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Clinical Analysis of Multidrug Resistance-Related Protein Expression in Gastric Cancer Cells

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Abstract: This study investigated the expression of multidrug resistance-related proteins in gastric cancer cells using archival paraffin blocks from 50 surgical specimens collected between 2021 and 2022. The analysis included P-glycoprotein (P-gp), glutathione S-transferase pi (GST-π), and B-cell lymphoma-extra-large (Bcl-xL). The positive expression rates and high expression levels of P-gp, GST-π, and Bcl-xL were significantly higher in gastric cancer tissues compared to normal gastric mucosa tissues. The expression of these multidrug resistance proteins did not show significant associations with clinical characteristics but revealed a positive correlation between Bcl-xL expression and tumor differentiation degree. These findings provide insights into the expression patterns of multidrug resistance-related proteins in gastric cancer tissues, contributing to the understanding of their relationship with multidrug resistance in gastric cancer.

Gastric cancer, as a common malignant tumor worldwide, has maintained high incidence and mortality rates. According to data from the World Health Organization, gastric cancer is the third leading cause of cancer-related deaths globally [1]. High-incidence areas for gastric cancer primarily include regions such as Asia, Latin America, Eastern Europe, and Africa. The occurrence of gastric cancer is closely linked to factors such as diet, environment, and genetics, and its early symptoms are often subtle, leading to many patients being diagnosed at an advanced stage. Surgical intervention remains the primary treatment method for gastric cancer [1, 2]. Multidrug resistance poses a significant challenge in the treatment of gastric cancer, severely impacting patient prognosis and treatment outcomes [3]. Multidrug resistance refers to the simultaneous resistance of cancer cells to multiple chemotherapeutic drugs [4], leading to treatment failure and recurrence. Currently, the study of multidrug resistance mechanisms has become a hot topic in the field of gastric cancer treatment; however, many aspects of the specific mechanisms and the expression of related proteins remain unknown. Analyzing the expression of multidrug resistance-related proteins within gastric cancer cells, exploring their roles and significance in the development of gastric cancer, and providing new targeted treatment strategies for gastric cancer are discussed in this study. The details are as follows:

1. Materials and Methods

1.1. General Information

In this study, archival paraffin blocks from 50 surgical specimens of gastric cancer, collected between 2021 and 2022 at our institution, were included. Each specimen consisted of both tumor lesion tissue and normal gastric mucosa tissue located more than 5 cm away from the cancer. These specimens were derived from 30 male and 20 female patients, with ages ranging from 26 to 88 years. Notably, the selected cases had not undergone any prior antitumor treatment. Patient demographics, including age, gender, and clinical pathological data related to gastric cancer, were thoroughly documented. This data encompassed the clinical stage of the tumor, depth of invasion, degree of differentiation, and lymph node involvement. According to the international TNM classification for cancer staging, our study sample included 7 cases of Stage I gastric cancer, 6 cases of Stage II gastric cancer, 22 cases of Stage III gastric cancer, and 15 cases of Stage IV gastric cancer. Based on the World Health Organization's histological classification, the study sample included 23 cases of poorly differentiated adenocarcinoma, 13 cases of moderately differentiated adenocarcinoma, 7 cases of well-differentiated adenocarcinoma, and 7 cases of signet ring cell carcinoma. Well-differentiated and moderately differentiated adenocarcinomas were categorized as the differentiated group, while poorly differentiated adenocarcinoma and signet ring cell carcinoma were classified as the undifferentiated group. Additionally, 35 patients had lymph node metastasis, while 15 patients did not.

1.2. Methods

1.2.1. Sectioning

Archival paraffin blocks were cut into 4-5 μm thick sections and placed on glass slides. The sections were baked at 56 ℃ for 1 hour to ensure their complete adhesion to the glass slides.

1.2.2. Deparaffinization to Water

The sections were immersed in xylene three times for 15 minutes each. Subsequently, they were sequentially placed in 100%, 95%, 75%, and 50% ethanol solutions for 2 minutes each to dehydrate. The sections were rinsed with phosphate-buffered saline (PBS) and soaked in PBS for 5 minutes.

1.2.3. Antigen Retrieval and Blocking

The sections were placed in EDTA antigen retrieval solution and microwaved for 5 minutes, followed by cooling to room temperature. 5% BSA was added to the sections, and they were incubated at 37 ℃ for 30 minutes.

1.2.4. Antibody Incubation

Specific antibodies against P-glycoprotein (P-gp), glutathione S-transferase pi (GST-π), and B-cell lymphoma-extra-large (Bcl-xL) were added separately to the sections and incubated at 37 ℃ for 2 hours. The sections were then washed with PBS to remove unbound antibodies. Corresponding secondary antibodies were added to the sections and incubated at 37 ℃ for 30 minutes. The sections were rinsed in PBS three times, each time for 5 minutes.

1.2.5. Staining and Observation

The prepared 3, 3'-diaminobenzidine (DAB) staining solution was dropped onto the sections, and

the reaction was stopped after an appropriate staining time. Hematoxylin counterstaining was performed for 40 seconds, followed by rinsing in water and immersion in bluing reagent for 1 minute. After gradient alcohol dehydration and xylene clarification, the sections were coverslipped with neutral resin. They were then observed and photographed under a microscope.

1.2.6. Result Analysis

The staining results were evaluated by considering the combined positive staining intensity and the proportion of positively stained cells. Positive staining intensity was graded as 0 (no cell staining), 1 (pale yellow granules), 2 (tan granules), and 3 (brown granules). Positive cell proportion was graded as 0 (≤10%), 1 (11%–50%), 2 (51%–75%), and 3 (>75%). The positive labeling score was calculated as the product of the positive staining intensity grade and the positive cell proportion grade, resulting in four levels: (-), (+), (++), and (+++), where (++ and +++) represent high expression.

1.3. Statistical Methods

Data were processed using SPSS 26.0 statistical software. Count data were expressed as mean ± standard deviation (±s) and analyzed using t-tests. Percentage data were analyzed with chi-squared tests to determine whether there were significant differences between two groups ($P < 0.05$).

2. Results

2.1. Expression of P-gp, GST-π, and Bcl-xL

The positive expression rates and high expression levels of P-glycoprotein (P-gp), glutathione S-transferase pi (GST-π), and B-cell lymphoma-extra-large (Bcl-xL) in gastric cancer tissues were significantly higher than those in normal gastric mucosa tissues ($p < 0.05$). Specific data are presented in Table 1 and Table 2.

Table 1: Specific expression profiles of P-gp, GST- π, and Bcl-xL

Group	Example number	P-gp				GST-π				Bcl-xL			
		-	+	++	+++	-	+	++	+++	-	+	++	+++
Gastric cancer tissue	50	30	10	8	2	20	15	7	8	7	15	15	13
Normal gastric mucosal tissue	50	48	2	0	0	45	5	0	0	40	10	0	0

Table 2: Specific expression profiles of P-gp, GST- π, and Bcl-xL, (%)

Group	Example number	P-gp		GST-π		Bcl-xL	
		Positive rate	High expression rate	Positive rate	High expression rate	Positive rate	High expression rate
Gastric cancer tissue	50	40	20	60	30	86	56
Normal gastric mucosal tissue	50	4	0	10	0	20	0
$\chi 2$		36.029	11.111	27.473	17.647	43.717	38.889
p		0.000	0.000	0.000	0.000	0.000	0.000

2.2. Occurrence of Nursing Risks

The expression of multidrug resistance proteins, P-glycoprotein (P-gp), glutathione S-transferase pi (GST-π), and B-cell lymphoma-extra-large (Bcl-xL), in gastric cancer was not associated with patient clinical characteristics, including tumor clinical stage, depth of invasion, degree of differentiation, and lymph node metastasis ($p > 0.05$). Detailed data are provided in Table 3.

Table 3: Relationship between Biological Behavior of P-gp, GST-π, and Bcl-xL in Gastric Cancer Tissues

Group	Example number	P-gp				P
		-	+	++	+++	
Sex						
Male	30	19	6	5	0	0.368
Female	20	11	4	3	2	
Age						
>62	26	15	6	5	0	
≤62	24	15	4	3	2	0.419
Degree of differentiation						
Differentiated	20	12	2	6	0	
Differential type	30	18	8	2	2	0.067
Infiltration depth						
The plasma membrane was not involved	18	9	6	3	0	
Involve the plasma membrane	32	21	4	5	2	0.251
lymphatic metastasis						
None	15	8	3	3	1	
Have	35	22	7	5	1	0.860
Clinical stages						
I	7	4	2	1	0	
II	6	4	1	1	0	
III	22	16	2	2	2	
IV	15	6	5	4	0	0.450
Group	Example number	Bcl-xL				P
		-	+	++	+++	
Sex						
Male	30	4	8	9	9	0.858
Female	20	3	7	6	4	
Age						
>62	26	1	7	8	10	
≤62	24	6	8	7	3	0.060
Degree of differentiation						
Differentiated	20	1	7	3	9	
Differential type	30	6	8	12	4	0.025
Infiltration depth						
The plasma membrane was not involved	18	5	4	7	2	
Involve the plasma membrane	32	2	11	8	11	0.057
lymphatic metastasis						
None	15	3	4	3	5	
Have	35	4	11	12	8	0.618
Clinical stages						0.665

I	7	2	2	2	1	
II	6	0	1	3	2	
III	22	4	5	7	6	
IV	15	1	7	3	4	
Group	Example number	GST-π				P
		-	+	++	+++	
Sex						
Male	30	14	6	5	5	0.858
Female	20	6	9	2	3	
Age						
>62	26	11	6	5	4	
≤62	24	9	9	2	4	0.249
Degree of differentiation						
Differentiated	20	8	5	3	4	
Differential type	30	12	10	4	4	0.888
Infiltration depth						
The plasma membrane was not involved	18	9	3	3	3	
Involve the plasma membrane	32	11	12	4	5	0.472
lymphatic metastasis						
None	15	8	3	1	3	
Have	35	12	12	6	5	0.440
Clinical stages						
I	7	3	2	1	1	
II	6	3	1	1	1	
III	22	12	6	2	2	
IV	15	2	6	3	4	0.591

3. Discussion

Multidrug resistance in gastric cancer cells refers to the phenomenon in which these cells develop resistance to multiple chemotherapy drugs simultaneously.[5] Understanding the mechanisms of multidrug resistance is crucial for improving the effectiveness of gastric cancer treatment and developing personalized treatment strategies.[6-7] The mechanisms of multidrug resistance primarily involve the action of drug efflux pumps and the influence of cellular signaling pathways. Drug efflux pumps play a significant role in gastric cancer cells by expelling chemotherapy drugs from the cells, reducing drug accumulation inside the cells, and decreasing drug toxicity. Drug efflux pumps can be categorized into active and passive transport. Active transport involves the expulsion of drugs from the cell through specific transport proteins, with members of the ABC transporter family being the most common. Overexpression of these transporters can increase resistance to various chemotherapy drugs.[8-9] Passive transport involves regulating cell membrane permeability and drug-binding protein expression, impacting the distribution of drugs within and outside the cells. Cellular signaling pathways play a crucial regulatory role in multidrug resistance in gastric cancer cells, with the abnormal activation of multiple signaling pathways leading to increased resistance to chemotherapy drugs. One significant signaling pathway is the PI3K/Akt/mTOR pathway,[10] which, when activated, promotes cell proliferation, survival, and transcription factor activity, ultimately reducing the cytotoxic response to chemotherapy drugs.[11-12] Another vital signaling pathway is the NF-κB pathway, a transcription factor closely associated with multidrug resistance in gastric cancer cells.[13] Activation of NF-κB can regulate the expression of anti-apoptotic proteins, making cancer cells resistant to the apoptotic effects of chemotherapy drugs.[14-15]

Multidrug resistance-related proteins include members of the ABC transporter family.[16-17] These transporters play a central role in multidrug resistance mechanisms, effectively transporting chemotherapy drugs inside and outside the cell, thus reducing drug accumulation within the cells.[18] Common ABC transporters include P-glycoprotein (P-gp, ABCB1), and breast cancer resistance protein (BCRP, ABCG2), both of which are closely associated with multidrug resistance in gastric cancer cells.[19-20] P-gp is one of the most widely studied transport proteins related to multidrug resistance in gastric cancer.[21] It transports chemotherapy drugs inside and outside the cell, reducing drug accumulation inside the cells. Overexpression of BCRP in gastric cancer cells increases resistance to several chemotherapy drugs. Additionally, drug-metabolizing enzymes play a vital role in multidrug resistance within gastric cancer cells.[22-23] These enzymes metabolize chemotherapy drugs, reducing their cellular toxicity and treatment efficacy. Drug-metabolizing enzymes mainly include members of the cytochrome P450 (CYP450) family and uridine diphosphate glucuronosyltransferases (UGT).[24-26] In gastric cancer cells, abnormal expression and activation of these multidrug resistance-related proteins mutually affect each other and collectively contribute to the development and maintenance of multidrug resistance. Understanding the mechanisms of action of these proteins can help identify new therapeutic targets and enhance the efficacy of chemotherapy in gastric cancer.

In this study, we utilized immunohistochemical staining to assess the expression of P-gp, GST-π, and Bcl-xL in gastric cancer tissues and normal gastric mucosa tissues. In gastric cancer tissues, the levels of P-gp, GST-π, and Bcl-xL expression were significantly higher than those in normal gastric mucosa tissues ($p < 0.05$). This suggests that P-gp, GST-π, and Bcl-xL are overexpressed in gastric cancer. In gastric cancer tissues, the expression levels of P-gp and GST-π were significantly higher than those in normal gastric mucosa tissues, typically displaying low to moderate positive expression as "+" or "++." Conversely, Bcl-xL exhibited relatively high expression levels, often presenting as high-intensity positive expression as "+++" or "++++." Multidrug resistance is a significant issue in gastric cancer treatment, greatly limiting the efficacy of chemotherapy drugs. Our research results indicate that the high expression of P-gp, GST-π, and Bcl-xL in gastric cancer tissues is closely related to multidrug resistance. P-gp contributes to multidrug resistance by actively transporting various chemotherapy drugs inside and outside the cell, reducing drug accumulation inside the cell. On the other hand, GST-π participates in drug metabolism and detoxification processes, reducing the cytotoxicity and therapeutic effects of chemotherapy drugs. The overexpression of Bcl-xL in gastric cancer tissues may be associated with aberrations in the apoptosis process, further increasing resistance to chemotherapy drugs.[27]

The expression of multidrug resistance-related proteins is closely related to the prognosis and treatment outcomes of gastric cancer. The results of this study provide a pattern of expression for multidrug resistance-related proteins in gastric cancer, offering critical insights for personalized treatment of gastric cancer. Given the close association between the overexpression of P-gp, GST-π, and Bcl-xL and multidrug resistance, inhibiting the function of these proteins may help improve the efficacy of chemotherapy drugs in gastric cancer treatment.[28-30] There are already inhibitors or antagonists developed for P-gp, GST-π, and Bcl-xL that can serve as candidate drugs for gastric cancer treatment, further enhancing treatment efficacy.[31-33] Additionally, in the analysis of their correlation with clinical characteristics, it was found that the expression level of Bcl-xL, one of the multidrug resistance-related proteins, was positively correlated with the degree of tumor differentiation. This suggests that the overexpression of multidrug resistance-related proteins in gastric cancer tissues may be related to the invasiveness and malignancy of tumors.

However, there are some limitations to this study. First, the sample size was relatively small, which may limit the reliability and generalizability of the results. Expanding the sample size and conducting multicenter and multiethnic clinical studies can better validate our results and enhance their reliability

and applicability. Second, this study only examined the expression of multidrug resistance-related proteins at the tissue level and did not explore their genetic aspects. Subsequent research can comprehensively investigate their functions and regulatory mechanisms by analyzing the gene expression or mutation status of these proteins. Additionally, all the research was based on a single assessment, and there was no tracking observation of the long-term treatment response and prognosis of the patients. Subsequent research can validate the relationship between the expression of these proteins and treatment outcomes through long-term follow-up.

In conclusion, through a clinical analysis of the expression of multidrug resistance-related proteins in gastric cancer cells, this study reveals the expression pattern of these proteins in gastric cancer tissues and elucidates their relationship with multidrug resistance in gastric cancer. This provides important insights for personalized gastric cancer treatment and the development of novel drugs. Future research can further expand the sample size and scope of the study, explore the regulatory mechanisms of multidrug resistance-related proteins, integrate them with other clinical information, and achieve more precise gastric cancer treatment. This will offer better treatment options and prognosis assessment for gastric cancer patients and provide more effective strategies and methods for gastric cancer treatment.

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