Research Article

Association of β-Catenin, APC, SMAD3/4, Tp53, and Cyclin D1 Genes in Colorectal Cancer: A Systematic Review and Meta-Analysis

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Objectives. Accumulating evidence indicates that the expression and/or variants of several genes play an essential role in the progress of colorectal cancer (CRC). The current study is a meta-analysis undertaken to estimate the prognosis and survival associated with CTNNB1/β-catenin, APC, Wnt, SMAD3/4, TP53, and Cyclin D1 genes among CRC patients. Methods. The authors searched PubMed, EMBASE, and Science Direct for relevant reports published between 2000 and 2020 and analyzed them to determine any relationship between the (immunohistochemically/sequencing-detected) gene expression and variants of the selected genes and the survival of CRC patients. Results. The analysis included 34,074 patients from 64 studies. To evaluate association, hazard ratios (HRs) were estimated for overall survival (OS) or disease-free survival (DFS), with a 95% confidence interval (CIs). Pooled results showed that β-catenin overexpression, APC mutation, SMAD-3 or 4 loss of expression, TP53 mutations, and Cyclin D1 expression were associated with shorter OS. β-Catenin overexpression (HR: 0.137 (95% CI: 0.131–0.406)), loss of expression of SMAD3 or 4 (HR: 0.449 (95% CI: 0.146–0.753)), the mutations of TP53 (HR: 0.179 (95% CI: 0.126–0.485)), and Cyclin D1 expression (HR: 0.485 (95% CI: 0.772–0.198)) also presented risk for shorter DFS. Conclusions. The present meta-analysis indicates that overexpression or underexpression and variants of CTNNB1/β-catenin, APC, SMAD3/4, TP53, and Cyclin D1 genes potentially acted as unfavorable biomarkers for the prognosis of CRC. The Wnt gene was not associated with prognosis.

1. Introduction

Globally, cancer is the second leading cause of death after heart disease, and it is a prominent health issue. More specifically, colorectal cancer (CRC) is the third leading cause of death among men and women [1]. Unlike many other types of cancer, the survival rate for CRC has not changed a great deal. Recent studies showed that the prognostication of CRC depends upon the clinicopathological factors and the stages of tumor characteristics and reported the association with survival times and clinical outcomes [2–4]. Several susceptibility studies on the association of a genetic variant and CRC have been reported [5]. The solid tumors of CRC have served as genetic and biological paradigms and instigated to conduct studies on early detection [6], prevention [7], risk stratification [8], and treatments [9]. However, a greater understanding and identification of genetic biomarkers involving molecular and genetic pathways with improved sensitivity and specificity could improve screening for and expedite the diagnosis of CRC, yielding better outcomes. Currently, the prediction of outcomes in CRC relies heavily on traditional cancer characterization methods, including clinicopathological characteristics, such as staging, tumor size, invasion, tumor sidedness, and metastasis. It contributes to CRC’s high mortality rate and tendency for poor prognosis with disappointing survival rates [10].

The uses of molecular prognostic biomarkers to forecast the progression of the condition and likely survival have interested scholars for some time [11]. However, CRC is
a very diverse disease, and it is associated with complex interactions between genetic biomarkers and environmental risk factors. In addition, transduction pathways, namely transforming growth factor β-suppressor of mothers against decapentaplegic (TGFB-SMADs), wingless/integrated (Wnt), and tumor suppressor protein (p53), play an essential role in the initiation and development of CRC [4]. The tumor protein p53 gene (Tp53) located at chromosome 17p13 consists of 90% of missense mutations. Furthermore, studies have reported that genetic variations, particularly at codon 72 Pro/Arg gene polymorphism of the Tp53 gene, could affect the prognosis and treatment of CRC [12]. The Wnt signaling pathway is of particular interest because of its vital function in embryogenesis and tissue homeostasis. Many studies have identified the excessive activation of Wnt signaling as playing a major role in CRC [13]. A genome-scale analysis has recognized that 90% of patients with CRC carried genetic variations in the Wnt signaling pathway, particularly the loss-of-functional variations of adenomatous polyposis coli (APC) and variations that activate the mutations of β-catenin [14].

The membranous expression of β-catenin applies a restrictive impact on the movements of tumor cells and their growth. The increases in cell motility, growth, and transformation promote tumorigenesis because of the loss of β-catenin expression on the cell surface [12]. Pre-existing intracellular β-catenin can cause abnormality in Wnt/β-catenin-TCF signaling, leading to the progression of CRC. The hyperactivation of Wnt/β-catenin signaling enhances the invasive and metastatic possibility of CRC cells, while the knockdown of β-catenin in CRC cells reduces cell proliferation and further invasion [15]. Studies have reported the detection of nuclear β-catenin expression using immunohistochemical methods, and they have reported an association with a high burden of tumor and poor CRC survival [15].

Somatic mutations at the APC gene are found in approximately 75% of CRC cases. Several studies have suggested worse outcomes for CRC patients with wild-type APC (APC-WT) in comparison to mutant-type APC (APC-MT) [16]. However, the prognostic implication of this genomic alteration is not well-defined, especially in metastatic CRCs. SMAD4/DPC4 is a tumor suppressor gene that regulates cell growth and a common intracellular mediator that could alter the TGFB signaling to promote tumor progression. Studies have reported an association of SMAD4 genetic variation with tumor invasion, metastasis, and prognosis in various cancers [17].

In light of inconsistent results in the literature, the authors perceived a need for a meta-analysis that would explore the prognostic value of selected genes in CRC. The objectives were to estimate the pooled risk (hazard ratio, HR) identified (between the years 2000 and 2020) for each of these genes for overall survival (OS) and disease-free survival (DFS) in CRC patients. Thus, this meta-analysis comprehensively explores the prognostic role of selected genes in the β-catenin and related pathway implicated in the development and progression of CRC.

2. Methods

2.1. Publication Search and Inclusion Criteria. The authors searched the databases of PubMed, EMBASE and Science Direct for relevant published articles. Search terms included medical phrases related to SMAD 3, SMAD 4, β-catenin, Catenin beta 1 (CTNNB1), APC, Wnt, Cyclin D1, Tp53, or p53 genes and their variants/polymorphisms, in combination with words related to CRC (tumor, neoplasms, carcinoma, CRC, colon cancer, or rectal cancer). In addition, terms related to prognosis (outcome or survival) were used to retrieve eligible studies from 2000 through to the end of 2020. Furthermore, the references in the selected published articles were searched to identify potentially relevant studies.

Eligible studies were selected based on the following criteria: (a) pathologically confirmed (i.e., via tissue samples) patients with CRC, (b) immunohistochemical/sequencing detection methods for the selected genes and OS, DFS, cancer-specific survival (CSS), or recurrence-free survival (RFS), (c) English language, and (d) full-text articles. Editorial letters, reviews, case reports, studies with duplicated/repeated data, and studies lacking essential information and animal studies were excluded.

2.2. Data Extraction. In accordance with the meta-analysis of observational studies in epidemiology (MOOSE) guidelines [18] and in compliance with PRISMA guidelines, the data were evaluated and extracted by two independent researchers, who entered them all onto the data extraction form. For data extraction, the details recorded were as follows: the first author, publication year, country, total number of cases, type of cancer, stages, reported genes, gene detection method, cut-off values used, hazard ratios (HRs) with their 95% confidence intervals (CIs), and P values. For inconsistencies, a consensus was reached on each item among the authors. The Newcastle–Ottawa scale (NOS) was used to evaluate the quality of the eligible studies.

2.3. Statistical Analysis. The meta-analysis was executed based on HRs calculated by the log-rank test for OS and RFS differences with different gene expression levels. Calculations were based on HRs from the original publications, including 95% CI, and subsequent back-calculation to log (HR) and standard error (SE) for overall estimates. Wherever available, HRs based on a multivariate analysis were used. Log (HR) and SE were entered in statistical software NCSS (NCSS, LLC, Kaysville, UT, https://www.ncss.com/), and meta-analyses were validated in the software Comprehensive Meta-Analysis (CMA; Biostat, Inc., Englewood, NJ, https://www.meta-analysis.com/). The heterogeneity of pooled results was analyzed using Cochran’s Q test and the Higgins I-squared statistic. The absence of heterogeneity is based on the Q test revealed P heterogeneity > 0.1 and I² < 50%. To estimate the summary HRs/ORs, a fixed-effects model (the Mantel–Haenszel method) was used [19]. Elsewhere, the arandom-effects model (the DerSimonian and Laird method) [20] was used. To examine the publication bias, Begg’s funnel plot and
Egger’s linear regression test were used, and $P < 0.05$ was considered statistically significant (i.e., an asymmetrical distribution). All of the results were presented with HRs, upper and lower limits, and $P$ values and were illustrated in forest plots for the individual studies with the weighted and pooled effects.

3. Results

3.1. Study Characteristics. Figure 1 shows the comprehensive process used to select articles in this study, which was based on PRISMA guidelines. After the removal of duplicates, the database search yielded 4,112 articles. Based on the inclusion criteria and after screening the titles, abstracts, figures, and key data, 82 articles were finalized for literature studies [21–40], [41–60], [61–80], [81–102]. However, only 64 articles [21–31, 33–36, 38–40, 42–56, 59–61, 64, 66, 68–70, 72, 73, 75, 76, 78, 81–86, 88, 90, 91, 93, 95, 97–102] were retrieved for meta-analysis with 105 data points of the selected genes. Of these, four studies had evaluated the prognostic value for RFS [47, 81, 88, 101]. Six studies included cancer-specific survival [26, 46, 48, 65, 98, 103], whereas three reported progression-free survival (PFS) [32, 76, 84]. All others reported either OS and/or DFS. Since the number of studies for the first three indicators was small, the data for CSS, PFS, and RFS were combined with DFS. Thus, 64 studies involving 34,074 patients evaluating OS and DFS were analyzed in the current meta-analysis.

3.2. Review of Eligible Studies. The 82 studies identified as having presented data on baseline genes and prognosis in CRC are listed in Table 1 [21–40], [41–60], [61–80], [81–102]. Most of these studies were from the USA ($n = 18$), followed by China ($n = 11$), Korea ($n = 7$), Sweden ($n = 6$), Japan and Greece ($n = 5$), Australia and Austria ($n = 4$), Norway ($n = 3$), Taiwan, Egypt, Germany, Hungary, Italy, Netherlands and Turkey ($n = 2$), and one each from Brazil,
| No. | Author et al. | Year | Region | Sample size | Male % | Sample type | Tumor type | Clinical stage of tumor | Tumor side (right %) | Gene | Method of gene expression | Elevated levels/abnormality | Cut-off value | Outcome | NOS rating |
|-----|---------------|------|--------|-------------|--------|-------------|------------|------------------------|--------------------|------|-------------------------|----------------------------|----------------|----------|------------|
| 1   | Rafael et al.  | 2014 | Spain  | 345         | 53.3   | Tissue      | CRC        | Duke A-D               | NA                 | Wnt  | SSCP                    | Mutations                  | NA             | β-Catenin mutation not associated with OS | 5           |
| 2   | Yoshida et al. | 2015 | Japan  | 201         | 59.7   | Tissue      | CRC        | Stage 1,2,3            | NA                 | Wnt  | IHC                     | High, low                  | >50%           | Nuclear β-catenin associated with poor OS and DFS | 6           |
| 3   | Ting et al.    | 2013 | Taiwan | 282         | 52.4   | Tissue      | CRC        | AJCC                   | NA                 | Wnt  | Genomic DNA sequencing, tagger algorithm | NA             | NA             | Wnt polymorphism associated with high risk in OS | 6           |
| 4   | Tzaloumis et al.| 2017 | Greece | 57          | NA     | Tissue      | Colon and rectal adenocarcinoma | TNM 1-4           | 33.3 | Wnt  | IHC                     | Negative, weak, intermediate, strong | Median | Nuclear β-catenin associated with poor OS | 4           |
| 5   | Kim et al.     | 2018 | Korea  | 194         | 65.5   | Tissue      | CRC        | NA                    | 22.2               | Wnt  | Genomic DNA extraction | Methylated/nonmethylated | NA             | Methylation observed in 32%, not associated with OS | 7           |
| 6   | Wangefjord et al.| 2011 | Austria | 527         | 47.6   | Tissue      | CRC        | TNM 1-4               | NA                 | Cyclin D1 | IHC                     | weak, moderate, strong | 0-75%          | High Cyclin D1 expression associated with poor survival in men | 7           |
| 7   | Bao et al.     | 2005 | Italy  | 160         | 47.5   | Tissue      | CRC        | Duke A-D              | NA                 | TP53 | PCR-SSCP                | Mutation                  | NA             | Associated with poor OS | 6           |
| 8   | Khan et al.    | 2018 | USA    | 1825        | 56.7   | Tissue      | CRC        | NA                    | 37.2               | TP53 | Genomic sequencing     | Mutation                  | 5-10%          | Associated with poor OS | 5           |
| 9   | Brandstedt et al.| 2014 | Sweden | 304         | 0      | Tissue      | CRC        | TNM 1-4               | NA                 | p53  | IHC staining and gene sequencing | Positive/negative | p53: >50%; β-catenin: 0-2; Cyclin D1: 0-75% | Associated with poor OS | 6           |
| 10  | Huemer et al.  | 2018 | Austria | 181         | 39.7   | Tissue      | CRC        | Grade 1-3              | 24                 | TP53 | Genomic DNA sequencing | Mutation                  | NA             | TP53 mutation not associated with shorter OS compared with TP53 wild type tumor. TP53 mutation not associated with shorter OS in right-sided tumors | 5           |
| 11  | Sun et al.     | 2014 | China  | 197         | 64.4   | Tissue      | CRC        | TNM 0-4               | NA                 | TP53 | IHC                     | High/low                  | 150            | Associated with poor OS p53+ > 65.3% tumors. Advanced T stage associated with p53 expression | 4           |
| 12  | Theodoropoulos et al. | 2008 | Greece | 165         | 67.8   | Tissue      | Colorectal adenocarcinoma | TNM stage 1-4       | NA              | TP53 | Nuclear immunostaining of positive cells | Overexpression | >10%           | Associated with poor OS | 5           |
| 13  | Warren et al.  | 2013 | USA    | 607         | 55.5   | Tissue      | Colon cancer | Stage 3               | NA                 | TP53 | Direct sequencing and hybridization | Mutation                  | NA             | TP53 mutations- 45% | 4           |
| 14  | Netter et al.  | 2014 | France | 68          | 75     | Tissue      | Colon ca., metastatic | NA | 67.6 | TP53 | PSA and sanger sequencing | Mutation                  | NA             | 10-15% | Associated with poor OS | 5           |
| 15  | Kandolfer et al.| 2015 | Austria | 389         | 53.1   | Tissue      | Colon cancer | Stage 3               | NA                 | TP53 | Sanger sequencing       | Mutations                   | <75%           | Associated with poor OS | 4           |
| 16  | Chen et al.    | 2013 | China  | 203         | 42.3   | Tissue      | CRC        | AJCC                   | NA                 | TP53 | TP53                    | Negative, positive, strong | >10%           | Associated with poor OS | 5           |
| 17  | Russo et al.   | 2014 | USA    | 222         | 26.12  | Tissue      | CRC        | Stage 1-4             | NA                 | TP53 | Clinical tumor genotyping | IHC and next generation sequencing | Weak expression associated with poor OS | 6           |
| 18  | Oh et al.      | 2019 | Korea  | 621         | 59.9   | Tissue      | CRC        | AJCC 2 and 3          | NA                 | TP53 | Negative, positive, strong | Weak expression associated with poor OS | 0%             | Weak expression associated with poor OS | 6           |
| No. | Author et al. | Year | Region | Sample size | Male % | Sample type | Tumor type | Clinical stage of tumor | Tumor side (right %) | Gene | Method of gene expression | Elevated levels/abnormality | Cut-off value | Outcome | NOS rating |
|-----|---------------|------|--------|-------------|--------|-------------|------------|------------------------|---------------------|------|--------------------------|------------------------|--------------|----------|------------|
| 19  | Wang et al.   | 2017 | China  | 124         | 50.8   | Tissue      | CRC        | TNM 1–4                | NA                  | TP53 | IHC                      | Expression            | >10%          | P53 positive: 58.8% | 7          |
| 20  | Zhang et al.  | 2014 | China  | 185         | 42.7   | Tissue      | CRC        | AJCC 1–4               | 40                  | TP53 | IHC                      | Negative/positive     | Negative; >10% cells with +ve nuclei: Positive | Associated with poor OS | 7          |
| 21  | Godai et al.  | 2009 | Japan  | 211         | 57.8   | Tissue      | CRC        | Duke Stage A-D         | NA                  | TP53 | Genomic DNA Sequencing   | Mutations             | NA            | TP53 mutations: 70% | 6          |
| 22  | Chun et al.   | 2019 | USA    | 408         | 55.6   | Tissue      | CRC        | AJCC                  | 24.6                | TP53 | APC, SMAD-4               | Low or high risk (EAp53 score) | NA            | Associated with poor OS | 8          |
| 23  | Tiung et al.  | 2014 | China Taiwan | NA    | NA     | Tissue      | CRC        | NA                    | NA                  | TP53 | IHC                      | Overexpression         | NA            | Associated with poor survival | 4          |
| 24  | Li et al.     | 2018 | China  | 315         | 57.1   | Tissue      | CRC        | TNM                   | NA                  | TP53 | Next gen mutational analysis | Mutation            | NA            | Double mutated P53 with PIK3CA associated with poor survival | 6          |
| 25  | Iacopetta et al. | 2006 | Multinational | 3583   | 52.3   | Tissue      | CRC        | Dukes Stage A-D        | NA                  | TP53 | PCR                      | Mutation              | NA            | TP53 mutation associated with distal colon cancer | 6          |
| 26  | Morikawa et al. | 2012 | USA    | 1060        | 39     | Tissue      | Colon and rectal cancer | Stages 1–4 | NA | TP53                      | IHC                    | Moderate and strong | Associated with poor OS | 8          |
| 27  | Kawaguchi et al. | 2019 | USA    | 490         | 58.3   | Tissue      | CRC        | AJCC Cat. T            | NA                  | TP53 | TP53/SMAD-4               | Expression             | >10%          | Associated with poor OS | 7          |
| 28  | Samowitz et al. | 2002 | USA    | 1464        | 50.2   | Tissue      | Colon cancer | AJCC | NA | TP53                      | NA                    | NA            | Associated with poor survival | 7          |
| 29  | Soong et al.  | 2000 | Australia | 995     | NA     | Tissue      | CRC        | Dukes Stage B & C      | 34                  | TP53 | NA                       | Mutation              | NA            | 39% mutations | 5          |
| 30  | Jurach et al. | 2006 | Brazil  | 83          | 56.6   | Tissue      | Rectal Cancer | Coller B & C | NA | TP53                      | IHC                    | >20%          | Associated with poor OS | 5          |
| 31  | Loses et al.  | 2016 | Norway  | 151         | 60.2   | Tissue      | CRC        | NA                    | TP53 | Sanger sequencing         | Mutations             | NA            | TP53 mutations: 60.4% | 4          |
| 32  | Iacopetta et al. | 2006 | Multinational | 3583   | 52.3   | Tissue      | CRC        | Dukes Stage A-D        | NA                  | TP53 | PCR                      | Mutation              | NA            | TP53 mutation associated with distal colon cancer | 6          |
| 33  | Salim et al.  | 2013 | Sweden  | 85          | NA     | Tissue      | Colon cancer | NA | β-Catenin (CRC) | IHC                    | Less expression | <50% | Associated with poor OS | 4          |
| 34  | Kampaosktas et al. | 2013 | Greece | 106         | 61.3   | Tissue      | CRC        | NA                    | NA | β-Catenin (CRC) | IHC                    | Overexpression | Moderate | Associated with poor OS | 7          |
| 35  | Gao et al.    | 2014 | China   | 181         | 58     | Tissue      | CRC        | TNM stages 1–4         | NA                  | β-Catenin | IHC                      | Overexpression         | >50%          | Associated with poor OS | 6          |
| 36  | Jang et al.   | 2012 | Korea   | 218         | 61.4   | Tissue      | Colon cancer | NA | 23.3 | β-Catenin, Cyclin D1 | IHC                    | Overexpression | >30% | Associated with poor survival | 5          |
| 37  | Lee et al.    | 2013 | Korea   | 305         | 61.9   | Tissue      | CRC        | AJCC stages 1–4        | NA                  | β-Catenin | IHC                      | Overexpression         | >30%          | Associated with poor OS | 6          |
| 38  | Wong et al.   | 2003 | China   | 60          | 65     | Tissue      | CRC        | NA                    | NA | β-Catenin (CRC) | IHC                    | Overexpression | >300 | Associated with poor survival | 4          |
| 39  | Chung et al.  | 2001 | USA     | 543         | NA     | Tissue      | CRC        | NA                    | NA | β-Catenin | IHC                      | Overexpression | Moderate | Associated with poor survival | 4          |
| No. | Author et al. | Year | Region | Sample size | Male % | Sample type | Tumor type | Clinical stage of tumor | Tumor side (right %) | Gene | Method of gene expression | Elevated levels/abnormality | Cut-off value | Outcome | NOS rating |
|-----|---------------|------|--------|-------------|--------|-------------|------------|-------------------------|-------------------|------|--------------------------|------------------------|--------------|----------|------------|
| 40  | Fernebro et al. [60] | 2004 | Sweden | 257      | 67.3 | Tissue | Rectal cancer | NA | NA | β-Catenin, p53 | IHC | Abnormal expression | Weak | Associated with poor survival | 5 |
| 41  | Bondi et al. [61] | 2004 | Norway | 162      | 45.6 | Tissue | colon cancer | NA | NA | β-Catenin | IHC | overexpression | >1% | Associated with poor survival | 4 |
| 42  | Kim et al. [62] | 2005 | Korea | 124      | NA | Tissue | CRC | Duke A-D | NA | β-Catenin | IHC | Abnormal expression | >5% | Associated with poor survival | 6 |
| 43  | Filiz et al. [63] | 2010 | Turkey | 138      | 60.1 | Tissue | CRC | NA | NA | β-Catenin, p53 | IHC | Expression levels | Weak | Associated with poor survival | 5 |
| 44  | Jung et al. [64] | 2013 | Korea | 349      | 59.5 | Tissue | CRC | NA | 21.7 | β-Catenin, p53 | IHC | Overexpression | >0% | Associated with poor survival | 7 |
| 45  | Wangelund et al. [65] | 2013 | Sweden | 527      | 47.4 | Tissue | CRC | TNM stages 1-4 | NA | β-Catenin | IHC | Overexpression | Moderate | Associated with poor survival | 5 |
| 46  | Balci et al. [66] | 2015 | Italy | 321      | 53.2 | Tissue | CRC | NA | NA | β-Catenin | IHC | Overexpression | Moderate | Associated with poor survival | 5 |
| 47  | Youssef et al. [67] | 2015 | Egypt | 72       | 48.1 | Tissue | CRC | TNM stages 1-4 and dukes A-C | 69.4 | β-Catenin | IHC | Overexpression | >10% | Associated with poor survival | 6 |
| 48  | Togo et al. [68] | 2008 | USA | 183      | 62.8 | Tissue | CRC | TNM stages 1-4 | 33.3 | β-Catenin, p53 | IHC | Overexpression | Moderate/strong expression | Associated with poor survival | 5 |
| 49  | Matsuoka et al. [69] | 2011 | Japan | 156      | 63.4 | Tissue | CRC | TNM stages 1-4 | NA | β-Catenin | IHC | Overexpression | >20% | Associated with poor survival | 7 |
| 50  | Morikawa et al. [70] | 2011 | USA | 955      | 39.9 | Tissue | CRC | NA | NA | β-Catenin | IHC | Overexpression | Moderate/strong expression | Associated with poor survival | 8 |
| 51  | Orguen et al. [71] | 2011 | Turkey | 60       | 33.3 | Tissue | CRC | NA | NA | β-Catenin | IHC | overexpression | >0% | Associated with poor survival | 5 |
| 52  | Stanczak et al. [72] | 2011 | Poland | 66       | 66.6 | Tissue | CRC | NA | NA | β-Catenin | IHC | Overexpression | >10% | Associated with poor survival | 6 |
| 53  | Toth et al. [73] | 2012 | Hungary | 79      | 50.6 | Tissue | CRC | NA | NA | β-Catenin | IHC | Overexpression | >10% | Associated with poor survival | 7 |
| 54  | Sun et al. [74] | 2011 | China | 67       | 64.2 | Tissue | Colon cancer | NA | NA | β-Catenin | IHC | Decreased expression | >10% | Associated with poor survival | 8 |
| 55  | Wang et al. [75] | 2020 | USA | 341      | 56.3 | Tissue | COAD | NA | 30.7 | APC TP53 CTNNB1 | DNA sequencing | Mutations | NA | APC mutations: 74.8% | 8 |
| 56  | Mondaca et al. [76] | 2020 | USA | 471      | 52.9 | Tissue | CRC | NA | 26.1 | APC TP53 CTNNB1 | DNA sequencing | Mutations | NA | APC mutations: 74.8% | 8 |
| 57  | Schell et al. [77] | 2016 | USA | 407      | NA | Tissue | CRC | NA | 41 | APC | DNA sequencing | AG vs. AA genotype | NA | AG genotype associated with poor survival | 5 |
| 58  | Gerami et al. [78] | 2020 | Iran | 57       | 77.2 | Frozen tissue | CRC | TNM stage 1 to 4 | 36.8 | APC | DNA sequencing | AG vs. AA genotype | NA | AG genotype associated with poor survival | 5 |
| 59  | Conlin et al. [79] | 2005 | Scotland | 107      | 60.7 | Tissue | CRC | Duke stage A-D | 14.9 | APC p53 | Genomic DNA extraction and sequencing | Mutations | NA | APC mutations: 56%; p53 mutations: 61%; not associated | 4 |
| 60  | Wang et al. [80] | 2020 | USA | 331      | NA | Microsatellite stable, tissue | CRC | 4 | NA | APC | Next-gen genomic analysis | APC-WT or APC-MT | NA | APC-WT associated with poor survival | 7 |
| No. | Author et al. | Year | Region | Sample size | Male % | Sample type | Tumor type | Clinical stage of tumor | Tumor side (right %) | Gene | Method of gene expression | Elevated levels/abnormality | Cut-off value | Outcome | NOS rating |
|-----|--------------|------|--------|-------------|--------|-------------|-----------|-------------------------|-------------------|------|--------------------------|----------------------------|--------------|----------|------------|
| 61  | Jorissen et al. [81] | 2015 | Australia | 746 | 55.4 | CRC MSI (unstable) and MSS (stable); validation cohort, tissue | CRC | Stage 1 to 4 | 42.2 | APC TP53 | DNA sequencing | APC-WT or APC-MT | NA | TP55: 55.4%; APC-WT associated with poor survival | 6 |
| 62  | Voorneveld et al. [82] | 2012 | Netherlands | 209 | NA | Tissue | CRC | NA | NA | SMAD-4 | IHC | Expression | NA | Associated with poor survival | 5 |
| 63  | Li et al. [83] | 2011 | China | 147 | NA | Tissue | CRC | NA | NA | SMAD-4 | IHC | Expression | NA | Associated with poor survival | 5 |
| 64  | Yoo et al. [84] | 2019 | Korea | 1370 | NA | Tissue | CRC | NA | NA | SMAD-4 | NA | SMAD-4 high vs. low | NA | Associated with poor survival | 5 |
| 65  | Su et al. [85] | 2016 | China | 251 | 57.37 | Tissue | CRC | Stages 1–4 | NA | SMAD-4 | IHC | Expression | NA | No association | 5 |
| 66  | Isaksson et al. [86] | 2006 | Sweden | 86 | 42 | Tissue | CRC | Duke A-C | 35 | SMAD-4 | NA | Expression | NA | Associated with poor survival | 6 |
| 67  | Fleming et al. [87] | 2013 | Australia | 744 | 55.6 | Sporadic CRCs, tissue | CRC | AJCC stages 1–4 | Stage 2 (18%) and 3 (23%) | NA | SMAD-4 | IHC detection | Loss of expression | NA | Associated with poor survival | 6 |
| 68  | Roth et al. [88] | 2012 | Switzerland | 1404 | NA | Tissue | CRC | NA | Stage 1 to 4 | NA | SMAD-4 | IHC | Expression | NA | Associated with poor survival | 6 |
| 69  | Lampropoulos et al. [89] | 2012 | Greece | 195 | NA | Tissue | CRC | Stage 1 to 4 | Stage 1 to 4 | SMAD-4 | NA | Expression | NA | Associated with poor survival | 4 |
| 70  | Isaksson et al. [90] | 2011 | Sweden | 441 | NA | Tissue | CRC | Stage 1 to 4 | SMAD-4 | NA | IHC | Weak expression, high | 0-5% | Loss of SMAD-4 associated with poor OS | 5 |
| 71  | Jia et al. [91] | 2017 | US | 209 | 53.7 | Tissue | CRC | Stage 1–4 | SMAD-4 | Genomic DNA sequencing | High, low | NA | Expression | NA | Associated with poor survival | 7 |
| 72  | Oyanagi et al. [92] | 2019 | Japan | 208 | 117 | Tissue | CRC | TNM 1–4 | 56 | SMAD-4 | NA | IHC | Loss of expression | NA | Associated with poor survival | 6 |
| 73  | Ionescu et al. [93] | 2014 | Romania | 39 | 66.6 | Tissue | CRC | Duke A-D | 25.6 | SMAD-3 | q-RT-PCR | Overexpression, under-expression | NA | No association with OS | 6 |
| 74  | Fukushima et al. [94] | 2003 | Japan | 100 | NA | Sporadic CRC and normal tissue | Sporadic CRC | NA | NA | SMAD3/SMAD4 | PCR-SSCP | Abnormal | NA | SMAD-3: no abnormality; SMAD-4: abnormal 5 cases | 4 |
| 75  | Chun et al. [95] | 2014 | Korea | 201 | 65.7 | Tissue | Rectal cancer | 3 | NA | SMAD4 | PCR | Nuclear or cytoplasmic expression | NA | SMAD-3 and SMAD-4 in tumor, effects on TGFβR2 pathway downregulation | 5 |
| 76  | Bacman et al. [96] | 2007 | Germany | 310 | 61 | Tissue | Colon cancer | Stage 2 (57.4%) and 3 (42.6%) | NA | SMAD3/SMAD4 | PCR | Expression | NA | SMAD-3 and SMAD-4 in tumor high or low | 4 |
| 77  | Meskar et al. [97] | 2009 | Netherlands | 135 | 54.4 | Tissue | CRC | Stage 1 (17.8%), 2 (77.8%), and 3 (4.4%) | SMAD4 | NA | Stroma high vs. stroma low | NA | Stroma high SMAD-4 associated with poor prognosis | 7 |
| 78  | Horst et al. [98] | 2009 | Germany | 142 | 50 | Tissue | CRC | UICC stage 2A | SMAD4 | NA | β-Catenin | IHC staining | Nuclear β-catenin | NA | Associated with poor survival | 6 |
| 79  | Bondi et al. [99] | 2005 | Norway | 219 | 47.9 | Tissue | Colon cancer | Duke A-D | NA | Cyclin D1 | Real time q-PCR and IHC | Low, high | Grade +2 | Cyclin not associated with survival | 6 |
| No. | Author et al. | Year | Region | Sample size | Male % | Sample type | Tumor type | Clinical stage of tumor | Tumor side (right %) | Gene | Method of gene expression | Elevated levels/abnormality | Cut-off value | Outcome | NOS rating |
|-----|---------------|------|--------|-------------|--------|-------------|------------|-------------------------|---------------------|------|--------------------------|----------------------------|--------------|---------|-----------|
| 80  | Bahnassy et al. [100] | 2004 | Egypt | 60 | 60.0 | Tissue | CRC | TNM 1–4 | NA | Cyclin D1 | DNA extraction and gene amplification, IHC | >75% | Associated with poor survival | 7 |
| 81  | Saridaki et al. [101] | 2010 | Greece | 144 | 56.94 | Tissue | CRC | Stages 1–4 | NA | Cyclin D1 | DNA extraction and IHC | ≥50% with weak and ≥20% with strong staining | Overexpression is not associated with poor outcomes | 6 |
| 82  | Ogino et al. [102] | 2009 | USA | 602 | 43 | Tissue | Colon cancer | AJCC stages 1–4 | NA | Cyclin D1 | IHC | No, weak, moderate, strong | Overexpression not associated with poor survival | 8 |

NA: not applicable; CRC: colon rectal cancer; COAD: colon adenocarcinoma; IHC: immunohistochemical; OS: overall survival.
Table 2: Hazard ratios of studies included in meta-analysis.

| No. | Author | Year | Gene | Outcome | HR | 95% CI |
|-----|--------|------|------|---------|----|--------|
|     |        |      |      |         |    | Lower  | Upper  |
| 1   | Wang et al. (COH/UCD) [75] | 2020 | APC  | OS      | 0.62 | 0.44   | 0.86   |
|     | Wang et al. (MSKCC) [75]  |      | APC  | OS      | 0.63 | 0.49   | 0.81   |
|     | Wang et al. (COH/UCD) [75] |      | CTNNB1 | OS | 0.95 | 0.35 | 2.55 |
|     | Wang et al. (COH/UCD) [75] |      | CTNNB1 | OS | 1.67 | 0.86 | 3.26 |
|     | Wang et al. (COH/UCD) [75] |      | TP53 | OS      | 1.33 | 0.93   | 1.88   |
|     | Wang et al. (MSKCC) [75]  |      | TP53 | OS      | 1.00 | 0.77   | 1.30   |
|     | Mondaca et al. [76]        | 2020 | APC  | Progression-free survival | 0.68 | 0.54 | 0.86 |
|     | Gerami et al. [78]         | 2020 | APC  | OS      | 0.56 | 0.42   | 0.75   |
|     | Jorissen et al. (MSI) [81] | 2015 | APC  | RFS     | 1.63 | 0.97   | 2.74   |
|     | Jorissen et al. (MSS) [81] | 2015 | APC  | RFS     | 1.18 | 0.64   | 2.19   |
|     | Jorissen et al. (Validation cohort, MSS) [81] | 2015 | APC  | RFS     | 3.24 | 1.21   | 8.68   |
|     | Voorneveld et al. [82]     | 2012 | SMAD-4 | OS | 0.56 | 0.37 | 0.84 |
|     | Li et al. [83]             | 2011 | SMAD-4 | OS | 2.47 | 1.02 | 5.48 |
|     | Yoo et al. [84]            | 2019 | SMAD-4 | Cancer-free survival | 7.04 | 3.88 | 12.82 |
|     | Su et al. [85]             | 2016 | SMAD-4 | DFS | 0.92 | 0.69 | 1.22 |
|     | Roth et al. [88]           | 2012 | SMAD-4 | DFS | 0.87 | 0.64 | 1.18 |
|     | Isaksson et al. [90]       | 2011 | SMAD-4 | RFS | 1.58 | 1.23 | 2.01 |
|     | Chun et al. [95]           | 2014 | SMAD-4 (nuclear) | OS | 1.47 | 1.19 | 1.81 |
|     | Chun et al. [95]           | 2014 | SMAD-4 (cytoplasmic) | OS | 1.81 | 1.09 | 3.00 |
|     | Meskar et al. [97]         | 2009 | SMAD4 | OS | 1.71 | 0.83 | 3.51 |
|     | Salim et al. [53]          | 2013 | β-catenin (membrane) | OS | 1.15 | 0.57 | 2.30 |
|     | Kamposioras et al. [54]    | 2013 | β-Catenin (membrane) | OS | 7.98 | 4.12 | 15.44 |
|     | Gao et al. [55]            | 2014 | β-Catenin (nucleus) | DFS | 6.57 | 3.43 | 12.56 |
|     | Jang et al. [56]           | 2012 | β-Catenin | OS | 1.98 | 1.01 | 3.89 |
|     | Jang et al. [56]           | 2012 | Cyclin D1 | DFS | 0.33 | 0.14 | 0.77 |
|     | Chung et al. [59]          | 2001 | β-Catenin, nuclear | OS | 0.11 | 0.06 | 2.05 |
|     | Fernebro et al. [60]       | 2004 | β-Catenin, phosphonuclear | OS | 0.71 | 0.38 | 1.70 |
|     | Fernebro et al. [60]       | 2004 | β-Catenin (cytoplasm) | OS | 0.41 | 0.19 | 0.85 |
|     | Bondi et al. [61]          | 2004 | β-Catenin (nucleus) | OS | 1.26 | 0.62 | 2.56 |
|     | Bondi et al. [61]          | 2004 | p53 | OS | 1.11 | 0.50 | 2.50 |
|     | Bondi et al. [61]          | 2004 | C-Myc | OS | 5.26 | 1.93 | 14.36 |
|     | Jung et al. [64]           | 2013 | β-Catenin, combined with C-Myc | OS | 0.68 | 0.39 | 1.19 |
|     | Wangefjord et al. [65]     | 2013 | β-Catenin | OS | 1.39 | 0.82 | 2.28 |
|     | Balzi et al. [66]          | 2015 | β-Catenin | OS | 0.70 | 0.51 | 0.97 |
|     | Togo et al. [68]           | 2008 | p53 | OS | 1.99 | 0.75 | 5.32 |
|     | Matsuoka et al. [69]       | 2011 | β-Catenin | OS | 1.94 | 0.86 | 4.38 |
|     | Togo et al. [68]           | 2008 | p53 | OS | 1.70 | 0.83 | 3.48 |

**Note:** The table includes hazard ratios (HR) and 95% confidence intervals (CI) for various genes and outcomes across multiple studies.
| No. | Author                  | Year | Gene                  | Outcome                      | HR  | 95% CI Lower | 95% CI Upper |
|-----|-------------------------|------|-----------------------|------------------------------|-----|--------------|--------------|
| 25  | Morikawa et al. [70]    | 2011 | β-Catenin (cytoplasm) | Cancer-specific mortality    | 0.82| 0.64         | 1.06         |
|     |                         |      | β-Catenin (nucleus)   | Cancer-specific mortality    | 0.80| 0.62         | 1.03         |
| 26  | Stanzak et al. [72]     | 2011 | β-Catenin             | OS                           | 2.48| 1.30         | 4.74         |
|     |                         |      |                       | OS                           | 0.58| 0.14         | 2.28         |
| 27  | Toth et al. [73]        | 2012 | β-Catenin (membrane)  | OS                           | 2.25| 0.61         | 8.32         |
|     |                         |      | β-Catenin (nucleus)   | DFS                          | 2.92| 1.30         | 6.53         |
| 28  | Horst et al. [98]       | 2009 | β-Catenin             | Cancer-specific mortality    | 7.46| 2.08         | 26.72        |
| 29  | Bazan et al. [27]       | 2005 | TP53                  | OS                           | 2.26| 1.21         | 4.21         |
|     |                         |      |                       | DFS                          | 2.14| 1.06         | 4.32         |
| 30  | Khan et al. [28]        | 2018 | CTNNB1, SMAD-4, APC,  | OS                           | 0.88| 0.78         | 1.00         |
|     |                         |      | p53                   | OS                           | 0.79| 0.44         | 1.44         |
|     |                         |      |                       | OS                           | 1.31| 1.09         | 1.57         |
|     |                         |      |                       | OS                           | 0.89| 0.79         | 1.01         |
| 31  | Brandstedt et al. [29]  | 2014 | β-Catenin             | CRC risk                     | 0.97| 0.66         | 1.41         |
|     |                         |      | Cyclin D1             | CRC risk                     | 0.07| 0.01         | 0.88         |
|     |                         |      |                       | OS                           | 1.22| 0.84         | 1.78         |
| 32  | Huemer et al. [30]      | 2018 | TP53                  | OS                           | 2.05| 1.26         | 3.34         |
|     |                         |      |                       | DFS                          | 0.71| 0.65         | 0.76         |
| 33  | Sun et al. [31]         | 2014 | TP53                  | OS                           | 0.60| 0.54         | 0.66         |
|     |                         |      |                       | DFS                          | 0.99| 0.53         | 1.55         |
| 34  | Warren et al. [33]      | 2013 | TP53                  | OS                           | 1.04| 0.60         | 1.79         |
|     |                         |      |                       | OS                           | 0.78| 0.47         | 1.28         |
| 35  | Netter et al. [34]      | 2014 | TP53                  | OS                           | 1.88| 1.17         | 3.04         |
|     |                         |      |                       | DFS                          | 1.73| 1.04         | 2.86         |
| 36  | Loes et al. [51]        | 2016 | TP53                  | OS                           | 1.58| 0.97         | 2.56         |
|     |                         |      |                       | DFS                          | 1.71| 1.03         | 2.86         |
| 37  | Kandioler et al. [35]   | 2015 | TP53                  | OS                           | 0.47| 0.27         | 0.83         |
|     |                         |      |                       | OS                           | 0.42| 0.24         | 0.73         |
| 38  | Chen et al. [36]        | 2013 | TP53                  | OS                           | 1.66| 0.88         | 3.14         |
|     |                         |      |                       | OS                           | 1.65| 0.81         | 3.38         |
| 39  | Oh et al. [38]          | 2019 | TP53                  | OS                           | 2.62| 1.41         | 4.87         |
|     |                         |      |                       | OS                           | 1.50| 1.05         | 2.14         |
|     |                         |      |                       | OS                           | 1.93| 1.17         | 3.19         |
| 40  | Wang et al. [39]        | 2017 | TP53                  | OS                           | 2.02| 1.04         | 3.91         |
|     |                         |      |                       | OS                           | 1.68| 0.98         | 2.87         |
| 41  | Zhang et al. [40]       | 2014 | TP53 (and CTNNB1)     | Cancer-specific survival     | 1.30| 1.02         | 1.65         |
|     |                         |      | Wnt 5A                | OS                           | 2.21| 1.49         | 3.28         |
| 42  | Chun et al. [42]        | 2019 | TP53 (double mutation | Cancer-specific survival     | 1.40| 1.11         | 1.78         |
|     |                         |      | with PIK3CA)           | OS                           | 1.82| 1.17         | 2.83         |
|     |                         |      |                       | OS                           | 1.62| 1.20         | 2.20         |
|     |                         |      |                       | OS                           | 1.34| 1.07         | 1.63         |
| 43  | Tiong et al. [43]       | 2014 | TP53 (and CTNNB1)     | Cancer-specific survival     | 1.10| 0.91         | 1.34         |
|     |                         |      | Wnt 5A                | OS                           | 1.40| 0.89         | 2.21         |
| 44  | Li et al. [44]          | 2018 | TP53                  | Cancer-specific survival     | 2.32| 1.34         | 4.03         |
|     |                         |      |                       | OS                           | 2.64| 1.19         | 5.83         |
| 45  | Morikawa et al. [46]    | 2012 | TP53                  | Cancer-specific survival     | 2.52| 1.28         | 4.93         |
|     |                         |      |                       | OS                           | 0.61| 0.50         | 0.73         |
| 46  | Kawaguchi et al. [47]   | 2019 | SMAD-4                | Cancer-specific survival     | 0.69| 0.49         | 0.96         |
| 47  | Samowitz et al. [48]    | 2002 | TP53                  | Cancer-specific survival     | 4.57| 1.17         | 17.8         |
| 48  | Soong et al. [49]       | 2000 | TP53                  | Cancer-specific survival     | 0.47| 1.17         | 17.8         |
| 49  | Jurach et al. [50]      | 2006 | TP53                  | Cancer-specific survival     | 1.52| 1.28         | 4.93         |
|     |                         |      |                       | OS                           | 0.61| 0.50         | 0.73         |
| 50  | Iacopetta et al. [45]   | 2006 | TP53                  | Cancer-specific survival     | 0.69| 0.49         | 0.96         |
|     |                         |      |                       | OS                           | 4.57| 1.17         | 17.8         |
The table represents 105 data points on genes where HR data were available. OS: overall survival, RFS: relapse-free survival, CFS: cancer-free survival, DFS: disease-free survival, PFS: progression-free survival, CRC risk: colorectal cancer risk.

### Table 2: Continued.

| No. | Author                | Year | Gene                      | Outcome | HR   | 95% CI |
|-----|-----------------------|------|---------------------------|---------|------|--------|
| 54  | Tonescu et al. [93]   | 2014 | SMAD-3                    | OS      | 1.09 | 0.30   |
| 55  | Jia et al. [91]       | 2017 | SMAD-4 (nuclear)          | OS      | 1.70 | 0.96   |
| 56  | Kim et al. [25]       | 2018 | Wnt                      | OS      | 1.25 | 0.87   |
| 57  | Veloudis et al. [24]  | 2017 | Wnt/β-catenin             | OS      | 3.86 | 1.24   |
| 58  | Ting et al. [23]      | 2013 | Wnt                      | DFS     | 1.50 | 0.80   |
| 59  | Yoshida et al. [22]   | 2015 | β-Catenin                 | DFS     | 2.10 | 1.10   |
| 60  | Rafael et al. [21]    | 2014 | Wnt                      | OS      | 0.36 | 0.05   |
| 61  | Bondi et al. [99]     | 2005 | Cyclin D1                | OS      | 0.57 | 0.33   |
| 62  | Bahnassy et al. [100] | 2004 | Cyclin D1                | OS      | 10.86| 1.05   |
| 63  | Saridaki et al. [101] | 2010 | Cyclin D1                | OS      | 1.1  | 0.6   |
| 64  | Ogino et al. [102]    | 2009 | Cyclin D1                | OS      | 0.74 | 0.57   |

### Figure 2: Forest plot of β-catenin gene and overall survival in CRC (a). Forest plot of β-catenin gene and disease-free survival in CRC (b).
Sixty-five studies, with 105 data points on Colorectal Cancer. 3.4. Prognostic Value of Gene Expression and Mutations in Colorectal Cancer. Sixty-five studies, with 105 data points on genes where HR data was available, were included in the meta-analysis. These are shown in Table 2. Twenty-eight enrolled studies provided the HRs, and 95% CI directly or indirectly reported the correlation between β-catenin overexpression and OS. The pooled HR of β-catenin overexpression in the nucleus, cytoplasm, or membranous with OS was 0.257 (95% CI: 0.003–0.511; Q = 51.76; P = 0.000) (Figure 2(a)), however, heterogeneity existed. The association of β-catenin overexpression with shorter DFS was analyzed. The pooled HR was 0.137 (95% CI: 0.131–0.406; Q = 48.832; P = 0.000) (Figure 2(b)). The above results suggested that β-catenin overexpression in the nucleus, membrane, or cytoplasm was associated with lower OS and DFS.

For the APC gene, the pooled HR for OS based on 8 studies was 0.035 (95% CI: 0.308–0.377; Q = 51.76; P = 0.000) (Figure 3(a)). This value suggested the association of the mutant variant with a lower OS compared with the wild type but not for DFS, where pooled HR = 0.387 (95% CI: 0.483–1.256; Q = 22.624; P = 0.000) (Figure 3(b)). For the SMAD3/4 genes, 13 studies were included. The pooled HR was 0.688 (95% CI: 0.403–0.974; Q = 47.689; P = 0.000) (Figure 4(a)). Their pooled HR for DFS was 0.449 (95% CI: 0.146–0.753; Q = 32.012; P = 0.000) (Figure 4(b)). These results implied a worse prognosis of CRC in the event of the loss of expression of SMAD-3 or SMAD-4.

Studies reporting the mutations of the Tp53 gene (n = 24) had a pooled HR of 0.319 (95% CI: 0.133–0.504; Q = 201.339;

| Study name | Log HR | Standard error | 95% Lower CI | 95% Upper CI | Percent Random Effects |
|------------|--------|----------------|--------------|--------------|------------------------|
| Wang et al. (COH/UCD) | -0.478 | 0.171 | -0.813 | -0.143 | 14.980 |
| Wang et al. (MSKCC) | -0.462 | 0.128 | -0.713 | -0.211 | 15.985 |
| Mondaca et al. | -0.580 | 0.148 | -0.870 | -0.290 | 15.542 |
| Gerami et al. | 1.176 | 0.503 | 0.190 | 2.161 | 7.139 |
| Khan et al. | -0.117 | 0.063 | -0.239 | 0.006 | 17.108 |
| Jorissen et al. (MSI) | -0.105 | 0.611 | -1.303 | 1.092 | 5.567 |
| Jorissen et al. (MSS) | 0.698 | 0.274 | 0.160 | 1.236 | 12.125 |
| Jorissen et al. (Validation cohort, MSS) | 1.105 | 0.303 | 0.512 | 1.698 | 11.464 |

| Combined Average | 0.035 | 0.175 | -0.308 | 0.377 |

| Study name | Log HR | Standard error | 95% Lower CI | 95% Upper CI | Percent Random Effects |
|------------|--------|----------------|--------------|--------------|------------------------|
| Jorissen et al. (Validation cohort, MSS) | 0.761 | 0.341 | 0.093 | 1.428 | 26.867 |
| Jorissen et al. (MSS) | 0.997 | 0.340 | 0.330 | 1.666 | 26.870 |
| Jorissen et al. (MSI) | 0.231 | 0.831 | -1.398 | 1.860 | 15.057 |
| Mondaca et al. | -0.386 | 0.119 | -0.618 | -0.153 | 31.206 |

| Combined Average | 0.387 | 0.444 | -0.483 | 1.256 |

Figure 3: Forest plot of APC gene and overall survival in CRC (a). Forest plot of APC gene and disease-free survival in CRC (b).


| Study name       | Log HR | Standard error | 95% Lower CI | 95% Upper CI | Percent Random Effects weights |
|------------------|--------|----------------|--------------|--------------|-------------------------------|
| Isaksson et al.  | 1.520  | 0.694          | 0.158        | 2.881        | 3.265                         |
| Tonein et al.    | 0.086  | 0.694          | -1.275       | 1.447        | 3.265                         |
| Voomneved et al. | 0.904  | 0.358          | 0.203        | 1.606        | 7.130                         |
| Li et al.        | 1.952  | 0.358          | 1.250        | 2.653        | 7.130                         |
| Su et al.        | -0.132 | 0.305          | -0.730       | 0.465        | 8.086                         |
| Roth et al.      | 0.457  | 0.125          | 0.212        | 0.703        | 11.459                        |
| Isaksson et al.  | 0.593  | 0.258          | 0.087        | 1.100        | 8.984                         |
| Chun et al.      | 0.538  | 0.356          | -0.179       | 1.256        | 6.993                         |
| Chun et al.      | 0.136  | 0.356          | -0.561       | 0.834        | 7.168                         |
| Meskar et al.    | 2.077  | 0.337          | 1.416        | 2.737        | 7.497                         |
| Kawaguchi et al. | 0.599  | 0.337          | -0.062       | 1.259        | 7.497                         |
| Khan et al.      | 0.270  | 0.225          | -0.172       | 0.712        | 9.634                         |
| Jia et al.       | 0.531  | 0.093          | 0.348        | 0.713        | 11.911                        |

| [Combined]       | Average| 0.688          | 0.146        | 0.403        | 0.974                         |

| Study name       | Log HR | Standard error | 95% Lower CI | 95% Upper CI | Percent Random Effects weights |
|------------------|--------|----------------|--------------|--------------|-------------------------------|
| Meskar et al.    | 1.883  | 0.331          | 1.234        | 2.531        | 10.664                        |
| Roth et al.      | 0.385  | 0.107          | 0.176        | 0.595        | 18.933                        |
| Su et al.        | -0.086 | 0.146          | -0.371       | 0.200        | 17.571                        |
| Yoo et al.       | -0.239 | 0.117          | 0.009        | 0.469        | 18.591                        |
| Yoo et al.       | 0.372  | 0.161          | 0.057        | 0.686        | 17.003                        |
| Kawaguchi et al. | 0.482  | 0.153          | 0.179        | 0.785        | 17.237                        |

| [Combined]       | Average| 0.449          | 0.155        | 0.146        | 0.753                         |

**Figure 4:** Forest plot of SMAD3/4 gene and overall survival in CRC (a). Forest plot of SMAD3/4 gene and disease-free survival in CRC (b).

$P = 0.000)$ (Figure 5(a)) for OS and 0.179 (95% CI: 0.126–0.485; $Q = 143.796; P = 0.000$) (Figure 5(b)) for DFS ($n = 14$). The results were widely heterogenous but implied significantly poor prognosis overall, as well as DFS, in CRC cases. Five studies showed a pooled HR of 0.671 (95% CI: 0.116–1.458; $Q = 10.746; P = 0.030$) (Figure 6) for the Wnt gene with OS, thereby showing no association of Wnt gene expression/mutation with survival in CRC. Since only one study [14] reported the hazard ratio for DFS, meta-analysis was not performed for the Wnt gene with shorter DFS. Five studies on Cyclin D1 were included in the meta-analysis. The pooled HR for OS was 0.362 (95% CI: 0.944–0.221; $Q = 5.421; P = 0.253$) (Figure 7(a)) and that for DFS was 0.485 (95% CI: 0.772–0.198; $Q = 5.810; P = 0.214$) (Figure 7(b)). High Cyclin D1, therefore, produced a worse prognosis in CRC, both in terms of OS and DFS.

3.5. Publication Bias. We assessed the publication bias for APC, SMAD, β-catenin, and Tp53 gene studies by constructing funnel plots (Figure 8(a)–8(f)) as more than ten studies were included in the meta-analysis. Egger's test indicated that publication bias existed for the evaluation of the impact of β-catenin, APC, and Tp53 with OS, however, Begg's test showed no significant publication bias (β-catenin and OS: $I^2 = 65.83%$, tau ($\tau$) = 0.047 ($P = 0.76$), β-catenin and DFS: $I^2 = 71.33%$, $\tau$ = 0.21 ($P = 0.25$), TP53 and OS: $I^2 = 88.82%$, $\tau$ = 0.153 ($P = 0.28$), TP53 and DFS: $I^2 = 89.12%$, $\tau = 0.25$ ($P = 0.13$), APC and OS: $I^2 = 86.48%$; $\tau = 0.28$ ($P = 0.32$), SMAD and OS: $I^2 = 83.17%$, and $\tau = 0.23$ ($P = 0.27$). It is notable that with Egger’s test, there is insufficient power of testing when the number of selected studies is below 20. It was, therefore, not attempted for the remaining genes.

4. Discussion

Colorectal carcinogenesis is a complex multistage process that involves multiple genetic variations. The aberrant activation of the Wnt/β-catenin pathway has been identified as being involved in the progression of CRC [104] and early colorectal tumorigenesis [103]. In several studies, the β-catenin accumulation in the nucleus or cytoplasm was identified as a marker for poor prognosis. The variations of the APC or CTNNBI genes are the main causes of the
accumulation of nuclear β-catenin [105]. In contrast, β-catenin expression in the nucleus was associated with noninvasive tumors and more favorable outcomes [106] but remains controversial.

The current meta-analysis has explored the cumulative prognostic significance of the different subcellular localizations of β-catenin expression among CRC subjects. The results indicated that the nuclear expression or decreased expression of β-catenin in the membrane was associated with lower OS, which is consistent with the published articles. Pooled data from a study [107] found that the reduced expression of β-catenin in the membrane to be significantly associated with poor survival among CRC patients, thus the majority of the selected studies are from nuclear β-catenin overexpression.

Wnt2 is an oncogene with the potential to activate canonical Wnt signaling during CRC tumorigenesis [21, 22]. The role of Wnt5 in the progression of CRC is quite complex and appears to be inconsistent in findings. Several studies [21–25] proved that Wnt5a was silenced in most CRC cell lines because of recurrent methylation in the promoter region. Wnt5a acts as a tumor suppressor by interfering with the canonical β-catenin signaling. However, it activates the noncanonical signaling pathways [100]. In this study, there

| Study name | Log HR | Standard error | 95% Lower CI | 95% Upper CI | Percent Random Effects weights |
|------------|--------|----------------|--------------|--------------|-------------------------------|
| Bazan et al. | 0.761 | 0.358 | 0.058 | 1.463 | 6.215 |
| Brandstedt et al. | -1.661 | 0.811 | -3.250 | -0.072 | 2.643 |
| Warem et al. | -0.511 | 0.051 | -0.611 | -0.410 | 9.163 |
| Netter et al. | 0.039 | 0.279 | -0.507 | 0.586 | 7.141 |
| Loes et al. | -0.248 | 0.256 | -0.749 | 0.252 | 7.412 |
| Kandiolser et al. | 0.549 | 0.256 | 0.047 | 1.050 | 7.406 |
| Chen et al. | 0.536 | 0.261 | 0.026 | 1.047 | 7.354 |
| Oh et al. | 0.997 | 0.269 | 0.469 | 1.525 | 7.251 |
| Wang et al. | 0.863 | 0.281 | -1.413 | -0.312 | 7.116 |
| Zhang et al. | 0.506 | 0.363 | -0.206 | 1.218 | 6.159 |
| Morikawa et al. | 0.262 | 0.123 | 0.022 | 0.503 | 8.753 |
| Kawaguchu et al. | 0.336 | 0.120 | 0.100 | 0.573 | 8.770 |
| Samowitz et al. | 0.095 | 0.099 | -0.098 | 0.289 | 8.923 |
| Jurach et al. | 0.971 | 0.405 | 0.176 | 1.765 | 5.693 |

| [Combined] Average | 0.179 | 0.156 | -0.126 | 0.485 |

**Figure 5**: Forest plot of TP53 gene and overall survival in CRC (a). Forest plot of TP53 gene and disease-free survival in CRC (b).
was no significant association of Wnt (2 and 5) to OS or DFS found among CRC patients, and it is well in accordance with the contradictory studies reported [23–25].

In our meta-analysis pertaining to SMAD genes, we found that the loss of SMAD 3 or SMAD4 staining was strongly associated with a worse prognosis for OS and DFS (including CSS/RFS). Several other individual reports are in alignment with our findings [87, 92, 93]. These studies reported SMAD-4 to have a stronger association compared with SMAD-3 or other SMAD genes.

Most studies have shown the predictive value of Tp53 for overall survival in CRC to be poor. Dong et al. [108] reported 53% of Tp53 gene variation as the susceptibility for the development of CRC. Another study reported that, in mouse models, a high rate of spontaneous tumors was noted because of p53-deficiency [109]. Moreover, the deletion of p53 and the Tp53 gene variation led to tumor progression and tumor cell death.

A meta-analysis of Asian patients indicates that an association between Tp53 Arg72Pro polymorphism CC genotype might contribute to an increased risk of CRC [110]. The current meta-analysis included diverse populations, and the results pertaining to the association of Tp53 with shorter overall and DFS in CRC may, therefore, be considered more generalizable.

In an independent study of 331 patients, the prognostic value of APC was evaluated, and the findings were validated on a public database of stage IV colon cancer from Memorial Sloan Kettering Cancer Center (MSKCC) [75]. The study found that APC-WT was present in 26% of metastatic CRC patients, and it was more prevalent in patients of younger age and those with right-sided tumors. APC-WT tumors
have been shown to be associated with other Wnt-activating alterations, including CTNNB1, FBXW7, RNF43, ARID1A, and SOX9. APC-WT patients in a study were found to have a worse overall survival (OS) than APC-MT pts (HR = 1.809, 95% CI: 1.260–2.596) [75]. Overall, in most studies, APC-WT is associated with poor OS. Additionally, APC-WT tumors were associated with other activating alterations of the Wnt pathway, including RNF43 and CTNNB1.

Cyclin D1 overexpression has been reported to occur in 40–70% of colorectal tumors [111]. Despite the well-established role of Cyclin D1 in cell cycle progression, previous data on Cyclin D1 and clinical outcomes in CRC have been conflicting. Cyclin D1 overexpression has also been significantly related to poor OS in Asian and non-Asian CRC patients [112]. Two mechanisms have been implicated, namely nuclear expression and cytoplasmic expression, wherein most studies found an association of the nuclear expression of Cyclin D1 with OS and DFS. Moreover, Cyclin D1 also has been shown as a poor prognosis marker when co-expressed with other genes, notably p53 [113]. These results are consistent with the present meta-analysis’s findings that shortened overall survival and DFS are associated with Cyclin D1 among CRC patients.

We acknowledge that this study has several limitations. Firstly, the element of bias cannot be ruled out because of the inclusion of retrospective studies. Secondly, all of the selected studies measured gene expression by immunohistochemistry and sequencing methods. Moreover, the cut-offs used in various studies differed between and across the genes studied. However, there was no subgroup analysis performed to investigate the potential effect of the technique on the combined results. Thirdly, some heterogeneity has been found because of location and the types of cancer. To eliminate variations across studies, a random-effects model was performed accordingly. Limited databases were used for article search, and only freely available full-text articles in the English language were used, which might affect the persuasive power of the pooled estimate, although to a limited extent. In addition, publication bias existed because only studies generating positive results or significant outcomes were suitable for publication. Future research might helpfully contribute further relevant analyses and well-designed extensive prospective studies, since they will address the limitations of the current meta-analysis.

5. Conclusion

The present meta-analysis has found that the genes associated with worst OS in CRC were β-catenin (cytoplasmic, membranous, and nuclear overexpression), APC (mutant type), Tp53 (mutated), SMAD-3 and SMAD-4 (loss of expression), and Cyclin D1 (high). The gene associated with shorter DFS in CRC patients was APC (mutant type). In
contrast, Wnt (2 and 5) genes were not associated with prognosis in CRC in this meta-analysis.

**Abbreviations**

APC: Adenomatous polyposis coli  
ARID1A: AT-rich interaction domain 1A  
CIs: Confidence intervals  
CRC: Colorectal cancer  
CSS: Cancer-specific survival  
DFS: Disease-free survival  
CTNNB1: Catenin beta 1  
FBXW7: F-box and WD repeat domain containing 7  
HRs: Hazard ratios  
OS: Overall survival  
p53: Tumor suppressor protein  
PFS: Progression-free survival  
RFS: Recurrence-free survival  
SMAD: Suppressor of mothers against decapentaplegic  
SOX9: SRY-box transcription factor 9  
Tp53: Tumor protein p53 gene  
TGFβ: Transforming growth factor β  
Wnt: Wingless/integrated.

**Data Availability**

The data extraction sheets used to support the findings of this study are available from the corresponding author upon request.

**Conflicts of Interest**

The authors declare no conflicts of interest.

**Authors’ Contributions**

Hongfeng Yan took part in conceptualization, methodology, resources, writing-original draft, writing-review, and editing. Jianwu Yang took part in conceptualization, methodology, data curation, resources, writing-original draft, writing-review, and editing. Fuquan Jiang took part in conceptualization, resources, writing-review, editing, and supervision. All authors have read and approved the manuscript. Fuquan Jiang and Jianwu Yang shared equal correspondence.

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