Introduction

• Skull development is a tightly regulated process that occurs along the osteogenic interfaces of the cranial sutures that allow rapid bone formation at the edges of the bone fronts (Opperman, 2000, 2011).

• Premature closure of cranial sutures can result in pathological conditions such as Craniosynostosis.

The mesenchymal proteins Polycystin 1 (PC1) and 2 (PC2) regulate skeletal development and potentially suture formation.

• Polycystin-1 (PC1), 420 kDa) sparsely covers the cell membrane, has a large extracellular domain and mediates mechanosensory signal together with Polycystin 2 (PC2, 120kDa).

• PCs play a central role in cellular mechanosensation and mechanotransduction processes (Dalagiorgou G et al. 2013).

• PC1 and PC2 was expressed in hPDL cells subjected to mechanical stretch for various time points (Dalagiorgou G et al., 2013).

• PC1 modulates osteoblastic gene transcription and bone cell differentiation through the calcitonin receptor-like receptor/NEAT signaling pathway (Dalagiorgou G et al., 2013).

• Mice subjected to midpalatal suture expansion in vivo, demonstrated that midpalatal force promoted cartilage formation (Woo B et al., 2007).

• PC1-deficient mice present restricted growth effects at the skull base and in cranialfacial sutures, without however knowledge of the underlying molecular mechanisms (Kolpakova-Hart E. et al., 2006).

Aim of research

To investigate the role of PC1/PC2 in suture development and suture fusion.

Methods

A. Western blotting: A1) Whole suture sagittal (SAG) bone tissue lysates were blocked and incubated with primary rabbit polyclonal antibodies for PC1 and PC2. B1) Similar procedures were followed for human samples.

B1. Primary Sagittal Suture cultures: Suture derived mesenchymal cells were harvested from 9-day-old Sprague Dawley rat:

1. SAG sutures with a bone margin on either side

2. Explants of SAG sutures were placed in 100-mm tissue culture dishes with the endocortical surface flush to the plate

3. Explants were then cultured in standard growth Medium. It was replenished every 2-days over the course of 1 week. tin cultures SAG-derived mesenchymal cells had migrated from tissue explants

4. At 7 days of primary culture, suture derived mesenchymal cells were passaged by trypsinization

B2. RT-PCR/PCR: RNA was extracted from primary SAG suture cells, post-natal day 9. Expression of PC1, SOX9 and RUNX2 was observed

C. Immunohistochemistry: Paraffin-embedded sections of SAG suture bone tissue from 0, 5, 15-day-old Sprague Dawley rats and H&E staining was used. Expression of PC1/PC2 was evaluated with primary rabbit polyclonal antibodies for PC1 and PC2.

Results

A1. Western Immunoblotting revealed a differential expression pattern for PC1 and PC2 in SAG sutures at p1/p5/p15 days

Activated form of PC1 (observed C-terminal) and PC2 levels were elevated at postnatal day 5

PC1, C-terminal: ~ 160 kDa

PC2 MW 120kDa

0 days: 160 kDa

15 days: 120, 160 kDa

A2. PC1 expression in human craniosynostosis samples was detected in the area of synostotic sutures

B1. In primary SAG cell cultures PC1 presence was associated with an elevated expression of the osteoblastic marker RUNX2 and a lower expression of the chondrocytic marker SOX9

C. Immunohistochemical analysis showed nuclear expression of PC1/PC2 in SAG sutures:

0 POST NATAL DAY Polycystin 1

0 POST NATAL DAY Polycystin 2

5 POST NATAL DAY Polycystin 1

5 POST NATAL DAY Polycystin 2

Conflict of Interest: None declared

References


• Zhang Q, Huen WS, Kipp S, Zhou J, Olsen BR, Tuhrim S, Kronenberg HM. Ongoing research will try to elucidate the potential cross-talk of PC1 signaling and Wnt-5c begin intracellular pathway in suture fusion processes: