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News and Commentary

Cellular senescence mechanisms in chronic wound healing

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Chronic Wounds and Cellular Senescence

Wound healing normally proceeds in a timely, sequential manner and can be broken down into four phases: inflammation, granulation, re-epithelialization, and tissue remodeling[1,2] (see Table 1) When this process is interrupted, a chronic wound is produced. Chronic wounds have been defined as wounds that have failed to return to functional and anatomical integrity in a timely fashion, or wounds that have proceeded through the repair process without a normal functional end result.[1] The vast majority of chronic wounds fall into one of three categories: pressure sores, diabetic ulcers, and venous ulcers.[2] Although these wounds all have very different etiologies, chronic wound development invariably stems from three factors; the cellular and systemic effects of aging, repeated ischemia–reperfusion injury, and bacterial contamination resulting in an inflammatory response.[2,3] The majority of chronic wounds respond well to conventional treatments; however, in a small population of chronic wound patients (estimated to be anywhere from 15 to 20% of all chronic wound sufferers) healing does not occur despite the best care.[1] Although there are many theories regarding the etiology of nonhealing chronic wounds, there are also similarities that are fairly consistent. Typically nonhealing wounds display decreased growth factors (EGF, KGF, PDGF, and IGF), decreased keratinocyte migration, increased reactive oxygen species (ROS), increased tissue proteases, and microbial contamination.[4]

Aged skin is more susceptible to developing chronic wounds than younger skin, possibly due to cellular senescence. In culture, most cells will only replicate a certain number of times before they become senescent, a phenotype characterized by enlargement and spreading of the cells, an accumulation of lipofuscine, expression of senescence associated β-galactosidase (SA-β-gal), and an increase in polynucleation.[5] These cells are cell cycle arrested in G_1, but are still metabolically active. Senescence in culture is frequently associated with shortening of the telomeres during repeated cell divisions eventually leading to cell cycle arrest, and is also known as replicative senescence.[5] Cell divisions in culture can be increased artificially through the expression of telomerase, which rescues the cell through the addition of telomeric sequences during cell division. In the chronic wound, replicative senescence is often mimicked, yet is not associated with telomere length. Factors that can cause a cell to develop a senescent phenotype include oxidative stress, activated oncogene supressor proteins, and cyclin-dependent kinase (cdk) inhibitors.[6] In the chronic wound environment, ROS attack DNA, causing an accumulation of lipofuscin (which is undegradable by the cell) and DNA damage-induced cell cycle arrest.[5]

Senescence in Fibroblasts

Fibroblasts in the dermis have the dual functions of being a synthetic cell, depositing the extracellular matrix, and being a signaling cell by secreting growth factors important for intercellular signaling and repair. Fibroblasts typically have a lifespan of 50 population doublings *in vitro*; however, fibroblasts isolated from chronic wounds have been shown to have a decreased or nonexistent replicative ability, which may or may not have anything to do with telomere length. Senescent fibroblasts appear to take on more of a wound healing phenotype, displaying an increase in gene expression consistent with activated fibroblasts.[5] The synthetic function of fibroblasts may also decrease with senescence, although Herrick *et al*[7] have shown that chronic wound fibroblasts show no decrease in matrix secretion and that the observed decrease appears to be related to increases in matrix remodeling enzymes. Mendez *et al*[8] saw an increase in fibroblast senescence in venous ulcers when compared to fibroblasts from the same site (nonwounded) on the opposite leg, characterized by a significant decrease in growth rate and an increase in SA-β-gal expression. Vande Berg and Robson[3] found that in both pressure and venous ulcers, fibroblasts displayed a senescent phenotype that decreased as the wound healed. Vande Berg *et al*[9] further showed a decrease in population doublings by half in pressure ulcer fibroblasts compared to adjacent normal fibroblasts. Dermal fibroblasts from diabetic mice (db/db) also showed characteristics of senescence; however, they showed no increases in matrix metalloproteases (MMPs) 2 and 9, the two MMPs most frequently associated with chronic wounds. Senescence-associated APA-1, a protein which induces matrix remodeling genes MMP-1 and PAI-1, is upregulated in senescent human fibroblasts.[5] Interestingly, the upregulation of APA-1 does not appear to be associated with telomere length as its expression remains elevated in telomerase rescued senescent fibroblasts.

Fibroblast senescence in chronic wounds appears to be more related to chronic inflammation than telomere length. When exposed to chronic wound fluid, normal fibroblasts in culture appear to switch to a senescent mode, showing

Table 1 The four phases of wound healing and the causes, results, and effector proteins involved

	Cellular actions	Effector proteins		Results
Inflammation	Keratinocyte damage Extravasastion of blood Bacterial load	IL-1 TNF-α CSF		Macrophage and neutrophil recruitment Fibrin clot formed
Granulation	Fibroblasts proliferate, migrate into wound, secrete extracellular matrix (ECM)	PDGF FGF EGF VEGF	MMPs	Fibrin, fibronectin, and hyaluronic acid provide a scaffold for cell migration, angiogenesis
Re-epithelialization	Keratinocytes proliferate, migrate into wound, myofibroblasts cause wound contraction	KGF TGF EGF IGF GM-CSF	MMPs uPA tPA	Re-epithelialization of wound surface, dissection of eschar, barrier formation
Tissue remodeling	Fibroblasts produce a collagenous matrix	TGF-b1	MMPs TIMPs	Formation of scar tissue, apoptosis of fibroblasts, switch from activation to differentiation of keratinocytes

Although each phase has distinct characteristics, the four phases are overlapping during normal wound healing (adapted from Singer and Clark[16] and references therein)

changes in morphology, growth, and SA-β-gal expression consistent with senescent fibroblasts.[10] Chronic wound fluid rapidly degrades exogenous growth factors, decreases the production of cyclin D1, phosphorylated RB (pRB), and increases p21.[3] Since this form of senescence seems to be telomere independent, treatments aimed at increasing telomere length such as telomerase would be ineffective.

Other Cell Types

Much attention has focused on the fibroblast senescence in the chronic wound, with little focus on the keratinocyte and other cells at the site involved in the wound healing process. Keratinocytes are activated during wound healing, which results in a change in the expression of various proteins. Human keratinocytes in normal skin express keratins 5 and 14 at the basal level, and switch to the expression of keratins 1 and 10 in the differentiation process suprabasally. Activated keratinocytes express keratins 6 and 16, a change that is characterized by increased migration at the wound edge and increased proliferation in the unaffected epidermis surrounding the wound site. Although there are few studies examining senescence in chronic wound keratinocytes, many of the same agents that cause abnormal changes in the fibroblast also affect the keratinocyte. Overexpression of cRel, a transcription factor in the Rel/NF-κB family, induces senescence in keratinocytes.[5] Inhibition of Rel/NF-κB decreases SA-β-gal, possibly resulting in a switch from the senescent phenotype.[5] Senescent keratinocytes also show increases in WT p53, p21, and p16.[11] Retinoic acid has been shown to delay the conversion to replicative senescence in keratinocytes by decreasing levels of p16.[11] Retinoid treatment has also been shown to stimulate fibroblast proliferation and collagen synthesis.[12] Treatment of diabetic skin (non-wounded) in organ culture with retinoic acid led to increased thickening, increased collagen production, and perhaps most importantly a decrease in MMP production.

For angiogenesis to occur, endothelial cells must receive a signal from the wound site (typically VEGF) and migrate to the site to establish a capillary network. Increases in proteolytic enzymes and inflammation lead to a gradual loss of endothelial cell homeostatic capacity mimicking replicative senescence.[13] Senescence-associated changes in endothelial cells include large increases in uPA and PAI-1, whose expression are normally modulated by signals from fibroblasts.[14] Therefore, induction of fibroblast senescence may have a direct effect on the induction of senescence in endothelial cells. Another cell type affected by aging is the macrophage, which has been shown to produce significantly less VEGF when isolated from aged skin compared to young controls.[15] Some theorize that the macrophages (along with other inflammatory cell types) are responsible for the chronic inflammatory environment present in venous ulcers.[10]

Matrix Metalloproteases

In acute wounds, there is a balance between protease activity and extracellular matrix (ECM) deposition. MMPs are a family of at least 23 zinc-dependent endopeptidases with the general role of breaking down ECM. MMPs are not expressed constitutively, cells require stimulation from mediators such as IL-1, TNF, and TGF-β to initiate transcription of these enzymes.[16] MMPs have an important role in the migration of fibroblasts (MMP-1, -2, -3, and -13) and keratinocytes (MMP-1, -2, -3, and -10).[16] MMPs have been implicated in the degradation of growth factors in the chronic wound.[17] In a recent clinical study, punch biopsies were obtained from chronic diabetic ulcers and acute wounds from nondiabetic patients.[18] The wounds were examined for presence of MMPs and the tissue inhibitors of metalloproteases (TIMPs). TIMPs

Table 2 Matrix metalloproteases and their inhibitors involved in wound healing

	Alternative names	Substrates	Biological effect	Chronic wounds
MMP-1	Fibroblast collagenase	Type I collagen, fibronectin, MMP-2, MMP-9	Cell migration, proliferation, regulation of MMPs 2 and 9 (chronic wound MMPs)	Large increase
MMP-2	Gelatinase A	Fibronectin, IGF-BP, FGF, MMP-1, MMP-9	Cell migration, proliferation	Large increase
MMP-3	Stromelysin-1	Collagens, gelatin, fibronectin, laminin, IL-1b, IGF-BP, fibrinogen, plasminogen, MMP-1, MMP-2/TIMP-2 complex, MMP-9, MMP-13	Cell migration, MMP regulation, proliferation	Increased
MMP-8	Neutrophil collagenase	Collagens, gelatin, fibronectin	Cell migration	Increased
MMP-9	Gelatinase B	Collagens, gelatin, fibronectin, plasminogen, IL-2R	Proinflammatory, reduction of IL-2 response	Large increase
MMP-10	Stromelysin-2	Collagens, gelatin, elastin, MMP-1, MMP-8	Similar to MMP-3 but with decreased proficiency	Increased
MMP-11	Stromelysin-3	IGF-binding protein-1, laminin, fibronectin, gelatin, and collagen IV	Increased IGF bioavailability	Increased
MMP-13	Collagenase-3	Perlecan, collagens, gelatin, plasminogen activator inhibitor 2, fibronectin, MMP-9	Increased FGF bioavailability	Increased
TIMP-1	NA	NA	Inhibits most MMPs (except 2 and 14), preferentially inhibits MMP-8	Absent
TIMP-2	NA	NA	Inhibits most MMPs (except 9), preferentially inhibits MMP-2 and -9	Decreased
TIMP-3	NA	NA	Bound to ECM, inhibits MMP-1, -2, -3, -9, and -13	Decreased
TIMP-4	NA	NA	Inhibits all MMPs, preferentially inhibits MMP-2 and -7	Decreased

Although at least 23 MMPs are known to exist, those listed above have been shown in multiple studies to have effects on wound healing, and to have been increased in the chronic wound. Only four TIMPs have been described thus far, and all of those are decreased in the majority of chronic wounds (adapted from Visse and Nagase[24] and references therein). NA, not applicable

are produced by most cells and inhibit MMPs in a 1 : 1 ratio. It was found that the MMPs examined (1, 2, 8, and 9) were greatly increased in the diabetic *versus* acute wound. TIMP-2 was decreased nearly twice that of the acute wound. Decreases in TIMPs have been shown in other studies as well.[19,20] Ladwig *et al*[21] also showed the importance of the ratio of MMP-9/TIMP-1 in healing pressure ulcers in a time-to-healing study. Interestingly, they showed a difference in MMPs 2 and 9 in wound biopsies compared to wound fluids, indicating the importance of examining both for an increased understanding of the processes in chronic wound healing (see Table 2).

Experimental Therapeutic Approaches

Treatment of the chronic wound will vary greatly depending on the type of wound, size, location, duration, and underlying etiology. In addition, external factors such as environment, pressure, and nutrition must also be taken into account. Experimental approaches to wound healing have met with limited clinical success. Chronic wound fibroblasts have a diminished capacity to react to growth factors that would normally stimulate a mitogenic response. Studies show a decreased response to bFGF, EGF, and PDGF, which does not appear to be related to a decrease in receptor quantity, but rather a disfunction in intracellular signaling.[4] Increased MMP levels are associated with many chronic wounds. Although MMP inhibitors exist (Hydroxamic acid derivatives, doxycyline[21]), inhibition of MMPs with a broad-spectrum inhibitor is reported to delay wound healing, thereby underscoring the importance for a balance between MMPs and TIMPs.[22] Most novel therapeutic approaches stress the need for a properly prepared wound bed, which includes debridement, vascularization, and a decrease in bacterial burden.[23] By removal of the senescent cells and their products, exogenously applied growth factors, chemicals, and biologics can stimulate the surrounding healthy tissue and promote wound healing.

Considering the varied causes and underlying health of individuals with chronic wounds, it is perhaps best to examine each wound in context and develop a treatment regimen for the individual. While there is currently no 'magic bullet' for healing all types of chronic wounds, the underlying themes appear to be balancing the factors associated with normal wound healing, so that none of the mediators becomes out of equilibrium. Future directions in chronic wound healing are best aimed at addressing all of the underlying pathologies, employing a multifaceted approach to this complicated problem.

1. Mulder GD and Vande Berg JS (2002) J Am Pediatr Med Assoc 92: 34–37
2. Mustoe T (2004) Am J Surg 187: 65S–70S
3. Vande Berg JS and Robson MC (2003) Surg Clin North Am 83: 509–520
4. Agren MS *et al.* (1999) J Invest Dermatol 112: 463–469
5. Bernard D *et al.* (2004) Cancer Res 64: 472–481
6. Shelton DN *et al.* (1999) Curr Biol 9: 939–945
7. Herrick SE *et al.* (1996) J Invest Dermatol 106: 187–193
8. Mendez MV *et al.* (1998) J Vasc Surg 28: 876–883
9. Vande Berg J *et al.* (2005) Wound Repair Regen 13: 76–83
10. Mendez MV *et al.* (1999) J Vasc Surg 30: 734–743
11. Min BM, Oh JE and Choi CM (2004) Int J Mol Med 13: 25–31
12. Lateef H, Stevens MJ and Varani J (2004) Am J Pathol 165: 167–174
13. Chang E *et al.* (2002) J Invest Dermatol 118: 752–758
14. Kipling D (2001) Maturitas 38: 25–37; discussion 37–8
15. Swift ME *et al.* (1999) Lab Invest 79: 1479–1487

16. Singer A and Clark R (1999) N Engl J Med 341: 738–746
17. Yager D and Nwomeh B (1999) Wound Repair Regen 7: 433–441
18. Lobmann R *et al.* (2002) Diabetologia 45: 1011–1016
19. Ashcroft GS *et al.* (1997) J Pathol 183: 169–176
20. Baker AH *et al.* (2002) J Cell Sci 115: 3719–3727
21. Ladwig GP *et al.* (2002) Wound Repair Regen 10: 26–37
22. Mirastschijski U *et al.* (2004) Exp Cell Res 299: 465–475
23. Falanga V and Brem H (2001) In *Cutaneous Wound Healing*, Falanga V, ed (London: Martin Dunitz Ltd) pp. 457–468
24. Visse R and Nagase H (2003) Circ Res 92: 827–839