Acta Transitional Medicine *An International Scientific Journal*	ISSN: 2521-8662	Volume 1	Issue 1	pp.19-34	March 2018

THECLINICAL SIGNIFICANCE OF SURVIVIN AND VASCULAR ENDOTHELIAL GROWTH FACTOR IN BREAST CANCER AND PRECANCEROUS LESIONS

Rui Wang[1], Lin Shi[2*]

[1]*Department of pathology, Rizhao People's Hospital, Affiliated Jining Medical University, Rizhao, China;*

[2]*Division of Cardiology, The First Affiliated Hospital of Soochow University, Suzhou City 215000, Jiangsu Province, China.*

E-mail: dkwlyrpq51687@sina.com

Abstract: Methods: Immunhistochemical *Ultra Sensitive*^{TM} S-P method was employed to detect the expression of Survivin and VEGF in 334 cases including 60 cases of UDH, 57 cases of atypical ductal hyperplasia (ADH), 89 cases of ductal carcinoma in situ (DCIS) and 128 cases invasive ductal carcinomas (IDC). The multiple biological parameters including the tumor size, grade, Lymph node status, tumor metastasis and stage were compared with and investigate the associations of Survivin and VEGF expressions in breast carcinoma. Results: ⑴ Survivin was maily distributed in cytoplasm in UDH, but also distributed in nucleus and cytoplasm in IDC, DCIS and ADH mammary tissues .⑵ The positive rates of Survivin and VEGF in IDC were 67.2% and 68.8%, in DCIS were 59.6% and 44.9%, in ADH were 57.9% and 36.8%, and in UDH tissues were 1.7% and 20.0%. Compaired with UDH tissues group, there were significant differences of the positive rates of Survivin and VEGF in IDC (χ^2=70.540, P=0.000; χ^2=38.993, P=0.000), DCIS (χ^2=51.967, P=0.000; χ^2=9.815, P=0.002) and ADH (χ^2=42.829,P=0.000; χ^2=4.095,P=0.043) group, P<0.05, respectively. There were significant differences in the positive expression rates of VEGF between DCIS and IDC (χ^2=12.298,P=0.001), ADH and IDC (χ^2=16.589, P=0.000), however, there were no significant differences of Survivin expression between IDC and DCIS (χ^2=1.330,P=0.249), IDC and ADH tissue (χ^2=1.484,P=0.223). Positive expression rates of Survivin and VEGF were found no significant between ADH and DCIS tissue (χ^2=0.039, P=0.0843; χ^2=0.938, P=0.333). ⑶There was positive correlation in over-expressions of Survivin and VEGF with histological grade (χ^2=10.631, 12.412), lymph node metastasis (χ^2=8.135, 7.677), distant metastasis (χ^2=17.732, 7.621) and stage (χ^2=6.992, 21.211) of IDC tumor. ⑷ The expression of VEGF was correlated positively with Survivin (r=0. 211, P=0.017). Conclusion: The results demonstrate that Survivin location changes from cell plasma to cell nucleus might participate in oncogenesis and development of breast cancer. The over-expression of Survivin and VEGF might be important biological markers for invasion and metastasis of breast invasive carcinomas. The combined detaction of Survivin and VEGF are the predictors for prognosis of breast carcinoma.

Keywords: Breast cancer, Survivin, vascular endothelial growthfactor, immunohistochemical, clinic, pathology.

1. INTRODUCTION

Cancer is a major public health problem all over the world. In recent years, the incidence of breast cancer is relatively high, and the peak incidence is in advance, a large number of patients died of breast cancer complications or serious organ metastasis each year [1, 2]. However, the pathogenesis of breast cancer is not fully clear, studies have shown that the proliferation and metastasis of breast cancer cells is a very complex, multistep process, affected by various factors including the common role of environmental, genetic and so on. Many studies have found that certain biological

indicators by detecting abnormal expression of molecules, such as ER, PR, HER2, Ki-67, etc, can guide clinical diagnosis and treatment activities prognosis [2-5]. There are several other molecular biomarkers, such as Survivin, CD105 and vascular endothelial growth factor (VEGF) and have been confirmed to participate in the evolution of breast cancer [2-5]. Survivin is one of the most important members of the inhibitors of apoptosis protein family, as it is expressed in most human cancers but is absent in normal, differentiated tissues [2]. Lending to its importance, survivin has proven associations with apoptosis and cell cycle control, and has more recently been shown to modulate the tumor microenvironment and immune evasion as a result of its extracellular localization. Survivin has been shown to localize in mitochondria, where it modulates tumor cell apoptosis similar to the Bcl-2 family [2]. Its localization to the nucleus and cytosol confers its role in mitosis regulation and apoptosis inhibition, respectively. Nuclear survivin is known to be a cell-cycle-associated protein. Investigations of cell division regulation during the depletion of survivin by small interfering (si)RNA demonstrated an increase in mitotic arrest and chromosomal misalignment. Furthermore, survivin is involved in microtubule assembly and centromere stabilization during mitosis. Vascular endothelial growth factor (VEGF) capable of promoting angiogenesis exerts an important effect in the process of genesis, development, metastasis and recurrence of various tumors. CD105 is an accessory receptor of transforming growth factor. The highest synthesis, as well as expression, of endoglin has been found in vascular endothelial cells. Upregulation of survivin has been found in many cancers including breast, prostate, pancreatic, and hematological malignancies, and it may prove to be associated with the advanced presentation, poorer prognosis, and lower survival rates observed in ethnically diverse populations [6, 7]. In this study, we investigated the prognostic significance of Survivin, VEGF and Microvessel density (MVD) based on the number of CD105-positive vessel in various breast tissues and epithelium surrounding area adjacent to the lesion and to evaluate the relationship between Survivin, VEGF, MVD and lymph node metastasis and tumor recurrence of breast cancer after surgery, in order to explore the above markers expressions and clinicopathological parameters in mammary cancer, and study their expressions with chemosensitivity in mammary carcinoma.

2. PATIENTS AND METHODS

2.1. Patients Selection

One handrened twenty-eight patients diagnosed invasive breast cancer (IDC), age ranged from 22 to 79 years, mean 48.7 years, were collected during excision surgery at Rizhao people's Hospital from June 2000 to June 2013. 89 cases of ductal carcinoma in situ (DCIS), and 57 cases of atypical ductal hyperplasia (ADH) and 60 cases of usual duct hyperplasia (UDH) lesions were selected as a control group. Permission was obtained from the Local Ethical Committee to collect mammary cancer tissues and all patients signed informed consents to the research. The patients had not been treated with hormone endocrine therapy, anti-neoplastic chemotherapy or radiotherapy during the last six months. The expression of Survivin and VEGF in mammary cancer were detected and their relationship with the multiple clinical biological parameters including the tumor size, grade, stage, region lymph node metastasis, distant metastasis and recurrence on files were also assessed in order to study the clinical and pathological characteristics associated with mammary cancer and improve the clinical diagnosis, monitor whether the cancer was indeed in regression due to the anti-cancer treatment, or reoccurring. Immunohistochemical *S-P* method was used to detect differences in tumor tissue Survivin and VEGF expression and the situation before and after chemotherapy in 52 cases of mammary cancer patients to neoadjuvant chemotherapy. According to the International Union against Cancer (UICC) TNM classification of solid malignant tumors standard (7th ed) and American Joint Committee on Cancer (AJCC) Cancer Staging Manual (7th ed) to evaluate curative effect of therapeutic effect [8, 9].

2.2. IMMUNOHISTOCHEMISTRY

2.2.1. QualityControl

One handrened twenty-eight patients diagnosed invasive breast cancer (IDC), age ranged from 22 to 79 years, mean 48.7 years, were collected during excision surgery at Rizhao people's Hospital from June 2000 to June 2013. 89 cases of ductal carcinoma in

©M&H ACADEMIC PUBLISHER

situ (DCIS), and 57 cases of atypical ductal hyperplasia (ADH) and 60 cases of usual duct hyperplasia (UDH) lesions were selected as a control group. Permission was obtained from the Local Ethical Committee to collect mammary cancer tissues and all patients signed informed consents to the research. The patients had not been treated with hormone endocrine therapy, anti-neoplastic chemotherapy or radiotherapy during the last six months. The expression of Survivin and VEGF in mammary cancer were detected and their relationship with the multiple clinical biological parameters including the tumor size, grade, stage, region lymph node metastasis, distant metastasis and recurrence on files were also assessed in order to study the clinical and pathological characteristics associated with mammary cancer and improve the clinical diagnosis, monitor whether the cancer was indeed in regression due to the anti-cancer treatment, or reoccurring. Immunohistochemical *S-P* method was used to detect differences in tumor tissue Survivin and VEGF expression and the situation before and after chemotherapy in 52 cases of mammary cancer patients to neoadjuvant chemotherapy. According to the International Union against Cancer (UICC) TNM classification of solid malignant tumors standard (7th ed) and American Joint Committee on Cancer (AJCC) Cancer Staging Manual (7th ed) to evaluate curative effect of therapeutic effect [8, 9].

2.2. Immunohistochemistry

2.2.1. Quality Control

Tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin. Tissue sections were deparaffinized and rehydrated using standard procedures. Immunoreactions were processed using the *Ultra Sensitive*^{TM} *S-P* Kit (Maixin-Bio, Fuzhou, Fujian, China) according to the manufacturer's instructions to detect differences in tumor tissue Survivin, VEGF and CD105 expression, and signals were visualized using the DAB substrate, which stains the target protein yellow. Localization of Survivin distributed was assessed. Positivity for VEGF was indicated by cytoplasmic staining. Histologically recognizable vessels within tissue sections served as internal controls for CD105 immunostaining.

2.2.2. Survivin Assay

In brief, a proportion score was assigned that represents the estimated proportion of positive tumor cells on the entire slide. For each histological section, the percentage of positive cells was scored as 0 (<5%), 1 (6%-25%), 2 (26%-50%), 3 (51%-75%) and 4 (>75%), and the staining intensity was scored as 0 (negative), 1 (weak), 2 (moderate) and 3 (strong). The immunoreactive score (IRS) was obtained by multying the percentage of positive cells and the staining intensity. Immunohistochemical results with an IRS of 0 were considered negative (-), 1-4 weak positive (+), 5-8 moderate positive (++) and 9-12 strong positive (+++). The negative controls were used. The primary antibody was replaced with PBS, containing 0.1% bovine serum albumin at the same concentration as the primary antibody. The positive controls were tissues known to express the antigen being studied. Localization of Survivin distributed was assessed. The multifaceted functionality of survivin was still being intensely scrutinized, and it appears that protein compartmentalization may be important. Survivin has been shown to localize in mitochondria. Its localization to the nucleus and cytosol confers its role in mitosis regulation and apoptosis inhibition, respectively. Survivin was maily distributed in cytoplasm, but also in nucleus and cytoplasm in mammary tissues. Assessment of the staining was evaluated by two independent pathologists without knowledge of the clinical status of the patients.

2.2.3. VEGF Assay

VEGF was localized in the cytoplasm and the membrane. Cells were classified according to the positive rate and color intensity as follows: negative, number of positive cells<25%; positive, brown particles, number of positive cells≥25%.

2.2.4. MVD Assay

The MVD recognized by CD105 was evaluated under light microscopy according to the procedure described by Kopczyńska et al [3]. Briefly, after scanning the sections at low magnifications, five tumor areas with the greatest number of distinctly highlighted micovessels were selected. The number of vessels was counted in the highlighted micovessels at high magnifications (400×), and the average counts of the fields were recorded. Each brown-stained endothelial cell or endothelial cell cluster, which was clearly separate from the adjacent micovessels,

tumor cells and connective tissue elements was considered a single, countable microvessel.Sections were considered positive for Survivin and VEGF when more than 25% of tumor cells were stained in the cytoplasm or cell membrane.

2.3. Pathologic Histopathology Analysis

The pathological diagnosis was verified by histological methods independently by two pathologists, and pathological categorization was determined according to the current World Health Organization classification system (WHO 2012) [1], and the pathological diagnosis was verified by histological methods independently by two pathologists, and the pathologists were blinded to the subject's clinical history and the results of the immunohistochemistry staining assay. The pathological reading was determined for each biopsy slide with an overall pathological diagnosis determined for each subject. The tumor grade was determined according to the modified Bloom-Richardson score. The grade was obtained by summing the scores for tubule formation, nuclear pleomorphism, and mitotic count, which were scaled as 1, 2, or 3. The final scores ranged between 3 and 9 and were then divided into three grades (I–III). The final grading scores were as follows: sum of points, 3–5, final grade I; 6–7, II; and 8–9, III.

2.4. Statistical Analysis

SPSS version 17.0 statistical software was used to analyze the data. The MVD results were expressed as the mean and standard deviation (mean±SD). Because the distribution of MVD was not Gaussian, the nonparametric Mann-Whitney *U*-test was used to determine differences between the benign and malignant groups, and the nonparametric Kruskal-Wallis test was used for the analysis of differences among more than two groups. Enumeration data with chi-squared (χ^2) test. The relationship of this dichotomous variable to other clinicopathological correlates was established using χ^2 or Fisher's exact tests, as appropriate. Kaplan-Meier overall survival curves was constructed to demonstrate the survival differences between the Survivin-positive and negative patients. A *P* value less than α=0.05 was deemed statistically significant in two groups, and α'=0.0083, $\alpha'=\alpha/N$, N=n (n-1)/2 among four groups. All other statistical tests were performed using Graphpad Prism 5.0.

3. RESULTS

3.1. The Immunohistochemical Survivin and VEGF Expression in Different Tissue

3.1.1. Immunohistochemical Expression Location

In different mammary tissues, Survivin immunoreactivity expression the percentage of cancer cells showed cytoplasm and nuclear reactivity shown in Fig. (**1**). Survivin was only distributed in cytoplasm, and there is no distributed in nucleus,which was shown in Fig. (**1a**), Survivin was maily distributed in cytoplasm in atypical ductal hyperplasia mammary tissues, ductal carcinoma in situ shown in Fig. (**1b**) and invasive ductal carcinomas, and also distributed in some degree in nucleus, and in high histological grade the expression of survivin in the nucleus ratio increased shown in Fig. (**1c** and **1d**). Therefor, our results support that Survivin location changes from cell plasma to cell nucleus might participate in oncogenesis and development of breast cancer. Survivin was referred to as a bifunctional protein, having essential roles in inhibiting apoptosis and controlling proper cell division. Nuclear survivin as a cell cycle regulator. Cytoplasmic/mitochondrial survivin as an apoptosis inhibitor; extracellular survivin as a modulator of tumor microenvironment. But VEGF expression were located in the tumor cell cytoplasm no matter in usual duct hyperplasia lesions or breast carcinomas.

3.1.2. Survivin Expressions

The expressions of Survivin in diffrent groups were shown in Table **1**. The positive rates of Survivin in invasive carcinomas was 67.2%, in ductal carcinoma in situ was 59.6%, in atypical ductal hyperplasia was 57.9%, and in usual duct hyperplasia lesions tissues was 1.7%. Compaired with usual duct hyperplasia tissues group, there were significant differences of Survivin in invasive ductal carcinomas (χ^2=70.540, p=0.000), ductal carcinoma in situ (χ^2=51.967, P=0.000) and atypical ductal hyperplasia (χ^2=42.829, p=0.000) group, P<0.05, respectively, which were shown in Table **2**. There were significant differences in the expression rates of Survivin between ductal carcinoma in situ and invasive carcinomas (χ^2=12.298, P=0.001), atypical ductal hyperplasia and invasive carcinomas (χ^2=16.589, P=0.000), however, there were no significant differences between invasive carcinomas and ductal carcinoma in situ, invasive carcinomas

and atypical ductal hyperplasia tissue. Positive expression rates of Survivin was found no significant between atypical ductal hyperplasia and ductal carcinoma in situ tissues. In high histological grade invasive, the survivin expression in the nucleus ratio increased shown in Fig. (**1c** and **1d**).

Table 1.Survivin expresses in different tissues.

Groups	No. of Cases	Positive	Negative
IDC	128	86	42
DCIS	89	53	36
ADH	57	33	24
UDH	60	1	59

Table 2.Comparison of Survivin expresses.

Comparison	χ^2	*P* Value	Significance
IDC vs DCIS	1.330	0.249	NS.
IDC vs ADH	1.484	0.223	NS.
IDC vs ADH	70.54	0.000	Sig.
DCIS vs ADH	0.039	0.843	NS.
DCIS VS UDH	51.97	0.000	Sig.
ADH vs UDH	42.83	0.000	Sig.
Sig.: Significance; NS.: no Significance.			

3.1.3. VEGF Expressions

The expressions of Survivin and VEGF in diffrent groups were shown in Table **3**. The positive rates of VEGF in invasive ductal carcinomas was 68.8%, in ductal carcinoma in situ was 44.9%, in atypical ductal hyperplasia was 36.8%, and in usual duct hyperplasia lesions tissues was 20.0%. Compaired with usual duct hyperplasia lesions tissues group, there were significant differences of the positive rates of VEGF in invasive ductal carcinomas (χ^2=38.993, p=0.000), ductal carcinoma in situ (χ^2=9.815, p=0.002) and atypical ductal hyperplasia (χ^2=4.095,p=0.043) groups, P<0.05, respectively, which were shown in Table **4**. There were significant differences of VEGF expression between ductal carcinoma in situ and invasive ductal carcinomas, atypical ductal hyperplasia and invasive ductal carcinomas, however, there were no significant differences of VEGF between atypical ductal hyperplasia and ductal carcinoma in situ tissue (P>0.05, respectively).

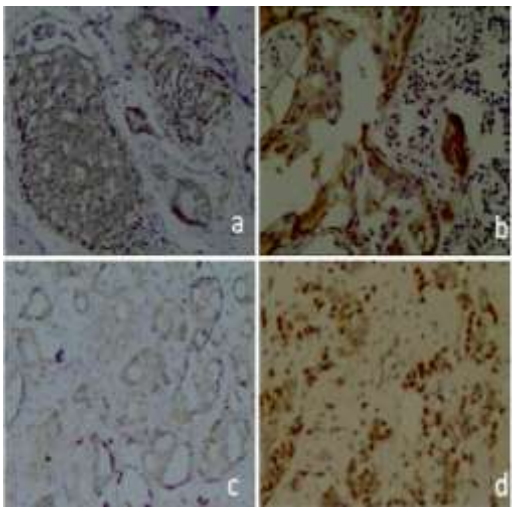

Fig. (1). Survivin expressions. (**a**) UDH tissue, weekly positive reactivity in cytoplasmic; (**b**) DCIS tissue, moderate positive in cytoplasmic; (**c**) Low grade breast carcinoma (grade I), maily distributed in cytoplasm, but also distributed in nucleus; (**d**) Highgrade (grade III) carcinoma, distributed in nucleus or cytoplasms trongly, and the nucleus expression percentage increased.

3.2. Relationship between Survivin, VEGF Expression and Biological Parameters in Breast Cancer

3.2.1. Survivin Express with Biological Parameters

The express of Survivin and VEGF and their relationship with clinical-pathological parameters in mammary cancer shown in Table **5**. There was positive correlation in over-expressions of Survivin and VEGF with high histological grade (III) with lymph node metastasis, distant metastasis and advanced stage (III+IV) of invasive ductal carcinomas tumor (P<0.05, respectively), and the expressions of Survivin were not related with age (≤50 yr vs 50yr) and size of tumor (≤5cm vs >5cm) (P>0.05, respectively). There was significantly difference in the mean express of Survivin frequency between ER or PR positive and negative groups (P<0.05, not shown in Table.5

3.2.2. VEGF Express with Biological Parameters

Relationship between COX-2 and clinical-pathological parameters of breast cancer was shown in Table **5** and Fig. (**2**)**.** There was positive correlation in over-expression of VEGF in breast carcinomas with histological grade (I or II *vs* III), lymph node metastasis and distant metastasis (P<0.05, respectively). However, the expressions were not related

with age (≤50 yr *vs* 50 yr) and size of tumor (≤5 cm *vs* >5 cm) (*P*>0.05, respectively).

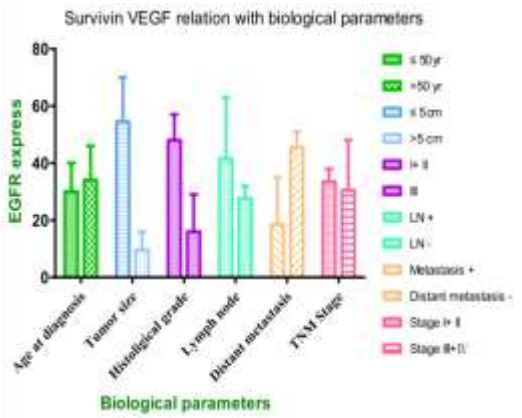

Fig. (2). VEGF express relation with biological parameters in breast cancer.

3.2.3. Correlation between Survivin and VEGF Expresses

The correlation between Survivin and VEGF Expresses in breast carcinoma was shown in Table **6**. The expression of VEGF was correlated positively with Survivin in breast carcinoma (r=0.211, P<0.05).

3.3. MVD in Different Tussue and Relationship with Clinicopathological Characteristics in Breast Cancer

3.3.1. MVD Expressions in Breast Carcinomas and Epithelium Adjacent Tussues

The various breast tissues and epithelium adjacent to the lesion were immunohistochemically stained for CD105, and MVD recognized by CD105 was assessed based on the number of CD105-positive vessels. Immunohistochemical results were shown and listed in Table **7** and Fig. (**3a**). Compaired with UDH groups, the MVD had statistical significance in IDC, DCIS, ADH, P<0.05, respectively. MVD in the peripheral area adjacent to the lesion was significantly higher than those central area within the lesion in every group (P<0.01 for each group), in Table **7** and Fig. (**3b, c**).

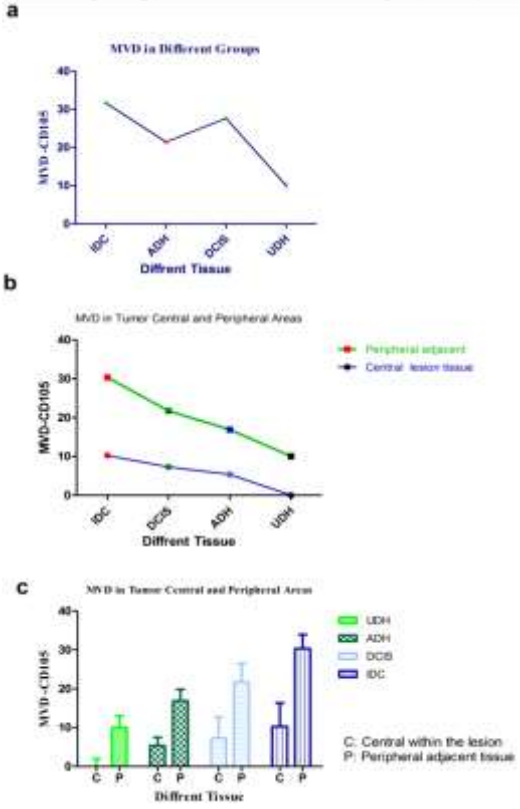

Fig. (3). The MVD in different groups (**a**) and comparison of MVD in tumor central and peripheral Areas (**b** and **c**).

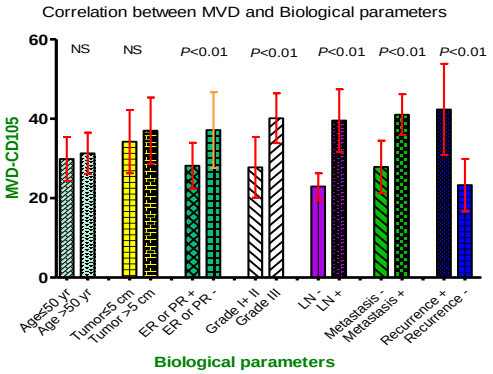

Fig. (4). Correlation between MVD and biological parameters of breast carcinoma.

Table 3.VEGF expresses in different tissues.

Groups	No. of	Positive	Negative
IDC	128	88	40
DCIS	89	40	49
ADH	57	21	36
UDH	60	12	48

Table 4.Comparison of VEGF expresses.

Comparison	χ^2	*P* Value	Significance
IDC vs DCIS	12.298	0.000	Sig.

IDC vs ADH	16.590	0.000	Sig.
IDC vs ADH	38.990	0.000	Sig.
DCIS vs ADH	0.938	0.333	NS.
DCIS VS UDH	9.815	0.002	Sig.
ADH vs UDH	4.095	0.0043	Sig.
Sig.: Significance; NS.: no Significance.			

Table 5. Survivin and VEGF express relation with biological parameters in breast cancer.

Biological parameters	Number of Cases	Survivin				VEGF			
		Positive	Positive rate	χ^2	*P* Value	Positive	Positive rate	χ^2	*P* Value
Age at diagnosis									
≤50 yr	60	40	58.8%	0.014	0.906	42	70.0%	0.082	0.774
>50 yr	68	46	67.6%			46	67.6%		
Tumor size									
≤5 cm	109	70	64.2%	2.933	0.087	73	67.0%	1.080	0.299
>5 cm	19	16	84.2%			15	78.9%		
Histoligicalgrade									
I+ II	96	57	59.4%	10.631	0.001	58	60.4%	12.412	0.001
III	32	29	90.6%			30	93.8%		
Lymph node metastasis									
Present	83	63	75.9%	8.135	0.004	64	77.1%	7.677	0.006
Absent	45	23	51.1%			24	53.3%		
Distant metastasis									
Present	37	35	94.6%	17.732	0.000	32	86.5%	7.621	0.006
Absent	91	51	56.0%			56	61.5%		
Stage									
I+ II	67	38	56.7%	6.992	0.008	34	50.7%	21.211	0.000
III+IV	61	48	78.7%			54	88.5%		

Table 6. Correlation between Survivin and VEGF expresses in breast carcinoma.

VEGF	n	Survivin		Kappa	*P* Value
		Negative	Positive		
Negative	40	19	21	0.211	0.017
Positive	88	23	65		

Table 7. MVD-CD105 in different groups and compariso n of MVD in tumor central and peripheral areas.

Groups	n	Lesions			Comparison of Tumor Central and Peripheral Areas			
		MVD ($\bar{x}\pm s$)	*t* value	*P* value	Central within the lesion	Peripheral adjacent to the lesion	*t* value	*P* value
IDC	128	31.691±8.621	-	-	10.234±6.127	30.363±3.591	47.275	0.00#
DCIS	89	27.633±6.879	3.696	<0.01*	7.275±5.443	21.751±4.732	12.496	0.00#
ADH	57	21.436±5.112	10.056	<0.01*	5.395±2.121	16.911±2.953	15.856	0.00#
UDH	53	10.038±3.976	22.105	<0.01*	0.000±0.000	10.038±3.976	-	-

*Compared with IDC, DCIS, ADH, UDH all *P*<0.01. # Comparison of tumor central and peripheral areas.

Table 8. Correlation between MVD and biological parameters of breast carcinoma.

Biological parameters	No	CD105-MVD		
		MVD ($\bar{x}\pm s$)	*t*	*P*
Age at diagnosis				
≤50yr	60	29.887±5.556	1.090	0.278
>50yr	68	31.282±5.223		
Tumor size				
≤5cm	46	34.245±7.984	1.818	0.07
>5cm	82	36.996±8.362		
ER or PR status				
positive	78	28.194±5.766	6.628	<0.01
negative	50	37.144±9.519		
Histoligical grade				
I+II	96	27.769±7.62	8.272	<0.0

		5		1
III	32	40.125±6.282		
Lymph node metastasis				
Absent	45	22.936±3.307	15.801	<0.01
Present	83	39.549±7.873		
Distant metastasis				
Absent	91	27.887±6.616	10.839	<0.01
Present	37	41.046±5.126		
Recurrence				
positive	22	42.366±11.497	23.639	<0.01
negative	106	23.273±6.618		

3.3.2. Relationship between MVD and Clinical pathological Parameters in breast cancer

The clinical-pathological parameters and their relation-ship with the express of MVD in IDC were shown in the Table **8** and Fig. (**4**). There was a significantly difference in the express of MVD frequency between ER/PR positive and negative IDC patients, histoligical grade (I+II) and grade III IDC, lymph node and distant metastasis (P<0.05). However, there was not difference in the mean express of CD105-MVD frequency between age at diagnosis (≤50yr vs >50yr) and tumor size (≤5cm vs >5cm), respectively (P>0.05).

3.4. Comparison of Survivin, VEGF Express with Chemotherapy and Survival Analysis

Survivin, VEGF expression and the situation before and after chemotherapy in 52 cases of mammary cancer to neoadjuvant chemotherapy was studied. Before and after chemotherapy, the expression of Survivin and VEGF has decreased significantly, P<0.05, respectively. Before and after chemotherapy, the expressions of Survivin and VEGF are positive correlation, P<0.05. According to International Union against Cancer, TNM classification of solid malignant tumors standard and American Joint Committee on Cancer Cancer Staging Manual to evaluate curative effect of therapeutic effect [8, 9]. Complete remission (CR): all known lesions disappeared at least up to 4 weeks; Partial response (PR) : measurable lesions, the total volume by 50% at least more than 4 weeks and no progression or other lesions; stable condition (SD): one or more of the measurement volume reduced less than 50% of the lesions or increased less than 25%, the time for at least four weeks; Disease progression (PD): one or more measurable lesion volume increases more than 25% or the emergence of new lesions. CR and PR for the total effective rate. Before neoadjuvant chemotherapy, the chemosensitivity of Survivin and VEGF positive expression was worse than the chemosensitivity of those nagetive expression (P<0.05). Survivin and VEGF can be used as a predictor of mammary cancer chemosensitivity to help develop individualized chemotherapy.

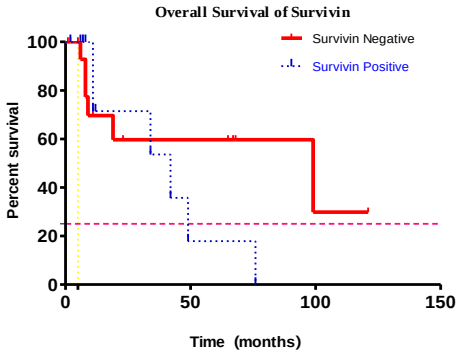

Fig. (5). Overall survival curves of the patient population under study (Kaplan-Meier).

Patients were monitored for survival from 2003 to 2015 through telephone communication and periodic returns to the Rizhao People's Hospital. Follow-up information was available for 86 of the patients. Seventeen (19.8%) of these patients relapsed, and nine (10.5%) died during the course of the follow-up period. Overall survival was defined as the period (months) between surgical removal and death caused by the neoplastic process. The patients whose deaths occurred from any other cause were not taken into account. Patients were censored if the follow-up period was less than 6 months. For Survivin and VEGF expression, the patients were divided into negative and positive two groups. For MVD, the patients were also classified two groups according to the median values: median 30 or below, median more than 31. Considering only the Survivin and VEGF expression, survival was found to be longer in Survivin negative group than that in positive group (P<0.05), which was shown in Fig. (**5**). When overall survival

and MVD were analyzed, patients with increased vascularization presented shorter overall survival (P<0.05).

4. DISCUSSION

Breast cancer is one of the common malignancies in women and its morbidity is on the rise year by year both in developed and developing countries [1]. It is a major public health problem throughout the world. Accounting for 23% of all cancers in women globally, it is more than twice as common as cancer at any other site [1, 2, 10]. Breast intraductal proliferative lesions (IDPLs) are a group of cytologically and architecturally diverse proliferations, typically originating in the terminal-duct lobular unit and confined to the mammary ductal-lobular system [1, 10]. Most invasive ductal carcinomas are associated with more than one histologic subtype of intraductal proliferative lesions and intraductal proliferative lesions are associated with an increased risk, albeit of different magnitudes, for the subsequent development of invasive carcinoma [1]. Some of these lesions are best considered as risk indicators whereas others are recognized as true precursors of invasive breast cancer [1, 10]. Precancerous lesions are associated with different levels of risk for development of breast invasive carcinoma that range from approximately l.5 times that of the reference population for UDH, to 3-5-fold for ADH, and 8-10-fold for DCIS [1]. The long-held notion of a linear progression from normal epithelium through UDH, ADH and carcinoma in situ to invasive cancer is overly simplistic. Breast precancerous lesions are characterized by an increase in the number of cells resulting in total alteration and distension of the normal unit structure of the breast without increasing in number. In fact, the interrelationship between these various intraductal proliferative lesions and IDCs is far more complex. DCIS is a segmental disease, originating in the terminal duct-lobular unit (TDLU) and progressing within the duct system toward the nipple and into adjacent branches of a given segment of the duct system, and involvement of the segment may be extensive and skipped areas may occur, especially in lesions of low nuclear grade compared to UDH diseases in many ADH, DCIS and IDC. In a small proportion of cases, high-grade DCIS may be sufficiently extensive and exhibit such an abundance of intraluminal necrosis or associated stromal reaction that it presents as multiple areas of round, pale comedo necrosis or a firm, gritty mass [1, 10]. Some of these lesions are coexist with IDC and often present more than one histologic architectural subtype (50.15%) [1, 10]. In this study, age of the breast precancerous lesions ranged from 21 to 78 years, mean 47.3 years, and the mean age at which DCIS and breast cancer diagnosed is in the fifth dacade. ADH shares many similarities with DCIS as neoplastic lesion in morphological, immunohistochemical and molecular features with ADH and DCIS. These data support that ADH and DCIS represent intraepithelial neoplasias. UDH is not a significant risk factor and in most cases is unlikely to represent a precursor lesion. However, there are some genomic data to suggest that a small proportion of UDH can harbour clonal populations of cells, which indicates that clonal lesions such as ADH may occasionally arise in this setting These emerge more immunophenotypical and molecular-genetic studies and the increasingly frequent detection of ADH and low-grade DCIS.

The incidence of breast cancer is signifcantly on the rise all over the world, hence, how to early diagnosis, early treatment, correctively evaluate the prognosis and find the postoperative recurrence of patients with breast cancer have been paid attention by more and more scholars allover the world [1, 2]. Early diagnosis, surgical treatment with systematic lymph node dissection and appropriate chemotherapy have improved the survival of patients with breast cancer. However, even after a curative resection, tumor recurrences are likely to assume a variety of forms in various organs. The prediction of risks for recurrences as well as recurrence patterns after surgery could help the design of better follow-up programmes and appropriate treatment strategies for breast cancer patients. However, in spite of advances in diagnostic techniques and surgical procedures, the prognosis after resection has remained unsatisfactory due to a high incidence of cancer lymph node metastases and cancer recurrence. The identification of variables in breast tumor biology may lead to a more precise assessment of outcome and response to therapy. The development of prognostic markers that can accurately predict outcome is crucial to identify patients who could benefit from aggressive therapy. Early

©M&H ACADEMIC PUBLISHER

diagnosis of breast cancer is challenging due to a lack of serum biomarkers and, inadequate as it is, performed through invasive means such as needle biopsy, scanning, and invasive pathological examination. Despite the availability of numerous diagnostic and prognostic methods, there remains a need for an easy, sensitive, and noninvasive way to track tumor activity. Numerous studies worldwide have sought to determine the most effective ways to treat breast cancer, assess the rapeutic effects, correctly evaluate prognosis, and identify postoperative recurrence in patients. Hypoxia, is a common feature of various cancers. Solid tumors are characterized by regions of low oxygen tension, which play a central role in tumor progression and resistance to therapy. Cells under hypoxic conditions develop numerous adaptive responses to hypoxic stress concurrently with altered expression of hundreds of genes that are regulated by hypoxia inducible factors [2,4,11-14]. Low oxygen tension affects mitochondrial function and for the cells to survive, mitochondria must functionally adapt to low oxygen tension to maintain the cellular bioenergetics [2, 4, 12]. HIF-1α is an important cellular survival protein under hypoxic conditions, regulating the cellular response to low oxygen tension via recruitment of a transcriptional co-activator, induces expression of multiple genes involved in cell survival, proliferation, angiogenesis, and tumor development [2,4,11-14]. Mammary cancer is one of the commonly-encountered solid malignant tumors, like most other forms of malignancy, occur as a result of HIF-1α of the effects of environmental and heritable factors. In this study, we analyze the expressions of Survivin and VEGF in mammary cancer, and the relationship between the expression and clinico-pathological parameters including stage, grade, lymph node metastasis, distant metastasisand recurrence, and the combined detecting MVD levels, and achieved a better application effect. We also detect differences Survivin and VEGF expression situation before and after chemotherapy of mammary cancer to neoadjuvant chemotherapy. In our study, the over-expression rates of Survival and VEGF in mammary cancer were significantly higher than benign leisions (P<0.01). Our result revealed that the cells of immunostaining for Survival and VEGF overexpression is more common in invasive mammary cancer than mammary cancer benign leisions.

Survivin is referred to as a bifunctional protein, having essential roles in inhibiting apoptosis and controlling proper cell division [2, 4]. The multifaceted functionality of survivin is still being intensely scrutinized, and it appears that protein compartmentalization may be important. Survivin has been shown to localize in mitochondria, where it modulates tumor cell apoptosis similar to the Bcl-2 family [2, 4, 15]. Our study shown the positive rates of Survivin in invasive ductal carcinomas was 67.2%, in ductal carcinoma in situ was 59.6%, in atypical ductal hyperplasia was 57.9%, and in usual duct hyperplasia lesions tissues was 1.7%. Compaired with usual duct hyperplasia lesions tissues group, there were significant differences of the positive rates of Survivin in invasive ductal carcinomas (χ^2=70.540, p=0.000), ductal carcinoma in situ (χ^2=51.967, p=0.000) and atypical ductal hyperplasia (χ^2=42.829, p=0.000) group, P<0.05, respectively. The positive rates of Survivin in invasive ductal carcinomas was 67.2%, in ductal carcinoma in situ was 59.6%, in atypical ductal hyperplasia was 57.9%, and in usual duct hyperplasia lesions tissues was 1.7%. Compaired with usual duct hyperplasia lesions tissues group, there were significant differences of the positive rates of Survivin in invasive ductal carcinomas (χ^2=70.540, p=0.000), ductal carcinoma in situ (χ^2=51.967, p=0.000) and atypical ductal hyperplasia (χ^2=42.829, p=0.000) group, P<0.05, respectively. The results demonstrate that Survivin might participate in oncogenesis and development of breast cancer. The over-expression of Survivin might be important biological markers for breast invasive carcinomas. The detaction of Survivin is the predictor for prognosis of breast carcinoma.

Its localization to the nucleus and cytosol confers its role in mitosis regulation and apoptosis inhibition, respectively [2, 4, 16]. This study our result revealed that Survivin was maily distributed in cytoplasm in UDH, but also distributed in nucleus and cytoplasm in IDC, DCIS and ADH mammary tissues Compaired with UDH tissues group, there were significant differences of the positive rates of Survivin in IDC, DCIS and ADH group, P<0.05, respectively. However, there were no significant differences of Survivin expression between IDC and DCIS, IDC and ADH tissue. Positive expression rates of

©M&H ACADEMIC PUBLISHER

Survivin was found no significant between ADH and DCIS tissue. Our results demonstrate that Survivin location changes from cell plasma to cell nucleus might participate in oncogenesis and development of breast cancer. Nuclear survivin is known to be a cell-cycle-associated protein. Investigations of cell division regulation during the depletion of survivin by small interfering (si)RNA demonstrated an increase in mitotic arrest and chromosomal misalignment. Furthermore, Survivin is involved in microtubule assembly and centromere stabilization during mitosis[2, 16]. Survivin have been shown to dramatically increase upon exposure to hypoxia [2,16-18]. Furthermore, survivin's promoter has been shown to contain three putative HIF-1 binding or response elements. Nuclear survivin was found to be distinctly involved in the prognosis of different cancers, as will be discussed in our specific cancers section. Survivin's ability to interfere with cellular death pathways appears to reside in the cell's cytoplasm. Survivin localizes to the mitochondria and therefore may provide, like Bcl-2, a role in mitochondrial stability [2]. Cellular stress was shown to modulate the expression and localization of surviving, with hypoxia-induced survivin found exclusively in the mitochondria. Furthermore, upon apoptotic stimulation, mitochondrial survivin is rapidly released to the cytosol where its cytoprotective effects prevent the activation of the initiator caspas [2]. Early studies showed that survivin and XIAP protected cells from undergoing caspase-dependent apoptosis. Subsequently, in vitro binding experiments showed that survivin, like XIAP and other IAPs, bound to the terminal effector cell death proteases, caspases 3 and 7, but not to initiator caspase 8[2]. Controversy in the field arose when a study showed that survivin did not inhibit caspase 3 activity, and where recombinant survivin failed to decrease recombinant caspase 3 activity in vitro. Current evidence suggests that survivin acts on caspases in an indirect manner by binding to the hepatitis B X-interacting protein (HBXIP) and forming a complex with procaspase 9, inhibiting the apoptosome formation. This survivin–HBXIP complex, not individual survivin or HBXIP proteins, binds to procaspase 9 and works to prevent recruitment of apoptosis activating factor 1 (Apaf1), thus suppressing intrinsic apoptosis. In addition, survivin binds to and regulates the stability of XIAP, which is a direct caspase 3 and 9 inhibitor.27 More specifically, the formation of a survivin–XIAP complex promotes increased XIAP stability, protecting XIAP from proteasomal degradation, resulting in a facilitated inhibition of caspase-dependent cell death[2]. Survivin has recently been shown to exist in the extracellular space, via 40-100 nm membrane vesicles called exosomes [2,19]. Various cell types, such as B- and T-lymphocytes, dendritic cells, neurons, intestinal epithelial cells, as well as tumor cells, release exosomes. In particular, it has been shown that both human and mouse tumor cells release tumor cell-derived exosomes (TEX) constitutively. Additionally, specific protein content found both on and within TEX give an indication of their functional and biological roles, and their cell of origin, making TEX excellent biomarkers [20]. Now we regard nuclear survivin as a cell cycle regulator, Cytoplasmic/mitochondrial survivin as an apoptosis inhibitor, extracellular survivin as a modulator of tumor microenvironment, and Survivin in cancer immunity evasion [2, 4, 20-23]. Survivin has been ascribed multiple roles not only in malignancy but also in immunity and differentiation. Survivin has been shown to be essential for T-cell maturation, homeostasis, and proliferation at various stages of development [24-26]. It has also been shown to modulate peripheral blood leukocytes when in the extracellular space by binding to leukocytes, thereby inducing molecular processes implicated in the pathogenesis of inflammation [2, 24-27]. The different subcellular pools of survivin in breast cancer appear to have distinct functions. Adamkov et al suggested that nuclear staining of the survivin antigen could be used as a marker of the degree of neoplasia[28], while Rexhepaj et al suggested that increased levels of nuclear surviving are associated with a proliferative phenotype [29]. One thing that is clear is that survivin plays a key role in the initiation and progression of breast cancer. High messenger (m)RNA expression was found to be an independent prognostic marker in breast cancer patients and survivin upregulation significantly correlated to lymph node involvement, tumor stage, and histological type[30-31].

In this study, Survivin, VEGF expression and the situation before and after chemotherapy in 52 cases of mammary cancer to neoadjuvant

©M&H ACADEMIC PUBLISHER

chemotherapy was studied. Before and after chemotherapy, the expression of Survivin and VEGF has decreased significantly, $P<0.05$, respectively. Before and after chemotherapy, the expressions of Survivin and VEGF are positive correlation, $P<0.05$. Our study have shown that high levels of its expression are associated with a beneficial response to chemotherapy.This could be due to alternative splicing of survivin. Multiple studies demonstrate that alternative splicing patterns are altered during cancer progression. Several different mechanisms contribute to changes in the regulation of alternative splicing including stress, stimulation of receptors by growth factors, cytokines, hormones, etc. Survivin, to date, has six different described variants with different apoptotic properties and intracellular localization [32]. Protein and Mrna levels of the pro- and antiapoptotic isoforms of surviving correlate with cancer prognosis. It is very important to specifically target survivin in a defined location for therapeutic purposes. Survivin is a unique inhibitor of apoptosis with triple functionality: in cell cycle regulation when it is present in the nucleus; inhibition of apoptosis when it is in the mitochondria; and resistance to chemotherapy when it exists in the tumor microenvironment packaged in exosomes. Survivin's upregulation in specific cancers, in addition to its presence in serum exosomes, makes it an important molecule as a diagnostic as well as prognostic marker. Unfortunately, controversy exists as to whether survivin expression is favorable or unfavorable in the outcome of cancer. Survivin expression is an unfavorable prognostic indicator in esophageal, hepatocellular, and ovarian cancers, cholangiocarcinoma, and endometrial cancers, but it has associated favorable outcomes in gastric, bladder, breast, ependymoma osteosarcoma, and pancreatic ductal adenocarcinomas. To validate its role, a large number of case-control studies need to be adapted. Subsequent studies exploiting the exosomal packaging of survivin may also 1 day be used in cancer therapeutics.

Vascular endothelial growth factor (VEGF) capable of promoting angiogenesis exerts an important effect in the process of genesis, development, metastasis and recurrence of various tumors. In the process of tumor genesis and development, tumor regenerative capillaries capable of providing nutrients for tumor cells and favorable conditions for distal metastasis are the precondition to induce the local growth, infiltration and distal metastasis of malignant tumors, hence, how to inhibit tumor angiogenesis is a new research hotspot at present [3,6,33,34]. VEGF, one of the key factors to promote tumor angiogenesis and with the strongest function and highest specificity, can not only promote the proliferation of endothelial cells, but also regulate and participate in angiogenesis. Due to an intimate association with genesis, development, metastasis and infiltration of breast cancer, it is an important indicator to judge the metastasis and infiltration of breast cancer in clinic. VEGF was localized in the cytoplasm and the membrane. In the study, VEGF expression in different pathological stagings of patients in observation group were analyzed. The results revealed that with pathological staging increasing, the levels of VEGF in observation group gradually increased, and the statistical significance was remarkably presented by comparison ($P<0.01$). There was significant difference regarding VEGF expression in different pathological stagings of patients ($P>0.05$). In the study, VEGF expression in the patients with and without lymph node metastasis were compared in observation group. Research results revealed the patients with lymph node metastasis were markedly higher than those without lymph node metastasis, and the difference had statistical significance ($P<0.01$). There was posive correlation in over-expression of VEGF with histological grade and lymph node metastasis and distant metastasis of breast cancer, and the expressions of VEGF were not related with age and size of tumor ($P>0.05$). VEGF, which promotes angiogenesis and promotes the proliferation of endothelial cells, also exerts an important effect in the genesis, development, metastasis, and recurrence of various tumors. In this study, the positive VEGF rate in recurrence group were obviously higher than in non-recurrence group ($P<0.01$). The result suggest that detection of VEGF indicator above can judge the prognosis better, which is of great importance to monitor recurrence and metastasis.

In our study, we investigated the prognostic significance of CD105 and MVD assessed based on the number of CD105-positive vessel in various tissues of breast invasive breast cancer and benign breast leisions and

©M&H ACADEMIC PUBLISHER

epithelium adjacent to the lesion. The results showed that the high expressions of CD105 and MVD are significantly associated with worse prognosis in all cases and histoligical grade (III), tumor invasion and lymph node metastasis. Tumor angiogenesis and its clinical significance have been studied in a variety of neoplasms [3,33,35,36]. Our results demonstrate that there was positive correlation in over-expressions of VEGF with histological grade, lymph node metastasis, distant metastasis and stage of IDC tumor, and the expression of VEGF was correlated positively with Survivin (r=0.211, P=0.017). Our study suggest that angiogenesis contributes to the pathogenesis of various cancer, and microvessel density may improve our ability to predict breast cancer extension. In breast carcinoma, abnormal express of CD105 and MVD is associated with poor differentiation, like higher grade lesions and metastatic disease. Microvessel densities are significantly greater in the primary tumors of patients with metastatic disease than in those without metastases [3,35]. In addition, an association between microvessel density peripheral area adjacent to the lesion and those central area within the lesion in every group has been observed. It is interesting that microvessel density peripheral area tissue adjacent to the lesion is significantly higher than those central area within the lesion in breast cancer. CD105 is a proliferation-associated and hypoxia-inducible glycoprotein abundantly expressed in angiogenic endothelial cells, and it is essential in angiogenesis. The intensity of staining for CD105 is greater in blood vessel endothelia within neoplastic than within normal tissues, indicating that CD105 is a powerful marker of neovascularization in solid malignancies. However, in spite of advances in diagnostic techniques and surgical procedures, the prognosis after resection has remained unsatisfactory due to a high incidence of cancer lymph node metastases and cancer recurrence. The identification of variables in breast tumor biology may lead to a more precise assessment of outcome and response to therapy.

Our results suggest that in mammary cancer, abnomlally expressions of Survivin, VEGF and MVD seem to as event in carcinoma development. The results in the study revealed that Survivin, VEGF and MVD are positive in the patients with mammary cancer. Our results support that the over-expression VEGF might be important biological markers for invasion and metastasis of breast invasive carcinomas, the combined detaction of Survivin and VEGF are the predictors for prognosis of breast carcinoma; MVD is closely relevant to lymph node metastasis and recurrence, and act as a valuable indicators of prognosis and as tumour angiogenesis markers, useful for cancer diagnostics and clinical application. The combination of biomarkers may improve the ability to identify cancer patients at high risk of disease. In our study, before and after chemotherapy, the expression of Survivin and VEGF has decreased significantly. Before and after chemotherapy, the expressions of Survivin and VEGF are positive correlation. Before neoadjuvant chemotherapy, the chemosensitivity of Survivin and VEGF positive expression was worse than the chemosensitivity of nagetive expression (P<0.05). The result suggest that detection of VEGF indicator above can judge the prognosis better, which is of great importance to monitor recurrence and metastasis. Survivin is referred to as a bifunctional protein, having essential roles in inhibiting apoptosis and controlling proper cell division. Nuclear survivin as a cell cycle regulator. Cytoplasmic/ mitochondrial survivin as an apoptosis inhibitor; extracellular survivin as a modulator of tumor micro-environment. Our results implicate the importance of Survivin and VEGF can be used as a predictor of mammary cancer chemosensitivity to help develop individualized chemotherapy. However, further study is needed to understand the exact pathogenic mechanism. How to treat mammary cancer, effectively assess the therapeutic effect, correctively evaluate the prognosis and find the postoperative recurrence of patients with mammary cancer have been paid attention by more and more and further study is needed to understand the exact pathogenic mechanism.

CONCLUSION

In this study, we analyze Survivin, VEGF and MVD, and MVD the peripheral area adjacent to the lesion and those central area within the lesion in every group, and analyze the relationship between the MVD and clinicopathological parameters including staging, grading, estrogen and progesterone receptors. Take integrated analysis of the

©M&H ACADEMIC PUBLISHER

above results, and the following conclusions can be drawn:

(1) Our results support that Survivin location changes from cell plasma to cell nucleus might participate in oncogenesis and development of breast cancer.

(2) The over-expression of Survivin and VEGF might be important biological markers for invasion and metastasis of breast invasive carcinomas. Meanwhile, the abnomlally expressions of Survivin, VEGF and MVD rate in the patients with metastasis, recurrence and prognosis are also conspicuously higher than those without.

(3) The combined detaction of Survivin, VEGF and MVD are the predictors for prognosis of breat carcinoma. The over-express Survivin, VEGF and MVD is correlated with worse prognosis.

(4) In our study, before and after chemotherapy, the expression of Survivin and VEGF has decreased significantly. Before and after chemotherapy, the expressions of Survivin and VEGF are positive correlation. Before neoadjuvant chemotherapy, the chemosensitivity of Survivin and VEGF positive expression was worse than the chemosensitivity of nagetive expression. Our results implicate the importance of Survivin and VEGF can be used as a predictor of mammary cancer chemosensitivity to help develop individualized chemotherapy. However, further study is needed to understand the exact pathogenic mechanism.

REFERENCES

[1] Lakhani S.R., Ellis I.O., Schnitt S.J. WHO classification of tumours of the breast. Lyon, France: IARC Press, 1-117, 2012.

[2] Khan S., Ferguson B.H., Asuncion V.M.M. Localization and upregulation of surviving in cancer health disparities: a clinical perspective. Biologics: Targets and Therapy.9(9): 57-67, 2015.

[3] Wang G., Qin Y., Zhang J., et al. Nipple Discharge of CA15-3, CA125, CEA and TSGF as a New Biomarker Panel for Breast Cancer. Int. J. Mol. Sci. 15, 9546-9565, 2014.

[4] Witteveen A., Kwast A.B., Sonke G.S. Survival after locoregional recurrence or second primary breast cancer: impact of the disease-free interval. PLoS One, 10(4): e0120832, 2015.

[5] Sueoka E., Sueoka-Aragane N., Sato A. Development of lymphoproliferative diseases by hypoxia inducible factor-1αlpha is associated with prolonged lymphocyte survival. PLoS One, 8: e57833, 2013.

[6] Kopczyńska E., Makarewicz R. Endoglin-a marker of vascular endothelial cell proliferation in cancer. WspolczesnaOnkol, 16: 68-71,2012.

[7] Kim M.J., Kim H.S., Lee S.H. NDRG2 controls COX-2/PGE2-mediated breast cancer cell migration and invasion. Mol Cells, 37(10): 759-765, 2014.

[8] Edge S.B., Byrd D.R., Compton C.C. eds. American Joint Committee on Cancer (AJCC) Cancer Staging Manual 7th ed. New York, Springer, 2009.

[9] Sobin L.H., Gospodarowicz M.K., Wittekind Ch. eds. International Union against Cancer (UICC): TNM classification of malignant tumors 7th ed. Wiley-Blackwell, Oxford, 2009.

[10] Wang G., Zhang H., Zhang Z. Clinical and pathological characteristics of intraductal proliferative lesions and coexist with invasive ductal carcinomas.Chinses-German J ClinOncol, 12: 574-580, 2013.

[11] Sueoka E., Sueoka-Aragane N., Sato A. Development of lymphoproliferative diseases by hypoxia inducible factor-1αlpha is associated with prolonged lymphocyte survival. PLoS One, 8: e57833, 2013.

[12] Semenza G.L. Defining the role of Hypoxia inducible factor 1 in cancer biology and therapeutics. Oncogene, 29: 625-634, 2010.

[13] Dewi F.R., Fatchiyah F. Methylation impact analysis of erythropoietin (EPO) Gene to hypoxia inducible factor-1α (HIF-1α) activity. Bioinformation, 9: 782-787, 2013.

[14] Liang Y., Li Z., Wang G. Expression of HIF-1α in breast cancer and precancerous lesions and the relationship to clinicopathological features. Chinese-German J ClinOncol, 13:23-28, 2014.

[15] Fortugno P., Wall N.R., Giodini A. Survivin exists in immunochemically distinct subcellular pools and is involved in spindle micro tubule function. J Cell Sci., 115(Pt 3):575-585, 2002.

[16] Church D.N., Talbot D.C. Survivin in solid tumors: rationale fordevelopment

of inhibitors. CurrOncol Rep., 14(2):120-128, 2012.

[17] Simpson R.J., Lim J.W., Moritz R.L., Mathivanan S. Exosomes: proteomicinsights and diagnostic potential. Expert Rev Proteomics. 6(3): 267-283, 2009.

[18] Greening D.W., Gopal S.K., Xu R., Simpson R.J., Chen W. Exosomes andtheir roles in immune regulation and cancer. Semin Cell Dev Biol. 40: 72-81, 2015.

[19] Khan S., Jutzy J.M., Aspe J.R., McGregor D.W., Neidigh J.W., Wall N.R. Survivin is released from cancer cells via exosomes. Apoptosis. 16 (1): 1-12, 2011.

[20] Alečković M., Kang Y. Regulation of cancer metastasis by cell-free miRNAs. Biochim Biophys Acta. 1855(1): 24-42, 2015.

[21] Khan S., Jutzy J.M., Valenzuela M.M. Plasma-derived exosomalsurvivin, a plausible biomarker for early detection of prostate cancer.PLoS One. 7 (10): e46737, 2012.

[22] Park J.O., Choi D.Y., Choi D.S. Identification and characterization of proteins isolated from microvesicles derived from human lung cancerpleural effusions. Proteomics. 13(14): 2125-2134, 2013.

[23] Shender V.O., Pavlyukov M.S, Ziganshin R.H. Proteome-metabolome profiling of ovarian cancer ascites reveals novel components involved in intercellular communication. Mol Cell Proteomics. 13(12): 3558-3571, 2014.

[24] Pietra G., Manzini C., Rivara S. Melanoma cells inhibit naturalkiller cell function by modulating the expression of activating receptors and cytolytic activity. Cancer Res. 72(6): 1407-1415, 2012.

[25] Jutzy J.M., Khan S., Asuncion-Valenzuela M.M., Milford T.A., Payne K.J.,Wall N.R. Tumor-released survivin induces a type-2 t cell response anddecreases cytotoxic T cell function, in vitro. Cancer Microenviron. 6(1): 57-68, 2013.

[26] Dehal A., Abbas A., Johna S. Racial disparities in clinical presentation,surgical treatment and in-hospital outcomes of women with breast cancer: analysis of nationwide inpatient sample database. Breast CancerRes Treat. 139(2): 561-569, 2013.

[27] Johnson R.H., Chien F.L., Bleyer A. Incidence of breast cancer with distantinvolvement among women in the United States, 1976 to 2009. JAMA. 309(8): 800-805, 2013.

[28] Adamkov M., Kajo K., Vybohova D., Krajcovic J., Stuller F., Rajcani J. Correlations of survivin expression with clinicomorphological parameters and hormonal receptor status in breast ductal carcinoma. Neoplasma. 59(1): 30-37, 2012.

[29] Rexhepaj E., Jirstrom K., O'Connor D.P. Validation of cytoplasmicto-nuclear ratio of survivin as an indicator of improved prognosis inbreast cancer. BMC Cancer. 10: 639, 2010.

[30] DedićPlavetić N., Jakić-Razumović J., Kulić A., Vrbanec D. Prognosticvalue of proliferation markers expression in breast cancer. Med Oncol. 30 (2): 523, 2013.

[31] Xu C., Yamamoto-Ibusuki M., Yamamoto Y. High survivin mRNA expression is a predictor of poor prognosis in breast cancer:a comparative study at the mRNA and protein level. Breast Cancer. 21(4): 482-490, 2014.

[32] Necochea-Campion R.D., Chen C.S., Mirshahidi S., Howard F.D., Wall N.R.Clinico-pathologic relevance of Survivin splice variant expression incancer. Cancer Lett. 339(2): 167-174, 2013.

[33] Thielemann A., Baszczuk A., Kopczyński Z. Clinical usefulness of assessing VEGF and soluble receptors sVEGFR-1 and sVEGFR-2 in women with breast cancer. Ann Agric Environ Med, 20: 293-297, 2013.

[34] Wang G.P., Qin M.H., Liang Y.A. The significance of biomarkers in nipple discharge and serum in diagnosis of breast cance. In: LiquanXie, Dianjian Huang, eds. Advanced Engineering and Technology. CRC Press: 665-671, 2014.

[35] Wang J.J., Sun X.C., Hu L. Endoglin (CD105) expression on microvessel endothelial cells in juvenile nasopharyngeal angiofibroma: Tissue microarray analysis and association with prognostic significance. Head Neck. 35(12): 1719-1725, 2013.

[36] Koyama Y., Okayama H., Kumamoto K. Over expression of endoglin (CD105) is associated with recurrence in radically resected gastric cancer. Experimental and Therapeutic Medicine, 1: 627-633, 2010.

©M&H ACADEMIC PUBLISHER