THAI NGUYEN UNIVERSITY
THAI NGUYEN UNIVERSITY OF MEDICINE AND PHARMACY

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RESEARCH ON THE EXPRESSION AND RELATIONSHIP OF IMMUNE MARKERS OF ALDEHYDE DEHYDROGENASE, KRAS IN GASTRIC ADENOCARCINOMA

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ABSTRACT OF MEDICAL PHD THESIS

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INTRODUCTION

Stomach cancer is a common cancer with the second highest number of deaths in the world. According to GLOBOCAN 2020 estimates, stomach cancer has caused about 800,000 deaths (accounting for 7.7% of total cancer deaths) and is the fourth leading cause of cancer death in the two sexes combined.

In the past 2 decades, the expression of Aldehyde dehydrogenase and KRAS have been known as important markers participating in the formation, development and metastasis of cancer. However, understanding of the role of ALDH in the progression and metastasis of gastric cancer is still limited. Besides, KRAS is known as a particularly important gene in cancer signaling pathway. Currently, there are very few studies on the relationship between KRAS expression levels in cancer in general and stomach cancer in particular.

In Vietnam, there have been a number of studies on immunohistochemistry, but no study has mentioned the relationship between co-expression of *ALDH* and *KRAS* with the clinical and histopathological features of stomach cancer. Therefore, we conduct this topic with the objectives:

1. Describe clinical and paraclinical characteristics and expression of immune markers Aldehyde dehydrogenase and KRAS in gastric cancer patients.

2. Analyze the relationship between the expression of immune markers Aldehyde dehydrogenase and KRAS with some clinical and paraclinical characteristics of gastric cancer patients.

NEW CONTRIBUTION OF THE THESIS

- This is the first study on the expression of ALDH and KRAS conducted in Vietnam.

- Research results have shown that the rate of patients with positive ALDH is 68.0%, positive KRAS is 55.3%.

- The expression of ALDH is highest in the tubular type (65.7%) according to WHO histopathological characteristics. The expression of ALDH was highest (35,7%) at low differentiation. KRAS expression was highest (33,3%) at low differentiation. Simultaneous expression of two markers has the highest rate (34,0%) at low differentiation levels.

THESIS STRUCTURE

The thesis consists of 123 pages (excluding references and appendices), 4 chapters (question 2 pages, overview 38 pages, object and research methods 23 pages, research results 29 pages, discussion 28 pages, conclusion 2 pages, recommendations 1 page), the results have 43 tables, 4 chart, 23 figures, 130 references (9 Vietnamese, 121 English), 4 appendices.

MAIN ABBREVIATIONS

1.	ALDH	Aldehyde Dehydrogenase
2.	KRAS	Kirsten Rat Sarcoma Viral Oncogene Homolog
3.	GC	Gastric cancer

CHAPTER I
OVERVIEW

1.1. Application of *ALDH* in GC

1.1.1. ALDH's role in protecting cancer cells

There is abundant evidence implicating *ALDH* in self-protection against endogenous and exogenous threats in cancer cells. Inactivation of antioxidants and substrate-specific drugs is one of the mechanisms controlling this ability. For example, *ALDH* has the ability to regenerate NADPH, which is consistent with its cellular antioxidant capacity. In addition, *ALDH* is also frequently co-expressed at high levels with antioxidant factors and intracellular drug transport channels. *ALDH1A1* and *ALDH1A3* have the ability to inactivate enzymes with alkyl groups such as oxazaphosphorines. Furthermore, *ALDH* is resistant to drugs and analogues such as doxorubicin, cisplatin, arbinofuranosyl citidine (Ara-C), temozolemide and taxanes although the mechanism is still unclear.

1.1.2. The role of ALDH in treatment resistance

ALDH is an enzyme involved in the detoxification process, protecting tissues from the toxic effects of aldehydes that have been known for a long time. *ALDH1A1* and *ALDH3A1* can protect cells against the toxic effects of drugs. The first observations several decades ago in hematopoietic and leukemic stem cells with *ALDH* were highly resistant to cyclophosphamide and its alkylates, *ALDH1A1* and *ALDH3A1* modified cyclophosphamide in active form and 4-hydroperoxycyclophosphamide into an inactive form for excretion. Therefore, *ALDH* can cause drug resistance and radiotherapy resistance.

1.2. Application of *KRAS* in GC

1.2.1. Role of KRAS in gastric cancer metastasis

Genes encoding the Receptor Tyrosine Kinase (RTK)-RAS signaling pathway and the tumor suppressor TP53 are altered in 60% and 50% of gastric adenocarcinomas, respectively. The RAS family of proteins (in humans, HRAS, *KRAS*, and NRAS) are small GTPases involved in cell signaling processes that support cell growth and survival. *KRAS* is amplified or mutated in 17% of gastric adenocarcinomas. Upon stimulation by upstream receptors, *KRAS* switches from an inactive, GDP-bound form to an active, GTP-bound form. This conformational change leads to its binding to the RAF. *KRAS* recruits RAFs to the membrane where RAF activation and dimerization are promoted. Activated RAF and activated MEK phosphorylation, and activated MEK phosphorylation and ERK activation.

There is some evidence that RTK-RAS signaling is important in epithelial-to-mesenchymal transition (EMT) and maintenance of gastric cancer stem cells (CSCs). CSCs, the existence of which is still controversial, share properties of normal stem cells such as the ability to self-renewal and differentiate, and may be a source of metastasis. Many of the phenotypic differences between CSCs and stem-deficient tumor cells may be due to epigenetic changes induced by the EMT program. Although the role of the RTK-RAS pathway in EMT and CSC has been studied more extensively in other cancer types, there have been few studies specifically on gastric adenocarcinoma.

1.2.2. The role of KRAS in treatment resistance

A meta-analysis by Hewitt, Lindsay C. reported that approximately 64 studies reported the prevalence of *KRAS* mutations in GC, with the majority of studies (61%) originating from Asia. Two studies compared *KRAS* mutations between GC patients from the East and the West (37,38). Forty-five (70%) studies investigated *KRAS* mutation status in patient groups that included fewer than 100 patients. The majority of studies (70%) investigated *KRAS* mutation status in less than 100 patients. Such small studies may not be representative of the GC patient population. Thus, two of the smallest studies with five and seven patients reported some of the highest *KRAS* mutation rates, 20% and 29%, respectively. Furthermore, twenty-two (34%) studies investigating *KRAS* mutations intentionally selected subgroups of GC patients to study *KRAS*/BRAF mutation status, such as progressive disease and/or metastasis and early disease.

The predictive value of *KRAS* and *BRAF* mutations in GC is much less clear. In vitro, several studies on *KRAS* wild-type GC cell lines have reported sensitivity to EGFR-targeted drugs. Other investigators reported that both mutant and wild-type GC cell lines were resistant to cetuximab. In GC xenografts, apoptosis was induced only in *KRAS* wild-type tumor cells treated with Cetuximab. Cetuximab has been shown to reduce tumor volume, spread, and angiogenesis in wild-type, EGFR-expressing xenografts.

CHAPTER II
SUBJECTS AND METHODS

2.1. Studying subjects

The study subjects were 103 patients with a confirmed diagnosis of stomach cancer and tumor removal surgery at K Hospital, facility 1, Quan Su, Hanoi, from May 2017 to May 2020.

2.1.1 Selection criteria for study patients

Patients were selected for the study when meeting the following criteria:

- The patient was diagnosed with primary gastric carcinoma by histopathology according to ESMO 2016 standards.
- The patient was treated with tumor resection surgery with regional lymph node dissection.
- Sufficient tissue samples for immunohistochemistry.
- Patients agree and voluntarily participate in the study.

2.1.2. Exclusive criteria

- GC metastasizes from other organs.
- GC recurrence.
- There is another cancer associated with GC.
- Have been treated with chemotherapy.

2.2. Reseach methodology

Cross-sectional descriptive research method.

2.3. The main criteria and classifications in the research

- Tumor location in the stomach on gastroendoscopy.
- Tumor morphology according to Borrmann:

+ Borrmann type I (Polyp form), Borrmann type II (Fungal form), Borrmann type III (Ulcerative form), Borrmann type IV

(Infiltrative form).

- Histopathological classification according to Lauren:

+ Intestinal type, diffuse type, mixed type.

- Histopathologic features according to the 2010 WHO classification, including: papillary adenocarcinoma, tubular adenocarcinoma, mucinous adenocarcinoma, signet-ring cell carcinoma, adenosquamous carcinoma, squamous cell carcinoma, small cell carcinoma, undifferentiated carcinoma, other carcinoma.

- Histopathological classification according to the degree of differentiation according to WHO:

+ Poor differentiation.

+ Moderate differentiation.

+ High differentiation.

- Diagnosis of TNM stage: According to the 7th AJCC 2009 system.

- Evaluate the expression level of the *ALDH* marker in cancer samples and control samples according to the levels: 0, 1+, 2+, 3+. The expression level of *ALDH* is 0 evaluated as negative, the expression level of *KRAS* is 1+, 2+ and 3+ evaluated as positive.

- Evaluate the expression level of *KRAS* markers in cancer samples and control samples according to the levels: 0, 1+, 2+, 3+. *KRAS* expression levels of 0 and 1+ are considered negative, *KRAS* expression levels of 2+ and 3+ are considered positive.

2.4. The method of data collection

2.4.1. Clinical examination and subclinical indications

The patients coming to the hospital were asked about the history, clinical examination, indicated hematology, biochemistry,

coagulation, and immunological tests. The patient is assigned to have a gastric endoscopy. When a stomach tumor is found, a biopsy will be performed during the endoscopy.

2.4.2. Gastroendoscopy with biopsy

- Put the scope through the mouth, throat into the esophagus, stomach, down the duodenum, inflate and observe. It may be necessary to pump water to clear the mucus in the areas to be observed, and to drain the esophagus and stomach.

- If lesions are detected, pump clean, then observe carefully with NBI mode and near focus to evaluate, biopsy for pathology.

- Specimens are fixed into tubes containing Formol 20% neutral buffer.

2.4.3. Tumor removal surgery and how to handle specimens

After being diagnosed as GC, the patient underwent surgery to remove the tumor at the Department of Surgery, K Hospital. Specimens from gastric cancer after surgery were transferred to the Pathology Department at K Hospital for treatment. dissected, fixed in 10% formalin solution, transferred and molded tissue samples in paraffin to form candle blocks for histopathological examination.

2.4.4. Methods of histopathological analysis

- Place of implementation: Inserm U1053 laboratory.

- Technique: Histopathological analysis by conventional HE staining method.

2.4.5. Immunohistochemistry and staining methods ALDH and KRAS

* *Place of implementation*

Laboratory Inserm U1312, University of Bordeaux, France.

* ***Technique:***

- Solutions and chemicals:

+ Xylene (Analytical chemicals, code 1330-20-7).

+ Ethanol (Analytical chemicals, code 64-17-5).

+ TBST cushion.

+ Citric pH6 buffer (Abcam, code: ab93678).

+ Mouse specific HRP/DAB (ABC) Detection IHC Kit (Abcam, code: ab64259).

+ Hematocylin staining solution (Abcam, code: ab220365).

+ Mouse monoclonal antibody against human *ALDH* (clone 44/*ALDH*; BD).

+ *KRAS* monoclonal antibody (Abacam).

- Device:

+ Tissue cutting machine (Leica).

+ Semi-adhesive glass slides (Leica).

+ Laminated enamel (Leica).

+ Histochemical staining tank (Leica).

+ Humidifying tank (thermofisher).

+ Pressure cooker (Philips).

+ Liquid Blocker Super Pap pen (Daido Sangyo – Japan) waterproof pen.

+ Olympus CX23 microscope and imaging microscope.

* ***Steps to take:***

- Step 1: Remove paraffin

+ Tissue slices were washed 3 times with xylene solution, for 5 minutes each time.

+ Wash twice with 100% ethanol, 10 minutes each time.

+ Wash twice with 95% ethanol, 10 minutes each time.

+ Wash twice with water, 5 minutes each time.

Caution: Always avoid tissue drying at any time during this process.

- Step 2: Expose the antigen

+ Place glass slides containing deparaffinized tissue sections in a box containing Citrate pH6 buffer solution. These cans are placed in a pressure cooker and securely closed. Turn on pressure mode 950C - 980C for 30 minutes. Next, place the buffer solution box containing the glass slides outside for 30 minutes to gradually reduce the temperature.

+ Wash with TBST1X buffer, once for 5 minutes.

+ Add a sufficient amount (2-4 drops, equivalent to about 50 µL) of Hydrogen Peroxide Block solution to cover the surface of the tissue section. Wash twice with 1X TBST buffer, 5 minutes each time.

+ Add 50 µl BlockK Protein and incubate for 10 minutes at room temperature to prevent non-specific staining. Wash once with 1X TBST buffer.

+ Add 50 µl of anti-1 antibody solution mixed in Protein Block solution, incubate for 1 hour at room temperature. Wash twice with TBST buffer, 5 minutes each time.

+ Add 50 µl of Biotinylated Goat Anti-Mouse solution and incubate for 10 minutes at room temperature. Next wash with 1X TBST buffer (twice, 5 minutes each).

+ Add 50 µl Streptavidin Peroxidase and incubate for 10 minutes at room temperature, then wash 4 times with TBST buffer, 5

minutes each time.

+ Add 1 drop of DAB Chromogen solution to 1.5 ml of DAB substrate, vortex well and pipet 50 µl of solution after mixing to cover the cut tissue. Incubate for 5 minutes at room temperature. Wash 4 times with 1X TBST buffer, 5 minutes each time.

+ Place the slide containing the tissue slice in Hematocylin solution for 3 minutes.

+ Dehydration.

+ Incubate the slide containing tissue slices in 95% ethanol solution, repeat 2 times, 3 minutes each time.

+ Incubate in 100% ethanol solution, repeat 2 times, 3 minutes each time.

+ Incubate in xylene solution 2 times, 3 minutes each time.

+ Mount the slide with SignalStain Mounting Medium solution.

** Interpretation of immunohistochemical test results:*

Interpretation of immunohistochemical staining results under an optical microscope at 100-400 times magnification was performed by an experienced pathologist.

2.5. Data analysis

The data were processed using the SPSS 22.0 medical statistical software.

CHAPTER III
RESEARCH RESULTS

3.1. Clinical and laboratory characteristics and expression of immune markers *ALDH*, *KRAS* in gastric cancer patients

- The majority of patients are 50 years old or older, in which the age group 60-69 accounts for the highest proportion (35.0%).

- 98.1% of patients were admitted to the hospital because of epigastric pain, the second most common reason for admission was weight loss with the rate of 32.0%.

- The most common tumor location is the antrum (52.4%), followed by the small curvature (29.1%). Other positions such as body, large curvature, and pylorus have low ratio.

- GC ulcer according to Borrmann classification accounted for the highest rate with 66.0%. This was followed by fungal and infiltrative GC.

- According to Lauren's classification system, in this study, intestinal type accounts for 67.0%, only 33.0% of GC cases are diffuse type.

- According to the WHO classification system, the tubular type accounts for the highest proportion with 57.3%, followed by the signet cell type with 30.1%.

- According to WHO's level of differentiation, low-differentiated GC accounts for the highest proportion with 52.4%, the lowest is highly differentiated GC with 10.7%.

- GC stage III has the highest rate with 63.1%, followed by stage II with a rate of 31.1%.

- The rate of *ALDH*-positive patients is 68.0%, *ALDH*-negative patients is 32.0%.

- The rate of *KRAS* positive patients is 55.3%, *KRAS* negative patients is 44.7%.

- 71.4% of cases co-express *ALDH* and *KRAS*. There were 28.6% of patients only positive for *ALDH* but negative for *KRAS*. There were 21.2% of patients only positive for *KRAS* but negative for *ALDH* ($p<0.05$).

Table 3.17. Co-expression ratio of *ALDH* and *KRAS* in GC

		ALDH expression				p
		Negative		Positive		
		n	%	n	%	
KRAS expression	Negative	26	78,8	20	28,6	0,001
	Positive	7	21,2	50	71,4	
Total		33	100,0	70	100,0	

3.2. Relationship between *ALDH*, *KRAS* and some clinical and paraclinical characteristics

3.2.1. The relationship between ALDH and some clinical and subclinical characteristics

Table 3.25. Expression of *ALDH* according to WHO histopathological characteristics

ALDH expression / WHO	Negative		Positive		p
	n	%	n	%	
Papillary	0	0	1	1,4	0,01
Tubular	13	39,4	46	65,7	
Mucinous	6	18,2	3	4,4	
Signet-ring cell	12	36,3	19	27,1	

Squamous cell	2	6,1	0	0	
Other	0	0	1	1,4	
Total	33	100,0	70	100,0	

Patients with tubular GC had the highest *ALDH* expression rate with 65.7%.

Table 3.2. Expression of *ALDH* according to the degree of differentiation

ALDH expression / Degree of differentiation	Negative		Positive		p
	n	%	n	%	
Low differentiation	8	24,2	25	35,7	0,012
Moderate differentiation	3	9,1	21	30,0	
High differentiation	2	6,1	1	1,4	
No differentiation	20	60,6	23	32,9	
Total	33	100,0	70	100,0	

Patients with low-differentiated GC had the highest expression rate of *ALDH* with 35.7%, $p < 0.05$.

Table 3.27. Expression of *ALDH* according to disease stage

ALDH expression / Stage	Negative		Positive		p
	n	%	n	%	
Stage I	1	3,0	3	4,3	0,77
Stage II	11	33,3	21	30,0	
Stage III	21	63,7	44	62,9	
Stage IV	0	0	2	2,8	
Total	33	100,0	70	100,0	

3.2.2. The relationship between KRAS and some clinical and subclinical characteristics

Table 3.35. Expression of *KRAS* according to differentiation level

KRAS expression / Degree of differentiation	Negative		Positive		p
	n	%	n	%	
Low differentiation	14	30,4	19	33,3	0,227
Moderate differentiation	7	15,2	17	29,8	
High differentiation	2	4,3	1	1,8	
No differentiation	23	50,0	20	35,1	
Total	46	100,0	57	100,0	

Patients with poorly differentiated GC have the highest *KRAS* expression rate with 33.3%.

3.2.3. Association between simultaneous expression of ALDH and KRAS with some clinical and paraclinical characteristics

Table 3.42. Simultaneous expression of 2 markers according to the degree of differentiation

Marker / Degree of differentiation	Number of positive markers			p
	0 marker	1 marker	2 marker	
Low differentiation	6 23,1%	10 37,0%	17 34,0%	0,047
Moderate differentiation	3 11,5%	4 14,8%	17 34,0%	
High differentiation	1 3,8%	2 7,4%	0 0%	
No differentiation	16 61,6	11 40,8	16 32,0	

Patients with low-differentiated GC had the highest rate of simultaneous expression of both markers with 34.0%. There is a difference in the simultaneous expression of both markers according to the degree of differentiation, $p < 0.05$.

Table 3.43. Simultaneous expression of two markers according to disease stage

Marker / Giai đoạn	Number of positive markers			p
	0 marker	1 marker	2 marker	
Stage I	1 3,8%	3 11,1%	0 0	0,23
Stage II	8 30,8%	7 25,9%	17 34,0%	
Stage III	17 65,4%	17 63,0%	31 62,0%	
Stage IV	0 0	0 0	2 4,0	

CHAPTER IV
DISCUSSION

4.1. Clinical and laboratory characteristics and expression of immune markers *ALDH*, *KRAS* in gastric cancer patients

4.1.1. Some clinical, endoscopic and histopathological features

4.1.1.1. Age and sex characteristics

In this study, I conducted a study on 103 patients and found that the majority of patients were 50 years old and older, in which the age group 60-69 accounted for the highest percentage (35.0%) (Table 3.1).

4.1.1.2. Clinical features

❖ Clinical Manifestations

Our research results show that depending on the stage of cancer detected, the number and extent of clinical symptoms are not the same. The main symptom, however, some common clinical features are epigastric pain, nausea and weight loss. In this study, the most common clinical manifestation was epigastric pain, accounting for 99.0%, other clinical manifestations were encountered but accounted for a lower rate (Table 3.5).

4.1.1.3. Endoscopic features

Our study results show that the most common tumor location is the antrum, accounting for 52.4%, followed by the small curvature at 29.2%. Other positions such as body, large curvature, pylorus were found but accounted for a low percentage.

4.1.1.4. Histopathological features

* Histopathological classification according to Lauren and WHO

According to Lauren's classification system, in this study, we determined that the intestinal form accounted for 67.0%, only 33.0% of GC cases were diffuse, so this rate was 2 ,03:1 (table 3.8).

Classified according to the WHO's level of differentiation, in our study, low-differentiated GC accounts for the highest rate of 52.4%, moderately differentiated type accounts for 36.9%, and the lowest rate is dedifferentiated type. 10.7% high (table 3.10). The results of our study are similar to previous research by Nguyen Quang Bo with the highest rate of low differentiation being 39.6%, 26.4% of moderate differentiation, and 24.5% of high differentiation.

4.1.2. Expression of immune markers ALDH, KRAS

4.1.2.1. Expression of ALDH in gastric cancer patients

Our study results show: *ALDH* expression rate is 68.0%. This rate is similar to other studies. As in Nguyen Khac Tan's study, the *ALDH* expression rate was 61.2%.

4.1.2.2. KRAS expression in gastric cancer patients

Our study results show that the *KRAS* expression rate is 55.3%. This rate is similar to other studies. As in Polom's study, K. analyzing the role of *KRAS* status in GC performed the analysis on 595 patients, using polymerase chain reaction (PCR) to screen for *KRAS* mutations (exon 2). *KRAS* mutations were seen in 24 patients. Older and predominantly female *KRAS* mutant patients exhibit more advanced T and N stages of the disease, more metastatic tumors, and more need for adjuvant therapy. The five-year survival rate was 72.2% for *KRAS* mutation patients. In multivariate analysis, *KRAS* had worse survival ($p = 0.304$).

4.2. Relationship between *ALDH*, *KRAS* and some clinical and paraclinical characteristics

4.2.1. The relationship between ALDH and some clinical and subclinical characteristics

Patients with ductal GC had the highest *ALDH* expression rate with 65.7%. In addition, low-differentiated GC patients had the highest *ALDH* expression rate with 35,7%, followed by moderately differentiated tumors with *ALDH* expression rate of 37.1%, patients with highly differentiated tumors had the highest rate of *ALDH* expression. the lowest expression rate with 1,4%. There is a difference in *ALDH* expression according to differentiation, $p < 0.05$.

Wakamatsu et al reported that *ALDH*1 was associated with advanced T stage, TNM stage, intestinal histology, and poor 5-year overall survival. Furthermore, Zhang et al. found that *ALDH*1 was associated with lymph node metastasis, tumor differentiation, tumor pTNM stage, and overall survival at 5 years. Although there are inconsistencies between the results of these studies, most findings are quite consistent. Therefore, *ALDH*1 may be a useful diagnostic and prognostic marker for GC.

Li et al. reported that *ALDH*1A1 was significantly associated with depth of invasion, lymph node metastasis and disease stage. In addition, the survival time (overall survival and recurrence-free survival) of gastric cancer patients with high *ALDH*1A1 expression was significantly shorter than that of those with low *ALDH*1A1 expression.

4.2.2. The relationship between KRAS and some clinical and subclinical characteristics

Meanwhile, there is a statistically significant difference in *KRAS* expression according to the level of differentiation: the lower the differentiation level, the higher the *KRAS* expression rate ($p<0.05$). In the meta-analysis by Lindsay C. Hewitt, 15 (23%) studies investigated the relationship between *KRAS* and tumor differentiation and showed conflicting results. One (7%) study showed a significantly higher prevalence of *KRAS* in histologically moderately differentiated gastric tumors (82), three (20%) studies showed a higher prevalence of *KRAS* in GC were well differentiated while nine (60%) studies reported a higher prevalence of *KRAS* mutations in poorly differentiated gastric cancers. Two studies (13%) showed similar rates of *KRAS* mutations in well- and poorly differentiated GC. When compared with our study, gastric cancer patients with poorer differentiation had higher *KRAS* expression rates ($p<0.05$). To explain this situation, further studies are needed on the relationship between the degree of tumor differentiation and the rate of *KRAS* in GC patients.

The rate of *KRAS* expression in patients with stage III (61.4%) was higher than in other stages, but the difference was not statistically significant ($p>0.05$). In essence, the *KRAS* (Kirsten rat sarcoma 2 viral oncogen homolog) gene encodes *KRAS* proteins that play a role in transmitting intracellular signals downstream from epithelial growth receptors (picture). This protein has serine/threonine activity with the function of transmitting intracellular signals downstream from epithelial growth receptors on

the cell surface to intracellular targets through signaling cascades. In cells, RAS protein is kept in balance through the formation of two complexes corresponding to the activated and inhibited states of RAS protein: RAS-GTP complex (activated RAS protein) and RAS-GDP complex (RAS protein is inactivated). RAS protein is activated by guanine nucleotide exchange factors (GEFs). RAS protein signaling is inhibited when the RAS-GTP complex is hydrolyzed to the RAS-GDP complex by GTPase-activating proteins (GAPs). Under normal physiological conditions, RAS-GTP concentration in the body is strictly controlled thanks to the rhythmic activity of two factors GEFs and GAPs (picture). They make the RAS-GTP complex protein resistant to hydrolysis by GTPase, thereby leading to an endless increase in *KRAS*-GTP activity, leading to signal-independent activation of downstream signaling pathways in the cells, stimulates proliferation, inhibits apoptosis and regulates growth and prolongs cell life resulting in.

4.2.3. Relationship between simultaneous expression of ALDH and KRAS with some clinical and paraclinical features

Up to now, there has been almost no research on the simultaneous expression of both *ALDH* and *KRAS* in GC patients, so the relationship between the simultaneous expression of these markers and clinical and internal characteristics is unclear. Endoscopy and histopathology are not available. After testing 2 markers *ALDH*, *KRAS* and analyzing the relationship, we found that the difference in the simultaneous expression of both markers in patients with low differentiation was highest (62.0%) compared to other differentiation levels, $p < 0.05$.

The results of studying the simultaneous expression of *ALDH*, *KRAS* in this study show that the above GC immunohistochemical markers are related to factors such as ductal glands according to WHO histopathology and low specificity, which is a valuable source of additional information to develop more effective therapies for GC patients.

CONCLUSION

Through research on some clinical and paraclinical characteristics, *ALDH* and *KRAS* immunohistochemical tests on gastric cancer patients treated at K Hospital, we draw the following conclusions:

I. Clinical and laboratory characteristics and expression of immune markers *ALDH*, *KRAS* in gastric cancer patients

- The age group of GC patients from 60-69 accounts for the highest proportion (35.0%), the average age is 57.9 ± 11.4. GC rate in men is 59.2%, in women is 40.8%.

- The most common clinical symptom is epigastric pain (99.0%).

- GC is most common in the antral area (52.4%), the ulcer type according to Borrmann accounts for the highest proportion (66.0%).

- Histopathological classification according to Lauren shows that intestinal type accounts for the highest proportion (67.0%), histopathological classification according to WHO shows that tubular type accounts for the highest proportion (57.3%), low differentiation According to WHO, it accounts for the highest rate (32,0%).

- The rate of *ALDH*-positive patients is 68.0%, *ALDH*-negative patients is 32.0%.

- The rate of *KRAS* positive patients is 55.3%, *KRAS* negative patients is 44.7%.

- *ALDH* expression in GC patients has a significant relationship with *KRAS* expression, $p < 0.05$.

II. The relationship between the expression of immune markers *ALDH* and *KRAS* with some clinical and paraclinical characteristics of gastric cancer patients

- The expression of *ALDH* and *KRAS* did not differ according to clinical symptoms or endoscopic characteristics, with $p > 0.05$.

- The expression of *ALDH* is highest in the tubular type (65.7%) according to WHO histopathological characteristics, with $p < 0.05$.

- There is no relationship between the expression of *Aldehyde dehydrogenase, KRAS* and histopathological classification (according to Lauren) ($p>0.05$).

- There is a relationship between the expression of *Aldehyde dehydrogenase* and the degree of differentiation ($p<0.05$).

- Simultaneous expression of two markers *Aldehyde dehydrogenase* and *KRAS* with a statistically significant level of differentiation ($p < 0.05$).

PROPOSALS

There has been research on the expression of single immune markers ALDH and KRAS on some types of cancer such as: stomach cancer (ALDH), colon cancer (KRAS). The study on co-expression of ALDH and KRAS in gastric cancer patients is the first study conducted in Vietnam.

Medical facilities and patients should do these two immune markers at the same time if possible, or can rely on factors such as tubular type and low differentiation to predict the expression of immune markers. Translated above.

From the results of the thesis, further studies should be conducted to evaluate the prognostic value of immunohistochemical markers ALDH and KRAS in gastric cancer patients.

LIST OF PUBLISHED ARTICLE RELATING TO THESIS

1. Le Viet An, Duong Hong Thai, Nguyen Phu Hung (2023), "Expression of immunohistochemical markers *ALDH* and *KRAS* in gastric cancer", *Vietnam Medical Journal*, volume 529, number 2, p. 352.

2. Le Viet An, Duong Hong Thai, Nguyen Phu Hung (2023), "Relationship between immunohistochemical markers *ALDH* and *KRAS* with histopathological characteristics in gastric cancer", *Vietnam Medical Journal*, volume 529, number 2, p. 362.

3. Le Viet An, Duong Hong Thai, Nguyen Phu Hung (2023), "Relationship between simultaneous expression of *ALDH* and *KRAS* with histopathological characteristics in gastric cancer", *Science and technology magazine*, number 2, p. 362.