Journal of Molecular Biomarkers & Diagnosis

Rubio-Hernández et al., J Mol Biomark Diagn 2019, 10:2

Research Article Open Access

Aberrant Expression of N-Glycolyl GM3 Ganglioside Correlates with Nuclear Grade in Stage II/III Resectable Estrogen and Progesterone Receptors Positive Breast Cancer

María Caridad Rubio-Hernández[1], Anabely Estévez-García[1], Adanays Calvo-Pérez[2], Damián Blanco-Santana[2], Orlando Valdés-Guerrero[1], Yenia Diaz-Prado[1], Janet Lamadrid-García[3], Patricia Lorenzo-Luaces[4], Carmen Viada-González[4], Rancés Blanco-Santana[5] and Luis Enrique Fernández[6*]

[1]Mastology Service, National Institute of Oncology and Radiobiology, Havana, Cuba

[2]Department of Cell Biology and Tissues Banking, National Institute of Oncology and Radiobiology, Havana, Cuba

[3]Department of Pathology, National Institute of Oncology and Radiobiology, Havana, Cuba

[4]Clinical Research Direction, Center of Molecular Immunology, Havana, Cuba

[5]Laboratory of Recognition and Biological Activity Assays, Center of Molecular Immunology, Havana, Cuba

[6]Innovation Direction, Center of Molecular Immunology, Havana, Cuba

[*]**Corresponding author:** Fernández LE, Center for Molecular Immunology, Cuba, Tel: + 53 7 214 3156; Fax: + 53 7 2720644; E-mail: luis@cim.sld.cu

Rec Date: February 28, 2019, **Acc Date:** March 20, 2019, **Pub Date:** March 27, 2019

Abstract

The expression of N-glycolyl GM3 ganglioside (NGcGM3) in breast cancer was previously reported. However, the role of this molecule in the biological behavior of breast cancer still remains unclear. The aim of the present work was to assess a possible relationship between the expression of the evolutionary-fixed tumor neoantigen NGcGM3 ganglioside with clinicopathological parameters and the aggressiveness of breast tumors. 126 stage II/III breast tumors, obtained from patients participating in a phase III clinical trial with the GlycoVaxGM3 cancer vaccine, were clinically, molecularly and pathologically classified. An immunohistochemical study was performed using the 14F7 antibody, specific for NGcGM3, in order to measure the staining intensity and percentage of positive cells. The NGcGM3 expression in estrogen and progesterone receptors positive tumors was not associated with age, race, menopausal status, tumor size, lymph node status and stage. Nevertheless a significant increase in the presence of this cancer neoantigen in tumor tissue samples was mostly found in tumors displaying higher nuclear grades II and III when compared with grade I ones. Overall these results indicate a relationship between NGcGM3 expression and a more aggressive biological behavior of estrogen and progesterone receptors positive breast tumors, additionally sustaining this molecule as an attractive target for breast cancer immunotherapy.

Keywords: N-glycolyl GM3 ganglioside; Immunohistochemistry; Estrogen receptor; Progesterone receptor; Nuclear grade; Molecular classification

Abbreviations: ANOVA: Analysis of variance; DAB: 3,3′ - Diaminobenzidine; ER: Estrogen Receptor; FFPE: Formalin-Fixed and Paraffin-Embedded; IHC: Immunohistochemistry; Mab: Monoclonal antibody; NGcGM3: N-Glycolyl GM3 Ganglioside; NSCLC: Non-Small Cell Lung Cancer; PR: Progesterone Receptor; TMA: Tissue Microarrays; WHO: World Health Organization.

Introduction

Breast carcinoma is one of the most common types of cancer worldwide [1]. Despite recent progresses in the treatment of primary tumors, about 20-40% of early-stage breast cancer patients eventually develop recurrences in distant organs [2,3]. As a result, research efforts are currently focused on the better understanding of the biology and genetics of breast tumors to select new targets, leading to more effective and personalized treatments for this often difficult disease [4,5]. Among these explored molecules, gangliosides are included [6,7].

Gangliosides are sialic acid-containing glycosphingolipids engaged in many biological events that take place at vertebrate's cell membrane [8,9]. In breast tumors, a differential ganglioside composition was observed when compared with normal mammary tissues [6], thus probably becoming attractive targets for active and passive immunotherapy. While malignant cells expressing aberrantly glycolylated sialic acid containing gangliosides have been identified in breast carcinomas by immunohistochemical methods [10-12] a more convincing evidence of the expression of N-glycolyl GM3 ganglioside (NGcGM3) in breast tumors was obtained by mass spectrometry analysis [6] and later corroborated by immunohistochemistry (IHC) with the 14F7 monoclonal antibody (Mab), an IgG1 specific antibody only reactive with NGcGM3. 14F7 Mab recognizes breast infiltrating ductal carcinoma, both in frozen [13] and formalin-fixed and paraffin-embedded [14] samples. A final validation of the NGcGM3 ganglioside expression in human breast tumors was achieved by radioimmunoscintigraphy with 99Tc labelled 14F7 Mab in breast cancer patients [15].

In addition an increased expression of NGcGM3 in lymph nodes metastasis of breast tumors were also observed [12,16]. Curiously, the presence of the ganglioside was also noted in lymphocytes localized around the metastatic cells in this organs, indicating a shedding to the tumor microenvironment to promote a potent immunosuppressive effect over effector immune cells, as was previously suggested [17,18].

More recently differences in the levels of NGcGM3 expression, according to the histological classification of breast carcinomas, were reported [19]. Nevertheless, this study was limited by a scarce number

of samples and the absence of information regarding the clinicopathological description of patients. Consequently, additional studies confirming the role of NGcGM3 in the biological behavior of breast cancer are needed. Here, the NGcGM3 expression in a large series of breast tumors, basically of the estrogen and progesterone receptors positive type, was determined and any possible association of this level of expression with clinicopathological parameters such as nuclear and histological grading, explored.

Materials and Methods

Patients and histopathological parameters

Formalin-fixed and paraffin-embedded (FFPE) breast tumor samples of 173 patients included in a phase III clinical trial with GlycoVaxGM3 vaccine were obtained from the pathology department of the National Institute of Oncology and Radiobiology. The study was performed according to the principles of the Declaration of Helsinki and the consent of both patients and institutional ethical committees were previously obtained.

The histological subtype classification of tumors was performed accordingly to the 2003 World Health Organization (WHO) guidelines. Nuclear grade was determined as previously described [20]. Histological grade was categorized as I, II or III by experienced pathologists, following the Nottingham modification of the Scarff-Bloom-Richardson criteria [21]. In addition, some clinicopathological features such as age at diagnosis, race/ethnicity, menopausal status, tumor size, lymph node status, were obtained from the clinical records.

Tissue microarrays construction (TMA) and immunohistochemical staining

Five-micrometer sections from each block were obtained in a micrometer (Leica, L2530) and they were mounted on poly-L-lysine coated slides (Thermo Scientifics). Slides were stained with hematoxylin and eosin and an experienced pathologist (JLG) confirmed the presence of malignant cells and selected the tumor area. Two mm diameter biopsies were taken from each donor block and they were inserted into the recipient block using a precision tissue array instrument (Thermo Scientifics, TMA-001).

Five-micrometer serial sections from each TMA block were obtained and mounted on poly-L-lysine coated slides (Thermo Scientifics). All sections were attached to the slide by heating in a 60°C oven for 1 h. Afterwards the slides were dewaxed in xylene and rehydrated in graded ethanol series in the usual way. The samples were maintained in tap water until they were stained.

HER2 expression was detected by mean of the HercepTestTM (Dako, K5207) commercial kit following the manufacturer instructions. For NGcGM3 expression, the 14F7 Mab (a highly specific IgG1 anti-NGcGM3 ganglioside) was used according to a previously described procedure [14]. For estrogen (ER) and progesterone (ER) receptors detection, the sections were pre-treated with 10 mM sodium citrate buffer pH 6.0 for 30 minutes at 95-100°C. Afterward, the tissues were incubated with the anti-ER (Anacrom Diagnosticos, SP1) and anti-PR (Anacrom Diagnosticos, SP2) Mabs in a humid chamber for 30 minutes at room temperature followed by streptavidin-biotin detection system (MLINK, Anacrom Diagnosticos) for 10 minutes each step. Between incubations, the slides were washed with Tris-Buffer Saline pH 7.6 (TBS).

For all samples the enzymatic activity was visualized with a 3,3′ -Diaminobenzidine (DAB) solution (Dako, K3465). Finally, the slides were counterstained with Mayer's Hematoxylin (Dako, S2020), dehydrated, and mounted (Figure 1).

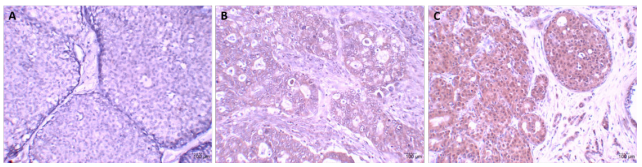

Figure 1: Examples of NGcGM3 expression patterns in breast cancer. A, B and C represent weak, moderate and intense expression of this molecule, respectively (Brown color). Counterstaining with Mayer's Hematoxylin (Blue color). Black bar=100 μm.

Immunohistochemical evaluation

ER and PR tissue sections showing more than 1% of positive cells were considered as hormone receptors positive as previously established [22]. HER2 overexpression was scored according to the protocol described in the manufacturer guidelines (HercepTestTM Interpretation Manual). NGcGM3 expression in each tissue section was judged as negative (0), weak (1), moderate (2) and strong (3). The percentage of tumor cells (0-100%) expressing this ganglioside was estimated using 10x optical microscopy fields selecting the most cellular regions of each section. In addition, an IHC score was calculated for each specimen by multiplication of the intensity of reaction and the percentage of positive cells, resulting in a score ranging from 0 to 300.

Statistical analysis

Statistical analysis was carried out using SPSS (version 15.0; SPSS Inc., Chicago, IL). The association between NGcGM3 expression and clinicopathological features was analyzed using the chi-square test. For the percentage of positive cells and IHC score, the one-way analysis of variance (ANOVA) was applied. Correlations were determined using Spearman's test. A p value <0.05 was considered statistically significant.

Results

Patient population

Table 1 shows a summary of the patient's characteristics from which tumor samples were obtained together with certain pathological features. The majority of patients were white (65.9%) and postmenopausal (60.7%) women with a median age of 52-years-old (from 31 to 77). While the most frequent disease stage was IIA (44.5%), the prevalent histological subtype was infiltrating ductal carcinoma (78.6%) as expected.

In the tumor sample the predominant nuclear grades were II (50.0%) and III (42.1%), with a predominance of histological grade III (58.7%). Nevertheless a significant correlation between nuclear and histological grades was found (Spearman's correlation coefficient 0.6198; $p<0.0001$).

NGcGM3 expression

A tissue expression of NGcGM3 was observed in all studied tumor samples (n=126), but with variations in intensity and the percentage of positive cells. The distribution of expression according to the 14F7 Mab staining intensity was as follows: 29/126 (23.0%) weak, 58/126 (46.0%) moderate and 39/126 (31.0%) intense (Table 2). In addition, most specimens exhibited more than 50% of positive cells (75.4%). A significant correlation was found between these two parameters (Spearman's correlation coefficient 0.5685; $p<0.0001$). According to the IHC score, the median value was 160 (ranging from 10 to 270).

Clinicopathological features	Number of cases (%)
Age (years)	
< 50	70 (40.5)
≥ 50	103 (59.5)
Race/Ethnicity	
White	114 (65.9)
Black	41 (23.7)
Mixed	18 (10.4)
Menopausal status	
Pre	57 (32.9)
Post	105 (60.7)
Unknown	11 (6.4)
Tumor size (cm)	
0-2	62 (35.8)
2.1-5	93 (53.8)
>5	12 (6.9)
Unknown	6 (3.5)
Lymph node metastasis	
0	58 (33.5)
01-Mar	66 (38.2)
≥ 4	49 (28.3)
Stage	
IIA	77 (44.5)
IIB	32 (18.5)
IIIA	31 (17.9)
IIIB	2 (1.2)
IIIC	31 (17.9)
Histological type	
Infiltrating ductal carcinoma	136 (78.6)
Infiltrating lobular carcinoma	17 (9.8)
Infiltrating ducto-lobular carcinoma	4 (2.3)
Others	16 (9.3)
Nuclear grade	
I	10 (5.8)
II	63 (36.4)
III	53 (30.6)
N.D.	47 (27.2)
Histological grade*	
I	9 (5.0)
II	43 (24.9)
III	74 (42.9)
N.D.	47 (27.2)
Number of cases, 173; N.D., not determined; *Bloom-Richardson criteria	

Table 1: Clinicopathological characteristics of patients.

NGcGM3 expression and clinicopathological features

No association between NGcGM3 expression in tumors and parameters like age, race, menopausal status, stage of the disease, tumor size and lymph node status was evidenced, though a tendency for a higher presence of this ganglioside in infiltrating ductal carcinomas, when compared with the rest of tumor types, was apparent but not statistically significant (Table 2). On the contrary, an increased expression of NGcGM3 (intensities 2 and 3) was found in tumors displaying higher nuclear grades II and III (80.2%) when compared with grade I (40.0%), consequently associating the intensity of the presence of the glycolipid with the nuclear grade in estrogen and progesterone receptors positive breast tumors ($p=0.015$).

Interestingly, a more precise comparison between specific groups showed that the major difference in the level of expression of the ganglioside arose from that of nuclear grade II and nuclear grade I tumors (Chi-square paired test, $p=0.0036$).

Similarly, tumors with histological grades II and III (77.8%) tended to display higher levels of NGcGM3 expression in comparison with grade I ones (66.7%), but again lacking statistical significance.

Further association of NGcGM3 expression with the clinicopathological parameters was not evidenced when the percentage of positive cells or the IHC score were analyzed. Only a tendency between the percentage of positive cells and the tumor size was observed ($p=0.062$).

Discussion

The aberrant expression of NGcGM3 has been demonstrated in a variety of human tumors including breast carcinomas [13-15]. Moreover, a possible relationship of this ganglioside with cancer progression and its immunosuppressive properties has been also suggested [16,23,24], while evidences of the association between its expression and a poor overall survival in colon adenocarcinoma [24] and non-small cell lung cancer (NSCLC) [25] were found. However, experimental data concerning to the role of this molecule in the biological behavior of breast cancer, particularly in estrogen and progesterone receptors positive tumors, is scarce.

Histopathological features	NeuGcGM3 expression no. cases (%)			P value
	Low	Medium	High	
Histological type				
Infiltrating ductal carcinoma	30 (22.1)	71 (52.2)	35 (25.7)	ns
Infiltrating lobular carcinoma	7 (41.2)	4 (23.5)	6 (35.3)	
Infiltrating ducto-lobular carcinoma	2 (50.0)	1 (25.0)	1 (25.0)	
Others	3 (18.8)	9 (56.3)	4 (25.0)	
Nuclear grade				
I	6 (60.0)	2 (20.0)	2 (20.0)	0.015
II	9 (14.3)	35 (55.6)	19 (30.2)	
III	14 (26.4)	21 (39.6)	18 (34.0)	
Histological grade*				
I	3 (33.3)	3 (33.3)	3 (33.3)	ns
II	9 (20.9)	22 (51.2)	12 (27.9)	
III	17 (23.0)	33 (44.6)	24 (32.4)	

*Bloom-Richardson criteria; ns, no significant.

Table 2: Distribution of NeuGcGM3 according to histopathological characteristics.

In the present study the aberrant expression of NGcGM3 in 126 breast carcinoma samples was assessed by IHC. An important practical issue in this type of study is the quantitative criteria of NGcGM3 expression. In previous studies different procedures for the quantitation of the ganglioside expression was applied, as the separate assessment of the percentages of positive cells and the staining intensity [14,23,26], or also different scores [24,25,27]. As divergent ways to measure this molecule in tumor samples could render contradictory results between distinct laboratories, we conducted the immunohistochemical analysis of NGcGM3 expression mainly using the intensity of the 14F7 Mab reaction, though the percentage of positives cells and an IHC score was also explored. Unfortunately, a universal criteria for the evaluation of NGcGM3 expression has not been established yet.

In this serial an association between the abundance of this glycolipid in tumor tissues with parameters like age, race, menopausal status, tumor size, lymph node status and stage was not observed. Curiously, in positive cases the presence of the ganglioside was detected in malignant cells´ membranes and cytoplasm as well.

Similarly, differences in the intensity of 14F7 Mab reaction within breast cancer histological subtypes was not detected, confirming previously described results in this type of malignancy [14]. Interestingly, previously reported differences between infiltrating ductal *vs.* lobular breast carcinomas by means of an IHC score [19] were not reproduced in the present study, probably due to differences in the number of cases, histological subtypes and cut-off points. A similar lack of association between the intensity of NGcGM3 expression and the histological subtype was described for NSCLC [25], while statistically significant differences of NGcGM3 expression within the histological subtypes of ovarian adenocarcinomas [14] and skin tumors [28] have been observed.

Nevertheless, a first evidence of the association of NGcGM3 expression with nuclear grade in breast tumors is the major finding in this research. Mostly, samples displaying nuclear grades II and III showed a relative preferential accumulation of NGcGM3. More specifically, a paired comparison between the intensity of NGcGM3 expression in tumor samples with nuclear grades I, II and III showed that the major difference in the level of expression of the ganglioside is between nuclear grade II and nuclear grade I tumors, suggesting a particularly higher accumulation in the intermediate stage of undifferentiation.

It is well known that nuclear grading is the cytological evaluation of size and shape alterations of tumor nuclei in comparison with normal mammary epithelial cells and this histopathological feature is strongly associated with the aggressiveness of breast carcinomas [29,30].

Furthermore, an increased accumulation of NGcGM3 was apparent in breast carcinomas displaying higher histological grades according to the Bloom-Richardson system (based on the evaluation of nuclear grading in conjunction with mitotic count and the degree of tubule or gland formation) [31]. A similar increase in the presence of NGcGM3, associated to a major histological grade, was reported for transitional cell bladder carcinomas and malignant gliomas [19].

Reasonably, a common increased expression of NGcGM3 in higher nuclear and histological grade estrogen and progesterone receptors positive breast tumors could be expected from the strong correlation between these two parameters found for the samples in the present study.

As one limitation of the tumor samples characterization was the lack of cell proliferation index evaluation, avoiding the differentiation between luminal A and B tumors [32], a further assessment of the NGcGM3 expression in these two subgroups of estrogen and progesterone receptors positive breast tumors is warranted.

Conclusion

In summary, a step forward in the understanding of the presence and possible biological significance of NGcGM3 ganglioside in estrogen and progesterone receptors positive breast cancer has been accomplished. Interestingly, a significant enhancement of the expression of this molecule correlated with an increase in nuclear grade, mainly for nuclear grade II tumors, which is strongly associated with the aggressiveness of breast carcinomas. On the other hand these results may be relevant for predicting which type of patient is more sensitive to be benefited by vaccination with GlycoVaxGM3, a NGcGM3 based cancer vaccine currently tested in advanced clinical trials in breast cancer patients.

Conflict of Interests

The authors declare that there is no conflict of interests.

Acknowledgements

The authors want to express grateful to Ms. Idelmis Curbelo-Heredia (Nursing Service, National Institute of Oncology and Radiobiology, Havana, Cuba) for her immense help. This work was supported by the National Institute of Oncology and Radiobiology and the Center of Molecular Immunology, Havana, Cuba.

References

1. Torre LA, Siegel RL, Ward EM, Jemal A (2016) Global Cancer Incidence and Mortality Rates and Trends--An Update. Cancer Epidemiol Biomarkers Prev 25: 16-27.
2. Roth BJ, Krilov L, Adams S, Aghajanian CA, Bach P, et al. (2013) Clinical cancer advances 2012: annual report on progress against cancer from the american society of clinical oncology. J Clin Oncol 31: 131-161.
3. Zhang HX, Giuliano M, Trivedi MV, Schiff R, Osborne CK (2013) Metastasis Dormancy in Estrogen Receptor–Positive Breast Cancer. Clin Cancer Res 19: 6389-6397.
4. McArthur HL, Page DB (2016) Immunotherapy for the Treatment of Breast Cancer: Checkpoint Blockade, Cancer Vaccines, and Future Directions in Combination Immunotherapy. Clin Adv Hematol Oncol 14: 922-933.
5. Athreya K, Ali S (2017) Advances on immunotherapy in breast cancer. Transl Cancer Res 6: 30-37.
6. Marquina G, Waki H, Fernandez LE, Kon K, Carr A, et al. (1996) Gangliosides Expressed in Human Breast Cancer. Cancer Res 56: 5165-5171.
7. Ruckhaberle E, Karn T, Rody A, Hanker L, Gatje R, et al. (2009) Gene expression of ceramide kinase, galactosyl ceramide synthase and ganglioside GD3 synthase is associated with prognosis in breast cancer. J Cancer Res Clin Oncol 135: 1005-1013.
8. Yamashita T, Wada R, Sasaki T, Deng C, Bierfreund U, et al. (1999) A vital role for glycosphingolipid synthesis during development and differentiation. Proc Natl Acad Sci USA 96: 9142-9147.
9. Birkle S, Zeng G, Gao L, Yu RK, Aubry J (2003) Role of tumor-associated gangliosides in cancer progression. Biochimie 85: 455-463.
10. Ikuta K, Nishi Y, Simizu Y, Higashi H, Kitamoto N, et al. (1982) Hanganutziu-Deicher type heterophyle antigen-positive cells in human cancer tissues demonstrated by membrane immunofluorescence. Biken J 25: 47-50.
11. Devine PL, Clark BA, Birrel GW, Layton GT, Ward BG, et al. (1991) The breast tumor–associated epitope defined by monoclonal antibody 3E1.2 is an O-linked mucin carbohydrate containing N-glycolyl neuraminic acid. Cancer Res 51: 5826-5836.
12. Vazquez AM, Alfonso M, Lanne B, Karlsson KA, Carr A, et al. (1995) Generation of a murine monoclonal antibody specific for N-glycolylneuraminic acid-containing gangliosides that also recognizes sulfated glycolipids. Hybridoma 14: 551-556.
13. Carr A, Mullet A, Mazorra Z, Vázquez AM, Alfonso M, et al. (2000) A mouse IgG1 monoclonal antibody specific for N-glycolyl GM3 ganglioside recognized breast and melanoma tumors. Hybridoma 19: 241-247.
14. Blanco R, Cedeño M, Escobar X, Blanco D, Rengifo CE, et al. (2011) Immunorecognition of the 14F7 Mab raised against N-glycolyl GM3 ganglioside in some normal and malignant tissues from genitourinary system. ISRN Pathol 2011: 953803.
15. Oliva JP, Valdés Z, Casacó A, Pimentel G, González J, et al. (2006) Clinical evidences of GM3 (NeuGc) ganglioside expression in human breast cancer using the 14F7 monoclonal antibody labelled with 99mTc. Breast Cancer Research and Treatment 96: 115-121.
16. Blanco R, Blanco D, Quintana Y, Escobar X, Rengifo CE, et al. (2013) Immunoreactivity of the 14F7 Mab raised against N-glycolyl GM3 ganglioside in primary lymphoid tumors and lymph node metastasis. Patholog Res Int 2013: 920972.
17. de León J, Fernández A, Mesa C, Clavel M, Fernández LE (2006) Role of tumour-associated N-glycolylated variant of GM3 ganglioside in cancer progression: effect over CD4 expression on T cells. Cancer Immunol Immunother 55: 443-450.
18. de León J, Fernández A, Clavel M, Labrada M, Bebelagua Y, et al. (2008) Differential influence of the tumour-specific non-human sialic acid containing GM3 ganglioside on CD4+CD25-effector and naturally occurring CD4+CD25+ regulatory T cells function. Int Immunol 20: 591-600.
19. Blanco R, Blanco D, Escobar X, Rengifo CE, Cedeño M, et al. (2015) Immunoreaction of 14F7 Mab Raised against N-Glycolyl GM3 Ganglioside Correlates with High Histological Grade in some Tumors of Neuroectodermal and Epithelial Lineage. J Mol Biomark Diagn 6: 252
20. Black MM, Opler SR, Speer FD (1955) Survival in breast cancer cases in relation to the structure of the primary tumor and regional lymph nodes. Surg Gynecol Obstet 100: 543-551.
21. Elston CW, Ellis IO (1991) Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 19: 403-410.
22. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, et al. (2010) American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer (unabridged version). Arch Pathol Lab Med 134: e48-e72.
23. van Cruijsen H, Ruiz MG, Van der Valk P, de Gruijl TD, Giaccone G (2009) Tissue micro array analysis of ganglioside N-glycolyl GM3 expression and signal transducer and activator of transcription (STAT)-3 activation in relation to dendritic cell infiltration and microvessel density in non-small cell lung cancer. BMC Cancer 9: 180.
24. Lahera T, Calvo A, Torres G, Rengifo CE, Quintero S, et al. (2014) Prognostic Role of 14F7 Mab Immunoreactivity against N-Glycolyl GM3 Ganglioside in Colon Cancer. J Oncol 2014: 482301.
25. Blanco R, Domínguez E, Morales O, Blanco D, Martínez D, et al. (2015) Prognostic significance of N-Glycolyl GM3 ganglioside expression in non-small cell lung carcinoma patients: new evidences. Pathol Res Int 2015: 132326.
26. Scursoni AM, Galluzzo L, Camarero S, Pozzo N, Gabri MR, et al. (2010) Detection and characterization of N-glycolylated gangliosides in Wilms tumor by immunohistochemistry. Pediatric and Developmental Pathology 13: 18-23.

27. Torbidoni AV, Scursoni A, Camarero S, Segatori V, Gabri M, et al. (2015) Immunoreactivity of the 14F7 Mab raised against N-Glycolyl GM3 Ganglioside in retinoblastoma tumours. Acta Ophthalmol 93: e294-e300.

28. Blanco R, Rengifo E, Rengifo CE, Cedeño M, Frómeta M, et al. (2011) Immunohistochemical reactivity of the 14F7 monoclonal antibody raised against N-glycolyl GM3 Ganglioside in some benign and malignant skin neoplasms. ISRN Dermatol 2011: 848909.

29. Yang Q, Mori I, Sakurai T, Yoshimura G, Suzuma T, et al. (2001) Correlation between nuclear grade and biological prognostic variables in invasive breast cancer. Breast Cancer 8: 105-110.

30. Madani SH, Payandeh M, Sadeghi M, Motamed H, Sadeghi E (2016) The correlation between Ki-67 with other prognostic factors in breast cancer: A study in Iranian patients. Indian Journal of Medical and Paediatric Oncology: Official Journal of Indian Society of Medical & Paediatric Oncology 37: 95-99.

31. Rakha EA, Reis-Filho JS, Baehner F, Dabbs DJ, Decker T, et al. (2010) Breast cancer prognostic classification in the molecular era: the role of histological grade. Breast Cancer Res 12: 207.

32. Coates AS, Winer EP, Goldhirsch A, Gelber RD, Gnant M, et al. (2015) Tailoring therapies--improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015. Ann Oncol 26: 1533-1546.