Temporal and Spatial Evaluation of Matrix Metalloproteinases Activity (MMPs -2 and -9) During Mice Endochondral Ossification.

Virginia Sant`Ana Cronemberger, Fabio Daumas Nunes, Katiucia Batista da Silva Paiva - Laboratory of Molecular Pathology, Depto Oral Pathology, Dental School, University of São Paulo

MMPs are zinc-dependent endopeptidases that, collectivelly, degrade all components of the ECM. They are able to remodelate the ECM during normal developmental processes such as embryogenesis and organogenesis, as well as in pathological processes such as tumoral invasion. The biological mineralization research looking for discovering the genes involved in the molecular mechanisms that control the endochondral ossification process. MMPs and their inhibitors (TIMPs and RECK) are responsible for bone matrix remodeling and, probably, determinate the level of its turnover. Our research group study the temporal-spatial expression of MMPs, TIMPs, and RECK protein in several biological mineralization processes, such as odontogenesis and amelogenesis, alveolar bone repair, palatogenesis, endochondral and intramembrabous ossification by imunohistochemistry, gel zymography, *in situ* hybridization and Real Time PCR techniques. Thus, our goal was evaluate the temporal-spatial enzymatic activity of MMP-2 and MMP-9 in mice embryos and newborns during endochondral ossification. Femurs (n=5/period) were collected from foetuses (E13-E20) and 1 day postnatal (PN1), stored at -80ºC, cryosectioned and processed for *in situ* zymography (DQ-gelatin, FITC-conjugated). At E15, gelatinolytic activity was observed at the center of cartilaginous anlagen in hyperthrophic chondrocytes and bone core. We also observed fluorigenic sites in hyperthrophic, prehyperthrophic and proliferative chondrocytes at bone extremities. At E19 and PN1, enzymatic activity was only detected in growth plate. In agreement of previous immunohistochemical results, we suggest that MMP-2 and -9 play an important role in the replacement of cartilaginous matrix by bone matrix.

Financial Support: FAPESP