Experimental and Molecular Pathology 109 (2019) 25–35

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

journal homepage: www.elsevier.com/locate/yexmp

High GD2 expression defines breast cancer cells with enhanced invasiveness

Maryam Mansoori[a,b], Raheleh Roudi[a], Ata Abbasi[c], Maryam Abolhasani[a,d], Isa Abdi Rad[e], Shariftabrizi A.[a,f,**], Zahra Madjd[a,b,*]

[a] Oncopathology Research Center, Iran University of Medical Sciences, Tehran, Iran
[b] Department of Molecular Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran
[c] Department of Pathology, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran
[d] Hasheminejad Kidney Center, Iran University of Medical Sciences, Tehran, Iran
[e] Cellular and Molecular Research Center, Urmia University of Medical Sciences, Urmia, Iran
[f] Department of Nuclear Oncology, Memorial Sloan Kettering Cancer Center, New York, USA

ARTICLE INFO

Keywords:
Breast cancer
Cancer stem cells
GD2
Tissue microarray
CD44
CD24

ABSTRACT

Introduction: Breast cancer is the most frequently diagnosed cancer among women. Cancer stem cells (CSCs) are suggested to be responsible for tumor initiation, progression, metastasis, recurrence and drug resistance. This study was conducted to evaluate the clinical significance of GD2, a newly suggested CSC marker and two other traditional CSC markers, CD44 and CD24 in breast cancer patients.

Material and methods: A total of 168 primary breast cancer tissues were evaluated in terms of GD2, CD44 and CD24 expression using tissue microarray. Then, the correlation of expression levels of these markers with patients' clinicopathological characteristics was assessed.

Results: Higher GD2 expression was mainly found in patients with advanced histological grade ($p = 0.02$), presence of lymph node invasion ($p = 0.04$), larger size of tumors ($p = 0.04$) and older age ($p = 0.04$). Breast cancer samples with advanced histological grade also showed higher CD44 ($p = 0.03$) and CD24 expression ($p = 0.05$). A significant positive association was found between increased CD24 expression and lymph node involvement ($p = 0.01$). Furthermore, GD2-high/CD44-high/CD24-low phenotype was frequently seen in breast cancer samples with positive lymph node involvement ($p = 0.05$).

Conclusion: In summary, increased expression of GD2 may define more aggressive tumor behavior in breast cancer. GD2 can well be considered as a diagnostic and prognostic marker in breast cancer.

1. Introduction

Breast cancer is the most frequent cancer among women and the second most commonly diagnosed cancer worldwide. The incidence of breast cancer in the last 30 years has been increasing in many countries (DeSantis et al., 2017). Strategies targeting the primary tumor have markedly improved, but systemic treatments to prevent metastasis are less effective; drug resistance and metastatic disease remain the underlying cause of death in the majority of patients with breast cancer (Hawley et al., 2018).

Cumulative evidence suggests that tumor growth is driven by a subpopulation of tumor cells, known as cancer stem cells (CSCs) or tumor initiating cells (TICs). These cells are thought to be responsible for cancer initiation, drug resistance, progression, recurrence and metastasis (Owens and Naylor, 2013). In an earlier study, CD44-high/CD24-low were suggested as breast CSCs which possessed high potential for tumor initiation, progression, metastasis and in vivo tumorigenesis (Al-Hajj et al., 2003). Subsequently, a panel of markers was introduced as potential breast CSC markers, including aldehyde dehydrogenase 1 (ALDH1) and CD133 (Ginestier et al., 2007; Wright et al., 2008). Therefore, identification of novel CSC markers with therapeutic applications to overcome drug resistance and tumor aggressiveness seems to be both necessary and challenging.

Ganglioside GD2 is a sialic acid-containing glycosphingolipid (GSL),

Abbreviations: CSC, cancer stem cell; ALDH1, aldehyde dehydrogenase; TMA, tissue microarray; IHC, immunohistochemistry; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; GD2S, GD2 synthase; GD3S, GD3 synthase

* Correspondence to: Z. Madjd, Oncopathology Research Center, Iran University of Medical Sciences (IUMS), Hemmat Street (Highway), Next to Milad Tower, Tehran 14496-14530, Iran.
** Correspondence to: A. Shariftabrizi, Department of Nuclear Oncology, Memorial Sloan Kettering Cancer Center, New York, USA.
E-mail addresses: sharifta@mskcc.org (A. Shariftabrizi), Majdjabari.z@iums.ac.ir (Z. Madjd).

https://doi.org/10.1016/j.yexmp.2019.05.001
Received 14 October 2018; Received in revised form 8 February 2019; Accepted 2 May 2019
Available online 08 May 2019
0014-4800/ © 2019 Elsevier Inc. All rights reserved.

Table 1
Association between the level of GD2 expressions (in terms of intensity of staining and H-score) with pathological parameters (*p*-value; Pearson χ^2). The bold value indicates a statistically significant difference with a p-value less than 0.05.

Patients characteristics	Total number (%)	Intensity of staining				*p*-value	H-score (Mean = 98)		p-value
		No staining	Weak	Moderate	Strong		Low	High	
Median age									
≤47	62 (53)	2(3)	37(60)	14(23)	9(14)	**0.04**	32(57)	30(43)	0.57
> 47	56 (47)	1(2)	20(36)	22(39)	13(23)		26(46)	30(54)	
Tumor types									
IDC[a]	106(89.8)	2(2)	51(48)	32(30)	21(20)	0.52	51 (48)	55 (52)	0.90
ILC[b]	2 (1.7)	0(0)	1(50)	1(50)	0(0)		1 (50)	1 (50)	
Mixed (IDC + ILC)	3 (2.5)	0(0)	2(67)	1(33)	0(0)		2 (67)	1 (33)	
Metaplastic	2 (1.7)	0(0)	1(50)	0(0)	1(50)		1 (50)	1 (50)	
Other	5 (4.2)	1(20)	2(40)	2(40)	0(0)		3 (60)	2 (40)	
Histological grading									
G1&G2	67 (57)	3(4)	29(43)	26(39)	9(14)	**0.02**	35(52)	32(48)	0.44
G3	51 (43)	0(0)	28(55)	10(20)	13(25)		23(45)	28(55)	
Mean tumor size (cm)									
≤4	60 (50.8)	2(3)	36(51)	20(29)	12(17)	0.87	41 (59)	29(41)	**0.04**
> 4	58 (49.2)	1(2)	21(44)	16(33)	10(21)		19 (40)	29 (60)	
Lymph node involvement									
Absence	37 (31.4)	1(3)	20(54)	12(32)	4(11)	0.48	13 (34)	24 (65)	**0.04**
Presence	81 (68.6)	2(2)	37(46)	24(30)	18(22)		45 (56)	36 (44)	
Vascular invasion									
Absence	55 (46.6)	1(2)	24(44)	20(36)	10(18)	0.6	31 (56)	24 (44)	0.14
Presence	63 (53.4)	2(4)	33(52)	16(25)	12(19)		27 (43)	36 (57)	
Stage[c]									
I	5 (16)	0(0)	0 (0)	4 (80)	1 (20)	1	3 (60)	2 (40)	**0.04**
II	11(36)	0(0)	9 (81.8)	2 (18.2)	0(0)		6 (54.5)	5 (45.5)	
III	14 (48)	0(0)	5 (35.7)	6 (42.9)	3 (21.4)		2 (14.3)	12 (85.7)	

[a] IDC = Invasive ductal carcinoma.
[b] ILC = Invasive lobular carcinoma.
[c] Stage: data for 30 out of 118 cases are available.

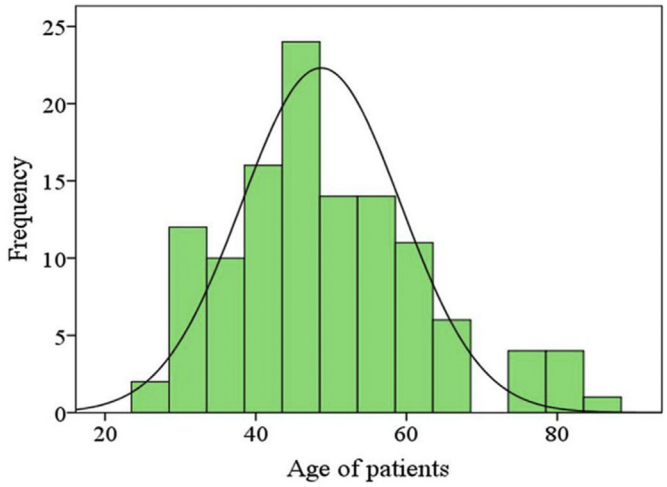


Fig. 1. The distribution of the studied population using histogram plot based on the age of breast cancer patients. Log transformation of the data, resulting in a normal distribution ($p = 0.936$).

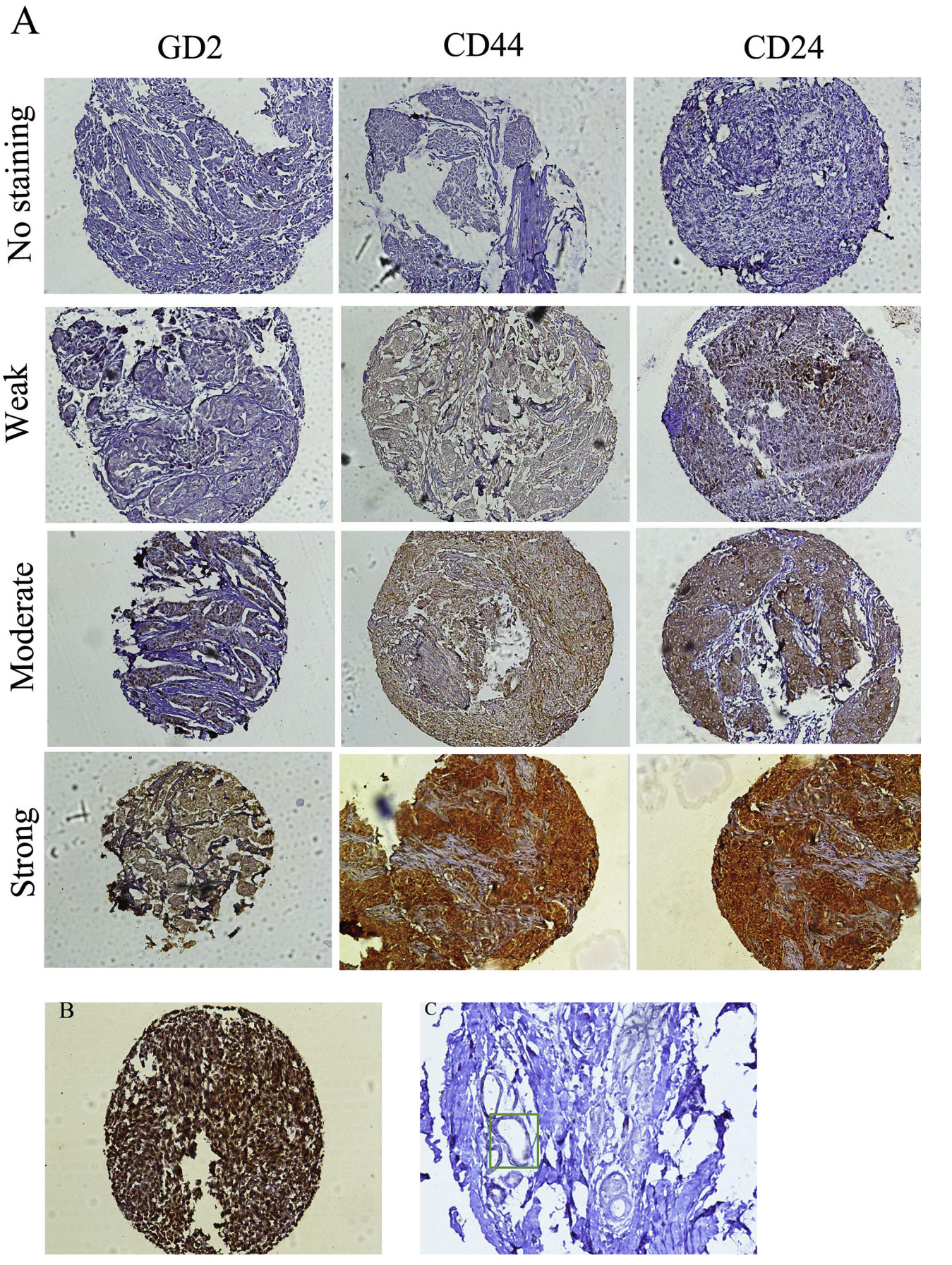
is synthesized from GD3 by GD2 synthase and is expressed on the cell surface of limited normal adult tissues (such as central nervous system) and on ectoderm-derived tumors such as neuroblastoma, melanoma, and breast cancer (Yamashiro et al., 1995; Groux-Degroote et al., 2015; Roth et al., 2014). In 2012, GD2 was introduced as a specific cell surface marker of CD44 high/CD24 low breast CSCs derived from cell lines and tissue samples (Battula et al., 2012). Direct ablation of GD2 synthase gene, B4GALNT1, induces conversion of CSC phenotype to a non-CSC phenotype, including inhibition of mammosphere formation and in vivo tumor formation (Liang et al., 2013). Down-regulation of GD2 mediated by ST8SIA1 knockdown inhibited mammosphere formation, and increased invasion and in-vivo tumor formation (Liang et al., 2013). Based on this evidence, ganglioside GD2 was proposed as a specific breast CSC marker (Battula et al., 2012; Liang et al., 2013). Interestingly, GD2 expression is shown to have restricted distribution to the cell membrane and provides a unique opportunity for cancer immunotherapy (Gholamin et al., 2018). At the present time, anti-GD2 antibody therapy is standard of care in high-risk neuroblastoma (Dobrenkov and Cheung, 2014).

Immunohistochemical analysis of GD2 expression on a limited number of breast cancer clinical samples by Orsi et al. showed upregulation of GD2 in breast cancer patients with older age (Orsi et al., 2017). To further enhance the diagnostic performance of GD2, we assessed for the first time the expression of GD2, in combination with breast CSCs markers CD44/CD24 on a panel of breast cancer samples using tissue microarray (TMA). Furthermore, the possible correlations between expression patterns of these markers with the patients' clinicopathological parameters were assessed.

2. Material and methods

2.1. Patients and tumors characteristics

A total of 168 formalin-fixed paraffin-embedded (FFPE) tissue blocks from patients with primary breast cancer who underwent in the Imam Khomeini Hospital of Urmia University of Medical Sciences from 2011 to 2016 were enrolled in this study. None of the patients had received treatment prior to the surgery. The clinicopathological parameters, including the tumor type, histological grade, tumor size, lymph node involvement and vascular invasion were obtained by reviewing the medical records. Tumors were graded according to the Bloom-Richardson grading system (Bloom and Richardson, 1957). Tumor size ranged from 1 cm to 18 cm with the mean size of 4 cm ± 2.64 cm which was considered as the cut-off for categorization of tumor size as small (≤4 cm) or large (> 4 cm) (Orsi et al., 2017). This study was approved by the Ethics Committee for Clinical Investigations of Iran



(caption on next page)

Fig. 2. Expressions of GD2, CD44, and CD24 in breast cancer tissues. (A) In each type of marker, the intensity of staining was graded as no staining (0), weak (+1), moderate (+2) and strong (+3) staining. (B) Melanoma tissues used for positive control of GD2 antibody has been shown. (C) Immunohistochemical staining of GD2 in normal breast tissue (the area within the green square is a normal duct). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

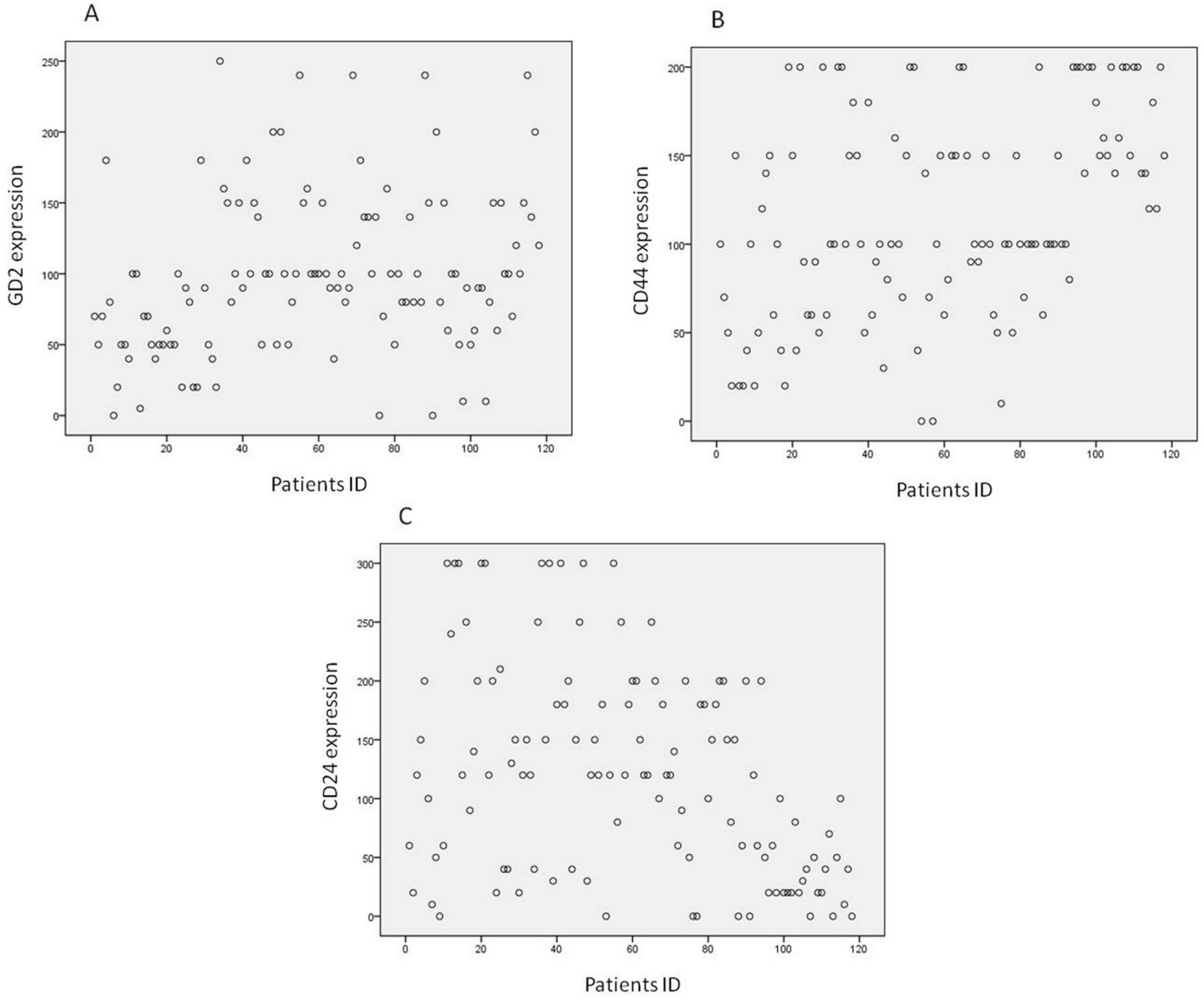


Fig. 3. Scatter dot plots of GD2, CD44 and CD24 based on the expression of each marker. Plots show the expression levels of each patient in all of the tissue samples. The X-axis represents the patients' ID and Y-axis for each marker expression.

University of Medical Sciences. The GD2, CD44 and CD24 staining of tissue sections were scored using a semi-quantitative scoring system in a blinded and coded manner without previous knowledge of clinical and pathological parameters. The study was approved by the ethics committee (no. IR.IUMS.REC1394.26349), each patient gets an ID number without mentioning their information.

2.2. Tissue microarray (TMA) construction

Breast cancer TMA blocks were prepared as described previously (Kalantari et al., 2017; Sedaghat et al., 2017). Briefly, haematoxylin–eosin slides were analyzed by a pathologist (A. A.) to select the representative tumor regions. The selected spots were then punched out using a precision arraying instrument (Tissue Arrayer Minicore; ALPHELYS, Plaisir, France) and placed into a recipient block. The validation study of TMAs showed that despite the variability of antigen expression between the cores, the analysis of a single readable disk would represent the staining pattern of the whole tissue section > 90% of the time, whereas the analysis of two readable disks achieved > 95% accuracy (Camp et al., 2000). In this study, three cores were evaluated from each breast tumor and scored individually to overcome the heterogeneity of antigen expression. In each TMA block, normal breast tissues were also included to compare the expression pattern and the distribution of stained markers GD2, CD44 and CD24 in a range of tissue specimens.

2.3. Immunohistochemistry (IHC)

The breast cancer TMA slides were immunostained using standard chain polymer-conjugated technique, as described previously (Erfani et al., 2016; Roudi et al., 2014). Briefly, the tissue sections were dewaxed for 20 min in 600C and rehydration process was started in

Table 2
Association between the level of CD44 expressions (in terms of intensity of staining and H-score) with pathological parameters (p-value; Pearson χ^2). The bold value indicates a statistically significant difference with a p-value less than 0.05.

Patients characteristics	Total number (%)	Intensity of staining				*p*-value	H-score (Mean = 116)		p-value
		No staining	Weak	Moderate	Strong		Low	High	
Median age									
≤47	62 (53)	1(2)	22(35)	32(52)	7(11)	0.42	31(50)	31(50)	0.33
> 47	56 (47)	1(2)	27(48)	21(37)	7(13)		33(60)	23(40)	
Tumor types									
IDC[a]	106(89.8)	1(9)	43(41)	48(45)	14(5)	0.23	57 (54)	49(46)	0.82
ILC[b]	2 (1.7)	0(0)	2(100)	0(0)	0(0)		2 (100)	0 (0)	
Mixed (IDC + ILC)	3 (2.5)	0(0)	1(33)	2(67)	0(0)		1 (33)	2 (67)	
Metaplastic	2 (1.7)	1(50)	0(0)	1(50)	0(0)		1 (50)	1 (50)	
Other	5 (4.2)	0(0)	3(60)	2(40)	0(0)		3 (60)	2 (40)	
Histological grading									
G1&G2	67 (57)	2(3)	33(49)	29(43)	3(5)	**0.009**	42(63)	25(37)	**0.03**
G3	51 (43)	0(0)	16(31)	24(47)	11(22)		22(43)	29(57)	
Mean tumor size (cm)									
≤4	60 (50.8)	0(0)	32(46)	28(40)	10(14)	0.14	42 (60)	28(40)	0.12
> 4	58 (49.2)	2(4)	17(35)	25(52)	4(9)		22 (46)	26(54)	
Lymph node involvement									
Absence	37 (31.4)	0(0)	13(35)	16(43)	8(22)	0.15	19 (51)	18(49)	0.67
Presence	81 (68.6)	2(2)	36(44)	37(46)	6(8)		45 (56)	36(44)	
Vascular invasion									
Absence	55 (46.6)	0(0)	25(45)	24(44)	6(11)	0.68	30 (54)	25(46)	1
Presence	63 (53.4)	2(3)	24(38)	29(46)	8(13)		34 (54)	29(46)	
Stage[c]									
I	5 (16)	0(0)	3 (60)	1 (20)	1 (20)	0.12	3 (60)	2 (40)	0.2
II	11(36)	0(0)	7 (63.6)	3 (27.3)	1(9.1)		8 (72.8)	3 (27.3)	
III	14 (48)	0(0)	7 (50)	5 (35.7)	2 (14.3)		9 (64.3)	5 (35.7)	

[a] IDC = Invasive ductal carcinoma.
[b] ILC = Invasive lobular carcinoma.
[c] Stage: data for 30 out of 118 cases are available.

Table 3
Association between the level of CD24 expressions (in terms of intensity of staining and H- score) with pathological parameters (p-value; Pearson χ^2). The bold value indicates a statistically significant difference with a p-value less than 0.05.

Patients characteristics	Total number (%)	Intensity of staining				*p*-value	H-Score (Mean = 122)		*p*-value
		No staining	Weak	Moderate	Strong		H-Score (Mean = 122)	H-Score (Mean = 122)	
Median age									
≤47	62 (53)	6(10)	18(29)	29(47)	9(14)	0.4	35(56)	27(44)	0.85
> 47	56 (47)	3(5)	22(39)	20(36)	11(20)		33(59)	23(41)	
Tumor types									
IDC[a]	106(89.8)	8(7)	37(35)	41(39)	20(19)	0.86	59 (56)	47 (44)	0.55
ILC[b]	2 (1.7)	0(0)	1(50)	1(50)	0(0)		1 (50)	1 (50)	
Mixed (IDC + ILC)	3 (2.5)	0(0)	1(33)	2(67)	0(0)		3 (100)	0 (0)	
Metaplastic	2 (1.7)	0(0)	0(0)	2(100)	0(0)		1 (50)	1 (50)	
Other	5 (4.2)	1(20)	1(20)	3(60)	0(0)		4 (80)	1 (20)	
Histological grading									
G1&G2	67 (57)	8(12)	23(34)	29(43)	7(11)	**0.05**	42(63)	25(37)	0.2
G3	51 (43)	1(2)	17(33)	20(39)	13(26)		26(51)	25(49)	
Mean tumor size (cm)									
≤4	60 (50.8)	7(10)	25(36)	24(34)	14(20)	0.2	44(63)	26 (37)	0.16
> 4	58 (49.2)	2(4)	15(31)	25(52)	6(13)		24 (50)	24 (50)	
Lymph node involvement									
Absence	37 (31.4)	3(8)	7(19)	21(57)	6(16)	0.07	15 (40)	22 (60)	**0.01**
Presence	81 (68.6)	6(7)	33(41)	28(35)	14(17)		53 (65)	28 (35)	
Vascular invasion									
Absence	55 (46.6)	3(5)	18(33)	26(47)	8(15)	0.62	31 (56)	24 (44)	0.79
Presence	63 (53.4)	6(9)	22(35)	23(36)	12(20)		37 (59)	26 (41)	
Stage[c]									
I	5 (16)	0(0)	3 (60)	1 (20)	1 (20)	0.09	3 (60)	2 (40)	0.11
II	11(36)	2(18.2)	5 (45.5)	1 (9.1)	3(27.3)		8 (72.8)	3 (27.3)	
III	14 (48)	1(7.1)	3 (21.4)	9 (64.3)	1 (7.1)		6 (42.9)	8 (57.1)	

[a] IDC = Invasive ductal carcinoma.
[b] ILC = Invasive lobular carcinoma.
[c] Stage: data for 30 out of 118 cases are available.

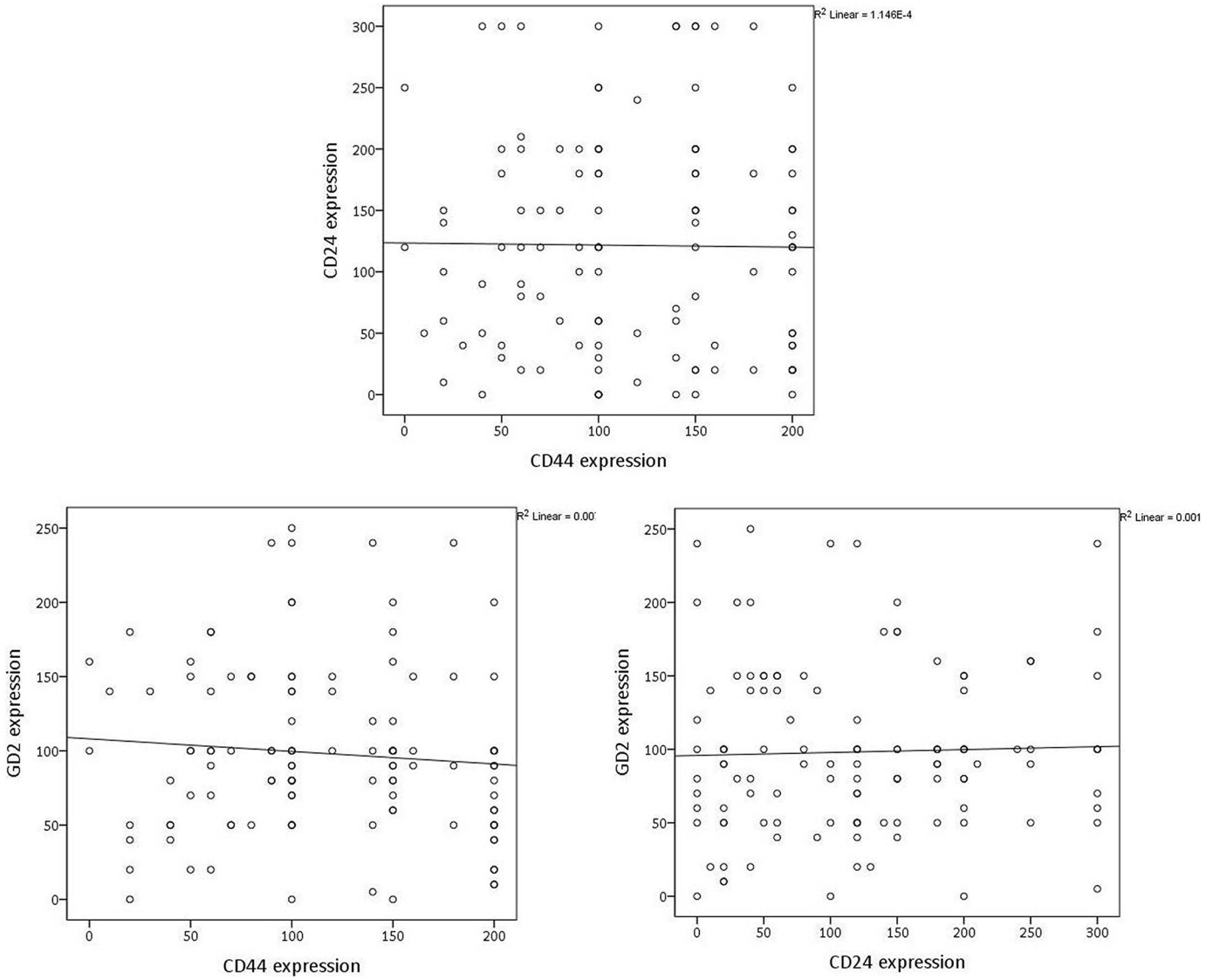


Fig. 4. The correlation between immunohistochemical expression of CD44 and CD24, GD2 and CD44 as well as GD2 and CD24 in breast cancer. A. the scatter dot plot of the correlation between CD24 and CD44 (R^2 = 1.146E – 4 and p = 0.42), B. the scatter dot plot of the correlation between CD44 and GD2 (R^2 = 0.007 and p = 0.56) and C. The scatter dot plot of correlation between CD24 and GD2 expressions in breast cancer (R^2 = 0.001 and p = 0.36).

xylene, followed by graded ethanol treatment. Endogenous peroxidase and non-reactive staining were blocked by 3% H2O2 for 20 min at room temperature. For antigen retrieval, tissue sections were autoclaved for 10 min in sodium citrate buffer (pH = 6). The sections were then incubated overnight at 4 °C with antibody against GD2 (sc-53,831, Santa Cruz Biotechnology Inc.) at 1:10, mouse monoclonal antibody against CD44 (ab51037, Abcam, Cambridge, UK) at 1/100, and CD24 (ab31622, Abcam, Cambridge, UK) at 1:1000 dilution. After washing, the sections were incubated with anti-rabbit/anti-mouse Envision (Dako, Denmark) as the secondary antibody for 1 h. TMA slides were treated with 3, 3′-diaminobenzidine (DAB, Dako) substrate as a chromogen (20 min at room temperature), lightly counterstained with haematoxylin, dehydrated in ethanol, and cleared with xylenes. For negative control samples, the primary antibodies were replaced with tris-buffered saline (TBS). Melanoma and neuroblastoma tissues were used as positive controls for GD2 staining. The antibody was commercially available and was validated, as described previously (Oliveira et al., 2018; Woodward et al., 2005; Choijamts et al., 2011; Neumeister et al., 2010). We also validated the antibody on melanoma tissues as the positive control (Detre et al., 1995).

2.4. Evaluation of immunostaining

Expression levels of GD2, CD44 and CD24 were analyzed using a semi-quantitative scoring system by two trained researchers (M. A. and Z. M.), which were blinded to the patients' information including patients' outcome and pathologic information. TMA slides were eyeballed at x 10 magnification to obtain an idea of the overall distribution of the tumor cells. The positive cells were then assessed semi-quantitatively at higher magnifications (x40 and x100) and final scores calculated. The level of expression of GD2, CD44 and CD24 in breast cancer was assessed by three scoring methods, namely the intensity of staining, the percentage of positive cells and the H-score. The intensity of immunostaining was classified into four categories: 0 (no immunostaining present), 1 (weak), 2 (moderate) and 3 (strong). The percentage of positive cells was assessed semi quantitatively and scored as 0% to 100%. The Histochemical score (H-score) of immunoreactivity was obtained by multiplying the intensity and percentage scores (0–100%), and a final score of 0–300 was given. The mean H-score was selected as the cut-off point (for GD2 H-score = 98, CD44 H-score = 116, and CD24 H-score = 122) and specimens were categorized into two groups, high- and low-expression, divided by the cut-off point, as used in the

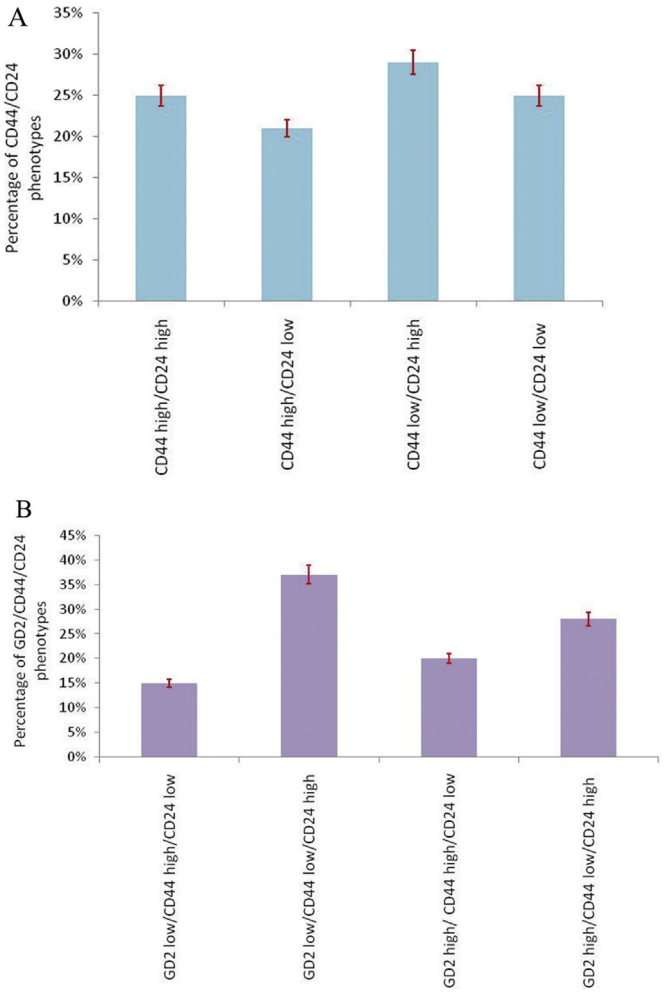


Fig. 5. Distribution of CD44/CD24 and GD2/CD44/CD24 phenotypes in breast cancer. A. the distribution of different phenotypes of CD44/CD24 including CD44+/CD24+, CD44+/CD24−, CD44−/CD24+ and CD44−/CD24-. B. the distribution of different phenotypes of CD44/CD24/GD2 including GD2−/CD44+/CD24−, GD2−/CD44−/CD24+, GD2+/CD44+/CD24− and GD2+/CD44−/CD24+. The standard deviation has been shown in the different phenotypes mentioned above.

previous studies (Mehrazma et al., 2013; Sabet et al., 2014). Various values were compared, including median and mean, then the value with maximum chi-squarestatistic significance, minimum *p*-value, or maximum relative risk was chosen. As there is no set method for the quantitation of immunohistochemical staining (Fedchenko and Reifenrath, 2014), in the current study, the mean of H-score was used as the cut-off value for semi-quantitative evaluation of immunostained samples.

2.5. Scoring system

Immunostaining of GD2, CD44 and CD24 was evaluated in the resected specimens by a trained observer (A. A.) after the series was examined on a double-headed microscope blinded to patient's outcome and other clinical findings. The obtained results were also re-assessed by two investigators (ZM and MA), and a consensus agreement was achieved. Initially, TMA slides were scanned at 10× magnification to obtain a general impression of the overall distribution of the tumor cells, and positive cells were then assessed semi-quantitatively at higher magnifications and final scores were given. The degree of staining was categorized based on the severity of staining by a comparative scale. The intensity of the GD2, CD44 and CD24 immunostaining was scored on a scale of 0 to 3+ as the score of 0 = no visible staining, 1 = faint staining, 2 = moderate staining and 3 = strong staining. To compare all of the available data, an overall Histochemical Score (*H*-score) was assigned to each case by multiplying the intensity score by the percentage of stained cells, and a final score of 0–300 was given. Cut-off points were chosen to categorize samples as high or low GD2 expressing samples in terms of the *H*-score (Cut-off = 98). The specimens with *H*-score ≤ 98 were regarded as low GD2 expressing specimens, and the specimens with *H*-score > 98 were regarded as high GD2 tissues. Cut-off points were chosen to categorize samples as high or low CD44 expressing samples in terms of the *H*-score (Cut-off = 116). The specimens with *H*-score ≤ 116 were regarded as low CD44 expressing specimens, and the specimens with *H*-score > 116 were regarded as high CD44 tissues. Cut-off points were chosen to categorize samples as high or low CD24 expressing samples in terms of the *H*-score (Cut-off = 112). The specimens with *H*-score ≤ 112 were regarded as low CD24 expressing specimens, and the specimens with *H*-score > 112 were regarded as high CD24 tissues.

2.6. Statistical analysis

Data were analyzed using SPSS software, version 20.0. To analyze the significance of the association between each marker expression and clinicopathological parameters, the Pearson's $\chi 2$ test was used. *P*-value of < 0.05 was considered statistically significant.

3. Results

3.1. Patient characteristics

Study population consisted of 168 breast cancer patients including 149 (89%) invasive ductal carcinoma (IDC), 8 (5%) invasive lobular carcinoma (ILC), 3 (2%) combined IDC and ILC, 2 (1%) metaplastic and 6 (3%) other types of breast cancer. Twenty-one (13%) of patients had G1 (grade 1) histological grade tumor, whereas 76 (45%) and 71 (42%) of patients were G2 and G3 (grade 2 and grade 3) histological grade tumors, respectively. One hundred and thirty (77%) of cases had tumors with size smaller than the mean (≤4 cm) and 38 (23%) of patients had tumors with size larger than the mean (> 4 cm). Presence and absence of lymph node involvement were reported in 116 (69%) and 52 (31%) of breast cancer cases, respectively. Seventy-nine (47%) of samples had a positive vascular invasion, while 89 (53%) of specimens were negative for vascular invasion (Table 1). The distribution of the studied population using histogram plot based on the age of patients has been shown in Fig. 1.

3.2. GD2 expression and its correlation with the patients' clinicopathological characteristics

Each breast tumor core was categorized into either low (≤ mean of H-scores) or high (> mean of H-scores) expression, according to the mean of H-scores which was 98 (Figs. 2 and3). High expression of GD2 was observed in 60 (51%) and low expression of GD2 was detected in 58 (49%) of samples. Increased expression of GD2 was more frequently seen in breast tumors with high grade ($p = 0.02$), larger tumor size ($p = 0.04$) and with lymph node involvement ($p = 0.04$) (Table 1). There was a significant correlation between higher expression of GD2 with older age in patients ($p = 0.02$). Considering the intensity of staining, there was a significant correlation between higher expression of GD2 with advanced grades ($p = 0.02$). There was a significant correlation between GD2 expression with a median of age ($p = 0.04$). The increased expression of GD2 was found in 12 (86%) while low expression of GD2 was only seen in 2 (14%) of cases. There was a significant correlation between higher GD2 expression and advanced stage (*p*-value = 0.04).

Table 4
The correlation between CD44/CD24 phenotypes and clinicopathological characteristics of breast cancer. The bold value indicates a statistically significant difference with a p-value less than 0.05.

Patients characteristics	Total number (%)	CD44/CD24 phenotypes				P-value
		CD44 high/CD24 high	CD44 high/CD24 low	CD44 low/CD24 high	CD44 low/CD24 low	
Median age						
≤47	62 (53)	12 (40)	15 (62.5)	19 (55.9)	16 (53.3)	0.4
> 47	56 (47)	18 (60)	9 (37.5)	15 (44.1)	14 (46.7)	
Tumor types						
IDC[a]	106(89.8)	28 (93.3)	22 (91.7)	29 (85.3)	27 (90)	0.9
ILC[b]	2 (1.7)	1 (3.3)	0 (0)	1 (2.9)	0 (0)	
Mixed (IDC + ILC)	3 (2.5)	0 (0)	1 (4.2)	1 (2.9)	1 (3.3)	
Metaplastic	2 (1.7)	0(0)	0 (0)	1 (2.9)	1 (3.3)	
Other	5 (4.2)	1 (3.3)	1 (4.2)	2 (5.9)	1 (3.3)	
Histological grading						
G1&G2	67 (57)	7 (23.3)	2 (8.3)	2 (5.9)	2 (6.7)	**0.04**
G3	51 (43)	23 (76.7)	22 (91.7)	32 (94.1)	28 (93.3)	
Mean tumor size (cm)						
≤4	60 (50.8)	19 (63.3)	16 (66.7)	23 (67.6)	12 (40)	0.1
> 4	58 (49.2)	11 (36.7)	8 (33.3)	11 (32.4)	18 (60)	
Lymph node involvement						
Absence	37 (31.4)	6 (20)	6 (25)	13 (38.2)	12 (40)	0.2
Presence	81 (68.6)	24 (80)	18 (75)	21 (61.8)	18 (60)	
Vascular invasion						
Absence	55 (46.6)	13 (43.3)	11 (45.8)	17 (50)	14 (46.7)	0.9
Presence	63 (53.4)	17 (56.7)	13 (54.2)	17 (50)	16 (53.3)	
Stage[c]						
I	5 (16)	2 (40)	0 (0)	1 (20)	2 (40)	0.2
II	11(36)	1 (9.1)	0 (0)	8 (72.7)	2 (18.2)	
III	14 (48)	5 (35.7)	0 (0)	7 (50)	2 (14.3)	

[a] IDC = invasive ductal carcinoma.
[b] ILC = invasive lobular carcinoma.
[c] Stage: data for 30 out of 118 cases are available.

3.3. CD44 expression and its correlation with patients' clinicopathological characteristics

The level of CD44 expression was subdivided into either low (≤mean of H-scores) or high (> mean of H-scores) expression where the mean of the H-scores was 116 (Figs. 2 and 3). Low expression of CD44 was seen in 64 (54%) and high expression of CD44 was detected in 54 (46%) samples. Higher expression of CD44 was more often found in breast tumors with advanced histological grade ($p = 0.009$) (Table 2). Considering the intensity of staining, there was a significant correlation between higher expression of CD44 with advanced grade ($p = 0.009$).

3.4. CD24 expression and its correlation with patients' clinicopathological characteristics

The level of CD24 expression was subdivided into either low (≤mean of H-scores) or high (> mean of H-scores) expression where the mean of H-scores was 122 (Figs. 2 and 3). Low expression of CD24 was detected in 68 (58%) and high expression of CD24 was observed in 50 (42%) samples. Increased expression of CD24 correlated with more advanced histological grade of breast tumors ($p = 0.05$) and with the presence of lymph node involvement ($p = 0.01$) (Table 3). Considering the intensity of staining, there was a significant correlation between higher expression of CD24 with advanced grade ($p = 0.05$).

3.5. Association between CD44/CD24 phenotypes and the patients' clinicopathological characteristics

The correlation between CSC markers CD44 and CD24 is shown in Fig. 4. In the current study, we defined four expression patterns in breast cancer samples; CD44 high/CD24 high, CD44 high/CD24 low, CD44 low/CD24 high, CD44 low/CD24 low. The CD44 high/CD24 low phenotype, the representative breast CSCs population (Battula et al., 2012), showed the lowest frequency (21%), whereas the CD44 low/CD24 high phenotype, as non-tumorigenic population, had the highest frequency (29%) (Fig. 5). The correlation of CD44/CD24 phenotypes with clinicopathological parameters was explored by one-way ANOVA and Tukey's post hoc analysis. A positive significant correlation was observed between tumor differentiation and CD44 high/CD24 low phenotype ($p = 0.04$). No significant correlation was seen between CD44 high/CD24 low phenotype and other clinicopathological characteristics (Table 4).

3.6. Association between GD2 expression with CD44/CD24 phenotypes and the patients' clinicopathological characteristics

We found an association between immunohistochemical expression of GD2 and with that of CD44 and CD24 (Fig. 4). Four phenotypes were determined by the status of GD2 expression relative to CD44 high/CD24 low and CD44 low/CD24 high phenotypes; i.e. GD2 high/CD44 high/CD24 low, GD2 low/CD44 high/CD24 low, GD2 high/CD44 low/CD24 high and GD2 low/CD44 low/CD24 high (Fig. 5). Evaluation of the GD2/CD44/CD24 phenotypes with clinicopathological parameters using one-way ANOVA and Tukey's post hoc analysis revealed a significant positive correlation between the tumor size and GD2+/CD44+/CD24- phenotype ($p = 0.009$). Furthermore, the GD2 high/CD44 high/CD24 low phenotype was more often detected in breast tumor specimens with lymph node involvement ($p = 0.05$) (Table 5).

4. Discussion

Early detection and proper care are currently the best available approaches for the treatment of breast cancer patients (Goldhirsch et al., 2003). Drug resistance, loco-regional and systemic recurrence and metastasis are the greatest challenges in this regard. Cancer recurrence originates from residual treatment resistant cells, which regenerate at least the initial breast cancer phenotype. CSCs are thought

Table 5
The correlation between GD2/CD44/CD24 phenotypes and clinicopathological characteristics of breast cancer. The bold value indicates a statistically significant difference with a p-value less than 0.05.

Patients characteristics	Total number (%)	GD2/CD44/CD24 phenotypes								p-Value
		GD2high/ CD44high/ CD24high	GD2high/ CD44high/ CD24low	GD2high/ CD44low/ CD24low	GD2high/ CD44low/ CD24high	GD2low/ CD44high/ CD24high	GD2low/ CD44low/ CD24high	GD2low/ CD44high/ CD24low	GD2low/ CD44low/ CD24low	
Median age										
≤47	66 (56)	6 (9)	14(21)	5 (8)	10(15)	10(15)	7(11)	7(10)	7(11)	0.36
> 47	52 (44)	6 (12)	6 (12)	8 (16)	3 (6)	7(14)	4(8)	7(13)	10(19)	
Tumor types										
IDC[a]	106(90)	11(10)	17(16)	12(11)	11(10)	15(14)	11(10)	13(11)	16(15)	0.9
ILC[b]	2 (1)	0(0)	0(0)	1(50)	0(0)	0(0)	0(0)	1(50)	0(0)	
Mixed (IDC + ILC)	3 (3)	0(0)	1(33)	0(0)	1(33)	1(33)	0(0)	0(0)	0(0)	
Metaplastic	2 (1)	0(0)	1(50)	0(0)	0(0)	1(50)	0(0)	0(0)	0(0)	
Other	5 (5)	1(20)	1(20)	0(0)	1(20)	0(0)	0(0)	1(20)	1(20)	
Histological grading										
G1&G2	67 (57)	6 (9)	10 (14.9)	10 (14.9)	9 (13.4)	7 (10.4)	3 (4.5)	9 (13.4)	13 (19.4)	0.08
G3	51 (43)	6 (11.8)	10 (19.6)	3 (5.9)	4 (7.8)	11 (21.6)	8 (15.7)	5 (9.8)	4 (7.8)	
Mean tumor size (cm)										
≤4	60 (50)	3(4)	12(17)	5(7)	9(13)	8(11)	7(10)	11(15)	14(20)	**0.009**
> 4	58 (50)	9(18)	9(17)	8(16)	4(8)	9(18)	4(8)	3(6)	3(6)	
Lymph node involvement										
Absence	37 (31)	3(8)	5(13)	2(5)	3(8)	9(24)	3(8)	9(22)	4(10)	**0.05**
Presence	81 (69)	9(11)	15(18)	11(13)	10(12)	8(10)	8(10)	6(7)	13(16)	
Vascular invasion										
Absence	55 (47)	8(13)	9(16)	7(12)	8(14)	7(12)	3(5)	8(14)	6(10)	0.62
Presence	63 (53)	5(8)	11(17)	6(9)	5(8)	10(16)	8(12)	6(9)	11(17)	
Stage[c]										
I	5 (16)	0 (0)	1 (20)	1 (20)	0 (0)	2 (40)	0 (0)	0 (0)	1 (20)	0.1
II	11(36)	1 (9.1)	5 (45.5)	0 (0)	0 (0)	1 (9.1)	0 (0)	3 (27.3)	1 (9.1)	
III	14 (48)	2 (14.3)	7 (5)	3 (21.4)	0 (0)	0 (0)	0 (0)	0 (0)	2 (14.3)	

[a] IDC = invasive ductal carcinoma.
[b] ILC = invasive lobular carcinoma.
[c] stage: data for 30 out of 118 cases are available.

to be the major cause of tumor recurrence and metastasis (Prieto-Vila et al., 2017). Finding new markers can lead to more accurate molecular profiling and better therapeutic strategies.

GD2 is a novel CSC-specific cell surface marker with potential application in improving survival and cure rates in patients with breast cancer. GM2, GD2, and GD3 are gangliosides, with high expressions in human tumors of neuroectodermal origin, such as melanomas, gliomas, and neuroblastomas, whereas they are absent or weakly expressed in normal tissues (Battula et al., 2012). In this regard, we examined the expression of GD2 in a well-characterized collection of breast cancer clinical samples. Our findings revealed that breast tumors with higher grades, larger size, and also with lymph node involvement and invasion expressed higher levels of GD2 marker. Orsi et al. evaluated GD2 expression and its correlation with clinicopathological characteristics in breast cancer FFPE samples, on sixty-three clinical tumor samples. They proposed challenging the scoring system in a larger cohort of patients in an effort to generate a standardized method for GD2 immunohistochemical evaluation in breast cancer (Orsi et al., 2017).

In this study, to our knowledge the largest breast cancer clinicopathologic study on this subject with 168 samples; we evaluated previously identified breast cancer stem cell markers including CD44 and CD24 together with GD2 as a novel breast cancer CSC marker. Higher expression of CD44 and CD24 was seen in breast cancer with poor differentiation and lymph node involvement. Horiguchi et al. have shown higher CD24 expression in breast tumors with larger size, positive axillary lymph node metastasis and higher stage (Horiguchi et al., 2010). Wan Seop Kim data revealed that the expression of CD24 significantly correlated with HER2-positive status ($P < 0.001$). The HER2-positive tumors are generally considered an aggressive form of breast cancer, associated with rapid tumor growth through enhanced angiogenesis and invasion in breast tumorigenesis (Jang et al., 2016). Honeth et al. indicated that HER2-positive tumors highly expressed CD24 (Honeth et al., 2008). Baumann et al. showed that CD24 expression increased tumor cell metastasis, in vivo proliferation and spreading, and enhanced cell motility and invasion (Baumann et al., 2005). Athanassiadou et al. revealed that CD24 expression correlated with adverse prognostic parameters, including advanced stage, higher tumor grade, positive lymph nodes and increased tumor size (Athanassiadou et al., 2009). Conversely, Schindelmann et al. observed that CD24 was significantly downregulated in invasive breast cancer cell lines, and this downregulation might be associated with more aggressive behavior of the tumor (Schindelmann et al., 2002).

The various combinations of surface markers used to identify breast cancer stem cells, all define subpopulations of cells that have been shown in limiting dilution xenograft transplantation assays to have increased tumorigenic potential (Lathia and Liu, 2017). The combination of CD44 and CD24 is a widely used cancer stem cell marker in breast cancer. However, no conclusion has yet been reached as to which marker is the best for characterizing cancer stemness. We also evaluated the correlation between potential breast CSC markers CD44 and CD24. Our findings confirm the previous study by Andreeff M. (Battula et al., 2012) indicating the lower frequency for CD44 low/CD24 high as non-cancer stem cell population and the higher frequency for CD44 high/CD24 low as cancer stem cell population. A positive correlation was also found between CD44/CD24 population in breast cancer with higher differentiation. In breast cancer, the first report identifying and isolating tumorigenic CSC from non-tumorigenic cancer cells used the combined expression of two cell surface markers: CD44 + /CD24 − /low. Interestingly, some studies revealed an enrichment of the CD44 + / CD24 − /low and CD44 − /CD24 + cell populations in basal-like and

luminal breast cancer cell lines, respectively, CD44 being positively associated with stem cell-like characteristics and CD24 expression related to differentiated epithelial features (Ricardo et al., 2011). Immunohistochemical evaluation using cancer stem cell markers is clearly less commonly used clinically than in basic experiments; and how the expressions of these markers relate to patients' outcomes remains controversial (Horimoto et al., 2016). In a study by Wan Seop Kim et al., they aimed to identify the correlation between the expression of CD44 and CD24 and clinicopathological parameters and overall survival. CD44 and CD24 expression did not have any correlation with the clinicopathological characteristics of patients. In the survival analysis, there was no statistical difference in overall survival based on the expression of CD44 and CD24. The results of this study suggest that CD44 and CD24 are clinically significant markers associated with breast tumorigenesis, but not the sole determinants of poor prognosis in invasive breast cancer (Jang et al., 2016). Ryspayeva D.E et al. aimed to validate the CSC surface markers such as CD44 and CD24 and their clinical significance in 45 patients' tumor blocks. Their analysis failed to detect a statistically significant relation between groups of patients with different prognosis, and their expression was not enough for the characterization of CSCs. Their obtained data demonstrating the worst clinical outcome in the absence of CD44+/CD24− expression apparently requires further investigation and validation of the immunohistochemical method with the determination of a valid cut-off line in defining the CD44 and CD24 status (Ryspayeva et al., 2017).

In the next step, we analyzed the correlation between GD2 expression and CD44/CD24 phenotype. GD2 high/CD44 high/CD24 low phenotype was mainly detected in breast cancer with larger size and presence of lymph node involvement. GD2 is a sialic acid-containing glycosphingolipid which is encoded by B4GALNT1 gene (Nagata et al., 1992). Sen-itiroh Hakomori et al. showed increased mRNA levels of GD2 in breast CSCs (Liang et al., 2012). Moreover, knockdown of B4GALNT1 significantly reduced the expression of GD2 causing a phenotype change from CSC to a non-CSC, which was detected by reduced mammosphere formation and cell motility. In a study of 12 different breast cancer cell lines and samples, Michael Andreeff et al. (Battula et al., 2012) showed that GD3 synthase (GD3S), not GD2S, is highly expressed in GD2+ and CD44high/CD24low cells. Moreover, they found that interference with GD3S expression, either by shRNA or using a pharmacological inhibitor, reduces the CSC population and CSCs-associated properties. GD2 has certain diagnostic advantages compared to other tumor-associated gangliosides, as this glycolipid is highly expressed in tumor cells and is not expressed at all or is expressed at a very low level in normal cells (Doronin et al., 2014). Several lines of investigation have evaluated the anti-GD2 antibody in preclinical and clinical applications (Dobrenkov and Cheung, 2014). Addition of anti-GD2 antibody to the treatment regimen of neuroblastoma patients has led to > 50% improvement in progression-free survival. Despite its potential relevance in cancer diagnostics and therapeutics, the significance of GD2 requires further investigation, especially in breast cancer (Orsi et al., 2017).

Increased expression of GD2 was found in 12 (86%), while low expression of GD2 was only seen in 2 (14%) of cases. Higher expression of GD2 positively correlated with larger tumor size and nodal invasion. Increased expression of CD24 also correlated with nodal invasion. Higher expression of GD2/CD44/CD24 phenotypes significantly correlated with higher tumor grade and larger tumor size. Our findings indicate that there was a significant correlation between higher GD2 expression and advanced stage (p-value 0.04); whereas, no significant correlation was detected between CD44 and CD24 with tumor stage (p-value > .05). Since the current study design was based on a retrospective analysis, ER/PR/Her2 information was available only for 10 out of 65 samples. Among them, all of the patients with increased expression of GD2, showed ER + and PR +, whereas the majority of cases with low expression of GD2 showed ER – and PR -. Overall, it seems that GD2 alone can be a potential valuable prognostic marker in breast cancer patients, whereas further high throughput studies on a larger size of population are required.

5. Conclusions

In summary, increased expression of GD2 may confer with more aggressive breast cancer. GD2 may be considered as an effective diagnostic and monitoring target in clinical applications, but further analysis on a larger set of breast tumors is warranted.

Acknowledgment

This study was supported by a grant from Iran University of Medical Sciences (Grant # 94-02-126-26349).

Conflict of interest

The authors declare that they have no conflict of interest.

References

Al-Hajj, M., Wicha, M.S., Benito-Hernandez, A., Morrison, S.J., Clarke, M.F., 2003. Prospective identification of tumorigenic breast cancer cells. Proc. Natl. Acad. Sci. U. S. A. 100 (7), 3983–3988.
Athanassiadou, P., Grapsa, D., Gonidi, M., Athanassiadou, A.M., Tsipis, A., 2009. E. P. CD24 expression has a prognostic impact in breast carcinoma. Pathol. Res. Pract. 205 (8), 524–533.
Battula, V.L., Shi, Y., Evans, K.W., Wang, R.Y., Spaeth, E.L., Jacamo, R.O., et al., 2012. Ganglioside GD2 identifies breast cancer stem cells and promotes tumorigenesis. J. Clin. Invest. 122 (6), 2066–2078.
Baumann, P., Cremers, N., Kroese, F., Orend, G., Chiquet-Ehrismann, R., Uede, T., et al., 2005. CD24 expression causes the acquisition of multiple cellular properties associated with tumor growth and metastasis. Cancer Res. 65 (23), 10783–10793.
Bloom, H.J., Richardson, W.W., 1957. Histological grading and prognosis in breast cancer; a study of 1409 cases of which 359 have been followed for 15 years. Br. J. Cancer 11 (3), 359–377.
Camp, R.L., Charette, L.A., Rimm, D.L., 2000. Validation of tissue microarray technology in breast carcinoma. Lab. Investig. 80 (12), 1943.
Choijamts, Batsuren, et al., 2011. CD133+ Cancer stem cell–like cells derived from uterine Carcinosarcoma (malignant mixed Müllerian tumor). Stem Cells 29 (10), 1485–1495.
DeSantis, C.E., Ma, J., Goding Sauer, A., Newman, L.A., Jemal, A., 2017. Breast cancer statistics, 2017, racial disparity in mortality by state. CA Cancer J. Clin. 67 (6), 439–448.
Detre, S., Saclani Jotti, G., Dowsett, M., 1995. A "quickscore" method for immunohistochemical semiquantitation: validation for oestrogen receptor in breast carcinomas. J. Clin. Pathol. 48 (9), 876–878.
Dobrenkov, K., Cheung, N.K., 2014. GD2-targeted immunotherapy and radioimmunotherapy. Semin. Oncol. 41 (5), 589–612.
Doronin, I.I., Vishnyakova, P.A., Kholodenko, I.V., Ponomarev, E.D., Ryazantsev, D.Y., Molotkovskaya, I.M., et al., 2014. Ganglioside GD2 in reception and transduction of cell death signal in tumor cells. BMC Cancer 14, 295.
Erfani, E., Roudi, R., Rakhshan, A., Sabet, M.N., Shariftabrizi, A., Madjd, Z., 2016. Comparative expression analysis of putative cancer stem cell markers CD44 and ALDH1A1 in various skin cancer subtypes. Int. J. Biol. Markers 31 (1), e53–e61.
Fedchenko, Nickolay, Reifenrath, Janin, 2014. Different approaches for interpretation and reporting of immunohistochemistry analysis results in the bone tissue–a review. Diagn. Pathol. 9 (1), 221.
Gholamin, S., Mirzaei, H., Razavi, S.M., Hassanian, S.M., Saadatpour, L., Masoudifar, A., et al., 2018. GD2-targeted immunotherapy and potential value of circulating microRNAs in neuroblastoma. J. Cell. Physiol. 233 (2), 866–879.
Ginestier, C., Hur, M.H., Charafe-Jauffret, E., Monville, F., Dutcher, J., Brown, M., et al., 2007. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell 1 (5), 555–567.
Goldhirsch, A., Colleoni, M., Domenighetti, G., Gelber, R.D., 2003. Systemic treatments for women with breast cancer: outcome with relation to screening for the disease. Ann. Oncol. 14 (8), 1212–1214.
Groux-Degroote, S., Guerardel, Y., Julien, S., Delannoy, P., 2015. Gangliosides in breast cancer: new perspectives. Biochem. Mosc. 80 (7), 808–819.
Hawley, S.T., Li, Y., An, L.C., Resnicow, K., Janz, N.K., Sabel, M.S., et al., 2018. Improving breast Cancer surgical treatment decision making: the iCanDecide randomized clinical trial. J. Clin. Oncol. 36 (7), 659–666.
Honeth, G., Bendahl, P.O., Ringner, M., Saal, L.H., Gruvberger-Saal, S.K., Lovgren, K., et al., 2008. The CD44+/CD24- phenotype is enriched in basal-like breast tumors. Breast Cancer Res. BCR 10 (3), R53.
Horiguchi, K., Toi, M., Horiguchi, S., Sugimoto, M., Naito, Y., Hayashi, Y., et al., 2010. Predictive value of CD24 and CD44 for neoadjuvant chemotherapy response and prognosis in primary breast cancer patients. J. Med. Dent. Sci. 57 (2), 165–175.
Horimoto, Y., Arakawa, A., Sasahara, N., Tanabe, M., Sai, S., Himuro, T., et al., 2016.

Combination of Cancer stem cell markers CD44 and CD24 is superior to ALDH1 as a prognostic Indicator in breast Cancer patients with distant metastases. PLoS ONE 11 (10), e0165253.
Jang, M.H., Kang, H.J., Jang, K.S., Paik, S.S., Kim, W.S., 2016. Clinicopathological analysis of CD44 and CD24 expression in invasive breast cancer. Oncol. Lett. 12 (4), 2728–2733.
Kalantari, E., Saadi, F.H., Asgari, M., Shariftabrizi, A., Roudi, R., Madjd, Z., 2017. Increased expression of ALDH1A1 in prostate Cancer is correlated with tumor aggressiveness: a tissue microarray study of Iranian patients. Appl. Immunohistochem. Mol. Morphol. AIMM 25 (8), 592–598.
Lathia, J.D., Liu, H., 2017. Overview of Cancer stem cells and Stemness for community oncologists. Target. Oncol. 12 (4), 387–399.
Liang, Yuh-Jin, Dinga, Yao, Levery, Steven B., Lobaton, Marlin, Kazuko Handa, Hakomori S-i., 2012. Differential expression profiles of glycosphingolipids in human breast cancer stem cells vs. cancer non-stem cells. PNAS 110 (13), 4968–4973.
Liang, Y.-J., Ding, Y., Levery, S.B., Lobaton, M., Handa, K., Hakomori, S-i., 2013. Differential expression profiles of glycosphingolipids in human breast cancer stem cells vs. cancer non-stem cells. Proc. Natl. Acad. Sci. 110 (13), 4968–4973.
Mehrazma, M., Madjd, Z., Kalantari, E., Panahi, M., Hendi, A., Shariftabrizi, A., 2013. Expression of stem cell markers, CD133 and CD44, in pediatric solid tumors: a study using tissue microarray. Fetal Pediatr. Pathol. 32 (3), 192–204.
Nagata, Y., Yamashiro, S., Yodoi, J., Lloyd, K.O., Shiku, H., Furukawa, K., 1992. Expression cloning of beta 1,4 N-acetylgalactosaminyltransferase cDNAs that determine the expression of GM2 and GD2 gangliosides. J. Biol. Chem. 267 (17), 12082–12089.
Neumeister, Veronique, et al., 2010. In situ identification of putative cancer stem cells by multiplexing ALDH1, CD44, and cytokeratin identifies breast cancer patients with poor prognosis. Am. J. Pathol. 176 (5), 2131–2138.
Oliveira, Rodrigo V., et al., 2018. Detection of putative stem-cell markers in invasive ductal carcinoma of the breast by immunohistochemistry: does it improve prognostic/predictive assessments? Appl. Immunohistochem. Mol. Morphol. 26 (10), 760.
Orsi, G., Barbolini, M., Ficarra, G., Tazzioli, G., Manni, P., Petrachi, T., et al., 2017. GD2 expression in breast cancer. Oncotarget. 8 (19), 31592–31600.
Owens, T.W., Naylor, M.J., 2013. Breast cancer stem cells. Front. Physiol. 4, 225.
Prieto-Vila, M., Takahashi, R.U., Usuba, W., Kohama, I., Ochiya, T., 2017. Drug resistance driven by Cancer stem cells and their niche. Int. J. Mol. Sci. 18 (12).
Ricardo, S., Vieira, A.F., Gerhard, R., Leitao, D., Pinto, R., Cameselle-Teijeiro, J.F., et al., 2011. Breast cancer stem cell markers CD44, CD24 and ALDH1: expression distribution within intrinsic molecular subtype. J. Clin. Pathol. 64 (11), 937–946.
Roth, Michael, Linkowski, Marissa, Tarim, John, Piperdi, Sajida, Sowers, Rebecca, Geller, David, et al., 2014. Ganglioside GD2 as a therapeutic target for antibody-mediated therapy in patients with osteosarcoma. Cancer. 120, 548–554.
Roudi, R., Madjd, Z., Korourian, A., Mehrazma, M., Molanae, S., Sabet, M.N., et al., 2014. Clinical significance of putative cancer stem cell marker CD44 in different histological subtypes of lung cancer. Cancer Biomark. Section A Dis. Markers 14 (6), 457–467.
Ryspayeva, D.E., Smolanka, I.I., Dudnichenko capital, A.C., Lyashenko, A.A., Grinevich, Y.A., Gurianov, V.G., et al., 2017. Are CD44(+)/CD24(−) cells the assumed cancer stem cells in breast cancer. Exp. Oncol. 39 (3), 224–228.
Sabet, M.N., Rakhshan, A., Erfani, E., Madjd, Z., 2014. Co-expression of putative cancer stem cell markers, CD133 and nestin, in skin tumors. Asian Pac. J. Cancer Prev. 15 (19), 8161–8169.
Schindelmann, S., Windisch, J., Grundmann, R., Kreienberg, R., Zeillinger, R., 2002. H D. expression profiling of mammary carcinoma cell lines: correlation of in vitro invasiveness with expression of CD24. Tumour Biol. 23, 139–145.
Sedaghat, S., Gheytanchi, E., Asgari, M., Roudi, R., Keymoosi, H., Madjd, Z., 2017. Expression of Cancer stem cell markers OCT4 and CD133 in transitional cell carcinomas. Appl. Immunohistochem. Mol. Morphol. AIMM 25 (3), 196–202.
Woodward, Wendy A., et al., 2005. On mammary stem cells. J. Cell Sci. 118 (16), 3585–3594.
Wright, M.H., Calcagno, A.M., Salcido, C.D., Carlson, M.D., Ambudkar, S.V., Varticovski, L., 2008. Brca1 breast tumors contain distinct CD44+/CD24- and CD133+ cells with cancer stem cell characteristics. Breast Cancer Res. BCR 10 (1), R10.
Yamashiro, S., Okada, M., Haraguchi, M., Furukawa, K., Lloyd, K.O., Shiku, H., et al., 1995. Expression of α2, 8-sialyltransferase (GD3 synthase) gene in human cancer cell lines: high level expression in melanomas and up-regulation in activated T lymphocytes. Glycoconj. J. 12 (6), 894–900.