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**Chapter**

# Control of Cytoskeletal Dynamics in Cancer through a Combination of Cytoskeletal Components

*Ban Hussein Alwash, Rawan Asaad Jaber Al-Rubaye, Mustafa Mohammad Alaaraj and Anwar Yahya Ebrahim*

## Abstract

The dynamic alterations in the cytoskeletal components actin and intermediate, etc. filaments are required for cell invasion and migration. The actin cytoskeleton is a highly dynamic structure that is governed by a delicate balance of actin filament formation and disassembly. To controlling the activities of key components of the epithelial mesenchymal transition (EMT) could be a viable solution to metastasis. Bioinformatics technologies also allow researchers to investigate the consequences of synthetic mutations or naturally occurring variations of these cytoskeletal proteins. S100A4 is S100 protein family member that interact with a variety of biological target. In study has shown that S100A4 interacts with the tumor suppressor protein p53, indicating that S100A4 may have additional roles in tumor development. The S100A4 and p53 interaction increases after inhibition of MDM2-dependent p53 degradation using Nutlin-3A. The main goal of this research was control of cytoskeletal dynamics in cancer through a combination of, actin and S100A4 protein. The investigate the molecular mechanism behind S100A4 function in (EMT) and indicating that S100A4 is promoting p53 degradation. Understanding the signaling pathways involved would provide a better understanding of the changes that occur during metastasis, which will eventually lead to the identification of proteins that can be targeted for treatment, resulting in lower mortality.

**Keywords:** cytoskeleton dynamics, actin, cancer, S100A4 protein and p53, bioinformatics

## 1. Introduction

A highly coordinated multistep process involving the stroma, blood vessels, and cytoskeleton is the leading cause of death in cancer. Invasion, migration, extravasation, and angiogenesis are all important factors in successful metastasis. Invasion is a limited process that occurs at the tumor-host interface, where tumor and stromal cells exchange enzymes and cytokines that modify local ECM and promote cell

movement [1]. The ability of cells to move and divide is controlled by dynamic changes. Most cancers are characterized by changes in the expression levels of numerous protein kinases. As a result, most cancer cells show dynamic alterations in cytoskeletal proteins. The capacity of cancer cells to divide, infiltrate, and generate distal metastases is complicated by their migratory nature, the plasticity of cell migration, and these dynamic alterations. The importance of dynamic alterations in the modulation of the function of various cytoskeletal polymers in cancer cells is highlighted in this work. Actin (which generates MF), myosin (mini-filaments), tubulin (MT), and several IF protein families, such as keratins, desmins, peripherin, vimentin, internexins, and others, are among these monomers [2]. The mesenchymal-to-epithelial transition (MET) theory was established to explain these phenomena when histological examinations revealed that macrometastases have epithelial phenotypes rather than mesenchymal phenotypes [3]. DTCs undergo MET to transition from a mesenchymal to an epithelial form, allowing them to multiply at the metastatic site and develop into macrometastases, according to this view. The involvement of the actin cytoskeleton, microtubules, and intermediate filaments in EMT is explored in this paper, as well as how these cytoskeleton proteins can be exploited as a possible biomarker. The S100 family is a subgroup of calcium-binding proteins with EF-hands that regulate a number of cellular processes by interacting with a variety of protein targets. S100A4 expression has been found in fibroblasts, blood cells, and endothelial cells, and it is thought to be one of the mesenchymal cell markers involved in the epithelial-mesenchymal transition (EMT) [4, 5]. The capacity to migrate efficiently in cell motility experiments is a characteristic trait of S100A4-positive cells, but ectopic production of S100A4 in S100A4-negative cells increases migration [6]. Monomers of folded 10S and unfolded extended 6S versions of Nom-muscle myosin (NM IIA) protein exist. The latter has the ability to form filaments [7]. In cancer, genetic changes that impact protein kinases are quite common [8, 9]. Mutations or deletions that induce loss of function or enhanced catalysis are the most common. Activating mutations might have unanticipated consequences for several cytoskeletal systems. Mutations in the small GTPase RhoA, for example, may result in enhanced activation of proteins that regulate minifilament production [10].

These events result in abnormal molecular activities in cancer cells, such as enhanced cell motility, invasion, division, and mechanosensing. The occurrence of many isoforms of these proteins, some of which have non-overlapping activities, complicates the investigation of these alterations. Actin, tubulin, and myosin are all isoforms, and IF comes in a variety of forms and variations. One of the main goals of this project is to present a broad, although incomplete, view of the field. Finding possible areas that could be targeted specifically to treat a variety of cancers in human cancer A431 cells, we show that S100A4 expression is increased during EMT mediated by the transcription factor ZEB2. In addition, we show the interaction between endogenous S100A4 and p53 in cells and that the interaction takes place within the cell nucleus. We also show that knockdown of S100A4 results in stabilization of p53 at the protein level. Further, knockdown of S100A4 is shown to increase the transcriptional activity of p53, resulting in p53-dependent growth arrest [11]. Transgelin (TAGLN) has been shown to have a role in the genesis of proteinuria, although the mechanism by which it does so is unclear. The goal of this research was to look at the involvement of TAGLN in the development of proteinuria. The study's distinctive feature is that it provides an updated, birds-eye view of the global changes in the cytoskeleton, which includes changes in tubulin and intermediate filaments as well as actin and actin binding proteins.

## 2. The cellular cytoskeleton's role in EMT

The cytoskeleton provides the mechanical strength and integrity that allows cells to maintain their shape and movement. **Figure 1** depicts the situation. As seen in the first step, epithelial cancer cells undergo EMT, losing their cell-cell connections and gaining the potential to penetrate the surrounding tissue parenchyma. These EMT-induced cells can subsequently intravasate into the systemic circulation and survive in the circulation before reaching the target site in the third stage. The cells must then extravasate into the tissue parenchyma in the fourth phase before going into dormancy or becoming micro metastases. MET activation in the fifth phase is required for subsequent improvement and potentially life-threatening mega metastases.

The epithelial cytoskeleton is remodeled during EMT, resulting in cell polarity loss and extracellular matrix remodeling (ECM). The cells then become motile and have the ability to invade [12]. The cytoskeleton's critical function in the EMT process is described in the following sections:

### 2.1 Cytoskeleton of actin

Actin filament remodeling is linked to EMT [13], and it is one of the most important components of the cytoskeleton. G-actin (globular actin) is a monomeric unit,
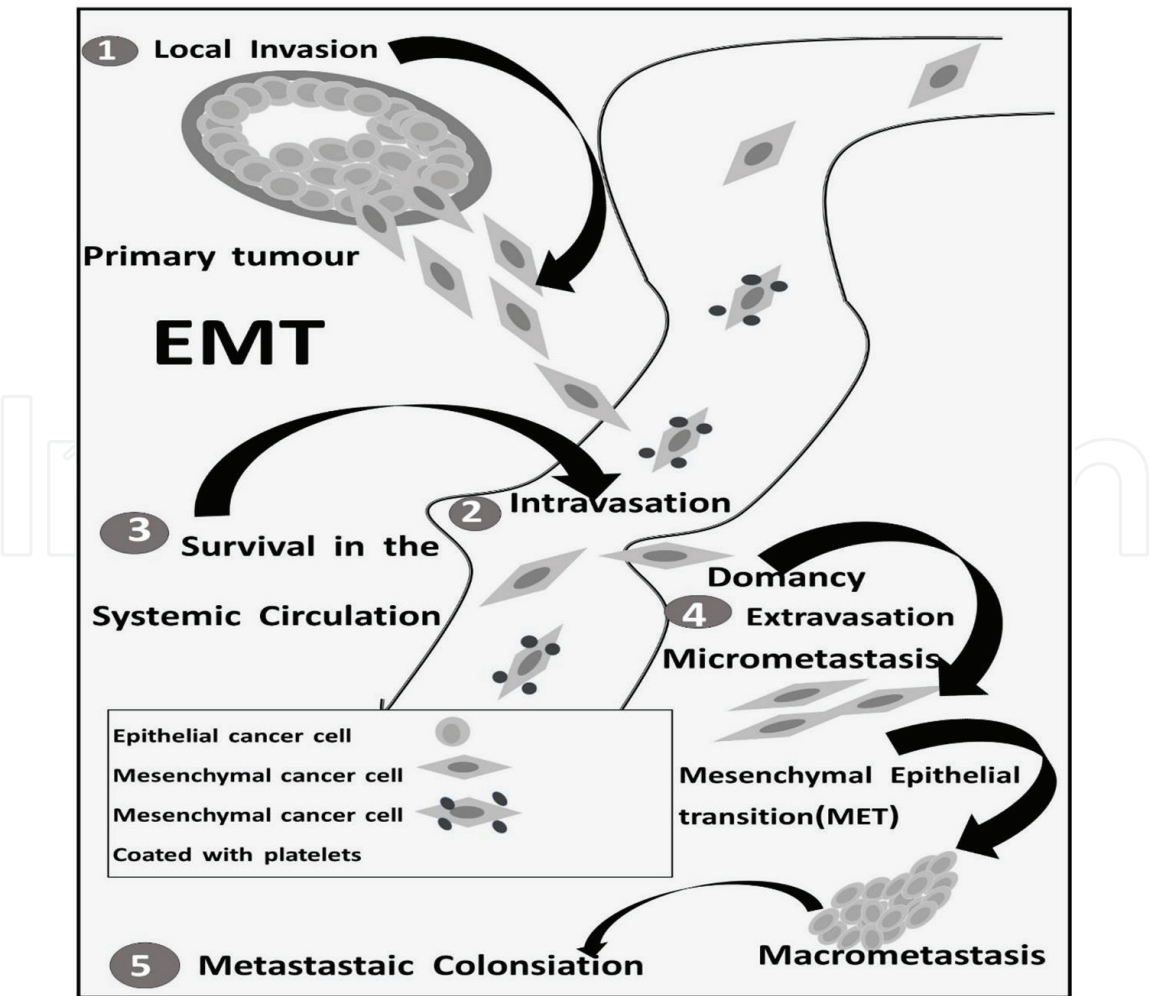


**Figure 1.**
*The metastatic cascade is represented by the EMT-MET model.*

while F-actin (fibrous actin) is a polymeric filament. G-actin is distributed uniformly throughout the cytoplasm and nucleus. With the simultaneous hydrolysis of ATP, G-actin rapidly polymerizes to create F-actin under specific physiological conditions. Actomyosin mediates cell spreading and adherence to the ECM by producing conspicuous bundles of F-actin known as stress fibers. Stress fibers attach to focal adhesions and have a function in cell adhesion and morphogenesis as a result. Within the leading cell edge, actin filaments engage with actin-binding proteins and myosin II to deliver F-actin. For cell migration, this is a crucial process. Through its ATP-dependent motor activity, myosin II is thought to play a key role in the construction and disassembly of the actin cytoskeleton [14]. Different biological activities such as cell motility, cell shape, and so on rely on actin organization [15]. Gene expression, post-translational protein modification, and cytoskeleton remodeling all play a role in the EMT process [16]. Recent research has discovered that cells in intermediate phases of EMT have increased tumor-cell spreading ability. E-cadherin complexes have also been demonstrated to be connected to the dynamic actin framework via -catenin and stabilized by inhibiting RhoA activity and activating Rac and cdc42 [13, 17]. Cell-surface receptors, such as integrins, bind to ECM components and play a vital role in altering cell attachment, which is necessary for motility and invasion. A multi-protein complex binds to the actin cytoskeleton and achieves integrin-mediated cell-matrix adhesion.

### 2.1.1 Proteins that bind to actin

The actin cytoskeleton is made up of actin microfilaments and a large number of actin-binding proteins (ABPs). ABPs are proteins that regulate the formation and disassembly of actin microfilaments. This is important for cell motility, division, and cancer growth, all of which require coordinated actin filament turnover and remodeling [18]. Actin filaments are grouped in a loosely ordered meshwork in lamellipodia, which is referred to as dendritic networks [19], whereas actin filaments are arranged in parallel bundles in filopodia [20]. The action of specific actin-organizing proteins is required for these two types of organizations. During migration, the depolymerization of actin and debranching allows for the dynamic remodeling of the actin network as well as the cyclic extension and retraction of lamellipodia, which generates the pushing force that propels the cell forward. The cell body follows the orientation of the front lamellipodia due to the contraction of actin filaments. Filopodia are made up of closely packed parallel actin filaments with tapered ends facing the plasma membrane. Small crosslinking actin-binding proteins like fascin are principally responsible for bundling filopodia filaments [13, 21].

Cells are thought to be able to penetrate the tissue barrier by forming invadopodia, which are F-actin protrusions that breakdown the ECM, allowing cell penetration [22]. Invadopodia are actin-rich protrusions that are engaged in cell penetration and are related with ECM degradation via local deposition of proteases. The Arp2/3 (actin-related protein2/3) complex is a seven-subunit protein that is regulated by the WAVE and WASP families of WH2 domain-containing proteins (WAVE1, 2, and 3, WASP, and N-WASP), which bind both the Arp2/3 complex and actin monomers [23]. Arp2/3 is a protein complex that aids in the polymerization of actin filaments. Arp2/3 is typically overexpressed in cancers such as breast and liver carcinomas, implying a link between dynamic actin rearrangement and cancer progression [24]. Cortactin, an actin-binding protein, also binds to Arp2/3, allowing active Arp2/3 complexes to be located on the sidewalls of existing actin filaments, resulting in branched arrays of F-actin. Cortactin overexpression has been discovered during metastasis [25, 26].

Facin, an actin-binding protein that stimulates the development of invadopodia and filopodia, is increased during migration [27]. Gelsolin is essential for the formation of lamellipodia and podosomes, both of which are critical protrusions for motile cells [28]. The actin nucleating proteins that regulate cell mobility and organization are known as formins. EMT has been shown to upregulate formin expression at the leading edge in mesenchymal-transformed cells [29]. The gene coding for ABPs has been found to have altered transcription or translation in several cancer types, according to studies. Because ABP expressions vary throughout cancer types, changes in the actin cytoskeleton are a common characteristic of tumor cells. In breast cancer tissues, ARPC2 (actin-related protein2/3 complex) expression is greater and ARPC2 expression is associated with EMT and metastasis [13, 30]. Filamin deficiency has been found to be prevalent in carcinomas such as colon, prostate, and breast cancer [31]. As a result, migration is boosted, which is linked to a bad prognosis [32]. Higher levels of-actinin (actin filament cross-linker) are linked to a bad prognosis in breast cancer, as well as the degree of clinical progression and lymph node status [33].

**2.2 Rho GTPases**

Rho GTPases play a role in a range of cellular activities, including cell migration, cell polarity, and cell cycle progression, by controlling actin, MT dynamics, and regulating cytoskeleton and cell adhesion dynamics. It has been established that increased expression of Rho GTPases genes associated with a metastatic phenotype in a variety of cancer types, and are tightly related to the actomyosin cytoskeleton's overall control [34]. Rac1, RhoA, and Cdc42 are members of the Rho family of GTPases, which regulate actin cytoskeleton organization such as cytoskeletal dynamics, cell-cell junction assembly/disassembly, and integrin-matrix adhesion. Controlling the activities of Rho GTPases is critical during the growth-factor-induced EMT. Rho signaling activity is controlled by guanine nucleotide exchange factors (GEFs) which catalyze the exchange of GDP to GTP. During growth factor-induced EMT, controlling the activities of Rho GTPases is crucial. Guanine nucleotide exchange factors (GEFs), which catalyze the conversion of GDP to GTP, regulate Rho signaling activity. GTPase-activating proteins (GAPs) facilitate intrinsic GTPase activity to re-form the GDP bound state, which inactivates Rho action. Finally, the inactive GTPase domains and their covalently linked lipid groups engage with the guanine nucleotide dissociation inhibitors (GDIs). As show in **Figure 2**, the GDIs prevent GDP from being dissociated from Rho GTPases, which could inhibit spontaneous activation [35].

Rho GTPase activity in cells is regulated by Rho-dependent factors, as shown in this diagram. GEFs can stimulate Rho-GTPases to engage with downstream actomyosin-regulating effectors by activating the exchange of GDP for GTP, whereas GAPs bind to the GTPase and boost the intrinsic GTPase activity by switching bound GTP to GDP. The GDIs interact with the GDP-bound version of the molecule, preventing GTP binding and thus activation. This illustration is based on Raftopoulou and Hall [36]. Rho GTPases function as molecular switches that cycle between a GDP-bound inactive form and a GTP-bound active form to govern signal transduction pathways [13, 36].

Rho governs cytoskeleton alterations and stimulates actin stress fiber production, impacting cell-cell or cell-matrix adhesion. Rho signaling is important in the regulation of actin-myosin contraction because it stimulates actin reorganization, which leads to the formation of stress fibers. Many of these regulatory mechanisms become unregulated in cancer cells, which contributes to invasive behavior during metastasis, according to recent research [37].
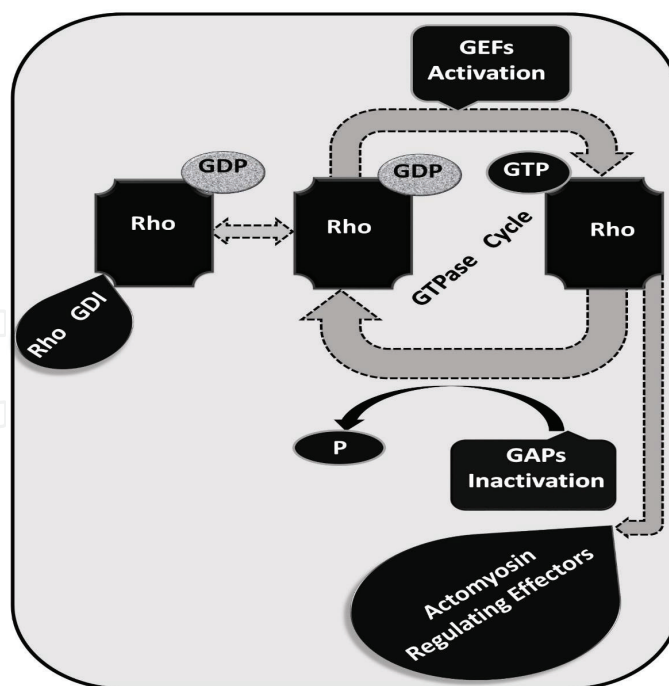
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**Figure 2.**
*The diagram depicted Rho GTPase cycle.*

## 2.3 Microtubule (MT)

In EMT, all aspects of the actin cytoskeleton and intermediate filaments are well identified, but the function of microtubules (MT) is still being explored. MTs are an important part of the cytoskeleton and play an important role in movement, intracellular transport and supporting cell shape [38]. MTs are composed of α and β-tubulin dimers, which mostly grow and shrink from the positive end and produce dynamic instability [39]. The function of MTs depends on their assembly and stability, which are regulated by post-translational modifications and interactions with various stable and destabilizing proteins [40]. Calmodulin regulated spectrin associated protein (CAMSAP3) is an MT-binding protein required to maintain MT tissue. It has been shown that the loss of CAMSAP3 promotes Akt dependent EMT through tubulin acetylation [41]. Studies have shown that the microtubule-interacting protein EB1 (end-binding protein) is located in one location and interacts with the microtubules. EB1 is a negative regulator of microtubule stability and promotes the migration of tumor cells. It modulates the dynamics of MT both in vitro and in vivo [42, 43]. Stathmin is an MT regulatory protein that depolymerizes MT and strengthens and regulates MT dynamics. MT destabilization is related to the phosphorylation of stathmin at its four serine residues [44]. In some human cancers, such as Wilms' sarcomas and tumors, stathmin levels have been elevated and have been associated with more aggressive metastases [45]. During EMT, MT plays a significant role in cell migration. Anti-MT drugs act on the one hand by inhibiting cell division, but also by inhibiting cell migration by stopping the formation of projections of MT-based membranes [46, 47]. Stability variability in MT regulates cortical F-actin by activating or inhibiting various Rho GTPases [13, 48]. Aside from their roles in cell division and migration, MT is also important for cell polarization. The creation of a polarized MT required for morphogenesis and cell migration is thought to be aided by cortical control of MT. Although MT indirectly contributes to cell-cell adhesion through

dynamic remodeling of the actin network, the role of MT in regulating migration or EMT by interacting with cell-cell adhesion is currently being investigated. Reveal that the MT-interacting protein stathmin is important in cell migration and metastasis via MT-actin cytoskeleton crosstalk [49]. Novel pharmaceutical techniques could be created using this relationship, in which the actin cytoskeleton is targeted via MT, to overcome the toxic effects associated with some actin-based medicines.

## 2.4 Intermediate filament (IF)

Intermediate filaments (IF) are important cytoskeletal components that provide structural support and mechanical strength. One of the largest gene families in the human genome encodes more than 50 different IF proteins, and this family contains five different IF classes. Types I-IV are located in the cytoplasm and include vimentin, which is a classic marker of EMT, and its expression is related to the aggressive phenotype of epithelial cancer. Compared with actin cytoskeleton and MT, IF also shows a different tissue expression pattern. Type I IF keratin is epithelial-specific and is essential for the mechanical stability of epithelial cells. During EMT, the reduction of keratin is generally considered to be the histological and biochemical characteristics of cancer cells [50, 51]. Type III IF, vimentin, is a typical marker of EMT. Vimentin expression is up-regulated during EMT of epithelial cells, and it has been reported to increase vimentin expression in various cancer cell lines. It is used as an indicator of poor prognosis [52]. During EMT, vimentin helps determine and maintain cell shape. Recent studies have shown that the expression of vimentin is related to active prostate cancer cell lines, and its knockdown significantly reduces the activity and invasiveness of tumor cells [13, 53]. It shows that vimentin is significantly increased in polyploid giant cancer cells (PGCCs). Vimentin intermediate filaments are responsible for expanding morphology and increasing migration [54]. In general, vimentin expression has significant characteristics during EMT, including tumor cell migration and invasion.

## 3. Materials and methods

The materials and methods are described in the following steps:

### 3.1 Evaluation of S100A4 and p53 interaction in cells

S100A4 interacts with p53 in the nucleus S100 family proteins have no known enzymatic activity, and therefore it is generally believed that S100 proteins function through interaction with other proteins to regulate their functions. Nuclear colocalization between S100A4 and p53 was however apparent both in untreated and cisplatin-treated A549 cells [11]. Therefore, to investigate the suggested interaction between S100A4 and p53. IP of endogenous S100A4 in A549 cells resulted in coprecipitation of endogenous p53 in untreated cells. In addition, the amount of coprecipitated p53 increased after treatment of the cells with the p53-stabilizing drug Nutlin-3A **Figure 3**. To validate the interaction between S100A4 and p53 and to retrieve information about the subcellular location of the interaction, using antibodies targeting S100A4 and p53 **Figure 3**. The results from PLA supported the interaction between S100A4 and p53 in cells, and also underscored the dramatic increase in the interaction after treatment with Nutlin-3A. In addition, in situ PLA clearly showed that the subcellular location of the interaction between S100A4 and p53 was in the nucleus **Figure 3**.
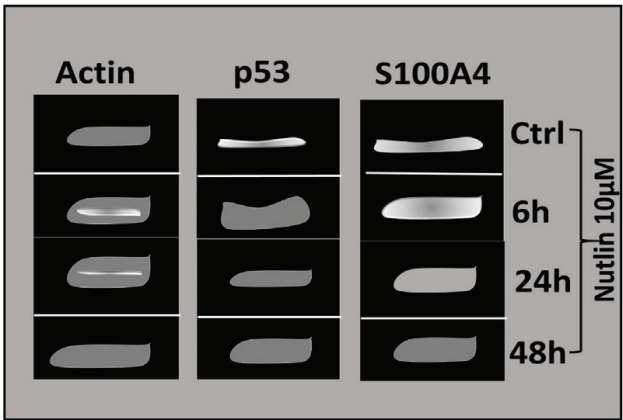
**Figure 3.**
*Immunoblot analysis of p53 and S100A4 protein levels in A549 cells in response to Nutlin-3A treatment at indicated time-points.*

## 3.2 Cytoskeleton protein transgelin developing proteinuria by bioinformatics

The cytoskeleton protein transgelin is designated in the following phases:

### 3.2.1 Immunity and TAGLN-related transcription factors (TFs) correlation analysis

For stratification of the immune milieu based on function and activity, a group of important immune-related genes that have been widely researched in carcinogenicity were discovered. A scatter plot was used to display statistically significant genes in each category, as well as all relationships within each categorization.

### 3.2.2 Analysis of the relationship between TAGLN and well-known genes involved in cell viability and apoptosis

According to their function and activity, a group of well-known cancer genes that have been widely examined in carcinogenicity were gathered and divided into cell cycle-related and apoptosis-related pathways. The apoptosis-related star genes were divided into two groups: G0-G1 and G2-M. The expression profile data for each class was used to determine the associations between TAGLN and the star genes.

Differentially expressed genes (DEGs) were identified using Gene Expression Omnibus microarray expression profiling datasets and processed using the short time series expression miner to cluster DEGs in proteinuria progression and build a gene interaction network [55].

## 3.3 Western blotting

Western blotting dry was used to determine the quantity of extracted P53. In one input, the total protein extracted from cells was displayed, whereas flow-through indicated unbound protein (4-A) [11]. This method was chosen to avoid the presence of antibodies, which could cause more P53 aggregation. To conduct the negative staining experiment, recombinant S100A4 protein was purified under natural conditions. Luciferase IIA immunoprecipitated from A431/ZEB2-WT cells was analyzed using Western blotting [11]. Elution displays the amount of protein that separated from the immunocomplex, while beads reflect the immunocomplex. To see if p53 stabilization

alone has an effect on cellular S100A4 levels. Nutlin-3A prevents p53 from interacting with MDM2, the ubiquitin E3 ligase that ubiquitinates p53 and sends it to the proteasome for destruction. We were unable to identify any changes in the messenger RNA (mRNA) level of S100A4, indicating that the increase in S100A4 in response to Nutlin-3A was due to protein stabilization. Knockdown of S100A4 results in increased cisplatin-induced apoptosis S100A4 knockdown by itself did not induce apoptosis, but still the increased p53 levels could prime the cells for apoptosis activation.

### 3.4 Microscopy with immunofluorescence

Cells were cultured on 9 mm glass coverslips (VWR), fixed with 4% paraformaldehyde (VWR), and permeabilized with 0.5% Triton X-100 (Sigma). Primary and secondary Alexa Fluor conjugated antibodies (Life Technologies) were used for 1 hour of staining. Nuclear staining was done with DAPI (Sigma). An inverted Nikon Eclipse Ti microscope and a custom-built prism-based TIRF microscope with 60× objectives were used for confocal and TIRF microscopy [56]. Samples were analyzed with the help of sample.

## 4. Result

### 4.1 In the nucleus S100A4 and p53 interaction

S100A4 interacts with p53 in the nucleus because S100 family members have no known enzymatic activity, it is usually assumed that they control their activities via interacting with other proteins [11]. Non-muscle myosin IIA and p53 have already been identified as possible S100A4-interacting proteins. As a result, we started to look into the possible relationship between S100A4 and p53. In untreated cells, IP of endogenous S100A4 resulted in coprecipitation of endogenous p53.

In addition, as shown in **Figure 4**, the amount of coprecipitated p53 increased after the cells were treated with the p53-stabilizing medication Nutlin-3A. We used
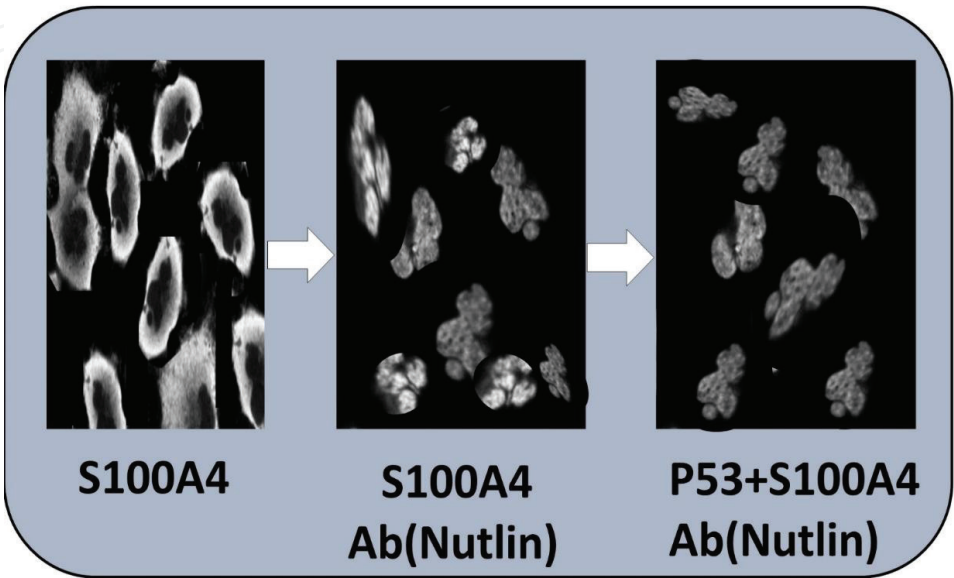


**Figure 4.**
*S100A4 interacts with p53 in the nucleus.*

antibodies targeting S100A4 and p53 to perform in situ PLA35 to confirm the interaction between S100A4 and p53 and to acquire information regarding the subcellular location of the interaction. PLA findings confirmed the contact between S100A4 and p53 in cells, as well as the substantial increase in the interaction following Nutlin-3A therapy. Furthermore, in situ PLA clearly demonstrated that the subcellular location of the interaction between S100A4 and p53 was in the nucleus as shown in **Figure 4**.

To utilizing cisplatin, a cytotoxic agent that promotes apoptosis in p53-dependent cells, to see if this was the case. We found higher cisplatin sensitivity in S100A4 shRNA cells relative to control cells using both a short-term cell viability assay and a clonogenic survival experiment as shown in **Figure 5**. We employed different assays to analyze cell mortality after S100A4 knockdown to learn more about the cisplatin response. S100A4 is significantly silenced as shown in **Figure 5**.

## 4.2 The actin cytoskeleton in EMT: clinical evidence and therapeutic implications

Recent research has revealed that scientists are concentrating their efforts on combination therapies that target numerous molecules in the same signaling pathway, multiple pathways in the same tumor, or both cancer cells and immune cells [57, 58]. Combination medicines are still being studied, and they will help us better understand drug resistance processes in the future. As a result, recent theories propose that targeting EMT and cytoskeletal proteins could be a unique way to battle cancer medication resistance. Normal cell physiology requires actin. As a result, despite their promise in vitro and in vivo, prospective actin-specific chemotherapeutics have yet to be tested. Due to their non-specific targeting of normal tissues, which causes cardiotoxicity and renal difficulties, they have not been successful [59, 60]. Increasing data suggests that the commencement of the EMT process and metastasis causes an increase in the number of EMT-related actin-binding proteins (ABPs) involved with actin cytoskeleton remodeling. As a result, controlling ABP expression may aid in preventing cancer cells from migrating and increasing their sensitivity to
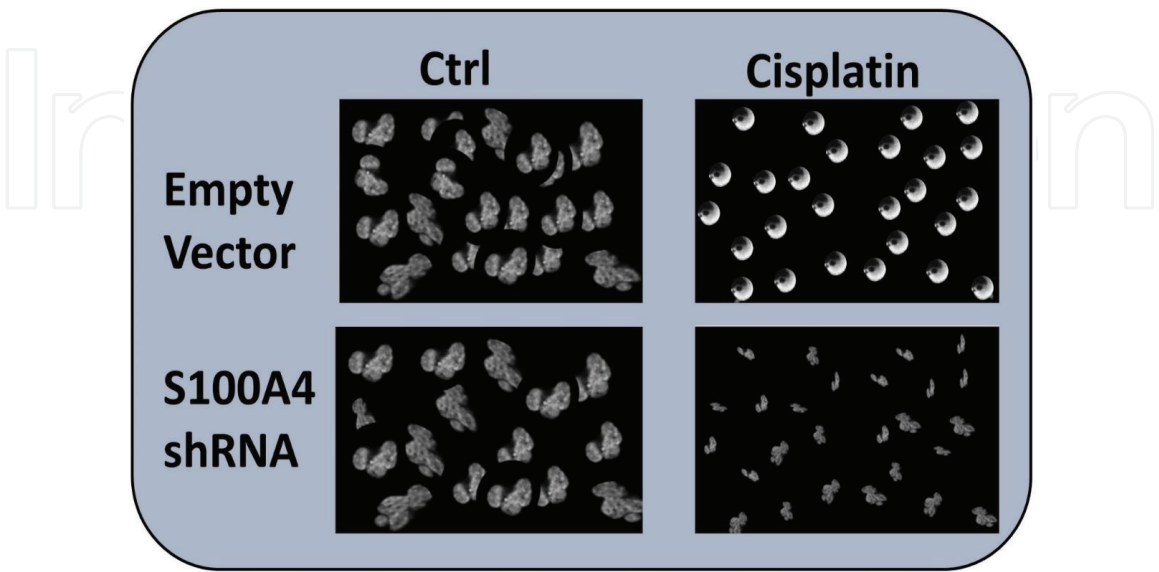


**Figure 5.**
*Knockdown of S100A4 results in increased cisplatin sensitivity.*

therapeutic therapies. Arp2/3, cortactin, formins, and fascin have all been studied extensively. Other ABPs, which could be potential targets in carcinogenesis, are, however, understudied. The actin cytoskeleton and ABPs are difficult to target for anti-cancer therapy, because ABPs are involved in the creation of contractile structures in cardiac and skeletal muscles [13, 61]. The intermediate filaments vimentin and nestin are linked to several cancers. When it comes to EMT, vimentin is a marker for mesenchymal cells. Anti-tumor medications have been discovered to change microtubule dynamics, which affect mitosis and apoptosis [62]. Microtubules have a big role in tumor migration and invasion during EMT. These anti-tumor medications stop cancer cells from dividing and forming membrane protrusions caused by network-based microtubules, which cause cell migration and invasion. Eribulin is a MI depolymerization medication that is used to treat metastatic breast cancer patients. In breast cancer, this medication suppresses angiogenesis, vascular remodeling, and EMT [63, 64]. The anti-tumor medication diaryloxazole PC-046 has a high oral bioavailability. It is a synthetically produced small molecule microtubule destabilizing agent. When compared to other microtubule destabilizing agents, this medication is reported to have a lower rate of MDR cross-resistance. Drug resistance in cancer cells is influenced by many signaling pathways involved in EMT and cytoskeletal proteins [65].

Anti-apoptotic effects and drug efflux pumps are increased in EMT cells. As a result, recent theories imply that focusing on EMT and cytoskeletal proteins could be a unique way to battle cancer treatment resistance. Chemotherapy is commonly used in the treatment of cancer, either alone or in combination with radiotherapy or surgery. Multiple breakthroughs in cancer treatment have been made in recent years, while medication resistance, which has been one of the leading causes of cancer death, has increased [66, 67]. In a drug-filled environment, EMT cells are thought to have the ability to develop selectively. While some studies imply that EMT may not totally contribute to cancer metastasis, others reveal that EMT is strongly linked to treatment resistance in cancer cells. Anti-microtubule drug resistance is thought to be caused by changes in the drug target, such as altered microtubule dynamics, tubulin mutations, modified tubulin isotype expression, and altered microtubule regulatory proteins, according to a large body of research. Other cytoskeletal proteins that can regulate microtubule regulation via signaling or structural links have also been discovered may be essential factors of anti-microtubule resistance [68, 69]. ADCs (antibody-drug conjugates) are a new type of targeted anticancer therapy that has been shown to be effective in MDR cancer. When a high-affinity antibody (Ab) binds with the drug and pushes a targeted drug delivery into the cell, this ADC causes apoptosis in tumor cells. In **Figure 6**, aside from producing a cytotoxic load paired with tumor cell death, this Ab-drug combination also blocks the cells' pro-survival receptor. The discovery of ADC could lead to the development of other combination medicines, such as immunotherapy. A lot of work is being done right now to improve the efficacy and targetability of ADCs in the treatment of cancers.

In **Figure 6**, (i) high-affinity antibody binds to the drug. ADC is formed when an antibody binds to a drug and enters the cell's double lipid-membrane layer, causing cell death. (ii) ADC attaches to a cancer cell's pro-survival receptor, blocking its function and triggering apoptosis. (iii) ADC binds to both the cancer cell's membrane-surface antigen and an immune system effector cell, causing cancer cells to be lysed by cellular cytotoxicity.
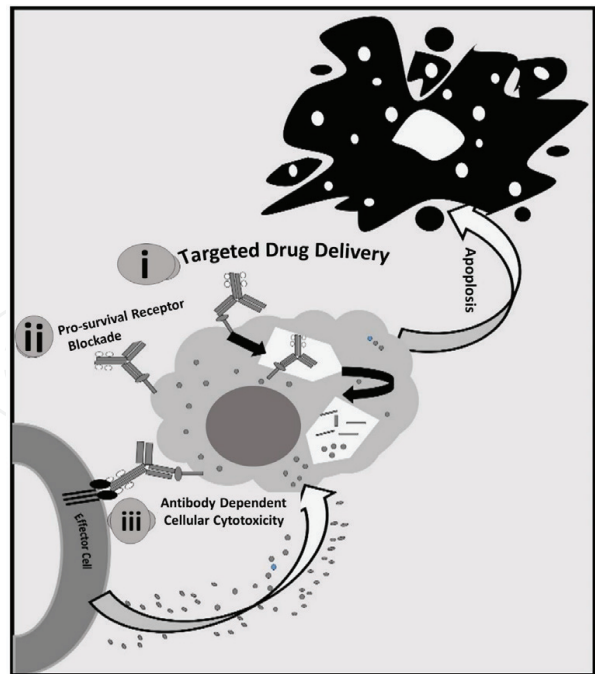
**Figure 6.**
*Diagram depicting the antibody-drug conjugate (ADC) mode of action in a cancer cell.*

## 5. Conclusions

It was necessary to conduct research. The plasticity of the cytoskeleton, motility, multi-drug resistance, and immunosuppressive properties have revealed a great deal about the plasticity of the cytoskeleton, motility, and immunosuppressive properties during the transformation of an epithelial cell to a mesenchymal cell. The cell's signaling systems, and how it adapts in order to live although there has recently been an emphasis on finding new cytoskeletal markers that can be used to detect cancer. Recent research suggests that cytoskeleton dynamics and EMT have a strong association, which can be used to find possible biomarkers. Epithelial cells lose their apical-basolateral polarity and adopt a fibroblast-like motility characteristic during EMT. S100A4 is a mesenchymal marker that is essential for improved mesenchymal cell motility. We chose to study the interactions between NMIIA and S100A4 in a cellular model of EMT because both proteins are expected to work together to generate the mesenchymal cell phenotype. There is less evidence for an S100A4-NMIIA complex in vivo. In this study, we report on control of cytoskeletal dynamics in cancer through a combination of actin and S100A4 protin. The interaction between S100A4 and p53 in the nucleus, and also that S100A4 negatively affects cellular p53 protein levels. In situ PLA was utilized to look at the interaction between p53 and S100A4. We were able to confirm not just the connection between S100A4 and wt p53, but also that it occurs in the cell nucleus, using this method. The difficulties in identifying the connection between p53 and S100A4 might be explained if the interaction between S100A4 and p53 represents a stage in the biological processes that leads to p53 ubiquitination and destruction. Our findings imply that S100A4 is involved in MDM2-dependent p53 ubiquitination and degradation, given the nucleus localization of the interaction between S100A4 and p53 and the fact that lower S100A4 levels result in enhanced p53 stability.

The findings provided here are particularly significant because p53 is one of the most well-known tumor suppressor proteins. An abundance of evidence suggests

that p53 inactivation is essentially required for tumor growth. S100A4, a protein that is commonly overexpressed in malignancies and has been linked to poor prognosis, may contribute to p53 degradation through its interaction with p53, according to the findings. These findings clearly indicate why high S100A4 expression is advantageous to tumor development, and they also explain why S100A4 has a poor prognostic impact in clinical trials. Taken together, the findings imply that, in addition to raising the risk of metastasis as previously demonstrated, increased S100A4 expression in malignancies has the ability to suppress p53 activity. This research also suggests that S100A4 expression in clinical samples should be investigated in connection to cisplatin sensitivity to see if S100A4 may be used as a predictor of cisplatin therapy response. Also TAGLN mediated regulatory network implicated in proteinuria development was used. These findings add to our understanding of the molecular pathways driving proteinuria etiology. Recent study has uncovered a significant feature of the protein that makes it a promising candidate for further investigation as a therapeutic target: its specific control of activity levels and expression in cancer cell lines. In both epithelial and mesenchymal cells, the Rho family GTPases play an important role in directing the dynamics of the actin cytoskeleton. There is strong evidence that EMT is linked to the production of the vimentin protein, which is phosphorylated and reoriented in cells, regulating cell contraction and focal adhesion assembly and disassembly. During metastasis, there is also crosstalk between distinct components of the cytoskeleton. The use of actin-binding proteins as new therapeutic targets has a lot of promise for the creation of specific cancer medicines, according to researchers also when employing phenotypic screening to get positive results, there are a lot of procedural concerns to keep in mind. In conclusion, in addition to the crucial role of the RLC phosphorylation in driving the myosin IIA's conformations. These novel findings and analyses are attracting a lot of attention because they have the potential to lead to ground-breaking outcomes in our fight against cancer and drug-resistant cancer cells by combining traditional cancer therapy with EMT-related mechanisms. The findings imply that the mix of cytoskeletal components plays a critical role in the modulation of cytoskeletal dynamics in cancer.

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