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Expression of T helper 1-associated lncRNAs in breast cancer

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ABSTRACT

Interferon gamma (IFN-gamma)-associated genes participate in the pathobiology of cancer and response of patients to immunotherapeutic modalities. This cytokine is regarded as a hallmark of T helper 1 type responses. In the current study, we estimated expression of this gene and a number of genes/ long non-coding RNAs (*IFNG-AS001* and *IFNG.AS003*, *AC007278.2* and *AC007278.3* and *IL18R1*) which are encoded from proximal genomic regions to *IFNG* in a larger cohort of Iranian patients with breast cancer. Both *IFNG.AS001* and *IFNG.AS003* were up-regulated in breast cancer tissues compared with nearby non-cancerous tissues (Ratios of Mean Expressions = 5.62 and 5.88, *P* values = 1.28E-03 and 1.47E-03, respectively). Finally, *IL18R1* was over-expressed in breast cancer tissues compared with nearby non-cancerous tissues (Ratio of Mean Expressions = 9.43, *P* values = 3.14E-03). Expression of *AC007278.3* was associated with breast feeding duration (P value = 2.65E-02). Positive significant correlations were detected between expression levels of all genes in both sets of samples. The most robust correlation in the nearby non-cancerous tissues was detected between *IFNG-AS003* and *AC007278.2* (*r* = 088, *P* value = 5.19E-23). In the tumoral tissues, the strongest correlation was found between *IFNG-AS001* and *IL18R1* (*r* = 0.86, P value = 3.79E-15). *AC007278.3* had the best diagnostic power among the assessed genes (AUC = 0.82). Both *AC007278.2* and *AC007278.3* were reported to be specific markers for differentiation of tumor tissues from nearby non-cancerous tissues. Combination of expression levels of genes increased specificity, sensitivity and AUC values to 0.97, 0.89 and 0.95, respectively. The current study accentuates the role of *IFNG*-associated genes in the pathogenesis of breast cancer.

1. Introduction

Interferons (IFNs) are a group of proteins with diverse functions including inhibition of replication and cell growth as well as regulation of cell differentiation (Borden and Balkwill, 1984). As a member of this family, IFNγ has a role in the suppression of growth of some cancer cells, potentiating it as an antitumor molecule (Harvat and Jetten, 1996; Mueller et al., 1996). In a cohort of patients with different breast lesions including *in situ* carcinomas as well as benign and infiltrating tumors, *in situ* carcinomas had the highest density of IFNγ expression (García-Tuñón et al., 2007). Recently, we have reported up-regulation of *IFNG* expression in a subset of breast cancer samples (Yaghoobi et al., 2018). Moreover, experiments in other types of cancers demonstrated correlations between expression levels of this gene in tumor samples and response of patients to therapeutic options (Higgs et al., 2018; Gao et al., 2016). Notably, a recent data mining and proximity analysis of genes involved in the T helper 1 differentiation and function have led to recognition of a number of long non-coding RNAs (lncRNAs) which are functionally or locally associated with IFNγ. Being located on the chromosome 12, *IFNG* gene resides inside an intron of the lncRNA *IFNG-AS1-001*. The lncRNA *IFNG-AS1-003* is located on the same chromosomal region albeit with a distance about 100 kbp from the *IFNG* gene. Two other functionally related lncRNAs, namely *AC007278.2* and *AC007278.3* are located on chromosome 2 within the introns of *IL18RAP* and *IL18R1* (Hosseini et al., 2019). *IL18R1* has a crucial role in the mediation of IL-18 signaling pathway. Expression of this cytokine

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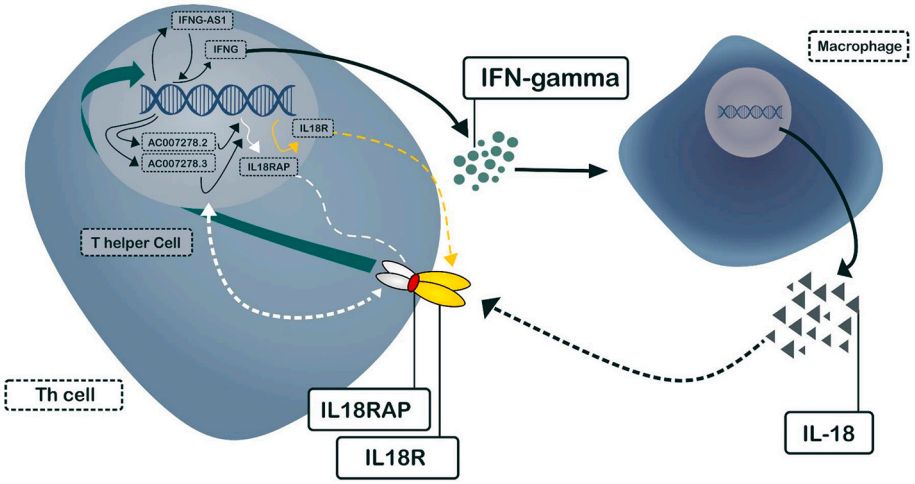

Fig. 1. Illustration of the functional interplay between IFNG, IFNG.AS001, IFNG.AS003, AC007278.2, AC007278.3 and IL18R1.
Based on the possible role of *IFNG* and *IL18R1* in the pathogenesis of breast cancer and the functional association between these gene and mentioned lncRNAs, we aimed to investigate their expression pattern in breast cancer samples and adjacent non-cancerous tissues (ANCTs).

receptor has been shown to be higher in T cells from invasive ductal carcinoma samples compared with *in situ* tumors, suggesting its role in immune escape of cancer cells (Del Alcazar et al., 2017). This gene has also been among the up-regulated genes in the breast cancer cells with stem cell-like features (Lee et al., 2016). Fig. 1 represents the functional interplay between these genes.

2. Materials and methods

2.1. Patients

Expressions of mentioned genes were measured in tissue specimens obtained from 69 patients with breast cancer. Tumor tissues and ANCTs were cut out during surgery. Patients were admitted to Farmanieh and Sina hospitals, Tehran, Iran during 2017–2020. The study protocol was approved by the ethical committee of Shahid Beheshti University of Medical Science (ethical code: IR.SBMU.RETECH.REC.1398.379). Patients signed informed consent forms. Samples were taken from patients who did not receive chemotherapy or radiotherapy.

2.2. Expression assays

RNA was isolated from both groups of samples using the RiboEx Total RNA extraction kit (GeneAll, Seoul, South Korea). Afterwards, cDNA was synthesized from approximately 75 ng of RNA using the ExcelRT™ Reverse Transcription Kit II (SMOBIO, Taiwan). Expressions of genes in all samples were quantified in the ABI step one plus PCR machine using the RealQ Plus 2× PCR Master Mix Green Without ROX™ PCR (Ampliqon, Odense, Denmark). *B2M* gene was selected as the normalizer. Primer sequences and PCR conditions were similar to the study conducted by Hosseini et al. (Hosseini et al., 2019).

2.3. Statistical methods

R software was used for statistical analyses. Transcript quantities of mentioned lncRNAs and mRNA coding genes were measured relative to the normalizer gene using the following formula: $\frac{amp_{gene}^{-CT_{gene}}}{amp_{B2M}^{-CT_{B2M}}}$. Afterwards, the calculated values were log2 transformed.

The significance of difference in the expression of T helper 1-associated genes between two sets of samples was assessed using the paired *t*-test. Correlations between expressions of genes were judged using Spearman correlation coefficients. Receiver operating characteristic

Table 2
General information of patients.

Parameters	Values
Menarche age (mean ± SD)	13.15 ± 1.56 (10–18)
Menopause age (mean ± SD)	49.47 ± 5.08 (38–60)
First pregnancy age (mean ± SD)	21.09 ± 4.69 (14–37)
Breast feeding duration (months) (mean ± SD)	47.85 ± 48.88 (0–240)
Cancer stage (%)	
I	18 (26.08%)
II	22 (31.88%)
III	20 (28.98%)
IV	5 (7.24%)
Unknown	4 (5.79%)
Overall grade (%)	
I	12 (17.39%)
II	32 (46.37%)
III	18 (26.08%)
Unknown	7 (10.14%)
Mitotic rate (%)	
I	23 (33.33%)
II	26 (37.68%)
III	7 (10.14%)
Unknown	13 (18.84%)
Abortion	
Positive	57 (82.60%)
Negative	12 (17.39%)
Oral contraceptive use	
No	35 (50.72%)
Yes	34 (49.27%)
Hormone replacement therapy	
No	58 (84.05%)
Yes	11 (15.94%)
Estrogen receptor	
Positive	52 (75.36%)
Negative	13 (18.84%)
Unknown	4 (5.79%)
Progesterone receptor	
Positive	48 (69.56%)
Negative	14 (20.28%)
Unknown	7 (10.14%)
Her2/neu expression	
Positive	13 (18.84%)
Negative	50 (72.46%)
Unknown	8.69 (7.5%)

Table 3
Detailed statistics of expressions of genes in breast cancer samples *versus* non-cancerous tissues.

	SE	Ratio of Mean Expressions	*P*-Value	95% CI	
IFNG	0.74	0.50	1.85E-01	−2.46	0.48
IFNG.AS001	0.74	5.62	1.28E-03	1.01	3.97
IFNG.AS003	0.77	5.88	1.47E-03	1.02	4.09
AC007278.2	0.61	11.75	1.39E-07	2.35	4.76
AC007278.3	0.58	33.03	1.35E-12	3.88	6.21
IL18R1	0.98	9.43	3.14E-03	1.21	5.27

(ROC) curves were depicted to assess the diagnostic power of genes. Bayesian Generalized Linear Model, Generalized Linear Model, and Linear Discriminant Analysis with 10-fold cross validation were used to compute the sensitivity and specificity values. The most efficient estimates were obtained from the Bayesian Generalized Linear Model (bayesGLM). Youden's J statistic was used for calculation of the optimum threshold. Association between patients' demographic/clinical data and expression levels of genes was assessed using the Chi-square test. Genes with log2FC ≥ 1 (tumor tissues *vs.* ANCTs) were described as up-regulated and those with log2FC ≤ -1 were considered as down-regulated. *P* values <0.05 were regarded as significant.

3. Results

3.1. General information of patients

Table 2 shows a summary of clinical data of patients.

3.2. Expression of genes

Both *IFNG.AS001* and *IFNG.AS003* were over-expressed in breast cancer tissues compared with ANCTs (Ratios of Mean Expressions = 5.62 and 5.88, P values = 1.28E-03 and 1.47E-03, respectively). Besides, expression levels of *AC007278.2* and *AC007278.3* were elevated in cancer tissues compared with controls (Ratios of Mean Expressions = 11.75 and 33.03, *P* values = 1.39E-07 and 1.35E-12, respectively). Finally, *IL18R1* was over-expressed in breast cancer tissues compared with ANCTs (Ratio of Mean Expressions = 9.43, P values = 3.14E-03). Table 3 displays the detailed statistics of expressions of mentioned genes in breast cancer samples compared with controls.

Fig. 2 depicts expression levels of genes in breast cancer tissues and controls samples.

We categorized patients to three categories based on the relative expression of each gene in tumor tissue *vs.* ANCT (Log2FC ≤ -1 and log2FC ≥ 1 were indicative of down-regulation and up-regulation, respectively. -1 < log2FC < 1 indicated similar levels of expressions). Then, we assessed association between these categories and clinical data using Chi square test. Expression of *AC007278.3* was associated with breast feeding duration (*P* value = 2.65E-02). However, expressions of other genes were not associated with any of recorded demographic or clinical information (Table 4).

3.3. Correlation analysis

Positive significant correlations were observed between expression levels of all genes in both sets of samples (Fig. 3). The most robust correlation in the ANCTs was detected between *IFNG-AS003* and *AC007278.2* (r = 088, P value = 5.19E-23). In the tumoral tissues, the strongest correlation was found between *IFNG-AS001* and *IL18R1* (r = 0.86, P value = 3.79E-15).

3.4. ROC curve analyses

Fig. 4 shows the ROC curves depicted by three predictive machine learning methods and those plotted by the best predictive model.

Table 5 shows the detailed statistics of ROC curve analysis for estimation of the power of *IFNG*, *IFNG.AS001*, *IFNG.AS003*, *AC007278.2*, *AC007278.3* and *IL18R1* in differentiation of breast cancer samples from ANCTs. *AC007278.3* had the best diagnostic power among the mentioned genes (AUC = 0.82). Both *AC007278.2* and *AC007278.3* were reported to be specific markers for differentiation of tumor tissues

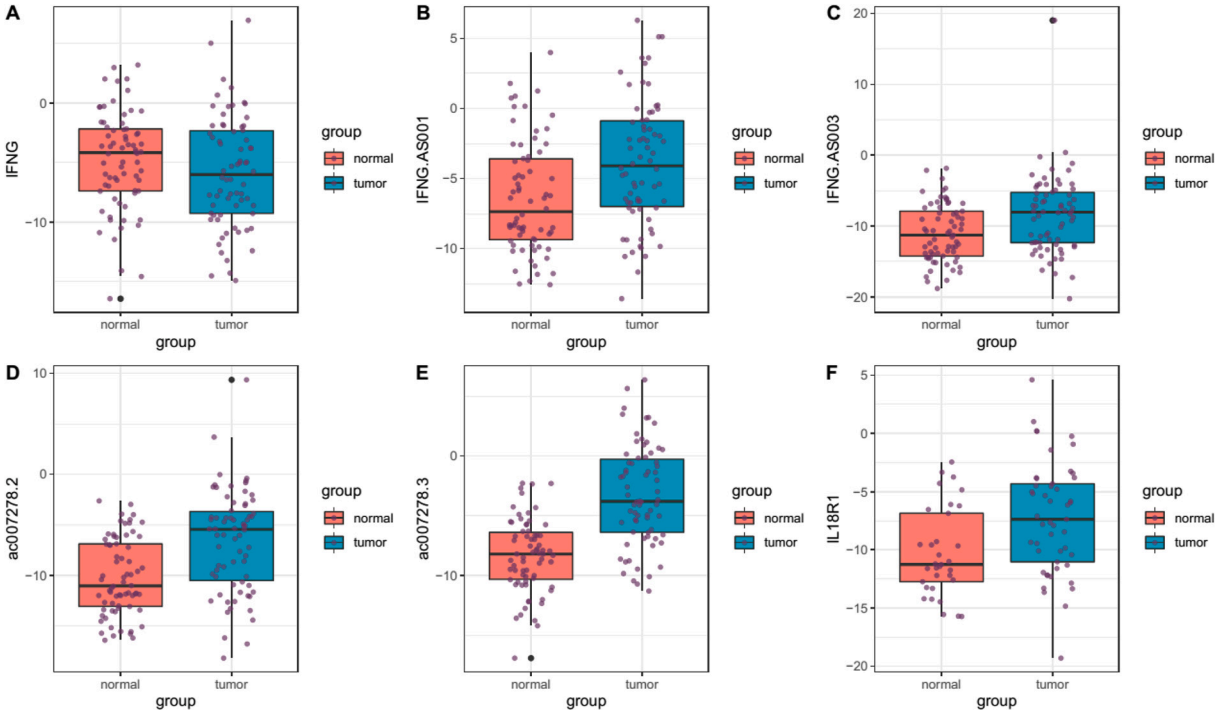

Fig. 2. Expression levels of *IFNG*, *IFNG.AS001*, *IFNG.AS003*, *AC007278.2*, *AC007278.3* and *IL18R1* in breast cancer tissues and paired non-cancerous samples. Median, upper and lower quartile values are shown.

Table 4
Association between expression of genes and recorded patients' information (Log2FC ≤ −1 and log2FC ≥ 1 were described as down-regulation and up-regulation, respectively. -1 < log2FC < 1 indicated similar levels of expressions).

		IFNG				*IFNG-AS001*				*IFNG-AS003*		
		Down- regulated	Same	Up- regulated	P-value	Down- regulated	Same	Up- regulated	P-value	Down- regulated	Same	Up- regulated
Age					4.91E-01				4.44E-01			
	Post-Menopause	46.88%	15.63%	28.13%		28.13%	9.38%	53.13%		3.13%	3.13%	3.13%
	Pre-Menopause	6.25%	3.13%	0.00%		6.25%	0.00%	3.13%		28.13%	6.25%	56.25%
Stage					6.64E-01				7.56E-01			
	0	4.55%	0.00%	1.52%		3.03%	0.00%	3.03%		4.62%	0.00%	1.54%
	1	10.61%	4.55%	10.61%		7.58%	3.03%	15.15%		4.62%	3.08%	18.46%
	2	21.21%	3.03%	7.58%		13.64%	1.52%	16.67%		10.77%	1.54%	18.46%
	3	13.64%	3.03%	13.64%		9.09%	4.55%	16.67%		7.69%	3.08%	20.00%
	4	1.52%	1.52%	3.03%		0.00%	1.52%	4.55%		3.08%	0.00%	3.08%
Grade					2.13E-01				3.23E-02			
	0	1.67%	0.00%	0.00%		0.00%	1.67%	0.00%		0.00%	0.00%	1.69%
	1	8.33%	1.67%	8.33%		6.67%	0.00%	11.67%		5.08%	1.69%	11.86%
	2	31.67%	6.67%	10.00%		21.67%	8.33%	18.33%		13.56%	5.08%	28.81%
	3	10.00%	3.33%	18.33%		8.33%	1.67%	21.67%		11.86%	1.69%	18.64%
Mitotic Rate					6.05E-01				1.76E-01			
	0	1.82%	0.00%	0.00%		0.00%	1.82%	0.00%		0.00%	0.00%	1.85%
	1	21.82%	5.45%	10.91%		14.55%	3.64%	20.00%		7.41%	5.56%	24.07%
	2	25.45%	3.64%	16.36%		18.18%	3.64%	23.64%		14.81%	3.70%	27.78%
	3	5.45%	0.00%	9.09%		3.64%	1.82%	9.09%		7.41%	0.00%	7.41%
Tumor Size					6.79E-01				5.38E-01			
	<2	11.67%	3.33%	11.67%		5.00%	5.00%	16.67%		3.39%	3.39%	20.34%
	2–5	36.67%	8.33%	25.00%		25.00%	5.00%	40.00%		23.73%	5.08%	40.68%
	>5	3.33%	0.00%	0.00%		1.67%	0.00%	1.67%		1.69%	0.00%	1.69%
ER Status					3.20E-01				9.72E-01			
	Positive	37.70%	13.11%	29.51%		6.56%	1.64%	11.48%		6.67%	1.67%	10.00%
	Negative	11.48%	0.00%	8.20%		27.87%	8.20%	44.26%		23.33%	6.67%	51.67%
PR Status					7.34E-02				2.57E-01			
	Positive	37.29%	13.56%	22.03%		5.08%	3.39%	18.64%		22.41%	8.62%	43.10%
	Negative	11.86%	0.00%	15.25%		30.51%	6.78%	35.59%		6.90%	0.00%	18.97%
Her2 Status					5.69E-01				5.55E-01			
	Positive	21.67%	5.00%	11.67%		16.67%	3.33%	18.33%		15.25%	3.39%	20.34%
	Negative	26.67%	8.33%	26.67%		18.33%	6.67%	36.67%		13.56%	5.08%	42.37%
Menarche Age					7.47E-01				3.95E-01			
	10–12	30.16%	6.35%	25.40%		6.35%	6.35%	19.05%		17.74%	4.84%	40.32%
	13–15	17.46%	4.76%	9.52%		23.81%	4.76%	33.33%		8.06%	1.61%	20.97%
	16–18	1.59%	1.59%	3.17%		1.59%	0.00%	4.76%		1.61%	1.61%	3.23%
Menopause Age					2.23E-01				7.29E-01			
	≤50	40.63%	15.63%	15.63%		28.13%	6.25%	37.50%		31.25%	3.13%	37.50%
	51–55	12.50%	3.13%	6.25%		6.25%	3.13%	12.50%		0.00%	6.25%	15.63%
	≥ 56	0.00%	0.00%	6.25%		0.00%	0.00%	6.25%		0.00%	0.00%	6.25%
Breast Feeding Duration					4.11E-01				3.16E-01			
	0	10.94%	4.69%	6.25%		7.81%	1.56%	12.50%		6.35%	0.00%	15.87%
	1–30	9.38%	1.56%	14.06%		7.81%	1.56%	15.63%		4.76%	1.59%	17.46%
	31–60	12.50%	3.13%	9.38%		7.81%	3.13%	14.06%		9.52%	1.59%	14.29%
	61–120	12.50%	1.56%	7.81%		6.25%	1.56%	14.06%		7.94%	3.17%	11.11%
	≥ 121	6.25%	0.00%	0.00%		3.13%	3.13%	0.00%		1.59%	1.59%	3.17%
Hormone Replacement Therapy	Yes	6.06%	3.03%	7.58%	5.25E-01	4.55%	0.00%	12.12%	3.33E-01	3.08%	0.00%	13.85%
	No	45.45%	9.09%	28.79%		28.79%	10.61%	43.94%		27.69%	7.69%	47.69%

from ANCTs. Combination of expression levels of genes increased specificity, sensitivity and AUC values to 0.97, 0.89 and 0.95, respectively.

4. Discussion

AC007278.2, *AC007278.3*, and *IFNG-AS1* are three T helper 1 lineage-specific lncRNAs which are located near to protein-coding genes participating in the differentiation of these cells (Hosseini et al., 2019). In the current investigation, we evaluated expression of these lncRNAs and *IL18R1* and *IFNG* protein coding genes. Previous studies have evaluated the influence of breast cancer cells on different populations of T cells showing the suppression of T helper 1 to T helper 2 ratio in sentinel nodes of patients I relation with tumor extension and lymph node metastasis (Ehi et al., 2008). However, expression patterns of T helper 1 lineage-specific lncRNAs and mRNAs in breast cancer tissues have not been elucidated. We reported over-expression of *IFNG.AS001*, *IFNG.AS003*, *AC007278.2*, *AC007278.3* and *IL18R1* in breast cancer tissues compared with nearby non-cancerous tissues. The observed over-expression of *IL18R1* in cancerous tissues is in line with the reported roles of this gene in the maintenance/ function of cancer stem cells and immune escape in breast cancer (Lee et al., 2016, Del Alcazar et al., 2017). The significance of over-expression of mentioned lncRNAs in the tumoral tissues should be investigated. Previous studies have shown association between low IFN-γ signaling in the breast cancer tissues and poor clinical outcome (Mehta et al., 2018). However, the impact of *IFNG.AS001*, *IFNG.AS003*, *AC007278.2* and *AC007278.3* lncRNAs on this signaling pathway has not been elucidated yet. These lncRNAs might also affect other signaling pathways. For instance, MAPK pathway, cell cycle, T cell receptor signaling and cancer-related pathways are among KEGG pathways being influenced by *AC007278.2* (Hosseini et al., 2019). In addition, both *IFNG.AS* and *AC007278.3* have

IFNG-AS003	*AC007278.2*				*AC007278.3*				*IL18R1*			
P-value	Down-regulated	Same	Up- regulated	P-value	Down- regulated	Same	Up- regulated	P-value	Down- regulated	Same	Up- regulated	P-value
3.00E-01				7.65E-01				6.05E-01				1.40E-01
	15.63%	3.13%	71.88%		12.50%	9.38%	68.75%		11.11%	44.44%	33.33%	
	3.13%	0.00%	6.25%		3.13%	0.00%	6.25%		11.11%	0.00%	0.00%	
5.54E-01				5.83E-02				3.58E-01				3.52E-01
	4.55%	0.00%	1.52%		1.52%	0.00%	4.55%		0.00%	4.35%	4.35%	
	1.52%	1.52%	22.73%		0.00%	3.03%	22.73%		0.00%	8.70%	17.39%	
	7.58%	6.06%	18.18%		3.03%	4.55%	24.24%		13.04%	0.00%	30.43%	
	3.03%	3.03%	24.24%		6.06%	0.00%	24.24%		4.35%	8.70%	4.35%	
	1.52%	0.00%	4.55%		0.00%	0.00%	6.06%		0.00%	0.00%	4.35%	
9.65E-01				8.69E-01				9.19E-01				5.10E-01
	0.00%	0.00%	1.67%		0.00%	0.00%	1.67%		0.00%	0.00%	0.00%	
	3.33%	1.67%	13.33%		0.00%	1.67%	16.67%		5.00%	5.00%	20.00%	
	10.00%	6.67%	31.67%		6.67%	3.33%	38.33%		5.00%	15.00%	25.00%	
	3.33%	1.67%	26.67%		3.33%	1.67%	26.67%		10.00%	0.00%	15.00%	
6.58E-01				8.26E-01				9.82E-01				8.49E-01
	0.00%	0.00%	1.82%		0.00%	0.00%	1.82%		0.00%	0.00%	0.00%	
	5.45%	5.45%	27.27%		3.64%	1.82%	32.73%		11.11%	11.11%	22.22%	
	10.91%	3.64%	30.91%		5.45%	3.64%	36.36%		5.56%	11.11%	33.33%	
	1.82%	0.00%	12.73%		1.82%	0.00%	12.73%		0.00%	0.00%	5.56%	
5.11E-01				2.22E-01				2.12E-01				3.00E-01
	1.67%	1.67%	23.33%		0.00%	3.33%	23.33%		0.00%	9.52%	9.52%	
	15.00%	8.33%	46.67%		8.33%	5.00%	56.67%		19.05%	14.29%	47.62%	
	0.00%	1.67%	1.67%		1.67%	0.00%	1.67%		0.00%	0.00%	0.00%	
8.60E-01				8.13E-01				9.03E-01				3.39E-01
	3.28%	3.28%	13.11%		1.64%	1.64%	16.39%		14.29%	23.81%	38.10%	
	13.11%	8.20%	59.02%		9.84%	4.92%	65.57%		4.76%	0.00%	19.05%	
3.32E-01				9.37E-01				7.07E-01				9.94E-01
	3.39%	3.39%	20.34%		1.69%	1.69%	23.73%		5.26%	5.26%	15.79%	
	11.86%	8.47%	52.54%		10.17%	5.08%	57.63%		15.79%	15.79%	42.11%	
3.57E-01				1.42E-01				1.26E-01				1.00E+00
	10.00%	5.00%	23.33%		8.33%	3.33%	26.67%		5.00%	5.00%	15.00%	
	5.00%	6.67%	50.00%		3.33%	3.33%	55.00%		15.00%	15.00%	45.00%	
7.69E-01				8.07E-01				3.13E-01				3.18E-01
	6.35%	3.17%	22.22%		0.00%	1.59%	30.16%		0.00%	9.09%	31.82%	
	11.11%	4.76%	46.03%		9.52%	4.76%	47.62%		13.64%	13.64%	22.73%	
	0.00%	0.00%	6.35%		0.00%	0.00%	6.35%		4.55%	0.00%	4.55%	
7.80E-02				4.77E-01				7.53E-01				1.99E-01
	18.75%	3.13%	50.00%		12.50%	9.38%	50.00%		11.11%	44.44%	22.22%	
	0.00%	0.00%	21.88%		3.13%	0.00%	18.75%		11.11%	0.00%	0.00%	
	0.00%	0.00%	6.25%		0.00%	0.00%	6.25%		0.00%	0.00%	11.11%	
7.16E-01				6.29E-01				2.65E-02				6.65E-01
	1.56%	4.69%	15.63%		0.00%	1.56%	20.31%		4.35%	0.00%	17.39%	
	3.13%	1.56%	20.31%		0.00%	4.69%	20.31%		8.70%	4.35%	8.70%	
	7.81%	1.56%	15.63%		1.56%	0.00%	23.44%		0.00%	8.70%	17.39%	
	4.69%	1.56%	15.63%		4.69%	1.56%	15.63%		4.35%	4.35%	13.04%	
	1.56%	0.00%	4.69%		3.13%	0.00%	3.13%		0.00%	4.35%	4.35%	
2.75E-01				4.47E-01				5.59E-01				4.71E-01
	3.03%	0.00%	13.64%		1.52%	0.00%	15.15%		0.00%	4.35%	17.39%	
	15.15%	10.61%	57.58%		9.09%	7.58%	66.67%		17.39%	17.39%	43.48%	

functional relationship with cell cycle regulating pathways (Hosseini et al., 2019). Therefore, these lncRNAs might exert their oncogenic roles in breast cancer through IFN-γ independent manners as well.

Expression of *AC007278.3* was associated with breast feeding duration. Prolactin has been shown to enhance production of IFN-γ (Matalka, 2003). Meanwhile, IL-18 expression has been recognized in actively secreting epithelial cells in lactating mammary gland (Takahata et al., 2001). However, other pieces of the puzzle of interaction between IL-18, IFN-γ and this lncRNA should be clarified in the context of breast cancer.

We also detected positive significant correlations between expression levels of all genes in both sets of samples supporting their functional interaction. However, the robustness of correlations was different among two sets of samples. While the most robust correlation in the ANCTs was detected between *IFNG-AS003* and *AC007278.2*, in the tumoral tissues, the strongest correlation was found between *IFNG-AS001* and *IL18R1*. Therefore, the presence of malignancy can affect the strength of correlations between these genes.

We also evaluated the diagnostic power of T helper 1-associated genes in breast cancer. *AC007278.3* had the best diagnostic power among the assessed genes. Both *AC007278.2* and *AC007278.3* were reported to be specific markers for differentiation of tumor tissues from ANCTs. Combination of expression levels of genes increased specificity, sensitivity and AUC values to 0.97, 0.89 and 0.95, respectively. Therefore, the panel of mentioned genes has an appropriate diagnostic power in differentiation of breast cancer tissues from ANCTs.

Taken together, the current study underscores the role of *IFNG*-associated genes in the pathogenesis of breast cancer and warrant functional investigations to verify the obtained results and clarify the underlying mechanisms. Our study has a limitation in the terms of lack of appraisal of expression of genes in tumor-infiltrating T cells.

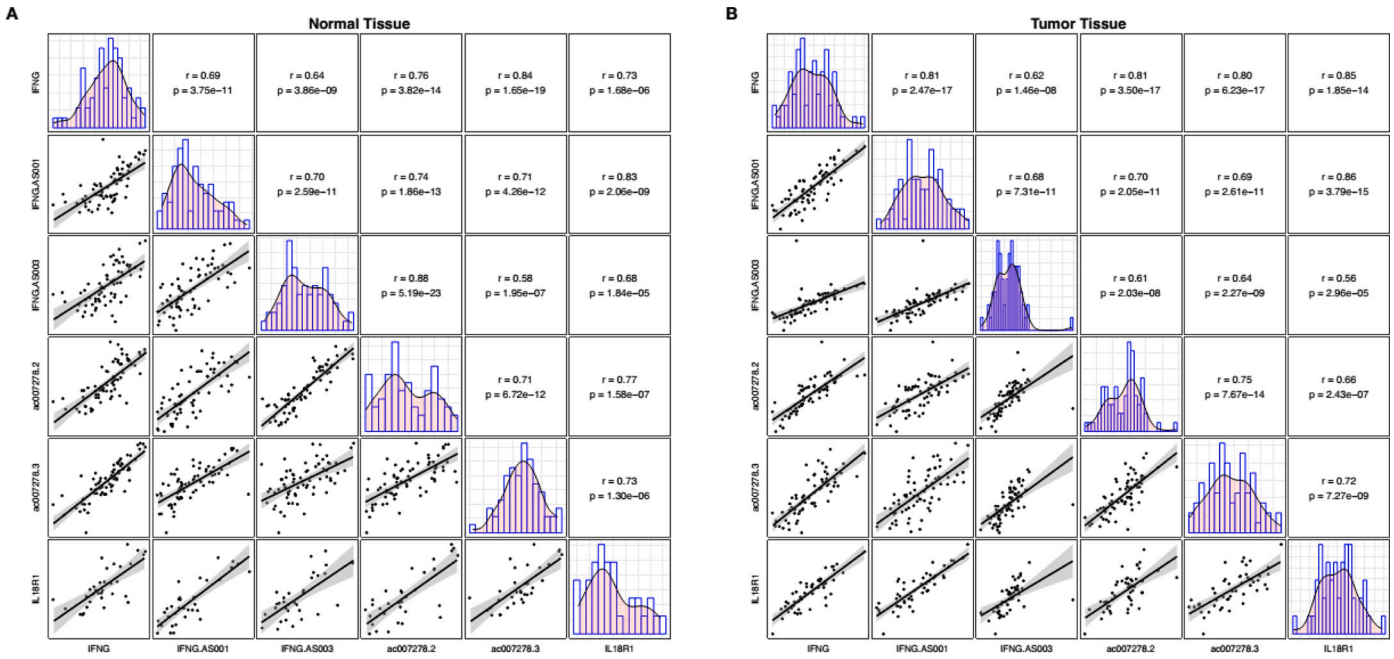

Fig. 3. Correlation matrix showing the correlation between expression levels of *IFNG*, *IFNG.AS001*, *IFNG.AS003*, *AC007278.2*, *AC007278.3* and *IL18R1* in non-cancerous samples (A) and breast cancer samples (B). Distribution of expression level of each gene is presented on the diagonal. Bivariate scatter plots with a fitted line are depicted in the bottom of the diagonal. The correlation coefficients and P values are displayed on the upper section of the diagonal.

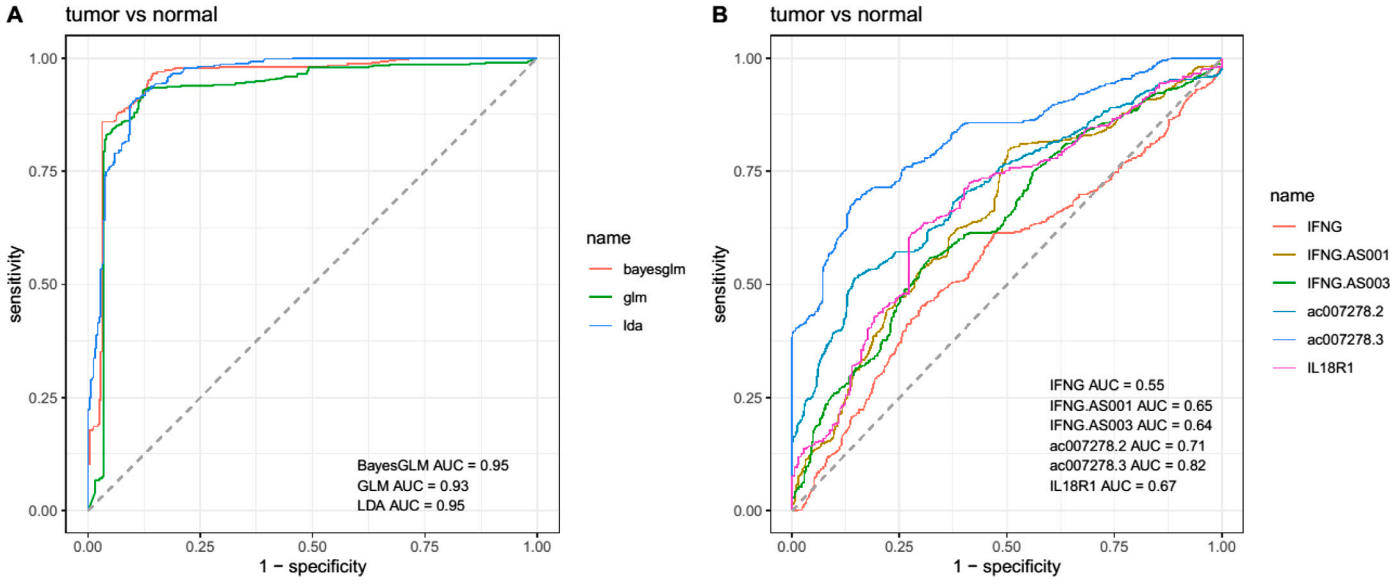

Fig. 4. Receiver Operating Characteristics curves illustrated by three predictive machine learning methods, namely Bayesian Generalized Linear Model (bayesGLM), Generalized Linear Model, and Linear Discriminant Analysis (A). BayesGLM resulted in the most efficient estimates (B).

Table 5
Detailed statistics of ROC curve analysis for estimation of the power of *IFNG*, *IFNG.AS001*, *IFNG.AS003*, *AC007278.2*, *AC007278.3* and *IL18R1* in differentiation of breast cancer samples from ANCTs.

	AUC	Sensitivity	Specificity
IFNG	0.55	0.45	0.70
IFNG.AS001	0.65	0.80	0.50
IFNG.AS003	0.64	0.56	0.68
ac007278.2	0.71	0.51	0.85
ac007278.3	0.82	0.69	0.85
IL18R1	0.67	0.61	0.72
All	0.95	0.86	0.97

5. Conclusion

The current study accentuates the role of *IFNG*-associated genes in the pathogenesis of breast cancer. These transcripts can serve as markers for distinguishing between breast cancer samples and neighboring tissues, facilitating molecular diagnosis in this kind of cancer.

Authors statement

MT and SGF wrote the draft and revised it. AS, AAH and BMH performed the experiment. MDO analyzed the data. AZ performed the bioinformatic analysis and designed the picture. All the authors are contributed equally and approved the submitted version.

Declaration of competing interest

The authors declare they have no conflict of interest.

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