นิพนธ์ต้นฉบับ • Original Article

การแสดงออกของ ESA ในผู้ป่วยมะเร็งเต้านม โรงพยาบาลศรีนครินทร์

กฤติกา ทรัพย์คณารักษ์[1], สัญญา ร้อยสมมุติ[1], ภารดี เอื้อวิชญาแพทย์[1], โกสินทร์ วิระยร[1], ชัยวัชร์ อภิวาทนสิริ[1], ปิยะฉัตร ศรีมันตะ[1], วิยะดา ปัญจรัก[1]
[1]ภาควิชาสรีรวิทยา [2]ภาควิชาอายุรศาสตร์ [3]ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

The Expression of Epithelial Specific Antigen (ESA) in Breast Cancer Patients, Srinagarind Hospital

Krittiga Sapkanarak[1], Sanya Roysommuti[1], Paradee Auervitchayapat[1], Kosin Wirasorn[2], Chaiwat Aphivatanasiri[3], Piyachat Srimunta[3], Wiyada Punjaruk[1]
Department of [1]Physiology, [2]Medicine, [3]Phathology, Faculty of Medicine, Khon Kaen University, Khon Kaen Province, Thailand

หลักการและวัตถุประสงค์: มะเร็งเต้านมเป็นสาเหตุการตายอันดับสองในผู้หญิงปัจจุบัน Epithelial Specific Antigen (ESA) มีบทบาทสำคัญใน signaling pathway ของมะเร็งเต้านม ระดับการแสดงออกของ ESA เกี่ยวข้องกับการพยากรณ์โรค การดื้อต่อการรักษาและระยะเวลาการรอดชีวิต ซึ่งขณะนี้ยังไม่มีข้อมูลระดับการแสดงออกของเซลล์ต้นกำเนิดมะเร็งในผู้ป่วยมะเร็งเต้านมโรงพยาบาลศรีนครินทร์
วิธีการศึกษา: ข้อมูลผู้ป่วยมะเร็งเต้านมจากโรงพยาบาลศรีนครินทร์ตั้งแต่ปี พ.ศ. 2546-2559 จำนวน 247 ราย ได้นำมาวิเคราะห์ข้อมูลพื้นฐานทางสถิติและศึกษาระดับการแสดงออกของ ESA ในกลุ่ม triple negative จำนวน 45 ราย ด้วยวิธี immunohistochemistry ในชิ้นเนื้อมะเร็งเต้านมที่ถูกฝังในพาราฟินเพื่อทดสอบหาความสัมพันธ์ระหว่างระดับการแสดงออกของ ESA และระยะเวลาการรอดชีวิตโดยเฉลี่ย (average survival time) ด้วย TIS score และ H-score โดยใช้การวิเคราะห์ตัวแปรเดียวและการวิเคราะห์พหุตัวแปร
ผลการศึกษา: อายุ ดัชนีมวลกายและระยะของมะเร็งมีความสัมพันธ์กับระยะเวลาการรอดชีวิตโดยเฉลี่ยในผู้ป่วยมะเร็งเต้านม โดยในผู้ป่วยมะเร็งเต้านมที่มีอายุมากกว่าหรือเท่ากับ 41 ปี มีภาวะอ้วนหรือมีระยะที่ 4 ของมะเร็งจะมีอัตราการรอดชีวิตโดยเฉลี่ยที่สั้น ผู้ป่วยมะเร็งเต้านมกลุ่ม triple negative มีการแสดงออกของ ESA ในระดับปานกลางถึงสูง แต่ไม่พบความแตกต่างกันอย่างมีนัยสำคัญใน 2 กลุ่มอายุ (ที่มีอายุมากกว่า หรือเท่ากับ 41 ปี เปรียบเทียบกับ 21-40 ปี)

Background and objectives: Breast cancer is the second most common of death in female. ESA plays an important role in signaling pathway of breast cancer. The levels of ESA expression associate with prognosis, resistance of therapy and short survival time. At present, there is no data of the expression levels of cancer stem cells in breast cancer patients in Srinagarind hospital.
Methods: The data of breast cancer patients (247 cases in total) in Srinagarind hospital collected during 2003-2016 were analysed. Formalin-fixed paraffin-embedded (FFPE) breast cancer tissues were investigated for the levels of ESA expression in 45 cases of triple negative subtype by immunohistochemistry. TIS score and H-score were performed to determine the levels of ESA expression.To evaluate the relationship of ESA expression levels and average survival time, the data were analysed by using univariate analysis and multivariate analysis.
Results: Age, BMI, TNM staging had influence on average survival time. Patients with age more than or equal 41 years, obesity or TNM stage IV has high fatality risk. The levels of ESA expression in triple negative subtype had overexpression but it was not significantly different between two age groups (≥ 41 years vs 21-40 years).
Conclusions: Age, BMI and TNM staging negatively related to average survival time in breast cancer patients and the effects were different in each subtype of breast

สรุป: อายุ ดัชนีมวลกายและระยะของมะเร็งมีความสัมพันธ์กับระยะเวลาการรอดชีวิตในผู้ป่วยมะเร็งเต้านม ปัจจัยเหล่านี้ส่งผลในแง่ลบกับอัตรารอดชีวิตโดยเฉลี่ยของผู้ป่วย โดยมีผลแตกต่างกันในแต่ละชนิดของมะเร็งเต้านม ผู้ป่วยมะเร็งเต้านมในกลุ่ม triple negative มีระดับการแสดงออกของ ESA ที่สูง

คำสำคัญ: ESA, EpCAM, มะเร็งเต้านม, surface marker

cancer. Patients with triple negative subtype had overexpression of ESA.

Keywords: ESA, EpCAM, breast cancer, surface marker

ศรีนครินทร์เวชสาร 2560; 32(6): 524-33. • Srinagarind Med J 2017; 32(6): 524-33.

Introduction

Breast cancer is heterogenous disease with different biological characteristics, histological features and clinical manifestations and the result in different treatment outcome found in different subtypes of breast cancer. Therefore, many breast cancer patients have tumor recurrence after the complete course of treatment. Currently, it is generally believed that cancer stem cells are responsible for resistance of treatment in many cancers including breast cancer[1,2]. Cancer stem cells, which have special characteristics in terms of indefinite proliferation, self renewal and multiple lineage differentiation, play an important role in distant metastasis, disease progression and tumor recurrence[3]. These cells also have better DNA damage repair process compared to normal cells and can efflux chemotherapeutic drugs resulting in avoiding from drug toxicity. Consequently, cancer stem cells can survive from chemotherapy and radiation leading to resistant to treatment and tumor recurrence. These cells can be identified by using surface markers, which specifically bind to cancer stem cell population but not other normal cells.

Presently, the expression of several surface markers is purposed that they can effectively indicate cancer stem cell population such as $CD44^{+}$, $CD24^{-/low}$, ESA^{+} and $ALDH1^{+}$[4-6]. In this study, ESA marker was used to identify cancer stem cells in formalin-fixed paraffin-embedded (FFPE) tissues of breast cancers. ESA plays an important role in signaling pathway of breast cancer that associates with ability of proliferation, migration and invasion of breast cancer cells[7-9]. Additionally, high levels of ESA expression correlate with poor prognosis, resistance of treatment and short overall survival rate studied in both *in vivo* and *in vitro*[10]. The different expression levels of each surface marker for cancer stem cells are detected in different subtypes of breast cancer. Therefore, it is important to know the characteristics of ESA expression in different subtypes of breast cancer. This information possibly indicates the prognosis and response of treatment and also provides useful data for choosing appropriate treatment option for individual breast cancer patients. At present, there is no data of the expression levels of cancer stem cells in breast cancer patients in Srinagarind hospital.

Methods

Samples of the study

Breast cancer patients included in this study were 247 cases, who were treated in Srinagarind hospital during 2003-2016. All breast masses surgically removed from patients were pathologically diagnosed by 2 independent pathologists. Formalin-fixed paraffin-embedded (FFPE) breast cancer tissues were obtained from 45 triple negative breast cancer patients for investigating the levels of ESA expression. This study was approved by Khon Kaen University Ethics Committee for human experiment research (HE601142).

Demographic data

The medical records of breast cancer patients, who were treated at Srinagarind hospital during 2003-2016,

were reviewed from OPD card to obtain the clinical data comprising age, BMI, TNM staging, performance status, comorbidity, pathological findings, tumor biological and treatment. Clinical data were analysed using STATA version 14.0 and average survival time and hazard ratio were also analysed.

Immunohistochemistry technique

ESA expression was investigated by using well-established immunohistochemistry technique. For primary antibody treatment, ESA was detected using 1:300 ESA; clone moc-31 (Pathology Ware International Co., Ltd) and UV HRP multimer (goat anti-mouse IgG, goat anti-mouse IgM and goat anti-rabbit) (Ventana Medical systems, Inc.) was used as the secondary antibody.

Score analysing of ESA expression levels

To determine the levels of ESA expression, two standard methods were used; total immunostaining score (TIS)[11] and H-score[10]. The score of ESA expression was determined by two independent pathologists. The details of each method are described below.

- Total immunostaining score (TIS)

IHC slides of breast cancer tissues stained with ESA were examined under the light microscope to investigate the percentage of positive staining of ESA and intensity of staining and 10 fields of ESA staining breast cancer tissues were evaluated. Total immunostaining score (TIS) is the levels of ESA expression, which was calculated by a proportion score (PS) multiplying with intensity score (IS) [TIS = PS x IS]. PS is levels of positive staining of ESA, which are classified into 5 grades; 0 = no staining of ESA, 1 = ESA staining <10%, 2 = ESA staining 10–50%, 3 = ESA staining 51–80%, 4 = ESA staining > 80% of total cells. IS is the levels of ESA staining intensity, which are classified into 4 grades; 0 = no staining, 1+ = weak staining (weak staining ≤ 60% or moderate staining ≤ 30% of total cells), 2+ = moderate staining (weak staining > 60% or moderate staining 31-70%, or strong staining ≤ 30% of total cells), 3+ = strong staining (moderate staining >70% or strong staining >30% of total cells). Finally, the results of multiplying between PS and IS were determined. If the results of multiplying are greater than 4, the expression of ESA was indicated as overexpression. Conversely, if the multiplying results are less than or equal 4, the expression of ESA was defined as weak expression.

- H-score

Slides were analysed under the light microscope to investigate the levels of ESA expression in breast cancer patients. Similar to TIS, 10 fields of breast cancer tissues were examined. For H-score, the levels of ESA expression was evaluated from the percentage of positive staining of ESA (0-100%) multiplying with intensity score (IS), which is mentioned earlier in TIS methods [H-score = % of staining x IS]. H-score classifies ESA expression levels into 3 levels; low ESA expression (H-score = 0-100), moderate ESA expression (H-score = 101-200) and high ESA expression (H-score = 201-300).

Statistical analysis

Statistical comparisons of the significant difference of the demographic data of patients from each subtype of breast cancers were performed by using Student's t-test. Prognostic factors, which significantly affected the average survival time of each breast cancer subtype, were analysed by using univariate and multivariate method. For comparing two different age groups of triple negative subtype, Fisher's exact test was used. The data have statistical significance when p value is less than 0.05

Results

The average age of breast cancer patients was 49 years. Interestingly, breast cancer was found at early age of life and the youngest age of patients was only 24 years whilst the oldest age was 82 years. The majority of breast cancer patients were in the age ≥ 41 years and obese. Importantly, the results showed that almost 30% of patients were found at the age less than 40

years. Most of patients presented with the performance status of 0 at the first diagnosis. For menopause status, most of breast cancer patients had pre-menopause status approximately 70% and 56% of breast cancer patients had no other co-morbidities. The pathological findings showed that almost 98% of breast cancer patients had invasive breast cancer and approximately half of breast cancer patients had stage II of disease at diagnosis. The characteristics of tumor biological marker expression were varied. Half of patients had positive estrogen receptor (ER) expression whereas positive progesterone receptor (PR) expression was detected only 36%. More than half of patients had negative HER2 expression while only 17% of patients had positive HER2 receptor expression. For treatment options, most of patients received chemotherapy (80.97%) and hormonal therapy (76.83%) whilst less than 40% of patients were treated with radiotherapy. The average age and average survival time was not significantly different compared among 4 subtypes. All variables studied in this project comprising BMI, performance status, pathological findings and staging of disease were not significantly different compared between 4 subtypes.

Prognostic factors for analysing average survival time are shown in Table 1 and analysis of hazard ratio of each prognostic factor influencing on average survival time are also shown in Table 2. The data showed that age factor in luminal A and triple negative breast cancer subtype significantly had an influence on average survival time. The age more than or equal 41 years group had short average survival time and high fatality risk compared to the age group ranging between 21-40 years. For BMI factor, BMI of patients only in luminal B subtype significantly had an influence on average survival time. Obese group had short average survival time and high fatality risk compared to non-obese group. For stages of breast cancer factor, the average survival time of patients in luminal A and luminal B subtypes was significantly different compared among 4 stages with in each subtype. The trends of average survival time were decreased with increasing stages and stage IV had high fatality risk compared to stage I. For HER2 group, the results were not complete because there were too small number of cases (only 6 patients) for analysis which are presented with grey color in Table 1.

ESA expression was investigated in 45 cases of triple negative subtype by using immunohistochemistry technique. The results showed that the average levels of ESA expression was 73% and most of triple negative breast cancer patients has moderate to high expression of ESA. The levels of ESA expression in this study were evaluated by using TIS score and H-score. For TIS score analysis, most of triple negative cases had ESA over expression. Old age, which had worse survival time compared to younger age group, had over expression of ESA compared to the younger age group. However, these results were not significantly different among these two age groups ($p = 1.000$) and the results are shown in Table 3 and Figure 1. For H-score analysis, the results showed that the majority of triple negative cases had moderate ESA expression and the results revealed no significant difference of ESA expression between the younger and older age group of triple negative subtype ($p = 0.683$) (Table 4 and Figure 2).

Table 1 Prognostic factors influencing on the average survival time investigated in 4 subtypes of breast cancer patients in Srinagarind Hospital.

Variable	luminal A			luminal B			HER2			triple negative		
	Average survival time (mo)	95% CI	p-value	Average survival time (mo)	95% CI	p-value	Average survival time (mo)	95% CI	p-value	Average survival time (mo)	95% CI	p-value
Age			0.007			0.06						0.04
21-40	148.63	137.09-160.17		111.20	94.15-128.26		134.73	99.82-169.63		134.91	118.37-151.47	
≥ 41	110.29	87.59-132.10		142.07	121.17-162.96					88.12	55.51-120.73	
BMI			0.21			0.01						0.089
Non-Obese	146.55	131.69-161.40		128.05	110.78-145.33		93.49	48.45-138.53		115.46	91.67-139.26	
Obese	135.78	119.72-151.85		101.36	79.73-122.99		154.23	154.23		142.66	123.83-161.50	
Staging TNM			0.004			0.001						0.124
Stage I	152.40	142.30-162.50		124.54	79.69-169.40					97.51	57.81-137.20	
Stage II	145.07	132.03-158.10		131.97	117.58-146.35					138.72	120.99-156.44	
Stage III	119.27	93.68-144.86		104.03	79.51-128.56					99.78	60.55-139.01	
Stage IV	36.9			39.26	11.01-67.52					98		

Table 2 The hazard ratio of prognostic factors significantly influencing on average survival time of 4 subtypes of breast cancer patients in Srinagarind Hospital by multivariate analysis.

Variable	luminal A			luminal B			triple		
	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value
Age	3.404	1.41-8.23	***0.007***	0.375	0.13-1.07	*0.069*	2.984	1.05-8.45	***0.04***
BMI	1.759	0.73-4.25	*0.210*	2.194	1.06-4.53	***0.033***	0.427	0.16-1.14	*0.089*
TNM staging	2.760	1.37-5.55	***0.004***	2.373	1.42-3.95	**0.001**	1.74	0.86-3.53	*0.124*

Table 3 Levels of ESA expression determined by TIS score studied in 2 different age groups of triple negative subtype.

TIS score	All case (n=45)		Age 21-40 (n=39)		Age ≥41 (n=6)		p-value
	N	%	N	%	N	%	
Weak expression	13	28.8	12	30.8	1	16.7	1.000
Overexpression	32	71.1	27	69.2	5	83.3	

Table 4 Levels of ESA expression determined by H-score studied in 2 different age groups oftriple negative subtype.

H- score	All case (n=45)		Age 21-40 (n=39)		Age ≥41 (n=6)		p-value
	N	%	N	%	N	%	
Low	12	26.7	11	28.2	1	16.7	0.683
Moderate	28	62.2	23	58.9	5	83.3	
High	5	11.1	5	12.9	0	0	

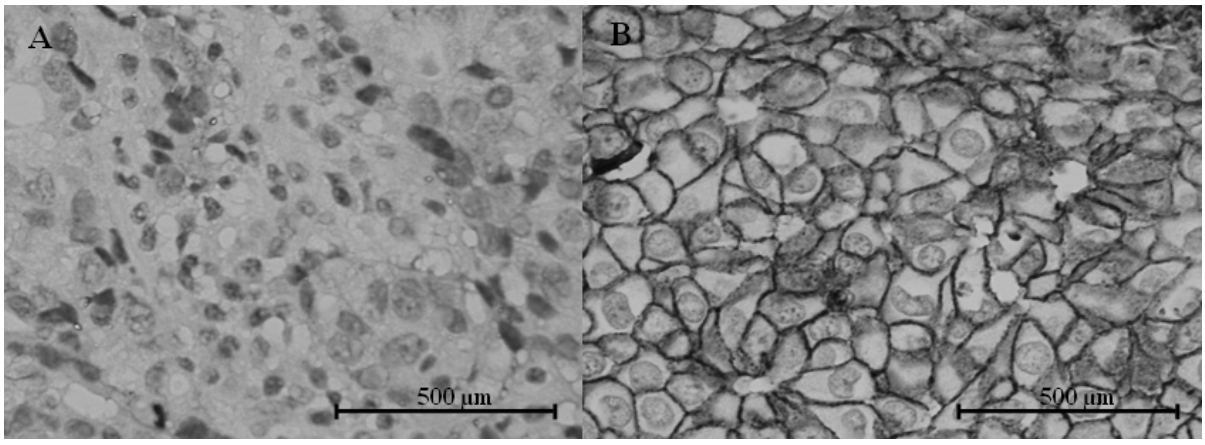

Figure 1 ESA expression determined by total immunostaining score (TIS). This Figure shows the representative results of ESA expression found in triple negative subtype patients. Triple negative subtype patients with weak ESA expression had TIS=2 (PS=2 and IS=1) as shown in Figure A. Triple negative subtype patients with ESA over expression, which had TIS=12 (PS =4 and IS=3), are shown in Figure B (Original magnification X600).

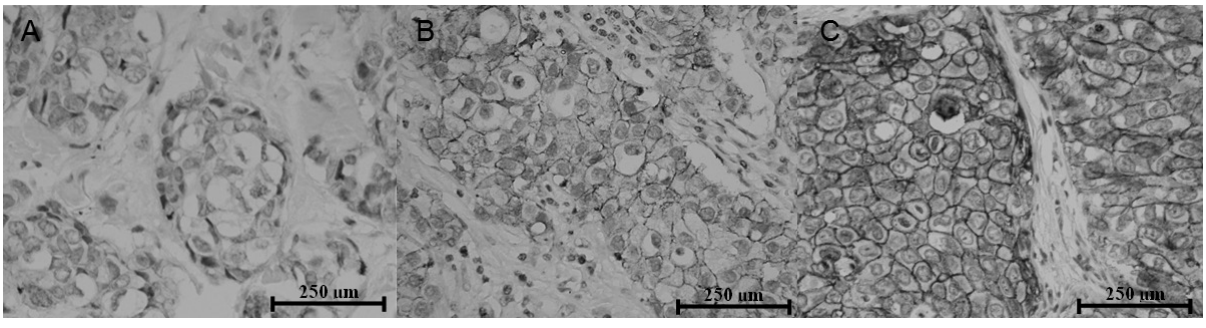

Figure 2 ESA expression determined by H-score. This Figure shows the representative results of ESA expression in triple negative subtype of breast cancer patients based on H-score analysis. Figure A shows low ESA expression of breast cancer patient (H-score =50) and moderate ESA expression (H-score = 120) is shown in Figure B. High ESA expression (H-score= 300) is shown in Figure C (Original magnification X 600).

Discussions

The main findings of this study are the relationship between prognostic factors (age, BMI and TNM staging) and average survival time that were studied in 4 subtypes (luminal A, luminal B, HER2 and triple negative subtype) of breast cancer. Old age, obese or advanced TNM staging of disease had negative influence on average survival time of breast cancer patients. Additionally, in triple negative subtype, which had poor average survival time, had overexpression of ESA.

For age factor, previous studies found that breast cancer patients under the age of 40 years had poor prognosis, high mortality rate, high recurrence rate and[12,13] low cumulative 5-year relative survival ratio (RSR) compared to older age group[14]. In this study, average survival time of breast cancer patients was studied in 2 different age groups and the results showed that age group more than or equal 41 years had short average survival time and high fatality risk compared to the age group ranging between 21-40 years and the results were significantly different in luminal A subtype and triple negative subtype. These results possibly indicate that younger age breast cancer patients has better average survival time and low risk of fatality compared to older age patients. This might be because

younger patients had good performance status with good health status. Consequently, these patients can well recover after treatment and have a chance to receive aggressive treatment resulting in having more effectiveness of treatment outcome compared to older age breast cancer patients, who have more comorbidity and decreased immune response. The results were not consistent with the previous studies because young age breast cancer patients included in the previous studies mostly had advanced stage with aggressive subtypes such as triple negative or HER2 subtype and delayed diagnosis and treatment resulting in worse prognosis but young age breast cancer patients included in this study had good performance status with no comorbidity. Additionally, some previous studies proposed that there was no significant difference in overall survival time compared between younger and older age breast cancer patients[15-17]. Therefore, there is still controversy about age factor influencing on prognosis of breast cancer. It might depend on many factors involving in the status of patients, which affects the outcome of treatment.

For BMI factor, several studies revealed that high BMI related to poor prognosis, increased distant metastasis, increased risk of mortality and short survival time[18,19]. Our results showed that obesebreast cancer patients had short average survival time and high fatality risk compared to non-obese patients. This might be because breast cancer patients with obesity have increased risk of insulin resistance, cardiovascular risk, risk of cancer development and poor response of neoadjuvant chemotherapy. It was proposed that prolong period of obesity results in changing abnormal adipose tissue distribution, cytokine and lipid secretion profiles[20]. Expansion of adipose tissues leads to fat accumulation and increases free fatty acid (FFA). FFA is a source for cancer development and associates with oxidative processes and activates pathway of insulin resistance[21]. Additionally, previous studies about inhibition of fatty acid synthase revealed that this could reduce capacity of cancer cell proliferation[22,23]. In addition, increasing adipose tissues result in increased aromatase enzyme, which leads to increased converting androgen, androstenedione and testosterone to estrogens leading to increasing risk of breast cancer[24]. Another pathway of adipose tissue involving in cancer promotion, when adipocytes have interaction with immune cells and this leads to inflammation and subsequently contributes to adipose tissue dysfunction, which influences on tumor promotion and progression[25,26]. However, this factor is preventable. To increase effectiveness of treatment and decrease treatment resistance, breast cancer patients should be suggested to control weight and have normal weight to have better prognosis and survival time.

TMN staging is certainly one of important prognostic factor in many cancers including breast cancer because advanced stage of disease correlates to worse survival time and poor prognosis. In China, previous studies found that TNM staging significantly had influence on 5-year overall survival rate in breast cancer patients. The results showed that at early TNM staging, patients had longer survival time compared to the late staging[27]. These results were consistent with data studied in Thailand and the results presented that the 5-year survival rate had decreased when the staging had risen[28]. In this study, the results showed that TNM staging significantly had an influence on the average survival time only in luminal A and luminal B subtype. Therefore, patients with stage IV had shorter average survival time and high fatality risk compared to patients with the early stage of disease. Advanced stage of breast cancer has aggressive disease with distant metastasis. Additionally, advanced stage normally has large size of tumor with spreading to lymph nodes and metastasising to multiple distant organs. Consequently, patients with advanced stage certainly have poor response of treatment resulting in short survival time compared to early stage of disease. Therefore, patients should be aware about the abnormality of breast and come to see the doctor as early as possible when abnormal signs or symptoms are detected. When the disease is detected at the early stage, patients will receive early treatment and the

disease is not aggressive with no spreading resulting in good prognosis and treatment outcome.

For ESA expression, many previous studies revealed that ESA plays an important role in many cancers such as breast cancer, hepatocellular carcinoma, renal cell cancer, colon cancer and pancreas cancer[29,30]. In our studies, triple negative breast cancer patients had high expression levels of ESA and higher levels of ESA expression were detected in the older age group compared to the younger age group but the data were not significantly different. The important roles of ESA in cancer cells are inducing cell proliferation, differentiation and migration via specific mechanism[7-9]. The previous study proposed that ESA can promote cancer metastasis via inhibiting the expression of cadherin, which mediates cell adhesion[31]. Therefore, this results in cancer aggressiveness, poor prognosis and short survival time. This is consistent with our study that triple negative subtype had worse average survival time after luminal B subtype. About 80% of basal-like breast cancer characteristics are similar to triple negative breast cancer and one of distinct characteristics of basal-like breast cancer is having high molecular weight of basal cytokeratin,[32] which has a link to the origin of ESA. Therefore, both basal-like breast cancer and triple negative breast cancer have the majority of characteristics in common. Triple negative subtype possibly also has basal cytokeratin; consequently, this might result in the high expression levels of ESA in triple negative subtype[33]. Additionally, triple negative subtype has a high chance to present in patients with obesity[12]; therefore, this factors certainly lead to aggressive tumor and poor prognosis that were mentioned earlier in BMI part. In addition, there is no target receptor for treatment resulting from no hormonal receptors detected in triple negative subtype and has overexpression of ESA, which enhance cancer aggressiveness, metastasis and resistance of treatment. Therefore, it is very interesting to study further in triple negative subtype of breast cancer to deeply investigate the specific characteristics and develop effective treatment in this subtype.

Conclusions

The results revealed that age, BMI and TNM staging were significant factors correlating to average survival time. Old age, obesity or advanced stage of breast cancer patients associated with worse average survival time. For triple negative subtype, these patients tended to have overexpression of ESA with poor average survival time. Older age group of triple negative subtype, who had poor survival time, had overexpression of ESA. Therefore, overexpression of ESA possibly correlates with poor prognosis of breast cancer patients.

Limitation and Suggestion of this study

1. There is too small number of breast cancer patients included in this study. Especially, when breast cancer patients were classified into 4 subtypes, there were only 6 patients classified as HER2 subtype in this study.

2. Cohort study should be performed via multicenter study to include more number of breast cancer patients; therefore, the data can be analysed more effectively and have statistical significance.

3. This study is pilot study of ESA expression in breast cancer patients in Srinagarind hospital. The effects of different ESA expression levels (weak and overexpression) should be studied further in aspects of tumor aggressiveness, tumor metastasis, lymph node metastasis, tumor recurrence rate and treatment response. Further study should be performed to prove the influence of ESA expression in prognosis and treatment outcome of breast cancer patients.

Acknowledgements

This study was supported by Invitation Research Fund (IN60310), Faculty of Medicine, Khon Kaen University, Thailand.

References

1. Lawson JC, Blatch GL, Edkins AL. Cancer stem cells in breast cancer and metastasis. Breast Cancer Res Treat 2009; 118: 241-54.

2. Jiang W, Peng J, Zhang Y, Cho WC, Jin K. The implications of cancer stem cells for cancer therapy. Int J Mol Sci 2012; 13: 16636-57.
3. Dalerba P, Cho RW, Clarke MF. Cancer stem cells: models and concepts. Annu Rev Med 2007; 58: 267-84.
4. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A 2003; 100: 3983-8.
5. Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, et al. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell 2007; 1: 555-67.
6. Azzam DJ, Zhao D, Sun J, Minn AJ, Ranganathan P, Drews-Elger K, et al. Triple negative breast cancer initiating cell subsets differ in functional and molecular characteristics and in gamma-secretase inhibitor drug responses. EMBO Mol Med 2013; 5: 1502-22.
7. Osta WA, Chen Y, Mikhitarian K, Mitas M, Salem M, Hannun YA, et al. EpCAM is overexpressed in breast cancer and is a potential target for breast cancer gene therapy. Cancer Res 2004; 64: 5818-24.
8. Cimino A, Halushka M, Illei P, Wu X, Sukumar S, Argani P. Epithelial cell adhesion molecule (EpCAM) is overexpressed in breast cancer metastases. Breast Cancer Res Treat 2010; 123: 701-8.
9. Munz M, Kieu C, Mack B, Schmitt B, Zeidler R, Gires O. The carcinoma-associated antigen EpCAM upregulates c-myc and induces cell proliferation. Oncogene 2004; 23: 5748-58.
10. Soysal SD, Muenst S, Barbie T, Fleming T, Gao F, Spizzo G, et al. EpCAM expression varies significantly and is differentially associated with prognosis in the luminal B HER2(+), basal-like, and HER2 intrinsic subtypes of breast cancer. Br J Cancer 2013; 108: 1480-7.
11. Spizzo G, Went P, Dirnhofer S, Obrist P, Simon R, Spichtin H, et al. High Ep-CAM expression is associated with poor prognosis in node-positive breast cancer. Breast Cancer Res Treat 2004; 86: 207-13.
12. Anders CK, Johnson R, Litton J, Phillips M, Bleyer A. Breast cancer before age 40 years. Semin Oncol 2009; 36: 237-49.
13. Kroman N, Jensen MB, Wohlfahrt J, Mouridsen HT, Andersen PK, Melbye M. Factors influencing the effect of age on prognosis in breast cancer: population based study. BMJ 2000; 320: 474-8.
14. Fredholm H, Eaker S, Frisell J, Holmberg L, Fredriksson I, Lindman H. Breast cancer in young women: poor survival despite intensive treatment. PLoS One 2009; 4: e7695.
15. Vostakolaei FA, Broeders MJ, Rostami N, van Dijck JA, Feuth T, Kiemeney LA, et al. Age at diagnosis and breast cancer survival in iran. Int J Breast Cancer 2012; 2012: 517976.
16. Crowe JP, Jr., Gordon NH, Shenk RR, Zollinger RM, Jr., Brumberg DJ, Shuck JM. Age does not predict breast cancer outcome. Arch Surg 1994; 129: 483-7; discussion 487-8.
17. Rapiti E, Fioretta G, Verkooijen HM, Vlastos G, Schafer P, Sappino AP, et al. Survival of young and older breast cancer patients in Geneva from 1990 to 2001. Eur J Cancer 2005; 41: 1446-52.
18. Montazeri A, Sadighi J, Farzadi F, Maftoon F, Vahdaninia M, Ansari M, et al. Weight, height, body mass index and risk of breast cancer in postmenopausal women: a case-control study. BMC Cancer 2008; 8: 278.
19. Litton JK, Gonzalez-Angulo AM, Warneke CL, Buzdar AU, Kau SW, Bondy M, et al. Relationship between obesity and pathologic response to neoadjuvant chemotherapy among women with operable breast cancer. J Clin Oncol 2008; 26: 4072-7.
20. Bray GA, Popkin BM. Dietary sugar and body weight: have we reached a crisis in the epidemic of obesity and diabetes?: health be damned! Pour on the sugar. Diabetes Care 2014; 37: 950-6.
21. Virtue S, Vidal-Puig A. Adipose tissue expandability, lipotoxicity and the Metabolic Syndrome--an allostatic perspective. Biochim Biophys Acta 2010; 1801: 338-49.
22. De Schrijver E, Brusselmans K, Heyns W, Verhoeven G, Swinnen JV. RNA interference-mediated silencing of the fatty acid synthase gene attenuates growth and induces morphological changes and apoptosis of LNCaP prostate cancer cells. Cancer Res 2003; 63: 3799-804.
23. Pizer ES, Pflug BR, Bova GS, Han WF, Udan MS, Nelson JB. Increased fatty acid synthase as a therapeutic target in androgen-independent prostate cancer progression. Prostate 2001; 47: 102-10.
24. Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet 2008; 371: 569-78.
25. Perez-Hernandez AI, Catalan V, Gomez-Ambrosi J, Rodriguez A, Fruhbeck G. Mechanisms linking excess adiposity and carcinogenesis promotion. Front Endocrinol (Lausanne) 2014; 5: 65.
26. Balkwill F, Charles KA, Mantovani A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell 2005; 7: 211-7.

27. Dong G, Wang D, Liang X, Gao H, Wang L, Yu X, et al. Factors related to survival rates for breast cancer patients. Int J Clin Exp Med 2014; 7: 3719-24.
28. Poum A, Kamsa-ard S, Promthet S. Survival rates of breast cancer: a hospital-based study from northeast of Thailand. Asian Pac J Cancer Prev 2012; 13: 791-4.
29. Schnell U, Cirulli V, Giepmans BN. EpCAM: structure and function in health and disease. Biochim Biophys Acta 2013; 1828: 1989-2001.
30. Spizzo G, Fong D, Wurm M, Ensinger C, Obrist P, Hofer C, et al. EpCAM expression in primary tumour tissues and metastases: an immunohistochemical analysis. J Clin Pathol 2011; 64: 415-20.
31. Spizzo G, Obrist P, Ensinger C, Theurl I, Dunser M, Ramoni A, et al. Prognostic significance of Ep-CAM AND Her-2/neu overexpression in invasive breast cancer. Int J Cancer 2002; 98: 883-8.
32. Badve S, Dabbs DJ, Schnitt SJ, Baehner FL, Decker T, Eusebi V, et al. Basal-like and triple-negative breast cancers: a critical review with an emphasis on the implications for pathologists and oncologists. Mod Pathol 2011; 24: 157-67.
33. Agboola AJ, Paish EC, Rakha EA, Powe DG, Macmillan RD, Ellis IO, et al. EpCAM expression is an indicator of recurrence in basal-like breast cancer. Breast Cancer Res Treat 2012; 133: 575-82.