Journal of Advanced Pharmaceutical Sciences
Journal homepage: https://japs.journals.ekb.eg/

Research article

The antiangiogenic activity of anti-IL-6 and anti-IL-8 in the tumor microenvironment of breast cancer (preclinical study)

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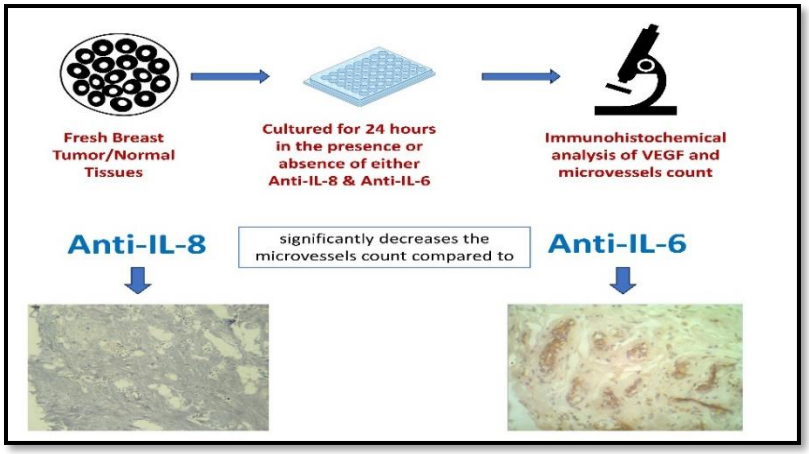
Abstract:



The tumor microenvironment of breast cancer is considered a vibrant, multifactorial milieu that contains different cell subsets that shape the tumor fate. In addition, cytokines play a vital role within the tumor microenvironment where different cytokines can promote inflammation, angiogenesis, and immune suppression. Interleukin-8 (IL-8) and Interleukin-6 (IL-6) are two of the most important cytokines that aid tumor progression and metastasis. Thus, this study aimed to compare the anti-angiogenic actions of neutralizing antibodies against IL-8 and IL-6. Tumor tissues from 30 patients having mastectomy supplemented with either monoclonal neutralizing antibodies against IL-8 or IL-6 were used to evaluate their antiangiogenic effect by evaluating (Vascular endothelial growth Factor) VEGF expression and microvessels count. It was demonstrated that Anti-IL-8 mAb declined significantly the number of microvessels in the tumor tissues compared to Anti-IL-6 and the control tissue cultures $p<0.0001$. In addition, the decrease in the microvessels count was insignificant within the untreated normal tissues ($p=0.46$). Therefore, the antiangiogenic activity of Anti-IL-8 monoclonal antibody is more potent than Anti-IL-6 according to the study conditions.

Keywords: Breast cancer, Angiogenesis, IL-8, IL-6, VEGF

Received: 16-5-2024 Accepted: 5-9-2024 Published: 6-12-2024

ISSN: 3009-7061 **DOI: 10.21608/japs.2024.287068.1025**

1. Introduction

Breast cancer is the most common malignancy worldwide in women [(1)]. Although it possesses a good prognosis in most cases, once it acquires metastatic properties the prognosis decreases significantly [(2)]. The breast cancer tumor microenvironment is a heterogenous environment that consists of different nonmalignant cells in addition to the cancer cells including fibroblasts, endothelial cells, and different immune cells, in addition to cytokines including interleukin-8 (IL-8), interleukin-6 (IL-6), interleukin-10 (IL-10), and tumor growth factor- β (TGF-β) that affect tumor immune surveillance, tumor invasion, metastasis, and therapy resistance [(3)].

Angiogenesis is a fundamental process in cancer progression, consisting of the formation of neovasculature from existing blood vessels [(4)]. Angiogenesis is a key step for tumor survival and metastasis. The development of the angiogenic blood micro-vessels supplies cancer cells with the required nutrients and metabolites for extensive tumor proliferation, progression, and expansion [(5)]. Different angiogenic stimuli including hypoxia, presence of reactive oxygen species (ROS), low pH, and nutrient deprivation initiate an angiogenic signaling cascade by inducing the secretion of proteases, heparinase, and other metalloproteinases such as matrix metalloproteinase 9 (mmp9) resulting in the dissolution of the extracellular matrix, proangiogenic factors release and the formation of new blood vessels [(6)].

Interlukin-8 (IL-8) is one of the most common proangiogenic factors studied. It is a pleiotropic cytokine that has a vital role in chemotaxis during the immune response, in addition, it was demonstrated that it has a tumor-promoting effect as it recruits myeloid-derived suppressor cells to the tumor microenvironment in addition to its pro-angiogenic properties [(6)]. IL-8 was demonstrated to promote the effect of vascular endothelial growth factor (VEGF) within the tumor microenvironment thus promoting angiogenesis and neovascularization. [(7)].

Interleukin-6 is a multifunctional cytokine that is vital in many processes such as inflammation, hematopoiesis, and immune response to infections. In addition, high serum levels of IL-6 have been associated with various cancer types such as lymphoma [(8)], prostate cancer [(9)], ovarian cancer [(10)], and breast cancer [(11)]. Moreover, elevated levels of IL-6 in breast cancer patients were reported to be associated with metastasis, invasion, and poor prognosis [(11)]. The angiogenic activity of IL-6 is thought to be due to its ability to induce the expression of the pro-angiogenic VEGF in many cell types including tumor cells [(12)]. Moreover, VEGF and its receptor are found to be highly expressed in the human epidermal growth factor-2 (HER-2) positive subtype compared to other breast cancer subtypes, highlighting the role of angiogenesis in the invasiveness of the HER-2 positive breast cancer and the potential efficacy of the antiangiogenic therapies [(13)].

Although angiogenesis inhibitors, including VEGF- monoclonal antibodies, tyrosine kinase inhibitors, and other types of VEGF pathway inhibitors, showed promising results in different types of cancer, they were insufficient for increasing the overall survival of breast cancer patients thus [(14)].

Bevacizumab is the first humanized anti-VEGF-F monoclonal antibody approved by the FDA. It was demonstrated to inhibit angiogenesis and limit tumor invasion and metastasis successfully. It was approved for the management of advanced colorectal cancer and lung cancer as an adjuvant therapy [(15)]. Moreover, it was studied for its angiogenic effect for use in metastatic breast cancer. However, its efficacy as a

ISSN: 3009-7061 **DOI: 10.21608/japs.2024.287068.1025**

neoadjuvant therapy is still controversial. Different studies failed to provide a significant effect of bevacizumab on the overall survival of breast cancer patients [16], shedding light on the importance of evaluating other non-VEGF antiangiogenic therapies. Thus, this work aimed to assess the anti-angiogenic properties of anti-IL-8 and anti-IL6 in breast cancer tumor microenvironment. Hopefully, it could be considered a promising anti-tumor strategy against breast cancer.

2. Subjects & methods

2.1.Subjects

Thirty Egyptian women scheduled for modified radical mastectomy for histologically proven breast cancer were recruited from the Department of Surgery, Medical Research Institute, Alexandria University. The current protocol was approved by the medical research institute ethical committee (5/2011) and confines the provisions of the declaration of Helsinki. Each patient signed an approval consent for their enrollment in the current study.

Patients underwent comprehensive clinical examination and a complete history taking, with particular attention to the disease's stage and lymph node involvement.

Tissue culture strategy

Following surgical excision, each patient's breast tumor was collected to get fresh tissue. Every tumor sample was split into two sections: one section was used for immunohistochemical examination for ER and PR, H&E staining, and the other portion was used for tissue slicing and culture. 0.2 cm thick tissue slices were prepared from each sample and then cultured in a 96-well culture plate with complete RMPI media alone or with either 1µg /ml anti-IL-8 neutralizing mab (Invitrogen USA Catalog #**MA5-23697)** or 1µg /ml anti-IL-6 neutralizing mab (eBioscience™.USA Catalog #**16-7069-81)**

Breast normal tissues were collected from the same excised breast and cultured as well with complete RMPI media alone or with either 1µg/ml anti-IL-8 neutralizing mab (Invitrogen USA Catalog # **MA5-23697)** or 1µg/ml anti-IL-6 neutralizing mab (eBioscience™.USA Catalog # **16-7069-81)**

Afterward, the culture plate was incubated for 24 hours at 37 degrees Celsius in a continuous environment of 5% CO2. Following the time of incubation, the tumor and normal tissues were preserved for 12 to 24 hours in 10% phosphate-buffered formalin (PH 7.4) and then processed to create microscopic slides [17]. According to the manufacturer's instructions, the Hematoxylin and Eosin stain (H&E) were applied to one slide [18].

2.2.Assessment of angiogenesis

Immunohistochemical staining of VEGF was performed using a rabbit polyclonal IgG ProSci-INCORPORATED using a labeled streptavidin-biotin immunoenzymatic antigen detection system, (UltraVision Detection System, Anti-Polyvalent, HRP/DAB) according to the manufacturer's manual.

After deparaffinizing and rehydrating the tissues, they were incubated in a 3% hydrogen peroxide block for fifteen minutes to minimize any non-specific background staining. Afterward, slides were placed in sodium citrate buffer 0.01M, PH: 6.0 for antigen retrieval, and heated for three minutes at 100°C. Followed by washing with phosphate buffer saline (PBS). After that, slides were incubated for 30 minutes in PBS diluted at 1:15 with normal goat serum. Diluted Rabbit Polyclonal Antibody VEGFA (5µg/ml) was then applied to the sections, and they were left to incubate for a whole night at 4°C in a humid environment. After that, biotinylated goat anti-polyvalent antibodies were added and incubated at room temperature for 10 minutes. Next, streptavidin peroxidase was added and incubated at room temperature for 10 minutes.

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Forty µl DAB Plus Chromogen was added to 2 ml of DAB Plus Substrate. Then 15 µl of the mixture was applied to tissues and incubated for 10 minutes. After counterstaining the tissue slices with Mayer's hematoxylin, they were covered with a permanent mounting medium [19].

Examination of the slides was done using low power magnification to detect (the hot spots); then counting microvessel count in 10 high power fields, and assessing the average count. The field area was equal to 0.74 mm^2 so calculating the average count/mm^2 was done using Weinder et al equation [20].

$$\text{MVC in mm}^2 = \frac{\text{average count of microvessels x 100}}{74}$$

2.3.Statistical analysis

Statistical analysis was done using the GraphPad Prism 8.0.2 Package. The descriptive analysis of samples included the assessment of Mean, median, and standard deviation. ANOVA test was used to test the significance of variance between sample means in the different tested groups. Tukey's post hoc test was used to compare the means of the different groups. The findings were deemed statistically significant at the 5% level ($p \leq 0.05$).

3. Results

3.1.Clinicopathological parameters

Patients' clinicopathological parameters are summarized in **Table 1**.

3.2. Histopathological examination (H&E)

Normal breast tissue H&E slides microscopic examination revealed the presence of different areas of fibrosis, apocrine metaplasia, and epithelioid regions with diffused adenosis associated with lymphocytic infiltration.

Anti-IL-8 treated normal tissue cultures investigation showed focal areas of aggregated acini and ductules compressed by the surrounding collagenic stroma with a minimal observation of visible blood vessels.

Table 1: Patients' clinicopathological data

Clinicopathological Parameter	No=30	%
Age		
<50	10	33.3
≥50	20	66.7
Min. - Max.	32.0 – 77.0	
Mean ± SD	52.53 ± 10.73	
Median	53.50	
Histopathologic type		
Invasive Ductal Carcinoma	28	93.3
Mucinious carcinoma	1	3.3
Invasive lobular carcinoma	1	3.3
Histologic grade		
IDC		
Grade I	1	3.3
Grade II	23	76.7
Grade III	5	16.7
ILC low grade	1	3.3
Pathological stage		
Stage I	1	3.3
Stage II	15	50.0
Stage III	14	46.7
Vascular invasion		
Positive vascular invasion	25	75
negative vascular invasion	5	25
Lymph node involvement		
NX Lymph node cannot be assessed	1	3.33
N0 Negative	7	23.33
N1 Positive	10	33.33
N2	8	26.67
N3	4	13.33
Tumor size		
T1	1	3.3
T2	22	73.3
T3	6	20.0
T4	1	3.3
Hormonal status		
ER		
-ve	3	10.0
+	12	40.0
++	7	23.3
+++	8	26.7
PR		
-ve	2	6.7
+	15	50.0
++	9	30.0
+++	4	13.3

Whereas in the anti-IL-6 treated normal tissue cultures, areas of fibrocystic disease

ISSN: 3009-7061 **DOI: 10.21608/japs.2024.287068.1025**

are observed, associated with increased manifestations of adenosis, and minor lymphocytic infiltration. **(Fig. 1).**

On the other hand, examination of the untreated cultured tumor tissues showed the infiltration of malignant cells within the collagenic stroma in the form of ducts and nests, evidently infiltrated by lymphocytes with the abundance of several blood vessels in the stroma. Moreover, tumor tissues cultured with anti-IL-8 showed attenuation & compression of several carcinoma cells by the surrounding extensively hyalinized stroma with the presence of tiny thick-walled occluded blood vessels. Nevertheless, tumor tissues cultured with anti-IL-6 showed clusters of malignant ductal cells within the desmoplastic stroma, accompanied by lymphocytic infiltration around the ducts and blood vessels with obvious malignant cells' apoptosis and degeneration. **(Fig. 1)**.

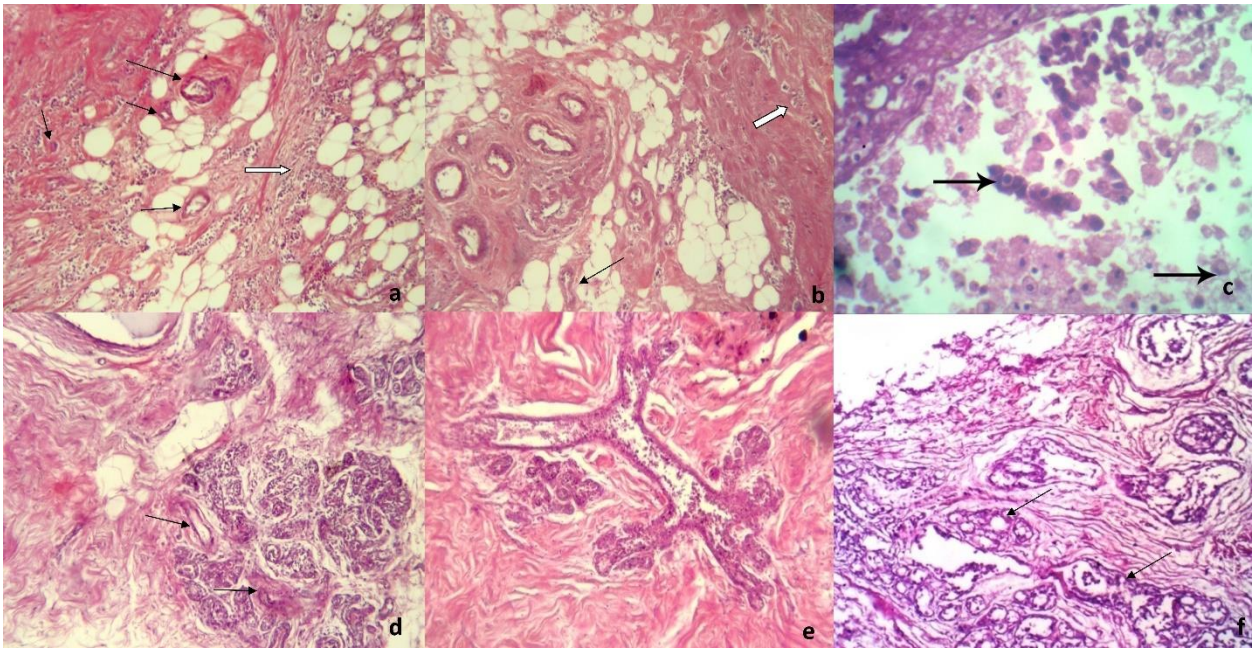

Fig. 1: H&E staining of the different designed normal and tumor tissue culture systems.

a: Untreated cultured tumor tissue (invasive ductal carcinoma) showing streaks and cords of hyperchromatic cells (white arrow) in a collagenic stroma. Four blood vessels can be identified in the stroma (black arrow) (H&E X100). b: Anti-IL-8 treated cultured tumor tissue (invasive ductal carcinoma) showing near vanishing of the tumor cells except for few residual cords of compressed ductal carcinoma cells (white arrow) among predominantly sclerosed and hyalinized stroma. Only one blood vessel can be identified in the field (black arrow) (H&E x100). c: Tumor tissue of invasive ductal carcinoma cultures with anti-IL-6 with obvious necrotic and apoptotic areas (H&E X100). d: Untreated cultured normal breast tissue section showing a lobule of compact acini with adjoining dilated duct. Two blood vessels are seen in the field (black arrow) (H&E X100). e: Anti-IL-8 treated cultured normal breast tissue section showing a branching terminal duct connected to acinar structures lying in collagenic stroma (H&Ex400). f: Anti IL-6 treated cultured normal breast tissue showing adenosis with almost normal breast lining and lymphocytic filtration (H&E X 400)

ISSN: 3009-7061 **DOI: 10.21608/japs.2024.287068.1025**

3.3. Assessment of angiogenesis (assessing microvessel count)

Assessment of angiogenesis was done immunohistochemically by the detection of vascular endothelial growth factor (VEGF) in 30 breast cancer patients, after cultivating tumor and normal tissues with either anti-IL-6 or anti-IL-8 monoclonal antibodies. Immunohistochemical staining using VEGF highlights vascular spaces. It stains vascular endothelial cells in brown color (the hot spots) then the microvessels were counted to assess the average count.

A significant difference was noticed between the number of microvessels observed in the untreated tumor tissues compared to the untreated normal ones ($p \leq 0.0001$). Moreover, treating the tumor tissues with Anti-IL-8 mab decreased the expression of VEGF and the number of microvessels significantly ($p \leq 0.0001$). On the other hand, although a high percentage of the microvessels in the anti-IL-6 treated tissues were closed or sclerosed, yet there is no significant difference between the mean of the microvessels count in untreated and treated breast tumor tissues ($p = 0.3421$).

Finally, no significant difference was noticed between the number of microvessels counted on the untreated normal tissues with normal tissues cultured with Anti-IL-8 and Anti-IL-6 ($p=0.9705$, $p = >0.9999$ respectively) **(Fig. 2, 3)**

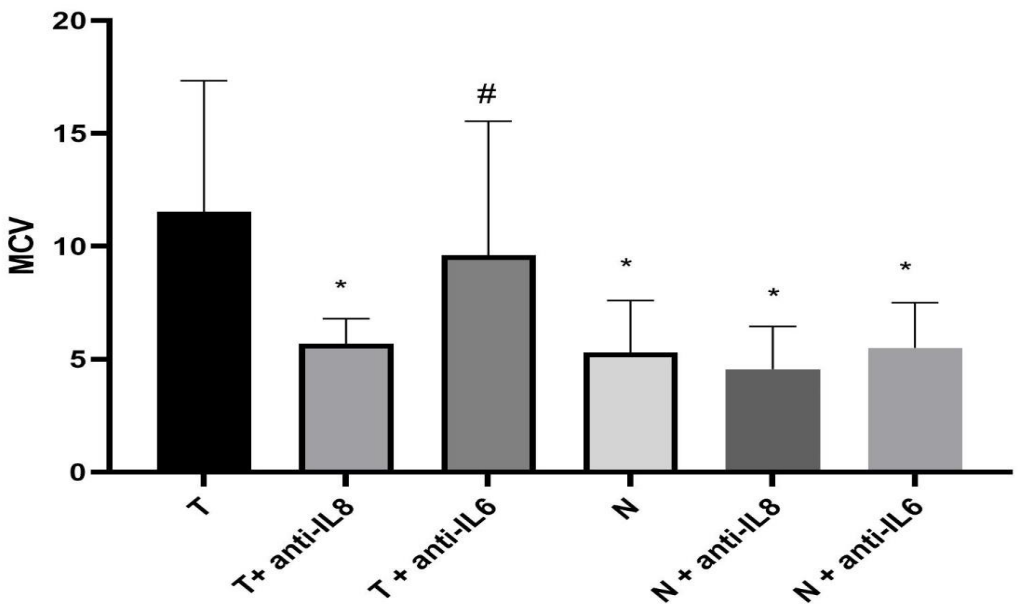

Fig. 2: Schematic presentation of the average microvessels count, detected by immunohistochemical staining of VEG-F in the different cultured tissue culture systems. Data presented as mean ± SD.

* Significant from control tumor tissue cultures (T) (T vs T + anti-IL8 $p \leq 0.0001$) & (T vs N $p \leq 0.0001$).

Significant from tumor tissue cultured with anti-IL8 (T + anti IL8 vs T + anti IL6, $p = 0.001$) No significant difference was observed between the MCV count in (T vs T + anti-IL6, $p = 0.3421$), (T + anti-IL8 vs N + anti IL8, $p = 0.843$), (N vs N + anti-IL8 $p=0.9705$), (N vs N + anti-IL6, $p>0.9999$) and (N+ anti-IL8 vs N + anti-IL6, $p=0.9212$).

ISSN: 3009-7061 **DOI: 10.21608/japs.2024.287068.1025**

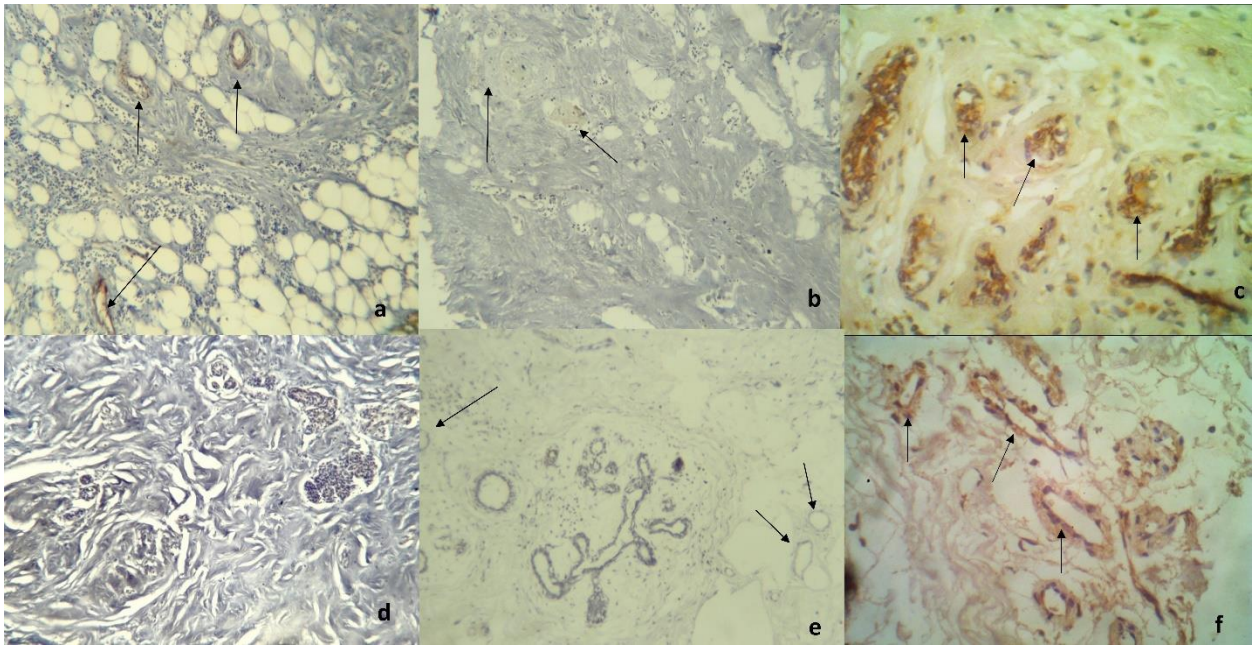

Fig.3: Microvessel count estimated by Immunohistochemical analysis of VEG-F a: Untreated cultured tumor breast tissue section showing 3 moderately stained opened microvessels (black arrow) (IHC X100). B: Anti-IL8 treated cultured tumor breast tissue section showing extensive stromal fibrosis including very few weakly stained collapsed vessels (black arrow) (IHC X100).c: Angiogenesis of anti-IL6 treated tumor tissue showing 10-12 closed vessels (IHC-VEGF X400) d: Normal breast tissue section cultured without either Anti-IL-8 or Anti-IL-6 showing undetectable blood vessels being encroached upon by the sclerotic stroma (IHC X100). e: Normal breast tissue cultured with Anti-IL8 showing a branching duct in a fibrofatty stroma including 3 microvessels with negative staining (arrow) (IHC X100). f: Angiogenesis of cultured normal breast tissue with anti-IL6. Average count 5-8 (IHC- VEGF x400).

4. Discussion

Targeting the tumor microenvironment (TME) for effective cancer therapy has gained increasing attention in the past decade, as the dynamic and heterogenous TME plays a vital role in tumor progression, invasion, and therapy resistance [(21)]. In cancer treatment, anti-angiogenic therapeutics are thought to be among the most effective immunotherapeutic approaches, as previous studies showed that angiogenesis contributes to other factors that support tumor progression other than increasing blood supply, which aids the tumor's need for oxygen and nutrition [(22)].

Angiogenic factors such as VEGF were reported to decrease lymphocyte infiltration within the TME and inhibit the activation and proliferation of different immune cells within the TME such as cytotoxic T cells. Moreover, VEGF was reported to increase the number and activity of intertumoral immunosuppressive lymphocytes such as myeloid-derived suppressor cells and regulatory T-cells thus promoting the immunosuppressive nature of the TME aiding the tumor immune escape [(23)].

Anti-IL8 mab is well known for its effective antiangiogenic properties and different studies assessed the efficacy of Anti-IL-8 antibodies for the treatment of different types

ISSN: 3009-7061 **DOI: 10.21608/japs.2024.287068.1025**

of cancer, where several researches demonstrated the role of IL-8 in tumor angiogenesis, tumor promotion, and induction of cancer stem cell survival signaling thus making it a promising therapy target [24]. In addition, a recent study of our previous work demonstrated the effective use of Anti-IL-8 for cancer stem cells and autophagy inhibition in breast cancer [25].

Anti-IL6 is an anti-inflammatory monoclonal antibody that has been studied extensively and approved for use in different infectious, inflammatory, and autoimmune disorders [26]. In addition, many studies demonstrated the possible antitumor effects of Anti-IL-6. It has been reported that Anti-IL-6 can induce apoptosis in different types of cancer, inhibit tumor progression, reprogram the tumor microenvironment to assess immune surveillance and decrease chemotherapeutics resistance [27-30].

Moreover, multiple pieces of evidence demonstrated the proangiogenic effects of IL-6 in different types of cancer via the promotion of VEGF/NFKβ signaling and JAK/STAT3 pathway [31,32]. In addition, it was demonstrated that cancer-associated fibroblasts have a vital role in the promotion of tumorigenesis, angiogenesis, and trastuzumab resistance in HER2+ breast cancer, by the activation of IL-6/VEGF/STAT3 pathway as well where STAT3 acts as a transcriptional activator of the VEGF promoter [33].

The inhibitory effect of Anti-IL-6 on VEGF signaling had been demonstrated previously in rheumatoid arthritis [34]. Regarding the insignificant difference in MVC between the treated and untreated tumor tissue cultures reported in the current study, it could be due to the short incubation period (24 hrs.) that seems to be insufficient for the complete inhibition of microvessels formation compared to the potent effect of Anti-IL-8. In addition, the immunohistochemical examination of the tumor tissue cultured with Anti-IL-6 showed that the microvessels are most of them closed or sclerosed, thus it is suggested for future studies to assess the effect of IL-6 inhibition within the breast cancer TME for a longer incubation period. Supporting this suggestion recent findings reported that Tocilizumab (Anti-IL-6 mab) inhibits angiogenesis in triple-negative breast cancer cell lines via inhibiting IL-8 production [35]. Thus, it is proposed that Anti-IL-6 treatment could need more time to inhibit angiogenesis compared to the well-established anti-angiogenic Anti-IL-8 immunotherapy. To overcome the limitations of the current study, suggestions for future work include extending the culture period for 72 hours, adding a positive control group for anti-VEGF antiangiogenic therapies, and adjusting the inclusion criteria for patients undergoing the study to include only 1 subtype of breast cancer.

5. Conclusion

Anti-IL-8 and anti-IL-6 monoclonal antibodies are considered promising immunotherapeutic strategies for targeting angiogenesis in breast cancer tumor microenvironments. Anti-IL-8 demonstrated a superior anti-angiogenic activity compared to Anti-IL-6 within the 24-hour incubation period, suggesting assessing the Anti-IL-6 angiogenic activities in longer incubation periods in future studies.

Highlights

- Breast cancer tumor microenvironment is a promising target for cancer immunotherapy.
- IL-6 and IL-8 have a vital role in angiogenesis promotion in breast cancer.
- Anti-IL-8 monoclonal antibody has a superior effect in angiogenesis inhibition compared to Anti-IL-6.

Statements and Declarations

Funding information:

This study is not funded by any institution or a funding body

Conflict of interest

All of the authors declared no conflict of interest

ISSN: 3009-7061 **DOI: 10.21608/japs.2024.287068.1025**

Ethical statement

The current study was approved by the ethical committee of the medical research institute, Alexandria University, Egypt, and confines the provisions of the declaration of Helsinki. Written informed consent was provided by all subjects under study.

Authors' contributions

Seham Abou Shousha designed the study, developed the methodology, and participated in the manuscript writing, Manal Sheta interpreted the histology and IHC data. Mohamed A. Motawea provided the surgical tissue samples; Suzan Baheeg and Ahmed Abo El-Wafa performed the experiments and participated in the interpretation of the results. Yasmine Shahine performed the experiments and the statistical analysis of the data and participated in the manuscript writing. All authors read and approved the final manuscript.

Data availability

The data supporting the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy.

6. References

(1) Arnold M, Morgan E, Rumgay H, Mafra A, Singh D, Laversanne M, Vignat J, Gralow JR, Cardoso F, Siesling S, Soerjomataram I. Current and future burden of breast cancer: Global statistics for 2020 and 2040. The Breast. 2022;66:15-23.

(2) Liu D, Wu J, Lin C, Andriani L, Ding S, Shen K, Zhu L. Breast subtypes and prognosis of breast cancer patients with initial bone metastasis: a population-based study. Frontiers in oncology. 2020;10:580112.

(3) Li JJ, Tsang JY, Tse GM. Tumor microenvironment in breast cancer—updates on therapeutic implications and pathologic assessment. Cancers. 2021 Aug 23;13(16):4233.

(4) Madu CO, Wang S, Madu CO, Lu Y. Angiogenesis in breast cancer progression, diagnosis, and treatment. Journal of Cancer. 2020;11(15):4474.

(5) Ghalehbandi S, Yuzugulen J, Pranjol MZ, Pourgholami MH. The role of VEGF in cancer-induced angiogenesis and research progress of drugs targeting VEGF. European Journal of Pharmacology. 2023;949:175586.

(6) David JM, Dominguez C, Hamilton DH, Palena C. The IL-8/IL-8R axis: a double agent in tumor immune resistance. Vaccines. 2016 Jun 24;4(3):22.

(7) Abou Shousha SA, Hussein B, Shahine Y, Fadali G, Zohir M, Hamed Y, Hemedah M, Baheeg SA, Ibrahim A, El Shannawy M, Osman EM. Angiogenic activities of interleukin-8, vascular endothelial growth factor and matrix metalloproteinase-9 in breast cancer. Egypt J Immunol. 2022;29(3):54-63.

(8) Bao C, Zhou D, Zhu L, Qian W, Ye X. Increased serum level of interleukin-6 correlates with negative prognostic factors in extranodal NK/T-cell lymphoma. Translational Cancer Research. 2020;9(4):2378.

(9) Katongole P, Sande OJ, Nabweyambo S, Joloba M, Kajumbula H, Kalungi S, Reynolds SJ, Ssebambulidde K, Atuheirwe M, Orem J, Niyonzima N. IL-6 and IL-8 cytokines are associated with elevated prostate-specific antigen levels among patients with adenocarcinoma of the prostate at the Uganda Cancer Institute. Future Oncology. 2022 Feb;18(6):661-7.

(10) Amer H, Kartikasari AE, Plebanski M. Elevated interleukin-6 levels in the circulation and peritoneal fluid of patients with ovarian cancer as a potential diagnostic biomarker: a systematic review and meta-analysis. Journal of Personalized Medicine. 2021 Dec 9;11(12):1335.

(11) Lin S, Gan Z, Han K, Yao Y, Min D. Interleukin-6 as a prognostic marker for breast cancer: a meta-analysis. Tumori Journal. 2015;101(5):535-41.

(12) Hegde M, Guruprasad KP, Ramachandra L, Satyamoorthy K, Joshi MB. Interleukin-6–mediated epigenetic control of the VEGFR2 gene induces disorganized angiogenesis in human breast tumors. Journal of Biological Chemistry. 2020;295(34):12086-98.

(13) Nasir A, Holzer TR, Chen M, Man MZ, Schade AE. Differential expression of VEGFR2 protein in HER2 positive primary human breast cancer: potential relevance to anti-angiogenic therapies. Cancer cell international. 2017 ;17:1-2..

(14) Ayoub NM, Jaradat SK, Al-Shami KM, Alkhalifa AE. Targeting angiogenesis in breast cancer: current evidence and future perspectives of novel anti-angiogenic approaches. Frontiers in pharmacology. 2022;13:838133.

(15) Garcia J, Hurwitz HI, Sandler AB, Miles D, Coleman RL, Deurloo R, Chinot OL. Bevacizumab (Avastin®) in cancer treatment: A review of 15 years of clinical experience and

ISSN: 3009-7061 **DOI: 10.21608/japs.2024.287068.1025**

future outlook. Cancer treatment reviews. 2020;86:102017.

(16) Hey SP, Gyawali B, D'Andrea E, Kanagaraj M, Franklin JM, Kesselheim AS. A systematic review and meta-analysis of bevacizumab in first-line metastatic breast cancer: lessons for research and regulatory enterprises. JNCI: Journal of the National Cancer Institute. 2020;112(4):335-42.

(17) Abou Shousha S, Baheeg S, Ghoneim H, Zoheir M, Hemida M, Shahine Y. The effect of Fas/FasL pathway blocking on apoptosis and stemness within breast cancer tumor microenvironment (preclinical study). Breast Disease. 2023;42(1):163-76.

(18) Grosset AA, Loayza-Vega K, Adam-Granger É, Birlea M, Gilks B, Nguyen B, Soucy G, Tran-Thanh D, Albadine R, Trudel D. Hematoxylin and eosin counterstaining protocol for immunohistochemistry interpretation and diagnosis. Applied Immunohistochemistry & Molecular Morphology. 2019;27(7):558-63.

(19) Hofman F. Immunohistochemistry. Current protocols in immunology. 2002;49(1):21-4.

(20) Weidner N. Current pathologic methods for measuring intratumoral microvessel density within breast carcinoma and other solid tumors. Breast cancer research and treatment. 1995;36:169-80.

(21) Singh SR, Rameshwar P, Siegel P. Targeting tumor microenvironment in cancer therapy. Cancer letters. 2016;380(1):203-4.

(22) Hu H, Chen Y, Tan S, Wu S, Huang Y, Fu S, Luo F, He J. The research progress of antiangiogenic therapy, immune therapy and tumor microenvironment. Frontiers in immunology. 2022;13:802846.

(23) Tamura R, Tanaka T, Akasaki Y, Murayama Y, Yoshida K, Sasaki H. The role of vascular endothelial growth factor in the hypoxic and immunosuppressive tumor microenvironment: perspectives for therapeutic implications. Medical Oncology. 2020;37:1-4.

(24) Alfaro C, Sanmamed MF, Rodríguez-Ruiz ME, Teijeira Á, Oñate C, González Á, Ponz M, Schalper KA, Pérez-Gracia JL, Melero I. Interleukin-8 in cancer pathogenesis, treatment and follow-up. Cancer treatment reviews. 2017;60:24-31.

(25) Abou Shousha S, Medhat E, Baheeg S, Shahine Y. Anti-IL-8 monoclonal antibodies inhibit the autophagic activity and cancer stem cells maintenance within breast cancer tumor microenvironment. Breast Disease. 2024;43(1):37-49.

(26) Ohsugi Y. The immunobiology of humanized Anti-IL6 receptor antibody: From basic research to breakthrough medicine. Journal of Translational Autoimmunity. 2020 ;3:100030.

(27) Sumida K, Wakita D, Narita Y, Masuko K, Terada S, Watanabe K, Satoh T, Kitamura H, Nishimura T. Anti-IL-6 receptor m A b eliminates myeloid-derived suppressor cells and inhibits tumor growth by enhancing T-cell responses. European journal of immunology. 2012;42(8):2060-72.

(28) Song L, Smith MA, Doshi P, Sasser K, Fulp W, Altiok S, Haura EB. Antitumor efficacy of the anti-interleukin-6 (IL-6) antibody siltuximab in mouse xenograft models of lung cancer. Journal of Thoracic Oncology. 2014;9(7):974-82.

(29) Wang D, Xu J, Liu B, He X, Zhou L, Hu X, Qiao F, Zhang A, Xu X, Zhang H, Wicha MS. IL6 blockade potentiates the anti-tumor effects of γ-secretase inhibitors in Notch3-expressing breast cancer. Cell Death & Differentiation. 2018;25(2):330-9.

(30) Wang Y, Miao X, Jiang Y, Wu Z, Zhu X, Liu H, Wu X, Cai J, Ding X, Gong W. The synergistic antitumor effect of IL-6 neutralization with NVP-BEZ235 in hepatocellular carcinoma. Cell Death & Disease. 2022;13(2):146.

(31) Briukhovetska D, Dörr J, Endres S, Libby P, Dinarello CA, Kobold S. Interleukins in cancer: from biology to therapy. Nature Reviews Cancer. 2021;21(8):481-99.

(32) Huang B, Lang X, Li X. The role of IL-6/JAK2/STAT3 signaling pathway in cancers. Frontiers in oncology. 2022;12:1023177.

(33) Wei LH, Kuo ML, Chen CA, Chou CH, Lai KB, Lee CN, Hsieh CY. Interleukin-6 promotes cervical tumor growth by VEGF-dependent angiogenesis via a STAT3 pathway. Oncogene. 2003;22(10):1517-27.

(34) Nakahara H, Song J, Sugimoto M, Hagihara K, Kishimoto T, Yoshizaki K, Nishimoto N. Anti–interleukin-6 receptor antibody therapy reduces vascular endothelial growth factor production in rheumatoid arthritis. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology. 2003;48(6):1521-9.

(35) Alraouji NN, Aboussekhra A. Tocilizumab inhibits IL-8 and the proangiogenic potential of triple negative breast cancer cells. Molecular Carcinogenesis. 2021;60(1):51-9.

ISSN: 3009-7061 **DOI: 10.21608/japs.2024.287068.1025**