## Regulation of odontogenic gene expression in dental stem cells

Angelo Leone<sup>1,2</sup>, Ana Angelova Volponi<sup>1</sup>, Ivan Diakonov<sup>1</sup>, Maria Buscemi<sup>2</sup>, Paul T. Sharpe<sup>1</sup>

<sup>1</sup> Department of Craniofacial Development, King's College University of London, Guy's Hospital Tower, London, UK

<sup>2</sup> BioNec, Biomedicina sperimentale e neuroscienze cliniche- Facoltà di Medicina e Chirurgia- Sezione di Istologia ed Embriologia Arcangelo Pasqualino di Marineo, Policlinico Universitario P. Giaccone, Palermo, Italy

**Introduction** Barx1, Msx1,Pax9, Lhx8 and Runx2 are genes expressed in mesenchymal cells during the odontogenesis. Our investigation aimed to study the expression of these genes in different adult dental mesenchymal stem cells. SHED(Stem Cells from Human Exfoliated Deciduous teeth), SCAP(Stem Cells from Tooth Apical Papilla),DPA (Adult Pulp Stem Cells) and HTM (Human Embryonic Dental Mesenchymal Cells) were used. Regulation of gene expression in these cells by FGF and Wnt signalling pathways together with the effects of tooth matrix proteins was investigated.

**Materials and Methods** Cells were grown in 6 well plates in 3 ml MSCGM medium (Lonza) containing Glutamax, 10% FBS and gentamicin Sulfate. Cells were grown to confluence and with 80% confluent, they were treated for 24 hours with either 20 ng per ml of BIO (Gsk3 inhibitor), 1.5µl per ml of porcine dental matrix proteins (EMD), 0.4µl per ml of Fgf8 and 4µl per ml of DMSO (control). RNA was extracted and purified according the RNeasy-kit-protocol from Qiagen. RNA quantitation was done using a Bio Photometer (Eppendorf) in 2:100 dilutions of the samples with nuclease-free water. Quantitative analysis was performed using Rotor Gene Q-serier softhware. 10µl of mixture was made with 5µl of Sensimix- syber green from Quantace, 0.2µl F- primer, 0.2µl R-primer and 4.6µl of cDNA.

**Results** QPCR for PEA3 and AXIN2 confirmed induction of FGF and canonical WNT signaling respectively. Complex patterns of changes in the levels of gene expression were observed that were both cell and treatment specific. One striking result was the induction of PAX9 expression in SHED cells following addition of EMD.

**Conclusions** Data obtained can help to understand and imitate the natural process of embryonic tooth development using adult dental cells. For this reason we believe that the induction of Pax expression after EMD treatment of SHED cells warrants further investigation.

Key word Odontogenic genes, dental stem cells