

# Detection Of P56 In Grouper (*Chromileptes Altivelis*) By Giving Peridinin Chlorophyll Protein (PCP) Using Immunohistochemical

Rima Oktavia Kusuma# Uun Yanuhar\* and Joni Johanda Putra\*

**Abstract:** P56 protein is a protein molecule that has immune activity, which inhibits the initiation of the translation of viral genetic material. The administration of Peridinin Chlorophyll Protein (PCP) is thought to be able to initiate the release of the P56 molecule, detection of protein 56 expression can be carried out using immunohistochemical techniques, namely painting by involving labeled antibodies. This study looked qualitatively at P56 expression in the brain tissue and eyes of grouper rats that had been given Peridinin Chlorophyll Protein (PCP) and infected with Viral Nervous Necrosis (VNN). Based on the results of painting anti-P56 antibodies, it can be seen that P56 is expressed in tissue of fish organs that have been given PCP, as well as in fish exposed to VNN

**Keywords:** P56, Immunohistokimia, PCP, *Chromileptes Altivelis*, VNN

## 1. INTRODUCTION

P56 is a cytoplasmic protein molecule and has no enzymatic activity. They are induced very strongly by interferon  $\alpha$ , the gene that encodes p56 is IFIT 1 [1]. IFIT 1 consists of several tetratricopeptide repeat (TPR), with a motif of 34 amino acids helix-turn-helix. P56 is often also referred to as IFIT 1, they can inhibit mRNA translation initiation by binding to multisubunit eukaryotic translation initiation factor 3 (eIF3) and interfering with the assembly of the preinitiation complex, consisting of the 40S ribosomal subunit, eIF3, eIF2/GTP/Met-tRNA<sub>i</sub> and eIF4F. That interaction caused slowdown virus replication by slowing down the overall cellular metabolism. In reporter, IFIT 1 inhibits HCV IRES –dependent translation even more strongly than it does cap- dependent translation. This inhibition derives from IFIT1 binding to eIF3e [2]. Peridinin Chlorophyll Protein (PCP) is unique light- harvesting antenna, instead of collecting light mainly by chlorophylls (Chl) or bacteriochlorophylls, the main light-harvesting pigment of PCP is Carotenoid, Peridinin (Per), which absorb light between 350 and 550 nm [3]. PCP homodimer or consists of one chloroplast and four rubberenoids measuring 16 Kda, while PCP complexes have a size of 36 Kda [4]. By using X-ray Crystallography it is known that in addition to consisting of pigments namely chlorophyll and carotenoid (peridinin), there is a bond with water, fat in the form of digalactoyl produced by glycerol (DGDG), DGDG binds very strongly with chlorophyll before the complex PCP form is formed. This DGDG is often found in thylakoid membranes. Protein is also involved in this complex bond, several types of amino acids are bound in PCP complexes.[5] In this study we were isolated PCP from the microalgae *Nannochloropsis oculata*. The aim to know whether crude PCP from *Nannochloropsis oculata* is able to induce p56 / IFIT 1. Detection of P56 expression using immunohistochemistry which is a technique which use the antibody-antigen complex that recognized by an enzyme label (peroxidase), resulting in a coloured product after reaction with a specific substrate and chromogenic substrate. These techniques are histologically used for visualization tissue specimen labeling, and to detect localization of antigen.[6]

## II. MATERIAL AND METHOD

Isolation of PCP. Paste of *Nannochloropsis Oculata* as much as 50 grams, placed in a mortar and crushed at a speed of  $8.3 \times 10^7$  rpm for 1 hour, then added liquid nitrogen and crushed again for 30 minutes to one hour. Add 8 ml of Glycine + KCL resuspension buffer. The sample was weighed 2 grams and put in ependof, then cold centrifuged  $4^\circ\text{C}$ , 17000rpm for 60 minutes. The supernatant is removed and placed on a sterile ependof. Supernatant is added with 30% solid ammonium sulfate (SAS) solution from the supernatant. Sentrifuse 15000 rpm;  $4^\circ\text{C}$ ; 30 minutes. Take the supernatant and transfer it to a sterile ependof. The results of the isolation process are then purified by entering the dialysis stage. The dialysis process is carried out for 2 times 24 hours. The dialysis results are filtered with a disposable filter and put in a sterile falcon bottle. Samples of *N. Oculata* were added with a solution of biurate with a ratio of 1:10, put in a sterile ependof then homogenized and covered with aluminum foil, put in an oven (Binder brand) at  $30^\circ\text{C}$  for 30 minutes. Meanwhile blank solution (Biurat solution) is prepared to calibrate the spectrophotometer. Then the sample is measured using a spectrophotometer with a wavelength of 550 nm. Protein concentration was calculated using absorbance values. In vivo test, PCP protein isolate (fwas given to mouse grouper fish orally with the help of feeding tube hose (figure 17B). Giving PCP is done 6 times with a span of every 5 days. Because Dorland (2000) states that the production of antibodies begins on day 5 after exposure until day 14. Begins with IgM production which will last for several days which is then immediately followed by the peak of IgG production. The production of antibodies decreases within a few weeks, but memory cells remain in circulation. VNN exposure was carried out 5 times, giving as much as  $140\mu\text{l}$  starting on the 14th day until the 22nd day. Immunohistochemical assay. Deparaze preparations (paraffin blocks) Grouper Tissue with xylene 3 times each of 3 minute. Rehydration preparations using ethanol 100%, ethanol 95% and ethanol 70% each for two minutes, two minutes, one minute and finally with water for one minute. Soak in peroxidase blocking solution at room temperature for 10 minutes. Incubation of preparations in prediluted blocking serum  $25^\circ\text{C}$  for 10 minutes. Soak the preparation in an  $25^\circ\text{C}$  monoclonal anti-p53 antibody for 10 minutes. Wash preparations with Phosphate Buffer Saline (PBS) for 5 minutes. Incubate preparations with secondary antibodies

(conjugated to horse radish peroxidase) 25 ° C for 10 minutes. Wash preparations with PBS for 5 minutes. Incubation of preparations with peroxidase 25 ° C for 10 minutes. Wash preparations with PBS for 5 minutes. Incubate preparations with DAB chromogen (Diaminobenzinidine) 25 ° C for 10 minutes. Incubation preparations with Hematoxylin Eosin for 3 minutes. Wash preparations with running water. Clean preparations and drops with mounting media. Cover the preparation with coverslip. Observe the expression of p53 (brown) in cells using a light microscope magnification of 1000 x. Documentation of each observation.

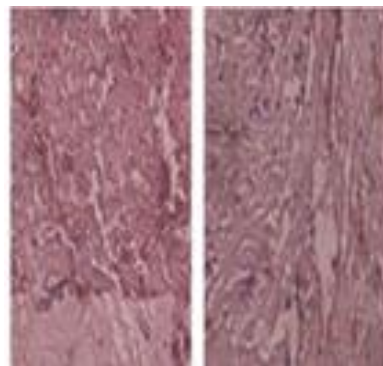
**III. RESULTS AND DISCUSSION**

Immunohistochemical assay were performed on normal grouper brain tissue, grouper fish with PCP *N. oculata* treatment, and grouper fish treated with PCP *N. oculata* with

VNN exposure. Base on Immunohistochemical, there is expretion of P56 at brain tissue of Grouper



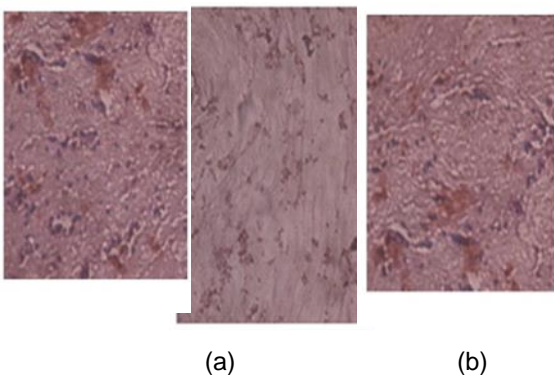
Control fish brain tissue showed P56 expression, although not as much as fish brain tissue given PCP. This shows that PCP is able to be accepted as a foreign body by cells, thereby being able to induce  $\beta / \alpha$  interferon.



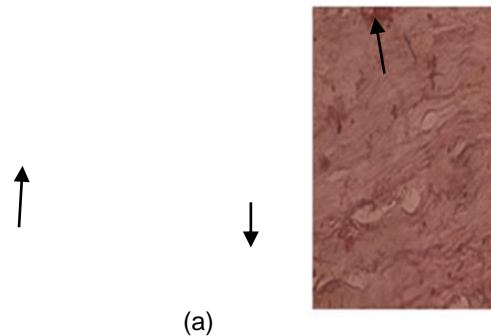
**Figure 1.** (a) brain tissue of grouper control, (b) brain tissue of grouper with PCP

PCP + VNN brain tissue shows more expression than PCP and control brain tissue. Likewise, VNN brain tissue shows the expression of P56 in large numbers. VNN is caused by Betanodavirus, a naked positive-sense single-stranded RNA virus belonging to the family Nodaviridae. The viral genome is made of two genetic segments containing three open reading frames (ORFs). The RNA1 gene of approximately 3.1 kb encodes the viral replicase and the RNA2 segment of ca. 1.4 kb encodes the capsid protein. A third transcript, known as RNA3 (0.4 kb), is cleaved from the RNA1 terminus during viral replication and encodes the B2 non-structural protein, an inhibitor of cell RNA silencing [7].

treatment, and they are mainly induced by IFN- $\alpha/\beta$  Under basal condition, ISGs are not expressed in most cell types. But they can be induced immediately to a high level after virus infection or IFN treatment. Their product stake on diverse roles such as enhancing innate immune capabilities, inhibiting virus infection and negativelyregulating 2380ignalling through the JAK-STAT pathway. Interferon-induced protein with tetratricopeptide repeats 1 (IFIT1) which is an effector molecule in antiviral pathways, locates in the cytoplasm[8].



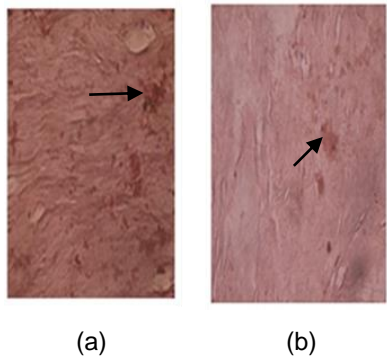
**Figure 2.** (a) brain tissue of PCP+VNN (b)brain tissue of VNN



**Figure 3.** (a) eye tissue of control (b) eye tissue of PCP

The results of the eye tissue in control did not appear P56 expression, while the eye tissue in fish with PCP showed the expression of P56.

Interferon stimulated genes (ISGs) are a subset of genes response to RNA- or DNA- virus infection or type I IFN



**Figure 4.** (a) eye tissue of PCP+VNN, (b) eye tissue of VNN

The PCP and VNN treatments showed a greater amount of expression compared to fish-eye tissue that was only exposed to VNN. This is due to the possibility that a combination of VNN and PCP induction can increase P56 expression. However, this must be investigated more deeply.

## CONCLUSIONS

Based on the results of immunohistochemical detection showed that P56 was expressed in the tissue of fish organs that were given PCP. This indicates that PCP is able to induce P56.

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## REFERENCES

- [1] Pidugo VK, WuMM, Yen AH, Pidugu HB. Chang KW, Liu CJ, Lee TC. IFIT 1 and IFIT 3 Promote oral squamous cell carcinoma metastasis and contribute to the antitumor effect of gemcitabine via enhancing- EGFR recycling. doi: 10.1038/s41388-018-0662-9.2019
- [2] D.I Young, Y Andrejeva, X.Li, Inesta, Vaquera, C. Dong, V.H. Crowling, S. Goodbourn, R.E. Randall. Human IFIT 1 inhibits mRNA Translation of Rubella Viruses but not other members of the paramyxoviridae family. Journal of virology. volume90.2016.
- [3] Sumie Shima, Robielyn P. Ilagan, Nathan Gillespie, Brandi J. Sommer, Roger G. Hiller, Frank P. Sharples. Harry A. Frank, and Robert R. Birge. Two- Photon and Fluorescence spectroscopy and the effect of environment on the photochemical properties of peridinin in solution and the peridinin- chlorophyll- protein from Amphidium Carterae, J Phys.A. 107.8052-8066.2003
- [4] Eckhard Hofmann, Pamela M. Werch, Frank P, Sharples. Roger G Hiller. Wolfram Welte, Kay Diederichs. Structural basis of light harvesting by carotenoids : peridinin- chlorophyll-protein from Amphidium Carterae. http://kops.uni-konstanz.de.1996
- [5] Donatella Carbonera, Marilena Di valentin. Riccardo Spezia and Alberto Mezzetti. The unique photophysical properties of the peridinin- Chlorophyll – Protein. Current protein and peptide science.15,332-350.2014

- [6] Larasati, Adam Hermawan. Muthi Ikawati, Edy Meiyanto. Pengecatan imunohistokimia p53. Cancer Chemoprevention research Center. Hlm 2. http://http://ccrc.farmasi.ugm.ac.id
- [7] Toyohiko Nishizawa, Koh-Ichiro, Mori, Toshihiro Nakai, Iwao Furusawa, Kiyokuni Muroga. Polymerase chain reaction amplification of RNA of striped Jack Nerveous Virus (SJNNV). Diseases of Aquatic Organism. Vol.18:103-107.1994
- [8] Dong Yi Xie, Shi- Ming Wang, Jin Min Yang, Liang- Hui Wang. IFIT 1 polymorphisms predict interferon- treatment efficiency for hepatitis B virus infection. World Journal of Gastroenterology. Doi: 10.3748/wjg.V221449813.2016