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Laboratory of Comparative Genomics

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

Radhika S. Ganu[1] , Timothy A. Garrow[1,2] , Monika Sodhi[3] , Lawrence B. Schook[1,3]

[1]Division of Nutritional Sciences, [2]Department of Food Science and Human Nutrition & [3]Department of Animal Sciences, University of Illinois at Urbana Champaign

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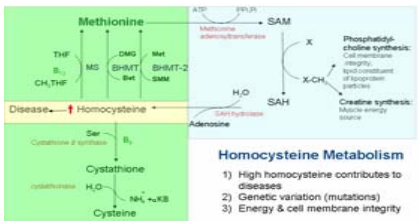
Abstract

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 BHMT in fetal lungs (aged days 30, 60, 84, 90, 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, consisting of 77 and 1,142 nucleotides, whereas BHMT-2 UTRs were composed of 17 and 893 nucleotides, respectively. The deduced amino acid sequences of BHMT and BHMT-2 contain 407 and 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 horseshoe fold, whereas wild type BHMT is a $(\beta\alpha)_8$ barrel. The function of this 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); and
- The tissue expression of BHMT varies among species but the reason for these differences are unknown
  - sheep - pancreas
  - humans & pigs - liver & kidney cortex
  - rat – liver



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:

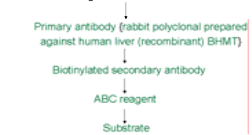
1) Compare the amino acid composition of human and pig BHMT and BHMT-2 and perform analysis to show that pig is closer to humans with respect to evolution of BHMT and BHMT2
2) Identify BHMT gene splice variants which contribute to differential regulation of BHMT
3) Compare structural changes in BHMT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

Materials & Methods:

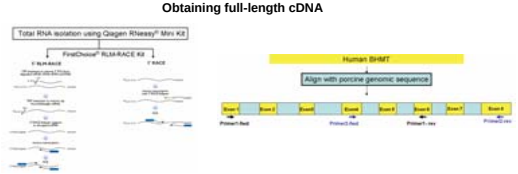
Sample collection & preparation: Fetal and adult tissues were collected
- **Stored 10% formalin for 24 hrs and then transferred to 70% ethanol (4°C)**
- **Snap-freeze in liquid nitrogen, then stored at -80°C**

Immunohistochemistry

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



Obtaining full-length cDNA



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Results

Fig 1a

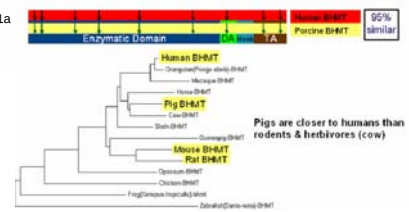

Fig 1b

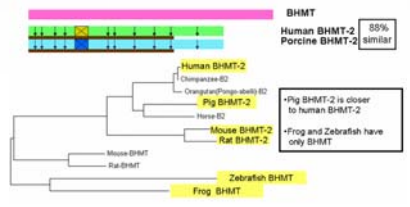

Fig1a & 1b: Pigs are an appropriate model to study the role of BHMT. The above figures show the amino acid alignment of porcine BHMT & BHMT2 with human BHMT & BHMT-2 using Biology Workbench. The evolutionary analysis is performed by using MEGA4 software

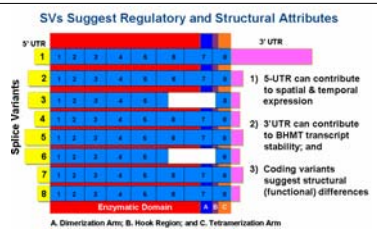

Fig 2: Splice variants of porcine BHMT using RLM-RACE

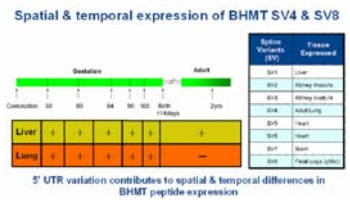

Fig 3 : Developmental changes in BHMT expression using immunohistochemistry

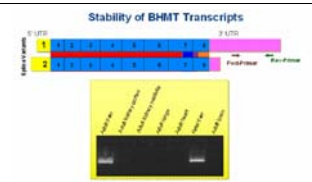

Fig 4: Length of 3' UTR provides stability to the transcript

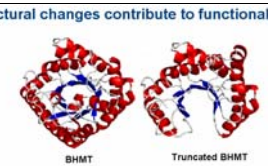

Fig 5: The structure of BHMT and the truncated BHMT variant (Phyre-generated model)

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:

1) Garrow TA. Purification, kinetic properties, and cDNA cloning of mammalian betaine-homocysteine methyltransferase(1996) J Biol Chem. Sep 13;271(37):22831-8
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3) M. A. Pajares and D. Pérez-Sala. Betaine homocysteine S- methyltransferase: just a regulator of homocysteine metabolism? (2006)*Cell. Mol. Life Sci*. 2792–2803