P2-2

Comparison of Matrisome Between Mandible and Tibia Osteoblasts

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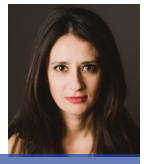


OVERVIEW

INTRODUCTION

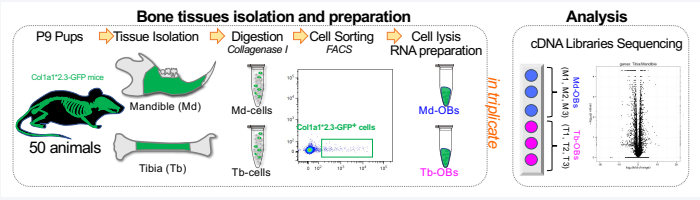
Local site-specific differences between bones from different anatomical regions may account for their different properties and functions (Wang 2020). Evidences that craniofacial bones differ from those forming the axial and appendicular parts of the skeleton come from the existence of skeletal diseases such as osteonecrosis of the jaws.

OBJECTIVE

To identify mechanisms behind these differences, we have performed a cross-study comparing RNA transcriptomes of in vivo mandible and tibia osteoblasts (OBs) on genes encoding extracellular matrix (ECM) proteins.

METHOD

Gene expression profiles were obtained from OBs isolated from mandible and tibia of P9 Col1a1*2.3-GFP mice using RNAseq sequencing technique. The transcripts were excluded from analysis if gene expression level was < 1. The transcripts within these groups were overexpressed if p≤0.05 and fold change (FC) ≥1.4 in mandible OB or tibia OB.



Nassif et al. JDR in press

RESULTS

1 ECM gene expression in OBs

→ A total of 614 OBs genes were expressed in the RNA-seq data.

ECM Proteins Category	Detected ECM Proteins in RNA-seq data	Expressed in RNA-seq data (RPKM ≥ 1)	Not Expressed in RNA-seq data
ECM Glycoproteins	213	159	54
Collagens	50	39	11
Proteoglycans	42	31	11
ECM-Affiliated Proteins	154	102	52
ECM Regulators	224	143	81
Secreted Factors	296	140	156
TOTAL OF PROTEINS	979	614	365

Table I. ECM-related gene expression (gene level expression ≥1) and not expression (gene level expression <1) in raw RNAseq data.

→ 34 transcripts were expressed only in the mandible and 10 transcripts were expressed only in the tibia.

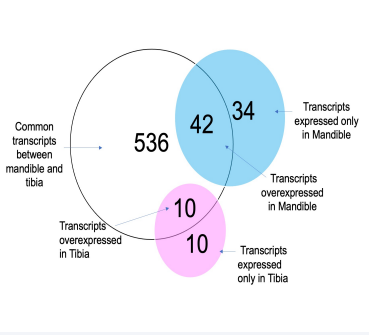


Figure 1. Venny diagram of transcripts expression increased in the Mandible and Tibia OBs.

2 The bone core: the common genes between mandible and tibia ECM

→ The common genes between mandible and tibia more expressed were Glycoproteins.

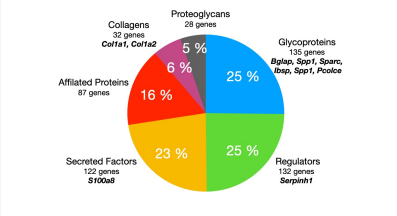


Figure 2. Common genes between mandible and tibia ECM with the top 10 genes more expressed.

→ The enrichment pathways in the bone core were related to development and wound healing.

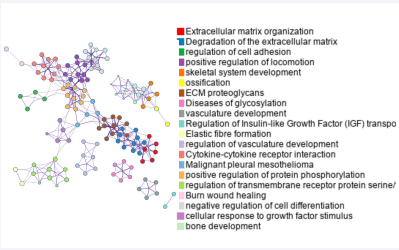


Figure 3. Network of enriched terms of the common genes between mandible and tibia ECM. Colored by cluster ID, where nodes that share the same cluster ID are typically close to each other.

3 Overexpressed genes: Mandible and Tibia sets

→ Overexpressed ECM profiles were site-specific

a) Mandible OBs **b) Tibia OBs**

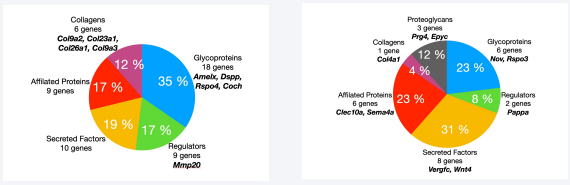


Figure 4. a) Genes expressed in Mandible; b) Genes expressed in Tibia with the top 10 genes more expressed in each bone type.

→ The enriched terms shows the associated pathways of overexpressed genes in mandible (figure 5), which are related to craniofacial disorders (Figure 6).

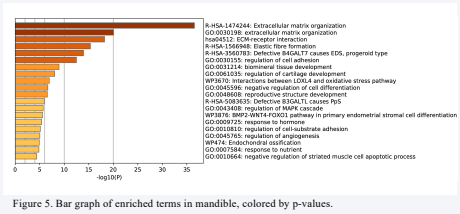


Figure 5. Bar graph of enriched terms in mandible, colored by p-values.

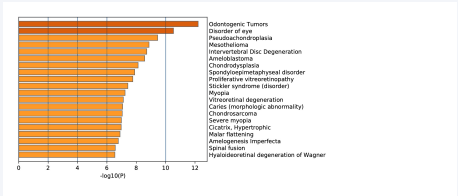


Figure 6. Summary of enrichment analysis in DisGeNET in mandible.

CONCLUSION

Our findings support significant differences in expression of genes encoding ECM proteins in mandible and tibia OBs. It strongly suggests functional differences in formation, resorption, and mechanical properties of these bones and may help us to understand the unique pathophysiology of jawbones. We are currently woking in validate the RNAseq data, the expression of ECM-encoding genes will be validated by RT-qPCR in raw bone tissue isolated from 9-week-old mice and further analyzed at various ages (preliminary results in Figure 7). Our following aim is to find the site-specifc matrisome signature of gingiva fibroblasts and to validate the protein expression with immunochemistry in mouse tissue and proteomics analysis in-vivo with human cells.

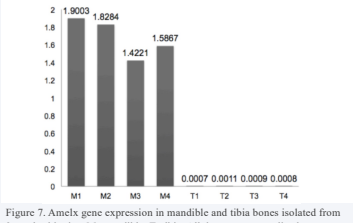


Figure 7. Amelx gene expression in mandible and tibia bones isolated from 9-week-old mice. M: mandible, T: tibia. All data were normalized to housekeeping genes Hprtrt and Tbp

REFERENCES

1. Nassif et al. Transcriptional regulation of jaw osteoblasts: development to pathology JDR in press.
2. Wang N, Niger C, Li N, Richards GO, Skerry TM. Cross-Species RNA-Seq Study Comparing Trasnscriptomes of Enriched Osteocyte. Populations in the Tibia and Skull. Front Endocrinol (Lausanne). 2020 Sep 24;11:581002.

FUNDING

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