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Extracellular dsRNA induces a type I interferon response mediated via class A scavenger receptors in a novel Chinook salmon derived spleen cell line

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ABSTRACT

Despite increased global interest in Chinook salmon aquaculture, little is known of their viral immune defenses. This study describes the establishment and characterization of a continuous cell line derived from Chinook salmon spleen, CHSS, and its use in innate immune studies. Optimal growth was seen at 14–18 °C when grown in Leibovitz's L-15 media with 20% fetal bovine serum. DNA analyses confirmed that CHSS was Chinook salmon and genetically different from the only other available Chinook salmon cell line, CHSE-214. Unlike CHSE-214, CHSS could bind extracellular dsRNA, resulting in the rapid and robust expression of antiviral genes. Receptor/ligand blocking assays confirmed that class A scavenger receptors (SR-A) facilitated dsRNA binding and subsequent gene expression. Although both cell lines expressed three SR-A genes: SCARA3, SCARA4, and SCARA5, only CHSS appeared to have functional cell-surface SR-As for dsRNA. Collectively, CHSS is an excellent cell model to study dsRNA-mediated innate immunity in Chinook salmon.

1. Introduction

Of the Pacific salmon species, Chinook salmon (*Oncorhynchus tshawytscha*) is the largest and most highly valued in North America (Christensen et al., 2018; Ohlberger et al., 2018). As a result, this species has historically been a focus for capture fishery production. Up until 1922, as many as 11 million kg of Chinook salmon were harvested annually before a decline in wild populations was noted. Unfortunately, native populations are in decline at such a rate that annual harvests are now approximately 2 million kg per year (Johnson et al., 2017). This has resulted in Chinook salmon's addition to the threatened species list despite concerted efforts to replenish wild populations via hatchery bred stocks (Knudsen et al., 2006, Paquet et al., 2011). Due to the inability of capture fisheries to meet the rising demands for Pacific salmon consumption, the culture of Chinook salmon is a promising solution to alleviate pressure from wild stocks. As a relatively undomesticated species, a deeper understanding of the Chinook salmon immune system is necessary to ensure successful aquaculture practices.

Viral pathogens present a formidable obstacle in aquaculture due to their rapid replication rate and persistence in the environment (Oidtmann et al., 2017). As every milliliter of seawater contains roughly 10^7 viral particles (reviewed in Suttle, 2005), interaction with these microorganisms is unavoidable and can be detrimental in aquaculture environments. Fortunately, vertebrates have developed an arsenal of pattern recognition receptors (PRRs) designed to recognize and initiate appropriate responses to viral pathogen associated molecular patterns (PAMPs). One such PAMP, double-stranded (ds) RNA, is produced by all viruses at some point during their lifecycle (Jacobs and Langland, 1996; DeWitte-Orr and Mossman, 2010). In response, fish and other vertebrates have evolved several nucleic acid binding PRRs including toll-like receptors (TLRs), RIG-I receptors (RLRs) and class A scavenger receptors (SR-As) to sense dsRNA (reviewed by Poynter et al., 2015a). Binding of dsRNA initiates a signal cascade culminating in the production of type I interferons (IFNs), such as IFN1 in fish, that can induce the expression of interferon-stimulated genes (ISGs) in both a paracrine and autocrine fashion. Many of the resulting ISGs are responsible for interfering with, and rendering host cells non-permissive to, viral infection often referred to as an "antiviral state" (Zhang and Gui, 2012). In fish, significant antiviral activity has been demonstrated for several ISGs including Vig3 (known in mammals as ISG15) and Mx1, but there is still limited information regarding their functional mechanisms (Poynter and DeWitte-Orr, 2016). As antiviral immune responses may vary depending on the host species (Heil et al., 2004; Kuzmann et al., 2017), appropriate model systems are required to

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further our understanding of viral pathogens and antiviral responses in aquatic species.

Eukaryotic cell lines offer a controlled, cost-effective method to explore antiviral immune function within a single-cell monoculture. Currently, the only cell line available for the study of Chinook salmon is CHSE-214, an embryonic epithelial-like cell line. CHSE-214 is commonly used in comparative virology due to its permissibility in supporting propagation of aquatic viruses (MacDonald and Kennedy, 1979). Interestingly, these cells are incapable of mounting an effective antiviral response when exposed to extracellular dsRNA (Jensen et al., 2002b). When CHSE-214 is transfected with the synthetic dsRNA, polyinosinic:polycytidylic acid (pIC) the innate antiviral IFN response is intact (Jensen et al., 2002b; Monjo et al., 2017), revealing the intracellular signaling pathways are not defective. CHSE-214 cells appear to lack surface receptors for dsRNA as the cells do not bind to extracellular dsRNA, however when a human dsRNA receptor, SR-AI, is expressed in the cells it enables binding capabilities (Monjo et al., 2017). This apparent absence of cell-surface receptors that can uptake dsRNA makes CHSE-214 an excellent model for transfection studies to understand dsRNA sensing. However, as most fish cells can sense extracellular dsRNA, a more biologically equivalent Chinook salmon cell line that uptakes extracellular dsRNA without transfection is desirable.

The present study describes the establishment and characterization of CHSS, an epithelial-like cell line derived from the spleen of a triploid Chinook salmon. Although CHSS presents a diploid phenotype, its creation provides a valuable tool for understanding the innate antiviral immune response in Chinook salmon. Following exposure studies to pIC, CHSS was observed to both take up and subsequently respond to extracellular dsRNA. As CHSS is the only known cell line created from an adult Chinook salmon, these cells are much more relevant for understanding species specific aspects of immunity, physiology, and cellular function.

2. Materials and methods

2.1. Primary cultures

A 200 g Chinook salmon was retrieved from a net-pen containing a 99–100% triploid population, based on the rate at which maturation was Impaired (John Heath, personal comm.), that was produced and maintained at Yellow Island Aquaculture Ltd (YIAL, Quadra Island, BC, Canada). The fish was euthanized with an overdose of clove oil prior to tissue collection. All procedures were performed following the guidelines of the Animal Care Committee at the University of Waterloo. The spleen, caudal fin and gills were collected and stored in L-15 media supplemented with 20% fetal bovine serum (FBS, Gibco) and 200 U/mL of penicillin and 200 U/mL streptomycin (Thermo Scientific). Upon arrival to the University of Waterloo, each tissue type was diced into small pieces in a laminar flow hood and rinsed three times with Dulbecco's buffered saline solution (DPBS, Lonza) containing the same antibiotics as noted above. Tissue pieces were then transferred to 25 cm^2 flasks (BD Falcon) where approximately 1–2 mL of L-15 supplemented with 20% FBS and the previously described antibiotic concentrations. Primary cultures were established by the explant outgrowth method as previously demonstrated with walleye spleens and fins (Vo et al., 2015a,b; 2016). Flasks were then incubated at 18 °C with media changes occurring every 2–3 days. Only the primary adherent cells from the spleen were able to outgrow and eventually developed into the CHSS cell line.

2.2. Maintenance of cell cultures

CHSS was routinely grown in L-15 supplemented with 20% FBS at 18 °C. Following the first passage, CHSS was subcultured at a 1:2 ratio on a weekly or bi-weekly basis using trypsin (Lonza). CHSS has been maintained for over two years and has undergone over 25 passages. The embryonic Chinook salmon cell line, CHSE-214 was used in this study for comparison. CHSE-214 was routinely grown in L-15 supplemented with 10% FBS at 18 °C and was subcultured at a 1:3 ratio every 1–2 weeks using trypsin.

2.3. Cryopreservation of CHSS

Approximately 3×10^6 CHSS cells at multiple passages were cryogenically frozen in L-15 containing 20% FBS and 10% dimethyl sulphoxide (DMSO, Sigma). Cells were initially frozen at −60 °C overnight and subsequently immersed in liquid nitrogen (−196 °C) for long-term storage. To determine cell viability upon thawing, a trypan blue (Sigma) exclusion test was performed using a haemocytometer under a phase contrast microscope (Leica).

2.4. Optimal growth conditions of CHSS

Optimal temperature of growth for CHSS was analyzed between 4 and 26 °C. CHSS cells were seeded into nine 6-well plates (Fisher Scientific) at a concentration of 2×10^5 cells/well and incubated overnight at 18 °C. Three wells of one plate were used to provide the day 0 cell counts. Two 6-well plates were incubated at each of the four temperatures studied (4, 14, 18 and 26 °C) for 2 weeks. On days 3, 7, 10 and 14, triplicate wells from each temperature were washed with 1 mL of DPBS and cells were dissociated using 300 µL of trypsin (Gibco). Cell counts for each well were determined using a hemocytometer under a phase contrast microscope (Leica). Cell counts for each time interval were averaged and calculated as a percentage of the day 0 growth to determine the percent growth.

To determine the optimal FBS concentration for maintenance of CHSS, nine 6-well plates were seeded with cells as described above. Following overnight adherence at 18 °C, media was removed from all plates and two 6-well plates received 2 mL of either 5% FBS, 10% FBS, 15% FBS or 20% FBS media. Plates were then returned to the 18 °C incubator. On days 3, 7, 10 and 14, triplicate wells from each FBS concentration were washed with 1 mL of DPBS so that cells could be dissociated and counted as described above. Statistical analysis for both optimum temperature and FBS concentration was conducted using a two-way ANOVA and Tukey's post-hoc test through the GraphPad Prism software (v7.0, GraphPad Software, Inc. USA).

2.5. Determining ploidy of CHSE-214 and CHSS

Although CHSS was believed to be created from triploid fish tissue, it was necessary to confirm this assumption through flow cytometric analysis. As a positive control for the diploid karyotype, the ploidy of CHSE-214 (McCain, 1970) was also examined. All cells analyzed were grown to 60% confluency and a media change was performed two days prior to flow cytometric analysis. Single cell suspensions were prepared in 500 µL of DPBS following cell detachment by trypsin-EDTA and two rinses with ice-cold DPBS. Seven milliliters of ice-cold 70% ethanol was used as a fixative and added drop-wise to the cell suspensions while simultaneously vortexing. Fixation was incubated at 4 °C for 30 min. Fixed cells were pelleted at 800 g for 5 min, rinsed once with 5 mL of ice-cold PBS and resuspended in 500 µL of 50 µg/mL RNAse A at 37 °C for 15 min (as performed by Rieger and Barreda, 2016). All contents were transferred to individual 5-mL BD Falcon polystyrene round-bottom test tubes and were then brought to a final volume of 1 mL with ice-cold DPBS. Twenty microliters of 1 mg/mL propidium iodide (Invitrogen) was added to the cell suspensions and incubated at 37 °C for 15 min. DNA content assessment was performed with the FACSAria Fusion Cell Sorter with an integrated 3-laser and 9-color detection unit (BD Biosciences). Data analysis was completed using the FlowJo® software (https://www.flowjo.com).