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Expression of tapasin in rainbow trout tissues and cell lines and up regulation in a monocyte/macrophage cell line (RTS11) by a viral mimic and viral infection

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ABSTRACT

Tapasin is a transmembrane glycoprotein that acts as a bridge between the transporter associated with antigen processing and the MHC class I receptor in mammals. Through the development of antibody against trout tapasin, this report demonstrates the detection of trout tapasin as a N-glycosylated 48 kDa protein. Tissue and cell line distribution revealed that tapasin protein is expressed mainly in immune system organs and in rainbow trout epithelial cell lines from gill (RTgill-W1), liver (RTL-W1), and intestine (RTgutGC). An additional 20 kDa band was observed in tissues and cell lines, and appeared to be most prominent in RTgutGC but was absent in peripheral blood leukocytes. Tapasin 48 kDa protein was most strongly expressed in RTS11 (monocyte/macrophage cell line) and its regulation following dsRNA stimulation was explored. Upon poly I:C treatment and Chum Salmon Reovirus (CSV) infection, tapasin protein expression was upregulated up to 3.5 fold and 3 fold respectively, in parallel with increased expression of the glycosylated MH class I heavy chain, whereas the expression of the 20 kDa form remained unchanged. Overall this work demonstrates the induction of tapasin protein by dsRNA stimulation, which implies its possible conserved regulation during viral infection in teleost cells.

1. Introduction

Major histocompatibility complex (MHC) class I receptors are expressed on the cell surface of all nucleated cells and present peptides derived from intracellular proteins to $CD8^+$ T lymphocytes. Peptides are generated by the proteasome and delivered into the lumen of the endoplasmic reticulum (ER) by the transporter associated with antigen processing (TAP), which associates with the MHC class I receptor through a endogenous antigen presentation pathway specific chaperone: tapasin also known as TAP binding protein (TAPBP) (Wearsch and Cresswell, 2008; Pamer and Cresswell, 1998; Rizvi and Raghavan, 2010).

Co-immunoprecipitation studies in human cells have identified tapasin as a 48 kDa protein associated with TAP, MHC class I and calreticulin (Sadasivan et al., 1996). Molecular cloning revealed its identity as a transmembrane glycoprotein containing immunoglobulin superfamily motifs and putative ER retention signal, encoded by a gene that is linked to the MHC (Ortmann et al., 1997). Evidence for its possible function came from a study using the human cell line .220, a lymphoblastoid cell line which does not express tapasin, which demonstrated a lack of any association between MHC class I and TAP, and displayed low levels of MHC class I on the cell surface. The normal phenotype was restored upon transfection with a gene producing recombinant tapasin (Ortmann et al., 1997). Later, studies with tapasin deficient mice showed that not only was the MHC class I surface expression impaired but CD8+ responses during viral infection were also compromised (Garbi et al., 2000). Additional insights into the functional role of tapasin during antigen presentation came from studies in mammals which focused on its role in optimizing the selection of peptides loaded into the MHC class I receptor, favouring peptides with longer half-life (Williams et al., 2002; Howarth et al., 2004), similar to the role of HLA-DM with MHC class II molecules (Brocke et al., 2002). However the mechanisms by which this role is carried out are still not completely understood.

In mammals, the expression levels of tapasin along with other molecules involved in MHC class I assembly such as TAP, β2 microglobulin and the proteasomal subunits were shown to be induced by the interferons (IFNs) which are cytokines involved in antiviral response, primarily by interferon gamma in APC, but by type I IFN in non-hematopoietic cells. This induction was shown to be coordinated by the presence of interferon factor binding elements in the promoter regions of these genes (Wright et al., 1995; Herberg et al., 1998). Tapasin expression in mammals was shown to enhance upon infection with the intracellular bacteria: *Listeria monocytogenes* and also during treatment with IFNγ in primary embryonic mouse fibroblasts (Abarca-Heidemann et al., 2002).

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In salmonid fish, the tapasin gene was shown to be upregulated during *in vivo* infection with two pathogenic viruses: infectious salmon anaemia virus (ISAV) and infectious hematopoietic necrosis virus (IHNV) (Landis et al., 2006; Jørgensen et al., 2007) coordinated with increased transcriptional expression of MH class I heavy chain and β2 microglobulin (Jørgensen et al., 2006; Hansen and La Patra, 2002). Furthermore, tapasin promoter reporter assays demonstrated that tapasin promoters respond to the interferon regulatory factors, Onmy-IRF1 and Onmy-IRF2 (Landis et al., 2006), which supports its transcriptional activation by the interferon response.

The chum salmon virus (CSV) is a non-pathogenic virus which belongs to the dsRNA aquareovirus family (Essbauer and Ahne, 2001; Winton et al., 1987). It has been demonstrated that pre-exposure of rainbow trout to CSV can offer protection up to eight weeks against subsequent infection with IHNV (La Patra et al., 1995). Later, it was shown that CSV infection can induce the expression of antiviral genes such as Mx and vig-1 in RTS11 and RTG-2 cells (DeWitte-Orr et al., 2007) which may contribute to the cells resistance to this virus.

In this report, polyclonal antibodies against trout tapasin were developed which allowed the characterization of its protein distribution in trout tissues and cell lines as well as the monitoring of its regulation during poly I:C stimulation and CSV infection in RTS11 cells.

2. Materials and methods

2.1. Fish

Rainbow trout were obtained from Silver Creek Aquaculture (Erin, ON) and kept in 200 l fresh-water flow-through tanks at the University of Waterloo. All fish were kept and handled under a permit from the University of Waterloo Animal Care committee according to CCAC guidelines. Blood was drawn from the caudal vein after anaesthesia with 0.01% MS222 and tissues samples were collected in RNAlater and stored at −80 °C for later use.

2.2. Cell lines

RTS11 is a rainbow trout spleen monocyte/macrophage cell line (Ganassin et al., 1998) and was maintained at 20 °C in Leibovitz's L-15 medium (ThermoFisher Scientific) with 20% fetal bovine serum (FBS, PAA) containing 150 U/ml of penicillin and 150 μg/ml streptomycin in 25 cm^2 culture Falcon flasks. RTS11 cells were sub-cultured every 10–14 days by passaging half of the growth-conditioned medium with the cells into new culture vessels containing equal volumes of fresh medium.

The following cell lines were maintained in L-15 with 10% FBS in 75 cm^2 culture Falcon flasks and sub-cultured biweekly by trypsin (Lonza): CHSE-214 from chum salmon embryo, RTL-W1 from trout liver (Lee et al., 1993), RTgill-W1 from trout gill (Bols et al., 1990) and RTgutGC from trout intestinal tract (Kawano et al., 2011) were cultured at 20 °C.

2.3. Propagation of chum salmon reovirus

Chum Salmon Reovirus (CSV) was obtained from ATCC and routinely propagated on permissible CHSE-214 cultures in L-15 media with 5% FBS at 18 °C as previously described (DeWitte-Orr et al., 2007; DeWitte-Orr and Bols, 2007). Virus-containing supernatants were collected on day 7 post-infection (p.i.). The supernatants were centrifuged at 4500 g for 5 min at 4 °C, filtered through 0.2 μm membranes (Pall Corporation), aliquotted and frozen at −80 °C until used. Viral titers were determined by $TCID_{50}$/mL assays using CHSE-214 reporter cell line as previously described in DeWitte-Orr and Bols (2007).

2.4. Infection of RTS11 cells with CSV

Approximately 3×10^6 RTS11 cells/well were seeded L-15 with 2% FBS in six well tissue culture plates and subsequently infected with 2.5×10^4 $TCID_{50}$/mL of CSV. Control cultures had the same number of cells without the virus. Both control and infected cultures were incubated at 14 °C for up to 14 days. On selected days post infection cells were collected, washed with ice-cold PBS three times and centrifuged at 500 g for 4 min at 4 °C. Cell pellets were immediately frozen at −80 °C. When all samples were collected, cell pellets were thawed on ice and lysed with 100 μL of 1% NP-40 protein containing protease inhibitor (Roche). Protein concentrations were determined by the BCA protein assay (ThermoFisher Scientific).

2.5. In vitro stimulation with poly I:C and phytohemagglutinin

RTS11 cultures were seeded to 2×10^6 in a 25 cm^2 flasks and treated for 24 h with 50 μg/ml of polyinosinic–polycytidylic acid (poly I:C) (Sigma Aldrich) or 15 μg/ml of phytohemagglutinin (Sigma Aldrich); for controls the same volume of vehicle (PBS) was added. For mRNA analysis, RTS11 cells were treated for 48 h only with poly I:C.

2.6. Production of polyclonal and affinity purification

Rabbit antisera for tapasin were raised against a synthetic peptide conjugated to keyhole limpet haemocyanin (KLH). The peptide was derived from the 21 amino acid C-terminus of trout tapasin (Gene bank ID: AAZ66042.1). Two New Zealand white rabbits (Charles River, ON, CA) were injected intramuscularly with 0.5 mg of tapasin c-terminal peptide with Freund's complete adjuvant (1:1) followed by subsequent peptide boosts of 0.5 mg with Freund's incomplete adjuvant given every three weeks. Rabbits were exsanguinated after 30 weeks and serum was collected and purified using SulfoLink immobilization kit (Thermo Fisher Scientific) according to manufacturer's instruction. In order to validate the specifity of the antibody, a western blot experiment was conducted as followed: incubating membranes with tapasin antibody pre-mixed with an excess of the peptide (immunogen) or with tapasin antibody alone. Our results showed that in the presence of the excess peptide no detection of the 48 kDa or the 20 kDa band was observed.

2.7. Deglycosylation

Approximately 80 mg of gill tissue was lysed with 1% NP-40 containing 150 mM NaCl, 50 mM Tris [pH 8.0] followed by centrifugation for 15 min at 13,000 rpm at 4 °C. Supernatants were collected and concentrated using vivaspin 500 with 3 kDa MWCO (GE healthcare) according to manufacturer's instruction. De-glycosylation with Endo H and N-glycosidase F (New England Biolabs) was performed as previously described (Kales et al., 2007). Enzyme treated and untreated protein lysates were loaded on a 15% gel SDS PAGE gel and blotted with 1:200 anti tapasin affinity purified antibody.

2.8. Western blot for tissue and cell line expression

Cell pellets and tissue samples were lysed in 1% NP-40 lysis buffer containing 150 mM NaCl and 50 mM Tris [pH 8.0] supplemented with 1× of protease inhibitor cocktail (Roche). Thirty μg of protein samples obtained from tissues or cell lysates were separated on a 12% acrylamide gel and transferred to nitrocellulose membranes (Bio-Rad) overnight. Membranes were blocked with