CALIFORNIA STATE SCIENCE FAIR
2002 PROJECT SUMMARY

Name(s)

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Project Number

22544

Project Title

Influence of rAm and rAb in Differentiation of Odontogenic Cells in vitro

Abstract

Objectives/Goals

An extract of pig enamel proteins is being used to repair tissues destroyed by periodontal disease without knowing what is the active ingredient. In this project I want to determine if the major enamel proteins (amelogenin (Am) or ameloblastin (Ab)) can function like growth factors to repair tissues.

Methods/Materials

Cells were grown in Petri dishes containing permissive media until they reach confluence and then placed on differentiation media containing recombinant Am or Ab protein or just the media. Cells were grownx for different days, media was changed every other day and changes in morphology were documented before mRNA was isolated. Cell differentiation was determined using Reverse Transcription # Polymerase Chain Reaction (RT-PCR). RT was done by adding a Poly-T, 4 deoxy-nucleotides and reverse transcriptase enzyme to the mRNA and incubated at 42oC for 1 hour. PCR was done by with 1 µl of cDNA, dNTPs, Ex Taq polymerase and specific primers for proteins associated with tooth formation. Thx PCR products are analyzed using agarose gel Electrophoresis and the genes expressed are visualized using UV light.

Results

The results indicate that there are no morphological changes in cells incubated with rAM or rAB as compared to the control. The results from the RT-PCR indicate that there is a band for β-actin primer for each of the cells used. Osteonectin primer: there is no differences at 7 days, the expression goes down at 14 days in culture, and at 28 days, rAm down regulates the expression of osteonectin, rAb just a little. BMP4: day 7, no effect, follows a similar profile as osteonectin except at 35 days rAm and rx down regulated its expression. NFI-X primer: at 14 days, rAb up-regulates expression of NFI-X, later it down regulates. For the DSPP primer bands do not appear.

Conclusions/Discussion

No morphological changes were found in any of the cells tested in the presence of rAb or rAm. All mRNAs obtained were of good quality to do the studies. rAm down regulates the expression of osteonectin and BMP-4 after 14 28 days in culture. rAb up-regulates the expression of NFI-X at 14 dax and then it down-regulates it. This suggest that Am and Ab might act as growth factors and induce cex differentiation besides being involved in the production of an enamel extracellular matrix.

Summary Statement

Influence of rAm and rAb in Differentiation of Odontogenic Cells In Vitro.

Help Received

Used lab equipment at the Center for Craniofacial Molecular Biology under the supervision of Dr. Margarita Zeichner-David