Biomaterials 47 (2015) 29–40

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Reengineering autologous bone grafts with the stem cell activator WNT3A

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ARTICLE INFO

Article history:
Received 21 July 2014
Accepted 16 December 2014
Available online 2 February 2015

Keywords:
Age/ageing
Biomimetic material
Bone regeneration
Liposome
Osteogenesis
Spinal surgery

ABSTRACT

Autologous bone grafting represents the standard of care for treating bone defects but this biomaterial is unreliable in older patients. The efficacy of an autograft can be traced back to multipotent stem cells residing within the bone graft. Aging attenuates the viability and function of these stem cells, leading to inconsistent rates of bony union. We show that age-related changes in autograft efficacy are caused by a loss in endogenous Wnt signaling. Blocking this endogenous Wnt signal using Dkk1 abrogates autograft efficacy whereas providing a Wnt signal in the form of liposome-reconstituted WNT3A protein (L-WNT3A) restores bone forming potential to autografts from aged animals. The bioengineered autograft exhibits significantly better survival in the hosting site. Mesenchymal and skeletal stem cell populations in the autograft are activated by L-WNT3A and mitotic activity and osteogenic differentiation are significantly enhanced. In a spinal fusion model, aged autografts treated with L-WNT3A demonstrate superior bone forming capacity compared to the standard of care. Thus, a brief incubation in L-WNT3A reliably improves autologous bone grafting efficacy, which has the potential to significantly improve patient care in the elderly.

1. Introduction

It is generally accepted that as we age, healing potential diminishes. This is especially obvious in the skeleton: compared to adolescent or adult skeletons, the geriatric skeleton is usually osteoporotic [1–3] and co-morbidities such as decreased vascularization, poor metabolism, and accumulated DNA damage contribute to slow bone healing in the elderly. Consequently, there is an increasing demand for biomaterials that take age-related skeletal changes into consideration.

The most common treatment for bony non-unions and delayed unions is autologous bone grafting, or autografting. Autografts are a heterogenous collection of marrow blood products, connective tissue stroma, bony extracellular matrix, and a variety of hematopoietic, vascular, and osteogenic stem cell populations [4–7]. The physical, biological, and chemical composition of autografts makes them an ideal bone-regenerating biomaterial in young patients [8,9]; in older individuals, however, autografts are unpredictable [10–12].

A number of bone graft substitutes have been developed to address this need [13]. For example, synthetic scaffolds such as ceramics (e.g., tricalcium phosphate, hydroxyapatite) and bioactive glass (silica and calcium oxide) have been fabricated to resemble the micro-porosity and compressive strength of bone [14,15]. While these synthetic materials are generally considered biocompatible, they exhibit no inherent osteogenic activity [16] and cannot adapt to changing physiologic conditions [17]. Cadaveric demineralized bone matrix (DBM) can replace the mineralized component of an autograft [18] and while DBM appears to support osteogenesis [19,20] the material is devoid of viable cells and disease transmission remains a concern [21]. Other engineered bone substitutes include allogeneic stem cell products [22] but whether they are

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http://dx.doi.org/10.1016/j.biomaterials.2014.12.014
0142-9612/

osteogenic still remains a matter of considerable debate [23,24]. None of these bone graft substitutes take into account the changing skeletal properties of the aging patient.

If we understood why autografts fail in older patients we might be in a position to improve this standard of care for bone regeneration. We know that the physical properties of autografts change with age: the marrow undergoes fatty degeneration [25] and the mineralized extracellular matrix component of an autograft is significantly reduced because of osteoporotic changes [22]. Aging also impacts the chemical properties of autografts: aged stem cells are less responsive to the growth factor stimuli in their environments [26,27] and accumulating evidence indicates that both local and systemic levels of growth factor stimuli decline in the elderly (reviewed in Ref. [28]).

Here, we tested the hypotheses that the osteogenic potential of an autograft is attributable to stem cells in the graft material, and that aging impacts the Wnt responsive status of these stem/progenitor populations. We focused on the role of Wnt signaling in this regard because the pathway is widely recognized as a key regulator of bone mass [29–31]. Experimental and clinical evidence both indicate that elevated Wnt signaling induces bone formation [32–34] whereas reduced Wnt signaling induces bone loss [35,36].

We posited that a reduction in Wnt signaling might be responsible for the loss in osteogenic potential of autografts. To counteract the age-related decline in Wnt signaling we supplied a chemical stimulus in the form of a Wnt protein to autografts from aged animals. In previous work we cataloged the distribution of canonical and non-canonical Wnt ligands in the intact and injured skeleton, and this analysis revealed that Wnt3a was most broadly expressed [37]. Further, the expression level of Wnt3a was the most severely affected by aging [38]. Consequently, our study here focused on delivery of WNT3A to autografts from aged animals.

2. Methods

2.1. Animal care

The Stanford Committee on Animal Research approved all procedures. Beta-actin-enhanced green fluorescent protein (*ACTB-eGFP*), and CD1 syngeneic hosts, as well as *Axin2*$^{CreERT2/+}$ and *R26R*$^{mTmG/+}$ mice were used; the latter were purchased (The Jackson Laboratory, CA). Mice <3 months old were considered young; >10 months were considered aged. Aged Lewis rats ("retired breeders", Charles Rivers, MA), were used for spinal fusion surgeries.

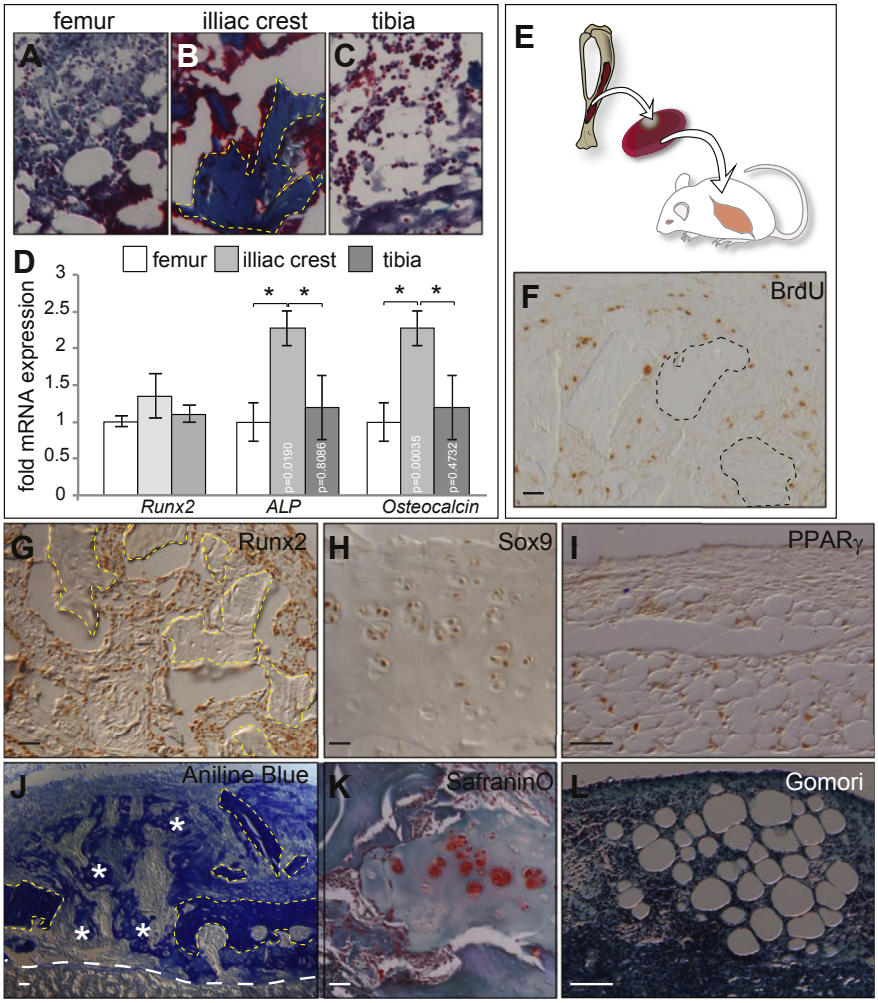

Fig. 1. Bone graft material contains stem and progenitor cell populations. (A) Gomori staining of BGM harvested from the rat femur, (B) the iliac crest (where trabecular bone chips are indicated with a dotted line), and (C) the tibia. (D) Quantitative RT-PCR analyses of endogenous osteogenic gene expression in BGM from the indicated sources. (E) Schematic of the experimental design, where autologous BGM is harvested from the iliac crest of a rat and transplanted into the SRC. On post-transplant day 7 (F) representative tissue sections were stained to detect BrdU, (G) Runx2, (H) Sox9, and (I) PPARγ expression. (J) Representative tissue sections were also stained with Aniline blue to detect osteoid matrix; asterisks indicate new bone matrix as opposed to old bone chips (dotted lines). The surface of the renal capsule is indicated with a dashed white line. (K) Representative tissue sections were stained with Safranin O/Fast green histology to detect proteoglycan-rich cartilage (red), and (L) Gomori trichrome staining to detect adipocytes. Abbreviations: BrdU, bromodeoxyuridine. Scale bars: 50 μm, asterisks: $p < 0.05$.