

## EXPRESSION PROFILING AND BIOCHEMICAL CHARACTERIZATION OF MOUSE AMELOTIN

S Cheon<sup>1</sup>, E Somogyi-Ganss<sup>1</sup>, Y Nakano<sup>2</sup>, E Bajenova<sup>1</sup>, V Nguyen<sup>1</sup>, R Kosnevitch<sup>1</sup>, MD McKee<sup>2</sup>, B Ganss<sup>1</sup>

<sup>1</sup>CIHR Group in Matrix Dynamics, University of Toronto, Faculty of Dentistry, Toronto, Ontario, CANADA and <sup>2</sup>Faculty of Dentistry, McGill University, Montréal, Québec, CANADA

**INTRODUCTION:** Dental enamel is formed in a biomineralization process under the influence of ameloblast-specific proteins, mainly amelogenins, which orchestrate the crystallization of hydroxyapatite ribbons, resulting in the hardest known bioceramic material. The roles of several additional structural (e.g. ameloblastin, enamelin) and proteolytic (enamelysin/MMP-20 and kallikrein-4) ameloblast proteins are currently being deciphered. We have recently discovered a novel ameloblast-specific gene, **amelotin** (AMTN<sup>1</sup>), whose mRNA is highly and transiently expressed in maturation-stage ameloblasts. AMTN appears to be secreted and was reported in the junctional epithelium and the basal lamina in erupted rodent teeth<sup>2</sup>, suggesting a potential involvement in soft tissue attachment to enamel. However, the AMTN expression profile during pre-eruptive tooth development has not been determined and the protein has not been characterized further.

**METHODS:** A polyclonal anti-AMTN antibody was raised in rabbits and affinity purified. After confirming its specificity for native AMTN protein in Western blots, it was used for immunohistochemical and immuno-gold TEM staining in mouse molars and incisors from various post-natal stages.

**RESULTS:** In these analyses AMTN shows a specific, transient and unique expression pattern: AMTN expression was first detected at postnatal day 1 (D1) in cells of the developing incisor tip and cuspal surfaces of molars. Expression levels increased to a maximum around D10, then declined until tooth eruption. In incisors, the protein was detected from D1 onwards and persisted until after eruption in a zone extending from the late secretory through the entire maturation stage to the zone of reduced enamel epithelium. The majority of the protein was found - in molars and incisors - at the interface between maturation stage ameloblasts and dental enamel and - to a lesser extent - in the enamel. AMTN is predicted to be post-translationally modified by O-glycosylation and/or phosphorylation. To confirm this, we have expressed the recombinant AMTN

protein from mouse and human in bacteria and compared it with native protein extracted from mandibular molars and incisors. SDS-PAGE comparison of the respective molecular weights has shown that the native protein is approximately 9kD larger than its recombinant counterpart. Immunostaining with specific antibodies to O-linked carbohydrate moieties has indicated that AMTN is a glycoprotein.

**DISCUSSION & CONCLUSIONS:** This work has shown that AMTN protein is highly and specifically expressed in ameloblasts, transiently in molars, and continuously in incisors. The protein accumulates at the ameloblast/enamel interface, and some AMTN is found deeper in the enamel. The murine protein appears to be post-translationally modified at least by O-glycosylation. The AMTN expression pattern is distinct from that of known structural and proteolytic enamel proteins and suggests a unique role for AMTN in cell-to-enamel attachment and/or enamel maturation.

**REFERENCES:** <sup>1</sup>K Iwasaki, E Bajenova, E Somogyi-Ganss et al. (2005): Amelotin - a Novel Secreted, Ameloblast-specific Protein. *J Dent Res.* 84(12):1127-32. <sup>2</sup> P Moffatt, CE Smith, R St-Arnaud et al. (2006): Cloning of rat amelotin and localization of the protein to the basal lamina of maturation stage ameloblasts and junctional epithelium. *Biochem J.* 399(1):37-46.

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