Review Article

The impact of somatic *SMAD4* mutations in colorectal liver metastases

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Abstract: Recent advances in cancer genomics have led to the identification of many molecular pathways involved in colorectal cancer (CRC) carcinogenesis. Pre-clinical and clinical data have shown that gene mutations involved in several of these pathways have an important prognostic impact, particularly on the outcomes of patients with metastatic CRC. Therefore, specific information on such gene mutational status can be potentially used as biomarkers to guide genome-oriented personalized treatment and ultimately improve patient outcomes. Drosophila protein, mothers against decapentaplegic homolog 4 (SMAD4) has a critical intermediate role in the TGFβ signaling pathway. Loss of SMAD4 expression is associated with both metastatic development and worse response to chemotherapy for patients with CRC. Additionally, it has been reported that the loss of SMAD4 function is independently associated with decreased recurrence-free (RFS) and overall survival (OS) for patients with CRC, especially for patients with advanced stages of disease. Furthermore, among patients who undergo hepatectomy for colorectal liver metastases (CRLM), *SMAD4* mutations are associated with a high likelihood of simultaneously carrying RAS mutations, which independently predict worse OS. Although recent evidence highlights the prognostic importance of somatic *SMAD4* mutations in CRLM, ongoing research is necessary to untangle the specific molecular mechanisms involved in the complex *SMAD4* regulatory network as well as the synergism with other mutations implicated in the pathogenesis of CRC. The detailed elucidation of such mechanisms may potentially aid the development of future trials in establishing novel, targeted therapeutic advances to further guide clinical decision-making for patients with CRC.

Keywords: Colorectal cancer (CRC); hepatectomy; liver resection; personalized medicine; biomarker; Drosophila protein mothers against decapentaplegic homolog 4

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Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related death and the third most frequently diagnosed cancer in the United States (1). While the prognosis for CRC varies widely, approximately 20% of patients with CRC have metastases at the time of diagnosis. For patients who develop colorectal liver metastases (CRLM), multimodality therapy consisting of contemporary chemotherapy and metastasectomy can lead to 5-year overall survival (OS) rates that exceed 50% (2,3). Nevertheless, more than half of these patients will develop recurrent disease within 2 years from surgery (4). Therefore,

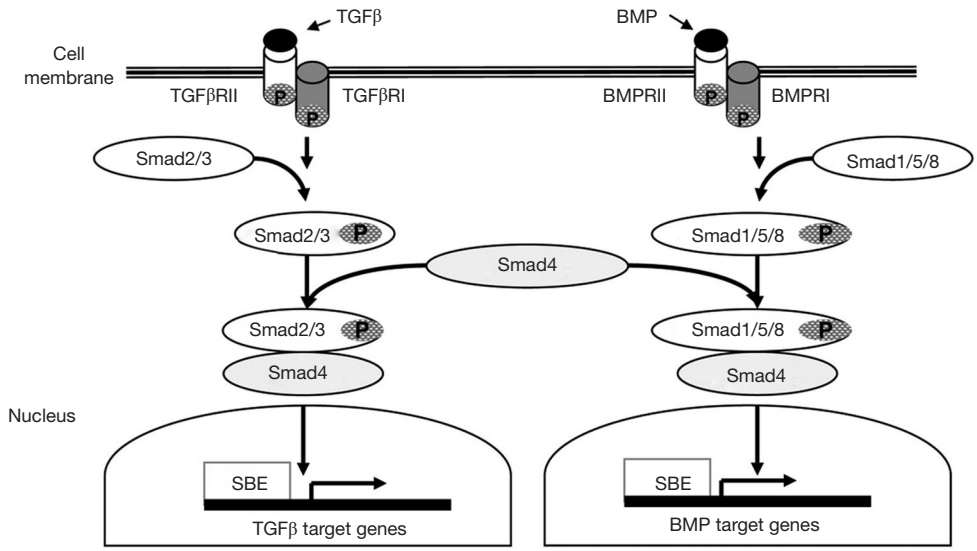

Figure 1 Schematic of TGFβ signaling pathways highlighting the role of SMAD family members' in TGFβ expression to influence cell proliferation, differentiation, apoptosis, extracellular matrix production and metastasis. Used with permission from Malkoski & Wang (6).

the accurate risk stratification of patients for recurrence, via the identification of relevant prognostic factors, may lead to improved outcomes through individualized treatment or specific surveillance strategies.

In recent years, significant advances have led to the discovery and characterization of specific genes and molecular pathways involved in carcinogenesis, disease progression, and mechanisms of treatment resistance in CRC biology. While these advances have led to the ability to provide improved prognostic information, novel biomarkers based on genomic profiling are required to further differentiate among the various subgroups of patients with CRC. More importantly, these genome-oriented biomarkers for CRC would facilitate the implementation of more personalized treatment, potentially leading to better prognosis and improved survival.

SMAD4 in CRC progression

Alterations of the transforming growth factor (TGF)-β signaling cascade play an essential role in carcinogenesis and disease progression of CRC given its critical involvement in cell proliferation, differentiation, apoptosis, and extracellular matrix production (5,6). While activation of TGFβ may be associated with tumor suppression in early stages (7), it is hypothesized to promote angiogenesis, epithelial-to-mesenchymal transition, and tumor metastasis in later stages of CRC development (8,9).

Drosophila protein, mothers against decapentaplegic homolog 4 (*SMAD4*) is an essential mediator in the TGFβ signaling pathway, which is located on chromosome 18q21 (5). Following the linking of TGFβ ligands to TGFβ transmembrane protein kinase receptors 1 and 2, SMAD2 and SMAD3 proteins are activated via phosphorylation which subsequently allows them to link to SMAD4 (10,11). The activated SMAD4 complex then relocates to the nucleus where it regulates TGFβ-related gene transcription (6,12-15). Among the components of this protein cascade, SMAD4 is an essential intermediator, exhibiting a critical role as a common downstream regulator and tumor suppressor gene (16,17) (*Figure 1*).

SMAD4 mutations that lead to decreased SMAD4 protein expression have been reported to occur in approximately 20% of patients with CRC (18-20). The loss of *SMAD4* expression has been implicated both in metastasis and in poor response to chemotherapy for patients with CRC (21-24). In fact, recent studies have demonstrated that decreased SMAD4 expression is independently associated with worse recurrence-free survival (RFS) and OS among patients with CRC, especially among those with advanced stages of disease (22,25-27). In contrast, greater levels of *SMAD4* expression are associated with improved disease-free survival (DFS) and OS (26). It is posited that *SMAD4* inactivation leads to unregulated TGFβ-induced growth (23) which may contribute to worse prognosis in CRC (28).

Recent work has also highlighted that *SMAD4* downregulation may occur in up to 60% of patients with metastatic CRC, which is significantly higher than the incidence of *SMAD4* mutations (29). Interestingly, there is increasing evidence that *SMAD4* expression is also regulated by several micro-RNAs (30). Specifically, previous studies using animal models have noted that *SMAD4* is influenced by miR-130a, miR-20a, miR-224, miR-34a and miR-19b (31-35). While the exact mechanism of action and role of these micro-RNAs in regulating *SMAD4* in metastatic CRC progression is not understood, there is evidence that micro-RNAs contribute to chemotherapy-resistance. For example, several studies have indicated that the upregulation of miR-20a, miR-224 and miR-19b is associated with decreased response to the commonly used chemotherapy agent 5-fluorouracil (5-FU) (36-38). Similarly, these same micro-RNAs reduce the sensitivity of CRC cells to oxaliplatin, another commonly employed chemotherapeutic in CRC (35,36,39). Therefore, ongoing research on the regulatory mechanisms of miRNAs to the downregulation of *SMAD4* could have important and relevant therapeutic implications to the management of patients with metastatic CRC.

It is equally important to note that somatic mutations, concurrent with *SMAD4*, may have an additive effect on the prognosis of patients with CRC. For example, concomitant *SMAD4* and *PTEN* mutations have been identified in a subgroup of patients with CRC who have more aggressive disease. This gene association is believed to yield significantly worse outcomes compared with patients with CRC who have only one of these mutations (40). Interestingly, there is evidence that micro-RNAs miR-130a (41), miR-20a (42) and miR-19b (43) regulate *PTEN* in addition to *SMAD4*. Nevertheless, the precise role of the specific markers that simultaneously decrease the expression of *SMAD4* and *PTEN* and contribute to the development of more aggressive CRC or how exactly they yield resistance to 5-FU and oxaliplatin treatments have not been explicitly elucidated (35,36,38).

Besides the association with poor prognosis in CRC, *SMAD4* mutations have been also found to be associated with colonic tumor location, female sex and mucinous histology type (44,45). In more recent studies, *SMAD4* mutation was found to be associated with high- versus low-grade mucinous adenocarcinomas, advanced stage of disease and aggressive phenotypes of CRC (22,46-48). However, as previously discussed, the worse outcomes observed in patients with CRC may be in part confounded by the relative resistance to 5-FU (26,49).

SMAD4 mutations in CRLM

The incidence of somatic *SMAD4* mutations in patients with isolated CRLM is approximately 15%. Recently published data on surgical outcomes for patients with CRLM have also highlighted the clinical relevance of *SMAD4* mutations among patients undergoing liver resection (50). Mizuno et al. retrospectively evaluated the outcomes of patients with known *SMAD4* gene mutation status following hepatectomy for CRLM. *SMAD4* mutations were found to be independently associated with worse OS following liver resection, independent of *RAS* mutation status. Furthermore, the negative prognostic impact of *SMAD4* gene mutation status was also confirmed in a validation cohort of patients who only received systemic chemotherapy for metastatic CRC (50). The results of this study highlight the utility of *SMAD4* mutation status in the surgical decision-making for patients scheduled to undergo surgical resection for CRLM, especially patients with initially unresectable disease or those who are scheduled to undergo a two-stage hepatectomy.

While the impact of *SMAD4* mutation status on recurrence rates or DFS in patients with CRLM undergoing hepatectomy has not been yet clearly established, it is notable that patients with *SMAD4* mutations were less likely to undergo repeat hepatectomy for recurrent disease after initial metastasectomy (50). Although the survival differences after recurrence between patients with *SMAD4* gene mutations versus *SMAD4* wild type tumor genotype may represent differences in ability to undergo repeat hepatectomy, the mechanisms driving this difference is unknown and warrants additional investigation (51,52). A plausible explanation is that *SMAD4* mutant recurrences are more likely to occur in an unresectable fashion due to their more aggressive tumor biology, or alternatively, they may become unresectable because of tumor progression during neoadjuvant chemotherapy.

It is also known that patients with *SMAD4*-mutant CRLM also have a higher incidence of somatic RAS mutations. This is presumably related to differences in the specific signaling pathways that each protein mediates: while SMAD4 regulates the TGFβ signaling pathway (5), RAS plays an important role in the mitogen-activated protein kinase signaling pathway (53). Nevertheless, the synergistic action between *SMAD4* and *RAS* should be further investigated since both gene mutations are important independent predictors of poor OS (50). Additional research into the mechanistic and prognostic importance of other somatic

mutations in CRLM, such as *APC*, *PIK3CA*, *BRAF* and *TP53*, will only enhance our understanding regarding the complex molecular pathways involved in CRC development, progression, prognosis, and response to treatment (54,55). In fact, emerging evidence suggests that mutation status of *RAS*, *TP53*, and *SMAD4* provides superior prognostic information following resection of CRLM compared to any single or double somatic mutation alone (56).

Future research

While the prognostic importance of somatic mutations in CRC and more specifically in CRLM continues to be highlighted, future research is necessary to address several unanswered questions. The distinct molecular mechanisms underlying the complex *SMAD4* regulatory network, including the specific mechanisms by which micro-RNAs downregulate *SMAD4* expression and leads to the disruption of important TGFβ signaling pathways, need to be better understood. A detailed elucidation of these mechanisms will aid the translation of foundational molecular concepts into the establishment of future novel, targeted, therapeutic advances for patients with CRC. The clinical significance of *SMAD4* and its influence on the use of diverse and individualized perioperative therapies for CRC need to be validated by future investigations. An important question to answer is whether the *SMAD4* gene can be specifically targeted as a novel therapeutic agent for patients with CRC. Given the evidence that concomitant mutations in *RAS*, *TP53*, *APC* and *PIK3CA* are associated with worse OS following hepatectomy for CRLM, the precise cooperative mechanisms of *SMAD4* with other genes of influence requires further examination. Future research is also warranted regarding the gene's impact on the recurrence rates for patients with CRLM undergoing hepatectomy.

Conclusions

In summary, *SMAD4* expression mediates an important role in the development and progression of CRC. Somatic mutations of *SMAD4* are associated with more aggressive tumor biology, poor response to chemotherapy, metastases and unfavorable OS among patients with resectable and unresectable CRC. Additionally, there is evidence that *SMAD4* mutations are significantly associated with worse OS, irrespective of *RAS* mutation status or other clinicopathological factors, in patients undergoing metastasectomy for CRLM. Given the relative frequency with which *SMAD4* mutations occur among patients with CRC, routine *SMAD4* testing may be appropriate. In the contemporary era of personalized treatment for CRC, further research on whether *SMAD4* represents a targetable mutation could have important implications for guiding clinical-decision making.

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Footnote

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

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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