Association Between c-Myc and Colorectal Cancer Prognosis: A Meta-Analysis

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Background: There is debate as to whether c-Myc predicts prognosis in colorectal cancer (CRC). In this study, we aimed to review the association between c-Myc and CRC prognosis.

Methods: Pertinent studies were identified by searching electronic databases and carefully reviewing the reference lists of pertinent studies until March 2016. The summary hazard ratio (HR) and corresponding 95% confidence interval (CI) were calculated to study the association between c-Myc and CRC prognosis.

Results: Eight cohort studies (including seven studies about overall survival [OS] and one study about disease free survival [DFS]) were included. The pooled HR of OS was 1.13 (95% CI: 0.66–1.95). In subgroup analysis, no significant association between c-Myc and CRC prognosis was found in the studies either from Western countries (HR: 0.87, 95% CI: 0.68–1.10) or Asian countries (HR: 1.89, 95% CI: 0.62–5.77). HRs were 0.86 (95% CI: 0.38–1.94) and 1.57 (95% CI: 0.73–3.39) for the studies using univariate analysis and multivariate analysis, respectively. HR from the studies that examined DNA level was significantly different (HR: 2.05, 95% CI: 1.22–3.46); while that about RNA level or protein level was not significantly different.

Conclusion: c-Myc was not associated with CRC prognosis in this meta-analysis. However, the conclusion is preliminary and should be examined in future studies.

Keywords: c-Myc, colorectal cancer, prognosis, biomarker, meta-analysis

INTRODUCTION

Colorectal cancer (CRC) is the third most frequent cancer worldwide and the fourth most common cause of cancer-related death (Torre et al., 2015). There are approximately 1.4 million new cases and 700,000 deaths from CRC each year (Torre et al., 2015). CRC is a heterogeneous and complicated disease affected by both environmental and genetic factors. A number of cancer-related genes are correlated with CRC prognosis, but the survival benefit associated with targeted therapies is only 4–5 months (Bokemeyer et al., 2012), indicating that the precise molecular mechanisms of CRC are unclear.
The Myc family encodes three highly related nuclear phosphoproteins: c-Myc, l-Myc, and n-Myc (Mukherjee et al., 1992). c-Myc functions as an oncogene, participating in cell growth, death, transformation, and therapy sensitivity (Hermeking and Eick, 1994; Lee K.B. et al., 2016; Wang et al., 2016). The c-Myc protein occupies regulatory regions of up to 15% of all genes and can both activate or suppress various target genes (Dang et al., 2006; Feng et al., 2016). The target genes of c-Myc are involved in various cellular functions, including survival, cell cycle, protein synthesis, cell adhesion, and non-coding RNA expression (Dang et al., 2006).

Aberrant expression of c-Myc was observed in many human cancers and was elevated in up to 70–80% of CRC (Erisman et al., 1985). Several studies have focused on the association between c-Myc and CRC prognosis. Bhatavdekar et al. (1997) reported that measurement of c-Myc expression in primary CRC tissue did not predict prognosis (Erisman et al., 1988). However, some studies showed that positive c-Myc expression had the strongest association with poor survival in CRC patients (Rowley et al., 1990; Bhatavdekar et al., 1997; Kakisako et al., 1998; Lee et al., 2015). For example, Lee et al. (2015) indicated that c-Myc was an independent factor for poor prognosis in consecutive CRC patients according to multivariate analysis. On the other hand, several studies found that c-Myc was correlated with a favorable prognosis of CRC patients (Smith and Goh, 1996; Bockleman et al., 2012; Toon et al., 2014). For example, Smith and Goh (1996) demonstrated that overexpression of c-Myc mRNA in CRC tumors was associated with a better prognosis. All these findings

![Flow chart of the literature search and study selection.](image-url)
suggested that the prognostic value of c-Myc in CRC remained controversial and inconclusive. Therefore, we conducted a meta-analysis to evaluate the association between c-Myc and CRC prognosis.

**MATERIALS AND METHODS**

**Literature Search**
The Research Ethics Committee of Guangzhou University of Chinese Medicine provided ethical approval. PubMed, EMBASE, ISI Web of Knowledge, and the Cochrane Database were searched for eligible studies up to March 14th, 2016. The search strategy was carried out using the following words: “colorectal” (large intestine, large bowel, colon, colonic, rectal or rectum), “cancer” (carcinoma, tumor, neoplasm or cancers), “c-Myc” and “prognosis” (prognoses, prognostic, predictive, biomarker, marker, survival, survive, cox, log-rank or Kaplan-Meier). The search strategy for the Pubmed database was shown in Appendix 1. The reference lists of pertinent publications were also checked for the eligible studies. Only studies published in English were included. In case of duplicate reports or of studies obviously reporting results from the same study, only the latest published studies were selected. This meta-analysis was performed according to the preferred reporting items for systematic reviews and meta-analysis (PRISMA statement) (Moher et al., 2009). The PRISMA 2009 Checklist was shown in Appendix 2.

**Selection Criteria**
The inclusion and exclusion criteria consisted of the following three aspects: (1) studies of colorectal cancer (including colon cancer, or rectal cancer) were included; (2) the relationship between c-Myc and patients’ prognosis [i.e., overall survival (OS), disease free survival (DFS), or relapse free survival (RFS)] was studied; and the hazard ratio (HR) and its 95% confidence interval (CI) were provided; and (3) studies were published in the English language. The eligible studies included cohort studies and randomized control trials.

**Data Extraction**
The titles and abstracts of all the studies were screened by two of three reviewers independently (X-tW, J-lW, and Y-kL). The eligible or uncertain studies were retrieved for the full texts. Two of three reviewers (X-tW, J-lW and Y-kL) read the full texts and identified the eligible publications. For each eligible study, the following information was extracted: first author, year of publication, country of origin, study type, sample sizes, the characteristics of the patients (gender, stage, differentiation, and treatment method), median follow-up time, the c-Myc information (proportion of positive c-Myc, test sample, test content, and analytic method) and prognosis. Country of origin was categorized as Western countries and Asian countries. Disagreements in data collection were resolved by consensus.
TABLE 2 | The c-Myc information and results of the included studies.

| Author        | Proportion of positive c-Myc | Test sample | Test content | Test method | Analytic method | Outcome | HR   | 95% CI          |
|---------------|------------------------------|-------------|--------------|-------------|-----------------|---------|------|----------------|
| Bhatavdekar JM| 64.6                         | Tissue      | Protein      | IHC         | Uni             | OS      | 3.60 | (1.05–12.39)   |
| Böckelman C   | 28.0                         | Tissue      | Protein      | IHC         | Uni             | OS      | 0.51 | (0.28–0.92)    |
| Erisman MD    | 68.4                         | Tissue      | RNA          | Northern blot | Multi          | OS      | 2.22 | (0.68–7.29)    |
| Kakisako K    | 60.0                         | Tissue      | mRNA         | RT-PCR      | Uni             | DFS*    | 5.81 | (1.02–32.96)   |
| Lee KS        | 82.8                         | Tissue      | DNA          | IHC         | Multi           | OS      | 2.35 | (1.45–3.80)    |
| Rowley S      | 73.7                         | Tissue      | DNA          | Flow cytometric | Uni       | OS      | 1.21 | (0.40–3.67)    |
| Smith DR      | 60.5                         | Tissue      | RNA          | Northern blot | Uni           | OS      | 0.43 | (0.20–0.90)    |
| Toon CW       | 69.0                         | Tissue      | Protein      | IHC         | Multi           | OS      | 0.91 | (0.69–1.20)    |

DFS, disease-free survival; HR, hazard ratio; IHC, Immunohistochemistry; Multi, Multivariate analytic method; OS, overall survival; Proportion of positive c-Myc, Proportion of patients with positive c-Myc; RT-PCR, reverse transcription PCR; Uni, Univariate analytic method. *which was not included for analysis (not OS).

FIGURE 2 | The association between c-Myc and overall survival in seven studies.

Statistical Analysis

The association between c-Myc and CRC survival was examined using HR with its 95% CI. DFS and OS were analyzed separately. The heterogeneity of the individual HR was calculated using Chi-square tests. A heterogeneity test with inconsistency index statistic ($I^2$) and $Q$ statistic were carried out (Handoll, 2006). The $Q$ test suggested lack of heterogeneity when $P > 0.10$, and summary HR was examined using fixed-effect model (Mantel and Haenszel, 1959). Otherwise, random-effect model was executed (DerSimonian and Laird, 1986). Subgroup analysis were conducted according to different countries (West [Europe and America], and Asia), analytic methods (univariate analysis, multivariate analysis) and test content (Protein, DNA, RNA). Meta-regression was performed to find out the factors related with the heterogeneity of the HRs. A sensitivity analysis was carried out to evaluate the stability of the results. In addition, Egger’s test and funnel plots were utilized to evaluate publication bias. All statistical analyses were conducted using STATA software (version 12.0).

RESULTS

Characteristics of Studies

The initial search strategy identified 780 potentially eligible studies. Thirty studies were excluded because of duplication. We excluded 719 studies after detailed review of the abstract. The remaining 31 studies were evaluated for the full texts. Four studies did not involve c-Myc, thirteen studies did not deal with prognosis, two included other genes, three were review articles, and one was about single-nucleotide polymorphism and was therefore excluded. Eventually, we included eight studies in our meta-analysis (Figure 1; Erisman et al., 1988; Rowley et al.,...
Three studies were from Asian countries (Smith and Goh, 1996; Bhatavdekar et al., 1997; Kakisako et al., 1998; Bockleman et al., 2012; Toon et al., 2014; Lee et al., 2015). The prognostic roles of c-Myc in CRC were summarized in Figure 2. Inconsistent HRs were observed among studies, suggesting either favorable or poor prognostic roles of c-Myc in CRC. A random-effects model was executed to obtain an unadjusted pooled HR of 1.13 (95% CI: 0.66–1.96, I² = 79.0%, P < 0.001).

### Subgroup Analysis

The pooled HR for studies from Western countries was 1.10 (95% CI: 0.63–1.92; I² = 63.5%, P = 0.027, Figure 3 and Table 3). For OS.

### Meta-Analysis About OS of c-Myc in CRC Patients

Seven studies including 2,712 CRC patients were involved (Table 2). The following results were based on OS.

### Table 3 | The results of the meta-analysis (OS).

|                | Number of studies | Patients | HR (95% CI) | Heterogeneity (I², P) |
|----------------|-------------------|----------|-------------|-----------------------|
| All            | 7                 | 2,712    | 1.26 (0.74–2.17) | 78.4%, <0.001          |
| Country        |                   |          |             |                       |
| Western        | 5                 | 2,226    | 1.10 (0.63–1.92) | 63.5%, 0.027           |
| Asian          | 2                 | 486      | 1.03 (0.19–5.46) | 93.0%, 0.001           |
| Analytic methods |              |          |             |                       |
| Multivariate analysis | 3         | 2,145    | 1.57 (0.73–3.39) | 83.8%, 0.002           |
| Univariate analysis | 4        | 767      | 0.86 (0.38–1.94) | 71.3%, 0.015           |
| Test content   |                   |          |             |                       |
| Protein        | 3                 | 2,009    | 0.97 (0.47–1.99) | 76.1%, 0.015           |
| RNA            | 2                 | 157      | 0.91 (0.18–4.56) | 81.3%, 0.021           |
| DNA            | 2                 | 546      | 2.05 (1.22–3.46) | 13.3%, 0.283           |
FIGURE 4 | Subgroup analysis for the association between c-Myc and overall survival in the studies using different analytic methods. Uni, univariate analysis; Multi, multivariate analysis.

FIGURE 5 | Subgroup analysis for the association between c-Myc and overall survival in the studies using different test content (including Protein, DNA, RNA).
TABLE 4 | The results of Meta-regression.

|                     | Coef. | SE   | t-value | P      | 95% CI            |
|---------------------|-------|------|---------|--------|------------------|
| Country             | 0.536 | 0.434| 1.23    | 0.217  | (−0.315–1.387)   |
| Proportion of c-Myc | 0.012 | 0.014| 0.83    | 0.407  | (−0.016–0.039)   |
| Test content        | 0.528 | 0.264| 2.00    | 0.045  | (0.012–1.045)    |
| Analytic method     | 0.273 | 0.427| 0.64    | 0.522  | (−5.64–1.111)    |

Coef, coefficient; SE, standard error.

studies from Asian countries, the pooled HR was 1.03 (95% CI: 0.19–5.46; I² = 93.0%, P < 0.001, Figure 3 and Table 3). Pooled HR was 1.57 (95% CI: 0.73–3.39) by combining three studies that provided multivariate analysis (P = 0.002, I² = 83.8%, Figure 4 and Table 3). In addition, the pooled HR from four studies providing univariate analysis was 0.86 (95% CI: 0.38–1.94) based on the result of random-effect model (P = 0.015, I² = 71.3%, Figure 4 and Table 3).

Three studies examined protein level of c-Myc, two studies examined RNA level, while two studies examined DNA level. Pooled HR was 0.97 (95% CI: 0.47–1.99, Figure 5 and Table 3) for protein level of c-Myc, and 0.91 (95% CI: 0.18–4.56) for RNA level. HR from three studies that examined DNA level was 2.05 (95% CI: 1.22–3.46).

Analysis of Heterogeneity
There was significant heterogeneity for OS among seven studies (Figure 2). Meta-regression was performed. The variable
“Test content” was related with the heterogeneity of the HRs (Table 4). Sensitivity analysis (Figure 6A) and funnel plot (Figure 6B) were carried out to evaluate the influence of potential publication bias. We did not observe significantly publication bias from egger’s test ($P = 0.368$). However, the shape of the funnel plot indicated some studies were out of the reference line (Figure 6B). Each study in sensitivity analysis was successively removed to evaluate the effect of individual study on the pooled HR (Figure 6A). The results showed that the studies conducted by Bockleman et al. (2012); Toon et al. (2014) were out of the reference line, which demonstrated that there might be publication bias for OS.

**DISCUSSION**

This study is the first meta-analysis to examine the association between c-Myc and CRC prognosis. We found that c-Myc was not significantly associated with CRC prognosis.

 c-Myc participates in cell proliferation, differentiation, metabolism, survival, and apoptosis by regulating human genes (Guo et al., 2016; Su et al., 2016; Subramaniam et al., 2016). The c-Myc gene can promote tumorigenesis in many types of cancers (Aprilikova et al., 2016; Richart et al., 2016) and plays an important role in the progression of CRC (Smith and Goh, 1996; Kriegl et al., 2012).

Several studies have reported c-Myc status in many cancers, including prostate cancer (Zeng et al., 2015), breast cancer (Elster et al., 2016), and CRC (Lee K.S. et al., 2016). Some cancers with c-Myc overexpression, including oesophageal squamous cell carcinoma, gastric carcinoma, and soft tissue leiomyosarcoma, are correlated with poor survival (Ninomiya et al., 1991; Tsiatis et al., 2009; Wang et al., 2011). Likewise, several cancers with c-Myc gene amplification were associated with poor survival (Dimova et al., 2006; Choi et al., 2012; Seo et al., 2014). However, the prognostic value of c-Myc in CRC patients is quite controversial. It was reported that overexpression of either c-Myc mRNA or c-Myc protein in CRC patients was associated with favorable survival (Smith and Goh, 1996; Toon et al., 2014), but these were opposite results to previous studies that showed that high expression of c-Myc in CRC predicted worse survival outcome (Erisman et al., 1988). The association between c-Myc expression and CRC patients’ prognosis remains debatable. Therefore, it is required to further estimate c-Myc expression in CRC to obtain a conclusion regarding its prognostic value. Therefore, a meta-analysis including 2,947 CRC patients was performed. It was demonstrated that the c-Myc was not significantly associated with CRC prognosis in the overall investigated populations.

In subgroup analysis by ethnicity, we did not detect significant association between c-Myc and survival in either Europeans or Asians, indicating that ethnic differences in genetic backgrounds and the lifestyle context do not influence the association between c-Myc and CRC prognosis.

Nevertheless, there were some limitations in our study. First, adjusted confounding factors, including BMI and environmental factors, varied among studies. What was more, the method of therapy greatly affected the survival time of the CRC patients. Although all of the included patients were diagnosed as CRC, the use of specific therapy differed among the included studies. Thus, the confounding effects of different therapies remain unclear. Second, publication bias was observed among the studies, it might be inevitable due to unpublished studies or original data. Third, test content and evaluation criteria of c-Myc varied among studies, possibly giving rise to significant heterogeneity. HR from three studies that examined DNA level was significantly different, while those about RNA level or protein level were not significantly different. Fourth, only eight studies were enrolled in the meta-analysis, and each study included a relatively small sample size.

Overall, the meta-analysis indicates that c-Myc is not associated with CRC prognosis. However, due to the potential limitations, conclusions must be drawn with caution, and additional larger studies, particularly studies with sub-groups for environmental-genetic interactions, should be performed to validate our findings.

**AUTHOR CONTRIBUTIONS**

W-LH and YP wrote the draft of the paper. X-TW extracted the data and helped to modify the manuscript. J-LW and Y-KL extracted the data. T-WL searched the databases. Q-YZ and YH analyzed the data. X-LC conceived the study, searched the databases and modified the manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2018.01549/full#supplementary-material
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