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# The multiple roles of tissue factor in wound healing

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## 1. ABSTRACT

The procoagulant role of tissue factor (TF) is well recognized. The ability to form a hemostatic clot is essential to normal healing of an injury. However, TF also has additional activities as a regulator of cellular processes. Both by production of coagulant molecules that also have cytokine and growth factor-like activities, and by directly mediating cell signaling events, TF has the potential to influence the course and tempo of healing. The literature in this are remains somewhat sparse, but suggests that modulation of TF expression plays a role in modulating the host response to injury.

## 2. INTRODUCTION

Tissue factor (TF) is a transmembrane protein that serves as the primary initiator of physiologic hemostasis. It has no enzymatic activity of its own, but binds coagulation factor VII (FVII), promotes its activation, and greatly enhances the proteolytic activity of FVIIa (1). The procoagulant activity in tissue extracts was assigned to a protein component in the 1960's (2, 3). The complete genomic sequence was published in 1989 (4). Interestingly, TF was found not to be homologous to any of the other coagulation factors. Instead, it has an overall structure that places it in the family of type 2 cytokine

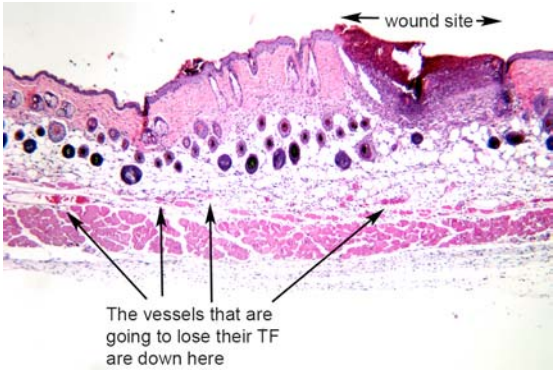


**Figure 1.** One day old punch biopsy wound on a wild type mouse stained with hematoxylin and eosin. The tissue defect is filled with a fibrin clot that is infiltrated by neutrophils. At this early time the tissue is not yet distorted by edema and cellular influx. The neutrophils are followed in the next 1-2 days by an influx of monocytes. Original magnification 40x

receptors (5). Thus, its lineage suggests that it should play a role in host defense in addition to hemostasis. TF has a very short intracytoplasmic tail, a fact which originally led researchers to believe that it was unlikely to mediate signal transduction events. However, more recent studies have made it clear that TF not only serves as a cofactor in coagulation, but also can mediate activation of several cellular signalling pathways (6, 7), and alter gene expression (8, 9). The burgeoning literature on the intracellular pathways impacted by FVIIa/TF is reviewed in (10). TF mediates signaling by two known mechanisms. First, the FVIIa/TF complex can cleave and activate protease activated receptor (PAR) 2 and, to a lesser degree, PAR-1 (11). This mechanism requires proteolytically active FVIIa, but does not require the cytoplasmic tail of TF. TF-mediated PAR signaling has been implicated in tumor metastasis (12) and tumor angiogenesis (13). Second, TF can transduce a signal via its intracellular domain when it binds FVIIa. The cytoplasmic domain of TF has also been implicated in tumor metastasis (14), angiogenesis (15), as well as vascular remodeling (16), leukocyte recruitment (17) and cell-mediated immune responses (18). It also appears that signaling by these two mechanisms can synergize in producing cellular responses.

Since expression of TF has been linked to several biological processes that are involved in restoring tissue structure and function after injury, it is reasonable to hypothesize that TF plays a role in mediating tissue repair. However, the amount of data that directly addresses the roles of TF in healing is quite limited. Many of the studies deal with the role of TF in the aberrant form of "healing" associated with malignancy, and others use *in vitro* models. Recent reports suggest that findings on the activities of TF in these settings do not necessarily apply to the role of TF in physiologic host responses to injury. The goal of this short review is to summarize the limited data available in this area and highlight some tantalizing findings suggesting that TF might indeed play an important role in wound healing beyond its procoagulant activity. Note that the vast majority of the data in this area are derived from studies in mice. Even where the data appear conclusive, their applicability to humans remains to be determined.

## 3. PHASES OF HEALING

Wound healing has been divided into four phases: 1) hemostasis, 2) inflammation, 3) proliferation and 4) remodeling or resolution. TF and active factors produced by its procoagulant activity have clear effects on each of the phases of healing. Receptor-triggered cellular responses that play a role in inflammation and healing are less well delineated, but maybe as important as the pro-coagulant activities of TF in modulating the host response to injury.

### 3.1. Hemostatic Phase

Hemostasis commences as soon as vessels are breached during wounding. The hemostatic process is important not only because it stops blood loss, but also because it delivers biologically active molecules to the wound site that influence subsequent inflammatory and proliferative responses. The hemostatic response to injury is clearly important for subsequent tissue repair. Our group has shown that wound healing is delayed and the histology of the wound site is abnormal in hemophilia B mice, which have a profound hemostatic defect (19). These studies used an excisional model of cutaneous wound healing in which a punch biopsy wound is placed on the back of a mouse and the progress of surface wound closure, as well as the histology of the tissue can be studied. A section of a one-day-old biopsy wound stained with hematoxylin and eosin is shown in Figure 1.

Of course, TF plays an important role in the hemostatic phase of wound healing because it is the major biological initiator of hemostatic clot formation. However, TF is not expressed equally by all tissues. Surprisingly, many sites have very low levels of expression, such as most fibroblasts and skeletal muscle. Highest levels are seen in the brain and lung, with high levels also in kidney and heart (20, 21). In the skin, TF expression is limited to the squamous epithelium and to the mesenchymal cells (pericytes) around blood vessels (20, 21). This variable expression led to the idea that TF is localized to sites where it is needed to rapidly initiate coagulation when significant injury – especially vascular injury – takes place.

The major sites of TF expression in the skin are shown in Figure 2. Tissue preparation and immunohistochemical staining for TF was conducted as described in (22). The brown color indicates the expression of TF in the squamous epithelium that lines the surface of the skin and hair follicles, as well as around the blood vessels in the dermis. TF around vessels has bound FVII(a) even in the absence of injury (23). Thus, small amounts of activated coagulation factors are likely formed at this site in the baseline state. However, when a vessel is breached, platelets adhere at the site of injury to provide additional surface to support the coagulation reactions. The procoagulant activity initiated by FVIIa/TF can then lead to a burst of thrombin production on platelet surfaces.

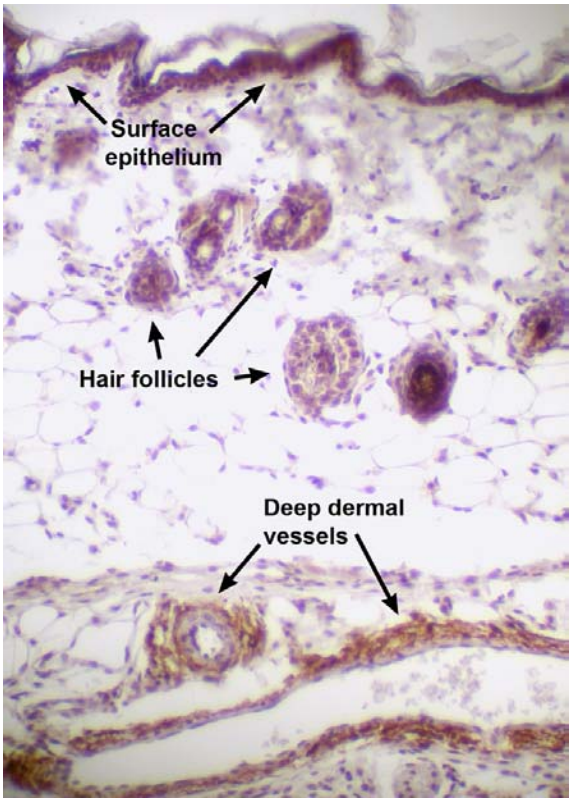


**Figure 2.** Immunohistochemical localization of TF in normal mouse skin. The brown color indicates expression of TF around vessels in the deep dermis, and by squamous epithelial cells of the surface epithelium and hair follicles. Original magnification 100x

Thrombin's key activities in hemostasis are in platelet activation and cleavage of fibrinogen to initiate formation of the fibrin clot. At completion of the hemostatic phase, a fibrin and platelet clot fills the wound site as indicated in Figure 1.

Mice that lack fibrinogen can still heal cutaneous wounds, but the initial wound strength is reduced and cellular migration into the wound bed is altered (24). Fibrinogen influences wound healing by several mechanisms. The fibrin clot forms a framework on which tissue repair takes place, and fibrin degradation products promote the influx of neutrophils and macrophages (25-27). Fibrin supports the influx of leukocytes and stromal cells and forms a substrate for keratinocyte migration during re-epithelialization (28). It is not sufficient simply that a fibrin clot is formed during hemostasis. The structural features of the fibrin clot affect fibroblast proliferation as well as expression of growth factors and interleukins (29). Mice that have a defect in fibrin stabilization (factor XIII knockout) also have defects in wound healing, including a delay in reepithelialization (30). A wound healing defect in humans with FXIII deficiency has long been recognized (31). Thus, the presence and structure of fibrin formed during hemostasis plays an important role in the speed and quality of healing.

Thrombin also has biological activities apart from its direct role in hemostasis. It influences subsequent wound healing by acting as a chemotactic agent and a mitogenic agent for macrophage influx as well as promoting angiogenesis (32-34). When it activates platelets it consequently promotes the secretion of a variety of cytokines and growth factors stored in platelet granules. Thrombin agonists have been found to speed the wound healing process in several animal models (34-37).

A major mechanism of thrombin's effects on cells is by cleavage of protease activated receptors (PARs) (38). Thrombin is able to cleave and transmit a signal via PAR-1, PAR-3 and PAR-4. The role of PAR's in hemostasis is different in humans and mice. In humans, the primary PAR involved in platelet activation is PAR-1, while in mice it is PAR-4 (39). However, the dominant mediator of thrombin's effects on non-platelet cells in both species appears to be PAR-1. Therefore, it seems reasonable that thrombin's role in wound healing might be mediated, at least in part, by PAR-1. However, PAR-1 knockout mice showed no defects in closure time of excisional wounds, tensile strength of healed wounds, or wound histology (40). One study did note a modest reduction in wound contraction and leukocyte infiltration in PAR-1 knockout mice (41). Furthermore, application of dressings impregnated with a specific PAR-1 agonist promoted more rapid closure of excisional wounds than dressing alone (37). These results suggest that while PAR-1-mediated signaling may not be essential for healing, it may be able to enhance healing as a pharmacologic effect.

Thus, the hemostatic blood clot that is initiated by TF plays a role in wound healing by halting blood loss and promoting inflammatory and reparative cell influx. The coagulation process also produces multifunctional molecules that link coagulation to tissue repair. However, TF can also function as a cell surface receptor, inducing both signaling events and changes in gene expression. There is currently a small body of tantalizing data to suggest that TF plays roles in inflammation and proliferative responses that are not due to its procoagulant activity.

### 3.2.Inflammatory Phase

Inflammation at sites of cutaneous wounding is characterized by the influx of neutrophils that begins within minutes and peaks about 1-2 days after injury. While neutrophils play an important first line of defense against bacterial invasion, a lack of neutrophils does not result in any defect in wound closure, cellularity or connective tissue formation (42). Neutrophil influx is followed by an influx of monocytes in the first day or two that peaks by 4-5 days after injury. Several findings support the idea that products of the hemostatic response play a significant role in inflammatory cell influx after cutaneous wounding. Neutrophil and monocyte influx in response to skin wounding is delayed and reduced in hemophilia B mice (19). The influx of monocytes is restored when hemostatic thrombin generation is temporarily restored at the time of injury (43). Thrombin likely plays a direct role in promoting leukocyte influx, since the defect in

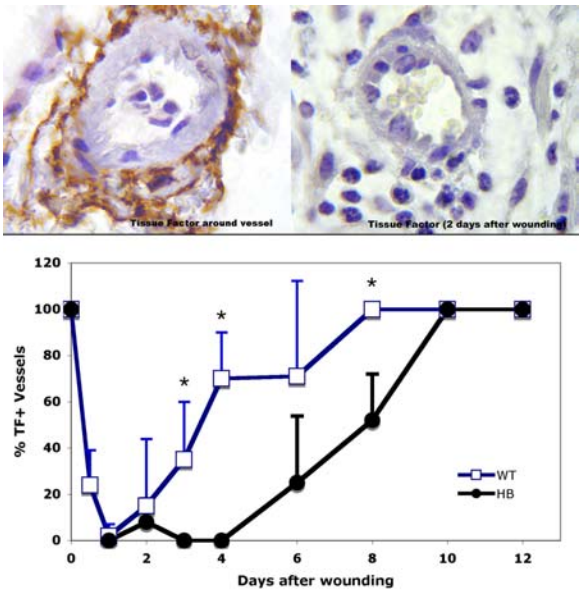


**Figure 3.** Tissue Factor (TF) immunostaining around dermal vessels near sites of wounding. Reproduced with permission from (39). Top panels - TF antigen is indicated by the brown color. Representative vessels from wild type mice are shown. Normal TF distribution around a dermal vessel is in the upper left, and 2 days after wound placement in the upper right. Original magnification 1000X. Bottom panel - TF expression around vessels within 2 high-power (40X) fields of the wound was scored for 3-4 mice per time point, and means with sd plotted.

inflammation appears to be more severe in hemophilia B mice than in fibrinogen deficient mice.

After monocytes migrate into the tissues, they rapidly mature into macrophages. Macrophages not only ingest and degrade debris, but also coordinate repair and immune responses through production of a broad spectrum of cytokines. Macrophages are not essential to repair of the epidermal defect, since mice that lack macrophages have normal wound closure times (44). However, formation of fibrous tissue (scar) and angiogenesis are strongly influenced by macrophage-derived factors. Macrophages are provoked to secrete such factors when they are activated by conditions and cytokines within the wound environment. Interestingly, even though macrophages do not seem to be essential for wound healing, the rate of healing of chronic skin ulcers can be enhanced by local injection or application of granulocyte-macrophage colony stimulating factor (GM-CSF) (45).

TF is expressed by macrophages, though its role in macrophage function is not well understood. It can modulate cell-mediated immune responses in a manner that appears to depend on signaling, rather than procoagulant function. For example, mice lacking the cytoplasmic domain of TF in their leukocytes show reduced cutaneous hypersensitivity, as well as reduced leukocyte-endothelial cell adhesion (46). This suggests that TF expression on infiltrating macrophages might play a role in regulating inflammation and immune responses.

TF expression is increased on a wide range of cell types in culture by inflammatory mediators. Not surprisingly, overall TF mRNA expression in skin is increased following cutaneous wounding (47). However, we recently made the surprising observation that TF goes away from its normal location around dermal vessels within 24 hours after cutaneous wounding (22). Immunostaining and the time course of downregulation of TF staining are shown in Figure 3. The cells that normally express TF around the adventitial side of blood vessels are termed pericytes. These cells have features of both fibroblasts and smooth muscle cells. They play a specialized role in supporting and stabilizing small vessels. They are active co-participants, along with endothelial cells, in the process of angiogenesis. Down-regulation of pericyte TF does not appear to be the result of inflammation and leukocyte influx per se, since a pure inflammatory stimulus (blister beetle toxin) causes a dramatic influx of leukocytes in both wild type and hemophilic mice, but does not lead to down-regulation of perivascular TF (48). This suggests that the loss of TF may be linked instead to the process of angiogenesis, which is not a part of the response to blister beetle toxin, but is prominent following cutaneous wounding.

### 3.3. Proliferative Phase

In skin wounds, the proliferative phase is characterized by epidermal regeneration, angiogenesis, and fibroblast ingrowth as the fibrin clot and debris filling the wound site are replaced by granulation tissue. The term "granulation tissue" comes from the fact that the surface of the reparative tissue has a granular appearance when it is not covered by an epithelial surface. It is very delicate and bleeds easily because of the great numbers of delicate new blood vessels it contains. This tissue is extremely cellular and generally has a greater bulk than the normal tissue that it replaces. TF expression on several of the cell types involved in tissue repair is modulated during the proliferative process.

#### 3.3.1. Keratinocytes

During cutaneous healing, epithelial cells both proliferate and migrate in from the edges of the wound to fill the surface defect. Squamous epithelial cells express TF constitutively. However, during healing the leading edge of the sheet of keratinocytes expresses particularly high levels of TF. The intense expression of TF at the edge of a healing skin wound is shown in Figure 4. Tissue preparation and staining was performed as in (22). The time allowed for color development of the immunohistochemical stain has been reduced to emphasize the higher level of TF expression in the migrating and proliferating keratinocytes compared to the surrounding quiescent epithelium. The active edge of squamous epithelium originates at the margin of the wound and migrates underneath the blood clot until the surface is again covered by an intact epithelial layer.

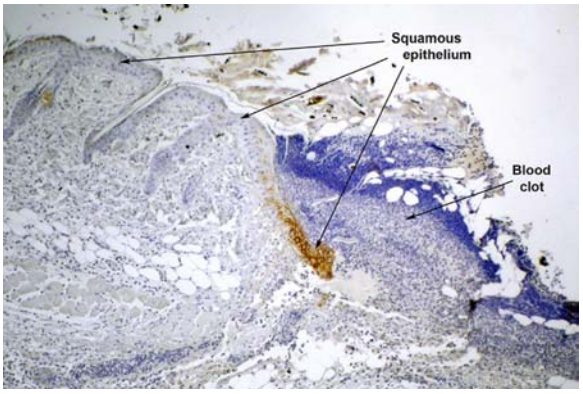


**Figure 4.** Three day old punch biopsy wound immunostained for TF. The squamous epithelium at the edge of the epidermal defect proliferates and migrates across the wound bed, underneath the blood clot. The leading edge of the squamous epithelium stains strongly for TF protein (brown color).

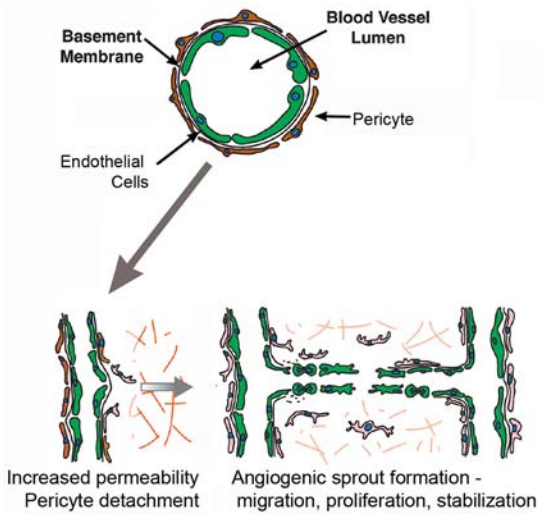


**Figure 5.** The process of angiogenesis - At the top of the figure is a schematic of a small cutaneous vessel. Under the influence of angiogenic factors, the vessel dilates, becomes leaky and its pericytes detach from around the adventitial surface. This is followed by degradation of the endothelial basement membrane and development of endothelial sprouts as a component of the granulation tissue filling the wound bed. The sprouts connect to form complete functioning capillary loops. The neovessels are stabilized as they are surrounded by pericytes.

TF has been hypothesized to mediate cellular adhesion and migration, based on the ability of TF ligands to support cell adhesion and migration in culture, and the association of the TF cytoplasmic tail with an actin binding protein (49). In addition, our group has some preliminary evidence on wound healing in mice expressing very low (1%) levels of TF (50). While there was only a slight increase in bleeding after wound placement, we found focal areas of separation between the epithelium and upper dermis at the sites of some healed wounds in these mice. Thus, while there is little data on this subject, TF might play a direct role in adhesion and migration of keratinocytes during re-epithelialization.

TF expressed by squamous epithelial cells, in contrast to TF around blood vessels, has NOT bound FVII(a) in the absence of injury (23). Binding of FVIIa to cultured squamous epithelial-like cells triggers expression of genes for several transcription factors, growth factors, inflammatory cytokines and proteins involved in cell migration (9). Some of the induced genes included connective tissue growth factor, isoforms of epidermal growth factor and fibroblast growth factor, interleukin-8, interleukin-1 beta and transforming growth factor beta-2. Effects of FVIIa on gene expression were evident by 30 minutes after binding. Thus, it seems likely that when FVII from the blood binds to epithelial TF at the site of an injury and becomes activated, it transduces a signal to the keratinocytes at the edge of the wound.

Squamous epithelial cells express a high level of PAR-2 (51), suggesting that PAR-2 cleavage by FVIIa/TF complexes could modulate some of these epithelial responses. PAR-2 has been shown to mediate other activities of keratinocytes, including secretion of cytokines (52), uptake of melanin pigment (53) and permeability barrier function (54). In addition, keratinocytes can produce substantial amounts of vascular endothelial growth factor (VEGF) (55). Thus, it is tempting to speculate that FVIIa/TF signaling to squamous epithelial cells rapidly promotes secretion of cytokines and growth factors that influence surface wound closure, leukocyte influx and angiogenesis during healing.

### 3.3.2. Vascular Pericytes

As noted above, TF expression by pericytes around dermal vessels is downregulated within 24 hours after a cutaneous wound (22) and remains depressed through most of the proliferative phase of healing. Another surprising finding in these studies was that the new angiogenic vessels in the granulation tissue that initially fills the dermal wound site also do not have a TF coat (TF is however visible in the squamous epithelium above the granulation tissue). We initially speculated that these new vessels lacked pericytes. However, staining for desmin (which stains pericytes but not endothelial cells) showed that pericytes were indeed present around vessels in the granulation tissue. Thus, during the period of active angiogenesis, both the vessels of origin and the neovessels themselves lack a layer of TF.

As illustrated schematically in Figure 5, vessels that will revascularize a site of injury first respond to angiogenic signals with increased permeability and the detachment of pericytes from around the vessel. The vessels progressively become more leaky as endothelial cells degrade the basement membrane and begin migration. Led by endothelial cells, angiogenic sprouts grow from their origins on pre-existing vessels into the granulation tissue of the wound bed. They eventually meet complementary sprouts and form functioning vascular loops that can then deliver blood to the healing tissues. As

the sprouts mature they are supported by pericytes that reestablish their positions around the outside of the new vessels. It appears that the loss of TF from around dermal vessels after wounding may closely follow the delivery of the first angiogenic signals.

TF expression by the endothelium of angiogenic vessels in malignancies has been reported by a number of workers (56), and has been considered by some to be a key driver of the angiogenic process (15, 57). However, we found that the angiogenic vessels in the healing skin wounds lacked TF expression by either the endothelial cells or pericytes. This may be one reason that granulation tissue is so prone to bleeding. In animal models of a defect in coagulation proteins or platelets, angiogenesis is associated with marked bleeding from the delicate and leaky neo-vessels. However, in hemostatically intact animals, only extravasation of small numbers of red blood cells is seen unless the granulation tissue is traumatized.

Vascularization of malignant tumors can be accomplished by several mechanisms (reviewed in (58)). Angiogenic sprouts from pre-existing normal vessels can occur as it does in normal adult tissues and wound healing. However, another mechanism involves malignant cells forming vascular channels in a process termed "vasculogenic mimicry" (59). Since the cells lining these "vessels" are derived from the tumor, it seems likely that these are the "endothelial" cells that express high levels of TF in the setting of malignancy. TF-expression in angiogenic vessels in malignancy is often associated with thrombosis. We hypothesize that a lack of TF expression around angiogenic vessels during normal healing is a protective mechanism to prevent thrombosis of the vessels during their early development while they are delicate and leaky.

In spite of the fact that TF is reduced or absent around vessels, the overall expression of TF near a skin wound is increased. Wound healing is delayed in diabetic humans and in animal models of diabetes. In diabetic mice, TF is not upregulated to the same extent as in wild type mice (47). When somatic gene transfer was used to increase TF levels in the wounds of these diabetic mice, more TF was seen in the leading edge of the migrating epidermal cells; fibrin deposition in the wound bed was increased; and wound healing time was restored to normal (47). In addition, vascular endothelial growth factor (VEGF) expression by the squamous epithelium was increased and this was associated with an increased number of angiogenic vessels in the wound bed. These results suggest that both coagulant and non-coagulant functions of tissue factor might be important in the proliferative phase.

### 3.4. Remodeling or resolution phase

Remodeling of the wound site can continue for weeks or months as proliferation stops, the overall cellularity of the site declines, the inflammatory infiltrate resolves, most of the newly formed vessels regress and the structure of the wound site collagen is reorganized. The bulk of the tissue at the wound site shrinks and the contour of the tissue returns to near normal or even smaller than its original size as the cells are replaced by acellular connective tissue (scar). As the proliferation declines and the remodeling phase ensues, TF expression in the squamous epithelium declines to baseline levels, and vessels in the deep dermis recover their surrounding coat of TF (22). Thus, along with the restoration of tissue structure, the balance of TF expression is also restored.

## 4. PERSPECTIVE

In summary, the data support the conclusion that TF plays multiple important roles in wound healing. These include the obvious role in coagulation, and also as a mediator of signals involved in proliferation, inflammation and immune responses. However, TF expression and activity is differentially regulated. It is upregulated at the leading edge of the healing epithelium, but downregulated around pre-existing and angiogenic vessels. The increased expression of TF in squamous epithelium seems to contribute to closure of the surface epithelial defect. Downregulation of TF around angiogenic vessels may serve to protect the leaky developing vessels from thrombosis. Importantly, TF seems to play a different role in normal healing than it does in tumor angiogenesis. In this setting TF expression by vascular lining cells seems to drive angiogenesis, but also contributes to thrombosis of tumor vessels. This suggests that a better understanding of the subtleties of TF's roles may allow development of more finely targeted anti-angiogenic therapies to treat malignancies and other pathologic conditions.

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## 6. REFERENCES

1. Y. Nemerson: The reaction between bovine brain tissue factor and factors VII and X. *Biochemistry*, 5(2), 601-8 (1966)

2. Y. Nemerson and T. H. Spaet: The Activation of Factor X by Extracts of Rabbit Brain. *Blood*, 23, 657-68 (1964)

3. M. Hvatum and H. Prydz: Studies on tissue thromboplastin--its splitting into two separable parts. *Thromb Diath Haemorrh*, 21(2), 217-22 (1969)

4. N. Mackman, J. H. Morrissey, B. Fowler and T. S. Edgington: Complete sequence of the human tissue factor gene, a highly regulated cellular receptor that initiates the coagulation protease cascade. *Biochemistry*, 28(4), 1755-62 (1989)

5. J. F. Bazan: Structural design and molecular evolution of a cytokine receptor superfamily. *Proc Natl Acad Sci U S A*, 87(18), 6934-8 (1990)

6. L. K. Poulsen, N. Jacobsen, B. B. Sorensen, N. C. Bergenhem, J. D. Kelly, D. C. Foster, O. Thastrup, M.

Ezban and L. C. Petersen: Signal transduction via the mitogen-activated protein kinase pathway induced by binding of coagulation factor VIIa to tissue factor. *J Biol Chem*, 273(11), 6228-32 (1998)

7. J.-A. Rottingen, T. Enden, E. Camerer, J.-G. Iversen and H. Prydz: Binding of human Factor VIIa to Tissue Factor Induces cytosolic Ca2+ signals in J82 cells, transfected COS-1 cells, Madin-Darby canine kidney cells and in human endothelial cells induced to synthesize Tissue Factor. *J. Biol. Chem.*, 270(9), 4650-4660 (1995)

8. U. R. Pendurthi, D. Alok and L. V. Rao: Binding of factor VIIa to tissue factor induces alterations in gene expression in human fibroblast cells: up-regulation of poly(A) polymerase. *Proc Natl Acad Sci U S A*, 94(23), 12598-603 (1997)

9. E. Camerer, E. Gjernes, M. Wiiger, S. Pringle and H. Prydz: Binding of factor VIIa to tissue factor on keratinocytes induces gene expression. *J Biol Chem*, 275(9), 6580-5 (2000)

10. L. V. Rao and U. R. Pendurthi: Tissue factor-factor VIIa signaling. *Arterioscler Thromb Vasc Biol*, 25(1), 47-56 (2005)

11. F. Schaffner and W. Ruf: Tissue factor and PAR2 signaling in the tumor microenvironment. *Arterioscler Thromb Vasc Biol*, 29(12), 1999-2004 (2009)

12. W. Ruf: Tissue factor and PAR signaling in tumor progression. *Thromb Res*, 120 Suppl 2, S7-12 (2007)

13. T. A. Hembrough, G. M. Swartz, A. Papathanassiu, G. P. Vlasuk, W. E. Rote, S. J. Green and V. S. Pribluda: Tissue factor/factor VIIa inhibitors block angiogenesis and tumor growth through a nonhemostatic mechanism. *Cancer Res*, 63(11), 2997-3000 (2003)

14. M. E. Bromberg, W. H. Konigsberg, J. F. Madison, A. Pawashe and A. Garen: Tissue factor promotes melanoma metastasis by a pathway independent of blood coagulation. *Proc Natl Acad Sci U S A*, 92(18), 8205-9 (1995)

15. M. Belting, M. I. Dorrell, S. Sandgren, E. Aguilar, J. Ahamed, A. Dorfleutner, P. Carmeliet, B. M. Mueller, M. Friedlander and W. Ruf: Regulation of angiogenesis by tissue factor cytoplasmic domain signaling. *Nat Med*, 10(5), 502-9 (2004)

16. I. Ott, B. Weigand, R. Michl, I. Seitz, N. Sabbari-Erfani, F. J. Neumann and A. Schomig: Tissue factor cytoplasmic domain stimulates migration by activation of the GTPase Rac1 and the mitogen-activated protein kinase p38. *Circulation*, 111(3), 349-55 (2005)

17. L. Sharma, E. Melis, M. J. Hickey, C. D. Clyne, J. Erlich, L. M. Khachigian, P. Davenport, E. Morand, P. Carmeliet and P. G. Tipping: The cytoplasmic domain of tissue factor contributes to leukocyte recruitment and death in endotoxemia. *Am J Pathol*, 165(1), 331-40 (2004)

18. Y. H. Yang, P. Hall, G. Milenkovski, L. Sharma, P. Hutchinson, E. Melis, P. Carmeliet, P. Tipping and E. Morand: Reduction in arthritis severity and modulation of immune function in tissue factor cytoplasmic domain mutant mice. *Am J Pathol*, 164(1), 109-17 (2004)

19. M. Hoffman, A. Harger, A. Lenkowski, U. Hedner, H. R. Roberts and D. M. Monroe: Cutaneous wound healing is impaired in hemophilia B. *Blood*, 108(9), 3053-60 (2006)

20. T. A. Drake, J. H. Morrissey and T. S. Edgington: Selective cellular expression of tissue factor in human tissues. Implications for disorders of hemostasis and thrombosis. *Am J Pathol*, 134(5), 1087-97 (1989)

21. R. A. Fleck, L. V. Rao, S. I. Rapaport and N. Varki: Localization of human tissue factor antigen by immunostaining with monospecific, polyclonal anti-human tissue factor antibody. *Thromb Res*, 59(2), 421-37 (1990)

22. A. G. McDonald, K. Yang, H. R. Roberts, D. M. Monroe and M. Hoffman: Perivascular tissue factor is down-regulated following cutaneous wounding: implications for bleeding in hemophilia. *Blood*, 111(4), 2046-8 (2008)

23. M. Hoffman, C. M. Colina, A. G. McDonald, G. M. Arepally, L. Pedersen and D. M. Monroe: Tissue factor around dermal vessels has bound factor VII in the absence of injury. *J Thromb Haemost*, 5(7), 1403-8 (2007)

24. A. F. Drew, H. Liu, J. M. Davidson, C. C. Daugherty and J. L. Degen: Wound-healing defects in mice lacking fibrinogen. *Blood*, 97(12), 3691-8 (2001)

25. V. J. Stecher: The chemotaxis of selected cell types to connective tissue degradation products. *Ann N Y Acad Sci*, 256, 177-89 (1975)

26. D. L. Richardson, D. S. Pepper and A. B. Kay: Chemotaxis for human monocytes by fibrinogen-derived peptides. *Br J Haematol*, 32(4), 507-13 (1976)

27. R. M. Senior, W. F. Skogen, G. L. Griffin and G. D. Wilner: Effects of fibrinogen derivatives upon the inflammatory response. Studies with human fibrinopeptide B. *J Clin Invest*, 77(3), 1014-9 (1986)

28. N. Laurens, P. Koolwijk and M. P. de Maat: Fibrin structure and wound healing. *J Thromb Haemost*, 4(5), 932-9 (2006)

29. S. Cox, M. Cole and B. Tawil: Behavior of human dermal fibroblasts in three-dimensional fibrin clots: dependence on fibrinogen and thrombin concentration. *Tissue Eng*, 10(5-6), 942-54 (2004)

30. A. Inbal, A. Lubetsky, T. Krapp, D. Castel, A. Shaish, G. Dickneitte, L. Modis and L. Muszbek: Impaired wound

healing in factor XIII deficient mice. *Thromb Haemost*, 94(2), 432-7 (2005)

31. F. Duckert, E. Jung and D. H. Shmerling: A hitherto undescribed congenital haemorrhagic diathesis probably due to fibrin stabilizing factor deficiency. *Thromb Diath Haemorrh*, 5, 179-86 (1960)

32. R. Bar-Shavit, A. Kahn, J. W. Fenton, 2nd and G. D. Wilner: Chemotactic response of monocytes to thrombin. *J Cell Biol*, 96(1), 282-5 (1983)

33. B. K. Pilcher, D. W. Kim, D. H. Carney and J. J. Tomasek: Thrombin stimulates fibroblast-mediated collagen lattice contraction by its proteolytically activated receptor. *Exp Cell Res*, 211(2), 368-73 (1994)

34. A. M. Norfleet, J. S. Bergmann and D. H. Carney: Thrombin peptide, TP508, stimulates angiogenic responses in animal models of dermal wound healing, in chick chorioallantoic membranes, and in cultured human aortic and microvascular endothelial cells. *Gen Pharmacol*, 35(5), 249-54 (2000)

35. J. Stiernberg, A. M. Norfleet, W. R. Redin, W. S. Warner, R. R. Fritz and D. H. Carney: Acceleration of full-thickness wound healing in normal rats by the synthetic thrombin peptide, TP508. *Wound Repair Regen*, 8(3), 204-15 (2000)

36. D. H. Carney, R. Mann, W. R. Redin, S. D. Pernia, D. Berry, J. P. Heggers, P. G. Hayward, M. C. Robson, J. Christie, C. Annable and *et al.*: Enhancement of incisional wound healing and neovascularization in normal rats by thrombin and synthetic thrombin receptor-activating peptides. *J Clin Invest*, 89(5), 1469-77 (1992)

37. S. M. Strukova, T. N. Dugina, I. V. Chistov, M. Lange, E. A. Markvicheva, S. Kuptsova, V. P. Zubov and E. Glusa: Immobilized thrombin receptor agonist peptide accelerates wound healing in mice. *Clin Appl Thromb Hemost*, 7(4), 325-9 (2001)

38. T. K. Vu, D. T. Hung, V. I. Wheaton and S. R. Coughlin: Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. *Cell*, 64(6), 1057-68 (1991)

39. G. R. Sambrano, E. J. Weiss, Y. W. Zheng, W. Huang and S. R. Coughlin: Role of thrombin signalling in platelets in haemostasis and thrombosis. *Nature*, 413(6851), 74-8 (2001)

40. A. J. Connolly, D. Y. Suh, T. K. Hunt and S. R. Coughlin: Mice lacking the thrombin receptor, PAR1, have normal skin wound healing. *Am J Pathol*, 151(5), 1199-204 (1997)

41. C. D. Major, R. J. Santulli, C. K. Derian and P. Andrade-Gordon: Extracellular mediators in atherosclerosis and thrombosis: lessons from thrombin receptor knockout mice. *Arterioscler Thromb Vasc Biol*, 23(6), 931-9 (2003)

42. D. M. Simpson and R. Ross: The neutrophilic leukocyte in wound repair a study with antineutrophil serum. *J Clin Invest*, 51(8), 2009-23 (1972)

43. A. McDonald, M. Hoffman, U. Hedner, H. R. Roberts and D. M. Monroe: Restoring hemostatic thrombin generation at the time of cutaneous wounding does not normalize healing in hemophilia B. *J Thromb Haemost*, 5(8), 1577-83 (2007)

44. P. Martin, D. D'Souza, J. Martin, R. Grose, L. Cooper, R. Maki and S. R. McKercher: Wound healing in the PU.1 null mouse--tissue repair is not dependent on inflammatory cells. *Curr Biol*, 13(13), 1122-8 (2003)

45. E. Jaschke, A. Zabernigg and C. Gattringer: Recombinant human granulocyte-macrophage colony-stimulating factor applied locally in low doses enhances healing and prevents recurrence of chronic venous ulcers. *Int J Dermatol*, 38(5), 380-6 (1999)

46. J. Apostolopoulos, M. J. Hickey, L. Sharma, P. Davenport, L. Moussa, W. G. James, J. L. Gregory, A. R. Kitching, M. Li and P. G. Tipping: The cytoplasmic domain of tissue factor in macrophages augments cutaneous delayed-type hypersensitivity. *J Leukoc Biol*, 83(4), 902-11 (2008)

47. J. Chen, M. Kasper, T. Heck, K. Nakagawa, P. M. Humpert, L. Bai, G. Wu, Y. Zhang, T. Luther, M. Andrassy, S. Schiekofer, A. Hamann, M. Morcos, B. Chen, D. M. Stern, P. P. Nawroth and A. Bierhaus: Tissue factor as a link between wounding and tissue repair. *Diabetes*, 54(7), 2143-54 (2005)

48. M. Hoffman and D. M. Monroe: Inflammation does not predispose to bleeding in hemophilia. *J Thromb Haemost,* 8(11), 2583-5 (2010)

49. I. Ott, E. G. Fischer, Y. Miyagi, B. M. Mueller and W. Ruf: A role for tissue factor in cell adhesion and migration mediated by interaction with actin-binding protein 280. *J Cell Biol*, 140(5), 1241-53 (1998)

50. D. M. Monroe, N. Mackman and M. Hoffman: Wound healing in hemophilia B mice and low tissue factor mice. *Thromb Res*, 125 Suppl 1, S74-7 (2010)

51. R. J. Santulli, C. K. Derian, A. L. Darrow, K. A. Tomko, A. J. Eckardt, M. Seiberg, R. M. Scarborough and P. Andrade-Gordon: Evidence for the presence of a protease-activated receptor distinct from the thrombin receptor in human keratinocytes. *Proc Natl Acad Sci U S A*, 92(20), 9151-5 (1995)

52. L. Hou, S. Kapas, A. T. Cruchley, M. G. Macey, P. Harriott, C. Chinni, S. R. Stone and G. L. Howells: Immunolocalization of protease-activated receptor-2 in skin: receptor activation stimulates interleukin-8 secretion by keratinocytes *in vitro*. *Immunology*, 94(3), 356-62 (1998)

53. M. Seiberg, C. Paine, E. Sharlow, P. Andrade-Gordon, M. Costanzo, M. Eisinger and S. S. Shapiro: The protease-activated receptor 2 regulates pigmentation via keratinocyte-melanocyte interactions. *Exp Cell Res*, 254(1), 25-32 (2000)

54. J. P. Hachem, E. Houben, D. Crumrine, M. Q. Man, N. Schurer, T. Roelandt, E. H. Choi, Y. Uchida, B. E. Brown, K. R. Feingold and P. M. Elias: Serine protease signaling of epidermal permeability barrier homeostasis. *J Invest Dermatol*, 126(9), 2074-86 (2006)

55. S. Frank, G. Hubner, G. Breier, M. T. Longaker, D. G. Greenhalgh and S. Werner: Regulation of vascular endothelial growth factor expression in cultured keratinocytes. Implications for normal and impaired wound healing. *J Biol Chem*, 270(21), 12607-13 (1995)

56. J. Contrino, G. Hair, D. L. Kreutzer and F. R. Rickles: *In situ* detection of tissue factor in vascular endothelial cells: correlation with the malignant phenotype of human breast disease. *Nat Med*, 2(2), 209-15 (1996)

57. P. M. Fernandez and F. R. Rickles: Tissue factor and angiogenesis in cancer. *Curr Opin Hematol*, 9(5), 401-6 (2002)

58. B. Dome, M. J. Hendrix, S. Paku, J. Tovari and J. Timar: Alternative vascularization mechanisms in cancer: Pathology and therapeutic implications. *Am J Pathol*, 170(1), 1-15 (2007)

59. A. J. Maniotis, R. Folberg, A. Hess, E. A. Seftor, L. M. Gardner, J. Pe'er, J. M. Trent, P. S. Meltzer and M. J. Hendrix: Vascular channel formation by human melanoma cells *in vivo* and *in vitro*: vasculogenic mimicry. *Am J Pathol*, 155(3), 739-52 (1999)

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