Objective: What parts of the ceramide pathway are present in oysters and what role does this pathway play in oyster physiology, especially in environmental response?

Introduction

The ceramide pathway is important in response to a variety of environmental stresses and also in development.

Summarize findings from molluscan apoptosis pathway gene discoveries – abalone and Pacific oyster

Apoptosis via ceramide is an important response to environmental stress: summarize stress responses (mostly vertebrate studies)

Ceramide and its enzymes are also implicated in other pathways (TNFa/PGE2, NfkB) and so characterization of the pathway will help to better understand how all these processes fit together and function.

This study: Characterize two important enzymes in the ceramide pathway that have not yet been described. Investigate the role these enzymes play in adult *C. gigas* response to environmental stress. Determine the extent of the ceramide pathway components in *C. gigas* with an *in silico* analysis.

Results

Both proteins are structurally similar (amino acids) to previously described sequences.

Transcript for *sptlc1* is expressed the most in the gill, which is the interface with the environment. Same expression pattern as *hsp70* another gene that is an important response to environmental stress.

*SMase* is expressed the most in the digestive gland and not at all in the other tissues. There are a variety of SMases that are expressed in different tissues and this one may only be expressed in the digestive gland.

Both genes are expressed more in oysters exposed to *Vibrio* when compared to controls. But weird that *hsp70* does not follow suit (PE2 does).

Discussion

1. *C. gigas* has full Sptlc1 and acid sphingomyelinase, which are essential enzymes in the ceramide pathway.
2. Serine palmitoyltransferase
   1. Structure
      1. Sptlc2 has a conserved motif that binds PLP, pyridoxal phosphate (Hanada 2003) – not in *C. gigas* (residue 311)
   2. Function
      1. De novo biosynthesis: catalyzes first step of condensation of serine and palmitoyl-CoA to 3-ketosphinganine, then reduces to dihydrosphingosine, then dihydroceramide, given trans-4,5-double bond to become ceramide (Hannun 1994, review)
      2. Regulation of sptlc is important for determining levels of ceramide and other sphingolipid intermediates (Hanada 2003)
      3. Sptlc is common in its function across taxa (hanada 2003)
      4. Ubiquitous expression of mRNA and protein but levels depend on tissue type and are developmentally correlated; sptlc activity can be affected by diet (Hanada 2003)
      5. mRNA and protein increase in response to external stress stimuli (Hanada 2003)
      6. apoptotic stimuli can activate sptlc post-transcriptionally increasing apoptosis (Hanada 2003)
3. Acid sphingomyelinase
   1. Structure
      1. Different isoforms of SMase depend on pH optima (Kolesnick 1998)
         1. Neutral (N)SMase operates in plasma membrane
         2. Acid (A)SMase operates in lysosome or endosome
      2. 3 types of transcripts (human), expressed differently in different tissues (Schuchman et al. 1991)
   2. Function
      1. Catabolic generation of ceramide (Ballou et al. 1996 review)
      2. Possible that radiation creates ROSs that trigger catabolic generation of ceramide (Haimovitz-Friedman 1994)
      3. Hydrolyze phosphodiester bond of sphingomyelin (Kolesnick 1998)
      4. Both forms of SMase are stimulated by external stimuli in seconds or minutes (Kolesnick 1998)
      5. A- and NSMase have distinct biological functions – ASMase is probably involved in TNF-mediated apoptosis (Kolesnick 1998)
      6. Loss of ASMase in embryonic mice causes apoptotic death early in development (Eliyahu et al. 2007)
4. *C. gigas* genes found *in silico*
   1. Ceramidases
      1. Deacylate ceramide to make sphingosine (Ballou et al. 1996)
      2. TNF/PGE2 pathway uses sphingosine generated from acid ceramidase activity; acid ceramidase upregulates PGE2 response to TNFa (Zeidan et al. 2006)
   2. Caspase proteases
      1. Activated by cytokine receptors and initiates apoptosis (Kolesnick 1998, review)
      2. In zebrafish caspase-3 induces apoptosis and is expressed at every developmental stage in tissue-specific manner (Yabu et al. 2001)
   3. Ceramide synthase catalyzes dihydrosphingosine -> dihydroceramide (inactive) in mitochondria and ER; enzyme is stimulus-responsive (Kolesnick 1998)
   4. TNFa
5. Other invertebrates with apoptosis genes
   1. *Bombyx mori*: 52 apoptosis-related genes (5 members of caspase fmily, 2 in TNF superfamily) (Zhang et al. 2010)
   2. Caspase-8 expression in adult disk abalone tissue (constitutive): gill > mantle > hepatopancreas > digestive tract > hemocytes (Lee et al. 2010)
   3. Immediate upregulation of caspase-8 in gills and hemocytes following bacterial and viral challenge of disk abalone (Lee et al. 2010)
   4. Apoptotic genes in *C. gigas* (Zhang et al. 2011)
      1. Cloned and sequenced 4 apoptosis genes: FADD, IAP (inhibitor of apoptosis), initiator and effector caspases
         1. IAPs bind to and inhibit caspases
      2. Increased complexity in gigas BIR (baculovirus IAP repeat) domains compared to other inverts – BIR necessary for interaction with pro-apoptotic factors
      3. Highest caspase expression in gill and mantle, lowest in gonad and digestive gland
      4. IAP highly expressed in hemolymph > gill > muscle
      5. FADD high in hemolymph
      6. Vibrio response (gene expression): all increased gradually until 12 h post-injection then decreased
      7. Gigas apoptosis complexity is greater than ecdysozoa but less than deuterstomes – gene loss in ecdysozoa or expansion in dueterstomes
      8. CgFADD is more similar to human sequence than to fly
      9. Effector amino acid sequence is more conserved than initiator caspases compared to human and fly
6. Important functions of ceramide and apoptosis
   1. Possible links to MAP kinase pathway; ceramide metabolites stimulate PGE2 production (Ballou et al. 1996)
      1. Modulates secretion of PGE2 in response to IL-1 (Hannun 1994)
   2. C2-ceramide has antiproliferative effects on leukemia cells (Hannun 1994)
   3. May be intracellular mediator of cytotoxicity (Hannun 1994)
   4. Activates transcription of cyclooxygenase (Hannun 1994)
   5. Stress-induced apoptosis (ionizing radiation, H2O2, UV radiation, and heat shock) is signaled by ceramide and requires SAPK/JNK cascade (Verheij et al. 1996)
   6. TNFa works through ceramide in apoptosis and inflammation (Verheij et al. 1996)
   7. (Catabolic) generation of ceramide in response to radiation is quick and sensitive (Haimovitz-Friedman 1994)
   8. abrupt change in salinity triggers changes in ceramide metabolism in sea bass – mediation of cellular rearrangemet after osmotic shock. Modulation of sphingomyelin metabolism is linked to environmental change (El Babili et al. 1996)