

Association of CCND1 Gene Polymorphism rs9344 with Grade and Invasion Degree of Colorectal Cancer at Prof. Dr. I.G.N.G. Ngoerah Central General Hospital

Nanang Masrani, Ni N. A. Dewi, Ni M. Linawati, Ni P. Ekawati, and I Made Mulyawan

ABSTRACT

The majority of colorectal cancer (CRC) are sporadic CRC that can be caused by genetic variations such as Single Nucleotide Polymorphisms (SNPs). The CCND1 gene polymorphism rs9344 could involve at the beginning and the development of CRC.

This study aimed to analyze the association between CCND1 gene polymorphism rs9344 with the grade and invasion degree of colorectal cancer in at Prof. Dr. I.G.N.G. Ngoerah Central General Hospital, Denpasar, Bali. This cross-sectional study was carried out at the Integrated Biomedical Laboratory Unit, Faculty of Medicine, Udayana University.

Data analysis of 32 samples showed majority age was above 50 years old as many as 28 (87.5%), with men as 21 samples (65.6%). Histopathology description was adenocarcinoma in 32 samples (100%). Grading histopathology low grade was 31 samples (96.6%). The degree of tumor invasion was high as 25 samples (78.1%). From the aspect of pathological stage pNx as much as 27 (84.4%). The majority location of tumors was on the left side as 21 samples (65.6%). The polymorphisms of CCND1 rs9344 genotype sequentially AA as 20 samples (62.5%), AG as 9 samples (28.1%), while GG as 3 samples (9.4%). The statistical analysis found that CCND1 gene polymorphism rs9344 was not associated with colorectal cancer grade ($p>0.05$), and not associated with invasion degrees of colorectal cancer ($p>0.05$).

Taken together, we conclude that no significant association between the CCND1 gene polymorphism rs9344 with grade and invasion degree of colorectal cancer at Prof. Dr. I.G.N.G. Ngoerah Central General Hospital, Denpasar, Bali.

Keywords: colorectal cancer, CCND1 gene, cyclin D1, polymorphism.

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N. Masrani

Biomedical Sciences Master Program,
Faculty of Medicine, Udayana
University, Denpasar, Bali, Indonesia
(e-mail: nanangmasrani@gmail.com)

N. N. A. Dewi *

Department of Biochemistry, Faculty
of Medicine, Udayana University,
Denpasar, Bali, Indonesia
(e-mail: ayu.dewi@unud.ac.id)

N. M. Linawati

Department of Histology, Faculty of
Medicine, Udayana University,
Denpasar, Bali, Indonesia
(e-mail: md_linawati@unud.ac.id)

N. P. Ekawati

Department of Pathology, RSUP Prof.
Dr. I.G.N.G. Ngoerah, Denpasar, Bali,
Indonesia
(e-mail: niputu.ekawati@ahoo.com)

I. M. Mulyawan

Department of Surgery, RSUP Prof.
Dr. I.G.N.G. Ngoerah, Denpasar, Bali,
Indonesia
(e-mail: mulyawanm@yahoo.com)

**Corresponding Author*

I. INTRODUCTION

Colorectal cancer (CRC) is one of the alarmingly health problems due to the high morbidity and mortality rates, especially in the elderly population. Majority of colorectal cancer (CRCs) type are adenocarcinomas, malignancy of epithelial tumors derived from the colon or rectum that exhibits glandular or mucinous differentiation [1]. Colorectal cancer ranks third in the world for the most common types of cancer. According to GLOBOCAN 2019, the number of new cases from CRC in the world reached 1.8 million while in Indonesia there were 30.017 cases [2].

Cancer is a genetic and cell cycle related disease. Cyclin D1 is a protein encoded by CCND1 gene which is the main regulator of the cell cycle [3], [4]. Single Nucleotide Polymorphisms (SNPs) are common genetic variation that can produce different functions and can cause susceptibility to disease. Polymorphisms can be considered for sporadic tumor risk prediction including CRCs. Polymorphisms play

an essential role in the genetic predisposition of CRC [5]. Some meta-analyses found that CCND1 gene polymorphisms rs9344 was significantly associated with an increased risk of CRCs. But descriptions related to ethnicity, location, study design, and family history vary widely around the world [3].

The most common and most explored CCND1 gene polymorphism is rs9344. Due to its important role in tumorigenesis, polymorphisms of the CCND1 gene rs9344 is thought to be involved in accelerating the development of CRC [6]. Meta-analysis by Qiu *et al.* [6] confirmed the CCND1 gene polymorphisms rs9344 related to CRC susceptibility among Asian. However, there are some limitations, including high heterogeneity in the meta-analysis. The meta-analysis of Xie *et al.* [7] provides stronger evidence on the association between CCND1 gene polymorphisms rs9344 and CRC risk. The polymorphisms of the CCND1 gene rs9344 with A allele changes from CCND1 which has a longer half-life compared to the G

allele pushes cells through the G1-S phase check point in the cell cycle and rapid proliferation, ultimately resulting in high cancer progression [7]. Risk factors such as age, environment, high-fat diet is recognized in the formation of CRC [3].

Meta-analysis by Li *et al.* [8] on 22 studies with 4150 CRC patients showed overexpression of CCND1 significantly attributed to severe of invasion degrees of CRC. Li *et al.* suggested that CCND1 gene as a poor prognostic factor for CRCs. Over expression of CCND1 may be associated with poor clinical outcomes as well as age and stage TNM in CRC.

Retrospective research by Albasri *et al.* [9] showed a significant correlation between over expression of cyclin D1 with the degree of differentiation or grade of tumor, lymph nodes involvement, lymph vascular invasion, distant metastasis and TNM staging. This suggests the role of CCND1 gene in carcinogenesis and worse prognosis of CRC.

Based on these, the CCND1 gene polymorphism rs9344 plays important role on development and prognosis of CRC. Clinicopathological characteristics such as cell differentiation or grade as well as the progressiveness of cancer or invasion degree of CRC are two important things to determining the prognosis and therapy of CRC. However, research between CCND1 gene polymorphisms rs9344 with the grade and invasion degree CRC in Indonesia, particularly in Bali still very limited. This research is important to conduct, especially to know the prognosis of CRC patients to improve the quality of life of CRC patients and for the therapy development in the future.

II. LITERATURE REVIEW

Most CRCs are non-hereditary CRC which can be caused by some variation of genetics such as Single Nucleotide Polymorphisms (SNPs) [5]. More than 90% of all CRCs are adenocarcinomas [1]. Colorectal cancer develops through a series of events that lead to the transformation from a normal mucosa to adenoma and then carcinoma. Three molecular pathways that play role in the pathogenesis of CRC are chromosomal instability (CIN), microsatellite instability. can involve processes that are more than one pathway [10].

The CIN pathway is the most common cause of genomic instability in the CRC. This pathway plays 65-70% role in sporadic CRC. The CIN otherwise known as adenoma-carcinoma sequence is one of genome instability that plays a role in carcinogenesis of CRC. Various mechanisms are involved in the formation of CIN such as chromosomal segregation errors, telomere dysfunction and DNA damage response mechanisms. The CIN pathway is divided into three lines including the RAS/BRAF/MAPK line, the Wnt/ β -Catenin and the p53 pathway. The Wnt/ β -catenin pathway plays an important role in metabolism and biological processes and in cells. In addition to acting as a regulator of homeostasis, this pathway also involved in various human diseases, especially cancer [11].

Abnormally activated Wnt/ β -catenin pathways found in the development of colorectal cancer. The abnormal Wnt/ β -

catenin pathway is characterized by the presence of mutations on chromosome 5q that encode APC proteins or adenomatous polyposis as negative regulators of β -catenin in the cytoplasm. Almost about 90% of colorectal cancer cases are caused by mutations in this pathway, specifically mutations in APC [10],[11].

The Wnt/ β -Catenin line plays a major role in supporting the renewal of the intestinal epithelium. The APC protein binds to β -catenin and induces degradation of β -catenin, thereby acting as a negative regulator of β -catenin. The loss of APC function (specifically through mutations) results in the accumulation of β -catenin in the cytoplasm, which promotes β -catenin bonds with the T cell factor (TCF) protein/lymphoid enhancer factor (LEF) in the nucleus [10].

The CCND1 gene, which encodes cyclin D1, is a prime target of the β -catenin/LEF bond complex. Cyclin D1 is necessary for β -catenin to drive the development of CRC [12]. The β -catenin also activates cyclin D1 transcription through TCF binding inside the promoter. The expression of cyclin D1 is highly dependent on β -catenin/TCF bonds and has a direct effect on cell proliferation. Increasing transcribing cyclin D1 by RAS and β -catenin played an important role in the development of CRC and other malignancies involving this pathway [13].

A. The CCND1 Gene

The CCND1 gene encodes the protein cyclin D1, weighing 36-kDa, located on chromosome 11q13. Cyclin D1 is expressed by most normal human cells except cells derived from bone marrow stem cells [4],[14]. The biological functions of cyclin D1 include as sensor and integrator between mitogen signals, plays a role in the G1-S phase transition, promotes, and inhibits cell proliferation, plays a role in apoptosis and cell survival, cell migration and neural regeneration [15].

Cyclin D1 is an important cell cycle regulator protein required for G1-S phase transition, binding to CDK (cyclin dependent kinase) enzymes 4 and 6. Increased expression of the Cyclin D1 protein can influence the evolution of malignancy through cell proliferation, differentiation, and apoptosis. Abnormal cell proliferation is an important step in carcinogenesis. There are various proteins in the cell cycle pathway affect cell proliferation and carcinogenesis. Among these proteins, cyclin has a very important role in this process [6], [16].

Increased expression of CCND1 prolongs G1 phase and interferes the normal control of cell cycle. The CCND1 gene encodes 2 main splice variants, namely CCND1a mRNA and CCND1b mRNA which are oncogenic isoform [17]. Cyclin D1b has five times longer half-life than transcript form of cyclin D1a. The expression of both increases in some neoplasms and plays an important role in carcinogenesis. However, research reveals that Cyclin D1b seems to be a stronger oncogene, as it can change cells more easily compared to Cyclin D1a. Changes in both CCND1 gene and the Cyclin D1 protein are often found in precancerous lesions and neoplasms [18].

Meta-analysis by Li *et al.* [8] showed over expression of CCND1 was significantly attributed to the high invasion degree T3-T4, lymph nodes metastasis and distant metastasis of CRC. Li's analysis suggests that CCND1 as a

poor prognostic factor for CRC. Over expression of CCND1 may be associated with poor clinical outcomes as well as some factors such as age and TNM stage in CRC [8]. The retrospective study of Albasri *et al.* [9] showed no significant correlation between over expression of cyclin D1 with age, sex, tumor size, type, and location. However, over expression of cyclin D1 saw a significant correlation with degrees of differentiation or tumor grade, lymph node involvement, lymph vascular invasion, distant metastasis, and staging. The study concluded over expression of Cyclin D1 increased successively starting from normal - adenoma - carcinoma. A significant association was observed between over expression of cyclin D1 with advanced tumor stage and low survival rate, it showed the role of cyclin D1 in carcinogenesis and progressivity of CRC [9].

B. The CCND1 Gene Polymorphisms rs9344 and Colorectal Cancer

Single nucleotide Polymorphisms (SNPs) are common genetic variations, which can produce different gene functions, thereby affecting susceptibility to disease. Polymorphisms can be considered as a biomarker for predicting the risk of sporadic tumors including CRC [5]. Polymorphisms can change the structure of the genome and affect protein expression and function, leading to the proliferation of abnormal cells exiting the apoptosis pathway, increasing the risk of cancer [3].

The most common polymorphisms of the CCND1 gene are rs9344 (G870A) which there is a change of guanine (G) base to adenine (A) on 870 nucleotide position [5], [3]. The change in bases G to A of codon 241 (pro-241-pro) on the donor splice site exon 4-intron 4 drives an alternate transcript splice of the CCND1 gene. Polymorphisms that lead to alternative splicing result in a variant of cyclin D1 called cyclin D1b. Cyclin D1b has a lower capacity phosphorylation of the retinoblastoma protein RB1 which is oncogenic [19]–[21]. The dominant A allele primarily transcribes truncated transcripts, which encode the cyclin D1 protein with a longer half-life, pushing cells through the G1-S phase check point in the cell cycle and rapid proliferation. Transcript b causes deregulation of cell proliferation because it does not have the PEST (proline–glutamate–serine–threonine) sequence necessary for degradation. Elevated levels of cyclin D1 protein may be associated with high risk and progressiveness of CRC. The polymorphisms of the CCND1 gene rs9344 has 3 different genotypes namely GG, AG, and AA. All genotypes synthesize similar proteins due to their identical biological functions. However, the difference among these genotypes is the ability of allele causes transcript cutting, which then increases the half-life of the resulting protein. The A allele, specifically homozygous AA genotype was associated with the increased risk of CRC [7], [22].

A case-control study by Huang *et al.* [23] analyzed the relationship of the CCND1 genotype to CRC risk in Taiwan. The results showed that the genetic frequency of CCND1 A870G was distributed differently between the control group and CRC patients. The AG+GG vs AA genotype model also showed that people from AG or GG had a lower risk of CRC than the AA genotype. As for the analysis of the frequency of alleles, those with G allele had a lower risk

of cancer than those who had A allele on the polymorphisms of the CCND1 gene rs9344.

Several studies have shown activation of the CCND1 gene and/or over-expression of Cyclin D1 is closely related to patient clinical outcomes in various types of malignancies. In parallel with this, it was found that the amplification of CCND1 affects the regulation of Cyclin D1, which leads to disruption of the cell cycle, promotes the growth and development of cancer. The CCND1 amplification is found in various tumor types in humans and is a key determinant of the nature of malignancy, such as aggressiveness and high tumor proliferation activity [24].

Loic *et al.* [25] conducted a case control study on multiethnic populations in Hawaii including Japan, white and native Hawaiians. Overall, A allele was associated with a 30% increase in CRC risk whereas CCND1 gene polymorphisms rs9344 of the AA genotype was associated with a 50% increased risk of CRC unless the Japanese participants were more into rectal cancer. The association of the CCND1 gene of the AA genotype with an increased risk of CRC is stronger at an advanced stage. In contrast, statistically no significant association was found between A allele for early-stage CRC among all participants.

A case control study by Yang and Shi [41], 164 digestive system cancer patients (including 82 patients with gastric cancer and 82 with colorectal cancer) and 82 healthy subjects (control group) in China were examined with PCR-restriction fragment length polymorphism (PCR-RFLP). The distribution of CCND1 gene G870A frequency in the 3 groups and its association with tumor staging and grading were analyzed. The study found that GA and AA genotypes were associated with a significantly higher risk of digestive system cancer risk than the GG genotype ($p < 0.05$), and their frequencies were significantly higher in patients with tumors of higher pathological grade and in those in advanced tumor stages ($p < 0.05$).

III. MATERIALS AND METHODS

The 32 Formalin-Fixed Paraffin-Embedded (FFPE) samples that were stored at Department of Pathology, Faculty of Medicine, Udayana University, Denpasar, Bali. This cross-sectional study was approved by the Ethics Committee of Faculty of Medicine, Udayana University (EC number: 2885/UN14.2.2.VII.14/LT/2022).

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

Colorectal cancer grade classification is grouped based on WHO 2019 grade criteria [1]. Low grade means well-differentiated cancer cells; the cells were abnormal but arranged very similar to normal cells. High grade means poorly differentiated or undifferentiated cancer cells; the cells were arranged very differently and unlike normal cells.

Classification of colorectal cancer invasion degree is based on AJCC 8th edition 2018 for pathological stage tumor criteria. In this study, T1 and T2 were grouped into low degree of invasion, T3 and T4 are grouped into high degree of invasion. The T1 means tumor invades submucosa, T2

means tumor invades muscularis propria, T3 means tumor invades subserosa or non-peritoneal pericolic or perirectal tissues, and T4 means tumor invades other organs or structures and/or perforates visceral peritoneum [1].

The DNA isolation performed using black FFPE PREP DNA kit, Analytic Jena. The concentration of DNA samples was measured so that each sample can be equated to 10 ng/ μ L. The CCND1 rs9344 gene polymorphism amplified with primer forward: 5'-CTTCCTGTCCTACTACCGCC -3' and primer reverse: 5'-TAGGAGCAGTGGAAGAAGCC -3'. Amplification was performed in a total volume of 35 μ L containing 17.5 μ L green master mix, 0.7 μ L each primer, 11.5 μ L ddH₂O, and 4.6 μ L of isolated DNA. Amplification of DNA samples used the following cyclical parameters: initial denaturation at 95°C for 5 minutes followed by 40 denaturation cycles at 95°C for 15 seconds, annealing at 52°C for 60 seconds, and at 72°C for 30 seconds, and final elongation at 72°C for 5 minutes. Cooling at 4°C for 10 minutes [40]. Furthermore, visualization of PCR results using 1.5% agarose gel was carried out. A 3 μ L each sample and DNA marker inserted into the gel hole. Electrophoresis 50 volts run from the cathode to the anode for 60 minutes. The gel then visualized using a UV transilluminator and documented. With this visualization, we can match whether the amplified DNA fragment is the CCND1 gene with an amplicon size of 238 bp. The PCR products that have visualization results according to the amplicon size of 238bp proceed to sequencing.

IV. STATISTICAL ANALYSIS

The statistical package for social studies (SPSS version 26.00) was used for data analysis. Descriptive statistics (numbers and percentages for categorical variables) and Fisher-exact tests was used to test the association between the polymorphisms of the CCND1 gene rs9344 AA, AG, GG genotype and the colorectal cancer grade and invasion degree. The $p < 0.05$ was considered statistically significant.

V. RESULT

This study used 32 DNA samples from patients diagnosed with colorectal cancer who were treated in 2018-2020 at Prof. Dr. IGNG Ngoerah Central General Hospital, Denpasar, Bali. The results of data analysis from medical records presented in Table I.

The eligible samples were processed by PCR and then electrophoresed. The analysis was continued with the BLAST program to match the base sequence of the CCND1 gene polymorphisms rs9344 which had been sequenced according to the base sequence listed in the BLAST program (Fig. 1).

TABLE I: CHARACTERISTICS OF CRC PATIENTS IN 2018–2020 AT PROF. DR. I.G.N.G NGOERAH CENTRAL GENERAL HOSPITAL

Variable	N=32 (%)
Age	
<50 years	4 (12.5)
≥50 years	28 (87.5)
Gender	
Male	21 (65.6)
Female	11 (34.4)
Histopathological Classification	
Adenocarcinoma	32 (100.0)
Grade	
High	1 (3.1)
Low	31 (96.9)
Invasion Degree	
Low	7 (21.9)
High	25 (78.1)
pN Pathological stage	
pN0	2 (6.3)
pN1	1 (3.1)
pN1b	2 (6.3)
pNx	27 (84.4)
Location	
Right	11 (34.4)
Left	21 (65.6)



Fig. 1. The representative sample where the SNP of CCND1 gene rs9344 is marked in red.

The base sequence was then checked on the electropherogram using Snappgene software. Below is an example of sequencing result shown on Snappgene (Fig. 2a, 2b and 2c).

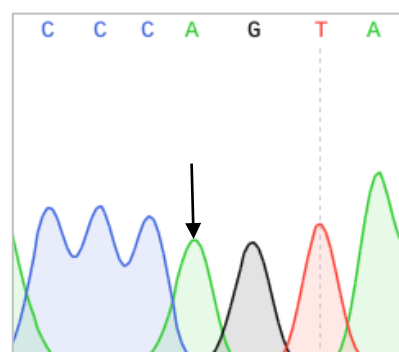


Fig. 2a. Sequencing of the CCND1 gene polymorphisms rs9344 Homozygous AA.

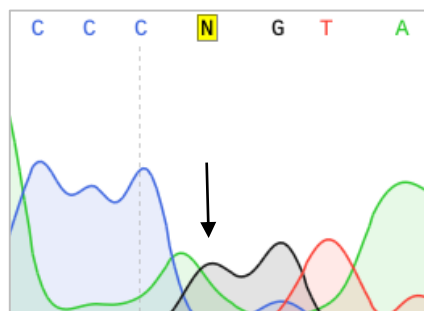


Fig. 2b. Sequencing of the CCND1 gene polymorphisms rs9344 Heterozygous AG.

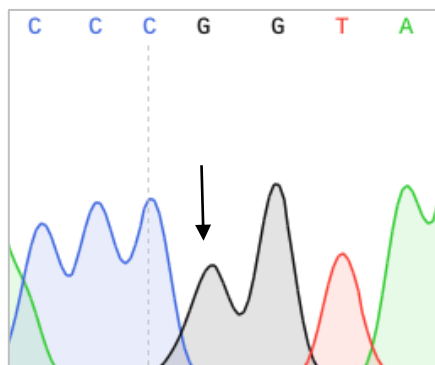


Fig. 2c. Sequencing of the CCND1 gene polymorphisms rs9344 Homozygous GG.

The polymorphism analysis of the CCND1 gene rs9344 found that AA genotype polymorphisms as 20 samples (62.5%), the AG genotype as 9 (28.1%), while the GG genotype as 3 samples (9.4%) (Table II).

TABLE II. GENOTYPE OF CCND1 GENE POLYMORPHISMS RS9344 SEQUENCING

Genotypes	N (%)
AA	20 (62.5)
AG	9 (28.1)
GG	3 (9.4)
TOTAL	32 (100.0)

Fisher's exact test results, p-value >0.05, meaning there is no association between the CCND1 gene polymorphisms rs9344 AA, AG, GG genotype with the colorectal cancer grade and invasion degree (Table III and IV).

TABLE III: ANALYSIS OF THE CCND1 GENE POLYMORPHISMS RS9344 ASSOCIATION WITH COLORECTAL CANCER GRADE.

Variable	Invasion Degree		p-Value
	Low n (%)	High n (%)	
Genotypes			
AA	4 (57.1%)	16 (64.0%)	0.906
AG	2 (28.6%)	7 (28.0%)	
GG	1 (14.3%)	2 (8.0%)	
Dominant Model			
AA+AG	6 (85.7%)	23 (92.0%)	0.536
GG	1 (14.3%)	2 (8%)	
Recessive Model			
AG+GG	3 (42.9%)	9 (36.0%)	1.000
AA	4 (57.1%)	16 (64.0%)	

TABLE IV: ANALYSIS OF THE CCND1 GENE POLYMORPHISMS RS9344 ASSOCIATION WITH COLORECTAL CANCER INVASION DEGREE

Variable	Grade		p-Value
	High n (%)	Low n (%)	
Genotypes			
AA	1 (100.0%)	19 (61.3%)	1.000
AG	0	9 (29.0%)	
GG	0	3 (9.7%)	
Dominant Model			
AA+AG	1 (100.0%)	28 (90.3%)	1.000
GG	0	3 (9.7%)	
Recessive Model			
AG+GG	0	12 (38.7%)	1.000
AA	1 (100.0%)	19 (61.3%)	

VI. DISCUSSION

A. Characteristics of Colorectal Cancer Patients

From this study, it was found that most samples were ≥ 50 years old. Research by Xi and Xu [26] also showed CRC number increased dramatically above the age of 50, similarly with Jun *et al.* [27] research on 495 years showed samples aged above 50 years old as many as 84.2%. The aging process closely related to molecular changes and cellular degeneration resulting an increase in the incidence of age-related cancers [28].

The prevalence of CRC in this study was highest in male. These results are in line with the research of Gunasekaran *et al.* [29], Jun *et al.* [27] which also showed that male participants were more than half of research subjects. The high prevalence of CRC in male can be attributed to lifestyles such as smoking and the alcohol habit more in male and these is one of the high-risk factors of CRC [26].

The histopathological grade or degree of cell differentiation is not only an important prognostic factor of CRC but is also a variable used for the selection of therapies [30]. In this study, most of cancer grades were found to be low grade. In line with several previous studies by Albasri *et al.* [9], Jun *et al.* [27], and Maker and Sriwidyani [31] whose showed low grade more than 90% of research subjects.

The tumor stage is the most important prognostic to predict CRC clinical outcomes. Histopathological examination of surgery specimen has an important role in determining the invasion degree of tumor (T) and lymph node metastasis (N) [27]. In this study, due to most of pN were pNx which mean lymph nodes metastasis cannot be analyzed, the stage analyzed from the invasion degree of tumor. Analysis of the invasion degree of tumors T in this study showed that most degrees were severe invasion (T3-T4) as many as 25 samples (78.1%). Almost similar results were found in the research by Maker and Sriwidyani [31]. As mentioned before, the aspect of lymph node metastasis in this study the pathological stage N or pN were mostly pNx, the regional lymph node could not be assessed, pNx as many as 27 samples (84.4%).

Most of CRC location in this study were on the left side. These findings were in line with two previous studies by Albasri *et al.* [9], Joshi and Chan [32] which also showed CRC incidence more on the left side. Left-sided colorectal cancers range from the descendent and sigmoid colons to 1/3 of the distal transverse colon, while the right side ranges

from the ascending colon to 2/3 of the proximal transverse colon. The left-side CRC is mostly through the chromosomal instability (CIN) molecular pathway. The right-side CRC is mostly via the microsatellite instability (MSI) pathway [33]. The CIN pathway is the most common cause of genomic instability in the CRC. It has 65-70% role in sporadic CRC [11].

B. The CCND1 Gene Polymorphisms rs9344 in Colorectal Cancer

Analysis of the CCND1 gene polymorphisms rs9344 at Prof. dr. I.G.N.G. Ngoerah Central General Hospital found most of the samples were AA genotype polymorphisms as many as 20 samples (62.5%), the AG genotype as many as 9 samples (28.1%), while GG genotype as samples (9.4%) which means more carriers of A allele. These results in line with the research of Rahimirad *et al.* [20] on Iran population. Their study showed that individuals carrying the AA genotype had higher risk in the development of CRC compared to the GG genotype. Logistic regression analysis showed that the influence of GG, AG, and AA genotypes did not change according to sex variable. These findings suggest that association between CCND1 gene polymorphisms rs9344 and sporadic CRC risk is not gender specific.

A meta-analysis by Xie *et al.* [3] found that CCND1 rs9344 polymorphisms GG, AA, AG, AA+AG genotypes was significantly associated with CRC risk. Meta-analysis by Xie *et al.* suggested that the polymorphisms of CCND1 G870A is associated with an increased risk of CRC, notably that carrier of A allele may be a major risk factor for CRC. Carriers of A allele (AA or AG or AA+AG) has a 1.19-fold increase in risk of CRC, especially in Asian and Caucasian populations.

Hong *et al.* [34] examined the polymorphisms of the CCND1 gene against CRC risk, age of onset and overall survival in 254 patients and 101 controls in the Chinese population in Singapore without family history of CRC. This found GG genotypes were associated with higher risk of CRC. In contrast to studies in the Caucasian population, many report that A allele is associated with an increased risk of CRC. The risk of CRC in AA genotype is lower than the GG genotype. Further, poorly differentiated AA and AG genotype patients and location on the left side were associated with a much lower risk than GG genotype patients. The findings contradict studies in Caucasian populations, GG (not AA) genotypes are associated with increased susceptibility and advanced CRC in patients in Singapore, this suggests a more complex association between SNP CCND1 risk and CRCs, possibly due to different population.

C. Association of CCND1 Gene Polymorphisms rs9344 with Colorectal Cancer Grade and Invasion Degree

The CCND1 gene polymorphisms rs9344 are thought to be closely related to the adverse effects of alternative splicing. The A allele of CCND1 gene rs9344 oversees encoding proteins in the truncated C-Terminal Domain with a higher level of mRNA expression than the G allele. The cyclin D1 variant with altered C-Terminal domain has a longer half-life than G allele, which can avoid the G1-S check point of cell cycle, causing abnormal cell proliferation

[23]. Over expression of cyclin D1 in cells is involved in the process of differentiation. Cyclin D1 in the nucleus increases cell cycle activity by regulating the G1/S transition through interaction with cyclin dependent kinase CDK2/4. If cyclin D1 is over-expressed and CDK2/4 complex levels increase, it not only increases proliferation, but also reduces cell differentiation without entering the G0 phase of the cell cycle. Cyclin D1 works with proteins other than CDK, it can control apoptosis, cell aging, invasion through transcription or DNA damage response [35].

Migration and cell invasion are characteristic of cancer that cause the expansion and spread of tumor cells through metastasis. Cyclin D1 increases cell motility by inhibiting Rho-activated kinase II (ROCKII) signaling and suppressing thrombospondin1 (TSP1), which is a metastasis of the repressor. This process requires the activity of cyclin D1/CDK4/6 and phosphorylation of cytoskeleton proteins involved in remodeling cell shape. Some cytoskeleton-related proteins are phosphorylated by cyclin D1/CDK4/6 complexes. Cytoplasmic cyclin D1 regulates cell invasion and metastasis through phosphorylation of paxillin and activation of RAC1 and promotes invasion. Cyclin D1 was found to be bound to paxillin on cell membranes in cells migration. Research shows the leading role of cytoplasmic cyclin D1 in invasion and metastasis [21].

In this study, cell merger was carried out between the AA+AG genotype (A allele) to analyze the dominant model, between the GG+AG genotype (G allele) to analyze recessive model according to research by Sameer *et al.* [22], Yang *et al.* [36], and Thakur *et al.* [37]. The A allele has an association with the increased risk of CRC progressiveness according to the research of Xie *et al.* [3], Xu *et al.* [7], Huang *et al.* [23], Loic *et al.* [25].

The results of this study on the association of CCND1 gene polymorphisms rs9344 with CRC grade in 2018-2020 at Prof. Dr. I.G.N.G. Ngoerah Central General Hospital showed that it was not significantly related ($p>0.05$) likely to be caused by the uneven distribution of CRC data between low and high grade. This is not in line with the meta-analysis of Yang *et al.* [36] which showed that there was a significant difference between the polymorphisms of the CCND1 gene rs9344 and low-grade of CRC.

The research of CCND1 gene polymorphisms rs9344 with CRC invasion degree in 2018-2020 at Prof. Dr. I.G.N.G. Ngoerah Central General Hospital showed no significant association ($p>0.05$). The results were not in line with a case control study by Yang and Shi [41], the study found that GA and AA genotypes were associated with a significantly higher risk of digestive system cancer risk than the GG genotype ($P<0.05$), and their frequencies were significantly higher in patients with tumors of higher pathological grade and those in advanced tumor stages ($p<0.05$).

The association between the CCND1 gene polymorphisms rs9344 and invasion degree of CRC in 2018-2020 at Prof. Dr. I.G.N.G. Ngoerah Central General Hospital showed that it was not significantly related to the degree of invasion of colorectal cancer tumors likely due to the uneven distribution of CRC invasion degree data between low invasion degree and high invasion degree. Most of the samples were likely to have high invasion

degrees likely because sampling was only collected at tertiary-level health facilities which is central of referral. There are variables that have influence on the degree of invasion but not studied such as the age and duration of the patient suffering from cancer.

About 60-65% of CRC cases are sporadic CRC which is CRC without family history. The onset of sporadic CRC largely associated with acquired somatic gene mutations or epigenetic changes caused by modifiable risk factors [38]. Non-hereditary CRC can be caused by some variations of genetics such as Single Nucleotide Polymorphisms (SNPs). The study of polymorphisms is important to improve alertness and early diagnosis CRC [5]. Due to different environmental influences, each country's population has different types of polymorphisms towards different types of diseases, thus each country must have polymorphisms research on its own population [39]. Research on CCND1 gene rs9344 polymorphism is expected to help in prevention and therapy of CRC according to the concept of Anti-Aging Medicine which hopefully can extend life expectancy and improve quality of life.

D. Research Limitations

The limitations in this study include the limited number of samples and only carried out at one health service facility, that is Prof. Dr. I.G.N.G. Ngoerah Central General Hospital during a period of 2018 - 2020, so it is likely to be less represented the genetic variations in different areas, population, and times. The absence of data on other variables such as the duration of the patient's suffering from CRC, family history, body mass index, diet, smoking, alcohol consumption habits and other progressive risk factors so the association of CRC with these progressive risk factors cannot be analyzed further.

VII. CONCLUSION

The results of this study can be concluded that majority age above 50 years old, male sex, adenocarcinoma, low grade, and high invasion degree. Most of tumor location were on the left side. The CCND1 gene polymorphisms rs9344 was sequentially AA, AG, and GG genotypes. No association between the polymorphism of CCND1 gene rs9344 with grade and invasion degree of colorectal cancer at Prof. Dr. I.G.N.G. Ngoerah Central General Hospital, Denpasar, Bali.

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CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

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