Int J Clin Exp Med 2015;8(12):22198-22203
www.ijcem.com /ISSN:1940-5901/IJCEM0016043

Original Article

Clinicopathological significance of NGX6 expression in breast cancer and its relationship to angiogenesis

Jidong Xiao[1], Yuyao Mao[1], Yuanquan Zhou[1], Kaimin Xiang[2]

Departments of [1]Diagnostic Ultrasound, [2]General Surgery, Third Xiangya Hospital, Central South University, Changsha 410013, Hunan Province, China

Received September 12, 2015; Accepted December 5, 2015; Epub December 15, 2015; Published December 30, 2015

Abstract: Objective: The aim was to explore clinicopathological significance of Nasopharyngeal carcinoma-associated gene 6 (NGX6) expression in breast cancer and its relationship to angiogenesis. Methods: Clinicopathological feature of 168 patients with breast cancer were analyzed. NGX6 expression and microvessel density (MVD) in tumor tissue were measured using immunohistochemistry methods. The association of NGX6 expression with MVD and clinicopathological features was assessed. Results: Among 168 cases of breast cancer, NGX6 positive expression were found in 92 (54.8%) cases and NGX6 negative expression were found in 76 (45.2%) cases. Incidence rate of large size tumor, high tumor node metastasis (TNM) stage and lymph node metastasis in NGX6 negative expression group were higher than NGX6 positive expression group in breast cancer (P=0.003, 0.007, and 0.003, respectively). MVD in NGX6 negative expression group is 22.5±4.8, MVD in NGX6 positive expression group is 15.2±4.2. MVD in the NGX6 negative expression group was significantly higher than the NGX6 positive expression group (P<0.05). Conclusion: The expression of NGX6 was closely associated with tumor size, TNM stage, lymph node metastasis and MVD. NGX6 is involved in metastasis and angiogenesis activity of breast cancer. The study may provide a theoretical basis for anti-angiogenic therapy of breast cancer.

Keywords: Breast cancer, nasopharyngeal carcinoma associated gene 6 (NGX6), angiogenesis, metastasis

Introduction

Breast cancer is one of the most frequent malignant tumors with invasive fast-growing, high recurrence rate and fatality. Angiogenesis is the basis of tumor growth, invasion and metastasis. Current studies suggest that the process of angiogenesis is involved in unbalance of angiogenesis factors and inhibitors. The balance is regulated by oncogene, suppressor oncogene and some regulatory peptide [1-4]. NGX6 is a novel tumor suppressor gene (GenBank accession no. AF188239). It located in chromosome 9 p21-22 areas and showed the loss of heterozygosity in allele for many tumors [5-8]. Little is known about the relationships between NGX6 expression and angiogenesis in breast cancer. The study discussed clinicopathological significance of NGX6 expression in breast cancer and its relationship to angiogenesis.

Materials and methods

Patients

Between January 2012 and April 2014, a total of 168 operable patients with breast cancer confirmed with pathological examination at the Third Xiangya Hospital were retrospectively collected for this study. All patients were female and the mean age of patients was 65 years (ranging from 28 to 76 years). None of the patients received radiotherapy, chemotherapy or immunotherapy prior to surgery. The clinical data were collected, including age, tumor features, histologic classification, TNM stage, lymph node metastasis. The study protocol was approved by the Ethics Committee of Third Xiangya Hospital of Central South University. An informed consent was acquired from each patient in this study.

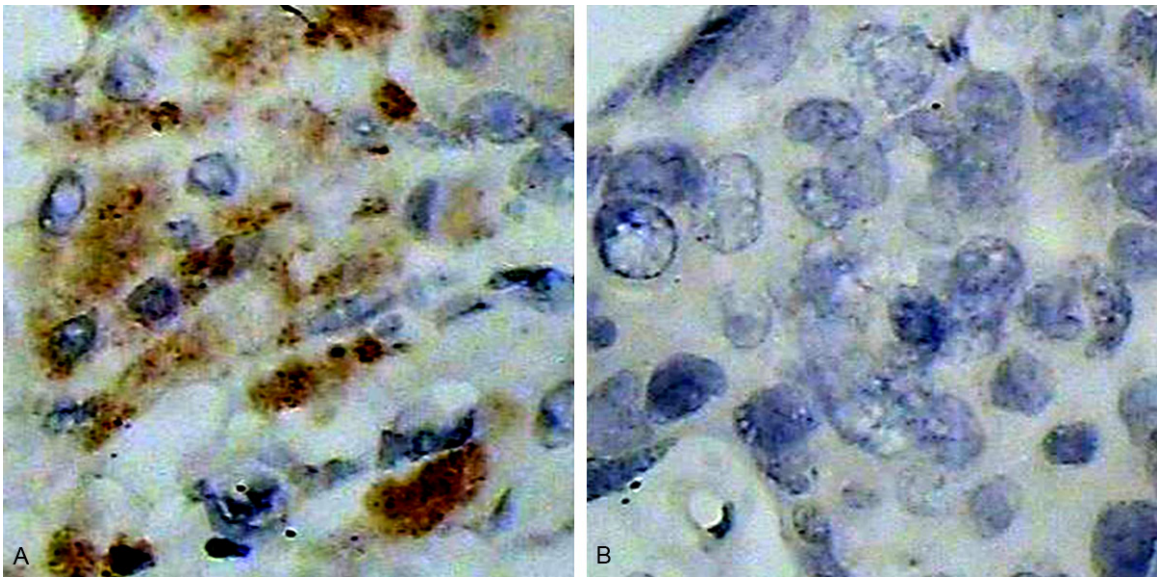

Figure 1. Representative immunohistochemical staining of NGX6 expression in breast cancer tissues. A. Positive expression of NGX6; B. Negative expression of NGX6. Representative images are shown at ×400 magnification.

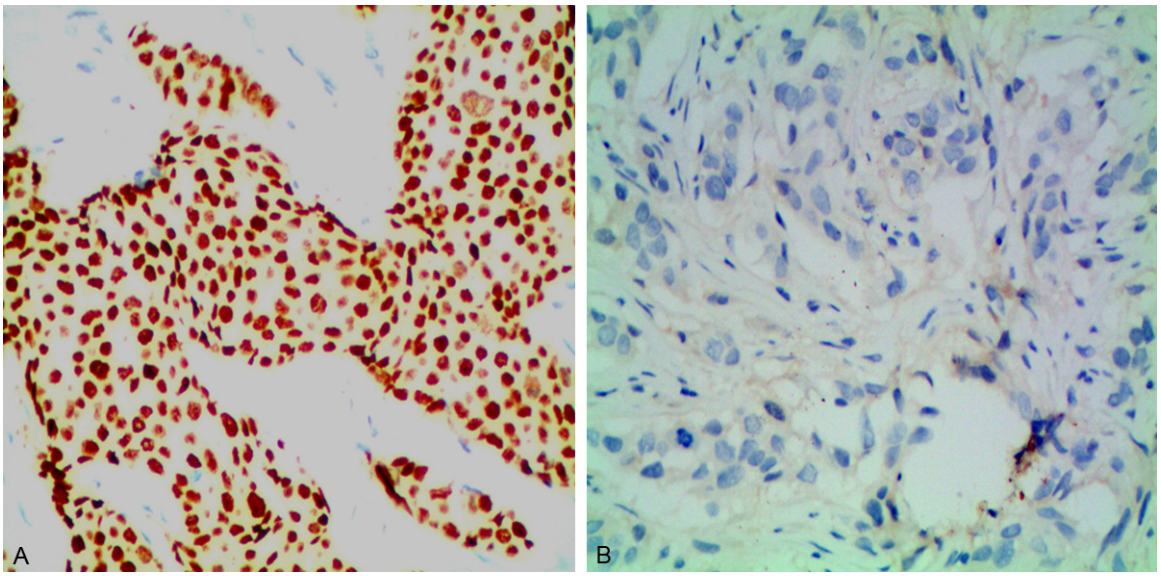

Figure 2. Representative immunohistochemical staining of ER expression in breast cancer tissues. A. Positive expression of ER; B. Negative expression of ER. Representative images are shown at ×100 magnification.

Immunohistochemistry (IHC)

IHC was performed as previously described [9]. The specimens were fixed in 10% formalin solution and then embedded in paraffin. Serial sections were cut and prepared for hematoxylin and eosin (H&E) staining and immunohistochemistry. The sections were incubated with Rabbit anti-NGX6 monoclonal antibodies (1:200 dilution, Abcam, USA) or CD34 antibody (1:100 dilution, Abcam, USA) overnight at 4°C, and then incubated at 37°C for 30 min with a secondary antibody against rabbit and mouse immunoglobulins (EnVision, DAKO, Denmark). Afterwards, the sections were stained with DAB for 5 min. The cytoplasm and cell membrane stained in yellow brown were the positive pattern. Classification is done according to the strength of cells staining and the proportion of the positive cell [10]. The measurements of MVD were performed according to a well established method by Weidner et al. [11]. All immu-

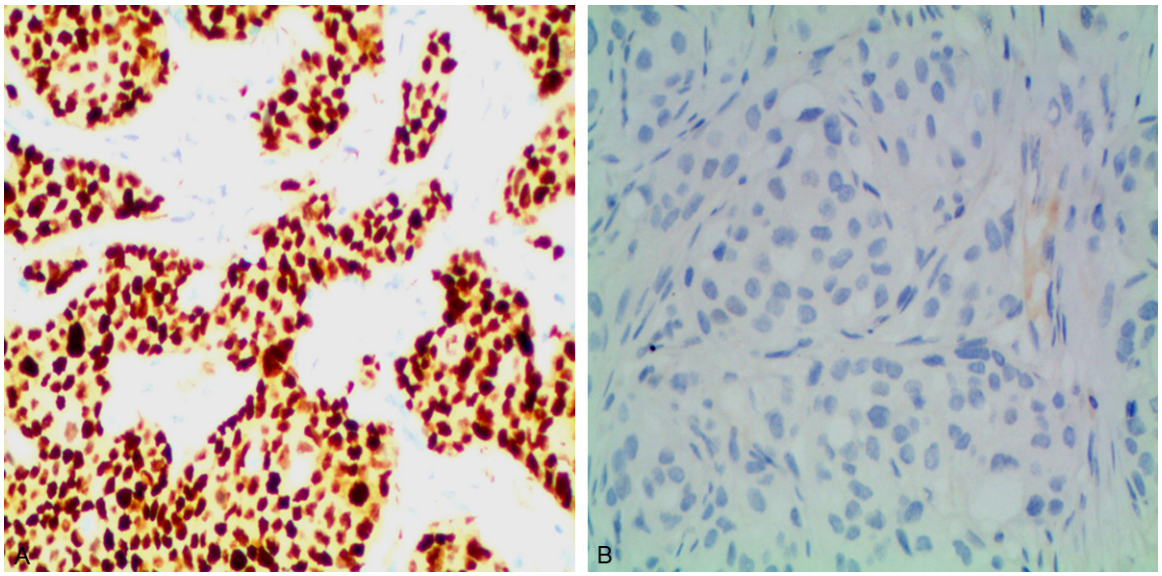

Figure 3. Representative immunohistochemical staining of PR expression in breast cancer tissues. A. Positive expression of PR; B. Negative expression of PR. Representative images are shown at ×100 magnification.

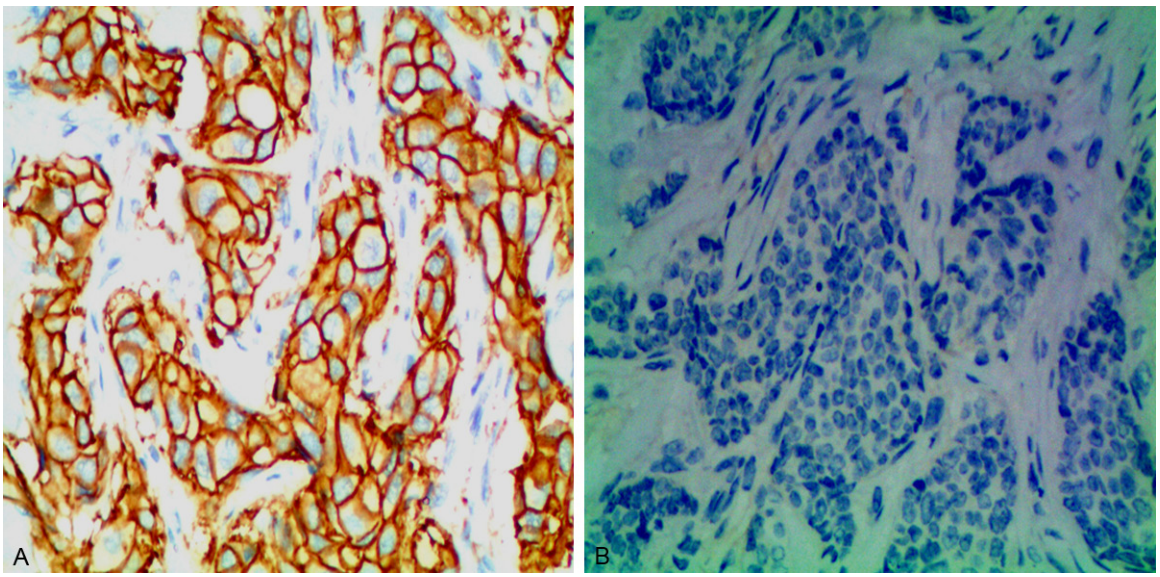

Figure 4. Representative immunohistochemical staining of HER-2 expression in breast cancer tissues. A. Positive expression of HER-2; B. Negative expression of HER-2. Representative images are shown at ×100 magnification.

nohistochemical results were independently assessed by two pathologists double-blindly.

Statistical analysis

Data were analyzed using SPSS 13.0. Contingency coefficients were calculated to measure the correlation between NGX6 expression and clinicophathological characteristics in patients with breast cancer. The t-test was used to compare the difference of micro-vessel density (MVD) between patients with NGX6 positive expression and those with NGX6 negative expression. A p-value of <0.05 was considered statistically significant.

Results

NGX6 expression in breast cancer

Among 168 cases of breast cancer, positive expression was found in 92 (54.8%) cases (**Figure 1A**) and negative expression was found in 76 (45.2%) cases (**Figure 1B**).

Table 1. Correlation between NGX6 expression with clinicopathological features in patients with breast cancer

Clinicopathologic features	n	NGX6 (+)	NGX6 (-)	χ^2	*P*	Contingency coefficient
Age						
<65 years	70	40	30	0.275	0.600	0.040
≥65 years	98	52	46			
Tumor size						
<2 cm	81	54	27	8.948	0.003	0.225
≥2 cm	87	38	49			
TNM stage						
I-II	58	40	18	7.214	0.007	0.203
III-IV	110	52	58			
Histological grade						
Well/moderately	73	45	28	2.468	0.116	0.120
Poorly	95	47	48			
ER						
Negative	64	39	25	1.592	0.207	0.097
Positive	104	53	51			
PR						
Negative	91	55	36	2.584	0.108	0.123
Positive	77	37	40			
HER-2						
Negative	83	48	35	0.624	0.430	0.061
Positive	85	44	41			
Lymph node metastasis						
Negative	67	46	21	8.685	0.003	0.222
Positive	101	46	55			

Correlation of NGX6 expression with clinicopathological features in patients with breast cancer

Incidence rate of large size tumor (≥2 cm), high TNM stage (III-IV) and lymph node metastasis in NGX6 negative expression group were higher than NGX6 positive expression group in breast cancer, NGX6 expression was associated with tumor size, lymph node metastasis and TNM stage. Age, estrogen receptor (ER) (**Figure 2**), progesterone receptor (PR) (**Figure 3**), human epidermal growth factor receptor 2(HER-2) (**Figure 4**) and histological grade were not associated with NGX6 expression, which were shown in **Table 1**.

Correlation of NGX6 expression with microvessel density (MVD) in patients with breast cancer

MVD in NGX6 negative expression group (**Figure 5A**) is 22.5±4.8 per 0.2 mm^2, MVD in NGX6 positive expression group (**Figure 5B**) is 15.2±4.2 per 0.2 mm^2. MVD in the negative group was significantly higher than the positive expression group ($P<0.05$).

Discussion

NGX6 proteins contain (EGF)-like domain structure and result in a variety of biological actions, such as tumor growth, invasion and metastasis [6-8]. These biological actions are closely related with angiogenesis. MVD is now widely used to evaluate the tumor angiogenesis [12-17]. The staining of endothelial cells for CD34 was used to evaluate MVD. To explore the association of NGX6 with MVD may contribute to understand the clinicopathological significance of NGX6 expression in breast cancer.

In this study, the incidence rate of the large tumor size, high TNM stage (III-IV) and lymph node metastasis in NGX6 negative expression group were higher than NGX6 positive expression group in breast cancer. MVD in the NGX6 negative group was significantly higher than that in the positive group. NGX6 expression was related with clinicopathologic features of breast cancer including tumor size, lymph node metastasis, TNM stage and MVD. All these suggest that NGX6 was involved in the invasion, metastasis and angiogenesis activity of breast cancer patients.

The limitation of our study is that only a small number of patients were examined in this study, making it difficult to reach statistical significance. We hope to study a greater number of patients in the future.

In conclusion, NGX6 is an important tumor suppressor gene. It is closely related with angiogenesis and metastasis. The study of the clinicopathological significance of NGX6 expression in breast cancer and its relationship to angiogenesis may provide a theoretical basis for anti-angiogenic therapy.

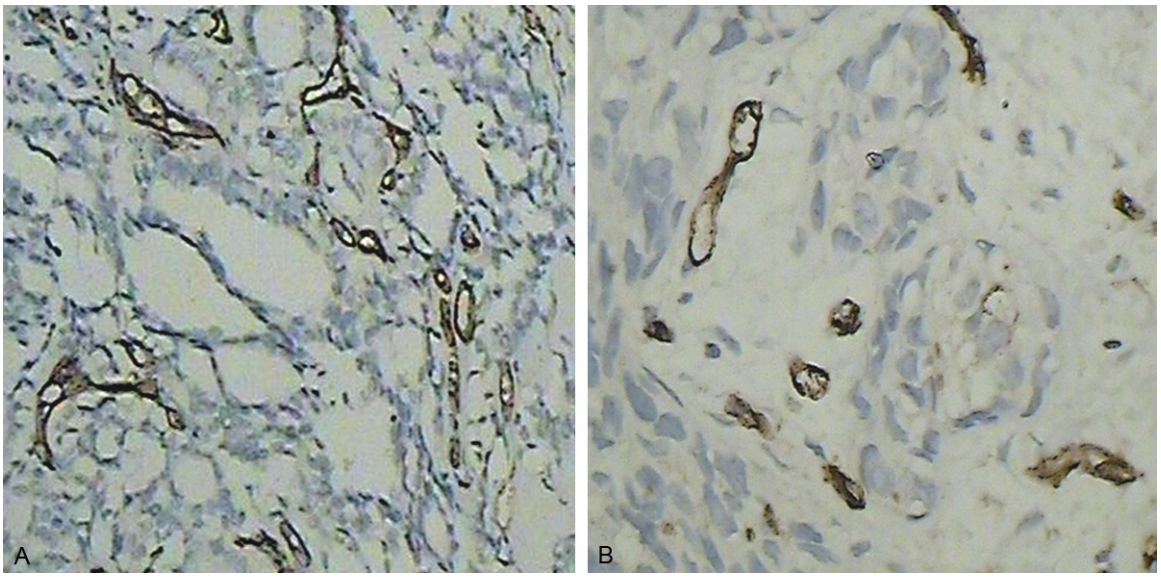

Figure 5. MVD in breast cancer tissues. A. MVD is 22 per 0.2 mm^2 in NGX6 negative tissues; B. MVD is 12 per 0.2 mm^2 in NGX6 positive tissues. Representative images are shown at ×100 magnifications.

Acknowledgements

This work was supported by grants from the Science Foundation of Hunan Province (2013FJ4094), the Medical Foundation of Hunan Province (B2013-023), High-level medical project (225) and the Educational Project of Hunan province (XJK013CGD081) China.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Kaimin Xiang, Department of General Surgery, Third Xiangya Hospital, Central South University, Changsha 410013, Hunan Province, China. E-mail: xiangkaiming2015@163.com

References

[1] Ming H, Lan Y, He F, Xiao X, Zhou X, Zhang Z, Li P, Huang G. Cytochrome b5 reductase 2 suppresses tumor formation in nasopharyngeal carcinoma by attenuating angiogenesis. Chin J Cancer 2015; 34: 42.

[2] Gahr S, Mayr C, Kiesslich T, Illig R, Neureiter D, Alinger B, Ganslmayer M, Wissniowski T, Fazio PD, Montalbano R, Ficker JH, Ocker M, Quint K. The pan-deacetylase inhibitor panobinostat affects angiogenesis in hepatocellular carcinoma models via modulation of CTGF expression. Int J Oncol 2015; 47: 963-970.

[3] Yu GR, Kim SH, Park SH, Cui XD, Xu DY, Yu HC, Cho BH, Yeom YI, Kim SS, Kim SB, Chu IS, Kim DG. Identification of molecular markers for the oncogenic differentiation of hepatocellular carcinoma. Exp Mol Med 2007; 39: 641-652.

[4] Mas VR, Maluf DG, Archer KJ, Yanek KC, Fisher RA. Angiogenesis soluble factors as hepatocellular carcinoma noninvasive markers for monitoring hepatitis C virus cirrhotic patients awaiting liver transplantation. Transplantation 2007; 84: 1262-1271.

[5] Wang L, Ma J, Li J, Li X, Zhang Q, Peng S, Peng C, Zhou M, Xiong W, Yang J, Zhou J, Fan S, Tan C, Yan Q, Shen S, Li G. NGX6 gene inhibits cell proliferation and plays a negative role in EGFR pathway in nasopharyngeal carcinoma cells. J Cell Biochem 2005; 95: 64-73.

[6] Ma J, Zhou J, Fan S, Wang L, Li X, Yan Q, Zhou M, Liu H, Zhang Q, Zhou H, Gan K, Li Z, Peng C, Xiong W, Tan C, Shen S, Yang J, Li J, Li G. Role of a novel EGF-like domain-containing gene NGX6 in cell adhesion modulation in nasopharyngeal carcinoma cells. Carcinogenesis 2005; 26: 281-291.

[7] Wu M, Li X, Li X, Li G. Signaling transduction network mediated by tumor suppressor/susceptibility genes in NPC. Curr Genomics 2009; 10: 216-222.

[8] Liu M, Peng Y, Wang X, Guo Q, Shen S, Li G. NGX6 gene mediated by promoter methylation as a potential molecular marker in colorectal cancer. BMC Cancer 2010; 27: 160.

[9] Xiao J, Zhou Y, Zhu W. Association of ultrasonographic features with NGX6 expression and prognosis in invasive ductal breast carcinoma. Int J Clin Exp Pathol 2015; 8: 6458-6465.

[10] Ma F, Ding X, Fan Y, Ying J, Zheng S, Lu N, Xu B. A CLDN1-negative phenotype predicts poor prognosis in triple-negative breast cancer. PLoS One 2014; 9: e112765.
[11] Weidner N, Folkman J, Pozza F, Bevilacqua P, Allred EN, Moore DH, Meli S, Gasparini G. Tumor angiogenesis: a new significant and independent prognostic indicator in early-stage breast carcinoma. J Natl Cancer Inst 1992; 84: 1875-1887.
[12] Kather JN, Marx A, Reyes-Aldasoro CC, Schad LR, Zöllner FG, Weis CA. Continuous representation of tumor microvessel density and detection of angiogenic hotspots in histological whole-slide images. Oncotarget 2015; 6: 19163-19176.
[13] Pöschinger T, Renner A, Eisa F, Dobosz M, Strobel S, Weber TG, Brauweiler R, Kalender WA, Scheuer W. Dynamic contrast-enhanced micro-computed tomography correlates with 3-dimensional fluorescence ultramicroscopy in antiangiogenic therapy of breast cancer xenografts. Invest Radiol 2014; 49: 445-56.
[14] Zhang X, Wang Z, Wang Z, Zhang Y, Jia Q, Wu L, Zhang W. Impact of acetylsalicylic acid on tumor angiogenesis and lymphangiogenesis through inhibition of VEGF signaling in a murine sarcoma model. Oncol Rep 2013; 29: 1907-1913.
[15] Zhai LL, Wu Y, Cai CY, Tang ZG. Overexpression of homeobox B-13 correlates with angiogenesis, aberrant expression of EMT markers, aggressive characteristics and poor prognosis in pancreatic carcinoma. Int J Clin Exp Pathol 2015; 8: 6919-6927.
[16] Fan HX, Chen Y, Ni BX, Wang S, Sun M, Chen D, Zheng JH. Expression of MMP-1/PAR-1 and patterns of invasion in oral squamous cell carcinoma as potential prognostic markers. Onco Targets Ther 2015; 8: 1619-1626.
[17] Calcinotto A, Ponzoni M, Ria R, Grioni M, Cattaneo E, Villa I, Sabrina Bertilaccio MT, Chesi M, Rubinacci A, Tonon G, Bergsagel PL, Vacca A, Bellone M. Modifications of the mouse bone marrow microenvironment favor angiogenesis and correlate with disease progression from asymptomatic to symptomatic multiple myeloma. Oncoimmunology 2015; 4: e1008850.