

INVITED MEDICAL REVIEW

Exploiting PI3K/mTOR signaling to accelerate epithelial wound healing

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The molecular circuitries controlling the process of skin wound healing have gained new significant insights in recent years. This knowledge is built on landmark studies on skin embryogenesis, maturation, and differentiation. Furthermore, the identification, characterization, and elucidation of the biological roles of adult skin epithelial stem cells and their influence in tissue homeostasis have provided the foundation for the overall understanding of the process of skin wound healing and tissue repair. Among numerous signaling pathways associated with epithelial functions, the PI3K/Akt/mTOR signaling route has gained substantial attention with the generation of animal models capable of dissecting individual components of the pathway, thereby providing a novel insight into the molecular framework underlying skin homeostasis and tissue regeneration. In this review, we focus on recent findings regarding the mechanisms involved in wound healing associated with the upregulation of the activity of the PI3K/Akt/mTOR circuitry. This review highlights critical findings on the molecular mechanisms controlling the activation of mTOR, a downstream component of the PI3K–PTEN pathway, which is directly involved in epithelial migration and proliferation. We discuss how this emerging information can be exploited for the development of novel pharmacological intervention strategies to accelerate the healing of critical size wounds.

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Introduction

The skin is the largest organ in the body, with remarkable implications in physiology and homeostasis. Its main purpose is to maintain a barrier function, which prevents the loss water and protects the body against biological, physical, and chemical insults. Moreover, a healthy skin plays a crucial esthetical role, and often contributes to our social interactions and overall feeling of well-being (Elias, 1983; Elias and Choi, 2005; Proksch *et al*, 2008; Fuchs, 2009). These key functions require the integrity of the epidermal barrier. Upon injury, the fast response of the immune system, the deposition of a provisional matrix, and the coordinated engagement of all cellular components of the epidermis, including their tissue repopulating stem cells are required for the rapid reestablishment of local homeostasis (Woodley *et al*, 1993; Singer and Clark, 1999; Elias and Choi, 2005; Fuchs and Nowak, 2008). Such capacity to heal injured tissues, however, may be compromised by large size wounds and can be aggravated by the presence of infections and medical conditions. This review presents an epitheliocentric view of the complex process of wound healing, with a special focus on the PI3K–mTOR signaling pathway, whose pharmacological perturbation can be exploited to promote accelerated epithelial healing.

The epidermis

The skin is formed during embryogenesis in a liquid interface with the amniotic fluid. Initial characteristics of skin are observed as early as 30 days after the estimated gestational age. By 6 weeks, the skin of a human embryo already shows a well-defined epidermis and dermis, tissue innervation and vascularization (Holbrook, 2011). The embryonic epidermis is composed by only two layers of epithelial cells separated from the dermis by a continuous basal lamina (Marinkovich *et al*, 1993). During the second trimester, the skin presents a much-improved organization with the development of appendances that progressively start to gain function.

During embryogenesis, studies using murine animal models have revealed two distinct phases of cell division

in the epidermis (Lechler and Fuchs, 2005). At embryonic day 12.5 (E12.5), mice present most of the epidermis as a single-cell layer, with the majority of the cellular divisions occurring laterally (92% of cells), thereby reflecting the direction of the rapidly growing embryo. Only a small percentage of the cells undergo a cellular division pattern with mitosis occurring perpendicular to the basal membrane; the resulting daughter cells therefore start to settle on top of the basal cells constituting the parabasal epithelial layer of the epidermis. Soon after, by day E15.5, there is a dramatic shift on the cellular division pattern with more than 70% of the cells presenting spindles perpendicular to the basal membrane. Such process results in asymmetrical cellular division, and the detachment of daughter cells from the basal membrane initiates the stratification process of the epidermis (Smart, 1970) (Poulson and Lechler, 2010).

The postnatal epidermis is organized in stratified layers presenting an outward progressive differentiation pattern (McGrath and Uitto, 2010). Epidermal stratification is essential in the development of a mechanical barrier against the external environment. The epidermis is composed by (i) the basal cellular layers responsible for conferring the regenerative capacity of the skin through the activation of symmetrical and asymmetrical cellular division during homeostasis or during the process of wound healing; (ii) the spinous cells located above the basal cells preceding the (iii) granular layer that is characterized by the intracellular accumulation of granules of keratohyalin. Finally, the (iv) stratum corneum is the outermost layer from the epidermis characterized by the absence of nuclei and cytoplasmic organelles presenting flat keratin and filaggrin filaments. The last two stages of differentiation observed in the epidermis (accumulation of keratohyalin granules and accumulation of cross-linked flat keratin filaments) are important steps in the formation of the protective barrier of the skin mediated by an irreversible proteolytic cascade (List *et al*, 2002) (Miller and List, 2012).

The PI3K/AKT/mTOR signaling circuitry in skin development and homeostasis

The serine/threonine kinase AKT is an important component of the phosphatidylinositol 3-kinase (PI3K) signaling pathway. Overexpression and persistent activation of AKT are driven by oncogenes, growth factors, and cytokines. AKT activation converges in the phosphorylation of its Thr308 and Ser473 residues and, in turn, active AKT controls many cellular functions, ranging from cell growth and survival, to cell metabolism and growth (Alessi *et al*, 1996) (Andjelkovic *et al*, 1997). AKT functions are negatively regulated by molecules capable of antagonizing the PI3K signaling. Perhaps one of the most studied regulatory mechanism impinging on PI3K is the phosphatase PTEN (for phosphatase and tensin homolog deleted from Chromosome 10) (Li *et al*, 1997) (Steck *et al*, 1997). PTEN negatively regulates the PI3K pathway by converting phosphatidylinositol 3,4,5-triphosphate (PIP₃) into phosphatidylinositol 4,5-bisphosphate (PIP₂), thus further restraining the activation status of AKT (Cantley and Neel, 1999).

The loss of function approaches has unveiled the important functions of AKT during skin development and maintenance. Double knockout for Akt1 and Akt2 genes present translucent skin due to a proliferation impairment of the basal epithelial layer. However, no signs of changes in the differentiation pattern were observed. Double knockout mice are viable presenting a Mendelian distribution of traits, which indicates that Akt signaling is not essentially required during embryogenesis, but important for the maintenance of the normal architecture and homeostasis of the skin (Peng *et al*, 2003). Double knockout mice for Akt1 and Akt2 also result in a deficient activation of the mammalian target of Rapamycin (mTOR) (Gingras *et al*, 2001) (Simpson and Parsons, 2001).

The function of AKT signaling during skin homeostasis has been also indirectly demonstrated by Santos and colleagues in 2002 with the development of an animal model expressing cytokeratin 10 targeted to the basal layer of the epidermis driven by the bovine keratin K5 promoter (Santos *et al*, 2002). Cytokeratin 10 is an intermediated filament protein directly associated to the differentiation of the skin and found within the spinous layer of the epidermis. When expression of cytokeratin 10 was targeted to the proliferative layer of the epidermis (basal layers), it resulted in downregulation of the Akt activity along with reduced cellular proliferation, epidermal hypoplasia, and hyperkeratosis. These results support the physiological role of AKT in the maintenance of a viable and proliferative epidermis with preservation of the skin architecture. Indeed, the role of AKT during skin homeostasis have been partially addressed by the work of Paramio *et al* where endogenous Akt was shown to interact with cytokeratin 10 (Paramio *et al*, 2001). Such interaction suggests that cytokeratin 10 regulates the translocation of AKT to the cell membrane, thereby preventing its activation by phosphorylation of the activation-loop threonine (Thr308), thus promoting the differentiation process of the spinous layer (CK10 positive cells) (Chou *et al*, 1998) (Kulik *et al*, 1997).

Gain of function assays have also helped the understanding of the role of the PI3K signaling in skin biology. Increased phosphorylation of AKT mediated by up regulation of the PI3K signaling subunits p110 α and p110 β results in the formation of a disorganized, hyperplastic, and poorly differentiated epithelium in organotypic skin cultures as a result of cytoskeleton reorganization, leading to an increased cellular migratory phenotype (Pankow *et al*, 2006). Interestingly, expression of an active AKT instead of the PI3K subunits results in growth arrest and differentiation of epithelial cells, while pharmacological interference of PI3K results in cell death and disruption of the machinery responsible for epithelial differentiation (Calautti *et al*, 2005). Thus, the modulation of the PI3K signaling pathway at the AKT level or upstream by the p110 α and p110 β PI3K subunits directly influences the epithelial response indicating a much broader function of the PI3K pathway in the control of skin homeostasis and probably wound healing.

Wound healing

As the largest organ in the human body, the skin homeostasis is constantly disturbed by injuries affecting its most

important function, the physical barrier. Such a role is maintained through a continuous self-renewal process that involves the maintenance of proliferative layer by epidermal stem cells (Oshima *et al*, 2001) (Tumbar *et al*, 2004) and the orchestration of a terminal differentiation process that involves the asymmetrical cellular division of basal keratinocytes (Lechler and Fuchs, 2005).

Disruption of tissue integrity with complete loss of continuity of epithelial cells results in the activation of an intricate process aiming at two distinct objectives: (i) quick covering and protection of wounded area; and (ii) regaining the skin protective functions by tissue proliferation, migration, and ultimately remodeling. This process is mediated by an intricate mechanism that involves the formation of the blood clot, deposition of extracellular matrix (ECM) by stromal cells, and the induction of a well-regulated process of epithelial migration. Overall, the process of wound healing involves multiple interposed phases, including inflammation, re-epithelialization, formation of granulation tissue, vascularization, contraction, and finally, tissue remodeling (Woodley *et al*, 1993).

The inflammation phase is directly associated to the extravasation of blood to the wounded site and the formation of a fibrin clot, responsible for reestablishing tissue homeostasis. Shortly, increased concentration of growth factors initially secreted by platelets induces the differentiation of monocytes into macrophages. These cells are responsible for cleaning the wounded bed by the phagocytosis of cellular debris, foreign bodies and pathogens, and the differentiation of fibroblasts into myofibroblasts, which are essential for the process of tissue contraction (Singer and Clark, 1999). Short after the inflammation phase, the process of re-epithelialization is initially observed by the increase proliferation of the interfollicular epidermis (Singer and Clark, 1999) and the mobilization of hair follicle stem cells that migrate from their niche toward the wounded area (Ito *et al*, 2007) (Levy *et al*, 2007). The collaborative effort of the interfollicular and the follicular components of the skin during the re-epithelialization phase are evident by the recent characterization of the hair follicle stem cells as responsible for approximately 25% of cells found in the healed skin (Ito *et al*, 2005). These cells are essential for the accelerated healing during the initial phase of re-epithelialization (Langton *et al*, 2008). Underneath the re-epithelialization process, a new wound bed is formed by the migration of newly recruited fibroblasts and endothelial cells along with macrophages that together form the granulation tissue. This process is essential for the continuous migration of the epithelial cells and the progressive accumulation of ECM and collagen deposition. The neovascularization process then nurtures this newly formed granulous tissue, with endothelial cells originally guided by the deposition of ECM and angiogenic factors released by the fibroblasts. Finally, the differentiation of fibroblasts into myofibroblasts marks the phase of wound contraction and tissue remodeling that alone can take several months to complete. Overall, the acute phase of wound healing is fast and provides a protective barrier early on in the process; however, full recovery of a wounded area including tissue remodeling is a longer

process, which is often marked by a suboptimal recovery of strength and appearance of the affected tissues.

The negative role of PI3K signaling in the control and maintenance of skin homeostasis is evident during the forced activation of oncogenes. Such process, known as oncogene-induced senescence (OIS) has been previously shown by our team (Castilho *et al*, 2009) to protect from the process of malignization of the epidermis through upregulation of mTOR function and the initiation of cell senescence programs. In this case, inducible upregulation of Wnt1 targeted to the basal layer of the skin using a cytokeratin 5 promoter results in activation of cellular senescence and differentiation of hair follicles, known to represent an important reservoir of stem cells in the skin. The process of cellular senescence in response to the conditional Wnt1 expression was initially documented by the overexpression of cytokeratin 10 in the hair follicles, which is not observed in control littermates reflecting increased cellular differentiation. Interestingly, mTOR function was also found upregulated in the hair follicle of transgenic mice expressing Wnt1 by the accumulation of the phosphorylated form of the ribosomal protein S6, an indirect downstream target of mTOR. Pharmacological interference of this pathway using the mTOR-specific allosteric inhibitor rapamycin resulted in complete abrogation of this phenotype, which demonstrated a new role of mTOR in the maintenance of skin homeostasis and its ability to trigger epithelial differentiation programs.

The PI3K/AKT/mTOR in wound healing

AKT

Numerous pathways and signaling networks have been studied and implicated in the control of skin response after injury. During normal wound healing in mice, we have observed a progressive upregulation of the Akt phosphorylated at serine 473 (ser473) toward the wound edge compared with the intact adjacent skin presenting a patched expression of active Akt in the differentiated layer of the epidermis (Squarize *et al*, 2010). Expression of Akt 473 subsequently extends to the spinous layer of the epidermis (cytokeratin 10 positive) in the transitional skin next to the epithelial tongue and is further expressed in all layers of the epidermis in the migratory epithelial tongue. Similar upregulation of Akt function during wound healing was also observed in a tail model of wound healing. In this model, overexpression of phosphorylated Akt was found confined to the proliferative and migratory areas of the wound, where Akt phosphorylation remains restricted to the outermost layer of the epidermis characterized by late stage cellular differentiation (Pankow *et al*, 2006). Activation of Akt resulting in augmented epithelial migration and proliferation is also observed upon disrupted contact of epithelial cells with the underlining connective tissue as observed in autoimmune blistering diseases (Pretel *et al*, 2009). Deletion of the ECM multidomain glycoprotein Emilin1, responsible for promoting $\alpha4\beta1$ integrin-dependent cell adhesion, results in the proliferation of epithelial cells and accelerated wound healing (Danussi *et al*, 2011). Similarly, overexpression of the zinc finger transcription factor Klf5 in epithelial cells result in enhanced cellular migration

through a direct interaction with integrin-like kinases, which activates Akt (Yang *et al*, 2008). These results indicate a central importance of AKT as a molecule acting downstream from multiple pathways involved in wound healing.

PTEN

The phosphatase and tensin homolog (PTEN) gene was identified in 1997 as a tumor suppressor gene capable of antagonizing proto-oncogenes as the PI3K (Maehama and Dixon, 1998) (Maehama, 2007) (Stambolic *et al*, 1998). This phosphatase has as a substrate PIP₃ that is dephosphorylated into PIP₂, which results in the controlling of the AKT signaling activation. As a major regulator of the PI3K signaling, the role of PTEN in the control of physiological and pathological processes is becoming increasingly clear. This is the case for autoimmunity and lymphomagenesis (Newton and Turka, 2012), innate immune responses in mouse liver ischemia (Kamo *et al*, 2012), normal lung morphogenesis, and the prevention of lung carcinogenesis (Yanagi *et al*, 2007). Of interest for this review, in skin homeostasis loss of *Pten* results in progressive changes in the hair follicles with increased thickness of the outer root sheet and the development of multiple hyperproliferative lesions throughout the skin, especially around the nose, mouth, eyes, paws, limbs, and mucosa (Squarize *et al*, 2008). Such loss of function over the control of cellular proliferation caused by deletion of *Pten* denotes the importance of this tumor suppressor gene for the maintenance of tissue homeostasis, yet also indicates that transient downregulation of this tumor suppressive pathway may be of interest for regenerative therapies. Indeed, we have previously shown that excision of *Pten* from the epidermis using a CRE recombinase driven by the cytokeratin 14 promoter can dramatically influence the recovery of a wounded skin by accelerating the healing process (Fig. 1) (Squarize *et al*, 2010). As expected, deletion of *Pten* results in over activity of the PI3K signaling pathway and activation of the phosphorylated Akt and pS6 (Fig. 2) in the wounded area. Expression of active Akt and pS6, typically observed in the superficial layers of the epidermis (stratum corneum), was promptly upregulated in the spinous and partially in the basal layer at the transition skin, which is localized between normal skin and the wound edge. Akt and pS6 (Fig. 2) were further upregulated at the epithelial tongue throughout the entire thickness of the epidermis. Skin wounds from control mice also demonstrate an upregulation of Akt and pS6 in the transition and epithelial tongue, however much better compartmentalized within the differentiated layers of the epidermis than mice presenting deletion of *Pten* (Fig. 2) (Squarize *et al*, 2010). These findings suggest that physiological downregulation of Pten may be required for proper skin healing and that further downregulation of other members of the PI3K signaling pathway, aiming at the activating mTOR, may also be a viable strategy to influence skin regeneration while preventing unwanted side effects.

The tuberous sclerosis proteins

The tuberous sclerosis genes *TSC1* and *TSC2* code for two proteins named hamartin and tuberin, respectively.

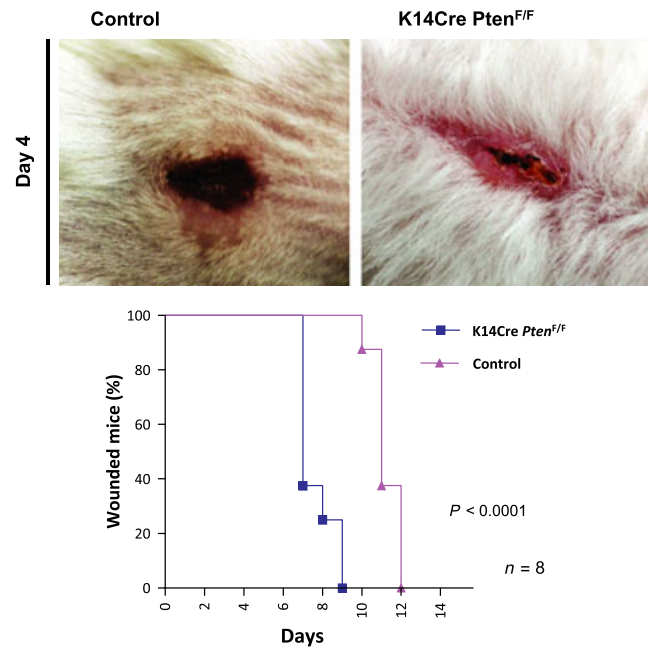


Figure 1 Absence of *Pten* from epithelial cells of the skin resulted in accelerated wound healing. *Pten* is the main regulator of the PI3K/mTOR pathway. With the excision of *Pten*, epithelial cells proliferate and migrate faster than the epithelial cells from control mice. Accordingly, skin healing from K14Cre *Pten*^{F/F} is dramatically accelerated after incisional surgical wounding. As seen here, wound closure of mice group with *Pten* epithelial-specific conditional deletion was completed by day 9, while control group exhibited wound closure by day 12 ($P < 0.0001$, $n = 8$) (data are from Squarize *et al*, 2010)

Their main function is to control cell growth and cell division. Both proteins also act as tumor growth suppressors by antagonizing the mTOR signaling pathway through the interaction with the Rheb1 GTPase. Loss of *TSC1* or *TSC2* gene function is caused by sporadic genetic mutations resulting in the genetic disorder known as tuberous sclerosis complex leading the accumulation of active Rheb1 and thereby constitutive activation of mTOR (Inoki and Guan, 2009). Therefore, transient suppression of the tuberous sclerosis complex constitutes a safe and attractive approach to induce mTOR signaling.

Indeed, we have shown that targeted excision of *Tsc1* using a genetically defined approach exclusively to the epidermis results in the *in vivo* activation of mTOR signaling demonstrated by the phosphorylation of S6 protein, a hallmark of mTOR activity (Fig. 2) (Squarize *et al*, 2010). Moreover, by inhibiting the *Tsc1* gene function and subsequently production of hamartin protein, we could observe an accelerated wound healing of conditional knockout mice with an overall reduction of the healing time of full thickness wounds similar to the phenotype observed in *Pten* conditional knockout mice (Fig. 3). Patients harboring mutations in the *TSC* gene develop hamartomas and non-neoplastic proliferative disorders; however, they fail to develop tumors such as those observed in patients with Cowden disease, which present loss of function mutations of the *PTEN* gene (Squarize *et al*, 2008). Similarly, long-term follow-up of mice presenting targeted mutation for

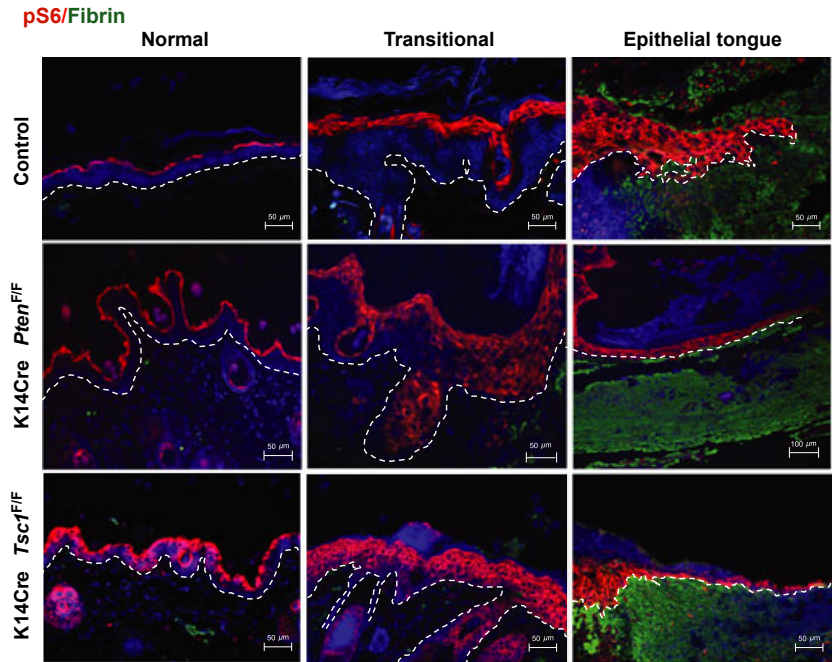


Figure 2 mTOR pathway is activated during the wound healing process. Upregulation of mTOR (pS6) is found in the transitional and epithelial tongue correspondent to the proliferative and migratory anatomical areas of normal wounds. In addition, histological analyses of the wounds demonstrate the upregulation of mTOR signaling on epithelial cells derived from *Pten* and *Tsc1* deletion (K14Cre *Pten*^{F/F} and K14Cre *Tsc1*^{F/F} mice, respectively), which resulted in increased reepithelization. (pS6, red (TRICT); fibrin clot, green (FITC); cells/nucleus, blue (DAPI) (data are from Squarize *et al*, 2010)

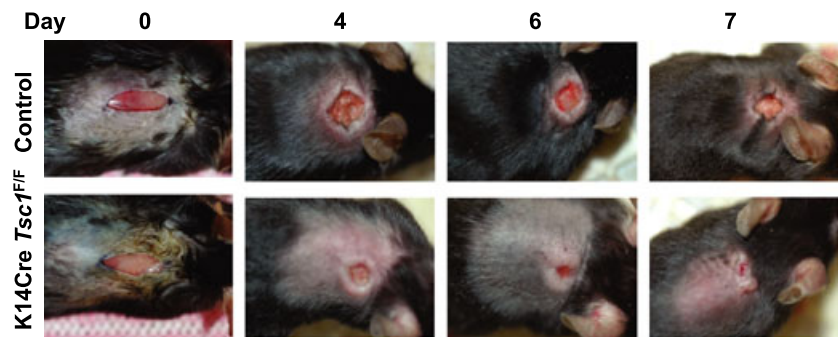
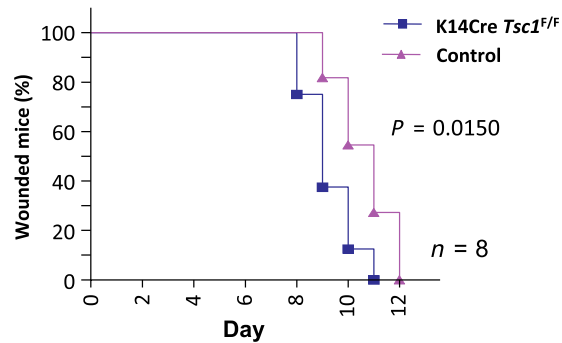


Figure 3 Deletion of *Tsc1* from the epithelial cells of the skin resulted in acceleration of healing. Tuberous sclerosis complex protein 1 (TSC1) forms a complex with TSC2. The inactivation of TSC2/TSC1 complex leads to the accumulation of the GTP-bound (active) form of Rheb1, which in turn promotes the direct activation of mTOR. Using a genetically defined approach, *Tsc1* excision from the skin (using K14Cre *Tsc1*^{F/F} mice) resulted in accelerated wound healing. The median wound closure time in K14Cre *Tsc1*^{F/F} mice was 9 days after wound, as compared to day 11 in littermate control mice ($P = 0.0150$, $n = 8$) (data are from Squarize *et al*, 2010)



Tsc1 shows normal skin development, and lack of tumor formation indicating that transient modulation of the PI3K signaling at the level of the tuberous sclerosis protein complex constitutes a viable and safe strategy to enhance tissue repair.

Whereas interfering with the ability of normal regulatory mechanisms involved in the control of epithelial growth and cellular division may increase cancer risk, and hence, it should be carefully taken in consideration, we have also observed that epithelial stem cells are endowed with protective mechanisms capable of inducing stem cell differentiation or senescence upon aberrant activation of

mTOR (Castilho *et al*, 2009). Thus, strategies aiming at the indirect activation of mTOR through pharmacological or genetic downregulation of *TSC* proteins may represent a suitable strategy to enhance epithelial migration into the wound bed, thereby reducing the overall healing time and the development of secondary complications, without increasing cancer risk.

Periostin

The process of epithelial migration during wound healing is controlled not only by intrinsic factors, but also by

extrinsic stimuli available in the wounded site produced during the process of blood clotting or released by the stromal cells involved in the process of re-epithelialization. Periostin is one of the matricellular proteins normally expressed in adult skin, which is highly upregulated during wound healing (Hamilton, 2008) (Zhou *et al*, 2010) (Nishiyama *et al*, 2011) (Elliott *et al*, 2012). In fact, fibroblasts exposed to environmental changes found during wound healing differentiate into myofibroblasts, which present an increased secretion of periostin (Zhou *et al*, 2010). This matricellular protein is endowed of unique properties capable of responding to mechanical stress, as initially observed in the periodontal ligament and periosteum, where mechanical stresses play a central role in the dynamic of bone remodeling (Horiuchi *et al*, 1999). As a component of the ECM, periostin is known to interact with integrin molecules, thereby activating the PI3K signaling pathway in tumor cells (Gillan *et al*, 2002) (Bao *et al*, 2004). Recently, Ohtsuka *et al* revealed that human dermal fibroblasts stimulated with recombinant periostin lead to the activation of several kinases including AKT, establishing the role of periostin in fibroblast-driven wound healing (Ohtsuka *et al*, 2012). Although many functions of periostin have been identified, the signaling pathways involved in accelerated wound healing are still unknown. We have recently characterized the effects of periostin over epithelial cells at the biological and molecular levels (L.K. Rosselli-Murai and R.M. Castilho, manu-

script in preparation). In this study, we found that periostin is capable of dramatically modulating the behavior of epithelial cells *in vitro* by increasing their migratory and proliferative capacity in a process that involves the upregulation of the AKT/mTOR signaling pathway. Therefore, the mitogenic and migratory capacity of epithelial cells observed during wound healing may not be solely driven by cell intrinsic signaling events, but it may rather result from the cooperative signaling from both the epithelial and mesenchymal components of the skin.

The emerging picture of the field is that a diversity of molecular signaling circuitries involved in the process of wound healing ultimately converges on the activation of the PI3K pathway resulting in activation of mTOR (Fig. 4). Although the process of healing is multifactorial and requires the cooperation between different cell types along with the deposition and maturation of the ECM, the sole enhancement of epithelial migration is feasible with obvious benefits for patients suffering of extensive wounds. Therefore, modulation of the PI3K pathway may represent a new therapeutic target for accelerating skin regeneration and the closure of critical life-threatening wounds.

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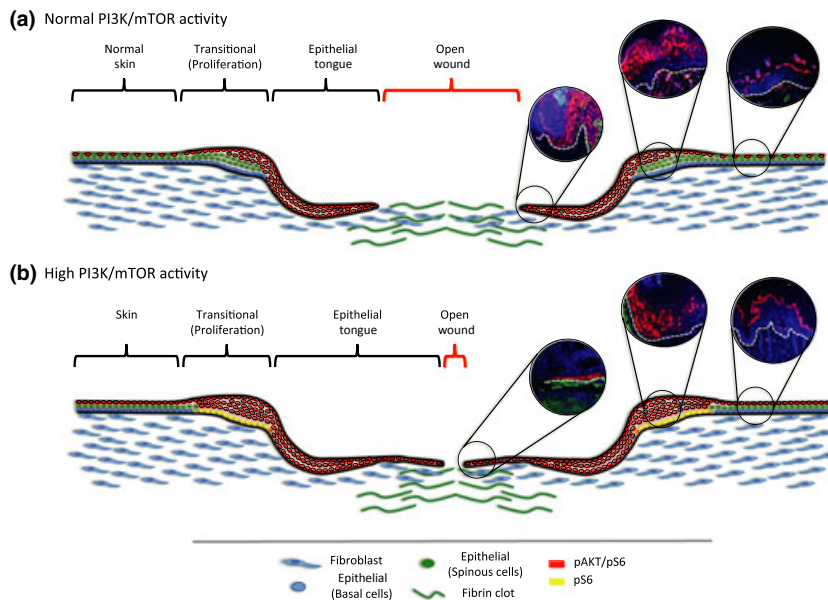


Figure 4 The PI3K signaling pathway is highly activated during the wound healing process. (a) During the healing process, proliferation of epithelial cells occurs at the transitional area adjacent to the injury site, which displays increased pAKT (phosphorylated AKT) (immunofluorescence inserts) and mTOR activity mainly restricted to the differentiated layers of the epidermis. Normal skin is characterized by a patched expression of pAKT and pS6 (Normal skin red cells). Accumulated epithelial cells migrate toward the center of the wound forming the epithelial tongue characterized by the co-overexpression of pAKT and pS6 (red cells). The epithelial tongue aims at covering the wounded area in the shortest time possible. Next, this thin layer of keratinocytes initiates a differentiation program that results in the stratification and formation of all skin layers comprised of basal, spinous, granular and cornified layers. (b) Upon upregulation of the PI3K/mTOR signaling pathway, epithelial cells proliferate and migrate faster, thereby accelerating the wound healing process. Upregulation of the PI3K pathway is characterized by overall increased expression of pAKT and pS6 in the skin. The proliferative anatomical area (transitional) of the wounded skin is now characterized by double expression of pS6 and pAKT in the spinous and parabasal layers of the epidermis, while persistent expression of pS6 is now observed in the basal layer of the epidermis (yellow cells). Overexpression of the PI3K pathway results in the elongation of the epithelial tongue characterized by double expression of pS6 and pAKT (red cells). While the transient activation of PI3K/AKT/mTOR accelerates wound healing, the epithelial stem cells initiate cell senescence program if this pathway is persistently activated, thus protecting from hyperproliferation and tumor initiation

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Author contributions

RMC, CHS, and JSG have contributed to the design, organization and writing of the review article.

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