The Effect of 17β Estradiol Exposure on Expression of Estrogen Receptor β in the Trophoblastic Cells of Normal Placenta and Hydatiform Mole

Tatit Nurseta, Sumarno, Agung Supriyanto and Eviana Norahmawati

Abstract—Objective: to compare of the effect of estrogen (17β estradiol) to the expression of estrogen receptor β in the culture of normal trophoblast cell and hidatiform mole cell.

Materials and Methods: This is an experimental study that was held on Physiology Laboratory in Faculty of Medicine, Brawijaya University used group of normal trophoblast cell and group of hidatiform mole and both of group receive various dose of 17β estradiol (5nm, 10nm, 20nm, 40nm), then each group made as microscopic preparation with immunohistochemistry staining. The expression of estrogen receptor was being observed through microscope in 400x magnifying. The data was analyzed using One-Way ANOVA test, Tukey Test, Correlation and regression test.

Results: Various dose of 17 beta estradiol decrease expression of estrogen receptor β in the mole hydatiform cell significantly (47.20± 7.530, 47.80 ± 12.814, 37.00 ± 7.106, 29.00 ± 9.274). By ANOVA, the expression of estrogen receptor β have significant difference on 17 beta estradiol 5 g/ml and 17 beta estradiol 40 g/ml. 17 beta estradiol 40 g/ml result in lower expression of expression of estrogen receptor β than 17 beta estradiol 5 g/ml. There are no difference of expression of estrogen receptor β between 17 beta estradiol 20 g/ml and 17 beta estradiol 40 g/ml. In group of normal trophoblast there are no difference between control and which received various dose of 17 beta estradiol. There are significance difference between both of group (p<0.005).

Summary: Experiments showed significant differences of estrogen receptors between group of normal trophoblast and group of hydatiform mole and also expression of estrogen receptor in group hydatiform mole with various dose of 17 beta estradiol (p<0.005).

Keywords—Expression of estrogen receptor β, group of normal trophoblast cell, group of mole hydatiform cell, 17 β estradiol.

I. INTRODUCTION

TROPHOBLAST cells from the placenta had proliferation, migration, and invasion of the uterus in order to maintain the development of the fetal trophoblast [1]. The cells only discovered when she was pregnant, while in trophoblastic disease there is a failure of differentiation of the placenta, hydropic degeneration of chorion that resemble bubbles called a hydatidiform mole [2]. The incidence of hydatidiform mole in the American 1:450-1:2,000 deliveries, Japan 3: 2000 deliveries. Epidemiological studies in the municipality of Bandung and Malang in 2002 (population-based study) showed that the incidence of hydatidiform mole, respectively 1:500 and 1: 400. Approximately 15-20% of patients with hydatidiform mole will undergo transformation into malignant gestational trophoblastic tumors (GTT) [3,4,5]. Several factors are known to play a role in the process of carcinogenesis include DNA ploidy GTT, phospholipid expression, oncogenes, tumor suppressor genes, nutrition and hormonal status [6].

The role of growth hormone on the process of malignant transformation of trophoblast raises the suspicion of the role of estrogen in this process of carcinogenesis. Natural estrogen hormones play a role in the process of carcinogenesis in several malignancies such as breast cancer, endometrial cancer, liver cancer, and several other cancer types. Estrogen receptor expression contribute to determine the process of carcinogenesis. This is supported by the fact that over-expression of some cancers. Shao (2000) proved the occurrence of carcinogenesis in MCF-7 Human Breast Cancer Cell-induced estrogen [7].

Normal trophoblast cells and pathological trophoblast are histologically similar on expression of the estrogen receptor [8]. In a previous study, there is a expression of estrogen receptor alpha (ER-α) is in the villous cytotrophoblast. By Western blot analysis, in chorionic villous (CV) estrogen receptor beta are limited only on the syncytiotrophoblast (ST) [9]. Until now there has been little research on the involvement of estrogen on the transformation of trophoblast cells into GTT. Studies in rats have shown that estrogen or its metabolic having carcinogenic properties in some tissues, such as kidney, liver, uterus and mammary where the direct effect of estrogen on DNA nuclei is binding of hormone to the nuclear estrogen receptor, which then serves as a dimer to the Estrogen Response Elements (ERE), on the regulation of estrogen responsive gene and is associated with the basal
transcription factors, coactivator corepressor to alter gene expression so that when the response is excessive, overcome into carcinogenesis process. In addition there are three estrogen receptor pathway to increase cell proliferation and inhibit apoptosis is genomic pathways, nongenomic, and, mitochondria [7].

Until now, not been known clearly why and how the trophoblast cells can become malignant and no studies on the effects of natural estrogen 17β estradiol on trophoblast of hydatiform mole. Trophoblast cell research is not possible in humans. Therefore, it is necessary to use trophoblast cell culture media in vitro that mimic the actual conditions that can be given different treatment which desired. Research on the role of estrogen in the transformation of trophoblast cells do not exist, then in this study the researchers wanted to determine the effects of estrogen (17β estradiol) on the expression of estrogen receptor β in normal trophoblast cell culture and cell culture hydatidiform mole.

II. MATERIALS AND METHODS

This is an experimental study that was held on Physiology Laboratory in Faculty of Medicine, Brawijaya University used group of normal trophoblast cell and group of hidatyform mole and both of group receive various dose of 17β estradiol (5nm, 10nm, 20nm, 40nm), then each group made as microscopic preparation with immunohistochemistry staining. The expression of estrogen receptor was being observed through microscope in 400x magnifying. The data was analyzed using One-Way ANOVA test, Tukey Test, Correlation and regression test.

III. RESULTS

A. Expression of estrogen receptor β in normal trophoblast control mean 20.80 (ER antibody immunohistochemical staining 400x magnification), estrogen receptor β indicated by arrows

B. Expression of estrogen receptor β in normal trophoblast culture that gets 17β estradiol 5 µg / ml on average 18.4, showing a decrease compared to the image A (ER antibody immunohistochemical staining 400x magnification), estrogen receptor β indicated by arrows

Fig 1. Expression of estrogen receptor β in culture of normal trophoblast

A. Expression of estrogen receptor β in mole trophoblast control mean 61.60 (ER antibody immunohistochemical staining 400x magnification), estrogen receptor β indicated by arrows

B. Expression of estrogen receptor β in normal trophoblast culture that gets 17β estradiol 5 µg / ml on average 47.2, showing a decrease compared to the image A (ER antibody immunohistochemical staining 400x magnification), estrogen receptor β indicated by arrows

Fig 2. Expression of estrogen receptor β in culture of mole trophoblast

In the above figure it appears that cells that express estrogen receptor β brown and cells that do not express estrogen receptor β bluish
TABLE I
ONEWAY ANOVA TEST ESTROGEN RECEPTOR $\beta$ IN CULTURE OF MOLE TROPHOBLAST

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
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<td>61.60</td>
<td>8.325</td>
<td>3.723</td>
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<td>70</td>
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<td>47.20</td>
<td>7.530</td>
<td>3.367</td>
<td>39</td>
<td>55</td>
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<td>17 beta estradiol 10 ug/ml</td>
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<td>47.80</td>
<td>12.814</td>
<td>5.731</td>
<td>30</td>
<td>62</td>
</tr>
<tr>
<td>17 beta estradiol 20 ug/ml</td>
<td>5</td>
<td>37.00</td>
<td>7.106</td>
<td>3.178</td>
<td>29</td>
<td>48</td>
</tr>
<tr>
<td>17 beta estradiol 40 ug/ml</td>
<td>5</td>
<td>29.00</td>
<td>9.274</td>
<td>4.147</td>
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<tr>
<td>Total</td>
<td>25</td>
<td>44.52</td>
<td>14.057</td>
<td>2.811</td>
<td>19</td>
<td>70</td>
</tr>
</tbody>
</table>

In the comparative test of the mean of five estrogen receptors in culture of trophoblast $\beta$ mole using the F test (ANOVA) gained the result that there is very significant difference mean of estrogen receptor $\beta$ in culture of trophoblast mole between the control group and the group given 17 beta estradiol various doses with the Sig (p) = 0.000 <= 0.05.

TABLE II
ONEWAY ANOVA TEST ESTROGEN RECEPTOR $\beta$ IN CULTURE OF NORMAL TROPHOBLAST

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>Minimum</th>
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<tr>
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<td>14.80</td>
<td>4.382</td>
<td>1.960</td>
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<td>22.60</td>
<td>6.580</td>
<td>2.943</td>
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<td>33</td>
</tr>
<tr>
<td>17 beta estradiol 40 ug/ml</td>
<td>5</td>
<td>20.20</td>
<td>5.070</td>
<td>2.267</td>
<td>13</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
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<td>19.36</td>
<td>5.461</td>
<td>1.092</td>
<td>8</td>
<td>33</td>
</tr>
</tbody>
</table>

In the comparative test of the mean of five estrogen receptor $\beta$ in normal trophoblast culture by using the F test (ANOVA) obtained results that there is no significant difference mean estrogen receptor in culture of trophoblast $\beta$ normal control group and groups of administration 17 beta estradiol in dose of 5, 10, 20, 40 $\mu$g / ml.

IV. DISCUSSION

Specific reaction of tissue to estrogen receptors due to intracellular protein. Estrogen receptor is the primary cause of gene transcription but also regulate post-transcriptional events and non-genomic effects. Estrogen receptors regulate gene transcription through multiple mechanisms, which do not all involve direct interaction with DNA [10]. Estrogen increases the target tissue response to estrogen and other steroid hormone through the receptor influence by increasing concentrations of estrogen receptors that also will increase the concentration of progestin and androgen receptors. Formation of the estrogen receptor involves the rapid degradation of the receptor that do not bind to the estrogen and a slower degradation of the receptor that binds to estrogen after gene transcription. Presence of estrogen is an important factor for continuous response[10].

This study emphasizes the effect of 17 $\beta$ estradiol administration various doses to the expression of estrogen receptor $\beta$ in culture of normal trophoblast and culture of hydatidiform trophoblast. Figure 1 and 2 can be seen that the expression of estrogen receptor $\beta$ in normal trophoblast cultures lower than mola trophoblast culture by administering various doses of 17 $\beta$ estradiol.

From the data above can be seen that a decrease in expression of estrogen receptor $\beta$ estradiol after increasing doses of 17 $\beta$ estradiol in culture of mole trophoblast on the mean of five comparative trials using the F test (ANOVA) obtained the result that there are significant differences in mean trophoblast mole between the control group treated with 17 beta-estradiol administration various doses by Sig (p) = 0.000 <= 0.05.

While in the normal trophoblast culture obtained no change in expression of estrogen receptor $\beta$ estradiol with comparative trials of mean estrogen receptor $\beta$ in the culture of normal trophoblast using the F test (ANOVA) by Sig (p) = 0.209 > = 0, 05 which means there is no difference the mean estrogen receptor $\beta$ normal control group and the group given 17 beta estradiol 5,10,20,40 ug / ml.

This is consistent with the hypothesis put forward by researchers in which increasing the estradiol dose, the more receptors that bind to estrogen so that when did immunohistochemical staining, will be less of expression of estrogen receptor $\beta$. With upregulation of estrogen receptor, it will play role of carcinogenesis mole trophoblast overcome trophoblastic tumor malignant which the hormone estrogen and its metabolites generate Reactive Oxygen Species (ROS)
that lead to gene mutations (initiation) then endogenous hormone E2 in the cells produce ROS that would spur the increased expression of estrogen receptors, which in the presence of up regulation of estrogen receptors will increase cell proliferation and decrease apoptosis in mole trophoblast cells [7].

As described in the literature review that the mechanism of action mediated through the estrogen receptor pathway between the other three genomic pathways, non-genomic pathway and mitochondrial pathway where estrogen has a variety of cross talk in a different pathway. In this study focused on genomic pathways in which the response occurred within a few hours to several days. Estrogen receptor that is not attached to the ligand and free in the cytoplasm or nucleus are bound by a set of receptor proteins. This protein will stabilize the receptor in an inactive state or the DNA binding domain is closed. Estrogen free to diffuse through the cell and bind to the ligand binding domain of the receptor. Estrogen and estrogen-receptor complex then spread in the cell nucleus. Estrogen and estrogen receptor complex it binds to specific DNA sequences called estrogen-response elements as a homodimer or heterodimer, which facilitates gene expression, and has a high affinity for the protein to activate transcription coactivator gene or a nearby gene. Gene transcription by RNA polymerase results mRNA. Ribosomal mRNA and then translated in the cytoplasm to produce a protein associated with expression of the desired function. These proteins will determine the behavior of cells (cell behavior)[11].

The existence results of this study support previous research by Maria (2009) and Subandi (2011) that transformation of hydatidiform mole into malignant through estrogen receptors, may also be via the estrogen metabolites which lead to increased oxidative metabolism increased with increased oxidative metabolism enhance the formation of Reactive Oxygen species (ROS). Increased ROS will cause oxidative stress that causes depurination that the loss of pyrimidine bases from the deoxyribose bond hydrolysis and deamination that the changes of cytosine into uracil in DNA chains. Deamination and depurination cause the formation of mutant DNA sequences. On the other hand, tumor suppressor gene is a protein that easily activated when DNA damage occurs. Gene p53 is the primary of tumor suppressor because its function is quite broad, including a role in inhibiting the cell cycle, differentiation, apoptosis and angiogenesis. At malignant gestational trophoblastic or choriocarcinoma it is found that p53 protein mutation that cell proliferation is not inhibited and the cells continue to proliferate. P53 overexpression is important in the pathogenesis of complete mole and choriocarcinoma and related to trophoblastic disease. P53 overexpression associated with p53-dependent apoptosis to modulate excessive proliferation of trophoblast [12]. P53 gene mutations is important in the pathogenesis gestational trophoblastic and progression in humans, the technique polymerase chain reaction (PCR) has been detected p53 gene mutations in 30% of hydatidiform mole, choriocarcinoma and 75% without p53 gene mutation in normal choriocinic villi [13].

V. SUMMARY

1. There was no significant difference in estrogen receptor expression in cultured trophoblast β normal with the provision of 17 beta-estradiol treatment of various doses indicated by the value of Sig (p) = 0.209> = 0.05.
2. Provision of 17 beta estradiol with various doses of estrogen receptor β can lower the molar trophoblast cultures significantly with the Sig (p) = 0.000 <= 0.05.
3. There are significant differences in the expression of estrogen receptor β normal trophoblast culture with hydatidiform mole trophoblast cultures.
4. There are significant differences in estrogen receptor expression in cultured trophoblast β normal with estrogen receptor expression in cultured hydatidiform β receiving various doses of estradiol 17 beta

VI. SUGGESTION

Need to do further research on several types of tissue culture of sunfish both spontaneous regression and transformed into GTT and identification of other markers of carcinogenesis processes such as enzyme telomerase.

REFERENCES