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We read with interest the article titled "Frequent LOH at Chromosome 12q22-23 and *Apaf-1* Inactivation in Glioblastoma" by Watanabe et al (1). We would like to bring your attention to the major site change of the location of *Apaf-1* gene on chromosome 12q.

The authors studied the LOH status of 12q22-23, the locus of *Apaf-1* gene, using 2 microsatellite markers D12S1657 and D12S393 according to a previously cited article (2). They described that these microsatellite markers were located at ends of the *Apaf-1* locus; D12S1657 centromeric and D12S393 telomeric. However, following the publication of the previous study in 2001 (2), a reassessment of the *Apaf-1* gene location was made. The current information of the National Center for Biotechnology Information database indicates that the *Apaf-1* gene is located more distal (>0.3 Mb) from the chromosome 12q centromere. Based on this corrected location, both markers D12S1657 and D12S393 are at the centromeric side. This information is directly accessible at the following uniform resource locator (URL) of the Internet, *http://www.ncbi.nlm.nih.gov/mapview/maps.cgi?ORG=human&CHR=12&MAPS=sts[96M:98M],genes[96M:98M]-r&compress=off*. The authors have not accordingly identified LOH affecting the *Apaf-1* locus. In the authors' results summarized in Table 2, cases #18 and #24 have LOH on D12S1657 but are heterozygous on D12S393. These cases were determined to be LOH positive, but because D12S393 is actually located between D12S1657 and *Apaf-1*, the cases should now be categorized as heterozygous. Therefore, a re-analysis of the results is mandated with attention to the additional microsatellite markers located at the telomere side of *Apaf-1* gene. Because the locations of multiple genes have changed with the conclusion of the human genome project, investigators need to accurately verify locations of genes in question.

The authors have clearly shown the relation between the LOH status, mRNA expression levels and tissue protein levels. However, the value of this study remains uncertain secondary to the incorrect location of the *Apaf-1* gene. For example, with corrections in cases #18 and #24, the statistical significance between LOH status and mRNA expression level would be lost. Therefore, we express our concerns regarding the change of the gene locus. Furthermore, in this study, the authors evaluated case #5 as heterozygous with non-informative results in both D12S1657 and D12S393. However, there is no basis for such evaluation. Cases with no information should be removed from the analysis or treated as missing values. We consider that it is necessary to re-investigate with additional markers and strongly hope that this important study will be accurately revised.

REFERENCES

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2. Soengas MS, Capodieci P, Polsky D, Mora J, Esteller M, Opitz-Araya X, McCombie R, Herman JG, Gerald WL, Lazebnik YA, Cordon-Cardo C, Lowe SW (2001) Inactivation of the apoptosis effector Apaf-1 in malignant melanoma. Nature 409:207-211.

AUTHOR'S RESPONSE

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We are grateful to Drs Umetani and Hoon for presenting new and important information about the location of the *Apaf-1* gene on chromosome 12q.

According to their new information, the LOH markers used in our study (1) are both located proximal to the chromosome 12q centromere. While our original results suggested a consistent correlation between the 12q22-23 LOH and inactivation of *Apaf-1*, the new information from Umetani and Hoon prompted us to perform several additional studies, including an LOH study using the correct distal marker located at the telomere site of *Apaf-1* gene.

In our study, cases #18 and #24 have an LOH on D12S1657 but not on D12S393. It remains unclear whether or not one allele of the *Apaf-1* gene is deleted in these cases. However, the correlation between LOH in *Apaf-1* gene and mRNA expression may still be statistically significant, as the distal markers can be expected to show LOH. In our relatively small-scale examination, the additional findings of several new cases could easily change the statistical result. For this reason, we have indicated in our article that a larger study will be needed in the future.

Our study and the previous study by Soengas et al (1) demonstrated the relationship between the locus of 12q22-23 LOH and *Apaf-1* inactivation. Some cases in these studies might show *Apaf-1* inactivation without correct LOH on the locus of the *Apaf-1* gene. As described in the discussion in our article, methylation might occur on the *Apaf-1* gene of both loci in these cases.

Progress in the new gene findings advances the frontiers of our pathology and oncology fields. We are collecting a large number of tumors and continuing to study genetic abnormalities associated with glioblastoma, including abnormalities of the *Apaf-1* gene. The methylation study will be ongoing, for example. We would like to add the LOH studies using some additional distal makers to the *Apaf-1* locus in our ongoing projects. We also would like to present the LOH findings in relation to the 12q22-23 LOH and *Apaf-1* gene status.

REFERENCES

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