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Abbreviations and Acronyms

CAR = vascular homing peptide CAR; sequence (CARSKNKDC)

CTGF/CCN2 = connective tissue growth factor

EGF = epidermal growth factor

TGF- β = transforming growth factor- β

Targeted Antiscarring Therapy for Tissue Injuries

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Significance: The healing of injuries, such as those caused by ischemia (myocardial infarction, stroke), trauma, surgery, and inflammation, tends to happen through undesirable and harmful scarring. Current options in reducing scar formation are largely limited to local intervention in accessible tissues.

Recent Advances: We have designed a systemically administered, injury-targeted decorin for scar prevention. The delivery of decorin to injured tissues is achieved by fusing recombinant decorin to a 9-amino acid peptide, CARSKNKDC (CAR), which specifically recognizes the vessels in injured tissues and extravasates into the tissue, delivering decorin with it.

Critical Issues: Decorin is known to prevent tissue fibrosis and promote tissue regeneration. This activity of decorin is based on inhibition of TGF- β and some other regulatory activities. In addition to serving as a delivery vehicle, the CAR component endowed decorin with much stronger neutralizing activity against TGF- β 1 *in vitro* than is obtained with nontargeted decorin. The CAR-decorin fusion protein promoted wound healing in a mouse skin wound model and suppressed scar formation at doses at which nontargeted decorin was inactive. These results show that selective targeting enhances specificity and potency of an antiscarring compound.

Future Directions: Targeted decorin provides a new, systemic option for the treatment of scarring and fibrotic diseases.

SCOPE

PERMANENT SCARS FORM UPON healing of tissue injuries not only in the skin but also in almost any other organ in response to a wide variety of insults ranging from ischemia (myocardial infarction, stroke), trauma, burn injury, surgery, to inflammation.

The response to tissue injury in mammals is focused on quick sealing of an injury (restoration of the tissue continuity). Rapid proliferation of fibroblasts and enhanced extracellular matrix production replace the damaged tissue with fibrotic tissue, the scar.¹ Transforming growth factor-

β 1 (TGF- β 1) is mainly responsible for the scar formation, and inhibiting TGF- β 1 activity is a major focus of efforts to prevent scar formation and fibrosis.^{1,2}

Growth factors and other biologicals could potentially enhance tissue regeneration with reduced scarring, but their therapeutic application in regenerative medicine has been limited for several reasons: lack of retention of the agent, poor tissue penetration, and instability of protein therapeutics in the protease-rich environment of the injured tissue. These difficulties make it nearly impossible to maintain the bioactivity of

locally applied therapeutics. Moreover, most injuries are inaccessible to topical application, and multiple sites of injury limit the use of local treatments. Clearly, systemic approaches to tissue repair would be valuable.

TARGET ARTICLES

1. Järvinen TAH and Ruoslahti E: Molecular profiling of vasculature in injured tissues. *Am J Pathol* 2007; **171**: 702–711.

2. Järvinen TAH and Ruoslahti E: Target seeking antifibrotic compound enhances wound healing and suppresses scar formation. *Proc Natl Acad Sci USA* 2010; **107**: 21671–21676.

TRANSLATIONAL RELEVANCE

Decorin, a small chondroitin/dermatan sulfate proteoglycan, is a natural TGF- β inhibitor that has substantial interest to clinical medicine owing to its ability to prevent scar formation and tissue fibrosis and to promote tissue regeneration.^{2–8} The TGF- β 1 blockade is likely the main factor in the well-established antiscarring activity of decorin,^{2–5} but inhibitory effects on some other scar-inducing growth factors as well as a regulatory role in collagen fibrillogenesis may also contribute to the antiscarring activity of decorin.^{6–8}

Because of its role in controlling scar formation and fibrosis, decorin has been proposed to be a physiological regulator of tissue healing.² Accordingly, decorin expression is induced in chronic inflammatory conditions.⁴ This physiological role may be negated by increased TGF- β production in chronic scarring situations. Another mechanism counteracting decorin activity is proteolysis, which can result in the formation of a truncated, inactive form of decorin in tissues.⁹ Moreover, decorin expression is also suppressed in skin wounds during scarring,¹⁰ and decorin null mice exhibit fragile skin, accentuated scarring,⁷ and impaired regenerative potential in response to injury.^{7,11}

The ability to use a physiological regulator as a drug has obvious benefits; many of the most successful biotechnology drugs have been physiological substances produced by means of recombinant DNA technology. Despite the positive results in animal models of scarring and fibrosis in numerous organs,^{3–8} decorin has not reached the clinic. One reason is that as a protein therapeutic, it is relatively difficult to manufacture. Enhancing the activity of decorin as well as targeting its delivery to the target organ would be potential solutions to

this problem, as it would produce the same result with a lower dosage.

CLINICAL RELEVANCE

Scarring is a major problem in clinical medicine. A systemic antiscarring therapy that, by preventing scarring, promotes tissue regeneration would be an important development. The antiscarring effect of the TGF- β inhibitor decorin has been known for a long time, but decorin treatment has not reached the clinic. We have now shown that delivering decorin to sites of injury by using a vascular homing peptide specific for the vessels in injured tissues makes systemic decorin therapy more effective than decorin alone. An enhanced anti-TGF- β activity further increases the potency. These enhancements may make decorin a viable molecule for clinical development.

EXPERIMENTAL MODEL OR MATERIAL: ADVANTAGES AND LIMITATIONS

To provide new tools for systemic treatment of tissue injuries, we have used *in vivo* phage display to identify vascular homing peptides that target the angiogenic vasculature in healing skin wounds.¹² For this purpose, we cloned and expressed random CX₇C (where “X” denotes any random amino acid) cyclic peptide library (diversity of about 1.0×10^9 peptides) at the end of the coat protein of T7 bacteriophage.¹² *In vivo* phage display offers a unique opportunity to screen for an almost unlimited number of potential peptidic drug candidates simultaneously in an *in vivo* setting.¹²

Having identified the potent wound homing peptide CARSKNKDC (CAR), we designed, cloned, and expressed a multifunctional recombinant protein consisting of the CAR peptide fused with decorin in a mammalian expression system.¹³ We have studied the biological activity of CAR-targeted decorin *in vitro* and *in vivo*, establishing its inhibition profile on different TGF- β isoforms, its homing ability to skin wounds, and its ability to inhibit scar formation and stimulate tissue regeneration in a mouse model of skin wound healing.¹³

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

The wound-targeted, systemically administered decorin treatment can significantly reduce scar formation in skin wounds.¹³ Our targeting scheme utilizes regenerating angiogenic blood vessels that differ in their molecular composition from pre-existing

vasculature,¹² providing an opportunity for treating tissue injuries with systemically administered molecules in a target tissue-selective manner^{12,13} (Fig. 1).

Our targeted decorin accumulates in the blood vessels and in the granulation tissue of wounded dermis, especially at the bottom of the wound, where the angiogenic blood vessels form.¹³ The fibroblasts in the bottom layers of a skin wound are the main cell type responsible for the permanent, hypertrophic scar,¹⁰ and these cells (which would be difficult to reach with locally applied therapy) produce more of the major scar-inducing TGF- β 1 than cells from the other layers of the wound.¹⁰ The production of endogenous decorin, in turn, is suppressed in these regions.¹⁰ Thus, our targeting scheme provides sustained delivery of decorin to the regions responsible for scar formation and lacking endogenous decorin.¹³

Correct timing of the CAR-targeted therapy may be important. In order not to interfere with the early effect of TGF- β 1, the recruitment of inflammatory cells into the wound, we delayed the introduction of the therapy until the inflammatory cells had arrived

into the wound.¹³ Although the inflammatory cells contribute to scar formation, they are also important in combating infection, removal of necrotic material, and other aspects of wound healing,¹ and it is likely that the benefits of preserving these activities outweigh any possible increase in scarring.

Decorin binds to all isoforms of TGF- β ,¹⁴ but CAR-targeted decorin only neutralizes TGF- β s 1 and 2, not TGF- β 3.¹³ A likely explanation for this selectivity is in the different heparan sulfate binding properties of CAR and the TGF- β isoforms. CAR,¹² TGF- β 1, and TGF- β 2 bind heparan sulfate, whereas TGF- β 3 does not.¹⁵ Moreover, the heparan sulfate binding increases the biological activity of TGF- β 1 and TGF- β 2.¹⁵ Thus, we have suggested⁴ that the CAR-mediated binding of CAR–decorin to heparan sulfate may enhance the neutralizing effect of the fusion protein by bringing it to the proximity of the heparin-binding TGF- β s. CAR–decorin could also interfere with the binding of TGF- β s to heparan sulfate, eliminating the enhancing effect of heparan sulfate binding on TGF- β activity (Fig. 1).

Figure 1. Schematic representation of the mechanism of action of the multifunctional therapeutic molecule CAR–decorin.¹³ (a) CAR–decorin is a systemically administered, target-seeking, multifunctional biotherapeutic that inhibits scar formation. (b, c) The molecule can be targeted to injury taking place at any organ of the body (or multiple organs simultaneously). (d, e) The CAR homing peptide targets angiogenic vasculature, which forms at the site of the injury. (f) The peptide (and any payload attached to it) then extravasates into surrounding tissue, (g) where it binds to its receptor(s) on the cell surface of the scar-producing fibroblasts. (h) CAR binding to heparan sulfate proteoglycans provides docking sites in the proximity of the main scar-inducing growth factors TGF- β 1 and TGF- β 2,⁶ facilitating the neutralization of these growth factors by the therapeutic part of the molecule, decorin. This mechanism results in a therapeutic response, which is seen as reduced scar formation in the skin.¹³ Picture by Helena Schmidt; reproduced with permission from Finnish Medical Journal *Duodecim* (originally published in *Duodecim* 2011;127:50–51).

The differential inhibitory activity of CAR–decorin against the TGF- β isoforms could have important implications; TGF- β 1 is the isoform responsible for scar formation, and TGF- β 2 augments the profibrotic activity of TGF- β 1. TGF- β 3, in contrast, has antiscarring activity.^{1,16} We propose that CAR–decorin inhibits scar formation by blocking TGF- β s 1 and 2, but leaving the beneficial TGF- β 3 untouched.¹⁶

Additional activities are likely to contribute to the antiscarring activity of decorin. We were able to show that CAR-targeted decorin significantly reduces the transformation of fibroblasts to myofibroblasts and the expression of connective tissue growth factor (CTGF/CCN2).¹³ The myofibroblasts transformation is a TGF- β –dependent process and responsible for converting granulation tissue to permanent scar tissue.¹ CCN2, in turn, is a downstream mediator of TGF- β activity in this process.¹⁷ Our result is in agreement with previous data showing that decorin can block the TGF- β –induced transdifferentiation of myogenic cells to myofibroblasts⁵ and provide a molecular mechanism for the CAR–decorin treatment that resulted in the reduced scar formation.¹³

The scar-inducing activities of TGF- β 1 in fibroblasts are mediated by CCN2 and epidermal growth factor (EGF) family receptors (ERBBs).¹⁷ CCN2 activity requires the presence of EGF.¹⁷ Interestingly, decorin also neutralizes both CCN2 and EGF by binding to ERBBs.^{18,19} The active sites for TGF- β , CCN2, and EGF neutralization reside in different parts of the decorin molecule.^{18,19} Thus, in theory, a single decorin molecule could simultaneously block all three mediators of scarring. Moreover, decorin also inhibits myostatin, an important contributor to scarring in some organs.⁸ Owing to this multifunctionality, decorin may be superior to therapeutic approaches that only inhibit TGF- β .

Finally, the CAR peptide has been recently shown to target inflammatory lesions in the lungs.²⁰ Thus, CAR-targeted decorin could also be useful in the treatment of other conditions in which non-targeted decorin has shown activity.

INNOVATION

Systemic treatment of injured tissues would be important; a systemically administered therapeutic agent will reach the site of injury regardless of the loca-

tion. Moreover, in locally accessible injuries, such as skin wounds, systemic delivery can bring the therapeutic agent to the deep layers of the skin wound that are responsible for permanent scarring.

We have used vascular targeting technology to devise an improved version of a physiological antiscarring agent, decorin, which has been extensively validated in preclinical testing but has not reached the clinic. The increased efficacy of our targeted decorin may make systemic treatment of tissue injuries a clinically feasible option. This opens up new possibilities not only for the treatment of skin wounds we used as the model, but also in traumatology, surgery, and the treatment of tissue injuries that result in permanent scar formation (myocardial infarction and stroke).

CAUTION, CRITICAL REMARKS, AND RECOMMENDATIONS

Our product is still in preclinical testing, that is, not approved for human use at the moment.

FUTURE DEVELOPMENT OF INTEREST

We are performing more preclinical testing in experimental scar and fibrosis models in other organs

TAKE-HOME MESSAGE

Basic science advances

- We have identified vascular homing peptides that target skin wounds at different stages of healing.
- These peptides can be used to deliver therapeutics to skin wounds and other injured tissues.
- Vascular homing peptide-targeted decorin is more active than native decorin against TGF- β 1.
- Systemically administered decorin can inhibit scar formation efficiently in skin wounds.

Clinical science advances

- Systemically administered, wound-targeted therapies are plausible treatment options for skin wounds.
- Targeted decorin is effective in reducing scarring in skin wounds.
- Sustained delivery of therapeutic molecules can be obtained using target-specific vascular homing peptides.

Relevance to clinical care

- We hope to advance our wound-targeted decorin to clinical trials as an antiscarring agent.
- Our results establish proof-of-principle for systemically administered, targeted therapies for regenerative medicine.

than skin to confirm the enhanced antiscarring activity of CAR-targeted decorin. We are also simultaneously taking steps to improve the manufacturing process to advance decorin to clinical trials.

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DISCLAIMER

The content of the information does not necessarily reflect the position or the policy of the government, and no official endorsement should be inferred.

AUTHOR DISCLOSURE AND GHOSTWRITING

T.A.H.J. and E.R. are shareholders of Vascular BioSciences Inc., which has rights to some of the technology described in this article. This research was conducted in compliance with the Animal Welfare Act Regulations and other Federal statutes relating to animals and experiments involving animals and adheres to the principles set forth in the Guide for Care and Use of Laboratory Animals, National Research Council, 1996. Ghostwriters were not used to write this article.

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