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Nuclear staining of fgfr-2/stat-5 and runx-2 in mucinous breast cancer☆,☆☆

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ABSTRACT

Mucinous carcinoma (MBC) is a rare subtype of breast cancer characterized by the production of variable amounts of mucin, with a prognosis better than that of non-mucinous carcinomas (NMBC). The aim of this project was to evaluate the expression of STAT-5, RUNX-2, and FGFR-2 in a cohort of MBC and compare it with that of NMBC using standard immunohistochemistry. STAT-5 and RUNX-2 are two transcription factors with cytoplasmic and/or nuclear localization that have been related to FGFR-2, a tyrosine kinase growth factor receptor that can interact with STAT-5 and with PR in the nuclei of breast cancer cells. Membranous, cytoplasmic, and nuclear staining were evaluated and expressed as the percentage of stained cells (0–100%) multiplied by the staining intensity (0–3), thus obtaining an index ranging from 0 to 300. Nuclear and/or cytoplasmic immunoreactivity of the three proteins were detected in a high number of NMBC. Nuclear FGFR-2 staining correlated with nuclear STAT-5 ($p < 0.05$) and nuclear RUNX-2 ($p < 0.01$) in both tumor types; however MBC had a significant higher expression of nuclear FGFR-2 ($p < 0.01$) and RUNX-2 ($p < 0.05$) than that of NMBC, and displayed positive immunoreactivity of the 3 proteins in 70.8% of the cases. These results suggest that these proteins may have a role in the progression of the mucinous phenotype, in which nuclear STAT-5 may inhibit RUNX-2 prometastatic effect.

1. Introduction

Mucinous carcinoma (MBC) of the breast is a rare subtype of cancer characterized by the production of variable amounts of mucin. The incidence was reported to range from 1 to 6% (Andre et al., 1995; Bae et al., 2011; Bal et al., 2008; Diab et al., 1999; Horlings et al., 2013; Louwman et al., 2007; Zhang et al., 2014). Pure MBCs are defined as those with a mucinous component of more than 90% and they have been shown to have a better prognosis than mixed or non-mucinous tumors (Barkley et al., 2008; Di Saverio et al., 2008; Fentiman et al., 1997). Fujii et al., reported that MBC exhibits less genetic instability than other forms of breast cancer and that its molecular pathogenesis appeared substantially different from that of the usual breast carcinoma (Fujii et al., 2002). Recently, Lacroix-Triki et al., supported these findings confirming that MBCs were not only a histological, but also a molecular distinctive entity, different from Non-Mucinous Breast Carcinoma (NMBC) (Lacroix-Triki et al., 2010).

We have reported that FGFR-2 and STAT-5 may co-localize with PR in the nuclei of breast cancer samples, and that FGFR-2 is involved in FGF-2-induced progesterone receptor (PR) activation (Cerliani et al., 2011). We have also established that STAT-5 participates in the progestin or FGF2-induced PR activation (Cerliani et al., 2011). Interestingly, a polymorphism in the FGFR-2 gene has been related to sporadic ER- and PR-positive breast cancer risk (Hunter et al., 2007), and the SNPs involved alter the binding affinity for the transcription factors OCT-1/RUNX-2 and EBP-β (Meyer et al., 2008). Taking into account the role of FGF-2 in regulating the expression and activity of RUNX-2 (Teplyuk et al., 2009), and the fact that a physical interaction between STAT-5 and RUNX-2 has been reported (Ogawa et al., 2008), we decided to investigate the expression of FGFR-2, STAT-5 and RUNX-2 in breast cancer samples. Interestingly, MBC seems to be unique in that the three proteins show a preferential nuclear localization, unlike NMBC.

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2. Patients and methods

2.1. Patients

One hundred and seven patients with histologically proven breast carcinoma were collected from the archives of the Department of Pathology in General Hospital "Magdalena V de Martínez" and from a private pathology practice in Buenos Aires (J.M.). We intentionally enriched the population of MC (n = 17) to compare our findings in a larger set of patients. The study was approved by the Institutional IRB (IBYME, #2011–12).

Expression of the classical prognostic factors such as estrogen receptors (ER), PR, HER-2 and ki-67 were reevaluated for all tissues and are summarized in Table 1. MBC was defined as having a mucinous component of more than 90% and three specialized pathologists (M.M., J.M. and A.M.) with extensive experience in breast pathology performed a slide review. Patients with a diagnosis of MBC were compared with the NMBC group. Histological grading and the Nottingham Prognostic Index (NPI) (Sinn and Kreipe, 2013) were also evaluated.

2.2. Antibodies

The antibodies, company and dilutions are summarized in Table 2. For immunohistochemistry, biotinylated rabbit and mouse secondary antibodies were used (Vector Lab, Burlingame, CA) and for immunofluorescence FITC mouse and Dylight rabbit secondary antibodies (Vector Lab, Burlingame, CA).

Table 1
Clinico-pathological features of Mucinous Breast Carcinomas versus Non Mucinous Breast Carcinomas.

	NMBC (N = 90)	%	MBC (N = 17)	%	*P*-value
NPI					
I	24	26.7	17	100	
II	32	35.6	0	0	
III	25	27.8	0	0	<0.0001
N/A	9	10	0	0	
ER					
0%	10	11.1	1	5.88	
1–25%	6	6.67	0	0	
26–50%	7	7.78	0	0	
51–100%	56	62.2	15	88.2	0.7432
N/A	11	12.2	1	5.88	
PR					
0%	19	21.1	1	5.88	
1–25%	18	20	0	0	
26–50%	13	14.4	2	11.8	
51–100%	34	37.8	14	82.4	0.0118
N/A	6	6.67	0	0	
HER2					
Negative	58	64.4	15	88.2	
Positive	14	15.6	0	0	0.0311
N/A	18	20	2	11.8	
Tumor Size					
T1	18	20	2	11.8	
T2	24	26.7	0	0	
T3	14	15.6	1	5.88	
T4	13	14.4	0	0	0.3146
N/A	21	23.3	14	82.4	
Node					
Negative	16	17.8	2	11.8	
Positive	33	36.7	2	11.8	0.5977
N/A	41	45.6	13	76.5	

Table 2
Antibodies.

Antibody	Company	Dilution
PR (PgR 636)	Dako	RTU
ER (EP-1)	Dako	RTU
FGFR-2 (C-17)	SCB	1:50
STAT-5 (sc-835)	SCB	1:100
RUNX-2 (sc-10758)	SCB	1:50
Ki-67 (ab-15581)	Abcam	1:500

2.3. Immunohistochemistry and scoring of slide sections

Immunohistochemical procedures were performed as previously described. (Vanzulli et al., 2002) Briefly, 5 μm sections were deparaffinized and the endogenous peroxidase inhibited with H_2O_2 in ethanol. Antigen retrieval was performed with 10 mM citrate buffer pH 6 in a thermal bath. The tissue sections were washed with PBS and then sequentially incubated with the primary antibody in 2.5% bovine serum albumin overnight, washed and incubated with the appropriate secondary antibody, and the ABC kit (Vector Lab, Burlingame, CA). After washing with PBS, the samples were developed using 3,3′-diaminobenzidine chromogen solution (Dako, Carpinteria, CA) according to the manufacturer's protocol. The samples were then washed with distilled water and counterstained with hematoxylin solution for 10 s at room temperature. Samples were rinsed with water and dried, and then visualized using bright field microscopy.

Membranous, cytoplasmic, and nuclear staining were differentially evaluated and expressed as percentage of stained cells (0–100%) times the intensity (0–3), in a scale of values ranging from 0 to 300. The score was graded for positive samples in a low/high scoring system, considering <100 or >100 respectively. We included negative controls for each marker avoiding the primary antibody.

2.4. Immunofluorescence and co-expression

Sections of breast cancer paraffin embedded samples were dehydrated, blocked in 2.5% bovine serum albumin (BSA) and incubated with the FGFR-2 + RUNX-2, or STAT5 + RUNX-2 antibodies at 1/100 dilutions O.N. at 4 °C. Slides were washed, incubated for 1 h with both secondary antibodies at 1/100 dilutions and mounted with Vectashield (Vector Labs). Negative controls lacked primary antibodies. Tissues were analyzed using a Nikon Eclipse E800 Microscope by using the Nikon DS-U1 with ACT-2 U software.

2.5. Statistical analysis

The intergroup comparisons of clinicopathological variables were performed with the χ^2 test for discrete variables. The accepted level of significance was $p < 0.05$. All data analysis was performed with SPSS for Windows version 20.0.

3. Results

3.1. Expression of STAT-5, FGFR-2 and RUNX-2 in NMBC and MC

Regardless of the histological type, we found a strong positive association between nuclear expression of STAT-5 with FGFR-2 (r: 0.476; $p < 0.05$) and with RUNX-2 (r:0.415; $p < 0.05$), and between FGFR-2 and RUNX-2 (r: 0.369; $p < 0.01$).

Sixty eight percent of NMBCs disclosed positive nuclear staining for STAT-5 (24.7% high and 43.8% low staining); in 97.7% of the samples the immunoreactivity was cytoplasmic and primarily high. Simultaneous nuclear and cytoplasmic staining was observed in 47.2% of the samples. Membrane staining was a rare event (See