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ORIGINAL RESEARCH

Impact of Caspase3/GSDME-Mediated Pyroptosis on Tumor Immune Microenvironment and Clinical Prognosis Across Multiple Cancers

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Background: Globally, the disease that has the greatest impact on human health and is the most difficult to overcome is cancer (tumor or malignant tumor is another name for it). Cancers currently known to us can arise from almost any organ or tissue in the human body. Its uncontrolled growth pattern and metastasis characteristics are the fundamental reasons for the high mortality rate of cancer and its current incurability. An increasing number of studies have found that pyroptosis, a mode of programmed cell death, may inhibit tumor growth by changing the tumor immune microenvironment (TIME).

Methods: Through a retrospective study, we selected 160 cases of different tumor tissues (including 40 cases each of esophageal cancer, gastric cancer, breast cancer, and cervical cancer), and identified the expression of caspase3/GasderminE in the tumor tissues through immunohistochemical staining and infiltration of tumor-related immune cells. And analyze its relationship with clinical parameters of tumor patients. In addition, we also marked caspase8 and caspase9 among the caspase family members to analyze the main factors upstream of caspase3.

Results: The results showed that the expression level of caspase3/GSDME in different tumor tissues was positively correlated with the infiltration degree of tumor-related immune cells (natural killer cells, CD8+T cells, macrophages, etc). In addition, the expression level of caspase3 was positively correlated with caspase8, but not caspase9.

Summary: The expression levels of caspase3 and GSDME exhibited significant impacts on the survival prognosis of patients with diverse tumors as well as alterations in the immune microenvironment of tumor tissues, demonstrating statistical significance. After Caspase3/GSDME triggers the pyroptosis pathway, it may change the components of the immune microenvironment of tumor tissue, thereby achieving the effect of inhibiting tumors.

Keywords: pyroptosis, caspase3, GSDME, tumor immune microenvironment, cancer

Introduction

Esophageal cancer, gastric cancer, breast cancer and cervical cancer are all common malignant tumors in most countries, and the age of onset is gradually getting younger. In particular, breast cancer and cervical cancer are the two most difficult factors affecting women's health problems, and esophageal cancer and Gastric cancer is one of the most aggressive cancers, and its morbidity and mortality worldwide are extremely harmful to human health. Since cancer can arise from any organ and tissue in our body and can grow uncontrollably, we have limited treatment options for cancer. Of course, with our continuous research on cancer, we have found that in addition to the unlimited proliferation and growth of tumor cells, the infiltration and loss of relevant immune cells in tumor tissue are also a major factor affecting prognosis.[1,2] The composition and changes in the components of the tumor immune microenvironment may have a certain impact on the growth of tumor cells. If we can analyze and regulate the tumor immune microenvironment and combine immune combination therapy to bring substantial survival benefits to cancer patients, it will bring good news to cancer treatment and global human health.

With the research and development of science and technology, we know more and more about the methods of programmed cell death. Pyroptosis, as a programmed and inflammatory death method, is gradually entering people's field of vision and becoming a new topic and direction in cancer research. It may intersperse throughout the process of carcinogenesis, thereby

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affecting every stage of the cancer process.[3] The cell pyroptosis that we are currently familiar with mainly relies on the caspase protein family, the classical pathway and the non-classical pathway programmed cell death mode mediated by the Gasdermin protein family. GasderminD (GSDMD), a member of the Gasdermin protein family, is also called As the "execution protein" of cell pyroptosis.[4,5] The main target of our research is GasderminE (GSDME), not GSDMD. Members of the Gasdermin protein family have 45% sequence homology. As a member of the Gasdermin protein family, GSDME has the same structural domain and membrane pore-forming function as GSDMD.[5–7] Different from this, GSDME relies on the cleavage of caspase3 to mediate the occurrence of pyroptosis signaling pathway.[6,8] Although the pace of exploration of GSDME has never stopped in recent decades, the related research and known fields of GSDME are still limited, which may be because the methylation or mutation of GSDME leads to its silent expression in most tumor tissues and loss of function, thus limiting our research.[2,9] Therefore, we selected different types of tumor tissues in this experiment, and comparatively analyzed the expression of GSDME in different tumor tissues and its impact on the clinicopathological parameters of patients. As another important player in mediating the pyroptosis signaling pathway, caspase3 is a major effector enzyme that, after being activated by upstream caspases, participates in the execution of cell apoptosis and the activation of other inflammatory mediators, plays an important role in the caspase cascade reaction.[5,10,11] As our understanding of the caspase family continues to deepen, we gradually find that caspase3 has the function of mediating both apoptosis and pyroptosis, and when the expression level of GSDME is high, caspase3 can convert cell apoptosis into cell pyroptosis.[5,8] This change in the death mode makes cell death no longer a simple independent programmed death, but a programmed inflammatory necrosis that recruits inflammatory cells to aggregate and trigger the body's local or systemic inflammatory response. These changes will undoubtedly increase the body's immune system's surveillance of tumor tissues, reduce the immune escape of tumor cells, and thereby exert a tumor-suppressing effect.

Although pyroptosis and apoptosis are both programmed cell death, their characteristics are different. The main difference lies in whether the cell membrane maintains its integrity. We already know that pyroptosis is a form of inflammatory death. The first step in triggering it is the formation of pores in the cell membrane and loss of integrity, which results in the release of intracellular factors outside the cell and triggers a secondary inflammatory response.[3,12,13] In this study, we found that after the passive release of nuclear factor high mobility group protein (HMGB1) into the outside of the cell with the loss of cell membrane integrity, it may recruit a large number of immune cells to accumulate and amplify the inflammatory response. The most infiltrated immune cells are CD8+ T cells and macrophages. The aggregation of these immune cells enhances the monitoring effect of the body's immune defense system on tumor cells, and inhibits the proliferation and invasion of tumor tissues to a certain extent.[14] The accumulation of excess immune cells amplifies local or systemic inflammatory responses, while destroying the immunosuppressive microenvironment that tumor cells may form. Therefore, the triggering of pyroptosis pathways in tumor tissues plays a double-edged sword effect on the body.

In this study, we are not simply limited to the impact of caspase3/GSDME-mediated pyroptosis triggering on various clinical parameters in different tumor patients, but also focus on the stroma of tumor tissues where pyroptosis reactions occur. The impact of the accumulation of inflammatory factors and immune cells on the tumor immune microenvironment can be observed and analyzed as a whole to analyze the impact of this series of changes on the clinical prognosis of tumor patients. It is hoped that through the regulation of pyroptosis signaling pathways, we can intervene in changes in the tumor immune microenvironment, strengthen the body's immune system to eliminate tumor cells, and bring substantial benefits to the treatment of tumor patients.

Materials and Methods

General Information

We collected the clinical and postoperative pathological data of 40 patients each with esophageal cancer, gastric cancer, breast cancer, and cervical cancer who were treated at the First Affiliated Hospital of Bengbu Medical University from January to June 2018 and were diagnosed by the pathology department of the hospital. Here we need to emphasize that the tumor types collected in this study include esophageal squamous cell carcinoma, gastric adenocarcinoma, non-specific invasive ductal carcinoma of the breast and cervical squamous cell carcinoma, which are the most common and frequently occurring tumor types. None of the patients received radiotherapy or chemotherapy before surgery for sample collection. The data of a total of 160 patients that we collected are detailed in Table 1. In order to analyze factors that may affect the five-year survival rate of patients, we strictly

Table 1 Basic Information Table

		Patient No. (%)			
		Esophageal Squamous Carcinoma	**Gastric Adenocarcinoma**	**Invasive Ductal Carcinoma of the Breast**	**Squamous Carcinoma of the Cervix**
Age	>60	33 (82.5%)	19 (47.5%)	6 (15%)	7 (17.5%)
	≤60	7 (17.5%)	21 (52.5%)	34 (85%)	33 (82.5%)
Gender	Male	24 (60%)	24 (60.0%)	0 (0%)	0 (0%)
	Female	16 (40%)	16 (40.0%)	40 (100%)	40 (100%)
Pathology grade	I, II	31 (77.5%)	21 (52.5%)	27 (67.5%)	28 (70.0%)
	III	9 (22.5%)	19 (47.5%)	13 (32.5%)	12 (30.0%)
TNM stage	I, II	23 (57.5%)	29 (72.5%)	31 (77.5%)	28 (70.0%)
	III, IV	17 (42.5%)	11 (27.5%)	9 (22.5%)	12 (30.0%)
Lymph node	Positive	18 (45%)	23 (57.5%)	19 (47.5%)	12 (30.0%)
	Negative	22 (55%)	17 (42.5%)	21 (52.5%)	28 (70.0%)
Survival status	Survival	23 (57.5%)	17 (42.5%)	28 (70.0%)	24 (60.0%)
	Death	17 (42.5%)	23 (57.5%)	12 (30.0%)	16 (40.0%)
Tumor size	>3cm	16 (40%)	28 (70.0%)	14 (35.0%)	25 (62.5%)
	≤3cm	24 (60%)	12 (30.0%)	26 (65.5%)	15 (37.5%)

conducted postoperative follow-up on these 160 patients until the patient's death or as of June 2023. This study was approved by the Ethics Committee of Bengbu Medical University and followed the ethical guidelines of the Declaration of Helsinki. [Lenke Pi Zi (2022) No. 121]. The informed consent of all cancer patients involved in this study or their families was obtained through the signing of informed consent forms during the initial background investigation phase of this study. And we guarantee the privacy of every participant.

Reagents

Rabbit polyclonal antibodies against human caspase-3, GSDME, HMGB1 and caspase8, caspase9 were purchased from Proteintech (Rosemont, IL, USA). Rabbit monoclonal antibodies against human CD8+ T lymphocytes, macrophages (CD68), and natural killer cells (CD56) as well as ElivisionTM PlusKit and DAB color development kits were purchased from Fuzhou Maixin Biotechnology Company (China).

Experiment

All tumor samples we collected were obtained by serial sectioning after being fixed in neutral formalin solution and embedded in paraffin. After hematoxylin/eosin and immunohistochemical staining, histological observations were performed under a light microscope. The four different types of tumor tissues we collected were clinically staged according to the American Joint Committee on Cancer (AJCC) Cancer Staging Manual (8th Edition). Immunohistochemical staining was performed using the ElivisionTM Plus kit according to the manufacturer's instructions.

Immune Response Assessment

Our evaluation of the expression intensity of GSDME, caspase3/8/9 and HMGB1 proteins mainly depended on the two decisive factors, the degree of positivity and the proportion of positivity. The product of the scores of these two major factors was used to define the high expression and low expression of proteins. The final score 0–6 was defined as low expression, and 7–12 was defined as high expression.[15] For the definition of the degree of tumor stromal immune cell aggregation, we used X-tile.3.6.1 (Yale University, New Haven, Connecticut, USA) to measure it. Assessment of all results was performed by two expert pathologists using an independent double-blind method.

Statistical Analysis

We analyzed the data through SPSS26.0, used Kaplan-Meier to draw univariate survival curves, further used the Logrank test for inter-group comparison, and used the Cox multiple regression model for multivariate analysis to estimate the hazard ratio (HR) and 95% confidence interval (CI). Spearman correlation and analysis of variance were used to compare the relationships between variables and clinical parameters. P values <0.05 were considered statistically significant.

Result

Expression Levels of Caspase3/GSDME in Different Tumor Tissues

In our current study, the expression levels of caspase3, GSDME protein and related proteins of their pathways in different tumor tissues were analyzed through immunohistochemical staining. (Figure 1 and 2). We found that the number of cases

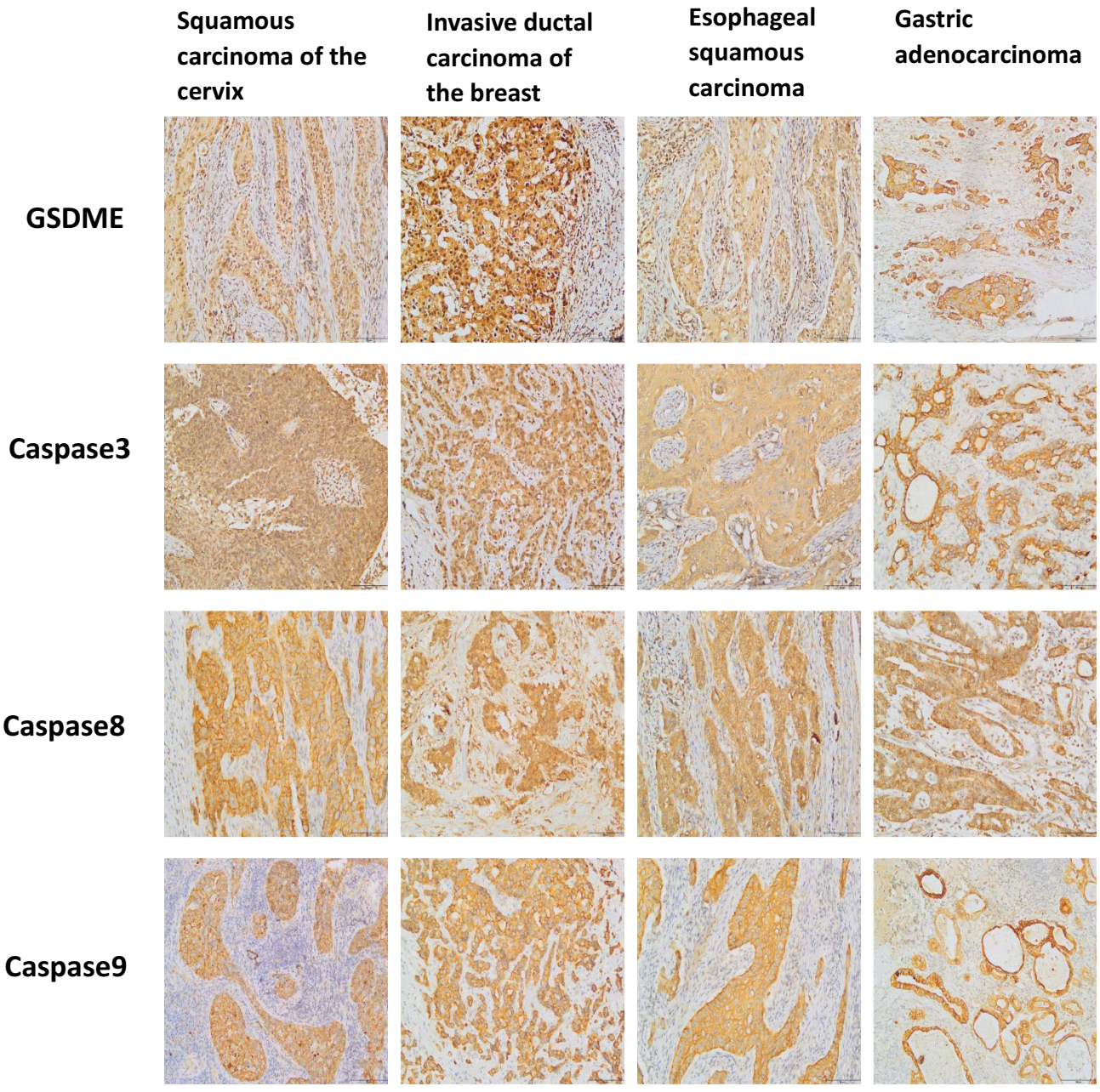

Figure 1 Continued.

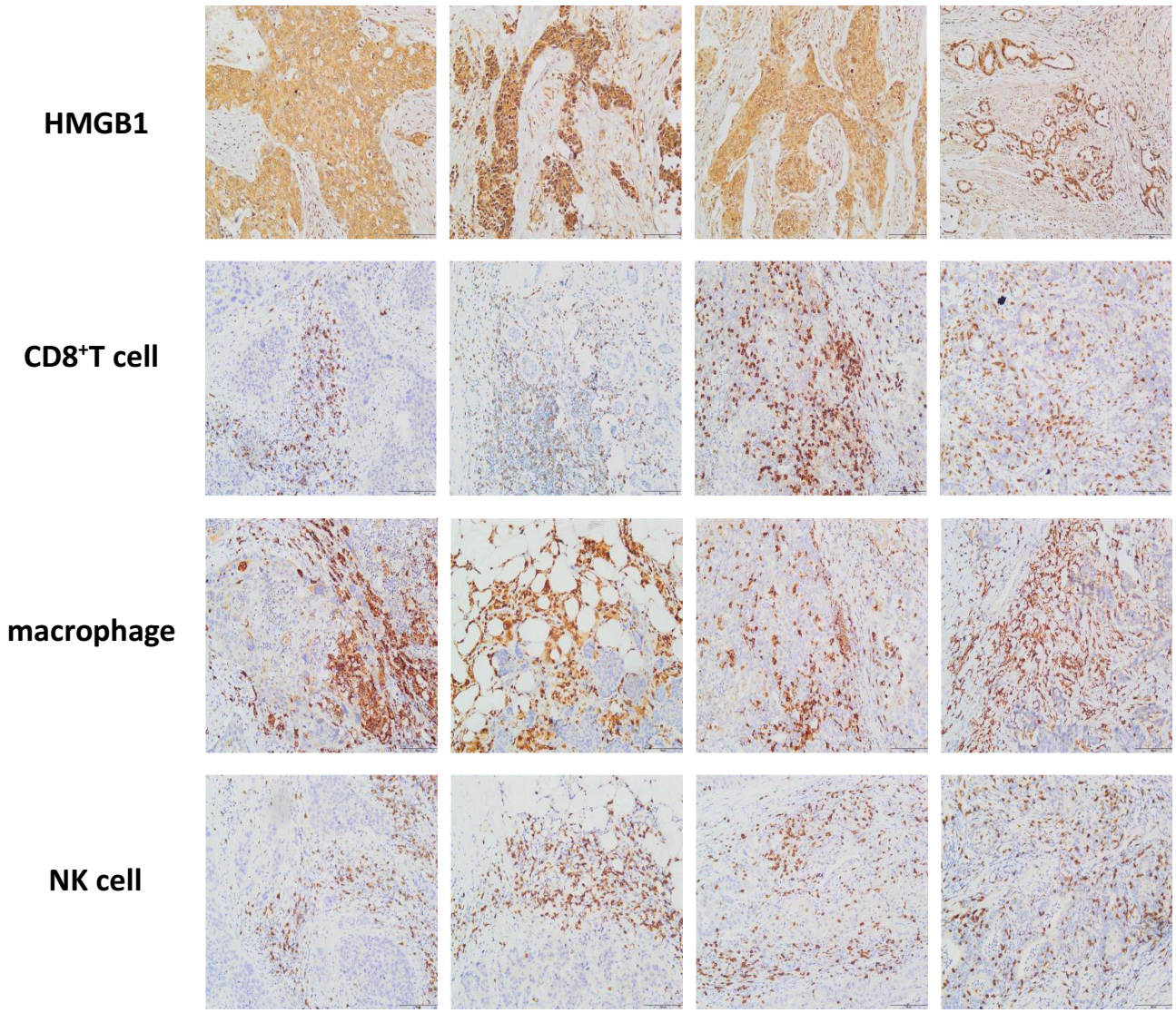

Figure 1 Images of high expression of GSDME/caspase3 in four different tumor tissues, as well as diagrams of high expression of different cytokines and proteins triggered by this pathway.
Note: The vertical array in the figure above corresponds to four different tumor types, and the horizontal array corresponds to different protein markers and staining markers of tumor-associated immune cells.

of high GSDME expression (Cell membrane positive) in cervical cancer and breast cancer was significantly higher than its expression in esophageal cancer and gastric cancer. This is consistent with reports in the literature that there are epigenetic silencing and/or gene mutations that lead to loss of GSDME function in cancer tissues. This also suggests that GSDME may play a role as a tumor suppressor.

Effects of Triggering the Pyroptosis Signaling Pathway on the Immune Microenvironment of Different Tumor Tissues

Through data analysis of this study and review of previous literature, we found that although the expression of GSDME is not the same in different types of cancer tissues, in some cancer tissues, there may be gene silencing or gene mutation that reduces the expression of GSDME protein. However, through data analysis, we found that cancer patients with high GSDME protein expression have a better prognosis. At the same time, we detected more intracellular factors and immune cell infiltration in the tumor tissue stroma of these patients, such as nuclear mobility protein (HMGB1), CD8+ T cells, macrophages, natural killer cells, etc. This suggests to us that it may be that the high expression of GSDME protein

causes tumor tissue to trigger the pyroptosis signaling pathway, which then triggers a cascade of amplified inflammatory responses and recruits a large number of immune cells. The aggregation and infiltration of a large number of immune cells changes the components of the tumor immune microenvironment. These changes may break the immunosuppressive microenvironment formed by the tumor tissue, thereby improving the body's circulating immune system's antagonism to the tumor tissue and playing a role in cancer inhibition and anticancer.

Analysis of the Correlation Between GSDME Expression Levels and Different Immune Markers

Through our research and analysis of 160 cases of different tumor tissues, we found that there was a certain relationship between the degree of immune cell infiltration in the tumor stroma and the expression intensity of caspase3/GSDME

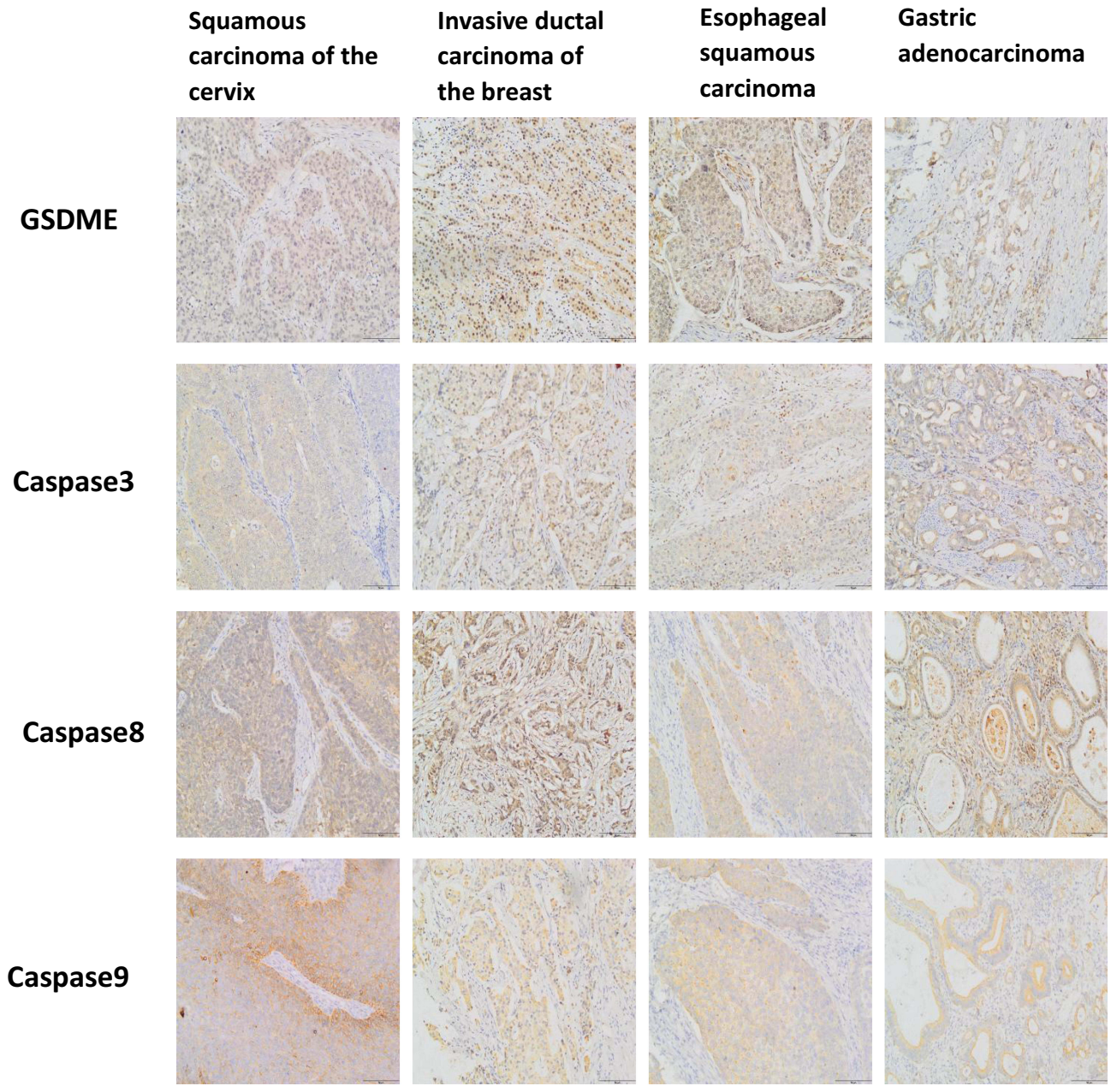

Figure 2 Continued.

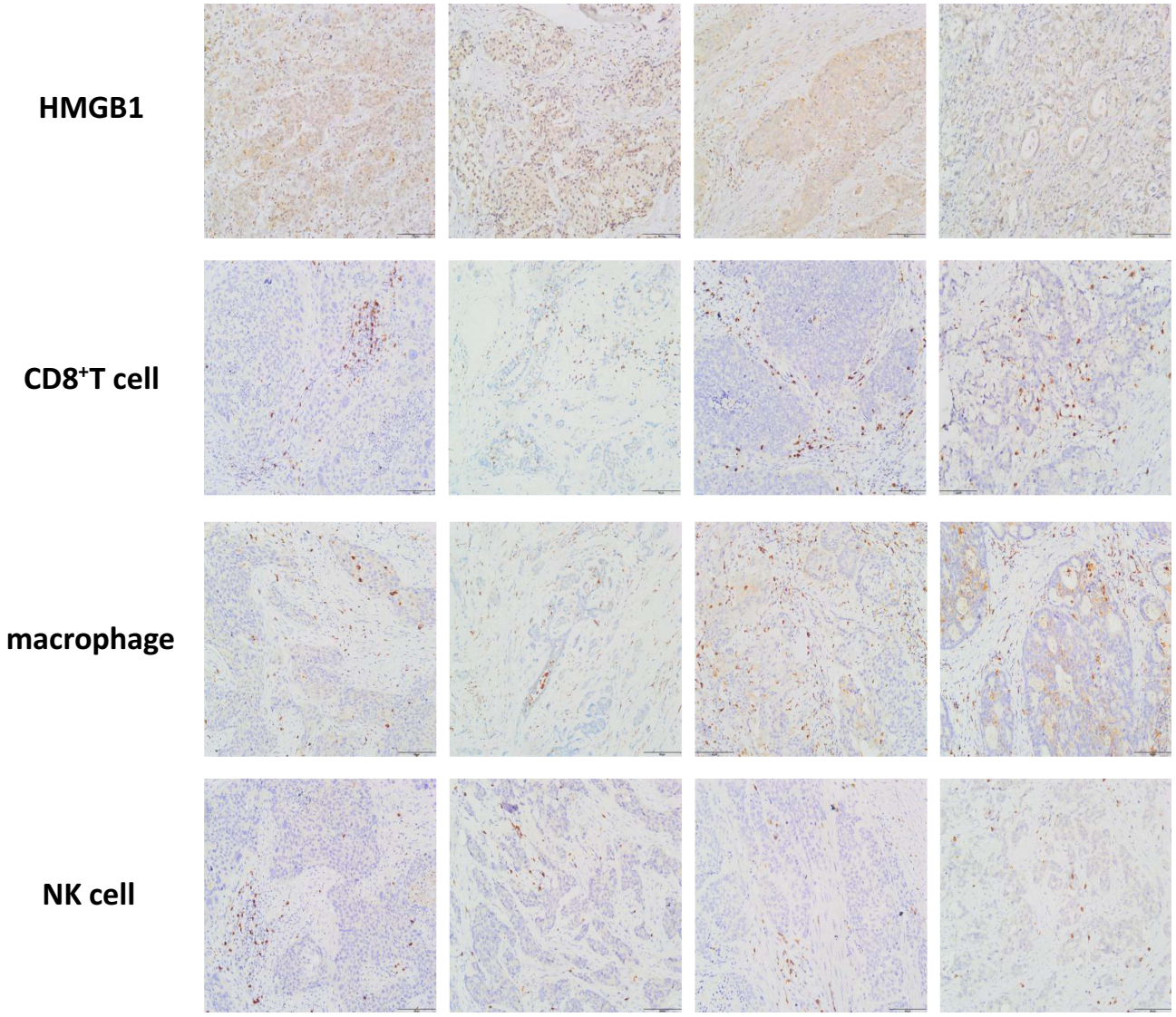

Figure 2 Images of low expression of GSDME/caspase3 in four different tumor tissues, and diagrams of low expression of different cytokines and proteins triggered by this pathway.
Note: The vertical array in the figure above corresponds to four different tumor types, and the horizontal array corresponds to different protein markers and staining markers of tumor-associated immune cells.

protein in tumor tissues where caspase3/GSDME mediated cell pyroptosis signaling pathway was triggered. In tumor tissues with high expression of caspase3 and GSDME, the degree of infiltration of CD8+T cells, macrophages and natural killer cells is correspondingly higher, and there is statistical significance in the differences in the degree of infiltration, which shows that there is a positive correlation between caspase3/GSDME and infiltrating immune cells (Tables 2–5). At the same time, we detected that the expression level of HMGB1 was also positively correlated with the expression of caspase3 and GSDME, which further verified that caspase3/GSDME mediates cell pyroptosis, destroys the integrity of the cell membrane, and causes the release of intracellular proteins to extracellular.

The Main Factor Upstream of Caspase3

Caspase3 is a well-known important factor in regulating apoptosis. We already know that the main cleaving functions upstream of it are caspase8 and caspase9. Caspase8 is an activating factor that triggers the extrinsic apoptotic pathway during the process of apoptosis. However, it has also been reported that caspase8 is a switch that regulates apoptotic cell pyrodeath and programmed cell necrosis.[16] In our current experiment, through the detection of tumor tissues from 160

Table 2 Relationship Between Expression of Various Proteins and Abundance of Tumor-Associated Immune Cells and Clinicopathological Parameters in Cervical Carcinoma

	Caspase3			GSDME			HMGB1			CD8+T cell			Macrophage			NK cell			Caspase8			Caspase9		
	Low	High	*P* value	Low	High	*P* value	Low	High	*P* value	Low	High	*P* value	Low	High	*P* value	Low	High	*P* value	Low	High	*P* value	Low	High	*P* value
Pathology grade																								
I, II	8	20	0.77	11	17	0.891	11	17	0.118	10	18	0.52	10	18	0.73	9	19	0.044	3	25	0.025	22	6	0.086
III	4	8		5	7		8	4		3	9		5	7		8	4		5	7		12	0	
TNM stage																								
I, II	9	20	0.822	10	19	0.259	13	16	0.594	7	22	0.07	11	18	0.929	7	22	<0.001	7	22	0.3	24	5	0.531
III, IV	3	8		6	5		6	5		6	5		4	7		10	1		1	10		10	1	
Lymph node																								
Negative	9	19	0.661	9	19	0.128	12	16	0.382	7	21	0.128	11	17	0.73	6	22	<0.001	7	21	0.238	23	5	0.452
Positive	3	9		7	5		7	5		6	6		4	8		11	1		1	11		11	1	
Survival status																								
Death	6	10	0.411	11	5	0.002	9	7	0.378	11	5	<0.001	9	7	0.047	12	4	<0.001	2	14	0.346	14	2	0.726
Survival	6	18		5	19		10	14		2	22		6	18		5	19		6	18		20	4	
Tumor size																								
≤3	3	12	0.297	5	10	0.517	6	9	0.475	2	13	0.046	4	11	0.285	4	11	0.123	2	13	0.427	10	5	0.011
>3	9	16		11	14		13	12		11	14		11	14		13	12		6	19		24	1	
Caspase3																								
Low				7	5	0.128	9	3	0.022	5	7	0.431	5	7	0.73	5	7	0.946	4	8	0.176	11	1	0.452
High				9	19		10	18		8	20		10	18		12	16		4	24		23	5	
GSDME																								
Low	7	9	0.128				14	2	<0.001	8	8	0.056	6	10	1	10	6	0.037	4	12	0.531	15	1	0.216
High	5	19					5	19		5	19		9	15		7	17		4	20		19	5	
HMGB1																								
Low	9	10	0.022	14	5	<0.001				7	12	0.588	6	13	0.475	10	9	0.228	6	13	0.086	19	0	0.011
High	3	18		2	19					6	15		9	12		7	14		2	19		15	6	

Abundance of CD8+T cells																							
Low	5	8	0.431	8	5	0.056	7	6	0.588				8	5	0.029	9	4	0.017	0	13	0.028	11	2	0.963
High	7	20		8	19		12	15					7	20		8	19		8	19		23	4	
Abundance of macrophage																							
Low	5	10	0.73	6	9	1	6	9	0.475	8	7	0.029				8	7	0.295	2	13	0.427	13	2	0.825
High	7	18		10	15		13	12		5	20					9	16		6	19		21	4	
Abundance of NK cells																							
Low	5	12	0.946	10	7	0.037	10	7	0.228	9	8	0.017	8	9	0.295				4	13	0.642	16	1	0.173
High	7	16		6	17		9	14		4	19		7	16					4	19		18	5	
Caspase8																							
Low	4	4	0.176	4	4	0.531	6	2	0.086	0	8	0.028	2	6	0.427	4	4	0.642				8	0	0.193
High	8	24		12	20		13	19		13	19		13	19		13	19					26	6	
Caspase9																							
Low	11	23	0.452	15	19	0.216	19	15	0.011	11	23	0.963	13	21	0.825	16	18	0.173	8	26	0.193			
High	1	5		1	5		0	6		2	4		2	4		1	5		0	6				

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Table 3 Relationship Between Expression of Various Proteins and Abundance of Tumor-Associated Immune Cells and Clinicopathological Parameters in Breast Cancer

	Caspase3			GSDME			HMGB1			CD8+T cell			Macrophage			NK cell			Caspase8			Caspase9		
	Low	High	*P* value	Low	High	*P* value	Low	High	*P* value	Low	High	*P* value	Low	High	*P* value	Low	High	*P* value	Low	High	*P* value	Low	High	*P* value
Pathology grade																								
I, II	5	22	0.744	7	20	0.211	8	19	0.146	12	15	0.324	9	18	0.096	12	15	0.588	6	21	0.623	21	9	0.623
III	3	10		6	7		7	6		8	5		8	5		7	6		2	11		11	2	
TNM stage																								
I, II	6	25	0.855	6	25	0.001	8	23	0.004	12	19	0.007	11	20	0.101	13	18	0.2	7	24	0.462	23	8	0.093
III, IV	2	7		7	2		7	2		8	1		6	3		6	3		1	8		9	0	
Lymph node																								
Negative	6	15	0.162	4	17	0.058	5	16	0.062	10	11	0.759	7	14	0.228	6	15	0.011	4	17	0.878	16	5	0.539
Positive	2	17		9	10		10	9		10	9		10	9		13	6		4	15		16	3	
Survival status																								
Death	1	11	0.238	10	2	<0.001	9	3	0.001	10	2	0.005	10	2	<0.001	11	1	<0.001	2	10	0.738	10	2	0.738
Survival	7	21		3	25		6	22		10	18		7	21		8	20		6	22		22	6	
Tumor size																								
≤3	6	20	0.52	6	20	0.087	8	18	0.242	13	13	1	9	17	0.178	10	16	0.125	5	21	0.872	20	6	0.52
>3	2	12		7	7		7	7		7	7		8	6		9	5		3	11		12	2	
Caspase3																								
Low				4	4	0.248	4	4	0.427	6	2	0.12	4	4	0.642	1	7	0.027	0	8	0.12	6	2	0.702
High				9	23		11	21		14	18		13	19		18	14		8	24		26	6	
GSDME																								
Low	4	9	0.248				11	2	<0.001	12	1	<0.001	11	2	<0.001	10	3	0.009	1	12	0.186	10	3	0.744
High	4	23					4	23		8	19		6	21		9	18		7	20		22	5	
HMGB1																								
Low	4	11	0.427	11	4	<0.001				12	3	0.003	11	4	0.002	11	4	0.01	3	12	1	10	5	0.108
High	4	21		2	23					8	17		6	19		8	17		5	20		22	3	

Abundance of CD8+T cells																							
Low	6	14	0.12	12	8	<0.001	12	8	0.003				12	8	0.025	11	9	0.355	3	17	0.442	17	3	0.442
High	2	18		1	19		3	17					5	15		8	12		5	15		15	5	
Abundance of macrophage																							
Low	4	13	0.642	11	6	<0.001	11	6	0.002	12	5	0.025				12	5	0.011	3	14	0.757	13	4	0.642
High	4	19		2	21		4	19		8	15					7	16		5	18		19	4	
Abundance of NK cells																							
Low	1	18	0.027	10	9	0.009	11	8	0.01	11	8	0.355	12	7	0.011				5	14	0.355	13	6	0.086
High	7	14		3	18		4	17		9	12		5	16					3	18		19	2	
Caspase8																							
Low	0	8	0.12	1	7	0.186	3	5	1	3	5	0.442	3	5	0.757	5	3	0.355				5	3	0.175
High	8	24		12	20		12	20		17	15		14	18		14	18					27	5	
Caspase9																							
Low	6	26	0.702	10	22	0.744	4	4	0.108	17	15	0.442	13	19	0.642	13	19	0.086	5	27	0.175			
High	2	6		3	5		11	21		3	5		4	4		6	2		3	5				

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Table 4 Relationship Between Expression of Various Proteins and Abundance of Tumor-Associated Immune Cells and Clinicopathological Parameters in Esophageal Carcinoma

	Caspase3			GSDME			HMGB1			CD8+T cell			Macrophage			NK cell			Caspase8			Caspase9		
	Low	High	*P* value	Low	High	*P* value	Low	High	*P* value	Low	High	*P* value	Low	High	*P* value	Low	High	*P* value	Low	High	*P* value	Low	High	*P* value
Pathology grade																								
I, II	8	23	0.295	14	17	0.594	11	20	0.635	14	17	0.594	18	13	0.482	12	19	0.381	7	24	0.462	27	4	0.164
III	4	5		5	4		4	5		5	4		4	5		5	4		1	8		6	3	
TNM stage																								
I, II	9	14	0.15	9	14	0.228	5	18	0.016	8	15	0.063	11	12	0.301	11	12	0.441	3	20	0.211	18	5	0.425
III, IV	3	14		10	7		10	7		11	6		11	6		6	11		5	12		15	2	
Lymph node																								
Negative	9	13	0.101	9	13	0.369	5	17	0.033	7	15	0.028	10	12	0.189	11	11	0.301	3	19	0.278	18	4	0.903
Positive	3	15		10	8		10	8		12	6		12	6		6	12		5	13		15	3	
Survival status																								
Death	4	13	0.456	12	5	0.011	11	6	0.002	14	3	<0.001	8	9	0.398	8	9	0.627	4	13	0.642	14	3	0.984
Survival	8	15		7	16		4	19		5	18		14	9		9	14		4	19		19	4	
Tumor size																								
≤3	9	15	0.215	10	14	0.378	9	15	1	10	14	0.378	11	13	0.161	11	13	0.612	4	20	0.531	21	3	0.32
>3	3	13		9	7		6	10		9	7		11	5		6	10		4	12		12	4	
Caspase3																								
Low				10	2	0.002	7	5	0.078	6	6	0.841	5	7	0.279	8	4	0.044	4	8	0.176	10	2	0.93
High				9	19		8	20		13	15		17	11		9	19		4	24		23	5	
GSDME																								
Low	10	9	0.002				13	6	<0.001	13	6	0.011	12	7	0.337	10	9	0.228	7	12	0.01	15	4	0.585
High	2	19					2	19		6	15		10	11		7	14		1	20		18	3	
HMGB1																								
Low	7	8	0.078	13	2	<0.001				10	5	0.062	10	5	0.262	9	6	0.087	5	10	0.108	13	2	0.602
High	5	20		6	19					9	16		12	13		8	17		3	22		20	5	

Abundance of CD8+T cells																							
Low	6	13	0.841	13	6	0.011	10	9	0.062				9	10	0.369	10	9	0.228	5	14	0.355	15	4	0.585
High	6	15		6	15		5	16					13	8		7	14		3	18		18	3	
Abundance of macrophage																							
Low	5	17	0.279	12	10	0.337	10	12	0.262	9	13	0.369				10	12	0.685	5	17	0.644	19	3	0.49
High	7	11		7	11		5	13		10	8					7	11		3	15		14	4	
Abundance of NK cells																							
Low	8	9	0.044	10	7	0.228	9	8	0.087	10	7	0.228	10	7	0.685				4	13	0.642	14	3	0.984
High	4	19		9	14		6	17		9	14		12	11					4	19		19	4	
Caspase8																							
Low	4	4	0.176	7	1	0.01	5	3	0.108	5	3	0.355	5	3	0.644	4	4	0.642				8	0	0.153
High	8	24		12	20		10	22		14	18		17	15		13	19					25	7	
Caspase9																							
Low	10	23	0.93	15	18	0.585	13	20	0.602	15	18	0.585	19	14	0.49	14	19	0.984	8	25	0.153			
High	2	5		4	3		2	5		4	3		3	4		3	4		0	7				

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Table 5 Relationship Between Expression of Various Proteins and Abundance of Tumor-Associated Immune Cells and Clinicopathological Parameters in Gastric Carcinoma

	caspase3			GSDME			HMGB1			CD8+T cell			Macrophage			NK cell			Caspase8			Caspase9		
	Low	High	*P* value	Low	High	*P* value	Low	High	*P* value	Low	High	*P* value	Low	High	*P* value	Low	High	*P* value	Low	High	*P* value	Low	High	*P* value
Pathology grade																								
I, II	1	20	0.502	12	9	0.149	10	11	0.759	9	12	0.209	8	13	0.565	7	14	0.228	3	18	0.2	19	2	0.919
III	2	17		15	4		10	9		12	7		9	10		10	9		6	13		17	2	
TNM stage																								
I, II	1	28	0.12	16	13	0.006	11	18	0.012	11	18	0.002	10	19	0.101	9	20	0.017	6	23	0.666	25	4	0.204
III, IV	2	9		11	0		9	2		10	1		7	4		8	3		3	8		11	0	
Lymph node																								
Negative	1	16	0.746	8	9	0.017	6	11	0.115	3	14	<0.001	7	10	0.888	4	13	0.038	1	16	0.031	14	3	0.174
Positive	2	21		19	4		14	9		18	5		10	13		13	10		8	15		22	1	
Survival status																								
Death	2	21	0.746	22	1	<0.001	15	8	0.025	18	5	<0.001	13	10	0.038	15	8	<0.001	7	16	0.171	21	2	0.757
Survival	1	16		5	12		5	12		3	14		4	13		2	15		2	15		15	2	
Tumor size																								
≤3	1	11	0.899	7	5	0.431	4	8	0.176	4	8	0.118	2	10	0.031	3	9	0.15	1	11	0.168	12	0	0.176
>3	2	26		20	8		16	12		17	11		15	13		14	14		8	20		24	4	
Caspase3																								
Low				2	1	0.975	2	1	0.56	2	1	0.62	2	1	0.392	2	1	0.392	2	1	0.059	3	0	0.56
High				25	12		18	19		19	18		15	22		15	22		7	30		33	4	
GSDME																								
Low	2	25	0.975				18	9	0.002	20	7	<0.001	15	12	0.015	17	10	<0.001	7	20	0.467	25	2	0.444
High	1	12					2	11		1	12		2	11		0	13		2	11		11	2	
HMGB1																								
Low	2	18	0.56	18	2	0.002				14	6	0.027	11	9	0.115	11	9	0.115	4	16	0.714	17	3	0.304
High	1	19		9	11					7	13		6	14		6	14		5	15		19	1	

Abundance of CD8+T cells																							
Low	2	19	0.62	20	1	<0.001	14	7	0.027				12	9	0.05	15	6	<0.001	6	15	0.346	20	1	0.257
High	1	18		7	12		6	13					5	14		2	17		3	16		16	3	
Abundance of macrophage																							
Low	2	15	0.392	15	2	0.015	11	6	0.115	12	5	0.05				12	5	0.001	5	12	0.381	17	0	0.073
High	1	22		12	11		9	14		9	14					5	18		4	19		19	4	
Abundance of NK cells																							
Low	2	15	0.392	17	0	<0.001	11	6	0.115	15	2	<0.001	12	5	0.001				5	12	0.381	17	0	0.073
High	1	22		10	13		9	14		6	17		5	18					4	19		19	4	
Caspase8																							
Low	2	7	0.059	7	2	0.467	4	5	0.714	6	3	0.346	5	4	0.381	5	4	0.381				9	0	0.267
High	1	30		20	11		16	15		15	16		12	19		12	19					27	4	
Caspase9																							
Low	3	33	0.56	25	11	0.444	17	19	0.304	20	16	0.257	17	19	0.073	17	19	0.073	9	27	0.267			
High	0	4		2	2		3	1		1	3		0	4		0	4		0	4				

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cancer patients, we found that there was a positive correlation between the expression of caspase8 and caspase3, but there was no obvious correlation between the expression level of caspase9 and caspase3. This suggests that caspase8 may play a major role upstream in triggering cell pyroptosis. However, a deeper understanding of the function of caspase8 remains to be explored in our follow-up studies.

The Expression Level of Caspase3/GSDME is Related to the Clinical Prognosis of Esophageal Cancer, Gastric Cancer, Breast Cancer and Cervical Cancer

Through analysis of the research results of 160 cases of different tumor tissues (including esophageal cancer, gastric cancer, breast cancer and cervical cancer), we found that the tumor tissues of patients with high GSDME protein expression had an abundance of infiltrating immune cells such as CD8+T and natural killer cells. The invasion and metastasis capabilities of tumor tissues in these patients are lower, and the clinical prognosis is better. We visually demonstrate the effect of various variables on the clinical patient survival analysis in this experiment through the data in Table 6 and Figure 3

Discussion

The relationship between pyroptosis and tumor development is not static. In different tumor tissues and different individual genetic backgrounds, pyroptosis has different effects on tumor tissues.[2,17] With the continuous exploration of pyroptosis in recent years, we have become familiar with the classical and non-classical pyroptosis signaling pathways mediated by GSDMD, as well as the newly discovered pyroptosis signaling pathway mediated by GSDME.[4,5,8] The purpose of our current study is to explore the impact of the caspase3-dependent pyroptosis signaling pathway mediated by GSDME on various clinical parameters and prognosis of tumor patients after being triggered in different tumor tissues and destroying the integrity of the cell membrane.

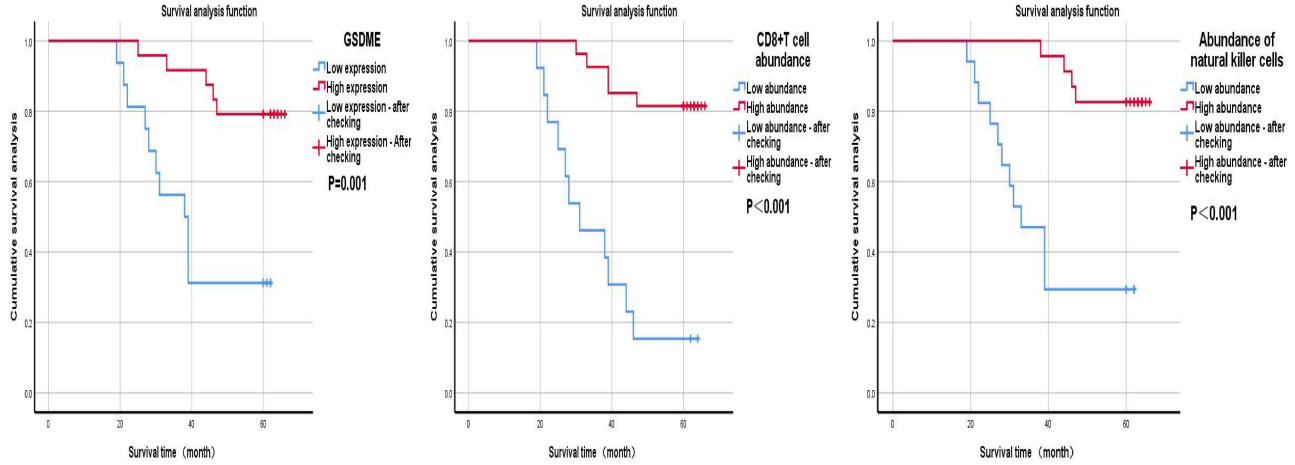
In our research results, the expression level of GSDME is positively correlated with the clinical prognosis of tumor patients. However, in patients with gastric cancer, we found that the number of cases with high GSDME protein expression was significantly less than that in other tumor tissues. We speculate that this may be the result of GSDME methylation or genetic mutation. At the same time, some studies have pointed out that GSDME is inhibited by methylation in primary gastric cancer and colorectal cancer.[18] At the same time, some reports that GSDME is a tumor suppressor gene in a large proportion of gastric cancer and colorectal cancer have attracted our attention.[12] The expression of GSDME in gastric cancer is indeed suppressed to a large extent, but if GSDME plays a role as a tumor suppressor gene in gastric cancer and colorectal cancer, can we inhibit GSDME methylation to make it highly expressed in tumor tissues? And then exert the effect of inhibiting tumors, which needs to be further explored in our follow-up research.

Table 6 Univariate Survival Analysis Table

	P value of Univariate Survival Analysis			
	Esophageal Squamous Carcinoma	**Gastric Adenocarcinoma**	**Invasive Ductal Carcinoma of the Breast**	**Squamous Carcinoma of the Cervix**
GSDME	0.006*	<0.001*	<0.001*	0.001*
Caspase3	0.585	0.641	0.29	0.31
HMGB1	0.002*	0.037*	0.001*	0.27
CD8+T cell	<0.001*	<0.001*	0.005*	<0.001*
NK cell	0.717	<0.001*	<0.001*	<0.001*
Macrophage	0.348	0.044*	0.001*	0.06
Caspase8	0.589	0.068	0.685	0.343
Caspase9	0.751	0.583	0.643	0.786

Note: *is a *P* value less than 0.05, which has statistical significance.

A. Squamous carcinoma of the cervix



B. Invasiveductal carcinoma of the breast cancer

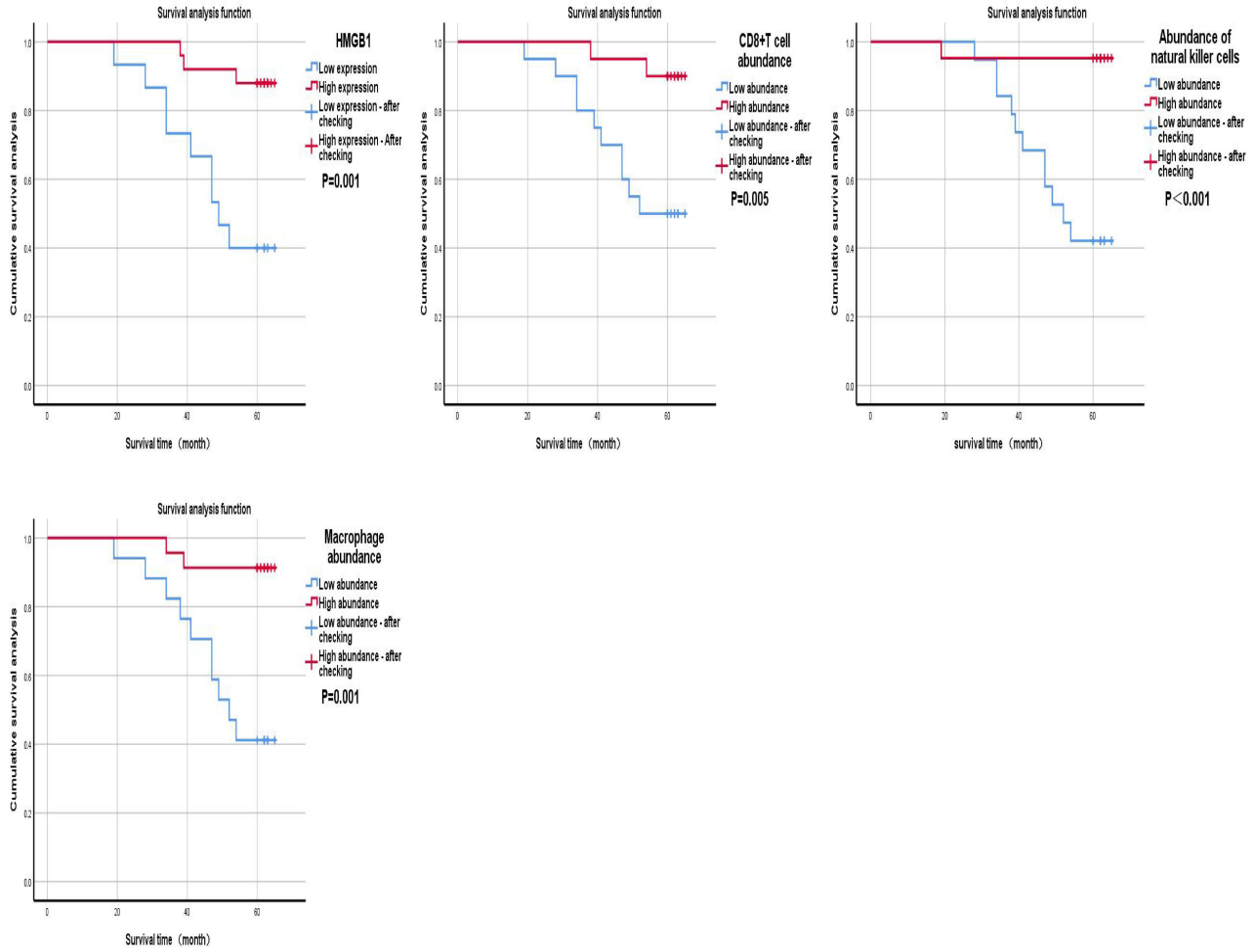
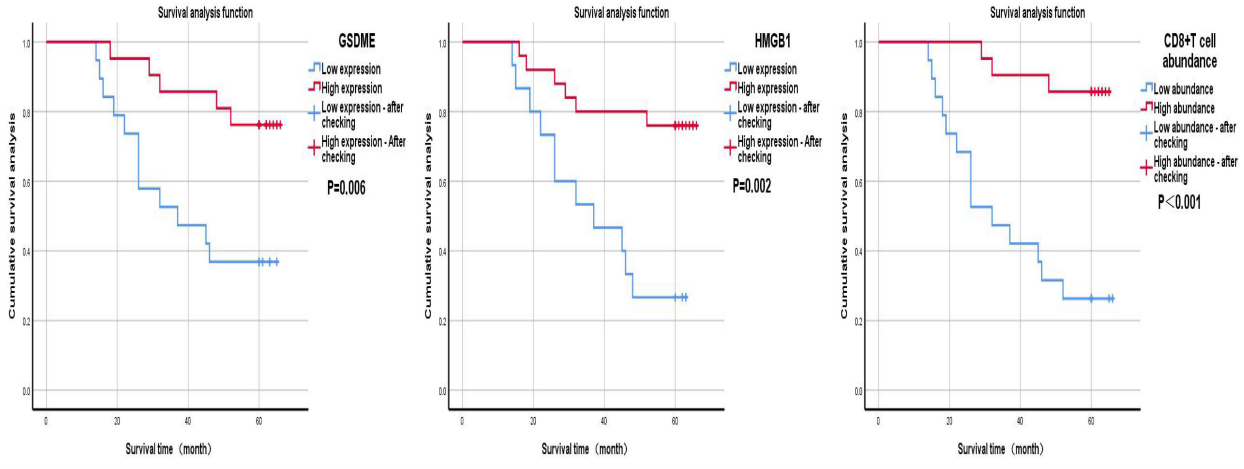


Figure 3 Continued.

C. Esophageal squamous carcinoma



D. Gastric adenocarcinoma

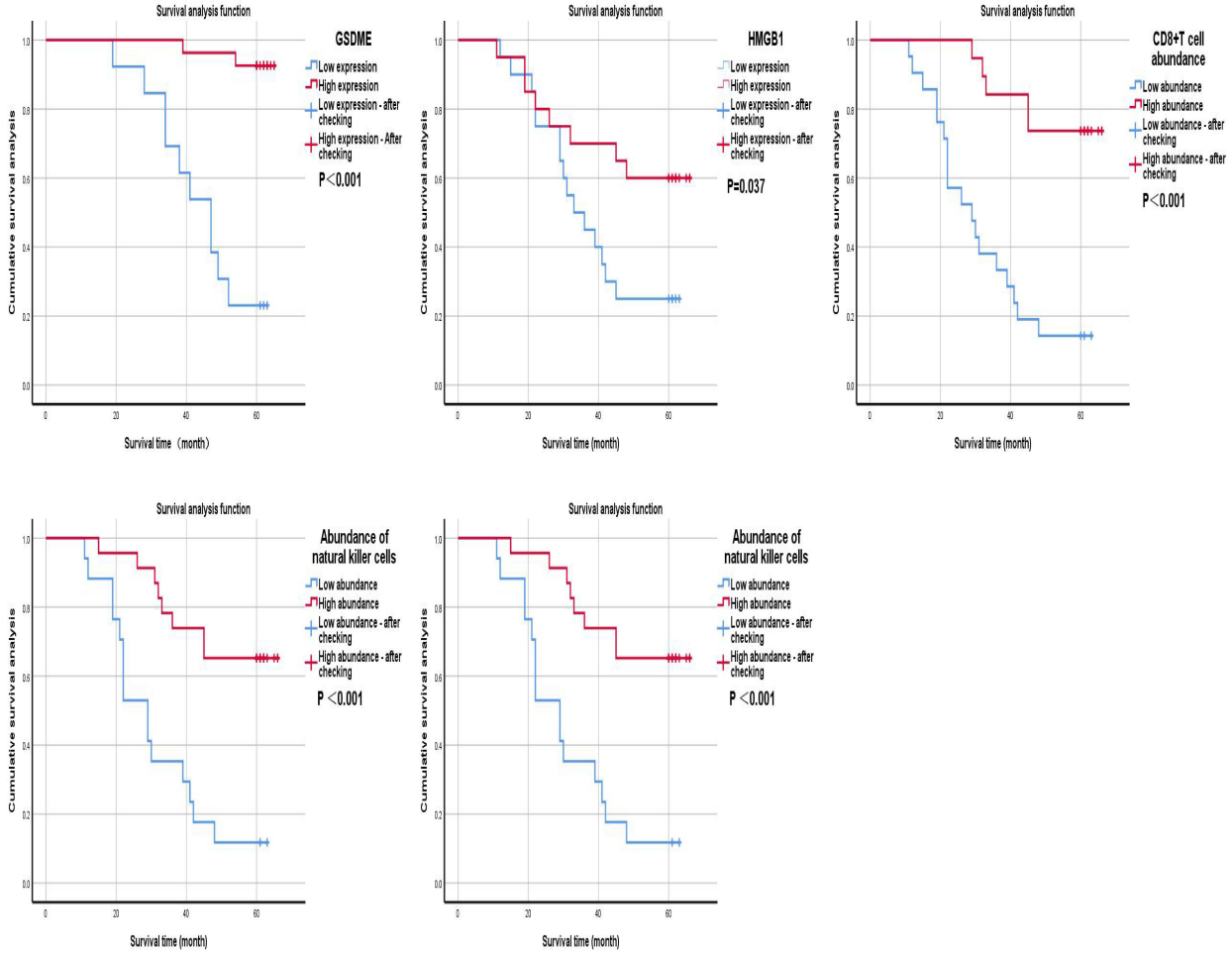


Figure 3 (**A**–**D**) is the K-M survival analysis curve with meaningful P-values for each variable in the univariate survival analysis for Squamous carcinoma of the cervix, Invasive ductal carcinoma of the breast, Esophageal squamous carcinoma and Gastric adenocarcinoma.

One of the characteristics of pyroptosis is that it destroys the integrity of the cell membrane. After triggering the pyroptosis pathway, we observe that intracellular factors are passively released outside the cell. High mobility group protein (HMGB1) is one of the representatives. As a nuclear factor, HMGB1 also plays dual functions. Intracellular HMGB1 is a highly conserved chromosomal protein that participates in DNA damage repair to maintain genome stability; while extracellular HMGB1 is related to inflammation and damage.[19] However, the impact of extracellular HMGB1 protein on tumor tissue has been controversial in academic circles. It is possible that the functions of HMGB1 in inhibiting tumors and promoting tumor proliferation and metastasis are also changing dynamically during the entire process of cancer changes.[20]

Our research results also show that HMGB1 has the effect of pro-inflammatory and recruiting immune cells. The degree of infiltration of CD8+ T cells, tumor-associated macrophages, and natural killer cells in the tumor stroma is positively correlated with the expression of HMGB1 protein. Moreover, the expression level of HMGB1 protein and the infiltration abundance of recruited immune cells reduce the invasion and metastasis ability of tumor tissue to a certain extent, and are significantly related to the prognosis of tumor patients. Despite this, we cannot directly conclude that HMGB1 plays a tumor suppressor role in tumor tissues. The process of tumor progression is constantly changing. Taking into account individual heterogeneity and temporal structural heterogeneity, the specific functions of HMGB1 in the process of tumor progression need to be further understood.

It is precisely because the triggering of pyroptosis reaction leads to the release of intracellular factors and the accumulation of extracellular inflammatory factors and related immune cells that we pay attention to possible changes in the tumor microenvironment. The tumor immune microenvironment is a part of the tumor microenvironment. The components of the tumor immune microenvironment are complex and are always changing during the progression of the tumor. We have known that some components of the tumor immune microenvironment can inhibit the growth of the tumor, but at the same time, there are also tumor promoting components.[21] For example, literature reports indicate that activated macrophages and NKT cells simultaneously play two-way tumor promotion and tumor suppression functions in tumor immunity.[22,23] Therefore, how to balance the anti-tumor and tumor-promoting effects of TIME is one of the issues that we need to further explore and solve.

We already know that in some tumor tissues, infiltrating tumor-associated lymphocytes are an independent factor affecting patient prognosis, and the most critical thing to fight against tumor tissues and the most infiltrated in tumor tissues are CD8+ T cells and macrophages.[14] In the results of our current study, the abundance of infiltrating CD8+ T cells in four different types of tumor tissues was positively correlated with the expression level of GSDME protein, and patients with higher abundance of CD8+T cells had fewer lymph node metastases and better prognosis, which also verifies the function of CD8+T cells as tumor-specific killer cells. Interestingly, however, the abundance of macrophage infiltration did not correlate positively with the prognosis of patients with all tumor types as we would expect. We know that macrophages are the most abundant cell types in the tumor microenvironment, and their plasticity and heterogeneity enable macrophages to be polarized into different subtypes according to changes in the microenvironment, thus playing different roles in various stages of disease development.[24] Unfortunately, our current study is limited in accurately measuring macrophages within the tumor immune microenvironment, thus preventing us from drawing definitive conclusions. However, the results of different associations between the abundance of macrophage infiltration in different types of tumor tissues and clinical prognosis indicate that in different cancer patients At different stages of the disease, macrophages always exist and play different roles, which provides a certain research basis for our subsequent research on macrophages.

In addition to focusing on the changes in the tumor immune microenvironment after tumor tissue triggers pyroptosis, we also pay attention to the integrity of the pyroptosis signaling pathway. We know that when caspase3 mediates cell apoptosis, its main upstream factors are different depending on the reasons for triggering the apoptosis mechanism.[25] However, in the pathway in which caspase3 cleaves GSDME to mediate cell pyroptosis, we found that caspase8 is positively correlated with caspase3 protein expression, but caspase9 has no correlation. Therefore, we speculate that caspase8 mainly plays a role upstream of caspase3 during pyroptosis, which is also different from apoptosis.

Conclusion

Through the observation and analysis of different tumor tissues this time, we speculate that tumor tissues with high expression of caspase3/GSDME trigger a pyroptosis response, leading to a large release of intracellular factors, thereby recruiting a large number of inflammatory factors and immune cells cause changes in the components of the tumor

immune microenvironment, fight against the tumor immunosuppressive microenvironment, inhibit or reduce the proliferation and invasion capabilities of tumor tissue, improve the body's immune surveillance and clearance of tumor cells. Changes in the immune microenvironment of tumor tissue play an important role in controlling the endless proliferation and distant metastasis of tumors. However, the specific impact of changes in the components of the immune microenvironment on tumor tissue remains to be further explored. The importance of the changes in the immune microenvironment of tumor tissues is self-evident, and we need to conduct more in-depth exploration of its change process. If the ability of tumor cell proliferation and invasion can be inhibited or reduced through the regulation of tumor immune microenvironment, then a better prognosis or a longer survival with cancer will become a reality for cancer patients. These changes will provide strong support for the treatment of solid tumors in the future, and will also bring substantial changes and benefits to patients with malignant tumors in the future.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Shigeta K, Matsui A, Kikuchi H, et al. Regorafenib combined with PD1 blockade increases CD8 T-cell infiltration by inducing CXCL10 expression in hepatocellular carcinoma. *J Immunother Cancer*. 2020;8(2):e001435. doi:10.1136/jitc-2020-001435
2. Hu J, Pei W, Jiang M, et al. DFNA5 regulates immune cells infiltration and exhaustion. *Cancer Cell Int*. 2022;22(1):107. doi:10.1186/s12935-022-02487-0
3. Wang YY, Liu XL, Zhao R. Induction of pyroptosis and its implications in cancer management. *Front Oncol*. 2019;9:971. doi:10.3389/fonc.2019.00971
4. Shi J, Zhao Y, Wang K, et al. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature*. 2015;526(7575):660–665. doi:10.1038/nature15514
5. Jiang M, Qi L, Li L, Li Y. The caspase-3/GSDME signal pathway as a switch between apoptosis and pyroptosis in cancer. *Cell Death Discov*. 2020;6:112. doi:10.1038/s41420-020-00349-0
6. Wang Y, Gao W, Shi X, et al. Chemotherapy drugs induce pyroptosis through caspase-3 cleavage of a gasdermin. *Nature*. 2017;547(7661):99–103. doi:10.1038/nature22393
7. Shi J, Gao W, Shao F. Pyroptosis: gasdermin-mediated programmed necrotic cell death. *Trends Biochem Sci*. 2017;42(4):245–254. doi:10.1016/j.tibs.2016.10.004
8. Zhang Z, Zhang Y, Xia S, et al. Gasdermin E suppresses tumour growth by activating anti-tumour immunity. *Nature*. 2020;579(7799):415–420. doi:10.1038/s41586-020-2071-9
9. Wang Y, Peng J, Xie X, Zhang Z, Li M, Yang M. Gasdermin E-mediated programmed cell death: an unpaved path to tumor suppression. *J Cancer*. 2021;12(17):5241–5248. doi:10.7150/jca.48989
10. Nagata S. Apoptosis and clearance of apoptotic cells. *Annu Rev Immunol*. 2018;36:489–517. doi:10.1146/annurev-immunol-042617-053010
11. Yang S, Thor AD, Edgerton S, Yang X. Caspase-3 mediated feedback activation of apical caspases in doxorubicin and TNF-alpha induced apoptosis. *Apoptosis*. 2006;11(11):1987–1997. doi:10.1007/s10495-006-0084-y
12. Wang Y, Yin B, Li D, Wang G, Han X, Sun X. GSDME mediates caspase-3-dependent pyroptosis in gastric cancer. *Biochem Biophys Res Commun*. 2018;495(1):1418–1425. doi:10.1016/j.bbrc.2017.11.156
13. Gaidt MM, Hornung V. Pore formation by GSDMD is the effector mechanism of pyroptosis. *EMBO J*. 2016;35(20):2167–2169. doi:10.15252/embj.201695415
14. Hu H, Yang M, Dong W, et al. A pyroptosis-related gene panel for predicting the prognosis and immune microenvironment of cervical cancer. *Front Oncol*. 2022;12:873725. doi:10.3389/fonc.2022.873725
15. Huang YL, Zhang GH, Zhu Q, Wu X, Wu LG. Expression levels of caspase-3 and gasdermin E and their involvement in the occurrence and prognosis of lung cancer. *Cancer Rep*. 2022;5(9):e1561. doi:10.1002/cnr2.1561
16. Li K, Qiu J, Pan J, Pan JP. Pyroptosis and its role in cervical cancer. *Cancers*. 2022;14(23):5764. doi:10.3390/cancers14235764
17. Huang Y, Zhang G, Zhu Q, Wu X, Wu L. Role of cytokines released during pyroptosis in non-small cell lung cancer. *Cancer Manag Res*. 2021;13:7399–7409. doi:10.2147/CMAR.S330232
18. Kim MS, Chang X, Yamashita K, et al. Aberrant promoter methylation and tumor suppressive activity of the DFNA5 gene in colorectal carcinoma. *Oncogene*. 2008;27(25):3624–3634. doi:10.1038/sj.onc.1211021
19. Kang R, Zhang Q, Zeh HJ, Lotze MT, Tang D. HMGB1 in cancer: good, bad, or both? *Clin Cancer Res*. 2013;19(15):4046–4057. doi:10.1158/1078-0432.CCR-13-0495
20. Zhang Z, Wang M, Zhou L, et al. Increased HMGB1 and cleaved caspase-3 stimulate the proliferation of tumor cells and are correlated with the poor prognosis in colorectal cancer. *J Exp Clin Cancer Res*. 2015;34(1):51. doi:10.1186/s13046-015-0166-1

21. Lv B, Wang Y, Ma D, et al. Immunotherapy: reshape the tumor immune microenvironment. *Front Immunol*. 2022;13:844142. doi:10.3389/fimmu.2022.844142
22. Anderson NR, Minutolo NG, Gill S, Klichinsky M. Macrophage-based approaches for cancer immunotherapy. *Cancer Res*. 2021;81(5):1201–1208. doi:10.1158/0008-5472.CAN-20-2990
23. Pan Y, Yu Y, Wang X, Zhang T. Tumor-associated macrophages in tumor immunity [published correction appears in Front Immunol. 2021 Dec 10;12:775758]. *Front Immunol*. 2020;11:583084. doi:10.3389/fimmu.2020.583084
24. Miao X, Leng X, Zhang Q. The current state of nanoparticle-induced macrophage polarization and reprogramming research. *Int J Mol Sci*. 2017;18(2):336. doi:10.3390/ijms18020336
25. Wu Y, Wang D, Wang X, et al. Caspase 3 is activated through caspase 8 instead of caspase 9 during H2O2-induced apoptosis in HeLa cells. *Cell Physiol Biochem*. 2011;27(5):539–546. doi:10.1159/000329955