



OLYMPUS SYMPOSIUM

Automated Tissue Microarray image analysis to identify and quantitatively determine tumour relevant proteins.

Daniel Göttel

Application Specialist, Olympus Soft Imaging Solutions GmbH, Berlin, Germany.

Stefanie.Conrad@olympus-europa.com

Abstract

Introduction: With the rise of high-throughput technologies and their application in the global analysis of tumour specific alterations of the genome, transcriptome or proteome, a multitude of candidate genes and proteins have been identified. For testing, large and clinically well annotated tumour collections need to be analysed. The tissue microarray (TMA) technique, has been proven to be particularly useful in such large scale testing. The technique permits immunohistochemistry (IHC) experiments to be carried out at well defined standard operating parameters. Problems using this technique arise with both, the misinterpretation introduced during the conventionally practiced, manual analysis by the pathologist and with the subjective component presented by the experimenters due to their varying level of expertise. A method for the automated analysis of TMAs was developed to overcome individual examination errors.

Methods: The TMA slides were digitised using a conventional microscope (for IHC) or a fluorescent scanner (for immunofluorescence - IF). Subsequently these images could be used as 'virtual slides' to perform various analyses. IHC labelled TMAs were analysed both manually and automatically and the results were compared. The method was also applied to tumour-biological research. A novel approach to automated TMA-analysis was developed by the introduction of the multi-fluor immunofluorescent technique. Here, two or more different fluorochrome-tagged antibodies were simultaneously used in IHC experiments to define a digital mask for specific tumour compartments (e.g. epithelial areas are labelled with epithelia-specific antibodies) and automatically analyse a second 'test-antibody' using heterogeneous tumour material. The work was performed at the German Cancer Research Institute (DKFZ) in Heidelberg Germany.

Results: The relevance of the method was demonstrated using a number of different examples, e.g. for expression levels of the anti-apoptotic proteins HMGB1 and c-IAP2 in colorectal carcinoma. The simultaneous use of phosphor-specific antibodies against STAT5 and STAT6, and their unphosphorylated forms respectively can be employed to analyse the activity of cellular components of signalling cascades on tumour-TMAs (e.g. JAK/STAT signalling).

Conclusion: Automated TMA-analysis is a big step towards the standardisation of large scale testing to identify tumour subgroups, which are characteristic of the activation of specific signal transduction pathways.