

Digital Imaging for Statistical Analysis of Tissue Microarrays

Daizo Yoshida and Akira Teramoto

Department of Neurosurgery, Graduate School of Medicine, Nippon Medical School

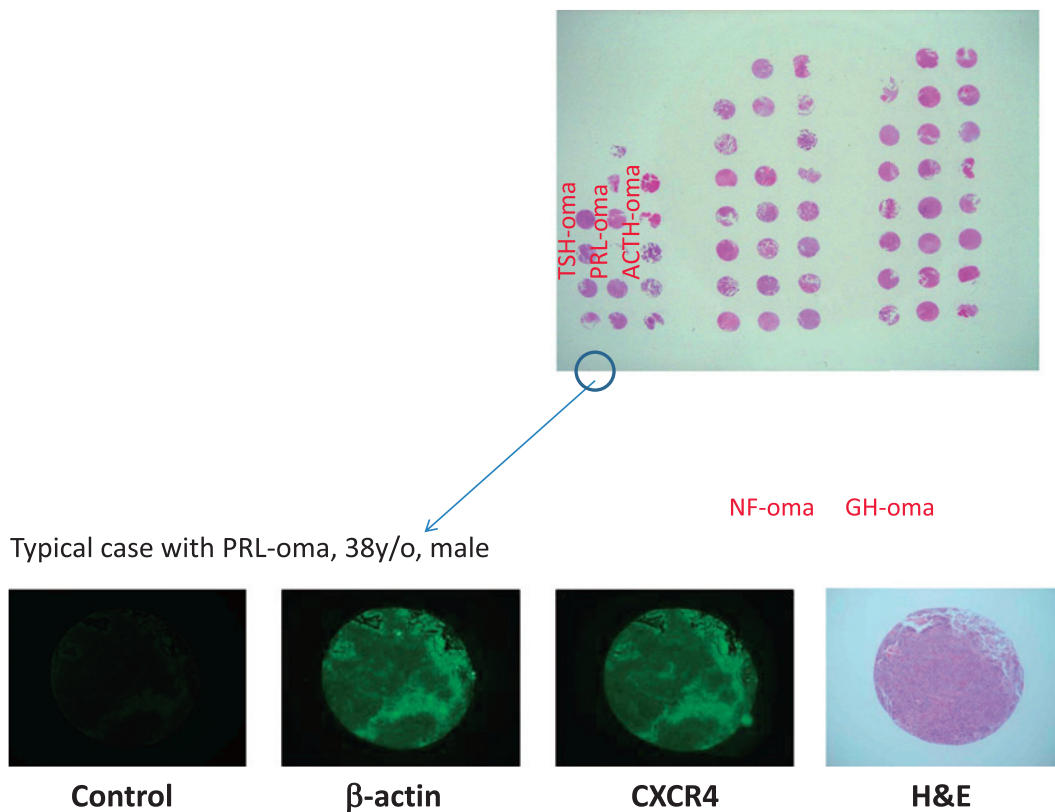


Fig. 1

Introduction

Immunohistochemical study includes morphological to determine the locations of target antigen in single specimens, and statistical analysis of multiple cases is often necessary to compare subgroups, particularly in tissue microarray analysis. However, the histochemical examination usually yields, at most semiquantitative data, therefore comparison by means of a grading system, such as (-) to (+++), to evaluate the extent of expression cannot be considered strictly statistical. The purpose of the present study was to perform a statistically valid analysis of issue microarray.

Material and Methods

Tissue microarrays were prepared of paraffin-embedded surgical specimens of 60 pituitary adenomas, 23 somatotrophinomas (GH-omas), 22 non-functioning pituitary adenomas (NF-omas), 6 prolactinomas (PRL-oma), 5 aderenocoroticotropinomas (ACTH-oma), and 4 thyrotropinomas (TSH-oma). Thin-sliced sections were confirmed with hematoxylin and eosin staining. Subsequently, the serial sections were examined with fluorescence immunohistochemistry for chemikine (C-X-C motif) receptor 4 (CXCR4), β -actin, and normal-host serum as a

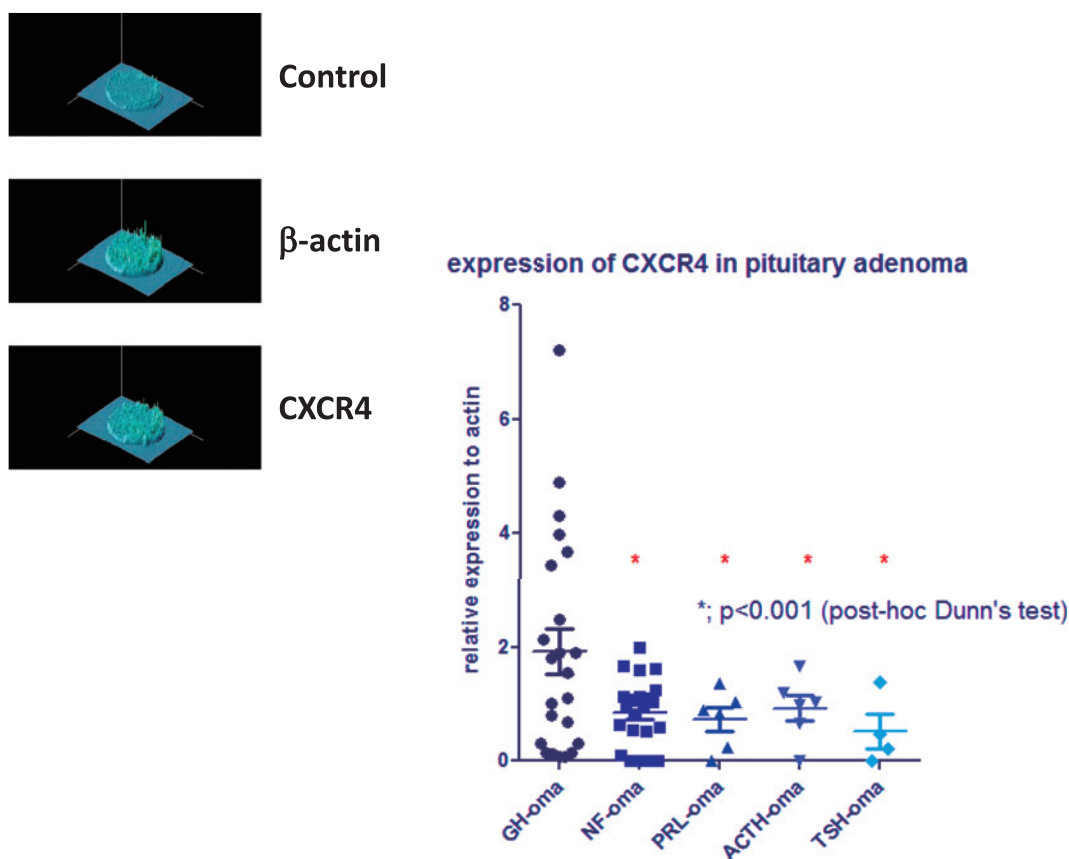


Fig. 2

control, and a fluorescein isothiocyanate (FITC)-conjugated second antibody was used. To analyze quantitative data, pixels of FITC expression were digitized with an imaging analysis software program (Image-Pro Plus, version 5.0, Media Cybernetics, Inc., Bethesda, MD, USA).

Results

Tissue microarray analysis showed that CXCR4 was clearly expressed in tumor parenchymal cells (**Fig. 1**). The CXCR4 expression was clearly demonstrable after digital image processing with Image-Pro Plus and the distribution of expression could then be statistically analyzed (**Fig. 2**).

Conclusion

In immunohistochemical analysis, quantitative clarification with digitized pixel data is highly accurate and is more beneficial than conventional analysis with a grading method, which is dependent on classification by each researcher.

Fig. 1 Immunohistochemical studies to detect expression with FITC and hematoxylin and eosin staining in tissue microarrays.

Fig. 2 Quantitative measurement of FITC pixels in each microarray. The relative expression of CXCR4 to β -actin was analyzed after subtracting control values.