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Short communication

A case of mandible hypoplasia treated with autologous bone graft from mandibular symphysis: Expression of VEGF and receptors in bone regeneration

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ABSTRACT

The vascular endothelial growth factor (VEGF)/VEGF receptor (VEGFR) system plays an important role in angiogenesis and osteogenesis during both skeletal development and postnatal bone growth and repair. Indeed, protein expression changes of this system could contribute to craniofacial defects commonly associated with a variety of congenital syndromes. Similarly to other craniofacial bones, mandible arises from neural crest cells of the neuroectodermal germ layer, and undergoes membranous ossification. Here, we report a case of left mandibular hypoplasia in a 42-year-old man treated with autologous bone graft from mandibular symphysis. After 3 months from surgical reconstruction, the protein expression of VEGF and receptors (VEGFR-1, -2 and -3) in regenerated bone tissue was evaluated by immunohistochemistry. At variance with the mandibular symphysis bone harvested for graft surgery, we observed *de novo* expression of VEGF and VEGFRs in osteoblasts and osteocytes from post-graft regenerating mandible bone tissue. In particular, while VEGFR-1 and VEGFR-3 immunopositivity was widespread in osteoblasts, that of VEGFR-2 was scattered. Among the three receptors, VEGFR-3 was the more intensively expressed both in osteoblasts and osteocytes. These findings suggest that VEGFR-2 might be produced during the early period of regeneration, while VEGFR-1 might participate in bone cell maintenance during the middle or late consolidation period. VEGFR-3 might, instead, represent a specific signal for ectomesenchymal lineage differentiation during bone regeneration. Modulation of VEGF/VEGFR signaling could contribute to graft integration and new bone formation during mandibular regeneration.

1. Introduction

The role of growth factors in bone repair and regeneration in response to injury or surgical treatment is widely recognized. Both growth factors embedded within the bone matrix and those secreted by bone and reactive cells contribute to bone reparative processes. A main factor acting on the skeleton is vascular endothelial growth factor (VEGF) (Devescovi et al., 2008). VEGF, which binds to target cell receptors, has a primary regulatory role not only in physiological angiogenesis, but also in osteogenesis during both development and postnatal life, participating in bone tissue formation, remodeling and healing processes (Zelzer and Olsen, 2005; Koerdt et al., 2014). Focusing on the maxillofacial area, the VEGF/VEGF receptor (VEGFR) system plays an important role especially in the mandible, where the dynamic interplay between the vasculature and bone has an initiating and regulatory function in osteosynthesis (Filvaroff, 2003; Zelzer and Olsen, 2005; Marini et al., 2012). In particular, neural crest cell-derived VEGF ensures adequate vessel growth and arterial stability within the mandible, and during mandibular development vessel-derived factors enable appropriate levels of chondrocyte proliferation and morphogenesis of Meckel's cartilage, as essential prerequisites for normal mandible extension (Wiszniak et al., 2015). Of note, an altered expression of VEGF provides insights into the etiology of a variety of craniofacial birth defects commonly associated with mandibular hypoplasia (Wiszniak et al., 2015).

To date, an autologous graft is considered the gold standard for bone regeneration (Du et al., 2015). Indeed, autologous bone grafts are commonly used for osteoconductive, osteoinductive, and osteogenic properties (Taşdemir et al., 2015). In this context, VEGF appears fundamental in bone reparative processes following graft insertion surgery (Rodella et al., 2010). In particular, autologous

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bone graft is widely used in oral and maxillofacial surgery to repair small and medium size defects and represents an auxiliary medical therapy for endosseous implants (Rodella et al., 2010). The embryologic origin of bone is another critical issue to consider in bone grafting. In fact, findings from different animal models and human clinical studies have clearly shown that membranous bone grafts (*i.e.* cranial bone) are preferable to endochondral bone grafts (*i.e.* iliac crest bone) because of less resorption over time (Hallman and Thor, 2008). For instance, in human maxillary sinus augmentation with autologous bone grafting, an elevated expression of VEGF was observed in the newly formed bone (Boëck-Neto et al., 2009). Moreover, several experimental data suggest that the expression pattern of VEGF/VEGFRs plays an important role during mandibular distraction osteogenesis, in particular through enhancing the production of VEGF (Bouletreau et al., 2002; Hu et al., 2003; Pacicca et al., 2003; Byun et al., 2007). Similar findings were reported in a study on human distracted mandible (Knabe et al., 2005).

In this context, this paper describes a case of mandibular hypoplasia treated with autologous bone graft harvested from the mandibular symphysis. The surgical procedure and the morphological features of post-graft regenerating mandible bone tissue are presented. In particular, we focused on possible differences in the immunohistochemical expression and localization of VEGF and VEGFRs between the harvested mandibular symphysis tissue and the regenerating bone collected 3 months after graft insertion surgery.

2. Materials and methods

2.1. Clinical case report

A 42-year-old man with atrophic left posterior mandible was referred to the Oral and Maxillofacial Surgery Service at the Careggi Hospital, Florence, Italy, for mandible reconstruction. The patient was subjected to general anesthesia in nasotracheal intubation. Intraoral and extraoral tissues were disinfected with 10% Iodopovidone (Betadine, Purdue Pharma LP, Stamford, CT, USA). The mandibular surgical sites were infiltrated with local anesthetic with vasoconstrictor (40 mg/ml D-Ultracain plus 5 mg/ml Suprarenin; Hoechst, Frankfurt am Main, Germany). The lateral wall of the mandible was exposed by continue crestal incision and a vertical incision in the vestibular fornix. After elevation of a mucoperiosteal flap, a hole was performed, with a bur, in the external cortical plate of the mandible to obtain bleeding. The bone graft was withdrawn from mandibular symphysis (Fig. 1A) and then fixed with screws on the receiving site (Fig. 1B). Finally, the flap was sutured without tension to prevent dehiscence using multiple points in horizontal mattress sutures with 3-0 absorbable thread (Vicryl Rapid, Ethicon, Johnson & Johnson, Roma, Italy). After 3 months from surgery, the patient was referred to removal of screws followed by fixed implant-prosthetic rehabilitation. During this procedure, mandibular tissue fragments from the regeneration area at the receiving site were collected and fixed in 4% buffered formalin solution, routinely processed and embedded in paraffin for morphological examination (hematoxylin-eosin staining) and immunohistochemical studies of VEGF, VEGFR-1, -2 and -3. As control tissue, mandibular symphysis bone fragments collected at the time of grafting were used. Written informed consent was obtained according to recommendations of the Local Ethics Committee (Marini et al., 2012).

2.2. Immunohistochemistry

According to previously published protocols (Marini et al., 2012; Marini et al., 2015), a standard immunohistochemical procedure for protein expression was performed on paraffin-embedded sections (5 μm thick) using the following primary antibodies: mouse monoclonal anti-human VEGF (1:80 dilution; clone C-1, catalog no. sc-7269, Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit polyclonal anti-human VEGFR-1 (1:80 dilution; clone H-225, catalog no. sc-9029, Santa Cruz Biotechnology), mouse monoclonal anti-human VEGFR-2 (1:80 dilution; clone A-3, catalog no. sc-6251, Santa Cruz Biotechnology), and rabbit polyclonal anti-human VEGFR-3 (1:100 dilution; clone C-20, catalog no. sc-321, Santa Cruz Biotechnology). Antibody specificity was controlled either by omitting the primary antibodies or by pre-absorption of the primary antibodies with the specific recombinant human protein (for VEGF) or specific peptides (for VEGFRs) at room temperature for 1 h, as described elsewhere (Marini et al., 2012; Marini et al., 2015). The slides were observed under a Leica DM4000B microscope (Leica Microsystems, Mannheim, Germany) and photographed using a Leica DFC310 FX 1.4-megapixel digital colour camera equipped with the Leica software application suite LAS V3.8 (Leica Microsystems). The immunostaining intensity was scored semiquantitatively as +++, ++, +, ± or − for strong, moderate, weak, very weak and negative staining, respectively.

3. Results

Histological examination of mandibular bone specimens collected 3 months after graft insertion surgery showed newly formed bone tissue with lamellar arrangements (Fig. 1C–F). Fibrous tissue was present between bone structures (Fig. 1C–F), and inflammatory infiltrate, represented by a few neutrophil granulocytes, was negligible (Fig. 1E, inset). Developing Haversian canals were also observed (Fig. 1F).

Immunohistochemical expression of VEGF and VEGFRs is shown in Fig. 2. In control mandibular symphysis tissue fragments harvested for graft surgery, VEGF, VEGFR-1 and VEGFR-2 were weakly expressed in fibroblast-like cells, whereas no immunostaining was detected in osteoblasts and osteocytes (Fig. 2A–C). A similar expression pattern was observed even for VEGFR-3 in osteoblasts and fibroblast-like cells, except for a weak and scattered immunopositivity in osteocytes (Fig. 2D). In regenerating mandibular tissue collected 3 months after graft insertion surgery, strong expression of VEGF was observed in osteoblasts lining the bone tissue structure surface and in fibroblast-like cells within the fibrous tissue, while weaker immunostaining was detected in osteocytes (Fig. 2E). The distribution of VEGFR-1, VEGFR-2 and VEGFR-3 appeared like that of VEGF (Fig. 2F–H). However, VEGFR-2 positivity was scattered in osteoblasts (Fig. 2G), while more intense VEGFR-3 signal was detected both in osteoblasts and osteocytes (Fig. 2H).

Results of semiquantitative analysis of the expression of VEGF and VEGFRs in the cellular components of control and post-graft regenerating mandibular bone tissue are summarized in Fig. 2I.

4. Discussion

Autologous grafts are the gold standard in bone reconstruction and, in particular, for the correction of mandibular bone defects (Çakır-Özkan et al., 2011). Quality of the reconstruction is an essential prerequisite to restore both the normal mandible function (*i.e.* sufficient alveolar thickness for dental implants, normal occlusion and mastication) and facial esthetics, thus improving the quality of life. A thorough knowledge of factors implied in skeletogenesis could help in understanding bone remodeling mechanisms after mandibular graft. Among multiple angiogenic and osteogenic factors involved during the multi-step process of bone development and repair (Kanczler and Oreffo, 2008), VEGF and VEGFRs play a pivotal role (Marini et al., 2012; Marini et al., 2015).