SMAD4 Levels and Allelic Imbalance in 18q21 in Colorectal Cancer

To the Editor: We read with interest Alhopuro et al.’s article in a recent issue of Clinical Cancer Research (1). They showed the significance of SMAD4 levels as an important predictor of poor prognosis in colon cancer, whereas allelic imbalance in chromosome 18q21 was of no prognostic significance. As Alhopuro et al. introduced our results, we have research on the similar topic but came to different conclusions (2). In Alhopuro et al.’s study, there are two major issues to be discussed.

The first point is about the relationship between allelic imbalance in chromosome 18q21 and survival. In their study, they studied only 21 patients with allelic imbalance in 18q and 22 patients without. In our previous study, we examined a total of 319 patients with regard to allelic imbalance in 18q and could show a significant difference in survival between patients with and without allelic imbalance in 18q (P = 0.005). Other studies also confirmed the importance of allelic imbalance in chromosome 18q21 as a prognostic marker with a large number of patients (3, 4). Considering the minimum number of patients needed to show any statistical difference, their sample size seems to be too small to draw any conclusion concerning the significance of allelic imbalance in 18q.

The second point is about SMAD4 level and survival. By both protein and mRNA levels of SMAD4, they showed a significant difference in disease-free survival between patients with high and low levels of SMAD4 (P = 0.03, P = 0.003). However, in this analysis also, the number of patients seems to be too small. They examined only 7 and 10 patients with low levels of SMAD4 mRNA and SMAD4 protein, respectively. In spite of this small number of patients, they showed a dramatic difference in disease-free survival depending on levels of SMAD4. Furthermore, there is a significant problem concerning how and when patients developed recurrence, especially in patients with low levels of SMAD4. In an analysis of SMAD4 mRNA, 80% of patients with recurrence developed recurrence within 1 year after surgery. Although they stated that potentially curative surgery was done, they provided no data concerning distant metastasis status at the time of surgery. Considering recurrence developed so early after surgery, many patients seem to have developed hematogenous metastasis, which might have been already present the time of surgery. Considering these points, there seems to be a significant selection bias, especially in patients with low levels of SMAD4.

In Response: We appreciate the attention given by Watanabe et al. to our recent publication “SMAD4 levels and response to 5-fluorouracil in colorectal cancer” (1). In this article, we described the potential value of SMAD4 tumor levels to predict the prognosis of Dukes C colorectal cancer patients that had potentially curative surgery and received 5-fluorouracil-based adjuvant chemotherapy. In addition, we compared the potential prognostic value of SMAD4 tumor levels and the presence of allelic imbalance in chromosome 18q, the genetic location of SMAD4, and a genetic marker that has been reported to be of prognostic significance in colorectal cancer patients.

In their letter, Dr. Watanabe et al. comment on two important issues: (a) the relationship between allelic imbalance in 18q21 and survival and (b) SMAD4 levels and survival. (a) The relationship between allelic imbalance in 18q21 and survival. In their study, Watanabe et al. (2) present survival analysis from a total of 221 stage III colorectal cancer patients, and, as mentioned in their letter, they found that patients whose tumors had genetic losses in 18q had significantly shorter survival than patients without allelic imbalance in 18q. Also, Watanabe et al. refer to earlier studies that would agree with their findings (3, 4). However, at least one of these larger studies (3) reported that allelic imbalance in 18q was associated with poor prognosis in Dukes B but not in Dukes C patients. This clearly illustrates the contention that we introduced in our study. Although most studies in the literature seem to show poorer prognosis in stage II colorectal cancer patients with loss of heterozygosity in 18q (4, 5), there is substantial disagreement regarding the value of allelic imbalance in 18q as a prognostic marker in stage III patient, with some studies showing improved survival for patients retaining heterozygosity in this region (2, 4, 5) and other studies showing no prognostic value (3, 6–8). Although, in our study, we analyzed 18q allelic imbalance in a smaller group of patients.

References
(43 patients) compared with the study of Watanabe et al. (221 patients), the results of our analysis seem to be in agreement with a number of earlier studies, including our own previous investigations (3, 6–9). However, we cannot rule out the possibility that increasing the number of patients in the study could reveal a significant difference in the survival of patients with and without genetic losses in chromosome 18q. In any case, the conclusions from our investigations (1, 9) clearly show that assessment of SMAD4 tumor levels is a better prognostic marker than the analysis of 18q allelic imbalance in this patient series.

(b) SMAD4 levels and survival. Watanabe et al. argue that the number of samples analyzed in this study is too small to support the conclusion that low tumor levels of SMAD4 protein and mRNA are associated with shorter survival. However, the significant $P$ values obtained when comparing the survival of patients with high and low SMAD4 tumor protein and mRNA levels using the log-rank test ($P = 0.03$ and $P = 0.003$, respectively) and Fisher’s exact test ($P = 0.019$) support the conclusions of the study. Moreover, the difference in survival between patients with high and low SMAD4 tumor levels is, to a large extent, independent of the cutoff selected to categorize SMAD4 levels as high or low (see Supplementary Table S1 and S2 in ref. 1). In addition, the results of this study are in good agreement with our previous investigations showing that low SMAD4 tumor levels correlate with shorter survival in Dukes C patients that had surgery as the only form of treatment (9).

Watanabe et al. also commented on what they consider to be a "significant problem concerning how and when patients develop recurrence." As they point out, the majority of the patients in the low SMAD4 group develop recurrence within the first 18 months following surgery. Watanabe et al. have not found any data in our publication concerning existence of distant metastasis at the time of surgery. In colorectal cancer, this information is provided by Dukes staging. All the patients in this study were staged as Dukes C and, therefore, no distant metastasis had been detected in any of the patients at the time of initial surgery. The authors are of the opinion that low SMAD4 levels in the primary tumor of these patients may be indicative of a higher probability of distant micro-metastasis that were undetectable at the time of surgery, and that this is likely to be responsible for the earlier recurrence and shorter survival in the low SMAD4 group. This is in good agreement with the results of our own previous study conducted with Dukes C patients that received no adjuvant chemotherapy following surgical removal of their primary tumors (9). Therefore, we do not think that there is "a significant selection bias" in the patients enrolled in the study, but rather believe that the ability of SMAD4 to identify patients that are likely to develop early recurrence represents a strength of SMAD4 as a marker of prognosis.

Pia Alhopuro
Lauri A. Aaltonen
Department of Medical Genetics, Haartman Institute, Biomedicum, University of Helsinki, Helsinki, Finland

References
Reply to the Letter to the Editor by Watanabe et al.

Diego Arango, Pia Alhopuro and Lauri A. Aaltonen


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/12/5/1654.1

Cited articles
This article cites 9 articles, 4 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/12/5/1654.1.full#ref-list-1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://clincancerres.aacrjournals.org/content/12/5/1654.1. Click on "Request Permissions" which will take you to the Copyright Clearance Center's Rightslink site.