

Attn: Dear Dr. Aisyah Elliyanti
Medical Physics and Radiology
Faculty of Medicine, Universitas Andalas
Padang, West Sumatra, Indonesia

August 31th, 2017

Dear Dr. Aisyah Elliyanti,

It is our great pleasure to inform you that the 12th Asia Oceania Congress of Nuclear Medicine and Biology (AOCNMB 2017) to be held from October 5 to 7, 2017 in Yokohama, Japan. in conjunction with:

The 57th Annual Scientific Meeting of the Japanese Society of Nuclear Medicine (JSNM)
The 37th Annual Scientific Meeting of the Japanese Society of Nuclear Medicine Technology (JSNMT)
The 7th Annual International Conference of the Asia Society of Nuclear Medicine (ASNMT)

Congratulations! Your abstract has been reviewed and accepted by the program committee of AOCNMB2017, as an oral presentation.

Submission Number : C000231

Title : Induction of Sodium Iodide Symporter Expression in Breast Cancer Cell Lines

Date : October 7th, 2017 11:20 -11:30

Venue : PACIFICO Yokohama 1-1-1, Minato Mirai, Nishi-ku, Yokohama, Japan

Your abstract will be published online in the program of AOCNMB2017.

We are sorry that AOCNMB is not providing any kind of financial assistance.
Thank you for your kind understanding.

Yours sincerely,



Tomio Inoue,
President, AOCNMB 2017
Professor, Yokohama City University Graduate School of Medicine



Tomohiro Kaneta,
Program Committee Chair, AOCNMB 2017
Yokohama City University Graduate School of Medicine

Date **October 5-7, 2017** Venue **PACIFICO Yokohama** President **Tomio Inoue** (Yokohama City University Graduate School of Medicine)

Theme **Connecting people for the bright future of Asia-Oceania nuclear medicine**

In Conjunction with The 57th Annual Scientific Meeting of the Japanese Society of Nuclear Medicine (JSNM)
The 37th Annual Scientific Meeting of the Japanese Society of Nuclear Medicine Technology (JSNMT)
The 7th Annual International Conference of the Asia Society of Nuclear Medicine Technology (ASNMT)

Congress Secretariat The 12th Asia Oceania Congress of Nuclear Medicine and Biology (AOCNMB2017)
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第57回 日本核医学会学術総会

The 57th Annual Scientific Meeting of the Japanese Society of Nuclear Medicine



第37回 日本核医学技術学会総会学術大会

The 37th Annual Scientific Meeting of the Japanese Society of Nuclear Medicine Technology



第12回アジア・オセアニア核医学会学術会議

The 12th Asia Oceania Congress of Nuclear Medicine and Biology



第7回アジア核医学技術学会国際会議

The 7th Annual International Asian Society of Nuclear Medicine Technology Conference

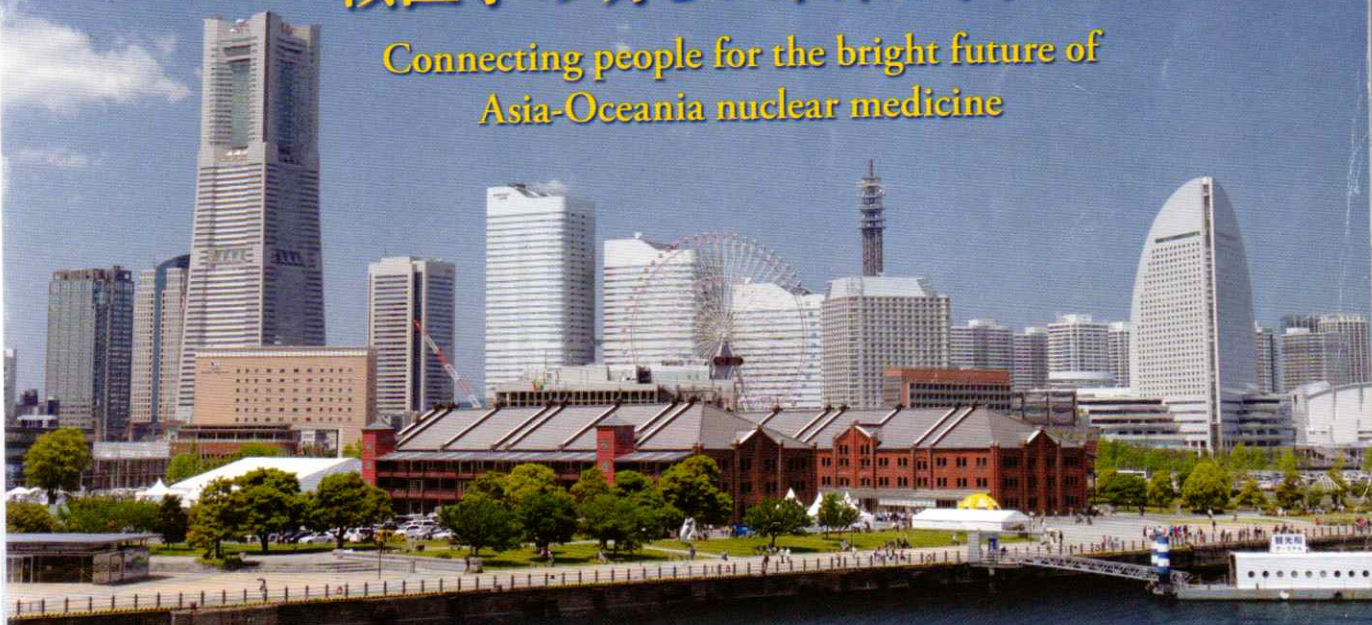
2017年10月5日(木) ▶ 7日(土)
October 5 (Thursday) - 7 (Saturday), 2017

パシフィコ横浜
PACIFICO Yokohama

Program

核医学の明るい未来に向けて

Connecting people for the bright future of
Asia-Oceania nuclear medicine



第57回 日本核医学会学術総会
第12回アジア・オセアニア核医学会学術会議
会長 井上 登美夫

第37回 日本核医学技術学会総会学術大会
大会長 渡邊 浩

第7回 アジア核医学技術学会国際会議
会長 片渕 哲朗

Tomio Inoue, Congress President of
The 57th Annual Scientific Meeting of the Japanese Society of Nuclear Medicine
The 12th Asia Oceania Congress of Nuclear Medicine and Biology

Hiroshi Watanabe, Congress President of
The 37th Annual Scientific Meeting of the Japanese Society of Nuclear Medicine
Technology

Tetsuro Katafuchi, Congress President of
The 7th Annual International Asian Society of Nuclear Medicine Technology Conference



**The 57th Annual Scientific Meeting of
the Japanese Society of Nuclear Medicine**



**The 37th Annual Scientific Meeting of
the Japanese Society of Nuclear Medicine Technology**



The 12th Asia Oceania Congress of Nuclear Medicine and Biology

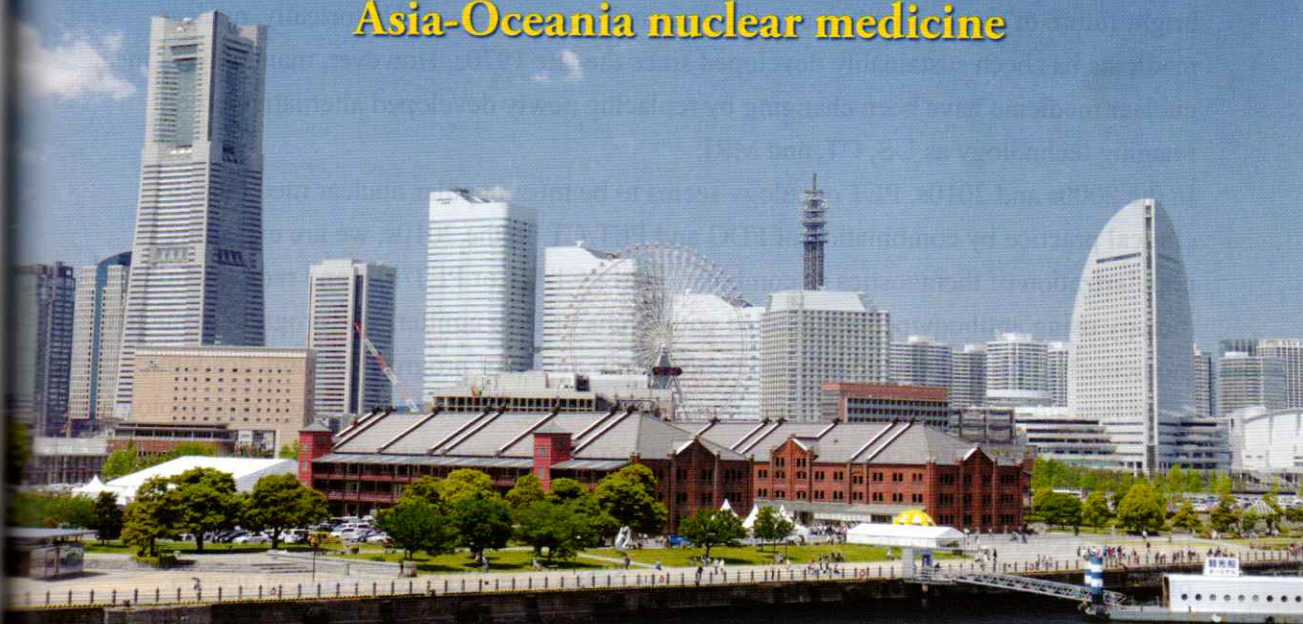


**The 7th Annual International
Asian Society of Nuclear Medicine Technology Conference**

**October 5 (Thursday) – 7 (Saturday), 2017
PACIFICO Yokohama**

Program

**Connecting people for the bright future of
Asia-Oceania nuclear medicine**



Tomio Inoue, Congress President of

The 57th Annual Scientific Meeting of the Japanese Society of Nuclear Medicine
The 12th Asia Oceania Congress of Nuclear Medicine and Biology

Hiroshi Watanabe, Congress President of

The 37th Annual Scientific Meeting of the Japanese Society of Nuclear Medicine Technology

Tetsuro Katafuchi, Congress President of

The 7th Annual International Asian Society of Nuclear Medicine Technology Conference

The 57th Annual Scientific Meeting of the Japanese Society of Nuclear Medicine
The 12th Asia Oceania Congress of Nuclear Medicine and Biology (AOCNMB 2017)

GREETING

“Sustainable Future Development of Nuclear Medicine in the Asia and Oceania Region”



Tomio Inoue MD

Congress President of JSNM and AOCNMB 2017

(Department of Radiology, Yokohama City University Graduate School of Medicine)

The reason that I determined “Connecting people for bright future of nuclear medicine in the Asia and Oceania region” as a key phrase of AOCNMB 2017 is that I believe Connecting people by academic activity of AOCNMB is the essential strategy to reach the bright future of nuclear medicine in the Asia and Oceania region. Historically, nuclear medicine has been sustainably developed since the late 1970s. However, main players in nuclear medicine have been changing by replacing newly developed alternative other imaging technology as US, CT, and MRI.

In the 2000s and 2010s, PET oncology seems to be most popular nuclear medicine in clinical practice by combination of FDG and PET/CT. In the 2010s, we are expecting popularization of theranostics as combination of PET/CT or PET/MR and radionuclide therapy using antibody/peptide labeled with Alpha or Beta emitter. The progress in nuclear medicine had been always supported by the combination of progress of many academic fields beyond nuclear medicine, including pharmacology, molecular biology, immunology, chemistry, engineering, computer science, and so on. In other ward, nuclear medicine essentially requires an interdisciplinary connecting people.

In 2015, General assembly of the United Nation adopts the following outcome documents of the United Nations summit for the adoption of the post-2015 development agenda: “Transforming our world: the 2030 Agenda for Sustainable Development”. The Agenda that is a grand plan of action for people, planet and prosperity is not directly related to the future of nuclear medicine in the Asia and Oceania region, but we should consider and understand the grand concept for human being of the 2030 Agenda to make a plan of future development of nuclear medicine in the Asia and Oceania region until 2030. Among 17 sustainable development goals(SDGs) defined in the Agenda 2030, goal 3 is to ensure

healthy lives and promote well-being for all at all age which nuclear medicine could contribute. In the 2030 Agenda for sustainable developments, emerging countries should play a certain role for keeping sustainable developments as well as the advanced countries. The strategy of AOFNMB should be to adapt this scheme for sustainable developments of nuclear medicine in the Asia and Oceania region. As the nuclear medicine in East Asian countries has been rapidly developed with economic growth in each country in the 2000s, a certain different advanced field and technology of nuclear medicine in each developed country should be introduced into the developing countries under the scheme of the Agenda 2030 of UN. While the nuclear medicine leaders in developing country must play a role of stakeholder for spreading the technology in their own country and to keep the sustainable development goals. For keeping SDGs in the field of nuclear medicine, AOFNMB must go forward in the three directions of standardized education, standardization of NM examinations procedures, and innovative research of imaging devices as total-whole body PET/CT and/or novel radiopharmaceuticals. As standardization of NM examinations procedures, the concept and profiles of Quantitative Imaging Biomarker Alliance (QIBA) under RSNA might be important to keep sustainable developments in the field of nuclear medicine in the Asia and Oceania region.

The 37th Annual Meeting of Japanese Society of Nuclear Medicine Technology

GREETING



Hiroshi Watanabe RT, PhD
Congress president of JSNMT2017
(Japan Organization of Occupational Health and Safety, Yokohama Rosai Hospital)

As the congress president of the 37th Annual Meeting of Japanese Society of Nuclear Medicine Technology (JSNMT2017), I would like to express my sincere gratitude to the members and people concerned.

This annual meeting will be held on October 5th (Thursday) to 7th (Saturday), 2017, in Yokohama. And, this annual meeting will be held jointly with the 57th Annual Scientific Meeting of the Japanese Society of Nuclear Medicine (JSNM), the 12th Asia Oceania Congress of Nuclear Medicine and Biology (AOCNMB) and the 7th Annual International Meeting of Asian Society of Nuclear Medical Technology (ASNMT).

The theme of this annual meeting is "Connecting people for the bright future of Asia-Oceania nuclear medicine". The environment surrounding the nuclear medicine is evolving day by day. Therefore, under such circumstances, we plan for various discussions with members and participants for the bright future of nuclear medicine, such as the JSNM and JSNMT Joint Symposium, the JSNMT Symposium, the basic course, the new image contest and the JSNMT and ASNMT joint international session etc. Also, the joint meeting of the JSNM and the JSNMT is the 10th year. We would like to collaborate with JSNM organizing committee members and hold a wonderful meeting for further development of nuclear medicine.

Furthermore, the Yokohama Minatomirai district where the venue is located is a worldwide tourist destination. There are many famous tourist sites such as Yokohama Red Brick Warehouse, Yokohama Landmark Tower, Yokohama Chinatown. Please enjoy sightseeing as well.

Finally, this annual meeting will be held in Yokohama (in Kanagawa prefecture) for the first time in a while. Therefore, we receive support from many people such as the Kanagawa society of nuclear medicine and the Kanto regional association of nuclear medicine technology, etc.. We appreciate all of you for your cooperation. we sincerely looking forward to seeing all of you at JSNMT2017 in Yokohama in 2017.

The 7th



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The 7th Annual International Conference of Asian Society of Nuclear Medicine Technology

President's Greeting

“Expect for nuclear medicine technology in Asia”



Conference Chair; Tetsuro Katafuchi, RT, PhD
(Gifu University of Medical Science)

We are pleased to announce that the 7th Annual International Conference of the Asian Society of Nuclear Medicine Technology (ASNMT) will be held at PACIFICO Yokohama on October 6, 2017. It is a great honor for us to host the conference.

ASNMT holds the annual conference for mutual exchange in academic and clinical nuclear medicine technology since 2011. Our objective is to contribute to the development of nuclear medicine technology in the Asian region through the many technologists who attend the annual conference from various countries.

The 7th ASNMT is jointed with the 57th Annual Scientific Meeting of the Japanese Society of Nuclear Medicine, the 37th Annual Meeting of the Japanese Society of Nuclear Medicine Technology and the 12th Asia Oceania Congress of Nuclear Medicine and Biology, which congresses will be precious meetings for academic exchanges. This conference program plans the oral presentations and poster presentations in academic study, and besides, special speaker is invited lecturer from Australia.

On behalf of the steering committees of ASNMT, I would like to ask you that you understand the purpose of ASNMT and give us great supports and guidance to make the conferences success.

**The 57th Annual Scientific Meeting of the Japanese
Society of Nuclear Medicine
The 12th Asia Oceania Congress of Nuclear Medicine
and Biology (AOCNMB 2017)**

Date October 5(Thursday) – 7(Saturday), 2017

Venue PACIFICO Yokohama
1-1-1 Minatomirai, Nishi-ku, Yokohama 220-0012, Japan

In conjunction with

The 37th Annual Meeting of the Japanese Society of Nuclear
Medicine Technology
The 7th Annual International Conference of the Asian Society of
Nuclear Medicine Technology

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Zhaohui Zhu (Peking Union Medical College Hospital, China)
Zvi Bar-Sever (Schneider Children's Medical Center of Israel, Israel)

RI therapy**Saturday Oct.7 10:00-11:00, Room 7**

Chair : Suresh C. Srivastava (Brookhaven National Laboratory, USA)

Tetsuya Higuchi (Department of Diagnostic Radiology and Nuclear Medicine, Gunma University)

- BM3VIIC-01** Bevacizumab radioimmunotherapy (RIT) in triple-negative breast cancer (TNBC) xenograft
(Radiology and Nuclear Medicine, Gunma University) **Ryan Yudistiro**
- BM3VIIC-02** ^{177}Lu -hydroxyapatite (Lu-177 HA) in Radiosynovectomy of knee joints due to Rheumatoid arthritis.
(Department of Nuclear Medicine and PET/CT, Kovai Medical Center and Hospital, India) **Kamaleshwaran Koramadai Karuppusamy**
- BM3VIIC-03** Preclinical study of α -RIT against liver metastasis of gastric cancer
(Graduate School of Medical and Pharmaceutical Sciences, Chiba University) **Huizi Keiko Li**
- BM3VIIC-04** Evaluation of Radiation Exposure Rate after I-131 Treatment of Patients
(Division of Nuclear Medicine, Chi Mei Medical Center, Taiwan) **Chien- hua Lu**
- BM3VIIC-05** Efficacy of 1110 vs. 3700 MBq RAI in Remnant Ablation of Low Risk Dtc: A Meta-Analysis
(Philippine Heart Center, Philippine) **Henry Gochoco Canizares**

Thyroid, Breast**Saturday Oct.7 11:00-11:50, Room 7**

Chair : Mei Tian (Zhejiang University)

Sze Ting Lee (Austin Hospital, Australia)

- BM3VIID-01** Response prediction of neoadjuvant therapy by dedicated-breast PET: compared with MR volumetry
(Diagnostic Imaging and Nuclear Medicine, Kyoto University Graduate School of Medicine) **Masako Kataoka**
- BM3VIID-02** The Effect of FDG-PET/CT in Response of Breast Cancer Therapy
(Faculty of Paramedicine, Semnan University of Medical Sciences, Semnan, Iran) **Mohsen Shoja**
- BM3VIID-03** Induction of Sodium Iodide Symporter Expression in Breast Cancer Cell Lines
(Medical Physics and Radiology, Universitas Andalas, Singapore) **Aisyah Elliyanti**
- BM3VIID-04** Importance of isolated raised thyroglobulin antibody in follow up and management of differentiated thyroid cancer
(Nuclear Medicine, Tata Medical Center, India) **Ray Soumendranath**
- BM3VIID-05** Can ^{18}F -FDG-PET predict ^{131}I therapeutic response in metastatic differentiated thyroid carcinoma?
(Department of Diagnostic Radiology and Nuclear Medicine, Gunma University) **Xieyi Zhang**

Oral 3rd Day

Induction of Natrium Iodide Symporter Expression in Breast Cancer Cell Lines

Aisyah Elliyanti¹, Andani Eka Putra², Yunia Sribudiani³, Noormartany⁴, Johan S. Masjhur⁵, Tri Hanggono Achmad³

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Natrium iodide symporter (NIS) is an iodide co-transporter, which plays a pivotal role in radioiodine uptake in breast cancer cells. The level of NIS expression in breast cancer tissues was reported to be varied between studies. Adenosine triphosphate (ATP) and epidermal growth factor (EGF) are proliferative agents which activate extracellular signal-regulated kinase (ERK). The signaling pathway has been known, play an important roles in NIS expression regulation. The aim of this study is to investigate the effect of ATP, EGF and combination of those two to the NIS expression in MCF7 and SKBR3 cell lines.

MCF7 cell line is representing the luminal type of breast cancer and SKBR3 cell line is representing the human epidermal receptor 2 (HER2) type. HacaT cell line, a non-cancer cell, is used as control. Cells were treated with ATP, EGF and combination of those two for 6, 12 and 24 hours. The expression of NIS *mRNA* was measured through quantitative reverse transcription-polymerase chain reaction (qRT-PCR). The NIS protein expression was confirmed by immunocytofluorescence.

Natrium iodide symporter was expressed in SKBR3 and in HacaT cells with or without ATP and EGF treatment. However the same treatment did not induce NIS expression in MCF7 cells. The combination of ATP and EGF treatment for 24 hours enhanced the level of NIS *mRNA* expression in SKBR3 cell line by 1.6 folds compared to that of untreated cells ($p < 0.05$). It also increased NIS protein expression at membrane cells in SKBR3 cell line.

The level of NIS expression is different among subtypes of breast cancer cell lines. ATP and EGF combination treatment increases NIS *mRNA* and protein expression in SKBR-3 cells line.

Keywords: Natrium iodide symporter, Breast cancer cell lines, Epidermal growth factor, Adenosine triphosphate

Introduction

Natrium Iodide Symporter is a plasma membrane glycoprotein. It transports two ions of natrium (Na^+) and one of iodide (I^-) and maintained by Na^+/K^+ ATPase^{1,2}. In thyroid cells, NIS plays a pivotal role to accumulate an iodine. Additionally, NIS is also expressed in breast cancer tissue and has been considered as a potential target

for radioiodine therapy. However, NIS expression in breast cancer tissues had been reported to be varied between studies. Furthermore, molecular mechanism of NIS expression in breast cancer remains unclear. Interestingly, it has been reported that NIS is highly expressed in invasive breast cancer tissues. Study by Tazebay *et al* reported that indeed there is a correlation between NIS expression and malignant transformation of human breast tissue^{3,4}.

Other than the variation of NIS expression level between molecular subtypes of breast cancer, the location of NIS expression is another factor that may influence the uptake of radioiodine by breast cancer cells. Sodium Iodide Symporter is supposed to be localized at membrane cell instead in the cytoplasm to be fully functional as iodine co-transporter^{5,6}. Several agents were used to increase NIS expression in breast cancer cells. The effect of those agents had been proven could increase radioiodine uptake and cells susceptibility toward the treatment. In T47D cell line, epidermal growth factor (EGF) treatment was reported to have an increase in NIS expression at membrane cell⁷. Another study reported that a combination of ATP with all-trans-Retinoic Acid (tRA) and hydrocortisone has increased NIS protein expression and cell membrane targeting in MCF7 cells⁸.

In this study, we examined the effect of EGF and ATP treatment for NIS *mRNA* and protein expressions in SKBR3 and MCF7 breast cancer cell lines. MCF7 and SKBR3 cell lines are representing the luminal and HER2 type of breast cancer respectively⁹. Hacat cell line, a human keratinocyte cell line, is used as a control.

Material and Methods

Cell lines and culture conditions

SKBR3, MCF7 and Hacat cell lines were used in this study. SKBR3 was obtained from the American Type Culture Collection (ATCC). MCF7 and Hacat cell lines were kindly provided by Dr. Ahmad Faried from faculty of Medicine, Universitas Padjadjaran, Bandung-Indonesia. MCF7 and Hacat cells were cultured in RPMI 1640 medium (Sigma-Aldrich). SKBR3 was cultured in McCoy's 5A medium (Sigma-Aldrich). Both cell culture mediums were supplemented with 10% fetal bovine serum (Sigma-Aldrich), 1% Penicillin, 1% Streptomycin and

1% amphotericin B. The cells were incubated at 37°C and supplied with 5% of carbon dioxide (CO₂) until 80% confluence. The cells were incubated in serum-free medium overnight, and followed by treatment of EGF 50ng/ml (Abcam #ab9697), ATP 100μM (Sigma-Aldrich # 1388), and a combination of EGF and ATP, for 6, 12 and 24 hours. The cells without any treatment were used as a control

Quantitative Real-time reverse transcriptase –PCR (qRT-PCR)

The cells were harvested at the appropriate time points by trypsinized, and then followed by centrifuged at 1000 rpm for a period of 4 minutes. The total RNA was isolated by using RNeasy mini kit (Qiagen #74106) following the manufacturer's instructions. RNA was quantified using Nanodrop 2000 and 5 ng of RNA was reverse transcribed and analyzed by one step real-time quantitative PCR using Rotor Gene Quantitect probe RT-PCR (Qiagen # 204443) using NIS and beta actin primers. The NIS expression fold change of treated cells to those untreated was analyzed using a method describe by Livak et al¹⁰. Three independent qPCR assays were conducted in triplicate.

Immunocytofluorescence

The cells were seeded on coverslips in 4 wells of a 24-wells culture plate and fixated by 4% paraformaldehyde for 15 minutes. This was followed by incubation with protein blocking agent fluorescein-isothiocyanate (FITC) for 15 minutes. The cells were rinsed twice with an ice-cold PBS and followed by an overnight incubation with 2μg/ml rabbit polyclonal antibody anti-NIS (Abcam, #ab83816) at 4°C. The cells were rinsed three times with PBS and incubated at room temperature with Goat anti-Rabbit IgG secondary polyclonal antibody, with dilution 1:1500 (Abcam, # ab6716) for one hour. The cells were rinsed three times with PBS. The coverslips were placed over slides and mounted with fluoroshield containing DAPI. The slides were inspected under immunofluorescence microscope (Olympus BX51) with 200x magnification. Cells which only incubated with secondary antibody were used as negative control.

Statistical analysis

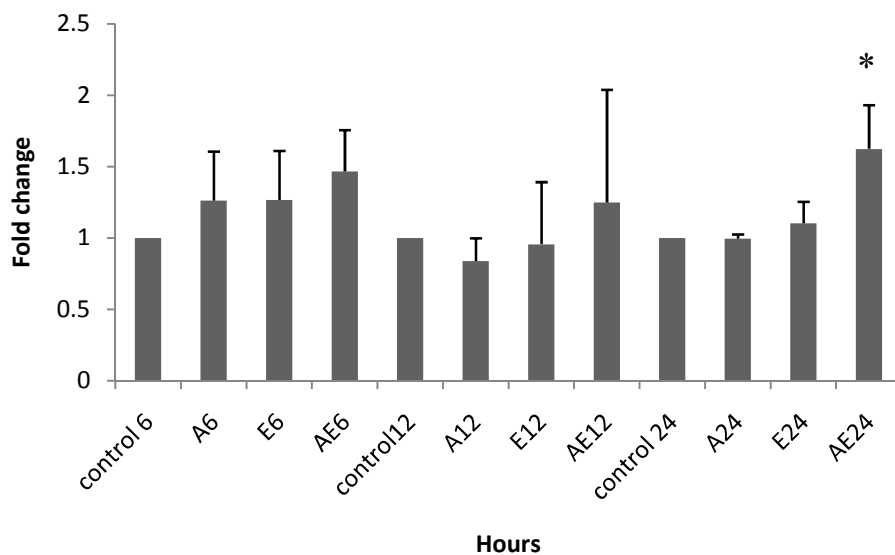
Collected results are expressed as mean \pm SD. Data were analyzed using ANOVA method followed by Tukey test. SPSS was used in all analyses. $P < 0.05$ was considered statistically significant.

Results

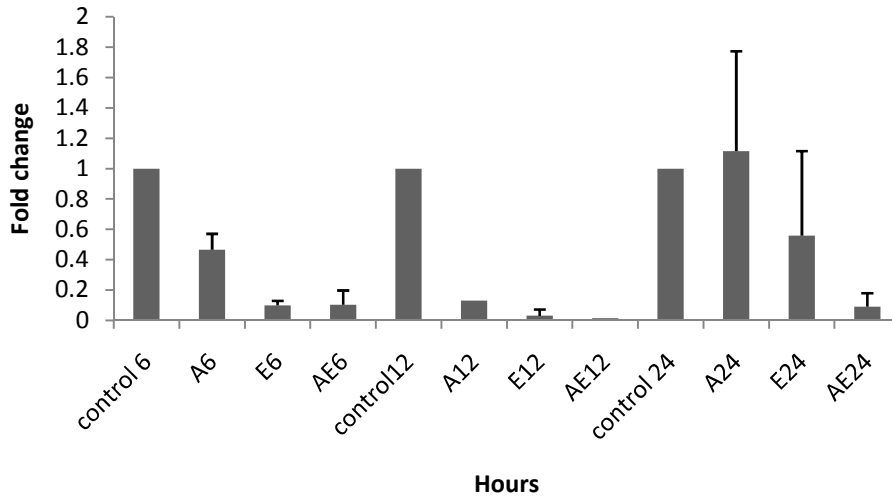
Effect of EGF and ATP treatment on NIS *mRNA* expression

We observed that NIS *mRNA* is expressed in SKBR3 and HacaT cell lines but not in MCF7. In order to examine the effect of EGF and ATP on NIS expression level, we treat all cell lines with ATP, EGF and the combination of both.

The levels of NIS *mRNA* expression, after treatment by EGF, ATP or the combination of both for 6 and 12 hours were not significantly different from those of untreated cells (figure 1A). However, the treatment by a combination of ATP and EGF for 24 hours increases the level of NIS *mRNA* expression by 1.6 fold higher than that of the untreated cells (1.6241 ± 0.3 , $p < 0.05$). The treatment of ATP, EGF and the combination of both in HacaT cells do not increase the level of NIS *mRNA* expression (figure 1B).



1A



1B

Figure 1:(A)represents the effect of ATP (A), EGF (E) and a combination of ATP and EGF (AE) for 6, 12 and 24 hours treatment on NIS *mRNA* expression. The combination of ATP and EGF (AE) for 24 hours treatment increases NIS *mRNA* expression of SKBR3 cell significantly when compared to untreated cells, $P < 0.05$. (B) NIS *mRNA* expression of Haca cells remains unchanged for both treated and untreated cells, $P > 0.05$.

Effect of EGF and ATP treatment on NIS protein expression

Results of immunofluorescence showed that NIS protein expression in SKBR3 (figure 2). The NIS expression in this cell is mostly found in cytoplasm. The treatments of EGF and the combination of both for 12 and 24 hours increase NIS protein expression at membrane cell (figure 2B-C). The cells which receive ATP only for 24 hours show a slight increase in NIS protein expression at membrane cell.

Moreover, NIS expression can be detected in Haca cells with and without treatments. Treatments of these cells by ATP, EGF and the combination of both induce increasing of the expression NIS protein only in cytoplasm (figure 3). On the other hand, we cannot detect NIS protein expression in MCF7 cell line. This result is in agreement with the results of qRT-PCR.

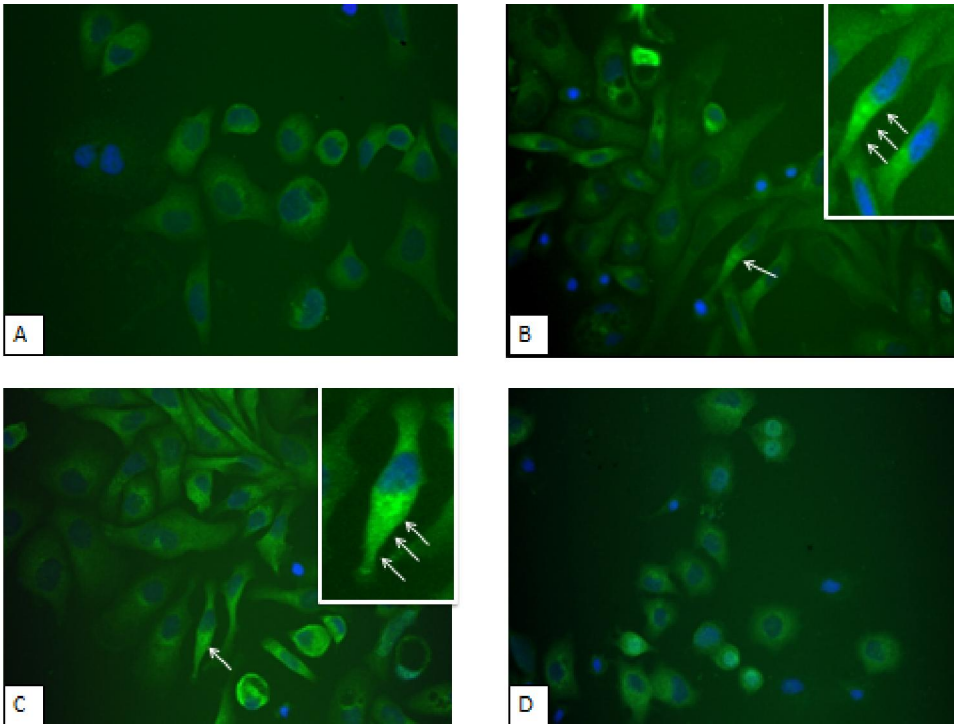


Figure 2: Immunofluorescence staining using primary antibody against NIS in SKBR3 cell line (green) and merging with DAPI staining (blue) at 200x magnification. (A,B,C) NIS is expressed after 24 hour treatments with ATP, EGF and the combination of both respectively. NIS expression in SKBR3 cells increase both in cytoplasm and membrane after 24 hours treatment by both ATP and EGF. (D) untreated cells

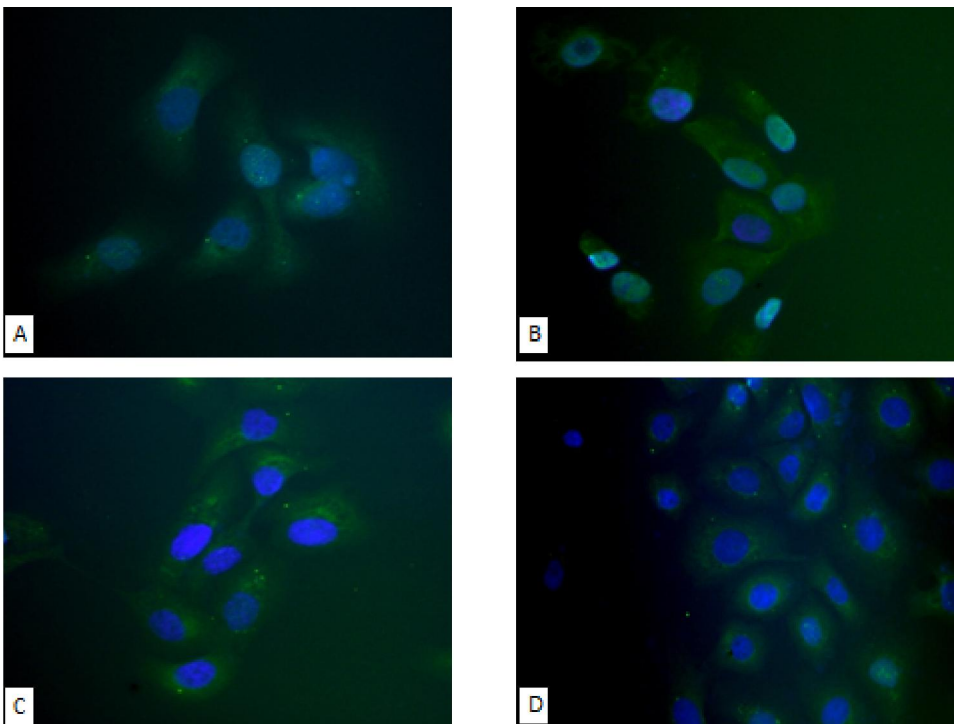


Figure 3: Immunofluorescence staining using primary antibody against NIS in Hacat cell line (green) and merging with DAPI staining (blue) at 200x magnification. (A,B,C) NIS is not expressed after 24 hour treatment of ATP, EGF and the combination of both, respectively and (D) untreated cells. NIS protein expression of cells that are either treated or untreated are expressed in cytoplasm.

Discussion

The expression of NIS in extra-thyroid tissues has been reported in some previous studies, however the results were vary. These findings have been considered as potential for radioiodine therapy in extra-thyroid tumor, including breast cancer. A study by Tazebay, et al reported that NIS was expressed in more than 80% of invasive breast cancer tissues. Another study by Wapnir, et al reported that the expression was found in 76% of invasive breast cancer tissues. Studies by other groups reported that 34% out of 44 breast cancer tissues express NIS. They also reported that 65.5% out of 23 triple negative breast cancer tissues express NIS^{11,12,13}.

In this study, we find that NIS *mRNA* and protein are expressed only in SKBR3 cells. Furthermore, the expression of NIS is not detected in MCF7 cells. As previously published studies on MCF7 cells for the detection of NIS expression, they reported that adding retinoid acid (RA) is mandatory^{8,14}. Thus, it can safely be assumed that NIS expression in MCF7 is RA-pathway dependent. Till date, the molecular mechanisms of NIS expression in breast cancer are not clearly defined. So that, further studies on targeted radioiodine therapy for breast cancer are needed. Meanwhile, it is important to determine the molecular breast cancer types which express NIS as potential candidate to receive radioiodine.

It has been established that MCF7 cell line type of breast cancer model is luminal A, with estrogen and progesterone receptors positive (ER⁺/PR⁺) and HER2 receptor negative (HER2⁻). SKBR3 is a breast cancer cell line with HER2 receptor positive (HER2⁺), estrogen and progesterone receptors negative (ER⁻/PR⁻)^{9,15}. A study by Oh et al, reported that NIS expression is not correlated with the level of hormonal receptors, such as ER/PR. Hormones that regulate NIS expression in breast lactating stage may not play a role in breast cancer¹⁶. Moreover, a study by Dohan et al reported that prolactin, oxytocin and estrogen did not induce NIS expression⁸. The efficacy of radioiodine therapy for breast cancer will be likely dependent on the level of NIS expression at the membrane cell. Therefore, stimulating of the NIS expression at membrane cell will increase iodine uptake. Several agents are used to increase NIS expression, such as retinoic acid, hormones and proliferative agents^{2,7,17}. Epidermal growth factor and ATP are classified into proliferative agent. Binding EGF with EGF receptor will stimulate the tyrosine kinase activity and induce cell proliferation¹⁸. Another study, by Tazebay et al, reported that there is a correlation between NIS expression and malignant transformation of human breast

tissue^{3,4}. This leads us to speculate that one of the molecular mechanism to induce cells proliferation is by EGF-EGFR pathway to up-regulate the NIS expression.

In this study, we observed that NIS *mRNA* expression and protein localization at membrane cell increases by the combination of ATP and EGF treatment in SKBR3 cells (*Figure 2*). Treatment of ATP and EGF alone do not increase the level of NIS *mRNA*; but, increases the membrane NIS protein after 12 and 24 hours treatments. A Study by Jung KH et al reported that EGF treatment augmented NIS mRNA and protein expression in T47D cell line, which transfected with NIS. However, further investigation is needed to understand the molecular mechanism of NIS stimulation in HER2 type by EGF and ATP treatment.

In our study, we are unable to detect NIS expression in MCF7 cells with or without treatment. These cells are negative or express low level of EGF receptors¹⁹. This may explain the absence of NIS expression under EGF induction in MCF7 cells. However, EGF is able to augment NIS expression in MCF7 cells which is transfected with NIS, even though the cells do not have EGF receptors. Further studies are still required to be performed in order to elucidate the mechanism of NIS expression in breast cancer and the differences with NIS expression between the molecular subtypes of breast cancer. Additionally, NIS expression can be detected in Hacat cells, even though the expression is within the cytoplasm. Neither ATP nor EGF treatment will increase NIS expression in Hacat cells.

Conclusion

The level of NIS expression varies among the different subtypes of breast cancer cell lines. MCF7 cell line is representing the luminal type of breast cancer do not express NIS as good as SKBR3 cell line, which represents the HER2 type. This type might be suitable to receive radioiodine therapy as those cells expressing NIS. A combination treatment of EGF and ATP increases the expression of NIS *mRNA* and protein at membrane in SKBR3 cells.

Acknowledgements

This work was accomplished by a grant from Indonesia Risbang Project 2013.

Conflict of Interest Statement

No potential conflicts of interest are disclosed

REFERENCES

1. Oh.JR, Ahn.BC. False Positive Uptake on Radioiodine Whole Body Scintigraphy Physiologic and Pathologic Variants Unrelated to Thyroid Cancer. *Am J Nucl Med Mol Imaging* 2012;2(3):362-385
2. Kogai T, Liu YY, Mody K, Shamsian DV, Brent GA. Regulation of Sodium Iodide Symporter Gene Expression by Rac1/p38 β Mitogen-activated Protein Kinase Signaling Pathway in MCF7 Breast Cancer Cells. *J.Bio.Chem.*2012;287:3292-3300.
3. Unterholzner S, Willhauck M J, Cengic N, Schutz M, Göke B, Morris J C, et al. Dexamethasone Stimulation of Retinoic Acid-Induced Sodium Iodide Symporter Expression and Cytotoxicity of ¹³¹I in Breast Cancer Cells. *The J of Clin Endocrinol Metab.*2006;91(1):69–78
4. Tazebay UH, Wapnir IL, Levy O, Dohan O, Zuckier LS, Zhao QH, et al. The Mammary Gland Iodine Transporter is Expressed During Lactation and in Breast Cancer. *Nature Medicine.*2000;6:871-878
5. Beyer SJ, Jimenez RE, Shapiro CL, Cho JY, Jhiang SM. Do Cell Surface Trafficking Impairments Account for Variable Cell Surface Sodium Iodide Symporter Level in Breast Cancer?. *Breast Cancer Res Treat.*2009;115:205-212
6. Beyer SJ, Zhang X, Jimenez RE, et al. Microarrays Analysis of Genes Associated with Cell Surface NIS Protein Levels in Breast Cancer.*BMC Research Notes.* (2011) 392
7. Jung KH, Paik JY, Ko BH, Lee KH. Mitogen-Activated Protein Kinase Signaling Enhances Sodium Iodide Symporter Function and Efficacy of Radioiodine Therapy in Nonthyroidal Cancer Cells.*J.Nuclear Medicine.*2008;49:1966-1972
8. Dohan O, De La Vieja A, Carrasco N. Hydrocortisone and Purinergic Signaling Stimulate Sodium/Iodide Symporter (NIS)-Mediated Iodide Transport in Breast Cancer Cells. *Mol Endocrinol.*2006;20(5):1121-1137

9. Holliday DL, Speirs V. Choosing The Right Cell Line for Breast Cancer Research. *Breast Cancer Research. BioMed Central* (2011) 215.
10. Ryan J, Curran CE, Hennessy E, Newell J, Morris JC, Kerin MJ, et al. The sodium iodide symporter (NIS) and potential regulators in normal, benign and malignant human breast tissue. *PLoS One*. 2011;6(1):e16023
11. Tazebay UH. Regulation of the Functional Na⁺/I⁻ Symporter (NIS) Expression in Breast Cancer Cells. In *Breast Cancer-Recent advances in Biology, Imaging and Therapeutics*. In Tech (2011) 103.
12. Reiner C, Yao C, Goris M, Ghosh M, Katznelson L, Nowles K. Endogenous NIS Expression in Triple-Negative Breast Cancers. *Annals of Surgery Oncology*. 2009;16(4):962-968
13. Wapnir IL, van de Rijn M, Nowels K, Amenta PS, Walton K, Montgomery K, et al. Immunohistochemical profile of the sodium/iodide symporter in thyroid, breast and other carcinoma using high density tissue microarrays and conventional section. *J. Clinical Endocrinology and Metabolism*. 2003;88:1880-1888
14. Kogai T, Taki K, Brent GA. Enhancement of sodium/iodide symporter expression in thyroid and breast cancer. *Endocrine-Related Cancer*. 2006;13:797-826
15. O'Brien KM, Cole SR, Tse CK, Perou CM, Carey LA, Foulkes WD, et al. Intrinsic Breast Tumor Subtypes, Race, and Long-Term Survival in the Carolina Breast Cancer Study. *Clin Cancer Res*. 2010;16 (24):6100-6110
16. Oh Jung H, Chung JK, Kang JH, Kang WJ, Noh DY, Park IA. The Relationship Between Expression of The Sodium/Iodide Symporter Gene and The Status of Hormonal Receptors in Human Breast Cancer Tissue. *Cancer Res Treat*. 2005;37(4):247-250
17. Arturi F, Ferretti E, Presta I, Mattei T, Scipioni A, Scarpelli D, et al. Regulation of Iodide Uptake and Sodium/Iodide Symporter Expression in the MCF-7 Human Breast Cancer Cell Line. *J Clin Endocrinol Metab*. 2005; 90(4):2321-2326
18. Wagstaff SC, Bowler WB, Gallagher JA, Hipkind RA. Extracellular ATP Activates Multiple Signalling Pathways and Potentiates Growth Factor-Induced c-fos Gene Expression in MCF-7 Breast Cancer Cells. *Carcinogenesis*. 2000; 21:2175-2181

19. Howlin.J, Rosenkvist.J, Andersson T. TNK2 Preserves Epidermal Growth factor Expression on The Cell Surface and Enhances Migration and Invasion of Human Breast Cancer Cells. Breast Cancer Research.2008;10:R36