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ER-α, HMGA2 and Wnt5a Correlates with the Grade of Mammary Phyllodes Tumor

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Abstract

Phyllodes tumor (PT) of the breast are fibroepithelial neoplasm, histologically characterized by more cellular stromal cell, stromal overgrowth and double-layered epithelial component arranged in clefts which in combination elaborate leaf-like structures. Unlike epithelial neoplasm of the duct and gland of the breast, there were inconsistencies of pathogenesis and unestablished indications for hormonal therapy of PT. The aim of this study is to analysis the immunoexpression of hormonal receptors (ER-α, ER-β, PR) and nonhormonal receptors (HMGA2 and Wnt5a) in mammary phyllodes tumor. We reviewed histology and performed immunohisto chemistry for paraffin blocks of PTs in Pathological Anatomic Laboratory at Hasan Sadikin general Hospital in 2011 until 2014 period. According to WHO Classification (2012), PTs were categorized into three groups benign, borderline and malignant. According to 62 cases of PT for patients aged between 16 and 66 years with mean value of 38.5, there are an issue that underwent bilateral and 6 cases of recurrence. PT mainly showed the distribution of ER-α immunoexpression >50 % in benign category is 35 patients or 87.5%. A significant correlation was observed between histoscore ER-α with histopathological grading of PT (p=0.001) in negative direction (R=-0.423), HMGA2 and Wnt5a in positive direction (R=0,439 and R=0,24). ER-α, HMGA2 and Wnt5a may play a role in the pathogenesis of phyllodes tumor, characterized by there were correlation between ER-α, HMGA2 and Wnt5a immunoexpression with the grade of the mammary phyllodes tumor.

Keywords: ER-α, HMGA2, Wnt5a, Grade, Mammary Phyllodes Tumor.

Introduction

Mammary phyllodes tumors (PTs) are fibroepithelial neoplasms, histologically characterized by more cellular stromal cell, stromal overgrowth and double-layered epithelial component arranged in clefts which in combination elaborate leaf-like structures[1]. PTs constitutes 0.3-1% of all breast tumors and 2.5% of fibroepithelial lesions of the breast, even in Singapore tumor incidence was reported up to 6.92%.[2,3]. Neoplastic component of the tumor is stromal cell, which determines its behavior. Although there is no grading scheme showing the accurate tumor behavior, histological features correlate with the biological attitude. In WHO classification PTs are divided into three subgroups; benign, borderline and malignant on the basis of histological criteria that include the degree of stromal cellularity, atypical stromal cell, mitotic activity, stromal overgrowth and the type of tumor margins (infiltrating/pushing)[4]. The tumor was first reported with much more details in 1883 by Johannes Müller, but the first report regarding to its metastases was reported in 1993 by Lee and Pack. Unlike epithelial neoplasm of the duct and gland of the breast, there were inconsistency in hormonal expression research in PTs. Tse on study found an inverse relationship between increasing expression of ER with improved gradation, and reinforced by the analysis of Lazaro who suspect estrogenic effect in the stroma of epithelial cells in the pathogenesis of phyllodes tumor[5,6]. Modern molecular pathology, especially genetic studies, have been pointed out biphasic behavior of some breast tumor (fibroadenomas, phyllodes tumors). These investigations cleared up the first steps of tumorous stromal/epithelial proliferations. Both histochemical and immunohistochemical examinations of numerous authors confirmed the suggestion that in the pathogenesis of these tumors, very important role have an inactivity of differentiation factors, like the factors in embryogenesis (Wnt signalization) as well as the tumor suppressor genes. On examination by in situ hybridization found any excessive expression Wnt5a mRNA in the epithelial cells, but not found over expression of β-catenin in epithelial cells both in normal breast tissue or in phyllodes tumor, thus allegedly Wnt5a only works on stromal cells. Sawyer et al found an interaction with the epithelial cells in the stroma of the Wnt pathway-APC-β-catenin, although also found cells that do not express wnt, so it also needs a lot of rethinking their other non-hormonal molecules in the pathogenesis of this tumor[7,8]. HMGA2 is one protein that is considered to play a role in the pathogenesis of sarcomas. HMGA is one of the proteins

that are found in undifferentiated cell in embryogenesis processes involved in activating stemcells[9]. The existence of the same element in sarcomas and phyllodes tumor primarily on malignant gradation can be used as the alleged involvement HMGA2 protein in the pathogenesis of these tumors. The aim of this study is to analysis the correlation of immunoexpression hormonal profile (Estrogen Receptor-Alpha (ER-α), Estrogen Receptor Beta (ER-β) and Progesterone receptor (PR)) and non hormonal (High mobility group AT-Hook 2 (HMGA2) and Wingless- integration 5a (Wnt5a)) with the grade of mammary phyllodes tumor.

Materials and Methods

We selected 62 paraffin blocks of phyllodes tumors from 64 patients. Cases were issued from the archives of the Department of Anatomical Pathology of Hasan Sadikin Hospital, Bandung, West Java, Indonesia, from 2011 until 2014 period. We divide phyllodes tumor into 3 grade: benign phyllodes tumor, borderline phyllodes tumor and malignant phyllodes tumor based on WHO classification. All cases that were diagnosed on the basis of conventional histological parameters were included in this study. The ages of the patients, tumor locations and the relevant details were obtained from the pathology reports. All cases were histologically regraded on the basis of stromal cytologic atypia, stromal hypercellularity, mitotic count, stromal overgrowth, tumor necrosis and tumor margins.

All tumor specimens were fixed in 10% buffered formalin and embedded in paraffin according to standard procedures. Serial 4-μm-thick sections were placed on positively charged slides. Immunohistochemical detection of ER-α (clone SP1, diluted 1:500, Thermo Scientific), anti-ER-β (clone Ab288, diluted 1:100, Abcam), anti-PR (clone 2F12B4, diluted 1:200, Thermo Scientific), Wnt5a (clone 6F2 diluted 1:300, Thermo Scientific) and HMGA2 (clone PA521320 diluted 1:500, Thermo Scientific) were performed. Sections of breast carcinoma and endometrium shown strong staining (3+) were used as a positive control. Negative control using a sample that does not use primary antibodies.IHC staining of the tumor nuclei were interpreted as negative when 0-20%, (+) when 20-50% and (+++) when >50% staining were detected.

Statistical analysis for numerical data, p value is calculated by using ANOVA test (normally distributed data) and Kruskall Wallis (non-normally distributed data). On the other hand, p value in categorical data is generated from statistical test of Chi-Square with Kolmogorov Smirnov as alternative test. Significance level based on $p<0.05$. Statistical analysis was performed with SPSS for windows (V.21.0) software.

Results and Discussion

Sixty and four phyllodes tumor samples obtained from various levels of gradation from 2011 to 2014. After the evaluation, two samples cannot meet the inclusion criteria for paraffin blocks that do not meet inspection standards immunohistochemistry, so the total sample were obtained as many as 62 samples. Table-1 describes the characteristics of the overall research subjects are age (years), tumor size (cm), the degree, location and recurrence. All patients have age range 19-66 years and tumor size between 2.2 to 30 cm, gradation most are benign as many as 40 patients, or 66.7%. Location of the tumor is slightly more common in the right breast as many as 33 patients, or by 53.2%, and there is one case with bilateral events and recurrence in 6 patients, or 9.7% (Table-1).

Comparison histoscore of ER-α, ER-β, PR, HMGA2 and Wnt5a immunoexpression with the grade of mammary phyllodes tumor can be seen in Table-2. Variable of ER-α immunoexpression was highest in benign group that is an average of 4.800 ± 1.856, and the lower the increase in gradation in borderline group average of 4.090 ± 1.814, and the malignant group average of 2.363 ± 1.911. There were no significant differences in the mean for ER-β and PR with grade. Immunoexpression of ER-α, can be seen in Figure-1.

Table-1
Clinical characteristics of subjects

Variable	**N= 62**
Age (years)	
Mean ± Std	40,11±12,06
Median	38,50
Range (min-max)	19,00-66,00
Size (cm)	
Mean ± Std	13,29±7,350
Median	11,00
Range (min-max)	2,20-30,00
Grade	
Benign	40 (64,6%)
Borderline	11 (17,7%)
Malignant	11 (17,7%)
Location	
Right	33 (53,2%)
Left	28 (45,2%)
Bilateral	1 (1,6%)
Recurrence	
Positive	6 (9,7%)
Negative	56 (90,3%)

Immunoexpression of Wnt5a in the borderline group is constant and HMGA2 immunoexpression in the malignant groups is constant for statistical analysis, HMGA2 and Wnt5a obtained information p-value less than 0.05 which means there are differences between the mean for all variables statistically significant or meaningful among the HMGA2 and Wnt5a immunoexpression variables with the grade of mammary phyllodes tumor. Immunoexpression of ER-α, HMGA2 and Wnt5a can be seen in Figure-1.

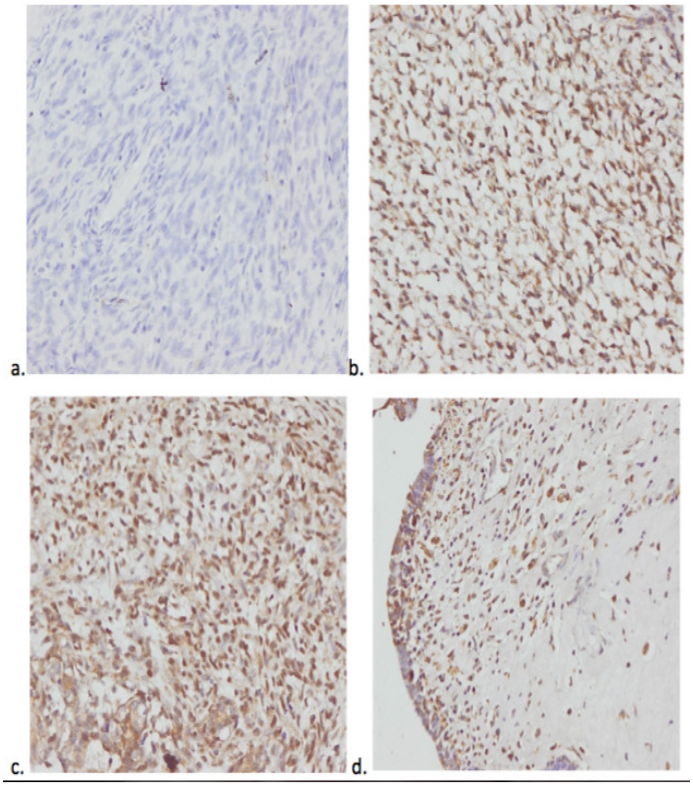

Figure-1
a) ER-α immunoexpression in malignant phyllodes tumor, showed no immunoreactive (0%)
b) ER-α immunoexpression in benign phyllodes tumor, showed strong immunoreactive >50%
c) HMGA2 immunoexpression in malignant phyllodes tumor, showed strong immunoreactive >50%
d) Wnt5a immunoexpression in benign phyllodes tumor, showed strong immunoreactive >50% in periductal

Phyllodes tumor is a fibroepitelial tumor of the breast which generally occurs at age 40-50 years, but the Asian country reported to occur at a younger age is 25-30 years[1,2,3].

In the present study found phyllodes tumor also occur at a younger age is 19-66 years of age, with a mean ± Std 40.11 ± 12.06 and a median of 38.9, according to the young age of the study conducted by Ben Hassouna J et al with a mean of 39.5 (14-71) and research Nurhayati in Malaysia mean 42[10,11].

Unlike breast malignancies of epithelial elements that has become standard operating procedures for immunoexpression of ER and PR, hormonal therapy for phyllodes tumor is still in controversy. In this study, it appears that there are significant differences in the proportion or statistically significant between variable intensity and histoskor ER-α with gradations phyllodes tumor with negative correlation (r=-0.423; p = 0.001). Nurhayati et al and Sapino, showed expression of ER-α with low value high in stromal cells phyllodes tumor, but the expression of ER-β are significant at the filodes stromal cells[11,12].

It is contrary to research conducted Ilic I et al, showed expression of ER is not expressed by stromal cells, but in the mast cell is suspected in a phyllodes tumor stromal cells.[13]

Kim et al also do not support their hormonal relationship to the development of phyllodes tumor, and just get the Ki-67 most indicate a relationship with gradations phyllodes tumor[14]. In contrast to the study by Tse, found differences in ER-α immunoexpression in epithelial cells of phyllodes tumor[15]. Bouris reported the role of estrogen receptor in the mechanism of epithelial-mesenchymal transition (EMT) in breast carcinoma cells and obtain significant results that decreased expression of ER-α influential in the process of EMT in breast carcinoma[16].

This is supported by research conducted by Gutilla concluding ER-α support the proliferation and differentiation of epithelial cells in breast carcinoma[17]. The change ER-α immunoexpression were significant negative correlations with the direction of giving the alleged existence of ER-α effect with the change of stromal cells in phyllodes tumor. The development of stromal cells in phyllodes tumor is supposedly binds to the activation of the EMT that causes increased cellularity stromal cells in the tumor. It continues to grow until a decline in the role of ER-α caused the transfer of the role of tumor cell activation by ER-α in mesenchymal stem cell. Mesenchymal stem cell activation will produce cells metaplastik on phyllodes tumor cells that will eventually lead to the development of the cells of malignant phyllodes tumor[18].

In contrast to Sapino and Nurhayati et al, this study did not reveal any significant value or statistical significance between the variable distribution, intensity and histoscore ER-β and PR with phyllodes tumor grading (p=0.216). Some differences ER-α and ER-β include the ER-α and ER-β have different biological functions, as shown by their different expression patterns. Estrogen receptor-α and ERβ have roles that overlap on estrogen signaling. When in normal breast ER-α almost mostly found in epithelial cells, Eβ can be found on epithelial cells and stromal cells.

This may imply, the presence of ER-β expression in phyllodes tumor is not a part of the pathogenesis of this disease, such as the ER-α were supposed to be part of the process of EMT.

Table-2
Comparison of ER-α, ER-β, PR, HMGA2 and Wnt5a immunoexpression with the grade of mammary phyllodes tumor

Variable	Grade			p	r
	Benign	Borederline	Malignant		
Histoscore ER-α				0,002*	0,428
Mean±Std	4,800±1,856	4,090±1,814	2,363±1,911		
Median	6,000	4,000	2,000		
Range (min-max)	0,000-6,000	1,000-6,000	0,000-6,000		
Histoscore ER- β				0,216	
Mean±Std	0,925±1,439	1,181±1,778	1,363±1,286		
Median	0,000	1,000	2,000		
Range (min-max)	0,000-6,000	0,000-6,000	0,000-4,000		
Histoscore PR				0,125	
Mean±Std	0,500±1,062	0,818±0,873	0,636±0,809		
Median	0,000	1,000	0,000		
Range (min-max)	0,000-6,000	2,000-6,000	6,000		
Histoscore HMGA2				0,001*	0,439
Mean±Std	4,025±1,967	5,272±1,348	6,000		
Median	4,000	6,000	6,000		
Range (min-max)	1,000-6,000	2,000-6,000	6,000		
Histoscore Wnt5a				0,040*	0,243
Mean±Std	5,150±1,610	6,000	5,818±0,603		
Median	6,000	6,000	6,000		
Range (min-max)	1,000-6,000	6,000	4,000-6,000		

*Value significance based on the value of p <0.05. Mark * indicates statistically significant or meaningful.*

In this research also found a significant correlation between wnt5a and HMGA2 immunoexpression with phyllodes tumor grading with the direction of the positive correlation, Wnt5a and HMGA2 immunoexpression getting stronger with the increase in gradation phyllodes tumor (p= 0,001 and 0,040, r= 0,439 and 0,243). HMGA2 protein is widely expressed on cells that have not differentiated in the process of embryology and decreased in adult tissues. HMGA2 their high expression in adult organs showed activation of progenitor cells in various tissues. Analysis HMGA2 in mice demonstrated an association HMGA2 with Wnt and resulted in increased cell proliferation and the number of progenitor cells. Progenitor cell that most strongly are pluripotent mesenchymal cell, so that HMGA2 expression is found in tumors with mesenchymal differentiation[19,20]. Henrisken examines the role HMGA2 on mesenchymal stem-like cells (MSC) line to get their resistance to processes of differentiation, reduced expression of markers of epithelial cells and increased expression of markers of mesenchymal, so

suspected amplification HMGA2 in sarcoma make tumor cells with the phenotype of stem-like cell[21,22]. In this study, HMGA2 immunoexpression very predominantly found in malignant phyllodes tumor ($p < 0.05$). Malignant phyllodes tumor stroma cells containing highly hypercellular with atypical cell nucleus, cell differentiation is already bad, and often the tumor cells were rahbdomyomatous, lipomasarcomatous even fibrosarcomatous. HMGA2 has been reported to be associated with distant metastasis in colon and pancreas cancers[23,24]. In previous studies, which evaluate the expression of proteins associated with the Wnt/β-catenin in cell phyllodes tumor, the increased expression of Wnt1 epithelial cells in accordance with the increase in gradation tumor and also increased expression of β-catenin in stromal cells[25]. Sawyer examines the epithelial-stromal interaction in the Wnt pathway-APC-β-catenin to assess the immunohistochemical expression of β-catenin and cyclin1 on phyllodes tumor. Obtained 72% of stromal cells expressing β-catenin accumulates in the area periductal on phyllodes tumor benign, while the other was found β-catenin immunoexpression weak around periductal in phyllodes tumor graded higher (borderline-malignant) so allegedly their dependence on glandular epithelial cells in the stromal cell proliferation in phyllodes tumor[26]. Pathway Wnt / β-catenin signaling pathway is one suspected of being involved in EMT. In a study of breast carcinoma, Wnt is considered responsible by the process of metastasis. Wnt signaling will cause the process of cell migration in the process of EMT. In this process the cells of epithelial mesenchymal will go into space. The process also involves E-cadherin which causes the epithelial cells separate from laminin. Snail is a zinc finger transcription factor, has a potential function in suppressing the expression of E-cadherin. These factors can work alone or together with the Wnt / β-catenin induces epithelial-mesenchymal transition[27,28]. Bo et al showed that Up regulation of Wnt5a was expressed in EMT and metastasis in pancreatic cancer models, which involves activation of β-catenin-dependent canonical Wnt signaling[29]. Several studies showed an increased Wnt5a in malignancy, Wnt5a is found elevated in gastric cancer cells, their correlation with improved gradation malignant melanoma, and this protein is also allegedly involved in the pathogenesis of colon carcinoma[30-32].

Conclusion

Our results indicate that the ER-α, HMGA2 and Wnt5a may play a role in the pathogenesis of phyllodes tumor, characterized by there were correlation between ER-α, HMGA2 and Wnt5a immunoexpression with the grade of the mammary phyllodes tumor.

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