An-Najah National University

Faculty of Graduate Studies

TELMISARTAN INHIBITS MELANOMA GROWTH AND METASTASIS THROUGH LRP1 SUPPRESSION

By

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Dedication

To everyone who supports and makes way for the science seeker. To everyone who is self-made and diligent and believes that his own success, realization, and achievement are largely dependent on him and not on others. I dedicate my thesis to my parents, who have given me unending love, support, and inspiration throughout my academic career. I hope this achievement will fulfill the dream they envisioned for me.

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Declaration

I, the undersigned, declare that I submitted the thesis entitled:

TELMISARTAN INHIBITS MELANOMA GROWTH AND METASTASIS THROUGH LRP1 SUPPRESSION

I declare that the work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

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Signature: 

Date: **11/3/2024**

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TELMISARTAN INHIBITS MELANOMA GROWTH AND METASTASIS THROUGH LRP1 SUPPRESSION

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Abstract

Melanoma is the most common type of skin cancer worldwide, which hasa poor prognosis becauseof its high metastatic rate and aggressive nature. Wild-type p53, which has been activated in response to cellular stressors such as oncogene activity, is commonly expressed in melanoma. It has been discovered that TP53 regulates the development of tumors mediated by the multifunctional scavenger protein LRP1 (CD91), which has been connected to the advancement of melanoma.Telmisartan, an antihypertensive medication, possesses anticancer properties that effectively treat melanoma. Moreover, Mir 107 is a crucial tumor suppressor factor that can inhibit the LRP1 oncogene.

Thisstudy aims to determine the effect of angiotensin receptor blocker telmisartan on melanoma growth and metastasis to evaluate it as a potential therapy option for patients with metastatic melanoma who wish to achieve a high survival rate.

An experimental study was carried out at the An-Najah Laboratory for Cancer and Stem Cell Research in Nablus. The B16F10 melanoma cell lineswere seeded in 12 well plates and treated with indicated concentrations of telmisartan to assess cell viability and migration.This study was conducted both in vitro and in vivo using C57BL6 mice to determine telmisartan's impact on tumor growth. To study the effect of LRP1 on melanoma cell proliferation, we utilized lentivirus to overexpress LRP1 and siRNA to knock down LRP1 in B16F10 cells. We also used lentivirus to express tumor suppressor miR107. The expressions of LRP1 (OE) LRP1(KD), and miR107 were verified by qPCR.

The study showed that telmisartan inhibits the proliferation and migration of B16F10 cells both in vitro and in vivo.Additionally, the research revealed thattelmisartan restores P53 by phosphorylation, which upregulates the expression of tumor suppressor miR107 and then leadsto downregulating LRP1 expression, inhibiting melanoma growth and metastasis.

There is currently no recognized cure for melanoma. Because telmisartan has anti-proliferative and anti-metastatic properties on melanoma, our work may provide information for future clinical trials repurposing it as an anti-cancer medication.

Keywords: Downregulation; Melanoma; Metastasis; MiR107; LRP1; Telmisartan; TP53; Upregulation.

Chapter One
Introduction

1.1 Background

The skin is the body's largest organ, accounting for about 16 % of adults' total weight [1]. Additionally, the skin is a multi-purpose barrier that regulates body temperature, produces vitamin D and removes waste by sweating. In addition, it safeguards internal organs from disease, chemical harm, dehydration, and ultraviolet (UV) radiation [2].

Skin cancer is one of the most common cancer types worldwide, especially among Caucasians.However, the most serious type of skin cancer is called cutaneous malignant melanoma. [3] This is due to its significant metastatic potential and therapeutic resistance [4]. Consequently, it has been associated with low patient survival rates.

Approximately 3 per 100,000 people are diagnosed with cutaneous melanoma worldwide[5]. By 2022, around 0.46 % of cases of melanoma were reported for the West Bank and Gaza Strips (Occupied Palestinian Territories) [6].The United States is expected to have 97,610 new instances of melanoma and 7990 fatalities from the disease in 2023[7]. Melanoma is the fifth most common type among men's cancers and the sixth most common type in women. Therefore,early detection of melanoma significantly lowers morbidity and mortality [8].

Melanoma is caused by an uncontrolled development of melanocytes, cells originating from the neural crest found in the deep layer of the epidermis, in between the basal cells. Throughout fetal development, they move to the epidermis and hair follicles [9]. The complex polymer melanin, produced by melanocytes, gives skin its color and acts as a scavenger for reactive oxygen species(ROS) [10]. This pigment protects the body from the sun's rays,but when UV radiation causessunburn, it will damage the DNA of skin cells and lead to the melanocytes' malignant transformation, eventually leading to cancer [11].

The most significant histological prognostic indicator for melanoma is tumor thickness, which is determined by the Breslow index.It is a useful measurement for determining the extent of melanoma invasion in the body since it calculates the vertical distance expressed in millimeters between the deepest point of tumor involvement and the top of the granular

layer or the base of superficial ulceration. Thus, a higher likelihood of metastasis and a worse prognosis can be predicted by increased tumor thickness [12].

1.2 Tumor microenvironment

The unique microenvironments that have molded the fate and behavior of cutaneous melanoma throughout its development explain why melanoma is becoming more common than any other type of cancer worldwide [13].

Targeting the tumor microenvironment has become important in treating malignancies due to its unique properties. It includes elevated ATP levels, overexpressed enzymes, a higher degree of hypoxia, a higher concentration of ROS, and an acidic environment [14].

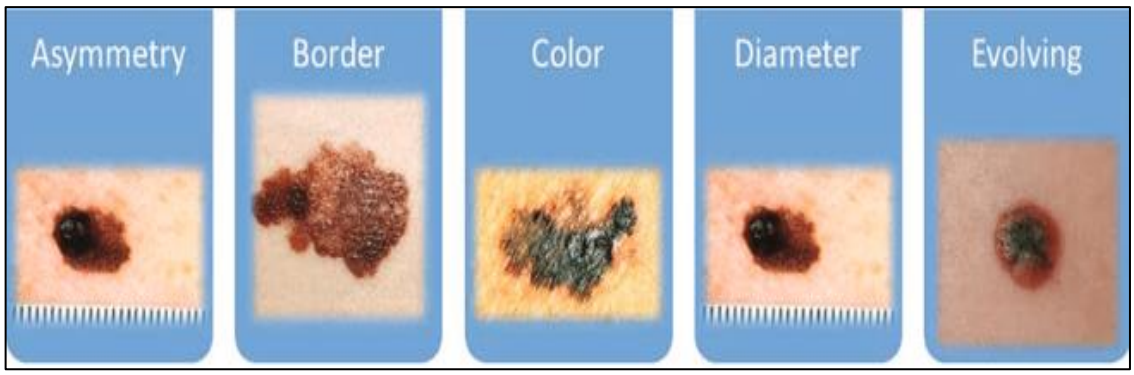
Cancer-associated fibroblasts (CAFs) are significant type of tumor-associated stromal cells because they promote extracellular matrix remodeling by secreting growth factors and chemokines.Transforming growth factor beta (TGF-β), platlet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF) are the most common CAF-released factors, which aid instimulating the growth of tumors, progression,angiogenesis, and metastasis [15]. Therefore, a poorer prognosis and a higher risk of metastasis are principally associated with a higher CAF abundance in the tumor stroma [16].

1.3 The ABCDE rule of melanoma

The ABCDE acronym for melanoma was created to make it easier for medical professionals to recognize characteristics in a skin lesion that may indicate an in situ melanoma.

Scheme 1

The ABCDE rule systematizes warning signs for malignant melanoma



Sources: Thomas, L., et al., . (1998).

Astands for Asymmetry: Normal nevi are normally symmetrical, but in the case of melanoma, the two halves will not line up when you draw a line through the middle of the lesion.

B is for border irregularity: The borders of spots are generally smooth and equal, but those that have an uneven border and scalloped or jagged edges may be signs of cancer [18].

Cis for Colorvariability: Typically, 96% of normal patches are one shade of brown, so any nevus that exhibits more than one color should be taken seriously. Skin affected by melanoma may exhibit different shades of brown or even black. Additionally, as it progresses, it can begin to form blue, white, or red regions [19].

D means for diameter: if a lesion is wider than a pencil eraser (6 millimeters), it should be taken seriously.

E stands for evolving: this name recognizes the dynamic character of this type of skin cancer. A lesion that changes in size, appearance, and texture and produces new symptoms, including pain, fluid, itching, redness, or bleeding, is said to be evolving [20, 21].

1.4 Risk factors of melanoma

The interaction of genetic, environmental, and host variables results in melanoma. UV exposure is the greatest environmental risk factor for melanoma, whereas the predominant host risk factor is an increase in nevi [22]. Additional host variables include having more freckles, fair skin, light-colored hair and eyes, lifestyle, gender, ethnicity, weakened immune system, and a family history of melanoma [18].

1.4.1 Sun exposure

A variety of risk factors contribute to melanomas, but exposure to ultraviolet (UV) light—which can occur via indoor tanning beds or the sun—is the primary risk factor due to the genotoxic effects of UV light [23].

Throughout previous decades, skin cancer incidence has progressively increased, especially in adolescent (15–20) age groups. Sun exposure is the main risk factor associated with melanoma development, even thoughmany other factors have contributed to this trend [24].

There are 3 types of UV radiation: UV-A, UV-B, and UV-C. UV-A and UV-B comprise 90% and 10% of the components of sunlight. The atmosphere absorbs the bulk of UV-C rays[25]. Due to its larger wavelength, UV-A is able to enter the deep layer (dermis) and produce free radicals. As opposed to this, Thiamine dimers are created when UV-B, which has a shorter wavelength, may pass through the epidermis and reach the stratum basal [26].

The CCTT and CT mutations are caused by the cyclobutane pyrimidine dimers that are formed when DNA's aromatic heterocyclic bases absorb a lot of UV-A and UV-B radiation [27]. UV-induced DNA damage has the potential to accelerate cancer by generating mutations throughout the genome and causing P53 activity to be lost, even though the tumor suppressor P53 is susceptible to pyrimidine mutagenesis. It is also important in controlling apoptosis if significant damage happens [28].

1.4.2 Indoor tanning increases melanoma risk

Indoor tanning beds emit concentrated UV radiation that is potentially more harmful than the sun's natural rays [29]. Almost one-third of Caucasian women between the ages of 16 and 25 are anticipated to use indoor tanning systems annually in the USA due to the growing popularity of tanning beds [30].

Indoor tanning beds, commonly seen in spas, gyms, and tanning salons, emit UV-A and UV-B rays to give users a cosmetic tan. The bulbs in these sun beds release a lot of light because they are only utilized for a short time, about 10 to 15 times more UVR than the sun [31].

Because the length and frequency of tanning bed used are linked to the risk of melanoma, those who tan when they are young are more likely to develop the disease, in part because their skin is still developing and when UV waves interfere with the DNA of melanocytes, the cells simply keep replicating at a rapid rate instead of dying [32]. The entire UVR spectrum and UV-emitting tanning devices are classified by the International Agency for Research on Cancer (IARC) as the first potent --group carcinogens, along with cigarette use and asbestos [33].

1.4.3 Having melanocytic nevi (mole)

The number of melanocytic nevi, which are benign aggregates of melanocytes or nevus cells that can be inherited or acquired, is one of the most important host risk factors. Approximately 25% of cases of melanoma coexist with a pre-existing nevus [34].

The dysplastic nevus typically measures at least 5 mm in diameter and has a flat component, varied coloring, rough, scaly surface, uneven asymmetric edges, and an ambiguous border [35].

Atypical nevi are not the only ones linked to a higher chance of developing melanoma. The risk is enhanced even by only one nevus having atypical features. Previous studies show that five atypical nevi raise the risk of developing melanoma by a factor of six [36].

1.4.4 Immunosuppression

Human Immune systems play a key role in cancer prevention, so People with weaker immune systems are more prone to acquire melanoma. For instance, People receiving organ transplants are commonly given immunosuppressive medications to help prevent the recipient from rejecting the new organ. Human immunodeficiency virus (HIV), which causes AIDS, commonly compromises an individual's immune system, increasing their risk of getting melanoma [37].

1.4.5 Family history

The probability of getting melanoma is increased if it runs in one or more first-degree families. Moreover,Genetic gene mutations that run in the family, a propensity for pale skin, or a history of regular sun exposure in the family could all contribute to the elevated risk [38].

Furthermore, melanoma risk is higher in people with family cancer syndromes, including familial retinoblastoma, Li-Fraumeni cancer syndrome, and Lynch syndrome type II [39].

1.4.6 Other risk factors

The American Cancer Society reports that the melanoma rate among White persons is more than 20 times greater than those among African Americans. For white people, the lifetime risk of acquiring melanoma is roughly 2.6%, compared to 0.1% (1 in 1,000) for dark color [40]**.**

Phaeomelanin isproduced in larger quantities in people with fair skin and red hair, and eumelanin, produced in greater quantities in those with a darker complexion, provides different levels of UV protection.

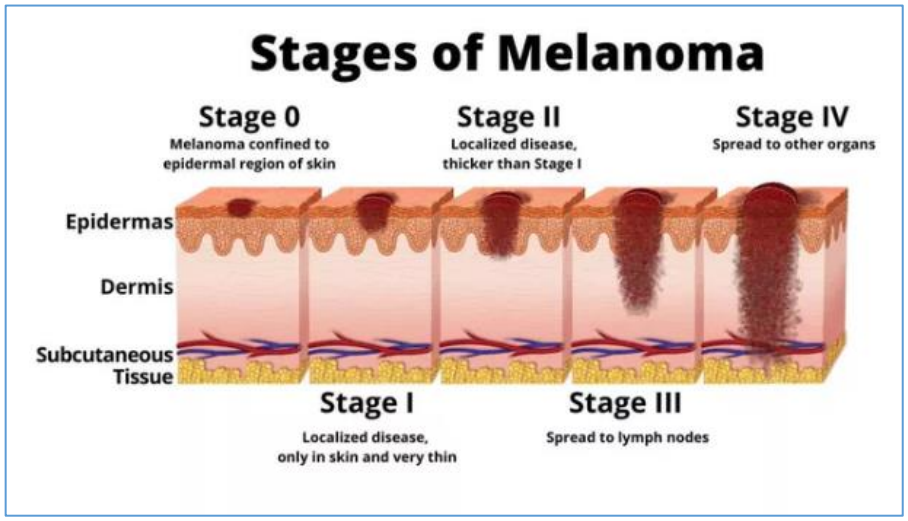
Those with dark complexions experience less harm from UV exposure due to their darker skin tone, which provides superior UV protection [41]. Nonetheless,the mean age for the two genders at melanoma diagnosis for non-Whites was significantly younger than that of Whites [42]. In general, women's tumors appear to arise more frequently on the lower extremities. At the same time, men seem to acquire tumors mostly on the trunk, particularly on the back [43].

1.5 Stages of melanoma

The epidermis's melanocytes, which produce the majority of cutaneous melanomas, normally go through two stages in their development. The name "radial development phase" (RGP) comes from the fact that a pigmented plaque that dramatically widens throughout the radii of an incomplete circle in the skin's horizontal axis can be used to identify a lesion in its early stages. The tumor then initiates the vertical growth phase and may either infiltrate the dermis or elevate the epidermis to generate a nodule along the vertical axis. RGP melanomas seem to have a better prognosis than VGP lesions, which are more likely to spread [44].

Scheme 2

This figure shows the differences between the five stages of melanoma which based on the progression of the tumor at the original site and the amount the melanoma has spread throughout the body



Sources: Cancer, A.J.C.o. (2017).

1.5.1 Stage 0 (Melanoma in situ"Tis (non-invasive melanoma)

Refers to cancer cells exclusively present in the epidermis, and there is no indication that the cancer has metastasized (M0) or spread to the lymph nodes (N0)TisN0M0 [45].

Surgery is the usual treatment for Stage 0 melanoma. According to National Comprehensive Cancer Network (NCCN) recommendations, the surgical margin should be between 0.5 and 1.0 cm.

1.5.2 Stage I (localized tumor)

Subdivided into IA and IB stages, exhibits no evidence of metastases or lymph node dissemination.

Stage IA (T1a) tumors are non-ulcerated lesions with a Breslow thicknessof less than 0.8 mm. In contrast, tumors in stage IB (T1b) might have a diameter of less than 0.8 mm with ulcers or a diameter of between 0.8 mm and 1.0 mm with or without ulcers. Tumors can also have a more than 1.0 mm diameter or up to 2.0 mm without ulceration [46].

Wide local excision surgery is the conventional treatment for Stage I melanoma. The goal of the surgery is to eliminate any malignancy that the biopsy did not detect. Based on the likelihood that the tumor will spread, your doctor might also perform a sentinel lymph node biopsy [47].

1.5.3 Stage II Melanoma (Localized tumor)

Stage II melanoma is thicker than stage I due to its deeper dermis and epidermis penetration. Metastases or lymph node spread are not present, and it is divided into three phases based on the two fundamental characteristics of ulceration and tumor thickness.

- In Stage IIA, themelanoma tumor is either 1.01 to 2.0 mm thick and ulcerated or 2.01 to 4.0 mm thick without ulcers.On the other hand, in Stage IIB, the tumor's Breslow depth is either more than 4 millimeters without ulceration or 2.1 to 4 millimeters thick with ulceration. The tumor exhibits ulceration and has a thickness greater than 4.0 mm in Stage IIC [48].

- For stage IIB melanoma, the possible treatment options include surgery, immunotherapy, and sentinel lymph node biopsy. Keytruda is classified as an immune checkpoint inhibitor because it blocks the pathway that connects cancer cell proteins

PD-L1 to programmed death-1 (PD-1)." Consequently, this immunotherapeutic medication aids the immune system in recognizing and getting rid of tumor cells once more. The use of this drug has been authorized for patients with Stage IIB and IIC melanoma after complete tumor excision [49].

1.5.4 Stage III Melanoma (regional spread)

In Stage III, cancer cells have not progressed to distant organs or other parts of the body; instead, they have moved to lymph nodes close to the melanoma's primary tumor or an area between the tumor and the surrounding lymph nodes [48].

This area is further divided by satellite metastases or in-transit metastases. Cancer cells are called satellite tumors when they move less than 2 centimeters from their original location. Meanwhile,they are called transit metastases when they spread more than 2 centimeters but before the closest lymph node.

Depending on the thickness of the tumor, the size, and number of lymph nodes affected by melanoma. Stage III, the presence of satellite or in-transit lesions and whether they appear ulcerated under a microscope, is divided into four subgroups: A, B, C, or D [45].

Neoadjuvant therapy is the first line of treatment for stage III melanoma. Following this, the main treatment surgery may involve sentinel lymph node biopsy, broad excision surgery to remove the primary tumor, and possibly full lymph node dissection. After that, for patients with a high risk of recurrence, adjuvant therapy—which includes combination signal transduction inhibitors (dabrafenib plus trametinib) and checkpoint inhibitors (nivolumab and pembrolizumab)—is recommended to eradicate any remaining cancer cells. Additional treatments, like radiation therapy, may then be necessary [50]

1.5.5 Stage IV melanoma (distant metastatic melanoma)

Three main characteristics define this advanced stage: number and size of tumors, location of distant metastases, and serum LDH [46]. In metastatic melanoma, LDH was the first prognostic blood biomarker identified by (the AJCC) staging system. Higher LDH levels are associated with a greater tumor burden and a worse chance of survival in patients with advanced stage [51].

This stage signifies melanoma that has metastasized or spread to distant areas on the skin or soft tissue (M1a), distant lymph nodes, or other organs such as the lung (M1b), gastrointestinal system (M1c), liver, brain (M1d) or bone [45].

Treatment options for stage 4 melanoma include surgery, intralesional therapy, which involves injecting a drug directly into the tumor, such as talimogene laherparepvec, laser surgery using CO2 laser, radiation therapy, chemotherapy, electrochemotherapy, and immunotherapy [52].

1.5.6 Brain metastasis

A subtype of Stage IV melanoma that can result in death in as many as 54% of instances is brain metastases, which affect nearly half of patients with advanced melanoma [53].

Brain parenchyma melanoma brain metastases (MBM) typically manifest as one or more well-confined solid or mildly cystic lesions. In terms of clinical presentation, MBM appears to be intracerebral hemorrhages surrounded by a significant amount of vasogenic edema. This edema profoundly affects the neurological system and causes symptoms such as convulsions and headaches [54]. The most frequent sites of MBM are the gray-white matter junction and the frontal lobe, which are positioned near the front of each cerebral hemisphere [55].

Melanoma brain metastases (MBMs) are among the most prevalent and challenging side effects of the disease to treat. Until recently, these patients had little hope, with a median overall survival of only five months.However, systemic medication and radiation advancements seem hopeful for this difficult condition, and some patients may even recover [56].

One of the treatment options for MBM is stereotactic radiosurgery (SRS). In fact, it is a modern methodwhich uses a gamma knife to target specific brain regions and significantly lower the risk of damaging healthy brain tissue [57].

Patients with brain metastases may benefit from checkpoint inhibitor-based immunotherapies as well as chemotherapeutic drugs like Temodar and Fotemustine, which can penetrate brain tissue. Furthermore, treatment for visible brain metastases and tumor cells too small to be picked up by an MRI or CT scan can be achieved with whole-brain radiation therapy (WBRT), which can also inhibit the growth of tumors [58].

1.6 Melanoma survival rates

The stage of melanoma when they are diagnosied determines the 5-year survival rate. In fact, patients who have been diagnosed with localized disease (stages I&II) have an (80-99%) relative 5-year survival rate, whereas those with regional spread (stage III) have a rate of 70%, and those with stage IV distant metastatic melanoma have a 30% survival rate. This decline in survival rates highlights the significance of early cancer detection and therapy to stop the disease's spread [59].

1.7 Treatment of Melanoma

1.7.1 Surgical treatment

Surgery is the most optimal and successful therapeutic option for localized melanoma, with a 92% 5-year survival rate. It is the principal treatment for melanoma at all stages [60]. An efficient treatment is wide local excision (WLE), which removes any possible microsatellites surrounding the melanoma to lower the loco-regional recurrence rate, avoid distant metastasis,and ultimately increase overall survival [61].

The appropriate clinical margins depend on the thickness of the tumor. A 1 cm excision margin should be utilized for T1-T2 melanoma and a 2 cm excision margin for T3-T4 melanoma, according to guidelines published in the Journal of the American Academy of Dermatology [62].

1.7.1.1 Sentinel lymph node biopsy (SLNB)

Sentinel lymph node biopsy is the most reliable method for identifying nodal metastases [63]. The surgeon injects a radioactive substance, blue dye, or both into the tumor site. The surgeon then looks for either lymph nodes that have been dyed or contain radioactive material [64].

After that,the node will next be examined by a pathologist to see whether cancer cells are present or not. The absence of surrounding lymph nodes affected by the malignancy is indicated by a negative SLNB test, allowing for the avoidance of more involved surgery on the lymph nodes and lowering the risk of consequences, including seroma and lymphedema [65]. On the other hand, a positive SNLB suggests that the surgeon might remove more lymph nodes by making a little skin incision and extracting the node [66].

1.7.2 Chemotherapy

1.7.2.1 Systemic chemotherapy

Systemic chemotherapy is an efficient treatment that targets and kills rapidly proliferating cancer cells by entering the bloodstream and spreading throughout the body. Its main goals are to control the spread of metastatic melanoma skin cancer and slow down the tumor's growth [67].

Dacarbazine and its analog, temozolomide, are alkylating agents that cause damage to DNA byincorporating alkyl groups into guanine bases, leading to cell death by apoptosis [68].One of the best chemotherapy drugs for metastatic melanoma is dacarbazine [69], which is injected intravenously, whereas temozolomide is taken orally [70]. Both drugs are effective therapies for brain metastases since they traverse the blood-brain barrier [71].

Microtubule targeting Agents such as Vinca Alkaloids (vindesine/vinblastine) and Taxens (paclitaxel) have been shown to exhibit mild efficacy in patients with metastatic melanoma [72]. Microtubule-destabilizing substances, such as vinca alkaloid, block tubulin polymerization and ultimately cause apoptosis by stopping cells in their metaphase [73]. On the other hand, taxanes that are thought to be mitotic inhibitors prevent mitosis by increasing tubulin polymerization, stabilizing microtubule dynamics, and stopping the cell cycle [74].

1.7.2.2 Regional chemotherapy

Regional chemotherapy with melphalan is the gold standard for treating unresectable recurrences of melanoma restricted to a limb [75]. This drug is directly injected into a specific arm or leg region when the patient is elderly, has large tumors that are refractory to surgical removal, or is at high risk of problems from systemic therapy [76].

1.7.3 Immunotherapy

Melanoma is an immune-related malignancy, making immunotherapy one of the most effective therapeutic options [77]. The FDA has approved interleukin-2 and interferon alfa-2b as two cytokines for adjuvant metastatic melanoma treatments [78] because they play an essential role in tumor immunosurveillance [79].

High doses of Interferon alfa-2b are given by injection for one year. Injections are typically administrated intravenously for a duration ofone month.Subsequently, three 10 MU/m2 subcutaneous injections are given for the rest of the year [80]. Interleukin-2,or intralesional treatment, is injected every eight hours a week, with a maximum of twelve to fifteen doses [81].

Immune checkpoint inhibitors are vital immunotherapy that prevents checkpoint proteins employed by melanoma cells to evade immune system attacks, enabling CD8-positive T cells to eradicate malignancy [82]. An immune checkpoint inhibitor called ipilimumab targets the cytotoxic T lymphocyte-associated antigen 4(CTLA4) [83]. Nivolumab, a fully human IgG4 monoclonal antibody, targets programmed cell death protein 1 (PD-1) and exhibits antitumor properties [84].

Combining ipilimumab and nivolumab has shown to have synergistic benefits on the immune response against advanced melanoma that go beyond what can be achieved with either medication alone [85]. Combining anti-CTLA4 and anti-PD-1 checkpoint suppression in brain metastases of melanoma has been demonstrated to produce an intracranial response in 57% of patients [86], making it the most successful therapy with the highest 5-year overall survival rate [82].

1.7.4 Radiation therapy

Treatment with radiation therapy is crucial for metastatic melanoma [87] because it triggers adaptive immunity and causes tumor cells to die immunogenically, which significantly lowers the tumor burden [88].

In order to eliminate the irradiated tumor and provide better antitumor activity, radiotherapy in conjunction with anti-PD-1 treatment can overcome immunosuppression and considerably promote T-cell infiltration in the irradiated region[89]. Additionally, this combination can enhance the abscopal impact [90].

1.8 Types of melanoma

1.8.1 Superficial spreading melanoma (SSM)

It is the most prevalent subtype of melanoma that is responsible for 70% of cases [91]. This particular subtype of melanoma is concentrated in body areas exposed to the sun through these tasks. Men tend to get melanoma on their chest and back, while women typically get it on the back of their legs [92].

Due to the fact that this type of melanoma is also linked to UV radiation from tanning beds, there is a major, albeit mild, epidemic of melanomas affecting primarily younger female people [93].

In SSM, BRAF is the driving oncogene that is most frequently mutated [94]. The most frequent mutation changes valine (V) to glutamic acid (E) at position 600, p. V600E [34].

1.8.2 Nodular melanoma

It is the second-most prevalent melanoma subtype, usually affecting men over 50, with most lesions appearing on the head and neck [95]. NM grows vertically into the deeper layer of the skin and is initiated by dermal stem cells, whereas the other types develop from epidermal stem cells [96].

NMs manifest as lesions that are advancing quickly since they are tumorigenic almost from the moment they begin [97]. They are created by melanocytes that have undergone oncogenic modification after initially losing the function of tumor suppressor genes [98]. Since nodular melanomas have been proven to act more aggressively and go through more mitoses, they have a worse prognosis [26].

The papulonodular symmetrical lesion of nodular melanomas is characterized by "a polypoid structure, necrosis, ulceration, may or may not be pigmented, infiltration of the inflammatory cells in the dermis (lichenoid inflammation) and substantial pleomorphic [99]. Since NM lesions do not meet the ABCD criteria, they are currently distinguished using the EFG rule, which stands for elevation, firmness on touch, and rapid growth lasting a month [100].

1.8.3 Lentigo maligna melanoma (LMM)

The third most prevalent melanoma subtype, lentigo maligna, appears as an irregular brown patch on sun-damaged skin in older people, notably on the head and neck [38]. Hutchinson's melanotic freckle was the name given to it when it was first documented in 1890. Due to its slow growth and histological similarity to benign lesions, it was misdiagnosed for the majority of the first two decades of the twentieth century, earning labels like "circumscribed precancerous melanosis" and "junctional nevus" that can increase mortality [39].

LM was classified as cancerous in the late 1970s and early 1980s. Nowadays, lentigo maligna melanoma (LM) is defined as a type of in situ melanoma that shows aberrant melanocyte proliferation in the basal epidermis and can potentially spread to the dermis [101].

UV radiation stimulates oxidative damage and results in C>T and CC>TT mutations in genes related to the PI3K and MAPK pathways, which constitute a crucial factor in the development of LM/LMM. Afterward, secondary mutations in the genes CCND1 and P53 lead to the tumor becoming malignant [39]. Furthermore, LM is more likely to occur in people with sun sensitivity-related hereditary diseases such as porphyria cutanea tarda, werner syndrome, and oculocutaneous albinism [40].

1.8.4 Acral lentiginous melanoma(ALM)

ALM is the least prevalent of the four basic kinds of cutaneous melanoma, accounting for approximately 2 to 3% of all melanoma cases [102].Among individuals with darker skin, this type of melanoma is the most commonly observed [103], and it is not influenced by sunlight or UV exposure. Typically, it affects the extremities, more commonly on feet than on hands (palms, under fingers, or toenails) [103].

Acral lentiginous melanoma is characterized by persistent asymmetrical development of melanocytes at the dermoepidermal interface, dermal invasion, and desmoplasia [104].The abbreviation "CUBED," which stands for colorful lesions, ambiguous diagnosis, bleeding, enlargement, and a delay in healing, has been proposed to replace the conventional "ABCDE" strategy for melanoma on acral surfaces [33].

1.8.5 Mucosal melanoma

Mucosal melanoma (MM) is an extremely rare tumor that arises from melanocytes found in the mucous membranes throughout the body.[105], particularly in the respiratory mucosa, oralcavity,esophagus, pharynx, vaginal canals, head and neck [106].Mucosal melanoma is predicted to account for 2% of new cancer cases in the United States in 2023, according to predictions of the incidence of cancer in the country [107].

Mucosal melanomas may develop as a result of an RGP exhibiting "ABCDE" characteristics. It causes pain and bleeding by infiltrating and destroying the surrounding tissues [108].Research suggests that women and elderly individuals are more likely to be affected compared to men. This data highlights the importance of taking into account potential gender and age-related differences. Additionally, it more commonly affects people with darker skin tones [109].

Globally, MMs differ from cutaneous melanoma in their etiology and clinical appearance.Furthermore, there is no association between exposure to UV radiation and MM carcinogenesis [110]. MMs are more aggressive and have a worse prognosis than CMs. Still, MMs and CMs are usually managed similarly [111].

1.8.6 Uveal melanoma

It is the rarest type of intraocular cancer in elderly people, which accounts for approximately 5% of all melanomas [112]. It is considered a life-threatening ocular condition Because it arises from melanocytes found in the highly pigmented ocular tract, the retina's primary source of nutrition and oxygen [113].

The development of uveal melanoma can be attributed to a variety of risk factors. Patients with BRCA1-associated protein 1 (BAP1) mutation are thought to have a higher chance of developing uveal melanomas at a younger age, including those with xeroderma pigmentosum, light-colored eyes, dysplastic nevus and ocular melanocytosis [114].

1.9 Prognostic factors for melanoma

1.9.1 Thickness of the tumor

An important factor for the likelihood of melanoma spread is the Breslow index [115]. Athicker tumor may indicate a more advanced tumor, which would raise the chance of metastasis and, consequently, worsen the prognosis [116].

1.9.2 Mitotic rate

Hot spot analysis is the most representative method for assessing the tumor's highly proliferative pool. This technique involves counting the number of mitoses in the invasive zone of the tumor per square millimeter to determine its mitotic rate [117].

Tumor mitotic rate is perceived to be the second most potent predictor of survival in localized cutaneous melanoma after tumor thickness [118]. A higher rate of mitosis is linked to a less favorable outcome for melanoma because it's a sign of an aggressive tumor with a higher proliferative index and a higher likelihood of metastasis [119].

1.9.3 Ulceration

The AJCC Melanoma Staging System lists ulceration as a third prognostic feature linked to higher death and recurrence rates [120]. Compared to nonulcerated melanoma of the same breslow thickness, those with ulcerated melanoma had a poorer prognosis [121] due to their lack of integrity in the epidermis [122].

1.9.4 Age, sex, and location of melanoma

The prognosis for melanoma patients is better for women and young people than for elderly and male patients [123]. This is because men are more prone to acquire melanoma in the head, neck, and trunk, and older patients have thicker tumors that are more likely to become ulcerated [123] due to greater proliferative activity and late detection [124].

1.9.5 Lymph node

The American Joint Committee on Cancer (AJCC) states that the melanoma patients with positive lymph node (LN) status got a higher risk of recurrence and metastasis. [125]. When melanoma develops outside the local lymph node, the survival rate drops to less than 10% [126].

1.10 Can Melanoma Be Prevented?

Over the past few decades, skin melanoma has been among the cancers that has increased in frequency among Caucasian people [127]. Nonetheless, the prognosis of malignant melanoma (MM) is mostly determined by prevention and early detection [128].

1.10.1 Mediterranean diet

A Mediterranean diet is linked to a decreased incidence of cutaneous melanoma due to its high bioactive substance content with strong anti-inflammatory, immunomodulatory,and antioxidant effects [129].For instance, it is wellknown for having an abundance of citrus fruits and carrots, which are the main sources of β-cryptoxanthin and high in β-carotene, respectively. [130]. Moreover, eating a lot of fish enriched in omega-3 polyunsaturated fatty acids has been associated with a 50% reduction in melanoma risk [131].

The Mediterranean diet is also well known for polyphenol-rich foods such as tea, cruciferous vegetables, and dark green veggies, which have anti-carcinogenic effects, including decreasing UV radiation-induced erythema, inducing apoptosis and inhibiting cell growth [132]. These foods also have an essential role in Photoprotection,which is achieved by scavenging ROS produced in photooxidative reactions [133].

1.10.2 High Vitamin D Intake

Vitamin D is an important preventive factor against melanoma, and this is due to its active form 1,25(OH)2D that can bind to the nuclear vitamin D receptor present in keratinocytes and melanocytes [134], causing apoptosis, cell cycle arrest, and suppression of metastasis [135]. Therefore, elevated levels of vitamin D in the blood have been connected to better survival rates in melanoma patients [136].Conversely, deeper tumors and later stages of cancer have been associated with low vitamin D levels [137] .

1.10.3 Limit exposure to ultraviolet radiation

Sun exposure has been implicated in melanoma progression [138]. Consequently, you should make an effort to avoid being outside during the midday sun, particularly between 10 a.m. and 4 p.m. Apply sunscreen with a skin protection factor (SPF) of 30 or higher, wear a hat and sunglasses, and reapply it often—usually every 1.5 hours, or more frequently if you swim or perspire. Additionally, avoid using tanning beds and sunlamps [139].

1.11 Problem Statement and Rationale

Cutaneous melanomacontinues to be a serious clinical challengedespite significant international efforts. Melanoma ranks first among the most prevalent cancer kinds, with one of the quickest rates in many countries worldwide. Until now, there have been few

effective systemic treatments for widespread melanoma, sothe development of new drugs is costly and may take years to obtain approval for use and enter clinics. On the other hand, it is possible to quickly incorporate medications into clinical research and medical treatment by repurposing previously approved medications for other medical uses.

There is still a gap or lack of definitive evidence of the relationship between telmisartan and its effect as an anti-cancer therapeutic for melanoma treatment, so this encourages us to perform many studies on the correlation between them.

1.12 Objectives

- To study the effect of telmisartan on melanoma growth and metastasis through LRP1 suppression in order to be considered as possible therapy option to patients with metastatic melanoma who wish to achieve a high survival rate.
- To find out how restoration of P53 by telmisartan will downregulate the expression of endocytic receptor LRP1 by upregulating the tumor suppressor miR -107.

1.13 Research question

Is there a relationship betweenthe antihypertensive drug telmisartan and suppression of LRP1 in the treatment of melanoma cells?

1.14 Hypothesis

There is a relationship between telmisartan and suppression of LRP1 oncogene through P53 regulating stress intensity –dependent MicroRNA feedback loopin the treatment of melanoma cells.

1.15 Significance of the study

Research has been done on the anti-cancer impact of telmisartan on a range of malignancies, including ovarian, breast, lung, and urological cancer. However, none of them investigated its effect on melanoma.So, this study aims to shed light on the importance of telmisartan in suppressing melanoma growth and metastasis through LRP1 suppression to achieve a high survival rate for melanoma patients.

1.16 Literature review

1.16.1 P53 function & role in melanoma

Human p53 is a highly conserved gene located on the short arm of chromosome 17(17p13.1)[140]**.** It encodes a phosphoprotein with a molecular weight of 53 kDa and 393 amino acids [141] Moreover, this P53 phosphoprotein is important for controlling the cell cycle and transcription of genes [142].

The P53 protein has several functional domains, including the N-terminal transactivation domain,which is crucial for P53 function [143].The proline-rich domain of p53 is crucial for its pro-apoptotic action. The DNA binding domain also interacts with DNA sequences and induces the transcription of p53 target genes.Finally, the carboxy-terminal tetramerization domain is required to stabilize the p53–DNA complex [144] and other regulatory domains [145].

P53 forms a tetrameric form with a molecular weight of approximately 200 kDa due to interactions between the protein's C-terminal domains[146]. These tetramers make P53's binding affinity to its DNA sequence 1000 times greater than the monomer's [144]. They also identify certain binding sites on target genes and activate them [147].

The tumor suppressor gene P53 is a powerful transcription factor that controls many genes and pathways that trigger cell cycle arrest and programmed cell death [148]. Additionally, depending on the kind of cell and the stressor it is exposed to, its activation might have varying impacts on metabolic alterations and the antioxidant response [149].

P53 is known as the "guardian of the genome" due to its preservation of stability through the prevention of genome mutation and the regulation of angiogenesis, cell division, DNA damage repair, and innate immunity [150]. Therefore, P53 functions as a decision node in stressed cells, weighing all inputs, such as the level of DNA damage and oxidation, before committing the cell to repair or apoptosis if the damage is irreversible [151].

Targeting oncogene is the main focus of efforts to produce innovative anti-cancer medications, while approaches to restore the function of the tumor suppressor p53 have received significantly less attention up to this point [152].

Due to that,a prior investigation concerning the restoration of wild-type p53 in human cancer revealed that employing compounds to reactivate the p53 function increased the

tumor's susceptibility to radiation or chemotherapy. This highlights the significance of developing novel drugs with reduced side effects and increased anti-tumor efficacy [153].

An in vivo study using "switchable" p53-engineered mice showed that p53 restoration results in an amazing regression of tumors that have already progressed, including lymphomas and hepatocellular carcinomas [154]. This finding supports the idea that p53 function restoration in tumors may be a desirable cancer treatment approach that blocks invasion and metastasis of the tumor [155].

While P53 mutations are common in many cancer types, melanoma mutational inactivation of P53 is uncommon, and wild-type P53 is frequently expressed at high levels [156]. The tumor suppressor wild-type P53 is frequently transcriptionally inactivated in melanoma due to malfunctioning signaling pathways downstream of P53 [157]. This clarifies why human melanoma with wild-type P53 is incapable of causing cell cycle arrest or death in response to ionizing radiation [158].

In contrast, according to some theories, the progression of nevi into melanoma is significantly prevented by the stabilization and activation of WT p53 due to post-translation alteration [159].

An investigation conducted in 2018 found that when lethal stress occurs, TP53 regulates the expression of the LRP1 oncogene and promotes cell death by activating the tumor suppressor miR-107 [160].

1.16.1.1 Activation of p53 by phosphprylation

The initial step in stabilizing P53 in response to stress is phosphorylation, which involves post-translational modifications carried outatseveral threonine and/or serine sites to stabilize the p53 and increase its activity[161]. It is also essential for controlling how activated p53 causes apoptosis and maintains cell cycle arrest [162].

One of most frequently phosphorylated sites in the C-terminus of the p53 protein is Ser392. It is thought that phosphorylation of Ser392 enhances p53's in vitro capacity to bind DNA, which leads to transactivation of its apoptotic function [163].

Research on hepatocellular carcinoma published in 2017, the findings showed that telmisartan provides anti-tumor impact and inhibits HCC cell proliferation via increasing

the levels of phospho-p53 (ser392), which induce apoptosis and controls the levels of apoptosis-related proteins [164].

1.16.1.2 The role of MDM2

Murine double minute 2 protein is a transcriptional ubiquitin ligase expressed by the MDM2 gene, which was cloned and found on chromosome 12q13–14. It has been demonstrated to have two promoter regions, P1 and P2 (p53 dependent)[165], which attach to the P53 transactivation domain [166].

WT P53 is maintained at low levels in the cytoplasm by its negative regulator MDM2 [167], but in response to stress, the physical complex between p53 and MDM2 is disrupted, MDM2 protein is downregulated, leading to a reduction in p53 nuclear export and subsequently increased nuclear stabilization [168].

Numerous studies have demonstrated that suppressing MDM2-P53 activates P53 tumor suppressor function, resulting in cell cycle arrest or programmed cell death, providing this a promising therapeutic option for cancer treatment [169].

1.16.2 LRP1 function

A multifunctional scavenger protein, LRP1, sometimes referred to asa cluster of differentiation CD91, controls the structure of the plasma membrane. The capacity of LRP1 to bind to apoE raises the likelihood that it participates in lipoprotein metabolism [170].

It was quickly found that the alpha 2 macroglobulin (α2M) receptor was identical to LRP1, demonstrating that LRP1 is a versatile receptor that has a high affinity for a variety of ligands, influencing a number of biological processes that support angiogenesis, cell proliferation, and differentiation [171].

LRP1 binds ligands via its extracellular domain and promotes internalization using its cytoplasmic tail.. As a result, it is essential for the growth of tumors because it participates in endocytosis, which is connected to the stimulation of cell signaling [172].

(LRP1) Low-density lipoprotein receptor-related protein 1 belongs to the LDL-receptor gene family[173].It isa type I glycosylated transmembrane receptorproduced in the endoplasmic reticulum with a massive molecular mass of 600Kd. After reaching the

trans- Golgi, Furin breaks it down into two chains, a large extracellular α chain (515 kDa) and a small intracellular β chain (85 kDa), which are non-covalently linked on the cell surface [174].

The N- N-terminal α chain is responsible for ligand bindingand contains EGF repeats and four clusters of ligand–binding–type repeats with cysteine-rich repeats. The ligand binding domains 2 and 4 can interact with 40 ligands [175].

The short C-terminal β chain has a cytoplasmic tail and a single hydrophobic transmembrane domain. The transmembrane domain ensures that LRP1 is embedded in the plasma membrane.

A single hydrophobic transmembrane domain and cytoplasmic tail are found on the small C-terminal β chain. The cytoplasmic tail contains five motifs: two dileucine motifs and one YXxL motif, which are in charge of LRP1 endocytosis, and two NPxY motifs, which regulate signal transduction pathways. Meanwhile, the transmembrane domain ensures that LRP1 is embedded in the plasma membrane [172].

1.16.2.1 The role of LRP1 in cancer

LRP1 is a transmembrane protein that performs two roles: signal receptor and cargo transporter[176]. It is crucial for the uptake of more than 30 different extracellular ligands[177], which explains why it is thought to be a significant factor inthe development of cancer, inflammation, and antigen presentation[178, 179].In prior studies, LRP1 has seen to support the invasion and metastasis of breast [180] as well as thyroid carcinoma cells [181].

In many tumors, LRP1 expression is downregulated, which is linked to a more advanced stage. Studies on colorectal and hepatocellular carcinoma have shown that high histological grade and an unfavorable prognosis are related to reduced LRP1 expression [182, 183].

The Notch signaling pathway, which has been preserved for a long time, coordinates cellular functions [184]. A prior study discovered a direct correlation between the occurrence of several cancers and the dysregulation of this system [185]. Through bioinformatics analysis, researchers discovered that LRP1 was an important protein in the Notch pathway's protein–protein interaction network map [186].

LRP1 forms a co-receptor complex with PDGF, which controls LRP1 tyrosine phosphorylation in the cytoplasmic tail and plays a crucial role in cell proliferation. Furthermore, it has been demonstrated that LRP1 is present in monocytes and macrophages, which play a critical role in cancer development by encouraging cell invasion and metastasis [187].

VEGF is a powerful angiogenic agent produced by macrophages that significantly impact the tumor's micro vessel density, encouraging carcinogenesis by altering LRP1 to increase angiogenesis [188].

A comprehensive network-based exploratory analysis demonstrated a strong correlation between LRP-1 and a multi-cancer gene expression biomarker that is thought to be extremely predictive of the clinical prognosis in twelve distinct cancer types [189].

1.16.2.2 LRP1 as a novel P53 target gene

The endocytic receptor LRP1 controls the growth and metastasis of melanoma cells through interacting with fibrinolytic factor tissue-type plasminogen activator (tPA). A crucial switch that encourages melanoma formation is the tPA-LRP1 pathway, which alters the tumor niche's cellular composition and proteolytic profile [190].

Recent research has revealed that plasminogen activators, which include plasminogen receptors and tPA (additionally referred to as PLAT), are TP53 targets that facilitate the growth and spread of tumors [191].

1.16.3 Telmisartan drug

Telmisartan (Micardis) is a white to slightly yellowish solid crystalline powder that is made into oral tablets and is also known as 2-[4-[[4-methyl-6-(1-methylbenzimidazol-2-yl)-2-propylbenzimidazol-1-yl] methyl] phenyl] benzoic acid. With a chemical formula of C33H30N4O2 and a molecular weight of 514.6, it is a non-peptide member of the benzimidazole family and is frequently used to treat hypertension [192].

It is apotent antihypertensive drug that functions as a partial agonist of the peroxisome proliferator-activated receptor gamma (PPAR γ) and aninhibitor of the angiotensin-receptor 1 (AGTR1). It has the highest AT1 receptor affinity and the lowest AT2 receptor affinity of all the commercially marketed angiotensin receptor blockers (ARBS) [193].

The nuclear receptor(PPAR γ) controls gene transcription and lipid metabolism [194]. Furthermore,according to several studies, it inhibits the growth of cancer cells both in vivo and in vitro, making it a promising molecular target for creating anticancer drugs [194].

According to the findings of a previous study, PPAR γ expression has been found in tumor cells of breast, colon, lung, gastric, pancreatic, prostate, and bladder cancer. The findings demonstrate that in vitro tumor cell growth was significantly suppressed by PPAR γ activation caused by PPAR γ agonist [195].

Angiotensin II is a crucial peptide in the renin-angiotensin system involved in neovascularization and angiogenesis, which are the processes leading to tumor growth and metastasis. Angiotensin II receptor blockers (ARBs), which is a potent antihypertensive drug, suppresses vascular endothelial growth factors (VEGF), hence preventing tumor angiogenesis [196].

In a study conducted in 2010 into the impact of ARBs (candesartan, valsartan, irbesartan, losartan, and telmisartan) on human urological cancer,the findings indicate that only 100 MM of telmisartan was found to be efficacious. Only telmisartan caused early apoptosis and a drop in cell viability with a half-maximal dosage of growth inhibition via PPAR-γ, whereas other ARBs had no impact,leading to its acceptance as a crucial anticancer therapy approach [197].

A study was conducted in 2023 to examine the potential effects of telmisartan on the breast cancer cell line MCF7. Based on both in-silico and in-vitro tests, the findings demonstrated that telmisartan, with an IC50 of 7.75 μM, exhibits the greatest affinity among several drugs, including prazosin, hydralazine, irbesartan, telmisartan, and candesartan, indicating that it has a strong anticancer effect on breast cancer.Consequently, it would seem that telmisartan might be a good choice for pharmacological repurposing in the management of different cancers [198].

In human melanoma tissues, Angiotensin II and AGTR1 are both expressed. A study published in 2019 found that Telmisartan, which is both an AGTR1 inhibitor and a PPAR agonist, has anti-melanoma effects and induces apoptosis [199].

1.16.4 MicroRNAs role in melanoma

MicroRNAs (miRNAs) are one of the most significant post-transcriptional regulators of gene expression. They are small, highly conserved, long, single-strand, non-coding RNAs with roughly 22 nucleotides[200]. They are found within the intron of protein-coding or non-coding transcriptional units or within exons of non-coding genes.These miRNAs either express together with their host genes and share their direction, or they have independent promoters that are not reliant on the host genes [201].

The majority of miRNAs are produced from DNA sequences as primary miRNAs (pri-miRNAs), which are then transformed into precursor miRNAs (pre-miRNAs) and mature miRNAs. The 3' untranslated region (3′ UTR) of target mRNAs is frequently bound by miRNAs to trigger translational inhibition and mRNA degradation [202].

MicroRNA regulates the target mRNA's expression by silencing their translationat the initiation or elongation steps, causing the 3′ polyA tail to be lost (deadenylation) by encouraging the activity of deadenylases enzyme to do this and removing the 5′ cap structure from the mRNA (decapping) followed by mRNA destruction by exonuclease [203].

MiRNAs are classified into two types: oncogenes (oncomiRs) and tumor suppressor (TS) miRNAs. OncomiRs, a kind of miRNA that promotes tumor growth, bind to the messenger RNAs of TS genes. Hence, thet are overexpressed in cancer. On the other hand, TS miRNAs inhibit oncogene messenger RNAs and are hence underexpressed in cancer [204].

Depending on the kind of tumor, different miRNAs have opposing effects. In some tumors, they serve as tumor activators, whereas in others, they serve as tumor suppressors [205]. On chromosome 10, miR 107 is involved in numerous biological processes [206] and found to be an oncogenic role in liver cancer [207] while acting as a tumor suppressor in breast, gastric cancer, and non-small cell lung cancer [208].

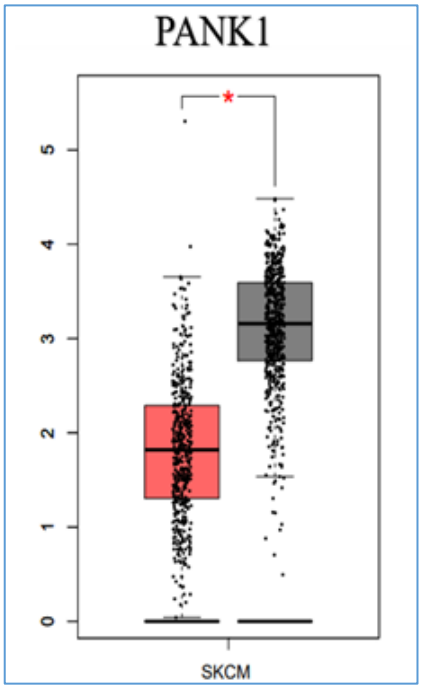
Various studies have suggested that p53 may be a factor in the induction of miR-107 expression. A 2018 study found that fatal levels of stress trigger p53-dependent overexpression of both miR-103 and miR-107, which in turn limit LRP1 translation and aid in cancer cell death [160].

Since miR-107 is expressed within an intron of the gene for pantothenate kinase enzyme 1, or PANK1, miR-103 is a paralog that differs from miR-107 just by a single nucleotide near the 3' end of the miRNAs. They had the same transcriptional start point and were being transcribed simultaneously [209].

In addition, we go back to the Gene Expression Profiling Interactive Analysis 2 "GEPIA2" database in order to study the expression of PANK1 both in normal and tumor tissues.In contrast to neighboring normal skin tissues, melanoma tumor tissues showed lower expression of the PANK1 gene (http://gepia2.cancer-pku.cn/#index).

***Scheme* 3**

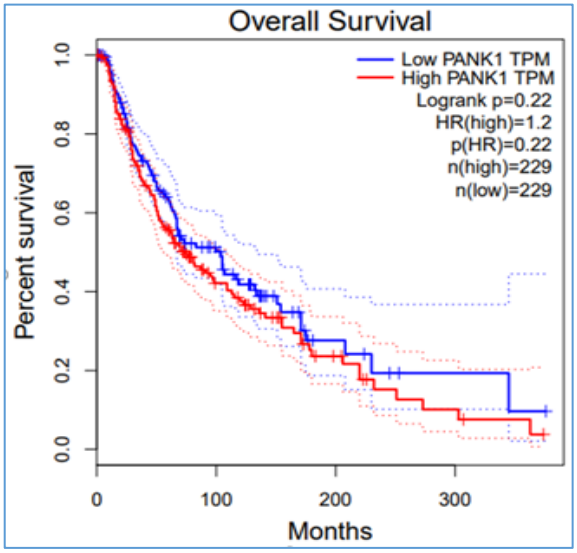
Human PANK1 expression levels in tumours (red) and normal tissues (black) of SKCM patients were retrieved from GEPIA2 database



Besides, we check out the GEPIA2 database to investigate the impact of PANK1 level on the survival rates of skin cancer patients (http://gepia2.cancer-pku.cn/#index).

Scheme 4

The survival rate of melanoma patients according to PANK1 in case of high level of PANK1 (red) and low level of PANK1 (blue) of human skin cutaneous melanoma (SKCM) patients were obtained from the GEPIA2 database



Mir 107 is crucial for controlling the cell cycle and is downregulated in melanoma and many malignancies. Salama reported that overexpression of miR-107 inhibited melanoma cell proliferation [210]. Furthermore,as previously shown by a study, over-expressing miR-107 targets numerous genes to stop renal cancer cells in their G2/M phase.

Several studies show that P53 controls the expression miR107, which contributes to cell cycle regulation. For instance, the results of a 2011 study show that P53 activates the PANK1/miRNA-107 gene, which subsequently results in the downregulation of CDK6 and p130, two cell cycle proteins [209].

A study on human colon cancer in 2006 used reporter constructs 2,500 bp upstream of the PANK1/miR-107 gene that produces firefly luciferase to show that p53 is transcriptionally controlled by miR-107 and to pinpoint the areas upstream of PANK1/miR-107 that drive p53 induction. The findings show that in HCT116 (WT) cells, genotoxic stress enhances the transactivation of the full-length reporter construct.Meanwhile, in HCT116 (p53 KO) cells, genotoxic stress does not increase the expression of the reporter construct [211].

In 2019, researchers looked into the role of miR107 as a tumor suppressor in ovarian cancer.The findings show that miR-107 reduces ovarian cancer cell proliferation by

targeting the 3'UTR of cyclin E1. This demonstrates that miR-107 functions as a tumor suppressor [212].

An in vivo study on the role of miR107 in the suppression of angiogenesis was carried out in 2011. The results showed thatstimulation of miR107, which is mediated by a p53-regulated miRNA gene pathway, suppresses hypoxia, downregulates HIF-1β,and eventually diminishes angiogenesis and tumor growth in mice [213].

1.16.5 BCl2/BAX as apoptotic gene

The apoptotic process is governed by two major mechanisms, the intrinsic and extrinsic pathways.The B-cell lymphoma 2 (BCL2) protein family, which is thought to operate as guardians at the mitochondrial gate, governs the activation of the intrinsic route.An apoptotic activator BAX (BCL2-associated X) and anti-apoptotic BCL2 (B-cell lymphoma protein 2) belong to the (BCL2) protein family [214].

The regulation of intrinsic pathway apoptosis was facilitated by the mitochondrial permeability transition pore (PTP). The pore's opening and shutting were regulated by the proteins Bax and Bcl-2. The Bax protein normally transforms from innocuous monomers into lethal oligomers that create pores in the mitochondrial outer membrane(MOM)[215]. These pores allow proapoptotic elements, such cytochrome c, to translocate to the cytoplasm,where they initiate a series of proteolytic events that ultimately result in cell death.In response to cytochrome c release, which often known as (MOM) permeabilization, the Pt.pore opens, and the Apoptotic protease activating factor-1 (Apaf-1) oligomerizes to create an apoptosome complex, which in turn activates procaspase-9 and triggers caspase-3 processing downstream. The Bcl-2 protein ordinarily binds to the mitochondrial Pt.pore in opposition to Bax's function, which blocks Bax-Bax oligomerization and causes the Pt.pore to close [216].

In a prior investigation on the impact of telmisartan on the NSCLC A549 cell line carried out in 2018, the results showed that telmisartan induced A549 cell apoptosis by increasing the expression of the pro-apoptotic protein Bax in comparison to the control group and decreasing the expression of the anti-apoptotic protein Bcl-2 [217].

Chapter Two
Methodology

2.1 Study time

After receiving approval from the faculty of graduated studies, the NNU's Institutional Review Board (IRB) in May 2022, and verbal consent from NNU laboratory for Cancer and Stem Cell research in Nablus, the work was conducted from July 2022 to September 2023. The deadline for additional research writing has been extended to November 2023. This writing will include an introduction, a literature review, data analysis, and outcomes**.**

2.2 Study design and setting

An experimental laboratory study was conducted at the Najah Center for cancer and Stem Cell Research. The research was performed in controlled conditions in the Lab, and the data acquired support the main idea that Telmisartan has a relationship with decreased melanoma growth and metastasis.

2.3 Materials and methods

2.3.1 Mice

We used male WT C57BL/6 mice aged 8–12 weeks from NNU in Nablus, for the tests. The protocols for animal treatments were approved by An-Najah National University's Institutional Animal Care and Use Committee.

2.3.2 Cell lines

The B16F10 melanoma cell line [CRL-6475; American Type Culture Collection (ATCC), Manassas, VA, USA] was maintained in “high-glucose Dulbecco's Modified Eagle's Medium (DMEM with L-glutamine, phenol red (Fuji Film Wako, Osaka, Japan), 10% fetal bovine serum (FBS; G.E. Healthcare, Chicago, IL, USA), and 1% penicillin/streptomycin (P/S) (Nacalai Tesque, Kyoto, Japan).” Human umbilical vein endothelial cells (HUVECs) were cultured at 37°C / 5% CO2 on 0.1% gelatin (Wako Pure Chemicals)-coated culture plates (Falcon) in endothelial growth medium-2 (EGM-2; Lonza; cc4176)

2.3.3 Drug treatment in vitro

Telmisartan was dissolved in sterile DMSO and diluted in DMED to make the desired concentrations.Telmisartan was added to *in vitro* cultures at concentrations ranging from 100 -500 μM.

2.3.4 Cell culture

Seeding B16F10 cells (4×105 cells/ml = 2*10^5 cells/well) in 12-well plates (TPP, Switzerland) allowed the cells to grow over night. Once Telmisartan was added to DMSO at three different doses (100 μM, 250 μM, and 500 μM) and as a control, the cells were cultured for an additional 24 and 48 hours. The number of live cells was then counted after trypan blue staining to determine the cells' viability.The cells were collected at relevant time points after telmisartan treatment.Trizol was used to extract the RNA in order to separate it from the protein and DNA.

2.3.5 Wound healing (Scratch) assay

B16F10motility was assessed using an in vitro wound healing assay, which is a simple and affordable way to investigate cell migration in vitro [218]**.** This assay creates a gap in a confluent cell monolayer of keratinocytes to simulate a wound (scratch). The key components of the scratch wound process are preparing the cell culture, performing the scratch wound assay, analyzing data, and taking photos in order to measure the cell migration rate [219].

B16F10 cells (4×10^5cells/ml) were planted into a six-well plate and grown to approximately 80% confluence. After the cells were confluent, the six-well plate was scratched or wound with two lines of comparable width in each well using a 1-mL pipette tip spearhead positioned perpendicular to the plate's bottom. Once again, complete DMEM was poured onto the plate after gently cleaning it with PBS three times.

A sterile white pipette tip wounded the confluent cell monolayers. Inverted microscopy examined four randomly selected fields for each well to quantify the cells that had migrated into the cell-free area. Three separate experiments were carried out.

2.3.6 In Vivo B16F10 Melanoma Model

The local tumor model involved inoculating B16F10 cells subcutaneously into C57/BL6 mice on day 0 (1×10^6/200 uL/mouse) after they had been twice washed with PBS (90%

viability, as assessed by trypan blue exclusion).Every day, the growth of the tumor was observed.

Mice that exhibited signs of severe pain, emaciation, or loss of more than 20% of their initial body weight 12 days after receiving subcutaneous. Intravenous tumor cell inoculation were euthanized by cervical dislocation. The tumors and surrounding conjunctive tissues were eliminated. The removed Tumors were weighed at d 12.

In vivo drug treatment scheme:On the fifth day following tumor cell inoculation, intraperitoneal injections of telmisartan (25 mg/kg bodyweight) or carrier (DMSO/PBS) were given every day.

2.3.7 RNA extraction using TRIZOL (TRI reagent)

We grew a monolayer of cells in a six-well plate, and then trypsin was added to detach cells. After that, we centrifuged the cells and added 1 ml trizol to the formed pellet, 0.2 ml of chloroform was added, RNA was formed as a colorless upper aqueous phase, we added isopropanol to form a white gel-like pellet of RNA, then the pellet was suspended in a 75% ethanol, and then RNAase-free water.

2.3.8 Quantitative reverse transcriptase-polymerase chain reaction (qPCR)

Using Trizol reagent, total RNA was isolated from cell lines. The RNA was then reverse-transcribed into complementary DNA (cDNA) using Prime Script RT Master Mix, a reverse transcription reagent kit, in accordance with the manufacturer's instructions. cDNA was kept cold at -30°C.

We extracted and purified miRNA using the miRNeasy Mini Kit (Qiagen, Germany).The “Mir-XTM miRNA First-Strand Synthesis Kit (cat no 638313, Takara, Japan)”was then used to reverse-transcribe miRNA into cDNA in accordance with the manufacturer's instructions.

The Step One Plus Real-Time PCR System (Applied Biosystems, USA) with the FastStart Universal SYBR Green Master was used to measure the mRNA expression levels by qPCR. Applied Biosystems StepOnePlus Real-time qPCR was used to perform comparative SYBR green qPCR in order to evaluate gene expression. Using the convenient 2-ΔΔCt technique, we were able to determine the relative fold mRNA

expression for each gene and miRNA. Every qPCR experiment was run in triplicate and was repeated twice on its own.

Unless stated otherwise, all qPCR findings for gene expression were normalized to b-actin mRNA expression. Using the 2−ΔΔCT method (ΔΔCt = ΔCt (treated sample) – ΔCt (control) sample), the expression levels of miR-107 were standardized to kit MRQ smart.The corresponding forward and reverse primers that were utilized are:

Table 1

The forward and reverse primers

Gene	Forward Primer	Reverse Primer
mLRP1	5'-GGACCACCATCGTGGAAA-'3	5'-TCCCAGCCACGGTGATAG-'3
mβ-actin	5′-CTAAGGCCAACCGTGAAAAG-′3	5′-ACCAGAGGCATACAGGGACA-′3
m18S rRNA	5′-CCATCCAATCGGTAGTAGCG-′3	5′-GTAACCCGTTGAACCCCATT′3
Mu-miR-107	5′- AGCAGCATTGTACAGGGCTATCA -′3	kit MRQ smart

LRP1 lentivirus generation for LRP1 OE. Cloning LRP1, as previously mentioned [190]. Briefly, we used mouse brain tissue to clone the mouse LRP1 coding sequence. Mouse LRP1 cloning sequence:

Table 2

Gene and sequences

Gene	Sequences
LRP1-XbaI	5′-TCTAGAGCCCACACCATGCTGACCCCGCCGTTGCTGCTGCT-3`
LRP1-HpaI	5′- GTTAACCTATGCCAAGGGATCTCCTATCTCGTCTTCAGGT-3`

miR-107 cloning: Using the genomic DNeasy Extraction Kit (Qiagen, Germany), genomic DNA was isolated from HUVEC cells. Using PrimeSTAR polymerase (Takara, Japan), the genomic sequence was amplified using genomic PCR. The PCR was conducted using the following primers.

Table 3

Primers for PCR

miR-107	`5-GGGCTCGAGAAGCAGGCTAAAATTCCAGTC-3`
miR-107	`5-CCCCCCGGGCCCAAAAGAACTTAGCAATCTT-3`

The "XhoI and SmaI sites of the eukaryotic expression vector LV-EF- L3T4-IRES2-EGFP" were used to insert the purified segment. Sanger sequencing (An-Najah University, Palestine) was used to sequence cloned plasmids, which then amplified in E. coli DH5α competent cells.

2.3.9 Cell Culture

After seeding B16F10 cells (1×10^5 cells/well) on 6-well plates (TPP, Switzerland), the cells were incubated for a full night before being transfected using Lipofectamine RNAiMAX (Invitrogen). B16F10 cells wild-type, LRP1 overexpressing (OE) or knockdown (K.D.) cells.

2.3.10 Small interfering ribonucleic acids (siRNA)-Based Gene Knockdown

Cells were seeded at a concentration of 2×10^5 cells/well in a 6-well plate for 16 h before transfection using siRNA against LRP1 or control sequences.Cancer cells were transfected with siRNA targeting LRP1 using Lipofectamine™ RNAiMAX Transfection Reagent (Invitrogen) at a final concentration of 100 nM. The transfection medium was changed out for new media after 12 hours. siRNAs obtained from Invitrogen (Thermo Fisher Scientific, Lafayette, CO, USA). The effectiveness of the knockdown was assessed in cells 12 hours after gene silencing using qPCR analysis. The following siRNAs were employed, which target mouse LRP1:

Table 4

Gene and sequences

Gene	Sequences
si-LRP1#1	5′-GCUCAUCUCGGGCAUGAUU-3′
si-LRP1#2	5′-GCAGUUUGCCUGCAGAGAUUU-3′
Si-Ctrl	5′-GCUCCACAGAGUAUACCUU-3′

2.3.11 Webserver GEPIA2

GEPIA2 is a universal online server and resource for systematically analyzing the relationship between gene expression and immune infiltrates in cancers of various types (http://gepia2.cancer-pku.cn/#index) [220] .

2.3.12 Statistical analysis

All analyses were performed using Microsoft Excel 2016 for Windows. Each experiment was done at least three times.We used the “mean” and “standard error of the mean (SEM)” to represent data. The Student's t-test was performed in the statistical analysis, and p-values less than 0.05 were considered significant. *, **, and *** represent $p< 0.05$, 0.01, and 0.001, respectively.

2.3.13 Ethical consideration

The institutional review board of Palestine's An-Najah National University permitted the study. The study was conducted in compliance with the standards of the Helsinki criteria. "The National Institutes of Health's guide for the Care and Use of Laboratory Animals was followed when conducting animal studies and euthanizing animals." The Committee on the Ethics of Animal Experiments at NNU gave its approval to the protocol.

Chapter Three

Results

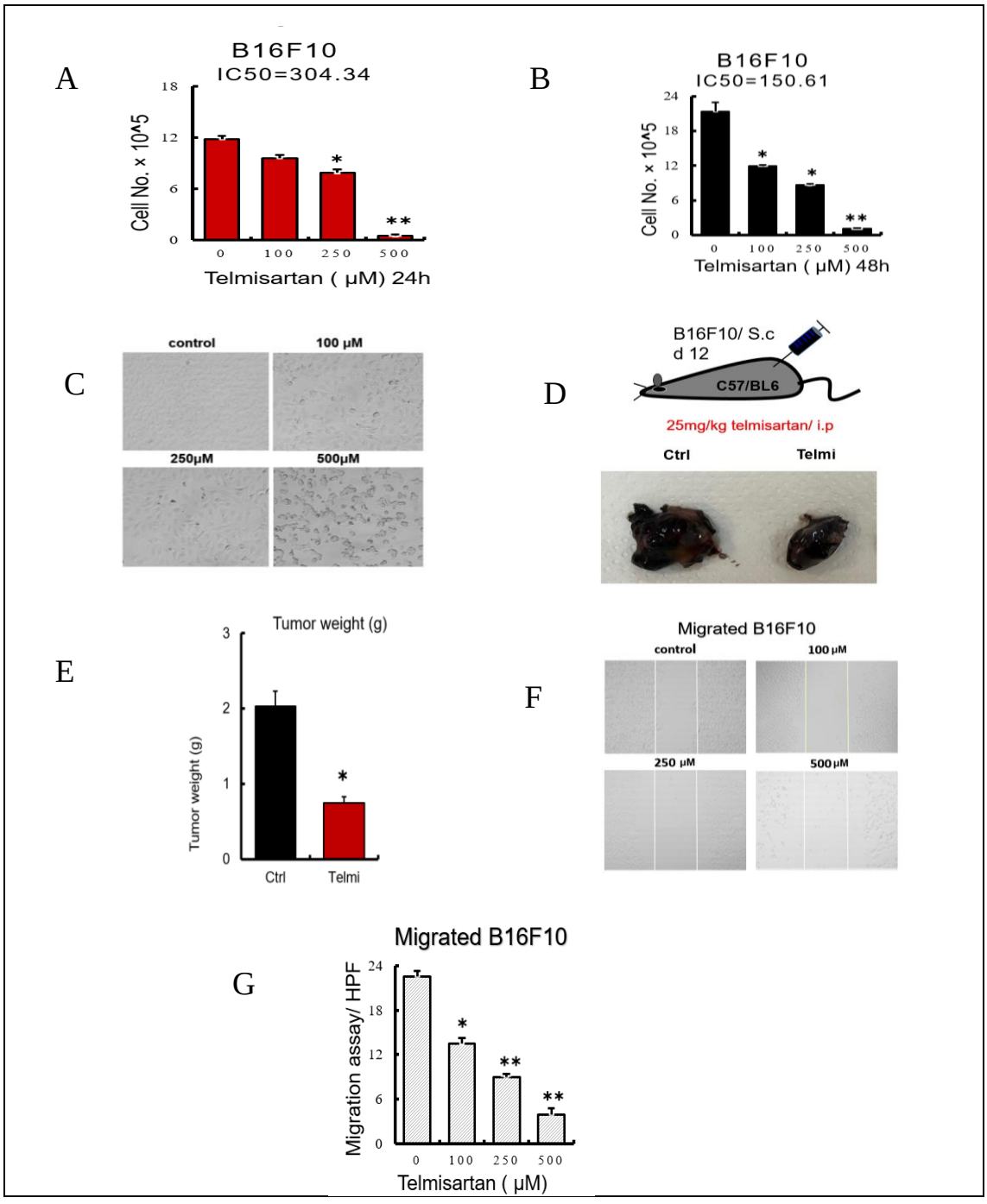
3.1 Telmisartan inhibits melanoma cell proliferation and migration in vitro and tumor growthin vivo

The proliferation of the human melanoma cell line B16F10 has been demonstrated to be affected by telmisartan. The cells have been treated with varying concentrations of telmisartan (0,100,250,500) µM for 24 and 48 h.When cells were counted 24 hours following the addition of telmisartan, a reduction in cell viability was observed.The half maximal inhibitory concentration (IC_{50}) which is calculated by the equation (Y = a * X + b, IC50 = (0.5 - b)/a) was 304.34 µM is indicated in (Figure 1A).Whereas, when the cells were counted after 48 hours,substantial inhibition of cell proliferation was especially detected and the (IC_{50}) of telmisartan was observed to be 150.61 µM (Figure 1B) .As a result of the findings, the cell viability rate decreased with increasing telmisartan concentration and incubation time, demonstrating that the growth of B16F10 cells was suppressed by telmisartan in a concentration- and time-dependent way. Images of B16F10 cells treated with or without telmisartan were obtained using a microscope. They show that the number of vible cells with spindle-shaped and epithelial-like cells decreases as the concentration of telmisartan increases(Figure 1C). The in vivo melanoma model offered more proof that telmisartan is efficient at slowing melanoma progression.The right flanks of C57/BL6 mice were injected subcutaneously with B16F10 cells (1×10^6/200 uL/mouse). Five days following the initial tumor inoculation,B16F10 wild-type tumor-bearing mice received telmisartan intraperitoneally once a day at a dose of 25 mg/kg. On day twelve, macroscopic tumor photos were taken (Figure1D). The weight of the tumor was then determined; the control group has a weight of 2 g, while the average tumor weight of mice treated with telmisartan is less than 1 g as shown in (Figure 1E). The findings demonstrate that telmisartan treatment significantlyreduced tumor growth when compared to a control group. A prior study on the esophageal squamous cell carcinoma cell line KYSE-180 demonstrated that high-dose (50 µg/day) telmisartan treatment inhibited tumor growth in a xenograft mouse model. The high-dose group had 74.2% reduced tumor volumes compared to the control group, while the low-dose group (10 µg/day) had smaller volumes [221].

The wound healing assay is a straightforward method for evaluating cell migration in vitro that is based on establishing a physical gapin a confluent cell monolayer of keratinocytes to mimic a wound andthen utilizing live cell imaging to collect images at different times to monitor the migration of cells into the gap.In this study, we used amigration assay to measure B16F10 motility. To assess the impact of telmisartan (0,100,250,500) μM on cell migration. B16F10 cells were exposed to the given doses for a duration of 24 hours.Changes in cell migration were seen using an inverted microscopeto examine the selected fields for each well to quantify the cells that had migrated into the cell-free area as shown in (Figure 1G). The cells in the scratched area were counted by employing high power fields (HPF) to count the migrating cells as seen in (Figure 1H). The findings show that the migration of B16F10 cells treated with telmisartan was dramatically reduced compared to the migration of cells in the control group.Additionally, the number of migratory cells decreases with increasing telmisartan concentration, suggesting that telmisartan has a deleterious effect on B16F10 cell migration ability as compared to the control.Our findings are in line with a prior investigation of the non-small lung cancer cell line A549. That study showed that telmisartan significantly reduced the migration of the A549 cell line by inhibiting the migratory capacity of cells in the telmisartan-expressing group relative to the control group [217].

Figure 1

Telmisartan inhibits melanoma growth in vitro as well as in vivo



Telmisartan inhibits melanoma cell proliferation and migration in vitro and tumor growth in vivo (A,B) Human melanoma cell line B16F10 were treated with indicated concentrations of Telmisartan (0,100,250,500) µM in a 5% CO2 incubator at 37C, then cells were counted using a hemocytometer after trypan blue staining to investigate the effect of telmisartan on the B16F10 cell line after 24 and 48 hours of incubation (C) Microscopic images of B16F10 cells treated with or without telmisartan were captured using inverted microscopy. (magnification 10x). (D) In vivo melanoma model, B16F10 cells were injected s.c. into the right flanks of C57/BL6 mice,On the fifth day, telmisartan with a dose of 25mg/kg was administered

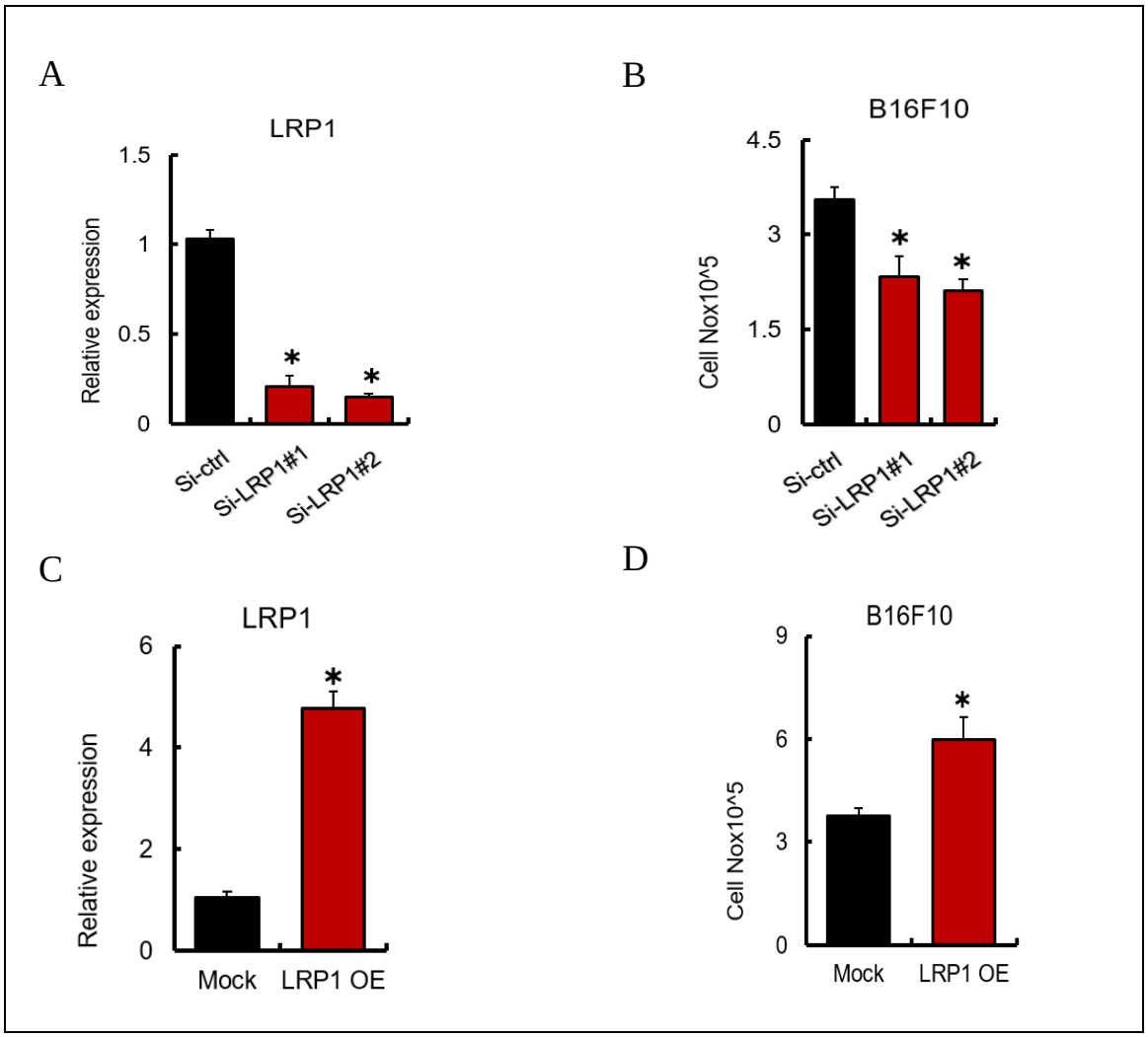
intraperitonially every day to B16F10 wild-type tumor-bearing mice and macroscopic tumor images were captured on day twelve ($n = 6$).(E) The weight of the tumor was determined on day twelve. (F) Changes in cell migration were seen using an inverted microscope following a 24-hour treatment of the B16F10 cells with gradient doses of telmisartan (0, 100, 250, and 500 μM). (G) In the scraped area (right panel), high power fields (HPF) were employed to count the migrating cells. The data is expressed as mean +/- SEM (*p <0.05, ** p <0.01, *** p < 0.001) using the Student's t-test.

3.2 LRP1 promotes B16F10 melanoma cell proliferation

Numerous studies have shown that LRP1 is involved in the development and spread of melanoma.[190].We used (siRNA) in order to knock down LRP1 (LRP1KD). The relative expression of LRP1 in LRP1(KD) was verified by qPC. The findings indicate that Knocking down LRP1 by siRNA will effectively decease LRP1 expression as shown in **(**Figure 2A). We also examine the impact of knocking down LRP1 on B16F10 cell counts. The results show that knocking down the expression of LRP1 will effectively reduce B16F10 cell proliferation and viability, as illustrated in (Figure 2B). We investigated the impact of LRP1 on melanoma cell proliferation by overexpressing LRP1 (LRP1 OE) using lentivirus. The expression of LRP1 in LRP1 (OE) was verified by qPCR(Figure 2C). The results show that LRP1 overexpression, as opposed to the control, increased B16F10 cell proliferation (Figure 2D).Consequently, LRP1 was linked to an advanced stage and badprognosis, suggesting that LRP1 may be a novel target for melanoma therapy and enhance survival.

Figure 2

LRP1 promotes B16F10 melanoma cell proliferation



Impact of LRP1 knockdown and overexpression on melanoma cell growth(A)In si-RNA gene silenced si-LRP1 or si-ctrl, a comparison was made between the expression of si-LRP1 and the si-ctrl. qPCR was used to measure the fold change in LRP1 transcript levels. (B) The proliferation of B16F10 cells in si-ctrl and LRP1(KD). (C) A comparison was made between the expression of LRP1 (OE) and the Mock controls. qPCR was used to measure the fold change in LRP1 transcript levels. (D) The proliferation of B16F10 cells in mock and LRP1 (OE). The data are presented as mean ± SEM (one-way ANOVA or unpaired Student's t-test; * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$).

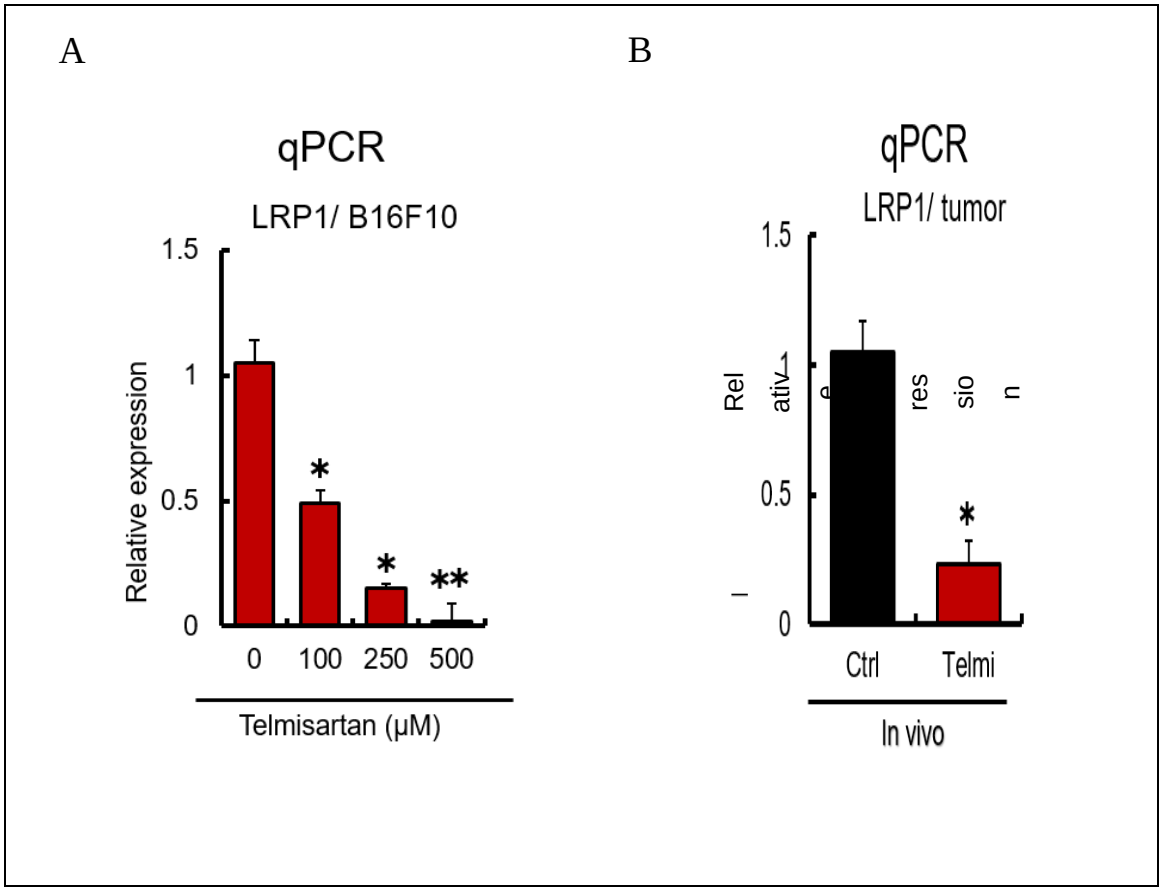
3.3 Telmisartan downregulates the expression of LRP1 on melanoma cell

Numerous investigations suggest that LRP1 regulates the development of melanoma cells. We looked at LRP1 transcript levels in response to telmisartan treatments at 0, 100, 250, and 500 μMand fold change in LRP1 expression was measuredby qPCR (Figure 3A).The findings show that telmisartan reduced LRP1 expression in B16F10 cells in a dose-dependent manner,indicating that telmisartan reduces LRP1 expression in

melanoma cells in vitro. We also examined the expression of LRP1 in vivo. The mice were split into groups and given either a placebo or telmisartan on the fifth day. On day 12, qPCR was used to measure LRP1 expression. Additionally, we confirmed that telmisartan treatment reduced LRP1's in vivo expression (Figure 3B).

Figure 3

Telmisartan suppresses LRP1 expression in melanoma cells both in vitro and in vivo



Telmisartan downregulates the expression of LRP1 on melanoma cell(A) Fold change in LRP1 expression was measured in B16F10 cells treated with various telmisartan concentrations (100, 250, and 500 μM). The expression was compared to a control group of untreated cells. (B) On the fifth day, mice were divided into groups and given telmisartan or placebo. On day 12, LRP1 expression was determined by qPCR. The data are presented as mean ± SEM (one-way ANOVA or unpaired Student's t-test; * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$).

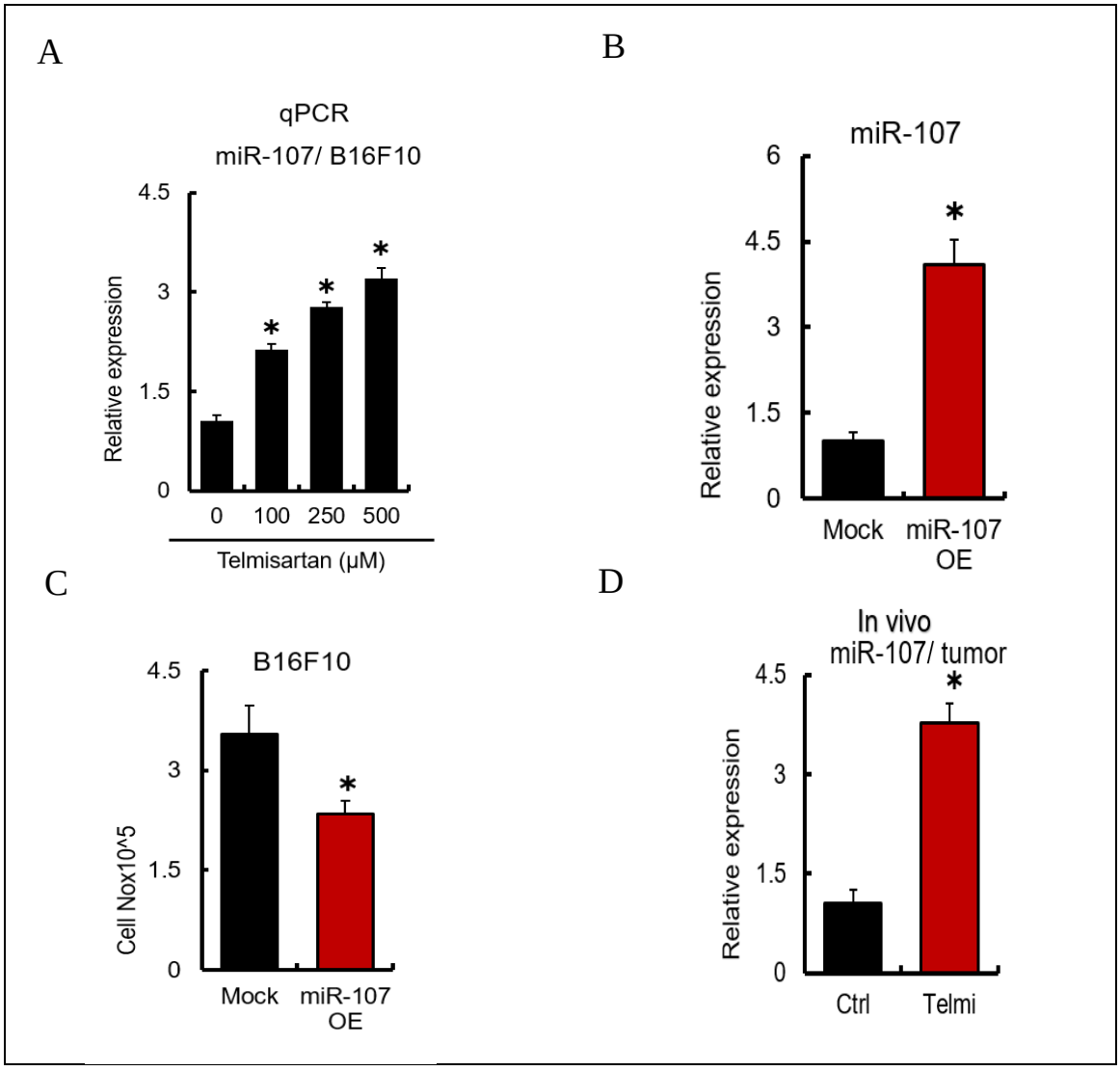
3.4 Telmisartan upregulatesthe tumor suppressor miR107 expression

In human and mouse melanoma cell lines, the tumor suppressor miR-107 and its host gene PANK1 were both downregulated according to Salama's report in a preveious study[210].Furthermore, the outcomes demonstrate that telmisartan treatment for 24h enhancesthe expression of miR107in B16F10 cells in a dose-dependent manner.Relative

expression of mir107 is verified by qPCR(Figure 4A).Lentivirus causes miR-107 overexpressionand qPCR was performed to measure the fold change in miR-107 expression in miR-107 OE cells compared to mock (Figure 4B).We also examine the impact of miR107 overexpression on B16F10 cell count.The outcomes demonstrated that B16F10 cell viability was reduced in the lentivirus-generated miR-107 OE cells compared to the control melanoma cells as illustrated in (Figure4C).Fold change in miR-107 expression as assessed by qPCR, between the tumor tissues of mice treated with telmisartan on day 12 and the control tissues obtained on day 0.The results show that tumors in telmisartan-treated mice exhibited high expression of miR-107, whereas control mice did not exhibit this same finding as shown in (Figure 4D).

Figure 4

Telmisartan upregulates miR107 expression



Telmisartan upregulates miR107 expressionin vitro as well as in vivo.(A) For 24 hours, B16F10 cells were maintained with appropriate concentrations of telmisartan (100,250,500) and control .Fold change in miR-

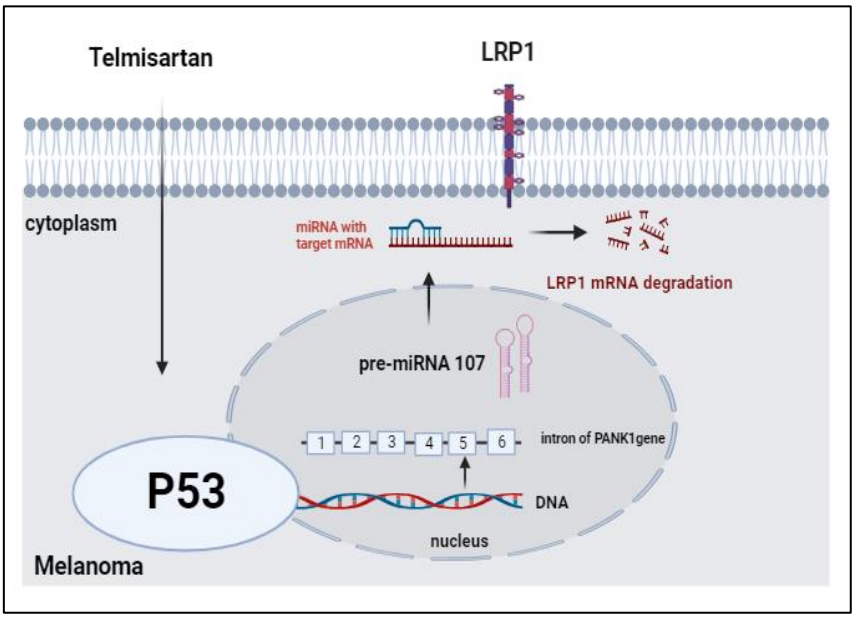
107 expressions were measured and compared to expression in control cells that had not received any treatment. (B) qPCR analysis revealed the fold change in miR-107 expression in miR-107 OE relative to mock (C) qPCR analysis revealed the fold change in miR-107 expression in miR-107 OE relative to its expression in mock B16F10 cells (n = 6/group). (D) Fold change in miR-107 expression, as assessed by qPCR, between the tumor tissues of mice treated with telmisartan on day 12 and the control tissues obtained on day 0. (n = 6/group). The information is shown as mean ± SEM (one-way ANOVA or unpaired Student's t-test; * $p < 0.05$, ** $p < 0.01$).

3.5 Telmisartan-induced miR107 expression downregulates LRP1 in melanoma cells

A new regulatory circuit, including Telmisartan, LRP1, TP53, and miR107, was found to be essential for melanoma cells. We demonstrate that the antihypertensive drug telmisartan exertsan anti-melanoma effect. It inhibits melanoma growth and metastasis by activation of P53 by phosphorylation. Restoration of P53 enhances the expressionmiR107, which is mapped to intron 5 of the PANK1 gene. Next, the tumor suppressor miR107 targets and downregulatesthe LRP1 transcript responsible for melanoma cell migration and invasion. This inhibits tumor growth in vivo as well as cell proliferation in vitro. So, it was shown thattelmisartan is recognized as a P53 inducer in melanoma cells, which consequently regulates the development of tumors mediated by LRP1(Figure5).

Figure 5

Telmisartan-induced miR107 expression downregulates LRP1 in melanoma cells



Proposed telmisartan mechanism of action in melanoma. Telmisartan upregulate P53 by phosphorylation which in turn activates PANK1/miRNA-107 gene, targeting LRP1 that responsible for melanoma migration

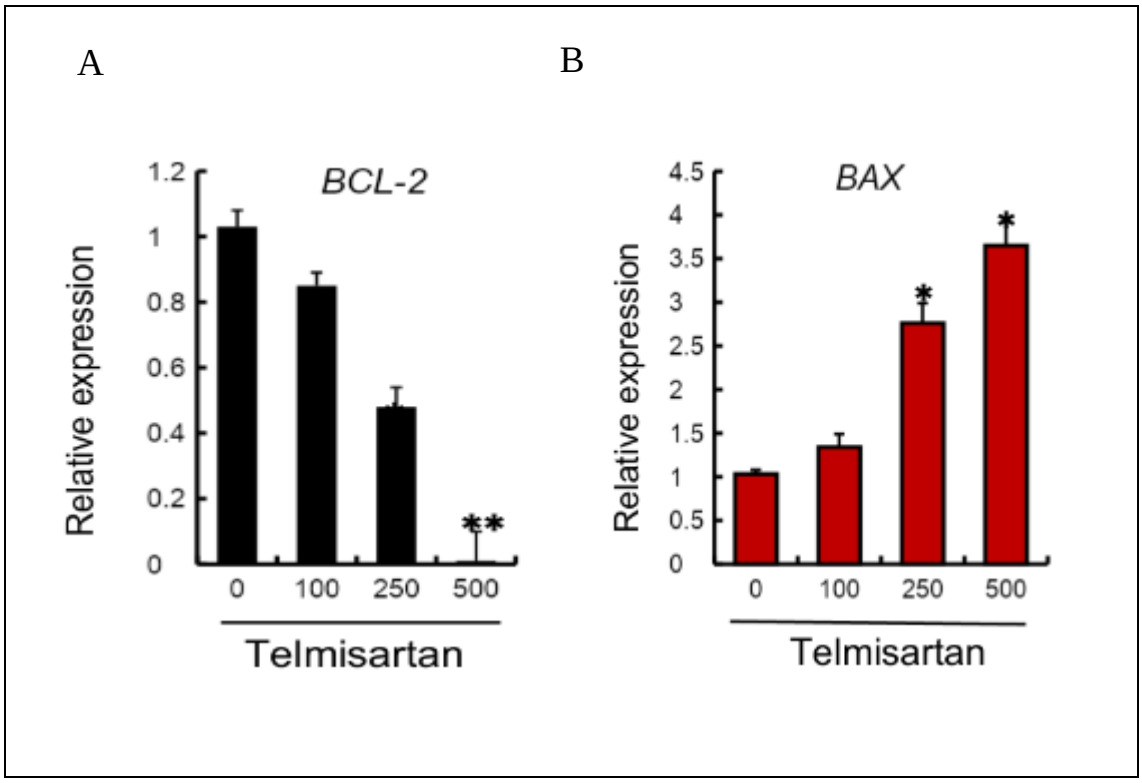
and invasion. Abbreviations: LRP1 stands for low-density lipoprotein receptor-related protein 1, PANK1 represents pantothenate kinase 1, and miR-107 refers to micro-RNA-107.

3.6 Telmisartan induces apoptosis in B16F10 cells

The BCL-2 family has been proven to be a key regulator of programmed cell death. In metastatic melanoma, it has been found that the anti-apoptotic BCL-2, which safeguards cells from apoptosis, is upregulated while the pro-apoptotic protein BAX, which encourages apoptosis to control the growth of abnormal cells and restrict their proliferation, is downregulated. In the present study, we examine the expression of these two apoptotic genes subsequent to telmisaeran treatment to assess the effectiveness of this drug on these proteins [222]. We investigate the expression of apoptosis-related genes (BCL-2 / BAX) in the B16F10 melanoma cell line treated with telmisartan.Telmisartan (100, 250, 500 μM) and a control were added to B16F10 cells and grown for a duration of 24 hours. The findings showed that as telmisartan levels increased, the expression of the anti-apoptotic protein BCL-2 dropped. By using qPCR, the BCL2 gene's expression was assessed (Figure 6A). Furthermore, we assessed the expression of the BAX apoptotic activator in B16F10 cells treated with telmisartan, and the fold change in BAX gene expression was determined using qPCR. The study found that telmisartan treatment resulted in greater levels of BAX expression compared to the control group, with a statistically significant value of $p<0.05$. Based on these findings, telmisartan could promote the apoptosis of B16F10 melanoma cells (Figure 6B).

Figure 6

Telmisartan promotes B16F10 cell apoptosis via downregulation of BCL-2 and upregulation of BAX



Telmisartan induces apoptosis in B16F10 cells By downregulating BCL-2 and upregulating BAX.**(A)**For 24 hours, B16F10 cells were cultured with recommended concentrations of telmisartan (100,250,500) and control. Expression of BCL-2 gene was evaluated by qPCR (B)Fold change in BAX gene expression was measured in B16F10 cells treated with various telmisartan concentrations (100, 250, and 500 μM). The expression was compared to a control group of untreated cells.By using qPCR, the expression of the BAX gene was evaluated; a value of $p<0.05$ was considered statistically significant.

Chapter Four

Discussion

The most recent forecasts from the American Cancer Society indicate that the prevalence and propagation of cutaneous melanoma are increasing every year,highlighting the pressing need to find efficient treatments [223].

The development of new pharmaceuticals is expensive, and it frequently takes between 10 and 15 years from initial discovery to get clinic approval. On the other hand, repurposing medications that have previously received approval for different uses permits their swift inclusion into clinical trials and healthcare [224].

The absence of biomarkers and targeted drug resistance impedes the achievement of successful cancer therapy. It may identify the multifunctional transmembrane protein LRP1 because of its versatility in recognizing various ligands.It is still unknown how LRP1 functions in the B16F10 melanoma cell line.

The endocytic receptor LRP1 has been linked to cancer progression. According to a study conducted in 2023 about the Impact of LRP1 on the gastrointestinal cell line, the findings revealed that knocking down LRP1 suppressed the growth and migration of GI cancer cell line, indicating that LRP1 is a useful therapeutic target for the management of GI cancers [225].

In the other hand,numerous investigations have revealed a strong correlation between low LRP1 expression and advanced tumor stages, as well as poor survival in melanoma [226]. Furthermore, several studies have shown that LRP1's interaction with apoE and tPA encourages the growth and metastasis of melanoma cells [227].

In more than 80% of melanomas, the transcription factor P53 is still present in its wild-type state [228]. It also has the nickname "guardian of the genome "due to its ability to bind to numerous genes' promotor regions and alter gene expression [229]. An earlier study demonstrated that P53 can cause cell cycle arrest and apoptosis and prevent nevi from developing into melanoma [159].

MiR107 is downregulated in melanoma. Our results are consistent with past studiesproviding evidence that miR-107 functions as a tumor suppressor in melanoma by

binding to the 3’UTR of POU3F2, which significantly reduces melanoma cell proliferation and migration [230].

According to computer analysis, the PANK1/miR107 transcription starting site contains a p53-binding site 1,811 base pairs upstream [231]. Chen and colleaguesshowed that P53 selectively targets miR107, connects to its promoter region, and boosts its expression, limiting the proliferation of glioma cells [232].

Telmisartan is an antihypertensive medicationthattargets the peroxisome proliferators-activated receptors γ (PPAR-γ) to influence lipid metabolism[233]. As reported in previous studies, telmisartan has anticancer properties by reducing cell proliferation and tumor progression in cholangiocarcinoma [234] and inducing apoptosis in human endometrial cancer [235].

Moreover, Tel inhibits the proliferation and migration of esophageal squamous cell carcinoma by inducing S-phase arrest in vitro and tumor growth in vivo, according to recent research conducted in 2019[236]. On the other hand, no studies have been conducted to determine how telmisartan inhibits the growth and metastasis of the B16F10 melanoma cell line.

Therefore, a novel mechanism for how telmisartan affects melanoma cell viability is proposed for the first time in this study. We demonstrate that telmisartan promotes its antiproliferative effect by phosphorylating TP53, and subsequently, p53 activates the PANK1/miRNA-107 gene, which in turn targets LRP1, downregulates LRP1 expression, and inhibits melanoma cell proliferation in vitro and tumor growth in vivo.

We demonstrate that the expression of miR-107 was increased by p53 induction caused by telmisartan, which led to the downregulation of the LRP1 oncogen. Also, Salama reported that P53 enhances the expression of miR107, which in turn downregulates LRP1 on melanoma, inhibits tumor growth, and induces apoptosis [210].

The outcomes revealed that telmisartan suppresses the B16F10 melanoma cell line's ability to proliferate in a way that is based on both time and concentration. As the concentration and incubation time of telmisartan increase, the viability of the cells decreases.Our findings further confirm that telmisartan inhibits the migration of B16F10 melanoma cell lines by reducing the number of migrating cells as its concentration

increases,suggesting that this antihypertensive drug can act as a multimodal anticancer agent in vitro.

We found that lentivirus-induced overexpression of LRP1 in the B16F10 cell line increased the cell line's metabolic capacity and sped up its proliferation. Moreover, LRP1 overexpression prevented melanoma-mediated melanoma growth inhibition. We used qPCR to verify the LRP1 expression. Consequently,thissupports our hypothesis regarding the function of LRP1 in melanoma progression.

We demonstrate that knocking down LRP1 by siRNA reduces the expression of LRP1 and changes the biological characteristics of melanoma cells (Viability, proliferation, and metastasis).The findings also show that LRP1 expression is reduced in vivo and in vitro by telmisartan-induced phosphorylation of P53 in melanoma.

We also identified telmisartan as powerful P53 inducer in the melanoma cell line. On the other hand, we found that overexpression of miR107 reduced melanoma cell survival in vitro and inhibited tumor growth in vivo when telmisartan treatment upregulated the expression of miR107 in B16F10 cells in a dose-dependent way.

Furthermore, changes in apoptotic gene expression have been linked to cancer progression and metastasis. Prior investigation has demonstrated that melanoma is characterized by overexpression of BCL-2.When compared to normal melanocytes, its level increased in the advanced stages of melanoma, causing tumor growth and metastasis. Conversely, it seems that BCL-2 protein dysregulation appears to be crucial for both melanoma survival as well as drug resistance [222]. In contrast, another study found that low expression of the pro-apoptotic BAX plays a substantial role in melanoma progression and is linked with a bad prognosis, whereas high expression correlates with a better prognosis. These prior papers, together with our data, suggest that high BAX expression and low BCL-2 expression result in more effective treatment strategies for this fatal malignancy [237].

Therefore,the study of these genes after treatments in the targeted way can assess the response of the treatment and the prognosis of the cancer. According to our analysis, telmisartan therapy is another potentially useful treatment option for melanoma.The findings indicate that telmisartan causes B16F10 cells to undergo apoptosis by

upregulating the expression of the protein BAX, which is pro-apoptotic, and downregulating the expression of BCL2, which is anti-apoptotic.

Last but not least, a previous study demonstrated that lethal stress doses upregulate p53-regulated miR-107, decreasing LRP1 translation [160]. This finding supports our idea that telmisartan inhibits melanoma growth and metastasis through LRP1suppression, and additional research is required to corroborate this mechanism.

4.1 Strength points and limitations of the study

4.1.1 Strength points

Pioneering study: This was the first study of its kind to be conducted about the role of the antihypertensive drug telmisartan on melanoma, representing a significant achievement in the field of melanoma cancer research.

Novel approach to treatment: The study paved the way for new, innovative approaches to treating melanoma, which could significantly improve patient outcomes.

Rigorous methodology: The experiments were conducted multiple times to ensure reliable and valid results, demonstrating a rigorous and significant approach to the research.

4.2 Limitations

In human therapy, telmisartan doses between 1 and 10 µM are indicated. However, in this in vitro trial, we are using higher doses of the medicine than recommended. So, Transferring these variables into a therapeutic setting might be difficult because the pharmacokinetics of the medicine may vary depending on the cultural context and the human body.

4.3 Recommendations

- Further investigations are recommendedbefore using telmisartan to treat malignant melanoma in clinical research. Since the telmisartan dosage used in our investigation was not clinically achievable.
- -In future studies, it is recommended to determine the precise telmisartan concentration to ascertain whether distinct p53 target genes exhibit inverted expression patterns

contingent on telmisartan concentration and that any dose variation can significantly impact protein expression and cell outcome.

– Further research on p53-regulated genes is also recommended since this may reveal a number of additional p53 targets that are regulated in the same way.

4.4 Conclusion

Telmisartan, an antihypertensive medication, has been shown in this study to have some therapeutic efficacy. It appears from our research that telmisartan has anti-proliferative and anti-metastatic effects on melanoma by inhibiting melanoma cell lines both in vitro and in vivo.

Telmisartan was found to be a P53 inducer. Telmisartan-induced phosphorylation of P53 increases the expression of the tumor suppressor miR107, which specifically targets LRP1, which is responsible for the proliferation of melanoma cells. For the therapeutic treatment of melanoma, telmisartan appears to be a promising candidate for pharmacological repurposing.

List of Abbreviations

Abbreviations	Meaning
AGTR1	angiotensin-receptor 1
AIDS	Acquired immunodeficiency syndrome
AJCC	American Joint committee on cancer
ALM	Acral lentiginous melanoma
α2M	alpha 2 macroglobulin
ARBS	angiotensin receptor blockers
Apaf-1	Apoptotic protease activating factor-1
BAP1	BRCA1-associated protein 1
BAX	BCL2-associated X
BCL2	B-cell lymphoma 2
CAFs	Cancer-associated fibroblasts
CD91	cluster of differentiation 91
cDNA	Complementary deoxynucleic acid
CT	computed tomography
CTLA4	cytotoxic T lymphocyte-associated antigen 4
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EGF	Epidermal growth factor
FBS	Fetal bovine serum
FDA	Food and drug administration
HCC	Hepatocellular carcinoma
HCT116	Human colorectal carcinoma
HIV	Human immunodeficiency virus
IARC	International Agency for Research on Cancer
IC_{50}	The half maximal inhibitory concentration
IRB	Institutional Review Board
KD	Knockdown
KDa	Kilodalton
LDL	Low density lipoprotein
LDH	Lactate dehydrogenase
LRP1	Low density lipoprotein receptor –related protein1
LMM	Lentigo maligna melanoma

MAPK	Mitogen activated protein kinase
MBM	Melanoma brain metastasis
MDM2	Murine double minute 2
miR	micro RNA
MM	Mucosal melanoma
μM	micro molar
MOM	Mitochondrial outer membrane
MRI	Magnetic resonance imaging
miR	micro RNA
mRNA	messenger ribonucleic acid
NCCN	National Comprehensive Cancer Network
NM	Nodular Melanoma
NSCLC	Non-small cell lung cancer
OE	Overexpression
PANK1	pantothenate kinase enzyme 1
PBS	Phosphate buffered saline
PD1	Programmed death 1
PDGF	Platelet derived growth factor
PI3Ks	Phosphoinositide 3-kinases
PPAR γ	peroxisome proliferator-activated receptor gamma
PTP	permeability transition membrane
qPCR	Quantitative reverse transcriptase-polymerase chain reaction
ROS	Reactive oxygen species
RGP	Radial growth phase
Ser 392	Serine 392
SRS	stereotactic radiosurgery
SSM	Superficial spreading melanoma
SEM	standard error of the mean
SLNB	Sentinel lymph node biopsy
siRNA	Small interfering ribonucleic acids
SKCM	Skin cutaneous melanoma
SLNB	Sentinel lymph node biopsy
SPF	skin protection factor
TP53	Tumor protein 53
tPA	Tissue- type plasminogen activator
3′ UTR	3′ untranslated region

UVR	Ultraviolet Radiation
VEGF	Vascular endothelial growth factor
VGP	Vertical growth phase
WBRT	Whole-brain radiation therapy
WLE	wide local excision

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جـامعـــة النجاح الوطنية

كليـــة الدراســـات العليـــا

دواء تيلميسارتان يثبط نمو الميلانوما وانتشارها من خلال تثبيط LRP1

إعداد

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قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في الكيمياء الحيوية السريرية، من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس– فلسطين.

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الملخص

الورم الميلانينيهو النوع الأكثر شيوعا من سرطان الجلد في جميع أنحاء العالم، والذي لديه تشخيص سيئ بسبب ارتفاع معدل انتشاره وطبيعته العدوانية. النوع البري p53 يتم التعبير عنه بشكل شائع في الميلانوما، الذي تم تنشيطه استجابةً للاجهاد الخلوي مثل نشاط الاونكوجينLRP1 (CD91). هو بروتين متعدد الوظائف تم ربطه بتطور سرطان الجلد .لقد تم اكتشاف أن TP53 ينظم تطور الأورام بوساطة LRP1. تيلميسارتان، وهو دواء خافض لضغط الدم، يمتلك خصائص مضادة للسرطان تجعله فعالا في علاج سرطان الجلد. علاوة على ذلك، يعد Mir 107 عاملًا حاسمًا في تثبيط الورم ولديه القدرة على تثبيط الجين الورمي LRP1

تهدف هذه الدراسة إلى تحديد تأثير حاصرات مستقبلات الأنجيوتنسين تيلميسارتان على نمو سرطان الجلد وانتشاره، من أجل تقييمه كخيار علاجي محتمل للمرضى الذين يعانون من سرطان الجلد (ميلانوما) والذين يرغبون في تحقيق معدل بقاء مرتفع.

أجريت دراسة تجريبية في مختبر النجاح لأبحاث السرطان والخلايا الجذعية في نابلس. تم زرع خلايا الميلانوما B16F10 ثم علاجها بتركيزات محددة من التيلميسارتان من أجل تقييم قابلية الخلية للنمو والهجرة. أجريت هذه الدراسة في المختبر وفي الجسم الحي باستخدام الفئران C57BL6 من أجل تحديد تأثير تيلميسارتان على نمو الورم. لدراسة تأثير LRP1 على تكاثر خلايا سرطان الجلد، استخدمنا الفيروس البطيء للإفراط في التعبير عن LRP1 وsiRNA لهدم LRP1 في خلايا B16F10، كما استخدمنا

الفيروس البطيء للتعبير عن مثبط الورم miR107، وتعبيرات LRP1 (KD) LRP1 (OE) وتم التحقق من miR107 بواسطة تقنيةٌ qPCR

أظهرت الدراسة أن التيلميسارتان يمنع تكاثر وهجرة خلايا B16F10 سواء في المختبر أو في الجسم الحي)الفئران). بالإضافة إلى ذلك، كشف البحث أن تيلميسارتان يستعيد P53 عن طريق الفسفرة والذي بدوره ينظم التعبير عن مثبط الورم miR107 ثم يؤدي إلى تقليل تنظيم تعبير LRP1، وبالتالي تثبيط نمو الورم الميلانيني وانتشاره

حتى الآن، لا يوجد علاج مثبت للورم الميلانيني. قد يقدم هذا العمل نظرة ثاقبة للتجارب السريرية المستقبلية التي تعيد استخدام تيلميسارتان كعلاج مضاد للسرطان بسبب آثاره المضادة للتكاثر والمضادة للانتشار على سرطان الجلد.(الميلانوما).

الكلمات المفتاحية: TP53، LRP1، miR107، تيلميسارتان، ورم خبيث، سرطان الجلد،زيادة نشاط، تنظيم أقل.