

# Wound closure and wound management

## A new therapeutic molecular target

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**W**ound closure and infection control are the primary goal of wound management. A variety of disinfectants and antimicrobial agents are widely available today and routinely achieve infection control. On the contrary, wound closure still remains a challenging goal. Cell adhesion, migration and contraction play significant roles in creating contractile force of patent wound margins and in contributing to wound closure. Modulations of these cellular behaviors have been investigated in the context of wound contraction; however, therapeutic strategy to achieve wound closure has not been established. Recently, we have reported that a previously unknown cytoskeleton molecule, wound inducible transcript-3.0 (wit3.0) also known as fibroblast growth factor receptor 1 oncogene partner 2 (FGFR1OP2), can significantly modulate fibroblast-driven wound closure *in vitro* and *in vivo*. The dynamic role of cytoskeleton in different experimental models may provide a novel platform for designing the therapeutic target of wound management.

Postoperative wound dehiscence and surgical site infections are common complications in surgical treatments. The severity of such complications may manifest from mild cases that require local wound care and antibiotics treatment to serious cases with repeated surgical interventions and a high mortality rate. When Eliason and McLaughlin (1934) published the classic review on postoperative wound complications, their focus was largely on surgical site infections.<sup>1</sup> The effective use of prophylactic antibiotics and the unidirectional

ventilation system for surgery and recovery suites are among the improvements developed in the past several decades, which have contributed to the reduction of surgical site infection risks.<sup>2,3</sup> On the contrary, the challenge to prevent surgical wound dehiscence remains problematic and wounds that dehisce frequently are left to heal by secondary or tertiary intention, which could lead to a large scar formation. Incisional and excisional wounds in adult tissues do not spontaneously close and thus remain patent during the critical initial healing stages.<sup>4,5</sup> Current approaches to achieve the initial wound closure employ sutures and adhesives that are essentially unchanged over a century.

### Fetal Wound Closure

It has been reported that skin wounds created in early mammalian embryos exhibit spontaneous wound closure and wound tissue regeneration.<sup>6,7</sup> In the adult wound, epithelial cells migrate into the wound center, but fetal wound epithelial cells remain blunt-faced and adherent to the underlying basal lamina. A thick cable of actin is formed in the basal epidermis at the leading edge of marginal cells surrounding the wound. The contraction of this actin cable is believed to provide the force required to draw the wound edges together in a “purse-string”-like manner.<sup>8,9</sup> In addition to a filamentous actin cable, other components of the contractile machinery, including myosin heavy chain II, are also assembled in a coordinated manner, as are the cytoskeletal proteins that enable the intracellular cable to link with neighboring cells through adherent

**Key words:** wound closure, cell migration, cytoskeleton, FGFR1OP2/wit3.0, therapeutic target

Submitted: 02/28/10

Accepted: 03/31/10

Previously published online:  
[www.landesbioscience.com/journals/celladhesion/article/11917](http://www.landesbioscience.com/journals/celladhesion/article/11917)

DOI: 10.4161/cam.4.3.11917

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junctions.<sup>10</sup> The purse-string wound closure appears to be unique in fetal wound healing and has not been found in wound healing of adult tissues.

Adult and fetal wound healing differs in cellular mediators, inflammatory cytokines, growth factors, and extracellular matrix (ECM) modulators.<sup>11,12</sup> The intense characterization of fetal wounds revealed that wound induced inflammation was less extensive, and profiles of cytokines and growth factors were different than adult wounds.<sup>11,12</sup> These observations provided the basis for the potential therapeutic targets such as neutralizing antibodies against TGF $\beta$ 1, TGF $\beta$ 2 and PDGF, as well as exogenous application of TGF $\beta$ 3.<sup>13,14</sup> Considering the favorable fetal wound healing, control of the inflammatory response or modulation of selected inflammatory cytokines may be therapeutically feasible to reduce post-wound scarring. However, these growth factor-related therapies intending to generate “fetal-like” wound healing phenotype in adult wounds show only mixed results to date.

### Alpha-Smooth Muscle Actin

Alpha-smooth muscle actin ( $\alpha$ -SMA) is a 42 KDa cytoskeleton molecule, found as a predominant actin species in aortic smooth muscle cells.<sup>15</sup> Gabbiani and Majno (1970) reported a group of fibroblasts in healing granulation tissue exhibiting ultrastructural features of smooth muscle cells such as microfilament bundles.<sup>16</sup>  $\alpha$ -SMA was identified in these so-called “myofibroblasts,” and immediately postulated to play a central role in wound contraction.<sup>17</sup> Furthermore, various growth factors and inflammatory cytokines were found to influence the  $\alpha$ -SMA expression,<sup>18</sup> supporting the postulated involvement of  $\alpha$ -SMA in wound contraction. Unlike cytoplasmic actin isoforms, which participate in the cytoskeletal apparatus and microfilaments contributing to cell motility,  $\alpha$ -SMA composes contractile sarcomeres and smooth muscle myofibrils in muscle tissues. In fact,  $\alpha$ -SMA null mutant mice showed the decreased vascular contractility and lowered basal blood pressure.<sup>19</sup> However, the role of  $\alpha$ -SMA in myofibroblasts is less established. The induction of cell traction force in corneal

fibroblasts did not require  $\alpha$ -SMA;<sup>20</sup> and the  $\alpha$ -SMA knockout mutation did not significantly affect fibroblast cell motility.<sup>21</sup> Cell traction force and cell migration play an important role in wound closure. These recent observations prompted us to search alternative therapeutic target molecules of wound management.

### Dorsal Closure of *Drosophila* Embryo

The progressive closure of a dorsal gap during *Drosophila* embryogenesis has been considered another model of wound closure.<sup>22</sup> The longitudinal epidermis margins on the either side of the dorsal opening close by approximation of the leading edges, which form seams at each canthus. It has been shown that dorsal closure is achieved by synchronized cellular forces generated by subcellular actin and non-muscle myosin II in each “purse string” of epidermal margins.<sup>23</sup> The dorsal opening is filled with specialized epithelial cells, amnioserosa, which undergo apoptosis during dorsal closure resulting in eliminating supernumerary cells.<sup>24</sup> Recently, Solon et al. (2009) demonstrated that amnioserosa cells generated the pulsed force, which effectively displaced the epidermis margins.<sup>25</sup> During this process, amnioserosa undergo extensive morphological changes from the initial squamous shapes to a narrow, tubular structure. Various molecules associated with the cytoskeleton and cell junctions have been postulated to regulate the dorsal closure. Further characterization of this model may contribute to the accelerated wound closure and wound management.

### Oral Wound Closure

The healing sequence of oral wounds is similar to that in adult skin wounds; hemostasis, inflammation, granulation tissue formation and remodeling of the connective tissue matrix. However, clinical observations and experimental animal studies consistently indicate that the extent of granulation tissue and scar formation in oral mucosa is small, and oral wound healing demonstrates faster wound closure compared to the equivalent wound in skin.<sup>26</sup> Both fetal and oral wounds are

exposed to a moist environment, which has been postulated to be favorable for wound healing.<sup>27</sup> Saliva also contains a group of cytokines and growth factors.<sup>28,29</sup> Saliva of NOD mice contains decreased concentrations of epidermal growth factor, and this type I diabetes mouse model has been reported to exhibit the impaired oral wound healing.<sup>30</sup> Certainly, the oral environment may assist better wound healing. However, because skin grafts transposed into the oral cavity maintain the skin wound healing phenotype,<sup>31</sup> the contribution of constitutive oral cellular and molecular components to the rapid wound closure cannot be overlooked.

Oral mucosa and gingiva are composed of a thin keratinocyte layer with underlying highly-vascularized connective tissue.<sup>32</sup> Epithelial cells of fetal wound and dorsal gap of *Drosophila*, which play the pivotal role in the “purse-string” closure, do not proliferate. In contrast, oral wound epithelium undergoes rapid proliferation. Oral keratinocytes constitutively express glucose transporter 1 (GLUT1) and contain fatty acids such as palmitate,<sup>33</sup> suitable for cell proliferation, which requires the high level of energy metabolism. While oral keratinocytes may quickly re-establish the epithelial continuity, epithelial “purse-string” mechanism does not seem to occur in the oral wound. Therefore, the rapid oral wound approximation may be accomplished by a different mechanism.

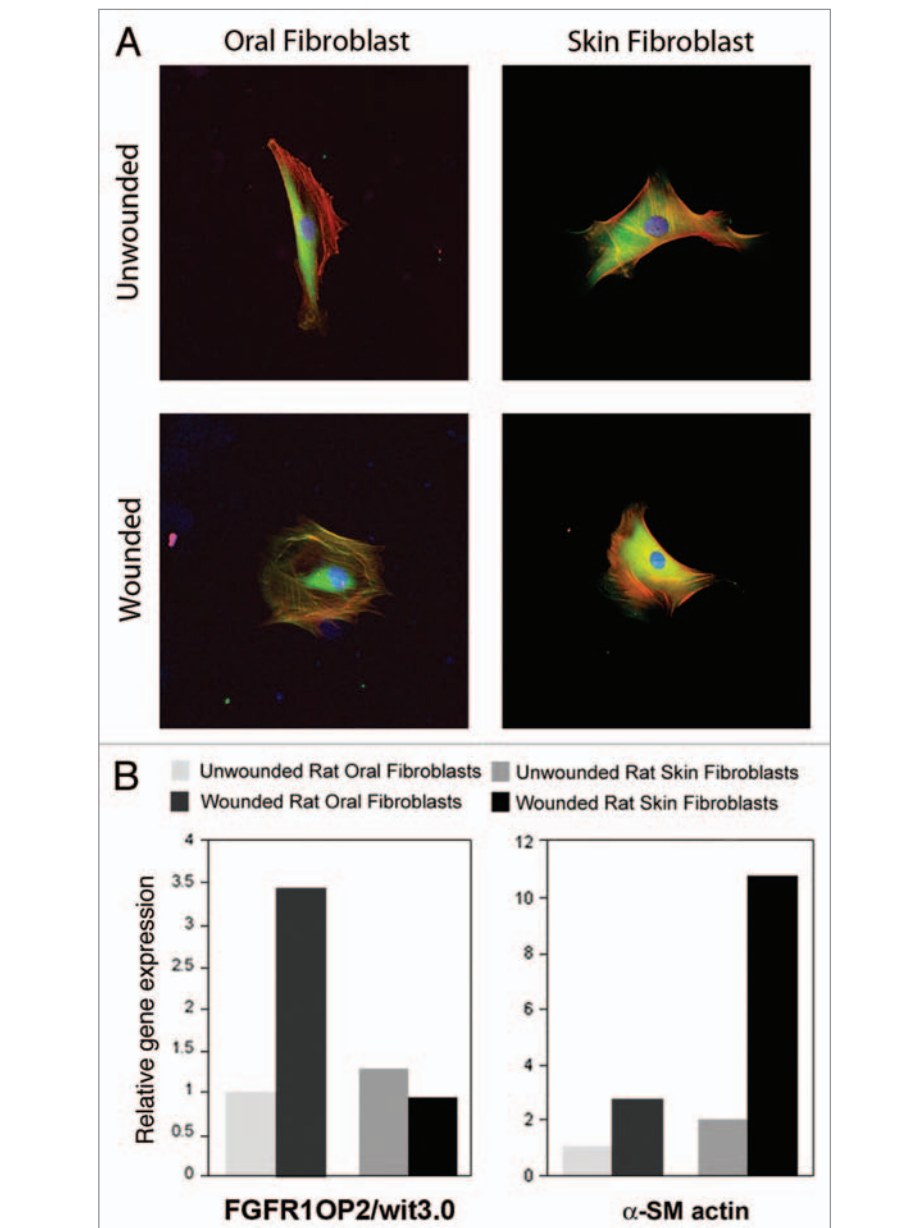
### FGFR1OP2/wit3.0

Collagen gel contraction by isolated oral fibroblasts occurs at a faster rate than with skin fibroblasts in vitro.<sup>34,35</sup> Therefore, it has been postulated that oral fibroblasts possess distinctive characteristics promoting accelerated wound closure. We have isolated a previously unknown cDNA from the oral wound library encoding Wound Inducible Transcript-3.0 (wit3.0).<sup>36</sup> The OMIM database identified the association of wit3.0 with 8p11 myeloproliferative syndrome, a case presented by Grand et al. (2004).<sup>37</sup> An unusual (12; 8) (p11; p11p22) chromosomal insertion was found in a 75-year-old male who suffered from a T-cell lymphoblastic lymphoma that progressed rapidly

to acute myelogenous leukemia (AML). The chromosomal abnormality facilitated the insertion of the wit3.0 N-terminal domain to the Fibroblast Growth Factor Receptor 1 (FGFR1) and resulted in the ligand-independent FGFR1 dimerization through coiled-coil structure derived from wit3.0. As such, the Genbank database registers this molecule as FGFR1 Oncogene Partner-2 (FGFR1OP2).

The highly conserved FGFR1OP2/wit3.0 sequence has been identified in *Homo sapiens*, *Rattus norvegicus*, *Mus musculus* and *Canis familiaris*. FGFR1OP2/wit3.0 is located on human chromosome 12, at 12p11.23, spanning almost 27.5 kb; rat chromosome 4q44; mouse chromosome 6G3; and dog chromosome 27. Among these species, the FGFR1OP2/wit3.0 allele contains highly conserved seven exons, with a start and stop codons located in exon2 and 7, respectively. In addition, exon5 is alternatively spliced, resulting in two isoforms. The NCBI database revealed that the FGFR1OP2/wit3.0 peptides in humans, dogs, mice, rats, chicken, fruit flies, and mosquitoes contain a conserved domain, DUF837, which belongs to the protein family pfam05769.3. This domain is consistently located at the N-terminal in all species, and has appeared in several eukaryotic proteins, although the function is still not clear. BLAST search identified a match with myosin heavy chain II, in particular, a conserved domain in a myosin tail family (pfam01576.8). The FGFR1OP2/wit3.0 peptide also matched with the pfam COG4372, an uncharacterized protein conserved in bacteria with the myosin-like domain.

In oral wound healing, FGFR1OP2/wit3.0 was significantly upregulated and oral fibroblasts have been identified as the primary cellular source.<sup>38</sup> Strikingly, oral fibroblasts expressing FGFR1OP2/wit3.0 were different from those expressing  $\alpha$ -SMA, suggesting that FGFR1OP2/wit3.0 expressing oral wound fibroblasts were not myofibroblasts. In immunocytological analyses, FGFR1OP2/wit3.0 peptide was amorously localized in cytoplasm, especially around the perinucleus area of both oral fibroblasts and skin fibroblasts. Skin fibroblasts derived from excisional wound exhibited the



**Figure 1.** Expression of FGFR1OP2/wit3.0 in rat primary oral and skin fibroblasts. (A) Immunocytological staining identified FGFR1OP2/wit3.0 (green) amorously in the cytoplasm surrounding nucleus (DAPI: blue) of oral and skin fibroblasts. In oral fibroblasts derived from gingiva of post-tooth extraction wounding, FGFR1OP2/wit3.0 localized in cytoskeletal network containing F-actin (red). In contrast, skin fibroblasts harvested after excisional wounding showed no change in FGFR1OP2/wit3.0 distribution. (Confocal laser scanning micrographs of oral fibroblasts derived from Lin et al., *Am J Pathol*, 2010, 176:108-121, with permission.) (B) The steady state mRNA levels of FGFR1OP2/wit3.0 and alpha smooth muscle actin ( $\alpha$ -SM actin) were characterized by real time reverse transcription polymerase chain reaction. Wounded oral and skin fibroblasts demonstrated the increase in  $\alpha$ -SM actin expression, albeit at different magnitudes. In oral fibroblasts, wounding increased the expression of FGFR1OP2/wit3.0 expression; whereas wounding did not modulate its expression in skin fibroblasts.

similar FGFR1OP2/wit3.0 distribution in cytoplasm (unpublished data, Fig. 1). On the contrary, oral fibroblasts derived from tooth extraction wound revealed the

dramatically modulated cytological distribution. Immunostaining of FGFR1OP2/wit3.0 was peripherally spread to form a cytoskeletal filamentous structure. F-actin

was also positive on the wit3.0-positive cytoskeletal filaments; however, these two molecules appeared to localize in a mutually exclusive fashion (Fig. 1).<sup>39</sup>

The three-dimensional collagen gel embedded with fibroblasts has been used as a reliable in vitro assay of wound contraction, in which volumetric changes of collagen gel substrate are thought to be generated by fibroblast migration and contraction force. Oral fibroblasts exhibit the greater rate of collagen gel contraction than skin fibroblasts. siRNA knockdown and overexpression of FGFR1OP2/wit3.0 resulted in the significant decrease and increase of oral fibroblast-embedded floating collagen gel contraction, respectively. The International Gene Trap Consortium (IGTC) represents all publicly available gene trap cell lines that are available on a non-collaborative basis, from which C57Bl/6 mouse ES cell lines carrying the gene trap mutagenesis in the FGFR1OP2/wit3.0 allele have been identified. The differentiated fibroblast-like cells from the mutant ES cells showed significantly decreased cell migration to the wound space in vitro.<sup>39</sup> Furthermore, a proof-of-concept study in a mouse skin wound model revealed that the application of lentiviral vector carrying FGFR1OP2/wit3.0 generated accelerated wound closure similar to oral wounds and resulted in less scarring.<sup>39</sup> Taken together, FGFR1OP2/wit3.0 may regulate the cell migration ability relevant to the fibroblast-driven wound closure; thus this newly characterized cytoskeleton molecule may present an alternative candidate for the new therapeutic target for wound management.

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