Targeting Microtubules for Wound Repair

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Significance: Fast and seamless healing is essential for both deep and chronic wounds to restore the skin and protect the body from harmful pathogens. Thus, finding new targets that can both expedite and enhance the repair process without altering the upstream signaling milieu and causing serious side effects can improve the way we treat wounds. Since cell migration is key during the different stages of wound healing, it presents an ideal process and intracellular structural machineries to target.
Recent Advances and Critical Issues: The microtubule (MT) cytoskeleton is rising as an important structural and functional regulator of wound healing. MTs have been reported to play different roles in the migration of the various cell types involved in wound healing. Specific microtubule regulatory proteins (MRPs) can be targeted to alter a section or subtype of the MT cytoskeleton and boost or hinder cell motility. However, inhibiting intracellular components can be challenging *in vivo*, especially using unstable molecules, such as small interfering RNA. Nanoparticles can be used to protect these unstable molecules and topically deliver them to the wound. Utilizing this approach, we recently showed that fidgetin-like 2, an uncharacterized MRP, can be targeted to enhance cell migration and wound healing.
Future Directions: To harness the full potential of the current MRP therapeutic targets, studies should test them with different delivery platforms, dosages, and skin models. Screening for new MT effectors that boost cell migration *in vivo* would also help find new targets for skin repair.

Keywords: microtubules, nanoparticle, siRNA, Fidgetin-like 2, cell migration

SCOPE AND SIGNIFICANCE

This review explores the role of microtubules (MTs), a major component of the cell's internal skeleton, in wound healing, especially during cell migration. The structure and function of MTs are spatially and temporally regulated by microtubule regulatory proteins (MRPs), which have diverse effects on cell migration depending on their role and the cell type being targeted. The review also presents alternative techniques to identify and examine new therapeutic wound-healing targets.

TRANSLATIONAL RELEVANCE

We recently characterized the role of fidgetin-like 2 (FL2), a novel MRP that can be targeted to enhance cell migration and healing both *in vitro* and *in vivo* wound-healing models. Identifying other MRPs that are involved in cell migration and wound healing !can elucidate how these proteins function and how they affect cell motility. As a faster and acute alternative to genetically modified animal models, RNA interference (RNAi) can be easily used to deplete these targets directly at the wound sites by utilizing nanotechnology and other technologies to deliver and protect small interfering RNA (siRNA).

CLINICAL RELEVANCE

Both chronic and acute wounds are costly and painful medical issues

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with scarce targeted therapies.[1] The ones that are available use pluripotent growth factors (GFs) that affect multiple different molecules and cell types and, thus, might have undesirable and serious side effects. Since MTs are tightly regulated structures in the cell and can affect cell migration differently in various cell types of the skin, MRPs offer an alternative set of therapeutic wound-healing targets that surpass the use of upstream regulators.

CELL MIGRATION IN WOUND HEALING

Rapid and efficient healing of cutaneous wounds is essential to protect against infectious agents while concomitantly properly restoring the structured layers and functional characteristics of the skin.[1] This extremely intricate and complex process relies heavily on the migration of diverse cell types into the wound (Fig. 1).[2,3] Initially, during the inflammatory stage, neutrophils and macrophages are rapidly recruited toward the concoction of platelets, extracellular matrix proteins, and GFs that initially plug the wound. While these immune cells migrate within the wound bed to combat microorganisms that enter the compromised integument, they also produce additional GFs and cytokines, which in turn stimulate the migration of endothelial cells and fibroblasts that revascularize and structurally stabilize the wound, respectively.[4] Subsequently, epithelial and stem cells migrate in from the wound edges and nearby hair follicles to form a protective barrier against the external environment.[3] These responses in mesenchymal and epithelial cell motility are part of the proliferative stage of wound healing.[5] Given the central role of cell migration to wound healing, the development of approaches to selectively, locally, and reversibly harness this process has immense therapeutic potential.

To date, most efforts to enhance wound healing via the stimulation of cell migration have focused on complex extracellular signaling cascades.[6,7] Unfortunately, these cascades are extremely pleiotropic and thus their manipulation can manifest in a multitude of unfortunate and difficult-to-predict side effects. For example, drugs such as Becaplermin (Regranex), a human platelet-derived GF, significantly increase the risk of cancer mortality when used over extended periods of time.[8] A promising alternative to broadly altering the extracellular signal milieu is to therapeutically target specific intracellular architectural and mechanical elements that control cell movements[9]—these include proteins that can be locally and reversibly manipulated within the wound by double-stranded RNAi.[10,11] This review specifically examines the evidence supporting the hypothesis that protein regulators of the MT cytoskeleton—key components of the cell's internal skeleton—are ideal in this regard. Indeed, we have just completed the first proof-of-principal study showing that a select MT regulator termed FL2 can be targeted by RNAi in animal models to effectively promote cutaneous wound closure and enhance tissue repair.[12]

WHAT ARE MTs?

MTs form complex and highly dynamic arrays involved in multiple aspects of cellular development and function, such as mitosis, vesicular trafficking, and cell motility.[13–15] They are made up of α and β tubulin heterodimers that polymerize and unite together to form a polar polymer with two distinctly dynamic ends. The plus-end is "the working end," where most of the tubulin dimers are added, polymerizing much more rapidly than the often capped and protected minus-end.[16] This fast growth at the plus-end is accompanied by depolymerization events termed catastrophes, making this end dynamically unstable with a series of intermingling growth/catastrophe cycles.[17,18] MTs are usually seeded from their minus-ends at MT-organizing centers (MTOCs),[19,20] which are most often located around the nucleus. The centrosome is the quintessential MTOC, whereby MT minus-ends cluster at this organelle while the plus-ends grow out into the cell periphery, innately polarizing the cell.[19]

In addition to the inherent dynamic instability of MTs, there are a number of proteins that tightly regulate MT organization and dynamics. The majority of MRPs either stabilize or destabilize the polymers, and examples of such MRPs include plus-end–tracking proteins (+TIPs), depolymerizing enzymes, and severing enzymes (Fig. 2).[21–23] Another class of MRPs, the force generating molecular motors, use MTs as directional railways for the movement of diverse cellular components as well as MT polymers (Fig. 2).[13,14,24] MT stabilizers, destabilizers, and motors are emerging as regulatory components of the migration machinery of cells.[9,25,26] A summary of key MRPs and their various functions is presented in Table 1.

MTs are also regulated through post-translational modifications (PTMs) that affect the organization and dynamics of the polymers as well as alter their susceptibility to certain MRPs. PTMs usually occur on long-lived MTs and include detyrosination, acetylation, and polyglutamylation.[27,28] MT motor proteins and destabilizers display differential preferences for certain modifications. Mitotic centromere-associated kinesin (MCAK), for example, mainly

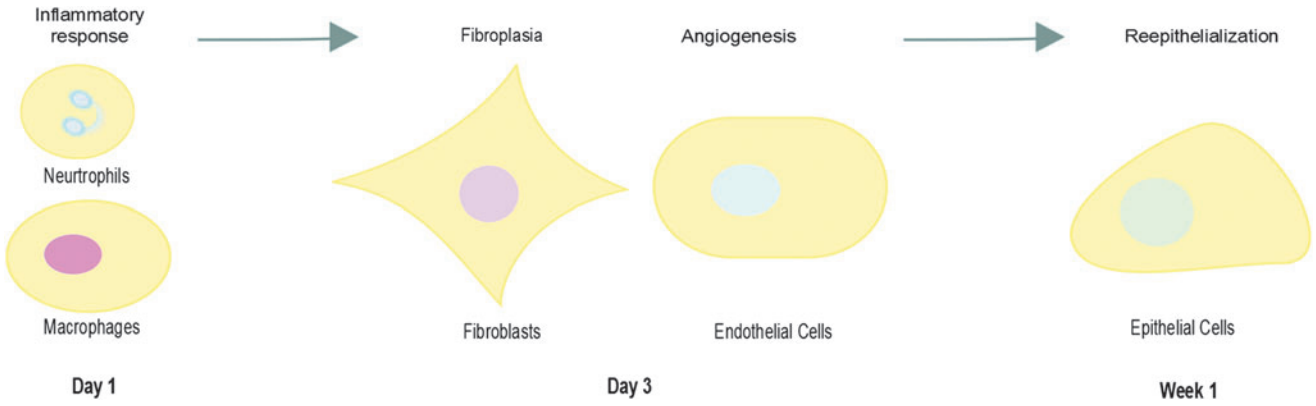

Figure 1. Timeline of the appearance of migrating cells involved in wound healing. At the early stages of wound healing, immune cells move in and secrete growth factors and cytokines, starting the inflammatory response. Fibroblasts and endothelial cells respond by migrating in and forming the granulation tissue that comprises the provisional matrix and blood vessels. The formation of this tissue is essential for reepithelialization, whereby epithelial cells migrate throughout the new matrix, resulting in wound closure. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

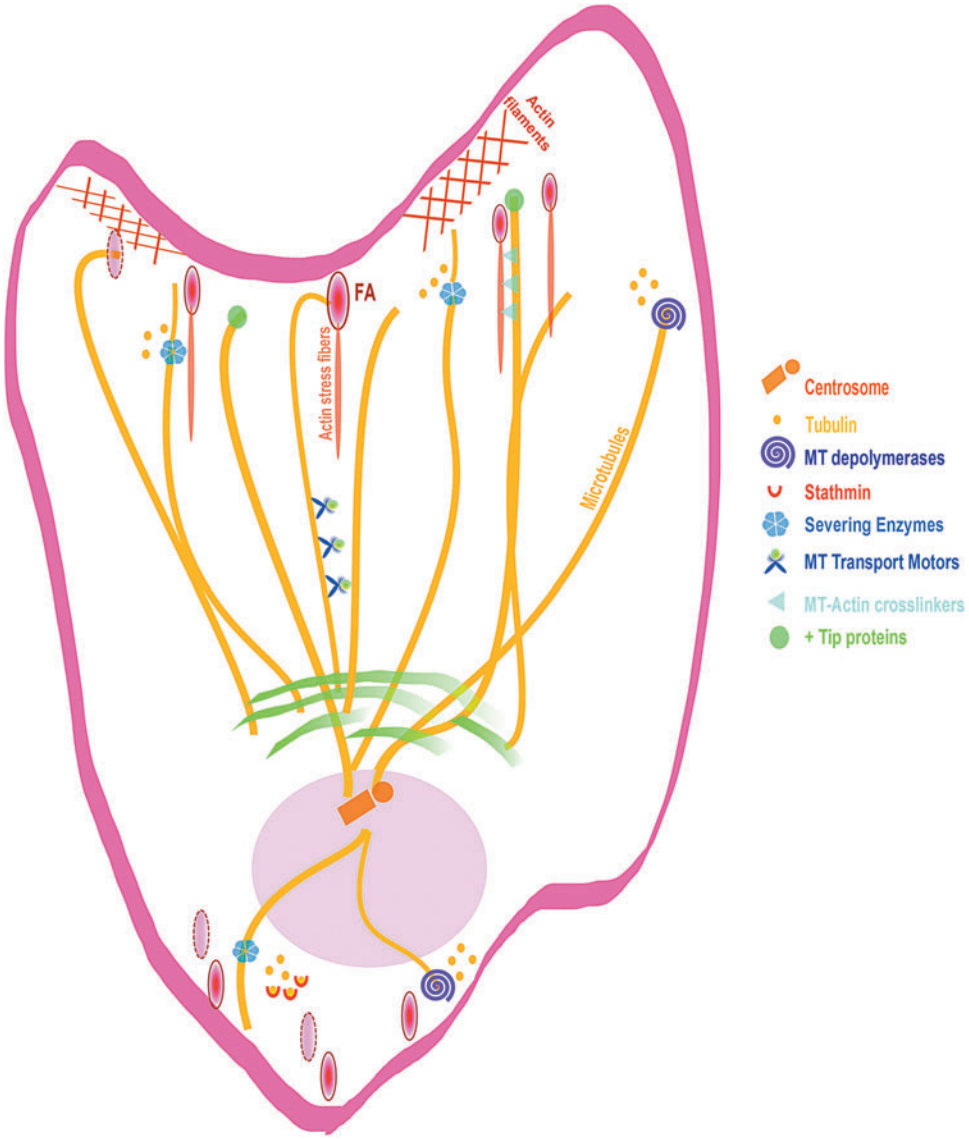

Figure 2. The MTs and their regulatory proteins. Tubulin dimers nucleate MT polymers at MT-organizing centers, such as the centrosome and the Golgi apparatus. MTs are robust but have dynamically unstable plus-ends that undergo repeating growth and shrinkage events. There are several MRPs that either control and/or take advantage of these traits. Depolymerases, severing enzymes, and stathmin destabilize MTs, either by disrupting the polymers or sequestering free tubulin. Alternatively, MT transport motors use the polymers as tracks to transfer vesicles and proteins, whereas +TIP proteins and MT-actin cross-linkers form stabilizing complexes at the plus-end. These complexes are known to help MTs target FA. FA, focal adhesion; MRP, microtubule regulatory protein; MT, microtubule; +TIP, plus-end–tracking protein. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Table 1. *An abbreviated list of various microtubule regulatory proteins and their assorted functions*

MT Stabilizers	*Examples*	*Key Functions*
Plus-TIPs	EB1,[82,83] CLASPs,[84,85] P150glue,[86] APC,[87] CLIP170[88]	Track growing MT plus-ends; stabilize MT plus-ends; deliver proteins
MT lattice binding and cortical proteins	CLIP170,[89] APC,[90] ACF7,[61] CLASPs[48]	Coordinate the interaction between MTs and actin; regulate FAs
MT Destabilizers	*Examples*	*Key Functions*
Depolymerases	Kif2A, Kif2B, Kif2C (MCAK)[91]	Depolymerize MTs
Severing enzymes	Katanin,[92] spastin,[93] fidgetin,[94] fidgetin-like 2[12]	Remove tubulin from MT lattice; depolymerize MTs
Antipolymerizer	Stathmin[95]	Sequesters tubulin
MT Motors	*Examples*	*Key Functions*
Plus-end–directed motors	Kinesins[96]: kinesin-1, kinesin-9, kinesin-5*[97]	Transport proteins and vesicles toward the cell periphery; *cross-links and slides antiparallel MTs
Minus-end–directed motors	Dynein[98]	Transports proteins and vesicles toward the cell interior; shifts MT orientation at the cell cortex

*means function specific to Kinesin-5 only.

ACF7, actin cross-linking factor 7; APC, adenomatous polyposis coli; CLASP, cytoplasmic linker-associated protein; EB1, end binding protein 1; FA, focal adhesion; MCAK, mitotic centromere-associated kinesin; MT, microtubule; +TIP, plus-end–tracking protein.

depolymerizes tyrosinated MTs while spastin preferentially attacks polyglutamylated MTs.[29,30] The different selectivities of these proteins and their varied effects on MT-associated processes can significantly impact cell migration.[31]

HOW DO MTs CONTRIBUTE TO CELL MIGRATION?

Cell migration is a cyclical activity that requires cells to polarize toward a chemotactic signal, leading to actin-based lamellipodial protrusions at the leading edge (*i.e.*, toward the signal) and actomyosin contraction in the back.[32] Actin polymerization and the formation of new integrin-based adhesions at these persistent membrane protrusions help cells attain traction, whereas contraction disrupts them in the back, leading to membrane retraction and cell movement.[33,34]

There is growing evidence that MTs exert spatiotemporal control over multiple parameters of cell movement. Dynamically growing MTs orient toward and crowd the cell front, leaving behind a small highly unstable MT population at the cell rear.[35] This asymmetry allows MTs and MRPs to interact with various components of the cell migration machinery, affecting the activity of Rho GTPases, cell polarity, vascular trafficking, and focal adhesion (FA) turnover.[36–38] The Rho family of GTPases controls actin dynamics and organization especially during cell migration. The three key Rho GTPases, Rho, Rac, and Cdc42, are involved in polarizing the cell through their different functions and activity gradients in the cytoplasm.[39] Rac is mainly active at the cell front and induces actin polymerization while Rho is mainly active at the cell rear and induces actomyosin contraction. The activity of both of these proteins has been shown to be partly regulated by MT dynamics.[38] For example, stable MTs at the cell front regulate Rho activity by sequestering its activator Rho-GEFH1,[40] while the instability in the back frees Rho-GEFH1 to activate Rho and in turn influence actomyosin contraction as well as adhesion turnover.[41] The opposite is true in the case of Rac, which binds free tubulin.[42] It is sequestered in the back portion of the cell but is free and active in the front, allowing for actin polymerization and FA.[43] In addition, the MTs and motor proteins of mesenchymal and epithelial cells, such as fibroblasts, are used as a delivery system to transport various components of the migration machinery, such as post-Golgi carriers and mRNA of an actin-related protein, to their target destination.[44,45] However, leukocytes, such as neutrophils, rely mostly on diffusion and not on the MT cytoskeleton for transport.

Therefore, mesenchymal and epithelial cells and leukocytes use MTs differently when they migrate. While MTs are not essential for the chemokinesis, that is, motility, of leukocytes, they do impact their chemotactic directionality toward the wound. In Zebrafish, the MT antipolymerizing agent, nocodazole, diminishes the recruitment of both neutrophils and macrophages to the wound bed.[46,47] Studies done in human neutrophils show similar effects on directionality *in vitro.* In *Drosophila melanogaster* embryos, specifically disrupting a subset of MTs in macrophages hinders their directionality, causing

them to reach the wound site later than controls.[48] These macrophages fair better than Rac mutants that generally fail to move at all during development.[49] Indeed, disrupting MTs in all these systems makes cells move at the same or even at a faster rate than controls, although aimlessly.

In contrast, large slow moving cells are too complex to depend on the simple diffusion of molecules. They utilize MTs to transport different components of cell motility.[38] Mesenchymal and epidermal cells require centrosome reorientation and asymmetrical MT organization to establish polarity and to properly deliver and traffic molecules and vesicles along its tracks.[44,45] Moreover, unlike leukocytes, epithelial and mesenchymal cells depend on strong adhesion complexes to stabilize and adjust their grip in the front and the rear for them to move.[38,50] Stable MTs at the front edge of the cell membrane are utilized as delivery docks for proteins and vesicles essential for adhesion and maturation.[38,51] For instance, in an *ex vivo* chick embryo cataract surgery model, exposure of the wounded lens epithelium to nocodazol collapses the lamellipodia of cells at the wound periphery and impairs healing.[52]

ROLE OF MRPs IN CELL MIGRATION

Studies have generally used harsh agents that disrupt the whole MT cytoskeleton, such as nocodazol and taxol, to study the role of MTs in cell migration. The use of these agents results in unspecific effects on cell behavior that impede the understanding of the true function of MTs in cell motility. Case in point, whether cells are subjected to stabilizing or destabilizing agents, their movement will be hindered.[53,54] Studies that pursue specific MRPs enable us to explore particular functions of MTs in cell migration to eventually fine-tune our desired effect on wound repair.

Role of MT destabilizers in cell migration

MT destabilizers can have antagonistic effects on cell migration. Our laboratory has previously shown that katanin, an MT severing and depolymerizing enzyme located at the cell cortex, is a negative regulator of cell migration in *Drosophila* cells.[55] The loss of katanin significantly increases cell motility but reduces cell directionality. Moreover, others have revealed that the overexpression of another severing enzyme, spastin, in hemocytes of *Drosophila* embryos results in defects in directional persistence during epithelial wound healing.[48] In human cells, spastin depletion leads to a drop in the rate of cell movement.[56] Likewise, stathmin, an antipolymerization protein, has recently been shown to support cell migration in cultured human keratinocytes and to be a positive regulator of cell migration and proliferation in murine cutaneous wounds. This is expected since stathmin is active at the cell rear, the hotspot for the unstable MTs needed for motility facilitated by actomyosin contraction.[57] In contrast, MCAK, a member of the MT depolymerizing kinesin 13s, has an interesting effect on cell migration: both its overexpression and knockdown impair endothelial cell movement. MCAK is mainly spatially inhibited when on the tips of MTs at the cell front, suggesting that there is a fine balance between its localization and activity to ensure proper cell motility.[58] These antagonistic functions have been further supported by an siRNA screen of different MT destabilizers assaying for effects on cell migration in cultured cells (D.J.S., data not shown), suggesting that MT dynamics and organization are coordinated by a highly intricate spatiotemporally controlled system.

Role of MT stabilizers in cell migration

MTs are known to interact with (Fig. 3), target, and dissolve mature adhesions by repeated growth/catastrophe cycles.[38,50] They are also captured and stabilized by young adhesions supplying tracks for MT-associated motors to deliver additional components. Several MT stabilizers involved in actin polymerization and FA turnover, such as adenomatous polyposis coli (APC), actin cross-linking factor 7 (ACF7), and cytoplasmic linker-associated proteins, have been identified.[59–62] Besides their interaction with MT +TIP protein end binding protein 1 (EB1), they are also targeted to adhesion neighboring regions at the cell membrane where they capture and stabilize MTs. Spectraplakin ACF7, for instance, cross-links MTs to filamentous actin (F-actin) and directs them to adhesion contacts.[61] The individual depletion of these stabilizers inhibited cell migration in various cell types, implying that these proteins positively affect motility by specifically regulating adhesions (Fig. 2). *In vivo* studies specifically targeting MT stabilizers also support the fact that the presence of a stable MT population at the front is essential for proper cell migration and wound healing. For instance, conditional ablation of ACF7 in the mouse epidermis hinders reepithelialization by disrupting MT growth along F-actin and toward FAs, interrupting their disassembly.[63] This further supports the idea that cells use these stable MTs at the front to dissolve adhesions, an essential step in the epithelial/mesenchymal cell motility cycle. In contrast, although a mutation in MT capping protein, EB1,

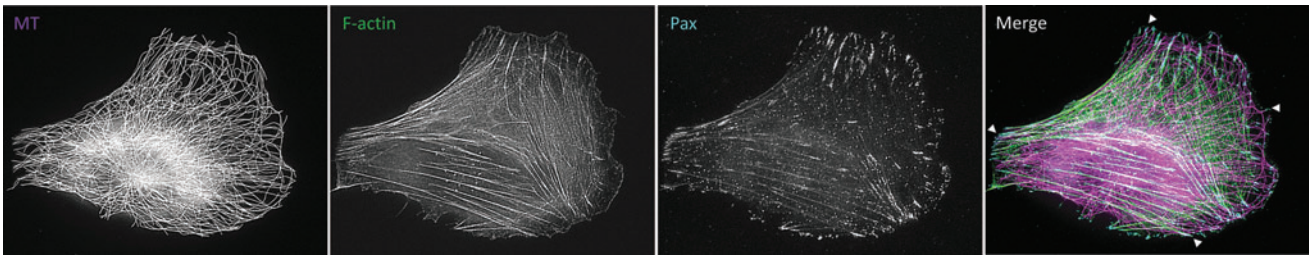

Figure 3. MTs interact with FAs. Fluorescent micrograph of a cultured human cell labeled for MTs in *magenta*, filamentous actin (F-actin) in *green*, and FA marker Paxillin (Pax) in *cyan*. *Arrowheads* indicate MTs interacting with FAs. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

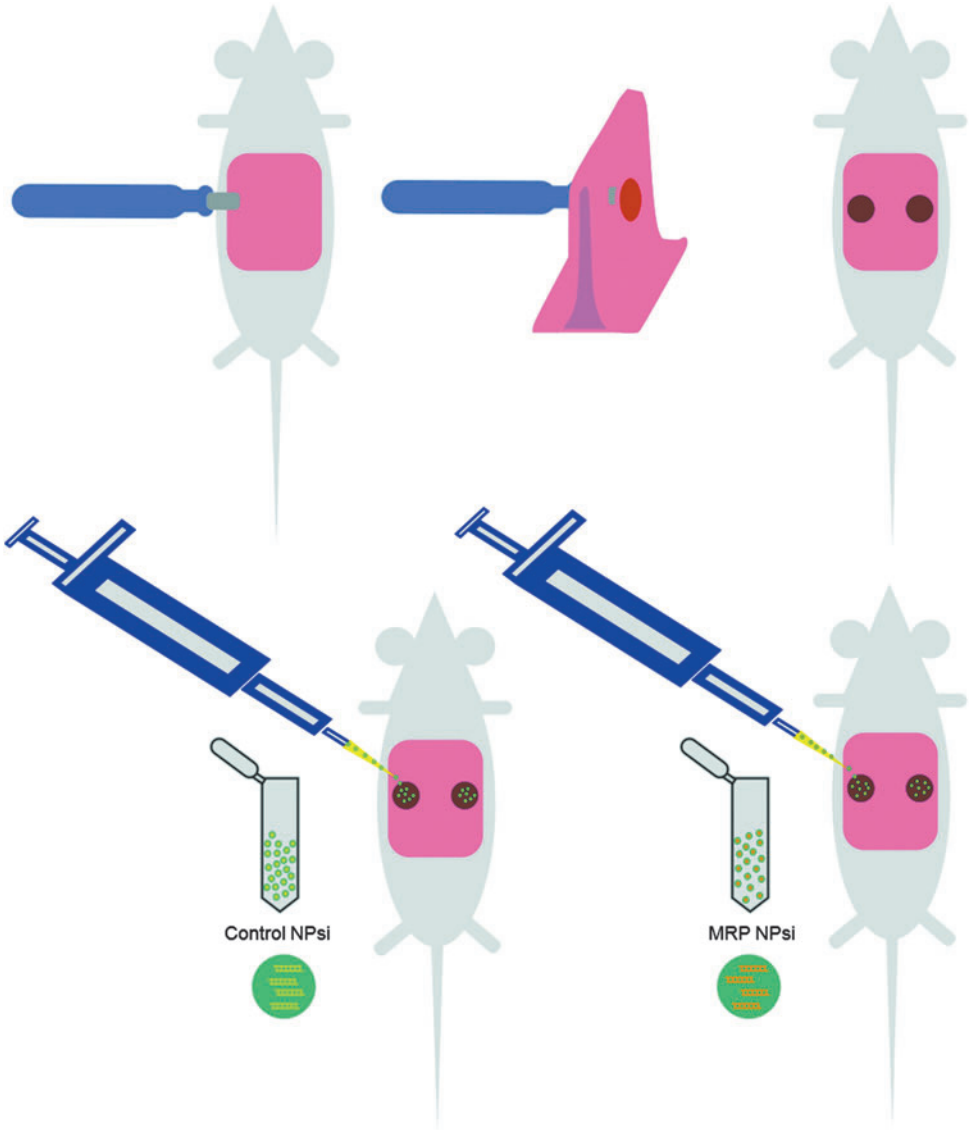

Figure 4. A simplified schematic representation of a wound-healing screen using topical nanoparticle siRNA treatment. Full-thickness excision wounds are inflicted on the flank of shaven mice (*top*). Wounds are treated topically with either control NPsi or a specific MRP NPsi (*bottom*). Healing progression is assayed through measuring wound size and histomorphic analysis. NPsi, nanoparticle-encapsulated siRNA; siRNA, small interfering RNA. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

does compromise MT dynamics, it only delays the initiation of the contraction stage with no overall effect on *Drosophila* embryonic wound healing.[64] It is unclear whether this specific delay is due to a disruption of cellular protrusions or an impairment in recruitment of repair components that ultimately form the actomyosin purse string at the wound edge, which are the two main mechanisms involved in the contraction phase of embryonic wound healing.[64]

Role of MT motors in cell migration

Even though they do not affect MT dynamics and organization *per se*, MT-associated motors significantly impact cell migration. Motor proteins, such as dynein and kinesins, "walk" along MTs and use them as directional highways to transport vesicles, proteins, and mRNA to the cell periphery.[14,44,45] In addition, the MT minus-end–directed motor, dynein, impacts fibroblast polarity and movement through tugging on the MT tips at any particular location on the cortex, forcing the centrosome to orient and the cell to polarize and move in that direction.[65]

UTILIZING ANIMAL MODELS AND RNAi TECHNOLOGY TO STUDY AND TARGET MT DYNAMICS

So how do we study and use these various MRPs for a more advantageous healing? Our knowledge about the consequences of the loss of various GFs, signaling molecules, extracellular matrix proteins, cytoskeletal components, and adhesion molecules during wound healing has been advanced through the use of model organisms. Conditionally disrupting those genes has been crucial in determining their functions and importance in wound healing.[66] Wound repair studies have been especially prolific in *Drosophila* and Zebrafish because of the ease of genetic manipulations and live microscopic imaging in these models.[46,48,49] They provide straightforward and rapid platforms to study different structural proteins during repair, many of which are further investigated in the more relevant mouse model that offers conditional skin/epidermal transgenic options.[66]

Thus, animal studies can be used to investigate the functions of the various MT stabilizers and destabilizers that have been reported to regulate cell migration in cultured cells and to look for new targets through assaying for differential gene expression of cytoskeletal components and their effectors in wounds. Generating conditional knockout animals can be one way to further help examine the importance and impact of these molecules, but utilizing a more rapid and transient technique, such as RNAi technology, to deplete gene expression is easier to perform.[67] In fact, using siRNA to specifically target and silence genes locally at the wound site can quickly and specifically assay what these proteins do and gauge their therapeutic potential. This approach can even facilitate the use of pigs, as they are the quintessential model for human cutaneous wound repair, but they are not a genetically malleable model organism.[68] However, delivering siRNA can pose a problem for *in vivo* studies because of various issues with the molecule's size, charge, and stability.[11]

Solutions to applying siRNA on cutaneous wounds

RNAi has been standardly used to target and deplete mRNA levels and, thus, protein levels in cultured cells or tissues, which has resulted in discoveries regarding the functions of a number of MRPs in cell migration. Since chemically unmodified siRNA is highly unstable, impermeable to cells, and can have off-target effects, it is hard to utilize "naked" or unpackaged siRNAs *in vivo* let alone as therapeutic oligomers.[11] However, recent advances in siRNA stability and specificity have led to an increased interest in utilizing RNAi approaches in clinical medicine. For instance, the use of better algorithms to predict siRNA specificity has limited off-target effects, making it an easier alternative to creating novel and specific small molecule inhibitors.[69] Moreover, chemical modifications of siRNA have resulted in the construction of hyperstable oligomers, called locked nucleic acid,[70] that are membrane permeable, bypassing the need of a vehicle in a process termed gymnosis.[71] In fact, self-delivering RNAi has been suggested to be ideal for studies in skin.[72] Alternatively, the utilization of various nanotechnology delivery platforms has further enhanced the stability and deliverability of therapeutic siRNA.[73–75] For example, different types of nanoparticles (NPs) can encapsulate and protect siRNA and acutely and locally deliver it to cells at the injury site.

The use of nanotechnology for siRNA delivery

Nanotechnology has been effectively utilized as a delivery platform for many biomolecules in various biological processes for either research or therapeutic purposes.[74] Depending on the physiochemical properties of these biomolecules, different types of NPs can package, protect, and deliver them to their intended target.[76] As mentioned above,

usage of siRNA in animal studies and as a therapeutic molecule is limited because of a number of different issues that hinder its delivery and cellular uptake, such as their susceptibility to nucleases, hydrophilicity, and negative charge.[77] Several natural and synthetic molecules have been used to consolidate or encapsulate siRNA and allow its uptake into cells. NPs can comprise sugar/polymers, saline/gel, a combination of both, or a number of different polymers/biomolecules mixed together.[78–81] Luckily, wound-healing therapeutics permit local administration of nanoparticle-encapsulated siRNA (NPsi) at the wound site, forgoing the need for NP-targeting conjugates such as ligands and antibodies. This advantage can be exploited in animal wound-healing studies screening various MRPs (Fig. 4). Most of the nontargeting NPs use cationic molecules that conceal the negative charge of siRNA and facilitate its entrance into the cell membrane.[77] The most efficient cationic molecules are synthetic polymers that can further protect siRNA from enzymatic degradation inside the cell. However, they are nondegradable and cytotoxic and, hence, unsuitable for pharmacological uses.[81] In addition to nontoxic and efficient cellular uptake, the solubility of NPs and the kinetics of the release of encapsulated siRNA are important in protecting the molecule and controlling its action.[79] After testing different mixtures and combination of polymers with hydrogel-based NPs, the laboratory of Dr. Joel Friedman found that a simple TMOS (tetramethyl orthosilicate)-based protocol obtained the most cohesive hydrogel NP-siRNA mixture. These saline-based hydrogel NPs were previously shown to be capable of encapsulating other biomolecules and to gradually release their load over time.[80] Our laboratory was able to successfully show that the NPsi crossed the cell membrane barrier (D.J.S., data not shown) and, as we will see below, targeted and depleted mRNA levels *in vivo*.

TAKE-HOME MESSAGES

- Cell migration is an essential process during wound healing.
- The MT cytoskeleton controls key components and processes in cell migration.
- MRPs tightly regulate MT structure and function, thus, influencing cell migration in a specific manner.
- MRPs offer viable targets for wound-healing therapies.
- siRNA can be used in translational studies to deplete and assess the therapeutic potential of specific MRPs in animal wound models.
- Nanotechnology and other siRNA protective and stabilizing technologies can be utilized to protect and deliver siRNA *in vivo*.

FL2 AS NOVEL THERAPEUTIC TARGET FOR WOUND HEALING: A CASE STUDY

Taking advantage of this nanotechnology delivery platform, we were able to successfully use NP-encapsulated siRNA to locally deplete the expression of FL2, an uncharacterized MT-severing enzyme, promoting *in vivo* wound repair in the process.[12] This discovery stemmed from an siRNA screen that was used to find the human equivalent of katanin, the severing enzyme whose depletion enhances the rate of *Drosophila* cell migration.[55] We assumed that if we could harness the same activity in human cells, we would have a potentially viable therapeutic target for enhancing wound healing. Indeed, when FL2 is locally and acutely depleted from the wounds, wound closure and repair are significantly enhanced through expedited reepithelialization and collagen depositions and remodeling. Therefore, cells not only enter the wound earlier than controls but also know how to behave once they arrive, probably because the signaling milieu has not been changed. *In vitro* studies support this concept since both mammalian keratinocytes and fibroblasts also exhibit this phenotype: After FL2 knockdown, these cells migrate faster and in a more productive manner. This is due to a decrease in MT acetylation, a rise in MT growth at the cell front, and a subsequent increase in FA size to an optimal area for cell movement. Interestingly, like katanin, FL2 localizes to the frontal cortex of the cell, further supporting the idea that MT destabilizers are also needed at the cell front.[12,55] Accordingly, equilibrium and asymmetry in MT organization/PTMs are not only present between the front and the back of the cell but are also more spatially dynamic and tightly regulated.

FUTURE DIRECTIONS AND CONCLUDING REMARKS

Since MTs are tightly and spatially regulated and function differently among cell types, we believe that targeting MT dynamics in different ways can impact wound repair early at two consequent stages: the inflammatory stage and the proliferative stage. We can target specific MRPs to slightly hamper the productive movement of leukocytes, which would help ease certain chronic wounds correlated with aberrant inflammation. In con-

trast, we can use MRPs that target particular subtypes of MTs, bypassing the need for GFs and cytokines to activate endothelial cells, fibroblasts, and keratinocytes. These approaches can cause the skin to heal in a manner analogous to embryonic wound healing by synergistically expediting dermal restoration and reepithelialization, while concomitantly reducing scarring. Ultimately, we would like to test whether FL2 and other similar MRPs are sustainable targets in clinical trials for both acute and chronic wounds. Meanwhile, we need to find and standardize the right siRNA dosages and delivery platforms for new targets that are able to shift the MT organization and equilibrium toward better wound healing through enhancing cell migration.

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AUTHOR DISCLOSURE AND GHOSTWRITING

DJS and RAC are co-inventors on US Patent #20130022667 entitled "Fidgetin-like 2 as a target to enhance wound healing", which has been licensed by MicroCures. DJS is currently CSO of MicroCures. MicroCures did not fund this work. No ghostwriters were used to write this article.

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Abbreviations and Acronyms

APC = adenomatous polyposis coli
ACF7 = actin cross-linking factor 7
CLASP = cytoplasmic linker-associated protein
EB1 = end binding protein 1
FA = focal adhesions
F-actin = filamentous actin
FL2 = fidgetin-like 2
GEFH1 = guanine nucleotide exchange factor H1
GF = growth factors
GTP = guanosine-5′-triphosphate
MRP = microtubule regulatory proteins
MT = microtubule
MTOC = microtubule-organizing center
MCAK = mitotic centromere-associated kinesin
NP = nanoparticle
NPsi = nanoparticle-encapsulated siRNA
PTM = post-translational modification
RNAi = RNA interference
siRNA = small interfering RNA
+TIP = plus-end–tracking protein