Faculté de médecine dentaire

LSCTB
LABORATORY FOR THE STUDY OF CALCIFIED TISSUES & BIOMATERIALS

SCAN ME

Université de Montréal

The junctional epithelium protein Odontogenic ameloblast-associated affects cell behavior

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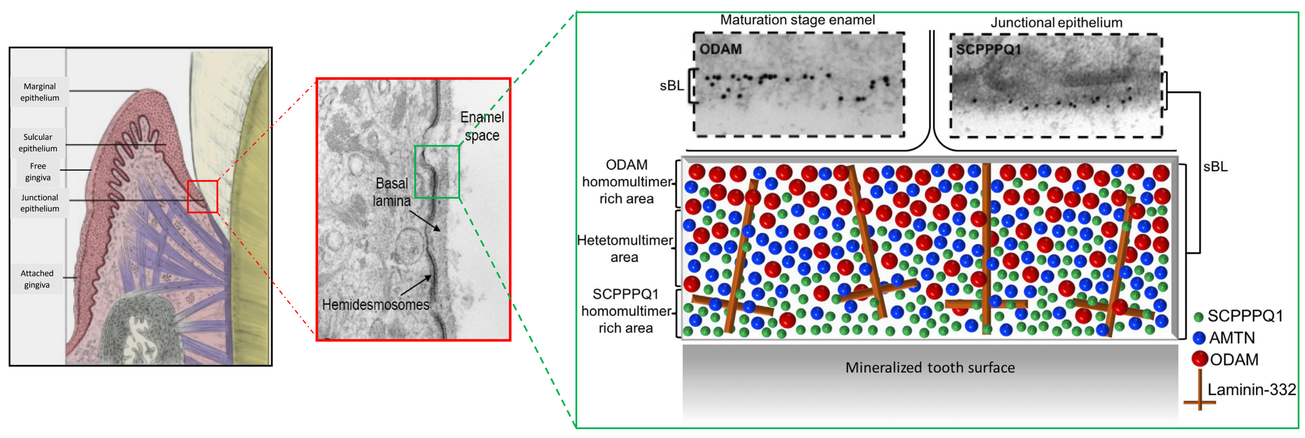
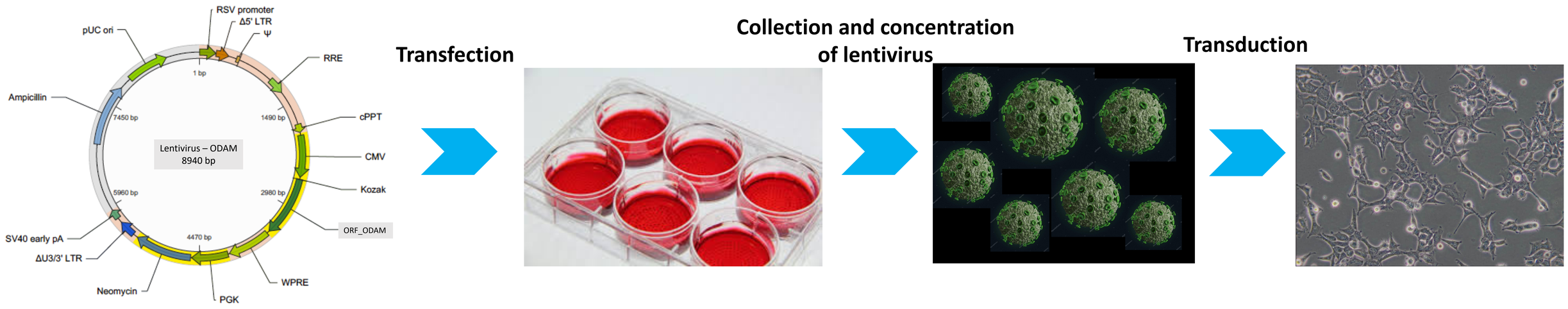
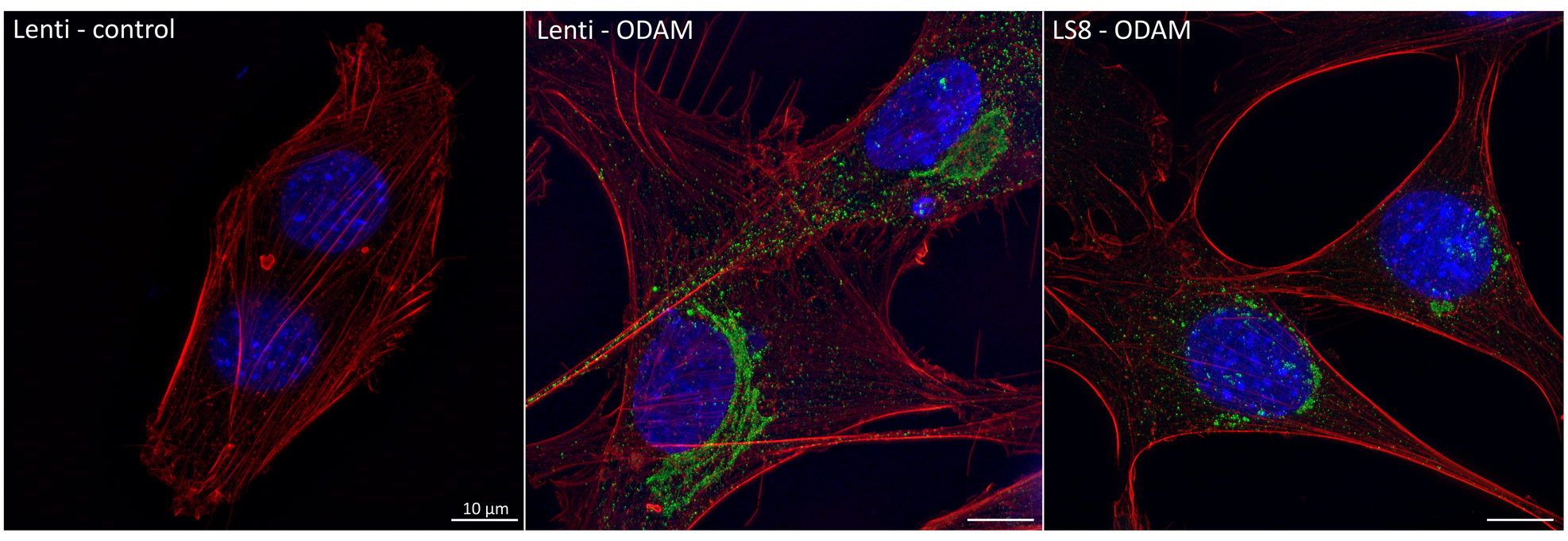
The junctional epithelium (JE), which initially derives from the enamel organ, produces a specialized basal lamina (sBL) that mediates its attachment to the tooth. This adhesive matrix is composed of four proteins. One of them, **Od**ontogenic **am**eloblast-associated (ODAM), stands out by being also continuously present among cells of the JE. However, the expression of ODAM is reduced in patients suffering from periodontal diseases (PD). This unique protein is also highly upregulated in epithelial neoplasia but is not found in healthy cells. The expression of ODAM in the incompletely differentiated cells of the JE and in dedifferentiated neoplastic cells has led to the hypothesis that ODAM influences cell status and behavior.



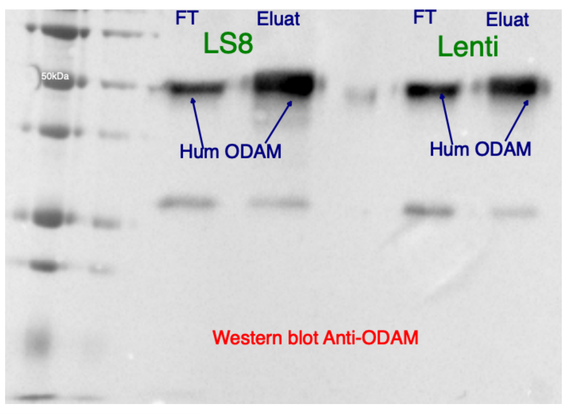
To test this hypothesis, we have expressed ODAM in lentiviral transducted HEK (lenti) and in LS8 ameloblast-related cells, and investigated its influence on cellular behavior by combining molecular and microscopic approaches.



Evaluation of protein production (Nucleus, ODAM, Actin)



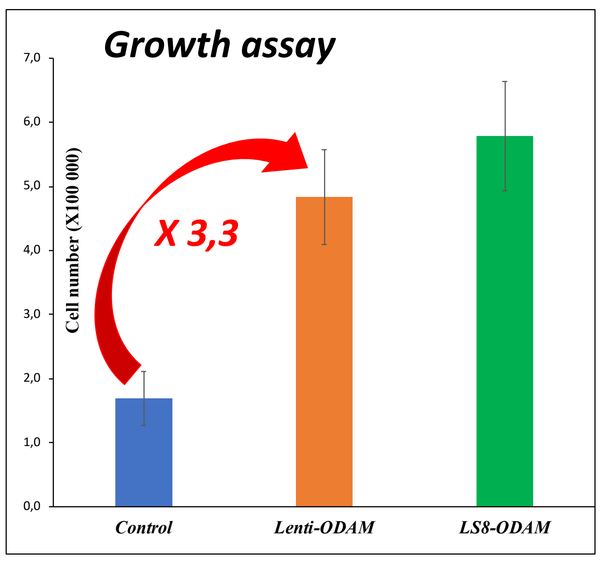
Western blot analysis to detect ODAM in LS8 or Lenti cells

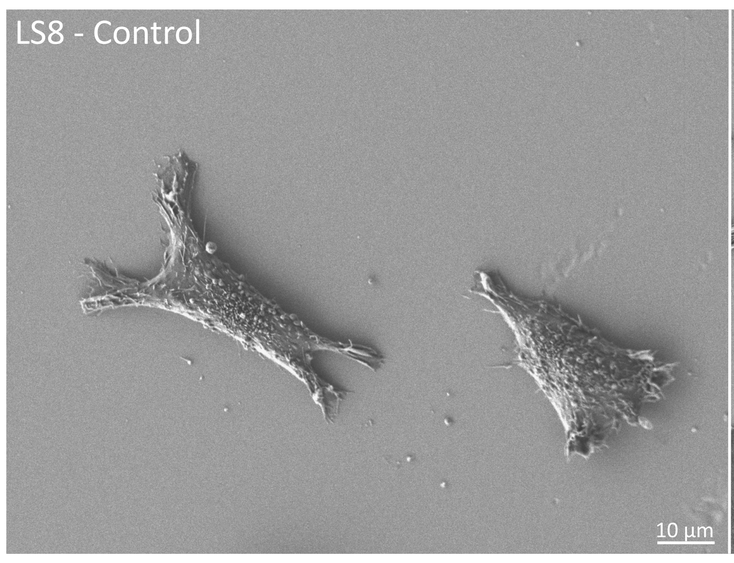


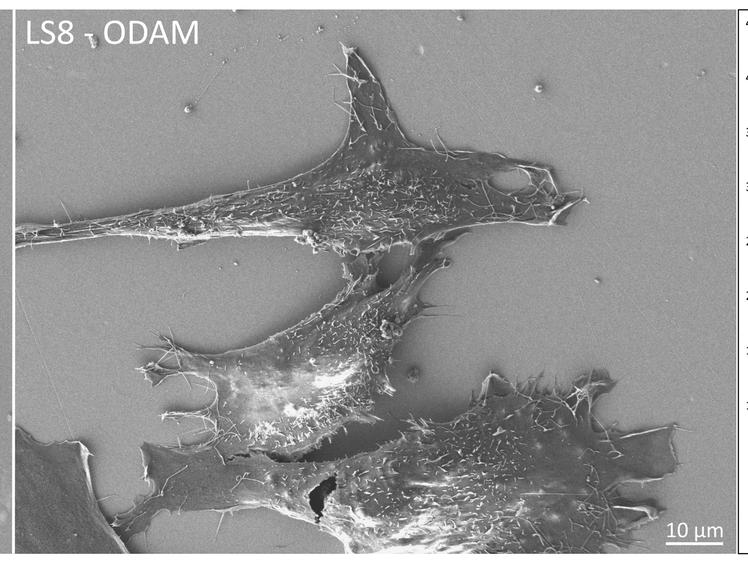
3D movie of the immunolabeling of ODAM

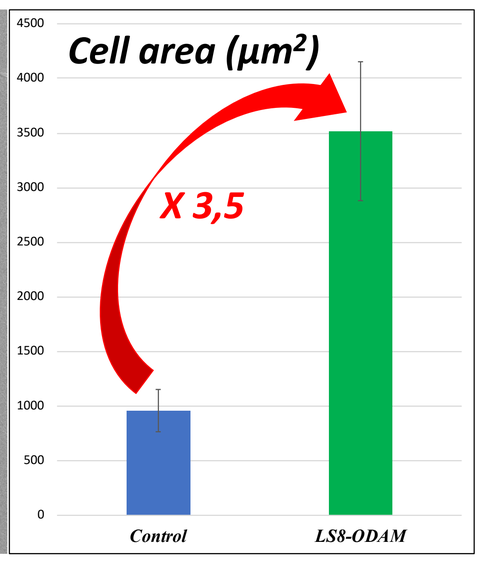
Western blot analysis and Immunofluorescence confirmed the successfully integration of the transgene and production of the protein in both cell types. Immunoluorescence additionally showed the localization of ODAM in the Golgi region. As assessed by cell counts, cells expressing ODAM divided ~3 times faster than control cells (non-infected and GFP-transducted). Both fluorescence and scanning electron microscopy revealed that cells were larger and generally more spread.

Evaluation of the behaviour of ODAM producing cell









RNA-Sequencing confirmed this cell behavior by highlighting the upregulation of genes implicated in **cell proliferation and differentiation pathways** (***e.g JAK/STAT3, MAPK***). Some genes related to **inflammation** were also upregulated (***e.g. NF-kB signaling***).

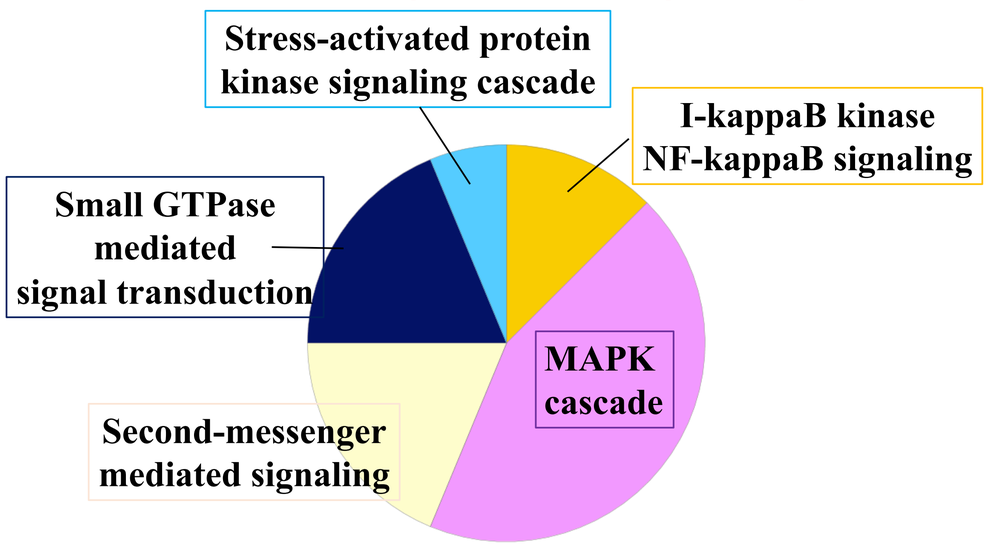
RNA Seq data treatment LS8 vs LS8-ODAM

55338 genes

- **153 upregulated**
- **1331 downregulated**

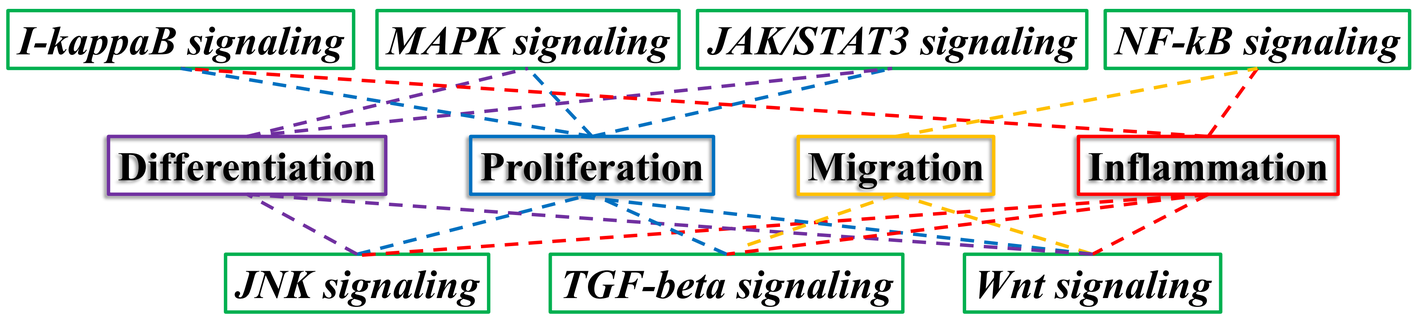
	log2FoldChange	pvalue	Gene
ENSRNOG00000023372-NM	10.1388511551037	5.08144633147113e-28	*Odam-rat*
ENSMUSG00000027656.7	2.93787094872597	8.25732612649346e-16	*Ccn5*
ENSMUSG00000006143.12	3.03959625101626	0.00446950574939687	*Upk3bl*
ENSMUSG00000018570.17	3.05295708561872	0.000326338972201319	*2810408A11Rik*
ENSMUSG00000033510.14	3.26141964427908	4.86402072650233e-06	*Otud7a*
ENSMUSG00000023039.17	3.50246251234712	5.53707288540622e-06	*Krt7*

PantherDB (data analysis – Pathways analysis)



GeneOntology (Data analysis)

Major pathways activated



<u>These data support the concept that</u> :

- ODAM plays a key role in JE specialization.
- The increase in cell number is consistent with the higher rate of cell division of the JE compared to the slower dividing adjacent gingival keratinocytes that do not express ODAM.
- The upregulation of inflammation-related genes makes a parallel with the perpetually inflamed state of the JE.

A better understanding of the role of ODAM may provide clues on how to revert the changes of JE during PD and on how it promotes epithelial neoplastic transformation.

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