JMedSci

Journal of the Medical Sciences
(Berkala Ilmu Kedokteran)

Volume 52, Number 3, 2020; 243-250
http://dx.doi.org/10.19106/JMedSci005203202006

Correlation between CD44 expression on histopathological grading, metastasis, survival overall (SO) and disease free survival (DFS) on women breast cancer

Ahmad Syaifudin[1], Septiman[2], Indra[2], Prihantono[2]

[1]Department of Surgery, [2]Division of Oncology Surgery, Department of Surgery, Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia

Submitted : 2020-04-01
Accepted : 2020-05-26

ABSTRACT

Breast cancer is one of the health problems in the world. In Indonesia, it was highest prevalence of cancer in 2018. CD44, a non-kinase transmembrane glycoprotein, plays an important role in cancer development and progression. It is responsible for mediating of the adhesion between adjacent cells andal so between cells and the extracellular matrix. The aim of study was to investigate the relationship between the CD44 expressionand histopathological grading, metastasis, overall survival (OS) as well as disease free survival (DFS) in breast cancer patients.This was a retrospective cross sectional study using data on medical records of breast cancer cases at Dr. Wahidin Sudirohusodo General Hospital, Makasar from 2016 to 2018. Examination of CD44 expression were performed from paraffin block samples. Data of the CD44 expression and medical records obtained were then presented in tables and statistically analyzed using chi-squared and Kaplan–Meier survival analysis. No significantly relationship between the CD44 expression and histopathological grading was reported ($p>0.05$). However, significantly relationship between the CD44 expression and metastasis war reported ($p<0.01$). Patients with metastasis having high CD44 expression was significantly higher (90%) than those patients without metastasis (60%) ($p<0.01$). No significantly relationship between the CD44 expression and OS or DFS was reported ($p>0.05$). In conclusion, there is relationship between CD44 expression and metastasis in the breast cancer patients. However, there is norelationship between CD44 expression and histopathological grading, OS as well as DFS.

Keywords:
breast cancer;
CD44 expression;
metastatic;
prognosis;
overall survival;

ABSTRAK

Kanker payudara merupakan salah satu masalah kesehatan di dunia. Di Indonesia, prevalensi kanker payudara paling tinggi pada 2018. CD44, glikoprotein transmembran non kinase, berperan penting dalam perkembangan dan progresivitas kanker. CD44 berperan dalam proses adisi antara sel yang berdekatan dan juga antara sel dan matrik ekstra selular. Penelitian ini bertujuan untuk mengkaji hubungan antara ekspresi CD44 dan nilai gambaran histopatologi, metastasis, keseluruhan kelangsungan hidup (OS), kelansungan hidup bebas penyakit (DFS) pada pasien kanker payudara. Ini adalah penelitian potong lintang retrospektif menggunakan data rekam medis kasus kanker payudara di Rumah Sakit Umum Dr. Wahidin Sudirohusodo, Makasar dari tahun 2016-2018. Pemeriksaan ekspresi CD44 dilakukan dari sampel blok parafin. Data ekspresi CD44 dan rekam medis yang diperoleh selanjutnya dipresentasikan dalam table dan dianalisis secara statistic menggunakan chi-square dan analisis keseluruhan hidup Kaplan-Meier. Tidak terdapat hubungan nyata antara ekspresi CD44 dan nilai gambaran histopatologi ($p>0,05$). Namun demikian, terdapat hubungan nyata antara ekspresi CD44 dan metastasis ($p<0,01$). Pasien metastasis yang mempunyai eskpresi CD44 tinggi (90%) lebih besar secara nyata dibandingkan pasien tanpa metastasis (60%). Tidak ada hubungan nyata antara ekspresi CD44 dan OS atau DFS ($p>0,05$). Dapat disimpulkan ada hubungan antara ekspresi CD44 dan metastasis pada pasien kanker payudara. Namun demikian tidak ada hubungan antara ekspresi CD44 dan nilai gambaran histopatologi, OS dan DFS.

*corresponding author: ahmadsyaifudin8@yahoo.co.id

INTRODUCTION

Breast cancer is one of the health problems in the world. It is the he most commonly occurring cancer in women and the second most common cancer overall. According to the Global Cancer Statistics 2018, 18.1 million new cancer cases were reported (17.0 million excluding nonmelanoma skin cancer) and 9.6 million cancer deaths (9.5 million excluding nonmelanoma skin cancer) in 2018. In both sexes combined, lung cancer is the most commonly diagnosed cancer (11.6% of the total cases) and the leading cause of cancer death (18.4% of the total cancer deaths), closely followed by female breast cancer (11.6%). According to the American Cancer Society (ACS) in 2012, breast cancer is the second leading cause of death in women (14%) after lung cancer (26%) in the United States.[1] In Indonesia, based on Basic Health Research (*Riset Kesehatan Dasar*/ Riskesdas) data in 2018, breast cancer is the highest prevalence of cancer in Indonesia that is suffered 58,256 patients or 16.7% from 348.809 cases.[2] Hospital based study conducted in Dr. Wahidin Sudirohusodo General Hospital, Makasar reported during a period 2008-2012 as much as 1,497 patients (299 patients a year) were diagnosed as breast cancer. About 39.4% of them aged between 40-49 years.[3]

In the developed countries, more breast cancer is diagnosed in early stages.[4] However, in the developing countries including Indonesia it is diagnoses in late stages. In Padang, the percentage of breast cancer patients with stage IIIA and IIIB were 43.4 and 14.3%, respectively.[4] In West Jakarta, it was reported the most of frequent of female breast cancer were in 45-49 years old, not other specific location subtopography (81.5%), the histopathology type was ductal and lobular neoplasm (51.8%), in stage III (44%), and with metastases to bone (34%).[5]

Various studies have proven the important role of tumor markers in assessing disease progression, monitoring therapy, recurrence and prognostic factors in malignancies.[6-8] CD44, a non-kinase transmembrane glycoprotein, plays an important role in cancer development and progression. It is responsible for mediating of the adhesion between adjacent cells and also between cells and the extracellular matrix. The CD44 is associated with prognostic factors in breast cancer.[9,10] The adhesion cells play an important role to construc the basic form of normal breast histology that are important for maintaining tissue integrity. This disruption of adhesion relationship causes loss of network architecture which is a sign of malignant transformation. This study aimed to evaluate the relationship between CD44 expression of histopathological grading, metastasis, overall survival (OS) and disease free survival (DFS).

MATERIALS AND METHODS

Design and subject

This was a retrospective cross sectional study using data on medical records of breast cancer cases at Dr. Wahidin Sudirohusodo General Hospital, Makasar from 2016 to 2018 who met the inclusion and exclusion criteria. The inclusion criteria were women with breast cancer who have been proven by histopathological examination, breast cancer patients with PA results showing histopathological grading by Nottingham Modification of Bloom Richardson System, patients with metastatic breast cancer evidenced by radiological examination (photo thorax, ultrasound, CT scan or MRI), and patient were willing to be involved in this study. The exclusion criteria were breast cancer patients with biopsy results do not show histopathological grading, and suffer

from other types of cancer (eg ovarian, liver, lung cancer). Subjects were selected using a randomized purposive sampling method taking members of the population. Sixty subjects who met the inclusion and exclusion criteria were involved in this study.

CD44 expression examination

The paraffin block sample was examined for CD44 expression using CD44 antibody (DF1485, Novocastra Laboratories Ltd., dilution 1:100) then count the percentage of positive cells and their intensity visually using a comparison light microscope. Immunostained samples were evaluated blind by one pathologist. CD44 was stained in cell membrane, with score 0 if < 10% positive tumour cells; 1 if 10-25%; 2 if 25-50%; and 3 if > 50%. Intensity was scored as 0 if unstained, 1 if weakly stained, 2 if intermediate, and 3 if strong. Interpretation of CD44 staining was determined based on multiplication of the percentage of positive cells and the intensity of staining. CD44 was scored as low if (-until +4) and high (+5 until +9). Data of CD44 expression and medical records were then processed, analyzed with chi square and presented in tables, graphs and narratives. This study was approved by the Joint Ethics Committee of Faculty of Medicine Hasanuddin University, Hasanuddin University Hospital, and Dr. Wahidin Sudirohusodo Hospital (Number 258/UN4.6.5.31/ PP36/2020).

Statistical analysis

Data were presented as frequency distribution or percentage. Chi Square test was used to evaluate the relationship between histopathological grading or metastasis with CD44 expression. Kaplan–Meiersurvival analysis was applied to evaluate relationship of overall survival (OS) or disease free survival (DFS) with CD44expression.

RESULT

The subjects characteristics of the study are presented in the TABLE 1. The largest number of subject saged between 40-49 years (63.7%) with moderate grading (55.0%), not metastatic (50.0%), multiple metastatic sites (15%), no DFS status (56.7%), and no OS status (60.0%). The subjects with high CD44 levels was 75.0% and low CD44 levels was 25.0%.

TABLE1. Frequency distribution of research subjects characteristics

Characteristics	Number	Percentage (%)
Age (year old)		
• <40	12	20.0
• 40-49	38	63.7
• 50-59	14	23.3
• 60-69	6	10.0
• >= 70	0	0.0
Hispathological grade		
• Low	4	6.6
• Moderate	33	55.0
• High	23	23.6
Metastatic amount		
• Multiple	9	15.0

• Single	21	35.0
• None	30	50.0
Metastatic site		
• Bone	3	5.0
• Lung	14	23.4
• Liver	2	3.3
• Brain	2	3.3
• None	30	50.0
• Multiple	9	15.0
DFS status		
• Yes	26	43.3
• No	34	56.7
OS status		
• Yes	24	40.0
• No	36	60.0
CD44 expression		
• High	45	75.0
• Low	15	25.0

TABLE 2 shows the relationship between CD44 expression with histopathological grading of the breast cancer patients. The high CD44 expression of patients with high, moderate, and low histopathological gradingwere 81.0%, 75.8% and 50.0%, respectively. No significantly relationship between CD44 expression with histopathological grading.

TABLE 2. Relationship between CD44 expression with histopathological grading

Histopathological grading [n (%)]	CD44 expression [n(%)]		Total
	Low	High	
High	4 (19.0)	17 (81.0)	21 (100.0)
Moderate	8 (24.2)	25 (75.8)	33 (100.0)
Low	3 (50.0)	3 (50.0)	6 (100.0)
Amount	15 (25.0)	45 (75.0)	60 (100.0)

Chi square test (p=0.300)

TABLE 3 shows the relationship between CD44 expression with metastasis in breast cancer patients. Patients with metastasis having high CD44 expression was significantly higher (90%) than those patients without metastasis (60%) ($p<0.01$). A significantly relationship between the CD44 expression and metastasis war observed ($p<0.01$).

TABLE 3. Relationship between CD44 expression andmetastatic

Metastatic [n (%)]	CD44 expression [n (%)]		Total
	Low	High	
Yes	3 (10.0)	27 (90.0)	30 (100.0)
No	12 (40.0)	18 (60.0)	30 (100.0)
Total	15 (25.0)	45 (75.0)	60 (100.0)

Chi square test (p=0.01)

FIGURE 1 shows the OS analysis using median with following up to 3 years for survival that could be divided into two groups. From month 0 to 5th month, the survival rate for OS with low and high CD44 expressions was similar with OS value of 75%. Decrease in 35% survival rate of patients with high CD44 expression was observed on the 10th month, where as for patients with low CD44 expression on the 17th month. Furthermore, the patients with high CD44 expression showed a progressive decrease in survival rate from month to month. In the 20th month, the survival rate decreased to be <15% and then 0-5% in the 25th month. The decrease in survival rate of patients with low CD44 expression in the 20th, 25th, 30th, and 40th moths were 20%, 15-20%, 10% and 5-10%, respectively. No significantly relationship between CD44 expression and OS was observed (p>0.05).

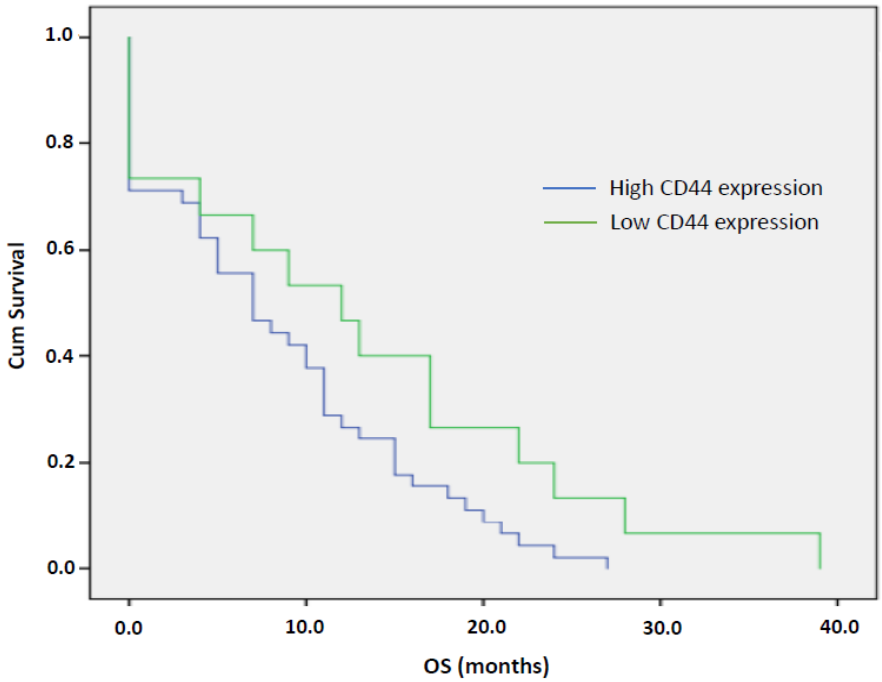


FIGURE1. Kaplan–Meier curve relationship of OS according to CD44 expression

FIGURE 2 shows the prediction test of patient's survival with low and high CD44 expressions using Kaplan-Meier analysis. The DFS degree of patients with high CD44 expression in the 5th, 10th, 20th were 60%, 20% and 5%, respectively. The longer the patients suffer, the more decreased the patient's ability to respond to the effects of the disease. The DFS degree of patients with low CD44 expression in the 5th, 10th, 20th were 65%, 40% and 20%, respectively. Although the DFS degree in low CD44 expression group was higher than that in high CD44 expression group, it was not significantly different.

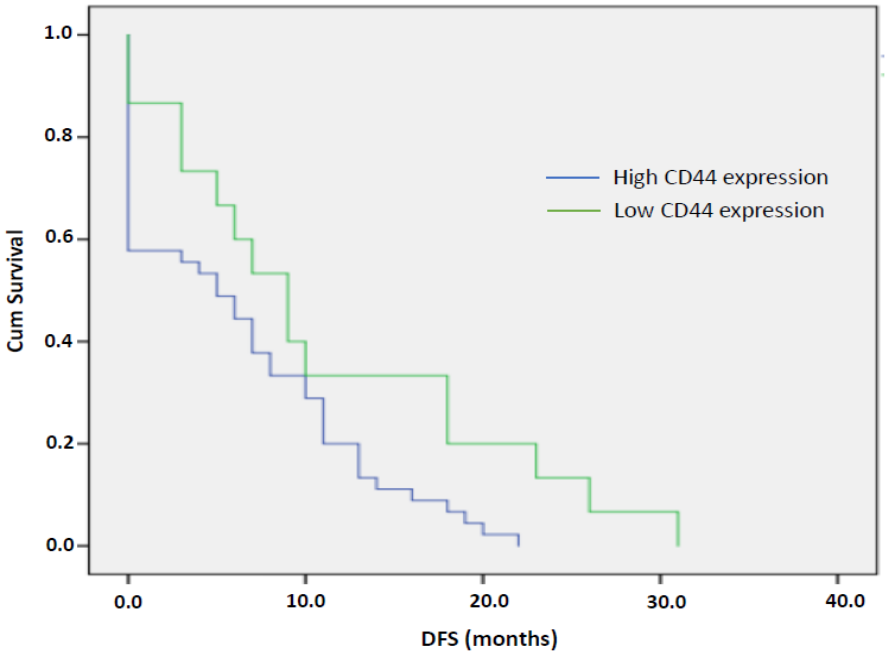
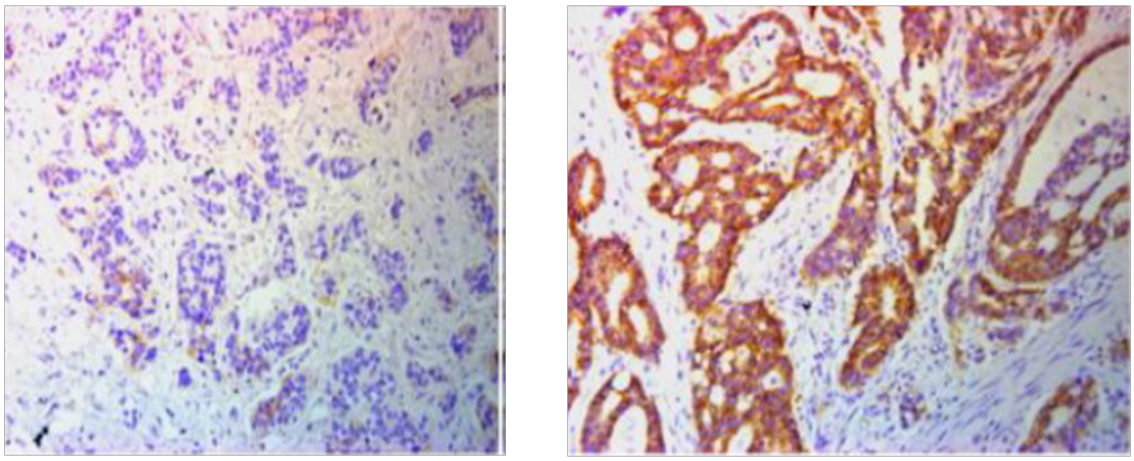


FIGURE 2. Kaplan–Meier curve relationship of DFS according to CD44 expression

FIGURE 3 shows the CD44 immunohistochemical staining of breast cancer tissues. Low CD44 expression was considered if less than 50%of the positive cells membrane or cytoplasmic were observed. High CD44 expression was considered if more than 50% of the positive cells membrane or cytoplasmic were observed.



A B

FIGURE 3. Imunohistochemistry CD44 Expression stain (scale 400x). A) negative or low expression; B) positive or high expression.

DISCUSSION

CD44 is involved in multiple physiological and pathological processes, such as angiogenesis, cell adhesion, inflammation, and cancer development. It is involved in tumorigenesis and metastasis in many cancer types such as colon, bladder, and gastric cancers. However, the role of the CD44 in breast cancer remains unclear.[11] Previous studies concerning the role of CD44 in tumorigenesis and metastasis gave different results.

In this study, no significantly relationship between the CD44 expression and histopathological grading was reported. The relationship between CD44 expression and histopathological grading has been reported in the previous studies with different results. Farida *et al.*[12] and Jang *et al.*[13] reported that CD44 expression is not correlated with histopathological grading in breast cancer. Otherwise, Moansoori *et al.*[14] and McFarlane *et al.*[15] reported a significant positive correlation between CD44 expression with histological grade. Breast cancer samples with advance histological grade also had higher CD44 expression. In addition, Qiao *et al.*[16] also reported that CD44v6, a variant isoform of CD44, is associated with histopathological grade.

A positive significantly relationship between the CD44 expression and metastasis was reported in this study. Breast cancer patients with metastasis have high CD44 expression (90%) higher than those patients without metastasis (60%). Studies concerning the role of CD44 in tumorigenesis and metastasis in many cancer types including breast cancers have been conducted. Qiao et al.16 reported that CD44v6 expression is significantly associated with poor prognosis, histological grade, and lymph node metastasis in breast cancer patients, especially among Asian patients. Moreover, McFarlane *et al.*[15] also reported that CD44 expressionis associated with metastasis-related phenotype.

Kaplan-Meier analysis revealed that no significantly relationship between CD44 expression and OS as well as DFS were observed in this study ($p>0.05$). Previous studies have been reported with different results. McFarlane *et al.*[15] reported that high CD44 expression correlated with the reduced DFS in lymph node-positive patients and patients with large tumor size. Furthermore, the correlation between CD44 overexpression and the poor OS rate(pooled OR =1.27, 95%CI: 1.04–1.55, Cochran's Q test p=0.505, and I2 =0.0%) was reported. However, CD44 expression was not associated with the RFS rate (pooled OR=1.04, 95% CI:0.89-1.23, Cochran's Q test p=0.417, and I2 =2.4%).11

CONCLUSION

In conclusion, there is a significant correlation between high CD44 expression and single or multiple metastatic of breast cancer patients indicating it can be used for metastatic prognostic factor. However, there is no significant correlation between CD44 expression and histopathological grading, OS and DFS.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2018 Nov; 68(6):394-424.
2. Balitbang Kemenkes RI. Riset kesehatan dasar; RISKESDAS. Jakarta: Balitbang Kemenkes RI. 2013; 2013:110-9.
3. Hamdani W. Analisis Kadar Her-2 mRNA CTC darah pada kemoterapi

neo adjuvant pasien KPD stad III, tipe Her-2 vs Non Her-2 dan analisis kadar Her-2 mRNA pada kemoterapi adjuvant sebagai predictor rekuren. [Dissertation]. Makasar: Universitas Hasanuddin; 2014.
4. Ramli M. Update breast cancer management diagnostic and treatment. Majalah Kedokteran Andalas 2015; 24(38):28-53.
5. Agustina J, Sinulingga D, Susana E, Andinata B, Ramadhan R, Kadir A. Epidemiology of female breast cancer in West Jakarta, Indonesia. J Global Oncol 2018; 4(2). https://doi.org/10.1200/jgo.18.63300
6. Kotowicz B, Fuksiewicz M, Gmyrek JJ, Bidzinski M, Kowalska M. The assessment of the prognostic value of tumor markersand cytokines as SCCAg, CYFRA 21.1, IL-6, VEGF and sTNF receptors in patients with squamous cell cervical cancer, particularly with early stage of the disease. Tumor Biol 2016; 37:1271–8. https://doi.org/10.1007/s13277-015-3914-0
7. Jing JX, Wang Y, Xu AQ, San T, Tian BG, Du LL, *et al.* Tumormarkers for diagnosis, monitoring of recurrence and prognosis in patients with upper gastrointestinal tract cancer. Asian Pasific J Cancer Prev 2014; 15(23):10267-72. https://doi.org/10/7314/APJCP.2014.15.23.10267
8. Kabel AM. Tumor markers of breast cancer: new prospectives. J Oncol Sci 2017; 3(1):5-11. https://doi.org/10.1016/j.jons.2017.01.001
9. McFarlane S, Coulter JA, Tibbits P, O'Grady A, McFarlane C, Montgomery N, *et al.* CD44 increases the efficiency of distant metastasis of breast cancer. Oncotarget 2015; 6(13):11465-76. https://doi.org/10.18632/oncotarget.3410
10. Kong Y, Lyu N, Wu J, Tang H, Xie Z, Yang L, *et al.* Breast cancer stem cell markers CD44 and ALDH1A1 in serum: Distribution and prognostic value in patients with primary breast cancer. J Cancer 2018; 9(20):3728-35. https://doi.org/10.7150/jca.28032
11. Xu H, Tian Y, Yuan X, Liu Y, Wu H, Liu Q, *et al.* Enrichment of CD44 in basal-type breast cancer correlates with EMT, cancer stem cell gene profile, and prognosis. Onco Targets Ther 2016; 9:431-44. htpps://doi.org/10.2147/OTT.S97192
12. Farida A, Wresnindyatsih, Yuliantini V. Correlation CD24 and CD44 expression against aggressiveness breast cancer. JPhys Conf Ser 2019; 1246(1):012012). https://doi.org/10.1088/1742-6596/1246/1/012012
13. Jang MH, Kang HJ, Jang KS, Paik SS, Kim WS. Clinicopathological analysis of CD44 and CD24 expression in invasive breast cancer. Oncol Lett 2016; 12(4): 2728–33. https://doi.org/10.3892/ol.2016.4987
14. Mansoori M, Roudi R, Abbasi A, Abolhasani M, Rad IA, Shariftabrizi A, *et al.* High GD2 expression defines breast cancer cells with enhanced invasiveness. Exp Mol Pathol 2019; 109:25-35. https://doi.org/10.1016/j.yexmp.2019.05.001
15. McFarlane S, Coulter JA, Tibbits P, O'Grady A, McFarlane C, Montgomery N, Hill A, *et al.* CD44 increases the efficiency of distant metastasis of breast cancer. Oncotarget 2015; 6(13):11465-76. https://doi.org/10.18632/oncotarget.3410
16. Qiao GL, Song LN, Deng ZF, Chen Y, Ma LJ. Prognostic value of CD44v6 expression in breast cancer: a meta-analysis. Onco Targets Ther 2018; 11:5451-7. https://doi.org/10.2147/OTT.S156101