INTRODUCTION

Craniosynostosis refers to premature ossification of cranial sutures, and occurs in approximately one of every 2,000 live human births. Children with craniosynostosis may suffer from craniofacial deformities, seizure and mental retardation. The primary treatment for craniosynostosis is surgical craniotomy, with a major goal to relieve abnormally high intracranial pressure. Craniotomy is a traumatic surgery, involving the reshaping of skull bones and removal of synostosed bone of empirical size (Mooney et al., 2004). Surgically corrected craniosynostosis may re-fuse, necessitating secondary surgeries. We have recently tissue-engineered a cranial suture-like structure and utilized TGFβ3 to modulate synostosed cranial sutures (Hong and Mao, 2004; Moioli et al., 2006), in consistency with the observation that TGFβ3 modulates the fate of both natural and synotosed cranial sutures (Opperman et al., 2002). TGFβ3 attenuates not only the osteogenic differentiation of bone marrow derived osteoblasts, but also osteoblastic matrix synthesis (Moioli et al., 2006). However, little is known whether other cytokines also regulate sutural morphogenesis. This is significant in that multiple growth factors likely co-regulate ossification or patency of cranial sutures. The purpose of this study is to modulate cranial suture morphogenesis ex vivo using connective tissue growth factor (CTGF).

METHODS

All animal procedure was approved by IACUC. Calvaria including the interfrontal, coronal, and sagittal sutures and their underlying dural tissues were harvested from post-natal day 10 (P10) male Sprague-Dawely rats (Harlan, Indianapolis, IN). The isolated calvaria were placed into the standard 12-well tissue culture plates and covered with 1 ml serum-free medium (Opperman et al., 1995). Medium was changed every 2 days and supplemented daily with 0 and 50 ng/ml recombinant human CTGF (BioVendr, Candler, NC) and 100 µg/ml ascorbic acid. 5 to 25 days following CTGF treatment, tenascin-C (Tn-C) contents, a marker for fibroblastic differentiation, were assayed using a commercially available ELISA kit (IBL-America, Minneapolis, MN). All calvaria cultured for 25 days were harvested and prepared for histological observation with H&E staining. Suture structures were reconstructed in 3D from scanned images by microCT (vivaCT 40; Scanco Inc., Southeastern, PA). The thickness of each IF suture was measured from the 3D reconstructed models. Conventional histology was performed to observe sutural morphology. All numerical data were compared by Student T tests at an alpha level of 0.05.

RESULTS

By 25 days in organ culture, exposure of 50 ng/ml recombinant human CTGF to rat suture explants induced significant increases in Tn-C contents in comparison with sutures cultured without CTGF (Fig. 1, p<0.05). Histological observations revealed marked suture fibrous tissue interface between mineralized bones in the representative IF suture upon treatment of 50 ng/ml CTGF for 25 days (Fig. 2A), in comparison with narrowed sutures without CTGF treatment (Fig. 2). Consistently, sutures in the presence of CTGF were maintained at a patent while sutures cultured without CTGF were narrowed as compared to P10 suture (Fig. 2). In addition to, 3D reconstructed calvarial structures from µCT scanned images showed marked sutural interface between two bones in the representative IF suture (Fig. 3A), in comparison with those with CTGF treatment (Fig. 3B). This was confirmed quantitatively by significantly wider IF sutures at 0.29±0.07 mm treated with 50 ng/ml CTGF than those without CTGF at 0.09±0.07 mm (data not shown).

DISCUSSION

The present findings demonstrate that CTGF delivery delayed the ossification of the interfrontal suture in organ culture. We previously showed that CTGF promotes the fibroblastic differentiation of human bone marrow derived mesenchymal stem cells (Lee et al., 2006). Taken together, CTGF may delay sutural ossification by stimulating fibroblastic differentiation in suture mesenchyme. The present observation that CTGF delays ossification may have implications in the treatment of craniosynostosis, and fine-tuning osteogenesis in the tissue engineering of ligaments, tendons, and periodontal ligament.

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REFERENCES