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Transcriptional analysis of human cranial compartments with different embryonic origins

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ABSTRACT

Objective: Previous investigations suggest that the embryonic origins of the calvarial tissues (neural crest or mesoderm) may account for the molecular mechanisms underlying sutural development. The aim of this study was to evaluate the differences in the gene expression of human cranial tissues and assess the presence of an expression signature reflecting their embryonic origins.

Methods: Using microarray technology, we investigated global gene expression of cells from the frontal and parietal bones and the metopic and sagittal intrasutural mesenchyme (ISM) of four human foetal calvaria. qRT-PCR of a selected group of genes was done to validate the microarray analysis. Paired comparison and correlation analyses were performed on microarray results.

Results: Of six paired comparisons, frontal and parietal compartments (distinct tissue types of calvaria, either bone or intrasutural mesenchyme) had the most different gene expression profiles despite being composed of the same tissue type (bone). Correlation analysis revealed two distinct gene expression profiles that separate frontal and metopic compartments from parietal and sagittal compartments. TFAP2A, TFAP2B, ICAM1, SULF1, TNC and FOXF2 were among differentially expressed genes.

Conclusion: Transcriptional profiles of two groups of tissues, frontal and metopic compartments vs. parietal and sagittal compartments, suggest differences in proliferation, differentiation and extracellular matrix production. Our data suggest that in the second trimester

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of human foetal development, a gene expression signature of neural crest origin still exists in frontal and metopic compartments while gene expression of parietal and sagittal compartments is more similar to mesoderm.

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1. Introduction

Calvarial bones are formed by intramembranous ossification and are divided by mesenchymal tissues called sutures. The human calvaria has four major sutures (metopic, coronal, sagittal, and lambdoid). The presence of unossified sutures facilitates foetal movement through the birth canal and functions as a growth centre to allow for brain growth.[1] Osteogenesis takes place at the osteogenic front, the leading edge of each bone.[2]

One important disorder of the cranial vault is craniosynostosis, the premature fusion of the sutures. Craniosynostosis occurs in 3–5 out of 10,000 live births and can cause malformations of the skull, increased intracranial pressure, and developmental delay.[3] Many studies have characterized the genetic and environmental aetiology of craniosynostosis,[4–6] but for the majority of cases the underlying molecular pathways are unclear. In order to understand these pathways, the basis of normal sutural development must be defined. The anatomic and developmental differences between the sutures suggest that distinct molecular mechanisms are controlling morphogenesis. These differences include a suture specific prevalence of synostosis[2]; predominance of coronal fusion in hereditary synostosis[7,8]; anatomic architecture (sutures with blunt vs. overlapping margins)[2]; the timing of physiologic fusion[9,10]; and distinct embryonic origins.[11–13]

The embryonic origins of the cranial compartments (bones of the calvaria and their intervening sutures), were not well understood until Jiang et al. used a Wnt1-Cre-recombinase LacZ reporter mouse model to identify craniofacial structures with neural crest (NC) origin. They demonstrated that in embryonic day 17.5 (E17.5) mice the frontal bone, the posterior frontal suture (equivalent to the metopic suture in humans), the sagittal suture, and the central portion of the interparietal bone were derived from NC cells while the parietal bone and coronal suture were of paraxial mesoderm origin.[12] This was later substantiated by Yoshida et al. who used the same strategy in Wnt1-Cre and Msp1-Cre mice to map cells of neural crest and paraxial mesoderm origin.[13] On the other hand, while supporting Jiang's studies regarding the origins of frontal and parietal bones and posterior frontal suture, Gagan et al. found that the sagittal sutures of neonatal day 1 (N1) Wnt1-Cre-recombinase LacZ reporter mice were of mesodermal origin. Additionally, their data suggests that on N10 cells in dura mater, which originate entirely from NC, migrate into the sagittal intrasutural mechencyme (ISM).[11] Deckelbaum et al. studied fate mapping using Wnt1, En1 and Gli1expression and demonstrated the complicated nature of cell mapping in the border of NC and mesoderm derived tissues due to cell mingling in some regions.[14] Taken together, there is agreement on the embryonic origin of frontal and parietal bones and metopic suture in mice but the embryonic origin of the sagittal suture remains unclear.

Based on the targeted expression and cell-level studies in the biology and pathology of cranial sutures, it is clear that developmental origins play an important role in the interactions of the adjacent tissues.[15–18] While these findings are enlightening, none of them investigate gene expression in human tissues. On the other hand, broad understanding of molecular pathways requires investigation of large array of genes. Recently, high throughput gene expression analysis (microarray analysis) has been used in investigating craniosynostosisaetiology[19,20] but not in studying human calvarial development. Therefore, we aimed to investigate the global gene expression profile of human calvarial compartments.

2. Materials and methods

2.1. Ethics statement

Samples used in this study were obtained from the Department of Pathology and the Birth Defects Research Laboratory at the University of Washington. The study participants (mothers) signed an informed consent. All procedures were approved by the University of Washington and Washington State University institutional review boards.

2.2. Study design and samples

Tissues samples were obtained from foetal crania of four normal human foetuses. We received two females ages 94 and 103 days and two males ages 97 and 98 days. Tissues were transported and cultured in Dulbecco's Modified Eagle Medium (DMEM) GlutaMAX (Life Technologies, Grand Island, NY) containing foetal calf serum (Life Technologies, Grand Island, NY) and antibiotic–antimycotic supplement (Life Technologies, Grand Island, NY) containing penicillin, streptomycin and amphotericin B.

2.3. Cell expansion

All dural and extracranial soft tissues were removed from the parietal and frontal bones. 2–3 mm tissue explants were excised from each compartment, frontal and parietal bones and metopic and sagittal ISM. To avoid contamination with osteoblasts, ISM tissue was dissected from the central portion of each suture. Similarly, bone was harvested at least 3 mm from the margin of the suture to avoid contamination with ISM. Media was changed every 3–4 days. After reaching 75–80% confluence, the cells were trypsinized with TrypLEExpress (Life Technologies, Denmark) and passaged. During the fourth and final passage, 180,000 cells were plated in triplicate

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