RESEARCH ARTICLE

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Characteristic Mutational Damages in Gastric and Colorectal Adenocarcinomas

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Abstract

Introduction: Gastric and colorectal adenocarcinomas are prevalent malignancies characterized by mutations in genes such as p53, RAS, and MDM2, which play crucial roles in tumorigenesis and cancer progression. Understanding the specific mutational patterns and their implications in these cancers was essential for identifying potential therapeutic targets. **Aim:** To identify the nature of mutational disorders in the *p53, p21Waf1, RAS* and *MDM2* genes, depending on the degree of cell differentiation by adenocarcinomas of the gastrointestinal tract. **Methods:** Genomic DNA was isolated from 200 samples of stomach tissue and 233 samples of colon and rectum adenocarcinomas. A total of 433 samples, including gastric adenocarcinomas, colon and rectum adenocarcinomas and adjacent tissues, were collected. **Results:** Genomic DNA was isolated, and mutational analysis of p53, RAS (HRAS, *KRAS*, *NRAS*), and *MDM2* genes was performed using polymerase chain reaction, gel electrophoresis, and restriction enzyme analysis. The deletion of p53 exon-intron 5-6, as well as HRAS 12 and HRAS 61 mutations, were detected in 78% of poorly differentiated adenocarcinomas. The deletions of p53 exon-intron 7-9 – in 100% of moderately differentiated adenocarcinomas and 50-60% of adjacent tissues. The loss of *WAF1* gene expression was registered in almost 90% of poorly differentiated adenocarcinomas and 20% of adjacent tissue samples. The *KRAS* and *NRAS* mutations in almost 63.9% of studied colon and rectal samples indicated autonomous cell growth. This explains the aggressive and metastatic growth of tumours and the ineffectiveness of growth factor inhibitors in colorectal cancer. Finding ways to influence specific substitutions in *RAS* genes could prevent and eliminate uncontrolled invasive tumour growth. **Conclusion:** By identifying specific gene mutations and differences in genetic markers, the study provided insights for the development of targeted diagnostic methods and personalised treatment strategies, ultimately improving the clinical outcomes in the field of oncology.

Keywords: Neoplasm – carcinogenesis – oncology – genetics – gastrointestinal – genes RAS

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Introduction

Gastric cancer is a globally common malignancy, with varying prevalence in different regions. Late-stage diagnosis often leads to high mortality rates associated with gastric cancer. Standard treatments include surgery, chemotherapy, radiation therapy, and targeted therapies tailored to individual cases. Molecular understanding is vital for developing targeted therapies and personalized treatment strategies. Colorectal cancer, prevalent in developed countries and increasing worldwide, also benefits from molecular insights for improved diagnosis and treatment outcomes.

The *p53* gene is critically involved in the initiation and progression of human carcinogenesis (Imashev et al., 2019). Point mutations and deletions in the *p53* gene are prevalent in every second case of malignancies. This disrupts the normal function of *p53*, compromising its ability to regulate cell cycle progression, DNA repair, and apoptosis, thereby promoting the development and persistence of cancer cells (Salimova et al. 2005). The types of mutations in the *p53* gene vary greatly among different types of cancer (Hollstein et al., 1991). When cells are exposed to DNA-damaging agents, such as ionizing radiation, chemicals or hypoxia, *p53* is activated by the DNA damage response (Svyatova et al., 2001; Liu & Bodmer, 2006). After discussing the *p53* gene, move on to the *RAS* genes, as they play a significant role in cancer development due to point mutations that trigger uncontrolled cell growth and division. J. K.

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M Lim and Leprivier (2019) explored that *RAS* genes (HRAS, *KRAS*, and *NRAS*) are among the most common human oncogenes. Point mutations in *RAS* genes are single-nucleotide changes that can result in uncontrolled cell growth and division. They are found in approximately 15% of all human cancers. *RAS* point mutations are the most common anomaly of human proto-oncogenes (Taukeleva and Toguzbaeva, 2014). *RAS* inhibitors are studied as anticancer agents since they exhibit profound anti-oncogenic effects in many cancer cell lines. However, more research is needed to determine their safety and efficacy (Downward, 2003). Dysregulation of RAS and its associated proteins in cancer disrupts the normal balance of cellular processes, resulting in several detrimental effects. This dysregulation contributes to enhanced invasiveness and metastasis of cancer cells (Tapbergenov et al., 2013). The aberrant RAS signalling also promotes cell survival and inhibits apoptosis, allowing cancer cells to evade cell death mechanisms that would normally eliminate them. This further contributes to the accumulation and survival of cancer cells, enabling tumour growth and progression (Blum et al., 2005; Rotblat et al., 2008).

The *p53, p21Waf1, RAS*, and *MDM2* genes play a pivotal role in regulating various signalling pathways that contribute to neoplastic aberrations. These genes exhibit a dual functionality in regulating signalling cascades, exerting inhibitory effects on certain pathways while concurrently activating others (Zharmakhanova et al., 2020). This contributes significantly to the process of cancer development and progression. These genes influence cellular behaviours such as proliferation, survival, and differentiation, ultimately shaping the malignant phenotype of cancer cells. (Grossman et al., 1998; Oda et al., 2000; McMurray et al., 2008; Warfel and El-Deiry, 2013). When these genes are damaged, various signalling systems activate cell growth and uncontrolled cell division. When cells lose their ability to differentiate properly, it disrupts the normal cellular hierarchy and function. This loss of differentiation promotes the invasive behaviour of cancer cells, enabling them to invade surrounding tissues and metastasize to distant sites (Benvenuti et al., 2007; Sakai et al., 2018).

The current study conducted an analysis of genes and transcripts associated with the regulation of cell growth and division control. The study's objective was to identify specific gene mutations that serve as triggers for neoplastic aberrations in various gastric neoplasms, including moderately differentiated adenocarcinomas (MDA) and poorly differentiated adenocarcinomas (PDA) in the gastric and colorectal regions. Through this analysis, the authors aimed to explore the molecular mechanisms underlying the initiation and progression of these malignancies, to better understand potential targeted therapeutic approaches. A more comprehensive understanding of how these genetic alterations contribute to the therapeutic resistance commonly observed in such cases would provide valuable insights into the development of targeted treatment strategies

Materials and Methods

A laboratory-based experimental study design with a descriptive and analytical approach was used. Samples of tissue tumours from gastric, colon and rectum adenocarcinoma patients were collected. Deoxyribonucleic acid (DNA) isolation, reverse transcription-polymerase chain reaction (RT-PCR), endonucleolysis, and real-time polymerase chain reaction (PCR) were used to analyse genetic mutations in specific genes. The study included patients aged 40 to 75 years with histologically confirmed cases of gastric adenocarcinomas and colorectal adenocarcinomas. Only patients who provided informed consent and had available tissue samples of both the tumour and adjacent normal tissues were included. Patients with a history of other primary cancers were excluded.

Cell DNA was isolated from 200 samples of stomach tissue (5 repeated series of 40 samples of gastric adenocarcinomas and adjacent tissues (AT)) using the Bioline ISOLATE II Genomic DNA Kit (DNA-Technology, Moscow, Russia). Each series contained 20 MDA and 20 AT samples, or 20 PDA and 20 AT samples. PCR was performed using a Hybaid Omn-E automatic thermal cycler (Franklin, Massachusetts, USA). The reverse transcription-polymerase chain reaction (RT-PCR) was carried out using the REVERTA-L-100 AmpliSens reagent kit (Moscow, Russia). The restriction enzyme EcoR1 was used for site-specific endonucleolysis of PCR products to analyze mutations in HRAS exons 2 and 3. Electrophoregrams were stained with ethidium bromide and analysed by light intensity in UV light using the Gel Analysis software (Kapelan Bio-Imaging, Leipzig, Germany).

The reverse transcription-polymerase chain reaction (RT-PCR) was carried out using the REVERTA-L-100 AmpliSens reagent kit (Moscow, Russia). 10 µl (1mcg) of the isolated RNA was incubated at 37°C for 30 minutes with 10 µl of the reaction mixture of hexanucleotide primers, RNA eluent, deoxynucleotide triphosphates (dNTP), ribonuclease inhibitor, and 200 U reverse transcriptase. The synthesized first-chain cDNA was added to the mixture to copy (amplify) the DNA fragments in PCR. PCR was performed using a Hybaid Omn-E automatic thermal cycler (Franklin, Massachusetts, USA): one 5-min cycle at 95°C, thirty cycles of 20 secs at 94°C, 20 secs at 55-56°C, and 40 secs at 72°C, and one 5-min cycle at 72°C. The reaction mixture (25 µl) contained 30 ng of each primer, 0.5 mm of each dNTP, 5 µl of DNA or cDNA, 2.5 units of Tag polymerase, 5 µl of 10-fold buffer for PCR. After electrophoresis in 1.5-2% agarose gels, ethidium bromide was added to detect PCR products in UV transilluminator. The restriction enzyme EcoR1 was used for site-specific endonucleolysis of PCR products to analyze mutations in HRAS exons 2 and 3. The medium that contained 5 µl of PCR product, 1.25 µl of H-buffer and 1 unit of restriction enzyme per 1 µl was brought up to 12.5 µl by water, incubated for 1 hour at 37°C, cooled in ice, and dispersed in 2% agarose gel electrophoresis. Electrophoregrams were stained with ethidium bromide and analysed by light intensity in UV light using the

Gel Analysis software (Kapelan Bio-Imaging, Leipzig, Germany).

Genomic DNA was isolated from 233 samples of colon and rectum adenocarcinomas using the Thermo Scientific GeneJET Genomic DNA Purification Kit (USA). The mutations in *KRAS* and *NRAS* exons 2, 3, 4 were detected using a Corbett Rotor-Gene 6000 (QIAGEN Shenzhen Co. Ltd., Shenzhen, China) amplifier and RAS mutation screening panel. The amplification product was detected using fluorescent probes that contained a FAM or VIC fluorophore at the 5-prime terminus and an extinguisher at the 3-prime terminus. PCR was carried out in a reaction mixture that contained 15 µl 2xPCR reaction mixture, 6 µl primer mixture (1-8), and 10-30ng (up to 9 µl) of DNA sample brought up to 30 µl by molecular water. A PCR plate that contained eight probes of each sample with different primers, one positive control, and one blank control was placed in a thermal cycler for real-time PCR for one 10-min cycle at 95°C, then forty 15-sec cycles at 95°C, and one 60-sec cycle at 60°C (Table 1).

Student's t-criterion, the significance level P, and the standard deviation (SD) between the series were employed to analyse the data. Pearson correlation coefficient was calculated for gastric cancer samples.

Results

Gastric adenocarcinoma samples

The difference observed between gastric MDA, PDA, and AT samples in the number of samples with PCR products of *p53*, HRAS gene fragments and *p53*, WAF1, and MDM2 mRNA were presented in Table 2.

Gastric MDA samples had fewer PCR products with HRAS codon 12 (16.7% vs. 80-100%, r_{xy}=+0.816), HRAS codon 61 (23 vs. 75%, r_{xy}=+0.813), and WAF1 mRNA (75% vs. 80%, r_{xy}=+0.816) than AT samples. All gastric MDA samples had a 5-6 exon/intron fragment. 7-9 fragment was absent in gastric MDA but present in all AT samples. *p53* gene transcription was preserved in 100% of MDA samples. WAF1 transcription and preserved *p53* trans-activating function were detected in 75% of MDA samples and 80% of AT samples. MDM2 transcription observed in 86% of gastric MDA and AT samples did not reduce the content and activity of *p53* protein. Gastric PDA samples had fewer PCR products with *p53* gene fragments with a 5-6 exon-intron site (22% vs. 43%, r_{xy}=+0.83), *p53* mRNA fragments (22% vs. 73%, r_{xy}=+0.85), WAF1 mRNA samples (11% vs. 80%, r_{xy}=+0.81), as well as HRAS codon 12 and HRAS codon 61 fragments (22% vs. 50%, r_{xy}= +0.79) than AT samples. All t-criterion values corresponded to the probability of error-free forecast of p>0.05. 5-6 exon-intron fragment was absent in 78% of PDA and 57% of AT samples. 7-9 exon-intron fragment was not amplified in 50% of PDA and AT samples. The absence of amplified *p53* fragments indicated the loss or damage of the relevant *p53* gene region in the samples. It seemed likely that the *p53* gene 5-6 exon-intron fragment was necessary for *p53* gene expression. WAF1 was expressed in 11% of PDA and 80% of AT samples. MDM2 transcription was detected in 100% of PDA and AT samples.

MDA and PDA varied in the number of samples with amplified *p53* and mRNA fragments. *p53* 5-6 exon-intron fragment was amplified in all 20 MDA and AT samples vs. 4/20 PDA and 10/20 AT samples. The difference in *p53* mRNA was even higher: *p53* mRNA was detected three times more often in adjacent tissues than in PDA samples (22% vs. 73%). 75-80% of PDA samples showed no cDNA *p53* amplification after RT-PCR. The resulting PCR product did not always possess a transactivating ability. In contrast, *p53* mRNA from MDA samples activated the *p53*, p21Waf1, and MDM2 transcription. The results obtained indicated the deletions or substitutions, which precluded the amplification of exon-intron 7-9 fragment in MDA samples. The lack of MDM2 mRNA expression in 14% of MDA samples indicated the lack of correlation between *p53* exon-intron 7-9 defect and MDM2 gene. In the present study, HRAS 12 and HRAS 61 fragments were found in 3-7 out of 20 PDA and MDA samples, which was 2-3 times less often than in AT samples.

Colon and rectal adenocarcinoma samples

In the current study, 149/233 (63.9%) colon and rectal adenocarcinoma samples had RAS mutations (Table 3,

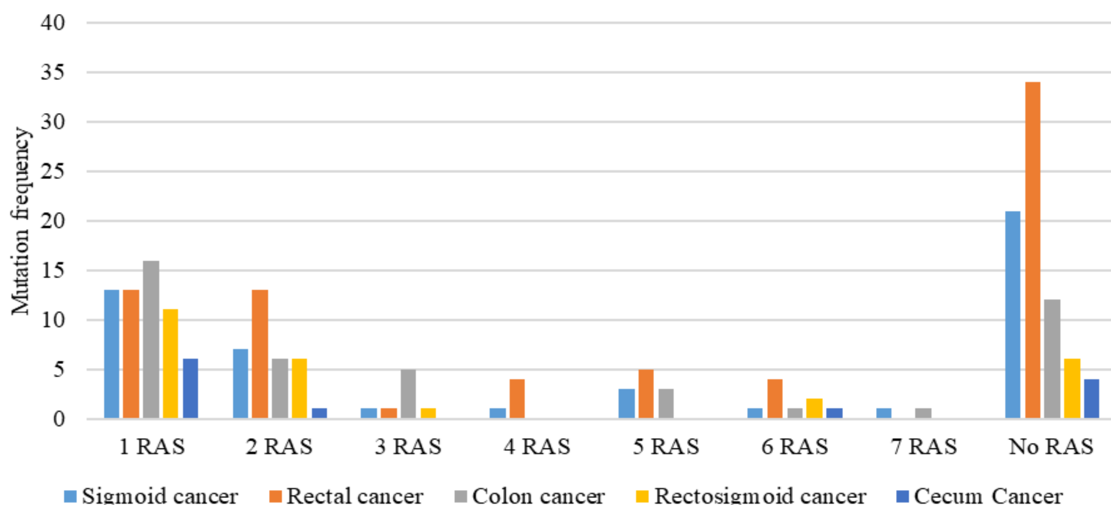

Figure 1. Distribution of Mutations in KRAS and NRAS Codons in Colorectal Cdenocarcinomas. Source: compiled by the authors.

Table 1. The List of *KRAS* and *NRAS* Mutations Detected Using the Kit for the Detection of Exon 2, 3, and 4 Mutations in *KRAS* and *NRAS* Genes and the Rotor-Gene 6000 (QIAGEN) Amplifier

Gene	Exon	aa change	nt change	Cosmic ID	Primer No.
KRAS	2	G12A	c.35G>C	522	1RAS
		G12D	c.35G>A	521	
		G12R	c.34G>C	518	
		G12V	c.35G>T	520	
		G13D	c.38G>A	532	
	2	G12C	c.34G>T	516	2RAS
		G12S	c.34G>A	517	
	3	Q61H	c.183A>T	555	3RAS
		Q61H	c.183A>C	554	
		Q61L	c.182A>T	553	
		Q61R	c.182A>G	552	
		A59E	c.176C>A	547	
		A59G	c.176C>G	28518	
		A59T	c.175G>A	546	
	4	K117N(AAC)	c.351A>C	19940	4RAS
		K117N(AAT)	c.351A>T	28519	
		K117R	c.350A>G	1178068	
		K117E	c.349A>G	1360831	
	4	A146T	c.436G>A	19404	5RAS
		A146P	c.436G>C	19905	
		A146V	c.437C>T	19900	
NRAS	2	G12D	c.35G>A	564	6RAS
		G12C	c.34G>T	562	
		G12S	c.34G>TA	563	
		G13R	c.37G>C	569	
		G13V	c.38G>T	574	
	4	K117R	c.350A>G		6RAS
	3	Q61H (CAC)	c.183A>C	585	7 RAS
		Q61H (CAT)	c.183A>T	586	
		Q61L	c.182A>T	583	
		Q61K	c.181C>A	580	
		Q61R	c.182A>G	584	
	3 and 4	A146T	c.436G>A	1237325	8RAS
		A59D	c.176C>A	253327	
		A59T	c.175G>A	578	

*, aa, amino acids; nt, nucleotide; AAC, Amino acid change; AAT, Amino acid substitution; CAC, Calcium channel; CAT, Catalase. Source: compiled by the authors

Figure 1). In colon adenocarcinomas (n=44), 12 samples had no RAS mutations. *KRAS* mutations were detected in exon 2, codons 12 and 13 in 16 cases at the tumour location in the ascending colon (n=6), the descending colon (n=5), the transverse colon (n=3), or in the colon with location unknown (n=2). Other *KRAS* mutations were detected in exon 2, codon 12 (n=6), exon 3, codons 61 and 59 (n=5), and exon 4, codon 146 (n=3). *NRAS* mutations (n=4) were detected in exons 2 and 4, codons 12, 13, and 117, and exon 3, codon 61. Among the 44 samples of colon adenocarcinomas analysed, 12 showed no RAS mutations. In 16 cases, *KRAS* mutations were observed at specific sites in the colon, including the ascending colon (n=6), descending colon (n=5), transverse colon (n=3), or cases where the exact location within the colon was unknown (n=2). These mutations were specifically identified in exon 2, affecting codons 12 and 13. Additional *KRAS* mutations were found in exon 2, codon 12 (n=6), exon 3, codons 61 and 59 (n=5), and exon 4, codon 146 (n=3). Similarly, *NRAS* mutations (n=4) were detected in exons 2 and 4, affecting codons 12, 13, and 117, as well as in exon 3, specifically at codon 61.

In rectal adenocarcinomas with or without metastasis (n=74 in total), 34 samples had no RAS mutations. *KRAS* mutations were found in codons 12 and 13 (n=26), 61 (n=1), 59 (n=1), 117 (n=4), and 146 (n=5); *NRAS*

Table 2. The Difference between Moderately and Poorly Differentiated Gastric Adenocarcinomas and Adjacent Tissues in Gene Mutations

Object	p53 5-6 exon-introns	p53 7-9 exon-introns	p53 mRNA	HRAS 12	HRAS 61	WAF1 mRNA	MDM2 mRNA
Moderately differentiated adenocarcinomas							
MDA*	20	0	20	3.4±2.2	5±1	15.4±3.8	17.2± 2.8
(5x20)	(100%)	0	(100)	(16.70%)	(23%)	(75%)	(86%)
AT*	20	20	20	16.6±7.2	15±2	16.4±4.2	17.2±2.8
(5x20)	(100%)	(100%)	(100%)	(80-100%)	(75%)	(80%)	(86%)
t-criterion*	*	**	*	2.44	2.42	2.44	*
r_{xy}*	*	**	*	0.816	0.813	0.816	*
Poorly differentiated adenocarcinomas							
PDA*	4.4±2	10±2	6±4	4±3	4±3.1	1.2±1	18±2
(5x20)	(22%)	(50%)	(22%)	(22%)	(22%)	(11%	(90-100%)
AT*	10±3	10±2	15.2±5	10±2	10±2	17.4±5	18±2
(5x20)	(43%)	(50%)	(73%)	(50%)	(50%)	(80%)	(90-100%)
t-criterion*	2.59	*	2.81	2.12	2.12	2.74	*
r_{xy}*	0.832	*	0.851	0.79	0.79	0.816	*

*, MDA, moderately differentiated adenocarcinoma; PDA, poorly differentiated adenocarcinoma; AT, tissue adjacent to the tumour; (5x20), 5 series, 20 samples in each; rxy, Pearson correlation coefficient; *, No difference between the two averages; **, The difference was significant and reliable in the complete absence of the p53 gene fragment in MDA. Source: compiled by the authors.

mutations were found in exons 2 and 4, codons 12, 13, and 117 (one mutation in each codon). Among the 12 cases of cecum adenocarcinomas with metastasis, four samples did not show any RAS mutations. *KRAS* mutations were identified in codon 12 (n=1) or codons 12 and 13 (n=6). Additionally, a *NRAS* mutation was detected in codons 12, 13, and 117 (n=1). In rectosigmoid adenocarcinomas with metastasis (n=26), 6 samples had no RAS mutations. *KRAS* mutations were detected in codons 12 (n=11), 13 (n=6), 61 (n=1), and 59 (n=1). *NRAS* mutations were found in codons 12, 13, and 117 (n=2 in each). In rectosigmoid adenocarcinomas with metastasis (n=26), 6 samples had no RAS mutations. *KRAS* mutations were detected in codons 12 (n=11), 13 (n=6), 61 (n=1), and 59 (n=1). *NRAS* mutations were found in codons 12, 13, and 117 (n=2 in each).

Thus, the presence of *KRAS* gene mutations, predominantly occurring in exon 2 (codons 12 and 13) and exon 4 (codons 117 and 146), was consistently observed in cases of sigmoid colon, colon, and rectal cancer, often with metastases to adjacent organs and tissues. In cancer of the descending, ascending and transverse colon, the mutations were found in exons 2 and 3, codons 12, 13 and 61, 59. In contrast, in rectosigmoid cancer with metastases

Table 3. Distribution of Mutations in *KRAS* and *NRAS* Gene Codons in Colon and Rectal Adenocarcinomas

Localization of the tumour	No RAS mutation	Primer No. for the detection of mutations in KRAS and NRAS genes*						
		1 KRAS exon 2, codons 12, 13	2 KRAS exon 2, codon 12	3 KRAS exon 3, codons 61,59	4 KRAS exon 4, codon 117	5 KRAS exon 4, codon 146	6 NRAS exons 2, 4, codons 12, 13, 117	7-8 NRAS exon 3, codons 59, 61
Sigmoid colon cancer with metastasis (n=48)	n=21	n=13 (incl. 2 G12D)	n=7 G13P	n=1	n=1	n=3 A146X	n=1	n=1
Colon cancer with metastasis (n=44)	n=12	n=16 G13D	n=6 G12V	n=5	-	n=3	n=1	n=1
Ascending colon cancer with metastasis (n=13)	n=4	n=6	n=2	n=1	-	-	-	-
Descending colon cancer (n=12)	n=2	n=5	-	n=3	-	-	n=1	n=1
Transverse colon cancer (n=4)	n=1	n=3	-	-	-	-	-	-
Cecum cancer with metastasis (n=12)	n=4	n=6 (incl. 1 G13D)	n=1 G12D	-	-	-	n=1	-
Rectal cancer with metastasis (n=74)	n=34	n=13 (incl. 1 G13D and 1 G12D)	n=13 (incl. 2G12V)	n=1	n=4	n=5 (incl. 1 A146T)	n=4	-
Rectosigmoid cancer with metastasis (n=26)	n=6	n=11 (incl. 1 G13D)	n=6 (incl. 3 G12D)	n=1 Q61H	-	-	n=2	-
Total (n=233)	n=84	n=73	n=35	n=12	n=5	n=11	n=10	n=3

*, The first digit indicates the No. of primer in Table 1. Source, compiled by the authors.

to the adjacent tissues and lymph nodes, the mutations were found in exon 2, codons 12 and 13. In sigmoid colon cancer and descending colon cancer, the *NRAS* gene mutations in codons 12, 13, and 117 in exons 2 and 4, and exon 3, codon 61 were found in isolated cases in almost all parts of the large intestine and rectum. No *KRAS* or *NRAS* mutations were found in the 2 studied samples of gastric adenocarcinomas. Acknowledging the molecular characteristics and mutations in gastric and colorectal adenocarcinomas could have potential implications for diagnosis, treatment, and further understanding of these cancers.

Discussion

In the present study, *p53* mRNA expression was 2-3 times less frequent in PDA than in adjacent tissues. Only every third PDA sample had an exon-intron 5-6 fragment of *p53*, which was twice less frequent than in AT samples. Shieh et al., (1997) examined the kinetics of induction of *p53* and MDM2 following DNA damage. According to scientists, DNA damage causes *p53* phosphorylation at ser15, that lead to a reduced interaction of *p53* and MDM2, which acts as its negative regulator. Besides, the MDM2 molecule has ubiquitin ligase activity and participates in *p53* ubiquitination (Honda et al., 1997). MDM2 complex with P300/SVR also plays a role in *p53* degradation (Grossman et al., 1998). The exact role of p21 in carcinogenesis is not established yet. In some types of tumours, the loss of p21 WAF1 indicates poor chances for survival. However, sometimes, the increased p21 concentration in cells positively correlates with the aggressiveness and metastatic propensity of the tumour. This is especially true when p21 accumulates in the cytoplasm rather than in the cell nucleus (Warfel and El-Deiry, 2013). A tumour cell with the HRAS fragment mutations with codons 12 and 61 can overcome several stages of tumour progression in one step and obtain such properties as the loss of differentiation, invasive growth, and the ability to metastasize (Downward, 2003). FGFR2 amplification leads to constitutive activation of the FGFR2 signalling pathway in gastric cancer. Inhibition of this pathway by a well-tolerated, potent, and selective inhibitor can lead to rapid and long-term tumour regression in fgfr2 xenograft models (Xie et al., 2013).

The findings align with those of Liu and Bodmer (2006), who conducted an analysis of the T*p53* gene and its protein status in 56 colorectal cancer (CRC) cell lines. In their study, they identified 46 mutations in 43 cell lines. Nearly half of those were truncated mutations. The authors suggested the dominant-negative effects of truncating mutations even when the truncated protein could not be detected by standard methods. In this study, protein-truncating mutations of the *p53* gene in exon-intron 7-9 were found in 100% of gastric MDA samples and 50% of PDA samples, in exon-intron 5-6 – in 78% of PDA samples. A significant proportion of studied samples (78-80%) contained mutations in the HRAS gene fragments with codons 12 and 61, that activated downstream effector genes. 1-8 RAS primers can detect or exclude all possible nucleotide substitutions in *KRAS* and *NRAS* genes, exons 2-4 (Table 1). The results presented in Table 3 can be useful for the development and selection of an appropriate method of suppressing the further development and invasion of tumour cells. McMurray et al., (2008) showed that a high share of genes synergistically controlled by the loss of *p53* function and RAS activation was critical for the malignant state of mouse and human colon cells and that synergistic control of gene expression by oncogenic mutations is a fundamental key to malignization which substantiates the search for targets for interference in gene networks after oncogenic mutations of gain and loss of function.

The results obtained in this study are consistent with other reports and also indicate the association of point mutations in *KRAS* codons 12 and 13 with CRC regardless of its stage and localization. It was found that those mutations in 63.9% of all studied colon and rectal tumours. According to Andreyev et al., (2001), *KRAS* point mutations occurred at early stages of progression from colorectal adenoma to carcinoma and were detected in 35-40% of patients regardless of the development stage. Oliveira et al., (2004) investigated *KRAS* in 158 HNPCC tumours from patients with germline hMLH1, hMSH2 or hMSH6 mutations, 166 MSI-H and 688 microsatellite stable (MSS) sporadic carcinomas. All tumours were characterized for MSI and 81 of 166 sporadic MSI-H colorectal cancer (CRCs) were analysed for hMLH1 promoter hypermethylation They found more than 90% of *KRAS* codon 12 (GGT) and 13 (GGC) mutations in such patients. Activating mutations in codons 61 and 146 were also reported in some of these tumours. The mutations in molecules involved in signalling pathways below EGFR, such as *NRAS*, *BRAF*, and *PIK3CA*, were also reported in CRC. *KRAS* mutations are prognostic markers of anti-EGFR antibody therapy efficacy in patients with metastatic CRC (Benvenuti et al., 2007). According to Kawamoto et al., (2012), *KRAS* mutation status is largely associated with primary and metastatic lesions. Still, it is not clear if genotoxic drugs can cause additional mutations during chemotherapy. Since prognostic biomarker genes do not change their mutation statuses after FOLFOX therapy, the samples of both primary tumours and tumour metastases after FOLFOX can serve as reliable sources of DNA for known genomic testing of biomarkers.

The activating point mutation in codon 12 of the HRAS gene was the first somatic point mutation identified in human cancer and has served to establish the role of somatic mutations as the general driver for oncogenesis. Edkins et al., (2006) identified over 11,000 mutations in the three *RAS* genes (*HRAS, KRAS*, and *NRAS*), codons 12, 13, and 61, as well as recurrent somatic missense mutations by alanine 146. 11 such mutations in CRC were identified. However, possible replacement of mutated stem cells with wild-type stem cells could explain the presence of mutations in the studied genes and gene products in adjacent healthy tissues. Such an explanation can be found in Vermeulen et al., (2013), who quantified the competitive advantage in the development of tumour clones with the loss of Apc (611731), activation of *KRAS* (190070), and *p53* mutation in the mouse gut. Their results showed that the fate of these mutations was

not deterministic, and many mutated stem cells were replaced by wild-type stem cells after the exposure to the influencing but still random events. In that study, *p53* mutations demonstrated a state-dependent advantage. The clones with mutated *p53* were dominant, especially in the colitis-affected intestine (Tamm et al., 2009). The authors agreed with the idea that the tissue architecture of the intestine suppressed the accumulation of mutated lines.

RAS activates several pathways, of which the mitogen-activated protein (MAP) kinase cascade is well understood. This cascade transmits signals downstream and leads to the transcription of genes involved in cell growth and division (Bos, 1989). Another RAS-activated signalling pathway is the PI3K/AKT/mTOR pathway, which stimulates protein synthesis, cell growth, and inhibits apoptosis (King et al., 2015). Lodish et al., (2002) have demonstrated that incorrect gene activation is a pivotal factor in disrupted signal transmission, proliferation, and the development of malignancies. Mutations in certain genes, including RAS, can induce this effect. Oncogenes like p210 BCR-ABL or the growth receptor erbB are situated upstream of RAS, so when they undergo constant activation, RAS will subsequently transmit their signals. The tumour suppressor gene NF1 encodes RAS-GAP; in the case of its mutation in neurofibromatosis, RAS is less likely inactivated. RAS can also be enhanced, although this only occurs in tumours. Finally, RAS oncogenes can be activated by point mutations (Downward, 2003).

Oda et al., (2000) discovered a gene responsible for promoting apoptosis, known as *p53*AIP1 (605426), which exhibited increased expression in response to wild-type *p53*. In cases of extensive DNA damage, ser46 phosphorylation of *p53* occurred, ultimately resulting in apoptosis induction. When ser46 was replaced, it hindered *p53*'s capability to initiate apoptosis and specifically suppressed the expression of *p53*AIP1. The authors concluded that p53AIP1 mediated p53-dependent apoptosis and that ser46 p53 phosphorylation regulated transcriptional activation of p53AIP1. That is, the p53 gene mutations could block apoptosis and stimulate the growth of damaged cells (Mamontov, 2016), as was observed in this research. Chiang and Massagué (2008) found that Chk2(604373)-/- mouse embryonic cells were defective in stabilizing p53 and inducing p53-dependent transcripts, such as p21, in response to gamma irradiation. The introduction of the chk2 gene restored p53-dependent transcription in response to gamma irradiation. Human CHK2 directly phosphorylated p53 by ser20, a modification known to inhibit MDM2 binding. The authors concluded that phosphorylation of p53 with CHK2 increased the stability of p53 by preventing ubiquitination in response to DNA damage. They established the mechanism of communication between CHK2 and *p53* to explain the phenotypic similarity of Li-Fraumeni-1 syndrome (LFS1; 151623) caused by mutations in *p53* and Li-Fraumeni-2 syndrome (LFS2; 609265) caused by mutations in CHK2. C.W. Reuter et al., (2000) found the tumour suppressor PML (102578) to regulate p53 response to oncogenic signals. Oncogenic RAS (HRAS; 190020) increased the PML expression, while PML overexpression caused p53-dependent aging. *p53* acetylated to lys382 with RAS expression essential for its biological function. RAS-induced *p53* and CBP acetyltransferase (CREB; 600140) localization in PML nuclear bodies and the formation of the *p53*-PML-CBP three-dimensional complex. RAS-induced *p53* acetylation, *p53*-CBP complex stabilization, and aging were lost in PML-/- fibroblasts. The authors suggested a relationship between PML and *p53* and concluded that *p53* acetylation and aging with oncogene expression required the integrity of PML bodies.

CRC is caused by the accumulation of driver mutations, but the contribution of specific mutations into different stages of malignant progression is not thoroughly known (Zharmakhanova et al., 2021). Sakai et al., (2018) have created mouse models harbouring different combinations of key CRC driver mutations (Apc, *KRAS*, Tgfbr2, Trp53, Fbxw7) in intestinal epithelial cells to comprehensively investigate their role in the development of primary tumours and metastases. Apc Δ716 mutation caused intestinal adenomas and combination with Trp53 R270H mutation or Tgfbr2 deletion induced submucosal invasion. The addition of *KRAS* G12D mutation yielded epithelial-mesenchymal transition (EMT)-like morphology and lymph vessel intravasation of the invasive tumours. On the contrary, the combinations of Apc Δ716 with *KRAS* G12D and Fbxw7 mutation were not sufficient for submucosal invasion but still induced EMT-like histology. Studies using tumour-derived organoids showed that *KRAS* G12D was critical for liver metastasis following splenic transplantation when this mutation was combined with either Apc Δ716 plus Trp53 R270H or Tgfbr2 deletion (Tamm et al., 2021). At that, the highest incidence of metastasis was observed in tumours with Apc Δ716, *KRAS* G12D, Tgfbr2-/- genotype. RNA sequencing of tumour organoids has determined different gene expression profiles characteristic of the respective combinations of driver mutations, with activated genes in Apc Δ716 *KRAS* G12D Tgfbr2-/- tumours found to be similarly positive in specimens of human metastatic CRC. These results evidence that Wnt and *KRAS* activation with the suppression of TGFβ signalling in intestinal epithelial cells was enough for CRC metastasis. This conclusion might be important for the development of metastasis prevention strategies. These findings show how key driver mutations in colon cancer cooperate to drive the development of metastatic disease. This information could influence the development of appropriate prevention strategies. The RAS inhibitor farnesylthiosalicylic acid (Salirasib) disrupts the spatiotemporal localization of active RAS: a potential treatment for cancer (Rotblat et al., 2008).

A comprehensive review of recent next-generation sequencing studies on gene mutations in gastric cancer reveals significant progress in understanding the molecular landscape of the disease, but there is a limitation in the interpretation of the data regarding the specific significance of genetic mutations (Lin et al., 2015). Study's Marbun et al., (2022) helps reveal the genetic basis of colorectal cancer in the Indonesian population. This is important because genetic characteristics can vary between different ethnic groups, and this can have an impact on the treatment

and prognosis of patients.

One of the key takeaways from this research is the potential for expanded molecular profiling in clinical settings. Understanding the specific genetic mutations and alterations that contribute to these cancers allows for more precise diagnosis and prognosis. This, in turn, can guide treatment decisions and enable a more personalized approach to patient care. Furthermore, the identification of actionable molecular targets presents an opportunity for the development of novel therapies. Targeted therapies that focus on specific genetic abnormalities, such as those in the *RAS* genes, hold promise in improving treatment outcomes. In the case of gastric cancer, where late-stage diagnosis is common, these targeted therapies could provide new avenues for intervention, potentially leading to better survival rates. Additionally, the molecular understanding of these cancers can inform the selection of existing treatments. For example, knowledge of specific mutations can guide decisions about the use of targeted drugs or immunotherapies, which may be more effective in certain patient populations. In summary, while this has shed light on the molecular intricacies of gastric and colorectal cancers, it's crucial to emphasize the practical implications for clinical practice. Expanded molecular profiling, the development of targeted therapies, and informed treatment decisions based on molecular insights all represent significant advancements in the battle against these cancers.

In conclusion, the study aimed to investigate the molecular characteristics and expression patterns of *p53*, *RAS, WAF1,* and *MDM2* genes in gastric and colorectal adenocarcinomas. Through the analysis, several key findings have emerged, shedding light on the role of these genes in cancer development and progression. The results of the present study indicated the damage of genes that control cell growth and division in gastric adenocarcinomas. This damage resulted in a decreased expression of truncated products of such major growth suppressors as *p53*, p21WAF1, and MDM2 proteins, the deletions of *p53* exons 5-6 and 7-9, and point mutations in functionally essential codons of *RAS* genes. The *KRAS* and *NRAS* mutations in almost 63.9% of studied colon and rectal samples indicated autonomous cell growth. All this explained the aggressive and metastatic growth of tumours and the ineffectiveness of growth factor inhibitors in CRC. The findings underscored the importance of these genes in tumorigenesis and provided directions for future investigations that could lead to improved therapeutic strategies. While the study has made contributions to the field, further research was needed to explore the functional implications of the mentioned gene alterations and their interactions in larger cohorts.

List of Abbreviations

p53, Tumour Protein 53; RAS, Rat Sarcoma gene; *KRAS*, Kirsten Rat Sarcoma gene; HRAS, Harvey Rat Sarcoma gene; *NRAS*, Neuroblastoma Rat Sarcoma gene; PCR, Polymerase Chain Reaction; RT-PCR, Reverse Transcription-Polymerase Chain Reaction; AT, Adjacent Tissues; DNA, Deoxyribonucleic Acid; FAM, Fluorescein Amidite; MDA, Moderately Differentiated Adenocarcinoma; PDA, Poorly Differentiated Adenocarcinoma; MDM2, Mouse Double Minute 2 gene; PI3K, Phosphoinositide 3-kinase; PKB, Protein Kinase B; AKT, Protein kinase B; RNA, Ribonucleic Acid; dNTP, Deoxynucleotide Triphosphates; cDNA, Complementary Deoxyribonucleic Acid; UV, Ultraviolet; WAF1, Wild-type Tumour Protein 53-Activated Fragment 1; AA, amino acids; NT, nucleotide; AAC, Amino acid change; AAT, Amino acid substitution; BCR-ABL, Breakpoint Cluster Region-Abelson tyrosine kinase; APC, Adenomatous polyposis coli gene; *BRAF*, v-raf murine sarcoma viral oncogene homolog B1; CAC, Calcium channel; CAT, Catalase; CBP, CREB-binding protein; CRC, Colorectal cancer; EGFR, Epidermal growth factor receptor; EMT, Epithelial-mesenchymal transition; FGFR, Fibroblast growth factor receptor; GAP, GTPase-activating protein; MAP, Mitogen-activated protein; NF1, Neurofibromin 1 tumour suppressor gene; *PIK3CA*, Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PML, Promyelocytic leukaemia protein; SVR, Subviral RNA; Δ716, Deletion of residue 716.

Author Contribution Statement

All authors contributed equally in this study.

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Availability of data

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

There is no conflict of interests to declare by authors.

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