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TOKAT GAZIOSMANPASA UNIVERSITY

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MEDICAL BIOLOGY MASTER'S PROGRAM

DETERMINATION OF *ARPC1A* GENE EXPRESSION IN KIDNEY CANCER

(MASTER'S THESIS)

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SCIENTIFIC ETHICS PAGE

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TO THE INSTITUTE OF GRADUATE STUDIES DIRECTORATE

I attest to the originality of the Master's thesis entitled "DETERMINATION OF *ARPC1A* GENE EXPRESSION IN KIDNEY CANCER" in this document. I confirm that I followed the guidelines of Tokat Gaziosmanpasa University – Graduate Education Institute while preparing this thesis. Moreover, I affirm that I gathered and presented all the information in accordance with academic regulations and ethical principles. To abide by these regulations and principles, I have cited all the data, thoughts, and findings that are not my own.

Ethical Committee for Clinical Research at Tokat Gaziosmanpasa University decision number: 22-KAEK-045.

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Lazgin Karim HUSSEIN

JURY ACCEPTANCE AND APPROVAL

The defense examination of the thesis study titled “Determination Of *ARPC1A* Gene Expression In Kidney Cancer’’ is prepared by Lazgin Kareem HUSSEIN was held on 24/09/2024 it has been accepted by jury members as a Master’s Thesis at Tokat Gaziosmanpasa University, Graduate Education Institute, Department of Medical Biology.

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Member: Prof. Dr. H. Omer ATES	…….
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CONFIRMATION

/ /2024

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ABSTRACT

DETERMINATION OF *ARPC1A* GENE EXPRESSION IN KIDNEY CANCER

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Master Thesis Department Of Medical Biology

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Renal cell carcinoma (RCC), in particular, is an important medical issue that is becoming more commonplace worldwide. Developing targeted therapeutics requires an understanding of the molecular pathways behind the disease's etiology. The *ARPC1A* gene is one of many genes linked to kidney cancer; however it has lately come to light due to its possible involvement in carcinogenesis and the advancement of the disease. Protein linked to actin the actin-related protein (ARP) family, which includes Subunit1A, is also known by the shortened form ARPC1A. This family of proteins is essential to many cellular functions, like the control of gene expression, intracellular transport, and cytoskeletal dynamics. The ARPC1A gene, located on chromosome 7q22.1, encodes the ARPC1A protein, a crucial component of the ARP2/3 complex. In order to enable cytoskeletal rearrangements that support cell motility, shape maintenance, and membrane dynamics—activities that include endocytosis, cell-cell adhesion, and cell motility—the ARP2/3 complex is a crucial regulator of actin filament nucleation and branching. This research analyzed gene expression rate samples in 11 effected cells and 11 control cells from the kidney of the same patient using quantitative reverse transcription PCR. In comparison to normal kidneys, the study found that kidney cancer tissue samples had 1.14 -fold higher *ARPC1A* gene expression; however, this increase is not statistically significant ($p = 0.75$). This abstract discusses the role of the ARPC1A gene in kidney cancer.

Keywords: Actin-Related Protein, *ARPC1A* Gene, Kidney Cancer, RCC.

ÖZET

BÖBREK KANSERİNDE *ARPC1A* GEN EKSPRESYONUNUN BELİRLENMESİ

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Özellikle renal hücreli karsinom (RCC), dünya çapında giderek yaygınlaşan önemli bir tıbbi sorundur. Hedefe yönelik terapötiklerin geliştirilmesi, hastalığın etiyolojisinin arkasındaki moleküler yolların anlaşılmasını gerektirir. *ARPC1A* geni böbrek kanseriyle bağlantılı birçok genden biridir, ancak son zamanlarda karsinogenez ve hastalığın ilerlemesindeki olası rolü nedeniyle gün ışığına çıkmıştır. Aktin İlişkili Protein Alt Birimi1A veya *ARPC1A*, gen ifadesinin düzenlenmesi, hücre içi taşıma ve hücre iskeleti dinamikleri de dahil olmak üzere bir dizi hücresel süreçte yer alan aktin ilişkili protein (ARP) ailesinin önemli bir üyesidir. ARP2/3 kompleksinin önemli bir parçası olan ARPC1A proteini, 7q22.1 kromozomunda bulunan *ARPC1A* geni tarafından kodlanır. Hücre hareketliliğini, şekil korumayı ve membran dinamiklerini (endositoz, hücre-hücre yapışması ve hücre hareketliliğini içeren faaliyetler) destekleyen hücre iskeleti yeniden düzenlemelerini sağlamak için ARP2/3 kompleksi, aktin filament çekirdeklenmesi ve dallanmasının çok önemli bir düzenleyicisidir. Bu araştırma, kantitatif ters transkripsiyon PCR kullanarak aynı hastanın böbreğinden alınan 11 kanser hücresi ve 11 normal hücredeki gen ifade oranı örneklerini analiz etti. Çalışma, normal böbreklerle karşılaştırıldığında, böbrek kanseri doku örneklerinin 1.14 kat daha yüksek *ARPC1A* gen ifadesine sahip olduğunu; ancak bu artışın istatistiksel olarak anlamlı olmadığını ortaya koymuştur ($p = 0,75$). *ARPC1A* geninin böbrek kanserindeki önemi bu özette vurgulanmaktadır.

Anahtar Kelimeler: Aktin İlişkili Protein, *ARPC1A* Gen, Böbrek Kanseri, RCC.

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ABHD3	:	Alpha beta hydrolase domain 3
ACTB	:	Actin B
ARP2/3	:	Actin-related proteins-2/3
ARPC1A	:	Actin-related protein complex subunit 1A
ASRD	:	Acquired renal cystic disease
ASRD	:	Acquired renal cystic disease
BMI	:	Body Mass Index
ccRCC	:	Clear cell renal cell carcinomas
cDNA	:	Complimentary DNA
cDNA	:	Complementary DNA
CK666	:	Arp2/3 complex inhibitor
CKD	:	Chronic kidney disease
CKD	:	Chronic kidney Disease
CLL	:	Chronic lymphocytic leukemia
crRCC	:	Chromophobe renal cell carcinomas
CT	:	Computed tomography
dH2O	:	Distillated water
DSBs	:	Double-strand breaks
dsDNA	:	Double-stranded DNA
HCC	:	Hepatocellular carcinoma

HNSCC	:	Head and neck squamous cell carcinoma
HR	:	Homologous recombination
IHC	:	Immunohistochemistry
LDH	:	Lactate dehydrogenase
mRNA	:	Messenger ribonucleic acid
NGS	:	Next-generation sequencing
NIH	:	National Institute of health
NPFs	:	Nucleation promoting factors
PCR	:	Polymerase chain reaction
qRT-PCR	:	Quantitative real-time polymerase chain reaction
RCC	:	Renal cell carcinoma
RNA	:	Ribonucleic acid
SEM	:	Standard error mean
ssDNA	:	single-stranded DNA
TCC	:	Transitional cell cancer
TSG	:	Tumor suppressor gene
UTUC	:	Upper tract urothelial carcinomas
UV	:	Ultraviolet radiation
WASP	:	Wiskott-Aldrich syndrome protein
WAVE	:	WASP family verprolin-homologous protein
WHO	:	World health organization

1. INTRODUCTION

The kidneys are a vital part of the urinary tract. Numerous biological processes, such as osmoregulation, blood pressure and volume regulation, red blood cell production, calcium absorption, toxin metabolism, and excretion, are regulated by the urinary system. The kidney's functional unit, the nephron, is composed of the glomerulus and renal tubules. Among the products of the kidney are urine, the hormones calcitriol and erythropoietin, and the enzyme hormone renin (Delaney et al., 2018).

Cancer is acknowledged as a worldwide health concern for which there is no proven treatment (Assembly., 2018). The formation and survival of cancer cells are multifactorial processes that include the body's defense mechanisms, physiological alterations in cancer cells, and genetic mutation of normal cells, may result in cellular longevity, proliferation, and carcinogenesis through mechanisms like oncogene gain-of-function mutations and tumor suppressor gene loss-of-function mutations. Apart from alterations in cells, the body's typical defensive systems can also be crucial in promoting or thwarting the development of cancer and the spread of tumors. The immune system's reaction to the growth and spread of cancer cells, as well as any possible involvement the immune system may play in the early stages of tumor formation, are particularly essential (Zamarron and Chen., 2011).

Kidney cancer is the 14th most common disease worldwide; accounting for approximately 400,000 cases in 2018 and 2.2% of all cancer diagnoses worldwide (Bray et al., 2018). Renal tubular epithelial cells are the source of renal cell carcinoma, or RCC, which makes up around 90% of every possible case of kidney cancer (Hsieh et al., 2017). According to reports, in 2016 the death rate from RCC accounted for about 2% of all cancer deaths. Furthermore, because cross-section abdominal imaging has improved recently, the incidence of RCC has grown. Despite being the most prevalent urogenital

cancer, RCC is typically discovered by mistake (Kane et al., 2008). Branching actin networks, which are necessary for numerous cellular functions, are formed by the Arp2/3 complex (Sindram et al., 2023). The seven-subunit complex known as actin-related proteins-2/3 (Arp2/3) is one of the three recognized actin nucleates in eukaryotes, which is special due to it can arrange filaments into branched networks. Precise regulation of actin polymerization start and filament organization is necessary for the actin cytoskeleton's biological functions. Actin-filament networks' polymerization, organization, and recycling are all regulated by the explosive (Arp2/3) complex, which is involved in both in vitro and live cells (Goley and Welch., 2006).

ARPC1A is one of the Arp2/3 complex family members. (Vinzenz et al., 2012). The Arp2/3 complex, consisting of seven proteins, was first identified in Acanthamoeba. It serves as essential for lamellipodia-mediated cell movement, which is required for cancer invasion and spread, because it may form branched actin filament networks (Vinzenz et al., 2012). Both the development of pseudopodia and the migration of bladder cancer cells are mediated by Arp2/3 (Kiuchi et al., 2011). It has also been demonstrated that Arp2/3-regulated cytoskeleton remodeling in gliomas is a crucial requirement for tumor spread and disease development (Liu et al., 2013). Furthermore, it has been documented that some tumor forms, such as gastric, breast, rectal, and lung tumors, have elevated expression of Arp2/3 complex components. Also, it is thought that the overexpression of these proteins contributes to the poor patient prognosis associated with these disorders via promoting the migration and invasion of tumor cells. However, previous studies have not shown the role of ARPC1A in PCa metastasis or its significance in PCa cells (Vinzenz et al., 2012).

The aim of this study is to examine the connection between the ARPC1A gene and kidney cancer by using quantitative real-time PCR to detect and identify the ARPC1A gene in patients with kidney cancer, as well as to differentiate between cancerous and healthy kidney tissue.

2. LITERATURE REVIEW

2.1 KIDNEY CANCER

2.1.1 Description of kidney

Each kidney is the size of a hand and is comprised of two bean-shaped organs. They are shielded from harm by the lower rib cage and joined to the upper back wall of the abdomen. The kidneys are positioned directly to the backbone's left and right. The words "superior pole" and "inferior pole" might be used to describe the top or lower part of the kidney. An adrenal gland is a small organ that sits atop each kidney. Gerota's fascia, a thin layer of fibrous tissue, surrounds the kidneys and adrenal glands (Kay and Pedrosa., 2018).

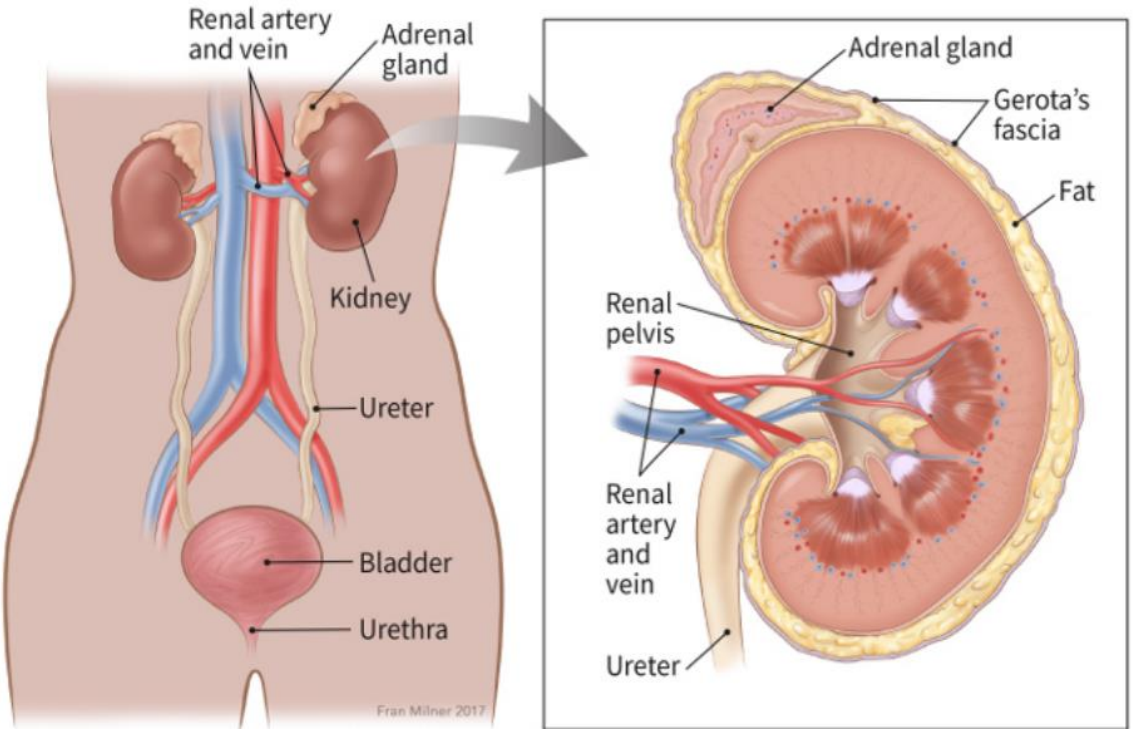


Figure 2.1. Kidney Structure

(Kay and Pedrosa,. 2018).

The kidneys' main carrier is to filter waste products, extra water, and salt out of blood that enters through the renal arteries and then they turn into urine. The renal pelvis which is located in the core of each kidney is where urine gathers before exiting the kidneys through the long, thin tubes known as ureters. The bladder is one last barrier before a person can urinate, and the ureters lead present (Kay and Pedrosa., 2018).

2.1.2 Types of kidney cancer

The majority of adult kidney malignancies that begin in the collecting system are transitional cell carcinomas, whereas renal cell carcinomas, also known as adenocarcinomas, are the most prevalent type that develops in the renal parenchyma. RCC is the cause of more than 90% of adult kidney carcinomas. Less than 10% of kidney carcinomas that have been histologically verified are renal transitional cell carcinomas (RTCCs), which originate in the renal pelvis. Nephroblastoma, commonly called Wilms tumor, is the most frequent type of kidney cancer in children, accounting for 1.2% of cases. Clear cell adenocarcinoma accounts for the majority of cases, with papillary, chromophobe, and RCC not otherwise defined subtypes following. Epidemiologic data on RCC subtypes are scarce and have not consistently indicated patterns of incidence or risk factors, despite the fact that histologic subtypes of the disease have been demonstrated to differ in clinical characteristics and genetic drivers. (Chow et al., 2010)

2.1.2.1 ***Renal Cell Carcinoma***

Renal cell carcinoma (RCC) is a type of cancer that develops in the lining of the proximal convoluted tubule. It can also be referred to as a hypernephroma, renal adenocarcinoma, or Grawitz tumor. It comprises 80% to 90% of malignant kidney tumors and 70% to 80% of all solid renal tumors (Hancock and Georgiades., 2016). The three most common types with an incidence of at least 5% are chromophobe RCC (chRCC), papillary RCC (pRCC), and clear cell RCC (ccRCC) (Figure 2). The remaining subtypes have a total

frequency of less than 1%, making them extremely unusualAn unclassified RCC tumor is one that does not correspond to any of the subtypes specified in the diagnostic criteria (uRCC; incidence of less than 4% total). Most kidney cancer deaths are caused by the most prevalent subtype, ccRCC. Options for treating localized RCC include radical or partial kidney removal (nephrectomy). Ablation, that involves using cold or heat to destroy cancerous tissue, or active surveillance, which involves routinely taking radiographic pictures to track the tumor's progress. In an attempt to cure their localized ccRCC condition, over 30% of patients later have metastases, even after receiving a nephrectomy.27–30. They have a high death rate and necessitate systemic therapy (Hsieh et al., 2017).

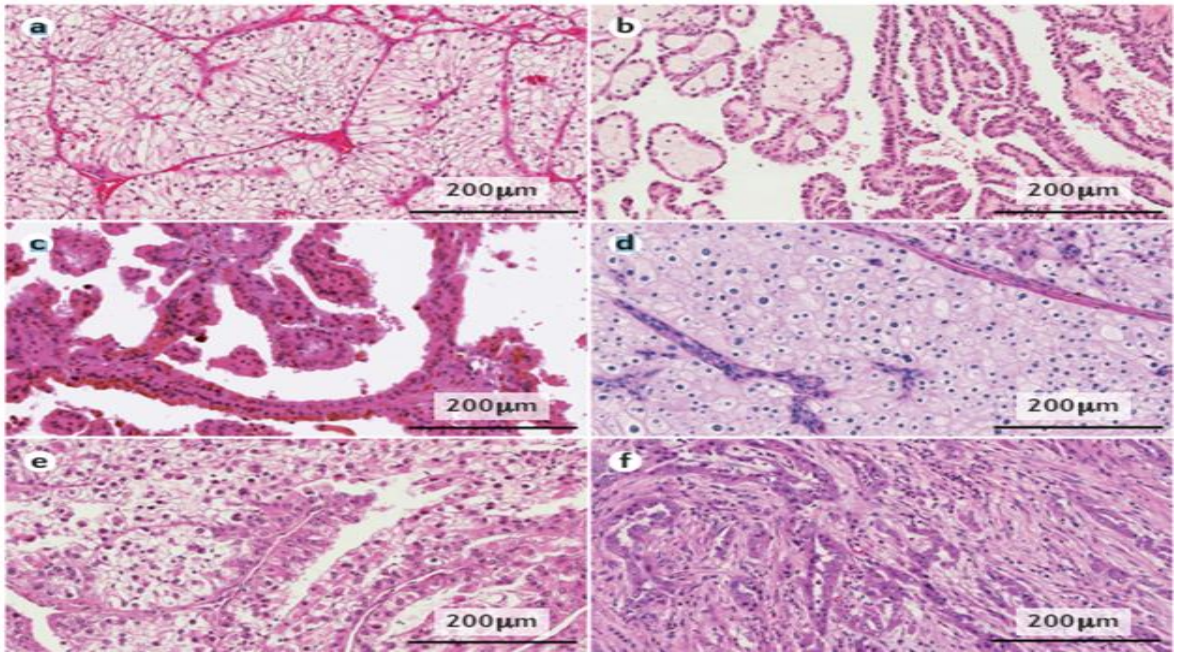


Figure 2.2. Distinct Subtypes of RCC

(Hsieh et al., 2017).

Clear cell renal cell carcinomas (ccRCCs) account for about 75% of all RCCs (part a). Papillary RCCs, which make up around 15% of kidney tumors, are divided into two

types according to the features of the staining: type 1 (basophilic; part b) and type 2 (eosinophilic; part c). Chromatophobe RCCs make up about 5% of kidney cancers (part d). MiT family translocation RCCs (part e) and collecting duct RCCs (part f) are two other minor subtypes. (Hsieh et al., 2017).

Clear cell RCC has a high lipid content, which gives it a golden yellow appearance when sliced. Microscopic analysis usually shows an alveolar, acinar, or solid architectural pattern with a delicate vascular network and clear or eosinophilic cytoplasm. On histology of the malignant epithelial cells that form papillae and tubules are a determining characteristic of papillary RCC. Type 1 tumors feature papillae covered with tiny cells with a single layer of widely dispersed cytoplasm, whereas type 2 tumors have papillae with eosinophilic cytoplasm and pseudostratified nuclei. Additionally, their prognosis is typically poorer than that of type 1 cancers due to their higher stages (Low et al., 2016).

The first hybrid oncocytoma/chromophobe RCC cases were found in people with Birt-Hogg-Dubé syndrome (BHD) syndrome. Lung cysts, spontaneous pneumothorax, renal tumors, and fibrofolliculomas are the hallmarks of this uncommon autosomal dominant disorder. A mutation on chromosome 17 of the tumor suppressor gene BHD is connected to this disorder. Hybridoma/chromophobe RCC, which is characterized by the morphologic features of both oncocytoma and chromophobe RCC in the same tumor RCC, is present in the renal tumors of these patients (Crumley et al., 2013).

2.1.2.2 ***Urothelial carcinoma***

Cancers of the bladder known as upper-tract urothelial carcinomas (UTUCs) originate from the urothelial lining of the urinary tract and progress from the calycle system to the distal ureter. Measuring 1-2 instances per 100,000 individuals, it is a comparatively uncommon occurrence that represents 5-7% of all renal tumors and 5–10% of all urothelial tumors. The male-to-female ratio is 2:1, meaning that males are more likely than women to experience UTUC (Soria et al., 2017).

Commonly seen in the bladder, transitional cell cancer (TCC) is typically detected during a cystoscopy. The ureter, renal pelvis, or calices are the source of 5% of urothelial malignancies, which make up 10% of upper tract neoplasms. The most common symptom of TCC patients is hematuria, which can be microscopic or frank. Approximately one-third of patients exhibit symptoms that are more commonly linked to calculi, such as acute renal colic or flank pain. Sometimes cancers present with far-off metastases, or they may be unintentionally found during radiologic screening (Browne et al., 2005).

2.1.2.3 ***Renal Sarcoma***

About 5% of renal tumors in children are clear cell sarcomas, which are the second most prevalent malignant kidney neoplasm. With a male predominance (male to female ratio of nearly 2:1) and a mean age of presentation of 36 months, the majority of cases occur between the ages of 2 and 3 years (Argani et al, 2000). There are just three examples with bilateral CCSK that have been reported in the literature, and it's not obvious from these cases if they genuinely indicate a second primary tumor or a metastatic condition (Parikh et al, 1998). The most often metastasized locations at presentation are the liver, lung, bone, and regional lymph nodes (Argani et al., 2000). Renal clear cell sarcoma has historically been linked to late recurrences, especially when cancer involves the bone.

Modern chemotherapy regimens, however, have reduced the incidence of late relapses, and the brain now accounts for more recurrences than bone (Furtwängler et al., 2013). Renal clear cell sarcoma usually appears as a big, unicentric, well-circumscribed tumor that can seem to be starting from the renal medulla. These tumors range in size from 2.3 to 24 cm, with a mean size of 11.3 cm. The tumors' sliced surfaces have a mucinous, bright resemble and are consistently brownish in color. Cystic foci are frequent and sometimes the defining characteristic. Discrete bleeding and necrosis foci are also frequently seen. About 5% of patients show gross extension into the renal vein (Argani et al., 2000).

2.1.3 Epidemiology

RCC, which causes a wide range of clinical signs and symptoms, is a complicated and variable cancer that accounted for around 140,000 deaths annually in 2013. It was the seventh most common cause of death due to cancer. RCC contributes about 2% to 3% of all cancers. With a fatality rate of almost 40%, compared to 20% for bladder and prostate cancers, it is the most deadly urogenital malignancy. Its incidence, which is constantly rising, differs globally and is more common in industrialized than in developing nations. Furthermore, The male to female ratio is 1.5:1, meaning that men are far more probable than women to die and to come up with RCC. Moreover, RCC primarily manifests between the ages of 60 and 70, and it becomes less common after that. This might be because fewer harsh diagnostic tests are used in this age group (Capitanio et al., 2019). The primary causes of this surge in RCC incidence are the development of diagnostic methods and increased public understanding of the importance of routine physicals. Consequently, more patients are receiving early diagnoses, which is another rising trend. The death rate from RCC has dropped quickly, even if the overall incidence has increased during the past three decades, particularly in wealthy nations because of early detection and treatment. However, many patients still experience locally advanced disease and distant metastases despite advancements in disease control and survival (Vasudev et al., 2020). Even after surgery, More than 40% of individuals with localized RCC get distant metastases, and 20–30% of patients already have metastases when they are first diagnosed. Thirty–five percent of patients advance to metastasis with local illness. Hemateuria, Typical symptoms of RCC include palpable abdominal bulge and flank pain, however these are only present in 4–17% of patients. Although the liver, opposite kidney, and adrenal glands can also be affected, the lungs, bones, and brain are the most often affected organs in distant metastases (Ather et al., 2010). While it is uncommon for RCC to spread to the pancreas and small intestine, patients may experience gastrointestinal bleeding, which could be the initial sign that these

organs are involved. Furthermore, about 40% of individuals with RCC eventually pass away (Cairns., 2011).

2.1.4 Risk factors

Gender and age are the two crucial risk factors for RCC. Location, ethnicity, history of tobacco product use, hypertension (HTN), and overweight are other possible risk factors (Johansson et al., 2019). However, According to new evidence, persons with RCC who are overweight might recover better, defying the hypothesis that obesity is a risk factor for the cancer and highlighting the need for further research. Several minor risk factors that could be causes of RCC are: consuming red and processed meat; exposure to cadmium and trichloroethylene; vitamin D deficiency; elevated triglycerides; sedentary lifestyle; prolonged use of palliative care; viral hepatitis; elevated CKD; end-stage renal disease (ESRD); and genetic syndromes (Choueiri et al., 2014).

2.1.4.1 ***Smoking***

Smoking has been identified as a risk factor for RCC based on the results of meta-analyses, case-control studies, and prospective data collecting. Nevertheless, risk classification based on length of smoking, intensity of smoking, and length of abstinence varies greatly between studies that are published. Numerous studies have noted this variability, which emphasizes the necessity of standardizing the reporting of smoking-related causes for RCC (NCI,. 2010). There are several compounds in tobacco smoke, more than fifty of which have been identified as carcinogens to humans (Li and Hecht,. 2022). While some of these substances can change gene expression or interfere with cellular signaling pathways, others have the ability to damage DNA. While some of these substances can change gene expression or interfere with cellular signaling pathways, others have the ability to directly destroy DNA, when these cancers are subjected to a metabolism, the chemicals are transformed into reactive metabolites, which have the ability to bind

DNA and cause structural alterations Although smoking rates have been falling globally, there is still significant worry about how smoking affects the development of RCC. Crucially, because tumor formation can take a while to manifest, those who quit smoking may still be at risk for developing RCC (Buendia et al,. 2015).

2.1.4.2 ***Obesity***

Obesity has been found to be one recognized risk factor for RCC. However, Several studies shown that patients with higher body mass index (BMI) had a higher prognosis for RCC in terms of survival benefit (Kim et al,. 2021). Obesity is defined by the world health organization as having a BMI of ≥30 kg/m2, which raises the risk of cancer in both men and women (Albiges et al,. 2016). Over the past ten years, Numerous investigations have shown a direct link between obesity and an increased risk of RCC. Overall, depending on the study, an increase in RCC risk in obese people has been documented, ranging from 1.32 to 1.76 fold higher. It was found that for every unit rise in BMI (1 kg/m2), the risk of RCC increased by 4-6% (Landberg, et al,. 2019). There is also evidence linking early adult obesity, particularly abdominal obesity (which is more common in men than in women), to a higher risk of RCC. It has been confirmed that being overweight increases the chance of developing RCC in both men and women. Nevertheless, some studies have found no correlation between being overweight and the incidence of RCC in women. Men's risk of RCC increased by 1.5 times for every kilogram of body mass, which explains why men's RCC incidence is higher than women's (Shen et al,. 2015).

2.1.4.3 ***Hypertension***

Millions of people worldwide suffer from hypertension (HTN), which is a major risk factor for several disorders including renal cancer. Numerous studies have shown a strong connection between RCC and hypertension, indicating that having high blood pressure may heighten the likelihood of acquiring this malignancy (Mariusdottir et al,.

2016). Investigations has repeatedly demonstrated a positive correlation between RCC incidence and HTN. There seems to be a connection between blood pressure and RCC occurrence, and RCC risk may be reduced with appropriate blood pressure treatment. The correlation between HTN and the prognosis of RCC is still poorly understood, and there is still variation in the definition of HTN and the methods used to account for confounders. (Liu, et al,. 2019).

2.1.4.4 ***Physical activity***

In general, it appears feasible that there is a weak inverse relationship between RCC incidence and physical activity, despite inconsistent research and inconsistent results. However, due to inconsistent confounder correction and highly variable study designs, physical activity may just be a stand-in for leading a healthy lifestyle. Furthermore, it is yet unclear how physical activity and exercise could influence the incidence and prognosis of RCC. No conclusions can be drawn on the impact of physical exercise on RCC mortality (Ihira et al,. 2019).

2.1.4.5 ***Diet and alcohol***

A varied diet that has a lot of different types of exposures, such consuming a lot of processed red meat and dairy products, or a diet heavy in saturated fats and low in fruits and vegetables, can make anybody a greater possibility to develop RCC. Conclusions about the relationship between diet and RCC are difficult to come to because these exposures also regularly interact with other aspects of lifestyle such as alcohol consumption, smoking, and physical exercise. Consuming fruits and vegetables has been demonstrated in a meta-analysis to reduce the probability to grow RCC (Huang, et al,. 2014). Likewise, a statistically significant correlation was discovered between the consumption of red meat and the risk of RCC in a meta-analysis that primarily included case-control studies (Zhang

et al,. 2017). There is a lower correlation between red and processed meat consumption and the risk of RCC in males than in women, according to a small number of prospective studies (Grieb et al,. 2009). Consuming fish, coffee, or poultry did not increase the chance to develop RCC (Daniel et al,. 2011). Since most of the included research is retrospective in nature, it is currently unknown how actual alcohol use affects the development of RCCs. Enduring conclusive outcomes may be hampered by the inaccurate nature of the objective assessment of alcohol consumption (Campi et al,. 2023).

2.1.5 Pathophysiology

RCC is a multifaceted, diverse illness with a range of clinical manifestations. RCC accounted for around 140,000 annual deaths and was the seventh most prevalent cause of cancer-related mortality in 2013. RCC accounts for 2% to 3% of all malignancies. With a fatality rate of almost 40%, compared to 20% for bladder and prostate cancers, it is the most deadly kind of urogenital cancer. Its incidence, which is constantly rising, differs globally and is more common in industrialized than in developing nations. In addition, men have a higher prevalence of RCC and a higher death rate than women (the male to female ratio is 1.5:1). Moreover, RCC primarily manifests between the ages of 60 and 70, and it becomes less common after that. This might be because fewer strong diagnostic tests are used in this age range (Capitanio et al., 2019).

The primary causes of this surge in RCC incidence are the development of diagnostic methods and increased public knowledge of the importance of routine health checks. Consequently, more patients are receiving early diagnoses, which is another rising trend. The death rate from RCC has dropped quickly, even if the overall incidence has increased during the past three decades, particularly in wealthy nations because of early detection and treatment. However, many patients still experience locally advanced disease and distant metastases despite advancements in disease control and survival (Vasudev et al., 2020).

Even after surgery, Distant metastases are present in more than 40% of patients with localized RCC, and 20–30% of patients already have metastases when they are first diagnosed. Thirty–five percent of patients advance to metastasis with local illness. Hemateuria, flank pain, and a palpable abdominal mass are typical signs of RCC, however these are only present in 4–17% of patients. Although the liver, opposite kidney, and adrenal glands can also be affected, the lungs, bones, and brain are the most often affected organs in distant metastases (Ather et al., 2010). While it is uncommon for RCC to spread to the pancreas and small intestine, patients may experience gastrointestinal bleeding, which could be the initial sign that these organs are involved. Furthermore, about 40% of individuals with RCC pass away (Cairns., 2011).

2.1.6 Genetics

One kind of cancer with a variety of genetic and epigenetic changes is RCC. Heidelberg carried out the first molecular genetic categorization of RCC in 1997. Subsequently, this classification was incorporated into the 2004 WHO tumor classification and the Vancouver ISUP from 2012 (Moch et al., 2016). A familial history with an autosomal predominance pattern is seen in about 3% of instances of RCC. In turn, there are two forms of RCC: sporadic and inherited (uncommon). Different mutations and epigenetic modifications in the subtypes of RCC are present in the genes that cause RCC. Many genes, including *VHL*, *MET*, *FH*, *BHD*, and *HRPT2*, are mainly accountable for inherited RCC. The biggest frequent mutation in the *VHL* gene that results in hereditary clear cell RCC (Maher., 2018).

Numerous genetic changes are present in clear cell RCC. The deletion of the short arm of chromosome 3, which codes for the *VHL* gene, is the first and most common of these (about 90%). Tumor suppressor *VHL* is observed in those susceptible to von Hippel-Lindau hereditary syndrome. Nevertheless, patients with clear cell RCC who have this kind of genetic abnormality have the greatest prognosis. (Kroeger et al., 2013). The initial stage

of growth, or inactivation of tumor suppressor genes may be attributed to additional loci on chromosome 3 such as 3p25–26, 3p12, 3p14.2, 3p21.1, 3p21.3, 3p22, and 3p26.2. Certain chromosomal abnormalities, such as 4p, 14q, and 9p, suggest that clear cell RCCs have a poor prognosis, While in high-grade clear cell RCC, acquiring 5q31 is linked to longer life. Clear cell RCC and distant metastases are frequently linked to Y chromosome deletions. Furthermore, chromosomal trisomy 7 is a common abnormality in clear cell RCC. Additionally, the c-Myc oncogene is coded by chromosome 8q, which is linked to metastatic disease and an increased chance of dying from cancer. A further twelve percent of the genes linked to clear cell RCC are *SETD2* (12%), *BAP1* (10%), *PBRM1* (around 40–50%), and *KDM5C* (5%). These genes play a role in histone methylation and chromatin remodeling. One gene linked to tumor growth and metastasis, *PBRM1*, may be a useful indicator for determining the prognosis of clear cell RCC (Wolf et al., 2020).

Papillary RCC type I and type II share similarities in their morphology, however they differ cytologically due to discrete gene changes in each type. At least three subgroups of papillary RCC type II have been identified by the national institute of health (NIH) based on genetic and phenotypic characteristics. Papillary RCC type one is an autosomal dominant disorder characterized by overexpression of the *MET* gene and several genetic defects. Chromosome 7q31 is where the oncogene *MET* is located. Some individuals with this kind of papillary RCC may present with solitary lesions that eventually proceed to an aggressive course, whereas other patients may present with bilateral, multifocal, and indolent clinical history. Although, papillary RCC type one has a better prognosis and a lower grade tendency than papillary RCC type II (Klatte et al., 2009). Papillary RCC type II is less likely to be related with MET gene mutation than papillary RCC type I. The majority of gene modifications observed in this kind of cancer include increased expression of the *NRF2*-antioxidant response element (*ARE*) pathway, mutations in the genes *SETD2*, *BAP1*, *PBRM1, TERT, NF2, FH*, and fusions of *TFE3*. Silencing of *CDKN2A* and *CPG* islands. Chromosomal Y deletion in men and trisomy of chromosomes 7q, 8, 12, 16, 17, and 20 are

commonly associated with papillary RCC type one Patients with RCC papillary type two often experience loss of 1p and 9p chromosomes and gain of an 8q chromosome (Furge et al., 2007). A rare kind of kidney cancer called chromophobe RCC develops in the distal part of the nephrons. The malignancy rate of this kind of cancer is about 5-6%. Two types of cellular components are present in chromophobe RCC: type 1 is composed of tiny, somewhat granular cytoplasm, while type 2 is numerous and has denser eosinophilic cytoplasm along the cell's periphery. Most RCC cases with chromophobes are sporadic (Nickerson et al., 2002).

A germline mutation in the *PTEN* gene also increases the risk of developing neoplasms resembling chromophobe or oncocytomas, which is another characteristic of Cowden syndrome. Furthermore, chromosomal abnormalities such as the loss of chromosomes 1, 2, 6, 10, 13, 17, and 21 are represented by chromophobe RCC. The classical type of chromophobe RCC has a higher frequency of chromosomal abnormalities in chromosomes 1, 2, 6, 10, 13, and 17 than the eosinophilic type, suggesting a higher degree of chromosome instability in this type. In addition to these chromosome losses, chromophobe RCC also shows gain copies of chromosomes 4, 7, 11, 12, 14q, and 18q (Tan et al., 2010). Furthermore, a mutation on the short arm of chromosome 7 is related to the deletion of the *mTOR* gene, a tumor suppressor and c-kit activator. A modest frequency of somatic mutation of *TP53* has been reported in chromophobe RCC, whereas germline mutations of the *PTEN* gene are the most frequent gene modification in this kind of renal cancer. Common mutations in *ZNF765*, *PRKAG2, ARID1A*, *ABHD3*, *FAAH2*, *PDHB, PDXD1*, and *ZNF765* are also associated with chromophobe RCC (Durinck et al., 2015).

2.2 Cell migration

The development of successful cancer treatments is facilitated by the regulation of cell migration, which is a crucial stage in the invasion and spread of malignancies. Cancer cells can move in a variety of ways, depending on their type and level of development. Different mechanisms govern the many types of cell migration. The actin cytoskeleton's rearrangement is the primary mechanism of cell motility and a prerequisite for the majority of cell migration processes (Yamazaki et al., 2005).

Since cancer cells spread into tissues during invasion and metastasis on their own, controlling cancer cell migration is a critical challenge in the treatment of tumors. Cell migration in non-neoplastic cells, such as fibroblasts and epithelial cells, has been thoroughly examined. The molecular pathways supporting cell migration are comparable in cancer and non-neoplastic cells. Cell migration involves multiple routes, each of which is regulated by a distinct signaling molecule. (Ridley et al., 2003).

For most cells, cell migration depends on the actin cytoskeleton and its regulatory proteins. The force required for cell migration is produced by the dynamic remodeling of the actin cytoskeleton occurring during cell migration. Understanding the molecular mechanisms behind actin rearrangement is important for developing effective cancer therapies, as blockage of these processes reduces cell motility. Actin polymerization that is dependent on the Arp2/3 complex and how it is regulated are particularly interesting (Pollard and Borisy., 2003).

As the polymers expand beneath the plasma membrane, motile cells form a branching network of actin filaments that generates physical force, creating a leading edge. Mathematical models of the constituent conditions predict the motion rate, and a core set of proteins (actin, Arp2/3 complex, profilin, capping protein, and ADF/cofilin) may be able to reproduce the process in vitro. The Arp2/3 complex is activated by signaling pathways that

focus on WASp/Scar proteins. This complex facilitates the beginning of new filaments as branches on existing filaments. After a brief growth burst, the capping protein stops the filaments from growing. ADF/cofilin proteins promote debranching and depolymerization after the filaments hydrolyze bound ATP and γ phosphate dissociation. In order to prepare it for elongation, profilin catalyzes the exchange of ADP for ATP, which refills the pool of ATP-actin monomers bound to profilin (Pollard and Borisy., 2003).

2.2.1 The actin cytoskeleton

Actin is the main component of the cytoskeleton of eukaryotic cells. Apart from providing the structural foundation for cellular morphogenesis and migration, it is also actively involved in processes such as cell adhesion, intracellular vesicular transport, extracellular material intake, and mechanical resistance to deformation. Actin also plays a role in the organization of complex cellular structures such the cytoskeleton, lamellipodia, filopodia, and podosomes (Azizi et al., 2020). The structural protein that forms microfilaments is called actin. It typically takes two forms: polymer and monomer. One of these is globular actin, also known as monomer actin, which is a polypeptide chain-based spherical molecule. Actin polymers are also known as fibroactin since they are responsible for the production of actin filaments. The actin cytoskeleton is regarded as an essential subcellular filament system. It reacts with several related proteins and regulatory cells that control vital processes such as muscle contraction, cell division, cell migration, and tissue integrity (Jiang et al., 2021).

Every cell's integrity depends on the actin cytoskeleton. The creation of fundamental cellular structures necessary for appropriate cell shape, motility, engulfment of particles and cellular fragments, and interactions between various cell types is supported by a dynamic balance between G-actin monomers and F-actin filaments. As the cytoskeleton is essential for both innate and adaptive responses, it is particularly essential for immune cells, specifically actin. To establish synapses that transfer instructions or eliminate infected

cells, leukocytes have to attach to both substrates and other cells. After the blood spreads, neutrophils efficiently reach the region of inflammation by actually squeezing the cell body. In addition, the cytoskeleton needs to be remodeled for immune cell development because it is dependent on transportation and adhesive interactions, among other mechanisms (Tur-Gracia et al,. 2021).

2.2.2 Arp2/3 complex

2.2.2.1 *Structure of Arp2/3 complex*

The process behind the advancement of the leading edge of motile eukaryotic cells is based on the Arp2/3 complex, a stable assembly of two actin-related proteins and five unique protein subunits. The structure and subunit organization of the Arp2/3 complex play significant roles in this process as well a flat spheroid measuring roughly 150 Å long, 140 Å broad, and 70 to 100 Å thick (Robinson et al., 2001).

Arp2 and Arp3, actin-related proteins, comprise two of these subunits. The complex's crystal structure reveals an inactive conformation in which the architecture supplied by the five additional subunits, referred to as actin related protein subunit 1 to 5, maintains Arp2 and Arp3 far apart (Figure 2.3) (Molinie and Gautreau., 2018). Arp2/3 complexes produce actin networks with branches. Electron microscopy revealed the structure of the branching junction formed by two actin filaments in the active conformation, which is facilitated by the Arp2/3 complex. Arp2 and Arp3 must be brought together within the complex in order for them to adopt the actin molecule configuration found in a filament. This is known as an active conformation (Madasu et al., 2015).

But in the absence of actin, this form can also be found in a population of soluble Arp2/3 complexes. Examples of nucleation promoting factors (NPFs) that dramatically raise the population of Arp2/3 complexes in the active conformation are N-WASP and

WAVE. The elongation of a lateral branch, also referred to as the daughter actin filament, may then be started by the conformationally active Arp2/3 complex interacting with an already-existing actin filament, also known as the mother filament. The widely used medicinal products CK-666 binds to both Arp2 and Arp3, inhibiting Arp2/3, thus blocking the conformational rearrangement required for Arp2/3 activation (Molinie and Gautreau., 2018).

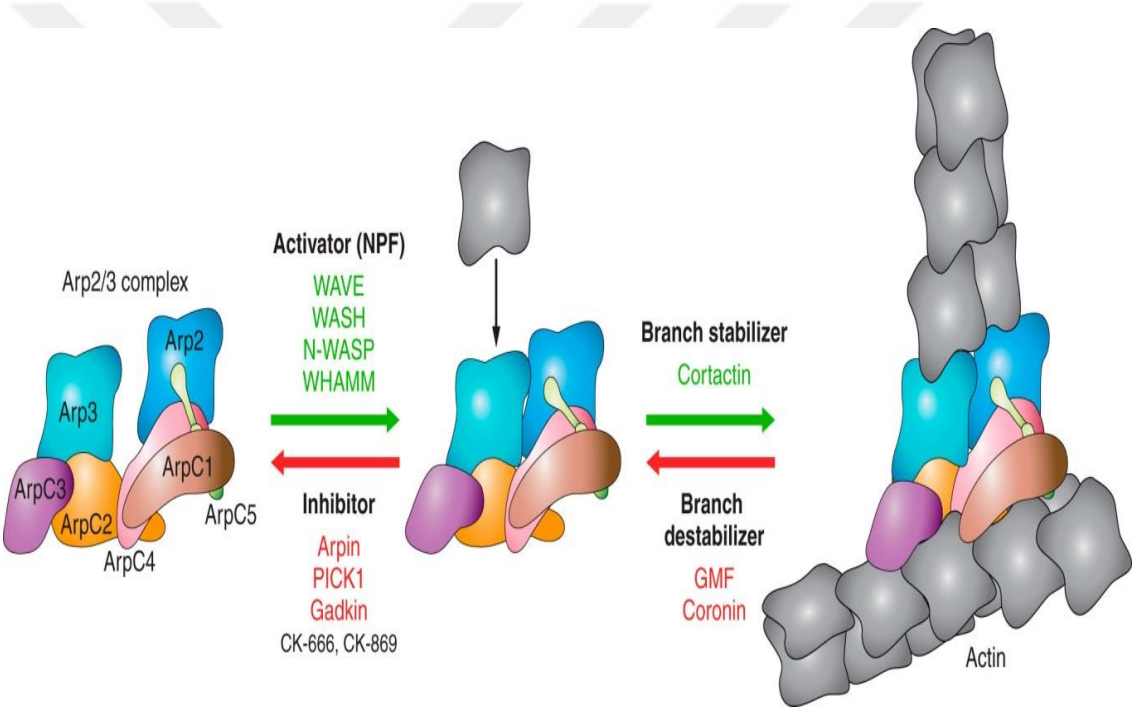


Figure 2.3. Arp2/3 Complex Conformations and The Regulators of Their Equilibria. (Molinie and Gautreau., 2018).

2.2.3 The Arp2/3 complex in cancers

Immunohistochemistry has demonstrated that overexpression of Arp2/3 subunits occurs in a number of cancer types, including lung (Semba et al., 2006), breast (Iwaya et al., 2007), colorectal cancers (lawya et al., 2007), and gastric (Zheng et al., 2008). When one Arp2/3 subunit is present, it probably means that the complex as a whole exists even if the subunits work together to form a complex. As an example, serial sections stained similarly when Arp2 and Arp3 antibodies were used. Nevertheless there has also been research into a free pool of *ARPC1B* linked to centrosomal homeostasis (Molli et al., 2010). In lung, breast, and colorectal carcinomas, in three articles, Arp2 and one of its NPFs, *WAVE2*, were simultaneously overexpressed at the same distinct cells. This shows that overexpression of both *WAVE2* and the Arp2/3 complex within tumors, which are often diverse, corresponds to a coordinated program. At the invasive front of colorectal and breast cancers, double-positive cells were frequently found (Lawya et al., 2007).

Breast cancer is the most common type of cancer in women, and invasion and metastasis are the two major ways that individuals with breast cancer pass away from their illness. The naturally occurring dimethylether analog of resveratrol, pterostilbene, has been shown to have anti-cancer properties. Pterostilbene successfully prevented serum-induced migration and invasion while maintaining the survival of breast cancer cells. Furthermore, pterostilbene greatly decreased the expression of *WASP*-family verprolin-homologous protein-2 (*WAVE*-2) and (Arp2/3). Taking everything into account, pterostilbene greatly reduced cell migration and invasion via blocking the Rac1/*WAVE*/Arp2/3 pathway and NF-κB-mediated synthesis of uPA (Ko HyunSuk et al., 2014).

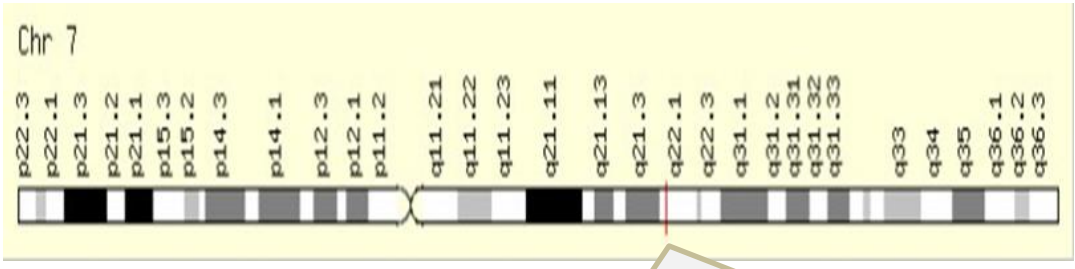
Gastric carcinomas, surrounding metaplasia, and gastritis were all included in tissue microarrays for immunohistochemistry (IHC) using antibodies against Arp2 and Arp3. The expression of these proteins was compared to the clinic pathological characteristics of carcinomas. Examining the Arp2 and Arp3 proteins in gastric cancer cell lines, most of the cell lines showed variable levels of protein expression. The degree of invasion, venous

invasion, and tumor size all showed favorable correlations with expression. The Arp2 and Arp3 proteins were found to be positively correlated ($p<0.05$). According to univariate analysis, patients with positive Arp2 or Arp3 expression did not have a different cumulative survival rate than patients without such expression. This suggests that abnormal expression of Arp2 and Arp3 may have a role in the etiology, development, invasion, and advancement of gastric carcinomas. The differentiation of intestinal and diffuse-type carcinomas is influenced by the expression of Arp3, which serves as a useful indicator of stomach cancer pathobiological characteristics (Zheng et al., 2008). The genome-wide gene expression analysis and bioinformatics research have suggested that miR-133a may target actin-related protein 2/3 complex subunit 5 (*ARPC5*). Furthermore, miR-133a directly regulates *ARPC5*, as demonstrated by the luciferase reporter test. Following ARPC5 silencing, significant decreases in cell migration and invasion were seen in HNSCC cell lines, SAS, HSC3, and IMC-3. Compared to non-cancerous tissues, *ARPC5* expression levels were significantly higher in HNSCC tissues. The results of immunohistochemistry demonstrated that invasive cancer cells expressed *ARPC5* at much higher levels. In HNSCC, miR-133a directly regulated *ARPC5*, which facilitated cancer cell motility and invasion. (Kinoshita et al., 2012).

One feature of directed cell migration is localized actin polymerization at the leading protrusions of the cell. At the leading edge of motile cells, the Arp2/3 complex creates the lamellipodia, or dendritic actin network. A favorable connection ($P=0.02$) was found by immunofluorescence and western blotting between the expression of Arp2/3 and the malignancy of glioma tissues. The Arp2/3 complex was also found to be concentrated in the lamellipodia of glioma cells, according to confocal recording. Additionally, the consequences of CK666, an Arp2/3 complex inhibitor, on glioma cells were studied. The Arp2/3 complex plays a crucial role in the invasion and migration of glioma cells also could be a target for therapeutic intervention (Liu et al., 2013).

2.2.4 ARPC1A gene

One of the seven subunits that comprise the human Arp2/3 protein complex is encoded by *ARPC1A* gene. The subunit, which belongs to the SOP2 protein family, is most like the protein that the gene *ARPC1B* encodes. These two proteins may be the p41 subunit of the human Arp2/3 complex, which regulates the actin polymerization process within cells, based on their similarity. The Arp2/3 complex's structural integrity may be assembled and maintained by the p41 subunit. The p41 subunit can exist in many forms, which could allow the complex to adapt its actions to various cell types or developmental phases. There are many isoforms of this gene encoded by alternatively spliced transcript variants that have been found. The *ARPC1A* gene is located on chromosome 7 (Figure 2.4). The protein that is produced by this gene is a component of the Arp2/3 complex, which regulates the movements of the actin cytoskeleton. The Arp2/3 complex is vital for various biological processes, for example cell motility, endocytosis, and cytokinesis (Laurila et al, 2009).



Gene location: *ARPC1A* is located on chromosome 7 (7q22.1).

Gene structure: The *ARPC1A* gene consists of 10 exons that encode a protein of 356 amino acids.

Figure 2.4. Genome Location

(Laurila et al, 2009).

The *ARPC1A* length is 43423 base pairs and this gene's mRNA includes 1582 base pairs (NM_006409).

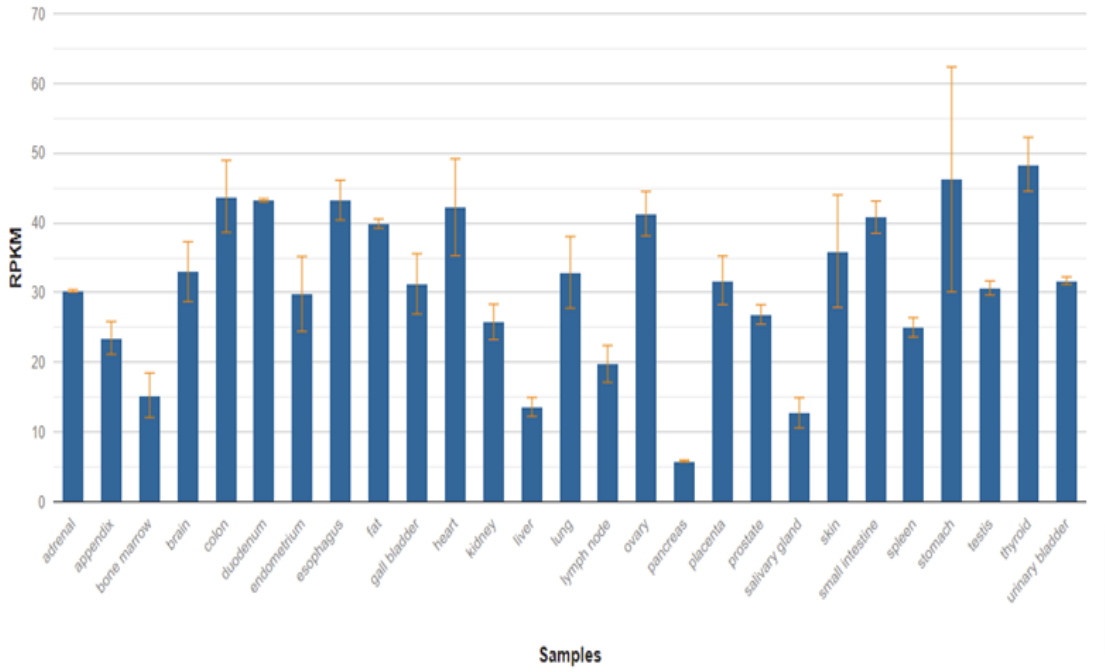
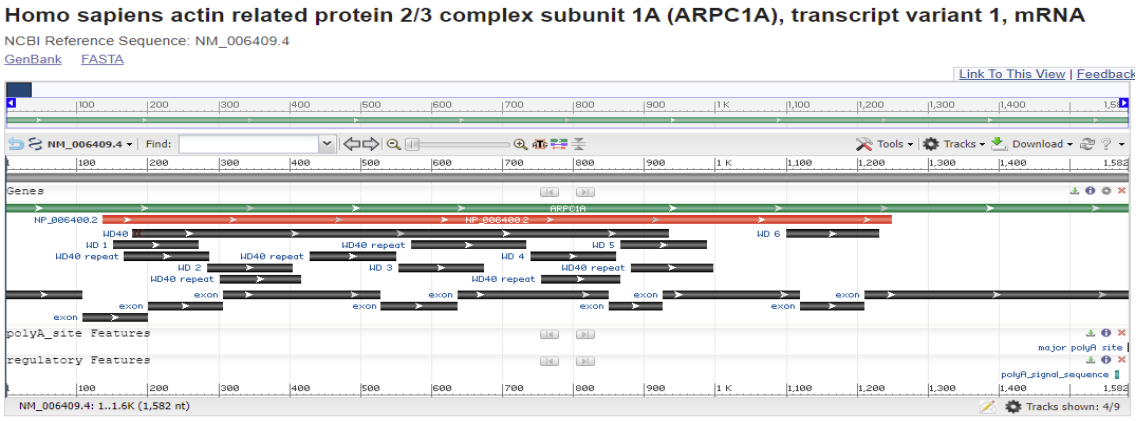


Figure 2.5. *ARPC1A* Gene Expressions in Difference



(Ji et al,. 2022).

Figure 2.6. *ARPC1A* mRNA

(https:///www.ncbi.nlm.nih.gov/gene).

2.2.4.1 ***Gene expression, regulation, and its importance***

One gene that codes for a protein is identified as *ARPC1A,* or actin-related protein 2/3 Complex subunit 1A. Actin dynamics control for the development of phagocytic caps and EPH-Ephrin signaling are two of the pathways involved. Actin filament binding is one of this gene's gene ontology (GO) annotations. *ARPC1A* has been knowned as a hub gene. Significant expression of *ARPC1A* was found in PCa tissues and cell lines, and it was found to be an independent predictor of both overall patient survival and biochemical recurrence after radical prostatectomy. Although ARPC1A knockdown had no effect on cell proliferation or cell cycle progression, it did reduce PCa cell invasion, migration, and cytoskeleton formation. While *ARPC1A* overexpression had little effect on tumor development in vivo, it did enhance lung metastasis of PCa. Moreover, it was found that one upstream regulator of *ARPC1A* was glutamine metabolism, which in turn stimulated PCa cell motility, invasion, and cytoskeletal alterations. These results revealed that *ARPC1A* regulates cytoskeletal alterations, cellular migration, and cellular invasion in PCa by acting downstream of glutamine metabolism, which is correlated with a poor prognosis in this disease (Chen et al., 2023).

Ferroptosis was made harder by *ARPC1A* knockdown. Luciferase reporter assays, Co-IP, ChIP, and signal transduction and activators of transcription 3 (STAT3) were used to clarify the transcriptional regulation mechanism of STAT3 on ARPC1A. The in vitro results have been verified by in vivo investigations. In a ferroptotic way, decreased *ARPC1A* expression prevents prostate cancer cells from developing and spreading. Prostate cancer patients' prognosis may be independently predicted by their *ARPC1A* level (Ji et al, 2022).

in an investigation, involving patients suffering from the inflammatory illness eosinophilia, microthrombocytopenia, Kahr et al. investigated the expression of Arp2/3 components in patient platelet lysates and in normal, unaffected family members using

immunoblot analysis. The findings demonstrated that the levels of *WASP* and other Arp2/3 components (*ARP2*, *ARP3*, *ARPC2*, *ARPC3*, and *ARPC5*) were normal in all patients and unaffected family members. The study suggested that the level of *ARPC1B* was found in less than 6% of cells and tissues, and it varied greatly between them. Also *ARPC1A* was expressed more in *ARPC1B*-deficient cells and less in normal platelets in comparison to *ARPC1B* (Kahr et al., 2017).

A study into the actin polymerization of the Vaccinia virus distinct functions for *ARPC1B* and *ARPC5B* were discovered. Actually, it was discovered that Arp2/3 complexes including ARPC1B and ARPC5B were strongly more successful in stimulating actin formation than those containing ARPC1A and ARPC5A. (Abella et al., 2016).

The seven-subunit Arp2/3 protein complex, a crucial player in actin polymerization and controls cell motility. This is supported by the complex's demonstrated expression in highly mobile cells like lymphocytes and macrophages. It's interesting to know that gastric cancer has been correlated with decreased expression of the Arp2/3 complex. In the other hand, higher levels of the complex has been frequently reported in a number of different cancers, such as lung, colorectal, and breast cancer. It is connected to the invasion and spread of cancer cells. Here, we argue that overexpression of two Arp2/3 complex subunit genes, *ARPC1A* and *ARPC1B*, is caused by amplification of the 7q21-q22 area and increases the motility of pancreatic cancer cells. It has been suggested that the p41 subunit that ARPC1A and ARPC1B encode controls the Arp2/3 complex's activity. Thus, it has been proposed that having too much of these proteins may significantly impact the complex's functionality, making ARPC1A and ARPC1B intriguing possibilities for anticancer treatment(Laurila,. 2012). The *ARPC1A* gene also belongs to a protein complex that regulates cell motility. It has been demonstrated that deleting this gene significantly decreases cell motility in pancreatic cancer cells, a crucial component linked to the spread of this cancer. (Laurila et al,. 2009). In a number of experimental scenarios, it has been

demonstrated that actin nucleation by the ARP2/3 complex is necessary for tumor cell invasion. Strong *ARP2* expression has been found in both tumor and stromal cells from colorectal tissues determined in human cancers. In addition, there was a significant decrease in cell invasion in pancreatic cancer cells when *ARPC1A* was silenced (Zucchini et al., 2014). The expression of multiple molecules essential for actin cytoskeleton remodeling is inhibited in osteosarcoma cells by transfection of CD99wt. These molecules include ezrin, an ezrin/radixin complex, Rho-associated, coiled-coil-containing protein kinase 2 (ROCK2), and (*ARPC1A*) (Khanna et al., 2004; Park et al., 2006). when RNA interference (RNAi) suppressed *ARPC1A*, which codes for the p41 component of the Arp2/3 protein complex, in AsPC-1 cells, Reduced migration, invasion, and proliferation of cells were seen, indicating that Arp2/3 plays an essential role in actamer polymerization (Aseervatham, 2020).

3. MATERIAL AND METHODS

3.1 Material

3.1.1 Working group

Eleven patients provided twenty-two samples. 11 kidney cancer samples and 11 control samples were obtained from the same individuals who applied to the Tokat Gaziosmanpasa University Faculty of Medicine, Department of Urology. These samples were used in the study. Kidney normal tissues from each participant's identical kidney constituting the control group, compared to kidney tumor tissue samples collected after surgical operations made up the patient group after the patients gave their agreement and awareness. The Clinical Studies department of Tokat Gaziosmanpasa University's Faculty of Medicine obtained the necessary approval for the investigation for this study. The investigation was conducted in the genetics and medical biology labs under project number: 22-KAEK-045.

3.1.2 Tissue Storage

Following the collection of 11 kidney cancer tissue samples and 11 control tissue samples from the same patients, part of the tissue samples were extracted, put into separate tubes, and immediately preserved for use in RNA isolation, cDNA synthesis, and ARPC1A gene expression analysis at -80 °C.

3.1.3 Tools and Equipment Used in the Study

1. -80 Deep Freezer (Nuaire, NU-9483E, USA).
2. Refrigerator (Arçelık, 570465 MB, Turkey).
3. Centrifuge (Hettich, D'78532, Germany).
4. Refrigerated Centrifuge (Hettich. Germany).

5. Microcentrifuge (Mikro120, Hettich Zentrifugen D- 78 532n).
6. Vortex (Velp Scientifica; F20220176, Italy).
7. Micropipette Set (Gilson, Thermo Scientific, Finnpipette).
8. PCR Device (MiniAmo Plus Thermal Cycle).
9. Qubit 3.0 Fluorometer (Invitrogen, Life Technology, Australia).
10. Real Time PCR (Applied Biosystems, UK).
11. Homogenizer (Heildolph Silent Crusher, Finnpipette).
12. Plate (Applied Biosystem, 4346907, UK).

3.1.4 Kits and chemical substances used

Isolation of RNA kit (Thermo Scientific GeneJet RNA Purification kit, Lithuania, LOT: (01125929)

- Washing buffer1
- Washing buffer2
- Lysis buffer
- Nuclease-free water
- Proteinase K
- Ethanol (Sigma-Aldrich Catalog No: E7023, USA)
- RNA purification column collection tube

cDNA synthesis kit (Thermo fisher scientific high-capacity cDNA reverse transcription kit, Lithuania, LOT: 01152470)

- Multiscribe™ Reverse transcriptase enzyme
- 10× RT buffer
- 25× dNTP mix (100 mM)
- 10× RT random primers
- Nuclease-free water

Qubit dsDNA assay kit Qubit™ 1X dsDNA HS assay kit (Invitrogen catalog No: Q33230, USA)

- Qubit™ 1X dsDNA HS working solution (component A) 50 µl
- Qubit™ 1X dsDNA HS RNA standard1 (component B) 1 mL
- Qubit™ 1X dsDNA HS RNA standard2 (component C) 1 Ml

For quantitative real time-PCR (qRT-PCR)

The 2× Master Mix Green with (High ROX™) (Enzo, Cat. No.: ENZ– NUC 1000, Lot No: 05272212) kit.

- Forward primer
- Reverse primer
- Template cDNA
- Nuclease-free water

3.2 Method

Tissues that we obtained from the patient we kept frozen at -80°C, and sections of those tissues are used to analyze gene expression. After taking out the specimens that were stored at -80°C. Then the samples are ready for processing:

3.2.1 RNA isolation and purification

The protocol of RNA isolation:

1. Frozen tissue samples were dissected into small segments using a medical scalpel and homogenized.

2. The tissues that had disintegrated were transferred directly into a 1.5 µl microcentrifuge (eppendorf) tube.

3. The 300 µl of Lysis Buffer were added to the samples and vortexed for 10 seconds to mix thoroughly.

4. After that, 600 µl of proteinase K that had been diluted were added and thoroughly mixed by vortex (Proteinase K (10 µl) was diluted in TE buffer (590 µl).

5. The mixture was incubated at 25 °C for 10 minutes.

6. The result mixture was then centrifuged for 5 minutes at 12000 × g.

7. After that, the supernatant was transferred by pipette into new RNAse-free micro centrifuge tubes.

8. Then, each of the sample tubes received 450 µl of ethanol (96%), and by pipetting, it was mixed.

9. After that, the tubes were changed, and the samples were moved into the collection tube. Transferred up to 700 µl of lysate to them (GeneJET RNA Purification) column putted in a collection tube.

10. The column was centrifuged for 1 minute at 12000 × g.

11. Next, the tubes storing the flow-through solution were thrown out of, and the filters for the GeneJET RNA purification column were placed into a new 2 µl collecting tube.

12. The 700 µl of Wash buffer 1 (enhanced with ethanol) was added to each of the GeneJET RNA purification column and centrifuged for 1 minute at 12000 × g.

13. Later that, the purification column was reinserted into the collecting tube and the flow-through solutions were disposed of.

14. The 600 µl of Wash Buffer 2 (enhanced with ethanol) was added to each of the GeneJET RNA Purification Column and centrifuged for 1 minute at 12000 × g.

15. The flow-through was discarded, and the purification column was placed back into the collection tube.

16. After that, the GeneJET RNA purification column (filters) was transferred into asterile 1.5 µl RNase-free microcentrifuge tube.

17. Then, 100 µl of nuclease-free water was added and mixtures were centrifuged for 1minute at 12000 × g.

18. At the end, the purification columns (filters) were discarded, and the RNA purification was prepared.

3.2.2 Synthesis of cDNA

The cDNA was created once the purified RNA was prepared. Following the instructions included with the kit, the High Capacity cDNA Reverse Transcription Kit (Lithuania, LOT: 01152470) was used in this step. All the components needed for the cDNA synthesis process are included in this kit (Table 3.1), which is divided into eight micro tubes. The RNA samples were then added to each tube, totaling 20 microliters, and the mixture was then put into the tubes. cDNA synthesis was done in two steps:

1- The 10 µl of 2× RT master mix was added to each tube.

2- Then, added the 10 µl of RNA samples that were already prepared to each tube, and it was mixed by pipetting twice.

3- The tubes were sealed.

4- Mixtures were centrifuged briefly until the contents spun down.

5- The tubes were put on ice until the next step.

Table 3.1. The Components for Reverse Transcriptase Reaction per Samples

Components	Volume
10× RT buffer	2 µl
25× dNTP mix (100 mM)	0.8 µl
10× RT random primers	2 µl
MultiScribe™ reverse transcriptase	1 µl
Nuclease- free water	4.2 µl
Isolated RNA sample	10 µl
Totality amount	20 µl

Eventually, the solution mixture, which contained RT master mix constituents and isolated RNA samples, was prepared and placed in a thermal cycler to undergo 33 cycles of reverse transcriptase PCR. These conditions were used for the thermal cycler program that displayed in Table 3.2.

Table 3.2. qRT-PCR Program Was Utilized for the Synthesis Of cDNA

RT reaction	Temp (C)	Time
Step 1 Annealing	25 °C	10 minutes
Step 2 Elongation	37 °C	120 minutes
Step 3 Termination	85 °C	5 minutes
Step 4 continuing	4 °C	∞

3.2.3 Measuring the concentration of cDNA

In this step, the kit that was used for measuring the cDNA concentration is the Qubit™ 1X dsDNA HS Assay Kits ((Invitrogen, Q33230). The Qubit 3.0 machine was used.

The steps were done as below:

1- According to the number of samples and the two standards, the essential number of 0.5 µl PCR tubes was prepared and labeled.

2- The 190 µl of Qubit™ working solution was added to these tubes, which were prepared to standards.

3- Then, 10 µl of standard 1 and standard 2 were added into the seemingly PCR tubes, and they were vortexed for 3 seconds. The terminal volume was 200 µl.

4- 190 µl of working solution with 10 µl of cDNA samples was added to the testing PCR tubes, so the terminal amount was 200 µl.

5- Then, they were mixed by vortex for 3 seconds.

6- For 2 minutes, all tubes were incubated at room temperature.

7-The Qubit 3.0 Fluorometer was used, and the tubes containing standards were inserted. First, standard 1 was read, and after that, standard 2.

8- The sample tubes were inserted into the sample chamber of the Qubit 3.0 device and read.

3.2.4 Quantitative real time polymerase chain reaction (qRT-PCR)

Real-time PCR is a well-researched method that consists of two forms: probe-based and intercalator dye-based. It is used to determine the PCR template copy number, such as DNA or cDNA. SYBR Green is an example of a free fluorescent dye that binds to deoxyribonucleic acid (dsDNA) and releases fluorescence when bound (Singh et al,. 2014). Every cycle, the fluorescence scale is measured to determine the quantity of amplified inventions (Narrandes and Xu, 2018).

In this step, to analyze the ARPC1A gene expression level, qRT-PCR was performed after the isolation of RNA to generate cDNA. The 2× Master Mix Green with (High ROX™) (Enzo, Cat. No.: ENZ – NUC – 1000, Lot No: 05272212) kit was utilized. The MsterMix was mixed with the forward and reverse primers Table 3.3. The *ARPC1A* gene as a target gene and *ACTB* gene as a control gene in cancer and healthy tissues of the human kidney were used, along with Master Mix and other components of the kit Table 3.4

Table 3.3. Primers for ARPC1A and ACTB Genes Used in qRT-PCR

ARPC1A-F	5’TGGGTGAGCAAGCAACCACATTAAA3’
ARPC1A-R	5’CACTGCCACCAAACTCTGAC3’
ACTB-F	5’ GCATGGGTCAGAAGGATTCC 3’
ACTB-R	5’ CACGCAGCTCATTGTAGAAGG 3’

Table 3.4. The Components for the Quantitative Real Time-PCR Reaction.

No.	qRT-PCR reaction components	Volume
1	2x Master Mix Green High ROX™	12.5 µL
2	Forward primer	0.5 µL
3	Reverse primer	0.5 µL
4	dH2O	8.5 µL
5	cDNA sample (Template)	3.0 µL
Total volume		**25.0 µL**

For the applied biosystems qRT-PCR device, the Reaction cycle was programmed as detailed in (Table 3.5).

Table 3.5. The Protocol of qRT-PCR.

Stages	Temperature	Time	Cycles
Stage 1 Denaturation	95.0 °C	15 minutes	X1
Stage 2 PCR Amplification	95.0 °C	15 second	X 40
	60.0 °C	1 minutes	
Melt Curve	95.0 °C	30 second	X1
	60.0 °C	15 second	
	95.0 °C	15 second	

3.2.5 Statistical evaluation of ARPC1A mRNA gene qRT-PCR data

Following the determination by RT-PCR of the *ARPC1A* gene expression at the mRNA level in kidney cancer and control kidney tissues. The Step One Plus software v2.3 program was used to quantify the RT-PCR data for the *ACTB* and *ARPC1A* genes. Absolute quantification, which employs a standard cure to ascertain the target gene's copy number, is employed when a precise number of amplifications are required. Comparative quantification, on the other hand, compares the data obtained from a reference gene (internal control gene) to the data obtained from the target gene. The *ACTB* gene, an internal control gene and a frequently used reference gene to normalize and reduce mistakes while amplifying with the target gene was utilized to normalize the data about the *ARPC1A* gene using relative quantification. The 2-ΔΔCt method, which computes the fold change, was applied in conjunction with comparative CT (Table 3.6). The mean ± SEM was used to show the data, and the 0.9–1.1 range was used to examine the findings. In kidney cancer tissue, the linked gene's expression level was lower than in normal kidney tissue if the values were less than 0.9. If it was in the range of 0.9–1.1, it did not differ from normal kidney tissue. Additionally, if the result exceeded 1.1, the gene's expression was higher than it was in normal kidney tissue (Livak & Schmittgen, 2001).

With SPSS 20.0, the expression data were statistically analyzed. The Clinical and demographic characteristics of kidney cancer patients were expressed as mean± SEM or percentage. The Paired t-test was used to ascertain the correlation of the *ARPC1A* gene between normal and abnormal tissues. The acceptable statistical significance threshold was set at 0.05; if P is less than 0.05, then the data was considered significant, indicating a substantial degree of difference. On the other hand, if $p>0.05$, there was no significant difference between the samples and the data value was deemed unimportant. Table 3.6. The $2^{-\Delta\Delta CT}$ equation for kidney cancer samples and control.

Table 3.6. The $2^{-\Delta\Delta CT}$ Equation for Kidney Cancer Samples and Control Samples (Livak & Schmittgen, 2001)

4.

- **Fold change = $2^{-\Delta\Delta CT}$**

  $2^{-\Delta\Delta CT}$ = [(C_T target gene – C_T reference gene) Cancer Sample **–**

  (C_T target gene – C_T reference gene) Normal tissue].

- **For Kidney cancer sample:**

  ΔC_T (cancer tissue) = Ct (*ARPC1A* gene) - Ct (*ACTINB* gene)

- **For control samples:**

  ΔC_T (Normal tissue) = Ct (*ARPC1A* gene) - Ct (*ACTINB* gene)

- **Each sample ΔΔCT value is obtained by subtracting both.**

  $\Delta\Delta C_T = \Delta C_T$ (cancer tissue) - ΔC_T (Normal tissue)

4. RESULTS

Diagnosis of kidney cancer was given to study participants who applied to the Tokat Gaziosmanpasa University Faculty of Medicine urology Department. Eleven of them have been found to have kidney cancer. qRT-PCR was used to examine the expression of the *ARPC1A* gene in both healthy and cancerous biopsies from research participants.

The patients' ages ranged from 39 to 75 years, with a mean age of 62 ± 11.61. As seen in (Figure 4.1), five of the patients were men and six were women. Six patients had cancer in stage I, one patient had cancer in stage II, and four patients had cancer in stage III. The study's sample of kidney cancer patients' clinical and demographic features are distributed below (Table 4.1).

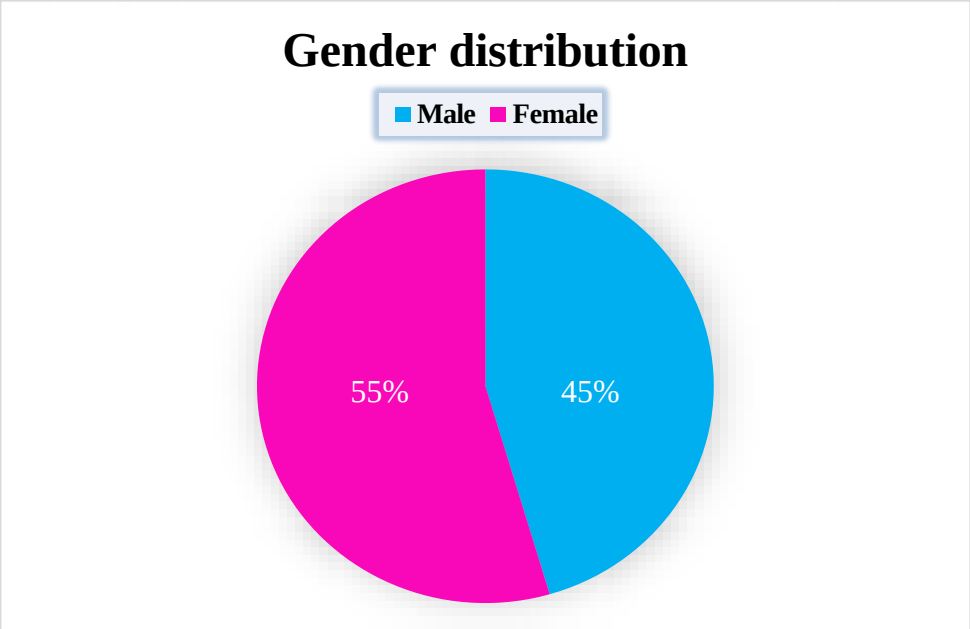


Figure 4.1. Showing Gender Distribution

Table 4.1. The Clinical and Demographic Features of Patients with Kidney Cancer (Demographic)

Number of cancerous kidney samples N=11	
Age, Mean. ± S.D.	62 ± 11.61
Genders	Male = 5 Female = 6
Stages	Stage I = 6 (54%) Stage II = 1 (9%) Stage III = 4 (37%)

Table 4.1. The Clinical and Demographic Features of Patients with Kidney Cancer (Clinical)

Sample ID.	**Genders**	**Age**	**Stage**
1	Male	67	1
2	Male	73	3
3	Female	67	1
4	Female	53	2
5	Female	39	1
6	Female	70	3
7	Male	66	1
8	Female	67	3
9	Female	45	1
10	Male	75	3
11	Male	60	1

4.1 The *ARPC1A* and *ACTB* gene figures from qRT-PCR

The phases of the melting and amplification curves for the data are shown in Figures 4.2 and 4.3, respectively. The derivative reporter view, also known as the melting curve plot, allows us to look at the maximum rate at which fluorescence changes as a function of temperature over time. The amplification plot concurrently displays the rise in product over the duration of the PCR operation. *ARPC1A* is the target gene, whereas *ACTB* is the internal control gene. The amplification curve and melting peak of the *ACTB* and *ARPC1A* genes can be found according to the RT-PCR technology (Figure 4.4) (Figure 4.5).

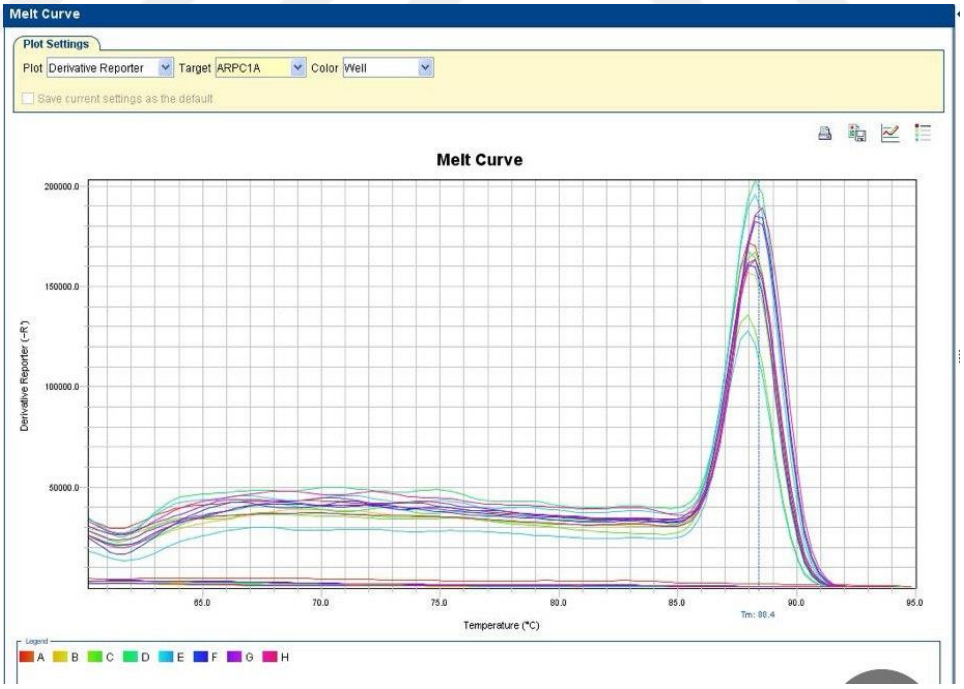


Figure 4.2. Melting Curve of *ARPC1A* in qRT-PCR

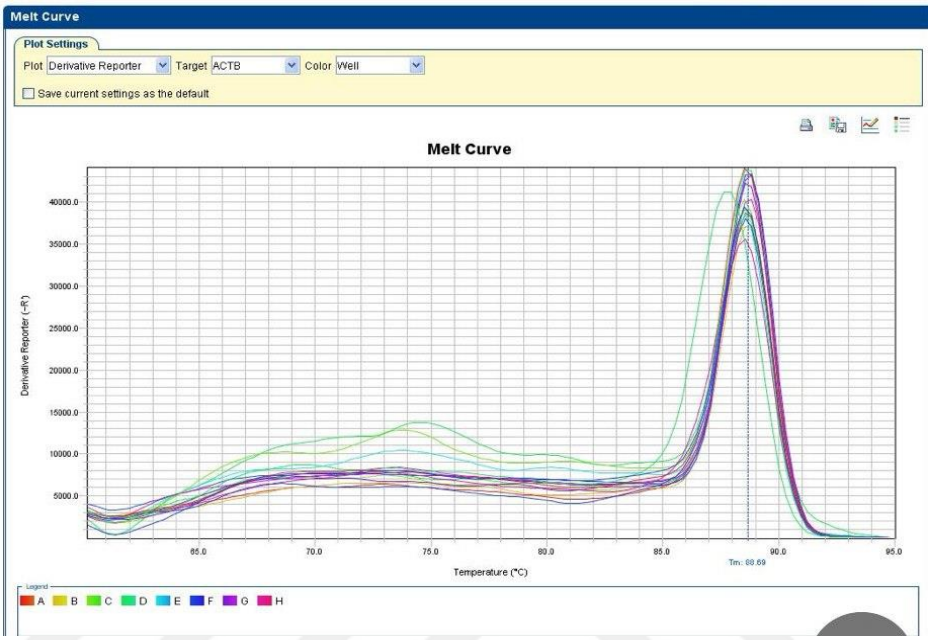


Figure 4.3. Melting Curve of *ACTB* in qRT-PCR

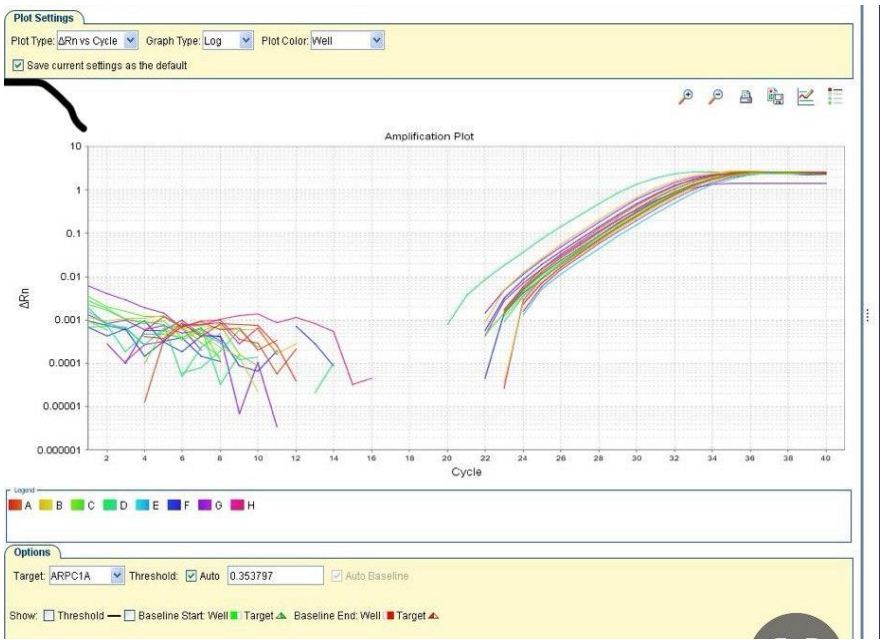


Figure 4.4. Amplification Plot Showing *ARPC1A* Gene CT

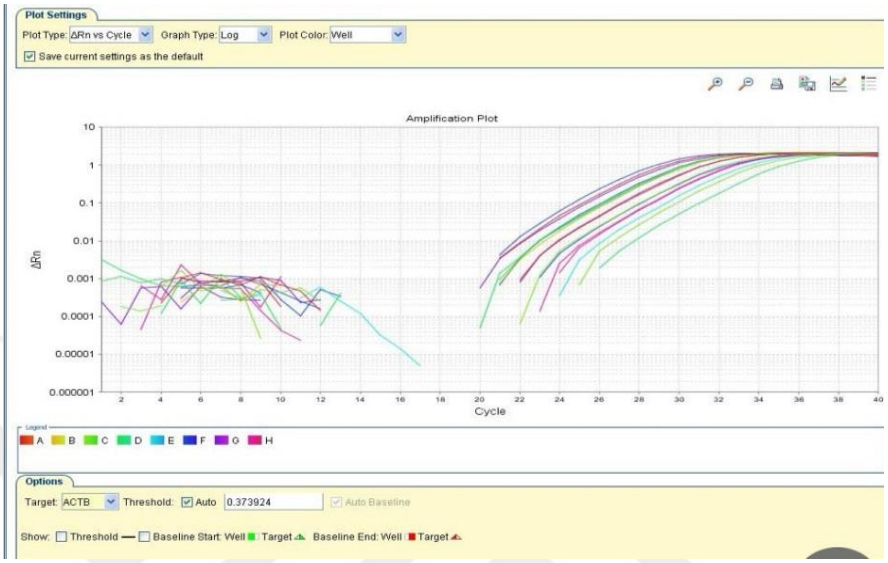


Figure 4.5. Amplification Plot Showing *ACTB* Gene CT

The current study found that the fold change in patients compared to the control group was 1.14 based on the statistical analysis of the RT-PCR results. An illustration of this result is shown in Figure 4.6. According to statistics, there was no discernible difference between the groups ($p = 0.75$). The analysis results are shown in Table 4.3'. The *ARPC1A* gene expression level in kidney cancer tissue was therefore found to be about comparable (not changed) to that of healthy kidney tissue, indicating that there is no difference in *ARPC1A* gene expression between kidney malignant and healthy tissues.

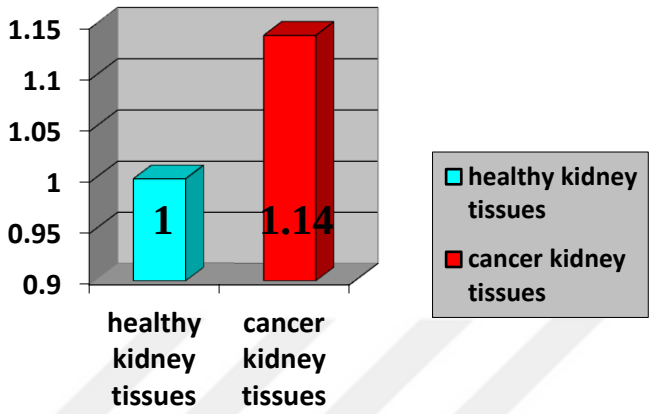


Figure 4.6. The Graph Displays the Fold Change of the *ARPC1A* Gene between Kidney Cancer Tissues (N=11) and Healthy Kidney Tissues.

The relative quantification values of the *ARPC1A* genes were ascertained through real-time PCR. The quantitative expression data of the *ARPC1A* gene was normalized against that of the *ACTB* gene. The fold change of kidney cancer tissues was found to be = 1.14, while the fold change of healthy kidney tissues was found to be = 1.

Table 4.2. The Statistical Analysis of the ARPC1A Gene in Kidney Cancer Tissue and Healthy Kidney Tissue. (SD: Standard Deviation; SEM: Standard Error Mean).

Samples	***ARPC1A* gene (Average)±(SEM)**	**P Value**
Kidney Cancer Tissues (n=11)	**1.14 ± 0.44**	**p=0.75**
Control tissue samples	**1**	

5. DISCUSSION AND CONCLUSION

RCC is a collective term for a range of genetically and clinically distinct disorders. Despite being uncommon, familial RCC disorders offer an attractive model for researching the molecular pathways behind renal carcinogenesis. With the identification of numerous tumor suppressor genes and oncogenes that cause cancer, genetic testing can now determine which carriers and those affected belong to. An understanding of these genes' molecular pathways will be crucial for both familial and sporadic RCC diagnosis and treatment (Cohen and Zhou., 2005).

Renal cell carcinoma (RCC) is a collective term for a collection of cancers originating from the renal epithelium. The histology, clinical background, and primary genetic mutations of these cancers are different. Since response to current treatment regimens is restricted to just a little of patients and cure rates are inversely correlated with stage, new therapeutic modalities and diagnostic techniques that enable early detection are needed. Growing understanding of the pathophysiology underlying renal cell carcinoma (RCC) has led to the identification of genetic changes implicated in the carcinogenesis of renal cell cancer. Promising medicines, some of which are already standard of treatment, are being developed to target these pathways, specifically the angiogenesis way. In recent decades, there has been an important development in our understanding of the role of epigenetics, in addition to genetics, in the process of renal carcinogenesis. The study of epigenetics is expected to contribute greatly to the creation of new treatments and could yield new markers for early diagnosis and prognosis (Baldewijns et al., 2008).

Previous investigation has indicated that the Arp2/3 complex is crucial for the functioning of several cancer types in both in vitro and in vivo conditions. Actin-related protein 2/3 complex subunit (*ARPC5*) is a component of the complex and plays a role in cell invasion and migratory processes. However, there is still much to learn about the expression pattern, biological function, and prognostic importance of *ARPC5* in all types of

cancer. *ARPC5* as the cut point in order to study a possible prognostic and immunological biomarker for malignancies (Huang et al., 2022). Despite the fact that *ARPC2* overexpression increased Brest cancer cell invasion, proliferation, and metastasis through EMT. The findings suggest that *ARPC2* might be an important target for breast cancer treatment or diagnosis (Cheng et al., 2019).

According to Semba et al., there is an independent predictor of tumour recurrence in lung adenocarcinoma that is the expression of *Arp2* and *WAVE2*. The coexpression of these genes was highly correlated with lymph node metastases and changed the overall survival rate of 115 individuals. The association between moderate chemotherapy and coexpression 56 was also examined in this study. Patients who underwent chemotherapy showed a higher disease-free survival rate than those who did not in 45 cases of positive coexpression. The difference between patients who got chemotherapy and those who did not in 32 cases with negative coexpression (Semba et al., 2006).

In PCa tissues and cell lines, *ARPC1A* was found to be highly expressed by Chen et al. (2023). This expression was found to be an independent predictor of both overall patient survival and biochemical recurrence after radical prostatectomy. *ARPC1A* knockdown decreased PCa cell invasion, migration, and cytoskeleton formation but had no effect on cell proliferation or cell cycle progression. While *ARPC1A* overexpression had little effect on tumour development in vivo, it did enhance lung metastasis of PCa. Moreover, it was found that the metabolism of glutamine serving as an upstream regulator of *ARPC1A*, which in turn increased the motility, invasion, and cytoskeletal modifications of PCa cells (chen et al.,2023).

Kahr et al. employed immunoblot analysis to find out at the expression of *Arp2/3* components in patients platelet lysates and normal, unaffected family members in the microthrombocytopenia, eosinophilia, and inflammatory infections investigation. *WASP* and other *Arp2/3* components (*ARP2*, *ARP3*, *ARPC2*, *ARPC3*, and *ARPC5*) were all within

normal ranges in all patients and unaffected family members, indicating that *ARPC1B* was not significantly expressed. *ARPC1B* level Isoform ratios and levels vary greatly throughout cell and tissue types, and it is found in less than 6% of platelets. In *ARPC1B*-deficient cells, we saw higher *ARPC1A* expression and decreased *ARPC1A* expression relative to *ARPC1B* in normal platelets. This suggested that *ARPC1A* was upregulated in *ARPC1B*-deficient platelet precursor megakaryocytes (MKs), as platelets do not have nuclei. This seemed to be consistent with the observed up-regulation of *ARPC1B* following the application of siRNA to inhibit the expression of *ARPC1A* in HeLa cells. The deletion of *ARPC1B* had no influence on the expression of other *Arp2/3* components, according to the same study (Kahr et al,. 2017).

The *ARPC1A* gene exhibited the statistically most significant link between amplification and higher expression, according to a qRT-PCR gene expression study. In AsPC-1 cells with high levels of amplification and expression, silencing *ARPC1A* by RNA interference led to a significant reduction in cell invasion and migration but only a little drop in cell proliferation. *ARPC1A* encodes the Arp2/3 protein complex's p41 component, which controls cell motility by acting as a requirement for actin polymerization. All findings point to *ARPC1A* as a potential target for antimetastasis therapy since, among other things; it regulates cell migration and invasion in pancreatic cancer and is a novel target for the 7q21-q22 amplification (chen et al,. 2023).

We examined the *ARPC1A* gene, which belongs to the actin-related protein ARP2/3 complex family, in kidney cancer tissue by analyzing all of these data. In the results of our study, we discovered that there was an increase in malignant tissue when the expression level of the *ARPC1A* gene in cancerous tissue was compared to normal tissue. However, this increase was not statistically significant ($p=0.75$, value $p>0.05$).

Considering all of the information, it can be concluded that while some patients in the studies done so far had the *ARPC1A* gene overexpressed, there was no discernible increase in other individuals. It is demonstrated that the results have various impacts. Only a small number of samples were examined in the majority of this research. In our investigation, there was not a significant rise in the *ARPC1A* gene with kidney cancer. The presence of an increase, however not statistically significant, implies that the *ARPC1A* gene could be involved in the development and forming of cells in the kidneys. To the best of our understanding, no particular study examining the connection between the *ARPC1A* gene and kidney development could be discovered in the available research. Our study is unique in that it is the first to examine the connection between the development of kidney cancer and the *ARPC1A* gene. Our study is the first of its kind in the world and in Turkey in this regard. Further research should be done to confirm the association between kidney cancer and the *ARPC1A* gene, given the novelty of our investigation and the limited sample size.

We believe that our study has importance since it is the first of its kind to be done in the literature in Turkish society and globally, and it serves as a model for future research projects.

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