Characterization of porcine betaine homocysteine methyltransferase (BHMT) and betaine homocysteine methyltransferase -2 (BHMT-2) genes

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Abstract
Betaine homocysteine methyltransferase (BHMT) and BHMT-2 methylate homocysteine to form methionine using betaine or S-methylmethionine, respectively. These enzyme activities are only observed in the liver of adult rodents, whereas in adult humans and pigs it is detected in the liver and kidney cortex. Because of this similarity, we have chosen the pig as a model to study the spatial and temporal expression of these enzymes and to determine whether the BHMT and BHMT-2 genes are transcribed into multiple mRNA isoforms. This report describes our progress to date. Immunohistochemical staining revealed the presence of BHMT in adult liver and kidney cortex, as reported earlier, but we also found immunodetectable levels of BHMT in fetal lungs (aged days 30, 60, 84, 90, and 105 of gestation). The BHMT and BHMT-2 cDNAs were subsequently cloned and sequenced, and their 5' and 3' UTRs were amplified using RLM-RACE. BHMT has a longer 5' and 3' UTR, 363 amino acids, respectively, and share 78% amino acid identity. Relative to BHMT-2, BHMT has two additional regions of amino acid sequence, a 9 amino acid sequence (86-94) in the N-terminal region, and a 34 amino acid sequence (373-407) at the carboxy terminus. Eight splice variants of porcine BHMT have been observed and one variant found in the kidney medulla and heart encodes a truncated form of BHMT. Although we do not know if this mRNA is efficiently translated and whether the resulting protein is stable, if it is, this protein is predicted to lack BHMT activity because it doesn’t have critical determinants for binding the enzyme’s catalytic Zn. We have modeled this truncated form of BHMT and the results show a dramatic change in tertiary structure when compared to wild type BHMT. The model predicts the truncated protein to adopt a truncated fold, whereas wild type BHMT is a βα barrel. The function of the hypothetical protein remains unknown.

Introduction
- Increased homocysteine is associated with vascular diseases, renal insufficiency, and adverse pregnancy outcomes (fetal development);
- BHMT converts homocysteine to methionine (50% of liver activity);
- BHMT represents ~ 1% of total liver protein (actin is ~10% of total);
- The tissue expression of BHMT varies among species but the reason for these differences are unknown:

Critical Questions:
1. Can an animal model that recapitulates human BHMT function(s) be identified to support developmental studies?
2. Do high BHMT levels indicate that the BHMT gene has additional functions than the enzymatic conversion of Hcy to Met?

Hypothesis:
Regulation of the BHMT gene (splice variants) contributes to multiple developmentally relevant functions (disease)

Aims:
1. Determine an appropriate model to study the role of the BHMT gene in development & diseases;
2. Identify BHMT splice variants and how their presence could contribute to the spatial & temporal expression of BHMT; and
3. Demonstrate whether splice variants would result in alternate BHMT function(s)

Approaches:
1) Compare the amino acid composition of human and pig BHMT and BHMT-2 and perform analysis to show that pig is closer to humans with respect to evolution of BHMT and BHMT2
2) Identify BHMT gene splice variants which contribute to differential regulation of BHMT
3) Compare structural changes in BHMT splice variants

Materials & Methods:

Sample collection & preparation: Fetal and adult tissues were collected and snap-frozen in liquid nitrogen, then stored at -80°C

Tissues were embedded in paraffin and 3mm sections were obtained on glass slide with microtome

Primary antibody: Rabbit polyclonal prepared against human liver enzyme (BHMT)

Secondary antibodies: ABC kit

Obtaining full-length cDNA

Storing 10% formalin for 24 hrs and then transferred to 70% ethanol (4°C)

Stability of BHMT Transcripts

Critical Questions: BHMT structural changes contribute to functional changes

Conclusions:
- Developmental regulation occurs in expression of BHMT and disruption could contribute to developmentally associated diseases;
- Regulation of BHMT (splice variants) suggest unique functions of BHMT during development; and
- Structural changes of truncated splice variant (coding region) suggest alternative function as a chaperone or an inhibitor.

Future Work:
- Quantify unique porcine BHMT transcripts, using qPCR, in different tissues during development.
- Determine the stability of the variant BHMT protein and further characterize biochemically.
- Determine the evolutionary history of BHMT and estimate time of gene duplication and estimate genetic divergence

References:

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