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Investigated the chemotherapy and prognosis of invasive ductal carcinoma patients based on the different molecular phenotypes.

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Abstract

Objective: It was designed to investigate and analyse the survival rate and prognosis of invasive ductal carcinoma patients in different molecular phenotypes groups.

Methods: The data collected from 347 invasive ductal carcinoma patients included symptoms, history and physical examination. Even they were discharged from hospital. Paraffin sections of carcinoma tissues were detected by the tissue chip method. It detected the expression of Estrogen Receptor (ER), Progesterone Receptor (PR), Ki-67, Human Epidermal Growth Factor Receptor 2 (HER-2), B-cell Lymphoma-2 (Bcl-2) and p53 on the tissues sections. Then it was taken systematic analysis.

Results: The incidences of invasive ductal carcinoma concentrated on the middle-age women in the First People's Hospital of Kunming Medical University from January, 2010 to December 2013. After Pearson chi-square and Spearman Rank sum testing, there were significant differences in the molecular phenotypes among age, tumor size, lymph node metastasis, menopause status and Ki-67 high expression, which could directly impact the prognosis and survival rate. Furthermore, the high level of Bcl-2 and p53 expression in the HER-2 like and triple negative subtypes suggested that they had high risk for tumor cells proliferated rapidly, enhancing invasiveness and poor prognosis.

Conclusion: The Bcl-2 expression closely related to the one and three year survival rate of invasive ductal carcinoma patients, to which should be paid more attention.

Keywords: Invasive ductal carcinoma, Molecular phenotype, Tissue microarray, Prognosis, Chemotherapy.

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Introduction

Breast cancer is a high mortality rate of tumor disease that develops from breast tissue [1]. Signs of breast cancer may include a lump in the breast, a change in breast shape, dimpling of the skin, fluid coming from the nipple, or a red scaly patch of skin. In the worldwide, breast cancer is the leading type of cancer in women, accounting for 25% of all cases [2]. In 2012 it resulted in 1.68 million cases and 522,000 deaths. It is more common in developed countries and is more than 100 times more common in women than in men. Outcomes for breast cancer vary depending on the cancer type, extent of disease, and person's age [3]. Survival rates in the developed world are high, with between 80% and 90% of those in England and the United States alive for at least 5 years. In developing countries survival rates are poorer.

Invasive carcinoma of No Special Type (NST) also known as Invasive Ductal Carcinoma (IDC), without otherwise specified (NOS) is a group of breast cancers that do not have the specific differentiating features [4]. The prognosis of IDC depends, in part, on its histological subtype, mucinous, papillary, cribriform, and tubular carcinomas have longer survival, and lower recurrence rates. Some rare forms of breast cancer (e.g., sarcomatoid carcinoma, inflammatory carcinoma) have a poor prognosis. Regardless of the histological subtype, the prognosis of IDC depends also on tumor size, presence of cancer in the lymph nodes, histological grade, presence of cancer in small vessels (vascular invasion), and expression of hormone receptors and of oncogenes like HER's-2 [5]. This study is planned to analyse the molecular phenotypes in histology. Molecular subtypes can be defined by genetic array testing to this classification using tissue microarray. These

subtypes have different epidemiological risk factors, different natural histories, and different responses to systemic and local therapies [6].

The treatment and chemotherapy is the core issue of all of us concern. The treatment options offered to an individual patient are determined by the form, stage and location of the cancer, and also by the age, history of prior disease and general health of the patient. It should point out that not all patients are treated the same way [7].

The approach to treatment within breast cancer molecular subtypes greatly simplifies the definition of therapy indications, since the subtypes themselves incorporate many of the risk and predictive factors used in previous consensus recommendations.

There are many kinds of molecular markers about invasive ductal carcinoma research. Human Epidermal Growth Factor Receptor 2 (HER-2) is a member of the human epidermal growth factor receptor (HER/EGFR/ERBB) family.

Amplification or over-expression of this oncogene has been shown to play an important role in the development and progression of certain aggressive types of breast cancer. In recent years the protein has become an important biomarker and target of therapy for approximately 30% of breast cancer patients [6,8].

In this study, we detected the molecular markers including Estrogen Receptor (ER), Progesterone Receptor (PR), Ki-67 and HER-2, according the comments of expert consensus on the primary therapy of early breast cancer 2011. Additional, after analysed a computer-based online system for search of related articles, it collected the data of expression of B-Cell Lymphoma-2 (Bcl-2) and p53 protein in these invasive ductal carcinoma tissues. Analysed the relationship between the treatment programs, expression of each molecular markers, histological grade and prognosis conditions for three years of clinical patients. It was plan to study clinical significance of Bcl-2 and HER-2 in the field of surgery, chemotherapy and adjuvant treatment methods.

Materials and Methods

Patients and diagnoses

Invasive ductal carcinoma tissues were collected from 347 patients in the Department of General Surgery, First People's Hospital of Kunming Medical University, from January, 2010 to December 2013. The patient cohort had an average age of 56.5 years (range: 16-78 y).

The patients' data were recorded, including symptoms, history and physical examination. Even discharged from hospital, the physicians performed a monthly telephone following-up for patients' condition. All samples were classified as invasive ductal carcinoma, according to the American Society of Clinical Oncology/College of American Pathologists Guideline [9].

All study participants provided written informed consent. The study was according to the declaration of Helsinki and world health organization guidelines to implement. It was under the supervision of the Ethics Committee of the Kunming Medical University (No.KMMU201101009167).

Sampled

After surgery, it excised tumor tissue, measured its size and weighted it freshly. Include but not limited lymph node involvement by lymphatic dissection as possible. Absence of cancer cells in the lymph nodes was a good indication that the cancer has not spread systemically. Presence of cancer in the lymph nodes indicates the cancer may have spread. In our studies, some women have had presence of cancer in the lymph nodes, before chemotherapy, and still did not have a systemic spread. Therefore, lymph node involvement was not a positive predictor of spread in some degree.

Histological observation

Inclusion criteria were including: more tendency to form tubular structures (Score 1), bigger nuclear size, irregular shape, and deep staining intensity (Score 2), high mitotic and division rate (Score 3), according to the appearance of cancer cells compared with normal duct cells under a microscope. We used the Bloom-Richardson grading system to make score of invasive ductal carcinoma tissues [10]. A total score of 5 and under was considered low. 6 to 7 was considered intermediate. 8 to 9 was considered high.

Tissue chip detection

Dissected tissues were washed with saline immediately following isolation. Then all the samples were fixed in 10% formalin and embedded in paraffin, immediately. Paraffin sections were detected by the methods of tissue chip, within thickness of 3 μm. The tissue chip detection experiment was carried out by Shanghai Zhuo Hao Super Biological Technology Co., Ltd.

According the comments of expert consensus on the primary therapy of early breast cancer 2011, it detected the expression of Estrogen Receptor (ER), Progesterone Receptor (PR), Ki-67 and Human Epidermal Growth Factor Receptor 2 (HER-2) on the tissues sections.

Furthermore, our research team analysed a computer-based online system for search of related articles from 2005 to 2016 which was conducted in PubMed and Springer link by inputting the key words of "invasive ductal carcinoma, molecular phenotypes", and the language was limited to English. Meanwhile, relevant Chinese articles between 2005 and 2016 were also searched in China Journal Full-text Database (CJFD) and Wanfang database with the key words in Chinese. Based on cell apoptosis and clinical application, it searched the Bcl-2 protein family and p53 at high rate, which had been detected in our study along with the detection of those receptors.

Statistical analysis of data

All data are represented as means ± SD ($\bar{x} \pm s$) of three or more independent experiments. The data are changed into normal distribution with logarithm if the original data are positive Skewness distribution. Comparison among the experimental groups, and the correlations between expression receptors protein and histological grade as well as pathological features in both tumor tissue and lymph nodes were analysed. If the data were homogenous, analysis of variance, the methods of Student-Newman-Keuls test and Pearson's correlation were used. If the data were not homogenous, the methods of Kruskal-Wallis and the Games-Howell test, as well as a Spearman's correlation analysis, were used. All the analyses were carried out using the SPSS20.0 software (SPSS Inc., Chicago, IL, USA). Values less than 0.05 were considered to be statistically significant.

Results

Clinical and pathological parameters

651 breast cancer cases were attended in Oncology Department of First Affiliated Hospital of Kunming Medical University from January, 2010 to December 2013, including 347 invasive ductal carcinoma patients. Its composition ratio was 53.30% in the four years.

Their age distribution was described as follows: 10 cases in 16-30 y olds (2.88%), 47 cases in 31-40 y olds (13.54%), 201 cases in 41-60 y olds (57.92%) and 89 cases in 61-80 y olds (25.65%). The incidences of invasive ductal carcinoma were concentrated in the middle-age women.

As shown in Table 1, patients with clinical stage distribution is concentrated on these clinical characteristics: advanced tumor stage (III/IV), large tumor size (>5 cm), poorly histological grades, postmenopausal, HER-2 negative and more than 4 lymph nodes affected.

Molecular phenotypes and pathological grading

According the Expert Consensus on the Primary Therapy of Early Breast Cancer 2011, we detected all samples by tissue microarray. There were four subtypes of the invasive ductal carcinoma molecular phenotypes (Table 2).

It found that 47.0% was the Luminal A subtype, which was consisted with Western country research (44.5% to 69.0%) [1]. After Pearson Chi-square and Spearman Rank sum testing, there were significant difference in the molecular phenotypes between age, tumor size, lymph node metastasis, menopause status and Ki-67 high expression.

On the other side, the Luminal A subtype was concentrated on low grade about the poorly differentiated patients, but triple negative on the high grade. And the high level of Bcl-2 and p53 expression in the HER-2 like and triple negative subtypes suggested that they had close relationship, further affected tumor cells proliferate rapidly, enhanced invasiveness and poor prognosis obtained.

Treatment of chemotherapy

The details of treatment programs were listed in Table 3. Total mastectomy surgery was implemented in triple negative and HER-2 like groups. These patients who were taken breast conserving surgery were accounting HER-2 positive rate less than 20%.

More patients were taken endocrine therapy after surgery in the (ER-PR-) group. As for radiotherapy and adjuvant chemotherapy were had no significant difference between four molecular typing groups, which had been applied to the vast majority of patients.

Survival survey

All the patients were followed up from 10 to 122 months, with a median follow-up of 67 months. This study follow-up rate was 99.7%, since 3 patients move away from the original address and lost contact. It surveyed their prognosis condition, collected the data of Overall Survival rate (OS) and Disease-Free Survival rate (DFS) by one and three years, respectively. 1 y and 3 y survival rates were found 94.9%, 71.1% and 87.2%, 67.1%, respectively.

One way analysis showed age, tumor size, Bcl-2 positive, TNM stage were related to survival rates; combined endocrine therapy, molecular phenotypes and surgical type were not related to survival rates. But the Bcl-2 and p53 had close relationship with the survival rates till the 3 years. Multivariate analysis showed age, Bcl-2 positive and combined treatment was related to survival rates (Table 4).

Discussion

We used the tissue microarray in this study to detect six molecular markers. As recommend by Kononen [11]. For its fast and high-throughput characteristics, it useful for molecular diagnosis of invasive ductal carcinoma. Each microarray block can be cut into 100-500 sections, which can be subjected to independent tests. Tests commonly employed in tissue microarray include immunohistochemistry, and fluorescent *in situ* hybridization. Tissue microarrays are particularly useful in analysis of cancer samples. This technique has important practical significance and broad application prospect in future scientific study.

The 12th St Gallen International Breast Cancer Conference Expert Panel adopted a new approach to the classification of patients for therapeutic purposes based on the recognition of intrinsic biological subtypes within the breast cancer spectrum [5]. They suggest that molecular classification subtypes may be approximated using clinicopathological rather than gene expression array criteria. Importantly, depending on the molecular subtypes, different chemotherapy and adjuvant chemotherapy regimens are required.

In general, systemic therapy recommendations follow the subtype classification. Luminal A disease generally requires only endocrine therapy, which is also useful for the treatment of Luminal B subtype. Chemotherapy is considered indicated

for most patients with HER-2 positive and triple negative disease, with the addition of trastuzumab inHER-2 positive disease.

According to research of Shanghai Fudan University (China), the patients who are under 50 years old and accept combined treatment have higher survival rates [12]. But, the low-age young patients have lower overall survival and disease-free survival than the other groups (P<0.05), with the high risk molecular phenotype as triple negative, in Tianjin Medical University Cancer Institute and Hospital (China) [13].

After treatment of breast cancer there is no evidence that lactation increases the risk of recurrence. The main prognostic factors associated with breast cancer are the number of lymph nodes involved, tumor size, histological grade, and hormone receptor status. At the same time, the scientist from University of Barcelona advise other prognostic and predictive factors have been studied in an effort to explain this phenomenon, some of which are more relevant than others: Ki-67, p53, Bcl-2 [14]. They were also more proliferative tumors measured by Ki-67. Breastfeeding history did not influence the subsequent behavior of the tumor regardless of histological subtype.

As for *p53*, *p53* is a tumor suppressor gene, one of the earliest discovered, is a multi-functional genes, not only plays a critical role in the regulation of the cell cycle, but also plays an important role in the regulation of apoptosis, it is mainly through the regulation of apoptosis. *P53* had close relationship with Bcl-2 Protein overexpression in the invasive ductal carcinoma patients, by Shanxi College of Traditional Chinese Medicine study [15].

The Armed Forces institute of Pathology (Pakistan) observed that Triple negative breast cancers are high grade aggressive tumors generally with a poor prognosis, not responding to hormonal and anti HER-2 Neu therapy.

Expression of the antiapoptotic Bcl-2 is associated with low grade, slowly proliferating hormone receptor positive tumors with improved survival. So, Bcl2 may be an important prognostic factor and its expression might be used for targeted therapy using Anti Bcl2 drugs [16]. That consistent with our findings.

But different opinions come from Kanagawa Cancer Center (Japan), they suggest that Bcl-2 expression might reflect a good prognosis in patients, rather than being a poor prognostic indicator, as it is in several types of neuroendocrine tumor [17]. However, to confirm this hypothesis, further investigation is required.

Table 1. *The baseline clinical characteristics of all patients (n=347).*

	Characteristics	Total patients	Luminal A (n)	Luminal B (n)	HER-2 like (n)	Triple negative (n)	P value
Age (y)	≤ 40 y	57	26	10	7	14	p<0.01
	>40 y	290	137	55	51	47	
Histologic grade	Well differentiated	89	122	25	1	0	0.01<p<0.05
	Moderately differentiated	99	22	13	7	7	
	Poorly differentiated	159#	19	27	50	54	
Tumor stage	I/II	116	83	26	3	4	p<0.01
	III/IV	231	80	39	55	57	
Tumor size (cm)	<2 cm	56	23	11	4	1	p<0.01
	2-5 cm	64	50	23	4	4	
	≥ 5 cm	227#	90	31	50	56	
Number of lymph node	0	54	36	17	1	0	p<0.01
	1/4	82	63	15	3	1	
	≥ 4#	211	64	33	54	60	
Menopause status	Premenopause	58	20	9	5	12	p<0.01
	Postmenopause	289	143	56	53	49	

Table 2. *The different molecular phenotypes in four groups.*

Phenotypes	Characteristics	Patients (n)	%	Bcl-2 expression	p53 expression
Luminal A	ER/PR+, HER-2-, Ki67 low	163	46.97%	43	43

Luminal B	ER/PR+, HER-2±, Ki-67 high	65	18.73%	32	32
HER-2 like	ER-, PR-, HER-2+	58	16.71%	44	44
Triple negative	ER-, PR-, HER-2-	61	17.58%	53	54

Table 3. *The treatment method for patients in different groups.*

Field in treatment		Luminal A (n)	Luminal B (n)	HER-2 like (n)	Triple negative (n)	P value
Type of surgery	Breast conserving surgery	74	24	0	0	p<0.01
	Total mastectomy	89	41	58	61	
Radiotherapy	Not done	69	27	26	25	p>0.05
	Done	94	38	32	36	
Adjuvant chemotherapy	Not done	93	37	35	36	p>0.05
	Done	70	28	23	25	
Endocrine therapy	Not done	120	34	14	16	p<0.01
	Done	43	31	44	45	

Table 4. *The survival survey was taken by multivariate analysis for each group.*

Factor	Over-one-year survival rate (OOS)						Over-three - year survival rate (OTS)					
	OS			DFS			OS			DFS		
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P	HR	95%CI	P
Tumor size	2.311	1.448-4.973	0.002	2.414	1.346-4.330	0.003	2.323	1.295-4.133	0.022	3.007	1.3445-4.779	0.012
Node metastasis	3.143	1.038-13.256	0.039	5.143	1.138-23.233	0.033	5.255	1.156-23.891	0.032	5.441	2.216-32.389	0.038
Endocrine therapy (done)	1.542	1.096-3.126	0.315	1.634	1.035-3.263	0.415	1.433	1.193-3.023	0.287	1.089	0.193-3.023	0.293
Type of surgery (total mastectomy)	1.96	1.164-4.526	0.726	1.76	1.091-4.757	0.834	1.775	1.167-4.604	0.745	1.625	1.064-3.987	0.698
Molecular typing	1.486	1.060-3.820	0.122	1.331	1.060-3.809	0.203	1.494	1.067-2.091	0.119	1.134	0.162-2.873	0.212
p53	2.376	1.323-6.132	0.0149	2.877	1.323-6.589	0.0211	1.335	1.343-8.163	0.014	1.496	0.643-8.41	0.007
Bcl-2	2.497	1.163-5.541	0.0219	2.994	1.163-6.534	0.022	1.489	1.161-7.484	0.027	2.489	1.993-7.883	0.032

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