Immunohistochemical expression of podoplanin in renal cell carcinomas

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IMMUNOHISTOCHEMICAL EXPRESSION OF PODOPLANIN IN RENAL CELL CARCINOMAS

Diploma thesis

Academic year:
2023/2024

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Split, June 2024

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Acknowledgement

Firstly, I want to thank my amazing mentor Assist. Prof. Sandra Zekić-Tomaš, MD, PhD, for her support and guidance throughout the process of writing my thesis.

Furthermore, I am extremely greatful for my parents and my brother, who enabled and supported me my entire life, especially the last six years, always believing in my goals.

I want to thank my friends. Without you, this journey would have only been half as fun, exciting and enjoyable.

Finally, many thanks to my girlfriend, for her patience, encouragement and trust. You were always there to help no matter what.

In memory of my dear grandmother.

LIST OF ABBREVIATIONS

CCRCC	Clear cell renal cell carcinoma
ChRCC	Chromophobe renal cell carcinoma
CT	Computerized tomography
EMT	Epithelial-to-mesenchymal-transition
JGA	Juxtaglomerular apparatus
MRI	Magnetic resonance imaging
PDPN	Podoplanin
PRCC	Papillary renal cell carcinoma
RCC	Renal cell carcinoma
WHO	World health organization

1. INTRODUCTION

1.1. Renal embryology

The development of the urinary tract in the fetus starts around the fourth week after conception. Embryonic folding of the mesoderm gives rise to the urogenital ridge. It can be divided into the nephrogenic cord, which is the starting point for the urogenital system, and the gonadal ridge, which will form the reproductive organs. Over the span of approximately three to four weeks, three kidney-like structures emerge, called the pronephros, mesonephros and metanephros, which are separated not only in time, but also in space, since they develop cranially to caudally (1).

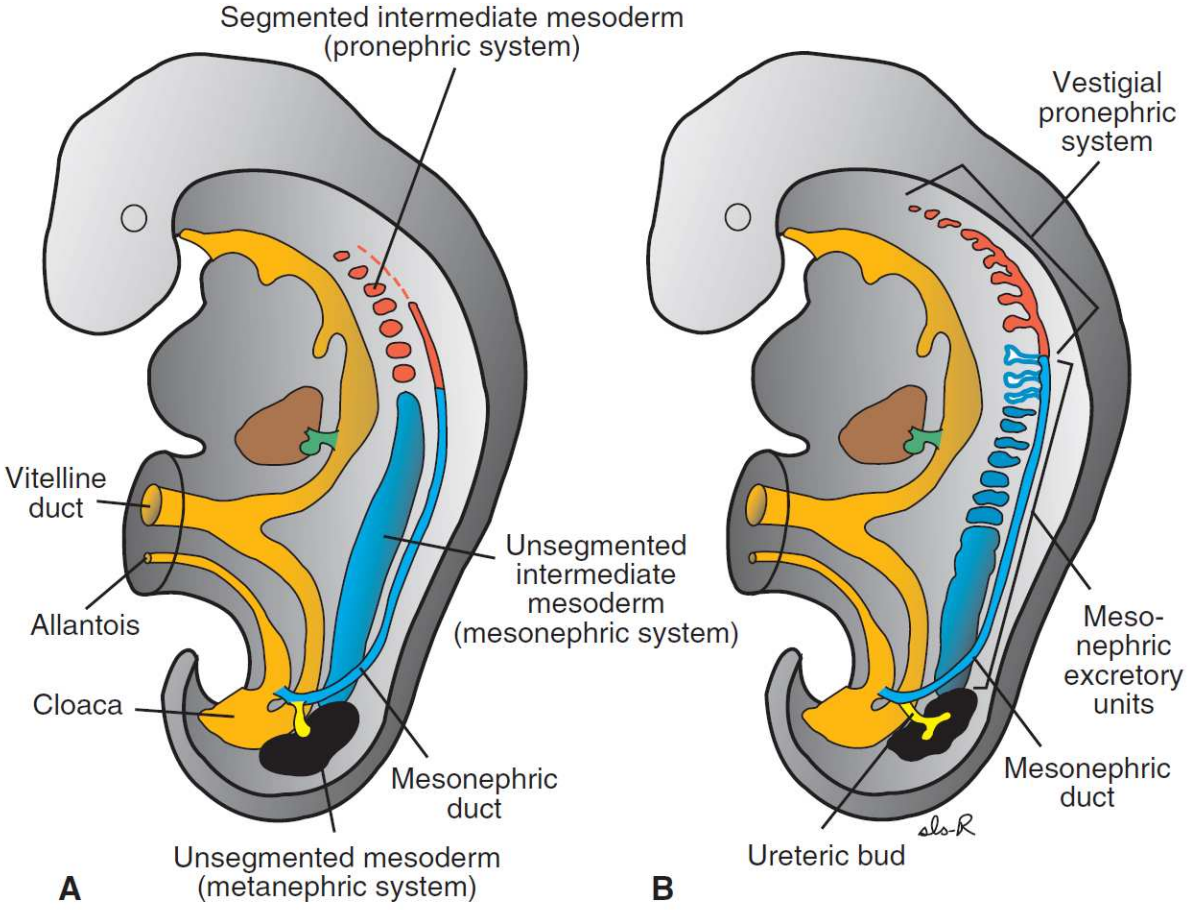

Figure 1. Embryonic relationship of pronephros, mesonephros and metanephros. Source: Sadler TW, Langman J. Langman's medical embryology. 12th ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2012. p. 232-42.

The pronephric kidney, which develops at the end of the third week of gestation, is only an intermediate form of the actual organ and serves little purpose. A connection with pronephric ducts forms to the cloaca of the embryo, but regress later on (2,3).

In the fourth to the sixth week of development, the next part of the nephrogenic cord starts forming, the so called mesonephros. Basic excretory functions are initiated in the so-called mesonephric tubules, consisting of 40 paired structures. They connect to capillaries and perform filtration. This filtrate drains into the mesonephric duct, also known as the Wolffian duct. Similar to the pronephric kidney, the mesonephric tubules will degenerate from rostrally to caudally once the development proceeds further to the actual functioning kidney (3,4).

Beginning in the fifth week of development, the metanephros starts to form, eventually developing into the actual kidney. Following the fusion of the mesonephric duct with the cloaca, the ureteric bud emerges from the lower portion of the duct, which will subsequentially differentiate into the kidney's collecting system. In the course of the next six to seven weeks, this bud bifurcates, forming the ureter, renal pelvis, calyces, and collecting. Hormonal secretions from the ureteric bud induce the mesoderm to differentiate into the metanephric blastema. Each tip of the ureteric buds attains metanephric blastemal cap. Further differentiation of these caps leads to the formation of a renal vesicles, which transform into an S-shape and associate with capillaries to form glomeruli. The elongation of the S-shaped structures forms the remaining parts of the renal tubules, thus completing a functioning nephron. This entire process occurs simultaneously, generating millions of nephrons that will constitute the adult kidney (5).

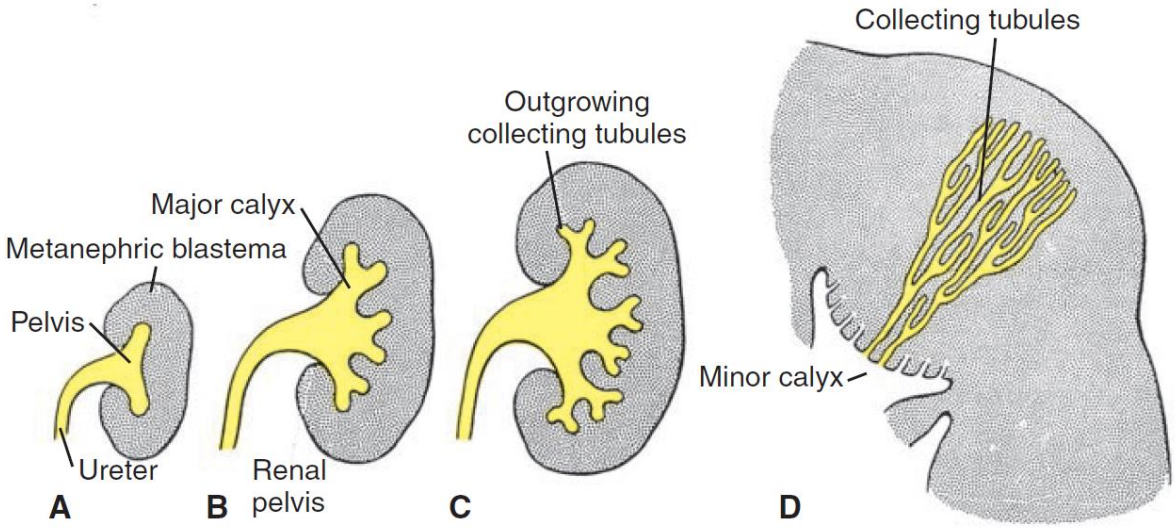

Figure 2. Development of renal collecting system. Source: Sadler TW, Langman J. Langman's medical embryology. 12th ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2012. p. 232-42.

The early kidneys initially form in more sacral regions, however, due to abdominal and pelvic growth, they migrate cranially and more laterally during the sixth to ninth weeks of development. In their final positions adjacent to the aorta and beneath the adrenal glands, the vascular supply is established through direct connections to the dorsal parts of the aorta (2,3).

1.2. Renal anatomy

The kidney is a bean shaped, paired organ located in the retroperitoneal space of the abdomen, on either side of the spine. The upper renal pole is bordered by the adrenal glands. The right kidney is positioned posterior to the right colic flexure, adjacent to the descending duodenum, and abuts the jejunum at its inferior pole. On the left side, portions of the kidney are situated beneath the greater curvature of the stomach and pancreas, laterally associate with the spleen, and in contact with the descending colon and jejunum. The kidneys span the levels of T12 to L3, with the left kidney situated higher than the right due to the liver's downward pressure on the right kidney. Each kidney weighs between 115 to 175 grams depending on the sex and age of the person, and measures 10 – 12 cm in length, 5 – 6 cm in width, and 3 – 4 cm in thickness (6-8). Vascular supply of the kidneys is ensured by the renal artery and renal vein, which branch from the abdominal aorta, and drain into the inferior vena cava, respectively. The renal plexus provides nervous supply to the renal structures, with sympathetic fibers originating from the splanchnic nerves and parasympathetic fibers from the vagus nerve (6).

Internally, the kidney consists of two main structures: the outer renal cortex and the inner renal medulla, both surrounded by the renal capsule. The cortex houses the filtering units of the kidney, the nephrons, which are responsible for various functions, including maintaining water and electrolyte balance, excreting waste products, preserving acid-base balance, and regulating blood pressure control. The primary function of renal medulla is to collect the urine produced inside the renal cortex and channel it towards the renal pelvis and ureter. The distal parts of the nephrons drain into the renal pyramids, whose apical regions, called the renal papillae, then open into the minor calyces. Collections of minor calyces converge to form major calyces. Ordinarily, up to three major calyces unite to create the renal pelvis. This structure marks the beginning of the ureter, which exits the kidney at the hilum (6,9).

1.3. Renal histology

Microscopically, the kidney is a highly complex organ, which is unsurprising, considering its important role in maintaining physiological balance for an overall healthy environment. As previously discussed, the renal cortex houses the primary functional units of the kidney, known as nephrons. Each kidney contains over one million nephrons, and each of them consisting of a renal corpuscle with a glomerulus and glomerular capsule, along with a renal tubule system comprising the proximal tubule, loop of Henle, distal tubule and collecting ducts (10).

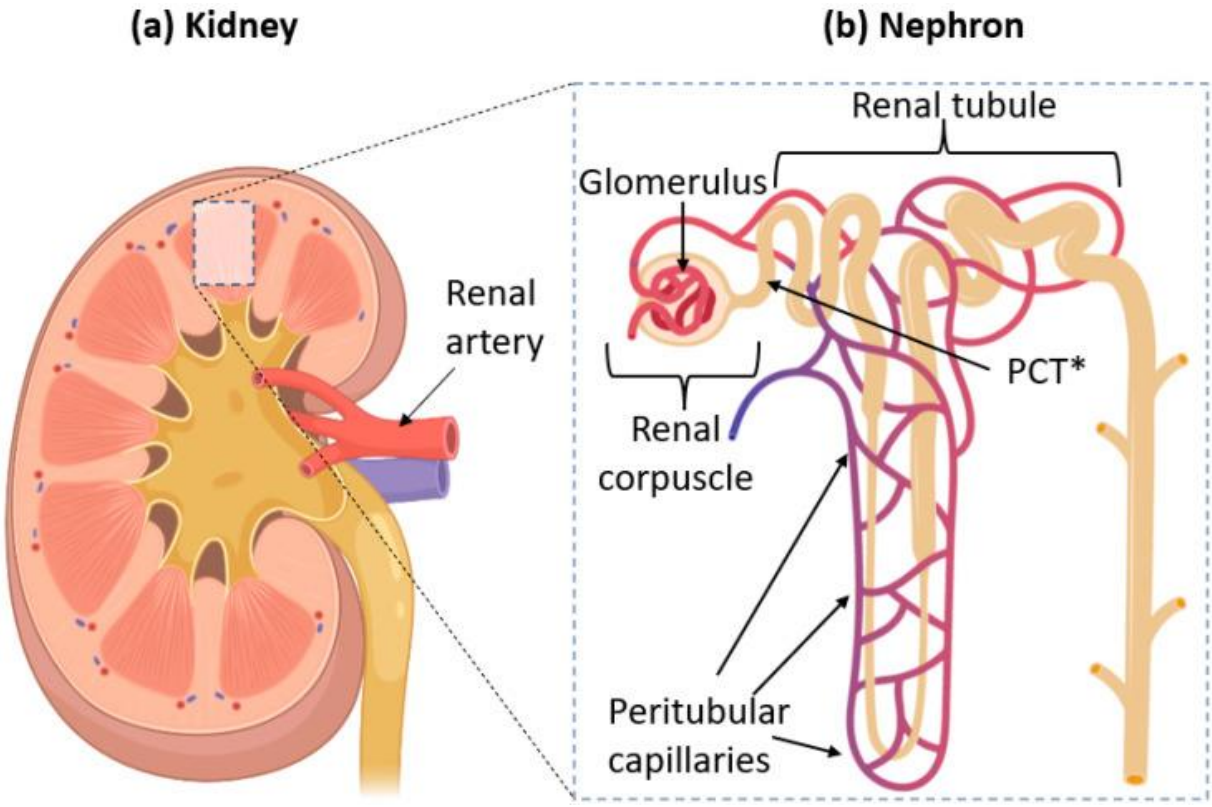

Figure 3. Anatomy of the kidney and structure of a single nephron. PCT: proximal convoluted tubule. Source: Adhipandito CF, Cheung SH, Lin YH, Wu SH. Atypical Renal Clearance of Nanoparticles Larger Than the Kidney Filtration Threshold. Int J Mol Sci. 2021;22(20).

The glomerulus, a network of small capillaries, is situated centrally within the renal corpuscle. These capillary's specific shape allows circulation and filtration of blood for more effective filtration, due to maximization of surface area within them, enhancing the potential for diffusion of various ions and molecules. Filtration processes are further regulated by the capillary endothelium, which features a porous surface and a negative charge, controlling the passage of particles. Furthermore, a layer of specialized cells called podocytes, covering the capillary walls act as another filtration barrier for larger molecules. Podocytes allow only for filtration of positively charged ions and particles smaller than 100 nm. Blood flow into and out of the glomerulus is managed by special resistance arterioles, which adjust blood flow and maintain intra-arteriolar pressure in response to factors such as blood pressure, hormonal effects, and direct renal control (11,12).

Mesangial cells occupy the space beneath the basement membrane of glomerular capillaries, where they secrete extracellular matrix, creating the so-called mesangium. This

matrix fills the intercapillary spaces not covered by podocytes, providing structural support to the capillaries. Besides their supportive role, mesangial cells contract in response to fluctuations in blood pressure and engage in phagocytosis of protein aggregates (12,13).

The glomerulus is encapsulated within the Bowman's capsule, which is comprised of two layers. The inner layer, known as the visceral layer, covers the glomerular capillaries, and is composed of specialized stellate epithelial cells previously mentioned as podocytes. The outer layer, termed the parietal layer, is made up of simple squamous epithelium. Between these layers lies the Bowman's space, where the filtered urine collects, before entering the renal tubule system. Here, the epithelial cells transition into cuboidal epithelium in the proximal tubule (14).

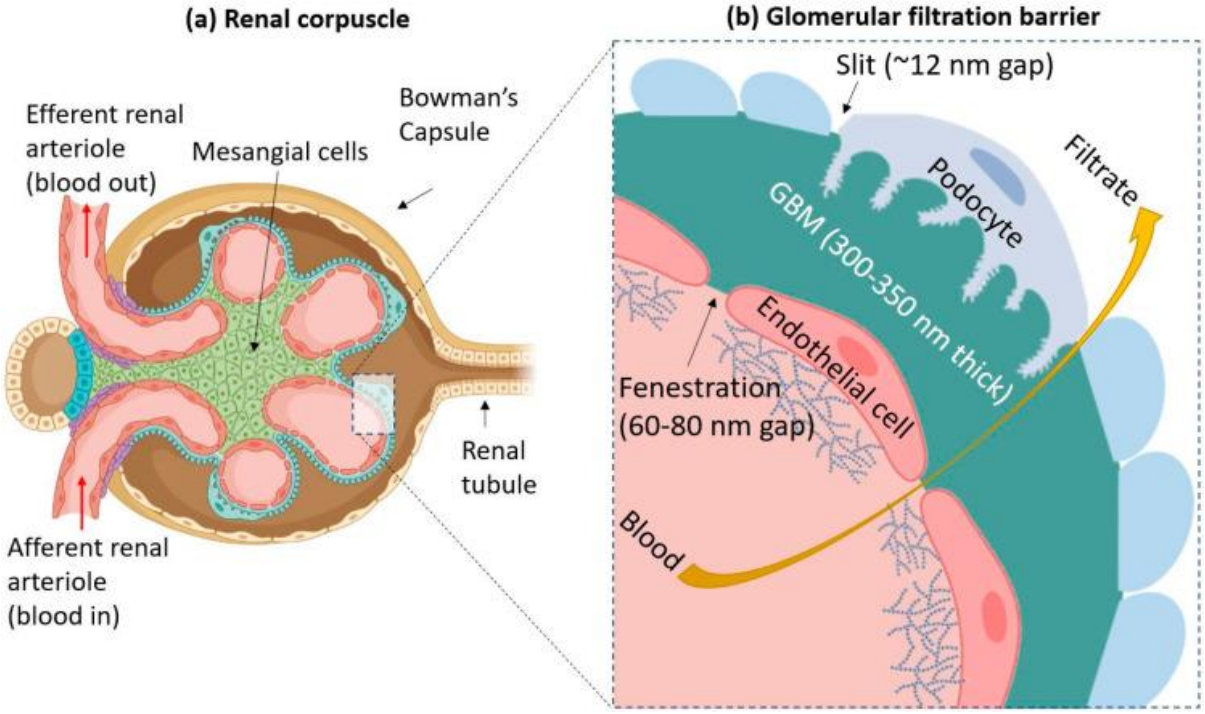

Figure 4. Cells in the renal corpuscle contributing to the filtration barrier. GBM: glomerular basement membrane. Source: Adhipandito CF, Cheung SH, Lin YH, Wu SH. Atypical Renal Clearance of Nanoparticles Larger Than the Kidney Filtration Threshold. Int J Mol Sci. 2021;22(20).

The renal tubular system comprises specialized segments, each with distinct roles and functions in mineral and nutrient exchange. While the glomerulus filters substances from the

blood, the tubular system performs secretion and reabsorption of substances to and from the filtered urine through processes involving the epithelium and adjacent capillaries. The renal tubular system is divided into four main sections: the proximal convoluted tubule, the loop of Henle, with its thin descending and thick ascending limbs, the distal convoluted tubule with the juxtaglomerular apparatus and the collecting duct (13).

The proximal tubule is lined by simple cuboidal epithelium, featuring a prominent brush border of microvilli on its luminal side. This structural adaptation significantly amplifies its surface area, allowing for more effective nutrient exchange. Here, more than half of the filtered water, electrolytes and organic matter undergo reabsorption. Additionally the proximal tubule facilitates the secretion of waste products such as bile salts and creatinine, as well as clearance of antibiotics and other substances. An abundance of mitochondria is essential for sustaining the energy demands of the high transporters density inside the proximal tubular cells (11,13).

Next in the nephron's structure is the loop of Henle, a U-shaped loop extending into the renal medulla. It consists of an ascending and a descending limb, each lined with distinct types of epithelia. The thin descending and thin ascending limbs are composed of simple squamous epithelium. In the descending limb, predominantly passive reabsorption of water and electrolytes occurs, while the ascending limb exclusively reabsorbs sodium chloride. Moving towards the distal end of the loop of Henle, the thick ascending limb characterized by simple cuboidal epithelium follows. This segment is rich in mitochondria, facilitating active transport of sodium, potassium and chloride ions. This active transport is crucial for generating a hypertonic medullary environment, essential for water reabsorption from the collecting ducts (13,14).

Continuing to the next segment, the distal convoluted tubule. This section lined with cuboidal cells similar to the proximal tubule, but it lacks a brush boarder, resulting in reduced surface area and lower absorptive capacity. This segment is flatter and contains fewer mitochondria compared to its proximal counterpart. Adjacent to the vascular pole of the renal corpuscle lies the juxtaglomerular apparatus (JGA) connecting to the distal convoluted tubule. The macula densa, part of the JGA, is characterized by apical nuclei and densely packed columnar cells. This structure influences glomerular blood flow through a specialized feedback mechanism, reacting to the salt concentration inside the distal convoluted tubule. Juxtaglomerular granular cells, modified smooth muscle cells found inside the walls of the efferent arterioles, are also part of the JGA. They secrete hormones in response to changes in

blood pressure. Extraglomerular mesangial cells, known as lacis cells, complete the JGA. Similar to intraglomerular cells, lacis cells provide support, contractile and defensive functions. Overall, the JGA plays a key role in the autoregulation of glomerular filtration rate, thereby influencing urine output and contributing to blood pressure control (12-14).

Lastly, the collecting ducts are responsible for reabsorption of additional water and transport of final urine into the minor calyces. Multiple connecting tubules converge into collecting ducts, which are lined with simple cuboidal epithelium in the cortex. As these ducts descend into the medulla, they increase in diameter, and the cuboidal cells gradually transition to columnar cells. At the apex of the renal pyramids, numerous collecting ducts merge, forming a single papillary duct, which delivers urine into the minor calyx. Inside the membranes of the pale-staining principal cells in the collecting tubules and ducts, specialized water channels called aquaporins facilitate water reabsorption. Intercalated cells, staining more darkly, are interspersed among the principal cells, which possess higher amounts of mitochondria, enabling active transport of acids or bases for the regulation of the body's pH (11,13,14).

1.4. Definition and main types of renal cell carcinoma

The kidney is a paired organ situated in the retroperitoneum. Beyond its main functions comprising waste removal and fluid regulation, the kidney is essential for maintaining acid-base balance and proper concentrations of sodium, potassium, and other vital minerals, ensuring body homeostasis. Additionally, renal function is crucial for hematopoiesis, as the kidneys secrete endogenous erythropoietin (EPO), which stimulates red blood cell production in the bone marrow.

Unfortunately, these processes do not always work as intended, particularly in patients with underlying illnesses. One of the most severe diseases to affect renal function is renal cell carcinoma (RCC). This malignant tumor arises when normal functioning cells begin to proliferate uncontrollably due mutations leading to the loss of cell cycle inhibitors and forming a mass that grows in disregard for other structures and tissues.

RCC constitute a diverse group of malignant tumors originating from the renal tubular epithelium. Accounting for over 90% of all renal cancers, RCC is the most prevalent malignancy in the kidney (15). Worldwide, they rank among the 15 most common cancer types, and for males, it is among the top 10 most common cancers (16,17). RCC encompasses over

ten different histological types which separate themselves by their unique cell structures and differentiation patterns. This classification laid the foundation for a proper understanding and the possibility for predicting RCC outcomes and application of tailored treatment options.

Renal carcinomas are broadly characterized by the uncontrolled proliferation of cancerous cells originating from normal renal tubular epithelium. Since 2004, the World Health Organization (WHO) has classified RCC into several distinct subtypes (18,19). The most prevalent of these subtypes, listed from highest to lowest frequency, are clear cell renal cell carcinomas (CCRCC) accounting for 75% of cases, papillary renal cell carcinomas (PRCC), constituting 15%, chromophobe renal cell carcinomas (ChRCC), representing around 5%, and various other subtypes, each with a very low incidence of less than 1% (17,18,20).

1.5. Epidemiology of renal carcinoma

Renal carcinomas represent a significant global health burden, with their incidence steadily rising over the past decades (21). Nowadays, it is the 14th most common cancer worldwide, with over 430,000 new cases in 2020, accounting for 2.4% of all cancers. The highest overall incidence rates for RCC are found six Eastern European countries, with Lithuania leading, followed by Czechia, Estonia, Slovakia, Latvia, and Belarus. Additionally, Western European countries like France and Ireland, as well as the United States and Uruguay, rank among the top ten countries for RCC incidence.

It is important to note the gender disparity in renal carcinoma incidence. Kidney cancer ranks as the 9th most common for men and 14th most common in women, indicating a clear male predominance (22). In fact, men are twice as likely to develop renal carcinoma compared to females, and exhibit a higher mortality rate from the disease (23). Age is also a significant risk factor, with the highest prevalence observed in individuals aged 60 to 70-year-olds (15).

New diagnoses of RCC often occur incidentally during magnetic resonance imaging (MRI), computerized tomography (CT), and ultrasound imaging. Only a tenth of all patients exhibit with pathognomonic symptoms such as hematuria, flank pain, an abdominal mass, or B-symptoms, including fever, night sweats, and unintentional weight loss. (21,24).

Survival rates for RCC are influenced by several factors, including gender, age, and overall physical condition, with the stage at diagnosis being the most critical determinant. The 5-year survival rates for early stages of RCC are notably high, ranging 91% to 74% for stages

I and II, and even 67% for stage III. However, stage IV renal cell carcinoma's specific survival rate drops down to just 32% (25). Between 2009 to 2015, the 5-year relative survival rate in the US was even 76%, despite that around 30 years prior, survival rates have been 46.8% (21). Despite the importance of staging factor, a noticeable discrepancy persists between male and female mortality rates. Specifically, from 1990 to 2013, there was an 11.3% decrease in female mortality, while in contrast male mortality increased by 9.9% (23).

1.6. Etiology of renal cell carcinoma

Concordant to other major diseases, lifestyle factors comprise the primary and most important contributors to the likelihood of developing any kind of illness. These factors also impact renal cancer, playing a major role in increasing the risk of renal cell carcinomas.

The primary cause of RCC, as well as for various urogenital diseases, is tobacco smoking. Toxic substances released during the burning process of a cigarette, such as polycyclic aromatic hydrocarbons (PAHs), N-nitrosamines, aromatic amines, and nicotine - the highly addictive neurotransmitter-modulating substance - disrupt of crucial enzymatic chains and DNA formation. This disruption can lead to the production of faulty DNA products, which are pivotal in carcinogenesis (26). Nicotine, contained in cigarettes, worsens the issue by causing addiction and increasing consumption, thereby introducing more toxic substances into the body. Breaking this vicious circle requires a multifactorial approach involving a combination of education, support, and strong personal resolve to quit tobacco use. Comparatively, individuals who smoke cigarettes face a 50% higher risk of RCC in men and a 20% higher risk in women compared to those who have never smoked (27).

Terminal renal insufficiency is a condition influenced by multiple causes, each of which can have an impact on renal cell cancer development. The primary factors leading to renal insufficiency and chronic kidney disease are hypertension, diabetes mellitus and obesity. These conditions induce oxidative stress within kidney tissue due to unfavorable circulatory changes (28). Oxidative stress, caused by reactive oxygen species can lead to DNA damage, causing alterations in cellular proliferation, survival, and migration.

Elevated glomerular pressure resulting from to hypertension is a cruical factor that can impact renal cells. According to a clinical study from the United States, researchers have

observed, that Caucasians with hypertension have double the risk of RCC than without hypertension (29).

Obesity poses another significant risk factor, creating an environment favorable for cancer development within the body. Adipose cells serve as ideal energy reservoirs for a growing tumor mass. Moreover, the accumulation of excess fat tissue in these individuals leads to increased levels of pro-inflammatory mediators and chronic tissue hypoxia. This further promotes the increased secretion of angiogenic factors, amplifying damage to genomic material under hypoxic stress. These factors not only facilitate cancer development, but also support the growth of RCC once it has developed. Hormonal changes and altered secretion patterns in obese individuals, involving sex hormones and insulin, have proven to change cellular proliferation, differentiation and even cell death (30).

Diabetes mellitus, especially type 2, is implicated in elevating kidney cancer risk. While the VITAL study (31) did not find an association, other research, such as the Nurses' Health Study (32), attribute a significant correlation between RCC and type 2 diabetes mellitus (33). Additionally, hypertension and obesity are recognized as comorbidities within the broader metabolic syndrome, which encompasses contains diabetes mellitus. Therefore, these conditions are interconnected and can either predispose to or result from one another.

The last major group of factors contributing to an increased risk of developing RCC are genetic syndromes. Changes in the genome, occurring in conditions like Von-Hippel-Lindau syndrome and tuberous sclerosis, can account for up to 5% of CCRCC cases (21). Additional familial syndromes include Birt-Hogg-Dubé syndrome and hereditary type 1 papillary RCC (HPRCC), each of which increases the risks for different renal cancers to a varying extend. For instance, Von-Hippel-Lindau syndrome displays an estimated 70% risk of RCC, notably clear cell RCC. Birt-Hogg-Dubé syndrome has a risk of 25% for various types of RCC (34).

Finally, certain toxins are directly implicated in causing RCC. For example, the industrially used agent trichlorethylene, which serves as a metal degreaser, is linked not only to RCC development but is also classified as an environmental pollutant (27). Other substances including cadmium and asbestos are known carcinogens affecting the kidney (21).

1.7. Diagnosis and management

Screening for RCC is generally not recommended, unlike in some other diseases. However, there are exceptions, such as when a familiar syndrome associated with RCC is present, as mentioned earlier. Primary prevention, on the other hand, is highly effective in reducing renal cancer risk. Educating patients about maintaining a healthy diet, controlling blood pressure, and advising against smoking are measures that may decrease the risk for developing renal carcinoma in later life (35).

Diagnosis and discovery of RCC often occur incidentally in more than half of cases during imaging originally thought for other diagnostic purposes (36). Key indicators for diagnosing advanced renal cancer include gross hematuria, flank pain, pyelonephritis, and a palpable mass in the upper abdomen (35). Common symptoms of neoplasia include B-symptoms such as night sweats, unexplained weight loss, and fever. Paraneoplastic symptoms namely hypercalcemia and erythrocytosis, can develop by secretion of hormones and cytokines by the cancerous tissue. These symptoms often disappear after tumor resection (17). Bone pain and pathologic bone fractures occur in metastatic disease. One specific sign for early disease may include right-sided varicocele or edema in both lower extremities, which can result from a tumorous mass compressing the right testicular vein draining into the inferior vena cava (35).

In case of suspected RCC, both ultrasound and CT imaging are effective tools for visualizing the kidney and surrounding structures to aid in diagnosis and metastasis evaluation in advanced stages of the disease. Ultrasound is advantageous due to its rapid and accurate detection of renal masses without exposing the patient to radiation. The advantage of CT imaging lies in its better resolution and spatial imaging allowing for differentiation between malignant and benign solid renal lesions. The use of contrast agents further improves diagnostic quality in both imaging techniques (36).

Laboratory diagnostics can also indicate an underlying renal malignancy through microscopic examination of the urine, with a special mark on red blood cells. It is essential to investigate hematuria accompanied by pus and red cell casts to exclude an infection of the genitourinary system (35).

Upon diagnosis of a renal mass, further management involves staging the carcinoma using the TMN system. The most important staging parameters include size, degree of invasion into renal and surrounding tissues, and the presence of lymph node and distant metastasis (17). Management of RCC is therefore highly dependent on the clinical stage of the tumor, guiding

treatment pathways accordingly. For localized disease (stage 1) surgical resection of the tumor is possible and provides a curative approach (37). However, in advanced stages, treatment becomes more complex. For stage 1b and beyond, nephrectomy, which involves resecting part or all of the kidney, is recommended, with follow-up care including monitoring with CT, MRI, and chest X-ray for at least three years. In stages 2 and 3, nephrectomy is still the preferred treatment, but requires a tightly planned follow-up (15). In stage 4, where lymph node or distant metastasis is present, the treatment strategies are complex, beginning with surgery aimed at the primary tumor, the extend depending on a curative or palliative intend. Additionally, immunotherapeutic and targeted molecular therapies provide chances for full tumor remission or life extending palliative measures. In cases, when no curative approach is possible, palliative nephrectomy alleviates symptoms such as pain, bleeding, or paraneoplastic effects in the terminal patients (15,38).

1.8. Histological classification

Since the invention of the microscope, people have been eager to find out what key features separate tissues from one another. Each cell group exhibits unique behaviors under microscopic examination, depending on their structure, staining capabilities and contents. Despite originating from the kidney, RCC subtypes are inherently different from one another. These variations become apparent not only in their incidence rates, growth patterns and aggressiveness, but also in their histological features, portraying distinct cellular structures and origins. Three primary subtypes of RCC - CCRCC, PRCC and ChRCC – show these distinctions. The WHO 2022 classification has introduced new subdivisions since its fourth edition in 2016, broadening the total number of renal cell neoplasms to 21 (18,39).

CCRCC, the predominant type comprising 60-70% of RCC cases, typically appears unilaterally and unicentrically in the renal cortex. These tumors often exhibit a yellowish appearance due to their high lipid content. Necrotic and hemorrhagic patches, giving the tumor a brownish-grey coloration, present as streaks within the mass (40). CCRCC commonly feature a pseudocapsule. Microscopically, the tumor is characterized by nests and sheets of cells filled with clear cytoplasm and surrounded by a membrane (41). “The tumor consists of solid to trabecular (cordlike) or tubular (resembling tubules), rarely cystic pattern” (42). Numerous branches of thin blood vessels intersperse between cells with granular eosinophilic cytoplasm, especially in cases of high-grade cancer. Calcifications inside the stroma may be observed (41).

Renal cell carcinoma of the papillary type (10-15%), the second most common type, are frequently detected incidentally or post-mortem. Unlike CCRCC, PRCC can manifest as multifocal and bilateral tumors in patients (42). Macroscopically, PRCC are well-defined with a surrounding pseudocapsule. They vary in color from yellow to brown or red. The granular surface texture corresponds with the papillary morphology, often displaying necrosis and cystic degeneration similar to CCRCC (43). Since 2022, the WHO no longer differentiates two distinct types of PRCC as previously described in the 2016 classification. PRCC is categorized under papillary renal tumors, together with papillary adenoma. Due to advances in molecular sciences, the PRCC previously described as type 2 was discovered to "not constitute a single well-defined entity, but rather individual subgroups with a different molecular background" (44). Histologically, PRCC exhibits papillae with vascular cores and tubular structures filled with cells staining basophilic in their cytoplasm. Nuclei are typically small and oval with even distribution. Infiltrations of foamy histiocytes and collections of psammoma bodies and hemosiderin pigments are also observed (45). The updated classification includes additional patterns such as biphasic PRCC with squamoid alveolar cells and solid phenotype. Biphasic PRCC consist of two cell lines, with nests of squamoid eosinophilic cells surrounded by smaller amphophilic cells to form an alveolar structure. "Papillary renal neoplasms with reversed polarity, previously described as "oncocytic low-grade PRCC", and Warthin-like PRCC that mimics salivary gland Warthin tumor" (18) are added to the morphological spectrum of PRCC as well. Papillary and tubulopapillary structures covered with a layer of eosinophilic cells, accompanied by peritumoral lymphoid aggregation, characterize papillary renal neoplasms with reversed polarity. Warthin-like PRCC's is distinguished by eosinophilic cells with lymphocytic infiltrate. Many tumors that have previously been described as type 2 PRCC are now categorized as independent entities (18,39,43,46).

Lastly, ChRCC, accounting for 5% of RCC cases, originates from intercalated cells of renal collecting ducts (42). Macroscopically ChRCC is well circumscribed, lacks a capsule and appears tan to brown in color. It may present with necrotic and hemorrhagic areas, small cystic regions, a central scar. While ChRCC can be multifocal in rare cases, it more commonly presents as a single nodule. Structurally, the cancer is a solid mass composed of nests, sheets, or alveoli of pale cells (47). Microscopically, ChRCC "are made up of basic chromophobe large polygonal cells that have a transparent, slightly reticulated cytoplasm and perinuclear halos with thick-walled vasculature" (42). The nuclei appear irregular and wrinkled and often

binucleated. Additionally, ChRCC can display non-conventional morphology, with trabecular, alveolar, papillary, microcytic, or cystic patterns (45,47).

1.9. Podoplanin protein

In the research of tumor cell-induced platelet aggregation, the protein Aggrus was first described in mouse and hamster trials in 1988 (48,49). The term podoplanin (PDPN) was later introduced for this transmembrane protein, first detected on the surface of renal glomerular podocytes in mice (50). This protein is also known by different names, due to its discovery in different mammalian species, including D2-40, T1α, E11 antigen, PA2.26 antigen, gp38 and more (48,51). PDPN is a mucin-type transmembrane protein of 162 amino acids, with a molecular weight ranging from 36- to 43-kDa (52). Due to its expression in various organs and specifically on lymphatic endothelium it serves as a marker for lymph angiogenesis (53). Under normal conditions in healthy human tissues and cells, PDPN is expressed in renal podocytes, placenta, alveolar epithelial cells of the lung and neuronal cells (48,52,54,55).

1.9.1. Physiological functions

PDPN has demonstrated a crucial role in developmental processes, as PDPN deficient mice show increased embryonic lethality due to respiratory failure and lymphedema resulting from malformation of lungs and lymphatics (52,56). Heart development is heavily influenced by PDPN, since in mice devoid of this protein cardiac issues appear, including hypoplasia in the pulmonary vein, left atrial wall and atrial septum (52).

In adults, PDPN is involved in lymph angiogenesis, production of platelets, and immunological response (57). Additionally, as its name suggests, PDPN is essential for the formation of podocyte foot processes and preserving glomerular permeability (58).

1.9.2. Pathophysiological expression

PDPN can be overexpressed in various pathological conditions. Advanced atherosclerotic lesions exhibit an increase in PDPN with, correlating with a higher propensity for thrombus formation and elevated risk for cardiovascular events (48).

There is a notable association between PDPN upregulation and autoimmune diseases. In these cases, either an increase in PDPN occurs within immune cells, mainly Th17 cells, or in within the tissues affected by the autoimmune reaction. Conditions such as multiple sclerosis, rheumatoid arthritis, systemic sclerosis and others are associated with PDPN upregulation, although the precise pathogenic role of PDPN remains unclear in some instances (57).

Overexpression of PDPN has been reported in in numerous carcinomas. Squamous cell cancers of the head and neck, respiratory system, oral and esophageal regions as well as various brain tumors like glioblastomas and mesotheliomas are affected (59). Additionally, bladder cancer, osteosarcoma, ovarian cancer, testicular tumors, and breast cancer exhibit PDPN overexpression (51,57,58,60,61). However, increased PDPN expression does not always correlate with a worse prognosis. In some cancers like squamous cell carcinomas PDPN expression is associated with a better outcome (62).

PDPN facilitates tumor metastasis through multiple mechanisms (63). By binding to blood platelets, PDPN is able to mask the cancer from the immune system, rendering it virtually invisible to immune cells during hematogenous metastasis (51). Cancer cells initiate platelet aggregation via PDPN binding to specific CLEC-2 receptors, resulting in tumor embolization and immune evasion (48,64).

Furthermore, PDPN is implicated to influence tumor progression by facilitating cancer invasion and metastasis (65). Invasion can occur through two primary mechanisms. PDPN expression at the leading invasive edge of cancers can downregulate of E-cadherin, causing Epithelial-To-Mesenchymal-Transition (EMT) (57,66,67). This leads to loss of the cell tight junctions and subsequently invasion of healthy tissues (68).

Alternatively, invasion may occur even in absence of EMT. PDPN influences actin structure remodeling and the induction of filopodial extensions, therefore enhancing cell motility and migration (57,63,69). PDPN binding to ERM proteins leads to the downregulation of RhoA-GTPase, resulting in filopodia formation (63,70,71).

2. AIMS AND HYPOTHESIS

The objective of our research was to assess the immunohistochemical expression of PDPN/D2-40 in the three most common types of renal cell carcinoma, CCRCC, PRCC and ChRCC. Additionally, we wanted to determine if there is a correlation between cancer aggressivity and metastatic potential and an increase of PDPN expression in the aforementioned tumors.

We propose that immunohistochemical expression of PDPN will be increased in CCRCC compared to non-clear cell RCC types of renal cell carcinoma.

There will be a higher number of PDPN positive lymphatics in the clear cell RCC compared to the non-clear cell RCC group.

We hypothesize a positive correlation of PDPN expression in tumor cells and presence of tumor necrosis and tumor size.

There will be a positive correlation between number of positive lymphatics in the tumor surrounding tissue and tumor size.

3. MATERIALS AND METHODS

3.1. Samples

Presented study included 15 RCC samples from patients operated at Urology Department of University Hospital Split and diagnosed at the Clinical Institute for Pathology, Forensic Medicine and Cytology at the same hospital, in the period from 1st of January 2018 till 31st of December 2018. The inclusion criteria was RCC diagnosis on operative material during the above listed period. In the case of insufficient patient's data or tumor material, or if the diagnosis was made on biopsy specimen, the samples were excluded from the study.

The patient's data, including patient's gender and age at the time of diagnosis, were collected from the database of Clinical Institute for Pathology, Forensic Medicine and Cytology. From the pathology report operation procedure, histological tumor type, size of the tumor, as well as presence of necrosis and lymphovascular invasion was noted.

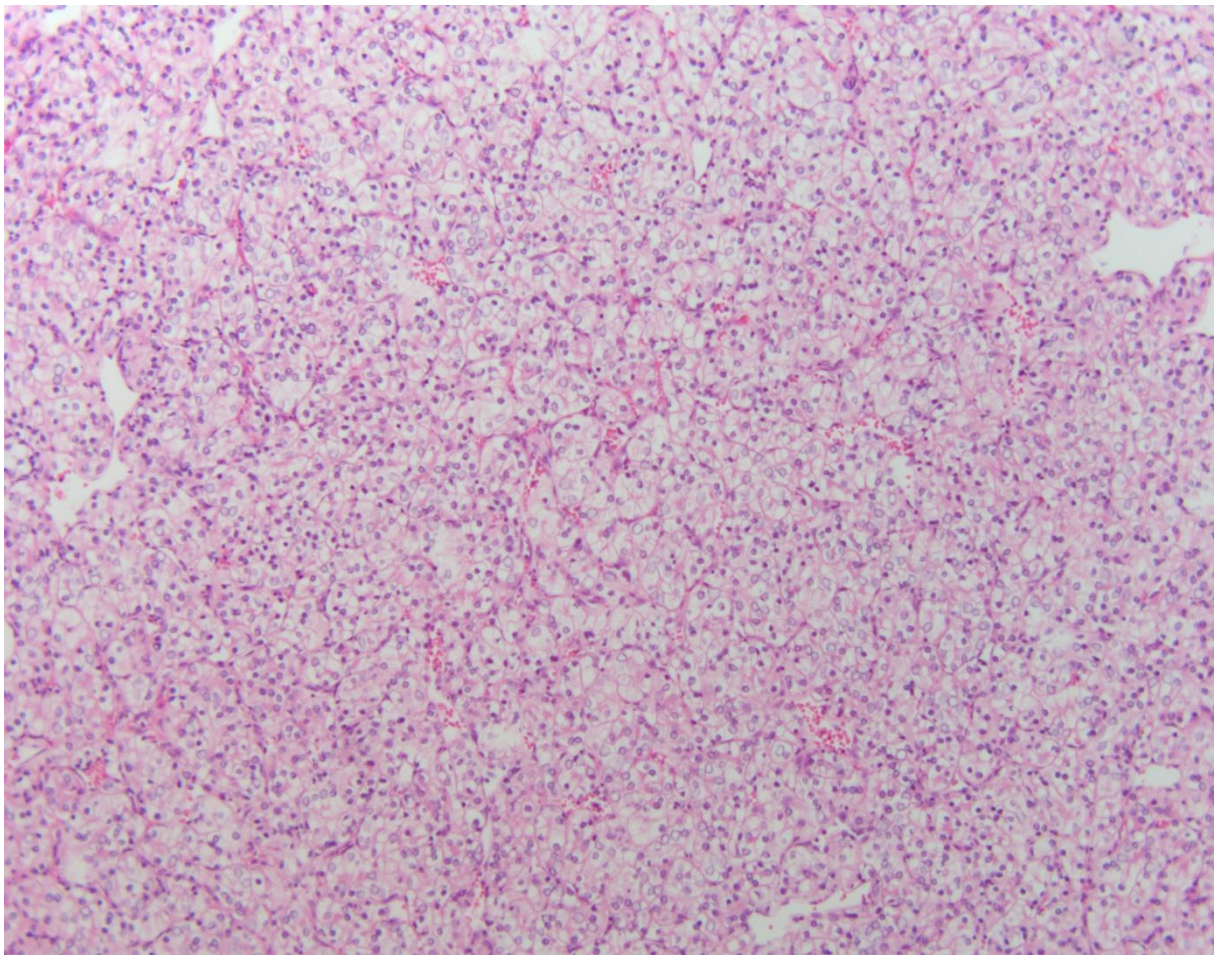

Figure 5. CCRCC: Image shows histology of clear cell renal cell carcinoma (H&E staining, magnification 100X, Olympus Image Analayzer). Source: Mentor's archive.

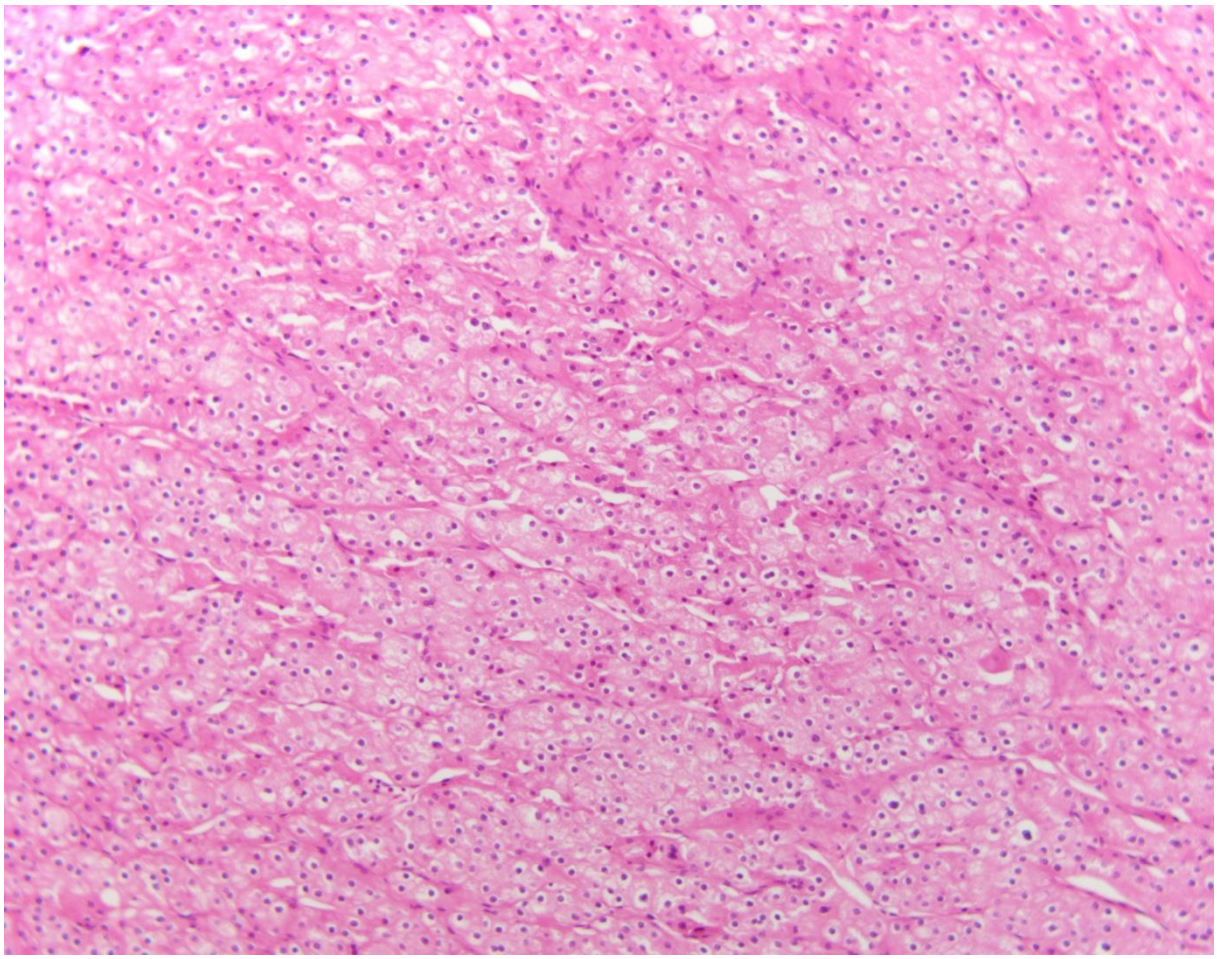

Figure 6. ChRCC: Image shows histology of chromophobe renal cell carcinoma (H&E staining, magnification 100X, Olympus Image Analayzer). Source: Mentor's archive.

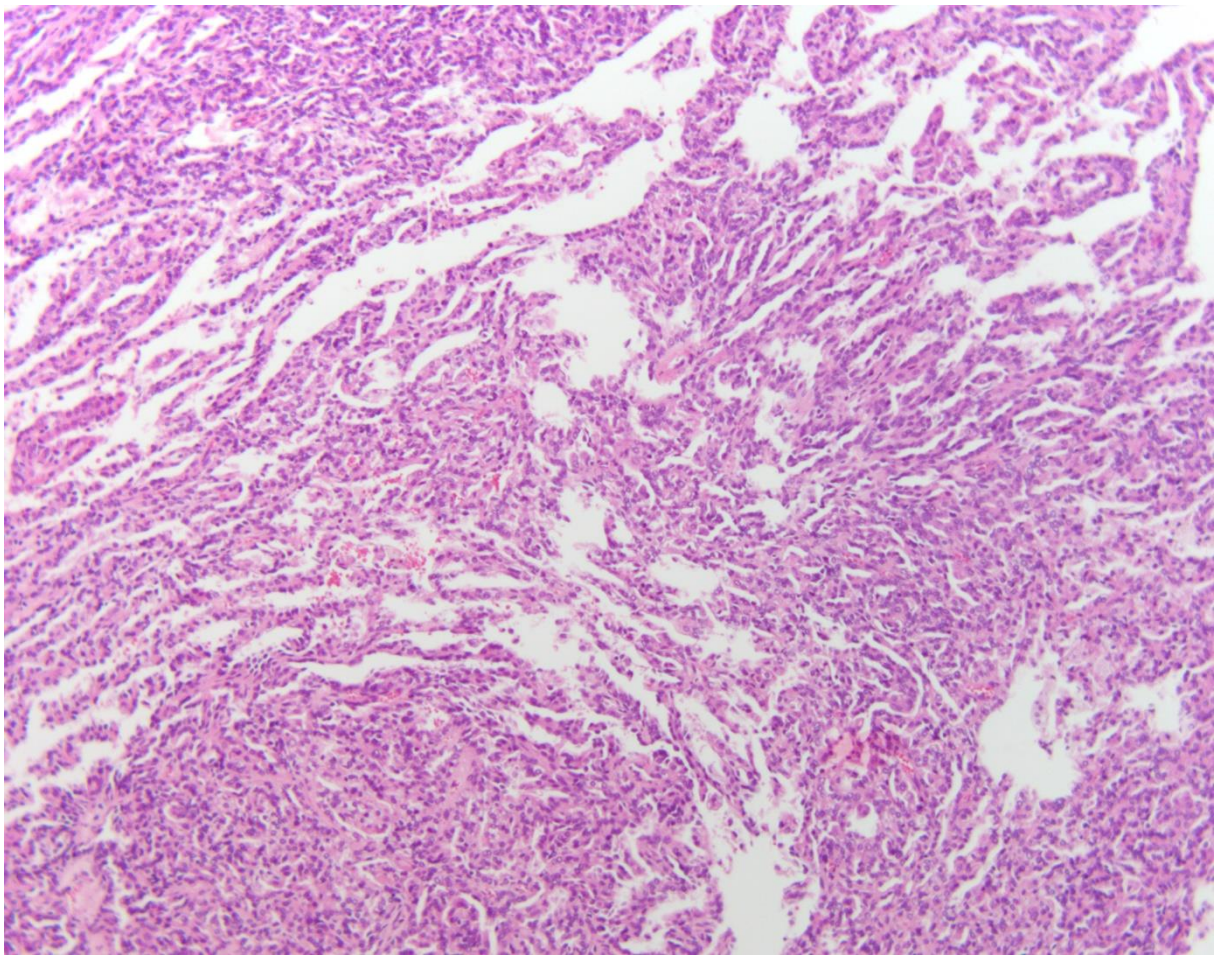

Figure 7. Papillary RCC: Image shows histology of papillary renal cell carcinoma (H&E staining, magnification 100X, Olympus Image Analayzer). Source: Mentor's archive.

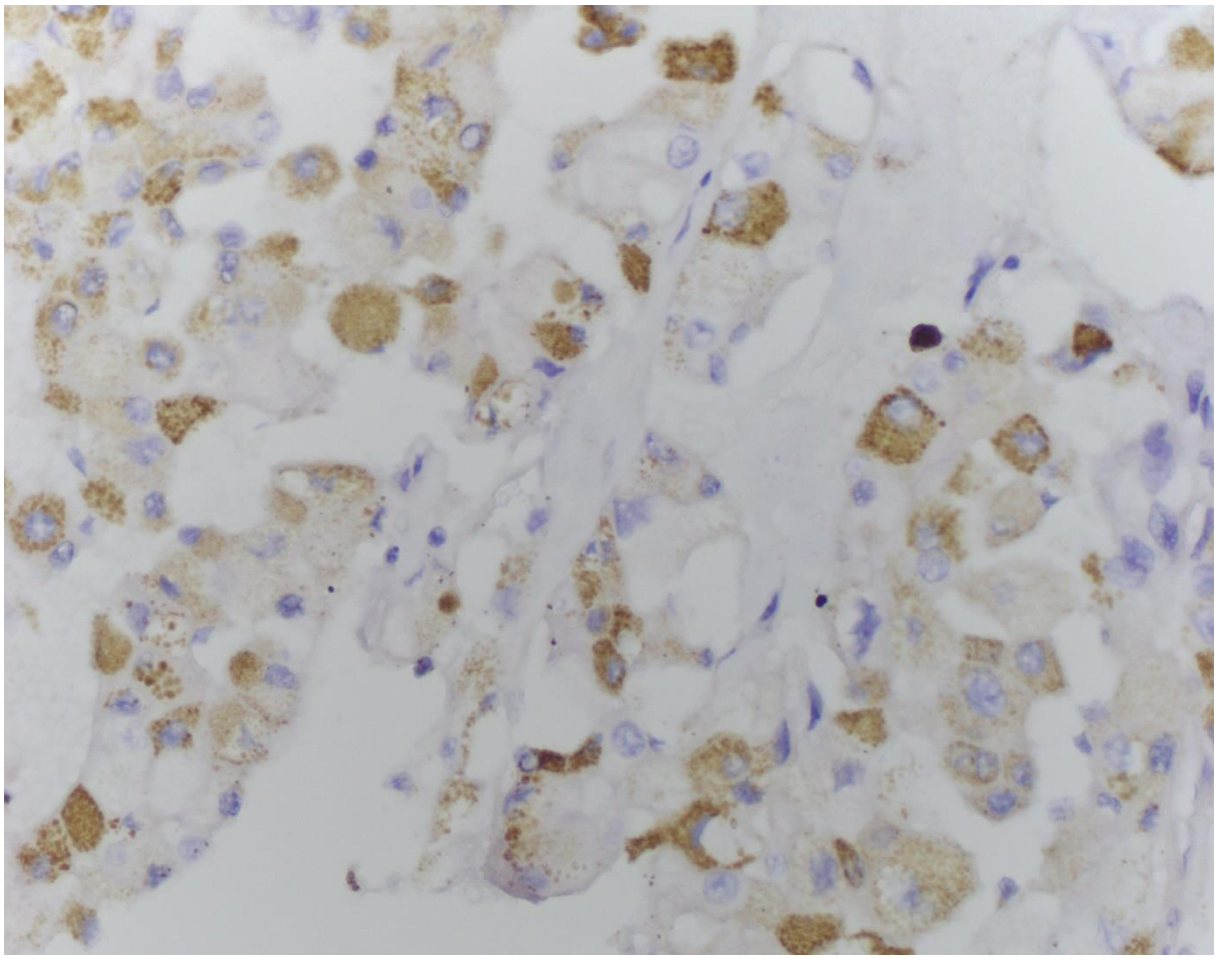

Figure 8. Podoplanin in ChRCC 40x: The image shows immunohistochemical expression of podoplanin in chromophobe RCC (Magnification 400x, Olympus Image Analayzer). The image from the present study.

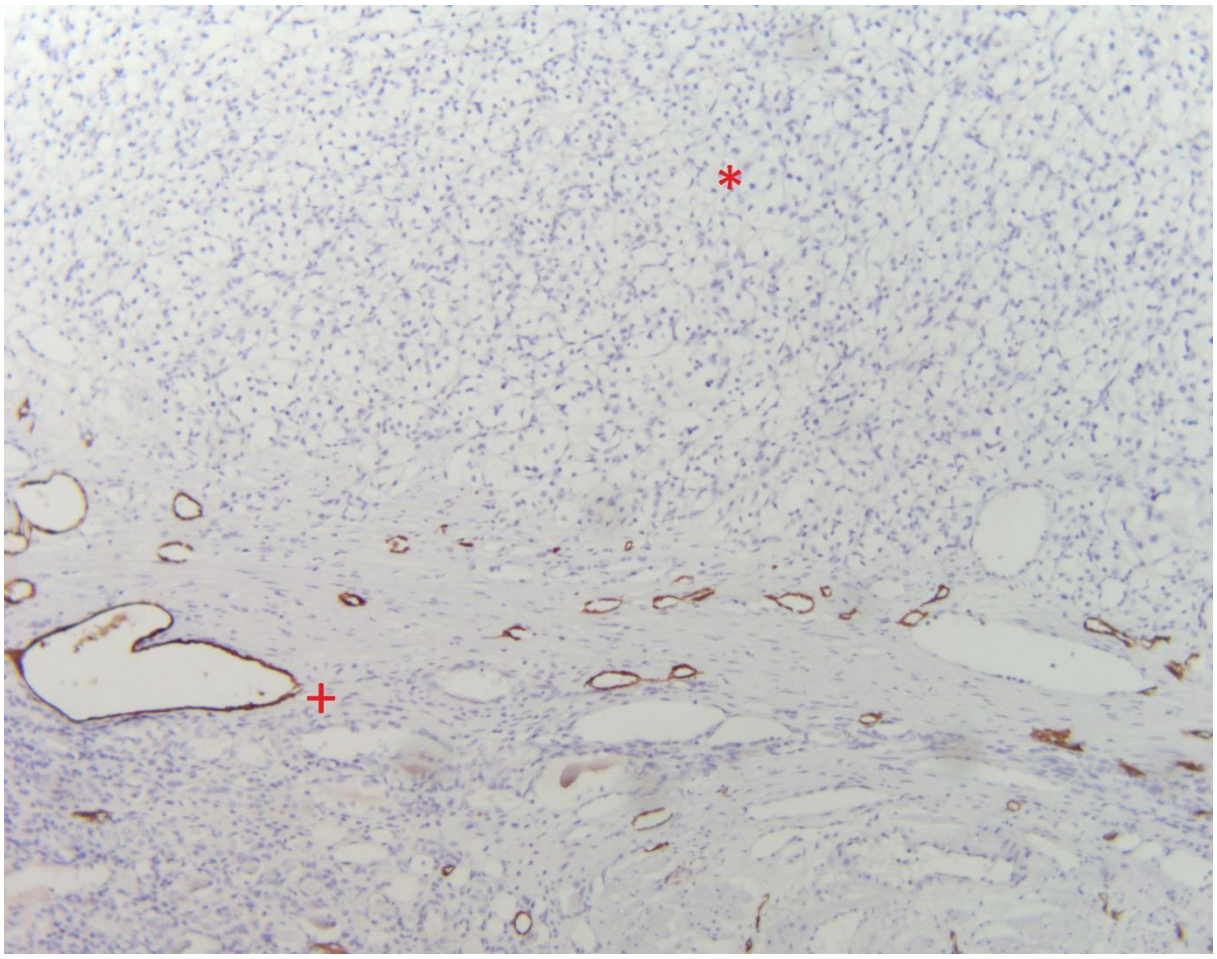

Figure 9. The image shows clear cell RCC (marked with *) negative for podoplanin immunohistochemical expression, and surronding healthy tissue (marked with+) with positive podoplanin immunohistochemial stainning of lymphatics (Magnification 100x, Olympus Image Analayzer). The image from the present study.

3.2. Immunohistochemical analysis of podoplanin

Podoplanin expression was determined via immunohistochemical analysis in the following manner; from each paraffin embedded tumor sample, a 4 µm-thick section was cut, mounted and dried at 37˚C, and processed automatically in BenchMark ULTRA IHC/ISH Staining Module (Ventana, Tucson, Arizona, USA). Primary antibody (podoplanin, D2-40) was ready to use antibody by Ventana, Tucson, Arizona, USA. Lymphatic vessels in a tumor surrounding healthy tissue served as a positive control. Ultra ViewDAB (Ventana, Tucson, Arizona, USA) and Ultra View RED (Ventana, Tucson, Arizona, USA) were use as a detection kit.

The podoplanin immunohistochemical expression in studied tumor was assessed via HSCORE method with the use of following equation HScore = Σ Pi (i+1), where the 'i' stands for intensity of staining with a value of 1 (weak), 2 (moderate), or 3 (strong), while the 'Pi' stands for percentage of stained cells of each intensity. Final HScore was calculated for each field of view after observing 10 representative fields of view for every sample and calculating arithmetic mean. To estimate the number of immunohistochemically podoplanin positive lymphatics in the normal non-tumor tissue in each sample, 1 mm2 of normal tissue bordering with tumor tissue was selected, and number of podoplanin positive lymphatics was counted.

3.3. Ethics approval

The study was approved by the Hospital Ethics Committee of the University Hospital Centre in Split, Croatia under the reference number 2181-147-01/06/M.S.-20-09. The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments.

3.4. Statistical analysis

Excel tables were used for obtained data input. Statistical data processing was done by MedCalc Statistical Software, Version 19.1.2 (MedCalc Software, Ostend, Belgium; medcalc.org; 2019, RRID:SCR_015044). Spearman correlation was used. The Chi-Squared test and Mann-Whitney U test were used. The statistical significance was set as $P<0.05$.

4. RESULTS

The study included 15 renal cell carcinoma samples, divided into two study groups: clear cell RCC and non-clear cell RCC. Clear cell RCC made up 40% and non-clear cell RCC comprised 60%. Among the non-clear cell RCC samples, there were five chromophobe RCC and four papillary RCC. The majority of samples, 73.3% were obtained via radical nephrectomy, followed by 20% of tumor excisions and 6.7% of partial nephrectomies.

Male patients comprised 80% of the study population. The average age of male patients at the time of diagnosis was 69 years compared to 37 years for female patients, which was statistically significant (P<0.001). However, there was no statistically significant difference in the patients' age between the two studied groups. The average age for clear cell RCC group was 69 years compared to 59 years in non-clear cell RCC group (P=0.248).

The gross and histological characteristics of studied tumors are presented in table 1. There was no statistically significant difference between studied groups in regard to tumor size (P=0.530), presence of tumor necrosis (P=0.489) or lymphovascular invasion (P=0.438).

Table 1. Descriptive characteristics of studied tumors

Parameter	**Clear cell RCC (N=6)**	**Non-clear cell RCC (N=9)**	**P**
Tumor size (largest diameter in cm)	4.37 ± 1.9	5.24 ± 2.9	0.530*
Tumor necrosis			
Present	1	3	0.489†
Not present	5	6	
Lymphovascular invasion			
Present	0	0	0.438†
Not present	6	9	

*Mann-Whitney test

†Chi-squared test

Abbreviations: RCC - renal cell carcinoma.

The podoplanin immunohistochemical expression in studied tumor was assessed via HSCORE method. The non-clear cell RCC group had slightly higher immunohistochemical expression of podoplanin than clear cell RCC group, 0.18 ± 0.4 and 0.08 ± 0.1 respectively. The difference wasn't statistically significant (P=0.554).

The number of immunohistochemically podoplanin positive lymphatics on 1 mm2 in the healthy tissue surrounding the tumor was calculated. The clear cell RCC group had higher number of podoplanin positive lymphatics (23 ± 9) compared to non-clear cell RCC group (14 ± 9), which wasn't statistically significant (P=0.141).

Negative correlation was noted between immunohistochemical expression of podoplanin in tumor cells and the presence of tumor necrosis and tumor size, but without statistical significance (r=-0.151; P=0.589) (r=-0.202; P=0.468).

There was statistically significant negative correlation between number of positive lymphatics in the tumor surrounding tissue and tumor size (r=-0.588; P=0.021).

5. DISCUSSION

Advancements in diagnostic techniques within pathology, coupled detailed research into organ systems down to the protein level, have expanded the capabilities of pathologists beyond traditional microscopy. Techniques such as immunohistochemical staining allow marking of specific proteins via specialized antibody markers, making them easily visible and distinguishable. This represents an essential tool for cancer-related pathology research.

A male predominance in renal RCC specimens correlates with the global RCC epidemiology. Although an 80% to 20% male-to-female ratio appears pronounced, literature commonly reports a ratio around 2:1, stressing the male predominance among RCC patients (17,72).

The average age of diagnosis of male patients compared to female patients in our study is significant and can be supported by other studies marking male gender and advanced age as a general risk factor for cancer development (17,73).

We found no statistically significant difference between clear cell RCC and non-clear cell RCC groups in regard to tumor size, presence of tumor necrosis and lymphovascular invasion.

Variations in RCC size were observed, depending on the cultural, geographical, and medical institutional context. In the review by Masood et al. (72) describing a specialized center over 10 years in Pakistan, mean tumor size of non-clear cell RCC was observed to be nearly 3 cm larger compared to our findings. Similarly, Aizer et al. (73), studying a United States population, reported larger sizes for non-clear cell RCC. In the case of Aizer et al., clear cell RCC were found to be larger than non-clear cell group, which is in line with our hypothesis, but isn't in conclusion with our results (74,75). This observation was not reflected in our results most likely due to our relatively small sample size.

Our study displays the presence of tumor necrosis as more frequently for non-clear RCC, although not statistically significant. Notably, literature reports a higher prevalence of tumor necrosis in more aggressive clear cell RCC, which is considered the less favorable histologic subtype of RCC (76,77). We found that tumors in the non-clear cell RCC group were larger in size compared to clear cell RCC, which could explain more pronounced tumor necrosis. It is well established, that larger tumors tend to exhibit more necrosis due to ischemic conditions during the rapid growth periods.

Lymphovascular invasion was absent in any of our samples. Since metastasis is a common sign of late-stage tumor disease, we expect higher incidences in a pool of larger samples and wider age groups with more advanced renal cancer cases. Research indicates that PDPN promotes lymphovascular invasion and facilitates cancer metastasis (48,55,64,68,69). Therefore, pathologic samples of late-stage cancers are expected to demonstrate more of aformentioned features.

Regarding the immunohistochemical expression of PDPN, our results reveal an elevated expression in the non-clear cell RCC group compared to clear cell RCC, which was not statistically significant and contradicts our hypothesis of increased PDPN expression in the more aggressive clear cell RCC group. Furthermore, our study displays a statistically significant negative correlation between number of positive lymphatics in the tumor-surrounding tissue and tumor size. These results suggest that factors other than PDPN may influence RCC growth and metastasis. Among these factors, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and others play a vital role in metastasis and tumor growth, due to their angiogenic and lymphangiogenic properties, facilitating tumor invasion. Additionally, Van-Hippel-Lindau gene mutation, found commonly in RCC, promotes VEGF production, but does not appear to affect PDPN expression (17,36,78-80). Although our findings regarding this correlation cannot be supported by existing literature, previous studies have investigated PDPN expression in other types of cancer. Research on PDPN in other tumors has shown higher expression in aggressive and invasive tumor types. For example, Xia et al. describes PDPN expression to correlate with poor clinical outcome, especially in CCRCC (81). The overall literature demonstrates that higher PDPN expression is associated with poorer prognosis and increased tumor malignancy (53,58,60,65,82-84).

Lymphatics immunohistochemically positive for PDPN were present in higher amounts in the clear cell RCC group, aligning with our earlier suggestion that PDPN may serve as a positive predictor for metastasis, especially in more aggressive tumors. However, this correlation was not significant. Reviewing existing research and literature reveals a connection in other cancers involving PDPN expression, due to its lymphangiogenic function (50,57,85,86). Missing lymph node excision and analysis limits our research outcome, not allowing for proper determination of lymph node invasion and metastasis of our renal carcinoma samples.

Especially aggressive tumors tend to outgrow their capacity for oxygen supply, potentially leading to necrosis within cancerous tissues (87). In our study, we observed a statistically insignificant negative correlation between PDPN expression and tumor necrosis and size. Typically, necrosis correlates with more aggressive and fast-growing cancers, leading us to expect higher PDPN levels in the clear cell RCC group (88-90). This statement is supported by aforementioned description of PDPN serving as a positive predictor for metastasis in many cancers listed in literature. It is also possible that in areas of necrosis with pronounced vascular proliferation, PDPN may not be a relevant marker for promoting angiogenesis. Instead, other factors like VEGF likely play a more important role, explaining this negative correlation. Considering the number of our samples, as well as sample size and lack of comparison with other metastasis-promoting factors, we might expect a different outcome if, under the inclusion of other angiogenic factors, greater sample size with larger number of samples was studied.

Further studies with larger sample counts, providing a more diverse picture of different tumor sizes and stages, are needed to evaluate the utility of immunohistochemical expression of PDPN as a biomarker for diagnosing and differentiating clear cell RCC and non-clear cell RCC.

6. CONCLUSION

- Our study results indicate, that there is no statistically significant correlation between PDPN expression and the histological characteristics of the tumor, lymphovascular invasiveness, tumor necrosis, or size.
- Our study was limited by a small sample size from a single hospital center, making us unable to determine whether PDPN expression in RCC influences the predictability of the tumors aggressivity and metastatic potential when comparing clear cell RCC and non-clear cell RCC subtypes.
- Lymphatics in RCC samples were more pronounced in PDPN absent tissues.
- Tumor necrosis and positive lymphatics surrounding the tumor both presented in smaller RCC.
- Additional studies are needed to clarify the role of PDPN in relation to RCC characteristics.

7. REFERENCES

1. Sadler TW, Langman J. Langman's medical embryology. 12th / T.W. Sadler. ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2012.
2. Lager DJ, Abrahams N. Practical Renal Pathology, A Diagnostic Approach E-Book: A Volume in the Pattern Recognition Series: Elsevier Health Sciences; 2012.
3. S R, D A. Embryology, Kidney, Bladder, and Ureter. 2024.
4. Ludwig KS, Landmann L. Early development of the human mesonephros. Anat Embryol (Berl). 2005;209(6):439-47.
5. Reidy KJ, Rosenblum ND. Cell and molecular biology of kidney development. Semin Nephrol. 2009;29(4):321-37.
6. Kenhub. Kidneys [Internet]. Vasković J.; 2023 [updated 2023 Nov 03; cited 2024 Feb 21]. Available from: https://www.kenhub.com/en/library/anatomy/kidneys.
7. TeachMeAnatomy. The Kidneys. [Internet]. Jones O.; 2022 [updated 2023 Jul 17; cited 2024 Feb 21]. Available from: https://teachmeanatomy.info/abdomen/viscera/kidney/.
8. News-Medical. Anatomy of the Kidney [Internet]. Laguipo A.; 2018 [updated 2018 Oct 24; cited 2024 Feb 21] Available from: https://www.news-medical.net/health/Anatomy-of-the-Kidney.aspx.
9. Soriano RM, Penfold D, Leslie SW. Anatomy, Abdomen and Pelvis: Kidneys. StatPearls. Treasure Island (FL): StatPearls Publishing; 2024.
10. Adhipandito CF, Cheung SH, Lin YH, Wu SH. Atypical Renal Clearance of Nanoparticles Larger Than the Kidney Filtration Threshold. Int J Mol Sci. 2021;22(20).
11. Kenhub. Kidney histology [Internet]. Pirie E; 2023 [updated 2023 Oct 30; cited 2024 Feb 23]. Available from: https://www.kenhub.com/en/library/anatomy/kidney-histology.
12. Murray IV, Paolini MA. Histology, Kidney and Glomerulus. StatPearls. Treasure Island (FL): StatPearls Publishing; 2024.
13. Mescher AL. Junqueira’s Basic Histology: Text and Atlas, 14e. Education M-H, editor2016. 573 p.
14. Southern Illinois University. Histology Study Guide - Kidney and Urinary Tract [Internet]. Carbondale SIU: King D; 2023 [updated 2023 Mar 12; cited 2024 Feb 23]. Available from: https://histology.siu.edu/crr/rnguide.htm.
15. Pandey J. Syed W. Renal Cancer [Internet]. Treasure Island (FL): StatPearls; 2023 [Updated 2023 Aug 8; cited 2023 Dec 9]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK558975/

16. World cancer research fund international. Worldwide cancer data [Internet]. London; [cited 2023 Dec 09]. Available from: https://www.wcrf.org/cancertrends/worldwidecancerdata/#:~:text=The%20top%20three%20%20lung%2C%20prostate,5%25%20were%20stomach%20and%20liver.
17. Hsieh JJ, Purdue MP, Signoretti S, Swanton C, Albiges L, Schmidinger M, et al. Renal cell carcinoma. Nat Rev Dis Primers. 2017;3:17009.
18. Alaghehbandan R, Siadat F, Trpkov K. What's new in the WHO 2022 classification of kidney tumours? Pathologica. 2022;115(1):8-22.
19. Lopez-Beltran A, Scarpelli M, Montironi R, Kirkali Z. 2004 WHO classification of the renal tumors of the adults. Eur Urol. 2006;49(5):798-805.
20. Muglia VF, Prando A. Renal cell carcinoma: histological classification and correlation with imaging findings. Radiol Bras. 2015;48(3):166-74.
21. Padala SA, Barsouk A, Thandra KC, Saginala K, Mohammed A, Vakiti A, et al. Epidemiology of Renal Cell Carcinoma. World J Oncol. 2020;11(3):79-87.
22. World cancer research fund international. Kidney cancer statistics [Internet]. London; 2022 [updated 2022 Mar 23; cited 2023 Dec 10]. Available from: https://www.wcrf.org/cancer-trends/kidney-cancer-statistics/.
23. Peired AJ, Campi R, Angelotti ML, Antonelli G, Conte C, Lazzeri E, et al. Sex and Gender Differences in Kidney Cancer: Clinical and Experimental Evidence. Cancers (Basel). 2021;13(18).
24. Cancer Council. Kidney cancer [Internet]. Australia: Cancer Council; 2023 [updated: 2023 Sep; cited 2023 Dec 10]. Available from: https://www.cancer.org.au/cancer-information/types-of-cancer/kidney-cancer.
25. Tsui KH, Shvarts O, Smith RB, Figlin RA, deKernion JB, Belldegrun A. Prognostic indicators for renal cell carcinoma: a multivariate analysis of 643 patients using the revised 1997 TNM staging criteria. J Urol. 2000;163(4):1090-5; quiz 295.
26. National Center for Chronic Disease Prevention and Health Promotion (US); Office on Smoking and Health (US). How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease. Atlanta (GA): Centers for Disease Control and Prevention (US); 2010.
27. Chow WH, Dong LM, Devesa SS. Epidemiology and risk factors for kidney cancer. Nat Rev Urol. 2010;7(5):245-57.
28. Saly DL, Eswarappa MS, Street SE, Deshpande P. Renal Cell Cancer and Chronic Kidney Disease. Adv Chronic Kidney Dis. 2021;28(5):460-8.e1.

29. Zhang GM, Zhu Y, Ye DW. Metabolic syndrome and renal cell carcinoma. World J Surg Oncol. 2014;12:236.
30. Gluba-Brzózka A, Rysz J, Ławiński J, Franczyk B. Renal Cell Cancer and Obesity. Int J Mol Sci. 2022;23(6).
31. Macleod LC, Hotaling JM, Wright JL, Davenport MT, Gore JL, Harper J, et al. Risk factors for renal cell carcinoma in the VITAL study. J Urol. 2013;190(5):1657-61.
32. Joh HK, Willett WC, Cho E. Type 2 diabetes and the risk of renal cell cancer in women. Diabetes Care. 2011;34(7):1552-6.
33. Capitanio U, Bensalah K, Bex A, Boorjian SA, Bray F, Coleman J, et al. Epidemiology of Renal Cell Carcinoma. Eur Urol. 2019;75(1):74-84.
34. Maher ER. Hereditary renal cell carcinoma syndromes: diagnosis, surveillance and management. World J Urol. 2018;36(12):1891-8.
35. Gray RE, Harris GT. Renal Cell Carcinoma: Diagnosis and Management. Am Fam Physician. 2019;99(3):179-84.
36. Bahadoram S, Davoodi M, Hassanzadeh S, Bahadoram M, Barahman M, Mafakher L. Renal cell carcinoma: an overview of the epidemiology, diagnosis, and treatment. G Ital Nefrol. 2022;39(3).
37. Barata PC, Rini BI. Treatment of renal cell carcinoma: Current status and future directions. CA Cancer J Clin. 2017;67(6):507-24.
38. Ball MW. Surgical management of metastatic renal cell carcinoma. Discov Med. 2017;23(129):379-87.
39. Caliò A, Marletta S, Brunelli M, Martignoni G. WHO 2022 Classification of Kidney Tumors: what is relevant? An update and future novelties for the pathologist. Pathologica - Journal of the Italian Society of Anatomic Pathology and Diagnostic Cytopathology. 2023;115(1):23-31.
40. Inamura K. Renal Cell Tumors: Understanding Their Molecular Pathological Epidemiology and the 2016 WHO Classification. Int J Mol Sci. 2017;18(10).
41. Nezami BG, MacLennan G. Kidney tumor, Adult renal cell carcinoma - common, Clear cell [Internet]. Bingham Farms MI: PathologyOutlines.com; 2021 [cited: 2024 Jan 14]. Avaiable from: https://www.pathologyoutlines.com/topic/kidneytumormalignantrccclear.html
42. Pandey J. Syed W. Renal Cancer [Internet]. Treasure Island (FL): StatPearls; 2023 [Updated 2023 Aug 8; cited 2023 Dec 9]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK558975/

43. Castillo VF, Saleeb R. Kidney tumor, Adult renal cell carcinoma - common, Papillary [Internet]. Bingham Farms MI: PathologyOutlines.com; 2021 [cited: 2024 Jan 15]. Available from: https://www.pathologyoutlines.com/topic/kidneytumormalignantrccpap.html.
44. Moch H, Amin MB, Berney DM, Compérat EM, Gill AJ, Hartmann A, et al. The 2022 World Health Organization Classification of Tumours of the Urinary System and Male Genital Organs-Part A: Renal, Penile, and Testicular Tumours. Eur Urol. 2022;82(5):458-68.
45. Tretiakova M. What's new in kidney tumor pathology 2022: WHO 5th edition updates. J Pathol Transl Med. 2022;56(6):383-4.
46. Angori S, Lobo J, Moch H. Papillary renal cell carcinoma: current and controversial issues. Curr Opin Urol. 2022;32(4):344-51.
47. Tretiakova M. Kidney tumor, Adult renal cell carcinoma - common, Chromophobe [Internet]. Bingham Farms MI: PathologyOutlines.com; 2020 [cited: 2024 Jan 15]. Avaiable from: https://www.pathologyoutlines.com/topic/kidneytumormalignantrccchromo.html.
48. Takemoto A, Miyata K, Fujita N. Platelet-activating factor podoplanin: from discovery to drug development. Cancer Metastasis Rev. 2017;36(2):225-34.
49. Watanabe M, Okochi E, Sugimoto Y, Tsuruo T. Identification of a platelet-aggregating factor of murine colon adenocarcinoma 26: Mr 44,000 membrane protein as determined by monoclonal antibodies. Cancer Res. 1988;48(22):6411-6.
50. Breiteneder-Geleff S, Soleiman A, Kowalski H, Horvat R, Amann G, Kriehuber E, et al. Angiosarcomas express mixed endothelial phenotypes of blood and lymphatic capillaries: podoplanin as a specific marker for lymphatic endothelium. Am J Pathol. 1999;154(2):385-94.
51. Suzuki H, Kaneko MK, Kato Y. Roles of Podoplanin in Malignant Progression of Tumor. Cells. 2022;11(3):575.
52. Astarita JL, Acton SE, Turley SJ. Podoplanin: emerging functions in development, the immune system, and cancer. Frontiers in Immunology. 2012;3.
53. Wicki A, Christofori G. The potential role of podoplanin in tumour invasion. British Journal of Cancer. 2007;96(1):1-5.
54. Wang Y, Sun J, Gu Y, Zhao S, Groome LJ, Alexander JS. D2-40/podoplanin expression in the human placenta. Placenta. 2011;32(1):27-32.

55. Martín-Villar E, Megías D, Castel S, Yurrita MM, Vilaró Sn, Quintanilla M. Podoplanin binds ERM proteins to activate RhoA and promote epithelial-mesenchymal transition. Journal of Cell Science. 2006;119(21):4541-53.
56. Schacht V, Ramirez MI, Hong YK, Hirakawa S, Feng D, Harvey N, et al. T1α/podoplanin deficiency disrupts normal lymphatic vasculature formation and causes lymphedema. The EMBO Journal. 2003;22(14):3546-56.
57. Quintanilla M, Montero-Montero L, Renart J, Martín-Villar E. Podoplanin in Inflammation and Cancer. Int J Mol Sci. 2019;20(3).
58. Raica M, Cimpean AM, Ribatti D. The Role of Podoplanin in Tumor Progression and Metastasis. Anticancer Research. 2008;28(5B):2997-3006.
59. Peterziel H, Müller J, Danner A, Barbus S, Liu HK, Radlwimmer B, et al. Expression of podoplanin in human astrocytic brain tumors is controlled by the PI3K-AKT-AP-1 signaling pathway and promoter methylation. Neuro Oncol. 2012;14(4):426-39.
60. Lee JA, Bae JW, Woo SU, Kim H, Kim CH. D2-40, Podoplanin, and CD31 as a Prognostic Predictor in Invasive Ductal Carcinomas of the Breast. J Breast Cancer. 2011;14(2):104-11.
61. Ugorski M, Dziegiel P, Suchanski J. Podoplanin - a small glycoprotein with many faces. Am J Cancer Res. 2016;6(2):370-86.
62. Shimada Y, Ishii G, Nagai K, Atsumi N, Fujii S, Yamada A, et al. Expression of podoplanin, CD44, and p63 in squamous cell carcinoma of the lung. Cancer Science. 2009;100(11):2054-9.
63. Wicki A, Lehembre F, Wick N, Hantusch B, Kerjaschki D, Christofori G. Tumor invasion in the absence of epithelial-mesenchymal transition: podoplanin-mediated remodeling of the actin cytoskeleton. Cancer Cell. 2006;9(4):261-72.
64. Fujita N, Takagi S. The impact of Aggrus/podoplanin on platelet aggregation and tumour metastasis. The Journal of Biochemistry. 2012;152(5):407-13.
65. Li YY, Zhou CX, Gao Y. Podoplanin promotes the invasion of oral squamous cell carcinoma in coordination with MT1-MMP and Rho GTPases. Am J Cancer Res. 2015;5(2):514-29.
66. Martín-Villar E, Scholl FG, Gamallo C, Yurrita MM, Muñoz-Guerra M, Cruces J, et al. Characterization of human PA2.26 antigen (T1α–2, podoplanin), a small membrane mucin induced in oral squamous cell carcinomas. International Journal of Cancer. 2005;113(6):899-910.

67. Tsuneki M, Yamazaki M, Maruyama S, Cheng J, Saku T. Podoplanin-mediated cell adhesion through extracellular matrix in oral squamous cell carcinoma. Lab Invest. 2013;93(8):921-32.
68. Shen Y, Chen C-S, Ichikawa H, Goldberg GS. Src Induces Podoplanin Expression to Promote Cell Migration. Journal of Biological Chemistry. 2010;285(13):9649-56.
69. Scholl FG, Gamallo C, Vilaró S, Quintanilla M. Identification of PA2.26 antigen as a novel cell-surface mucin-type glycoprotein that induces plasma membrane extensions and increased motility in keratinocytes. J Cell Sci. 1999;112 (Pt 24):4601-13.
70. Masi I, Caprara V, Bagnato A, Rosanò L. Tumor Cellular and Microenvironmental Cues Controlling Invadopodia Formation. Front Cell Dev Biol. 2020;8:584181.
71. Aseervatham J. Cytoskeletal Remodeling in Cancer. Biology. 2020;9(11):385.
72. Alzubaidi AN, Sekoulopoulos S, Pham J, Walter V, Fuletra JG, Raman JD. Incidence and Distribution of New Renal Cell Carcinoma Cases: 27-Year Trends from a Statewide Cancer Registry. J Kidney Cancer VHL. 2022;9(2):7-12.
73. Usher-Smith J, Simmons RK, Rossi SH, Stewart GD. Current evidence on screening for renal cancer. Nat Rev Urol. 2020;17(11):637-42.
74. Masood Y, Adnan S, Fiaz S, Hanif S, Cheema ZA, Mir K. Renal Tumours Of Non-Clear Cell Histology; 10 Years' Experience In A Specialized Centre. J Ayub Med Coll Abbottabad. 2023;35(2):231-4.
75. Aizer AA, Urun Y, McKay RR, Kibel AS, Nguyen PL, Choueiri TK. Cytoreductive nephrectomy in patients with metastatic non-clear-cell renal cell carcinoma (RCC). BJU Int. 2014;113(5b):E67-74.
76. Karlo CA, Kou L, Di Paolo PL, Kattan MW, Motzer RJ, Russo P, et al. Renal cell carcinoma: A nomogram for the CT imaging-inclusive prediction of indolent, non-clear cell renal cortical tumours. Eur J Cancer. 2016;59:57-64.
77. Oh S, Sung DJ, Yang KS, Sim KC, Han NY, Park BJ, et al. Correlation of CT imaging features and tumor size with Fuhrman grade of clear cell renal cell carcinoma. Acta Radiol. 2017;58(3):376-84.
78. Sasaki T, Hiroki K, Yamashita Y. The role of epidermal growth factor receptor in cancer metastasis and microenvironment. Biomed Res Int. 2013;2013:546318.
79. Chu C, Lu C, Zhang Z, Zhao C, Gu M, Yang A, et al. Correlation of VEGF and EGFR in peripheral blood with clinical stage and pathological grade of renal cell carcinoma and analysis of prognosis. J buon. 2018;23(4):1097-102.

80. Hu J, Tan P, Ishihara M, Bayley NA, Schokrpur S, Reynoso JG, et al. Tumor heterogeneity in VHL drives metastasis in clear cell renal cell carcinoma. Signal Transduct Target Ther. 2023;8(1):155.
81. Xia Y, Liu L, Xiong Y, Bai Q, Wang J, Xi W, et al. Podoplanin associates with adverse postoperative prognosis of patients with clear cell renal cell carcinoma. Cancer Sci. 2016;107(9):1243-9.
82. Hoshino A, Ishii G, Ito T, Aoyagi K, Ohtaki Y, Nagai K, et al. Podoplanin-positive fibroblasts enhance lung adenocarcinoma tumor formation: podoplanin in fibroblast functions for tumor progression. Cancer Res. 2011;71(14):4769-79.
83. Pula B, Jethon A, Piotrowska A, Gomulkiewicz A, Owczarek T, Calik J, et al. Podoplanin expression by cancer-associated fibroblasts predicts poor outcome in invasive ductal breast carcinoma. Histopathology. 2011;59(6):1249-60.
84. Shindo K, Aishima S, Ohuchida K, Fujiwara K, Fujino M, Mizuuchi Y, et al. Podoplanin expression in cancer-associated fibroblasts enhances tumor progression of invasive ductal carcinoma of the pancreas. Mol Cancer. 2013;12(1):168.
85. Bieniasz-Krzywiec P, Martín-Pérez R, Ehling M, García-Caballero M, Pinioti S, Pretto S, et al. Podoplanin-Expressing Macrophages Promote Lymphangiogenesis and Lymphoinvasion in Breast Cancer. Cell Metab. 2019;30(5):917-36.e10.
86. Hu L, Zhang P, Sun W, Zhou L, Chu Q, Chen Y. PDPN is a prognostic biomarker and correlated with immune infiltrating in gastric cancer. Medicine (Baltimore). 2020;99(19):e19957.
87. Lam JS, Shvarts O, Said JW, Pantuck AJ, Seligson DB, Aldridge ME, et al. Clinicopathologic and molecular correlations of necrosis in the primary tumor of patients with renal cell carcinoma. Cancer. 2005;103(12):2517-25.
88. Ito K, Seguchi K, Shimazaki H, Takahashi E, Tasaki S, Kuroda K, et al. Tumor necrosis is a strong predictor for recurrence in patients with pathological T1a renal cell carcinoma. Oncol Lett. 2015;9(1):125-30.
89. Zhang L, Zha Z, Qu W, Zhao H, Yuan J, Feng Y, et al. Tumor necrosis as a prognostic variable for the clinical outcome in patients with renal cell carcinoma: a systematic review and meta-analysis. BMC Cancer. 2018;18(1):870.
90. Alzumaili B, Xu B, Spanheimer PM, Tuttle RM, Sherman E, Katabi N, et al. Grading of medullary thyroid carcinoma on the basis of tumor necrosis and high mitotic rate is an independent predictor of poor outcome. Mod Pathol. 2020;33(9):1690-701.

8. SUMMARY

Objectives: The aim was to assess the immunohistochemical expression of PDPN/D2-40 in the three most common types of renal cell carcinoma, clear cell renal cell carcinoma (CCRCC), papillary renal cell carcinoma (PRCC) and chromophobe renal cell carcinoma (ChRCC). Additionally, we wanted to determine if there is a correlation between cancer aggressivity and metastatic potential and an increase of PDPN expression in aforementioned tumors.

Materials and methods: Our study included 15 RCC samples from patients operated at Urology Department of University Hospital Split and diagnosed at the Clinical Institute for Pathology, Forensic Medicine and Cytology at the same hospital, in the period from 1st of January 2018 till 31st of December 2018. The patient's data, including patient's gender and age at the time of diagnosis were collected and pathology report operation procedure provided information on histological tumor type, size of the tumor, as well as presence of necrosis and lymphovascular invasion. PDPN expression was determined via immunohistochemical analysis.

Results: 80% of patients were male. The average age of male patients at the time of diagnosis was 69 years and 37 years for female patients, which was statistically significant ($P<0.001$). There was no statistically significant difference between studied groups in regard to tumor size ($P=0.530$), presence of tumor necrosis ($P=0.489$) or lymphovascular invasion ($P=0.438$). The non-clear cell renal cell carcinoma group had slightly higher immunohistochemical expression of PDPN which was not statistically significant ($P=0.554$). The clear cell RCC group had higher number of PDPN positive lymphatics (23 ± 9) compared to non-clear cell RCC group (14 ± 9), which wasn't statistically significant ($P=0.141$). There is a negative correlation between immunohistochemical expression of PDPN in tumor cells and the presence of tumor necrosis and tumor size, but without statistical significance ($r=-0.151$; $P=0.589$) ($r=-0.202$; $P=0.468$). There was statistically significant negative correlation between number of positive lymphatics in the tumor surrounding tissue and tumor size ($r=-0.588$; $P=0.021$)

Conclusion: No statistically significant correlation between PDPN expression and the histological characteristics of the tumor, PDPN invasiveness, tumor necrosis, or size were found. Additional studies are needed to clarify the role of PDPN in relation to renal cell carcinoma characteristics.

9. CROATIAN SUMMARY

Ciljevi: odrediti imunohistokemijski izražaj PDPN/D2-40 u tri najčešća histološka tipa karcinoma bubrežnih stanica: svijetlo stanični karcinom (CCRCC), papilarni karcinom (PRCC) i kromofobni karcinom (ChRCC), te odrediti postoji li korelacija između metastatskog potencijala ispitivanih histoloških tipova tumora i PDPN imunohistokemijskog izražaja.

Materijali i metode: u studiju je uključeno 15 uzoraka karcinoma bubrega, operiranih na Klinici za urologiju Kliničkog bolničkog centra Split, a čija patohistološka dijagnoza je postavljena na Kliničkom zavodu za patologiju, sudsku medicine i citologiju iste bolnice, u razdoblju od 01.01.2018. godine do 31.12.2018. godine. Podaci o pacijentima, uključujući spol, dob u vrijeme postavljanja dijagnoze, tip operacijskog zahvata, histološki tip i veličina tumora, te prisutnost nekroze i limfovaskularne invazije, zabilježeni su iz patoshistološkog nalaza. Imunohistokemijski izražaj PDPN/D2-40 određen je uporabom HSCORE metode.

Rezultati: 80% pacijenata je bilo muškoga spola. Prosječna dob muških pacenata u vrijeme postavljanja dijagnoze je bila 69 godina, a za žene 37 godina što je bilo statistički značajno (P<0,001). Nije bilo statistički značajne razlike između ispitivanih skupina obzirom na veličinu tumora (P=0,530), prisutnost nekroze (P=0,489) ili limfovaskularne invazije (P=0,438). U skupini ne-svijetlostaničnih RCC imunohistokemijski izražaj PDPN/D2-40 je bio veći u usporedbi sa CCRCC skupinom, ali bez statističke značajnosti (P=0,554). U skupini CCRCC bilo je više PDPN/D2-40 pozitivnih limfnih žila (23 ± 9) u usporedbi s ne-svijetlostaničnom RCC skupinom (14 ± 9), bez statističke značajnosti (P=0,141). Postoji negativna korelacija između imunohistokemijskog izražaja PDPN/D2-40 u tumorskim stanicama i tumorske nekroze i veličine tumora, bez statističke značajnosti (r=-0,151; P=0,589) (r=-0,202; P=0,468). Postoji statistički značajna negativna korelacija između broja PDPN/D2-40 pozitivnih limfnih žila u tkivu koje okružuje tumor i veličine tumora (r=-0,588; P=0,021).

Zaključak: Nije nađena statistički značajna povezanost imunohistokemijskog izražaja PDPN/D2-40 i histoloških karakteristika tumora, limfovaskularne invazije, tumorske nekroze ni veličine tumora. Dodatna istraživanja su potrebna kako bi se rasvijetlila uloga PDPN u razvoju i metastatskom potencijalu RCC-a.