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The Expression and Significance of Angiopoietin-2 and Glucose Regulated Protein78 in Colorectal Cancer

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***Abstract*: Colorectal cancer (CRC) is a severe health problem all over the world. To detect expression of Angiopoietin-2 (Ang-2) and Glucose Regulated Protein78 (GRP78) in colorectal carcinoma tissues, and their correlation with clinical pathological features, we examine expression of Ang-2 and GRP78 by immunohistochemical staining of paraffin-embedded tumor specimens from 70 colorectal cancer patients who underwent curative surgery at Qingdao Central Hospital. Through this experiment, the correlation of Ang-2 and GRP78 expression with clinicopathological parameters of colorectal cancer was analyzed. Ang-2 and GRP78 expression were detected in 68.57% and 74.29% of colorectal carcinoma respectively (P<0.05). Expression of Ang-2 and GRP78 were highly related to differentiation degree, lymph node metastasis, and clinical stages (P<0.05), but no significant correlation to the patients' age, gender or the site and size of the lump was detected (P>0.05). Ang-2 and GRP78 are over-expressed in colorectal cancer, representing a molecular marker for malignant degree and infiltration metastasis of colorectal cancer. They play an important role in the occurrence and progression of colorectal cancer, thus providing new targets for targeted therapy of colorectal cancer.**

Keywords: Colorectal cancer; Targeted therapy; Angiogenesis; Angiopoietin-2; Glucose Regulated Protein78

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1. Introduction

Formation of new blood vessels is a major process allowing cancer progression and tumor spread. Studies have demonstrated that Angiopoietin-2 (Ang-2), a ligand of Tie2 receptor [1], was able to induce an anarchical blood vessel organization during cancer progression [2,3]. Ang-2 may play an important role in the growth of colorectal cancer metastasis. Tumor cells abnormally express high level of GRP78, a member of Heat shock protein 70 family and the key regulator of endoplasmic reticular (ER) function. Studies have revealed that GRP78 plays an important role in tumor cell proliferation, invasion and metastasis [4]. On the basis of Pathology, this study analyzed the expression pattern of Ang-2 and GRP78, discussed the growth and metastasis of colorectal cancer in different signal pathway, and provided the theoretical basis for clinical treatment.

2. Materials and Methods

2.1. Patient data

70 patients with colorectal cancer were recruited from Qingdao Central Hospital from February 2012 to May 2015. All the patients had integrated clinical data and underwent surgical operation as their initial treatment. The patients were aged from 30 to 73 with a median of 62, and no patients had any other anti-tumor treatment before surgery. Postoperative specimens of all patients were confirmed by pathology. Distribution of tumor location: rectum (n=31); sigmoid colon (n=23); ascending colon (n=5); transverse colon (n=3); descending colon (n=8). The TNM staging standard of colorectal cancer was based on Union for International Cancer Control [5]: stage I (n=10); stage II (n=26); stage III (n=18); stage IV (n=16). Lymph node metastasis: positive (n=34); negative (n=36).

2.2. Reagents

Rabbit anti-human Ang-2 antibody and GRP78 antibodies were purchased from Beijing Biosynthesis Biotechnology Co., Ltd. S-P immunohistochemistry kit and DAB kit (Dako) were purchased from Fuzhou new Biotechnology Development Co., Ltd.

2.3. Immunohistochemical staining

70 cases of colon cancer tissues and 20 cases of normal tissues pathology wax block, re-create the paraffin thickness 4μm, were SP immunohistochemical staining. Experimental procedure was finished according to kit instructions. Experimental antibody concentration: Anti-Ang-2, 1:200, Anti-GRP78, l:100. PBS instead of primary antibody as a negative control. After PBS washing DAB color 15min, hematoxylin, were mounted observation.

2.4. Immunohistochemistry judgment

PBS instead of a resistance is a negative control (Figure 1, after the directory), with a known colorectal cancer positive biopsy as positive control. Ang-2, GRP78 are cytoplasmic expression of tumor cells

under an optical microscope containing brown or tan particles are counted as positive cell cytoplasm. Comprehensive scoring method in accordance Fromowitz [6]: The percentage of positive staining cells with a cell occupied by both color intensity combined with determination, the average percentage of positive cells was 10 Vision percentage of positive cells in these 10 Vision cell count: negative recorded as 0 points, 1 point is positive cells ≤25%, 2 points is 26% to 50%, 3 points 51% to 75%, four points is >75%. Cell staining intensity: colorless to 0 points, pale yellow for 1 point, brown to 2 points, tan for three points. Both points are added: 0-1 points to (-), 2-3 points to (+), 4-5 points for the (+), 6-7 points to remember (+ +); (-) and negative (-), (+) and (+ +) is positive; (-) and (±) ruled negative, (+) and (++) judged as positive.

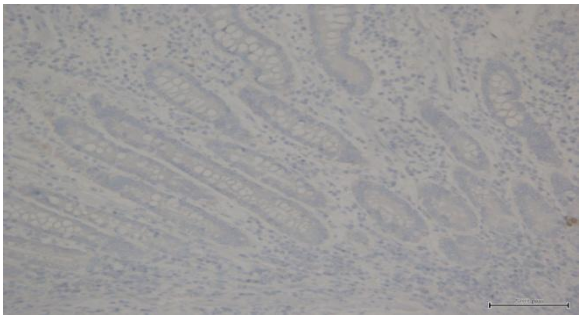

Figure1. The expression of Ang-2 in Normal Tissues (SP×100).

2.5. Statistical Analysis

Chi square test was performed with P<0.05 considered statistically significant. All statistical analyses were performed using SPSS software version 17.0 (SPSS, Inc., Chicago, Illinois, USA).

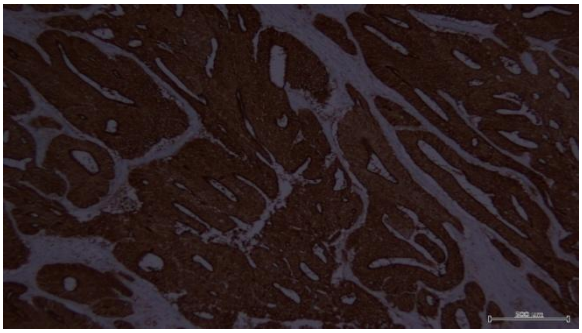

Figure2. The expression of Ang-2 in Tumor Tissues (SP×200).

3. Result

3.1. Overexpression of Ang-2 and GRP78 in Tumor Tissues

A variable staining pattern was noted across cases of colorectal carcinoma and normal tissue. Moderate to strong staining Ang-2 protein was detected in 68.57% (48/70) of colorectal carcinoma cases while only 30.00% (6/20) of the normal tissue cases showed positive Ang-2 expression (Figure 1 and 2. Meanwhile GRP78 is weakly expressed in normal colorectal mucosa (15.00%, 3/20), but highly expressed in carcinoma tissue (74.29%, 52/70), as displayed in Figure 3 and Figure 4. The differences were significant for both proteins (P <0.05, Table 1).

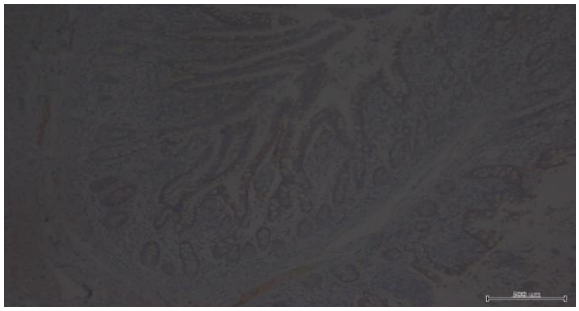

Figure3. The expression of GRP78 in Normal Tissues (SP×100).

3.2. Factors Associated with High Expression of Ang-2 and GRP78

Univariate analysis showed that Ang-2 and GRP78 expression were not correlated with age, gender, tumor size and location of the colorectal cancer patients (p>0.05), but were closely related to the differentiated degree, lymph node metastasis, and clinical stages (P ＜0.05） Table 2.

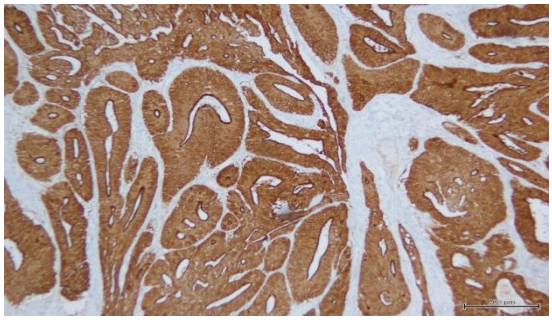

Figure4. The expression of GRP78 in Tumor Tissues (SP×200).

Table 1 The expression of Ang-2 and GRP78 in Tumor Tissues and Normal Tissues.

Variable	n	Ang-2		Positive rate	P	GRP78		Positive rate	P
		(+)	(-)			(+)	(-)		
Normal Tissue	20	6	14	30.00%	<0.05	3	17	15.00%	<0.05
Carcinoma Tissue	70	48	22	68.57%		52	18	74.29%	

Table 2 The relationship between the expression of Ang-2 or GRP78 and colorectal cancer clinicopathologic features.

Variable	n	Ang-2		Positive rate	P	GRP78		Positive rate	P
		(+)	(-)			(+)	(-)		
Gender					>0.05				>0.05
Male	46	33	13	71.74%		35	11	76.09%	
Female	24	15	9	62.50%		17	7	70.83%	
Age(years)					>0.05				
<60	23	15	8	65.22%		18	5	78.26%	>0.05
≥60	47	33	14	70.21%		34	13	72.34%	
Site of lesion					>0.05				>0.05
Colon	39	26	13	66.67%		31	8	79.49%	
Rectum	31	22	9	70.97%		21	10	67.74%	
Tumor size					>0.05				>0.05
<5cm	38	25	13	65.79%		29	9	76.32%	
≥5cm	32	23	9	71.88%		23	9	71.88%	
Clinical stages					<0.05				<0.05
I	10	3	7	30.00%		4	6	40.00%	
II	21	11	10	52.38%		12	9	57.14%	
III	24	19	5	79.17%		21	3	87.50%	
IV	15	15	0	100%		15	0	100%	
Lymph metastasis					<0.05				<0.05
-	31	15	16	48.39%		17	14	54.84%	
+	39	33	6	84.62%		35	4	89.74%	
Differentiation					<0.05				<0.05
Moderately or well	28	12	16	42.86%		13	15	46.43%	
Poorly or undifferentiated	42	36	6	85.71%		39	3	92.86%	

4. Discussion

Colorectal Colorectal cancer has become a serious threat to human health. The incidence and mortality of colorectal cancer in the world are increasing with time. In 2012, about 1.36 million new cases of colorectal cancer occurred, which was the third highest incidence among all malignant tumors in the world, ranking No. 3 in males and No. 2 in females; about 690 000 colorectal cancer patients died, ranking No. 4 among all malignant tumors. In china, the number of new cases of colorectal cancer had exceeded 270 000, causing more than 130 000 deaths in 2010 [7]. Early diagnostic markers and novel therapeutic targets can significantly improve clinical management of colorectal cancer. In this study, we examined the expressions of Ang-2 and GRP78 in 70 colonic cancer samples by immunohistochemistry to explore the relationship between the expression and clinical pathological features.

Formation of new blood vessels is a major process allowing cancer progression and tumor spread. Several studies showed that angiogenic molecular regulation is linked to the multistep oncogenesis leading to activation of an increasing number of angiogenic-related growth factors during the course of the disease [8]. Ang-2 (496 amino acids) is related to Angiopoietin-1 and was identified by homology screening. It is specifically expressed in the vascular reconstruction region of the margins of multiple human tumors, and is involved in the initiation and extension of tumor angiogenesis, which influences the growth and metastasis of tumor [1,9]. Further studies demonstrated that Ang-2, a ligand of Tie2 receptor that counteracts blood vessel maturation/stability mediated by Angiopoietin-1and appears to be a natural antagonist for Angiopoietin-1 and Tie2, was able to induce an anarchical blood vessel organization during cancer progression [1,10]. Ang-2 also is an important facilitator of inflammatory carcinogenesis and catastasis [11]. Overexpression of Ang-2 in tumor cells is associated with tumor progression and has been recognized in lung, breast, colon, esophageal cancer and gastric cancer [12, 13]. Ang-2 expression plays an important role in tumor initiation, progression, and distant-organ metastasis. In this study we found that Ang-2 is overexpressed in colorectal carcinoma tissues and is significantly associated with the differentiated degree, lymph node metastasis, and clinical stages. This is consistent with Yuji's results [14]. Thus, Ang-2 may play an important role in the development and metastasis of colorectal cancer.

Glucose-regulated protein 78 kDa (GRP78) is an endoplasmic reticulum (ER)-resident molecular chaperone that is essential for correct protein folding and assembly in the ER lumen. The unfolded protein response (UPR) is coordinated by GRP78. This pathway may regulate cell fate in response to chemotherapy by regulating cell survival. They can

either be through direct regulation of apoptosis or by promoting other pathways that permit such chemically stressed cells to survive [20]. Multiple studies [15-19] show that GRP78 overexpression occurs in numerous human cancers, including colorectal, breast, thyroid, lung, ovarian, and prostate cancers, which is consistent with our results.

Expression of Ang-2 and GRP78 has been proved to be related to the occurrence and progression of various tumors. Our data show that Ang-2 and GRP78 are highly expressed in colonic cancer, and are highly correlated with differentiated degree, lymph node metastasis, and clinical stages of tumors. But the mechanism of their occurrence and the relationship require further confirmation.

In conclusion, Ang-2 and GRP78 play important roles in the development and progression of colorectal cancer. They could be the molecular markers for judging the malignant degree of colorectal cancer and infiltration metastasis of it. They provided new targets for targeted therapy of colorectal cancer.

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