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LETTER TO THE EDITOR

Pregnancy and breastfeeding: a new theory for sHLA-G in breast cancer patients?

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Abstract It is now widely recognized that HLA-G molecule is implicated in immune tolerance and particularly in immune subversion of tumor cells. In this study, we explored levels of soluble HLA-G (sHLA-G) in plasma samples obtained from women with breast cancer (BC). Additionally, we correlated sHLA-G concentration with pregnancy and breastfeeding history. We reported in this preliminary work significant differences in sHLA-G levels between BC patients with/without breastfeeding experience ($p = 0.04$). Interestingly, among women with BC, only those without previous pregnancy experience present significant increase in sHLA-G ($p = 0.02$). Of relevance, we demonstrated that patients without both pregnancy and breastfeeding history have advanced SBR III grade, associated with significant enhancement in tumor size compared with patients who had both experiences ($p = 0.028$). Taken together, our results indicate the potential implication of previous pregnancy and breastfeeding experience in sHLA-G expression during BC. We theorized that having pregnancy and breastfeeding history may protect against advanced BC stages.

Keywords sHLA-G · Breast cancer · Pregnancy · Breastfeeding

Breast cancer (BC) is a leading cause of mortality in women [1, 2]. Several molecules predispose women to increased risk of BC, especially classical HLA (human leukocyte antigen) class I molecules [2, 3]. Interestingly, nonclassical HLA-I molecules have been highly implicated in the control of BC progression [3].

Among nonclassical HLA-I molecules, HLA-G molecules behave as an immune-tolerance mediator avoiding fetus rejection [4, 5] and promoting successful pregnancy [6]. In this context, HLA-G molecules are expressed as membrane-bound molecules as well as soluble ones (sHLA-G) [5]. Reciprocally, the reduced levels of HLA-G expression are involved in pregnancy complications [7–10].

It is well known that HLA-G molecules are implicated in tumor evasion through the regulation of immune cell activation [11]. In particular, HLA-G molecule inhibits different immune cell functions including NK lysis, LTCD8 + cytotoxicity, and cell presenting antigens' maturation and proliferation [12]. These functions are associated with HLA-G interaction at least with its specific ligands including ILT2 (immunoglobulin-like transcript), ILT4, and KIR2DL4 (killer cell immunoglobulin-like receptor) [12].

To the best of our knowledge, our preliminary study is the first exploring levels of HLA-G molecules in plasma samples of BC patients and evaluating their implication in

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BC disease progression in women with/without pregnancy/breastfeeding history.

Study participants are 36 confirmed Tunisian breast cancer patients from Salah Azaiz Hospital (Table 1). All participants satisfied the World Health Organization Classification of Tumours (WHO 2012) classification criteria. They were chemotherapy-naïve for at least 3 months before accrual. Twelve patients have low to intermediate TNM stage (I and II) and 15 presented an advanced TNM stage (III and IV). Patients' characteristics are listed in Table 1. This study was approved by scientific ethics.

Table 1 Clinical and demographic characteristics of BC participants

	N
No. of patients	36
Mean age (years)	47.19 (SEM = 1.47; range 30–67)
Breastfeeding experience	
No	18
Yes	18
Pregnancy experience	
No	16
Yes	20
Menopause	
No	19
Yes	13
Unknown	4
TNM stage	
Stage I	2
Stage II	10
Stage III	6
Stage IV	9
Unknown	9
Mean tumor size (mm)	38.41 (SEM = 3.21; range 15–80)
SBR grade	
SBR I	1
SBR II	24
SBR III	6
Unknown	5
Estrogen receptor expression	
Negative	8
Positive	28
Progesterone receptor expression	
Negative	11
Positive	25
HER2 expression	
Negative	21
Positive	11
Unknown	4

HER2 human epidermal growth factor receptor 2, *NS* not significant, *SBR* Scarff Bloom Richardson, *SEM* standard error of the mean, *TNM* tumor node metastasis

Patients were divided into two groups according to their previous history of pregnancy and breastfeeding: Group 1 are BC patients without both previous experience of pregnancy and breastfeeding, and group 2 are BC patients with previous experience of pregnancy and breastfeeding. sHLA-G dosage was performed in duplicate by sHLA-G ELISA assay kit (Exbio, Praha, Czech Republic) according to the manufacturer's instructions [13, 14].

Interestingly, statistical evidence of negative correlation was reported between sHLA-G levels and either pregnancy experience (R Spearman $= -0.416$, $p = 0.012$) or breastfeeding experience (R Spearman $= -0.341$, $p = 0.042$). This means that patients with history of pregnancy and breastfeeding should present low sHLA-G levels. This was validated by sHLA-G dosage reported here. Indeed, patients with previous pregnancy experience have lower sHLA-G (7.70 Units/ml) compared to patients without pregnancy experience (40.14 Units/ml; two-tailed Mann–Whitney test: $p = 0.018$). This is in accordance with the results showing decreased levels of sHLA-G after delivery [15, 16]. Furthermore, results showed for the first time that patients with no previous breastfeeding experience had higher sHLA-G levels (35.68 Units/ml) compared to women who have breastfed (8.13 Units/ml; two-tailed Mann–Whitney test: $p = 0.045$).

Interestingly, frequencies of SBR I/II grades are increased in group 2 patients (82.35 %) compared to group 1 patients (75 %). Inversely, SBR III frequency is increased in group 1 patients (25 %) compared to group 2 patients (17.65 %). sHLA-G levels are decreased in group 2 patients compared to group 1 patients (group 2 vs group 1; SBR II: 10 vs 39.39 Units/ml; SBR III: 5.47 vs 41.71 Units/ml).

Of relevance, group 1 patients showed an enhanced tumor size (mean = 43.82 mm) compared to group 2 patients (mean = 34.8 mm). This difference is statistically significant (Mann–Whitney: $p = 0.028$, Table 2).

We hypothesize that decreased sHLA-G in BC patients with previous pregnancy and/or breastfeeding experience may be a direct or indirect protection against complicated stages of the disease. Further studies are still needed to elucidate the mechanism under this regulation. Elsewhere, sHLA-G enhancement in patients without history of pregnancy and breastfeeding provides a possible explanation of worse outcome of BC. This may be corroborated by studies on membranous HLA-G that was reported in advanced stages of BC [17–20].

Overall, present results provided evidence that sHLA-G level is influenced by the previous pregnancy and, for the first time, by the previous breastfeeding experience. BC patients with both pregnancy and breastfeeding

Table 2 sHLA-G dosage characteristics in patients with/without pregnancy and breastfeeding experiences

	Patients without pregnancy and breastfeeding experiences (group 1) ($N = 16$)	Patients with pregnancy and breastfeeding experiences (group 2) ($N = 18$)	p value[a]
Mean tumor size ± SEM (mm)	43.82 ± 2.60	34.8 ± 5.78	0.028
TNM stage (%)			
Stage I	0	14.29	–
Stage II	41.67	28.57	0.52
Stage III	25	21.43	–
Stage IV	33.33	35.71	–
SBR grade (%)			
SBR I	0	5.88	–
SBR II	75	76.47	0.12
SBR III	25	17.65	0.35
Estrogen receptor expression (%)			
Negative	43.75	22.22	–
Positive	56.25	77.78	0.18
Progesterone receptor expression (%)			
Negative	31.25	16.66	0.44
Positive	68.75	83.34	0.040
HER2 expression (%)			
Negative	64.29	68.75	0.049
Positive	35.71	31.25	0.80

Patients with unknown data concerning the variables were excluded from the analysis

TNM tumor node metastasis

[a] Mann–Whitney test comparing sHLA-G means or the mean tumor size

experiences have lower sHLA-G compared to patients without these experiences. This cohort has also low advanced tumor stages. Further studies with large number of patients are still needed to confirm our preliminary findings.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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