We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

Mechanical Force and Actin Dynamics during Cutaneous Squamous Cell Carcinoma (cSCC) Progression: Opportunities for Novel Treatment Modalities

Sarah Boyle and Zlatko Kopecki

Abstract

Cutaneous squamous cell carcinoma (cSCC) accounts for 25% of cutaneous malignancies diagnosed in the Caucasian population. Surgical removal in combination with radio- and chemotherapy is an effective treatment; however, prognosis for patients suffering from aggressive cSCC is still relatively poor. Increasing prevalence coupled with high mortality and morbidity in aggressive metastatic forms of cSCC highlights the need for development of novel targeted therapeutics. Metastasis is a complex process requiring dramatic reorganization of the cell cytoskeleton. Recent studies have highlighted the importance of mechanical forces and actin dynamics in cancer cells' intrinsic ability to invade adjacent tissues, intravasate into vasculature, and ultimately metastasize. Tight regulation of the biochemical and mechanical properties of the actin cytoskeleton drives cellular processes involved in cSCC progression including polarity establishment, morphogenesis, and motility. Here we will provide a short introduction to disease pathogenesis, give an overview of the role of key regulatory proteins governing the mechanical forces and actin dynamics critical to cSCC progression, and describe the contribution of actin remodeling and actomyosin signaling to cSCC progression. We will also discuss how targeting protein regulating mechanical force and actin dynamics may have clinical utility in development of novel treatment modalities for patients suffering from aggressive cSCC.

Keywords: cutaneous squamous cell carcinoma, actin cytoskeleton remodeling, mechanical force, contraction, systemic therapy

1. Introduction

Cutaneous squamous cell carcinoma (cSCC) most commonly arises in actinically damaged skin and accounts for 25% of cutaneous malignancies diagnosed in the Caucasian population [1]. The incidence of cSCC continues to rise annually, with an estimated 50–200% increase in incidence in the last three decades in USA alone, and is predicted to increase in future years due to an aging global population [2]. Solar ultraviolet radiation is the primary environmental extrinsic cause of cSCC. Intrinsic

immunosuppression, the second most common cause, leads to the formation of aggressive cSCC in organ transplant patients, patients on immunomodulatory therapies, and those suffering from recessive dystrophic epidermolysis bullosa, a genetic skin blistering disease [3–5]. The incidence of cSCC is higher in individuals who are fair-skinned and have a sun-sensitive phenotype; however, the aggressive forms of cSCC are more common in men and the elderly [3]. Despite its prevalence, the relatively low fatality rate of cSCC means that its health and economic burden is often substantially underestimated [3], albeit latest data showing that in addition to significant morbidity cSCC accounts for up to 8000 deaths per year and costs approximately $4.8 billion annually in USA alone [6].

cSCC generally presents as a scaly, red or bleeding abnormal lesion on sun-exposed areas, and is associated with relatively benign outcomes and a low risk of metastasis. However, cSCC can demonstrate dramatic histopathological heterogeneity, resulting in a wide range of clinical outcomes [7]. Histopathologic subtypes of cSCC are broadly divided into low-grade SCC (**Figure 1A**) that are well-differentiated but have low metastatic potential (keratoacanthomas, SCC *in situ* and verrucous carcinoma), or high-grade SCC (**Figure 1B**) that are poorly differentiated, have high potential of metastasis and recurrence, and are associated with a poor prognosis for patients (desmoplastic cSCC, adenosquamous cSCC and cSCC associated with non-healing ulcers or scarring processes arising from chronic wounds) [2, 7]. Once developed, the natural history of untreated SCC is one of local invasion followed by metastasis via the lymphatic system, blood or perineural invasion, which can lead to death [7]. In most cases, cSCCs are detected early and can be successfully eradicated by surgical excision. However, if not detected and/or left untreated, disease progression to high-grade cSCC will often lead to mortality. Clinically, the most powerful predictor of disease pathogenesis is nodal metastasis and size, followed by invasion beyond fat, location, and lastly perineural invasion. These parameters are used in clinical staging systems for cSCC [3]. Management of cSCC is primarily surgical, with adjuvant chemoradiation approaches based on risk factors, patient and tumor features, as well as care features including access to treatment and associated costs [2].

With increasingly longer life expectancy, the health and economic burden of cSCC is likely to continue to increase significantly. Hence, a better understanding of factors contributing to cSCC progression and metastasis is necessary to aid development of novel therapies, aimed at combatting cSCC in the community. Despite
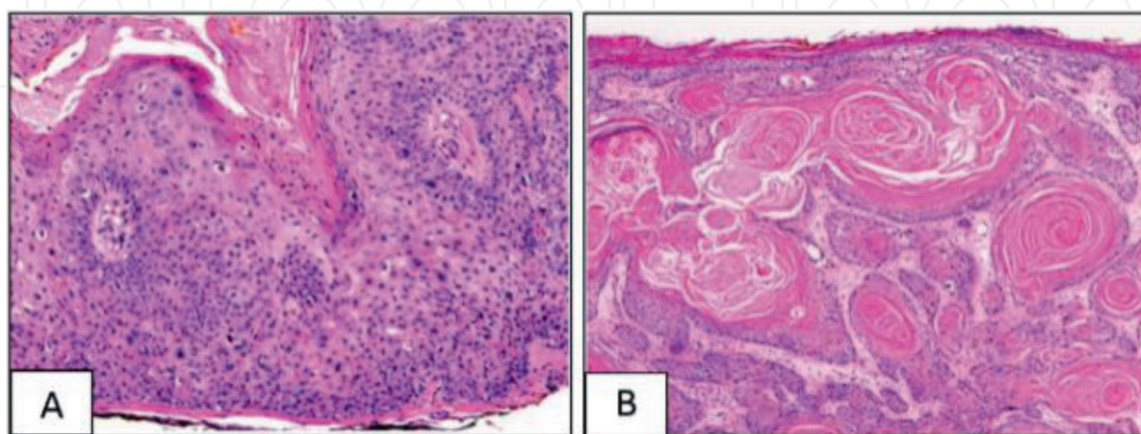

Figure 1.
Representative histopathological features of low-grade and high-grade cSCC. (A) Low-grade cSCC in situ with prominent dyskeratosis and aberrant mitosis at all levels of the epidermis, with marked parakeratosis and intact basement membrane. (B) High-grade poorly-differentiated cSCC lesions showing prominent keratinization and the formation of "pearl like" structures where dermal nests of keratinocytes attempt to mature. Adapted from Yanofsky et al. [7] and modified with approval.

recent advances in gene expression screening technologies that have begun to identify candidate genes commonly mutated in patients with cSCC (including TP53, CDKN2A, Ras and NOTCH1), which may be responsible for regulating motility and invasion in cSCC, a comprehensive understanding of the factors contributing to cSCC invasion including mechanical tension and actin dynamics is still emerging [8]. One thing that is clear is that patient outcome directly correlates with the degree of local and regional invasion, and coordinated regulation of the actin cytoskeleton is critical to cell motility, invasion and metastasis [9]. Consequently, the signaling pathways involved in mediating chemotactic cues from the extracellular environment that regulate the actin cytoskeleton and mechanical forces, guiding cancer cell invasion and metastasis, have been and continue to be an area of intense study.

Recent studies have revealed a number of proteins and molecules that are aberrantly expressed in cSCC. These proteins link cell migratory signals to the actin cytoskeleton, thereby playing an instrumental role in the ability of cancer cells to resist chemotherapy and/or metastasize [10]. In this chapter we will describe the actin dynamics and mechanical force governing tumor cell migration, invasion and metastasis. We will outline the main signaling pathways governing the formation of invasive protrusions by cancer cells with regard to the function of key regulatory proteins involved in actin cytoskeleton remodeling in cSCC.

The metastatic spread of aggressive cancers, including cSCC, is a highly selective process involving a series of sequential and orchestrated steps in the so-called "metastatic cascade": detachment from the primary tumor site, cell migration and invasion of the surrounding extracellular matrix (ECM), intravasation into vasculature, extravasation at a secondary site, and interaction with the extracellular environment to form metastatic tumors [11]. Each of these steps offers the potential for design of different therapeutic approaches to combat aggressive cSCC. Indeed, a number of recent studies have identified novel therapeutic approaches including both adjuvant and neoadjuvant treatments, with clinical trials utilizing epidermal growth factor receptor inhibitors and immune checkpoint blockers (nivolumab, pembrolizumab, and ipilimumab) showing promising early results as potential treatments of cSCC [12]. Recent trials using cytotoxic chemotherapy have, however, shown limited advances for the treatment of cSCC, and trials investigating combined immune checkpoint inhibitor and radiation therapies, which may have synergistic effects in treatment of cSCC, are still pending [13]. This highlights the need for increased research to close the gaps in our knowledge of cSCC biology, including better understanding of the factors that lead to aggressive cSCC, the role of microbiomes and HPV infection, the role that mechanical force and actin dynamics plays in this process, prediction of clinical response to therapies including immune checkpoint blockade, and how to tailor better prevention and treatment strategies to individual risk factors and needs [6]. Emerging evidence on the crosstalk between different components of the cytoskeleton in metastatic progression combined with clinical data illustrating strong relationships between cytoskeletal alterations and metastasis in various cancers pinpoints important opportunities for potential therapeutic targets [11]. Later in this chapter we will describe current research that has attempted to identify the steps of the metastatic cascade suitable and most amenable for therapeutic intervention, with a focus on harnessing our knowledge of actin cytoskeleton remodeling and mechanical forces to postulate therapeutic strategies targeting cytoskeletal and cytoskeletal-associated proteins critical in cSCC.

2. Cytoskeletal dynamics and regulation during cSCC progression

The skin is exposed to and responds to a wide range of mechanical signals throughout homeostasis and through to malignancy. Mechanical forces have been

shown to regulate these normal cellular processes including stem cell renewal, lineage differentiation and proliferation, wound healing, as well as transformation through changes in the actin cytoskeleton—the ability to protrude, adhere to the ECM, migrate through tissue and invade into the underlying basement membrane.

The types of mechanical forces exerted upon the skin can vary depending on the context. In homeostasis, tensile/stretch forces and compressive forces arise as a result of muscle and joint movements, and physical location—skin stretched over bone is under significantly more stress than when over fat or muscle [14]. Tensile forces cause cells to elongate and expand, and therefore are generated at sites of wounding as epithelial cells migrate in and contract to close the wound. This can generate scarring and fibrosis, which can lead to skin cancer including cSCC [15]. Compressive forces generate different biomechanics in skin cells compared to tensile force [16]. Compressive forces are able to activate Rho-ROCK signaling (described below) in the skin, which has been shown to play a role in tumor progression [17]. In melanoma, it was found that stress-bearing areas of the foot were more conducive to cancer development due to increased mechanical compressive stress [18]. Changes in substrate stiffness underlie these mechanical signals (**Figure 2**), and it has been shown that stiff stroma can lead to an activation of integrin signaling and subsequent cSCC development [19].

Cells have the ability to sense these changes in their environment (process referred to as "mechanosensing"). The mechanical signal is then converted to a biochemical signal in a process called mechanotransduction, and the biochemical signals initiate a cascade of changes within the cell at the transcriptional, translational and post-translational levels that result in a cell that can appropriately and reciprocally respond to the extracellular signals (process referred to as "mechanoreciprocity") [20]. In disease states including cSCC, the heightened and/or constitutive extracellular signals generate a detrimental loop of ever-increasing mechanoreciprocal signaling, hence leading to enhanced tumor progression, invasion and eventually metastasis [20].

Triggered by the changes in the cell microenvironment, the actin cytoskeleton undergoes a number of changes that allows a cell to become more motile and/or invasive. The ability of a cell to undergo directed migration is essential to its ability to metastasize, and is characterized by an ordered process (**Figure 3**) of membrane protrusion at the leading edge (filopodia and lamellipodia) and sides (invadopodia)
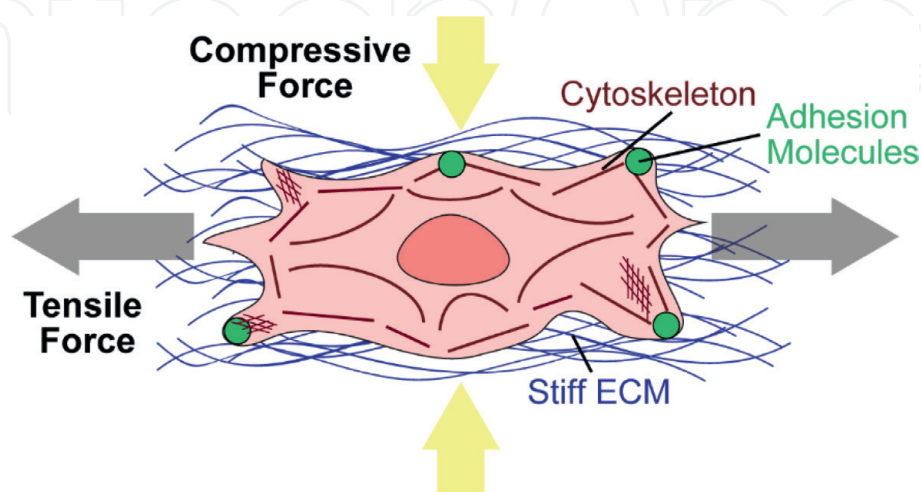

Figure 2.
Mechanical forces acting upon skin cells. The major types of mechanical forces experienced by skin cells are compressive (inward pushing) and tensile (stretching) forces, which in cSCC progression are generated by an increase in extracellular matrix stiffening. These are sensed by the cell, which then is able to respond accordingly.

of the cell, contact and adhesion between the protrusion and the matrix, movement of the main cell body, and retraction of the trailing edge [21, 22]. Lamellipodia are flat membrane protrusions containing dendritic arrays of actin filaments that branch out like a sheet from the leading edge of a cell. This particular form of protrusion is thought to have a major role in cell migration as their morphology allows the cell to make multiple contacts with the underlying substrate and pull the cell forward. Filopodia are narrow protrusions made up of bundled and cross-linked actin filaments that also stretch out from the leading edge. Invadopodia, unlike lamelli- and filopodia, are protrusions branching out from the sides of a cell, which have increased membrane remodeling and matrix degradation proteins [21]. These particular membrane protrusions are often seen in cancer cells including during cSCC progression [23].

Adhesions between the cancer cells and matrix are necessary for invasion and are largely mediated by integrins (discussed in detail below) [22]. During cSCC progression, there is an increase in cellular attachment to the ECM and, concurrently, a decrease in attachment to neighboring epithelial cells signified by a reduction in levels of E-cadherin expression. Following matrix adhesion, the trailing edge of the cell contracts, allowing the cell to move forward. Myosin II is required for this actin filament contraction, and is largely regulated by signaling through the small G-protein Rho. Once the cell contracts, its tail detaches as focal adhesion complex components are cleaved [22]. Actin remodeling proteins are essential in these processes, and often regulate cell-cell and cell-stroma attachment and turnover of focal adhesions, allowing cell traction and movement to take place. The coordination of actin polymerization and contraction allows the cSCC cell the ability to migrate through dense, stiff ECM and stroma to metastasize to lymph node or surrounding organs.

A number of different pathways are activated downstream of mechanical signals, causing changes in the actin cytoskeleton. Signaling pathways involved in cytoskeletal dynamics during cSCC progression are also activated as a result of constitutive or heightened growth factor signaling [24]. Together, the combination of mechanical and biochemical signals can trigger a multitude of intracellular signaling cascades that ultimately affect cell morphology. In this section, we will discuss the broad families of proteins regulating actin dynamics and mechanical forces in cSCC, an overview of which is illustrated in **Figure 4**. The overview provided in this chapter will not cover all the pathways or actin remodeling proteins involved but focus on those most relevant to cSCC progression, invasion and metastasis.
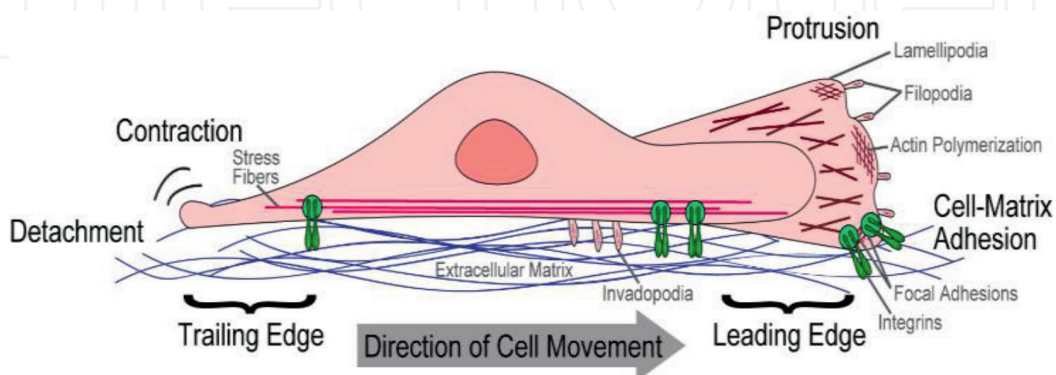

Figure 3.
Cell motility as controlled by the actin cytoskeleton. Upon sensing of extracellular cues, intracellular signaling cascades generate cytoskeletal protrusions (lamellipodia and filopodia) at the leading edge containing actin filaments, as well as invasive protrusions (invadopodia) at the sides of the cell, necessary for cSCC invasion. The cell adheres to the matrix, forming integrin-mediated focal adhesions. The nucleus and cell body are then pushed forward as the trailing edge contracts, via stress fibers. The rear of the cell then detaches, allowing the cell to migrate forward.

2.1 Mechanosensing: integrins

Integrins are well-characterized as the first point of contact for mechanical signal transduction. Inactive heterodimers on the cell surface, they are partially activated by intracellular proteins ("inside-out" signaling) before full activation upon binding to extracellular ligands ("outside-in" signaling) [25]. Binding to the extracellular ligand results in full activation of the integrin receptor and leads to the formation of either an intracellular focal adhesion complex to link the ECM to the actin cytoskeleton, or hemidesmosomes, linking the ECM to intermediate filaments. The integrin that is activated is context-dependent in regards to the particular focal adhesion complexes that are formed, broadly encompassing a range of adaptor proteins including: talin, vinculin, paxillin, Flightless I (Flii), focal
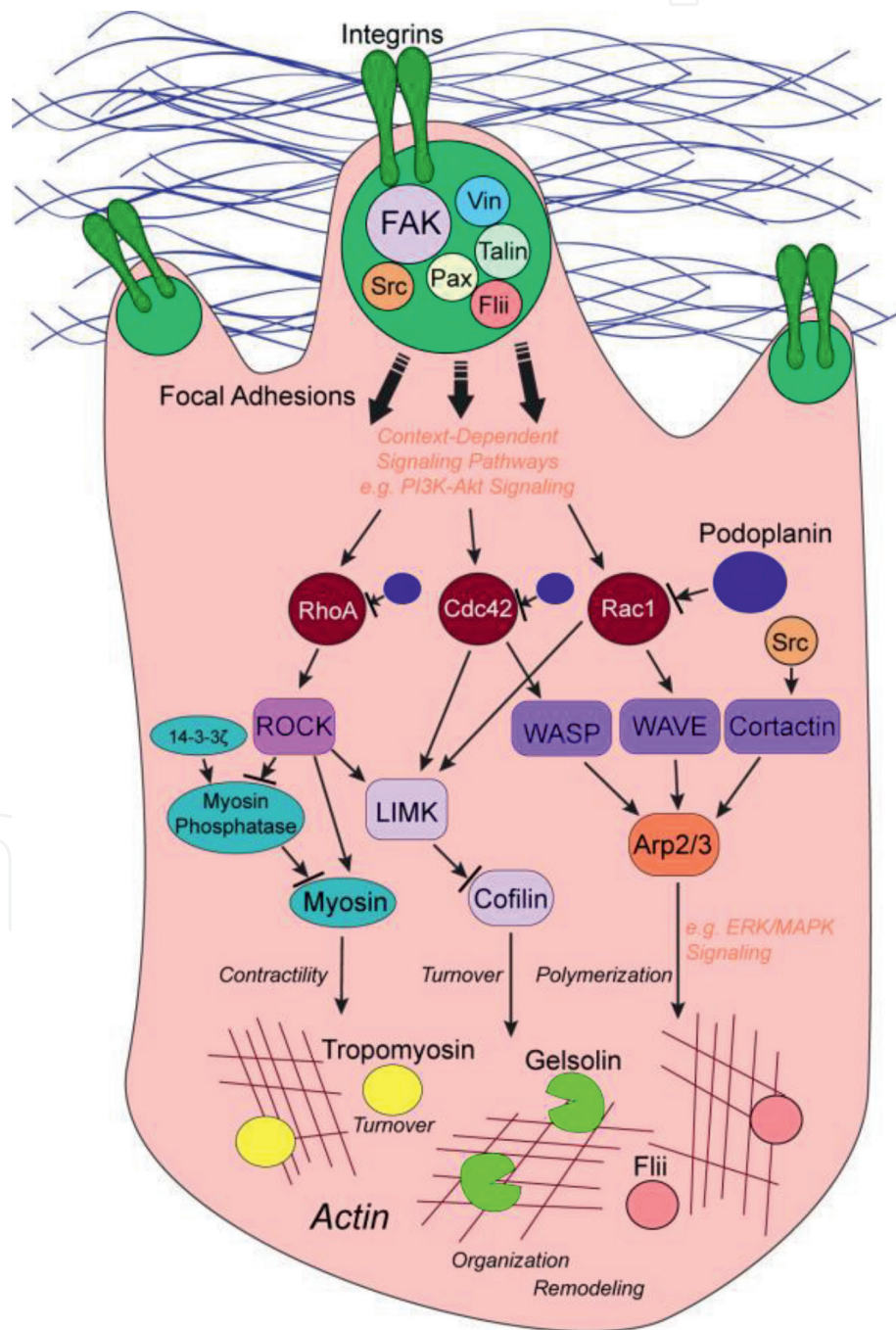

Figure 4.
Major pathways regulating cytoskeletal dynamics downstream of mechanical signals in cSCC. Graphical representation of signaling pathways which respond to mechanical force and govern downstream actin remodeling during cSCC progression.

adhesion kinase (FAK) and integrin-linked kinase (ILK). FAK is rapidly recruited to focal adhesions upon integrin activation and is auto-phosphorylated, driving downstream signaling. FAK phosphorylation, stimulated by integrin activation, allows binding of Src-family kinases that are then able to trans-phosphorylate FAK. This leads to activation of ERK/MAPK signaling, and this complex is therefore able to control cell shape and regulate focal adhesions [26]. In cSCC, a step-wise increase in activation of FAK from unaffected margin skin to hyperproliferative skin and invasive cSCC [27] results in elevated integrin-FAK-Src signaling that stimulates keratinocyte migration [28] and drives progression of benign papillomas to aggressive cSCC [29, 30]. In cSCC, it has been demonstrated that FAK function is required for cancer stem cell maintenance, regulating cSCC initiation, growth, regression, and progression [31]. Actin remodeling protein Flii, which will be discussed in detail in Section 2.4, has been shown not to directly bind integrin receptors but form focal adhesion complexes with adaptor proteins and regulate integrin activation, downstream Src/paxillin signaling and focal adhesion turnover in a Rac1 dependent manner [25, 32]. Additionally, latest research has shown that actin remodeling proteins Flii and gelsolin, which have always been thought to be intracellular, have also both been shown to be secreted where they function to sequester extracellular actin post tissue injury, modulate inflammation and affect collagen VII anchoring fibril formation. Flii is able to modulate inflammation via toll-like receptor 4 (TLR-4) signaling, as Flii leucine-rich repeat (LRR) domains have 50% similarity to LRR domains of TLR-4, by which the immune system is able to detect infection or injury. The binding of LRRs to PAMP and DAMP molecules activates intracellular TLR signaling and ultimately results in the release of proinflammatory cytokine secretion [33]. The extracellular roles of Flii and gelsolin in respect to mechanosensing and cSCC progression are still to be examined. Nevertheless, it is clear that the coordinated activation of integrin receptors, focal adhesion complex formation and downstream signaling stimulation is essential for cytoskeletal changes that are necessary for cell migratory and invasive capability during cSCC progression.

2.2 Mechanotransduction: Rho GTPases

Downstream of integrin activation are the Rho small GTPases, which are part of the Ras superfamily and key regulators of cell cytoskeletal dynamics through both actin polymerization and organization, hence driving cancer cell motility [34]. Of this subfamily, RhoA, Rac1 and Cdc42 are the best-characterized.

RhoA is involved in actomyosin contractility, formation of actin stress fibers and assembly of focal adhesion complexes. The main regulator of cytoskeletal dynamics leading to formation of stress fibers is myosin II, and its regulatory subunit myosin regulatory light chain-2 (MLC2) can be activated by RhoA signaling leading to contraction of actin fibers. Rho-associated kinases ROCK1 and ROCK2 are serine/threonine kinases that contain a Rho-binding domain and are activated by RhoA in its active GTP-bound form, directly activate MLC2 via phosphorylation. Due to its roles in cell contractility and movement, Rho-ROCK signaling has been implicated as a driver for invasiveness during cSCC progression but also plays a positive role in physiological normal wound healing processes [35]. In human cSCCs, ROCK is not only highly expressed but also activated in the hyperproliferative skin and invasive regions of the tumor, as shown by phosphorylation of the ROCK substrate myosin phosphatase (MYPT1) [27]. In the skin, this ROCK-mediated actomyosin contractility is required for proliferation of the epidermis, as ROCK activation stabilizes β-catenin through phosphoinositide 3-kinase (PI3K), Akt, and inhibition of its phosphorylating kinase GSK3β [36]. During cancer progression from normal skin through to hyperproliferative and invasive cSCC, nuclear localization of active

β-catenin and inactivation of GSK3β is increased, accompanied also by a progressive increase in FAK activation [27, 36]. It has further been demonstrated that a negative regulator of ROCK signaling, 14-3-3ζ, is significantly down-regulated in human cSCCs. As genetic deletion of 14-3-3ζ results in significantly larger papillomas in the two-stage chemical carcinogenesis (DMBA-TPA) mouse model of SCC, this suggests that uncontrolled ROCK signaling can drive cSCC tumor growth [35].

Cdc42 is involved in formation of F-actin microspikes and filopodia in both normal and cSCC cells, by via actin polymerization at the leading edge and at the sides of the cells, contributing to cSCC invasion. Traf6 has been demonstrated to regulate Cdc42 to induce these F-actin microspikes in SCC cells [37]. When Cdc42 is absent in keratinocytes, cells are no longer able to properly process and deposit ECM components or integrin receptors, hence halting cellular migration [38]. Taken together, these studies demonstrate roles for Cdc42 in both skin cell migration and invasion, necessary cellular processes for progression of cSCC. Cdc42 is necessary for proper cellular polarity in normal and migratory cells [34], and this in turn activates G-proteins of the Rac subfamily of Rho small GTPases [39].

Rac1 is hyperactivated in cSCC via integrins including α3β1, and is important for keratinocyte cell proliferation [40, 41]. Rac1 stimulates polymerization of actin via multiple kinase signaling cascades including that of MAP kinase (elevating the transcription factors AP-1, NFκB and CRE). This therefore allows the cell to form a branched actin network, necessary for leading-edge lamellipodia formation and membrane ruffling [42]. Indeed, actin remodeling protein, Flightless I (Flii), has been shown to regulate focal adhesion by inhibition of paxillin phosphorylation via a Rac1 dependent pathway [32]. Rac1 can be activated by Tiam1, and it was shown in the two-stage chemical carcinogenesis cSCC model that genetic deletion of Tiam1 significantly reduced tumor incidence, burden and growth. However, SCC tumors that did arise in Tiam-null mice were significantly more invasive and malignant, potentially due to a loss of cell-matrix adhesion [43]. This highlights the dual homeostatic and tumor-promoting roles that actomyosin regulatory pathways can play during cSCC progression.

Rho-associated kinase ROCK can also phosphorylate and activate LIM kinases 1 and 2, which are then able to phosphorylate and inactivate cofilin, an F-actin severing protein, resulting in F-actin filament stabilization [44]. Accordingly, it has been shown that LIMK1 levels are increased in cSCC tumor tissue compared to normal skin, and that LIMK1 silencing can suppress cell growth and invasion in cSCC cell lines [45]. In addition, it has been suggested that LIMK is required in the microenvironment in leading fibroblasts, to allow for efficient remodeling of the ECM and subsequent cSCC invasion [46]. It has been demonstrated that cofilin phosphorylation can be abolished by treating cSCC cells with LIMK inhibitors. This reduces β-catenin accumulation and epidermal proliferation via reversing actomyosin contractility [36], however clinical trials using these inhibitors are still pending. The involvement of Rho GTPases in cellular migration and invasion in cSCC due to cytoskeletal rearrangement implicates this family of proteins as drivers of cancer initiation, progression and metastasis. Hence, targeting Rho pathway signaling, in particular that of RhoA-ROCK signaling, is an attractive therapeutic option that will be explored later in this chapter.

2.3 Actin polymerization: WASP, cortactin, and Arp 2/3

Actin nucleation promoting molecules are activated downstream of Rho GTPases and growth factor receptors. Wiskott-Aldrich syndrome protein (WASP) family members, via Erk, paxillin, and Src signaling together with cortactin, act to stimulate the actin-related protein (Arp) 2/3 complex which in turn mediates actin polymerization [47].

The WASP family consists of WASP proteins and WASP-family verprolin-homologous (WAVE) proteins. WASP proteins interact with Rho GTPases in order to form cellular protrusions and allow the cell to migrate, for example, N-WASP is involved in formation of filopodia and invadopodia upon its activation by Cdc42. WASP proteins bind to G-actin and Arp2/3 resulting in their activation and hence triggering actin filament production. WASP and WAVE proteins also bind profilin, which transports actin monomers onto the growing ends of actin filaments, and is therefore also an important factor in cell motility [47]. As a loss of N-WASP in keratinocytes causes epidermal hyperplasia and a reduction in epithelial cell tight junctions [48], this highlights the need for proper control of these processes. The formation of cellular protrusions also relies on the Src protein cortactin, which binds to and activates the Arp2/3 complex independently of WASP, thereby regulating actin filament nucleation. Cortactin binds to F-actin, stabilizing actin filaments and allowing it to properly activate Arp2/3 [49, 50]. In head and neck SCC, overexpression of cortactin increases cancer cell proliferation and increases cell survival in anchorage-independent conditions [51], while in oral SCC, silencing of cortactin was shown to significantly impair invasiveness and downregulate the levels of epithelial markers, indicating an epithelial to mesenchymal transition (EMT) [52], a process by which epithelial cells lose their adhesion to one another and acquire a migratory and invasive mesenchymal phenotype (discussed in further detail below). Indeed, Arp2/3 complex proteins are required for cell proliferation and migration in other forms of SCC [53, 54], and based on the Arp2/3 role in actin filament polymerization, it is clear that Arp2/3 is also critical for actin cytoskeletal remodeling leading to cancer cell motility and invasion.

2.4 Actin remodeling: tropomyosin, Flightless I, and podoplanin

The actin cytoskeleton is composed of three distinct elements including microfilaments, microtubules and intermediate filaments. Tight regulation of cytoskeletal elements must be coordinated, and latest research has shown that interplay between actin and microtubules is bidirectional [55]. Actin-based motility is also dependent on the balanced activity of number of specific actin remodeling proteins. In this section we will highlight main actin remodeling proteins that have been shown to have specific functions in cSCC progression, including members of the tropomyosin family of actin-associated proteins, the gelsolin family of actin remodeling proteins, and Podoplanin, a simple glycoprotein with important roles in cSCC progression.

Members of the tropomyosin family of actin remodeling proteins display a tissue- and time-specific expression, while their association with actin filaments impairs isoform-specific regulation of actin filament dynamics [56]. Tropomyosin proteins assemble as polymers in the major groove of the polymerized actin filament and their association drives actin filament turnover, hence playing an important part in a number of cellular functions including motility and metastasis [57]. There are over 40 different isoforms of tropomyosin and few have been described as having an important role in cSCC progression. High expression of Tm5NM1, a specific cytoskeletal tropomyosin isoform, has been shown to inhibit cell migration and invasion as well as impair normal wound healing via its effects on Src activation, focal adhesion stabilization, increased actin filament tension, and paxillin phosphorylation [58]. Current research is examining the effect of Tm5NM1 inhibitor TR100 on cSCC progression (see Section 3). On the other hand, downregulation of tropomyosin-1 and complete loss of β-tropomyosin has been identified in human esophageal SCC, while α-tropomyosin has been shown to be preferentially

expressed in keratinocytes of the multistage model of murine cSCC, collectively suggesting isoform specific functions [59–61].

The dynamic remodeling of the actin cytoskeleton is also tightly regulated by the gelsolin family of actin remodeling proteins, which includes: gelsolin, villin, adseverin, capG, advillin, supervillin, and Flightless I (Flii) [62]. These actin binding proteins function in the cytoplasm of the cells where they control actin organization by severing pre-existing filaments, capping the fast growing filament ends and bundling filaments, enabling filament reassembly into new cytoskeletal structures that are required for cell motility, invasion and metastasis [63]. Studies have shown that downregulation of gelsolin proteins counteracts cancer cell invasion *in vitro* [64], however in cSCC, gelsolin and Flii have been the most studied to-date. Gelsolin over-expression has been shown to promote cell growth and motility in oral SCC [64, 65], while Flii, through its effects on apoptosis, has been linked to promotion of breast cancer progression and invasion and progression of cSCC [23, 66]. Flii is an important regulator of cell adhesion, migration and proliferation and a number of previous studies have described the role of Flii protein in wound healing and demonstrated the therapeutic effect of Flii neutralizing antibodies (FnAb) in acute and chronic wounds, skin blistering diseases and inflammatory skin conditions [25, 32, 67–72]. Flii modulates cell adhesion and paxillin signaling, and regulates actin polymerization, tight junction formation and ECM production during wound repair suggesting that similar roles may govern Flii activity in cSCC progression [23, 25, 32, 72]. Indeed, altering Flii levels both genetically and using Flii neutralizing antibodies significantly augments cSCC progression [23]. Therapeutic approaches targeting Flii in cSCC are described in Section 4.

The expression of Podoplanin, a small mucin-like protein, has also been linked to remodeling of the actin cytoskeleton in cSCC. Podoplanin is upregulated in the invasive front of a number of human carcinomas including cSCC and has been shown to induce collective cell migration by filopodia formation, via downregulating the function of Rho small GTPases [73, 74]. Podoplanin has also been linked to an increase in the migration of cancer-associated fibroblasts as well as endothelial network formation [75]. Collectively these findings suggest that Podoplanin is able to induce an alternative pathway of tumor cell invasion in the absence of traditional epithelial-mesenchymal transition.

Taken together, these studies highlight the important role of actin remodeling in cSCC progression and outline the importance of bidirectional stimulation of actin remodeling by both intrinsic factors and the microenvironment, critical to tumor invasion/metastasis. These findings provide a rationale for development of novel therapeutic strategies that target tumor invasion and metastasis.

3. Physiological effects of actin remodeling

Changes in the actin cytoskeletal structure result in changes to cell morphology, creating a cell more conducive to invasion. One of the commonly recognized requirements of metastasis is a cellular transition from epithelial to mesenchymal phenotype (EMT). This transition is characterized by upregulation of genes including vimentin, SNAI1 (snail), SNAI2 (slug) and Zeb1, and a downregulation of epithelial genes including cadherins, as well as concurrent loss of cell-cell junctions [76]. For example, in head and neck SCC an increase in matrix stiffness and hence increased mechanical signaling caused an increase in EMT markers in tumor-initiating cells [71]. Cells undergoing EMT develop an elongated spindle-like morphology, due to the enhanced membrane protrusion formation [77]. It has been shown in A431 cells, a human epidermal SCC cell line, that loss of T-cadherin induces elongation of cells and formation

of lamellipodia and multiple leading edges via changes in EGF-stimulated motility and invasion, as T-cadherin influences EGFR localization and responsiveness [78]. Of note, RhoA activation was also increased upon the loss of T-cadherin [79]. Likewise, Podoplanin is also capable of transforming cells to an invasive state without having to undergo EMT, due to rearrangements of the actin cytoskeleton [74].

The remodeling of the actin cytoskeleton also creates intracellular reciprocal forces that balance out the forces received by the cell from the extracellular microenvironment. Actin polymerization extends the filament network, and as filaments in the leading edge are compressed between transient associations with the cell membrane and the bulk of the actin cytoskeletal network behind them, intracellular force is generated. As protrusions are extended and retracted, actin filaments experience tension from transient bonds with the membrane, becoming bent or compressed [80]. Activation of the mechanotransduction pathways described above, downstream of ECM stiffness in cSCC, can also increase the propensity for augmented interactions with the stroma, and generate a tumor-promoting environment that enhances mechanoreciprocal signaling [20, 36].

4. Therapeutic approaches targeting actin cytoskeletal regulatory pathways

Metastasis is a complex process requiring significant reorganization of the actin cytoskeleton and coordinated involvement of number of key proteins. These proteins interact directly and indirectly with both actin and microtubule networks, hence significantly influencing migratory and metastatic cell phenotypes. Strong clinical relationships between actin cytoskeletal alterations and cutaneous cancer metastasis have been previously described [11], offering potential opportunities for therapeutic intervention. For example, up-regulation of cortactin, an actin-binding adaptor protein in melanoma, has been directly linked to increased distal metastasis and reduced disease-free survival, while up-regulation of Ras mRNA has been directly linked to Stage III and Stage IV disease in head and neck SCC [81, 82]. The complex nature of cellular migration and invasion presents challenges in developing therapeutic approaches, as compensatory pathways may overcome the effects of specific inhibitors. This highlights the need for development of combinational and adjuvant therapies targeting multiple pathways that are involved in actin dynamics to treat aggressive cSCC. Pharmacological inhibitors of actin have failed clinical development due to non-specific effects on normal actin function in tissue, resulting in high levels of cardiotoxicity. Hence, research efforts have centered on therapeutic approaches that can modify signaling pathways regulating the actomyosin cytoskeleton and/or target cytoskeletal and cytoskeletal-associated proteins [11].

Increasingly it has been recognized that microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) are involved in regulating cytoskeletal dynamics through regulation of gene expression. lncRNAs have been shown to regulate lamellipodia formation by downregulating integrin expression in cSCC [83]. A number of miRNAs have also been shown to play a role in regulating cell cytoskeletal dynamics and interactions with stroma in cSCC, including: miR-340 [84], miR-20a [45], miR-31 [85] and miR-125b [86]. These miRNAs act via inhibiting RhoA, LIMK1, WAVE3 and matrix metalloproteinase (MMP)-13 respectively. The use of miRNAs in the clinic has clear potential, however clinical trials are yet to be undertaken.

One of the signaling pathways participating in regulation of cancer cell motility, invasion and metastasis is the ROCK signaling pathway, described in detail above. Hyperactivation of this pathway promotes cancer cell invasion in many solid tumors and studies have shown that Rho signaling through ROCK promotes the rounded

bleb-associated mode of amoeboid motility, thereby promoting tumor cell metasta-sis [87]. Earlier reports have shown that treatment with the selective ROCK inhibi-tor Y-27632 increases SCC cell adhesion, upregulates expression of E-cadherin, and decreases the phosphorylation of cofilin (thereby activating it), resulting in altered actin cytoskeleton rearrangement [88]. In the two-stage chemical carcinogenesis model, Y27632 treatment resulted in significantly smaller and fewer papillomas, with a reduced rate of cSCC conversion. This was associated with reduced collagen deposition in the ECM, which would indicate a decrease in mechanical signaling due to ECM stiffness [36]. This illustrates that inhibition of ROCK is a potential strategy for treatment of solid cancers including cSCC.

A potential upstream regulator of ROCK-mediated cell migration is gamma-actin. Modulation of gamma-actin changes the directional cell migration via effects on microtubule dynamics and cell polarity, hence highlighting the crosstalk between actin cytoskeleton and microtubule signaling as a potential modality for targeting specific components of the network [89]. More recent studies, using newer genera-tions of ROCK inhibitors and pharmacological small molecule inhibitors of the downstream effectors of ROCK that have micro-tubule stabilizing effects, are also showing some promise in regulating tumor metastasis, however no compounds are yet clinically approved [90]. Another potential therapeutic strategy is harnessing the ability of 14-3-3ζ, a negative regulator of ROCK signaling, to moderate mechanoreci-procity in cSCC [35]. These approaches are particularly enticing due to the negative effects that clinical targeting of ROCK itself can potentially have [91].

Interestingly, studies investigating the interactions between SCC cells and cancer-associated fibroblasts have shown that ROCK activity is also an important requirement for adjacent stromal fibroblasts. ROCK activity positively influences the JAK1-STAT3 signaling pathway resulting in increased actomyosin contractility and proinflammatory cytokine secretion, favoring cSCC cancer cell invasion [92]. Consequently, these studies suggest that approaches aimed at inhibiting ROCK sig-naling have the potential to interrupt both intrinsic and microenvironment-derived signals during cSCC progression.

Actin remodeling proteins have long been implicated in cSCC, as a dysregu-lated actin cytoskeleton and an aberrant tumor microenvironment is a hallmark of aggressive cSCC [11, 93]. One particular actin remodeling protein, Flightless I (Flii), has been identified as a tumor promoter with transcriptional activity in colorectal, breast and hepatocellular carcinoma cell lines [66]. However, recent studies have also shown that Flii is significantly increased in human and mouse cSCC tissue samples, while secreted Flii is elevated in the sera of patients with cSCC and is increased in different cSCC cell lines established from human primary, recurring and metastatic cSCC as well as immortalized keratinocytes [23]. Human cSCC samples show positive staining for Flii in invading keratinocytes, surround-ing tumor stroma and the outer hyperkeratotic layer of cSCC nodules present in the deep dermis [23]. Together, these data suggest that Flii is not only an important regulator of the actin cytoskeleton involved in cSCC progression but also a potential therapeutic target and diagnostic marker of cSCC severity. Indeed, overexpression of Flii resulted in severe cSCC development via evasion of apoptosis, while reducing Flii expression using intradermal injections of FnAb during cSCC initiation and progression significantly reduced Flii expression in both the tumor microenviron-ment and in the serum, and led to significantly smaller tumor size (**Figure 5**) and decreased cellular sphere formation and invasion *in vitro* [23].

Remodeling and polymerization of actin filaments is critical during cSCC inva-sion and formation of invadopodia. Increased Flii levels have shown to weaken cell-stroma and cell-cell adhesions via alteration of GTPase and Src/paxillin signaling pathway activity [32] and augmented integrin-facilitated cell migration [25]. This
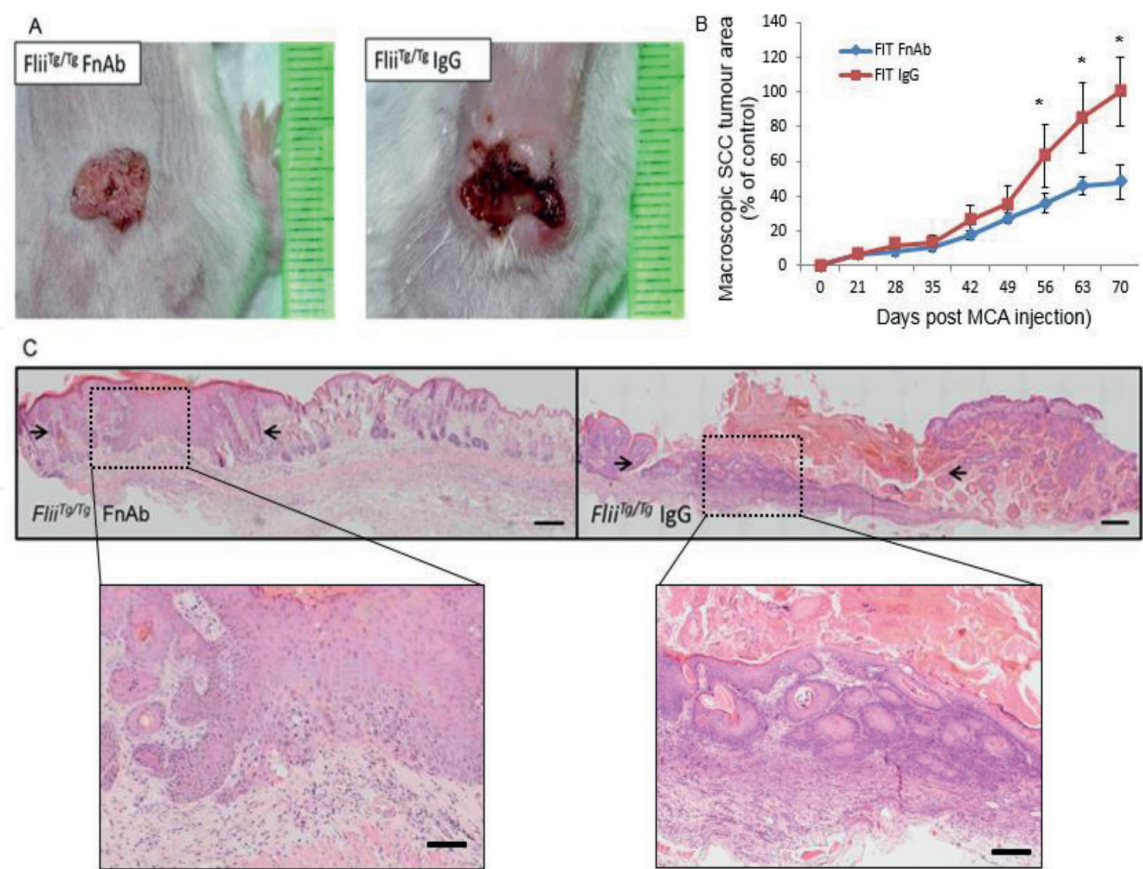
Figure 5.
Reducing Flii expression prior to initiation and development of cSCC using FnAb results in decreased cSCC progression. As Flii is increased in human SCC samples, the effect of preventative FnAb treatment prior to SCC induction was investigated in Flii$^{Tg/Tg}$ mice. Mice were treated with FnAb 2 weeks prior to cSCC induction and every second week throughout the trial and SCC development. (A and B) Reducing Flii levels in mice skin using preventative FnAb treatment prior to cSCC induction and during development resulted in decreased tumor progression and size relative to IgG control mice that have significantly larger and more developed necrotic and ulcerated tumors. (n = 12/treatment) Mean ± SD $^*p < 0.05$. (C) Representative images of H&E stained tumors treated with FnAb or IgG control show more severe ulcerated tumor pathology in the IgG control group (black arrows). Scale Bars = 500 µm and 100 µm.

promotes tumor progression and facilitates invadopodia formation and subsequent tumor invasion into surrounding tissue [23]. Indeed, Flii is significantly increased in invading cSCC and has been demonstrated to associate with cortactin at leading edges of invadopodia and to regulate the invasive properties of cSCC keratinocytes [23]. Systemic and topical therapeutic approaches using FnAb are currently in development with FnAb as a therapy for wound healing now entering the final pre-clinical validation stage [68, 94]. Flii has been shown to colocalize with structural (Claudin-1, -4 and -6) and adaptor (ZO-1 and -2) tight junction proteins and its overexpression in keratinocytes results in an altered F-actin/G-actin ratio, which can be restored using FnAb [72]. Therefore, taken together, these studies suggest that therapies targeting Flii may be a potential strategy for reducing the severity of cSCC in the community, however clinical trials using FnAb are still pending.

Pharmacological inhibition of actin-associated proteins aimed at compromising the survival and invasion of tumor cells may also have clinical benefit. One example of this strategy is harnessing TR100 inhibition of the tropomyosin isoform Tm5NM1. Tm5NM1 belongs to a family of actin-associated proteins that regulate the activity of several effectors of actin filament dynamics [95], as described above. The TR100 inhibitor has been shown to preferentially disrupt the actin cytoskeleton of tumor cells, impairing tumor cell motility and viability, and reducing melanoma growth both *in vitro* and *in vivo*. This therefore provides a pathway for development of a novel class of anti-actin compounds for the potential treatment of wide variety of cancers including cSCC [96].

Microtubule targeting agents of both synthetic and natural design, and microtubule stabilizing and destabilizing agents, have been the focus of anti-cancer therapy in the last decade and remain one of the most successful group of agents in the clinic [97, 98]. Their ability to regulate the tubulin-microtubule equilibrium disrupts the mitotic spindle, halting the cell cycle and resulting in cell death. They have been shown to be effective in combination with anti-angiogenic and anti-vascular properties and in some cases have demonstrated the ability to overcome multi-drug resistance, supporting their utilization as a chemotherapy [99]. Epothilones are a new class of anti-microtubule agents currently in clinical trials. Epothilones have shown activity in cSCC cell lines and in melanoma clinically, however clinical trials on cSCC patients are still pending [100, 101]. Other examples of microtubule-targeting agents, which have shown clinical promise in different subtypes of SCC including metastatic and recurrent disease, include semisynthetic compounds docetaxel and eribulin and a natural compound called rhizoxin [102–104]. While microtubule-targeting compounds are widely used as chemotherapeutic agents, they do have variability in different cancers, cancer cells frequently develop resistance to them, and they can be toxic to normal tissue, highlighting the need for better research and refinement of these compounds as well as a need to further understand their interactions with microtubule-associated proteins [105]. It is possible that microtubule-targeting agents also exert broader effects on tumor cell migration, invasion and metastasis and future studies should explore their effects on cSCC in combination with actin pathway inhibitors. Gaining a better understanding on the interplay of regulatory proteins governing the mechanotransduction and actin cytoskeletal remodeling involved in tumor cell migration, invasion and metastasis will lead to increased efforts to exploit therapeutic avenues targeting the actin cytoskeleton to treat aggressive cSCC.

5. Conclusions

The contribution of actin cytoskeletal remodeling and actomyosin signaling during SCC progression is significant and cannot be undervalued in the search for new treatment modalities. Recent research has identified a number of potential novel therapeutic targets within regulatory actin and microtubule signaling pathways that should be explored as potential therapeutic adjuvants to immunomodulatory therapies currently in clinical trials. A comprehensive understanding of the regulatory network of cutaneous mechanotransduction, mechanical forces and actin dynamics in cSCC, as discussed in this chapter, will facilitate the development of novel approaches to curb the incidence and progression of aggressive cSCC in the community, generating new inroads toward development of novel, individually personalized and efficient therapeutic approaches.

Acknowledgements

Conflict of interest

The authors declare no conflict of interest.

Nomenclature

Akt	protein kinase B—serine/threonine-specific protein kinase
AP-1	activator protein 1—transcription factor that regulates various cellular processes downstream of stimuli
Arp2/3	actin-related proteins 2/3 complex—protein complex regulating the actin cytoskeleton
Cdc42	cell division control protein 42—protein involved in regulation of cell cycle
CDKN2A	cyclin-dependent kinase inhibitor 2A
CRE	cAMP-response element
cSCC	cutaneous squamous cell carcinoma
DAMPs	damage-associated molecular pattern molecules
DMBA	7,12-dimethylbenz[a]-anthracene—polycyclic aromatic hydrocarbon that acts as a carcinogen
ECM	extracellular matrix
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
EMT	epithelial to mesenchymal transition
Erk	extracellular signal-regulated kinase—kinase involved in regulation of cellular processes such as meiosis and mitosis
FAK	focal adhesion kinase—a focal adhesion-associated protein kinase involved in cellular adhesion
Flii	Flightless I
FnAb	Flii neutralizing antibody
GSK3β	glycogen synthase kinase 3-beta—serine/threonine protein kinase
GTPase	enzyme that binds and hydrolyzes guanosine-5′-triphosphate
H&E	hematoxylin and eosin
HPV	human papillomavirus
ILK	integrin-linked kinase
JAK	janus kinase
LIMK	LIM-domain kinase
lncRNA	long non-coding RNA
MAPK	mitogen-activated protein kinase
miRNA	microRNA
MLC2	myosin regulatory light chain-2—subunit of myosin, which regulates cell contractility
MMP	matrix metalloproteinase—endopeptidases that degrade various ECM proteins
MYPT1	myosin phosphatase target subunit 1—subunit of myosin phosphatase, which dephosphorylates MLC and thereby opposes contractility
NFκB	nuclear factor kappa-light-chain enhancer of activated B cells—protein complex that regulates transcription and other cellular processes
PAMPs	pathogen-associated molecular pattern molecules
PI3K	phosphoinositide 3-kinase—intracellular signal transducer enzymes ROCK: Rho-associated coiled-coil containing kinases
SNAI1	snail—transcription factor promoting repression of E-cadherin
SNAI2	slug—transcription factor promoting repression of E-cadherin
STAT	signal transducer and activator of transcription proteins—intracellular transcription factor

Tiam1	T-lymphoma invasion and metastasis-inducing protein 1—regulates Rho-like proteins and transduces extracellular signals
TP53	tumor protein p53
TPA	12-0-tetradecanoylphorbol-13-acetate—tumor promoting phorbol ester
TLR-4	toll-like receptor 4—transmembrane receptor that activates NFκB signaling
Tm5NM1	tropomyosin isoform 5NM1
Traf6	tumor necrosis factor (TNF) receptor associated factor 6
WASP	Wiskott-Aldrich syndrome protein
WAVE	WASP-family verprolin-homologous
Zeb1	zinc-finger E-box-binding homeobox 1—transcription factor that induces EMT by repression of E-cadherin and other genes

Author details

Sarah Boyle[1] and Zlatko Kopecki[2,3]*

1 Centre for Cancer Biology, University of South Australia and SA Pathology, Adelaide, South Australia

2 Future Industries Institute, University of South Australia, Adelaide, South Australia

3 School of Pharmacy and Medical Sciences, University of South Australia Cancer Research Institute, Adelaide, South Australia

*Address all correspondence to: zlatko.kopecki@unisa.edu.au

IntechOpen

References

[1] Yanagi T, Kitamura S, Hata H. Novel therapeutic targets in cutaneous squamous cell carcinoma. Frontiers in Oncology. 2018;**8**:79

[2] Waldman A, Schmults C. Cutaneous squamous cell carcinoma. Hematology/Oncology Clinics of North America. 2019;**33**(1):1-12

[3] Green AC, Olsen CM. Cutaneous squamous cell carcinoma: An epidemiological review. The British Journal of Dermatology. 2017;**177**(2):373-381

[4] Mellerio JE et al. Management of cutaneous squamous cell carcinoma in patients with epidermolysis bullosa: Best clinical practice guidelines. The British Journal of Dermatology. 2016;**174**(1):56-67

[5] Collins L, Quinn A, Stasko T. Skin cancer and immunosuppression. Dermatologic Clinics. 2019;**37**(1):83-94

[6] Nagarajan P et al. Keratinocyte carcinomas: Current concepts and future research priorities. Clinical Cancer Research. 2018. DOI: 10.1158/1078-0432.CCR-18-1122

[7] Yanofsky VR, Mercer SE, Phelps RG. Histopathological variants of cutaneous squamous cell carcinoma: A review. Journal of Skin Cancer. 2011;**2011**:210813

[8] Que SKT, Zwald FO, Schmults CD. Cutaneous squamous cell carcinoma: Management of advanced and high-stage tumors. Journal of the American Academy of Dermatology. 2018;**78**(2):249-261

[9] Kelley LC, Shahab S, Weed SA. Actin cytoskeletal mediators of motility and invasion amplified and overexpressed in head and neck cancer. Clinical & Experimental Metastasis. 2008;**25**(4):289-304

[10] Yamaguchi H, Condeelis J. Regulation of the actin cytoskeleton in cancer cell migration and invasion. Biochimica et Biophysica Acta. 2007;**1773**(5):642-652

[11] Fife CM, McCarroll JA, Kavallaris M. Movers and shakers: Cell cytoskeleton in cancer metastasis. British Journal of Pharmacology. 2014;**171**(24):5507-5523

[12] Sacco AG, Daniels GA. Adjuvant and neoadjuvant treatment of skin cancer. Facial Plastic Surgery Clinics of North America. 2019;**27**(1):139-150

[13] Borradori L et al. Rescue therapy with anti-programmed cell death protein 1 inhibitors of advanced cutaneous squamous cell carcinoma and basosquamous carcinoma: Preliminary experience in five cases. The British Journal of Dermatology. 2016;**175**(6):1382-1386

[14] Hsu CK et al. Mechanical forces in skin disorders. Journal of Dermatological Science. 2018;**90**(3):232-240

[15] Alconchel MD, Olivares C, Alvarez R. Squamous cell carcinoma, malignant melanoma and malignant fibrous histiocytoma arising in burn scars. The British Journal of Dermatology. 1997;**137**(5):793-798

[16] Wang Y et al. Hyperelastic material properties of mouse skin under compression. PLoS One. 2013;**8**(6):e67439

[17] Boyle ST et al. Acute compressive stress activates RHO/ROCK-mediated cellular processes. Small GTPases. 17 Feb 2018:1-17

[18] Minagawa A, Omodaka T, Okuyama R. Melanomas and mechanical stress points on the plantar surface of the foot. The New England Journal of Medicine. 2016;**374**(24):2404-2406

[19] Mittapalli VR et al. Injury-driven stiffening of the dermis expedites skin carcinoma progression. Cancer Research. 2016;**76**(4):940-951

[20] Boyle ST, Samuel MS. Mechano-reciprocity is maintained between physiological boundaries by tuning signal flux through the Rho-associated protein kinase. Small GTPases. 2016;**7**(3):139-146

[21] Ridley AJ. Life at the leading edge. Cell. 2011;**145**(7):1012-1022

[22] Ridley AJ et al. Cell migration: Integrating signals from front to back. Science. 2003;**302**(5651):1704-1709

[23] Kopecki Z et al. Cytoskeletal protein Flightless I inhibits apoptosis, enhances tumor cell invasion and promotes cutaneous squamous cell carcinoma progression. Oncotarget. 2015;**6**(34):36426-36440

[24] Canueto J et al. Epidermal growth factor receptor expression is associated with poor outcome in cutaneous squamous cell carcinoma. The British Journal of Dermatology. 2017;**176**(5):1279-1287

[25] Kopecki Z et al. Flightless I regulates hemidesmosome formation and integrin-mediated cellular adhesion and migration during wound repair. The Journal of Investigative Dermatology. 2009;**129**(8):2031-2045

[26] Mitra SK, Schlaepfer DD. Integrin-regulated FAK-Src signaling in normal and cancer cells. Current Opinion in Cell Biology. 2006;**18**(5):516-523

[27] Ibbetson SJ et al. Mechano-transduction pathways promoting tumor progression are activated in invasive human squamous cell carcinoma. The American Journal of Pathology. 2013;**183**(3):930-937

[28] Guasch G et al. Loss of TGFbeta signaling destabilizes homeostasis and promotes squamous cell carcinomas in stratified epithelia. Cancer Cell. 2007;**12**(4):313-327

[29] McLean GW et al. Specific deletion of focal adhesion kinase suppresses tumor formation and blocks malignant progression. Genes & Development. 2004;**18**(24):2998-3003

[30] McLean GW et al. Decreased focal adhesion kinase suppresses papilloma formation during experimental mouse skin carcinogenesis. Cancer Research. 2001;**61**(23):8385-8389

[31] Schober M, Fuchs E. Tumor-initiating stem cells of squamous cell carcinomas and their control by TGF-beta and integrin/focal adhesion kinase (FAK) signaling. Proceedings of the National Academy of Sciences of the United States of America. 2011;**108**(26):10544-10549

[32] Kopecki Z et al. Regulation of focal adhesions by Flightless I involves inhibition of paxillin phosphorylation via a Rac1-dependent pathway. The Journal of Investigative Dermatology. 2011;**131**(7):1450-1459

[33] Cowin AJ et al. Lysosomal secretion of Flightless I upon injury has the potential to alter inflammation. Communicative & Integrative Biology. 2012;**5**(6):546-549

[34] Sahai E, Marshall CJ. RHO-GTPases and cancer. Nature Reviews. Cancer. 2002;**2**(2):133-142

[35] Kular J et al. A negative regulatory mechanism involving 14-3-3zeta limits signaling downstream of ROCK to regulate tissue stiffness in epidermal homeostasis. Developmental Cell. 2015;**35**(6):759-774

[36] Samuel MS et al. Actomyosin-mediated cellular tension drives increased tissue stiffness and beta-catenin activation to induce epidermal hyperplasia and tumor growth. Cancer Cell. 2011;**19**(6):776-791

[37] Chaudhry SI et al. Autocrine IL-1beta-TRAF6 signalling promotes squamous cell carcinoma invasion through paracrine TNFalpha signalling to carcinoma-associated fibroblasts. Oncogene. 2013;**32**(6):747-758

[38] Wu X, Quondamatteo F, Brakebusch C. Cdc42 expression in keratinocytes is required for the maintenance of the basement membrane in skin. Matrix Biology. 2006;**25**(8):466-474

[39] Kawakatsu T et al. Trans-interactions of nectins induce formation of filopodia and lamellipodia through the respective activation of Cdc42 and Rac small G proteins. The Journal of Biological Chemistry. 2002;**277**(52):50749-50755

[40] Chen R et al. Rac1 regulates skin tumors by regulation of keratin 17 through recruitment and interaction with CD11b+Gr1+ cells. Oncotarget. 2014;**5**(12):4406-4417

[41] Choma DP, Pumiglia K, DiPersio CM. Integrin alpha3beta1 directs the stabilization of a polarized lamellipodium in epithelial cells through activation of Rac1. Journal of Cell Science. 2004;**117**(Pt 17):3947-3959

[42] Kwei KA et al. The role of Rac1 in maintaining malignant phenotype of mouse skin tumor cells. Cancer Letters. 2006;**231**(2):326-338

[43] Malliri A et al. Mice deficient in the Rac activator Tiam1 are resistant to Ras-induced skin tumours. Nature. 2002;**417**(6891):867-871

[44] Ohashi K et al. Rho-associated kinase ROCK activates LIM-kinase 1 by phosphorylation at threonine 508 within the activation loop. The Journal of Biological Chemistry. 2000;**275**(5):3577-3582

[45] Zhou J et al. MiR-20a inhibits cutaneous squamous cell carcinoma metastasis and proliferation by directly targeting LIMK1. Cancer Biology & Therapy. 2014;**15**(10):1340-1349

[46] Scott RW et al. LIM kinases are required for invasive path generation by tumor and tumor-associated stromal cells. The Journal of Cell Biology. 2010;**191**(1):169-185

[47] Takenawa T, Suetsugu S. The WASP-WAVE protein network: Connecting the membrane to the cytoskeleton. Nature Reviews. Molecular Cell Biology. 2007;**8**(1):37-48

[48] Kalailingam P et al. Conditional knock out of N-WASP in keratinocytes causes skin barrier defects and atopic dermatitis-like inflammation. Scientific Reports. 2017;**7**(1):7311

[49] Weaver AM et al. Cortactin promotes and stabilizes Arp2/3-induced actin filament network formation. Current Biology. 2001;**11**(5):370-374

[50] Uruno T et al. Activation of Arp2/3 complex-mediated actin polymerization by cortactin. Nature Cell Biology. 2001;**3**(3):259-266

[51] Timpson P et al. Aberrant expression of cortactin in head and neck squamous cell carcinoma cells is associated with enhanced cell proliferation and resistance to the epidermal growth factor receptor inhibitor gefitinib. Cancer Research. 2007;**67**(19):9304-9314

[52] Yamada S et al. Overexpression of cortactin increases invasion potential in oral squamous cell carcinoma. Pathology Oncology Research. 2010;**16**(4):523-531

[53] Kinoshita T et al. Actin-related protein 2/3 complex subunit 5 (ARPC5) contributes to cell migration and invasion and is directly regulated by tumor-suppressive microRNA-133a in head and neck squamous cell carcinoma. International Journal of Oncology. 2012;**40**(6):1770-1778

[54] Moriya Y et al. Tumor suppressive microRNA-133a regulates novel molecular networks in lung squamous cell carcinoma. Journal of Human Genetics. 2012;**57**(1):38-45

[55] Smyrek I et al. E-cadherin, actin, microtubules and FAK dominate different spheroid formation phases and important elements of tissue integrity. Biology Open. 2019;**8**(1). DOI: 10.1242/bio.037051

[56] Gunning P, O'Neill G, Hardeman E. Tropomyosin-based regulation of the actin cytoskeleton in time and space. Physiological Reviews. 2008;**88**(1):1-35

[57] Gunning PW et al. Tropomyosin—master regulator of actin filament function in the cytoskeleton. Journal of Cell Science. 2015;**128**(16):2965-2974

[58] Lees JG et al. Tropomyosin regulates cell migration during skin wound healing. The Journal of Investigative Dermatology. 2013;**133**(5):1330-1339

[59] Jazii FR et al. Identification of squamous cell carcinoma associated proteins by proteomics and loss of beta tropomyosin expression in esophageal cancer. World Journal of Gastroenterology. 2006;**12**(44):7104-7112

[60] Zare M et al. Downregulation of tropomyosin-1 in squamous cell carcinoma of esophagus, the role of Ras signaling and methylation. Molecular Carcinogenesis. 2012;**51**(10):796-806

[61] Dong G et al. Genes differentially expressed with malignant transformation and metastatic tumor progression of murine squamous cell carcinoma. Journal of Cellular Biochemistry. Supplement. 1997;**28-29**:90-100

[62] Kopecki Z, Cowin AJ. Flightless I: An actin-remodelling protein and an important negative regulator of wound repair. The International

Journal of Biochemistry & Cell Biology. 2008;**40**(8):1415-1419

[63] Sun HQ et al. Gelsolin, a multifunctional actin regulatory protein. The Journal of Biological Chemistry. 1999;**274**(47):33179-33182

[64] Van den Abbeele A et al. Downregulation of gelsolin family proteins counteracts cancer cell invasion in vitro. Cancer Letters. 2007;**255**(1):57-70

[65] Deng R et al. Gelsolin regulates proliferation, apoptosis, migration and invasion in human oral carcinoma cells. Oncology Letters. 2015;**9**(5):2129-2134

[66] He JP et al. Flightless-I blocks p62-mediated recognition of LC3 to impede selective autophagy and promote breast cancer progression. Cancer Research. 2018;**78**(17):4853-4864

[67] Chong HT et al. Reducing Flightless I expression decreases severity of psoriasis in an imiquimod-induced murine model of psoriasiform dermatitis. The British Journal of Dermatology. 2017;**176**(3):705-712

[68] Jackson JE et al. Flii neutralizing antibodies improve wound healing in porcine preclinical studies. Wound Repair and Regeneration. 2012;**20**(4):523-536

[69] Kopecki Z et al. Overexpression of the Flii gene increases dermal-epidermal blistering in an autoimmune ColVII mouse model of epidermolysis bullosa acquisita. The Journal of Pathology. 2011;**225**(3):401-413

[70] Kopecki Z et al. Topically applied Flightless I neutralizing antibodies improve healing of blistered skin in a murine model of Epidermolysis bullosa acquisita. The Journal of Investigative Dermatology. 2012;**133**(4):1008-1016

[71] Kopecki Z et al. Flightless I alters the inflammatory response and autoantibody

profile in an OVA-induced atopic dermatitis skin-like disease. Frontiers in Immunology. 2018;**9**(1833). http://doi.org/10.3389/fimmu.2018.01833

[72] Kopecki Z et al. Flightless I over-expression impairs skin barrier development, function and recovery following skin blistering. The Journal of Pathology. 2014;**232**(5):541-552

[73] Wicki A, Christofori G. The potential role of podoplanin in tumour invasion. British Journal of Cancer. 2007;**96**(1):1-5

[74] Wicki A et al. Tumor invasion in the absence of epithelial-mesenchymal transition: Podoplanin-mediated remodeling of the actin cytoskeleton. Cancer Cell. 2006;**9**(4):261-272

[75] Suchanski J et al. Podoplanin increases the migration of human fibroblasts and affects the endothelial cell network formation: A possible role for cancer-associated fibroblasts in breast cancer progression. PLoS One. 2017;**12**(9):e0184970

[76] Nakamura M, Tokura Y. Epithelial-mesenchymal transition in the skin. Journal of Dermatological Science. 2011;**61**(1):7-13

[77] Zhang W et al. E-cadherin loss promotes the initiation of squamous cell carcinoma invasion through modulation of integrin-mediated adhesion. Journal of Cell Science. 2006;**119**(Pt 2):283-291

[78] Pfaff D et al. T-cadherin loss induces an invasive phenotype in human keratinocytes and squamous cell carcinoma (SCC) cells in vitro and is associated with malignant transformation of cutaneous SCC in vivo. The British Journal of Dermatology. 2010;**163**(2):353-363

[79] Kyriakakis E et al. T-cadherin is an auxiliary negative regulator of EGFR pathway activity in cutaneous squamous cell carcinoma: Impact on cell motility. The Journal of Investigative Dermatology. 2012;**132**(9):2275-2285

[80] Romet-Lemonne G, Jegou A. Mechanotransduction down to individual actin filaments. European Journal of Cell Biology. 2013;**92**(10-11):333-338

[81] Xu XZ et al. Cytoskeleton alterations in melanoma: Aberrant expression of cortactin, an actin-binding adapter protein, correlates with melanocytic tumor progression. Modern Pathology. 2010;**23**(2):187-196

[82] Schmalbach CE et al. Molecular profiling and the identification of genes associated with metastatic oral cavity/pharynx squamous cell carcinoma. Archives of Otolaryngology-Head and Neck Surgery. 2004;**130**(3):295-302

[83] Piipponen M et al. Long non-coding RNA PICSAR decreases adhesion and promotes migration of squamous carcinoma cells by downregulating alpha2beta1 and alpha5beta1 integrin expression. Biology Open. 2018;**7**(11). DOI: 10.1242/bio.037044

[84] Wang H et al. MicroRNA-340 inhibits squamous cell carcinoma cell proliferation, migration and invasion by downregulating RhoA. Journal of Dermatological Science. 2018;**92**(2):197-206

[85] Wang A et al. MicroRNA-31 is overexpressed in cutaneous squamous cell carcinoma and regulates cell motility and colony formation ability of tumor cells. PLoS One. 2014;**9**(7):e103206

[86] Xu N et al. MicroRNA-125b down-regulates matrix metallopeptidase 13 and inhibits cutaneous squamous cell carcinoma cell proliferation, migration, and invasion. The Journal of Biological Chemistry. 2012;**287**(35):29899-29908

[87] Sahai E, Marshall CJ. Differing modes of tumour cell invasion have

distinct requirements for Rho/ROCK signalling and extracellular proteolysis. Nature Cell Biology. 2003;**5**(8):711-719

[88] Wang L et al. Effects of ROCK inhibitor, Y-27632, on adhesion and mobility in esophageal squamous cell cancer cells. Molecular Biology Reports. 2010;**37**(4):1971-1977

[89] Shum MS et al. Gamma-actin regulates cell migration and modulates the ROCK signaling pathway. The FASEB Journal. 2011;**25**(12):4423-4433

[90] Patel RA et al. Identification of novel ROCK inhibitors with anti-migratory and anti-invasive activities. Oncogene. 2014;**33**(5):550-555

[91] Feng Y et al. Rho kinase (ROCK) inhibitors and their therapeutic potential. Journal of Medicinal Chemistry. 2016;**59**(6):2269-2300

[92] Rath N, Olson MF. Rho-associated kinases in tumorigenesis: Re-considering ROCK inhibition for cancer therapy. EMBO Reports. 2012;**13**(10):900-908

[93] Friedl P, Alexander S. Cancer invasion and the microenvironment: Plasticity and reciprocity. Cell. 2011;**147**(5):992-1009

[94] Haidari H et al. Development of topical delivery systems for Flightless neutralizing antibody. Journal of Pharmaceutical Sciences. 2017;**106**(7):1795-1804

[95] Bach CT et al. Tropomyosin isoform modulation of focal adhesion structure and cell migration. Cell Adhesion & Migration. 2010;**4**(2)

[96] Stehn JR et al. A novel class of anticancer compounds targets the actin cytoskeleton in tumor cells. Cancer Research. 2013;**73**(16):5169-5182

[97] Dumontet C, Jordan MA. Microtubule-binding agents: A dynamic field of cancer therapeutics. Nature Reviews. Drug Discovery. 2010;**9**(10):790-803

[98] Zhao Y, Mu X, Du G. Microtubule-stabilizing agents: New drug discovery and cancer therapy. Pharmacology & Therapeutics. 2016;**162**:134-143

[99] Fanale D et al. Stabilizing versus destabilizing the microtubules: A double-edge sword for an effective cancer treatment option? Analytical Cellular Pathology (Amsterdam). 2015;**2015**:690916

[100] Cheng KL, Bradley T, Budman DR. Novel microtubule-targeting agents—The epothilones. Biologics. 2008;**2**(4):789-811

[101] Forli S. Epothilones: From discovery to clinical trials. Current Topics in Medicinal Chemistry. 2014;**14**(20):2312-2321

[102] Verweij J et al. Phase II study of rhizoxin in squamous cell head and neck cancer. The EORTC Early Clinical Trials Group. British Journal of Cancer. 1996;**73**(3):400-402

[103] Arnold SM et al. Phase II evaluation of eribulin mesylate (E7389, NSC 707389) in patients with metastatic or recurrent squamous cell carcinoma of the head and neck: Southwest Oncology Group trial S0618. Investigational New Drugs. 2011;**29**(2):352-359

[104] Goto Y, Yoshida T, Kimura M. Higher efficacy and complete response with administration of eribulin for recurrent squamous cell breast carcinoma: A case report. Molecular and Clinical Oncology. 2018;**9**(3):318-320

[105] Bhat KM, Setaluri V. Microtubule-associated proteins as targets in cancer chemotherapy. Clinical Cancer Research. 2007;**13**(10):2849-2854