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Relationship between TGF-Beta 1 rs1800469 Gene Polymorphism with Degree of Tumor Invasion of Colorectal Cancer at RSUP Prof. Dr. I.G.N.G Ngoerah Denpasar Bali

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ABSTRACT

The incidence of cancer, including colorectal cancer (CRC), increases with age. The Transforming Growth Factor-Beta 1 (TGF-B1) rs1800469 gene polymorphism is associated with the pathogenesis of CRC. The T allele is associated with increased transcriptional activity compared to the C allele, meaning that individuals with TT homozygotes will produce higher TGF-B1 protein concentrations. This study aimed to analyze the relationship between the TGF-B1 rs1800469 gene polymorphism and degree of tumor invasion of CRC in Bali, especially at the RSUP Prof. Dr. I.G.N.G. Ngoerah Denpasar.

This hospital study was a cross-sectional study using formalin-fixed-paraffin embedded (FFPE) samples from CRC patients, stored in the Department of Pathology Prof. Dr. I.G.N.G. Ngoerah Denpasar Bali. Identification of TGF-B1 rs1800469 polymorphism gene was performed using polymerase chain reaction (PCR) and sequencing methods. Data on age, sex, histopathological grading, degree of tumor invasion, pathological staging N and tumor location were recorded from the patient's medical record, carried out descriptive analysis and hypothesis testing of bivariate Chi Square analysis.

Out of 48 samples, majority was over 50 years old (83.3%), male (54.2%), low grade histopathology (93.8%), adenocarcinoma histological classification (97.9%), high degree of tumor invasion (81.2%), staging pathology N with regional lymph nodes cannot be assessed (93.7%), left tumor location (58.3%) with TGF-B1 rs1800469 gene polymorphism CT genotype of 23 samples (47.9%), followed by TT genotypes of 17 samples (35.4%) and CC genotypes of 8 samples (16.7%). The results of the Chi Square statistical analysis test found no association between the TGF-B1 rs1800469 gene polymorphism in any genotype and degree of tumor invasion of CRC (p=0.688).

It can be concluded that there was no significant relationship between the TGF-B1 rs1800469 gene polymorphism and degree of tumor invasion of CRC patients at RSUP Prof. Dr. I.G.N.G. Ngoerah, Denpasar, Bali.

Keywords: Colorectal Cancer, Gene Polymorphism, SNP, Tumor Invasion

INTRODUCTION

Aging is characterized by a progressive loss of physiological integrity, which is a major risk factor for the occurrence of human pathologies, including cancer[(1)]. Colorectal cancer (CRC) is defined as cancer of the colon and rectum. Most CRC are sporadic, which can be caused by several genetic variations, such as single nucleotide polymorphisms (SNPs)[(2)]. Transforming Growth Factor Beta (TGF-B) is a

multifunctional cytokine that plays an important role in controlling tissue development, proliferation, differentiation, apoptosis and homeostasis in cells[3]. Disruption of the TGF-B signaling pathway has been implicated in many human diseases, including cancer. In normal tissues, TGF-B signaling inhibits epithelial growth, but in tissues with advanced cancer, TGF-B signaling promotes tumor cell development. This phenomenon is known as the TGF-B paradox[4]. Disruption of TGF-B signaling in the colon will promote tumor development in two ways, epithelial cell transformation and tumor-stromal interaction.[5]. The TGF-B1 rs1800469 gene polymorphism is a high quality single nucleotide polymorphism (SNP) that can act as a promising genetic biomarker for screening susceptibility to sporadic colorectal cancer (SCRC) clinically and its functional role in the development of SCRC.[2]. The rs1800469 polymorphism, also known as -509 C>T, is a SNP in the promoter region of the Transforming Growth Factor-Beta 1 gene. SNP rs1800469 alters TGF-B1 protein levels[6]. TGF-B1 rs1800469 gene polymorphism consists of genotype CC homozygous, CT heterozygote, and TT homozygote[7]. The TGF-B1 rs1800469 gene polymorphism has an impact on the carcinogenesis process of CRC, the expression of the TGF-B1 gene is significantly associated with inhibiting the tumor size and depth of tumor infiltration of CRC[8]. Other researchers found decreased circulating TGF-B1 serum levels produced by the CC genotype, is a possibility of accelerating tumor progression[9]. At this time there is no data regarding the relationship of the TGF-B1 rs1800469 gene polymorphism with the degree of tumor invasion of CRC at the RSUP Prof. Dr. IGNG Ngoerah, Denpasar, Bali. Based on this background review, it is necessary to conduct research that proves the relationship between the TGF-B1 rs1800469 gene polymorphism and the degree of tumor invasion of CRC in Bali.

LITERATURE REVIEW

Cancer is a multifactorial, multigenetic and multistage disease because of the complex interaction of environmental and genetic factors. Cancer heritability is related to gene defects and genetic variations in DNA sequences. SNPs are a common cause of human genetic variation[10]. TGF-B1 rs1800469 gene polymorphism as one of the high quality SNPs that has a significant relationship to sporadic CRC in the Asian subgroup[2].

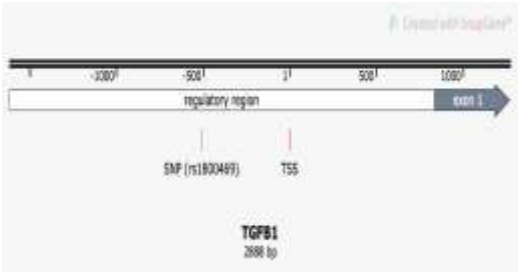

Figure 1 TGF-B1 rs1800469 gene[7]

The TGF-B1 rs1800469 gene polymorphism is the causative SNP code rs1800469 in the form of a non-coding SNP that occurs in the Transforming Growth Factor Beta 1 (TGF-B1) gene in the promoter section, which changes TGF-B1 gene expression in a transcription regulator to produce TGF-B1 protein. This SNP occurs on chromosome 19, locus NG-013364.1 (q13.1-13.3), nucleotide number 4536 (variation on "t"). TGF-B1 rs1800469 gene polymorphism consists of CC homozygous, CT heterozygote, and TT homozygote[7]. SNP rs1800469 changes the amount of TGF-B1 protein, but not change the nature of the TGF-B1 protein. The T version will increase the amount of TGF-B1 protein production by preventing activator protein 1 (AP1) from binding to the promoter region.[6]. The expression product of the TGF-B1 gene is a pro-TGF-B protein with a molecular weight of 44.3 kDa and consisting of 390 amino acids. Pro-TGF-B is the precursor of the protein LAP (latency related peptide) and TGF-B which belongs to the TGF-B superfamily ligand[8]. TGF-B1 gene mRNA expression in CRC tissue is

related to tumor progression and metastasis[9].

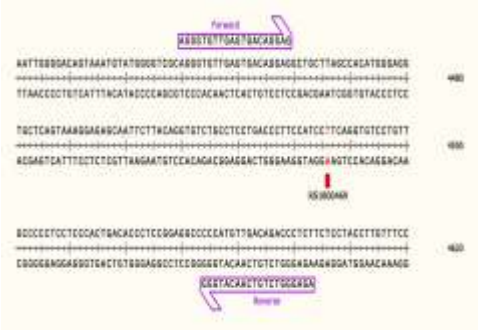

Figure 2 TGF-B1 rs1800469 gene polymorphism[7]

TGF-B is released by platelets (main) and activated macrophages, lymphocytes, and neutrophils[8]. The TGF-B protein is important cytokine in the regulator of tissue development and homeostasis[11], an important regulator of cellular proliferation, apoptosis, extracellular matrix remodeling in cells[12] and differentiation[3], also plays a role in angiogenesis and inflammation[13].

TGF-B activates the Smad-dependent canonical and Smad-independent noncanonical signaling pathways, starting with TGF-B binding to TGFBR2, then activating TGFBR1. TGFB1-phosphorylated Smad2/3 forms a complex with Smad4, enters the nucleus and regulates transcription of different target genes for early and late stages of tumor development, can be tumor suppression or tumorigenesis. Smad7 antagonizes TGF-B signaling by blocking Smad2/3 activation and interfering with the formation of the Smad2/3/4 DNA complex. In noncanonical signaling pathways, the TGF-B receptor initiates signaling via MAPK, PI3K, and the Rho family of small GTPases and others. Active JNK/p38/ERK can interact with SMAD or induce its individual transcriptional programs directly to influence cancer cells. Rho-activated Rho-associated protein kinase is involved in cytoskeleton modification in the EMT process. Through the PI3K-AKT pathway, TGF-B can activate mammalian target rapamycin to regulate protein translation. TGF-B activation of tumor necrosis factor receptor–associated factor proteins can induce NF-KB signals for inflammatory responses[11].

TGF-B has another pathway, namely TGF-B in the tumor microenvironment activates pro-tumor and anti-inflammatory mechanotransduction pathways through ECM remodeling, cytoskeletal alteration, and DNA damage repair.[14]. Release of proangiogenic factors MMP-9, VEGF and TGF-B from tumor cells alone leads to intensive invasion and cancer development. [15]

TGF-B1 rs1800469 Gene Polymorphism and Degree of Colorectal Cancer Tumor Invasion

In a case-control study in America, there was a significant association between the TGF-B1 rs1800469 gene polymorphism and colon cancer, but not for rectal cancer.[16]. A meta-analysis study concluded that the TGF-B1 rs 1800469 gene polymorphism allele C is a risk factor for the development of CRC in Asian races[17]. A case-control study in Saudi Arabia found the highest number of genotypes CT, TT and CC respectively[18].

Another meta-analysis study of the TGF-B1 rs1800469 gene polymorphism found that the T allele was associated with increased transcriptional activity compared to the C allele, because plasma TGF-B1 concentrations were found to be higher in individuals with T homozygotes compared to CT heterozygotes[10]. A cross-sectional study in Poland, found a decrease in the expression level of the TGF-B1 gene to be one of the factors contributing to the development of colorectal cancer. The relative mRNA expression level of the TGF-B1 gene is significantly related to the T feature (tumor size and infiltration depth) of the TNM classification. A high TGF-B1 gene expression level has an effect on inhibiting tumor size and infiltration depth.[8]. The T allele is associated with preventing the development and progression of CRC to an advanced stage[9].

Other studies have found that increased TGF-B expression promotes a more aggressive tumor phenotype. TGF-B itself is known to be involved in EMT, invasion and metastasis and influences the microenvironment[19]. This is the same as what was found in other meta-analysis studies which stated that the TGF-B1 rs1800469 gene polymorphism allele C has higher TGF-B expression, higher mRNA expression, higher TGF-B1 serum concentrations thereby increasing the development tumor of CRC[20]. A case-control study in China found that CRC with aggressive tendencies were more frequently associated with the C allele. However, this study found no significant association between the TGF-B 1 rs1800469 gene polymorphism in all genotypes and all alleles with tumor stage.[21]. A case-control study in Bulgaria, found the same thing, namely there was no relationship between the TGF-B1 rs1800469 gene polymorphism pathological stage of T[22].

MATERIALS & METHODS

Study Design and Sample Preparation

A cross-sectional analytical study to see the relationship between the TGF-B1 rs1800469 gene polymorphism and the degree of tumor invasion of colorectal cancer in RSUP Prof. Dr. IGNG Ngoerah, Denpasar, Bali.

The sample size was 48 samples. The sample used Archived Biological Materials in the form of tumor tissue stored in the form of Formalin-Fixed Parrafin-Embedded (FFPE) from colorectal cancer patients being treated at RSUP Prof. Dr. IGNG Ngoerah, Denpasar, Bali from 2018-2020 which is stored in the Department of Pathology RSUP Prof. Dr. IGNG Ngoerah, Denpasar, Bali.

The data collected includes demographic parameters and characteristics of CRC. Demographic parameters include age and gender. Characteristic parameters of CRC include histopathological grading, histological classification, degree of tumor invasion, pathological stage regional nodes and tumor location of CRC.

Classification of the degree of tumor invasion is grouped based on the criteria for pathological stage tumor from WHO. In this study, T1 and T2 were grouped into low degree of invasion. T3 and T4 are grouped into high degree of invasion. T1 means tumor invades submucosa, T2 means tumor invades muscularis propria, T3 means tumor invades subserosa or non-peritonealized pericolic or perirectal tissues, and T4 means tumor invades other organs or structures and/or perforates visceral peritoneum. This study was approved by Ethics Committee of Faculty of Medicine Udayana University (EC number: 2637/UN14.2.2.VII.14/LT/2022).

Sample Assessment

Formalin-Fixed Parrafin-Embedded (FFPE), begins with DNA isolation and calculates DNA concentrations at the LBT Unit of the Faculty of Medicine, Udayana University. The TGF-B1 rs1800469 gene polymorphism was evaluated by PCR, using the forward primer: 5'AGGGTGTTGAGTGACAGGAG3' and the reverse primer: 5'AGAGGGTCTGTCAACATGGG3'. Genotyping was performed by BLAST and Snapgene software.

STATISTICAL ANALYSIS

The Statistical package for social studies (SPSS version 26) was used for data analysis. Descriptive statistics (numbers and percentages for categorical variables) and Chi Square Fisher's Exact Tests was used to test the association. The $p \leq 0.05$ was considered statistically significant. Odds ratio (OR) with 95% confidence interval (95% CI) were generated to quantify relationships with each risk factor. Prevalence genotype of TGF-B1 rs1800469 gene polymorphism were identified by using descriptive statistics.

RESULT

The characteristics of the research subjects are listed in Table 1.

Table 1 Characteristics of the Research Subject

Variable	N = 48 n (%)
Age	
≤50 years	8(16.7)
>50 years	40(83.3)
Gender	
Man	26 (54.2)
Woman	22 (45.8)
Grading Histopathology	
Low grade	45 (93.8)
High Grade	3 (6.2)
Classification Histopathology	
Adenocarcinoma	47 (97.9)
Neuroendocrine Carcinoma	1 (2.1)
Degree of Tumor Invasion	
Low (T1-T2)	9 (18.8)
High (T3-T4)	39 (81.2)
Stadium Pathological Regional Lymph Nodes (N)	2 (4.2)
No metastasis (N0)	1 (2.1)
Metastasis 1-3 nodes (N1)	45 (93.7)
Nodes cannot be assessed (Nx)	
Tumor Location	
Right	20 (41.7)
Left	28 (58.3)

Eligible samples were processed by PCR and then electrophoresed. The analysis was continued with the BLAST program to match the base sequence of the TGF-B1 rs 1800469 gene polymorphism which had been sequenced according to the base sequence listed in the BLAST program (Figure 3). The Snapgene application is used to read the sequencing results (Figure 4).

Figure 3 Representative of BLAST Result for one sample

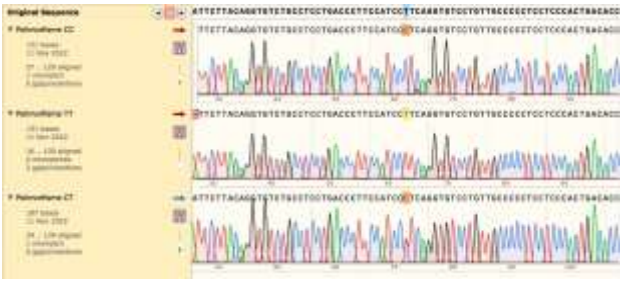

Figure 4 Visualization of Sequencing Results by Snapgene Application

Sample results of sequencing graphs in blue indicate cytosine bases (C) and sequencing results in red graphs indicate thymine bases (T). In Figure 6 the results of the C base sequencing with the single peak graph at the position of the SNP TGF-B1 rs1800469 are concluded as CC homozygous polymorphism. The sequencing results showed that the T base with a single peak graphic at the position of the SNP TGF-B1 rs1800469 was concluded as a TT homozygous polymorphism. The sequencing results showed C and T bases with a double peak graph at the position of the SNP TGF-B1 rs1800469 which was concluded as CT heterozygous polymorphism.

Table 2 Genotypes of the sequencing results of the TGF-B1 rs1800469 gene polymorphism

TGF-B1 rs1800469 gene polymorphism	N (%)
TT	17 (35.4)
CT	23 (47.9)
CC	8 (16.7)
Total	48 (100)

The TGF-B1 gene polymorphism rs1800469 was found in the study subjects, with the most CT genotypes of 23 people (47.9%), followed by TT genotypes of 17 people (35.4%) and CC genotypes of 8 people (16.7%).

Correlation between TGF-B1 rs1800469 Gene Polymorphism and Degree of Colorectal Cancer Tumor Invasion

Table 3. Results of Relationship Analysis of TGF-B1 rs1800469 Gene Polymorphism with Degree of Tumor Invasion

Variable	Degree of Tumor Invasion		P
	Low n (%)	High n (%)	
TGF-B1 rs1800469 Gene Polymorphism			
CC	2 (25)	6 (75)	
CT	5 (21.7)	18 (78.3)	0.667
TT	2 (11.8)	15 (88.2)	

Fisher's exact test results, p value> 0.05, meaning that there is no relationship between the TGF-B1 rs1800469 gene polymorphism CC genotype, CT genotype and TT genotype with the degree of tumor invasion CRC.

DISCUSSION

CRC cases continue to increase and various studies on CRC have been developed to date, but research related to the relationship of the TGF-B1 rs1800469 gene

polymorphism with CRC has not been explored in depth. In fact, the description of the relationship between the TGF-B1 rs1800469 gene polymorphism and CRC can be a consideration in evaluating the progressivity of CRC. The results of this study can provide an overview of the relationship of the TGF-B1 rs1800469 gene polymorphism to the characteristics of CRC, especially the size of the degree of tumor invasion, in Indonesia, especially in RSUP Prof. Dr. IGNG Ngoerah Denpasar, Bali.

Characteristics of the Samples

Based on the results of this study, colorectal cancer patients were diagnosed with CRC, most were over 50 years old (83.3%). In accordance with research conducted by Bray et al. (2018), most patients with sporadic CRC are over 50 years old[(23)]. This is because older patients experience many biological changes related to age, multimorbidity, pharmacological and non-pharmacological treatments, and the influence of lifestyle and environmental and psychosocial factors, all of which are likely to have an impact on their physiological reserves and susceptibility.[(1)]. Berben et al. (2021) found reasons for higher cancer incidence in old age. There are several things that can explain this. First, there is an accumulation of oxidative stress and DNA damage that accumulates over the years of life, due to exposure to both endogenous (e.g., free radicals) and exogenous (e.g., ultraviolet radiation, diet) factors. Ultimately leading to cell transformation and tumor initiation. Second, senescence cells accumulate during the aging process and exhibit a senescence-associated secretory phenotype (SASP). SASP secrete inflammatory mediators (e.g., interleukin (IL)-6, IL-8, monocyte chemoattractant protein (MCP)-2, growth-regulated oncogene alpha (GROα)) that can promote tumor growth by creating a tumorigenic environment. Finally, progressive impairment of immune function occurs with advancing age,[(24)]. According to Maugeri et al. (2021), SCRC occur most often over the age of 50. In addition, old age is an unmodifiable risk factor that is associated with an increased incidence of CRC[(25)].

Based on the results of this study, colorectal cancer patients were dominated by male sex (54.2%). This is in accordance with research conducted by Siegel et al. (2020), new cases of CRC are higher in men than in women[(26)]. Like Alsanea et al. (2015) found the number of new cases of CRC among Saudis in 2010, males were higher than females[(27)]. Gunasekaran et al. (2019) also found that the number of CRC in males was higher than in females[(28)]. Wahidin et al. (2012), found that men were more often affected by CRC (4.13 per 100,000) than women.[(29)]. Kwon et al. (2013) found that the high CRC in men can also be caused by the habit of consuming alcohol and smoking by 32 times more in men than women, so that it can trigger malignancy in the large intestine[(30)].

Histopathological grading results with the majority being low grade (93.8%), meaning that the cancer cells are well differentiated. The cells are not normal but look and are arranged very much like normal cells. Cancer tends to grow slowly and is less likely to spread. Grading at CRC serves to compare visible cancer cells with normal and healthy cells. Knowing the grading can give an idea of how fast CRC can grow and how likely it is to spread. This is very helpful in the treatment of CRC and the prognosis of CRC patients[(31)].

From the results of the study, adenocarcinoma was the most common histopathological classification of adenocarcinoma (97.9%). This is in accordance with the research by Gunasekaran et al. (2019) who found 90% of CRCs were adenocarcinomas. Adenocarcinoma being the majority histopathological classification in this study may be because the percentage of adenocarcinoma cases increases with age, namely at the age of 59 years at 79–96%. In line with this study which found the majority of patients aged over 50 years (83.3%)[(28).] The histopathological

classification of CRC according to WHO shows that the majority are adenocarcinomas. The application of neoadjuvant therapy such as radiotherapy or radiochemotherapy or systemic therapy for CRC is influenced by this histopathological classification[(32)].

The degree of tumor invasion is a pathological description of how far the cancer has grown into the walls of the colon and rectum. From the results of the study, the majority of patients diagnosed with CRC had the high degree of invasion tumor T3-T4 (81.2%), meaning that patients diagnosed with CRC had a tumor that had invades the subserosa or non-peritonealized pericolic or perirectal tissues, or had perforates the visceral peritoneum. and/or invades other organs or structures[(32)]. This reflects the need to increase CRC screening in Bali to diagnose CRC quickly and to increase awareness education for the Balinese people in assessing the symptoms of CRC. Examination of the fecal occult blood test as an initial screening for those over 50 years of age, needs to be started in the community, especially at the primary health care level. Pathological stage of regional nodes (N) is the result of histological examination of regional lymphadenectomy specimens. In this study, the most found were pNx (93.7%), meaning that histological examination of regional lymphadenectomy specimens from 12 or more lymph nodes could not be determined[(32)].

From the results of the study, there were more CRC on the left (58.3%) than on the right (41.7%), meaning that in this study it was found that more CRC occurred in the descending colon, splenic flexure, sigmoid, distal third of the transverse colon and rectum. According to Baran et al. (2018), left-sided tumor is associated with mutation-related chromosomal instability pathways. In addition, the left-sided tumor has a polypoid-like morphology. Patients with left-sided tumor have a better response to adjuvant chemotherapy such as 5-fluorouracil (5-FU) and to targeted therapy such as anti-epidermal growth factor receptor (EGFR) therapy. Tumor in the left location have a better prognosis than the right location. Whereas right-sided tumor are more responsive to immunotherapy[(33)]. According to Thrumurthy et al. (2016), left-sided tumor usually present with altered bowel habits, such as diarrhea, increased frequency of bowel movements, and intestinal obstruction secondary to progressive narrowing of the lumen, rectal or mucous bleeding, or tenesmus. Right-sided tumor may present more insidiously, with weight loss, abdominal pain, or a mass in the right abdomen, and iron deficiency anaemia[(34)].

The results of the TGF-B1 rs1800469 gene polymorphism with the most genotypes were CT (47.9%), followed by TT (35.4%) and CC (16.7%). This is in accordance with the results of research Althubyani et al. (2020) on Saudi Arabian sufferers from CRC and the results of Qi et al.'s research. (2010) on Chinese suffering from CRC. In contrast to the results of research by Gulubova et al. (2018), it was found that the highest number of CT, CC and TT genotypes were found in Bulgarians suffering from CRC. This was possible due to differences in the populations studied, where Althubyani was in the Saudian population, Qi was in the Chinese population and Gulubova was in the Bulgarian population. In addition, the difference in the number of samples from each researcher, where Althubyani was 70 cases and 70 controls, Qi was 150 cases and 503 controls, Gulubova was 120 cases and 176 controls.

Correlation between TGF-B1 rs1800469 Gene Polymorphism and Degree of Colorectal Cancer Tumor Invasion

Aging is a major risk factor for cancer, especially colorectal cancer[(35)]. The main cause of aging is the accumulation of extensive time-dependent cellular damage[(36)]. At the same time, cell damage can give certain cells an undue advantage, and can eventually result in cancer.

Therefore, aging and cancer can be considered as two different manifestations of the same basic process, namely the accumulation of cell damage that is dependent on time[37]. This is an illustration of the close relationship between aging and cancer that are inseparable.

One of the hallmarks of cancer is maintaining the signal of proliferation. The most fundamental property of cancer cells is the ability of cancer cells to maintain chronic proliferation[38]. The TGF-B1 rs1800469 gene polymorphism will change the expression of the TGF-B1 gene in the transcription regulator to produce TGF-B1 protein, but does not change the nature of the TGF-B1 protein.[7]. TGF-B1 protein is one of the most important cytokines in the regulator of cellular proliferation[12].

TGF-B1 protein is known to have many influences on hallmarks of cancer, including angiogenesis, tissue invasion, immune suppression, and metastasis.[39]. This illustrates the link between cancer, the TGF-B1 rs1800469 gene polymorphism and the TGF-B1 protein. TGF-B1 protein inhibits epithelial growth in normal tissue, but in cancer tissue, TGF-B1 protein promotes tumor cell development[4]. The role of the TGF-B1 protein in tumor progression and metastasis can be described by the characteristics of the degree of tumor invasion of CRCs. In line with research conducted by Wodzinski et al. (2022) who found a relationship between increasing TGF-B1 gene expression in inhibiting the size and depth of tumor infiltration[8].

The polymorphism rs1800469 is a polymorphism in the TGF-B1 gene in the promoter region, this polymorphism will change the expression of the TGF-B1 gene in the transcription regulator to produce TGF-B1 protein. The T allele will increase the amount of TGF-B1 protein production by preventing activator protein 1 (AP1) from binding to this region.[6]. So that the amount of TGF-B1 protein produced by the TGF-B1 rs1800469 gene polymorphism TT genotype is greater than the CT genotype and the CT genotype is greater than the CC genotype[10]. The involvement of the TGF-B1 protein in the progression of CRC has been described previously. Under physiological conditions, the TGF-B1 protein acts as a tumor suppressor by activating apoptosis and decreasing the expression of genes encoding vascular endothelial growth factor.[8]. Thus, many case-control studies have found that the higher the production of TGF-B1 protein (at the T allele), the lower the risk of developing CRC.

However, in cancer tissue, overexpression of the TGF-B1 gene is associated with increased neoplastic stem cell formation in the tumor stroma and increased EMT towards metastatic formation. In addition, over-expression of the TGF-B1 gene also causes a decrease in the immune response against cancer cells which will then increase the process of carcinogenesis. The T allele (CT+TT genotype) is associated with increased mRNA expression so that there is an increased level of TGF-B1 protein[21]. This may be related to the results of this study, the description of the TGF-B1 rs1800469 gene polymorphism in colorectal cancer patients at Prof. Dr. IGNG Ngoerah Denpasar Bali, the largest sequentially the CT genotype (47.9%), TT (35.4%) and CC (16.7%), meaning that the largest majority are alleles T. Wodzinski et al (2022) who found a significant relationship between TGF-B1 gene expression and tumor size and degree of invasion, increased TGF-B1 gene expression will inhibit the development of tumor size and degree of invasion[8]. Further research is needed to prove the relationship between the T allele and increased TGF-B1 protein levels in colorectal cancer patients at RSUP Prof. Dr. IGNG Ngoerah Denpasar Bali.

In this study, no correlation was found between the TGF-B1 rs1800469 gene polymorphism genotype CC, CT or TT with the degree of tumor invasion of CRC. The insignificant results may be due to the uneven distribution of tumor invasion degree data between mild and severe degrees. The results of this study are in line

with the case-control study of Gulubova et al. (2018) in the Bulgarian population, who found no association between the TGF-B1 rs1800469 gene polymorphism and pathological stage (T1-T2 and T3-T4)[(22)]. Qi et al. case control study. (2010) found the same thing, that there was no relationship between the TGF-B1 rs1800469 gene polymorphism in all genotype types and all alleles with tumor stage in the Chinese population.[(21)].

The results of this study are not in line with the results of Wodzinski et al. (2022). This may be due to many factors, including the expression of the TGF-B1 gene in producing TGF-B1 protein does not directly affect CRC progress, but through the TGF-B signaling pathway, in which there are 40 different proteins that play a role in the TGF-B signaling pathway.[(8)].

The results of this study are also not in accordance with the research by Stanilova et al. (2018) who found the T allele to be associated with preventing the development and progression of CRC to an advanced stage[(9)].

In this study, an interesting finding was found, namely that as many as 33 samples (68%) of the T allele (TT+CT) out of a total of 48 samples experienced a tendency for a severe degree of tumor invasion, although the results were not significant. This means that there is a need for vigilance for patients with the T allele. This certainly requires further research. However, this study is in line with research conducted by Gulubova et al. (2018), that the TT genotype has the shortest survival rate[(22)].

In this study, the majority of histopathological grades were found to be low grade (93.8%), however, the majority of tumor invasion degrees were found to be high (81.2%). This can be caused by the duration of the patient suffering from cancer, perhaps due to a late diagnosis, or waiting for the health service referral system, so that when the patient is operated on, it has reached high degree of invasion.

The TGF-B1 rs1800469 gene polymorphism is a genetic variation that has been carried since birth. The development of colorectal cancer is an interaction of environmental factors and genetic factors. Environmental factors that have a large influence on the development of colorectal cancer are lifestyle factors, most of which can be modified. This research is more directed at preventing CRC and improving the prognosis of CRC through a healthy lifestyle according to the concept of Anti Aging Medicine, which will ultimately improve quality of life, extend life expectancy and provide a better prognosis for CRC patients.

The limitation of this research is the sampling process that uses stored samples, so it cannot choose a sample from a particular ethnicity. This can cause bias to occur whether the sample is a pure Balinese population. However, this study has provided preliminary data regarding the role of the TGF-B1 rs1800469 gene polymorphism in colorectal cancer and the possibility of a high prevalence of the T allele in the Balinese population with a cross-sectional study design. However, this needs to be followed up with studies in larger populations through case-control studies where the number of case samples are colorectal cancer patients and the control sample is a balanced healthy population, with an in-depth evaluation of the TGF-B1 protein blood serum examination. so that the relationship between the TGF-B1 rs1800469 gene polymorphism and the risk of CRC occurrence can be investigated. The confounding factors of this study, which were not investigated, however, significantly influenced the results of the degree of tumor invasion, such as the patient's duration of cancer, which could be another limitation of the study.

CONCLUSION

From the results of this study, it can be concluded that the description of the characteristics of colorectal cancer at Prof. Central General Hospital. Dr. IGNG Ngoerah, majority age >50 years, male sex, majority histopathological grading is low

grade, histopathological classification is mostly adenocarcinoma, the degree of tumor invasion is the majority is high (T3-T4), the pathological stage of the majority of nodes cannot be examined (Nx) and the location of the majority of tumor left. The largest TGF-B1 rs1800469 gene polymorphism was sequentially the CT, TT and CC genotypes. There is no relationship between the TGF-B1 rs1800469 gene polymorphism and the degree of colorectal cancer tumor invasion at Prof. Dr. IGNG Ngoerah, Denpasar, Bali. Description of TGF-B1 rs1800469 gene polymorphism in a sample of CRC patients at Prof. Dr. IGNG Ngoerah, with the T allele tend to high degree of invasion compared to the CC genotype, but the result is not significant.

Declaration by Authors

Ethical Approval: Approved

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