A MURINE MODEL FOR NON-STABILIZED MANDIBULAR FRACTURE REPAIR

Introduction: A majority of the knowledge of bone repair results from the analyses of long bone fracture models. During adult long bone healing, mesenchymal cells from the surrounding tissues enter the wound site, where they proliferate, condense and differentiate into cartilage or bone in response to cytokines and growth factors. This process is influenced by the mechanical environment, where motion at the fracture site results in a cartilage callus and stability is associated with direct bone formation (1). Although advances have been made in understanding the cellular and molecular mechanisms of repair in the appendicular skeleton, a similar understanding of fracture repair in the cranial skeleton is lacking. One hypothesis is that given the differing embryonic origins of the cells that form the long bones (mesoderm) and the jaw bone (neural crest), the mechanisms of repair of these skeletal elements may be distinct. As a first step toward testing this hypothesis, we developed a mouse model of non-stabilized mandibular fracture healing and evaluated it using histologic and molecular techniques.

Methods and materials: Surgical procedure: All protocols were approved by the Institutional Committee on Animal Research. Twenty-one adult mice between the ages of 10-12 weeks were anesthetized by intraperitoneal injection of 2.5% Avertin. After shaving, an incision was made over the inferior portion of the right posterior mandible and the masster muscle was divided along its length and elevated. The posterior aspect of the mandible was exposed and a high-speed dental drill fitted with a 0.2mm diamond disc was used to create a complete transverse osteotomy just proximal to the 3rd molar (Fig. 1). The soft tissues and skin were then closed with non-absorbable suture. Following surgery, mice were given a ground diet and allowed to eat, which typically occurred within 24 hours. Their ability to consume food and water, surgical site, activity, weight, appearance, and behavior were monitored closely. Molecular and cellular analyses: The mice were allowed to heal and were sacrificed at time points corresponding with the inflammatory (3 and 5 days), soft-callus (7 and 10 days), hard callus (14 days), and remodeling (21 and 28 days) phases of healing. The skin was removed and the head was harvested and prepared for molecular and cellular analyses as previously described (2). Tissues were prepared for histology and in situ hybridization. 5'-labelled sense and antisense UTP-labeled riboprobes were synthesized from plasmids corresponding to osteocalcin (oc), collagen type II (col2) and collagen type X (col10). The histologic staining (Safranin-O, SFO and Hall Brunt Quadruple, HBQ) was performed following standard protocols.

Results: All fractures exhibit significant displacement of the bone ends, indicating instability (Fig. 2). By day 7, which corresponds to the soft callus phase of fracture healing, histologic (SFO and HBQ staining) and in situ hybridization data (oc, a molecular marker for osteogenic cells) demonstrate that new bone has begun to form at both proximal and distal bone ends, particularly along the periosteal aspects of the bone. Col2, a marker of chondrogenesis, is not detectable within any region of the stabilized fracture site. Later in the soft callus stage of fracture repair (day 10), SFO and HBQ staining and oc expression data show continued new bone formation at the fracture site. At the same time, col2 expression continues to be undetectable at the site of injury. By d14, which corresponds to the hard callus phase of healing, bone continues to form, but cartilage is detectable in limited aspects of the fracture callus as confirmed by histologic staining (Figure 2, box). Small col2-expressing cell domains appear between the bone segments, revealing the presence of immature chondrocytes, and large oc-expressing domains are expressed throughout the calluses (Fig. 2, arrows). By d21, regenerated bone bridges the fracture segments in the majority of the fractures with little remaining cartilage (Fig. 2, box). At this time, col2 expression is minimally detectable, whereas oc transcripts are abundant (Fig. 2, arrows). By day 28, the fractures exhibit bone healing.

Discussion: To begin to study membranous bone repair, we developed a mouse model of non-stabilized mandibular fracture healing. The bone is regenerated by intramembranous ossification during the early stages of fracture repair. Cartilage is not present until approximately two weeks post-fracture and healing is usually complete by one month post-fracture. The mouse has several distinct advantages over existing fracture models in other animals. Although creation of the fracture is technically more demanding, this model provides for improved molecular and cellular analyses. The clearest advantage is the ability to study fracture repair in genetically manipulated animals. Future studies will use this murine model to explore the cellular and molecular processes that govern mandibular fracture repair and the effects of the local mechanical environment on membranous bone healing.

References:

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