Tikrit Journal of Veterinary Sciences (2024) 3(1): 140-148
DOI : *10.25130/tjvs.3.1.12*

IRAQI Academic Scientific Journals

Tikrit Journal of Veterinary Science
http://tjvs.tu.edu.iq/

Immunohistochemical of Glycoprotein Cluster of Differentiation 44 (CD44) Expression in Breast Cancer

Entedhar Rifaat Sarhat [1], Zubaida N.M Albarzanji[2], Siham Agmee Wadee[3], Nawar J. Ali[4]

[1]Department of Biochemistry, Faculty of Medicine, University of Tikrit, /Iraq
[2]Department of Basic science, Faculty of Dentistry, University of Kirkuk, Iraq
[3]Department of Physiology, Pharmacology and Biochemistry ,Collage of Veterinary Medicine, University of Tikri, Iraq.
[4]Department of pathology and poultry disease, Collage of Veterinary Medicine, University of Tikrit, Iraq

ARTICLE INFO.

Article history:
-Received: 1/16/ 2023
-Accepted: 16/ 4/ 2024
-Available online: 30/6/2024

Keywords
CD44s; Breast cancer; Immunohistochemical staining.

Corresponding Author:
Name: Siham Agmee Wadee
E-mail: sihamwadee@tu.edu.iq

Tel: :

ABSTRACT

Breast cancer (BC) is the most frequently diagnosed cancer in women worldwide, and it is ranked as second in terms of cancer related death in women. The aim of this study several stem markers have been described for the various histological subtypes of breast cancer, stem cells (CSCs) of breast cancer have been found to strongly express adhesion molecule glycoprotein cluster of differentiation 44 (CD44). CD44 is expressed in the membrane of the cells, so it differs from the other studied markers which were cytoplasmic expressed markers. Negative expression scored (0) were observed in 15 out of 31samples (48.3%), while score (+) found in 5 out of 31samples (16.12%), score (++) found in 2 out of 31samples (6.4%)and score (+++) found in 9 out of 31samples.

1. Introduction

Cancer is characterized by loss of control of cellular growth and development leading to excessive proliferation and spread of cells. Breast cancer (BC) is the second most commonly diagnosed cancer and it still remains one of the major causes of death for cancer in world[1-2]. At present, breast cancer detection relies mostly on mammography, which has been associated with decreased breast cancer mortality [3-4]. Cancer stem cells (CSCs) make up a small subpopulation of cells within a tumor,

possess two main properties, the ability to self-renew and the ability to differentiate into heterogeneous lineages of cancer cells that comprise the tumor [5-6 -7].

Breast cancer is said to originate from breast cancer stem cells (BCSCs) that were first discovered by Al-Hajj and colleagues[8], and Phuc Van *etal* [9], are defined as a small fraction of cells capable of self-renewal and propagation of the heterogeneous populations of breast cancer cells[10] ,which possess the capacity for self-renewal and differentiation, are responsible for tumor initiation, proliferation and progression[11] characterized by high expression of aldehyde dehydrogenase (ALDH); and a quiescent, invasive, mesenchymal-like (M) state, characterized by $CD24^{-}CD44^{+}$ expression[12].

The glycoprotein cluster of differentiation 44 (CD44) also known as lymphocyte homing receptor, HUTCH-1, Hermes, H-CAM, and Ly-24, is an 85-90 kD a a family of non-kinase, single span transmembrane glycoproteins encoded by a single 20 exon gene located on the short arm of chromosome 11,, which are a class of oligonucleotides with a three-dimensional structure, can bind to CD44 in nanomolar range[13].

CD44 is a major receptor for binding hyaluronic acid (HA) in the extracellular matrix[14] ,that regulates cell adhesion and cell-cell, as well as cell-extracellular matrix interactions[15. It can be detached from cell surface by proteolytic enzyme activities found in blood serum which is used as a diagnostic tool to differentiate between malignant and non-malignant tumors[16].

2. Material and Methods:

2.1 Immunohistochemical staining procedure for detection of CD44,

2.1.1 General guidelines:

All incubations were carried out in a humidified chamber to avoid drying of the tissue. Drying at any stage led to non-specific binding and ultimately high background staining. A shallow, plastic box with a sealed lid and wet tissue paper in the bottom was an adequate chamber, just as long as the slides are kept off the paper and can lay flat so that the reagents don't drain off. Dilutions of the primary and secondary antibodies were listed on the datasheets or determined by testing a range. Dilutions were adjusted appropriately from the results obtained. All incubation times were restricted to the protocol[17] .

2.1.2 IHC staining method:

1. Slide baking: placed in a 45° angled inclined position in a hot air oven at 60°C over night.

2. Deparaffinization: the slides were immersed in xylene for 15 minutes two times at room temperature.

3. Rehydration: the slides were immersed sequentially in the following solutions at room temperature starting with:

-Twice in absolute ethanol for 5 minutes.

-95% ethanol for 5 minutes.

-90 % ethanol for 5 minutes.

-80 % ethanol for 5 minutes.

-70 % ethanol for 5 minutes.

-Distilled water for 5 minutes.

4. Enough drops of hydrogen peroxide block were added to slides and incubated in humid chamber at 37ºC for 10 minutes, then socked 2 times in buffer (5minutes for each).

5. Enough drops of protein block were added to slides and incubated at 37ºC for 10 minutes. Then washed 2 times in phosphate buffer PH:7 (5minutes for each), finally drained and blotted gently.

6. Diluted primary antibody was applied to each slide, incubated in humid chamber at 37ºC overnight .Early in the next day the slides were washed in buffer 4 times (5minutes for each), finally drained and blotted gently as before.

7. Enough drops of secondary antibody (link antibody yellow drops) reagent were added and incubated in humid chamber for 20 minutes at37ºC.After that, the slides were washed 4 times in phosphate buffer PH:7 (5minutes for each), finally drained and blotted gently.

8. Streptavidine-HRP antibodies (red drop) were applied on tissue and incubated for 20minutes at37ºC. After that, the slides were washed 4 times in phosphate buffer PH: 7 (5minutes for each), finally drained and blotted gently.

9. Diluted DAB was applied on tissue(this process was done in dark room) and incubated in humid chamber for 10 minutes at37ºC.Then slides washed carefully in tap water for 5 minutes

10. The slides were bathed in hematoxylin counterstain for 1-2 minutes then they were rinsed with tap water for 10 minutes.

11. Dehydration: the slides were dehydrated by immersing them in ethanol and xylene containing jars as follows:

-70 % ethanol for 1 minute.

-80 % ethanol for 1 minute.

-90 % ethanol for 1 minute.

-95 % ethanol for 1 minute.

-Twice in absolute ethanol for 1 minute each.

-Xylene for 6 minute.

12. one to two drops of DPX mounting medium was applied to the xylene wet sections and covered with cover slips and left to dry for 30 minutes.

2.1.3 Evaluation of Immunohistochemistry results

Positive reading was indicated when the cells display a brown cytoplasmic pigmentation staining, while negative reading was indicated for absence of immunostaining. Cut off values for all the antibodies used in the study were done with the help of a pathologist. The scoring was done under light microscope to evaluate the immunostaining of the antibodies; positively stained cells were counted at 5 representative fields (400X).

2.1.4 Immunohistochemical scoring of CD44:

CD44 expression was seen in the cell membrane of breast cancer cell and benign cells and the scoring of positive tumor cell was considered as follows[18].

0 =0-10%

1+ =10-25%

2+ =25-50%

3+ =more than 50%.

2.2 Statistical Analysis:

The values of the investigated parameters were given in terms of mean ± standard error, and person correlation using SAS computer program version 7.5. Differences in results were considered significant at probability value equal or less than 0.05 and 0.001[19].

3. Results and Discussion

CD44 is expressed in the membrane of the cells, so it differs from the other studied markers which were cytoplasmic expressed markers.

Negative expression scored (0) were observed in 15 out of 31samples (48.3%), while score (+) found in 5 out of 31samples (16.12%), score (++) found in 2 out of 31samples (6.4%)and score (+++) found in 9 out of 31samples (29%). While the benign breast lesions revealed positive expression in (5.26%) of lesions as in Table (1). Statistical analysis of the CD44 expression showed high significant difference between the malignant breast samples expression and the benign samples (P <0.001).

The figure (1).show the expression of CD44 in the membrane of ductal carcinoma stained by IHC (Brown stained membrane which indicates the positive expression CD44 and the negative expression showed no membrane staining).

Table (1): Immunohistochemical of CD44 Expression in ductal carcinoma.

Score group	0	+	++	+++	Total positive
Benign	18 (94.73%) A	0 (0%)B	0 (0%) B	1 (5.26%) C	1 (5.26%) from (19)
Malignant	15 (48.38%) B	5(16.12%) C	2 (6.45%) C	9 (29.03%) C	16 (51.6%) from(31)

*** P <0.001

Difference letters mean presence of significant difference.

Same letters mean there is no significant difference.

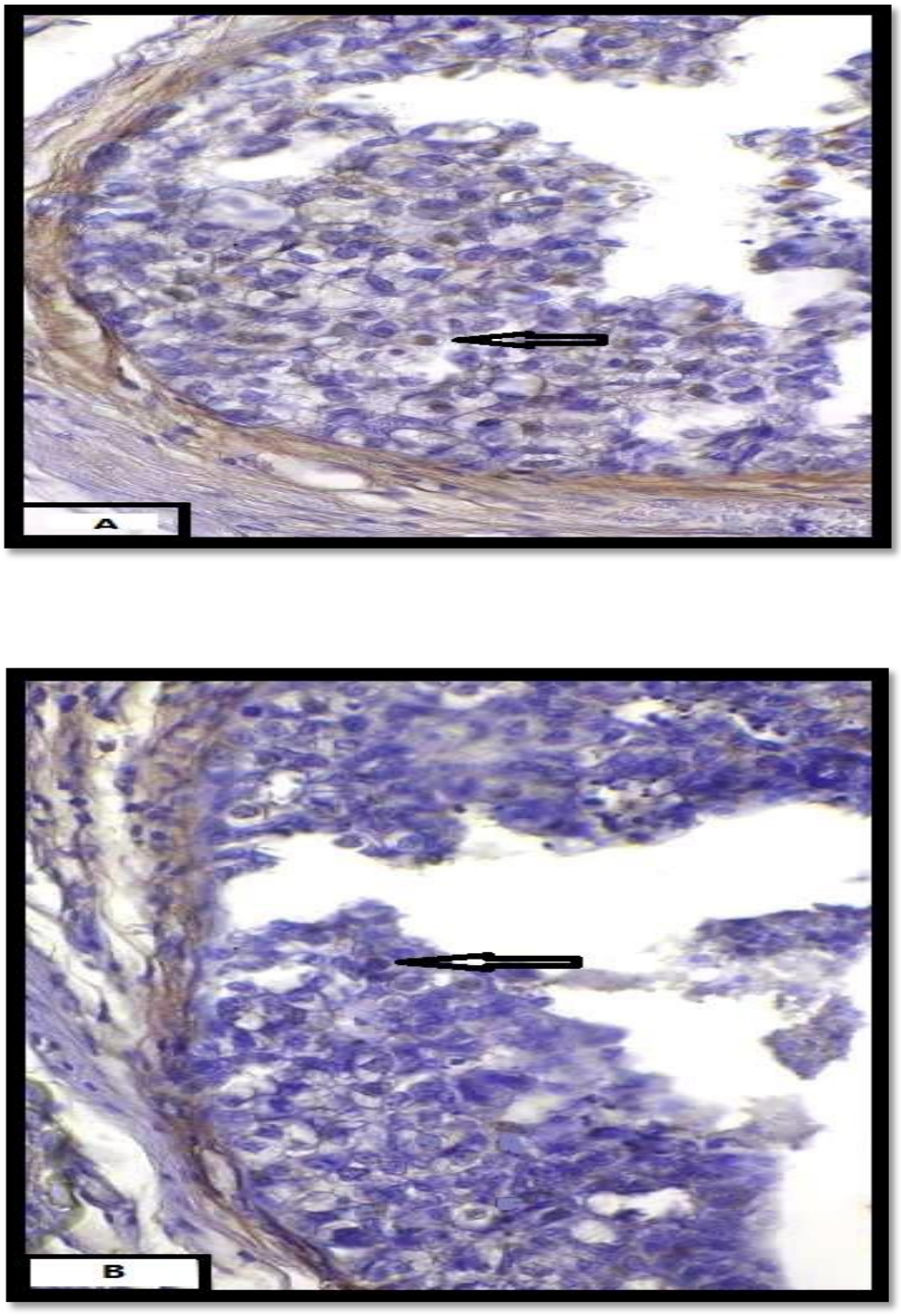

Figure 1:**Immunohistochemicalstaning in breast cancer sections. Immunostaining by peroxidase/ DAB (brown) counterstained with heamatoxyline (blue) , (A) positive CD44 expression(400X),(B) negative (no expression) for CD44 (40X).**

CD44 cells have been identified as putative cancer stem cells marker (CSCs) in breast cancer. However, the expression of this marker, as well as the CSCs role in the tumor microenvironment of breast cancer, remains largely unknown. In our study, we examined the expression of CD44 in human breast tumor tissues and assessed these clinicopathological correlations with other markers. Recent evidence has suggested that breast cancer originates from CSCs, which strongly express adhesion molecule CD44[8-20-21]. The cell adhesion molecule CD44 is the principal cell surface receptor for extracellular matrix glycosaminoglycan hyaluronan (HA), which is involved in a variety of important biological events, such as embryogenesis, hematopoiesis, lymphocyte homing and activation, inflammatory reactions, and tumor dissemination by interactions between CD44 and HA[22].

Our results relatively agreed with the results obtained from Wang et al., [23] ,who study the expression of CD44 in invasive ductal carcinoma which expressed in (43.6%). And also they completely agree with Ricardo et al., [18]. who revealed the CD44 CSCs marker was commonly expressed among primary breast carcinomas (51.2% of positive cases).

Cancer treatments are now being considered to focus on the tumor microenvironment as a therapeutic target, as the non-malignant cells are more genetically stable and less likely to evolve into drug resistant phenotypes.

Several recent reviews underscore the contribution of the microenvironment to tumor development[24-25]. While the normal cellular microenvironment can inhibit malignant cell growth, the modifications that occur in the tumor microenvironment synergistically support cell proliferation. Tumors shape their microenvironment and support the development of both tumor cells and non-malignant cells. An extensive review by Polyak, et al., [26]. concludes with a cautious summary, noting “although the importance of an altered microenvironment in tumorigenesis is no longer disputed, the nature of the molecular alterations underlying these changes remains unclear[26].

Conclusion: concludes with a cautious summary, noting “although the importance of an altered microenvironment in tumorigenesis is no longer disputed, the nature of the molecular alterations underlying these changes remains unclear.

References

1. Shaimaa Essa Ahmed , Entedhar R. Sarhat , Neda Awni , Thuraia Rifaat Sarhat& Kasim Sakran Abass(2021). Altered Serum Marker of Adipokines Profile in Breast Cancer Women.Indian Journal of Forensic Medicine & Toxicology,;15(3): 2598-2604.
2. Mahde S. Hamad , Entedhar R. Sarhat, 3Salim J. Khalaf , Thuraia Rifaat Sarhat& Kasim Sakran Abass(2020). Characteristic Abnormalities In Serum Biochemistry In Patients With Breast Cancer. Sys Rev Pharm;11(11):1967-1971.
3. Jin, J., Krishnamachary, B. & Mironchik, Y. et al (2016). Phototheranostics of CD44-positive cell populations in triple negative breast

cancer. Sci Rep 6, 27871 https://doi.org/10.1038/srep27871.

4. Kazarian A, Blyuss O, & Metodieva G, et al.(2017). Testing breast cancer serum biomarkers for early detection and prognosis in pre-diagnosis samples. Br J Cancer.;116(4):501–508. doi:10.1038/bjc.2016.433
5. Entedhar R. Sarhat. (2019).Practical Medical Biochemistry.1st rev. ed. Tikrit. Tikrit University Press.;425p.
6. Saeg F, Anbalagan M. (2018).Breast cancer stem cells and the challenges of eradication: a review of novel therapies. Stem Cell Investig;5:39.
7. Sarhat ER, Abid IM, Kamel NA, Sarhat TR, Abass KS. Changes of serum Interleukin and Chemerin levels in patients with Polycystic Ovary syndrome. J Adv Pharm Educ Res. 2021;11(4):11-4. https://doi.org/10.51847/XP8rpqX3Jx.
8. Al-Hajj M, Wicha MS, & Benito-Hernandez A. et al. (2003).Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A. 100:3983–3988.
9. Phuc Van Pham, Binh Thanh Vu, Nhan Lu-Chinh Phan, Hanh Thi Le, &Ngoc Kim Phan.(2015). In vitro spontaneous differentiation of human breast cancer stem cells and methods to control this process. Biomedical Research and Therapy.
10. Zhou J, Chen Q, Zou Y, Chen H, Qi L & Chen Y (2019) Stem Cells and Cellular Origins of Breast Cancer: Updates in the Rationale, Controversies, and Therapeutic Implications. Front. Oncol. 9:820. doi: 10.3389/fonc. 00820.
11. Yang F, Cao L, Sun Z, Jin J, Fang H, Zhang W, & Guan X.(2016). Evaluation of Breast Cancer Stem Cells and Intratumor Stemness Heterogeneity in Triple-negative Breast Cancer as Prognostic Factors. Int J Biol Sci; 12(12):1568-1577. doi:10.7150/ijbs.16874.
12. Ming Luo, Li Shang, Michael D. Brooks, Gary D. Luker, Douglas R. Spitz, &Max S. Wicha. (2018).Targeting Breast Cancer Stem Cell State Equilibrium through Modulation of Redox Signaling. Cell Metabolism.;28(1): P69-86.
13. Zhiyuan Xu, Ronghua Ni & Yun Chen. (2019).Targeting breast cancer stem cells by a self-assembled, aptamer-conjugated DNA nanotrain with preloading doxorubicin. ; (14) :6831—6842.
14. Covert H, Mellor LF, Wolf CL, Ankenbrandt N, Emathinger JM, Tawara K, Oxford JT, Jorcyk CL. Cancer Management and Research.2019;2019(11): 7721—7737
15. Calaf, G.M., Ponce-Cusi, R., & Abarca-Quinones, J. (2018). Effect of curcumin on the cell surface markers CD44 and CD24 in breast cancer. Oncology Reports, 39, 2741-2748. https://doi.org/10.3892/or.2018.6386
16. Anand M T, Kumar S. CD44: A key player in breast cancer. Indian J Cancer 2014;51:247-50
17. Khashman, B.M. (2008).Molecular and virological study of HPV on Iraqi patients with OSCC.Master thesis/ college of medicine, Baghdad University.
18. Ricardo, S.; Vieira, A.F.; Gerhard, R.; Leita˜o, D.; Pinto, R.; Cameselle-Teijeiro, J.F.; Milanezi, F.; Schmitt,F. and Paredes, J.(2011). Breast cancer stem cell markers CD44, CD24 and ALDH1: expression distribution within intrinsic molecular subtype.J. Clin .Pathol .,64:937- 946.
19. SAS users (2004). Guide personal computer (ver.7) inst. Inc. Cary. Nc., USA.
20. Abraham, B.K.; Fritz, P.; McClellan, M.; Hauptvogel, P.; Athelogou, M. and Brauch, H. (2005). Prevalence of CD44+/CD24−/low cells in breast cancer may not be associated with clinical outcome but may favor distant metastasis. Clin. Cancer Res., 11:1154–1159.

21. Lucinei Roberto OliveiraStefanie S JeffreyAlfredo Ribeiro- SilvaAlfredo Ribeiro-Silva. Stem cells in human breast cancer. Vieira and Paredes Molecular Cancer (2015) 14:178.
22. Murphy, J.F.; Lennon,F.; Steele, C.;Kelleher, D.; Fitzgerald, D. and Long, A.C.(2005). Engagement of CD44 modulates cyclooxygenase induction, VEGF generation, and proliferation in human vascular endothelial cells, FASEB J., 19:446–448.
23. Wang Z, Shi Q, Wang Z, et al. Clinicopathologic correlation of cancer stem cell markers CD44, CD24, VEGF and HIF-1α in ductal carcinoma in situ and invasive ductal carcinoma of breast: an immunohistochemistry-based pilot study. Pathol Res Pract. 2011;207:505–513.
24. Jain, R. K. (2005). Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science,307:58–62.
25. Shojaei, F. and Ferrara, N.(2008).Role of the microenvironment in tumor growth and in refractoriness/resistance to anti-angiogenic therapies. Drug Resistance Updates,11:219–230.
26. Polyak، K.; Haviv, I. and Campbell, I.G. (2009).Co-evolution of tumor cells and their microenvironment (Review).Trends Genet., 25: 30-38.

Tikrit Journal of Veterinary Sciences (2024) 3(1): 140-148
DOI : *10.25130/tjvs.3.1.12*

الكيمياء المناعية لمجموعة البروتين السكري للتمايز 44 (CD44) المعبر عنه في سرطان الثدي

أنتظار رفعت سرحت [1]، زبيدة نجاة البرزنجي [2] ، سهام عجمي وادي [3] نوار علي جاسم [4]

فرع الكيمياء الحياتية السريرية ، كلية الطب ، جامعة تكريت، تكريت، العراق[1]
فرع العلوم الاساسية ، كلية طب الاسنان ، جامعة كركوك، كركوك، العراق[2]
فرع الادوية والفسلجة والكيمياء الحياتية، كلية الطب البيطري، جامعة تكريت، تكريت، العراق[3]
فرع الامراض، كلية الطب البيطري، جامعة تكريت، تكريت، العراق[4]

الملخص

سرطان الثدي هو أكثر أنواع السرطان التي يتم تشخيصها لدى النساء في جميع أنحاء العالم، ويحتل المرتبة الثانية من حيث الوفيات المرتبطة بالسرطان لدى النساء. الهدف من هذه الدراسة هو وصف العديد من العلامات الجذعية للأنواع الفرعية النسيجية المختلفة للخلايا الجذعية لسرطان الثدي.

CSCs وقد وجد أنها تعبر بقوة عن مجموعة البروتين السكري لجزيء الالتصاق للتمايز 44 (CD44). يتم التعبير عن CD44 في غشاء الخلايا، لذلك فهو يختلف عن العلامات الأخرى التي تمت دراستها والتي كانت عبارة عن علامات معبر عنها السيتوبلازم. تمت ملاحظة التعبير السلبي (0) في 15 من أصل 31 عينة (48.3٪)، بينما تم العثور على النتيجة (+) في 5 من أصل 31 عينة (16.12٪)، والنتيجة (++) وجدت في 2 من أصل 31 عينة (6.4٪) و النتيجة (+++) وجدت في 9 من أصل 31 عينة.

الكلمات المفتاحية :سرطان الثدي ،التصبيغ المناعي ،البروتين السكري 44