Review Article

Interactions of multiple gene alterations in colorectal liver metastases

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Abstract: The advancements in next generation sequencing have expanded the information available in clinical practice. Alterations of *TP53*, *APC*, *RAS*, *PIK3CA*, and *SMAD4* are present in more than 10% of patients with colorectal liver metastases (CLM). Of these genes, *TP53*, *RAS* and *SMAD4* were associated with worse survival in patients undergoing CLM resection. Testing multiple gene alterations provides a more precise prognosis for patients undergoing CLM resection and may be useful for better clinical decision making.

Keywords: Somatic gene alteration; colorectal liver metastasis (CLM); liver resection; survival

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Introduction

Colorectal cancer is genetically heterogenous (1). Approximately 15–30% of patients with colorectal cancer develop colorectal liver metastases (CLM). Liver resection is regarded as a potentially curative treatment for patients with CLM and provides 5-year overall survival (OS) rates of approximately 40–60% (2-4). However, we experience clinical heterogeneity in patients undergoing CLM resection. Some patients have a good prognosis after CLM resection and achieve a prolonged cancer-free interval or a cure, while others have a progressive fatal disease. The recent development of the next generation sequencing has led to increased availability of gene alteration information in clinic practice. *RAS* alteration initially became the main focus because it determines resistance to anti-epidermal growth factor receptor (EGFR) agents (5-7). Furthermore, the prognostic role of alterations in *RAS* and *BRAF* has been well studied in patients with colorectal cancer (8-13). However, prognostic roles of gene alterations other than *RAS* and *BRAF* remain unclear. This article reviews the association of multiple gene alterations with prognosis after CLM resection to be useful for clinical decision-making.

Frequency of somatic gene alteration in patients with CLM

We recently reported 507 patients who had genetic sequencing data of 50 cancer-related genes and underwent CLM resection (14). Of the 50 genes analyzed, alterations of the following 13 genes were found in more than 1% of patients: *TP53*, *APC*, *KRAS*, *PIK3CA*, *SMAD4*, *FBXW7*, *NRAS*, *ERBB2*, *ATM*, *BRAF*, *PTEN*, *RB1*, and *CTNNB1* (*Table 1*). The frequencies of gene alterations are similar to a project from the Cancer Genome Atlas Network (1). Alterations in *TP53*, *APC*, *RAS* (*KRAS* + *NRAS*) occur in more than 50% of patients with colorectal cancer. *PIK3CA*, *SMDA4*, and *FBXW7* are the second most frequently altered gene group, ranging from 5% to 20% of alterations in this patient group.

Table 1 Frequency of gene alterations in 507 patients who underwent resection of colorectal liver metastases

Gene	Data, n (%)
TP53	359 (70.8)
APC	271 (53.5)
KRAS	237 (46.7)
PIK3CA	80 (15.8)
SMAD4	56 (11.0)
FBXW7	30 (5.9)
NRAS	22 (4.3)
ERBB2	16 (3.2)
ATM	13 (2.6)
BRAF	10 (2.0)
PTEN	9 (1.8)
RB1	9 (1.8)
CTNNB1	8 (1.6)

Alterations in RAS and BRAF and prognosis after CLM resection

Chemotherapy regimens, including anti-EGFR agents, have demonstrated improved survival in patients with unresectable metastatic colorectal cancer (15). Alterations in the *RAS* gene family (*KRAS*, *NRAS*, and *HRAS*) were found to be an important biomarker and used clinically to determine response to anti-EGFR therapy (5-7). The advancements in molecular-targeted therapy have quickly drawn attention to information on somatic alterations in colorectal cancer.

Studies have assessed an association of *RAS* alteration with prognosis in patients undergoing CLM resection (9,11,14,16-24). Most studies show worse OS and recurrence-free survival (RFS) in *RAS* altered patients than in *RAS* wild-type patients (9,11,14,23). In contrast, other studies have not shown significant differences between *RAS* altered patients and *RAS* wild-type patients in OS (17,18,21), or in RFS (20,21), using a multivariable Cox model.

Approximately 2–5% of patients undergoing CLM resection have *BRAF* alteration (12-14). Studies have reported that *BRAF* alteration occurs in approximately 10% of all patients with colorectal cancer (25). Moreover, *BRAF* altered patients with colorectal cancer were associated with worse prognosis (8,10). However, the rarity of *BRAF* altered patients undergoing CLM resection makes it hard to assess the prognostic role of *BRAF* alteration with sufficient statistical power. Recent multi-institutional studies evaluated 35 patients with *BRAF* alteration out of 1,497 total patients (12), and 45 patients with *BRAF* alteration out of 853 patients (13). Both studies demonstrated that OS and RFS were significantly worse in *BRAF* altered patients than in *BRAF* wild-type patients (12,13).

Alterations in TP53 and prognosis after CLM resection

TP53 is a tumor suppressor gene in the p53 pathway and has a role to inhibit tumor cell growth (26,27). The prognostic role of *TP53* alteration in patients undergoing CLM resection has been previously studied in early 2000s (28-34). While four studies failed to show the association of *TP53* alteration with prognosis (30,31,33,34), there are four other studies which showed that *TP53* altered patients were associated with worse OS than *TP53* wild-type patients (28,32,35). One study has shown better OS and RFS in *TP53* altered patients than in *TP53* wild-type patients (29). Because the type of gene alteration influences the change of proteins and functions, Chun *et al.* analyzed patients with *TP53* altered patients (24) by classifying a risk of missense *TP53* alteration into high and low risk groups on the basis of the evolutionary action score (36,37). *TP53* alteration with a high evolutionary action score is associated with worse survival (24). OS and RFS were worse in patients with co-alteration in *RAS* and *TP53* than in patients with one alteration of the two genes and in patients with no alteration (24,38).

Multiple alterations and prognosis after CLM resection

We recently analyzed prognostic roles of five frequently altered genes (*TP53*, *APC*, *RAS*, *PIK3CA*, *SMAD4*) as well as *BRAF* in 507 patients undergoing CLM resection (14). According to a multivariable Cox proportional hazards model analysis, alterations in *RAS*, *TP53*, and *SMAD4* were significantly associated with worse OS and RFS, together with other clinicopathologic factors. *BRAF* alteration was an independent risk factor for OS but not for RFS. Since alterations in *RAS*, *TP53*, and *SMAD4* were independently associated with OS and RFS, we calculated multivariable hazard ratios (HR) focusing on number of alterations among these three genes. Co-existing alterations of all three genes (triple alteration) was significantly associated

with worse OS than co-existing alteration in any two of the genes (double alteration) [HR 3.21, 95% confidence interval (CI): 1.72–5.99, P<0.001]. Triple alteration was also significantly associated with worse OS than alteration in only one of the genes (single alteration) (HR 6.04, 95% CI: 3.21–11.3, P<0.001) and no alteration in any of the genes (all wild-type) (HR 8.61, 95% CI: 3.80–19.5, P<0.001). Similarly, triple alteration was significantly associated with worse RFS than double alteration (HR 2.06, 95% CI: 1.28–3.29, P=0.003), single alteration (HR 3.17, 95% CI: 1.97–5.07, P<0.001), and all wild-type (HR 3.72, 95% CI: 2.14–6.46, P<0.001). Furthermore, OS and RFS did not differ significantly between patients with single alteration *vs.* patients with all wild-type (OS, HR 1.43, 95% CI: 0.77–2.63, P=0.256; RFS, HR 1.18, 95% CI: 0.82–1.68, P=0.378). Based on this finding, we compared OS and RFS between patients with *RAS* alteration and wild-type *TP53* and *SMAD4* and patients with *RAS* wild-type. OS and RFS did not differ significantly between both patient groups: OS, HR 0.95, 95% CI: 0.55–1.65, P=0.858; RFS, HR 1.06, 95% CI: 0.77–1.44, P=0.729. Our findings likely explain why some studies found prognostic role of *RAS* alteration (9,11,14,23) while others failed to show an association of *RAS* alteration with prognosis (17,18,20,21). Overall, the testing of multiple gene alterations, including the analysis of co-existing alterations, may provide additional prognostic information in patients undergoing CLM resection.

RAS, *TP53*, and *SMAD4* belong to different cancer-related signaling pathways (i.e., MAPK pathway, p53 pathway, and TGFβ pathway). Our recent study suggests that through the corresponding signaling pathways, the deleterious effects on survival are accumulated from single to double alterations, and also from double to triple alterations. However, our study cannot explain the interactions that may exist between these alterations. Studies report that there may exist an interaction and synergism for progression of carcinoma between MAPK pathway, p53 pathway, and TGFβ pathway. These interactions may in part account for the worse prognosis of patients with multiple gene alterations (39-41).

Conclusions

In patients with colorectal cancer, alterations in *RAS* and *BRAF* have been increasingly examined as biomarkers for testing resistance to anti-EGFR agents. Advancements in next generation sequencing have made multiple gene testing clinically available. Because *RAS* alteration status alone is not sufficient to predict survival after CLM resection, information on multiple gene alteration status may aid in predicting prognosis more precisely; and therefore, influence the clinical decision-making process. Further developments in other molecular targeted therapies may further highlight the importance of information on gene sequencing.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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