High Serum Levels of Interleukin-18 Are Associated With Worse Outcomes in Patients With Breast Cancer

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Abstract. *Background/Aim:* Interleukin (IL)-18, which belongs to the IL-1 superfamily of cytokines, is a known interferon-gamma (IFN- γ)-inducing factor. Since IFN- γ plays an essential role in anticancer immunity mediated through cytotoxic T cells, IL-18 may also contribute to the function of immunosurveillance. The aim of the study was to examine the association of IL-18 with the outcomes of patients with breast cancer. *Patients and Methods:* Serum IL-18 levels were determined at baseline in 270 patients operated for breast cancer, and the relapse-free survival (RFS) was compared between IL-18-high and -low groups. The relationships between IL-18 and tumor-infiltrating lymphocytes (TILs) or the neutrophil-to-lymphocyte ratio (NLR) were also investigated. *Results:* The RFS of patients was significantly better in the IL-18-low group than in the IL-18-high group ($p=0.032$). According to the multivariate analysis, IL-18 was a significant and independent predictive factor for RFS (hazard ratio(HR)=0.336; 95% confidence interval(CI)=0.147-0.727; $p=0.0053$). No association was observed between the IL-18 levels and TILs or NLRs. *Conclusion:* IL-18 levels may be useful for predicting the prognosis of patients who have received surgical treatment for breast cancer.

Interleukin (IL)-18, which belongs to the IL-1 superfamily of cytokines, was identified by Okamura *et al.* (1, 2) as an interferon-gamma (IFN- γ)-inducing factor. IL-18 is known

to act on both Th1 and Th2 inflammatory responses, eliciting various reactions, such as anticancer immunity, defense against intracellular microbial infections, and autoimmune diseases (3). The inflammasome, which is a multimolecular complex, plays an essential role in the innate immune system that includes IL-1 β and IL-18 (4). When the enzyme caspase-1 is activated, it processes pro-IL-1 β and pro-IL-18 proteins into active forms following induction by nuclear factor-kappa B (NF- κ B) (5). Both IL-1 β and IL-18 are inflammatory cytokines involved in signaling pathways, including the NF- κ B, mitogen-activated protein kinase (MAPK), and phosphoinositide 3-kinase (PI3K) pathways (6). These inflammatory reactions are not only essential for immune reactions toward various pathogens but also have a potential influence on tumor progression. Given that IL-18 functions in intestinal tissue remodeling, intestinal inflammation, and gut microbiota, its deficiency could lead to intestinal epithelial remodeling, resulting in tumorigenesis of inflammation-associated colon cancer (7). The studies indicating that IL-18-deficient mice have increased tumorigenesis and that IL-18 induction inhibited tumor progression in colitis-associated cancer (8, 9) suggest a protective effect of IL-18 against inflammation-associated colon cancer.

Contrary to the situation in inflammation-associated colon cancer, inflammasomes have been reported to promote the initiation and progression of various tumors, including skin cancer, breast cancer, lymphoma, and hepatocellular carcinoma (3, 10, 11). It has been reported that the inflammatory cytokine IL-1 β and its downstream cytokines are upregulated in melanomas, where the elevated IL-1 β levels promoted melanoma cell growth, invasion, and metastasis by autocrine and paracrine mechanisms (12). Immunity against breast cancers is critical in terms of prognosis during the treatment course of afflicted patients, and IL-18 may play an important role in the growth and

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Key Words: Breast cancer, IL18, prognosis, cytokine, cancer immunity.

progression of cancer in these patients. However, this issue has yet to be well disclosed.

Tumor-infiltrating lymphocytes (TILs) are well established as a predictive indicator of chemotherapy efficacy in patients with breast cancer, where those with high TIL levels generally have a significantly better prognosis. In a meta-analysis of 23 studies, breast cancers with high TIL levels were significantly associated with an improved pathologic complete response (odds ratio=2.81; $p<0.001$) (13). In addition, the neutrophil-to-lymphocyte ratio (NLR) is also a known immune-related peripheral blood marker for the prognosis of patients with breast cancer. According to a meta-analysis of 15 studies, high NLR is significantly associated with a worse disease-free survival (DFS) (hazard ratio (HR)=1.74; 95% confidence interval (CI)=1.47-2.07; $p<0.001$) and overall survival (OS) (HR=2.56; 95%CI=1.96-3.35; $p<0.001$) (14).

Since serum IL-18 levels did not correlate with either TIL levels or the NLRs, the serum levels of this cytokine are unlikely to influence prognosis mediated by the cytotoxic T-cell-induced immunity evaluable by TILs or NLRs and may instead modulate the prognosis through other mechanisms.

Patients and Methods

Patient recruitment and blood sampling. This retrospective study was conducted on patients who received surgical treatment for breast cancer at the Hyogo College of Medicine Hospital between November 2009 and November 2016. We recruited 270 patients who had been consecutively diagnosed pathologically with invasive breast cancer and whose sera were available for this study. The blood samples were obtained during the surgical procedure, except in the case of 36 patients treated with neoadjuvant chemotherapy (NAC) whose blood samples were taken before the start of the preoperative treatment. Adjuvant chemotherapies were administered to 68 patients.

Recurrence at any site, ipsilateral breast cancer, and death due to any reason occurred in 30 patients during the 37.1-month median follow-up period (range=1.0-94.4 months). We defined relapse-free survival (RFS) as the time from the operation to the first recurrence at any site, ipsilateral breast cancer, or death for any reason. This study was approved by the ethics committee of the Hyogo College of Medicine (Approval No. 106) and was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from each patient.

Determination of serum IL-18 levels. Serum levels of IL-18 were measured using the Enzyme-Linked Immuno Sorbent Assay Kit (Code No. 7620, Medical & Biological Laboratories Co. Ltd., Nagoya, Japan), according to the manufacturer's instructions. The assay uses two monoclonal antibodies against two different epitopes of human IL-18. After serum samples were diluted with assay diluent (1:4) and 150 μ l of each sample and standards were added to the 96-well polyvinyl plate precoated with the anti-human IL-18 monoclonal antibody and incubated 60 min at room temperature. After washing with the wash solution provided in the kit, a peroxidase-conjugated anti-human IL-18 monoclonal antibody was

Figure 1. Receiver operating characteristic curve for IL-18 for relapse-free survival. The cut-off value was determined to be 142.29 pg/ml (area under the curve=0.581; $p=0.0992$).

added to the microwells and the plate was incubated for 60 min at room temperature. After another wash with the provided wash solution, the peroxidase substrate mixed with a chromogen was added to the cells and incubation was carried out for 30 min at room temperature. An acid solution was then added to each well to terminate the enzyme reaction and to stabilize the developed color. The optical density of each well was measured at 450 nm using an ELISA reader (Model 680, Bio-Red, Hercules, CA, USA). The concentration of human IL-18 was calibrated from a dose-response curve based on reference standards. The sensitivity of this assay was 12.5 pg/ml.

Evaluation of tumor-infiltrating lymphocytes and neutrophil-to-lymphocyte ratios. TIL levels in breast cancer tissue samples obtained during the operation or from preoperative biopsy samples (from the patients on NAC) were determined according to a previously described method (15). The percentage of areas occupied by TILs within the tumor was evaluated and classified as either low (<10%) or high ($\geq 10\%$). NLRs were calculated as the ratio of neutrophil count to lymphocyte count in peripheral blood, adjusted to the collection timing for IL-18 measurement. We used the cut-off NLR value of 2.72 obtained in a previous study (16).

Public database. The RFS of patients with high ($n=1972$) and low ($n=1979$) IL-18 gene expression levels (Affymetrix ID, 206295_at) divided by the median value was obtained from the Kaplan-Meier Plotter database (<http://kmplot.com/analysis/>) (17). The co-expression of the IL-18 gene with the *INF- γ* , cluster of differentiation 4 (*CD4*), or *CD8A* genes, as calculated by Spearman's correlation analysis, was obtained from the cBioPortal website (<http://www.cbioportal.org/>) (18, 19) for 1084 invasive breast carcinoma samples (The Cancer Genome Atlas, Pan-Cancer Atlas).

Table I. Relationship between serum interleukin-18 levels and clinicopathological characteristics in patients with breast cancers.

	n	IL18 high ^a	IL18 low ^a	p-Value
Menopausal status ^b				
Pre-	93	37 (39.8%)	56 (60.2%)	0.040
Post-	176	94 (53.4%)	82 (46.6%)	
Tumor size ^c				
≤2 cm	180	84 (46.7%)	96 (53.3%)	0.517
>2 cm	89	46 (51.7%)	43 (48.3%)	
Lymph node metastasis ^d				
Negative	193	94 (48.7%)	99 (51.3%)	1.000
Positive	73	36 (49.3%)	37 (50.7%)	
Tumor grade ^e				
1	155	80 (51.6%)	75 (48.4%)	0.300
2 and 3	97	43 (44.3%)	54 (55.7%)	
Estrogen receptor status				
Positive	216	105 (48.6%)	111 (51.4%)	1.000
Negative	54	26 (48.2%)	28 (51.8%)	
Progesterone receptor status ^f				
Positive	170	82 (48.2%)	88 (51.8%)	0.892
Negative	80	40 (50.0%)	40 (50.0%)	
HER2 status				
Negative	232	112 (48.3%)	120 (51.7%)	0.863
Positive	38	19 (50.0%)	19 (50.0%)	
Ki67 expression levels ^g				
Low	158	85 (53.8%)	73 (46.2%)	0.043
High	104	42 (40.4%)	62 (59.6%)	
Chemotherapy administration				
No	202	98 (48.5%)	104 (51.5%)	1.000
Yes	68	33 (48.5%)	35 (51.5%)	

^aInterleukin-18, high: ≥142.29 pg/ml, low: <142.29 pg/ml; ^bOne male patient was excluded; ^cOne tumor was undetermined; ^dLymph node examination was not performed in three patients; ^eTwenty tumors were unknown; ^fTwenty-two tumors were undetermined; ^gKi67 low: <25%, high: ≥25%, four tumors were undetermined.

Ethics approval and consent to participate. This study was approved by the ethics committee of the Hyogo College of Medicine (Approval No. 106) and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from each patient.

Statistical analysis. The differences in clinicopathological factors between patients with high or low serum levels of IL-18 were calculated by Fisher's exact test or the Wilcoxon rank-sum test. The DFS of patients in the different subgroups was compared using Kaplan–Meier plots and log-rank tests. HRs and 95% CIs for univariate and multivariate analyses were calculated using a Cox proportional-hazards model for RFS. Serum levels of IL-18 were compared among subgroups according to TIL levels or NLR values, using the Wilcoxon rank-sum test. Statistical significance was set at $p < 0.05$. All statistical calculations were performed using JMP Pro 13 software (SAS Institute Inc., Cary, NC, USA).

Results

Determination of the IL-18 cut-off value for RFS. The IL-18 cut-off value for RFS was determined to be 142.29 pg/ml according to the receiver operating characteristic curve, which was

calculated using the Youden index of 0.581 for the area under the curve as shown in Figure 1 ($p = 0.0992$). On the basis of this cut-off value, patients were divided into IL-18-high (≥142.29 pg/ml, $n = 131$) and IL-18-low groups (<142.29 pg/ml, $n = 139$).

Clinicopathological characteristics of serum IL-18-high and IL-18-low groups. The relationships between IL-18 levels and clinicopathological characteristics are shown in Table I. Significantly more patients with high IL-18 levels were postmenopausal (53.4%) as opposed to premenopausal (39.8%) ($p = 0.040$). In addition, in the IL-18-high group, there were significantly more breast cancers with low levels of Ki67 protein (53.8%) than with high levels (40.4%) ($p = 0.043$). However, there was no significant association between IL-18 levels and tumor size, lymph node metastasis, tumor grade, estrogen receptor status, progesterone receptor status, human epidermal growth factor receptor 2 (HER2) status, or chemotherapy administration.

Serum IL-18 levels and patient outcomes. RFS of patients with low IL-18 ($n = 139$; 5-year RFS, 0.92) was significantly longer

Figure 2. (A) Relapse-free survival (A) and (B) overall survival (B) of patients with high (n=131) and low (n=139) levels of IL-18.

Figure 3. Subgroup analysis of relapse-free survival according to IL-18 levels. Hazard ratios and 95% confidence intervals (CIs) in each subgroup are shown. The hazard ratio (0.445) and (95%CI=0.199-0.929) of all patients is indicated with a dashed line.

Table II. Univariate and multivariate analyses of the relapse-free survival of all patients.

	n	Univariate		Multivariate	
		HR (95%CI) ^a	p-Value	HR (95%CI) ^a	p-Value
Menopausal status					
Pre-	93	1.000	0.114		
Post-	176	1.911 (0.863-4.819)			
Tumor size					
≤2 cm	180	1.000	0.081		
>2 cm	89	1.938 (0.919-3.989)			
Lymph node metastasis					
Negative	193	1.000	0.0006	1.000	0.0013
Positive	73	3.689 (1.773-7.820)		3.478 (1.637-7.539)	
Tumor grade					
1	155	1.000	0.025	1.000	0.233
2 and 3	97	2.281 (1.108-4.744)		1.783 (0.697-4.815)	
Estrogen receptor status					
Positive	216	1.000	0.141		
Negative	54	1.850 (0.804-3.921)			
Progesterone receptor status					
Positive	170	1.000	0.063		
Negative	80	1.997 (0.961-4.101)			
HER2 status					
Negative	232	1.000	0.055		
Positive	38	2.348 (0.979-5.075)			
Ki67 expression level ^b					
Low	158	1.000	0.0085	1.000	0.154
High	104	2.637 (1.281-5.631)		2.087 (0.758-5.683)	
Chemotherapy administration					
No	202	1.000	0.687		
Yes	68	1.184 (0.494-2.558)			
Serum IL18 level ^c					
High	131	1.000	0.031	1.000	0.0053
Low	139	0.445 (0.199-0.929)		0.336 (0.147-0.727)	

^aHazard ratio (95% confidence interval); ^bKi67 low: <25%, high: ≥25%; ^cInterleukin-18, high: ≥142.29 pg/ml, low: <142.29 pg/ml.

than that of patients with high IL-18 levels (n=131; 5-year RFS, 0.80; $p=0.032$) (Figure 2A). There was no significant difference in OS between the two patient groups with respect to IL-18 levels ($p=0.261$; Figure 2B). In the subgroup analysis, patients in the IL-18-low group had a consistently better RFS in all the subgroups; namely, tumor size, lymph node metastasis, nuclear grade, tumor subtype, and Ki67 levels (Figure 3). The better RFS in the IL-18-low group was consistent for patients in both the high and low groups of TILs or NLR.

Univariate and multivariate analyses of prognostic factors for relapse-free survival. RFS was significantly associated with lymph node metastasis ($p=0.0006$), tumor grade ($p=0.025$), Ki67 levels ($p=0.0085$), and serum IL-18 levels ($p=0.031$) according to the univariate analysis (Table II). According to the multivariate analysis, lymph node metastasis positivity (HR=3.478; 95%CI=1.637-7.539; $p=0.0013$) and low serum IL-18 levels (HR=0.336;

95%CI=0.147-0.727; $p=0.0053$) were significant and independent prognostic factors for RFS. With regard to OS, lymph node metastasis positivity was exclusively the significant prognostic factor (HR=11.686; 95%CI=2.923-77.491; $p=0.0003$) (Table III).

Correlations between serum IL-18 levels and TIL levels or NLRs. Serum levels of IL-18 were not significantly different ($p=0.34$) between the TIL-low (n=154; median, 132.48 pg/ml; range=14.68-3119.08 pg/ml) and TIL-high groups (n=73; median, 150.23 pg/ml; range=11.82-771.43 pg/ml) (Figure 4A). Similarly, there was no significant difference ($p=0.44$) in IL-18 levels between the NLR-high (n=81; median, 134.66 pg/ml; range=11.82-1364.95 pg/ml) and NLR-low groups (n=169; median, 134.88 pg/ml; range=3.23-3119.08 pg/ml) (Figure 4B).

Relapse-free survival according to IL-18 gene expression levels and co-expression between IL-18 and other genes in the

Table III. Univariate analysis of the overall survival of all patients.

	n	HR (95%CI) ^a	p-Value
Tumor size			
≤2 cm	180	1.000	0.471
>2 cm	89	1.596 (0.416-5.343)	
Lymph node metastasis			
Negative	193	1.000	0.0003
Positive	73	11.686 (2.923-77.491)	
Tumor grade			
1	155	1.000	0.457
2 and 3	97	1.578 (0.454-5.252)	
Estrogen receptor status			
Positive	216	1.000	0.211
Negative	54	2.282 (0.596-7.577)	
Progesterone receptor status			
Positive	170	1.000	0.118
Negative	80	2.588 (0.779-8.988)	
HER2 status			
Negative	232	1.000	0.111
Positive	38	2.938 (0.761-9.857)	
Ki67 expression levels ^b			
Low	158	1.000	0.224
High	104	2.092 (0.629-7.273)	
Chemotherapy administration			
No	202	1.000	0.873
Yes	68	1.116 (0.244-3.869)	
Serum IL18 level ^c			
High	131	1.000	0.260
Low	139	0.499 (0.130-1.663)	

^aHazard ratio (95% confidence interval); ^bKi67 low: <25%, high: ≥25%;

^cInterleukin-18, high: ≥142.29 pg/ml, low: <142.29 pg/ml.

public dataset. The public mRNA expression dataset of breast cancers was used for correlating the levels of *IL-18* gene expression with RFS. The RFS of patients with high levels of *IL-18* gene expression was significantly better than that of patients with low levels of expression of the gene (HR=0.8; 95%CI=0.71-0.89; $p<0.001$) (Figure 5). Next, data on the co-expression of several other genes with *IL-18* were obtained, focusing on genes related to immune reactions. As shown in Figure 6, the expression of the CD4 (Spearman's correlation, 0.641; Figure 6A), CD8A (Spearman's correlation, 0.514; Figure 6B), and *IFN-γ* (Spearman's correlation, 0.509; Figure 6C) genes correlated significantly with that of *IL-18*.

Discussion

In the present study, we demonstrated that patients with a high serum levels of IL-18 had a shorter RFS than those with a low IL-18 level. It has been reported that serum IL-18 levels in 44 patients with breast cancer (831.5±73.5 pg/ml) were significantly higher than those in 15 healthy individuals (157.1±27.5 pg/ml) ($p<0.001$) (20). Eissa *et al.* (21) have reported that serum IL-18 levels in patients with metastatic

disease (441.6±193 pg/ml) were significantly higher than in those without cancer metastasis (318.6±133 pg/ml) or in non-cancerous controls (253±106 pg/ml) ($p<0.001$). Similarly, in another cohort of patients with breast cancer, serum IL-18 levels were significantly higher in patients with metastatic disease (n=38; 390.3±20 pg/ml) than in those with non-metastatic disease (n=26; 235.8±53 pg/ml; $p<0.001$) or in healthy controls (n=16; 188.6±66 pg/ml; $p<0.001$) (22). In accordance with our results, another study has shown that high serum IL-18 levels were significantly associated with shorter RFS and OS, except in the hormone receptor (HR)-positive/HER2-negative subtype (23). These data seem to be in line with the hypothesis that IL-18 contributes to the promotion or progression of breast cancers. Although there was no significant association between RFS and IL-18 levels in the HR⁺/HER2⁻ subtype in the study by Park *et al.* (23), our data indicated that the association was consistent irrespective of subtypes. Although the exact reason for these different results is currently unknown, the different cut-off values used in the two studies (352.9 pg/ml for the Park study vs. 142.29 pg/ml for our study) may partly explain the discrepancy. In the study reported by Metwally *et al.* (20), serum IL-18 levels were significantly higher in tumors with lymph node metastasis positivity or estrogen receptor negativity. Since our data demonstrated that serum IL-18 levels were a significant and independent prognostic factor for RFS among the other prognostic factors (including lymph node metastasis, tumor grade, and Ki67 expression levels), we surmise that the prognostic significance of IL-18 was not mediated through these biological factors.

Originally identified as an inducer of IFN-γ, IL-18 plays a role in Th1 inflammation synergistically with IL-12, thus resulting in the activation of both cytotoxic T lymphocytes and natural killer (NK) cells that produce IFN-γ (1). Consistently, in the public database shown in the present study, patients with breast cancers and high expression levels of *IL-18* gene had a better RFS than those with low levels of *IL-18* expression. The expression levels of *IL-18* were significantly associated with the *IFN-γ*, *CD4*, or *CD8A* gene expression levels. Thus, the levels of *IL-18* gene expression and serum levels of IL-18 were both inversely associated with the prognosis of patients with breast cancer. Since serum IL-18 levels correlated with neither TIL levels nor the NLRs, serum levels of IL-18 are unlikely to influence the prognosis by cytotoxic T cells evaluable by TILs or NLRs and may instead modulate prognosis through other mechanisms.

In spite of the tumor suppressive effects induced by the immune reaction, it has been reported that tumor-derived IL-18 increased immunosuppression (24). In that model using melanoma and colon carcinoma cells, tumor-derived or exogenous circulating IL-18 suppressed the immunosurveillance role of NK cells in a programmed cell death protein-1 (PD-1)-dependent manner and enhanced metastases. In contrast,

Figure 4. Serum levels of IL-18 in the tumor-infiltrating lymphocyte (TIL)-low ($n=154$) and TIL-high groups ($n=73$) (A), and in the neutrophil-to-lymphocyte ratio (NLR)-high ($n=81$) and NLR-low groups ($n=169$) (B).

Figure 5. Relapse-free survival of patients according to high ($n=1,972$) and low ($n=1,979$) IL-18 gene expression levels in the public dataset. The cut-off value was set to the median value.

A CD4 gene expression

B CD8A gene expression

C IFN-γ gene expression

depletion of IL-18 sufficiently stimulated NK cell-dependent immunosurveillance. The immunosuppressive effects of IL-18 on NK cells through PD-1 upregulation have also been demonstrated in triple-negative breast cancer (23). Since immunosuppressive and PD-1-positive fractions of NK cells were expanded by IL-18 in that study, the unfavorable prognosis of patients with high levels of IL-18 would seem to be mediated, at least in part, by such an immunosuppressive effect induced by NK cells. Although IL-18 has been shown to elicit TILs in hepatocellular carcinoma, high levels of circulating IL-18 were correlated with a poor prognosis (11). In that study, the authors indicated differences in the expression of IL-18 in tumor and non-tumor tissues. IL-18 is produced not only by tumor cells but by peritumoral stromal cells as well. In addition, mesenchymal stem cells have been reported to express IL-18 *in vitro* as well as in a mouse model (25, 26). Thus, both the immune-activating and immunosuppressive events induced by IL-18 seem to be involved in the progression of breast cancer.

Some reports have demonstrated the relationship between IL-18 and the sensitivity to antitumor treatments. By screening doxorubicin resistance factors in MCF-7 parental cells and doxorubicin-resistant cells, Yao *et al.* (27) have identified 89 differentially expressed proteins, where increased expression of IL-18 was confirmed in the doxorubicin-resistant cell lines and breast cancer tissues. Serum levels of IL-18 were found increased in patients with metastatic triple-negative breast cancer who had been administered chemotherapy (23). Furthermore, serum IL-18 levels were significantly decreased after tamoxifen treatment in patients with breast cancer (28). These data indicate a possible role of IL-18 in the efficacy of breast cancer treatments, although this issue has yet to be well disclosed.

Figure 6. Correlations between IL-18 gene expression levels (log2) and (A) CD4 (log2), (B) CD8A (log2), and (C) IFN-γ (log2) expression levels.

In conclusion, our results demonstrated that high levels of serum IL-18 serve as an indicator of poor prognosis in patients with surgically treated breast cancer. Since, IL-18 is associated with neither TILs nor NLR, the cytokine may be involved in cancer immunity by mechanisms not mediated by TILs or the NLR. Nonetheless, serum levels of IL-18 may be useful for predicting the prognosis of patients with surgically treated breast cancer.

Conflicts of Interest

The Authors declare that they have no competing interests regarding this study.

Authors' Contributions

NI and WL analyzed the C-C motif chemokine ligand 5 (CCL5) expression in the tumors. YF was involved in the data collection. YM and TK analyzed the public database. HO and YM designed and supervised the study. NI, YF, and YM analyzed the data and prepared the manuscript. All Authors read and approved the final manuscript.

Acknowledgements

The Authors thank Editage (<https://www.editage.jp>) for the English language editing. This study was supported by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (No. 15K10077).

References

- Okamura H, Kashiwamura S, Tsutsui H, Yoshimoto T and Nakanishi K: Regulation of interferon-gamma production by IL-12 and IL-18. *Curr Opin Immunol* 10: 259-264, 1998. PMID: 9638361.
- Nakanishi K, Yoshimoto T, Tsutsui H and Okamura H: Interleukin-18 is a unique cytokine that stimulates both Th1 and Th2 responses depending on its cytokine milieu. *Cytokine Growth Factor Rev* 12: 53-72, 2001. PMID: 11312119.
- Esmailbeig M and Ghaderi A: Interleukin-18: a regulator of cancer and autoimmune diseases. *Eur Cytokine Netw* 28: 127-140, 2017. PMID: 29478963. DOI: 10.1684/ecr.2018.0401
- Ting JP, Duncan JA and Lei Y: How the noninflammasome NLRs function in the innate immune system. *Science* 327: 286-290, 2010. PMID: 20075243. DOI: 10.1126/science.1184004
- Gross O, Yazdi AS, Thomas CJ, Masin M, Heinz LX, Guarda G, Quadroni M, Drexler SK and Tschopp J: Inflammasome activators induce interleukin-1 α secretion via distinct pathways with differential requirement for the protease function of caspase-1. *Immunity* 36: 388-400, 2012. PMID: 22444631. DOI: 10.1016/j.immuni.2012.01.018
- Mora J and Weigert A: IL-1 family cytokines in cancer immunity – a matter of life and death. *Biol Chem* 397: 1125-1134, 2016. PMID: 27410464. DOI: 10.1515/hsz-2016-0215
- Pages F, Berger A, Lebel-Binay S, Zinzindohoue F, Danel C, Piqueras B, Carriere O, Thiounn N, Cugnenc PH and Fridman WH: Proinflammatory and antitumor properties of interleukin-18 in the gastrointestinal tract. *Immunol Lett* 75: 9-14, 2000. PMID: 11163860.
- Salcedo R, Worschech A, Cardone M, Jones Y, Gyulai Z, Dai RM, Wang E, Ma W, Haines D, O'hUigin C, Marincola FM and Trinchieri G: MyD88-mediated signaling prevents development of adenocarcinomas of the colon: role of interleukin 18. *J Exp Med* 207: 1625-1636, 2010. PMID: 20624890. DOI: 10.1084/jem.20100199
- Zaki MH, Vogel P, Body-Malapel M, Lamkanfi M and Kanneganti TD: IL-18 production downstream of the Nlrp3 inflammasome confers protection against colorectal tumor formation. *J Immunol* 185: 4912-4920, 2010. PMID: 20855874. DOI: 10.4049/jimmunol.1002046
- Goto N, Tsurumi H, Kasahara S, Kanemura N, Hara T, Yasuda I, Shimizu M, Murakami N, Sawada M, Yamada T, Takemura M, Seishima M, Kito Y, Takami T and Moriawaki H: Serum interleukin-18 level is associated with the outcome of patients with diffuse large B-cell lymphoma treated with CHOP or R-CHOP regimens. *Eur J Haematol* 87: 217-227, 2011. PMID: 21575062. DOI: 10.1111/j.1600-0609.2011.01649.x
- Markowitz GJ, Yang P, Fu J, Michelotti GA, Chen R, Sui J, Yang B, Qin WH, Zhang Z, Wang FS, Diehl AM, Li QJ, Wang H and Wang XF: Inflammation-dependent IL18 signaling restricts hepatocellular carcinoma growth by enhancing the accumulation and activity of tumor-infiltrating lymphocytes. *Cancer Res* 76: 2394-2405, 2016. PMID: 26893476. DOI: 10.1158/0008-5472.CAN-15-1548
- Dunn JH, Ellis LZ and Fujita M: Inflammasomes as molecular mediators of inflammation and cancer: potential role in melanoma. *Cancer Lett* 314: 24-33, 2012. PMID: 22050907. DOI: 10.1016/j.canlet.2011.10.001
- Wang K, Xu J, Zhang T and Xue D: Tumor-infiltrating lymphocytes in breast cancer predict the response to chemotherapy and survival outcome: A meta-analysis. *Oncotarget* 7: 44288-44298, 2016. PMID: 27329588. DOI: 10.18632/oncotarget.9988
- Ethier JL, Desautels D, Templeton A, Shah PS and Amir E: Prognostic role of neutrophil-to-lymphocyte ratio in breast cancer: a systematic review and meta-analysis. *Breast Cancer Res* 19: 2, 2017. PMID: 28057046. DOI: 10.1186/s13058-016-0794-1
- Watanabe T, Hida AI, Inoue N, Imamura M, Fujimoto Y, Akazawa K, Hirota S and Miyoshi Y: Abundant tumor infiltrating lymphocytes after primary systemic chemotherapy predicts poor prognosis in estrogen receptor-positive/HER2-negative breast cancers. *Breast Cancer Res Treat* 168: 135-145, 2018. PMID: 29168063. DOI: 10.1007/s10549-017-4575-z
- Fujimoto Y, Ozawa H, Higuchi T, Miyagawa Y, Bun A, Imamura M and Miyoshi Y: Improved prognosis of low baseline neutrophil-to-lymphocyte ratio is significantly exclusive in breast cancer patients with high absolute counts of lymphocytes. *Mol Clin Oncol* 10: 275-284, 2019. PMID: 30680208. DOI: 10.3892/mco.2018.1783
- Györfy B, Lanczky A, Eklund AC, Denkert C, Budczies J, Li Q and Szallasi Z: An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. *Breast Cancer Res Treat* 123: 725-731, 2010. PMID: 27716027.
- Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y, Reva B, Goldberg AP, Sander C and Schultz N: The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov* 2: 401-404, 2012. PMID: 22588877. DOI: 10.1158/2159-8290.CD-12-0095
- Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Singa R, Larsson E, Cerami E, Sander C and Schultz N: Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal* 6: pii, 2013. PMID: 23550210. DOI: 10.1126/scisignal.2004088
- Metwally FM, El-mezayen HA and Ahmed HH: Significance of vascular endothelial growth factor, interleukin-18 and nitric oxide in patients with breast cancer: correlation with carbohydrate antigen 15.3. *Med Oncol* 28: S15-21, 2011. PMID: 20725807. DOI: 10.1007/s12032-010-9657-2
- Eissa SA, Zaki SA, El-Maghraby SM and Kadry DY: Importance of serum IL-18 and RANTES as markers for breast carcinoma progression. *J Egypt Natl Canc Inst* 17: 51-55, 2005. PMID: 16353083.

- 22 Günel N, Coşkun U, Sancak B, Günel U, Hasdemir O and Bozkurt S: Clinical importance of serum interleukin-18 and nitric oxide activities in breast carcinoma patients. *Cancer* 95: 663-667, 2002. PMID: 12209760.
- 23 Park IH, Yang HN, Lee KJ, Kim TS, Lee ES, Jung SY, Kwon Y and Kong SY: Tumor-derived IL-18 induces PD-1 expression on immunosuppressive NK cells in triple-negative breast cancer. *Oncotarget* 8: 32722-32730, 2017. PMID: 28415798. DOI: 10.18632/oncotarget.16281
- 24 Terme M, Ullrich E, Aymeric L, Meinhardt K, Desbois M, Delahaye N, Viaud S, Ryffel B, Yagita H, Kaplanski G, Prévost-Blondel A, Kato M, Schultze JL, Tartour E, Kroemer G, Chaput N and Zitvogel L: IL-18 induces PD-1-dependent immunosuppression in cancer. *Cancer Res* 71: 5393-5399, 2011. PMID: 21724589. DOI: 10.1158/0008-5472.CAN-11-0993
- 25 Liu X, Hu J, Sun S, Li F, Cao W, Wang YU, Ma Z and Yu Z: Mesenchymal stem cells expressing interleukin-18 suppress breast cancer cells *in vitro*. *Exp Ther Med* 9: 1192-1200, 2015. PMID: 25780408.
- 26 Liu X, Hu J, Li Y, Cao W, Wang Y, Ma Z and Li F: Mesenchymal stem cells expressing interleukin-18 inhibit breast cancer in a mouse model. *Oncol Lett* 15: 6265-6274, 2018. PMID: 29725393. DOI: 10.3892/ol.2018.8166
- 27 Yao L, Zhang Y, Chen K, Hu X and Xu LX: Discovery of IL-18 as a novel secreted protein contributing to doxorubicin resistance by comparative secretome analysis of MCF-7 and MCF-7/Dox. *PLoS One* 6: e24684, 2011. PMID: 21931812. DOI: 10.1371/journal.pone.0024684
- 28 Coskun U, Gunel N, Sancak B, Onuk E, Bayram M and Cihan A: Effect of tamoxifen on serum IL-18, vascular endothelial growth factor and nitric oxide activities in breast carcinoma patients. *Clin Exp Immunol* 137: 546-551, 2004. PMID: 1532 0904.

Received June 28, 2019

Revised July 13, 2019

Accepted July 15, 2019