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TOKAT GAZIOSMANPASA UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

MEDICAL BIOLOGY DEPARTMENT

MASTER'S PROGRAM

ANALYSIS OF *ACTR3* GENE EXPRESSION IN RENAL CELL CARCINOMA

MASTER'S THESIS

Parzhin Muhajir ABDULHADI

Supervisor: Prof. Dr. H. Omer ATES

TOKAT - 2024

SCIENTIFIC ETHICS PAGE

According to the thesis writing guide of Tokat Gaziosmanpaşa University Institute of faculty of medical biology. My Master's thesis named “Analysis of *ACTR3* Gene Expression in Renal Cell Carcinoma” that I have prepared under the consultancy of “Prof. Dr. H. Omer ATES” is based on scientific ethical values and rules. I hereby declare that it is an appropriate, original work, and I will accept any legal sanctions if it is determined otherwise.

24/09/2024

Parzhin Muhajir Abdulhadi

JURY ACCEPTANCE AND APPROVAL

The defense examination of the thesis study titled "Analysis of *ACTR3* Gene Expression in Renal Cell Carcinoma'' is prepared by Parzhin Muhajir ABDULHADI was held on. I will schedule some time for us to connect. 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.

Jury Members (Title, Name Surname)	**Signature**
Assist. Prof. Dr. Nihan BOZKURT	…..…….
Prof. Dr. H. Omer ATES	……...
Asst. Prof. Dr. Saime SEZER SONDAS	……..

CONFIRMATION

/ /

Assoc. Prof. Dr. Yusuf TEMUR

Director of Institute of Graduate Studies

ACKNOWLEDGEMENTS

In the beginning, I thank God in advance for making this research practical and allowing me to complete it successfully. All praise belongs to God.

I would like to convey my gratitude to my supervisor, Prof. Dr. Haci Omer ATES. Who assisted and encouraged me in performing my study, as well as providing assistance in an area we were unfamiliar with. I am grateful to the Institute of Health Sciences for allowing me to do research at Tokat Gaziosmanpaşa University Department of Medical Biology. Thank you to kind teachers Assist. Prof. Nihan BOZKURT, Dr. Kubra SAHIN and Research Assistant Sadegul SAVKIN for their support and guidance during my research on performing investigations.

I would like to convey my great love and respect to my parents for their spiritual support, prayers for me, and belief in me, as well as to all of my brothers and sisters for being a source of pleasure for me and keeping me.

ABSTRACT

ANALYSIS OF *ACTR3* GENE EXPRESSION IN RENAL CELL CARCINOMA

Parzhin Muhajir ABDULHADI

Master Thesis Department of Medical Biology

Thesis Supervisor: Prof. Dr. H. Omer ATES

September 2024, xiii, + 57 pages

Renal cell carcinoma (RCC), in particular, is a major health concern that is becoming more commonplace worldwide. Developing targeted therapeutics requires an understanding of the molecular pathways behind the disease's etiology. The Actin-Related Protein 3 (*ACTR3*) gene has drawn interest among the many genes linked to kidney cancer because of its possible involvement in tumor growth and disease progression. A crucial component of the actin-related protein (ARP3) group, *ACTR3* is involved in a number of biological processes including intracellular transport, cytoskeletal dynamics, and gene expression regulation. The purpose of the study is to compare the role of *ACTR3* gene expression level between renal cell carcinoma tissue and control renal tissue. Quantitative Real-time polymerase chain reaction (qRT-PCR) was employed in the study to analyze gene expression. The study analyzed gene expression levels by examining ten renal carcinoma tissue samples and ten corresponding control renal tissue samples, both collected from the same group of patients using quantitative Real-time polymerase chain reaction (qRT-PCR). The research demonstrated a fold change with 1.147 in *ACTR3* gene expression in kidney cancer tissue samples relative to normal kidney tissues; however, this increase did not meet statistical significance, since ($p = 0.578$). Therefore, we think that more research using larger sample sizes is necessary to examine and comprehend the connection between kidney cancer and *ACTR3* expression levels.

Keywords: ACTR3 Gene Expression, Actin Related Protein 3, Cytoskeleton, Kidney Cancer

ÖZET

BÖBREK HÜCRELI KARSINOMDA *ACTR3* GEN EKSPRESYONUNUN ANALIZI

Parzhin Muhajir ABDULHADI

Master Tezi Biyoloji Anabilim Dalı

Tez Danışmanı: Prof. Dr. H. Ömer ATEŞ

Eylül 2024, xiii+ 57 sayfa

Böbrek hücreli karsinom (RCC), özellikle dünya genelinde giderek daha yaygın hale gelen önemli bir sağlık sorunudur. Hedefe yönelik tedavilerin geliştirilmesi, hastalığın etiyolojisindeki moleküler yolların anlaşılmasını gerektirir. Böbrek kanseriyle ilişkili birçok gen arasında, tümör büyümesi ve hastalığın ilerlemesindeki olası rolü nedeniyle Actin-Related Protein 3 (*ACTR3*) geni ilgi çekmiştir. *ACTR3*, hücre içi taşıma, iskelet sistemi dinamikleri ve gen ekspresyonunun düzenlenmesi gibi birçok biyolojik süreçte rol alan aktinle ilişkili protein (ARP3) grubunun önemli bir bileşenidir. Çalışmanın amacı, böbrek hücreli karsinom dokusu ile kontrol böbrek dokusu arasında ACTR3 gen ekspresyonu seviyesini karşılaştırmaktır. Çalışmada gen ekspresyonunu analiz etmek için Kantitatif Gerçek Zamanlı Polimeraz Zincir Reaksiyonu (qRT-PCR) kullanılmıştır. Araştırma. Çalışmada, 10 böbrek karsinomu doku örneği ve aynı hastalara ait 10 kontrol böbrek doku örneğini incelenerek gen ekspresyon seviyelerini analiz edildi. Kantitatif Gerçek Zamanlı Polimeraz Zincir Reaksiyonu (qRT-PCR) ile analiz etmiştir. Araştırma, böbrek kanseri doku örneklerinde normal böbrek dokularına göre *ACTR3* gen ekspresyonunda 1.147 kat değişim göstermiştir; ancak bu artış istatistiksel olarak anlamlı bulunmamıştır ($p = 0.578$). Bu nedenle, böbrek kanseri ve *ACTR3* ekspresyon seviyeleri arasındaki ilişkiyi incelemek ve anlamak için daha büyük örneklem boyutlarıyla daha fazla araştırma yapılması gerektiğini düşünmekteyiz.

Anahtar Kelimeler: *ACTR3* Gen Ekspresyonu, Aktin İlişkili Protein 3, Böbrek Kanseri, Sitoiskelet

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LIST OF ABBREVIATIONS

ACKD: Acquired Cystic Kidney Disease

***ACTB*:** Beta-actin

***ACTR3*:** Actin-related protein 3

ARP2/3: Actin-Related Protein 2/3 Complex

ARP3: Actin-Related Protein 3

ARPC5: Actin-related protein 2/3 complex subunit 5

BHD: Birt-Hogg-Dubé Syndrome

BRCA1: Breast Cancer 1 gene

BRCA2: Breast Cancer 2 gene

ccRCC: Clear Cell Renal Cell Carcinoma

cDNA: Complementary Deoxyribonucleic Acid

CKD: Chronic Kidney Disease

Cq values: Threshold Cycle Values

CT: Computed Tomography

Ct: Cycle Threshold

dH2O: Deionized Water

DNA: Deoxyribonucleic Acid

dsDNA: Double-stranded DNA

EDTA: Ethylenediaminetetraacetic acid

EMT: Epithelial-Mesenchymal Transition

F: Forward primer

FH: Fumarate Hydratase gene

HIF: Hypoxia-Inducible Factor

HIFs: Hypoxia-Inducible Factors

HLRCC: Hereditary Leiomyomatosis and Renal Cell Cancer

HNRNPK: Heterogeneous nuclear ribonucleoprotein K

HPRC: Hereditary Papillary Renal Carcinoma

HPV: Human Papillomavirus

HS assay: High Sensitivity Assay

kbp: Kilobase Pairs

MET: MET gene (no full form given, typically refers to a gene implicated in various forms of cancer)

MRI: Magnetic Resonance Imaging

mRNA: Messenger RNA

PCR: Polymerase Chain Reaction

PDAC: Pancreatic ductal adenocarcinoma

PI3K/AKT: Phosphoinositide 3-Kinase/Protein Kinase B

PKD: Polycystic Kidney Disease

qPCR: Quantitative Polymerase Chain Reaction

R: Reverse primer

RCC: Renal Cell Carcinoma

Rho GTPase: Rho Family of GTPases

RNA: Ribonucleic Acid

RNase: Ribonuclease

ROX: Passive Reference Dye

RT: Reverse Transcription

RT-PCR: Reverse Transcription Polymerase Chain Reaction

SD: Standard Deviation

SEM: Standard Error of Mean

SPSS: Statistical Package for the Social Sciences

SYBR Green: A fluorescent dye used in molecular biology for DNA detection

SYBR: SYBR Green I (a nucleic acid stain used in molecular biology)

TaqMan: A fluorescence-based reagent used in qPCR

TCC: Transitional Cell Carcinoma

TE buffer: Tris-EDTA buffer

VHL: Von Hippel-Lindau gene

WT1 and WT2: Gene mutations associated with Wilms Tumor

1. INTRODUCTION

Cancer is a highly complex disease that has been the focus of extensive research in the scientific community. It's now widely recognized that cancer is not a single disease, but rather a diverse collection of conditions, each presenting distinct characteristics and challenges (Hanahan & Weinberg, 2011; Derbal, 2018). The genetic diversity and dynamic nature of tumor cells are crucial factors contributing to the intricate nature of cancer (Hanahan, 2022).

Cancer development is influenced by a range of factors that can be classified into several categories, including genetic elements, environmental exposures, and lifestyle choices, among others (Obidiro et al., 2023). Genetic mutations in oncogenes, tumour suppressor genes, and DNA repair genes are pivotal, as these abnormalities can trigger uncontrolled growth and division of cells, thereby elevating cancer risk (Sinkala, 2023). Environmental factors also play a crucial role, with carcinogens such as tobacco smoke, ultraviolet radiation, asbestos, and specific chemicals posing significant threats by damaging DNA and fostering the proliferation of cancer cells (Guterres et al., 2022). Lifestyle choices are equally important; smoking, excessive alcohol intake, poor nutritional habits, sedentary behaviour, and obesity are all recognized as increasing the likelihood of cancer through mechanisms like inflammation, oxidative stress, and hormonal imbalances (Guterres et al., 2022). Moreover, infections from viruses, bacteria, and parasites have been linked to heightened cancer risk; for instance, the human papillomavirus (HPV) increases the risk of cervical cancer, and Helicobacter pylori infection is a known risk factor for stomach cancer. Age is another critical factor, as the risk of developing cancer tends to increase with age due to a higher probability of genetic mutations and longer exposure to various risk factors (Sharma, 2022). Genetic predisposition, through inherited mutations like those in the BRCA1 and BRCA2 genes associated with breast cancer, significantly affects cancer risk as well. Hormonal factors, like elevated levels of estrogen and testosterone, have also been implicated in the development of certain cancers, such as breast and prostate cancer, respectively (Acharya, Chacko, Bose, Lapenna & Pattanayak, 2019). Lastly, an impaired immune system, whether due to certain health conditions or immunosuppressive treatments, may compromise the body's ability to fight off cancer cells, thus increasing the risk of cancer (Zhang et al., 2023). These factors intertwine in complex manners, affecting cancer

development, and highlight the importance of comprehensive strategies for prevention, early detection, and treatment (Lustberg et al., 2023).

Kidney cancer, also recognized as renal cell carcinoma (RCC), represents a primary cancer form starting within the kidney cells. It stands as a frequently encountered cancer type targeting the kidneys, emanating from different kidney regions, such as the renal tubules, renal pelvis, and renal calyces. Among kidney cancer instances, RCC is predominant, with clear cell carcinoma emerging as the most familiar subtype (Xu et al., 2024). The significance of gene expression concerning kidney cancer is critical for grasping the molecular dynamics at play in both the disease's genesis and its advancement. An exhaustive discourse on gene expression's impact on kidney cancer includes several key aspects (Ding et al., 2021): Firstly, the patterns of gene expression in cancer cells of the kidney can ignite tumour formation by catalyzing unrestrained cellular growth and division. The disruption of genes responsible for cell cycle control, programmed cell death, and cell signaling pathways may culminate in the emergence of malignant tumours (Zhao et al., 2019). On the other hand, alterations in gene expression within kidney cancer cells are known to foster angiogenesis, which is the generation of new blood vessels to feed the tumour. These changes in gene expression can also amplify the cancer cell's ability to metastasize or spread to additional organs, heightening the disease's severity (Quintero-Fabián et al., 2019).

The *ACTR3* gene, which codes for the Actin-related protein 3, is involved in various critical cellular processes such as cytoskeletal structuring, cellular movement, and actin polymerization. It is a key component of the Arp2/3 complex, essential for actin polymerization and the organization of filaments within the actin cytoskeleton (Lai & Wong, 2020). Its role in cancer development is particularly notable, with significant implications for cancer progression and metastasis, especially in kidney cancer. Dysregulated expression of *ACTR3* has been associated with poor prognoses in various cancers, including kidney cancer, highlighting its vital function (Chen et al., 2021)

The investigation seeks to assess the expression levels of the *ACTR3* gene in RCC tissues as compared to normal kidney tissues. Additionally, the study aims to comprehend the underlying mechanisms by which *ACTR3* gene expression influences RCC pathogenesis, including its effects on cell proliferation, migration, and invasion.

2. LITERATURE REVIEW

2.1 Kidney Anatomy and Physiology

The kidneys are essential, bean-shaped organs that are part of the renal system and are located in the posterior part of the abdomen, behind the peritoneum. These vital organs are about the size of a human fist and surrounded by a tough, fibrous renal capsule and layers of fat that act as protective cushions (Sampaio, 2018). Positioned above the kidneys are the adrenal glands, and deep within the kidneys, there are pyramid-shaped lobes known as renal pyramids (Zawada, 2020). The primary function of the kidneys is to maintain homeostasis by filtering and excreting waste products from the bloodstream (Duguma, 2016). Each kidney contains over a million filtration units called nephrons, which play a pivotal role in removing toxins and regulating various substances such as nitrogen waste (urea), muscle waste (creatinine), and acids (Scott & Quaggin, 2015). The filtered blood is returned to circulation via the renal vein, while urine is transported through the ureters to the bladder for storage and eventual elimination.

Furthermore, the kidneys produce essential hormones such as calcitriol, which is a biologically active form of vitamin D, responsible for promoting calcium absorption in the intestines. Additionally, they secrete erythropoietin, a hormone that stimulates the production of red blood cells in the bone marrow, playing a crucial role in maintaining the body's oxygen-carrying capacity (Ongkum et al., 2016).

Cancer arises from alterations in the normal functioning of cells, resulting in unchecked growth and division (Younoussi et al., 2021). In the case of kidney cancer, these alterations primarily occur in the renal cells, which play essential roles in kidney functions such as filtration and waste excretion. A key factor in the development of kidney cancer is genetic mutations, which can be either inherited or acquired through environmental exposures to carcinogens (Feng et al., 2020). Notably, specific genetic changes, such as mutations in the VHL gene, have been associated with the onset of renal cell carcinoma (Nabi et al., 2018). These mutations interfere with the regulation of the cell cycle, leading to uncontrolled cell proliferation. The uncontrolled growth of these abnormal renal cells results in the formation of a tumor, which can then spread to other parts of the body if left untreated. Understanding the underlying genetic and cellular mechanisms of kidney cancer is crucial for developing effective treatment strategies and improving patient outcomes (Feng et al., 2020).

2.2 Renal Cell Carcinoma

Renal cell carcinoma (RCC), also referred to as kidney cancer, is a relatively uncommon form of cancer, constituting about 80% of all adult cancers. RCC encompasses various subtypes, including clear cell renal cell carcinoma, papillary renal cell carcinoma, and chromophobe renal cell carcinoma (Nabi et al., 2018). The diagnosis of RCC involves a thorough review of medical history, physical examination, and a series of imaging tests such as ultrasound, computed tomography (CT) scans, and magnetic resonance imaging (MRI), along with confirmatory tests like biopsies (Shi & Zhang, 2023). Early detection is pivotal in improving patient outcomes and enhancing the likelihood of successful treatment (Huang et al., 2023). The severity of RCC varies based on factors such as tumor size, type, stage, and the presence of metastasis, which is quite common in RCC and typically affects the lymph nodes (Peabody et al., 2019). Treatment options for RCC may encompass surgery, targeted therapy, immunotherapy, radiation therapy, and sometimes, chemotherapy. The choice of treatment is determined by factors such as cancer stage, overall health of the patient, and specific tumor characteristics (Nabi et al., 2018). In summary, a comprehensive understanding of the different RCC subtypes, early detection through accurate diagnostic methods, and personalized treatment strategies are vital for effectively managing renal cell carcinoma (Casuscelli et al., 2017).

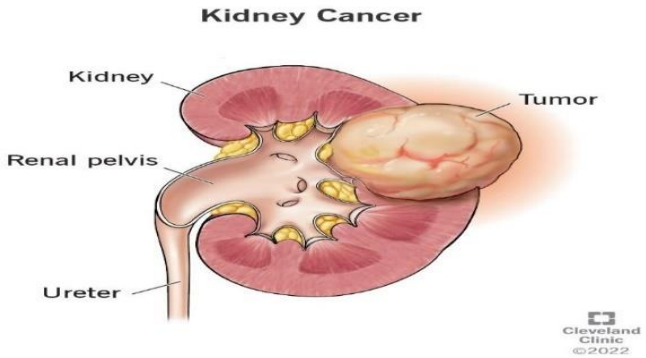

Figure 2. 1. Kidney Cancer in Kidney Cells

(Nath et al., (2022).

2.3 Kidney Cancer Etiology and Epidemiology

The study of the causes and origins of diseases, known as etiology, reveals that kidney cancer development can be attributed to several factors, including genetic

predispositions and lifestyle or environmental influences (Kentsis, 2020). Genetic factors play a significant role in hereditary syndromes like Von Hippel-Lindau (VHL) Syndrome, Hereditary Papillary Renal Carcinoma (HPRC), Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC), and Birt-Hogg-Dubé (BHD) Syndrome increasing the risk of kidney cancer due to mutations in specific genes (Nguyen et al., 2017). Moreover, lifestyle and environmental factors such as smoking, obesity, hypertension, and occupational exposure to certain chemicals further elevate the risk, alongside medical conditions like Chronic Kidney Disease (CKD) and Polycystic Kidney Disease (PKD) (Nithish, 2018). The disease also has a notable correlation with age and gender, being more common in older adults and men (Kövesdy, 2022).

In examining the epidemiology of kidney cancer, it becomes clear that this disease, the 14th most common cancer globally, varies widely in incidence and prevalence worldwide. Developed countries like those in North America and Europe report higher incidence rates compared to Asia and Africa (Bafadni et al., 2023). There has been a noticeable increase in the incidence of kidney cancer over recent decades, likely due to better diagnostic methods, an increase in imaging use, and a rise in risk factors such as obesity and hypertension. Demographically, kidney cancer risk escalates with age, predominantly affecting individuals between 50-70 years old, and is twice more likely to develop in men than in women, possibly due to differing levels of exposure to known risk factors (Zheng et al., 2019). Racial and ethnic disparities also exist, with varying incidence rates observed among different groups. Despite a significant mortality rate associated with kidney cancer, the prognosis can be relatively optimistic for localized cases diagnosed early, boasting a 5-year survival rate of about 93%. However, this rate dramatically drops for advanced, metastatic stages. By understanding both the etiology and epidemiology of kidney cancer, research and healthcare initiatives can better target prevention strategies, highlighting the importance of addressing modifiable risk factors such as smoking, obesity, and hypertension to reduce incidence rates (Padala et al., 2020).

2.4 Classification of Kidney Cancer Cells

Renal neoplasms, or kidney cancers, comprise a diverse group of malignancies categorized by their cellular origin and molecular characteristics. Among these, Renal Cell Carcinoma (RCC) represents the most prevalent form, accounting for approximately

85-90% of all renal neoplastic cases. RCC originates from the renal tubules and is further subdivided into distinct subtypes based on histological and molecular attributes (Xu et al., 2024).

The predominant subtype of RCC is Clear Cell Renal Cell Carcinoma (ccRCC), which constitutes about 70-80% of RCC cases. Characterized by their optically clear cytoplasm under microscopic examination, due to high concentrations of lipid and glycogen, these cells are frequently associated with mutations in the VHL gene, indicative of Von Hippel-Lindau disease (Zhu et al., 2020). Papillary Renal Cell Carcinoma stands as the second most common subtype, accounting for 10-15% of RCC cases. This subtype is characterized by the presence of projections resembling fingers at the cellular level, and is further divided into Type 1, which typically exhibits a less aggressive pathology, and Type 2, associated with a more aggressive course and poorer prognosis. Mutations in the MET gene are commonly implicated in the development of this form of carcinoma (Bohnenberger & Ströbel, 2021).

Chromophobe Renal Cell Carcinoma, comprising about 5% of RCCs, is distinguished by cells with larger dimensions, pale cytoplasms, and pronounced cell membranes. This subtype is frequently associated with multiple chromosomal losses, including chromosomal regions 1, 2, 6, 10, 13, 17, and 21(Bao et al., 2020). Collecting Duct Carcinoma, a rare entity constituting less than 1% of RCCs, arises from the renal collecting ducts and is noted for its highly aggressive nature and consequentially poor prognosis (Bellini duct carcinoma, 2021). Medullary Carcinoma, an exceedingly rare variant, predominantly affects younger individuals, particularly those with the sickle cell trait. This form is recognized for its aggressive behavior, often leading to a poor prognosis due to the advanced stage at diagnosis (Davis et al., 2023). Transitional Cell Carcinoma (TCC) or Urothelial Carcinoma originates from the renal pelvis' lining, contributing to 5-10% of kidney cancers. These cells bear resemblance to bladder cancer cells, with similar etiological factors such as tobacco use and exposure to specific industrial chemicals (Xu et al., 2022).

Wilms Tumor, or Nephroblastoma, mainly a pediatric kidney cancer, is typically diagnosed in children under the age of 5. Highlighted by a diverse cellular composition, it suggests embryonic origin and is associated with mutations in the WT1 and WT2 genes on chromosome 11. With adequate treatment, including surgery, chemotherapy, and

sometimes radiation therapy, the prognosis for Wilms Tumor is generally positive (Brady et al., 2020). Renal Sarcoma represents an extremely rare category of kidney cancer, encompassing less than 1% of cases. It includes various subtypes such as Leiomyosarcoma and Liposarcoma, originating from smooth muscle and fat cells, respectively. Due to its aggressive nature and the challenges of early diagnosis, renal sarcoma frequently results in a poor prognosis (Bao et al., 2020). In addition to malignant neoplasms, there exist benign renal tumors that can simulate the presentation of malignant counterparts. These include Renal Adenoma, slow-growing tumors; Oncocytoma, typically benign tumors necessitating surgical excision under certain conditions; and Angiomyolipoma, composed of blood vessels, muscle, and fat, often associated with tuberous sclerosis (Horst & Brenn, 2017). Furthermore, specific cystic kidney diseases predispose to the development of renal cancer, including Acquired Cystic Kidney Disease (ACKD), prevalent among individuals on long-term dialysis, and Polycystic Kidney Disease (PKD), a hereditary condition that elevates the risk for renal cell carcinoma (Sirohi et al., 2018).

2.5 Kidney Cancer Stages

Kidney cancer is a complex disease that progresses through different stages, each characterized by specific tumor size and spread. The stages of kidney cancer are an important factor in determining the appropriate treatment approach and predicting the overall prognosis for patients (Lin et al., 2020). Furthermore, understanding the kidney cancer stages can help healthcare professionals and patients make informed decisions about treatment options and facilitate effective communication between the medical team and the patient. The classification of kidney cancer stages is crucial for proper diagnosis and treatment planning. Accurate classification of kidney cancer stages is essential for guiding appropriate therapy and determining the prognosis of the disease. Accurate staging of kidney cancer allows healthcare professionals to determine the extent of tumor growth and spread, which helps in tailoring treatment options to individual patients. Additionally, accurate classification of kidney cancer stages allows for effective communication between the medical team and the patient, ensuring that both parties have a clear understanding of the (Mayce & Hernandez, 2021). Figure 2.1 illustrates the stages of kidney cancer.

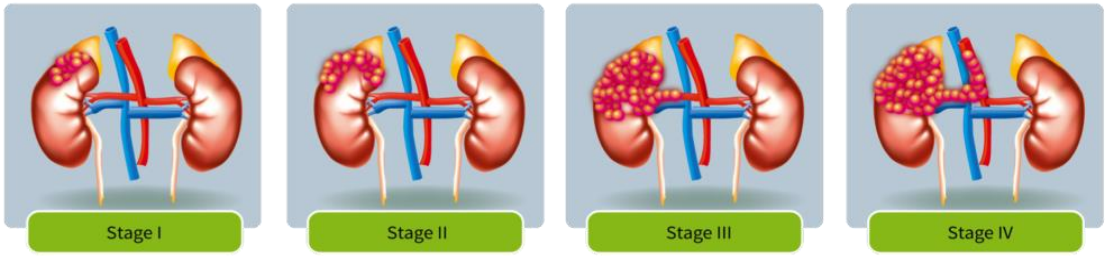

Figure 2. 2. The Stages of Kidney Cancer

(Kastrati, et al., (2023)

Figure 2.2 shows the stages of kidney cancer. In the classification of kidney cancer, Stage I is characterized by the tumor being confined within the kidney and having a diameter of less than 7 cm, with no lymph node involvement or distant metastasis, leading to a high five-year survival rate with early detection and surgical intervention. In Stage II, the tumor remains within the kidney but exceeds 7 cm in diameter. Stage III is marked by the cancer growing beyond the kidney capsule and potentially invading nearby tissues, with possible lymph node involvement but no spread to distant organs. Lastly, Stage IV indicates that cancer cells have metastasized to distant locations outside the genitourinary system, with treatment approaches and prognosis varying based on the cancer's specific characteristics and the patient's overall health (Kastrati, et al., (2023).

2.6 Genetic basis of Kidney Cancer

The relationship between the genetic factors of kidney cancer and the regulation of cell movement is highlighted by various molecular pathways crucial for tumour development, spread, and cellular dynamics (Nabi et al., 2018). Genetic mutations, such as those in the VHL gene, can significantly impact cell movement in kidney cancer. For example, the mutation in the VHL gene results in the accumulation of hypoxia-inducible factors (HIFs), promoting the expression of genes essential for angiogenesis and supporting cell migration and invasion. Similarly, mutations in the MET gene activate pathways that enhance cell adhesion and migration. In contrast, mutations in the FH gene create a pseudo-hypoxic environment that impacts cell adhesion and motility (Davidson et al., 2014).

Delving further into the molecular mechanisms, the process of epithelial-mesenchymal transition (EMT) becomes significant. Genetic mutations influence this transition from epithelial to mesenchymal cells in VHL, MET, and FH, which provoke

signaling cascades, leading to the upregulation of transcription factors that facilitate a mesenchymal phenotype (Ren et al., 2023).

Genetic mutations also influence the reorganization of the actin cytoskeleton, cell motility, and the remodeling of the extracellular matrix critical for cancer cell invasion and migration (Panciera et al., 2020). Moreover, genetic alterations in kidney cancer can affect the expression and functionality of adhesion molecules, such as integrin's and cadherin's; highlighting their deep interconnection with cell migration and adhesion processes (Valdembri & Serini, 2021). Figure 2.3 shows the genetics of the cancer.

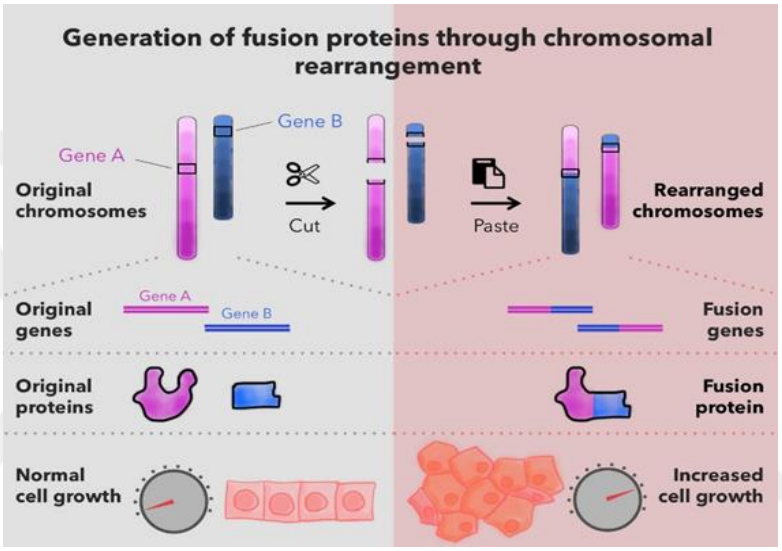

Figure 2. 3. Genetics of Cancer.

(Znaor et al., (2017)

Figure 3 indicates the formation of fusion proteins resulting from chromosomal rearrangement. Fusion proteins are atypical proteins that emerge when segments of two distinct genes are fused together. This fusion commonly arises from a chromosomal rearrangement, where a fragment of one chromosome detaches and connects to another chromosome (Znaor et al., (2017).

2.7 Cell Migration

Cell migration plays a crucial role in cancer progression, including kidney cancer. In cancer, cell migration refers to the ability of cancer cells to move from the primary

tumour site to other parts of the body, leading to metastasis, which is a significant factor in cancer progression and patient outcomes (Ma et al., 2023). The spread of cancer cells from the primary tumour in the kidney to other organs, such as lymph nodes, brain, bone, kidney, and liver. Metastasis in kidney cancer is a common occurrence and is associated with a worse prognosis, highlighting the significance of understanding and targeting cell migration in the context of this disease (Wei et al., 2021). The process of cell migration in cancer involves various molecular mechanisms that allow cancer cells to detach from the primary tumour, invade surrounding tissues, enter the bloodstream or lymphatic vessels, and establish secondary tumours in distant sites. These steps are critical for cancer progression and the formation of metastases (Fares et al., 2020). By understanding the molecular pathways and mechanisms involved in cell migration in kidney cancer, researchers and healthcare professionals can identify potential therapeutic targets to impede the spread of cancer cells and improve patient outcomes (Shu et al., 2023).

Ultimately, cell migration is essential in the progression of cancers, including kidney cancer, as it enables metastasis and the spread of cancer cells to other parts of the body. Grasping the mechanisms behind cell migration in kidney cancer is vital for creating targeted treatments to prevent metastasis and enhance patient outcomes (Cai et al., 2023).

2.8 Cell Cytoskeleton and kidney cancer

The cell cytoskeleton is a highly intricate and dynamic network of filamentous structures that plays a pivotal role in the structural integrity, organization, and functional processes of eukaryotic cells. This interconnected system predominantly comprises three major components: actin filaments, microtubules, and intermediate filaments, each possessing unique characteristics and specialized functions (Hartman & Spudich, 2012; Zhang et al., 2017). Actin filaments, also referred to as microfilaments, are the most abundant and versatile elements of the cytoskeleton (Hartman & Spudich, 2012). They are implicated in a diverse array of cellular activities, encompassing the maintenance of cell morphology, organization and anchoring of organelles, regulation of cell division, and facilitation of intracellular transport and signaling (Chemeris et al., 2017). The actin cytoskeleton is capable of generating both pushing and pulling forces through the coordinated polymerization and depolymerization of actin filaments, as well as the sliding of actin filaments along bipolar filaments of myosin II (Svitkina, 2018).

The cytoskeleton plays a crucial role in regulating cell migration, which is a vital step in the advancement of most metastatic cancers (Bordeleau et al., 2014). Cell invasion and migration processes depend on the dynamic changes within the cytoskeletal components, such as actin, tubulin, and intermediate filaments. Recent studies have emphasized the significance of the interplay between the extracellular matrix and the cytoskeleton in three-dimensional environments. Tumor tissue contains regions with varying levels of compliance, dense matrix, and interstitial spaces, where fibers and pores of different sizes are present (Aseervatham, 2020).

In the realm of cancer, actin rearrangement is closely linked to cancer cell migration, invasion, and metastasis. Cancer cells often display altered actin dynamics, leading to changes in cell morphology and motility that enhance their ability to infiltrate surrounding tissues and migrate to distant sites in the body (Cai et al., 2023). Actin is a fundamental component of the cytoskeleton, a complex network of filaments and proteins that provide structural support, facilitate cellular movement, and enable intracellular trafficking within eukaryotic cells. The dynamic reorganization of the actin cytoskeleton is a critical mechanism that underpins a wide range of cellular processes, including cell migration, division, and response to environmental cues (Jiang et al., 2023).

In the context of cancer, dysregulation of the actin cytoskeleton has been closely linked to the hallmarks of malignant transformation and metastasis. The actin rearrangement mechanism is a key driver of cellular motility, enabling cancer cells to break free from their primary tumor site, invade surrounding tissues, and disseminate to distant organs (Ionescu et al., 2022). Dysregulated actin dynamics can boost the migratory and invasive properties of kidney cancer cells, facilitating the spread of the disease (Li et al., 2022; Kim et al., 2021). Understanding the mechanisms that drive actin rearrangement in kidney cancer is crucial for developing targeted therapies that can disrupt the cytoskeletal changes associated with cancer progression. By targeting actin dynamics and the signaling pathways that regulate actin rearrangement, researchers hope to curb cancer cell migration and invasion, ultimately limiting metastasis and bolstering patient outcomes (Jiang et al., 2021).

Actin-Related Protein 3 (ARP3) is a crucial constituent of the Arp2/3 complex, which plays a vital role in governing actin polymerization and cytoskeletal dynamics in eukaryotic cells (Zheng et al., 2023). The Arp2/3 complex, comprised of multiple subunits

including ARP3, is instrumental in nucleating new actin filaments from existing ones, leading to the formation of branched actin networks that are essential for critical cellular processes such as cell motility, vesicular trafficking, and cell shape maintenance (Zheng et al., 2023).Within the Arp2/3 complex, ARP3 is responsible for promoting actin filament nucleation and facilitating the branching of existing filaments, both of which are crucial for the formation of cellular structures like lamellipodia and filopodia in migrating cells (Yang et al., 2022). These structures are pivotal for cell motility, membrane protrusion, and cell-cell interactions, emphasizing the significance of ARP3 in various cellular functions. Studies have revealed that ARP3 is intricately involved in cancer progression, with increased expression observed in several cancer types, including gastric cancer, squamous cell carcinoma, colorectal cancer, and breast cancer (Huang et al., 2022). In cancer cells, ARP3 has been implicated in promoting tumor development, metastasis, and epithelial-mesenchymal transition (EMT), a process essential for cancer cell invasion and dissemination (Zheng et al., 2023). Moreover, ARP3 has been associated with poor prognosis in cancer patients, highlighting its potential as a prognostic marker and therapeutic target in cancer treatment. The dysregulation of the Arp2/3 complex, in which ARP3 plays a crucial role, has also been linked to various mental disorders, emphasizing the importance of ARP3 in cellular processes beyond cancer due to its three-dimensional structure, ARP3 can interact with other proteins and contribute to complex cellular processes. Its role in actin polymerization and cytoskeletal organization underscores its significance in maintaining cell shape, motility, and intracellular transport processes (Casalou et al., 2020). In essence, ARP3, as a critical component of the Arp2/3 complex, holds a pivotal role in regulating actin dynamics, cell motility, and cancer progression. The intricate functions of ARP3 within the Arp2/3 complex underscore its importance in cellular physiology and pathology, making it a subject of ongoing research and interest in the fields of cell biology and cancer biology (Huang et al., 2022).

2.9 *ACTR3* Gene

The *ACTR3* gene is positioned on the forward strand of chromosome 2, approximately 90 kilobase pairs (kbp) downstream from the *ACTR3* protein-coding gene. This genomic location classifies the *ACTR3* gene as an intergenic region, indicating that it is not part of the coding region of any other known gene (Maroni et al., 2023). The proximity of the *ACTR3* gene to neighbouring genes on chromosome 2 suggests potential

functional associations with these genes or regulatory elements within the surrounding genomic region (Karagüzel et al., 2022).Given its genomic location and its involvement in critical cellular processes such as cell shape and motility maintenance (Chen & Jiang, 2022), understanding the genomic locations of the *ACTR3* gene and its neighbouring genes is crucial for elucidating their functional roles and potential interactions (Karagüzel et al., 2022).Comprehensive knowledge of the genomic locations of genes is essential for gaining insights into their roles in biological processes and their potential regulatory relationships with neighbouring genes or elements. This understanding is pivotal for a deeper characterization of gene function. Additionally, the genomic location of the *ACTR3* gene on chromosome 2, downstream from a protein-coding gene, indicates potential regulatory interactions and functional relationships with neighbouring genes or elements (Wang et al., 2022). The analysis of these genomic associations provides valuable insights that aid in achieving a more comprehensive understanding of gene functionality. Additionally, the genomic placement of the *ACTR3* gene downstream from a protein-coding gene highlights its potential regulatory interactions and functional connections with neighbouring genetic elements or factors (Li et al., 2023). For a visual representation of the genomic location of the *ACTR3* gene, refer to Figure 2.4.

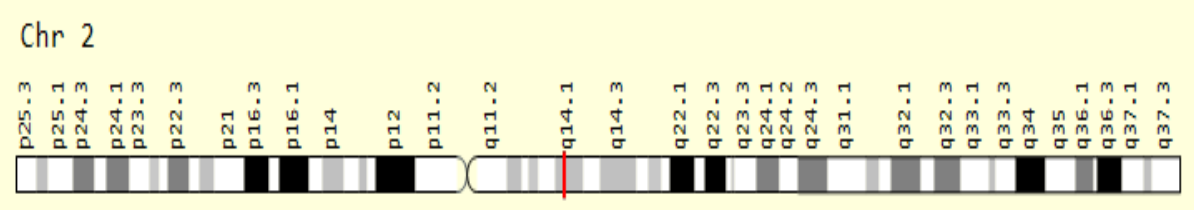

Figure 2. 4 *ACTR3* Gene in Genomic Location

(Ellis et al., 2018).

2.10 Relationship between *ACTR3* Gene and the Cytoskeleton

The interaction between the cytoskeleton and the *ACTR3* gene, especially the ARP3 protein, significantly influences kidney cancer progression by affecting cell migration, invasion, and metastasis (Serwe et al., 2023). The *ACTR3* gene encodes the ARP3 protein, a key component of the ARP2/3 complex. This complex is essential for nucleating new actin filaments, creating a dense and branched actin network crucial for various cellular processes. In the context of kidney cancer, the role of *ACTR3* is

multifaceted, significantly affecting cell migration, invasion, and metastasis (Chen et al., 2021).

ACTR3 influences actin polymerization through the ARP2/3 complex, promoting the development of cellular projections and facilitating cell movement. These dynamics enable kidney cancer cells to navigate the extracellular matrix, aiding in tissue invasion and metastatic spread. Additionally, *ACTR3* and the ARP2/3 complex play a critical role in the epithelial-mesenchymal transition (EMT), enhancing cell motility and aiding in the reorganization of the cytoskeleton (Serwe et al., 2023).

Molecular pathways, including Rho GTPase signaling, the Hypoxia-Inducible Factor (HIF) pathway, and the PI3K/AKT pathway, interact with *ACTR3* in kidney cancer, influencing the activation of the ARP2/3 complex and ultimately enhancing cell migration, invasion, and metastasis. These findings underscore the critical role of the *ACTR3* gene and its interactions within the cytoskeleton structure in kidney cancer progression (Serwe et al., 2023).

2.11 Exploring the Multifaceted Role of *ACTR3*

The *ACTR3* gene has been the focus of several previous studies aimed at understanding its function and its role in various biological processes. These studies have provided valuable insights into the role of the *ACTR3* gene in cellular organization, cytoskeletal dynamics, and cell migration (Widomska et al., 2023). One study investigated the obligatory function of the *ACTR3* gene in cell division and growth. The researchers found that the *ACTR3* gene is involved in cell division and growth, and they speculated that this function may be either specific to certain cell types or conditions, or it may have a more general function in all cells and tissues (Li et al., 2023). The *ACTR3* gene has been the subject of previous studies investigating its role in cellular organization, cytoskeletal dynamics, and cell migration (Sambandam et al., 2023).

Another study examined the potential role of the *ACTR3* gene in nuclear envelope rupture and breakdown. The researchers suggested that the deformed nucleus phenotype observed may be consistent with a specific function of the *ACTR3* gene in this process (Birjandi et al., 2022). However, further research is needed to determine whether the function of the *ACTR3* gene in cell division and growth is dependent on the Arp2/ 3 complex-dependent actin remodeling or if it has a broader, Arp2/3 complex-independent

function across all cell types and tissues. In addition to its role in cell division and growth, the *ACTR3* gene has also been implicated in various diseases and conditions (Chen & Jiang, 2022). For instance, previous studies have shown that the expression of *ACTR3* is increased in gastric cancer, squamous cell carcinoma, and colorectal cancer (Hu et al., 2021). These studies suggest that *ACTR3* may play a role in promoting tumor development in these types of cancer. Furthermore, it has been found that *ACTR3* can interact with other proteins, such as profilin-1 and LIM domain only 2, to regulate the formation of lamellipodia and filopodia in breast cancer (S15B). Additionally, *ACTR3* and its subunit ARPC5 have been identified as hub genes in the human Arp2/3 protein complex, which is crucial for actin regulation (Essletzbichler et al., 2023). Overall, previous studies on the *ACTR3* gene have highlighted its involvement in various cellular processes, including cell division and growth, nuclear envelope rupture and breakdown, and its association with cancer development (Asenjo et al., 2023).

2.12 *ACTR3* Gene role in Cancer Studies

The study has reported mutations in the *ACTR3* gene in cancer patients, suggesting that these mutations may contribute to the development and progression of cancer (Wang et al., 2022). The specific mechanisms by which *ACTR3* gene mutations contribute to cancer pathogenesis are not yet fully understood and require further investigation. However, it has been proposed that these mutations may disrupt the normal function of *ACTR3* in regulating actin dynamics, leading to aberrant cell division and growth (Sohn, 2022).

Another study has identified the involvement of *ACTR3* in various types of cancer, including lung cancer and cholangiocarcinoma (He et al., 2022). In recent study, *ACTR3* has been confirmed to play key roles in the early immunodiagnostic of lung cancer and cholangiocarcinoma (Wang et al., 2020). Furthermore, *ACTR3* has been found to be highly expressed in immune cells of different types of lung cancer, suggesting its potential as a candidate gene for immunotherapy strategies in cancer treatment (Liu & Deng, 2022). One study examined the expression of candidate genes in immune cells of lung cancer patients and found that *ACTR3*, along with ARPC5 and HNRNPK, were highly expressed in almost all types of lung cancer immune cells (Shen et al., 2021). These findings suggest that *ACTR3* may be involved in regulating actin polymerization, which is significant in the development of cancer (Liu & Deng, 2022; Shen et al., 2021 and Wang et al., 2020).

Another study explored the expression of *ACTR3* in different types of cancer, including gastric cancer, squamous cell carcinoma, and colorectal cancer (Hu et al., 2021). In these cancers, increased expression of *ACTR3* has been observed and shown to promote tumor development. Furthermore, *ACTR3* has been shown to interact with other proteins, such as profilin-1 and LIM domain only 2, in breast cancer, leading to the regulation of lamellipodia and filopodia formation. The findings from these studies collectively demonstrate the involvement of *ACTR3* in various types of cancer and highlight its potential as a biomarker for early diagnosis and immunotherapy strategies in cancer treatment (Yingjie et al., 2022).

Another study examined the function of the actin-related protein 3 (*ACTR3*) in pancreatic ductal adenocarcinoma (PDAC), an aggressive cancer with limited treatment options (Zheng et al., 2023). The researchers determined that *ACTR3* expression was significantly elevated in PDAC tissues and cell lines, and higher *ACTR3* levels were linked to poorer prognosis in PDAC patients (Huang et al., 2023). Reducing *ACTR3* expression inhibited the invasive and migratory abilities of PDAC cells, and also led to changes in cell morphology, decreased mesenchymal markers, increased epithelial markers, and altered F-actin distribution. These findings suggest that *ACTR3* promotes epithelial-mesenchymal transition, a process involved in cancer metastasis. Consequently, *ACTR3* may represent a potential therapeutic target and prognostic indicator for PDAC (Gasca et al., 2020). Rashid et., al (2022) studies about the expression of *ACTR3* has been found to be increased in multiple types of cancer, including gastric cancer, squamous cell carcinoma, colorectal cancer, lung cancer, and cholangiocarcinoma (Rashid et al., 2022). Hu et al., (2021) study suggest that *ACTR3* may play a crucial role in tumor development and progression. *ACTR3* has been found to be involved in various processes related to cancer, such as cell migration, invasion, and metastasis. Specifically, *ACTR3* has been shown to promote the epithelial-mesenchymal transition process, which is crucial for cancer cells to acquire invasive and metastatic properties (Ali et al., 2022). In addition, *ACTR3* has been implicated in regulating the formation of lamellipodia and filopodia, which are important for cancer cell migration and invasion (Hu et al., 2021). *ACTR3* has been observed to interact with other proteins, including profilin-1 and LIM domain only 2, in the context of breast cancer, thus further emphasizing its involvement in cancer development (Kang et al., 2023).

However, targeting the downstream processes and molecular pathways regulated by *ACTR3* may hold promise for therapeutic interventions. For instance, targeting the epithelial-mesenchymal transition process or the formation of lamellipodia and filopodia, both of which are promoted by *ACTR3*, could potentially inhibit cancer cell migration, invasion, and metastasis (Hu et al., 2021).

These studies have suggested a relationship between *ACTR3* and various cancers. However, there remains a gap in our understanding of how *ACTR3* specifically impacts kidney cancer progression and patient outcomes. *ACTR3* plays a pivotal role in cell movement, invasion, and cancer spread. Despite this, detailed molecular pathways and its function in kidney cancer remain largely unexplored. Moreover, kidney cancer-specific research on *ACTR3* is limited compared to other cancers (Shou et al., 2023; Lü et al., 2023).

3. MATERIAL AND METHOD

3.1 Material

3.1.1 Working Committee

In this study, we worked with a total of 20 samples, including 10 samples of renal cancer tissues and 10 samples of healthy renal tissues. These samples were gathered from patients who visited the outpatient clinic of the Department of Urology at Tokat Gaziosmanpasa University, Faculty of Medicine. With the informed consent and awareness of the participants, kidney cancer samples obtained during surgical biopsy procedures were utilized as the experimental group, while the healthy kidney tissues from the identical patients' kidneys were employed as the control group. The study got the required clearance from the Clinical Research and Ethics Committee at Tokat Gaziosmanpasa University's Faculty of Medicine, under study number 22-KAEK-045. The research was conducted in the laboratories of Medical Biology and Genetics.

3.1.2 Tissue Preservation

We kept the containers in which we stored the tissues at an internal temperature of -80°C. We performed gene expression analysis by performing real-time PCR using cDNA obtained from tissue samples.

3.1.3. Instruments and Devices Applied to the Research

1. -80 Degree Celsius Deep Freezer (Nuaire, NU-9483E, USA).
2. A fridge (Arçelık, 570465 MB, Turkey)
3. The centrifuge (Hettich, D'78532, Germany).
4. Cold-stored Centrifuge (Hettich. Germany).
5. Mikrocentrifuge (Mikro120, Hettich Zentrifugen D- 78 532n).
6. A vortex (Velp Scientifica; F20220176, Italy).
7. Micropipette Set (Gilson, Thermo Scientific, Finnpipette).
8. PCR Device (MiniAmp 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 (Aplllid Biosystem, 4346907, UK).

3.1.4 Utilization of Chemical Substances and Kits

1.Thermo Scientific Gene JET RNA Purification Kit (Lot. No 01125929, REF K0731).
2. cDNA Reverse Transcription Kit with High Capacity (Catalog Numbers 4368814).
3. Qubit™ 1X dsDNA HS Assay Kits (Invitrogen Catalog No: Q33230, Q33231).

4.The 2X Master Mix Green with (High ROXTM) (ENZ-NUC-1000, Lot No:05272212).

3.2 Method

After preserving the surgically obtained tissues at -80°C, we employed the tissue segments for examining gene expression.

3.2.1 Purification and Isolation of RNA

For RNA separation, we used the Gene JET RNA purification kit (REF - K0731) LS (Table 3.1). It outlines the sequence of steps in the process that involve RNA isolation.

Table 3.1. RNA Purification Kit.

No	GeneJET RNA Purification Kit	50 preps #K0731
1	Proteinase K	600 µL
2	Lysis Buffer	40 mL
3	Wash Buffer 1 (concentrated)	40 mL
4	Wash Buffer 2 (concentrated)	23 mL
5	Water, nuclease-free	30 mL
6	GeneJET RNA Purification Columns pre-assembled with Collection Tubes	50
7	Collection Tubes (2 mL)	50
8	Collection Tubes (1.5 mL)	50

Prior to initiating:

To make the required quantity of Proteinase K solution, we diluted 10 µL of the provided Proteinase K with 590 µL of TE buffer (10 mM Tris HCl, pH 8.0, 1 mM EDTA).

3.2.1.1 RNA isolation Procedure

1. To determine the sample weight, we used up to 30 mg of frozen tissue. After chopping the tissue sample into tiny pieces, we added 300µL of lysis buffer and mixed for ten seconds to mix thoroughly (lysis buffer; with 2-MERCAPTOETHANOL).

2. We added 600 µl of proteinase K that had been diluted. After mixing by vortexing, we placed the mixture in an incubator set at 25°C for 10 minutes.

3. Five minutes were spent centrifuging at 12,000 × g. By using a fresh microcentrifuge tube free of RNase, we transferred the supernatant.

4. We was utilized pipit to mix an added 450 µL of 96% ethanol.

5. We relocated700 µl of lysate into a GeneJET RNA purification column. The column underwent to centrifugation with a force of 12000 × g for 1 min. We discarded the container holding the liquid and utilized a filtration column.

6. We put 700 µL of Wash Buffer1 added (450 µL of 96% ethanol) into the GeneJET RNA Purification Column and put it to centrifugation at a force of 12000 × g for a duration of 1 minute. Once we got rid of the excess material, we inserted the cleaning column into the collection tube.

7. We inserted 600 µL of Wash Buffer2 added (450 µL of 96% ethanol) into the GeneJET RNA Purification Column and placed it to centrifugation at a force of 12000 × g for a duration of 1 minute. Once we got rid of the extra substance, we inserted the cleaning column into the collection tube.

8. We introduced 100 µl of water that is free from enzymes capable of degrading nucleic acids into the membrane medium of the GeneJET RNA purification column, then we applied the centrifuge at 12000 × g for 1 minute to extract RNA.

9. We discarded the packing column and kept RNA at a temperature of -80.

3.2.2 Conversion of RNA to cDNA

We employed a High-Capacity cDNA Reverse Transcription Kit to convert RNA into cDNA by utilizing the reverse transcriptase enzyme. The cDNA synthesis kit comprises all the necessary components for the production and generation of cDNA (Table 3.2).

Table 3. 1. The Components Required for The Reverse Transcriptase Reaction in Each Sample.

No	The components for the Reverse Transcriptase Reaction	Volume
1	10X RT Buffer	2.0 µL
2	25X dNTP Mix (100 mM)	0.8 µL
3	10X RT Random Primers	2.0 µL
4	MultiScribe™ Reverse Transcriptase (50 U/µL)	1.0 µL
5	Nuclease-Free water	4.2 µL
6	RNase Sample	10.0 µL
	Total volume	**20.0 µL**

We meticulously prepared the RT master mix on ice, each component was thoroughly blended. The total volume of the mixture was 20 microliters, initially we combined all components except for the RNA sample. Following this, we adjusted the quantity of each component to accommodate the sample size. Subsequently, we dispensed 10µl of this prepared mixture into 20 fresh tubes, labeling them accordingly. Concurrently, we transferred RNA samples into separate tubes and labeled identically to those containing only RNA. These tubes, now containing a mix of RNA samples and reverse transcriptase reaction components, then were we placed into the Thermocycler, following the specified settings detailed in Table 3.3

Table 3. 2. To Synthesize cDNA, A PCR Program Was Employed.

Setting	Temperature	Time
Step 1	25°C	10 minutes
Step 2	37°C	125 minutes
Step 3	85°C	5 minutes
Step 4	4°C	Hold

Lastly, the obtained cDNA can either be used immediately, without purification, for endpoint or Real-time PCR, or it can be stored in a freezer at -80 for subsequent use.

3.2.3 Measurement of cDNA Concentration

We conducted cDNA measurements using the Qubit™ 1X dsDNA HS assay with careful adherence to protocol. Initially, we ensured uniformity by equilibrating all components to room temperature. We were prepared Test tubes, both for standards and samples, with each tube containing 10 µL of Qubit™ standards and user samples. Following this, 190 µL of Qubit™ 1X dsDNA working solution were added to each tube to achieve a total volume of 200 µL. To guarantee thorough mixing, samples we were vortexed for 3 seconds, and subsequently incubated at room temperature for two minutes. The amounts of cDNA were equalized to ensure equal amounts of cDNA participate in the real-time PCR reaction. We did serial dilutions then performed to assess the expression levels of *ACTR3* as the target gene and using beta-actin *ACTB* as the reference gene. By using serial dilution process, we accurately assessed cDNA samples at different concentrations, particularly for evaluating the expression levels of the *ACTR3* target gene, with *ACTB* as the reference gene.

3.2.4. Determination of ACTR3 Gene Expression by qRT -PCR

In our study based on the SYBR Green dye we used Real-Time PCR for measuring the expression of *ACTR3* gene by utilizing the Applied Biosystems StepOnePlus apparatus to conduct the experiment with kidney tissue samples from 10 kidney cancer patients who took part in the study, as well as cDNA produced that extracted from RNA kidney cancer, the cDNA samples were subjected to serial dilution, with varied volumes by using (*ACTB*) as the reference gene (internal control gene) and *ACTR3* as the target gene. We obtained data from the StepOnePlus device that manufactured by Applied Biosystems. Then renal cancer samples of typical nature, control renal tissues, and complementary DNA (cDNA) were analyzed. At the end The $2^{-\Delta\Delta CT}$ values of the *ACTR3* and *ACTB* genes were acquired.

3.2.5. Real-Time PCR

Real-Time PCR (qPCR), also known as quantitative PCR, is a highly refined molecular technique used to precisely amplify and measure the amount of specific DNA or RNA sequences in a sample (Čermáková et al., 2023). This method is based on the principles of traditional PCR but incorporates real-time monitoring of the amplification process. It employs the use of fluorescent dyes such as SYBR Green or specific probes like TaqMan to observe the accumulation of PCR product as it occurs. These fluorescent signals are captured in real time during each cycle of amplification. During the thermal cycling process, the fluorescence intensifies as the target nucleic acid undergoes amplification. The level of fluorescence is directly proportional to the amount of PCR product generated (Zhang et al., 2020). This allows for the determination of the starting amount of the target nucleic acid in the sample. One notable aspect of qPCR is the use of the threshold cycle (Cq) values, which refers to the PCR cycle at which the fluorescent signal of the amplified product becomes detectable above background levels. These threshold cycle values can be correlated with the initial amount of the target nucleic acid in the sample. The qPCR is widely regarded as a highly sensitive and specific molecular technique. It is commonly utilized in various applications, including gene expression analysis, pathogen detection, and quantification of gene copy number. Its ability to provide quantitative information about nucleic acids makes it an indispensable tool in molecular biology and biomedical research. (Čermáková et al., 2023).

Table 3.4 provides information about primers used in real-time polymerase chain reaction (PCR) experiments. Specifically, it lists four primers along with their respective sequences for two genes: *ACTR3* and *ACTB*. These primers play a crucial role in amplifying specific DNA segments related to the *ACTR3* and *ACTB* genes during real-time PCR.

Table 3. 3. Primers for *ACTR3* and *ACTB* Genes Used in Real-Time PCR.

ACTR3-F	5’ TTCAACCATGTTCAGGGACTTTG 3’
ACTR3-R	5’ ACCACCACTCAATTCCTCACT 3’
ACTB-F	5’ GCATGGGTCAGAAGGATTCC 3’
ACTB-R	5’ CACGCAGCTCATTGTAGAAGG 3’

In table 3.4 we provide the genetic targets for amplification and table 5 shows practical setup for achieving that amplification in a real-time PCR experiment. Both tables are interconnected, as the primers from Table 4 are integral components of the PCR reaction mixture defined in Table 3.5.

Table3. 4. Displays The Constituents Required for The Real-time PCR Reaction.

No.	PCR Reaction Components	Volume
1	2x Master Mix Green High ROXTM	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**

The experiment was carried out using a 25 µL volume and the specific components mentioned in the *ACTR3* gene expression study. *ACTB* gene functioned as the internal control gene. We have formulated two solutions: one for the *ACTR3* gene and another for the *ACTB* gene. Before merging, the volume of each component was multiplied by a ratio of 22. Subsequently, all solutions were combined, with the exception of the cDNA sample.

We introduced the combination into the PCR plate, which housed two genes: the target gene (*ACTR3*) and the control gene (*ACTB*). Subsequently, we added 3.0 µL of cDNA samples into the plate for each gene, along with the corresponding pre-prepared forward and reverse primers. Following that we placed plate in the real-time PCR machine, in accordance with the specified criteria.

Table 3.6 shows stages form a comprehensive process that is integral for obtaining accurate and PCR results, playing a crucial role in various applications such as gene expression analysis, disease diagnosis, and genetic research.

Table3. 5. We Utilized a PCR Application to Perform cDNA Synthesis.

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

3.2.6. Statistical Analysis of *ACTR3* mRNA Gene qRT-PCR Data

After using qRT-PCR to determine the expression of the *ACTR3* gene at the mRNA level in both kidney cancer and control kidney tissues. The Step One Plus software v2.3 was utilized to quantify the qRT-PCR data for the *ACTB* and *ACTR3* genes. Absolute quantification is used when an exact number of amplifications is needed. It involves using a standard curve to determine the copy number of the target gene. Comparative quantification involves comparing the data collected from a reference gene (also known as an internal control gene) to the data obtained from the target gene. The *ACTB* gene, which serves as an internal control gene and is commonly used as a reference gene to minimize errors during amplification with the target gene, was employed to normalize the data regarding the *ACTR3* gene utilizing relative quantification. The $2^{-\Delta\Delta Ct}$ technique, which calculates the fold change, was used in combination with comparative CT (Table 7). The mean ± standard error of the mean (SEM) was employed to present the data, whereas the range of 0.9–1.1 was utilized to analyze the findings. If the results were less than 0.9, the expression level of the associated gene in kidney cancer tissue was decreased compared to normal kidney tissue. If the value fell within the range of 0.9-1.1, it indicated that there was no discernible difference compared to normal kidney tissue. Furthermore, if the outcome is above 1.1, the gene's expression is elevated compared to that of normal kidney tissue (Livak & Schmittgen, 2001).

The expression data was subjected to statistical analysis using SPSS 20.0. The clinical and demographic parameters of kidney cancer patients were presented as the

mean ± standard error of the mean (SEM) or as a percentage. The Paired t-test was employed to determine the difference in the expression of the *ACTR3* gene between normal and diseased tissues. The predetermined statistical significance threshold was established at 0.05. If the p-value is lower than 0.05, the data is deemed significant, suggesting a notable difference in gene expression. Conversely, if the p-value is greater than 0.05, it indicates that there is no statistically significant difference between the samples, and the data is considered not significant.

Table 3. 6. Final Formula of $2^{-\Delta\Delta CT}$ for Kidney Cancer Control Samples.

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

➢ **For Kidney Cancer Sample:**

ΔC_T (Cancer Tissue) = Ct (*ACTR3* gene) - Ct (*ACTB* gene)

➢ **For Control Samples:**

ΔC_T (Normal Tissue) = Ct (*ACTR3* gene) - Ct (*ACTB* gene)

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

$\Delta\Delta C_T = \Delta C_T$ (Cancer Tissue) - ΔCT (Normal Tissue)

(Livak & Schmittgen, 2001)

4. RESULTS

4.1 Demographic Information

Ten patients who were diagnosed with kidney cancers at Urological Department of Tokat Gaziosmanpasa University Hospital. The entire study conducted in Tokat Gaziosmanpasa University, Faculty of Medicine, Medical Biology and Genetics Laboratories.

Table 4.1 shows the distribution of patients by gender in a study. There are a total of 10 patients in the study, 5 of whom are male (50%) and 5 of whom are female (50%).

Table 4.1. Demographic Details.

Patients of the study	**Gender**	**N.**	**Percentage**
	Male	5	50 %
	Female	5	50 %
	Total	10	100 %

Table 4.2 shows the distribution of cancer stages among a sample of 10 patients. Stage 1 affects 5 patients (50 %), Stage 2 affects 1 patient (10 %), and Stage 3 affects 4 patients (40 %).

Table 4.2. Kidney Cancer Stages.

Kidney Cancer Stages	**N.**	**Percentage**
Stage 1	5	50 %
Stage 2	1	10 %
Stage 3	4	40 %
Total	10	100 %

4.2 Melting and Amplification Curves of *ACTR3* and *ACTB* Genes from qRT-PCR

Both the melting curve and amplification curve phases of the data are displayed (Figure 4.1 and Figure 4.2). The melting curve plot, shown in the derivative reporter view, allows us to examine the maximum rate of change in fluorescence as temperatures vary over time. This helps in assessing the specificity of the PCR products by distinguishing between specific and non-specific products based on their melting temperature (Zhang et al., 2020). Concurrently, the amplification plot illustrates the increase in product concentration during the PCR reaction, providing insight into the efficiency and dynamics of the amplification process (Kim et al., 2023).

In this study, *ACTB* is used as the internal control gene, while *ACTR3* is the target gene. The qRT-PCR technique facilitated the observation of both the amplification curves and melting peaks for the *ACTB* and *ACTR3* genes (Figure 4.3 and Figure 4.4). These plots are crucial for validating the accuracy and reliability of the RT-PCR results, ensuring that the observed gene expression levels are precise and specific to the target sequences.

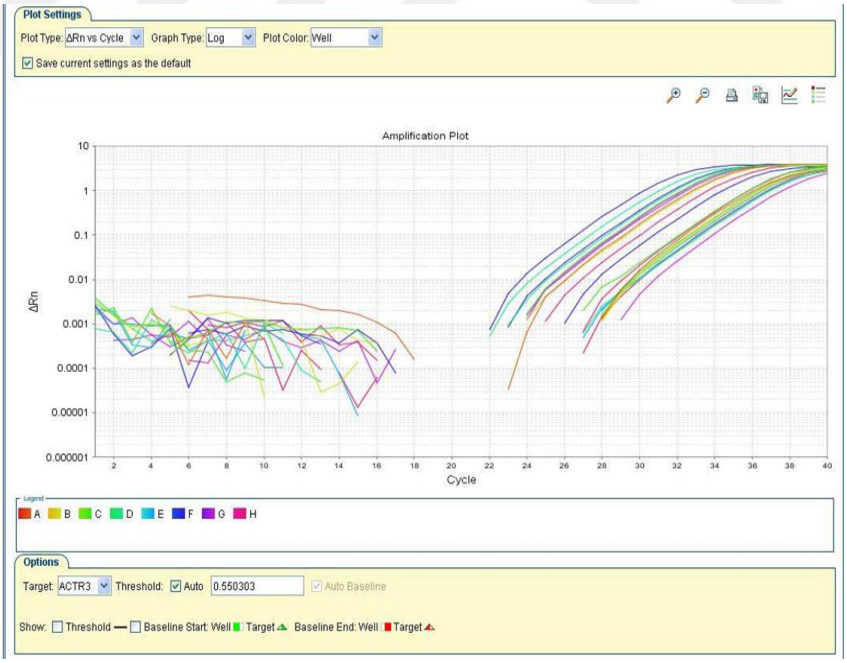

Figure 4.1. *ACTR3* Gene Amplification Plot

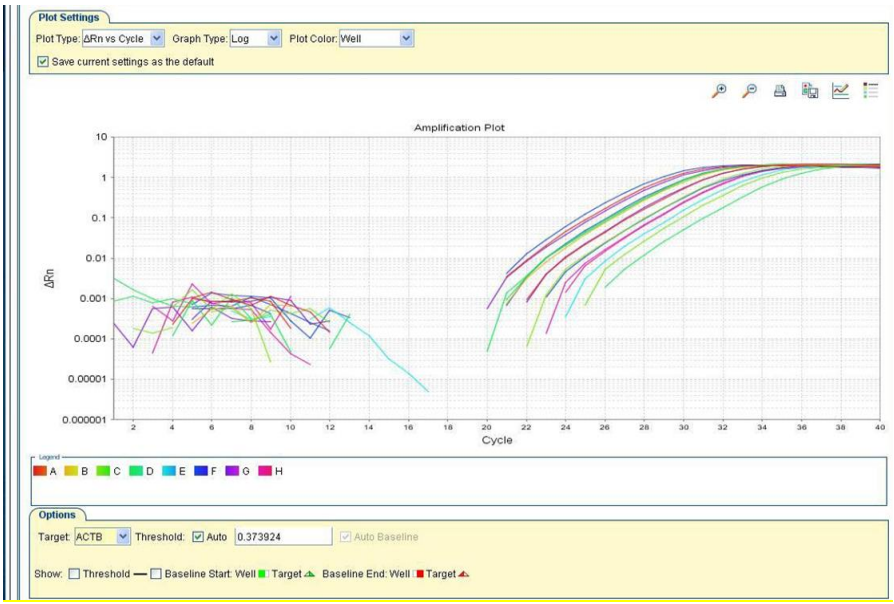

Figure 4.2. *ACTB* Gene Amplification Plot

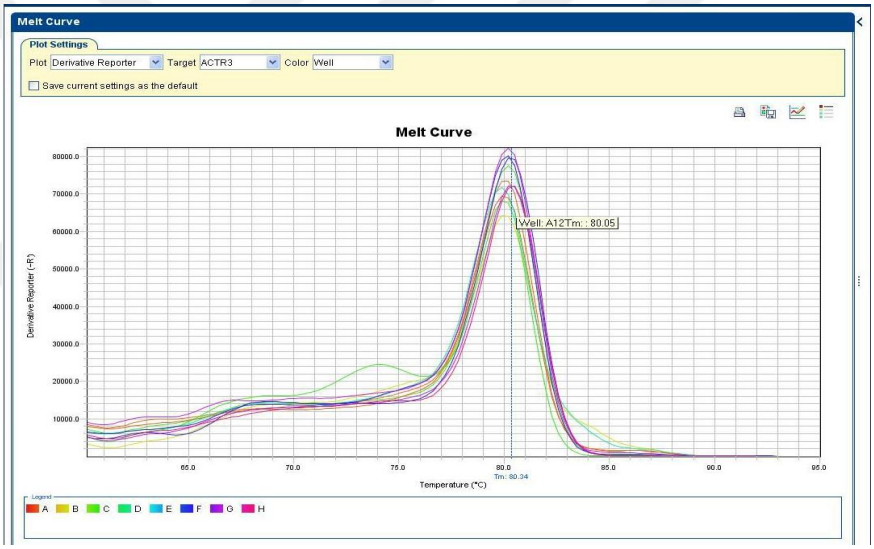

Figure 4.3. Melting Curve of *ACTR3*

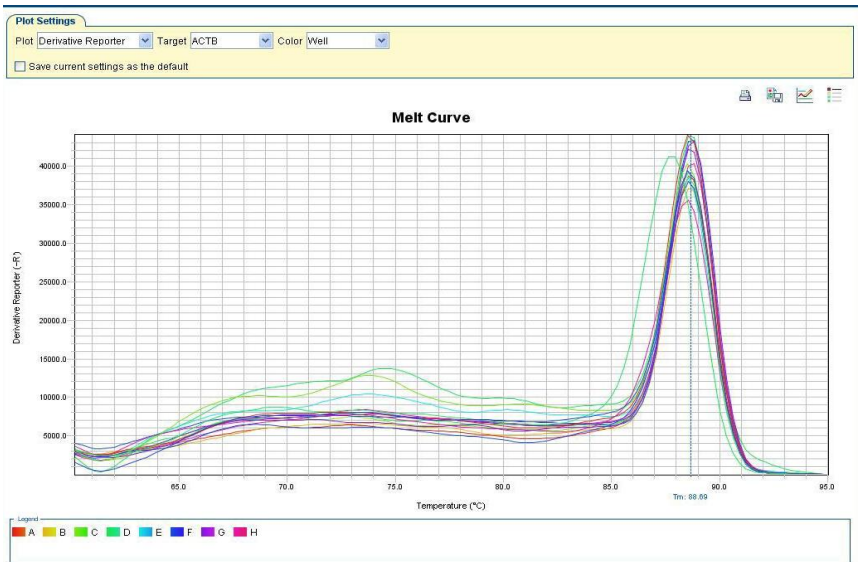

Figure 4.4. Melting Curve of *ACTB*

Table 4.3 shows the ΔΔCT values of the *ACTR3* and *ACTB* genes in 10 samples. The expression level of a target gene is compared in various samples by normalizing it to a reference gene and subsequently to a control sample. We indicated the fold change in *ACTR3* gene expression within kidney cancer tissue compared to its expression in normal kidney tissue, which is 1.147. The expression of the *ACTR3* gene is increased in the affected tissue compared to the control condition tissues in this test.

Table 4.3 The ΔΔCT Values of *ACTR3* and *ACTB* Genes.

Sample group	**$\Delta\Delta C_T$**	**$2^{-\Delta\Delta ct}$**
P1	-0.8604	1.815542
P2	0.9912	0.503059
P3	-1.477	2.783693
P4	1.5864	0.333001
P5	-0.2505	1.189619
P6	-1.011	2.015308
P7	0.4024	0.756599
P8	0.1563	0.897323
P9	1.3341	0.396639
P10	0.3508	0.784149

Table 4.4 provides the results of a paired sample t-test that compares the *ACTR3* gene expression levels in kidney cancer tissues. The fold change of 1.147 and Standard Error Mean (SEM) of 0.255, indicating a minor upregulation of the *ACTR3* gene in kidney cancer tissue compare to normal tissue. The study produces a p-value of 0.578, suggesting that the observed difference in the *ACTR3* gene expression levels in kidney cancer tissues statistically not significance.

Table 4.4 Statistical Analysis on The *ACTR3* Genes in Kidney Samples, Including Both Normal and Malignant Samples.

Samples	***ACTR3* gene (FC ± SEM)**	**P- Value**
Kidney Cancer Tissue (N=10)	1.147 ± 0.255	0.578
Control Kidney Tissue (N=10)	1	

5. DISCUSSION AND CONCLUSION

Kidney cancer, which is also called renal cell carcinoma, is a type of cancer that develops in the cells of the kidneys. It is a common form of cancer that accounts for approximately 80% of all adult cancers (Ferlay et al., 2018). Renal cell carcinoma is a serious illness that can have a profound impact on one's physical, emotional, and social health. The growth of tumors in the kidneys can cause a range of physical symptoms such as pain, discomfort, fatigue, weight loss, and changes in urinary function (Manavalan et al., 2017). The manifestation of kidney cancer symptoms is contingent on the stage and advancement of the ailment. Indications of kidney cancer in its early stages may comprise blood in the urine, discomfort or pain in the lower back or side, a mass or lump in the abdomen, inexplicable weight loss, fatigue, and recurrent fevers. Moreover, hypertension (high blood pressure), anemia (low red blood cell count), and swelling in the legs or ankles may also be experienced by some individuals with kidney cancer (Russo, 2023).

ACTR3 is a gene that plays a crucial role in cellular processes like cytoskeletal organization and cell migration. It is a significant component of the Arp2/3 complex, which regulates actin polymerization and the organization of filaments in the actin cytoskeleton. Dysfunctions in this complex have been implicated in various mental disorders. ARP3 is involved in various cellular processes related to the organization and dynamics of the actin cytoskeleton (Chen & Jiang, 2022). Previous studies indicate that *ACTR3* is highly expressed in immune cells of different lung cancer types, indicating its potential as a candidate gene for immunotherapy strategies in cancer treatment (Liu & Deng, 2022). The *ACTR3* gene has been extensively researched in relation to cancer. It plays a role in various types of cancer, such as lung cancer and cholangiocarcinoma (He et al., 2022). Additionally, more recent research has confirmed that *ACTR3* plays critical roles in the early immunodiagnosis of these types of cancer (Wang et al., 2020).

Another study explored the significance of *ACTR3* in the context of pancreatic ductal adenocarcinoma (PDAC), a cancer known for its high mortality rate and limited treatment options. It was discovered that PDAC tissues and cell lines exhibit a significant increase in *ACTR3* expression, and elevated levels of *ACTR3* were linked with a worse prognosis in patients with PDAC. When *ACTR3* was knocked down, PDAC cells showed a reduced capacity for invasion and migration, alongside alterations in cell shape, a decrease in mesenchymal markers, an increase in epithelial markers, and a redistribution

of F-actin. These changes indicate that *ACTR3* plays a role in promoting the epithelial-mesenchymal transition (EMT), a key process in cancer metastasis, suggesting its potential as a therapeutic target and prognostic marker for PDAC. The research adhered to ethical standards and received support from various funding sources (Jagannatha, et al., 2022).

According to Hu et al., *ACTR3* may play a crucial role in tumor development and progression (Hu et al., 2021). The expression of *ACTR3* has been found to be increased in multiple types of cancer, including gastric cancer, squamous cell carcinoma, colorectal cancer, lung cancer, and cholangiocarcinoma (Rashid et al., 2022). *ACTR3* plays a pivotal role in the pathogenesis of cancer, as highlighted by its involvement in critical processes such as cell migration, invasion, and metastasis (Zheng et al., 2023). Research illustrates that this protein is key in the formation of lamellipodia and filopodia, essential structures for the mobility and invasiveness of cancer cells. (Hu et al.,2021). Furthermore, it has been shown to facilitate the epithelial-mesenchymal transition, a vital process for cancer cells to develop invasive and metastatic capabilities (Ali et al., 2022). In addition, interactions of *ACTR3* with other proteins, including profilin-1 and LIM domain only 2, in breast cancer underscore its significant contribution to cancer progression (Hu et al., 2021).

Studies have underscored the significance of *ACTR3* gene mutations in the onset and advancement of cancer. These mutations are believed to hinder the gene's capacity to regulate actin dynamics, resulting in atypical cell proliferation and division (Sohn, et al., 2022). However, despite the mounting evidence pointing to the association between *ACTR3* mutations and cancer, the precise mechanisms by which they impact cancer pathogenesis are not yet fully understood and necessitate further exploration. Moreover, no cancer treatments have been designed to specifically target the *ACTR3* gene (Wang et al., 2022).

Research has shown that all members of the Arp2/3 complex, including *ACTR3*, are significantly overexpressed in HCC tissues compared to normal tissues. This overexpression correlates with the aggressiveness of the cancer and its prognosis, suggesting that *ACTR3* might serve as a potential biomarker for HCC (Huang, et al.,2021). ACTR3 is implicated in promoting cell migration and invasion through inducing epithelial-mesenchymal transition (EMT), a key process in cancer metastasis.

Studies using cell cultures have demonstrated that silencing ACTR3 can reduce cancer cell invasiveness, making it a potential target for cancer therapy (Hu, et al.,2021).

In our investigation, 10 patients had been investigated, to evaluate the expression levels of *ACTR3* in both renal cell carcinoma and healthy tissue. Quantitative real-time PCR (qPCR) analysis revealed notable variations in *ACTR3* expression, which correlated with the progression of kidney cancer stages. The results of a paired-sample t-test indicated that the levels of *ACTR3* expression in kidney cancer tissues (1.147 ± 0.255) did not exhibit a statistically significant difference compared to those in control kidney tissues, with a p-value of 0.578. Furthermore, the expression patterns of *ACTR3* in different subtypes of renal cell carcinoma (RCC) varied across stages, as confirmed by melting curve analysis during quantitative polymerase chain reaction (qPCR) for specific amplification. These findings suggest that although *ACTR3* may play a role in RCC, its expression levels do not show significant differences between cancerous and normal kidney tissues. Despite the limited sample size, the study observed relatively stable expression of the *ACTB* gene among samples. However, the presence of *ACTR3* in kidney cancer tissue highlights the need for further research with larger samples to refine these findings, especially considering the potential influence of environmental, genetic, or lifestyle factors on the results.

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