Public Abstract

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Graduation Term:SS 2008

Department:Animal Sciences

Degree:PhD

Title:AN ANALYSIS OF ASPARTIC PEPTIDASES EXPRESSED BY TROPHOBLASTS AND PLACENTA OF EVEN-TOED UNGULATES

The Pregnancy Associated Glycoproteins (PAGs) represent a large family of proteins expressed exclusively by trophoblast of the placenta of even-toed mammals, such as ruminants (cattle, sheep), pig, etc. From the analysis of cattle genome, we found that there are 18 distinct functional and 14 non functional genes. These PAGs in cattle, can be classified into ancient and modern PAGs based on their coding sequence. In addition to their differences in coding sequence, there are differences in the time of expression as well as the cell type of placenta in which they are expressed. Besides, it was also found that, there are differences in their ability to cleave substrates (proteolytic acitivity). Some of the modern PAGs have accumulated changes in sequence that results in loss of proteolytic activity. In contrast, all of the ancient PAGs of ruminants and swine, have all the hallmarks of typical aspartic peptidases (APs). When we investigated the proximal promoter sequence (500 base pairs upstream of translational start codon) of the PAG genes, we identified pockets of regulatory sequence that are different between ancient and modern PAGs, that could explain for observed differences between these two groups. We gathered evidence by Real-time PCR and global analysis of expressed sequence tags that confirm that, boPAG-2 is the most abundant of all boPAGs. We therefore selected boPAG-2 and its closest relative boPAG-12, as well as poPAG-2 the only ancient PAG found in pigs, as the candidates for investigation of proteolytic activity. From our experiments we found that, boPAGs -2 and -12, and poPAG-2 are proteases with optimal activity under acidic pH conditions. We also illustrated differences in proteolytic activity towards substrates, and in their relative affinity towards an AP inhibitor (pepstatin A). We found that, in comparison to the two bovine paralogs, boPAGs -2 and -12, poPAG-2 was found to be a more robust enzyme. Finally, we demonstrated that APs secreted by embryos such as PAGs (and likely others) can be objectively measured in the medium conditioned by the culture of pig embryos either individually or in pools for variable lengths of time in acidic conditions. We also observed that such activity seemed to correlate with stage and quality of embryos (assessed morphologically) in vitro. We, therefore believe that this proteolytic activity potentially could serve as a marker for developmental competence of the embryos.