Oral Presentation (PAT-3)

# In Vitro Growth Inhibition Activities of Natural (nCaIFN) and Recombinant (rCaIFN) Canine Interferons on Three Different Tumor-Derived Cell Lines.

Bambang Pontjo Priosoeryanto[1*], Rizki Rostantinata[1], Eva Harlina[1], Lina Noviyanti[2]

[1]Division of Veterinary Pathology, Division of Veterinary Pharmacy; Department of Veterinary Clinic, Reproduction & Pathology; Faculty of Veterinary Medicine;
Bogor Agricultural University (IPB), Kampus IPB Dramaga 16680 Bogor, Indonesia
[*]Corresponding author: bpontjo4@gmail.com

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## INTRODUCTION

Recently, from many kinds of diseases one could be counted is a tumor disease. Tumor is a degenerative disease that involved many biological pathways within the host. A tumor or neoplasm can be defined as a disturbance of growth characterized by excessive, abnormal and uncontrolled proliferation of transformed or altered cell(s) at one or more primary points within the host, and frequently at one or more metastatic sites (Priosoeryanto, 1994).

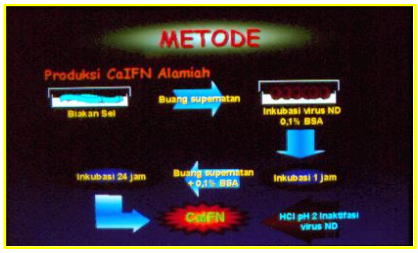
The treatment of tumor is mostly by medical surgery which usually combined with chemotherapeutic agent(s), unfortunately the using of chemotherapeutic agent can develop a seriously side effect to the treated-patients. Biological substances like interferon (IFN) known as anti-virus agent is also one of promising candidate for treating or preventing of tumor disorders.

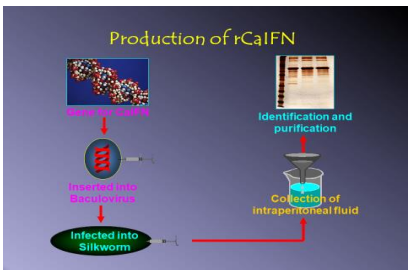
Feline and canine squamous cell carcinoma is one of tumor type that often found in cat and dog, and mostly develop to a very aggressive disorder. The using of in vitro cell culture is a way to avoid the use of live animals on the study of tumors especially the study of antitumor agent due to can mimic the in vivo condition. The aim of the present study is to examine the growth inhibition activity of natural and recombinant canine interferon in order to find the suitable biological substances for combating tumor disorder especially in the field of veterinary medicine and also as an information for the development of tumor treatment in the human medical side.

## MATERIAL AND METHODS

### Interferon Production

The method of natural and recombinant canine interferons production was used of our previous study (Priosoeryanto et al, 2000). For the production of natural canine interferon (nCaIFN), briefly the canine MDBK cells were infected with the Kumarov strain of New Castle Disease virus and incubated for 3-4 days. The culture medium was then added by HCl acid until pH 2 and incubated for another 4 days. The medium that contain interferon were then collected, extracted, purified and stored in the refrigerator until assays was performed. The production of recombinant canine interferon (rCaIFN) was conducted based on our previous result (Priosoeryanto et al, 2000). The bioreactor method using silkworm larvae (*Bombyx mori*) that infected with a Baculovirus recombinant CaIFN containing plasmid was used to produce a sufficient amount of rCaIFN. The same assays as nCaIFN to analyze the rCaIFN was also performed.




### Growth Inhibition Assay

Growth inhibition assay of natural and recombinant canine interferon were performed using three tumor-derived cell lines Canine Squamous Cell Carcinoma (CSCC), Feline Squamous Cell Carcinoma (FSCC) and MCM-IPB/B3 cell lines (Priosoeryanto et al, 1995a,b). Cells were cultured using microplate 24 wells in a complete culture medium (DMEM, 10% FCS, and antibiotics). The cells were treated with 6 different concentrations of nCaIFN and rCaIFN started from

$10^0$, $10^1$, $10^2$, $10^3$, $10^4$ $10^5$ U/mL and Doxorubicin as a control positive. The cells were cultured in three replicates. Cultivated cell line was incubated in an incubator 37 ° C, 5% CO2. After 4 days in culture, the total number of cells were counted using a Neubauer Hemacytometer with Trypan Blue dye exclusion Freshney (2010). The cell number were then averaged and analyses for the growth inhibition activity (Priosoeryanto et al, 2009).

## RESULT AND DISCUSSION

Two kinds of Canine interferon were successfully produced by in vitro and in vivo methods. Both interferons were assayed for their growth inhibition activity in three different tumor-derived cell lines. There is an activity of growth inhibition by these two types of interferons (Figure 1 & 2). The growth inhibition activity of these two interferons was quietly similar in each tumor cells (canine and feline cells), even the activity seems more sensitive to the canine cells compared to the feline cells. These phenomenon was agreed to our previous study stated that type of the interferon more sensitive to the tumor cells from the same species, even there are still some significant activity to the different closer species (Priosoeryanto et al, 1995).

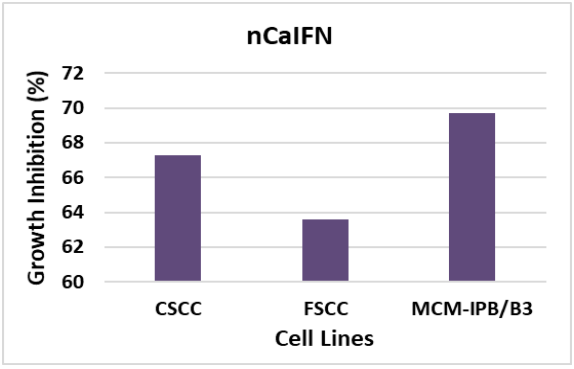



**Figure 1.** The growth inhibition activity of natural canine interferons on three different tumor derived cell lines.

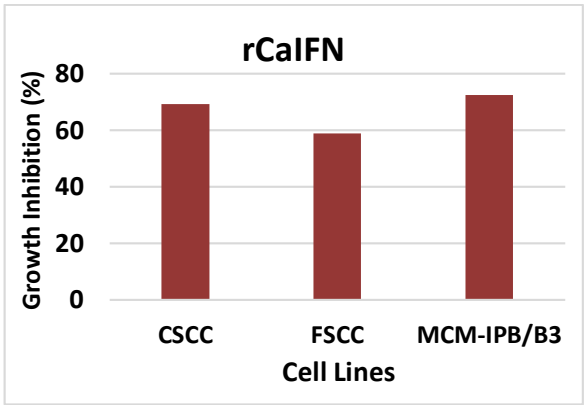



**Figure 2.** The growth inhibition activity of recombinant canine interferons on three different tumor derived cell lines.

Interferon known as an antiviral to some infectious diseases in human and animals (Srivasta et al, 2014) There are three types of interferons; type I interferon including alpha, beta and omega, binding IFN-alfa/beta receptor 1 (IFNAR1) and IFNAR2 subunits, type II (gamma) that binds IFN-gamma receptor 1 (IFNGR1) and type III (lambda) which binds the IFN-lambda receptor 1 and IL10 receptor subunit beta-heterodimeric receptor (Parker et al, 2016).

Interferon can indeed affect cell proliferation in tumor cells both by prolonging or blocking the cell cycle (Hobeika et al, 1997), IFN can also regulate the apoptotic machinery by controlling the extrinsic and intrinsic apoptotic pathways (Choie et al, 2003; Thyrell et al, 2002).

## CONCLUSION

Natural and recombinant canine interferons has a potential effect to be develop as an antitumor substance for treatment of tumor disorders in animals. We suggest a further study that these cytokines can be combine with some bioactive compound of medicinal herbs to increased its antitumor activity.

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