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ANALYSIS OF THE LEVEL OF *WAVE1* GENE EXPRESSION IN KIDNEY CANCER

MASTER'S THESIS

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Supervisor: Prof. Dr. Haci Omer ATES

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ETHICS CONTRACT

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I hereby reiterate that all data contained within the present thesis has been carefully compiled and presented in conformity with ethical standards and academic guidelines, and as outlined in these instructions, I have referred to all extraneous knowledge, ideas, and findings contained in this work.

25/07/2024

The Student Who Prepared the Thesis

RENAS NAJIB MOHAMMED

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In the name of God, the most gracious and merciful.

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ABSTRACT

ANALYSIS OF THE LEVEL OF THE *WAVE1* GENE EXPRESSION IN KIDNEY CANCER

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Master Thesis Department of Medical Biology

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Cancer is characterized by uncontrolled cell proliferation and is clearly the most destructive groups of human pathology. Renal cancer was previously thought to exist as a single illness, but it is currently recognized as several distinct cancer types that develop from kidneys, any one of them vary from histological features, disease progression, treatment responses, and genetic basis factors. Several of risk factors influenced renal cancer, involving lifestyle factors, medical condition factors, environmental factors, as well as genetic factors. The WASP family member 1 (*WASF1*) gene codes for WASP verprolin-homologous protein1 *(WAVE1)* which is an essential actin cytoskeleton control protein that is highly abundant in the brain. Particularly, *WAVE1* is one of the subunits of the *WAVE* regulatory complex (WRC) that permits activation of the complex of the actin-related protein 2/3 (ARP 2/3) and results in inducing the polymerization of actin that is important in diverse cell events like cellular migration and cellular adhesion. In this paper, we looked at the *WAVE1* (also known as *WASF1*) expression levels within renal cancer. For our work, 11 tissue samples of renal cancer as well as 11 healthy renal tissue samples from the same patient were evaluated. The renal cancer tissues were composed of 6 samples at stage I, 4 samples at stage III, and 1 sample at stage II. To analyze the *WAVE1* gene expression level in renal cancer and healthy tissues of any samples, we employed the quantitative real-time polymerase chain reaction (qRT-PCR) method. The findings of this study exhibited that the level of *WAVE1* gene was overexpressed in renal cancer tissue samples compared with healthy tissues (Fold change=5.08), which was statistically significant ($p = 0.030$). As a result, with all of these data, our research findings recommend that the *WAVE1* gene does have a significant role in renal cancer progress and growth.

Key Words: Actin Cytoskeleton, Kidney Cancer, qRT-PCR, *WAVE1*.

ÖZET

BÖBREK KANSERİNDE *WAVE1* GEN EKSPRESYON DÜZEYİNİN ANALİZİ

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Kanser, kontrolsüz hücre çoğalması ile karakterize edilir ve insan patolojisinin en yıkıcı gruplarından biridir. Böbrek kanserinin önceleri tek bir hastalık olarak var olduğu düşünülmekteydi, ancak günümüzde böbreklerden gelişen ve her biri histolojik özellikler, hastalığın ilerleyişi, tedaviye yanıtlar ve genetik temel faktörler açısından farklılık gösteren birkaç farklı kanser türü olarak kabul edilmektedir. Böbrek kanseri, yaşam tarzı, tıbbi durum, çevresel ve genetik faktörleri içeren bir dizi risk faktöründen etkilenmektedir. WASP ailesi üyesi 1 (*WASF1*) geni, WASP verprolin-homolog protein1 (WAVE1) proteinini kodlar ve beyinde oldukça bol miktarda bulunan temel bir aktin hücre iskeleti kontrol proteinidir. Özellikle WAVE1, ARP 2/3 kompleksini aktive eden WRC'nin alt birimlerinden biridir ve hücresel göç ve hücresel yapışma gibi çeşitli hücresel olaylarda önemli olan aktin polimerizasyonunun indüklenmesine neden olur. Bu çalışmada böbrek kanserinde *WAVE1* ekspresyon seviyelerine baktık. Çalışmamızda aynı hastaya ait 11 adet böbrek kanseri doku örneği ile 11 adet sağlıklı böbrek dokusu örneği değerlendirildi. Böbrek kanseri dokuları 6 evre I, 4 evre III ve 1 evre II örneğinden oluşuyordu. Böbrek kanseri ve sağlıklı dokulardaki *WAVE1* gen ekspresyon seviyesini analiz etmek için kantitatif gerçek zamanlı polimeraz zincir reaksiyonu (qRT-PCR) yöntemi kullandı. Çalışma sonuçlarımıza göre, *WAVE1*'in böbrek kanseri doku örneklerinde sağlıklı dokulara kıyasla ekspresyonunun arttığı (Kat artışı=5.08) ve bunun istatistiksel olarak anlamlı olduğunu tespit edilmiştir ($p = 0.030$). Sonuç olarak, araştırma bulgularımız *WAVE1*geninin böbrek kanserinin ilerlemesi ve büyümesinde kritik bir role sahip olabileceğini düşündürmektedir.

Anahtar Kelimeler: Aktin Hücre İskeleti, Böbrek kanseri, qRT-PCR, *WAVE1*.

TABLE OF CONTENT

LIST OF TABLES

Page

LIST OF FIGURES

page

LIST OF ABBREVIATIONS

ABI: Abl-interacting protein

ABPs: Actin-binding proteins

ARP: Actin related protein

BHD: Birt-Hogg-Dube Syndrome

CCRCC: Clear Cell Renal Cell Carcinoma

CD4: Cluster of Differentiation 4

CD8: Cluster of Differentiation 8

cDNA: Complementary DNA

CHRCC: Chromophobe Renal Cell Carcinoma

CNS: Central Nervous System

CRIB: CDC42/Rac-Interactive Binding

CTLA-4: Cytotoxic T-lymphocyte associated protein 4

CYFIP1: Cytoplasmic FMR1-interacting protein 1

DNA: Deoxyribonucleic Acid

dNTP: Deoxynucleotide Triphosphate

ECM: Extracellular Matrix

EMT: Epithelial to Mesenchymal Transition

EPO: Erythropoietin

F-actin: Filamentous-actin

G-actin: Globular-actin

GBD: GTPase-Binding Domain

GTPases: Guanosine Triphosphatases

HIF: Hypoxia Inducible Factor

HLRCC: Hereditary Leiomyomatosis-Renal Cell Carcinoma

HPRC: Hereditary Papillary Renal Cell Carcinoma

HSPC300: Hematopoietic Stem and Progenitor Cells 300

IGF2: Insulin-like Growth Factor 2

JMY: Junction-Mediating and Regulatory Protein

LAG-3: lymphocyte-Activation Gene 3

MMP: Matrix Metalloproteinase

mRNA: Messenger Ribonucleic Acid

NCKAP1: Nck-Associated protein 1

NPFs: Nucleation-Promoting Factors

N-WASP: Neural-WASP

PD-1: Programmed Cell Death

PDGF: Platelet-Derived Growth Factor

PDGFR: Platelet-Derived Growth Factor Receptor

PHD: Prolyl-4-Hydroxylase Domain

PRCC: Papillary Renal Cell Carcinoma

qRT-PCR: Quantitative Real Time Polymerase Chain Reaction

RCC: Renal Cell Carcinoma

RNA: Ribonucleic Acid

SCAR: Suppressor of cyclic AMP repressor

SHD: SCAR homology domain

TIME: Tumor Immune Microenvironment

VCA: Verprolin-Cofilin-Acidic

VEGF: Vascular Endothelial Growth Factor

VEGFR: Vascular Endothelial Growth Factor Receptor

VHL: Von Hippel-Lindau

WASF1: Wiskott-Aldrich syndrome protein family member 1

WASH: WASP and SCAR homologue

WASP: Wiskott-Aldrich Syndrome Protein

WAVE1: WASP-family verprolin homologous protein 1

WHAMM: WASP Homologue associated with Actin, Membranes, and Microtubules

WHD: Verprolin homology domain

WRC: WAVE regulatory complex

1. INTRODUCTION

The majority of cells within the body undergo a life cycle whereby their genetic material is maintained, fixed, and then transmitted to their daughter cells in an extremely controlled mechanism. Nevertheless, throughout the cell's lifecycle, sometimes the cycle or a controlling mechanism provides an error, resulting in uncontrolled proliferation. These are the characteristic properties of cancer cells (Mercadante and Kasi, 2022).

Classifying tumors as benign or malignant is the primary challenge in cancer pathology. The tumor that remains in its original position is the benign tumor; is not transmitted to other parts of the body, and also doesn't spread to proximal or distal structures in the body. Benign tumors develop slowly and exist clear borders. Malignant tumors are cancerous, meaning that cells in malignant tumors develop unregulated and disperse proximal and to distal places via the circulation or lymphatic system. This is known as metastasis (Patel, 2020).

Throughout the world, cancer occurrence and mortality rates are increasing. In 2018, there were 18.1 million new cases of cancer and 9.6 million fatalities. (Sung et al., 2021). On a global scale, cancer ranked as the number two killer. In terms of new cases, breast cancer was the most widespread kind of cancer, ; subsequently, other cancer kinds include: pulmonary cancer, large intestine/rectum cancer, prostate gland cancer, cutaneous cancer, gastric cancer, hepatic cancer, cervical cancer, esophageal cancer, thyroid cancer, urinary bladder cancer, non-Hodgkin's lymphoma (NHL), pancreatic ductal adenocarcinoma, blood cancer, and renal cancer (Kim et al., 2022).

Renal cancer is a prevalent urological neoplasm, and the rate of development and death ranks 15th among all cancers worldwide (Wang et al., 2022). Renal cancer is the third prevalent urological cancer and the one with the greatest death rate (Kim et al., 2022). Throughout 185 countries, based on the assessment given by Global Cancer Statistics (GLOCAN), renal cancer was responsible for 179,368 (1.8%) deaths out of the 9.9 million deaths from cancer and 431,288 (2.2%) cases out of the 18.1 million new cancer patients in the 2020 year (Sung et al., 2021).Renal cell carcinoma (RCC) is the most widespread sort of renal cancer. It starts in the inner layer of the tiny tubes within the renal, which filter blood and excrete waste. The glomerulus, tubular apparatus, and collecting duct make up the kidney's cortex, where the majority of RCCs develop. Cancer of the renal pelvis is another sort of kidney carcinoma that is called transitional cell carcinoma (TCC) (cancer that grows within the kidney's center where urine accumulates

and resembles in histology and behavior urothelial cancer) (Thakur and Jain, 2011). One percent of all renal cancer are renal sarcomas, which are extremely paucity tumors(Bakhshi et al., 2012) Wilms tumor (also called nephroblastoma) is another infrequent sort of renal cancer observed mainly among children (Ikhuoriah et al., 2023).

RCC can be an inherited or sporadic occurrence. In numerous investigations, having a close family member with renal cancer was related to a greater risk that has been 2–4 times higher (Noordzij and Mickisch, 2004). Compared to sporadic forms of the disease, hereditary RCC typically develops earlier in life (Choyke et al., 2003). The following are the general risk factors that were foreseen earlier: Tobacco smoking (Wang et al., 2019) and history of high blood pressure (it is unknown if the medicines or the high blood pressure are the reason for the increased risk) (Thakur and Jain, 2011), chronic renal illness or chronic renal failure, (a greater risk of renal cancer exists among individuals with severe renal disease who require dialysis) (Ciorcan et al., 2022), some medicines (including a previously recognized painkiller called (phenacetin) have been linked to renal cancer) (International Agency for Research on Cancer, 2012), gender (males are diagnosed with renal cancer approximately twice as frequently as females), race (renal cancer is more common in African Americans than in whites, the causes of this are equally obscure). Other risk factors are body size, family history, and hereditary factors, some uncommon hereditary syndrome are Von Hippel-Lindau syndrome (VHL), Birt-Hogg-Dube syndrome (BHD), Hereditary Leiomyoma-RCC (HLRCC), Hereditary Papillary RCC (HPRC), and familial renal oncocytomas. Most likely, a mix of sporadic genetic occurrences, exposure to the environment, and individual factor leads to renal cancer (Thakur and Jain, 2011).

Sick people with renal cancer, develop metastatic spread in nearly one-third of cases (Gong et al., 2016). Based on an investigation that performed by D'Elia and colleagues the organs most often affected by metastasis are the lungs, lymph glands (lymph nodes), liver (hepatic system), bone (osseous tissue), and adrenal glands(suprarenal glands); skeletal muscle metastasis occurs infrequently (D'Elia et al., 2013). Research has previously identified that metastatic renal cancer growth is facilitated by dysregulation of the actin cytoskeleton and many actin-binding proteins (also called ABPs) in both actin assembly, disassembly pathways, including Cofilin, Profilin, the ARP2/3 complex, WASP family proteins, and others (White et al., 2014). In humans, the WASP family member 1 (*WASF1*) gene encodes WASP-family verprolin homologous

protein-1 (WAVE1) protein, an actin-binding protein that is a part of the family of WASP and WAVE. It controls the actin cytoskeleton linked to the actin-related protein-2 and 3 ARP2 and ARP3 and results in encouraging the process of actin polymerization (Sowalsky et al., 2015). Despite its role, WAVE1's function in cancer progression and invasion has been indicated in numerous investigations (Zhang et al., 2012). It follows that WAVE 1 activity is essential for the movement of normal cell and may also have a function in many cancers. In light of this, lots of research has focused on the WAVE1 proteins and their crucial function in cancer development (Frugtniet et al., 2015). The list includes melanoma (Yamaguchi and Condeelis, 2007), leukemia (Kang et al., 2010), breast cancer (Fernando et al., 2007), prostate cancer (PC), and epithelial ovarian carcinoma (EOC) (Lu et al., 2023).

Presently, there is indeed no investigation in the scientific literature concerning the expression of the *WAVE1* gene with renal cancer. In this regard, the aim of this research is to investigate the *WAVE1* gene expression level in renal cancer. In addition, it may become a predictive marker for renal cancer and may help future researchers target the *WAVE1* gene as a treatment for renal cancer.

2. LITERATURE REVIEW

2.1. An Overview of Cancer and It's Prevalence

A healthy (or normal) cell would change into a dysplastic or abnormal cell, which would then become an invader or a cancerous cell, according to the classic model of cancer (Idikio, 2011). Cancer is clearly the most destructive groups of human pathology. It is a complex disease of disrupted signaling and metabolism (Upadhyay, 2021) and is deemed the main reason for mortality worldwide in the twenty-first century. Based on the World Health Organization (WHO), during the year 2015, malignant disease was assessed as the top or second factor of mortality for those under seventy years old within several nations (Bray et al., 2018). Also cancer is expected to result in 13.2 million fatalities and 21.4 million annual morbidity cases until 2030 (Alzahrani et al., 2021).

Many scientists believe that cancer is a hereditary disorder that starts with a sequence of molecular processes that change typical cells in the way they divide and multiply (Vogelstein and Kinzler, 2004). Cells that proliferate uncontrollably are referred to as tumors, and tumors have two types: benign and malignant. Benign tumors are well differentiated, develop slowly, exhibit expansile development with encapsulation, and stay restricted to their aboriginal place with no invading neighboring healthy tissues or causing widespread. Conversely, malignant tumors are frequently poorly differentiated, develop quickly with numerous mitoses, are without a capsule, and have capacity to transmit to both proximal healthy tissue and migrate (metastasize) to distant tissue in the body through blood and lymphatic circulation. (Jang et al., 2011; Alzahrani et al., 2021). Thus, malignant tumors obtain metastasis, which happens partly because of up-regulation of receptors that promote cell mobility and downregulation of receptors for adhesion of cells required for tissue-particular cell-to-cell adhesion. Furthermore, the stimulation of membrane metalloproteases supplies a physical mechanism for metastasis cancerous cells to disseminate (Sarkar et al., 2013). Solely malignant tumors are considered to be cancers, and cancer can be life-threatening due to its potential to spread, which also often results in resistance to therapy (Alzahrani et al., 2021). In general, benign tumors are not life-threatening. However, the growth of benign cells may turn hazardous if they transform widely or, occasionally, develop into cancers (Valet and Narbonne, 2022).

2.1.1 Classification of cancer

Most cancers are categorized in four manners: (1) according to tissue or organ of origin; then by (2) particular kind, and (3) grade on the basis of WHO classifications; (4) lastly according to spread on the basis of the system of tumor node metastasis (TNM) (Carbone, 2020).

Under the general categorization of cancer arranged by tissue or organ of origin, blood cancers are segregated from solid cancers, which have been more commonly categorized as 3 major types: Carcinomas, Sarcomas, and lymphomas. Carcinomas represent solid tumor types that arise in cells of the epithelium of the skin, digestive and intestinal systems, inner organ surfaces, and other anatomic places and compose almost 90 percent of human malignant tumors; sarcomas represent a paucity form of solid tumors that emerge in skeletal and connective tissues, or blood vessels; and lymphomas are a type of blood cancer that begin in lymph nodes and immune system tissue. Moreover, there are several cancer histotypes described in addition to these principal types (Carbone, 2020; Cooper, 2000).

Cancer grading and staging are then used to predict the clinical behavior of malignancies. Cancer grading is represented by numbers, often ranging from a low grade of 1 (considered well differentiated) to a high grade of 3 (described as poorly differentiated). The TNM cancer staging may identify the disease's degree by measuring the main tumor's size (T); checking for involvement of lymph node (N), and counting the occurrence of remote metastasis (M) (Carbone, 2020).

A precise categorization of the cell of origin of cancers would significantly improve our biological comprehension of cancer formation, and this would help construct new avenues for early therapy and prevention (Waldum et al., 2008).

2.2. Renal Cancer and It's Prevalence

The kidneys are an important and complicated structure for preserving the usual functions of the human body (Madrazo-Ibarra and Vaitla, 2023); structurally, both kidneys are covered with a dense layer of fibrous tissue known as a kidney capsule that protects the kidneys in position. Underneath this capsule is the kidney cortex, which is the extrarenal section; this is where the filtration of the blood starts. And the inner section of

the kidney is the kidney medulla. It has numerous conical structures identified as medullary pyramids. Urinary fluid streams from the papillae at the apices of every the pyramids to the minor calyx and major calyx, which drain it into the renal pelvis, and eventually the ureter within the renal pelvis carries the excess waste into the bladder (Figure 2.1) (Davidson, 2010). The cortex and medulla are both made up of nephrons; nephrons are the fundamental component and functional units in the kidney, which is divided into two parts: renal corpuscle (including Glomerulus and Bowman's capsule) and renal tubule. Nephrons exist in two forms: cortical (superficial) nephrons and juxtamedullary nephrons (Figure 2.2) (Toribio, 2007). The kidney is well recognized for playing a particular role in preserving osmotic homeostasis through ion transfer and waste material filtration. As a matter of fact, the nephron creates a number of gradients that control and react for blood sugar (glucose), urea, oxygen (O2), and other essential metabolism aspects. Furthermore, the medulla is extremely sensitive to alterations in blood oxygen levels, but the kidney preserves systems that ensure constant levels of oxygen in the near and distant regions of the nephron. Therefore, it is conceivable that the kidney is a tissue that can develop into cancer in a particular way (Figure 2.3) (Rathmell et al., 2018). In light of this, it can be said that renal cancer is an illness of cell metabolism (Linehan and Ricketts, 2013).

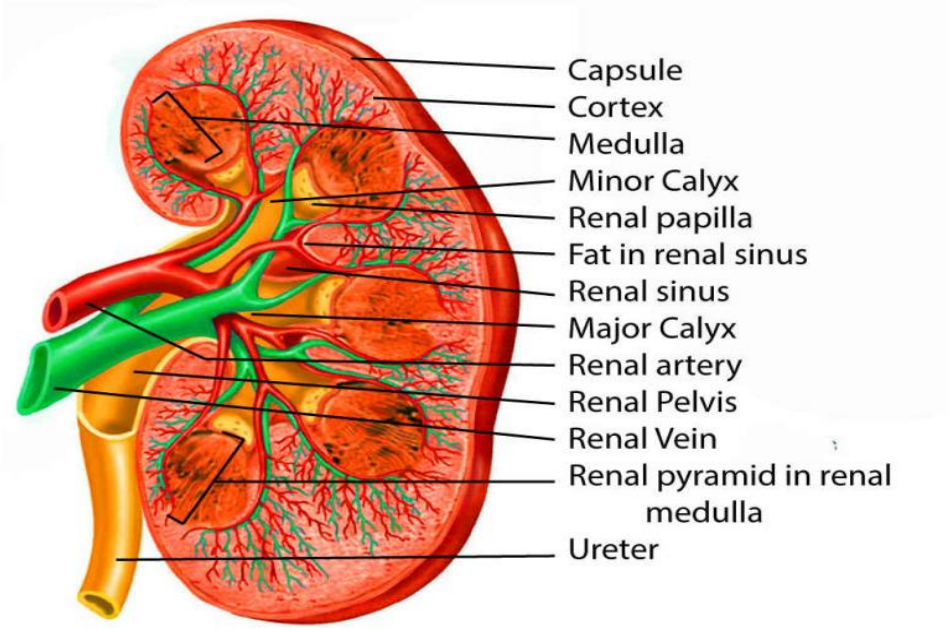

Figure 2.1. Renal structures (Leslie and Sajjad, 2017).

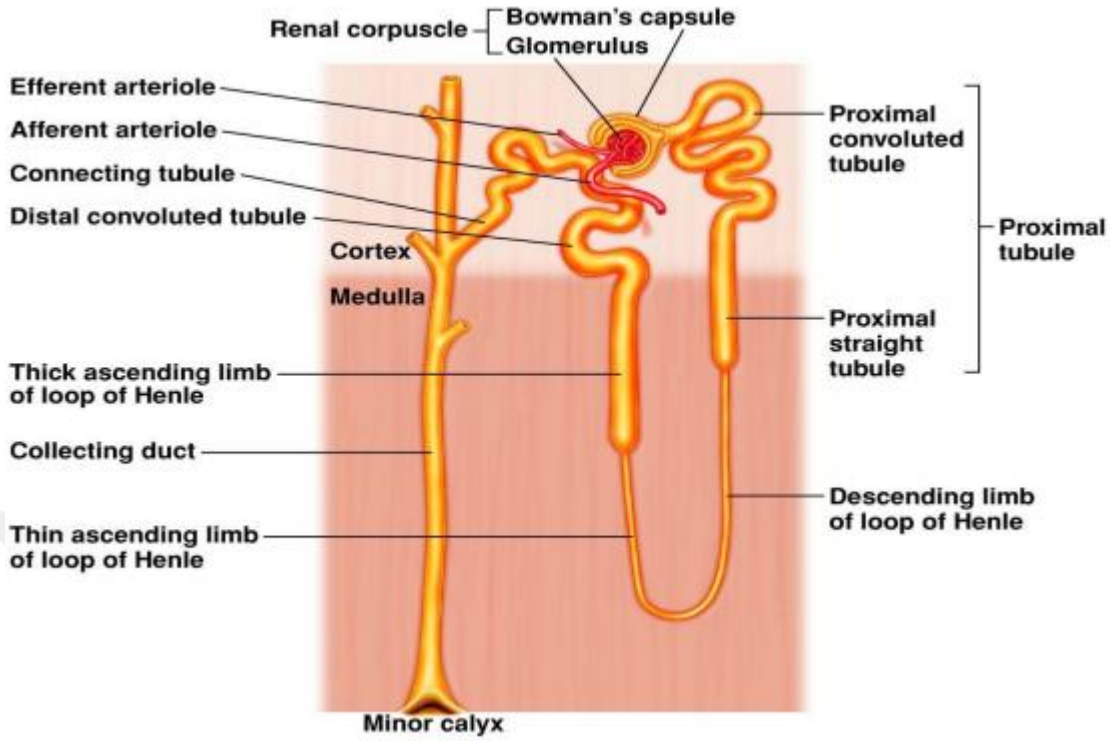

Figure 2.2. The nephrons diagram (Hoban, 2008).

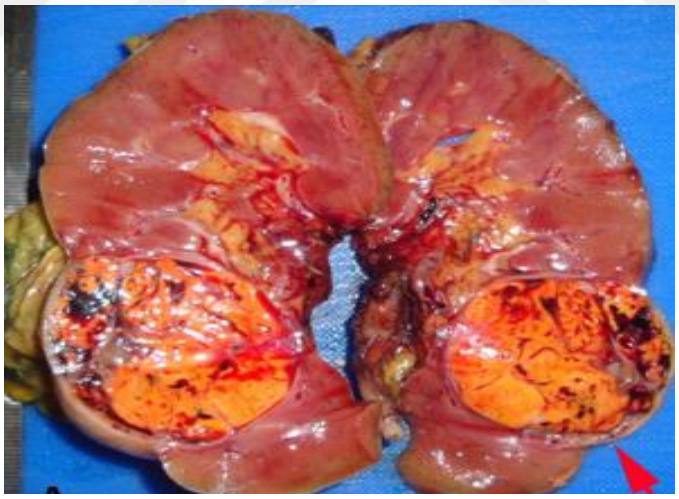

Figure 2.3. Renal cancer (Samaratunga et al., 2014)

Renal cancer is a common urological cancer (Li et al., 2022). Urothelial cancers involve cancer of the urinary bladder, prostate, testes, and renal (Barber and Ali, 2022). Due to its high prevalence and mortality rate, renal cancer ranks third in the occurrence of cancer cases in the urinary tract, after prostate and bladder cancers (Pascual and Borque, 2008). Renal cancer was previously thought to exist as a single illness, but it is currently recognized as several distinct cancer types that develop from renal cells, any

one of them presents vary from their histological features, disease progression, and that seem to react differentially for treatment and is caused by mutations in distinct genes, meaning that each one has a unique genetic basis (Linehan and Ricketts, 2013). Alterations in any of these genes cause metabolic pathway disruptions involved in oxygen, glucose, and other critical metabolic features. Focusing on its metabolism pathways within renal cancer supplies a novel therapeutic strategy to this disease (Linehan et al., 2019).

The 15th most prevalent cancer in the globe in 2020 was renal cancer (Marconi et al., 2023). Renal cancer incidence rates differ among countries. Developed countries (like the US and Europe) tend to have a greater renal cancer incidence rate than less developed countries (like Asia and South America) (Chow et al., 2010). Furthermore, men have double the chance of females for developing renal cancer throughout their lifetimes. It would indicate that the mortality rate is falling for females at a faster pace than for men. In addition, almost 50 percent of all instances of renal cancer are detected prior to 65 years old globally. The incidence rates continue to rise with age and reach a peak around the age of 75. (Scelo and Larose, 2018). 2.2 percent of cancer cases are renal cancer detects, based on GLOBOCAN statistics from 2018, and is thought to affect 403,000 individuals annually. Of these, around 254,500 instances in men and 148,800 in women are detected, indicating that males have a 1.7-fold higher relative risk (RR) than females do. In 2018, GLOBOCAN figures show that 175,000 individuals died as a result of renal cancer. This number accounts for 1.8% of cancer-related deaths worldwide. Because of this illness, about 61,000 females and 114,000 males died; the relative risk of death for males is 1.87, much higher than for females (Padala et al., 2020).

2.2.1. Histopathology

Renal cancer cases in adults develop within the renal parenchyma and renal pelvis. Approximately below 10% of the renal cancers with microscopic confirmation are renal pelvis carcinomas, of which the majority are urothelial cancers, TCCs. Approximately more than 90 percent of renal cancers originated from the renal parenchyma are RCCs. The prevalent form of renal cancer in children is wilms tumor, or nephroblastoma, which accounts for around 1.1 percent of renal cancer (Chow et al., 2010). And another rare renal cancer is renal sarcoma, constituting approximately 0.8–2.7% of renal cancer (Öztürk, 2015) (Figure 2.4).

RCCs are the predominant renal cancer kind that affects the renal of adults; they make up around 90 percent of all cancer that occurs in the renal. Furthermore, it manifests itself in sporadic (nonhereditary) forms (96 percent) as well as hereditary (four percent) forms, both of which originate in the renal parenchyma region (Pandey and Syed, 2020; Ikuemonisan et al., 2023). Particularly from the many cell types that are found throughout the length of the nephron, which is composed of the glomerulus, tubular apparatus, and collecting duct, it is possible to further categorize the nephron into histological subtypes (Kabaria et al., 2016; Madrazo-Ibarra and Vaitla, 2020). A great number of research have shown that the histological categorization of RCCs is quite important. This is due to the fact that the categorization of the subtypes of this cancer possesses a significant effect on the therapy and prognosis. In accordance with its morphological and molecular genetic characterisation, the WHO has classified RCC into distinct of subgroups. Histologically, RCC may be divided into four subclasses: conventional (clear cell) RCC (ccRCC), type 1 papillary, type 2 papillary, and chromophobe RCC (chrRCC). These subclasses are the prevalent types of RCC (Bahadoram et al., 2022). The other subclasses consist of unclassified RCC (uRCC), which accounts for around 4 percent of all cases, and other less common types of RCC, which are less than 1 percent. These include medullary RCC (mdRCC) and collecting duct RCC (cdRCC) (Chen et al., 2016). The prevalent subcategory of RCC is conventional (ccRCC), which comprises about seventy five percent and eighty percent of RCC cases. pRCC, which comprises about ten to fifteen percent of RCC cases, and chromophobe RCC, which comprises about five percent of cases (Cairns, 2011). ChrRCC is associated with the distal convoluted tubule (DCT) and the collecting tubule epithelium, particularly intercalated cells. ccRCC and pRCC are both linked to the proximal convoluted tubule (PCT) (Bahadoram et al ., 2022) and when seen via a microscope, ccRCC tumors are highly vascularized in a staghorn pattern and often consist of transparent cytoplasm. The cause of the transparent appearance of the cytoplasm is the buildup of glycogen and lipids. Certain cases of ccRCC, on the other hand, have tumor cells that include granular eosinophilic cytoplasm (Abu Haeyeh et al., 2022). pRCC, are made up of cells that are configured in a spindle-like form. Small basophilic cells with pale cytoplasm are characteristic of type 1 pRCC, while bigger cells with eosinophilic cytoplasm are characteristic of type 2 pRCC. (Muglia and Prando, 2015). chRCC is made up of cells that have a transparent reticulated cytoplasm and irregular nuclei with a perinuclear clear halo (Figure 2.5) (Abu Haeyeh et al., 2022). Moreover, ccRCC has the

highest rate of metastasis and a poorer prognosis than the majority of common subtypes (Bahadoram et al., 2022).

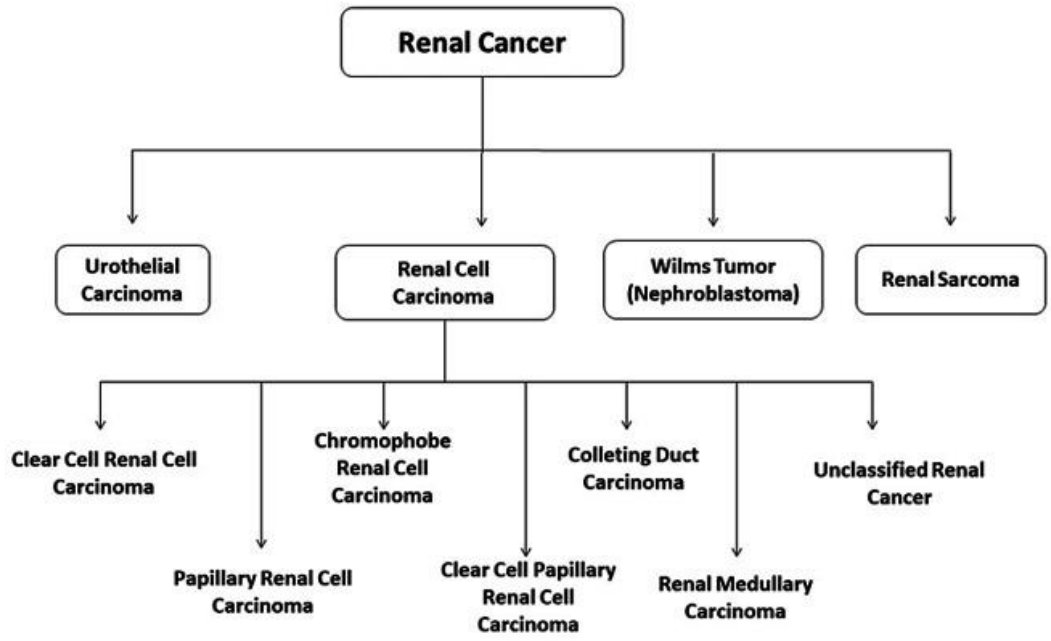

Figure 2.4. Categories of renal cancer (Mohd et al., 2022)

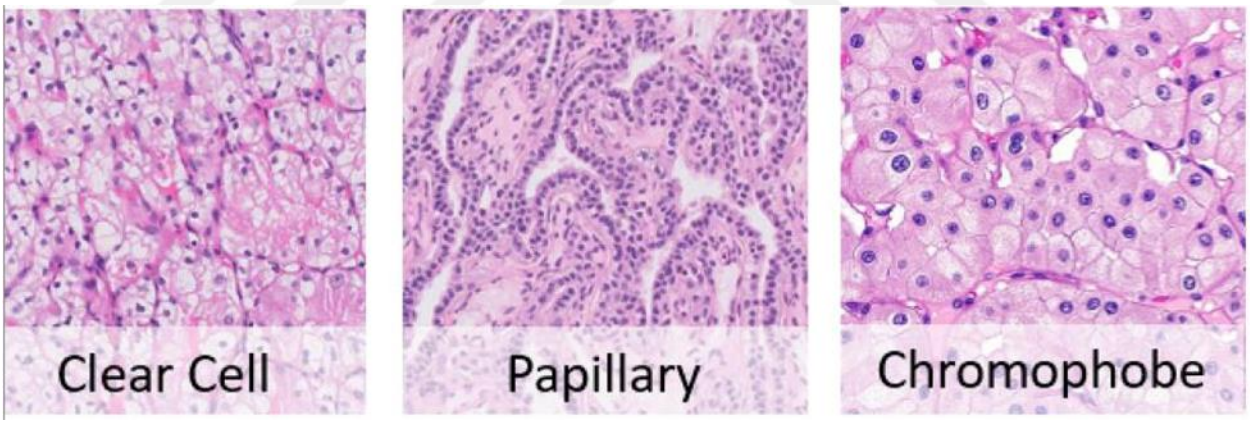

Figure 2.5. The main renal cell carcinoma (RCC) subtypes' morphology (Abu Haeyeh et al., 2022).

Furthermore, the majority of RCC instances are frequently without apparent symptoms and are incidentally diagnosed via medical imaging such as CT, MRI, and abdominal ultrasound. The classic clinical triad, which includes back pain, hematuria, and flank lumps, is observed in just 10% of cases. Fever, loss of weight, and leukocytosis are other typical symptoms (Padala et al., 2020).

2.3. Renal Cancer Risk Factors

The exact reason for renal cancer is largely unelucidated, even though causes are believed to be multifactorial. Up to now, researchers have identified numerous factors of risk that elevate renal cancer risk, some of which may be modifiable and offer a chance for main prevention. Potential factors contributing to the risk associated with renal cancer that are listed previously are as follows: lifestyle factors, medical condition factors, exposure to certain environmental and occupational carcinogens, and genetic factors (Safiri et al., 2020).

2.3.1. Lifestyle-related risk factors

2.3.1.1. Smoking

Cigarette is the major factor of risk for RCC and is considered modifiable (Campi et al., 2023). Cigarette smoke includes a number of carcinogenic compounds, including N-nitrosamine, which may damage DNA and raise the likelihood of RCC. Also, nicotine is a key tobacco substance that is found to promote carcinogenesis by affecting angiogenesis (Kabaria et al., 2016) and according to estimates, smoking is responsible for nearly 17% of renal cancer cases globally. About 15–20% of renal cancer cases smoke actively at the time of diagnosis (Sheikh et al., 2023).

One study found that, compared to never-smokers, the risk associated with renal cancer was thirty-nine percent higher in smokers at present and twenty percent higher in smokers at previous (Wang et al., 2022). Moreover, the risk also rises with chronic smoking duration and the number of cigarettes smoked per day (Sims et al., 2018). Furthermore, it appeared that cigarettes influenced men more than women (Peired et al., 2021) about 50% of men, but only 20% of women, are estimated to be attributed to cigarettes for renal cancer (Bai et al., 2020).

A study in the varied state of Florida demonstrates that cancer-specific survival (CSS) results in renal cancer cases vary according to the smoking status at diagnosis (Smokers are classified as never smokers, former smokers, and current smokers at the time of diagnosis) (Figure 2.6). (Baral et al., 2023). According to the preliminary findings of the research, the chRCC subkind of RCC is not influenced from tobacco smoking. It has been accomplished as a risk factor for both ccRCC and pRCC (Sims et al., 2018). Individuals who have given up smoking, particularly those who have done so for more

than ten years, have shown a reduction in risk (Chow et al., 2010).

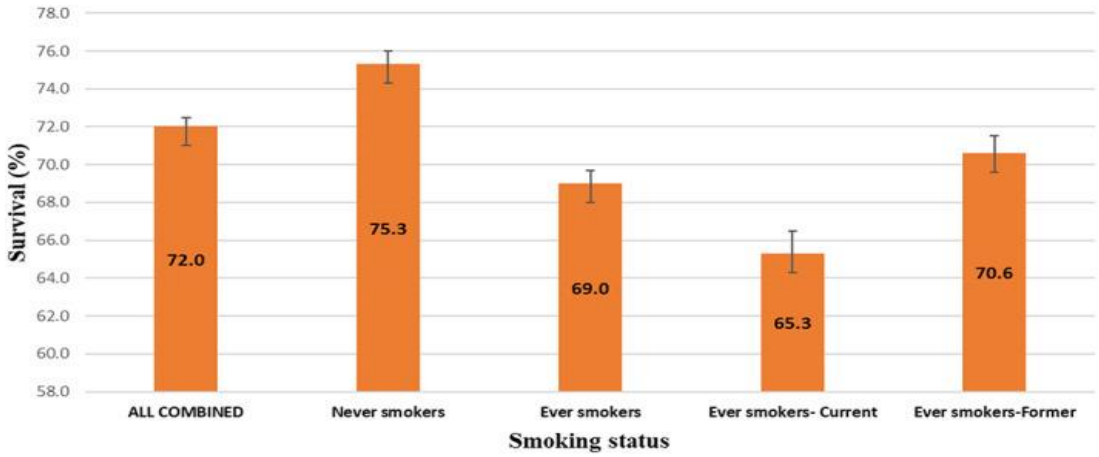

Figure 2.6. Estimates of five-year age-standard relative survival for renal cancer utilizing 95% confidence intervals (CI) according to smoking cigarette levels at diagnosis (Baral et al., 2023)

2.3.1.2. Obesity

Excess body weight as clarified by a body mass index (BMI), is another main proven risk for the growth of renal cancer (Wilson and Cho, 2016), which is attributable to elevated insulin/insulin-like growth factor signal -1 (IGF-I) that stimulates unchecked tumor progress, and dysregulates the secretion of adipocytokines such as adiponectin, which causes an excess of DNA damaging free radicals. Although the exact process behind obesity in the pathogenesis of renal cancer is still not clear (Padala et al., 2020). It was assessed that around 18.5% of renal cancer is caused by obesity (Safiri et al., 2020). According to meta-analyses, it has been demonstrated that a raised BMI had a consistent positive correlation with RCC, which was stronger than in males; men and women with a BMI ≥35 had a RR of achieving renal cancer of 2.47 and 2.59, respectively (Peired et al., 2021). One investigation revealed that a rise of 5 kg/m^2 in BMI elevated the risk of RCC by 25% among males and 35% among females (Padala et al., 2020). Although, papillary, chromophobe, and other RCC subtype individuals all exhibited a little lower rate of obesity than individuals with ccRCC (Sims et al., 2018).

2.3.1.3. Alcohol consumption

Another potential risk factor is alcohol intake. Moderate levels of alcohol intake seem to decrease the risk of RCC relative to abstinence (Makino et al., 2022). Whereas, heavy drinking has been definitively related to increased risk for RCC (Sims et al., 2018). In fact, an inverse association has been suggested between alcohol intake and the risk of RCC for all alcoholic beverages, consisting of beer, wine, and liquor, among men and

women in extensive prospective cohort research. Beer and liquor consumption decrease RCC risks in men, but the risk in women particularly decreases with wine consumption (Kabaria et al., 2016). Contrarily, no relation between RCC risk and drinking of either alcohol beverage was recorded in the United States in the study of VITAL. Despite the fact that a recent meta-analysis reported that drinking all types of alcohol linked with reduced RCC risk (Makino et al., 2020).

2.3.1.4. Exercise

An extensive meta-analysis of prospective cohorts has linked physical exercise to a slight lowering of the risk of renal cancer. Considering all forms of physical activity, the highest activity group showed a 13% lower risk than the least activity group. However, the mechanism is not clear (Scelo and Larose, 2018). According to a recent meta-analysis of physical exercise and the risk associated with RCC, an opposite connection in risk was reported (Behrens and Leitzmann, 2013).

2.3.2. Medical history related risk factors

2.3.2.1. Hypertension

Hypertension (HTN) is a major factor in the risk of RCC, and studies indicate that high blood pressure elevates the likelihood of the progression of RCC. Recently, a meta-analysis within the VITAL investigation of eighteen prospective investigations indicated a direct link high blood pressure and the risk of RCC. And showed RCC risk was sixty-seven percent higher within those individuals who have hypertension histological background, and with each ten mmHg increase in hypertension relating to a 10 to 22 percent higher likelihood of RCC (Makino et al., 2022). The risk of RCC may be increased in females with HTN. Recently, meta- analysis revealed that the risk of RCC is 54% greater in females with hypertension compared to males (RR = 63 vs. 29). However, the variance was significantly lessened. In addition, investigations on the association between RCC likelihood and high blood pressure in adolescents have shown opposite results. Nevertheless, users of substances that promote urine flow and other anti-hypertensive medications in individuals with HTN were associated with an elevated likelihood of developing RCC, in accordance with recent Korean cohort research. Although the various antihypertensive medication classes have varying impacts on the risk of RCC (Ba et al., 2022).

There is no clear biological mechanism for the association between elevated HTN and increased RCC risk. But it is speculated that HTN may induce a state of chronic kidney hypoxia, which results in cell growth dysregulation and angiogenesis through a transcription factor called hypoxia-inducible factor. Also increased lipid peroxidation with the fabrication of oxygen radicals, which is associated with the pathogenesis of RCC (Capitanio et al., 2019).

2.3.2.2. Renal diseases

A history of renal disease, including renal stones, renal cysts, renal infections, and end-stage kidney disease (ESKD), was implicated as a factor for RCC risk. Washio and Mori, in their study that was conducted across the Japanese population, found that renal disease was associated with an elevated RCC risk. However, they indicated that another study has found that renal disease was not shown to be an important risk factor for dying from RCC (Washio and Mori, 2009).

2.3.2.3. Diabetes

Despite the fact that the Nurses' Health research discovered that females with type 2 diabetes had a higher chance of developing RCCs, the VITAL inquiry discovered that there was no connection between the two conditions. There is a significant correlation between RCCs and diabetes, on the basis of nine cohort studies's meta-analyses. (Makino et al., 2022).

2.3.3. Occupation and environment related risk factors

There are various environmental and occupational exposure factors that have been proposed. Trichloroethylene, an organic solvent that has been investigated the most, is commonly used as a cleaning and degreasing agent for metals, and it is also used to make other chemicals. Several studies have linked raised RCCs risk to higher exposure to occupational and environmental risk factors. Nevertheless, an exact description of the mechanism involved is still unclear (de Leon and Pedrosa, 2017).

2.3.4. Genetic risk factors of renal cancer

RCC has sporadic (nonhereditary) forms (96 %) and hereditary forms. Scientific research has discovered that innate pathogenic germline variants are the main reason for

the inherited risk of RCC in some affected families. Inherited conditions are responsible for around 4% of all RCCs, according to a 2014 study by Byler and Bratslavsky. They also noted that they have a tendency to develop at an early age, be bilateral, and be multicentric. Karami and colleagues, in their research, indicated that first-degree relatives of individuals with inherited renal cancer in Caucasians and Black Americans are at an increased likelihood of getting renal cancer (Karami et al., 2015). The study of families with specific types of hereditary syndromes related to an elevated likelihood of developing renal cancer causes the recognition of those genes that were altered, which is significant for this disease (Haas and Nathanson, 2014).

Developing RCC is more likely in families with four main syndromes: (1) BHD syndrome; (2) HLRCC syndrome; (3) VHL syndrome; and (4) HPRC syndrome. The most prevalent hereditary syndrome is VHL syndrome, which is highly penetrant and has an autosomal dominant pattern. RCC is the leading cause of mortality for over half of the people with VHL illness. This syndrome is caused by a germline variant of the tumor suppressor gene (TSG), called the *VHL* gene, which has been seen in cystic kidney disease and ccRCC. An autosomal dominant pattern is seen in the hereditary renal cancer condition known as HPRC syndrome. Bilateral multifocal renal carcinoma papillary type 1 is a potential outcome for patients with HPRC. Hereditary *MET* proto-oncogene mutations on 7q31 are linked to HPRC. HLRCC is a hereditary syndrome inherited via those chromosomes that are not sex chromosomes. Potential complications in patients with HLRCC include uterine leiomyomas, cutaneous leiomyomas, and renal cancer. The majority of diseases connected with this syndrome are collecting duct, ccRCC, and renal carcinoma papillary type 1. It happens because of mutations that render inactive a tumor suppressor gene called the fumarate hydratase (*FH*) gene. This gene is essential for a Krebs cycle enzyme. There is strong evidence linking the hereditary BHD syndrome to an elevated risk of certain malignant renal tumors, like chRCC and ccRCC. Tumor suppressor gene mutations involving BHD , also called folliculin (*FLCN*), are associated with all cases of BHD syndrome (Sims et al., 2018; Pfaffenroth and Linehan, 2008).

2.4. Genetics of Renal Cancer

2.4.1. VHL loss and HIF-α stabilization

As was noted before, ccRCC is the histologic subcategory of RCC that is identified as the most prevalent and vascular tumor (Baldewijns et al., 2010). *VHL* gene has been identified as a tumor suppressor and is situated on chromosomes 3p25–26 (Dwyer and Tu 2017). The lack of expression of the *VHL* gene, that is possible the result of an inactivating mutation and/or epigenetic modification, is the defining characteristic of ccRCC, whether it is inherited or spontaneous (non-inherited) (i.e., aberrant methylation) (Rathmell and Chen, 2008). It is believed that around ninety percent of people who have sporadic ccRCC have a deletion of the *VHL* gene. Fifty percent of these tumors are caused by mutations, and ten to twenty percent are caused by aberrant methylation. Hypoxia-inducible factors (HIF) are activated when the *VHL* gene is lost, which causes hypoxia. The activation of HIF leads to modifications in the signaling pathways of receptor tyrosine kinase (RTK), which include the phosphoinositide 3-kinase (PI3K), protein kinase B (AKT), mechanistic target of rapamycin (mTOR), Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and mitogen activated protein kinase (MEK), extracellular signal-regulated kinases (ERK) pathways. Thus, this induces cellular proliferation as well as metastasis (Kumar et al., 2018).

HIFs are complexes made up of two subunits, one of which is an α subunit (HIF-1α/ HIF-2α/ HIF-3α), and the other is a β subunit (HIF-β: ARNT) (Gkotinakou et al., 2022). During normal oxygen state, the α subunit of HIF gets hydroxylated on a critical proline residue through the action of a group of proteins of the O2-dependent prolyl-4-hydroxylase domain (PHD) (PHD-1/PHD-2/PHD-3), which permits the complex of VHL to bind and mark it for degradation via polyubiquitination in the proteosome.

Alternatively, when the *VHL* gene is functionally inactivated during hypoxic conditions, prolyl hydroxylation of the HIFα subunit does not occur and leads to aberrant accumulation of HIF-α. HIFα then migrates into the nucleus and associates with HIFβ and coactivators, including p300 and CBP, to form transcriptionally active HIF that attaches to hypoxia response elements (HREs), and hence regulates the expression of over 60 HIF target genes implicated in important tumorigenesis pathways, including angiogenesis and growth factors, glucose metabolism [glucose transporter1 (*GLUT-1*), erythropoiesis, enhanced glycolysis, pH regulation, apoptosis suppressor, and cell cycle progression]

(Kumar et al., 2018). Cowey and Rathmell showed that ccRCC acquires its characteristics of resistance to chemotherapy, vascularity, and invasiveness through activation of these hypoxia target genes (Cowey and Rathmell, 2009), also Alchahin et al. supported that ccRCC are characterized by their hypoxic, immunogenicity, and angiogenesis tumors (Alchahin et al., 2023).

2.4.2. Angiogenesis and growth factors

Angiogenesis is a physiological mechanism that provides nutrients and O2 to every part of the body along with the steady equilibrium between several pro- and anti-angiogenic elements that govern it (Heidegger et al., 2019). The "angiogenic switch" describes the equilibrium between substances that promote angiogenesis and those that prevent it. (Kasherman et al., 2022). But when that equilibrium is out of whack, aberrant blood vessel development occurs, which is associated with the growth and spread of tumors. The overabundance of proangiogenic factors typically causes an imbalance in the angiogenic balance (Heidegger et al., 2019). There exist several distinct pro-angiogenic growth factors that have been discovered to significantly impact the ccRCC pathogenesis, including vascular endothelial growth factor (VEGF), insulin like growth factor 2 (IGF2), platelet derived growth factor (PDGF), and erythropoietin (EPO) (Mennitto et al., 2020). VEFG regulates neovessels (angiogenesis), cell proliferation, cell migration, and increased capillary permeability; it is considered a major and potent pro-angiogenic factor in ccRCC (Fujita et al., 2014; Guillaume et al., 2022). Additionally, Paradis and colleagues have indicated that VEFG is a key factor in ccRCC angiogenesis (Paradis et al., 2000).

VEFG-A/VEFG-B/VEFG-C/VEFG-D/VEFG-E/placenta growth factor (PGF) -1 and -2 are the seven components that make up VEGF. along with three receptors known as VEGFR1, VEGFR-2, and VEGFR-3. Indeed, VEGF-A was identified as a major function in the regulation of new blood vessel growth (angiogenesis), whereas VEGFC and VEGFD are evident in regulating lymphatic vessel growth (lymphangiogenesis) (Guillaume et al., 2022). Also, Li et al. supported that overproduction of VEGF-C and VEGFR3 may have a key function in ccRCC development by stimulating lymphangiogenesis (Li et al., 2021).

2.4.3. Immunogenicity

In addition to its angiogenesis characteristics, ccRCC has been shown to be a strong immunogenic tumor and is linked to a sophisticated tumor immune microenvironment (TIME) (Monjaras-Avila et al., 2023). However, the relationship between ccRCC and its TIME is not completely clear (Pan et al., 2020). It's previously been reported that the infiltration of several cells in immune structure is one of the prominent properties of the TIME of ccRCC, like natural killer (NK) cells, myeloid-derived suppressor cells (MDSCs), T and B lymphocytes (T and B cells), and dendritic cells (DC) (accessory cells). The most abundant immune cell infiltrate type noted in conventional RCC is T cells (Wang et al., 2021; Monjaras-Avila et al., 2023). Also, Su et al. supported the idea that CD8 and CD4+ T-cells are considered to be the predominant immune infiltration within ccRCC (Su et al., 2021).

Nonetheless, an immunosuppressive milieu is another property that has been noted in the TIME of conventional RCC, which causes immune cell dysfunction via a number of processes that involve the inhibition of effector T cells and antigen-presenting cells by elevated expression levels of immunosuppressive factors (immune checkpoint molecules) consisting of cytotoxic CTLA-4, lymphocyte-activation gene 3, (LAG-3), and programmed cell death (PD-1) or programmed cell death ligand 1 (PD-L1). The tumor is able to evade the immune system's detection and destruction because these proteins may hinder T cell function (Wang et al., 2021; Monjaras-Avila et al., 2023).

2.5. Actin Cytoskeleton and It’s Regulation

The cytoskeleton, which is present in all eukaryotic cells, consists of various filamentous proteins that are responsible for the morphogenesis and contribute to the regulation of critical dynamic cellular processes. It is also acts as a structural framework, or "scaffolding," for the activation, inhibition, and localization of various cytoplasmic signaling pathways. Exclusively, the cytoskeleton is significant to the cellular immune system because multiple of its functions rely on its motility, which encompasses T-cell activation, interaction between cells, phagocytosis, migration, and secretory processes (Wickramarachchi et al., 2010). These functions are regulated by three different types of proteins, including actin filaments (also called microfilaments), intermediate filaments, and microtubules (Bai et al., 2023).

Actin filament is a key cytoskeletal protein, located primarily within the cytoplasm, which exist as two kinds: monomeric globular actin (G-actin), and when it assembles head-to-tail create filamentous actin (F-actin) by polymerization. Within a balanced state, subunits disassemble at the slow-growing (pointed or minus) end and assemble at the fast-growing (barbed or plus) end. This mechanism of actin polymerization/depolymerization is known as treadmilling (MacTaggart and Kashina, 2021; Bai et al., 2023). In turn, the polymerization and depolymerization of filamentous actin require a variety of actin-binding proteins (ABPs), which have various roles depending on their properties to promote or inhibit actin polymerization, such as initiating the polymerization process, nucleating or sequestering the monomer, filament severing, filament cross-linking, and other constituents of cells (Pollard, 2016). In addition, signaling pathways involving Rho GTPase members (Cdc42, Rho, and Rac) tightly ensure the spatiotemporal regulation of polymerization and depolymerization of actin within the cytoplasm by controlling the activity of ABPs (Bai et al., 2023). Therefore, regulation and remodeling of the actin filament are critical to numerous physiological systems in cells, like membrane traffic pathways, cell movement, and cytokinesis (Lee and Dominguez, 2010). Nevertheless, alteration or dysfunction of proteins that regulate the actin cell skeleton may induce various diseases, including cancer progression (Sossey-Alaoui,2013).

2.5.1. ARP2/3 complex

ARP2/3 complex is an ABP and an actin filament nucleation factor, which have a major function in the polymerization of filamentous actin within cells. A group of 7 subunits make up this complex: 2 actin-related proteins (Arp2 and Arp3) and 5 other protein subunits (ARPC1 to ARPC5) (Paluck et al., 2021). Significantly, the complex of ARP (2/3) manages the actin cell skeleton via interacting with a preexisting (mother) filament at a constant angle of about 78° from the mother to create a new filament branch. Commonly, these new filamentous actin are concentrated at the front-edge membrane during the nucleation process, and this results in the generation of protrusive forces that drive the elongation of cellular membranes, including lamellipodia or filopodia, which mediate cell motility (Swaney and Li, 2016; Paluck et al., 2021). This process is facilitated by numerous proteins known as nucleation-promoting factors (NPFs), including the WASP/WAVE family of proteins (Swaney and Li, 2016). Thereby, the complex of ARP (2/3) and the family of WASP/WAVE are appearing as key contributors in both the

cytoplasmic and nuclear processes. Moreover, an alteration or disturbance in the expression level of the complex of ARP (2/3) and the family of WASP/WAVE was detected in many types of cancers (Campellone et al., 2023).

2.6. WASPs and WAVEs Family

2.6.1. Discovery and family members

The family members of the WASP serve as nucleation-promoting factors that participate in the transduction of several signals from receptor molecules on the cell membrane for the complex of ARP (2/3) to facilitate the generation of branched filaments of actin within numerous significant cellular activities like vesicle traffic, nerve cell development, and migration (Kramer et al., 2022). Three decades ago, the first gene discovered among the *WASP/WAVE* family genes was the *WASP* gene itself, in which mutations in this gene were identified as a genetic disorder responsible for an inherited, X-linked, recessive disease called Wiskott-Aldrich syndrome (WAS), defined by microthrombocytopenia, eczema, and immune deficiency, clinical trait resulting from imperfection in the function of clotting (platelets) and white blood cells (lymphocyte) (Kurisu and Takenawa, 2009). Rivers and Thrasher have also noted that the *WASP* gene has a function with both nonspecific (inherent) and specific immune systems by reorganizing the actin cell skeleton and relying on cell activates like immunological synapse development, signal transduction, and cytokine (Rivers and Thrasher, 2017). The second gene of the WASP subfamily was later discovered and became known as neural WASP (*N-WASP*), which is present in large amounts in the brain. Besides the WASP and N-WASP proteins, a novel subfamily of the WASP family was found, referred to as the WASP verprolin-homologous proteins (WAVEs), which are composed of *WAVE1, WAVE2, WAVE3*, also known as *SCAR1, 2* and *3*, and *WASF1, 2* and *3* (Kurisu and Takenawa, 2009). Lately, other distinct members associated with the WASP family have been recognized, including *WASH*, *WHAMM*, *JMY*, and *WHIMP* (Kramer et al., 2022). The genes belonging to the *WASP/WAVE* family appear on distinct chromosomes, each of which has a distinct pattern of expression (Kurisu and Takenawa, 2009).

2.6.2. Characteristic and structural features

All proteins of the family WASP and WAVE are defined by the existence of three common domains at the C-terminus, called the domain of VCA, involving the domains of (1) verprolin homology, (2) cofilin homology, and (3) the acidic region. Together, they make up the VCA domain. All proteins belonging to (the family of WASP and WAVE) also share general characteristics that possess a basic domain, called the domain of prolin-rich. In addition, CRIB and GBD domains and WASP homology domain 1 (WH1) are also contained in WASP and N-WASP. In comparison, the WAVE sub-family of proteins does not contain the GBD and CRIB domains, but has the WAVE homology domain (WHD), also called the SCAR homology domain (SHD) (Figure 2.7.). Within cellular environments, the WAVE subfamily of proteins is comprised of heteropentameric complexes called the WAVE regulatory complex (WRC). These complexes include NCKAP1 (NAP1), HSPC300, ABI, WAVE1/2/3, and CYFIP1 (SRA1) (Kurisu and Takenawa, 2009; Frugtniet et al., 2015). Hence, branched F-actin is generated via the co-oprate activities of distinct WASP and WAVE proteins and the complex ARP 2/3 (Campellone et al., 2023).

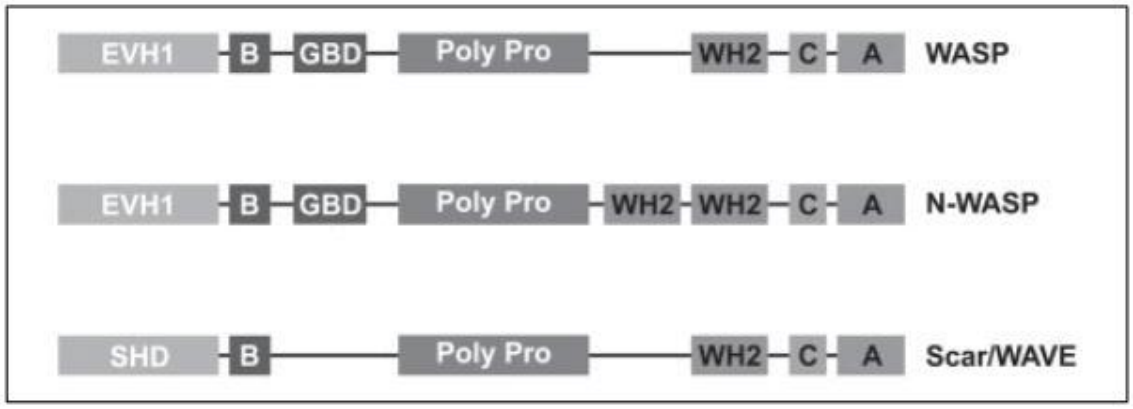

Figure 2.7. Domain structure of WASPs and SCAR/WAVEs (Kelly et al., 2006)

2.6.3. Activation and inactivation of WAVE sub-family proteins

In the resting condition, the WAVE sub-family is present within an autoinhibited closed conformational state, in which the VCA domain is at the C terminal linked to the complex made up of multi-proteins of the N terminus at the WHD. The complex of ARP (2/3) is prevented from being activated by this interaction. Receiving above signals from Rac-GTPase via bridging molecules such as SRA1 is necessary to activate WAVE proteins, which cause an open conformation, release the VCA region, and permit ARP

(2/3) and G-actin binding to it, leading to the process of assembling and polymerizing actin and creating actin branches. Within the physiological state, this mechanism is tightly regulated. But mutations or disorders in its regulation are immediately associated with development and invasion of cancer (Figure 2.8) (Loveless and Teng, 2021). Therefore, any proteins within this family are essential in physiological and pathological processes; one of the critical members of the WAVE family is WAVE1 (Chen et al., 2014).

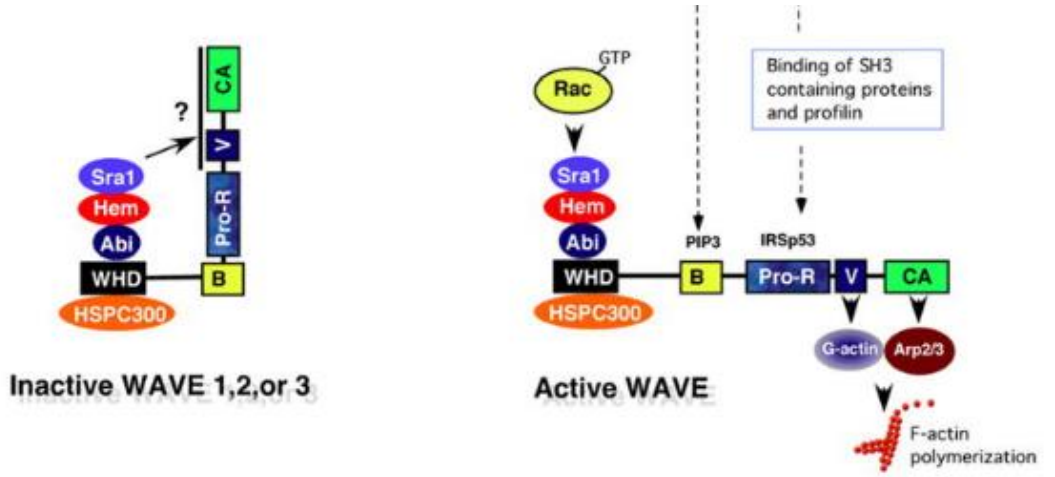

Figure 2.8. Activation and inactivation of WAVE sub-family proteins(Park et.al,2010)

2.7. *WAVE1* Gene and It's Protein

For humans, the WASP family member 1 (*WASF1*) gene, which is an official name also referred to as *WAVE1*, *SCAR1*, that codes for WASP-family verprolin homologous protein 1, more often known as WAVE1. This gene has eleven exons and is located on chromosome 6q21 (Figure 2.9). The *WAVE1* (also known as *WASF1*) gene length is 79860 base pairs, and this gene's mRNA includes 2886 base pairs (NM_003931) (Figure 2.10). It produces a protein consisting of 559 amino acids via the translation process (Lane et al., 2014). Functionally, WAVE1 plays a key role downstream of Rho/Rac GTPases via the participation of its WAVE complex in controlling the actin cell skeleton by binding to ARP (2/3), which is necessary for the synthesis of membrane protrusions based on actin, and that is important for cellular motility (Rafi and Butler, 2020). Sweeney and colleagues supported the idea that WAVE1 plays two distinct roles in network generation: suppressing the elongation of filamentous actin independently of the complex of ARP (2/3) and enhancing the nucleation of filamentous actin through the complex of ARP (2/3) (Sweeney et al., 2015). The study of Tang and colleagues also displayed that WAVE1

functions as a suppressor of elongation of the actin filament (F-actin) instead of an activator (Tang et al., 2020). WAVE1 is expressed highly within the cerebrum (brain) and testis; however, it is expressed moderately within other various tissues, such as heart (Figure 2.11) (Paolillo et al., 2022).

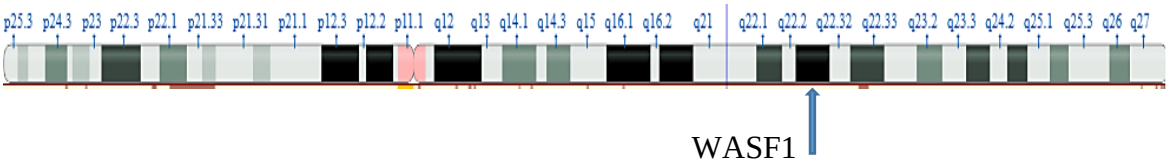

Figure 2.9. The location of *WAVE1* (also known as *WASF1*) gene on the chromosome 6

(https://www.ncbi.nlm.nih.gov/gene/8936)

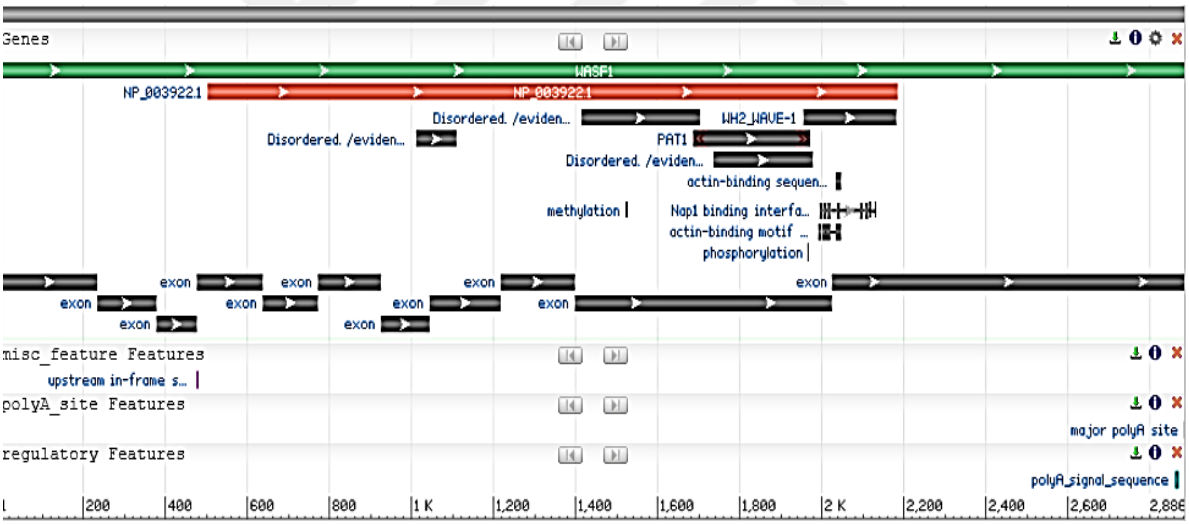

Figure 2.10. The diagram of mRNA of *WAVE1* gene

(https://www.ncbi.nlm.nih.gov/gene/8936)

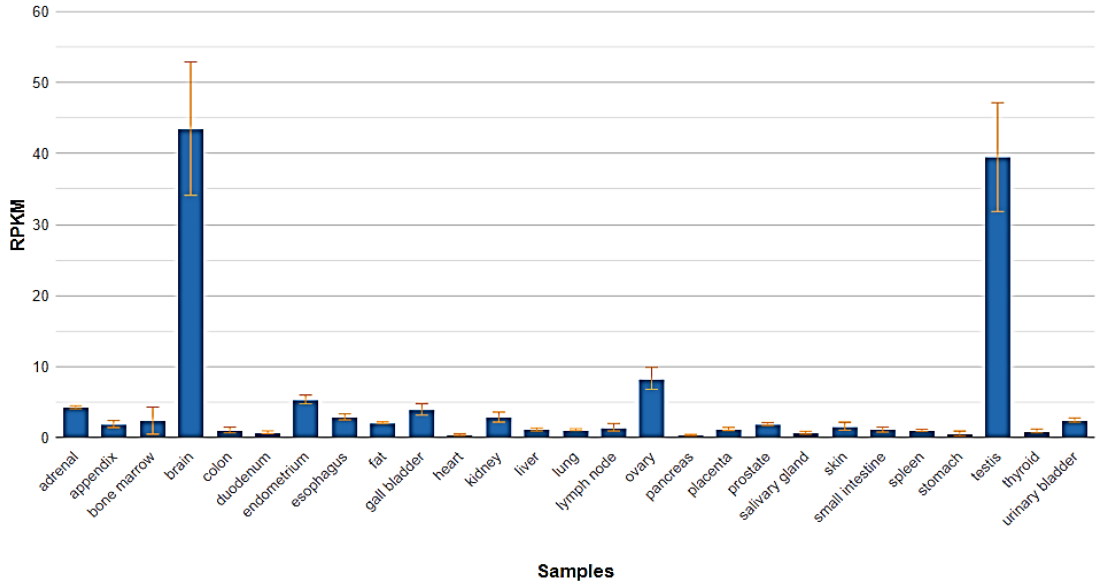

Figure 2.11. Diagram of *WAVE1* gene expression in body

(https://www.ncbi.nlm.nih.gov/gene/8936)

In addition, *WAVE1* has an important effect on central nervous system (CNS) functioning and development, and *WAVE1* has a crucial function in the morphology of dendritic spines, growth cones, mitochondrial dynamics, the growth of neuronal processes, and synaptic plasticity. It follows that, in neurons, *WAVE1* localizes to the mitochondria, dendritic spine (or spines), and dendritic growth cone (Ceglia et al., 2010). Moreover, Sloane and Vartanian indicated a function for WAVE1 in oligodendrocyte development and myelination. They also reported that the absence of WAVE1 leads to deterioration oligodendrocyte development (Sloane and Vartanian, 2007). Ceglia et al. have declared that WAVE1-deficient mice exhibit CNS dysfunction like motor sensory delay, disorders of the nervous system, behavioral disorders like weakness of the limbs, and decreased anxiety. They also demonstrated that WAVE1 knockout homozygote mice drop off viability and lose body mass. (Ceglia et al., 2010). In another investigation by Dahl et al. of WAVE1-deficient murine, they found that the lack of WAVE1 throughout growth caused neonatal death (Dahl et al., 2003).

Ito and colleagues, in 2018, found that de novo truncating mutations within the gene *WAVE1* lead to neurodevelopmental disorders (NDDs), an autosomal dominant disease with seizures and speech impairment (Ito et al., 2018). Also Shimojima Yamamoto and colleagues, in 2021, substantiated that de novo truncating mutations cause

neurodevelopmental disease characterized by dysmorphic facial features, delays in skill development, and epilepsy (Shimojima Yamamoto et al., 2021).

Furthermore, *WAVE1* is significant in the invasiveness, motility, as well as metastatic features in cancerous cells, as reported in several investigations (Zhang et al., 2012). An investigation performed by Suetsugu and associates demonstrated that in dorsal ruffles, MMP-2 is an extracellular matrix (ECM)-degraded enzyme protein co-located with WAVE1. Therefore, matrix metalloproteinase (MMP) activity is needed for migrations that rely on WAVE1. (Suetsugu et al., 2003). In this respect, He and colleagues indicated that the simultaneous location of MMP-2 and WAVE1 in leukemia cells means they worked together in their roles, which implied that WAVE1 may be related to the invasion and motility of leukemia cells via managing the MMP-2 expression level (He et al., 2009). Numerous investigations have been conducted and demonstrated that WAVE1 deficiency causes reduced development with invasiveness within epithelial ovarian cancer and prostate cancer, as well as stimulating leukemia cell apoptosis without depending on drug-induced processes (Lu et al., 2023). An investigation conducted by Kang and colleagues also confirmed that in leukemia cells there is a high expression of WAVE1, which also negatively serves in apoptosis regulation (Kang et al., 2010). The finding by Lu and colleagues also clarified the reduced invading and proliferation of ECO in deficient WAVE1 through the p38/MAPK and PI3K/AKT pathways, indicating that increased WAVE1 is linked to an unfavorable and poor prognosis for epithelial ovarian cancer (Lu et al., 2023). Another study about the roles of WAVE activity in metastatic and invasive cancer showed that the overexpression of WAVE1 and WAVE2 levels, as well as Rac activity, were associated with murine melanoma invasiveness (Frugtniet et al., 2015). Accordingly, the *WAVE1* gene was revealed to have a role in invasion and mobility in some types of cancer by previous studies (Zhang et al., 2013). In the context of renal cancer, there has not been related research regarding the impact of the role of the *WAVE1* gene on renal cancer.

Ali et al. affirmed that renal cancer is an extremely complex cancer composed of various types that vary in disease progression, treatment response, and genes (Ali et al., 2023). Under normal conditions, the kidneys are well known to maintain blood sugar homeostasis, synthesize and secrete hormones, control blood pressure, produce urine, etc. (Ray and Reddy, 2023). The nephron is the renal functional unit, and the nearest part of

the nephron is the glomerulus (Gounden et al., 2018). The structural preservation of the renal glomerulus depends largely on the actin cytoskeleton's integrity, and key controllers of the dynamics of the actin cytoskeleton are RhoGTPases. RhoGTPases activate the WASP/WAVE family, which permits activation of the ARP (2/3) complex (Steichen et al., 2022). When the activation of the complex of ARP (2/3) is triggered, it generates membrane protrusions based on actin, which is important for cellular motility and invasion of cancerous cells. Hence, it appeared that the control of RhoGTPases has a role in normal kidney cell function through the WASP/WAVE family (Steichen et al., 2022). Therefore, deformity in the WASP/WAVE proteins causes cancer disease (Kurisu and Takenawa, 2010). In this regard, in the present study, to ascertain the WAVE1 activity in renal cancer, we sought to analyze the role of the *WAVE1* gene in renal cancer development.

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Formation of working groups

The present study was conducted with 22 samples consisting of 11 renal cancer tissues and 11 healthy renal tissues that was taken in the same patient, which were recruited from the outpatient clinic of Tokat Gaziosmanpaşa University, Faculty of Medicine, Department of Urology. With written and verbal awareness consent obtained from participants that consisted of 6 females and 5 males, intraoperatively obtained kidney tumor tissue was utilized as the patient group. And utilized healthy renal tissue from the same renals that was taken from the same patient were used as a control group. Permission necessary for the present study was acquired by Clinical Research Ethics Committee from Tokat Gaziosmanpasa University, Faculty of Medicine with project number 22-KAEK-045. The whole study was conducted in the Laboratory of Medical Biology and Genetics.

3.1.2. Collection and storage of tissues

After the collection of the tissue samples, which were stored by freezing them at -80 degrees Celsius for later study, were applied to RNA isolation, process synthesis of cDNA, and determining expression levels of the *WAVE1* gene by using quantitative real time Polymerase Chain Reaction (qRT-PCR).

3.1.3. Devices and tools used in the study

1. Real-time PCR device (Applied Biosystems, UK)
2. PCR device (MiniAmo Plus Thermal Cycle)
3. Centrifuge (Hettich, D'78532, Germany)
4. Refrigerated Centrifuge (Hettich. Germany).
5. Vortex (Velp, Scientifica, F20220176, Italy)
6. Qubit 3.0 Fluormeter (Invitrogen, Life Technology, Australia)
7. Plate (Applied Biosystems, 4346907, UK)
8. Microcentrifuge (Mikro120, Hettich Zentrifugen D- 78 532n).
9. Micropipette Set (Gilson, Thermo Scientific, Finnpipette).

10. Refrigerator (Arcelik, 570465, Turkey)
11. -80°C Deep Freezer (Nuaire,Nu9483e, USA)
12. Homogenizer (next advance storme 24, BBY24M-CF, ABD)

3.1.4. Chemical substances, kits and kit contents used in the study

-RNA Isolation Kit (Thermo Scientific GeneJet RNA Purification kit, lithuania, LOT: 01125929)

- RNA Purification Columns with Collection Tubes (2 ml, 1.5 ml)
- Nuclease-free water
- Proteinase K
- Lysis Buffer
- Washing Buffer 1
- Washing Buffer 2

- cDNA Reverse Transcription Kit for cDNA synthesis (Applied biosystems brand High Capacity, lithuania, LOT: 01152470)

- Reverse Transcriptase Enzyme
- 10X Reaction Buffer (RT Buffer)
- Mix dNTP (100 mM)
- 10 X RT Random primers
- Nuclease-free water

Qubit dsDNA Assay Kit Qubit ™ 1X dsDNA HS Assay Kit (Invitrogen Catalog No: Q33230, USA)

• Qubit ™ 1X dsDNA HS Working Solution (Component A)

• Qubit ™ 1X dsDNA HS Standard #1 (Component B)

• Qubit ™ 1X dsDNA HS Standard #2 (Component C)

- Master mix used in Real-Time PCR 2x Master Mix Green (High ROX™) kit (Enzo, Cat. No: ENZ – NUC104- 1000, Lot No: 05272212)

- 2x Master Mix
- Primer Forward
- Primer Reverse
- Template cDNA
- PCR-grade H2O

-Other materials:

- TE Buffer
- Caps with Plan Tubes, 10-ml
- Tips (white, Yellow, Blue)
- Ethanol (Sigma-Aldrich Catalog No: E7023, USA)
- Distilled water
- Eppendorf tube
- Lancet
- Disposable gloves
- Slide

3.2. Method

Tissue samples obtained during the operation were stored at −80°C. The stored tissue pieces were used for RNA extraction and the determination of gene expression.

3.2.1. RNA isolation from tissues

In RNA extraction, Thermo Scientific GeneJet RNA Purification Kit lithuania, Lot: 01125929 was used and the isolation process was performed according to the kit protocol as follows:

1) Tissue specimens (30 mg) were thoroughly ground with a scalpel and homogenized.
2) Immediately, the fragmented tissues were inserted into eppendorf tubes (1.5 ml) using a pipette.
3) Then, for each sample tube, 300 µl of lysis buffer with beta-mercaptoethanol (also called 2-mercaptoethanol or BME) was added and mixed well for 10 seconds using a vortex.
4) Following that, 600 µL of Proteinase K Solution (10 µL of Proteinase K dissolved with 590 µl of TE buffer.) were increased for each tube and scrambled via vortex during 15–30 seconds.
5) Vortexed samples were incubated at 25°C for 10 minutes.
6) Centrifugation was implemented at 12000 x g for 5 minutes.
7) The centrifuged supernatants were segregated and transferred into another RNAase-free tube.
8) 96% ethanol in the amount 450 µL was increased and carefully scrambled with pipetting.
9) Amounts of up to 700 µl of lysate were added to collection tubes.
10) For 1 minute at 12000 x g the ingredient mixture was centrifuged.
11) After that, each tube was disposed of, and the filter was taken out and attached in a new collection tube (2 ml).
12) This step was performed continuously until the whole lysate was transmitted into the filter (column) and centrifuged, then again each tube was disposed of, and the filter was taken out and inserted in a new collection tube (2 ml).
13) Wash Buffer I (added with ethanol) was increased in the amount of 700 µL and centrifuged in 1 minute at 12000 x g.
14) After that, each tube was disposed of, and the filter was taken out and attached in another collection tube (2 ml).

15) Wash Buffer II was increased in the amount of 600 µL and centrifuged in 1 minute at 12000 x g.
16) After that, each tube was disposed of, and the filter was taken out and attached in another collection tube (2 ml).
17) Following that, the filtered container and column were centrifuged for an extra minute at maximum speed.
18) Subsequently, each tube was disposed of, and the filters were transmitted into eppendorf tubes (1.5 ml), and then nuclease-free water in the amount of 100 µL was increased to tubes. After that, with a 1 minute at 12000 x g. were centrifuged.
19) Finally, the filters were disposed of, and the purified RNA was ready.

3.2.2. cDNA synthesis

The process of cDNA synthesis was started after the isolation of RNA. For cDNA synthesis, the Applied Biosystems High Capacity cDNA Reverse Transcription Kit (4368814, Lot: 01152470, Lithuania) was employed in accord with the kit protocol. In order to achieve optimal performance in the cDNA synthesis kit, we ensured that PCR inhibitor, reverse transcription inhibitor, RNase activity, and genomic DNA were not present in the RNA samples. All the components of the kit were blended together in a microtube according to the recommended quantities listed in Table 3.1. This mixture in the amount of 10 µl was increased for each of the 11 new tubes. Subsequently, we transferred 10 µl of purified RNA samples into each of them, pipetting up and down twice for mixing. Following this, the mixture has been briefly centrifuged to avoid any droplets and the contents were instantly rotated. Finally, the tubes were inserted into a Thermocycler so that 33 cycles of reverse transcription PCR would occur in accordance with the specified conditions demonstrated in Table 3.2.

Table 3.1. The components and their amounts used in cDNA synthesis

Components (20 µl)	**Volume (µl)**
10X RT Buffer	2.0 µl
25X dNTP mix	0.8 µl
10X RT Random primer	2.0 µl
Reverse Transcriptase Enzyme	1.0 µl
Nuclease Free Water	4.2 µl
RNA Sample	10.0 µl
Total Volume	**20.0 µl**

Table 3.2. PCR program used in cDNA synthesis

RT reaction	**Stage names**	**Temp (°C)**	**Duration**
Stage 1	Annealing	25	10
Stage 2	Elongation	37	120
Stage 3	Termination	85	5
Stage 4	Hold	4	∞

3.2.3. Measurement of cDNA concentrations

For measuring the concentrations of cDNA, the Qubit 3.0 fluorometer and the Qubit ™ 1X dsDNA HS Assay Kit (Invitrogen, Q33230) were used.

1. For the Qubit™ 1X dsDNA HS Assay, two standards are required. The sufficient amount of 0.5-ml test tubes for standards and samples were collected.
1. For the standards, working solution in the amount of 190 µl was increased to PCR tubes (0.5 ml). 10 µL of Standard 1 and Standard 2 were placed in distinct tubes, and the final amount was completed to 200 µL with working solution. Subsequently, each tube was thoroughly vortexed for 3 sec.
2. For sample measurement, depending on the amount available, each cDNA sample in the range of 5 µl was added to tubes, and working solution in the range of 195 µL was increased to achieve a final amount of 200 µL. Subsequently, each sample tube was thoroughly vortexed for 3 sec.
3. For 2 minutes at room temperature, all tubes have been incubated and are ready for measurements..
4. Following that, the concentrations of standard 1 and then standard 2 were read on the device.
5. The concentration of each cDNA in the patient and control tissues was determined and recorded, and each of the reaction was measured to 60 ng. Thereby, the samples whose measurements were completed were ready for the next steps of the experiment.

6. For both the *WAVE1* gene and the *ACTB* housekeeping gene, samples of various cDNA concentrations (1/10, 1/100, 1/1000, and 1/10000) were made ready, and serial dilution was conducted.

3.2.4. Determination of *WAVE1* gene expression by qRT-PCR

We employed the qRT-PCR analysis test to analyze our hypothesis; qRT-PCR is a measurable technique for measuring the amount of PCR copy templates, like cDNA and DNA, and works in two forms: probe-based qRT-PCR and the intercalator-based dye SYBR green qRT-PCR. SYBR green dye is one of the most widely utilized fluorescence dyes that attaches to dsDNA throughout qRT-PCR, creating fluorescence when linked to newly produced dsDNA (Mo et al., 2012). It implies that qRT-PCR permits the coinciding

monitoring of cDNA amplification, is a procedure based on the measurement of a fluorescent signal that rises in proportion to the quantity of DNA. Hence in qRT-PCR analysis the simplest technique for detecting newly synthesized PCR products includes the use of fluorescent dyes (such as SYBR Green) or probe sequences that bind to the DNA helix and produce signals because of the degradation process. Therefore, in this study, the analysis of the *WAVE1* gene expression levels was conducted utilizing qRT-PCR based on the SYBR Green method on the applied Biosystem Step One Plus device (Appiled Biosystems, UK). For qRT-PCR 2x Master Mix Green (High ROX™) kit (Enzo, Cat. No: ENZ – NUC104- 1000, Lot No: 05272212) was used according to the kit protocol. The MasterMix was mixed with the forward primer and reverse primer (Table 3.3). In order to analyze the gene expression precisely, the applicable reference gene should be chosen to prevent some problems. It must work with a wide range of samples and be localized across all tissues and physiological states. Thus, we used the Actin Beta (*ACTB*) gene as a reference gene for qRT-PCR. In tables 3.4 and 3.5, the components and protocol used for qRT-PCR are given.

Table 3.3. *WAVE1* and *ACTB* qRT-PCR primers

***WAVE1* F**	**5' –GAACGTGTGGACCGTTTATCT-3'**
***WAVE1* R**	**5' –GGAATAGGCAAAGTCTTGCGA-3'**
***ACTB* F**	**5'-GCATGGGTCAGAAGGATTCC-3'**
***ACTB* R**	**5'-CACGCAGCTCATTGTAGAAGG-3'**

Table 3.4. Reaction components and amounts used in (qRT-PCR)

Components (20 µl)	Volume (µl)
2X Master Mix Green High ROX	12.5 µl
Primer F (forward)	0.5 µl
Primer R (reverse)	0.5 µl
cDNA sample	3.0 µl
PCR-grade H2O	8.5 µl
Total Volume	**25.0 µl**

Table 3.5. The qRT-PCR protocol

Stages Name	Cycles	Temp (°C)	Time
Initial Denaturation	X1	95.0	15 minutes
PCR Amplification	X40	95.0	15 second
		60.0	1 minutes
Melt Curve	X1	95.0	30 second
		60.0	15 second
		95.0	15 second

3.2.4.1. Statistical analysis of qRT-PCR findings of WAVE1 mRNA gene

The qRT-PCR outputs of the *WAVE1* gene and the *ACTB* gene were quantified with the use of step one plus the software version 2.3. The levels of the *WAVE1* gene expression have been normalized with the *ACTB* gene, which is statistical analysis was carried out using the threshold $2^{-\Delta\Delta CT}$ formula data to calculate the fold change amount (Livak & Schmittgen, 2001) (Table 3.5). Data were provided as Mean± SEM, and the evaluation of the results was conducted based on a range of 0.9–1.1. Values less than 0.9 indicate reduced levels of *WAVE1* gene expression in renal cancer tissues compared with

healthy renal tissues; values greater than 1.1 indicate increased levels of *WAVE1* gene expression in renal cancer tissues in comparison to healthy renal tissues; and values in the range 0.9–1.1 show no change in expression level compared with healthy renal tissue (Schmittgen & Livak, 2008). Statistical analysis was carried out with SPSS software version 20. Analysis of *WAVE1* mRNA expressions from renal cancer and healthy renal tissues was made by Paired Sample Test. Here, the significance levels of the data were analyzed by paired T test. The statistical significance level was established at 0.05; therefore, p values less than 0.05 were regarded as significant, and p values greater than 0.05 were regarded as not significant differences.

Table 3.6. $2^{-\Delta\Delta CT}$ Method

$2^{-\Delta\Delta CT}$ method	
ΔCt Renal cancer Tissue	CT (*WAVE1* gene)- CT (*ACTB* gene)
ΔCt Healthy control tissue	CT (*WAVE1* gene)- CT (*ACTB* gene)
ΔΔCt	ΔCt (Renal cancer Tissue) - ΔCt (Healthy control Tissue)
$2^{-\Delta\Delta CT}$	Its value demonstrates its logarithmic fold change.

(Livak & Schmittgen, 2001)

4. RESULTS

For this research, 22 samples consisting of 11 renal cancer tissues and 11 healthy renal tissues from the same patients were collected from the Urology Department of Tokat Gaziosmanpasa University Hospital. The entire investigation was carried out at Tokat Gaziosmanpasa University, Faculty of Medicine, Laboratory of the Department of Medical Biology and Genetics. The research patients consisted of 5 (45.5%) males and 6 (54.5 %) females with an average age of 62 ± 11.6 years, ranging in age from 39 to 75 years. A total of 11 samples of renal cancer tissue were composed of 6 samples at stage I, 4 samples at stage III, and 1 sample at stage II. The studied patient demographic details and the renal cancer sample stages are demonstrated in Tables 4.1 and Table 4.2, and the chart of the gender distribution is demonstrated in Figure 4.1.

Table 4.1. Demographic details of renal cancer patients

Renal cancer n=11	
Age	62 ± 11.6
Gender	**6 Females** **5 Males**

Table 4.2. Details of renal cancer sample stages

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

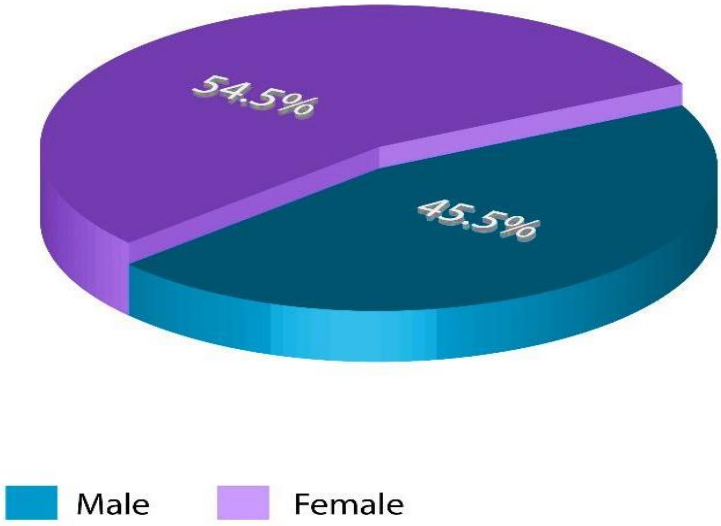

Figure 4.1. Gender distribution of renal cancer patients included in the study

4.1. Images From qRT-PCR and Evaluation of the *WAVE1* Gene.

Initially, for RNA isolation from renal cancer and healthy renal tissues, the Thermo Scientific GeneJet RNA Purification Kit was used. The process of cDNA synthesis was started after the isolation of RNA utilizing the Applied Biosystems High Capacity cDNA Reverse Transcription Kit. After that, the evaluation of the levels of the *WAVE1* gene expression within renal cancer and non-cancerous tissues was determined using SYBR Green-based qRT-PCR method (Applied Biosystems StepOnePlus device). *ACTB* was selected as the housekeeping gene (a reference gene). The amplification plot and melt curve of *WAVE1* and *ACTB* genes were obtained by qRT-PCR results. A graph of *WAVE1* gene amplification plot was demonstrated in Figure 4.2; a graph of *ACTB* gene amplification plot was demonstrated in Figure 4.3; a graph melt curve of *WAVE1* gene was demonstrated in Figure 4.4; a graph melt curve of *ACTB* gene was demonstrated in Figure 4.5.

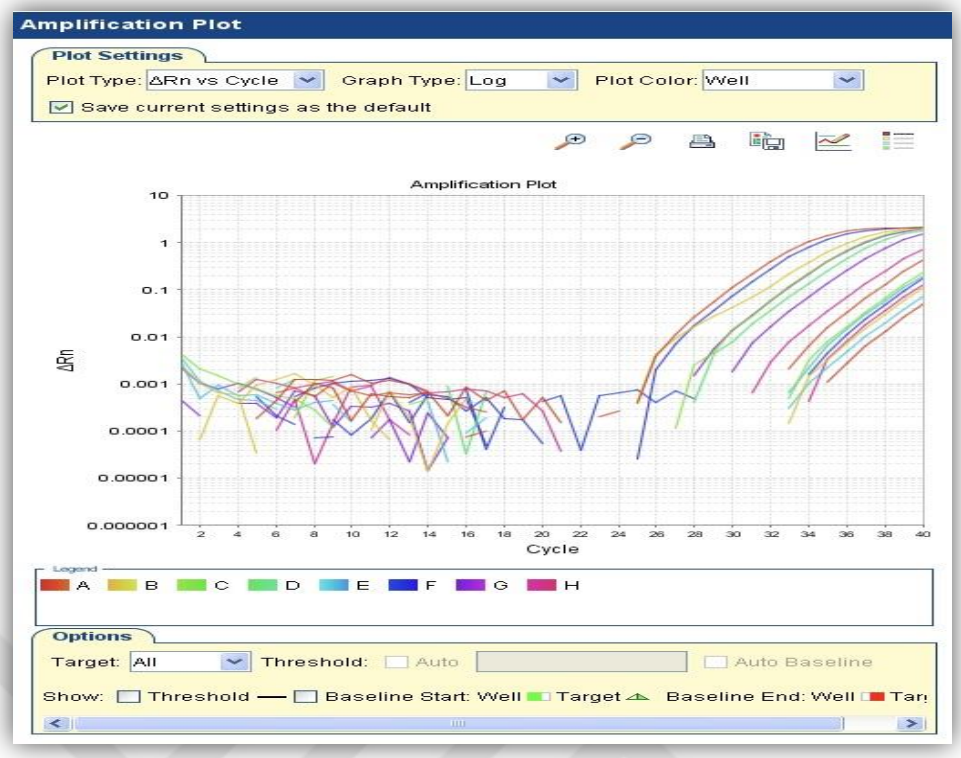

Figure 4.2. Graph of *WAVE1* gene amplification plot

Figure 4.3. Graph of *ACTB* gene amplification plot

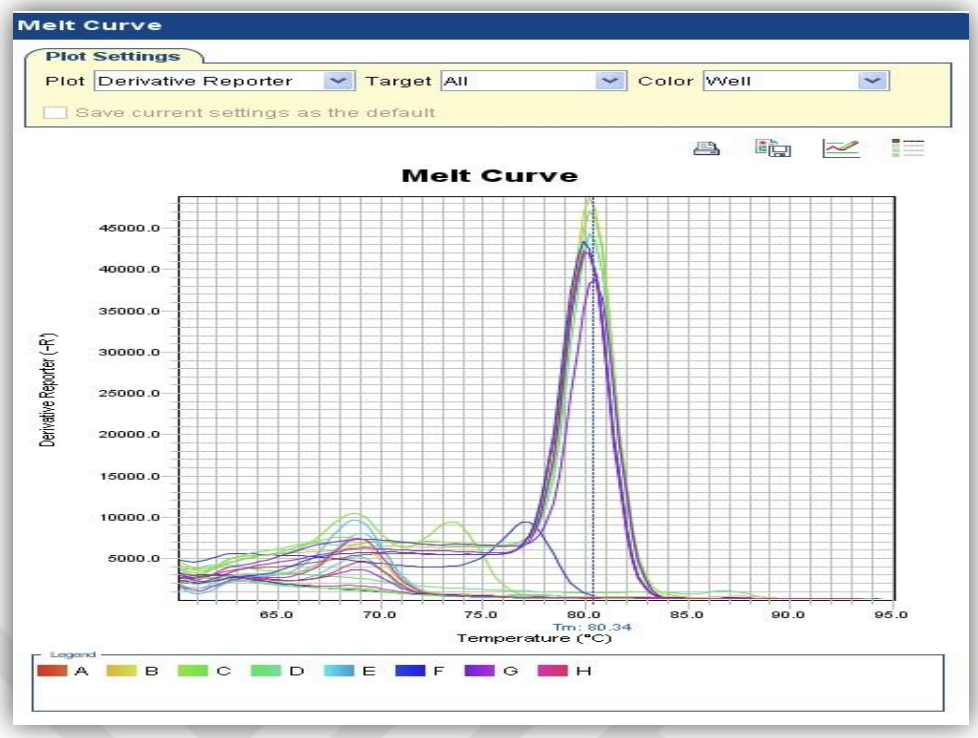

Figure 4.4. Melt curve of *WAVE1* gene

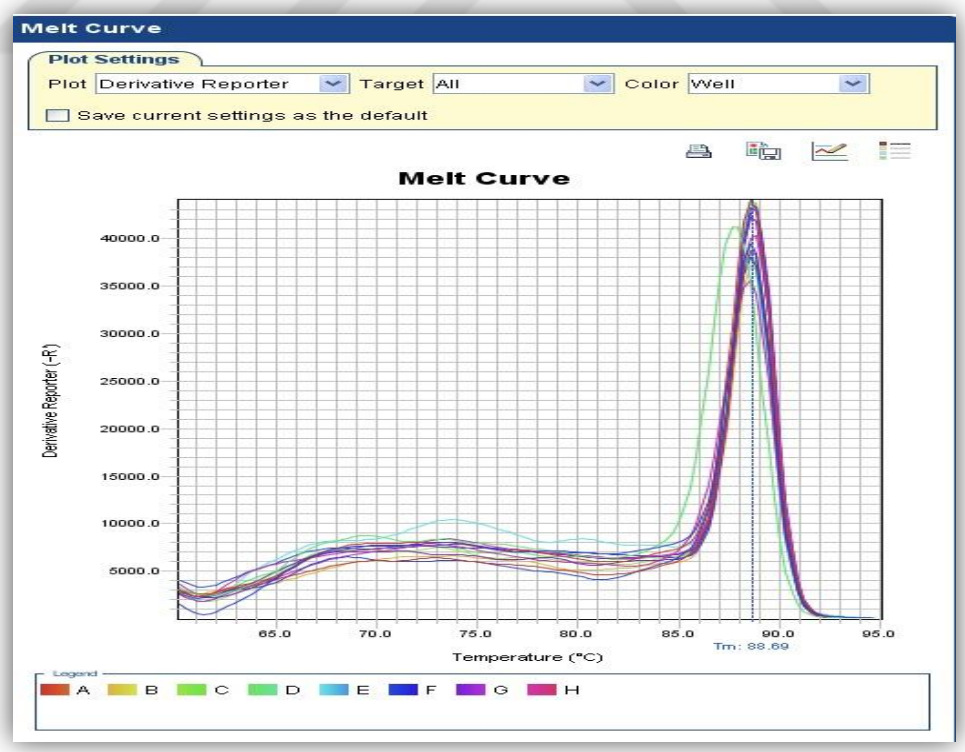

Figure 4.5. Melt curve of *ACTB* gene

Here, we found the *WAVE1* gene expression increased 5.08-fold in patients tissues compared to healthy tissues; this increase is shown in Figure 4.6. and this increase was statistically significant (p-value = 0.030). Analysis results are shown in Table 4.2. According to our results, the level of *WAVE1* gene expression was overexpressed in renal cancer tissues when this high expression was compared to the level of *WAVE1* gene expression in healthy renal tissues, and the result of our study suggests that the *WAVE1* gene may have a significant role in renal cancer progression and growth.

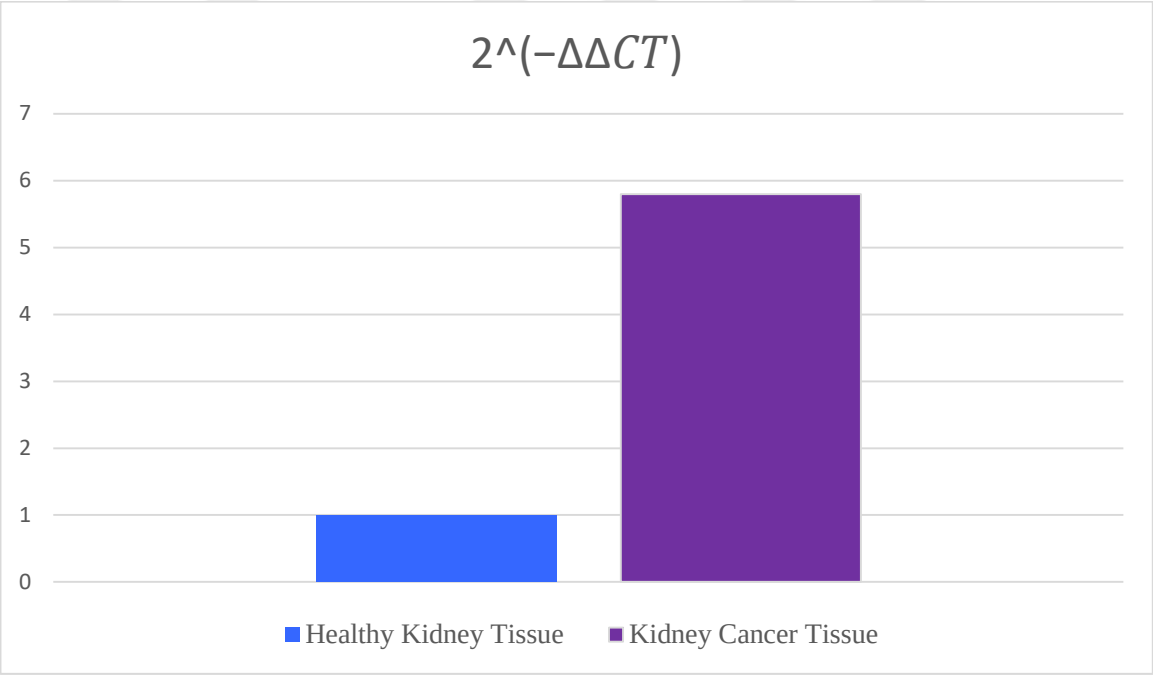

Figure 4.6. A graph of the fold change of the *WAVE1* gene in the renal cancer tissue (n=11) compared to healthy renal tissue

(The *WAVE1's* genes relative quantification values were obtained via qRT-PCR. Quantitative expression data of the *WAVE1* gene were normalized to the *ACTB* gene. The fold change of renal cancer tissues was measured to be 5.08, while healthy renal tissue was measured to be 1).

Table 4.3. Statistical analysis of the *WAVE1* gene in renal cancer and healthy renal tissues

Samples	*WAVE1* gene (SD±SEM)	*P*-value
Renal Cancer Tissue (n=11)	5,36± 1.61	*p= 0.030
Healthy Renal Tissue (n=11)	1	

(SD: standard deviation; SEM: standard error mean; *:0, 05 significant level)

5. DISCUSSION

Renal cancer was previously thought to exist as a single illness, but it is currently recognized as several distinct cancer types that develop from kidneys and vary in histological features, disease progression, treatment responses, and genetic basis factors (Linehan et al., 2003). Several of risk factors influenced renal cancer, involving lifestyle factors, medical condition factors, environmental factors, as well as genetic factors (Scelo and Larose, 2018). Renal cancer is the third prevalent urological cancer (Kim et al., 2022). As well as the rate of development and death of renal cancer ranks 15th among all cancers worldwide (Wang et al., 2022). Furthermore, the widespread type RCC is frequently without symptoms; however, after the mass enlarges, certain symptoms could arise, including back pain, hematuria, and flank lumps, which are observed in just 10% of cases. Fever, loss of weight, and leukocytosis are other typical symptoms (Padala et al., 2020). Renal cancer develops metastatic spread in nearly one-third of cases (Gong et al., 2016). And those organs most often affected by metastasis are the lungs, lymph glands (lymph nodes), liver (hepatic system), bone (osseous tissue), and adrenal glands (suprarenal glands) (D'Elia et al., 2013). The investigation of renal cancer-associated genes and the functions of molecular mechanisms are crucial because it will aid in the determination of new approaches for treatment in order to improve survival rates (Lim et al., 20007). However, until now, the molecular mechanisms responsible for the pathogenesis renal cancer are still unknown (Lin et al., 2023).

An extensively used molecular method for approving nominates gene roles is the pattern of gene expression (Wang et al., 2024). Gene expression profiling is an effective technique to create complete genome-scale information on disease situations like cancer (Narrandes and Xu, 2018). Numerous investigations have explored gene expression profiles in healthy renal tissues and compared them to renal cancer tissues. This has helped in the determination of genes that participate in these tissues and revealed comprehensive biological pathway organization within tumors (Sültmann et al., 2005). Researching the gene expression pattern connected to the spread of renal cancer is one way to find potential prognostic indicators according to the scales of expression (Apanovich et al., 2023). Studies efforts are still performed to investigate the expression of potential genes engaged in renal cancer, with the aim of estimating their effects and further investigating the possibility of treatment targeting these genes.

Structural preservation of the renal glomerulus (the nearest part of the nephron, which is a renal functional unit) depends largely on the actin cytoskeleton's integrity (Steichen et al., 2022). The actin cell skeleton is a crucial component in several aspects of cell processes, including morphogenesis, cellular proliferation, and mobility. Incomplete or abnormal expression of regulating actin cytoskeleton-related proteins leads to the invasion of cancer cells, such as the WASP/WAVE family (Sossey-Alaoui, 2013). With the realization that the family proteins WAVE and WASP essentially participate in cellular motion by regulating the polymerization of actin, it became apparent that this family could contribute to cellular cancer motion and probably invasion metastasis. As a consequence, this protein family has gained considerable attention in recent years. Until now, there has been no other research examining the association between WAVE1 or each protein in the WASP family and renal cancer. In the present study, we sought to analyze the role of the *WAVE1* gene expression level in renal cancer development.

WAVE1 shows maximum abundance in mouse brain tissue and hematological cancer cell and has been shown to be barely expressed in other tissues such as heart (Zhang et al., 2016). In a study by Paolillo et al., it has been confirmed that WAVE1 is expressed highly within the cerebrum (brain) and testis; however, it is expressed moderately within other various tissues, such as the heart (Paolillo et al., 2022). WAVE1 appears to have a role downstream of Rho/Rac GTPases via the participation of its WAVE complex in controlling the actin cell skeleton by binding to ARP2/3, which is necessary for the synthesis of membrane protrusions based on actin and that is important for cellular motility (Rafi and Butler, 2020). The protein WAVE1 is not well-researched. A study indicated that WAVE1 functions as a suppressor of elongation of F-actin instead of an activator (Tang et al., 2020). Sweeney and colleagues supported the idea that WAVE1 plays roles in suppressing the elongation of filamentous actin independently of the complex of ARP (2/3) and also plays roles in enhancing the nucleation of filamentous actin through the complex of ARP (2/3) (Sweeney et al., 2015). The study by Mughees and colleagues also confirmed that WAVE1 functions as a repressor of elongation and not a promoter of F-actin (Mughees et al., 2021).

Furthermore, *WAVE1* is significant in the invasiveness, motility, as well as metastatic futures of cancerous cells, as reported in several investigations.

Zhang and his colleagues also asserted that WAVE1 deficiency causes reduced development as well as invasiveness in cancer cells in the prostate and may even prevent metastases. *WAVE1* is overexpressed in malignant melanoma cells and controls apoptosis, as well as being implicated in multidrug resistance in leukemia cells. The study included 320 tissue specimens and explored the level expression of *WAVE1* with epithelial ovarian cancer (EOC) and its implications for prognosis. Results show that *WAVE1* expression is overexpressed in EOC (Zhang et al., 2012). The finding by Lu and colleagues also clarified the reduced invading and proliferation of ECO in deficient WAVE1 through the p38/MAPK and PI3K/AKT pathways, indicating that increased WAVE1 is linked to an unfavorable and poor prognosis for ECO (Lu et al., 2023). Another study about the roles of WAVE activity in metastatic and invasive cancer showed that the overexpression of *WAVE1* and *WAVE2* levels, as well as Rac activity, were associated with murine melanoma invasiveness (Frugtniet et al., 2015). A study found that people who developed breast cancer had an elevated level of WAVE1 (Fernando et al., 2007). In another experiment performed, which included 30 samples, 13 women and 17 men appeared to have WAVE1 extremely expressed within primary BMMCs, leukemia cell lines, as well as childhood leukemia (Zhang et al., 2016). Accordingly, some of the research confirms that WAVE1 participates within cancer cell invasion and development (Zhang et al., 2013). And also inhibiting WAVE1 activity leads to reduced aggressiveness of cells with some cancers.

This current study, conducted on the Turkish residents in the province of Tokat, investigated the contribution of WAVE1 gene expression levels in human renal cancer tissue development, which is being done for the first time, and the results of our study are compatible with the results of the former studies. The findings of our qRT-PCR analysis revealed that, when compared to control tissue samples, the *WAVE1* gene was found to be overexpressed in renal cancer tissue samples. And this overexpression of the *WAVE1* gene in tissues with renal cancer was statistically significant ($p = 0.030$). As a result, our research findings recommend that the *WAVE1* gene may have a role in renal cancer progress and growth despite the fact that further research with a larger sample is needed to confirm our results.

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