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MASTER'S PROGRAM

ANALYSIS OF *WAVE3* GENE EXPRESSION IN KIDNEY CANCER

MASTER'S THESIS

SOZI TAHIR AHMED

Supervisor: Prof. Dr. Haci Omer ATES

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ETHICS CONTRACT

T.C.

TOKAT GAZIOSMANPASA UNIVERSITY

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With this document, I proclaim that the Master's thesis named "Analysis of *WAVE3* Gene Expression in Kidney Cancer" is an authentic work that I prepared in conformity with the guidelines of Tokat Gaziosmanpasa University - Institute of Graduate Studies and under the supervision of Prof. Dr. Haci Omer ATES. I herewith declare that all the information in the written thesis have been assembled and handed over in accordance with academic rules and ethical principles, and in accordance with these standards and principles, I have cited all data, thoughts, and results that do not belong to me.

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The Student Who Prepared the Thesis:

Sozi Tahir Ahmed

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ABSTRACT

Analysis of *WAVE3* Gene Expression in Kidney Cancer

Sozi Tahir Ahmed

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Cancer is a disease distinguished by uncontrollable cell proliferation that can develop in almost any organ or tissue, such as the kidney. Kidney cancer is a complex illness. Multiple distinct subtypes of kidney cancer develop in the kidney's cells; these subtypes vary in their histology, clinical manifestations, therapeutic responses, and underlying genetic causes. The Wiskott-Aldrich syndrome protein family verprolin homologous protein 3 (*WAVE3)* gene, whose official name is WASP family member3 (*WASF3)*, belongs to the WASP-WAVE family and is an actin cytoskeleton remodeling protein. *WAVE3* is required for many regulatory functions in cells, including organizing cell shape, regulating actin-filament polymerization of the cell membrane, motility, migration, and cell metastasis. Furthermore, it is associated with cellular proliferation and cytokinesis. Nonetheless, *WAVE3* has a major function in cancer cell invasion and metastasis. Thus, it acts as a metastasis-enhancing protein. This present investigation on the Turkish population is the first to examine the relationship between the *WAVE3* gene and kidney cancer. The aim of present study was to determine the expression level of the *WAVE3* gene in kidney cancer tissues relative to healthy tissues. The study involved 11 kidney cancer tissues and 11 healthy tissues from the same participants. The gene's expression level was measured by the quantitative real time polymerase chain reaction technique (qRT-PCR). According to the findings of the present study, there is no difference in *WAVE3* gene expression in kidney cancerous tissues when compared with healthy tissues (fold change = 0.93), and regarding this finding, statistically, no significant correlation was found (p = 0.87). Accordingly, further studies with large samples are needed to analyze and clearly understand the *WAVE3* expression level relationship to kidney cancer.

Key Words: Kidney cancer, Real-Time PCR, *WAVE 3*.

ÖZET

Böbrek Kanserinde *WAVE3* Gen Ekspresyonunun Analizi

Sozi Tahir Ahmed

Yüksek Lisans, Tıbbi Bioloji Ana Bilim Dalı

Tez Danışmanı: Prof. Dr. Hacı Ömer ATEŞ

Temmuz 2024, xi + 60 sayfa

Kanser, böbrek gibi hemen hemen her organ veya dokuda gelişebilen, kontrol edilemeyen hücre çoğalması ile ayırt edilen bir hastalıktır. Böbrek kanseri karmaşık bir hastalıktır. Böbrek hücrelerinde çok sayıda farklı böbrek kanseri alt tipi gelişir; bu alt tipler histolojileri, klinik belirtileri, terapötik yanıtları ve altta yatan genetik nedenleri bakımından farklılık gösterir. Resmi adı WASP aile üyesi 3 (*WASF3*) olan Wiskott-Aldrich sendromu protein ailesi verprolin homolog protein 3 (*WAVE3*) geni, WASP-WAVE ailesine aittir ve bir aktin hücre iskeleti yeniden şekillendirme proteindir. *WAVE3*, hücre şeklinin düzenlenmesi, hücre zarının aktin-filament polimerizasyonunun düzenlenmesi, hareketlilik, göç ve hücre metastazı dahil olmak üzere hücrelerde birçok düzenleyici işlev için gereklidir. Ayrıca, hücresel proliferasyon ve sitokinez ile ilişkilidir. Bununla birlikte, WAVE3 kanser hücresi invazyonu ve metastazında önemli bir işleve sahiptir. Böylece, metastazı artırıcı bir protein olarak görev yapar. Türk toplumu üzerinde yapılan bu araştırma, *WAVE3* geni ile böbrek kanseri arasındaki ilişkiyi inceleyen ilk çalışmadır. Bu çalışmanın amacı, sağlıklı dokulara kıyasla böbrek kanseri dokularında WAVE3 geninin ifade düzeyini belirlemektir. Çalışmaya aynı katılımcılardan alınan 11 böbrek kanseri dokusu ve 11 sağlıklı doku dahil edilmiştir. Genin ifade düzeyi kantitatif gerçek zamanlı polimeraz zincir reaksiyonu (qRT-PZR) tekniği ile ölçülmüştür. Bu çalışmanın bulgularına göre, sağlıklı dokularla karşılaştırıldığında böbrek kanserli dokularda *WAVE3* gen ifadesinde bir fark yoktur (kat değişimi = 0.93) ve bu bulguyla ilgili olarak istatistiksel olarak anlamlı bir korelasyon bulunmamıştır ($p = 0.87$). Buna göre, *WAVE3* ifade düzeyinin böbrek kanseriyle ilişkisini analiz etmek ve net bir şekilde anlamak için büyük örneklerle daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Böbrek kanseri, Gerçek Zamanlı PZR, *WAVE 3*.

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ABBREVATIONS LIST

ARP: Actin-related protein

BC: Breast Cancer

BHD: Birt-Hogg Dubé

cc-RCC: clear-cell Renal Cell Carcinoma

CD4+: Cluster of Differentiation-4

CD8+: Cluster of Differentiation-8

ch-RCC: chromophobe Renal Cell Carcinoma

CYFIP1: Cytoplasmic FMR1-interacting protein-1

DNA: Deoxyribonucleic Acid

dNTP: Deoxynucleotide Triphosphate

ECM: Extracellular Matrix

EMT: Epithelial to mesenchymal transition

FGF: Fibroblast Growth Factor

FGFR: Fibroblast growth factor receptors

HIF: Hypoxia-Inducible Factor

HIF-1α: Hypoxia-Inducible Factor-1-alpha

HIF-2α: Hypoxia-Inducible Factor-2-alpha

HIF-β: Hypoxia-Inducible Factor -beta

HL-RCC: hereditary-leiomyomatosis Renal Cell Cancer

HPRC: hereditary Papillary Renal Cancer

HSP70: Heat-shock protein 70

HSP90: Heat-shock protein 90

HSPC300: Hematopoietic stem and progenitor cells 300

LAG3: Lymphocyte Activating 3

miRNA: MicroRNA

MMPs: Matrix metalloproteinase

mRNA: Messenger Ribonucleic Acid

NCKAP1: NCK associated protein 1

PDGF: Platelet derived/growth factor

PDGFR: Platelet derived/growth factor receptors

PD-L1: Programmed death ligand 1

PI3K: Phosphoinositide-3-kinase

PIP3: Phosphatidylinositol(3,4,5)-triphosphate

pRCC: Papillary Renal Cell Carcinoma

qRT-PCR: Quantitative/Real-Time Polymerase Chain Reaction

RCC: Renal Cell Carcinoma

RNA: Ribonucleic Acid

STAT3: Signal transducer and activator of transcription-3

TGF-α: Transforming Growth Factor-alpha

TILs: Tumor-infiltrating lymphocytes

VCA: Verprolin-cofilin-acidic

VEGF: Vascular-Endothelial Growth Factor

VEGFR: Vascular-Endothelial Growth Factor Receptor

VHL: Von Hippel-Lindau

WAS: Wiskott-Aldrich Syndrome

WASF3: WASP Family Member3

WAVE3: Wiskott-Aldrich syndrome protein family member 3

WHAMM: WASP-homologue-associated with actin, golgi membranes, and microtubules

WHD: Verprolin homology domain

1.INTRODUCTION

Cancer is a disease distinguished by uncontrollable cell proliferation that can develop in almost any organ or tissue such as lung, colon, breast or kidney tissues, and result in a mass or tumor (Cooper and Hausman, 2018).

A tumor is an aberrant mass of cells in the body; it's caused by cells that divide more than normal or don't die when they're supposed to (apoptosis) and may either be malignant or benign (Patel, 2020). A tumor that remains contained in its original location and is neither invading nor disseminating belongs to a benign tumor, which is one that does not penetrate neighboring healthy tissue or spread to other areas within the individual. However, cancerous tumors may metastasize (spread) via the lymphatic or blood circulation systems as well as invade neighboring healthy tissue (metastasis). The term "cancer" is properly reserved for malignant tumors (Cooper and Hausman, 2018).

The processes that control healthy cell proliferation, differentiation, and survival frequently exhibit aberrations in cancer cells. These features of cancer cells present the definition of cellular malignancy (Victor et al., 2020).

Previously, it have been mentioned by studies that cancer is correlated with several causes, which are known as risk factors. Both intrinsic and extrinsic variables contribute to the development of cancer. Extrinsic causes include smoke, pollutants, ionizing radiation, and pathogenic microbes such as viruses; intrinsic causes include immune conditions, hormones, inherited mutations, and random mutations. On top of that, there are a plethora of other recognized carcinogens, such as unhealthy food choices, certain illnesses, lack of exercise, obesity, and environmental contaminants. Substances that cause cancer, referred to as carcinogens (Anand et al., 2008).

One of the organs that suffers from cancer is the kidney. The kidneys are intricate organs that are essential for maintaining healthy body processes. The system of the renal-urologic comprises kidneys, ureters, urinary bladder, and urethra (Wallace, 1998). The kidney's primary function is to regulate the fluid, electrolyte, and acid-base balances and glucose resorption inside the body to establish a stable environment for the metabolism of tissue and cells (Scott and Quaggin, 2015).

The condition known as kidney cancer is a complex illness. Multiple distinct subtypes of kidney cancer develop in the kidney's cells; these subtypes vary in their histology,

clinical manifestations, therapeutic responses, and underlying genetic causes (Linehan et al., 2003). The incidence and death rates of kidney cancer, the third most frequent cancer among urogenital malignancies, vary greatly by region, sex, and age (Pençe et al., 2019). In 2020, there will likely be 431,288 new cases and 179,368 fatalities globally. Kidney cancer ranks as the fourteenth most prevalent cancer globally, according to the Global Cancer Observatory (Bukavina et al., 2022). Earlier studies have mentioned that there are several types of kidney cancer. With over 90% of all renal malignancies in adults being renal cell carcinoma (RCC), this kind of kidney cancer is by far the most common. Other names for it include hypernephroma and the Grawitz tumor. It has a number of histological subtypes and makes up more than 3% of all adult cancers. It is an older age group tumor that most frequently affects people between the ages of 50 and 70. It is seen twice in male than in female. Renal pelvis transitional cell carcinoma is another type that has characteristics similar to cancer of bladder. Another uncommon tumor of the kidney is renal sarcoma (Siegel et al., 2020). Also, Wilms tumor is a rare kind of the condition and most common in children. Among malignancies for which a documented cause-effect etiology is lacking, RCC stands out. With respect to RCC, we can only make an effort to determine a few clinical, occupational, and tumor-related factors (Pascual and Borque, 2008). For RCC, history of cigarette smoking and history of using tobacco products are significant risk factors. Obesity (mainly in female) is a crucial risk factor as well. Some other factors are hypertension (HTN), chronic kidney disease, family history, dialysis, diet, life style, and chemical carcinogens like trichloroethylene (TCE) (Pandey and Syed, 2020). Certainly, for the occurrence of a few instances of kidney cancer, genetics have a great role due to mutation in gene, such as some genetic syndromes (Bahadoram et al., 2022).

The metastatic spread of cancer cells from their original site of development to other parts of the body is the main cause of cancer-related deaths for cancer patients (Guan, 2015). About 35% of kidney cancers, or RCC, eventually develop into metastatic (mRCC) (Lin et al., 2021). With the majority of locations being the lung, liver, bone, lymph nodes, adrenal gland, and brain (Umer et al., 2018). Metastasis is an extremely cohesive, multi-stage process involving the stroma, blood arteries, and cytoskeleton. At this level, deformation of the cytoskeleton and alteration in cytoskeleton-linked gene and protein expressions occur (Strube et al., 2020). Various studies showed that these molecules, which provide overexpressing migratory signals to the actin cytoskeleton, are seen in metastatic cancer cells and invasive cells. These proteins, are the actin-related protein 2/3

(Arp2/3) complex, cofilin, cortactin, and members of the WASP family (Yamaguchi and Condeelis, 2007).

The Wiskott-Aldrich syndrome protein family verprolin homologous protein 3 (*WAVE3*) gene, whose official name is WASP family member 3 (*WASF3*), belongs to the WASP-WAVE family and is an actin cytoskeleton remodeling protein. This group of proteins is known to influence cell shape, motility, cytokinesis, invasion, and cytoskeletal architecture, among other biological processes. They are structurally related proteins (Lu et al., 2017).

The WAVE3 protein helps cancer cells move, invade, and spread to other areas of the body, by activating the actin-related protein Arp2/3 complex. Its activity regulates the actin cytoskeleton assembly (Cory and Ridley, 2002). Therefore, WAVE3 acts as a metastasis-enhancing protein (Sossey-Alaoui et al., 2011).Among multiple WASP and WAVE proteins, *WAVE3* is one of the major agents that promote metastasis in different cancer subtypes. The phenotypic and invasive features of cancer cells linked to their potential for metastasis and governed by the mechanism that controls the expression of *WAVE3* in cancer cells (Sossey-Alaoui et al., 2009).

As aforementioned, in healthy cells, WAVE3 is an actin-binding protein that acts as a signal transducer that results in alterations in cytoskeletal structure and cell shape through regulatory mechanisms, and this gene in the nervous system is mostly expressed (Sossey-Alaoui et al., 2007). Prior research on *WAVE3*'s function in cancer cell invasion and metastasis shown that it may play a role in the crucial early phase of the invasion-metastasis cascade, the epithelial to mesenchymal transition (EMT). And in a wide variety of human cancers—from non-small cell lung cancer to hepatocellular, colorectal, gastric, esophageal, bladder, pancreatic, and intrahepatic cholangiocarcinoma—the *WAVE3* gene is overexpressed and causes an increase in cancer cells that are invasive and metastasized (Wu et al., 2014 ; Zhu et al., 2016 ; Wang et al., 2020).

The aim of the current study was to determine the expression level of the *WAVE3* gene in kidney cancer. As well, to learn about the role of the *WAVE3* gene mechanism in kidney cancer. In addition, the findings of this investigation may help researchers highlight the potential of targeting *WAVE3* as a therapy for prevention and as a prognostic indicator for kidney cancer.

2. LITERATURE REVIEW

2.1. Cancer Overview and It is Prevalence

Cancer is widely identified as a universal problem that, unfortunately, lacks a universal solution. Behind the cardiovascular disease, cancer is the second-most common cause of mortality around the world. A definition of cancer includes cells that begin to grow abnormally and maintain altered cells, as well as acquiring metastatic characteristics that leading to death (Sarkar et al., 2013). There are several kinds of cancer, but the most prevalent ones are breast, prostate, lung, and colorectal, which together account for about half of all cases. In addition, approximately the deadliest type of cancer is lung cancer (25% of all-cases) (Cooper and Hausman, 2018).

In 2022, the rates of cancer occurrence and fatality classified by age groups and gender for 36 locations of cancer across 185 countries or regions globally were evaluated. The global estimates for cancer in 2022 included 20.0 million new cases and 9.7 million deaths for men and women together. Also in 2022, the highest cancer fatality rate and nearly half of all cases were accounted for in Asia (Bray et al., 2024). In addition, in 2020, on a global scale, the three most common malignancies were prostate, lung, and breast cancer in women. The most common malignancies that resulted in death were those of the liver, stomach, and lungs (Ferlay et al., 2021).

Accumulated cells in the body, as a result of abnormal growth, produce a mass called a tumor. The first step in the discovery of tumors is to distinguish them from healthy biological tissue by comparing them according to their physical variations (differences in cell or tissue size, structure, and shape) and biological variations (variances in growth, metabolism, and angiogenesis related to genetic alterations), and tumors inside their host tissues stimulate these physical and biological changes to provide tumor pathogenesis and progression (Jiang et al., 2023). According to studies, early tumor diagnosis results in improved cure rates and longer life expectancies.

Tumor could be either benign or malignant. The following characteristics distinguish benign from malignant tumors: (1) development; (2) pace of growth; (3) pattern of growth; and (4) disseminate to other area of the body (metastasis) (Jang et al., 2011). In most cases, a benign tumor is completely noncancerous and does not spread to other area of the body. Typically have a more moderate pace of growth, a good degree of differentiation,

and are encapsulated. Some types of benign tumors may be harmful to human health and can be removed by surgery (Patel, 2020). As opposed to a benign tumor, a malignant tumor is cancerous and invades neighboring tissue or metastasizes through the blood and lymphatic systems. As well as being characterized by: fast growth, non-capsulated, frequently unwell differentiation, cell cycle speedup (mitosis), genomic alterations, invasive growth, increased cell motility, cellular surface modifications, and a large nucleus (Jang et al., 2011).

The capability of cancer tumors is to metastasize from their original place to nearby tissues and distant organs, which creates a risk to life. However, cells of the cancer without new vasculature are unable to spread or develop larger than 2-3 mm3 and these two mechanisms—angiogenesis and lymphangiogenesis are terms used to describe the creation of new lymph and blood vessels. The angiogenesis is a physiological mechanism that has a fundamental function in the creation of a new vascular network that transports waste materials and supplies oxygen and nutrients. But in cancer cell it is overactivated and poorly regulated. Cancer angiogenesis is a multistep process caused by hypoxia and the release of matrix metalloproteinases(MMPs), which permit endothelial cells to invade neighboring tissues as well as create new vessels (Ravi et al,. 2022).

2.1.1. Cancer classification

The classification of cancer decides the course of treatment in the clinic. As per the Health Organization of the World (WHO) and the Union for the International Control of Cancer (UICC)'s classification, cancer is classified depending on four methods: histological type, primary location of disease, tissue form grade, cancer metastasizing inside the body (Carbone, 2020).

There are over a hundred types of cancer in the world (Song et al., 2015). Currently, the studies have mentioned that cancer types can be classified based on the anatomical location in which they arise (tissue or organs) into three major groups: carcinoma, sarcoma, leukemia, or lymphoma. About 80% to 90% of all cancer cases are carcinomas, making them the most frequent kind of cancer. Which develops from the epithelial tissue of any organs of the human body, such as the stomach, intestines, skin, and other organs within the body. Sarcoma is a tumor that develops from connective tissue and thus forms in bone,

cartilage, muscle, fat, and vascular. Lymphomas are any tumors that arise in the lymphatic system (Carbone, 2020).

2.2. Kidney Cancer

The kidneys are intricate organs with a bean shape that are essential for maintaining healthy body processes. The system of the renal-urologic comprises kidneys, ureters, urinary bladder, and urethra (Wallace, 1998). The kidneys exist on each side of the spinal column, behind the peritoneum in the abdomen, and usually they stretch from T12 to L3. The liver's presence causes the right kidney to be slightly lower. For women, the kidneys weigh between 120 and 135 g, but for men, it is nearly 150 and 200 g. A closed fist roughly represents the size of each kidney (Soriano et al., 2018). The kidney consists of two distinct regions: the kidney cortex and the kidney medulla, each with multiple parts (Figure 2.1) (McMahon et al., 2023). The kidney's structural functional unit is the nephron, which is located in the cortex and medullary sections (Soriano et al., 2018).

The kidney's primary function is to regulate the fluid, electrolyte, and acid-base balances and glucose resorption inside the body to establish a stable environment for the metabolism of tissue and cells (Scott and Quaggin, 2015).

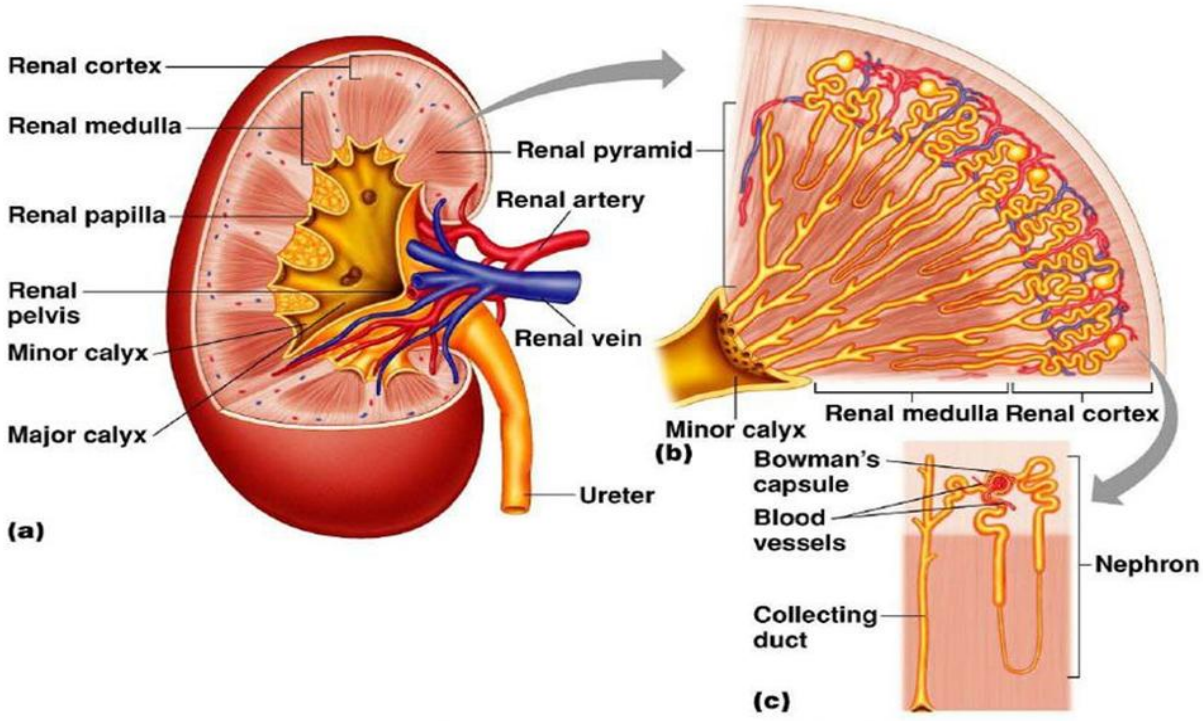

Figure 2.1. Kidney anatomy at a macroscopic scale (Hoban, 2008)

Cancer of the kidney is a complex illness, really a collection of sub-types that develop in the kidney's cells; these sub-types have distinct histologies, clinical manifestations, treatment reactions, and underlying genetic causes (Figure 2.2) (Linehan et al., 2003). In addition, some types of kidney cancer grow very slowly and are indolent, while others are very aggressive and can spread very quickly (Linehan, 2012). Marston Linehan has suggested that more than twelve different genes can lead to kidney cancer, such as receptor tyrosine kinase gene (*MET),*Fumarate hydratase gene *(FH),* von Hippel-Lindau (*VHL*) tumor suppressor gene*,* and folliculin gene *(FLCN)*. Moreover, it was discovered that kidney cancer is essentially a metabolic illness for the reason that all of these genes affects the cell's capability to perceive changes in nutrition, energy, oxygen, and iron (Lerner et al., 2012). Also, it is suggested in many studies that *VHL* gene is inactivated in majority of this disease. Thus, being aware of the genetic basis of kidney cancer can provide us with the chance to increase efficient therapy forms and learn how to manage this cancer (Linehan et al., 2003).

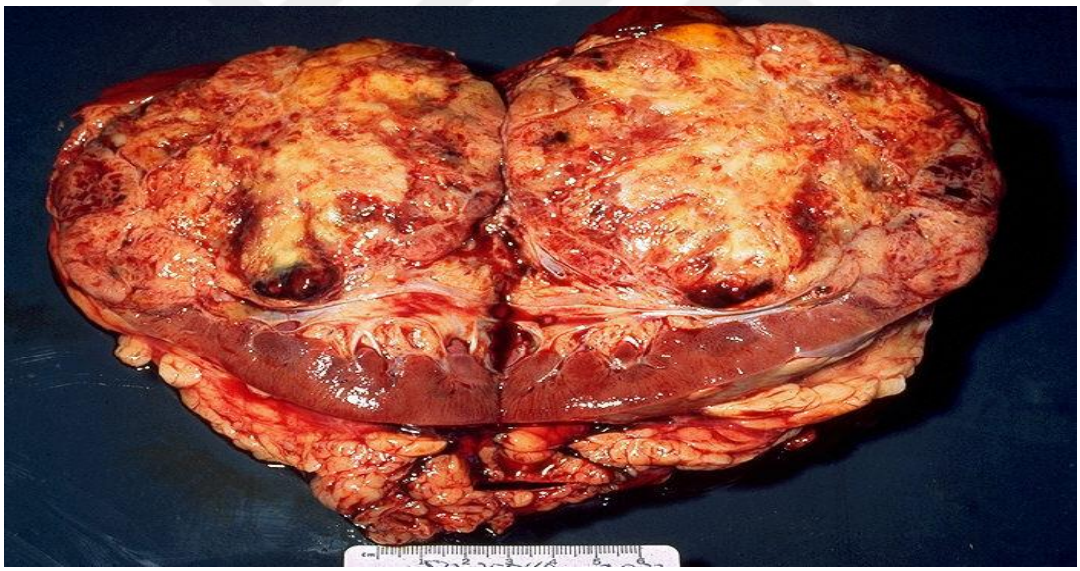

Figure 2.2. A large tumor of the renal cell carcinoma (RCC) tissue (Pandey and Syed, 2020)

There are many studies that suggest various risk factors that cause kidney cancer. The strongest risk factor for the condition is smoking and using tobacco products. Obesity, HTN, and chronic kidney diseases are also crucial factors. Nevertheless, other probability factors are geography, sex (males are more affected than females), age (adults are more affected than young people), ethnicity or race, environmental factors such as TCE exposures, reduced physical activity, diabetes, middling use of alcohol (more than 2 drinks daily), and genetic factors (Scelo and Larose, 2018). Also, it has been suggested that level

of vitamin D and human height have an effect on the occurrence of this disease (Scelo and Larose, 2018; Bahadoram et al., 2022).

Usually, the majority of kidney cancers are asymptomatic, not showing any signs until they have migrated outside the kidneys. When the tumor mass grows at an advanced stage, the symptoms include hematuria, loin or back pain, flank mass, fatigue, weight loss, hypertension, and anemia. Based on the tumor's size and distribution throughout the body, there are four stages of kidney cancer (Figure 2.3). Grade 1: the tumor is smaller than 7cm, remains inside the kidneys, and has not migrated. Grade 2: In this stage, the tumor is bigger than 7cm. In addition, tumors in stages 1 and 2 can be eliminated by one of two radical or partial nephrectomy surgeries. Grade 3: During this stage, either the tumor has begun to grow outside of the kidney into the surrounding adipose tissue, has migrated to a nearby lymph node, or has spread to the kidney's main blood vessels. Grade 4: This stage is called metastatic because the tumor has invaded several lymph nodes or has significantly migrated to other organs such as the lungs, bone, or brain (Thakur and Jain, 2011).

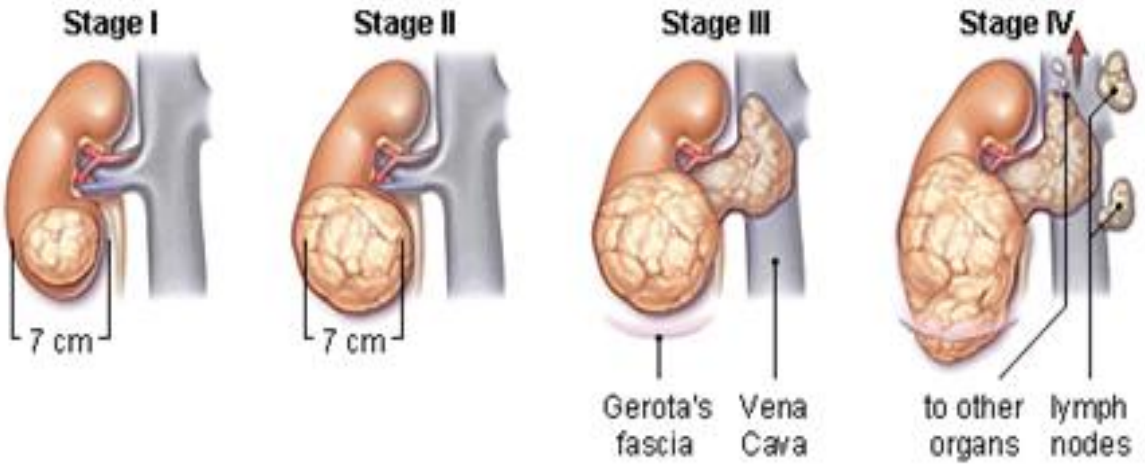

Figure 2.3. Stages of kidney cancer tumor development (Thakur and Jain, 2011)

2.3. Epidemiology

As mentioned above, cancer of kidney is a complex and metabolic disease that often occurs in old people and this disorder is more common in male than female (Schmidt et al.,

2010). Studies have indicated that kidney cancer cases have risen globally since 1975 (Luciani et al., 2000). Also, the mortality rate for this condition increased (Chow et al., 1999; Cai et al., 2020). However, the rates of incidence and mortality vary from country to country; for example, the rate is greater in developed countries than in non-industrialized countries. According to studies, it has been shown that North US, Europe, Japan and Australia-New Zealand have the higher prevalence of kidney cancer, with the Czech Republic having the highest rates among all countries (Scelo and Larose, 2018). Nevertheless, most people in Africa and Asia have the lowest possible rates, excluding Israel (Znaor et al., 2015). Recently, there have been 400,000 new kidney cancer cases worldwide annually, and around 175,000 people die because of the condition every year (Cirillo et al., 2024). In 2020, there will likely be 431,288 new cases and 179,368 fatalities globally, kidney cancer ranks as the fourteenth most prevalent cancer globally, according to the Global Cancer Observatory (Bukavina et al., 2022).

According to research, the five year relative survival in total associated with those individuals that were identified early in the course of the disorder ranged from 20% to 90%. While five-year relative survival ranged from 0% to 10% for those with advanced (metastatic) stage (Linehan et al., 2019).

2.4. Histopathology

Kidney cancer is composed of multiple distinct subtypes of cancer. These subtypes vary in their histology, clinical manifestations, therapeutic responses, and underlying genetic causes. Similar to cancers of the bladder, colon, prostate, and breast, kidney cancer can be sporadic (not inherited) or familial (inherited). Furthermore, according to a study, the sporadic form often manifests as a solitary lesion, while bilateral and multifocal of the condition often tend to be inherited (Linehan et al., 2003). The kinds of kidney cancer include RCC (>90%), renal pelvis carcinoma (8%), nephroblastoma or Wilm's tumor, and sarcoma (Pandey and Syed, 2020)

RCC is the most prevalent form of kidney cancer and constitutes more than 90% of all the conditions. It is also a heterogeneous form that originates from renal tubular epithelial cells (Hsieh et al., 2017). These renal tubular are the tiny tubes in the kidney that eliminate waste material and filtrate blood (Thakur and Jain, 2011). Furthermore, the most

frequent locations for metastatic RCC are the lung, liver, bone, and lymph nodes, which affect around 35% of all cases and lead to death(Lin et al., 2021). RCC is histologically categorized into several distinct subgroups; the prevalent subtypes are clear cell RCC (75%), papillary RCC (pRCC) (15%), chromophobe RCC (5%), and oncocytoma RCC (5%) (Figur 2.4) (Linehan et al., 2003).

It is believed that the source of cc-RCC and p-RCC is the proximal tubule epithelium (kidney cortex) carcinoma, but chromophobe and oncocytoma RCC, are occur from the collecting tubule epithelium (Cairns, 2011). ccRCC represents the majority of subtypes, making up around 75% of the entire condition. Macroscopically, ccRCC is a solid, yellowish lesion with internal necrosis, bleeding, and cystic degeneration that is frequently discovered in large-volume, quickly-expanding tumors. It frequently displays clear cells with eosinophil granular cytoplasm. Moreover, it is characterized by having a cytoplasm with rich lipids and glycogen (DeCastro and McKiernan., 2008).

pRCC and chromophobe RCC subtypes are the second and third most prevalent, respectively. There are two kinds of pRCC, types 1 and 2, and they both make up around 15% of all cases. In addition, the basal membrane of Type 1 pRCC is encased in a single basophilic tissue layer, whereas subtype 2 has cytoplasm that is granularly eosinophilic. Type 1 is generally discovered sooner and at a lower grade than type 2. In addition, pRCCs often have a better prognosis than ccRCCs, and they are stable, clearly defined, and slowly expanding lesions (Lubensky et al., 1999).

Chromophobe RCC nearly constitutes 5% of RCC. It exhibits a milder level of aggressiveness compared to ccRCC and has the greatest prognosis. It typically occurs most frequently in individuals in their sixties. Furthermore, microscopically, they show big, light-colored cells with cytoplasm containing a reticulated appearance and perinuclear halos. Additionally, it is closely related to oncocytomas (Muglia and Prando, 2015).

Other varieties of kidney cancer comprise renal pelvis transitional cell carcinomas (8%), which have bladder cancer-similar characteristics and originate from the central region of the kidney where urine accumulates (Thakur and Jain, 2011). Moreover, other remaining types of kidney cancer are rare, such as renal sarcoma (<1%) (Siegel et al., 2020). And Wilms tumor, which is a rare form of the condition, is especially prevalent among children.

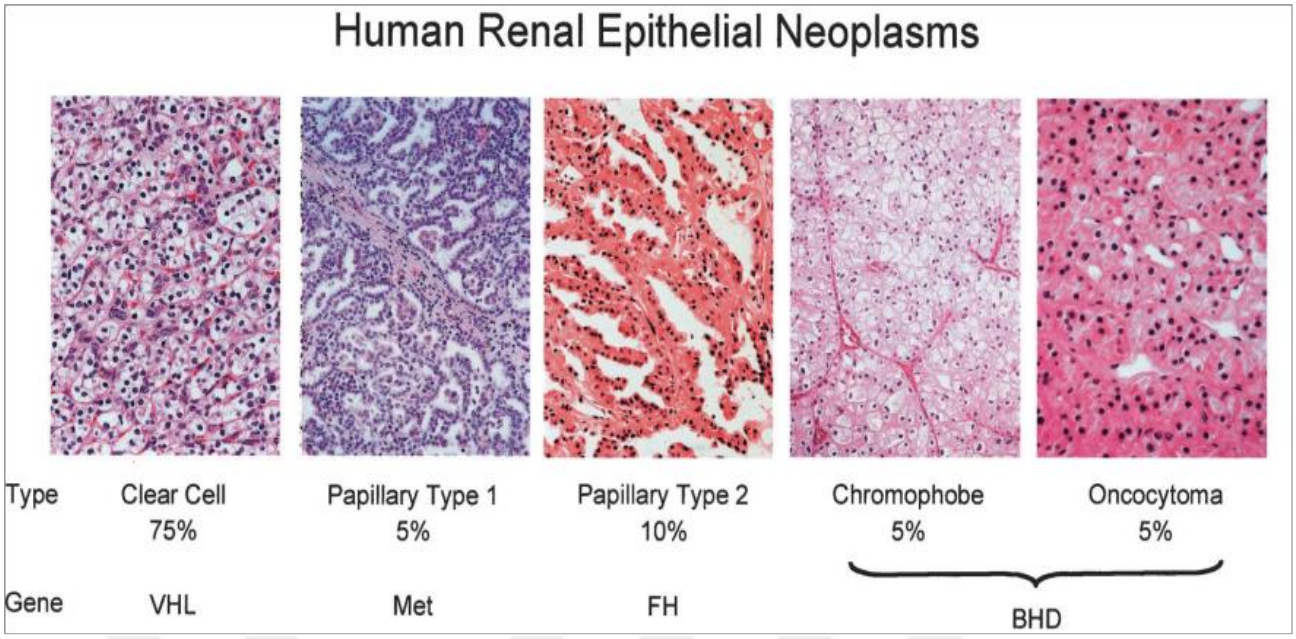

Figure 2.4. Kidney cancer is a complex disease composed of various types, each of which is influenced by many genes, including clear, papillary types (1 and 2), oncocytoma, and chromophobe (Linehan et al., 2003)

2.5. Risk Factors

2.5.1. Smoking

In the view of the United States Surgeon General and the IARC or International Agency for Research on Cancer, it is considered that, for RCC, smoking is a causative risk factor (Kabaria et al., 2016). Based on a meta-analysis of 24 investigations composed of nineteen control cases and five cohort investigations from Europe, Australia, and the north of the United States, the total relative risk for both sexes is around 1.38 for ever-smokers in contrast to never-smokers. And the risk is directly correlated with smoking duration and rises with the total amount given (amount of cigarettes smoked daily). However, it decreases after more than ten years of continuous abstinence (Hunt et al., 2005). Furthermore, in another big cohort investigation, which was performed on 250,000 veterans of America for more than twenty six years, the relative risk of kidney cancer was considerably raised by daily cigarette consumption (McLaughlin et al., 1990). Other investigations have also demonstrated that the risk of kidney cancer increases when nonsmokers receive exposure to tobacco smoke in the environment (Kabaria et al., 2016).

2.5.2. Obesity

Obesity is connected to a higher risk of developing kidney cancer, and there have been many epidemiologic investigations in Norway, America and the Netherlands that have approved it (Lipworth et al., 2009). The World Health Organization has defined obesity as a body mass index (BMI) ≥30 kg/m2, which raises the probability of acquiring the disease in both males and females (Kabaria et al., 2016). Nevertheless, prior investigations have suggested that females may have a higher relationship between kidney cancer risk and total body mass, than males. Also, in a study involving more than 340,000 Europeans, it was discovered that in females, the higher BMIs, body weights, and hip circumferences increased the RCC risk. Nevertheless, after adjusting for body weight, in males, the elevated risk of RCC is connected to low hip circumference. This indicates that in males, the distribution of body fat is perhaps more predictable, especially the hip circumference (Pischon et al,. 2006).

The advanced tumor stage may be related to the increased BMI. Furthermore, about United States adults, there was a big longitudinal study that composed more than 900,000 people and showed a related-to-dose link between the general mortality of RCC patients and raising body mass (Calle et al., 2003). Recently, the prevalence of obesity is rising globally, which may be a factor in the 40% to 30% rise in RCC cases in the United States, Canada, and Europe (Lipworth et al., 2009).

2.5.3. Hypertension

According to the available data, one of the known risk factors for kidney cancer is hypertension (Macleod et al., 2013). Conversely, it has been demonstrated that some types of kidney tumors and cancer treatments can result in hypertension (Chow et al., 2010). Also, many studies point out that antihypertensive drugs are linked with an elevated risk of the condition; however, it is hard to distinguish the condition effect from the treatment effect (Yoon et al., 2011). In a cohort investigation from Sweden about over 300,000 men, it was found that hypertension increases kidney cancer risk while decreasing with reduction, suggesting that effective blood pressure control could reduce this risk (Chow et al., 2000). Moreover, according to a cohort study in Europe, hypertension, both systolic and diastolic, increases the risk of the condition, irrespective of gender, BMI, or smoking,

as well as antihypertensive use, with similar results in several other European and American investigations (Kabaria et al., 2016).

2.5.4. Kidney disease

It has been demonstrated that kidney disease is linked to the risk of RCC (Macleod et al., 2013). The kidney cancer risk is two to three times higher in people with chronic renal disease (Hofmann et al., 2015). Further, kidney cancer risk was shown to be higher in individuals with advanced-stage renal disorder both during extended hemodialysis and after receiving a kidney transplantation (Figure 2.5) (Stewart et al., 2009). Moreover, it has been suggested that the risk of RCC increases in people with acquired kidney cysts disorder (Bonsib, Stephen, 2009).

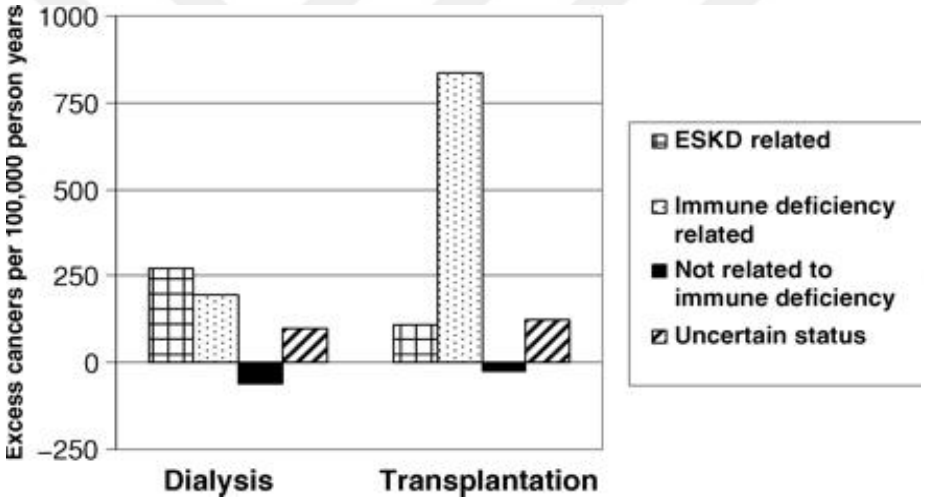

Figure 2.5. The responsibility of additional cancer occurrences according to the category of cancer and type of kidney replacement therapy (Stewart et al., 2009)

2.5.5. Diabetes mellitus, sex and age

There is some evidence that diabetes comorbidities, such as obesity and hypertension, have their own physiological impact, and prior research has connected diabetes mellitus to an elevated risk of kidney cancer. An additional 40% increased risk of kidney cancer is linked to a family history of diabetes (Larsson and Wolk, 2011).

Furthermore, kidney cancer is observed at twice the rate in males relative to females after controlling for age. Furthermore, adults between the ages of 55 and 74 receive more than 50% of RCC diagnoses (Kabaria et al., 2016).

2.5.6. Alcohol

In studies, it has been shown that there is an inverse kidney cancer, association with the consumption of alcohol (Karami et al., 2015 ; Rumgay et al., 2021). Furthermore, according to several North American, European, Asian, and Australian studies, the risk of RCC is reduced by moderate alcohol consumption (Kabaria et al., 2016). While, some other investigations found no result for an association between RCC and alcohol drinking (Washio et al., 2013). However, excessive use of alcohol has been firmly connected to the raised risk of various different cancer forms, like liver, colon, and maybe rectum, and pharyngeal, laryngeal, esophageal, and breast cancers. Alcohol is also a proven human carcinogen (Bagnardi et al., 2001). Further, it is unclear what biological mechanism might account for these interaction effects.

2.5.7. Physical activity

There has been conflicting evidence on the link between exercise and kidney cancer risk in prior investigation. A negative correlation was discovered in most of these researches (Chow et al., 2010). The risk of kidney cancer may be decrease by physical exercise for many reasons, including decreasing blood pressure, weight, chronic inflammation, enhancing insulin sensitivity, and lowering oxidative stress (Richardson et al., 2008; Solomon et al., 2009). According to several cohort investigations that have identified the kidney cancer risk of certain outcomes, the risk decreases further as activity levels increase. This assessment considers factors such as current exercise, regular physical activity, recreational pursuits, and daily energy expenditure (Setiawan et al., 2007; Chow et al., 2010). Further, the same result found in a comprehensive review of prospective cohorts (Behrens and Leitzmann, 2013).

2.5.8. Environmental and occupational exposure

TCE is most often used as a degreaser and cleaner for metal. Based on the IARC, TCE is a chemical that is known to cause kidney cancer, and occupational exposure to it is classified as carcinogenic to humans (IARC, 2014). Furthermore, a meta-analysis points out that working with TCE raises kidney cancer risk by 30% to 40% (Karami et al., 2012). A study for kidney cancer risk and TCE exposure also suggested the same result in 2022 (Andrew et al., 2022). Another environmental exposure is aristolochic acid, which can raise the risk of the condition (Turesky et al., 2016).

2.5.9. Genetics of kidney cancer

As it mentioned previously, cancer of kidney, can be sporadic (not inherited) or familial (inherited). The majority of RCC instances are sporadic; only approximately 4% or 5% of all cases are hereditary. In addition, according to several investigations, having a RCC family history is linked to a double-greater chance of RCC development (Kabaria et al., 2016). In the studies, there have been numerous autosomal dominant hereditary kidney cancers syndromes identified because of germline mutations in many tumor suppressor genes and oncogenes such as *MET, FLCN, FH, VHL,* and others. Familial kidney cancers develop at an earlier age (37–39 years), in contrast to sporadic forms that develop at an older age (63–64 years) (Testa et al., 2020).

Hereditary kidney cancer syndromes range from very frequent to very rare, with VHL syndrome, Birt hogg-dubé syndrome (BHD), Hereditary-Leiomyomatosis RCC syndrome (HLRCC), and Hereditary-Papillary RCC syndrome (HPRC) being the four most prevalent types (Pfaffenroth and Linehan, 2008; Board, 2019). The VHL syndrome is attributed to a germline-mutation in the *VHL* tumor suppressor gene, which manifests as a consistent kind of ccRCC. The HPRC syndrome, which is attributed to a change in the oncogene *MET*, is the most common form of type-I papillary. BHD patients are in threats of growing oncocytic tumors, oncocytomas, and chromophobes, as well as ccRCC, and have been discovered to have the tumor suppressor *BHD* mutation. HLRCC syndrome is most frequently identified as type 2 papillary because of the *FH* gene's germline alteration and serve as a gene that suppresses tumor growth (Pfaffenroth and Linehan, 2008).

2.6. Pathophysiology of Kidney Cancer

2.6.1. Loss of the *VHL* gene and HIF pathway

It is believed that the renal epithelium is the site of genesis for ccRCC, the most common kind of RCC (Mazumder et al., 2023). The inactivation of the *VHL* gene, which is involved in the genesis of ccRCC, is thought to be the main cause of the illness, according to research. The inactivation of the *VHL* gene is linked to certain epigenetic alterations or genetic mutations (Majo et al., 2020).

During both forms (sporadic and inherited) of ccRCC, the *VHL* gene plays an essential role in progressing the condition, and it is identified as a tumor-suppressor gene and positioned on chromosome 3 (3p25) (Pandey and Syed, 2020; Mazumder et al., 2023). A tumor suppressor protein known as pVHL is encoded by the *VHL* gene. The protein hypoxia-inducible factor (HIF) is one of several cellular proteins that pVHL regulates (Nabi et al., 2018). Also, cells respond differently to low oxygen levels depending on the presence or absence of a transcription factor called HIF. The HIF factor, which consists of a consistent beta-subunit (HIF-β) and an unstable alpha-subunit (HIF-1α and HIF-2α), may control several target genes that are engaged in various processes. Several biological processes are impacted by the genes that HIF controls, including cell division, matrix metabolism, angiogenesis, cell death, glucose transport, and metastasis (Haase, 2006).

In conditions of normal oxygen, the polyubiquitination of HIF1α is performed by the pVHL complex, demonstrating it via the proteasome for the destruction process. In conditions of low oxygen or pVHL lacking inside the renal cells, accumulation of HIF1a occurs, binds with HIF1β, and transcriptionally expression of hypoxia-inducible genes occurs (Nabi et al., 2018). Also, the studies have shown that, these incidents induce receptor tyrosine kinase signaling pathways, such as the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway, and Nuclear factor kappa B (NF-κB) pathway which is significant in the development of kidney cancer (Kumar et al., 2018). Consequently, reduction in the role of pVHL, accumulation and stability of HIF1a affect many physiology and pathophysiologic mechanism in kidney and increase renal tumor genesis, including angiogenesis and growth factor such as vascular-endothelial growth factor (VEGF) stimulate, increase of cellular proliferation, cell growth, cell cycle progression, suppression of apoptosis, glycolysis, erythropoiesis, extracellular matrix degradation, and metastasis (Kumar et al., 2018).

Furthermore, in addition to the *VHL* gene, the etiology of ccRCC involves additional genes, including the polybromol (*PBRM1*) gene, the BRCA1 associated protein-1 (*BAP1*) gene, the histone-modifying enzyme (*SETD2*) gene, and the lysine-specific demethylase 6A (*KDM6A*) gene (Petejova and Martinek, 2016).

2.6.2. Extracellular matrix degradation and MMPs in kidney cancer

Kidney cancer has abnormal extracellular matrix (ECM) remodeling. As mentioned previously, *VHL* gene inactivation and activation of the HIF cause major types of kidney cancer, and these events induce ECM aberrations in kidney cancer. Throughout every tissue and organ, the ECM is the essential structural element. ECM is composed of a highly dynamic network of protein filaments which surrounds cells and provides structural support and interaction between malignant cells and the stromal environment. It has been suggested that a distinguishing characteristic of cancerous tumors is the cells ability to infiltrate the ECM, start the invasion process to neighboring cells and tissues, and migrate to remote locations (metastasis) (Hashmi et al., 2021).

In RCC, the ECM of the tumor is constitute of various collagens, fibronectin, elastin, laminin, glycoproteins, and proteoglycans (Majo et al., 2020). In an investigation that reviewed many studies, showed that ECM components have an effect on kidney cancer development and migration (Oxburgh, 2022). In addition to it, Collagen 1 which is the principal ECM element, stimulate the invasion and metastasis of RCC tumor cells (Majo et al., 2020).

The lumps of kidney cancer have an increased level of matrix metalloproteinases (MMPs), a group of zinc-dependent proteinases that breakdown ECM proteins. A raised level of MMPs promotes tumor invasion, angiogenesis, growing tumors, and metastasis (Hashmi et al., 2021). The majority of MMP family members have been shown to be overexpression in kidney cancer subtypes, including MMP9 in all three main RCC subtypes, MMP2 and MMP9 in ccRCC, MMP11 and MMP14 in both ccRCC and PRCC, and MMP13, MMP15, MMP24, and MMP26 overexpressed in chromophobe RCC (Gobin., 2019).

2.6.3. Growth factors

Growth factors are molecules released in the body that play crucial roles in various essential processes, including cell growth, the stimulation or suppression of mitosis, cellular growth, and the recovery from injuries (Stone., 2017).

It is true that most cancer of kidney etiology involves overexpression of several growth factors, such as transforming growth factor alpha (TGF-α), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and platelet-derived growth factor beta (PDGF) (Nabi et al., 2018; Volkova et al., 2020). In a study, these growth factors were more prevalent in kidney cancer tumors, including VEGF, PDGF, and FGF, and their tyrosine kinase receptors, such as VEGFR, FGFR, and platelet-derived growth factor receptors (PDGFR), than in normal cells (Volkova et al., 2020). In this condition, binding growth molecules with their tyrosine receptor kinase promoted cellular proliferation, reduced apoptosis, and enhanced angiogenesis (Koul et al., 2011).

For instance, between the most important growth factors in the common form of renal malignance is VEGF (Nathan et al., 2006). In addition, in kidney cancer, the VEGF family regulates and promote the angiogenesis and lymphangiogenesis. Therefore, the ccRCC is identified as a highly vascular tumor as a result of growth factor overexpression which stimulates angiogenesis (Petejova and Martinek, 2016). Angiogenesis is a physiological mechanism that has an essential role in the creation of a new vascular network due to ones that already exist (Ravi et al,. 2022). Also, it has a crucial role in growth and progress of the tumor of the RCC (Mennitto et al., 2020).

2.6.4. Immune escape in kidney cancer

In its pathophysiology, it is likely that the immune system is connected to the development of kidney cancer. The main type of kidney cancer, ccRCC, exhibits an extremely immunogenic feature that is linked to the intricate microenvironment of the tumor. The progress of the kidney cancer is connected with the important roles of immune cells and cytokines, as well as immune escape and tumor growth, which occur through modifications of the immune microenvironment. The tumor immune microenvironment (TIME) plays a highly significant role in evading immune reactions, which are composed of immunosuppression and immunostimulation factors.

In cancer of kidney, there have been many raised immunity biomarkers (factors that both stimulate and inhibit the immune system). In kidney cancer, the presence of tumor-infiltrating lymphocytes (TILs) correlates with a more positive prognosis. Furthermore, in kidney cancer, immunological infiltrates are mainly composed of T-cells with an elevated ratio of cytotoxic T cells. Throughout kidney cancer, it may inhibit the antitumor immunological reaction because of increased myeloid-derived suppressor cells (MDSCs) density. Also, the level of immunological checkpoint molecules is highly expressed in kidney tumors, like programmed death ligand-1 (PD-L1), and this leads to tumor immunoevasion encouragement and prevents activation of T-cells. Furthermore, additional molecules of immunological checkpoints like lymphocyte-activating-3 (LAG3) and cytotoxic T lymphocyte associated protein 4 (CTLA-4) were similarly elevated in the condition (Monjaras-Avila et al., 2023).

The highest intensity masses of immune cells in the kidney cancer tissues have been identified as T lymphocytes. Especially, as reported by The Cancer Genome Atlas research programme which made examination for 19 types of cancers, ccRCC demonstrate the highest score for T-cell infiltration (Şenbabaoğlu et al., 2016).

2.7. Actin Cytoskeleton

The cytoskeleton is the most complex and functionally comprehensive component of animal cells (eukaryotic), and is vital for many mechanism including cell form adjustment, adherence and protection, endocytosis, division of cell (mitosis), transportation within the cells, provide ability to movement, response to outside force. The cytoskeleton, is mostly contained of three different macromolecules, which include intermediate filaments, microtubules, and filamentous-actin (F-actin) (Hohmann and Dehghani, 2019).

The actin filament, as a significant part of the cytoskeleton, can provide many support and functions to body cells (Lodish et al., 2000). To perform its functions such as (cellular proliferations, membranes trafficking and motility), numerous events are needed, including accurate regulation of the assembly, dynamics, and framework of actin filaments constructing the central part of the actinic cytoskeleton. Nevertheless, disruptions in actin organization occur during human diseases, such as the metastasis stage of cancer. The

protein family WASP/WAVE and the Arp2/3 complex are two of those genes that are linked with the adjustment of actin-cytoskeleton organization (Sossey-Alaoui, 2013).

2.7.1. Arp2/3 complex

The Arp2/3 complex has an influential role in regulating the nucleation of actin and the polymerization of actin filaments (Zheng et al., 2023). The members that compose the Arp2/3 complex include ARPC1, 2, 3, 4, and 5, as well as the actin related proteins Arp2 and Arp3. The activation of this complex occurs through nucleation-promoting factor (NPF) families: *WAVE* 1, 2, and 3, *N-WASP, WASP, WASH, JMY*, and *WHAMM* (Cao and Way, 2024).

After binding to the WASP family proteins, the Arp2/3 complex is active and serves as a nucleation site (factor) for new actin filaments, which is essential for constructing branched filament networks, which perform numerous functions, including cell motility, invasion by pathogens, and endocytosis (Smith et al., 2013). Subsequently, the development of lamellipodia, or projections of actin near the cell's leading edge, and invadopodia, structures that degrade the actin matrix occurs, which is caused by this branched nucleation process and is utilized to assist cellular motility and matrix reorganization (Frugtniet et al., 2015). During healthy conditions, the creation of these components is tightly regulated to protect tissues from disturbance as well as injury. However, during pathological states, cancerous cells utilize this mechanism to start invasion and spread to other bodily areas (Loveless and Teng, 2021).

2.8. *WAVE3* Gene

2.8.1. *WAVE3* gene identification

The WASP-WAVE family includes a set of molecules that provide an essential connection between the actin cytoskeleton and GTPases. A significant function of the WASP-WAVE family is regulating actin polymerization by activating the Arp2/3 complex, which is important to protrusion of the membrane and leads to invasion and cell movement (Frugtniet et al., 2015). The WASP-WAVE family, according to their resemblance, separated into WASPs and WAVEs sub-families (Loveless and Teng, 2021). Presently, it

is approved that the WASP and WAVE subfamilies in mammals have five individuals (genes), including *WAVE1/SCAR1, WAVE2, WAVE3, N-WASP, and WASP*. Each of these genes in the WASP-WAVE family of humans is placed upon the difference chromosomes and any of them demonstrates a distinct pattern of expression. For members of this family, the stimulation of phosphoactivation via extracellular components (e.g., growth factor or cytokines) results in their motifs of the C-terminus being revealed and afterward interacting with numerous factors, including the Arp2/3 complex (Kurisu and Takenawa, 2009).

The production of the WASP protein is associated with *WAS* gene signals. This protein exists in all blood cells (surface). The engagement of WASP involves the transmission of signals via cellular surface receptors to the actin cell skeleton. Cell migration and connection to other parts of the body (adhesion) are performed by signals from WASP. Consequentially, this communication creates the interplay between cells and foreign invaders in white blood cells through the actinic cell skeleton (Thrasher and Kinnon, 2000).

The *WAVE3* gene, belongs to the (WASP-WAVE family) and is an actin-cytoskeleton remodeling protein. (Sossey-Alaoui, 2013). The *WAVE3* gene is a protein-coding gene that contains 15 exons and is located on chromosome 13 (13q12.13) (Figure 2.6). The sequence of the *WASF3* gene is around 149810 base pairs. In addition, 4875 bps (NM_006646.6) are in the mRNA of the *WAVE3* gene (Figure 2.7), which translates to a protein of 502 amino acids. The multiprotein complex is constructed through the *WAVE3* gene product, which connects actin to receptor kinase. Binding all members of the family to actin is performed via the domain of C-terminal verprolin homology. The multiprotein complex acts as a signal transducer that involves alterations in the shape of a cell, function or motility. Widely, *WAVE3* has expression inside the human body, which includes the brain, fat, and other fifteen tissues (Figure 2.8) (Loveless and Teng, 2021).

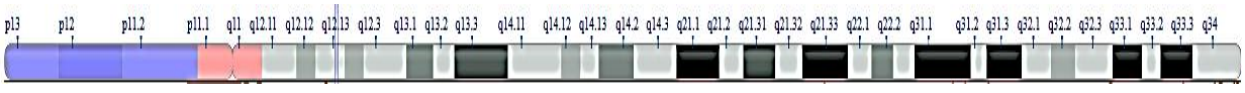

Figure 2.6. *WAVE3* gene location on chromosome 13

https://www.ncbi.nlm.nih.gov/gene/10810

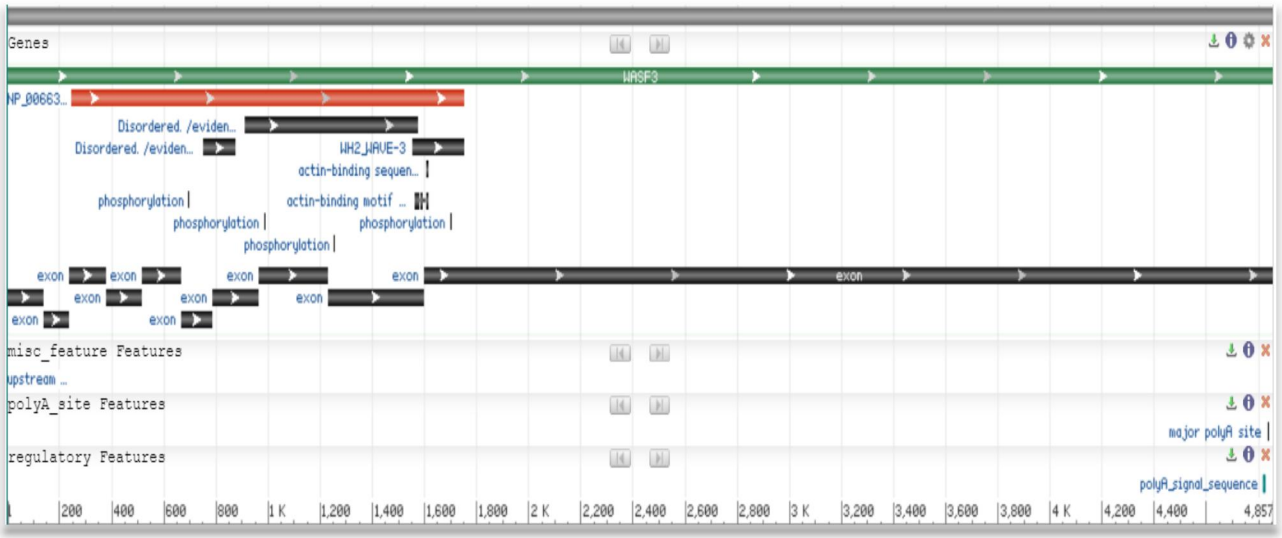

Figure 2.7. mRNA of the *WAVE3* gene

https://www.ncbi.nlm.nih.gov/gene/10810

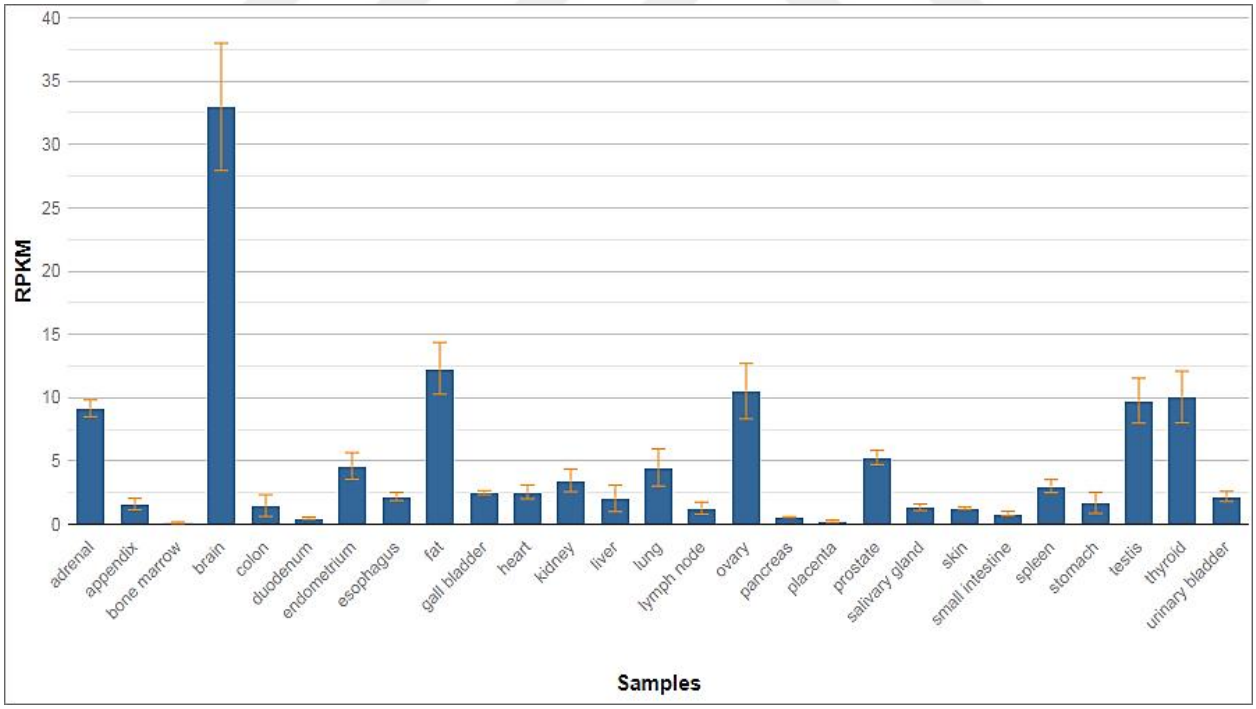

Figure 2.8. *WAVE3* gene expressions in difference tissues

https://www.ncbi.nlm.nih.gov/gene/10810

The Wiskott-Aldrich syndrome is an uncommon genetic (x-linked chromosome) disorder due to over 350 genetic alterations within the *WAS* gene (the production of the WASP protein is associated with *WAS* gene signals), which are predominantly noticed in men (Malik and Masab, 2019). The features of this syndrome are atopic dermatitis (eczema), persistent inflammation, microthrombocytopenia, high rate occurrence of autoimmunity and cancers. Wiskot-Aldrich syndrome patients possess microthrombocytopenia, which is an aberration in the normal structure and shape of blood cells involved in blood clotting and wound healing (platelets). And this condition, present from congenital, can cause bloody diarrhea, prolonged bleeding such as nose bleeds or small trauma, as well as thrombocytopenic purpura. In the case of prolonged bleeding, if it is not treated, it can be life-threatening and cause death (Massaad et al., 2013).

2.8.2. Arp2/3 complex activation by the *WASP-WAVE* protein family

Recent evidence suggests that the WASP and WAVE family of proteins activate the Arp2/3 complex to control actin cytoskeleton filament remodeling in response to environmental cues. Each of these WASP-WAVE proteins, such as the *WAVE3* protein (Figure 2.9), has an acidic (A) region at the C-terminus, a verprolinhomology (V) domain, and a cofilin-homology (C) domain (Takenawa and Miki, 2001).

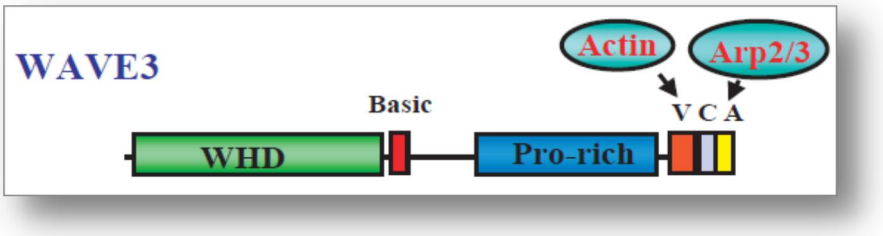

Figure 2.9. The WAVE3 protein domains (Takenawa and Miki, 2001)

The process of activating and inactivating WASF domain conformations. In a state of inactivation, to maintain the auto-inhibited closed conformational form of WASFs, the members of the WASF homology domain (WHD), which is positioned at the N-terminus, interact with the C-terminus. At WHD, protein complex members include *WAVE1, WAVE2,* and *WAVE3*, ABI, CYFIP1 (SRA1), HSPC300, and NCKAP1 (NAP1). In

contrast, because of Rac, the activation state occurs, and interaction of the Arp2/3 complex and G-actin (monomeric actin) is possible with the verprolin-cofilin-acidic (VCA) area due to WHD interconnection disruption and the WASFs open conformational state. Additionally, the WASF complex can also be attracted to the membrane by Phosphatidylinositol (3,4,5)-triphosphate (PIP3), which binds to the basic domain (B) of WASFs (Figure 2.10) (Loveless and Teng, 2021).

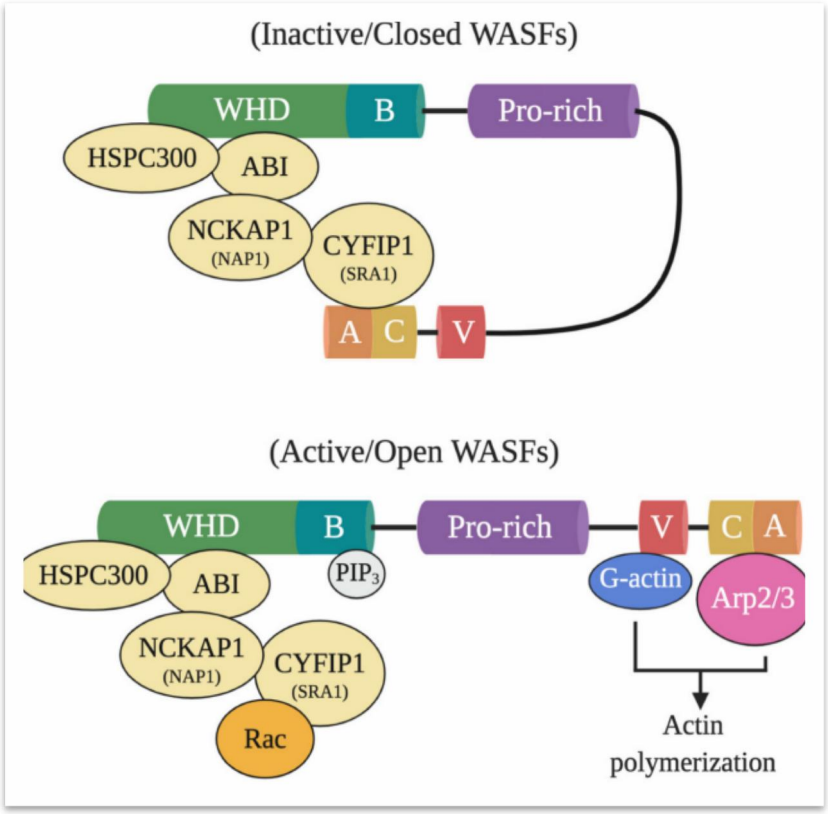

Figure 2.10. Inactivation and activation of *WASF* domain confirmations (Loveless and Teng, 2021)

For activation and stableness, the *WAVE3* gene during cancer, it requires some other proteins such as HSP70 and HSP90. HSP90 inactivation (loss) leads to the prevention of phosphorylation of *WASF3*, not its stability, and HSP70 deactivation causes the destabilization of *WAVE3* protein. Furthermore, in the various types of cancer, HSP90 and HSP70 deactivation results in a decline in the invasion of a range of tumor cell lines, probably due to the molecular chaperone cells' destabilization that affect this phenotype. It has been established that in advanced tumors, the *WAVE3* gene was overexpressed, and the process of invasion and metastasis was lost when its expression was decreased (Teng et al., 2012).

2.8.3. *WAVE3* gene connection to cancer progression

The WASP family is composed of crucial actin-binding proteins, which are required for many regulatory functions in cells, including organizing cell shape, regulating actin-filament polymerization of the cell membrane, motility, migration, and cell metastasis. In earlier studies, it was shown that any imperfections in WASP family proteins can lead to aberrant morphological structure and cellular migration (Li et al., 2020). There is a confusing aspect, which is that some members of the WASP family function as either promoters or suppressors of cancerous tumor according to tumor types as well as the stage of cancer (Zhang et al., 2012).

Among all of the actin regulator proteins, the WASP/WAVE family was first discovered, and in humans (mammals) five of them were expressed. Which, they are *WAVE 1, 2, 3, N-WASP, and WASP* (Thrasher and Burns, 2010).

WAVE3 is one of the most critical of the *WASP* family proteins for metastatic encouragement (Kansakar et al., 2020). And according to the studies, it is mostly expressed (Thrasher and Burns, 2010). Also, WAVE3 is rich in proline protein, which in normal cells has a tight effect on alteration in cellular migration, protects cellular shape, and regulates actin-filament polymerization (Li et al., 2020). As well, depending some studies, *WAVE3* is associated with cellular proliferation and cytokinesis. Nonetheless, *WAVE3* has a major function in cancer cell invasion and metastasis (Huang et al., 2018).

According to the majority of research, *WAVE3* has shown over-expression in numerous specific types of human cancer, including breast cancer (Teng et al., 2014), cancer of prostate (Moazzam et al., 2015), ovarian (Lu et al., 2017), colorectal (Zhang et al., 2012), pancreatic cancer, bladder cancer (Huang et al.,2018), esophageal (Li et al., 2020), hepatocellular carcinoma (Ji et al., 2015), gastric cancer (Nie et al., 2019), Intrahepatic Cholangiocarcinoma (Zhu et al., 2016), and non-small cell lung cancer (Wu et al., 2014). The investigations of *WAVE 3* have demonstrated its upregulation (overexpression) in each examined cancer type.

Furthermore, it has been reported that the *WAVE3* is engaged in numerous signaling pathways, such as the PI3K and AKT pathways, the NF-κB signaling pathway, and the p38 pathway, and is involved in regulating various processes in cells like cell invasion, migration, and proliferation (Li, X et al., 2020). Further, growing cancer cells and their progression are controlled by JAK-STAT3 signaling via activating numerous crucial

metastasis-stimulating genes, like the *WAVE3* gene, which encourage invasion and metastasis (Teng et al., 2014). Additionally, it has been shown that inducing the EMT process is connected with overexpression of *WAVE3* in some cancers especially in breast cancer which promotes invasive as well as metastatic cell growth (Taylor et al., 2013). Also, *WAVE3* has been linked with controlling the production of MMP, which is important in remodeling ECM. The MMP-1, -3, and -9 expression levels were notably decreased with reduced *WAVE3* (Sossey-Alaoui et al., 2005). Currently, medical studies demonstrate *WAVE3* as a developing and metastatic breast cancer (BC) biomarker and, more importantly, as the primary factor in the genesis of triple-negative breast cancer (TNBC), the most severe breast cancer subtype (Sossey-Alaoui, 2013).

As a mentioned above, kidney cancer is a complex disease composed of various types, each of which is influenced by many genes (Linehan et al., 2003). Moreover, this condition is highly deadly, with a tendency for widespread metastasis. And distinguished by the possibility of metastatic spread to liver, lymph glands, lungs, kidney in opposite site, bone, brain, and suprarenal glands (Sountoulides et al., 2011). In a study demonstrated that RCC metastasis to uncommon site, including breast, parotid, forearm, scalp, jaw, and skeletal muscle (Singla et al., 2022).

Furthermore, the *WAVE3* gene and its roles in cancer have been investigated in several studies that include how it affects cell invasion, migration, and motility in various cancer kinds (Li, 2020). The *WAVE3* is an actin-cytoskeleton remodeling protein (Taylor et al., 2013). Moreover, the *WAVE3* gene has a fundamental role in regulating cell morphology, polymerizing actin, and remodeling the cytoskeleton, which are connected to invasion and motility of cells (Davuluri et al., 2014). Evidence demonstrated role of *WAVE3* as a protein that promotes metastasis. Also, *WAVE3* has shown over expression in several specific types of human cancer. Furthermore, a study reported a highly significant relationship between breast cancer in its advanced stages and *WAVE3* gene expression levels (Sossey-Alaoui et al., 2011). Additionally, angiogenesis promotes the primary development and metastatic of tumors, and a study has discovered that it is regulated by the expression of *WAVE3*, which facilitates metastasis and chemoresistance in TN-breast cancer (Davuluri et al., 2014). Based on all of these data and research, we examined *WAVE3* gene expression level in cancer of the kidney.

3. MATERIALS AND METHODS

3.1. Material

3.1.1. Constituting the working groups

Twenty-two samples were taken from eleven patients. 11 samples with kidney cancer and 11 control samples that were taken from the same participants who submitted an application to the outpatient clinic of the Tokat Gaziosmanpasa University Faculty of Medicine, Department of Urology, were included in the investigation. After obtaining consent and awareness from the patients, kidney tumor tissue samples extracted during surgical procedures constituted the patient group, and the kidney normal tissues from the same kidney of each participant were used as the control group.

For this study, the required permission for the investigation was acquired by the Clinical Studies department at Tokat Gaziosmanpasa University's Faculty of Medicine with project number 22-KAEK-045. The study was performed in the laboratories of Medical Biology and Genetics.

3.1.2. Preservation of tissues

When the 11 kidney cancer tissue samples and 11 control tissue samples were collected from the same patients, a portion of the tissue samples was extracted and placed into distinct tubes and the samples immediately saved for later usage at -80 °C, in RNA isolation, synthesis of the cDNA, and analysis of the *WAVE3* gene expression.

3.1.3. Tools and devices used in the study

1. -80 Deep Freezer (Nuaire, NU-9483E, USA)
2. Refrigerator (Arçelık, 570465 MB, Turkey)
3. Vortex (Velp Scientifica; F20220176, Italy)
4. Homogenizer (Heildolph Silent Crusher, D91126, Germany)
5. Micropipette Set (Gilson, Thermo Scientific, Finnpipette)
6. Microcentrifuge (Mikro120, Hettich Zentrifugen D- 78 532n)
7. Plate (Applied Biosystems, 4346907, UK)
8. PCR Machine (MiniAmo Plus Thermal Cycle)
9. Qubit 3.0 Fluormeter (Invitrogen, Life Technology, Australia)

10. Real-Time PCR machine (Applied Biosystems, UK)

11. Centrifuge (Hettich, D’78532, Germany)

12. Refrigerated Centrifuge (Hettich. Germany)

3.1.4. Kits, kit components and chemical substances

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

▪ Lysis Buffer

▪ Proteinase K

▪ Wash Buffer 1

▪ Wash Buffer 2

▪ H_2O, nuclease-free.

▪ Ethanol (Sigma-Aldrich Catalog No: E7023, USA).

▪ RNA Purification Column Collection Tube

- cDNA synthesis kit (The Applied Biosystems™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

▪ H_2O, nuclease-free

-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 Standard 1 (Component B) 1 mL

▪ Qubit™ 1X dsDNA HS RNA Standard 2 (Component C) 1 mL

- For quantification Real-time PCR (qR-T 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

- Other materials:

▪ TE Buffer

▪Filtered Microcentrifuge Tubes and Collection Tubes. (GeneJET RNA Purification Columns pre-assembled with Collection Tubes).

▪ Plan Tubes with Caps, 10-mL.

▪Tips (Yellow, White, Blue)

▪ Distilled water

▪ Eppendorf tube

▪ Disposable gloves

▪ Lancet

▪ Slide

3.2. Method

3.2.1. Isolation of the RNA from the tissue samples

After taking the sample tissue from the patients in the operation and keeping it at -80 °C, the stored tissues were utilized to examine the gene expression. In this step, for the isolation of RNA, the Thermo Scientific GeneJET RNA Purification Kit was utilized. As mentioned below, the stages of the isolation of RNA are performed according to kit protocol.

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 mL microcentrifuge (eppendorf) tube.
3. The samples received 300 µl of Lysis Buffer and were completely mixed by 10 seconds of vortexing (lysis buffer; with 2-MERCAPTOETHANOL or DTT).
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 result mixture was incubated at 25 °C for 10 min.
6. The result mixture was then subjected to centrifugation at 12,000 × g for 5 min.
7. After that, the supernatant was transferred by pipette into new RNAse-free microcentrifuge tubes.
8. Then, each of the sample tubes received 450 µl of ethanol (96%); also, through a pipette, it was mixed.
9. After that, the tubes were changed, and the samples were moved into the collection tube. Transferred as much as 700 µl of lysate to them (GeneJET RNA Purification) column putted in a collection tubes.
10. The column underwent centrifugation at 12,000 × g for 1 minute.
11. Then, the collection tubes, which contained the (flow-through) solution, were discarded, and the GeneJET RNA Purification Column (filters) were set within a new 2 mL collection tube.
12. For each GeneJET RNA Purification Column, 700 µl of Wash Buffer 1 (enhanced with ethanol) was administered and underwent centrifugation at 12,000 × g for 1 minute.

13. After that, the flow-through solutions were discarded, and the purification column was returned to its collection tube.
14. For each GeneJET RNA Purification Column, 600 µl of Wash Buffer 2 (enhanced with ethanol) was administered and underwent centrifugation at 12,000 × g for 1 minute.
15. The solutions were discarded, and the filters were returned to its collection tube.
16. After that, the Column (filters) was relocated into a aseptic 1.5 mL RNase-free eppendrof tube.
17. Then, 100 µl of water nuclease-free was introduced, and the mixtures underwent centrifugation at 12,000 × g for 1 minute.
18. At the end, the purification columns (filters) were discarded, and the RNA purification was prepared.

3.2.2. cDNA synthesis

After preparing the purified RNA, the cDNA was synthesized. In this step, High-Capacity cDNA Reverse Transcription Kit (Lithuania, LOT: 01152470) was utilized, based on the protocol of the manufacturer's kit. This cDNA synthesis kit is composed of all the constituents that are required for this process that displayed in Table 3.1, and all of these ingredients were mixed together and transferred into microtubes, The RNA samples were then added to any of these tubes, and all of them equaled 20 µl.

cDNA synthesis was done in two steps:

First step:

1- Each tube received 10 µl of 2× RT master mix.

2- Then, added the 10 µl of RNA samples that were already prepared to all tubes, and through a pipette, it was mixed twice.

3- The tubes were sealed.

4- Mixtures were centrifuged briefly until the contents spun down and terminated all of the bubbles.

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
Water, nuclease-free	4.2 µl
Isolated RNA Sample	10 µl
Totality amount	20 µl

Second step:

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. PCR program was utilized for the synthesis of cDNA

RT reaction	Temp (C)	Time
Step 1 Annealing	25 °C	10 min
Step 2 Elongation	37 °C	120 min
Step 3 Termination	85 °C	5 min
Step 4 (Hold)	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 samples quantity and the two standards, the essential number of 0.5 mL PCR tubes was prepared and labeled.

2- The 190 µl of Qubit™ working solution was introduced 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- 195 µl of working solution with 5 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.

9- Recorded each cDNA concentration.

10- In the patient and control tissues, cDNA concentrations were measured, and any of the reactions were calibrated to 60 ng. And they were ready for the next steps of the study.

11- For *WAVE3* as a target gene and Beta-Actin (*ACTB*) as a reference (housekeeping) gene, the serial dilution was prepared, and at various concentrations (1/10, 1/100, 1/1000, and 1/10000) the cDNA samples were produced.

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

Real-time PCR is a well-established technique for detecting the PCR template copy number, e.g., DNA or cDNA, and is composed of two shapes: probe-based and intercalator dye-based. The DNA intercalating dye, such as SYBR Green, is a free fluorescent dye that attaches to dsDNA and generates fluorescence when bound (Mo et al., 2012). The fluorescence scale indicates the number of amplified creations and is measured in every cycle (Narrandes and Xu, 2018).

In this step, to analyze the *WAVE3* 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 *WAVE3* 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. qRT-PCR primers for the *WAVE3* and *ACTB gene*

WAVE3 F	5’ -TTGGTGAGTTGTTTAATGAGGCT-3’
WAVE3 R	5’-GCAAGGCGATCAATTCTGTCT-3’
ACTB F	5’-GCATGGGTCAGAAGGATTCC-3’
ACTB R	5’-CACGCAGCTCATTGTAGAAGG-3’

Table 3.4. The reaction components and amounts utilized in qRT-PCR

Components	Amounts
2× Master Mix	12.5 µl
Primer F	0.5 µl
Primer R	0.5 µl
PCR-grade H2O	8.5 µl
Template cDNA	3 µl
Total Amount	25 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 analysis of qRT-PCR data of *WAVE3* mRNA

After determining the expression of the *WAVE3* gene at the mRNA level in both kidney cancer tissue and healthy kidney tissue by qRT-PCR, the results of the *WAVE3* gene and *ACTB* were obtained and quantified by utilizing the Step One v2.3 program. The *ACTB* gene normalized the *WAVE3* gene data, and the amounts of fold change were calculated by using the $2^{-\Delta\Delta CT}$ method (Table 3.6). Due to the data collected from this process, statistical analysis was carried out. All the data are demonstrated as the mean ± SEM. For the results evaluation, the 0.9–1.1 range was utilized. If the values are below 0.9, it means the target gene expression level has decreased in kidney cancer tissue compared to healthy kidney tissue. But if the result was between 0 and 1.1, it means there is no alteration between kidney cancer tissue and healthy kidney tissue. And if the expression level of the target gene increased in kidney cancer tissue compared to healthy tissue, it would mean the value was above 1.1 (Schmittgen & Livak, 2008). The SPSS 20 software was used to carry out the statistical analysis of expression data. And from the results of this SPSS, the paired t-test was utilized to investigate the data's level of significance. For the level of statistical significance, 0.05 was accepted. If the result of the p-value was ≤ 0.05, the data was considered significant; if the result of the p-value was > 0.05, the data was considered not significant.

Table 3.6. The method of $2^{-\Delta\Delta CT}$

Rule number 1 ΔCt	ΔCt (Kidney cancer Tissue): *WAVE3* (CTort) - *ACTB* (CTort)
	ΔCt (Healthy Kidney Tissue): *WAVE3* (CTort) - *ACTB* (CTort)
Rule number 2 ΔΔCt	ΔΔCt: ΔCt (K.C Tissue) - ΔCt (Healthy kidney Tissue)
Rule number 3 Fold change = $2^{-\Delta\Delta CT}$	$2^{-\Delta\Delta CT}$ Its value demonstrate its logarithmic fold change

(Livak & Schmittgen, 2001)

4. RESULTS

In this investigation, specimens of kidney cancer were collected from eleven individuals who had been diagnosed with kidney cancer, along with samples of healthy renal tissue obtained from the same participants through collaboration with the Department of Urology at Tokat Gaziosmanpasa University Hospital. All examinations were conducted within the genetics and molecular laboratories of the Department of Medical Biology. qRT-PCR was applied to analyze the *WAVE3* gene expression level in these 11 patients with kidney cancer. The study encompassed 6 female (54.5%) and 5 male (45.5%) participants, with ages ranging from 39 to 75 years and an average age of 62 ± 11.6 years (Figure 4.1). The comparison of gender, age and stages of cancer has displayed in (Table 4.1). The demographic information about patients with kidney cancer is demonstrated in (Table 4.2).

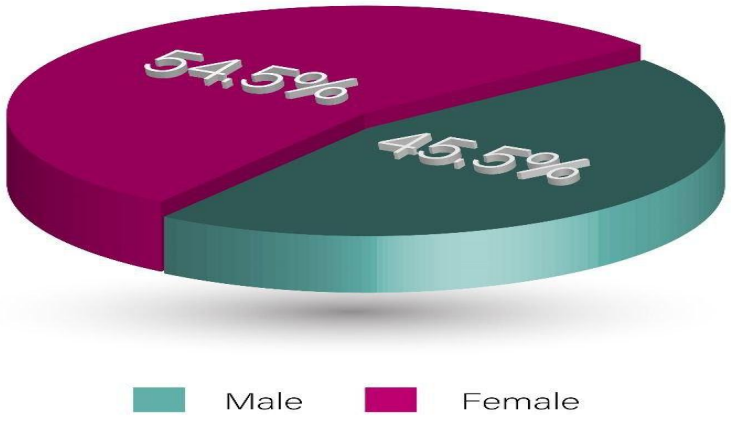

Figure 4.1. The distribution chart of the gender of kidney cancer patients constitutes the study

Table 4.1 Comparison of gender, age and stages of cancer.

Sample ID	Gender	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

Table 4.2. The demographic information of the kidney cancer patients

Kidney Cancer n=11	
Gender	6 females
	5 males
Age	62 ± 11.6

4.1. qRT-PCR Images and Analysis of the *WAVE3* Gene Results

After the RNA isolation step, which obtained total RNA from 11 samples of kidney cancer and healthy kidney tissues of the same patients, the MiniAmp Thermal Cycler device was used with the cDNA obtained. Finally, to evaluate the level of *WAVE3* gene expression in kidney cancer tissue and healthy kidney tissue, a SYBR-Green-based qR-T PCR method with an Applied Biosystems Step One Plus device was utilized.

WAVE3 amplification plot at Figure 4.2, *WAVE3* Melt Curve at Figure 4.3, *ACTB* Amplification Plot at Figure 4.4, and *ACTB* Melt Curve at Figure 4.5 were demonstrated, which were found using qRT-PCR.

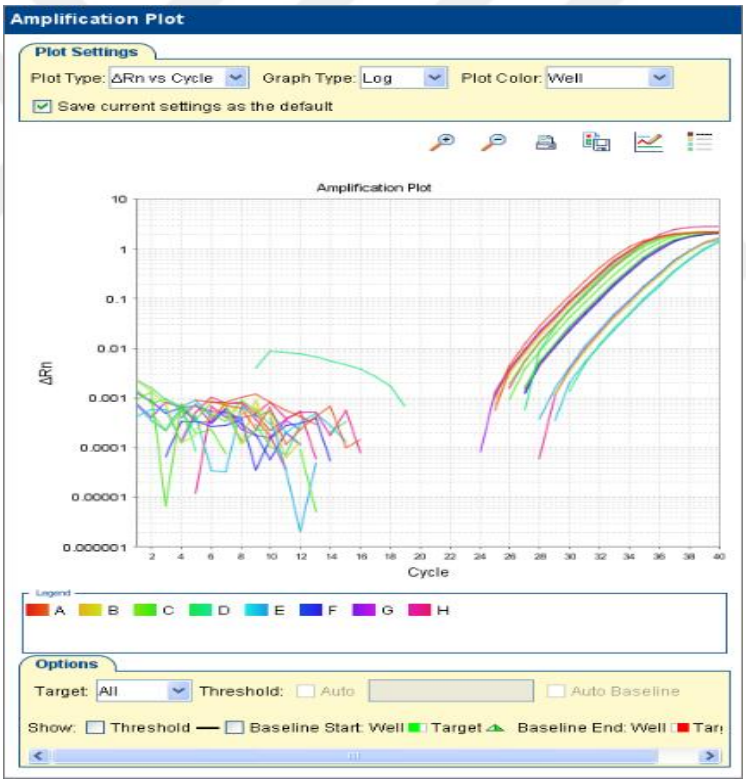

Figure 4.2. *WAVE3* Amplification Plot

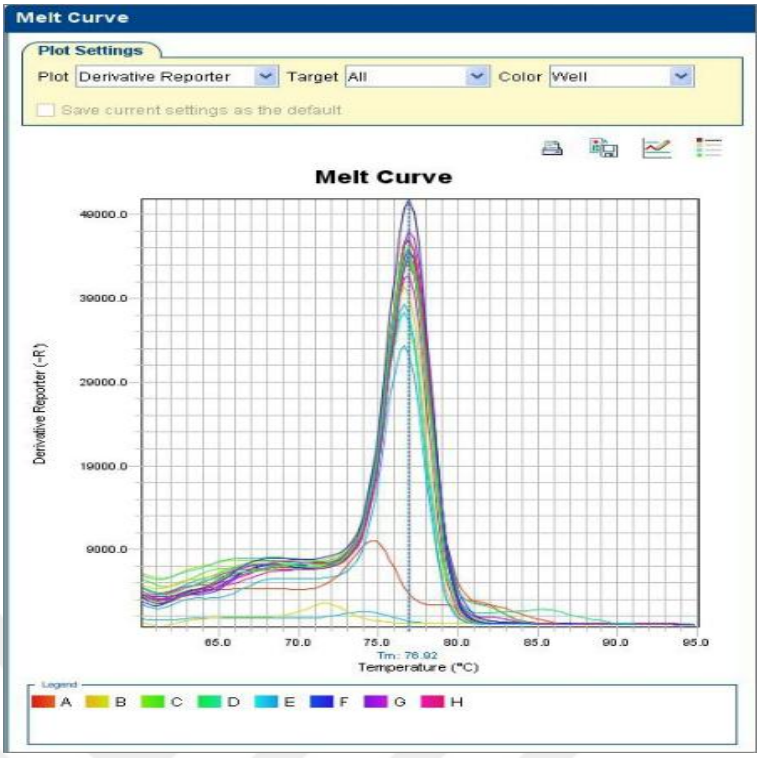

Figure 4.3. *WAVE3* Melt Curve

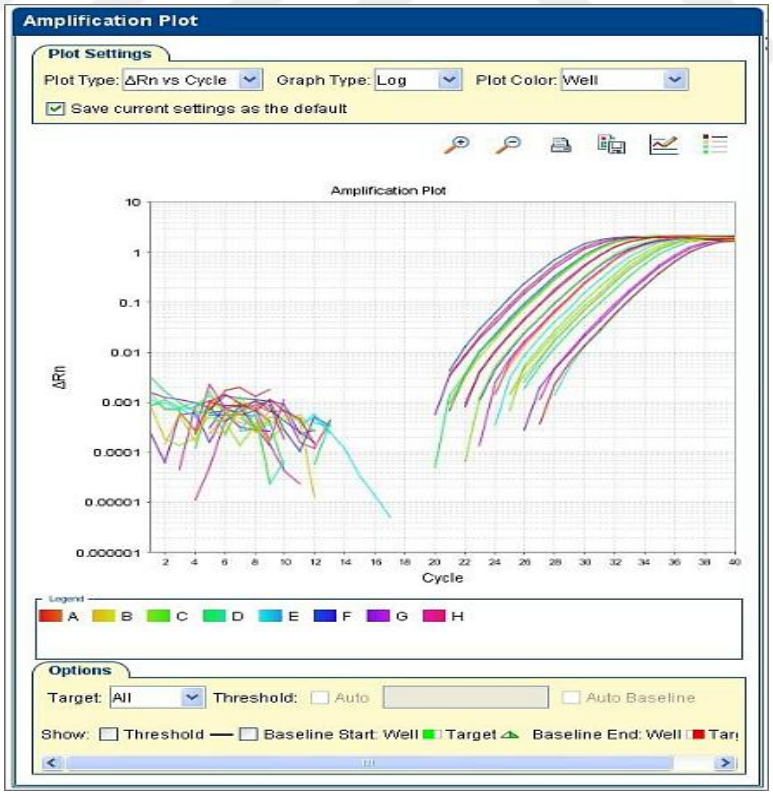

Figure 4.4. *ACTB* Amplification Plot

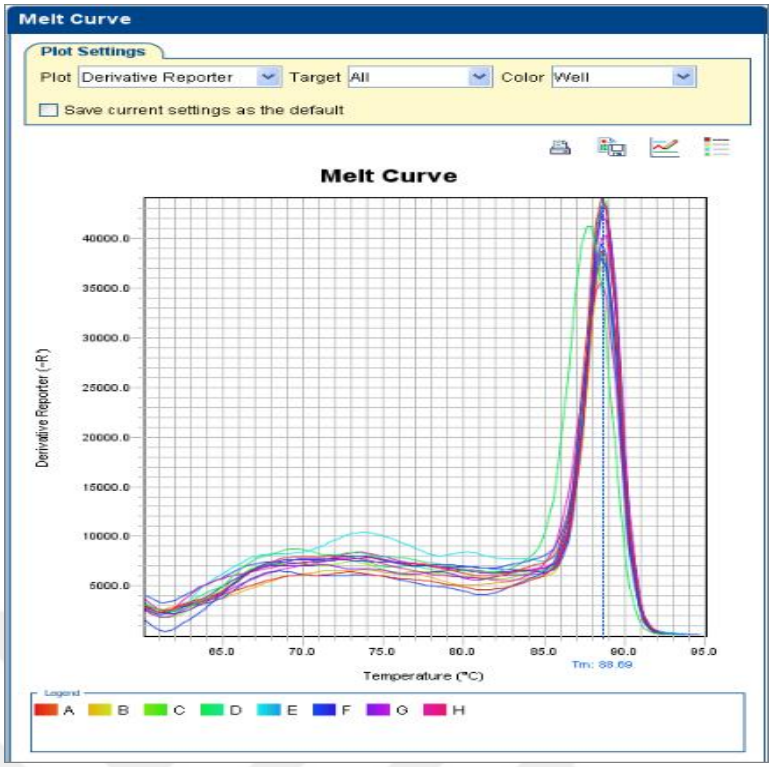

Figure 4.5. *ACTB* Melt Curve

According to the statistical analysis of the results of qRT-PCR, the present investigation discovered that the fold change was 0.93 in patients in comparison to the control group. This result is demonstrated in Figure 4.6. Statistically, no significant difference was found between groups ($p = 0.87$). In Table 4.3' the analysis results are demonstrated. Hence, it was determined that the *WAVE3* gene expression level in kidney cancer tissue was approximately equal (not changed) to that of healthy kidney tissue, and the result was that there is no difference in *WAVE3* gene expression in kidney cancerous tissues when compared with healthy tissues.

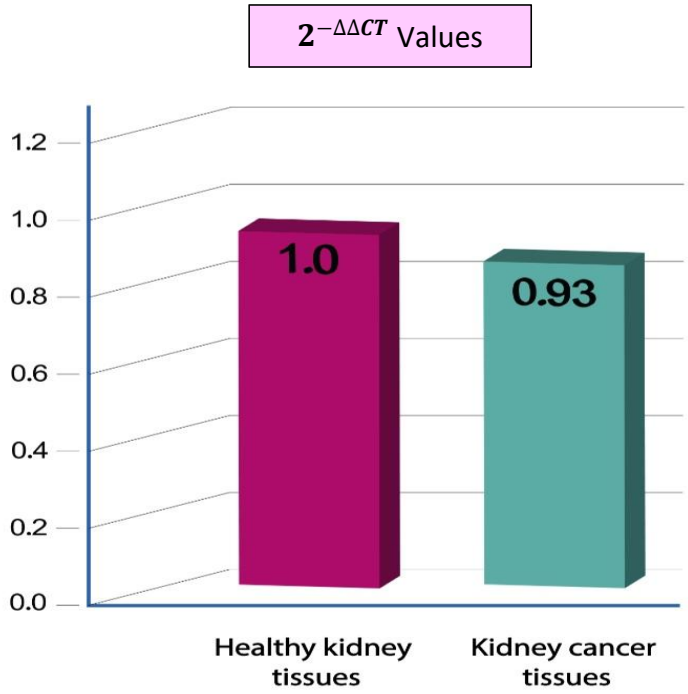

Figure 4.6. The graph displays the fold change of the *WAVE3* gene between kidney cancer tissues (n=11) and healthy kidney tissues.

(Real-time PCR was used to determine the relative quantification values of the *WAVE3* genes. *WAVE3* gene quantitative expression data were normalized with that of *ACTB* gene. The fold change of tissues with kidney cancer was determined to be = 0.93, whereas the fold change of healthy kidney tissues was found to be = 1)

Table 4.3. The statistical analysis of the *WAVE3* gene in kidney cancer tissue and healthy kidney tissue

Samples	***WAVE3* gene SD±SEM**	**P Value**
Kidney Cancer Tissues (n=11)	1.20± 0.40	p=0.87
Healthy Kidney Tissues (n=11)	1	

(SD: standard deviation; SEM: standard error mean)

5. DISCUSSION

Kidney cancer is not a single disease; it has multiple distinct subtypes that, due to gene alterations, come in genetically and histologically different varieties that react differently to treatment. The incidence and death rates of kidney cancer, the third most frequent form among urogenital malignancies, vary greatly by region, sex, and age (Pençe et al., 2019). And surgical excision serving as the primary therapy (Chen et al., 2020).

The disease is dependent on several risk factors, including history of cigarette smoking and history of using tobacco, obesity, hypertension, chronic kidney disease, family history, dialysis, diet, lifestyle, chemical carcinogens, and genetics (Scelo and Larose, 2018). Kidney cancer is highly deadly, especially the RCC type, with a tendency for widespread metastasis. And distinguished by the possibility of metastatic spread to liver, lymph glands, lungs, kidney in opposite site, bone, brain, and suprarenal glands (Sountoulides et al., 2011).

In the past, kidney cancer has been extensively investigated, and to date, many genes are involved in the condition (Lerner et al., 2012 ; Chen et al., 2020). Gene expression profiling is a useful technique for producing whole genome-level information on pathologic illnesses like cancer, rheumatoid disorders, and other diseases. Utilizing gene expression profiling in cancer diagnosis, prognosis, and therapy prediction, as well as determining which patients might benefit from therapeutic intervention to better understand the biology of the disease, is all made possible by this technique (Narrandes and Xu, 2018). There have been several assay methods developed and used for analyzing gene expression levels, such as qRT-PCR (Wong and Medrano, 2005 ; Narrandes and Xu, 2018).

A study has examined alterations in the expression levels of several genes between a specific type of 29 kidney cancer samples and a healthy tissue samples via utilizing 21,632 cDNA microarrays, and they have displayed that the majority of patients with this condition have gene expression mutations (Takahashi et al., 2001). Ongoing research is being performed to examine the expression levels of candidate genes for kidney cancer in order to evaluate their influence and further study the possible usage of these genes as therapeutic targets. This present study tried to examine the expression levels of the WAVE3 gene in kidney cancer.

The actin cytoskeleton is required for a variety of cellular processes such as cell shape changes, cytokinesis, motility, proliferation, and membrane traffic (Svitkina, 2018). Actin regulatory defects can result in human disorders such as cancer metastasis and Wiskott-Aldrich syndrome. The protein family WASP/WAVE and the Arp 2/3 complex are two of those genes that are linked with the adjustment of actin-cytoskeleton organization (Sossey-Alaoui, 2013).

WAVE3 gene belongs to the (family of WASP and WAVE); this group of proteins is known to influence cell shape, motility, cytokinesis, invasion, and cytoskeletal architecture, among other biological processes. They are structurally related proteins (Lu et al., 2017). WAVE3 is an actin-binding protein that acts as signal transducers that result in alterations in cytoskeletal structure and cell shape through regulatory mechanisms and this gene in the nervous system is mostly expressed (Sossey-Alaoui et al., 2007).

Prior research on *WAVE3*'s function in cancer cell invasion and metastasis shown that it may play a role in the crucial early phase of the invasion-metastasis cascade, that is epithelial to mesenchymal transition (EMT) (Wang et al, 2020). It has been detected that *WAVE3* is associated with several signaling-pathways, such as the PI3K and AKT pathways, the NF-κB signaling pathway, and the p38 pathway, acting as a regulator of cellular activities such as migration and proliferation (Li, X., 2020). The WAVE3 protein helps cancer cells move, invade, and metastasize to other bodily areas. Furthermore, by activating the Arp2/3 complex through *WASF3*, its activity regulates the dynamic assembly of the actin cytoskeleton (Cory and Ridley, 2002).

Previously, the *WAVE3* gene and its roles in cancer have been investigated in several studies that include how it affects cell invasion, migration, and motility in various cancer kinds, such as prostate, breast, colorectal cancer (Li, X., 2020).

Sossey-Alaoui and colleagues discovered that the expression of the *WAVE3* gene is associated with the progression of breast cancer. This assertion is supported by results from immunohistochemical staining, which revealed an overexpression level of *WAVE3* during grade III in contrast to absent *WAVE3* or minimal levels noted in normal breast tissue or grade I tumors among human patients (Sossey-Alaoui et al., 2007).

The identical investigators suppressed *WAVE3* in a stable form in MDA-MB-231 cells by utilizing the shRNA method. When *WAVE3* was repressed the invasive capacity

of MDA-MB-231 cells was exhibit to be roughly five times lower in a Matrigel invasion experiment. In addition, this suppressed *WAVE3* plays an inhibitory role in the metastatic probability of these cells. This was demonstrated by reducing lung surface metastases following the injection of reduction cells into the SCID mice's tail veins compared to control cells. Eventually, researchers also implanted the stable suppressed *WAVE3* in SCID mice's breast fat pads. The result was that tumor occurrence was 60%–80% lower in *WAVE3*-deficient cells than in control groups, and the development and angiogenicity of tumors occurred at a slower pace (Sossey-Alaoui et al., 2007 ; Frugtniet et al., 2015).

In an investigation by Lu et al., the expression level of *WAVE3* and protein was examined by utilizing qRT-PCR and western blotting in various cell lines of human ovarian cancer. They discovered that the *WAVE3* gene was upregulated in ovarian cancer tissue compared to healthy ovary, and this upregulation has been linked with the motility of cells, oncogenesis, and invasion. Further, NF-κB, MMPs, VEGF, COX-2, and the p38 MAPK pathway all have an association with *WAVE3* overexpression (Lu et al., 2017).

In the study by Huang et al., high expression of *WAVE3* was reported in pancreatic cancer tissue and was associated with low tumor differentiation, lymphatic metastasis, promote proliferation, migration, invasion and elevated level of CA19-9 before surgery (Huang et al., 2018). According to study findings by Li and associates, *WAVE3* may function as an oncogene in esophageal squamous cell carcinoma, and tumor cell growth was reduced when *WAVE3* was inhibited by utilizing miRNA 200b (Li et al., 2020). Another study was conducted and indicated that in the HCC's cytoplasm, the *WAVE3* level was significantly overexpressed, which was not present in normal tissue (Ji et al., 2015). Furthermore, it has been shown that *WAVE3* is upregulated in prostate cancerous tissues compared to non-cancerous tissues (Mughees et al., 2021). According to a study carried out at Peking-University Cancer, the expression of *WASF3* and MMP-9 was explored in the 216 individuals diagnosed with CRC by utilizing the SYBR-Green Master Mix through real-time PCR. The study conclusions indicate that there was an overexpression of *WASF3* and MMP-9 in tissues from colorectal cancer relative to healthy tissues ($p<0.001$) (Zhang et al., 2012).

Based on Yue et al and Xuebing et al studies, the expression level of the *WAVE3* gene in ESCC and stomach cancer was significantly associated with the TNM stages of cancer. In a study composed of 80 ESCC patients and a control group of about 30 individuals, the

outcomes of this research showed that patients with ESCC in stage III/IV of TNM (36.4%) had greater levels of positive *WAVE3* expression than those at TNM stage I/II (12.5%) (Yue et al., 2014; Xuebing et al., 2020).

In an investigation that Jie et al. (2014) conducted, the *WASF3* mRNA expression levels were evaluated in 38 individuals diagnosed with non-small cell lung cancer (NSCLC) and corresponding healthy tissues through qPCR by utilizing the SYBR Green method. And in another 96 patients, the expression levels of protein were examined through immunohistochemistry. The outcomes of this investigation revealed that the expression levels of *WASF3* mRNA were about 5 times greater in cancer tissues (4.8373±0.3142) than in healthy tissues (1.000), and a significant association was discovered between *WASF3* expression and the stages of the tumor (P value = 0.013). Also, the expression levels of its protein in NSCLC were similar to the results of mRNA expression (Wu et al., 2014).

Evidence demonstrated that *WAVE3* is a key regulator for characteristics linked to metastasis and that deactivating *WAVE3* is sufficient to prevent the metastasis and invasion of cancer (Teng et al, 2021). Therefore, based on study findings, targeting the *WAVE3* gene in cancer patients might be a useful therapeutic approach.

This current study on the Turkish population in Tokat Province is the first to examine the relationship between *WAVE3* gene and kidney cancer. The WAVE3 gene expression levels in human kidney cancer tissue were examined. For this work, it used the qRT-PCR test to evaluate the hypothesis for eleven patients diagnosed with kidney cancer. According to the findings of the present study, there is no difference in *WAVE3* gene expression in kidney cancerous tissues when compared with healthy tissues, and regarding this finding, statistically, no significant correlation was found ($p = 0.87$). There may be two possible reasons for the lack of intercourse for this investigation: the presence of a small number of patients and the absence of advanced-stage patients (metastasis). Accordingly, we believe that further studies with larger samples are needed to analyze and clearly understand the *WAVE3* expression level relationship to kidney cancer.

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