Pathology-2013

OMICS Group Conferences
Accelerating Scientific Discovery

2nd International Conference and Exhibition on
Pathology

August 05-07, 2013 Embassy Suites Las Vegas, USA

Phenotyping TILs *in situ*: Automated enumeration of FOXP3+ and CD69+ T cells in follicular lymphoma

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Abstract

In many cancers, tumor-infiltrating lymphocytes (TILs) indicate levels of tumor immunogenicity and predict survival. In particular, increased levels of regulatory T cells (Tregs) are associated with poorer prognosis, whilst CD69+ T-cells may also be prognostic. Understanding the phenotype and pattern of TILs in situ within tumors would be advantageous. However, visual TIL assessment cannot easily determine the type of lymphocyte in situ and multimarker quantitation is difficult with standard methods. We present a multi-marker, computer-aided event-counting method for determining the phenotypes of lymphocytes in follicular lymphoma using a multispectral imaging (MSI) automated tissue segmentation and counting approach.

Material and methods: A tissue microarray containing follicular lymphoma (FL) cores from 70 patients was chromogenically immunostained for CD3, CD69 and FOXP3, counterstained with hematoxylin, of which 40 cores were informative for both triplex staining and clinical follow-up. Each core was imaged using MSI and the individual staining of each marker separated from each other using spectral unmixing. Images were analyzed using software trained to recognize different tissue compartments based on morphology, specifically based on CD3 rich (extra-follicular) and poor (intra-follicular) areas. The FOXP3 or CD69 status of each CD3+ TIL was then determined and number Treg (FOXP3+/CD3+) and CD69+ T-cells counted in the intra- and extra-follicular areas.

Results: The intra-follicular (CD3 poor) and extra-follicular (CD3 rich) regions were accurately recognized within each core, based on abundance of CD3 cells. MSI enabled the accurate quantitation of CD3, CD69 and FOXP3 without crosstalk. The number of FOXP3+/CD3+ Tregs and CD69+ T-cells were counted in each core and used in Kaplan-Meier survival analysis, which demonstrated association of FOXP3+/CD3+ Tregs with favourable outcome in both the intra- ($p=0.0173$) and extra-follicular ($p=0.0173$) areas, as well as CD69+ T-cells in intra-follicular ($p=0.0175$) areas; CD69+ T-cells were not prognostic in extra-follicular areas ($p=4509$).

Conclusions: This study demonstrates use of an automated method for counting Tregs in follicular lymphoma, showing association of FOXP3+ Tregs with good outcome. Given the generic nature of the method automated multiplexed tissue cytometry analyses are feasible for routine clinical studies and work with many multiplexed IHC staining methodologies, of importance for translational cancer studies in general.

Biography

James R. Mansfield is a scientist with over 20 years of experience in in-vivo spectroscopy, spectral imaging and applied data analysis, directed towards finding of novel optical methods for the diagnosis and monitoring of medical conditions. He is currently the Director of Tissue Analysis at PerkinElmer where he is the senior applications scientist for their multispectral imaging and digital pathology systems, which are being used in a wide range of fluorescence and bright field microscopy applications. Before PerkinElmer he worked at Cambridge Research & Instrumentation, where he helped develop their MSI systems. Prior to that he worked at the National Research Council of Canada as a research scientist and at several small companies developing non-invasive spectroscopic methodologies. His research has included projects ranging from the objective classification of skin cancer spectra using mid-infrared spectral imaging, to developing methods for the non-invasive determination of the severity of rheumatoid arthritis, to the development of the first of several spectral imaging systems able to map out skin oxygenation levels. He holds 6 patents, has numerous pending patent application and 48 publications in these fields and has served as an invited speaker, session chair and organizer at a variety of international conferences.