathologists) the papillary renal cell tumors originate from the differentiated mature cells of the renal tubules, similarly to conventional renal cell carcinoma. A different theory states, that the development of papillary renal cell carcinoma follows a sequence of developmental disorder – precursor lesion – adenoma – carcinoma (Kovacs, 1993 a,b). The most important tool in the differentiation between adenoma and carcinoma is genetic analysis.

# 2. Objectives

Our hypothesis is, that papillary renal cell tumors originate from poorly or partly differentiated persisting embryonal cell-groups, and follows a sequence of developmental disorder – precursor lesion – adenoma – carcinoma. Earlier studies

show, that the chromosomal and genetical alterations (trisomy of 7, 17, loss of Y) that appear in the early phase of tumor development have an important role in the embryonal kidney development and thus lead to precursor lesions that may become malignant in adulthood. The goal of our work is to investigate these specific genetical alterations during kidney development, in precursor lesions and in papillary renal cell tumors to be able to prove the unusual origin of papillary renal cell carcinoma.

# 3. Materials and methods

## 3.1. Tissue samples

For the RNA based examinations fetal kidneys were collected were collected at the Department of Obstetrics and Gynecology of the University of Pécs, Medical School. The samples were stored in TRIzol at minus 80 degrees Celsius. A similar storage was used for the samples for MET gene analysis and the precursor lesion samples. All tissue samples were fixated in 4% formaldehyde and evaluated using the hematoxylin-eosin stained slides.

For the evaluation of tumors, specimen collected between the January of 2000 and December of 2014 from radical or partial nephrectomies were evaluated and cases of papillary renal cell carcinoma were selected. The blocks and slides were provided by the Department of Pathology, Universiy of Pécs, Medical School. The collection and investigation of the samples was approved by the regional comitte of ethics (reference number: 5343/2014). The histological diagnosis was confirmed in every case by a uropathologist following the Heidelberg classification (Kovacs et al., 1997).

## 3.2. Domain analysis of the MET tyrosine kinase

The exons 16,17,18, and 19 from the tyrosine kinase domain of MET were amplified (Fischer et al., 1998). The PCR products were sequenced with IR800 and IR700 signaled primers Thermosequenase Cycle Sequencing kit (Amersham Pharmacia, Freiburg, Germany). The marked PCR products were evaluated using laser detector LICOR Long ReadIr 4200 sequencer (MWG-Biotech, Ebersberg, Germany) aand BaseImageIR software. The mutations were shown when comparing the results with a wild type sequence (J02958).

### 3.3. SSCP (single-strand conformation polymorphism) examination of exon 19

Using the genomic AC004416 MET sequence, primers were created for the regions nighboring exon 19, with which a 198 base pair (bp) segment could be created, with a middle part containing the A3954C mutation. Combining the K2d (nt:24839-24860) “cca cgg gta ata att ttg tcc” intron primer with the K1r (nt:25019-25036) “cca cat ctg act tgg tgg tgg” primer found on the 3’ end of exon 19. The 20 µl PCR-reaction contained 100 ng DNA, 200 µM dNTP, 1,5 mM $MgCl^2$, 2 pmol of each primers (K2d radioactively marked) and 0,5 ng Taq-polimerase. After 2 minutes of denaturation at 94 degrees Celcius, 27 cycles were permormed (30sec 94 °C, 30sec 55 °C and 40sec 72 °C). The final phase lasted 5 minutes at 72°C.For all PCR reactions 20 µl running buffer, 2 µl 1M NaOH and 2 µl 20 mM EDTA were given. The samples were denaturated on 94 °C for 2 minutes and 3 µl was filled to 10% glicerol MDE gel. The electrophoresis was performed on 6 W over night, the gel was then dried and exposed to X-ray film over-night.

### 3.4. RT-PCR of mutated and wild type MET gene

RNA was isolated from frozen tissue samples or cell cultures using TRIzol (Invitrogen GmbH, Karlsruhe, Germany). The synthesis of the first strand of the cDNA from the 2µg RNA sample, was done with Superscript II reverse transcriptase (Invitrogen). The real time PCR reaction was done with DNA Engine Opticon system (MJ Research Inc., Watertown, USA), Platinum SYBRGreen qPCR SuperMix-UDG (Invitrogen), ß actin was used as control. All reactions were performed two separate occasions, the final results were calculated as an average of the two results.

The experiments listed in the 3.2-3.4 were done in the Molecular Oncology Laboratory of the Ruprecht-Karls University of Heidelberg, Germany as a scientific collaboration.

### 3.5. Tissue microarray (TMA)

Paraffin embedded samples were used to make TMA. HE stained slides were precisely evaluated to select areas of tumor sampling. Manual Tissue Arrayer (MTA1, Beecher Instruments, Inc., Sun Prairie, USA) system was used to extract 0.6mm cylinders from the tissue blocks. The samples included papillary renal cell tumors and also healthy fetal and adult kidney tissue for controls. More samples were extracted from tumors containing parts with different morphology or grade. The samples selected were then embedded in a block containing up to 150 different tissues on one slide.

### 3.6. Immunohistochemistry

For the immunohistochemistry slides and TMAs of papillary renal cell tumors, fetal and adult kideneys and pre neoplastic lesions were used. The 4 μm thick slides were put in low melting point paraffin for epitope conservation. The slides were deparaffinated with xylol and rehydrated in a series of alcohol solutions. Antigen retrieval was performed in boiling 10 mM sodium citrate buffer solution (pH 6.0) using 2100-Retriever (Pick-Cell Laboratories, Amszterdam, Hollandia). Blocking of non-specific binding sites and endogenous peroxidase activity was achieved with 0.3% hydrogen peroxide mixed with 1% horse serum for 10 minutes at room temperature. The slides were then incubated with the primary antibody overnight at 4°C. The list of antibodies used are summarized in Table 1. Following 30 minutes incubation with HRP-conjugated anti-rabbit secondary antibody (DAKO) AEC (3-amino-9-ethylcarbazol) substrate was added. Additional staining was done with Mayer's hematoxilin. Evaluation was performed with Leica LaborluxS microscope, photos were taken with Leitz DMRBE microscope and ProgRes C14 camera.

| Antibody | Origin | Catalog No | Buffer pH | Dilution |
|---|---|---|---|---|
| anti-HNF1B | rabbit | HPA002083 | citrate pH 6.0 | 1:200 |
| anti-MET | rabbit | SC-12 | citrate pH 6.0 | 1:100 |
| anti-KRT17 | rabbit | PA5-29919 | citrate pH 6.0 | 1:200 |
| anti-PCDH11Y | rabbit | PA5-38781 | citrate pH 6.0 | 1:50 |

Table 1. Antibodies used in immunohistochemistry studies

# 4. Results

## *4.1.* Histology and occurrence of papillary pre-neoplastic lesions

During the careful evaluation of kidneys containing different types of renal cell tumors we evaluated hundreds of slides to identify potential pre-neoplastic lesions (Table 2). As it could be estimated, we have found the most of these precursor lesions in kidneys bearing papillary renal cell tumors. These were 1-2mm large lesions with tubule-papillary structure. In 15 kidneys with papillary renal cell tumors we have found an average of 42 lesions per kidney. In these, various forms of small “blue cells” and large cell papillary structures were found. This conversion from small to large cells mimics the steps of embryonal differenctiation of the kidney tissue, thus the conversion from metanephrogen blasthema to tubular epithelium. Some subcapsular lesions have gone through regressive changes with stromal fibrosis, regression of epithelial cells and calcification. In kidneys with conventional renal cell carcinoma, only 0.4 lesions per kidney were observed. In case of renal oncocystoma only 0.8 lesions. In case of choromofobe tumors no lesions were found. In a previous study of hereditary papillary renal cell carcinoma however, more than 1000 lesions per kidney could be found (Ornstein et al., 2000).

| Tumor type | Nº of PNL/kidney |
|---|---|
| Conventional (clear cell) RCC | 0.4 |
| Chromophobe RCC | 0.0 |
| Renal oncocytoma | 0.8 |
| Sporadic papillary RCC | 42.0 |
| Hereditary papillary RCC | >1000* |

Table 2. Number of microscopic precursor lesions (PNL) per kidney with different types of kidney tumors (*Ornstein et al. 2000)

### 4.2. Histological heterogeneity of papillary renal cell tumors

The papillary renal cel tumors showed histological heterogeneity, however they showed a similarity in many cases with the pepillary pre-neoplastic lesions found in the same kidney. Often in one specific tumor more cell growth pattern can be identified. The well differentiated tumor can show moderate grade cells on an irregular stroma, but another part of the tumor shows clear cell papillary pattern. We have identified a case, where the tumor showed large basphil cells, but the lymph node metastasis showed a small cell pattern. There are cases when the papillary tumor can be diagnosed as a renal cyst with thick wall, but after years, it can suffer a sarcomatoid change and show a rapid progression and metastasis. The diagnosis of papillary renal cell tumor has been confirmed with genetical analysis in all cases.

### 4.3. Duplication of chromosome 7 and alteration of MET in hereditary renal cell carcinoma

To investigate the role of the MET TKR we have studied a hereditary MET mutated case. The 37 year old male had multiple papillary renal cell carcinomas. When comparing the mutated MET gene with the wild type MET gene, an A-C mutation was found. Mutation specific primers were used to show, that the mutated allele as well as

the wild type allele could be found in normal kidney cells of the patient. In the tumors however the SSCP examination showed, that the mutated gene was duplicated.

### 4.4. Expression of MET in fetal kidney, pre-neoplastic lesions and papillary renal cell tumors

Using immunohistochemistry we have seen MET expression in the fetal kidney in the following pattern: the ureteric bud, the cap metanephric mesenchyme, in the comma shaped body and in the distal compartment of the S-shaped body. This exoression was sustained in the pre neoplastic lesions in hereditary papillary tumors and also the ones that we have observes in connection with sporadic papillary renal cell tumor. In adult kidneys, MET expression was only seen in the distal tubules. In the investigated 76 papillary renal cell tumors 43 cases (56,6%) showed positive MET staining.

### 4.5. The expression of HNF1B in connection with duplication or amplification of chromosome 17 in in fetal kidney, pre-neoplastic lesions and papillary renal cell tumors

We have investigated the HNF1B transcription factor expression on the same samples. In the fetal kidney the end of the ureteric bud, the comma shaped body, the distal part of the S-shaped body, in some differentiating tubules a strong immune reaction was seen, and the cells of the proximal tubules, and the loop on Henle showed only moderate nuclear staining. Preneoplastic lesions showed strong reaction with the HNF1B antibody. In the adult kidney proximal and distal tubules showed mild nuclear HNF1B positivity. Positive nuclear reaction was seen in 57 (75%) of papillary renal cell tumors. A papilláris vese tumorok 75%-a (n=57) mutatott pozitív sejtmag festődést a HNF1B antitesttel. This positivity was the strongest in the peripheral growth zones of the tumors, and only mild reaction was seen in the central areas. The co-expression of MET and HNF1B could be pbserved in 32 (42.1%) of papillary renal cell tumors.

### 4.6. KRT17 expression associated with chromosome 17 duplication in fetal kidney, pre-neoplastic lesions and papillary renal cell tumors

KRT17 first appears in the ureteric bud and the collecting tubules of the fetal kidney. At the tip of the ureteric bud and the distal part of the S-shaped body show some expression, with the signal getting stronger throughout the medullary collecting tube in the direction of the kidney papilla. In the adult kidney, the connecting tubules show membrane-attenuated and selective (most of the cells, but no all cells) reaction. The medullary collecting tubules show similar selective staining. The KRT17 positive cells are likely principal cells in the connecting and collecting tubules. The expression of KRT17 in papillary renal cell tumors was investigated using a TMA with 151 tumor smaples. We have also studies 17 pre neoplastic lesions seen in kidneys with sporadic papillary renal cell tumors. All 17 pre neoplastic lesions were positive with KRT17. In the 151 tumor samples 116 (76.8%) showed diffuse cytoplasmic reaction, which was stronger near the cell membrane. The KRT17 showed strong condensation of KRT17 on the apical surface of the cells. KRT17 positivity was observed in the samples irrespectively of the cellular morphology (small and large cells, also calssified as type 1 and type 2) of papillary renal cell tumors.

### 4.7. Alterations of the X and Y chromosomes in papillary renal cell tumors

#### 4.7.1. Loss of chromosome Y in hereditary papillary renal cell tumors

The loss of chromosome Y was showed in earlier studies with karyotyping, Southern blot and BAC-array experiments (Kovacs et al., 1994; Szponar et al., 2011). The patient with hereditary papillary tumor discussed above had loss of chromosome Y in all samples, along with typical chromosome alterations. All 9 tumors had duplication of chromosomes 7 and 17. One tumor had chromosome 10, two others has chromosome 12 duplication.

#### 4.7.2. Expression of the PCDH11XY gene in fetal kidney, pre-neoplastic lesions and papillary renal cell tumors

In the Yp11.2 region two genes can be found, the PCDH11Y and homeobox gene TGIF2LY. From these genes PCDH11Y might have a connection with carcinogenesis.

PCDH11Y shows a 13bp deletion compared to the homologus PCDH11X found on chromosome X, which causes an altered signalization. This 13 bp difference could be used to find X and Y specific PCDH11 sequences with PCR studies. Both PCDH11X and PCDH11Y genes have multiple isoforms. Both the loss of chromosome Y and the loss of the expression of the PCDH11X isoform 4 could be found in papillary renal cell tumors. This isoform was found in large quantities in healthy kidney tissue along with placenta, fetal brain and testicular tissues. Isoforms 1, 2, and 3 can be found in most papillary renal cell tumors.

With immunohistochemistry we have found strong PCDH11XY stainging in fetal kidney cortex. The protein is expressed on the ureteric bud, but the differenctiated collecting tubules are negative for PCDH11XY. The cap metanephric mesenchyme shows strong positive cytoplasmic reaction with PCDH11XY, that later on after the mesenchy-epithelial transformation mostly on the surface of the cells is seen. In the S-shaped body, especially the distal compaertment PCDH11XY is mostly found on the surface of cells. In the adult kidney PCDH11XY is seen the loop of Henle, the distal tubules nd the macula densa. All pre neoplastic lesions and papillary renal cell tumors is our study did not stain using PCDH11XY antibody.

# 5. Discussion

## 5.1. The origin of papillary renal cell carcinoma

A recommendation was made 25 years ago for the development of papillary renal cell tumors from embryonal rests (Kovacs and Kovacs, 1993; Kovacs 1993). It was reported that in case of hereditary papillary renal cell carcinoma thousands of microscopic precursor lesions are present in the kidney (Ornstein et al., 2000). We have shown that in the case of sporadic papillary renal cell tumors an average of 42 pre neoplastic lesions per kidney are present (Bányai, Sarlós et al., 2018). The significance of this finding is raised by the fact that kidneys containing conventional RCC only 0.4 simila precursor lesions can be found (Bányai, Sarlós et al., 2018). The morphology of the pre neoplastic lesions can be similar to the nephrogenic rests (NR) associated with Wilms tumor (WT). The distribution of the lesion is similar to perilobular NR. In kidneys with papillary renal cell tumors perilobular nephrogenic rests are the dominant findings. In connection with this, it has to be noted, that according to histological and genetical

findings, the morphology of perilobular and interlobular NR determines the morphology of the developing WT (Beckwith et al., 1990; Fukuzawa et al., 2008).

Our results show evidence, that there is a connection between the embryonal developmental disorder and the development of papillary renal cell tumor. According to previous results, the trisomy of chormosomes 7 and 17 are found in the 1-2mm large pre neoplastic lesions (Kovacs, 1989). The papillary renal cell tumors developing predominantly in males show the loss of chromosome Y in addition to these changes (Kovacs et al., 1994). These data suggest, that the genes found on these chromosomes and the loss of chromosome Y contributes to the development of papillary renal cell tumors in the early phase of carcinogenesis.

The results of the detailed histological examinations and the high number of pre neoplastic lesions predict the association of these two. The cytological similarity is also an important finding. It has been a significant finding, that the pre neoplastic lesions have shown similar immune reactivity to the papillary tumors. The MET, HNF1B and KRT17 immunohistochemistry was positive in both pre neoplastic lesions and papillary renal cell tumors.

Cortical adenomas described in older scientific reports can be the macroscopically identifiable form of pre neoplastic lesions. Keyes (1890) has reported more than a hundred years ago on the frequency of these lesions

- “we do not regard it as extremely rare, since, owing the small size and inoffensive character of the growths, they are liable to be passed over without notice”. Cristol et al. (1946) have described 37 adenomas with papillary growth pattern in connection with the detailed examination of 22 tumorous kidneys. Apitz (1944) has reported 725 macrosopically detectable adenomas from around 4000 autopsies. Since the papillary renal cell tumors are more frequent in males, and the ratio of males and females was 10:1 and 4.2:1 in Cristol’s and Apitz’s studies, we can conclude, that in both series the so called adenomas might have been associated with papillary renal cell tumors.

These data strengthen the connection between pre neoplastic lesions and papillary renal cell tumors, the theory, that papillary tumors develop from embryonal precursor lesions. Despite these findings, the literature does not recognize this association, the origin of papillary renal cell carcinoma is still the differentiated tubular epithelium (Moch et al., 2016).

### 5.2. Duplication/amplification of chromosome 7 and the expression of MET in the development of papillary renal cell tumors

The sequencing of the MET gene lead to the discovery of the connection of germ line mutation and hereditary papillary renal cell tumors (Bentz et al. 1996; Schmidt et al., 1997). In both hereditary and sporadic papillary renal cell tumors a basic genetical alteration is the amplification and increased expression of the MET gene. Germline mutation of MET causes the development of thousands of pre neoplastic lesions in the kidney and the development of papillary RRC in early adulthood (Ornstein et al., 2000, Schmidt et al., 1997, Fischer et al. 1998). The patient with germline MET mutation involved in our study had multiple bilateral papillary renal cell tumors at the age of 37. Apart from the clinically significant tumors, many precursor lesions were found. These data suggest, that the amplification and increased expression of the MET gene has an important role in embryonal differentiational disturbance and the formation a pre neoplastic lesions. Similarly to the papillary tumors, the irregularity of the MET gene was also associatead with the development of Wilms' tumor. The nephrogenic rests 22%, in Wilms tumor 54% showed the increased expression of MET (Vuononvirta et al., 2009). The ligand of MET is HGF. Its gene is also located on chromosome 7. The duplication of chromosome 7 gives one more copy of the HGF gene, however no further amplification of this gene was found so far. The MET gene is frequently amplified in addition. In case of high cell surface desity of MET, an autophosphorilation can also occur, leading to activation in the absence of HGF (Fischer et al., 1998; Zhuang et al., 1998; Glukhova et al., 2000). MET positivity was found in tumors and pre neoplastic lesions with immunohistochemistry. A vast expression was noted on the peripheral part of the tumor.

### 5.3. The duplication of chromosome 17 and the role of the HNF1B (TCF2) transcription factor in the development of papillary renal cell tumors

The HNF1B gene is located on the long arm of chromosome 17 (17q12) in a 58817 bp DNA segment. The amplification of this DNA segment in papillary renal tumors lead to the discovery of this gene (Szponar et al., 2011). HNF1B plays a role in the branching of the ureteric bud and the initiation of the development of nephrons (Massa et al.,

2013). HNF1B is only expressed on polarized epithelial calls in adulthood (Fisher and Pontoglio, 2008). The kidney is a tubular system, the nephron develops from the mesodermal blathema and the polarization of the tubular cells appears secondary to the effect of the ureteric bud. Inactivation or expression of the dominant negative form of HNF1B leads to cystic malformations of renal tubules (Gresh et al., 2004; Hiesberger et al., 2004). The loss of HNF1B function can lead to multiple congenital malformations of the urogenital tract (Nakayama et al., 2010). Our studies show, that HNF1B first appears during the epithelialization in the renal vesicle and the distal compartment of the S-shaped body. The The distal tubules show strong HNF1B expression, whereas the ureteric bud and the proximal tubules show only mild positivity with the HNF1B antibody. Pre neoplastic lesions are formed from embryonal cells, that have already gone through the mesenchyme-epithelia transformation. Our studies showed HNF1B positivity in pre neoplastic lesions.

### 5.4. The duplication of chromosome 17 and the expression of KRT17 in pre neoplastic lesions and papillary renal cell tumors

Keratins are intermediate filaments that play key role in cellular intergrity and stability. The expression of KRT17 has been previously shown in gastric adenocarcinoma, ovarian carcinoma, breast cancer, papillary thyroid carcinoma, but in normal circumstances it is not expressed in the tissues of these organs. In this case, a neo-expression of KRT17 is present. The appearance of KRT17 correlates with progression and metastasis of tumors (van de Rijn et al., 1996; Ide et al., 2012; Escobar-Hoyos et al., 2014; Mockler et al., 2011; Wang et al., 2011; Kim et al., 2015; Hu et al., 2018). We have found that KRT17 is present in the uretric bud, the collecting tubes of the fetal kidney and the connecting tube of the adult kidney. The presence of KRT17 was described for the first time in simple layeres epithelium in our study. In this study 77% of papillary renal cell tumors showed KRT17 positivity, irrespective of cell morphology. Taken into consideration, that the majority of papillary renal cell tumors express KR17 and only 6% metastasise in 5 years of follow up, the role of KRT17 in the progression is these tumors has low probability. Finding the expression of KRT17, the marker of basal epithelial and myoepithelial merker in a simple layered epithelium is itself a great discovery. Furthermore, the continuous expression of KRT17 in the

developing kidney, the pre neoplastic lesions and papillary renal cell tumors drives us to a conclusion, that it might play a role in carcinogenesis

### 5.5. The absence of chromosome Y and the expression of PCDH11XY in pre neoplastic lesions and papillary renal cell tumors

The PCDH11Y, and the PCDH11X, located in the Xq21.3 region are members of the family of the protocadherin genes, which play a role intercellular communication and was previously found be important in the development and functioning of the brain (Hirano et al., 1999; Blanco et al., 2000). Our immunohistological study concludes, that it also has a function in the early phase of kidney development. It appears first in condensated blasthemal cells and during the mesenchyme-epithelium transformation its expression is more intense. We can draw a conclusion that it is important in the epithelialization of blasthemal cells thus the development of the distal tubular system. The absence of PCDH11Y can contribute to the developmental disorder of the tubular epithelium, leading to the formation of pre-neoplastic lesions.

### 5.6. Model for the carcinogenesis of papillary renal cell tumors

Based on previous results and the presented studies we recommend a different sequence of on the development of papillary renal cell carcinoma. The first step is the developmental disorder (Kovacs, 1993; Bányai, Sarlós et al., 2018). This leads to formation of pre neoplastic lesions, which persist in the kidneys and later on progress into adenoma and finally carcinoma. From the perspective of molecular pathology. the currently accepted classification, where only the size differentiates between adenoma and carcinoma is incorrect (Moch et al., 2016). The precise characterization of papillary renal cell tumors can only be based on the appearance of specific genetical alterations, such as the trisomy of chromosomes 7 and 17, the loss of chromosome Y, and further alterations, such as amplification of chormosomes 8, 12, 16, 20.

# 6. Conclusion

Accordind to the WHO ans ISUP, the origin of papillary renal cell tumors is the differentiated tubular cells, and the size of the tumor is used for differentiating between adenoma and carcinoma:

*"Papillary adenomas are uncapsulated tumours with papillary or tubular architecture of low grade (ISUP) and a diameter <15 mm" (WHO, 2016).*

*"Papillary renal cell carcinoma is a malignant tumour derived from renal tubular epithelium. It has papillary or tubulopapillary architecture". (WHO, 2016)*

The aim of the study was to determine the origin of papillary renal cell tumors. Taken into consideration the specific genetical changes, the continuous expression or the absence of different gene products such as MET, HNF1B, KRT17, PCDH11XY, we find the theory of papillary renal cell carcinoma development based on developmental disorder proven.

In summary, we state, that differentiational disorder - pre neoplastic lesion – adenoma – carcinoma is the correct sequence for the development of papillary renal cell tumors.

## 8. Acknowledgement

I would like to thank my mentors, Prof. Gyula Kovács for the opportunity to join the research team of molecular genetics for helpfully leading my research activity, and Árpád Szántó for his great support in my work. I would like to express gratitude to the Molecular Oncology Laboratory of the Ruprecht-Karls University of Heidelberg for performing important expreiments. I thank many colleagues working at the Department of Pathology of the University of Pécs, Medical School for their help (Tamás Tornóczki, Endre Kálmán, Dávid Semjén, Anita Pap, András Fincsur, Csaba Gyömörei, Zsuzsanna Halas, Judit Szilágyi Imréné). I thank my previous mentors, Prof. László Czirják, Prof. Péter Balogh, and Attila Vástyán. I thank all my colleagues at the Department of Urology, University of Pécs, Medical School for helping me every day in scientific and clinical work.

I thank my family for their great support.