



Integration of digital image data to TMA databases for high-throughput analysis of EGFR expression in giant cell tumors of bone (GCTB)

Levente Ficsor¹, Peter Balla¹, Linda Moskovszky¹, Vivien Angeli², Tibor Krenacs², Miklos Szendroi¹, Zoltan Sapi¹, Laszlo Kopper¹, Béla Molnár¹

1 2nd Department of Internal Medicine, Semmelweis University, Budapest, Hungary.

2 3DHISTECH Ltd, Budapest, Hungary.

krenacst@gmail.com

Abstract

Introduction: Tissue microarrays (TMA) allow for high-throughput biomarker analysis of large number of tumor samples in a cost- and time-efficient way under standardized conditions. However, linking and management of the enormous amount of clinical, pathological, epidemiological and image data of the samples requires database management. Digital TMA slides are ideal to be integrated in such a complex database system.

Methods: In this work we used the comprehensive TMA system developed by 3DHISTECH Ltd. for producing, scanning and analyzing of TMA slides of 450 samples of 275 GCTB cases tested for epidermal growth factor receptor (EGFR) expression. TMA blocks containing 70 or 80 tumor samples of 2 mm diameter each were created using the novel computer driver arrayer, TMA Master, allowing Excel database import and linking of core and patients data. EGFR protein was detected using both the Ventana Confirm (Tucson, AZ, USA; clone: 3C6) mouse monoclonal antibody and the standardized EGFR PharmDx kit (clone: 2-18C9), and EGFR gene copy number was tested with FISH (ZytoVision, Bremerhaven, Germany). Immunostained slides were digitalized with the Mirax Scan instrument (3DHISTECH; Zeiss, Gottingen, Germany) and analyzed with the TMA Module software of the Mirax Viewer platform using both empirical scoring and the HistoQuant image analysis software.

Results: 47% of studied GCTB cases were either moderately (2+), or strongly (3+) positive for EGFR, further 25% were weakly positive and only 28% were fully negative, which data were validated with image analysis. Double immunolabeling showed that the EGFR positive cells, located usually adjacent to osteoclasts, were consistent with the potentially malignant stromal cells. Recurrence and transformation into osteosarcoma were significantly more frequent in strongly EGFR positive cases. As opposed to the female predominance of GCTB, EGFR positive cases were significantly more frequent in male patients (M/Fm=1.5).

Conclusion: Our results suggest that about half of the studied GCTB cases are under the influence of EGFR signaling, which may contribute to their growth and progression including recurrence, and/or transformation. The advanced digital technology used in the project proved to be highly efficient in saving us enormous amount of time and effort, while supporting validated scoring and analysis.

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