Chapter-I

TARGETING TUMOR MICROENVIRONMENT IN METASTATIC CANCER

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Abstract--- Cancer is the second leading cause of global mortality, accounting for approximately one in six deaths worldwide, with an estimated 9.6 million deaths predicted in 2018. Disproportionately, 70% of cancer-related deaths occur in low- and middle-income countries. At its core, cancer is characterized by the uncontrolled, abnormal growth of cells, resulting from accumulated DNA alterations, which ultimately form a tumor. Accumulated oncogenic mutations allow tumor cells to proliferate and migrate without restriction, aid in invasion and colonization, trigger widespread angiogenesis, and spread. Malignant neoplasms, another name for cancer, are characterized by unchecked cell proliferation that has the capacity to penetrate and spread to other organs. Breast cancer is the most frequent cancer in women and the second leading cause of death globally, accounting for 9.6 million deaths in 2018. The number of instances is expected to rise in the near future. Recent studies have shown that the overexpression of metastasis-associated protein (MTA1), which stimulates angiogenesis, is closely linked to the metastasis of breast cancer and other malignant tumors. The use of the tumor microenvironment as a therapeutic target for cancer has greatly increased in both research and clinical interest. Here, we highlight the difficulties in focusing on the tumor microenvironment to attain therapeutic efficacy and provide an overview of the recent developments in this area in both drug development and clinical trials. address ways to influence the pro-tumor milieu and optimize therapeutic outcomes, as well as investigate novel technologies and methodologies to better understand the tumor microenvironment.

Keywords--- Drug, Resistance, Target, Therapy, Tumor Microenvironment.

1. INTRODUCTION

MTA1, a gene associated with tumor metastasis, is known to regulate other metastasis-related genes. Higher MTA1 levels are linked to several tumor types. Unfortunately, because TNBC patients lack ER, PR, and HER2/Neu receptors, their therapy options are limited, and they have the worst prognosis. Positively, chemotherapy usually works well for this kind of breast cancer. Additionally, angiogenesis is necessary for the promotion and maintenance of tumor growth in malignant tumors that are growing aggressively (Anderson & Simon, 2020). Consequently, it is well known that anti-angiogenic therapy in conjunction with chemotherapy is a crucial cancer treatment approach. Consequently, there is a great demand for novel anti-cancer drugs with few adverse effects.

In this study, we examined the impact of several new chemicals, both synthetic and derived from plants, on their ability to prevent cancer. The Soxhlet extraction method was used to purify Dioscorea bulbifera root extracts in an activity-guided manner. Only the ethyl acetate, acetone, and methanol extract out of the eight solvent extracts were determined to be the most promising. Additionally, we created nine new benzisoxazole (7a-i) compounds and evaluated their potential for biological activity. 3- (1- ((3- (3- (Benzyloxy) -4-methoxyphenyl) -4, 5- dihydroisoxazole- 5- yl) methyl) piperidine-4-yl)6-fluorobenzo[d] isoxazole (7e) was determined to be the most promising of the synthesized compounds. The chemical (7e) and D. bulbifera active extracts significantly inhibited the migration and proliferation of breast cancer cells stimulated by recombinant MTA1. It is well established that MTA1-protein is essential for both angiogenesis and cancer. In order to serve as a positive inducer in each experiment, a recombinant MTA1 protein was produced and purified. In vivo, the chosen drugs demonstrated angio-inhibitory and anti-tumor effects. Additionally, nuclear labeling and flow cytometry analysis were used to visualize the inducing apoptotic process. These results demonstrate that D. bulbifera root active extracts have the potential to be a rich source of bioactive compounds, and compound 7e's reported biological activity makes it an appealing therapeutic candidate.

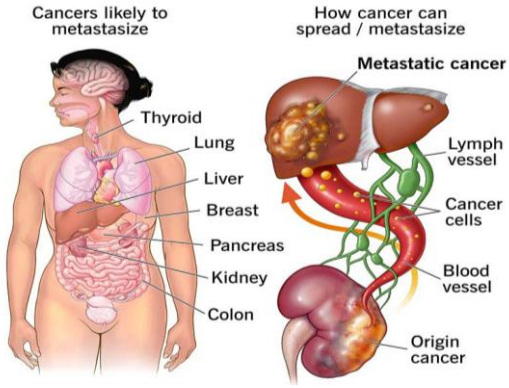

Figure 1: Metastatic Cancer (source: web)

When combined, our findings show that MTA1 promotes breast cancer survival and progression. For this reason, the third objective involved using RNA interference technology to silence the MTA1 gene and creating stable transfected clones with MTA1-shRNA using the mammalian transfection vector pCDNA-3. Using western blotting, immunofluorescence, and qPCR analysis, the complete suppression of MTA1 expression in silenced clones was confirmed. Additionally, the migratory ability of these clones was evaluated using the scratch assay, where MTA1-shRNA successfully blocked the wound closing while the scramble wound was fully closed. Additionally, it was clear from real-time PCR study of EMT regulatory genes that MTA1 knockdown resulted in lower expressions of EMT transcription factors TWIST and SNAIL and increased expression of E-cadherin. These results support novel strategies for the treatment of breast cancer (Aswathy et al., 2020).

2. CANCER AND ITS BRIEF HISTORY

Hallmarks of Cancer

According to Hanahan and Weinberg (2000), cancer cells are generally distinguished by their capacity to produce their own growth signals, disregard growth-inhibitory signals, prevent cell death, multiply endlessly, maintain angiogenesis, and infiltrate tissues through capillary walls and basement membranes. Finally, one of the characteristics of cancer is the avoidance of immunosurveillance (Dunn, Old, & Schreiber, 2004). When a tumor does not spread to the surrounding tissues, it is considered benign. On the other hand, the tumor is considered malignant if it spreads to distant tissues and invades nearby tissues

(Almeida & Barry, 2011). The etiology of certain tumors is not fully understood (Kilmister et al., 2022). Nonetheless, a number of risk factors that increase an individual's vulnerability to cancer have been found. According to the National Cancer Institute, these include heavy smoking, exposure to UV rays, drinking alcohol, eating poorly, being sedentary and obese, having chronic infections, having a family history and genetic predisposition, having hormones, and being around dangerous substances or radiation. A limitless supply of chemically varied chemicals can be obtained from natural plant products, either as pure compounds or as plant extracts.

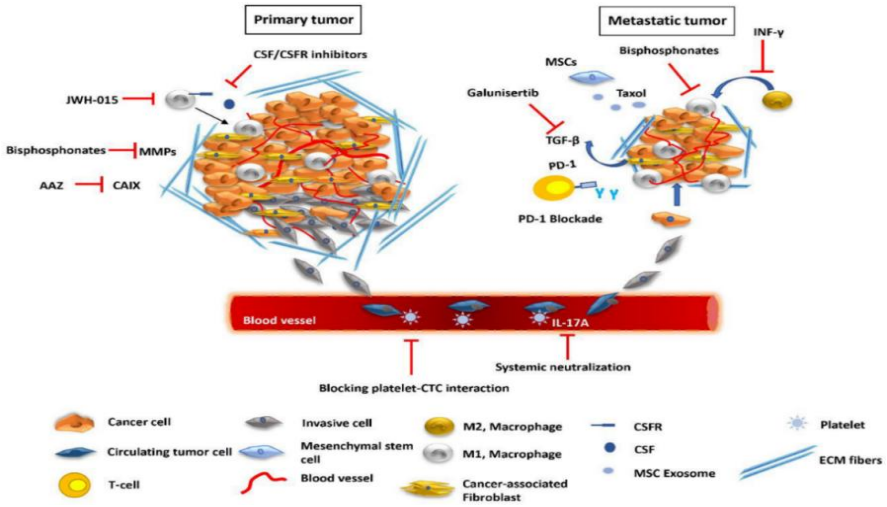

Figure 2: Metastatic Tumour Environment (source: web)

Traditional medicine continues to be the mainstay of medical care in Asia and other regions of the world (Greenwell & Rahman, 2015a). Numerous compounds found in plants are used to cure a variety of illnesses. As a result, studies have been conducted to look into the possible anti-cancer properties of many plants that are utilized in traditional herbal therapy. Plant phytochemicals have a solid track record of being used to treat cancer. A study claims that over half of the anticancer medications on the market come from plant origins (Shaikh, Pund, Dawane, & Iliyas, 2014). However, because chemotherapy resistance is a significant barrier to effective treatment, they are linked to negative side effects, and often, the effects are short-lived. Therefore, to lessen the health burden of this debilitating condition, there is a great need for novel anticancer drugs with few adverse effects. The air

yam, or Dioscorea bulbifera, is a traditional medicinal plant that is classified within the Dioscoraeceae family. It contains the steroidal saponin diosgenin and is composed of organic acids and polyphenols, some of which may be antioxidants.

Transformation and Isolation of Plasmid pGEX-5X-2-MTA1

The MTA1 insert-containing plasmid pGEX-5X-2 was obtained by aseptically inoculating fresh cultures or transforming competent cells from glycerol stocks. The transformation of chemically competent E. coli followed the manufacturer's instructions. One vial of cells was briefly thawed on ice. The bottle was then gently combined with 100ng of the plasmid DNA (pGEX-5X-2-MTA1). After 30 minutes of ice incubation, the cells received a brief heat shock treatment for 30 seconds at 42°C. The vial was immediately put on ice for two minutes. A Qiagen kit from Germany was used to further inoculate a subset of colonies from each plate in order to isolate the plasmid. In short, 500ml of an overnight LB culture was centrifuged at 4000g for 10 minutes in order to extract cells. After that, the pellet was reconstituted in Buffer (P2) with RNase A already added. After that, the cell suspension was lysed for five minutes at room temperature using Lysis Buffer (P3). After centrifuging the mixture for 10 minutes at 12000g, the clear supernatant was transferred to a column that had been pre-equilibrated with QBT buffer and drained using gravity flow. Wash Buffer (QC) was used to clean the column after the lysate filtration process was finished. Elution Buffer (QF) was then added to the column to elute the plasmid DNA (Belli et al., 2018). The DNA pellet was centrifuged at 15000g for 30 minutes at 4°C, and isopropanol was eliminated by washing it with 70% ethanol. After carefully removing the supernatant, the DNA pellet was allowed to air dry before being dissolved in the smallest amount of TE buffer. UV spectroscopy was used to evaluate the plasmid DNA's purity and concentration.

Agarose Gel Electrophoresis to Confirm Plasmid

TAE buffer (39 mM Tris, Acetic Acid, 0.5 M EDTA) was used to make a 0.8% Agarose solution. Ethidium bromide (0.5µg/ml) was then added to the solution and left to settle. The gel was put in the TAE buffer-filled electrophoresis tank. In a different lane, 1µg of plasmid and a conventional 1 kb DNA ladder were added. The gel was recorded and the electrophoresis was run at 50V.

Expression and Purification of Recombinant MTA1-Protein

IPTG induction was used to produce MTA1 protein in BL21 strains of E. Coli culture that carried the plasmid pGEX-5x-2-MTA1. After being inoculated into 5 milliliters of LB media, transformed colonies were cultured with ampicillin-containing media for the entire night. After moving the culture into bulk media and growing it till the OD reached 0.3, 50µM IPTG was added, and the shaker incubator was left to incubate it overnight. Processing of the expressed protein downstream: The culture was centrifuged for 10 minutes at 40C at 6000 rpm. PBS with a protease inhibitor cocktail and triton X-100 was used to resuspend the pellet. After being sonicated, the liquid was centrifuged. After centrifuging once again, the obtained supernatant was combined with PBS that included 2% triton X-100 N-Lauryl sarcosine and protease inhibitor. Protein-containing supernatant was measured and put through an affinity column for further purification.

Purification of MTA1-Protein by GST Tag Column

Glutathione S-transferase (GST) tag, which possesses affinity for recombinant MTA1-protein, is present in the affinity column. The binding buffer was used to equilibrate the column. Using 1% triton X-100, the bacterially expressed crude protein was put onto the column. Binding buffer was used to re-equilibrate the column until the OD reached 0.05. All of the unattached proteins are eliminated in this stage. Next, elution buffer containing reduced glutathione was used to elute the bound protein sample, and fractions were collected until the optical density (OD) reached zero. The absorbance at 280 nm was used to pool all of the peak fractions.

SDS-PAGE and Detection by Silver Staining

To perform SDS-PAGE, a 1 mm thick polyacrylamide gel was prepared. A gel cassette was made up of three vacuum-greased spacers that separated two glass plates that were 1 mm thick. They clamped the glass panels together. A resolving gel was put into the gel cassette, and then a stacking gel was put on top. Ten microliters of TEMED were added to the mixture. A plastic comb was placed into the stacking gel to create slots for loading the samples after the contents had been poured over the resolving gel. Once the gel had polymerized, the comb was taken out. After removing the bottom spacer, the glass cassette was put onto the

electrophoretic device. The running buffer was added to the electrophoresis chamber. Protein samples were heated to a boiling water bath for 5 to 8 minutes after being combined with 5X sample buffer with mercaptothions and bromophenol blue.

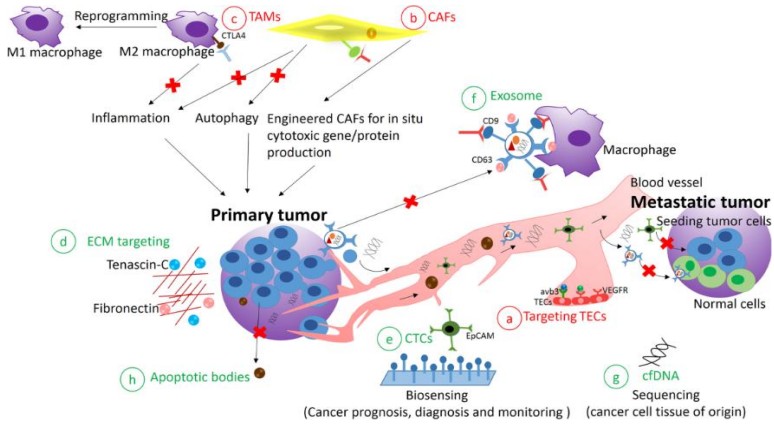

Figure 3: Tumor Targeting Environment (source: web)

Silver Staining

The gel was fixed overnight in a fixative solution (50µl formaldehyde, 12% acetic acid, and 50% v/v ethanol) following electrophoresis. After being pre-treated with 0.02% sodium thiosulphate for two minutes, they were then rinsed three times in distilled water after being cleaned three times with 50% ethanol for 20 minutes each. After 45 minutes of soaking in a 0.2% silver nitrate solution with 75µl of formaldehyde solution, the gel was carefully cleaned with distilled water. After developing the gel in a developing buffer (6% sodium carbonate and 50µl formaldehyde), the reaction was stopped with acetic acid after protein bands were visible.

Western Blotting

In short, Lowry's protein estimate was used to quantify the concentration of the recombinant protein MTA1 following purification. A protein sample of about 50 µg was separated on a 12.5% SDS-PAGE, electrotransferred to a PVDF membrane, and then left overnight in blocking buffer (5% skim milk solution). The following day, it was incubated for two hours with a 1:500 dilution of the anti-MTA1 primary

antibody, and then with a 1:5000 dilution of the HRP-tagged secondary antibody. The Enhanced Chemi Luminescence (ECL) method (Bio-Rad, USA) was used to create the immunoblot in a BioRad Gel document (Tsai et al., 2014).

Trypan Blue Dye is Used in an Exclusion Experiment for Different Solvent Extracts

24 well culture plates with full DMEM medium were planted with 1 x104 cells per well. After 12 hours, the media was swapped out with a full medium that contained various chemicals (plant extracts 100µg/ml and benzoisoxazole derivatives (7a-i) 100µM) and the corresponding controls. The incubator was then humidified and kept at 370C with 5% CO2. Following incubation, cells were extracted using centrifugation for five minutes at 3000 RPM.

3. MTT ASSAY

After being seeded with full DMEM media in a 96-well tissue culture plate (Nunc MicroWellTM), MDA-MB-231 cells (3x10^4) after 4 hours of incubation at 37°C. Medium blanks, treatment controls, and vehicle controls (0.1% DMSO) were all used in triplicate for the assay.

Proliferation Assay

After that, 10 ng of MTA1 protein and various solvent extracts of D. bulbifera (100 g/ml) and compound 7e (1, 5, 10, 50, and 100 mM) were given to the cells individually or in combination. The mixture was left to incubate for 48 hours following the addition of 3 [H]-thymidine (1 Ci/ml). Trypsinization of the cells occurred following a wash with PBS. 10% (w/v) trichloroacetic acid and one milliliter of cold water were used to treat the cell pellet. Following the extraction of DNA Radioactivity was measured in each sample after five millilitres of scintillation cocktail fluid were added. The percentage of triplicate data presented as the control mean SEM.

Wound Healing Assay

After being seeded in six-well plates, the cells were cultivated to confluence. Mitomycin-C (10 ng/ml) was given to the cells for three hours before the chemicals were added. A new tip was used to make a small scratch when the cells were 80–90

percent confluent. Medium containing various solvent extracts of D. bulbifera (100 ng/ml) and compound 7e either alone or in combination with MTA1 protein (10 ng/ml) was added to the wells after the cells had been washed with PBS. After that, the cells underwent a full day of incubation. At a 20X magnification, photographs were taken with a Zeiss phase-contrast inverted microscope following incubation.

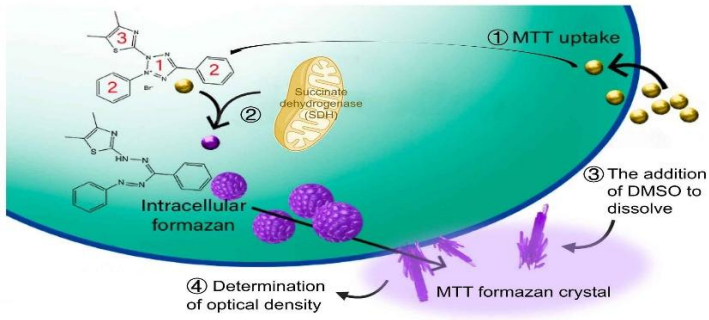

Figure 4: Cell Proliferation Inhibition Assay (source: web)

Peritoneal Angiogenesis in Vivo Assay

The test was conducted using an Ehrlich ascites tumor (EAT)-bearing mouse model to evaluate the anti-angiogenic effects of various solvent extracts and chemical 7e. Female Swiss albino mice were transplanted with EAT cells and treated with compound 7e and different solvent extracts daily from the sixth day onwards, with daily growth measurements recorded. The mice received intraperitoneal injections of EAT cells, and on the sixteenth day, the peritoneal cavity was examined for neovasculature, with photographs taken of the inner lining of the peritoneum to assess angiogenesis (Ji et al., 2013).

Cellular Staining for Apoptosis

It is likely that substances with the ability to alter planned cell death will be beneficial to medicine. In order to examine how D. bulbifera solvent extracts altered the morphology of EAT cells following treatment, Giemsa staining was employed. To sum up, cells from the in vivo test were extracted and washed with PBS after being treated and controlled with different solvent extracts (25 mg/kg b.w.). After being fixed in methanol and stained with a 10% Giemsa solution, the cells were examined

at 40X magnification using an optical light microscope and recorded using a Zeiss AxioVert camera.

Flow Cytometric Analysis

In order to investigate the distribution of cell cycle phases, EAT cells (1x106 cells/ml) were subjected to treatment with or without the chemical 7e (100 M) for a range of times, including 0 hours, 45 minutes, 3 hours, and 6 hours. After the cells were taken, the cell cycle was looked at. For the overnight fixation at 40C, cells were quickly resuspended in ice-cold 70% ethanol. Following centrifugation, the cells were dyed with 10 g/ml propidium iodide, allowed to sit at room temperature for half an hour, and then rehydrated with PBS. The Beckman Coulter Cell Lab QuantaTM SC Flow Cytometer was utilized for the analysis.

4. APOPTOSIS USING NUCLEAR STAINING

Morphological changes in EAT cells undergoing apoptosis after treatment with chemical 7e were analyzed using dual labeling with acridine orange (AO) and ethidium bromide (EtBr). AO stains both living and dead cells, while EtBr specifically stains nuclei of cells with compromised membrane integrity and examined under a Zeiss AxioVert fluorescent microscope after staining with AO/EtBr, allowing for the imaging of apoptotic cells and detection of nuclei.

Chemotherapy is the most common treatment for cancer worldwide. Depending on the stage of the tumor, it may be combined with surgery or radiotherapy. Since the identification of a number of significant mutations that contribute to carcinogenesis. Even though it has become increasingly clear that the TME's influence is important for tumor growth and MDR, cancer research has focused on tumor cells. For a late-stage solid tumor, the tumor microenvironment is extremely diverse and intricate. First, the genomic profiles of the tumor cells alter the majority of the surrounding tissue. The rapid growth of the tumor cells initiates a number of processes, including TME adaptation to the altered environment and hypoxia-induced metabolic reprogramming. The inflammation that occurs at the tumor site frequently leads to the interaction of cancer cells with nearby cells like immune system cells and stromal cells. The extracellular matrix (ECM) is a complex, three-dimensional framework comprising collagen, elastin, fibronectin, hyaluronic acid,

proteoglycans, and glycoproteins that provides structural support to tissues, maintains moisture and pH balance, and serves as a reservoir for growth factors. The heparanase inhibitor ronespartat (SST0001), which has a completed Phase 1 clinical trial for the treatment of multiple myeloma (Clinical Trial NCT01764880), had encouraging results in preventing tumor growth when administered either alone or in conjunction with other TME targeted medicines (Dmello et al., 2021). However, medicines that break down and/or deconstruct extracellular matrix (ECM) must be administered with caution due to the possibility that they will cause metastasis rather than halt the progression of the tumor.

5. THERAPEUTIC STRATEGIES TARGETING TME

For a long time, research on cancer has focused primarily on the cancerous tumor cells themselves, with little attention paid to the significance of the tumor microenvironment (TME). In the past ten years, the TME has received a lot of attention due to advancements in technology and growing evidence linking the TME to the emergence of cancer and treatment resistance. The tumor microenvironment (TME), a complex ecosystem that surrounds the tumor, serves as the tumor's supportive environment. Both cellular and molecular components make up TME. Immune cells, endothelial cells, and stromal cells make up the majority of TME's cellular components, while signaling molecules and extracellular matrix (ECM) proteins make up the majority of its molecular components. The immunological component encompasses all immune cells, both resident and infiltrating, including T cells, B cells, macrophages, and other immune cells. The support niches that are necessary for tumor growth are created by the stromal component, and the vascular component provides the infrastructure that transports the chemicals that are necessary for tumor cell survival. Carcinogenesis, progression, metastasis, and treatment outcomes may be improved by comprehending the TME's properties and interactions with tumor cells. For example, the vascular endothelial cells in the TME release angiogenic factors, which help tumors get oxygen and nutrients they need and encourage the formation of blood vessels. In the TME, fibroblasts and stromal cells secrete matrix metalloproteinases (MMPs), which aid in tumor invasion and metastasis. As a result of these discoveries, new pharmacological targets and drug development avenues have emerged, indicating that focusing on the TME offers

effective therapeutic approaches for enhancing cancer treatment outcomes. The TME's significance in therapeutic target research and development has become increasingly apparent in recent years. 6. Focusing on the TME and its remarkable immunological cells, for instance, has revolutionized cancer treatment. The successful discovery of immune checkpoint inhibitors (ICIs), such as inhibitors of cytotoxic T-lymphocyte associated protein 4 (CTLA4), programmed cell death ligand 1 (PD-L1), and programmed cell death 1, marked a significant turning point in the treatment of cancer. The prognosis of patients with advanced cancers has been improved by these ICIs' impressive efficacy in treating a variety of solid tumors and hematological malignancies. Because TME controls treatment/drug efficacy, responsiveness, and resistance, its significance in drug development is further emphasized (Wood et al., 2014; Neophytou et al., 2021; Ganesh & Massagué, 2021; Bejarano et al., 2021; Roma-Rodrigues et al., 2019; Zhou et al., 2022).

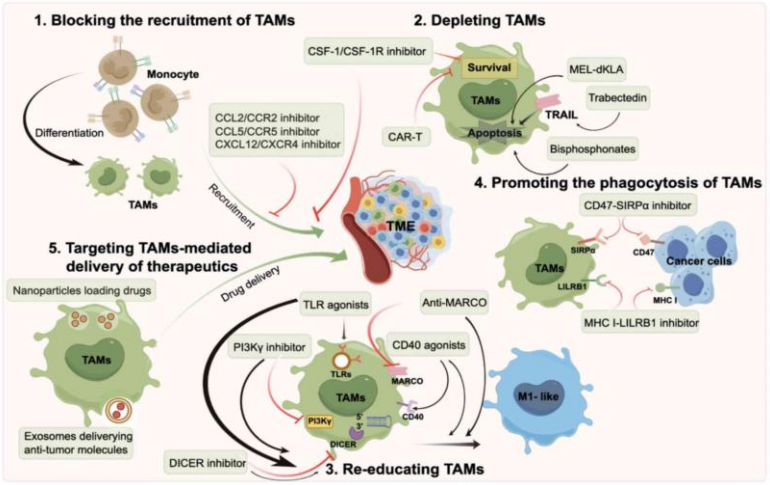

Figure 5: Therapeutic Strategies Targeting TME

It is now widely acknowledged that TME components significantly influence drug absorption, distribution, metabolism, and, ultimately, response. Immune cells in the TME can decrease the efficacy of immunotherapy and patients' responsiveness to ICIs, and the TME can restrict the administration of chemotherapy medications, which leads to drug resistance. In addition, more and more people are realizing that the TME can be used as a biomarker to predict how well a treatment will work.

6. ROLE OF TME IN CANCER PROGRESSION AND METASTASIS

Through a series of events known as metastasis, tumors move from their original site to a secondary one in order to form autonomous colonies, ultimately resulting in the patient's death. Metastasis is responsible for around 90% of cancer-associated morbidity, which also makes cancer treatment challenging and inefficient. Many factors contribute to the facilitation of metastasis. By breaking down the extracellular matrix around the tumor, matrix metalloproteinases (MMPs), which are important participants in the metastatic cascade, aid in the tumor cells' escape from the original location.

The zinc-dependent serine protease families known as matrix metalloproteinases play a role in the invasion, migration, and metastasis of tumors. Numerous cancers have been found to overexpress MMP-9, a well-known MMP linked to metastasis. Patients with higher MMP-9 expression levels had a worse outcome. Various investigations have indicated the significance of MMP-9's function in promoting metastasis. Serum MMP-9 levels were significantly higher in patients with various stages of colorectal cancer than in healthy people. Patients in the fourth stage of cancer had the greatest expression levels of MMP-9. In a similar vein, malignant brain tumors were found to express more MMP-9 than normal brain tissues. MMP-9 is a key target in anti-metastatic therapy because of its crucial involvement in causing metastases. According to several studies, metastasis can be prevented by specifically targeting MMP-9. When MMP-9 was inhibited by ribozyme in a rat sarcoma model, its capacity to spread was totally eliminated without compromising its tumorigenicity. Similarly, decreased tumor development, angiogenesis, and invasion were the outcomes of down-regulating MMP-9 in breast cancer cells.

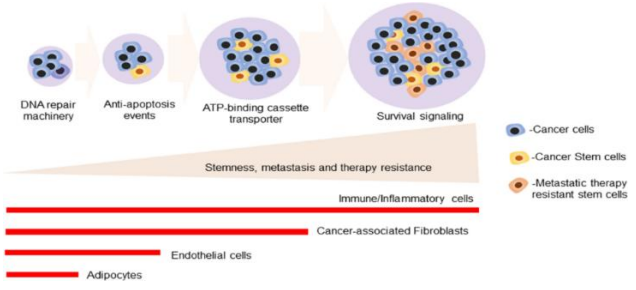

Figure 6: Role of TME

Inhibitor of KappaB Kinase beta (IKK2/IKKβ) phosphorylates and degrades Inhibitor of kappaB (IkB), hence modulating the NFθB pathway. When IkB is degraded, p50/p65 NF-B subunits are released. MMP-9 expression is down-regulated and cellular invasion occurs when the NF-B pathway is attenuated. When the NFøB pathway is activated by phorbol 12-myristate 13-acetate (PMA), MMP-9 expression rises and cell migration increases in tandem. Therefore, the NF-B pathway is a useful target to control MMP-9-mediated cancer cell invasion.

Despite the fact that metastasis is thought to be a primary cause of cancer-related death, anti-cancer therapy currently lacks an efficient therapeutic agent to target the metastatic cascade. When it comes to creating new medications to treat various illnesses, natural ingredients are always a great resource. It has been observed that a number of substances derived from natural resources suppress MMP-9 expression, hence preventing metastasis. One well-researched flavonoid, quercetin, has been demonstrated to suppress MMP-9 production, which lowers the ability of cancer cells to migrate. To find strong inhibitors of MMP-9 expression, seven less researched quercetin analogues were examined in this investigation.

7. TUMOR MICROENVIRONMENT (TME) COMPOSITION AND FUNCTION

This study highlights the roles of TGF-β and VEGF signaling in promoting angiogenesis and extracellular matrix remodeling, facilitating metastasis, and investigates how metabolic reprogramming of tumor and stromal cells drives cancer growth, influenced by nutrient availability in the TME. The study also assesses treatment strategies targeting these interactions to prevent metastasis (Tseng et al., 2018; Hansen et al., 2016).

Recent clinical findings have led to a greater understanding of the mysterious connection between the TME and cancer spread. The influence on clinical procedures and treatment development has grown as we learn more about the complexities of molecules. Treatment approaches have been transformed by the recognition of the TME as a critical component of metastasis. Novel strategies have sought to disrupt the TME rather than just target cancer cells. Due to this change,

medications that modify the TME have emerged, such as immune checkpoint inhibitors and procedures aimed at restoring normalcy to tumor blood vessels.

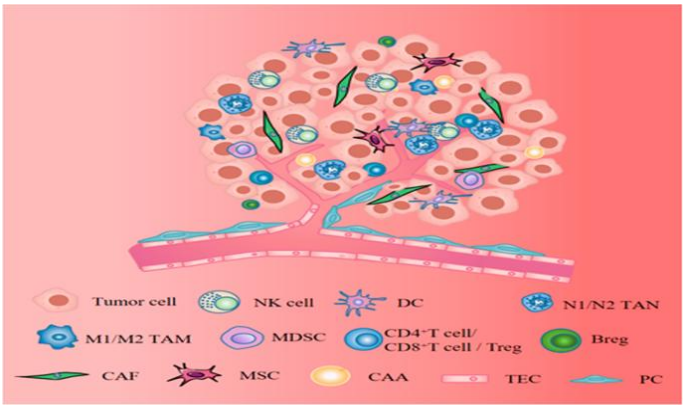

Figure 7: TME Composition and Function

A complex system, the TME is essential to the treatment of cancer. Better patient outcomes and the creation of targeted medicines are possible outcomes of this complex interaction. Future research should leverage single-cell technologies and spatial transcriptomics to elucidate the complex heterogeneity within the tumor microenvironment (TME), pinpointing the unique roles of diverse stromal components and deciphering the intricate cellular and molecular pathways that govern TME dynamics, ultimately informing the development of more targeted and effective cancer therapies. Although immunotherapy is already a game-changing treatment in oncology, each patient responds differently to it. In order to create more potent immunomodulatory treatments, future studies should focus on comprehending how cancer cells and the immune system interact within the TME. This might entail creating biomarkers that forecast immunotherapy response or resistance, allowing for individualized treatment regimens. Another promising topic is metabolic reprogramming within the TME. Comprehending these metabolic changes may aid in the creation of medications that disrupt the tumor's energy source or limit its metabolic adaptability, which is especially useful in addressing cancer cells that have spread to other locations. Research on how microbes, cancer cells, and the immune system interact could also identify novel therapeutic targets or improve on current therapies. The ethical, legal, and societal ramifications of TME research must also be continuously addressed. To guarantee that the

advantages of TME research are realized in an ethical and fair manner, and treatments.

Currently, cancer therapy primarily focuses on surgical removal of the tumour followed by chemotherapy in most cases of breast cancer. But, patients with advanced stage of breast cancer are associated with poor prognosis mainly due to relapse of disease after the standard surgical treatment. Hence, a better understanding of the molecular mechanisms involved in metastasis of breast cancer is needed to control the disease. Natural compounds and many synthetic derivatives contain a wide range of substances which find its uses in treating various diseases. Thus, research has developed in investigating and synthesizing the potential anti-cancer compounds. Phytochemicals of plants have a good history of use in the treatment of cancer. According to a report more than 50% of available anticancer drugs are derived from plant sources. Currently, chemotherapeutic agents for breast cancer such as doxorubicin, paclitaxel, docetaxel, vincristine, vinblastine, vinorelbine, podophyllotoxin and its derived compounds are in use (Shabani, 2016). However, they are associated with adverse side effects and in most cases the effect is not long lasting because resistance to chemotherapy is a major obstacle for effective treatment. Recent developments in targeted therapy have shown advantages when compared to conventional chemotherapy, which have high toxicity. Anti-angiogenic therapy is one such strategy in cancer therapeutics which targets angiogenesis-related signalling molecules, including vascular endothelial growth factor (VEGF), have significantly improved the clinical outcome of cancer patients. As there is no indication for endocrine therapy or any clinically proven effective targeted therapy available for TNBC, novel and alternative therapeutic strategies are crucially needed.

8. CONCLUSION

The ethyl acetate and acetone fractions of D. bulbifera roots were shown to be a potentially rich source of bio-active chemicals that might be employed in combinatorial therapies for the treatment of breast cancer after screening for plant extracts with anti-angiogenic and pro-apoptotic qualities. Benzisoxazole derivative (7e) was one of the chemically produced compounds that shown pro-apoptotic, anti-angiogenic, and anti-proliferative properties in MDA-MB-231 and mouse mammary

cancer cells. According to the SAR investigations, the biological activity was significantly impacted by the substitution position on the phenyl ring and compounds having electron-donating moiety, such as methoxy. Additionally, this is the first research demonstrating that benzoisoxazole compounds suppress MTA1-induced angiogenesis, migration, and proliferation. Compound 7e is a desirable therapeutic candidate due to its documented biological action. Using the cloning vector pGEX-5X-2-MTA1, a recombinant MTA1 protein of 80 K Da was generated, and GST-tag affinity column chromatography was used to purify it. A different new benzisothiazole derivative (5c) successfully stopped the growth of tumors caused by MTA1. At nanomolar concentrations, it was recognized as a strong anti-cancer lead structure. Particular changes in the MAPK signaling and VEGF-R2-mediated EMT pathways are ascribed to compound 5c's anti-angiogenic properties. This is the first mechanistic proof that the benzisothiazole derivative (5c) targets breast cancer mediated by MTA1. molecule 5c is acknowledged to show significant potential as a lead molecule for the treatment of breast cancer, as seen by the results above. Another tactic was to use RNA interference technology to silence the MTA1 gene. Stable transfected clones harboring MTA1-shRNA were then created, and the results were confirmed using the migration test and real-time qPCR analysis.

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