Tissue quality continues as significant obstruction to international comparison of tumor subtypes using biomarkers: Lymphoma classification by the Sub-Saharan Africa Lymphoma Consortium

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Abstract

Introduction

Collection, fixation and processing of tissues have profound effect on subsequent antibody and probe biomarker testing using the common clinical detection techniques of immunohistochemistry (IHC), chromogenic in situ hybridization (CISH) and fluorescent in situ hybridization (FISH). Large numbers of markers can be surveyed for purposes of international comparative tumor phenotype using archived clinical formalin-fixed paraffin-embedded tissues (FFPET). We collaborated within the international Sub-Saharan Africa Lymphoma Consortium (SSALC/NCI) to analyze biomarkers for classification of a large number of lymphomas from South, East and West regions of Africa.

Method

Malignant lymphoma cases submitted to the SSALC from South, East and West and reference collaborators were assembled into multiple tissue microarrays (TMA; Beecher Instruments, Silver Spring, MD). One to two (1 mm diameter) tissue cores from each FFPET block were placed in a recipient paraffin TMA block and sections stained with antibodies to CD20, CD10, CD3, CD5, Bcl-2, Bcl-6, MUM-1 and Ki-67 (IHC, Dako), EBV status (CISH, Vantana) and c-MYC (FISHba). Core retention and stains were evaluated for strength and consistency of antibody/probe reactions. Study was at the Pathology Core Laboratories (PCF), The Ohio State University. Stain results were evaluated by authors.

Results

Quality of fixation was reflected in histologic detail, fragility of the tissue cores with fracture and biomarker clarity and reactivity. Stains were positive within many of the tissue cores. Ki67 and Bcl-6 were most adversely affected by poor tissue fixation; CD20 was least affected. Other markers were variable and FISH signals were sometimes absent. CISH for EBV generally performed well.

Conclusions

Tissues vary in histomorphology and biomarker clarity by IHC, CISH and FISH due to tissue preservation quality, particularly among international sites. While TMA technology offers a rapid and economical method for evaluation of biomarkers in large collections, tissue quality remains the greatest obstruction to accurate international comparative evaluations of tumor phenotypes.

Background

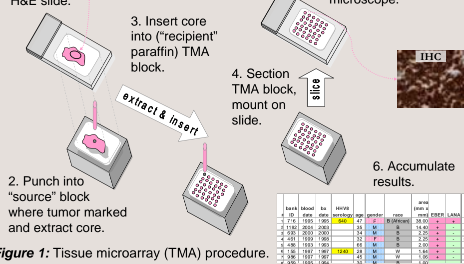

Figure 1: Tissue microarray (TMA) procedure.

Figure 2: (top) Instruments used for TMA construction: (left) hand-held Quick-Ray (UNITMA Co., Ltd., Seoul, Korea), (center) manual bench top MTA I (Beecher Instruments, Sun Prairie, WI, USA), (right) semi-automatic TMArrayer (Pathology Devices, Westminster, MD, USA). (bottom) All can be used to construct equally useful TMA blocks. Photos courtesy of the manufacturers.

Method

- One or two (1 mm diameter) tissue cores were removed from 1200 lymphoma cases from three geographical regions, East, West and South sub-Saharan Africa and organized into tissue microarray recipient blocks using a manual tissue-arraying instrument (Beecher Instruments, Silver Spring, MD). TMAs were examined for lymphoma subtype and immunophenotype.
- Sections of the TMA were cut, mounted on adhesive slides and stained with antibodies to CD20, MUM-1, CD10, CD3, CD5, Bcl-2, Bcl-6, and Ki-67. EBV tissue status was defined by EBER (CISH)
- Stains were evaluated for strength and consistency of antibody reactions, and concordance with expectations from whole tissue H&E stained sections. TMA construction and staining was at the Pathology Core Laboratories, The Ohio State University Comprehensive Center's Innovation Centre, Columbus, Ohio, USA.

Results

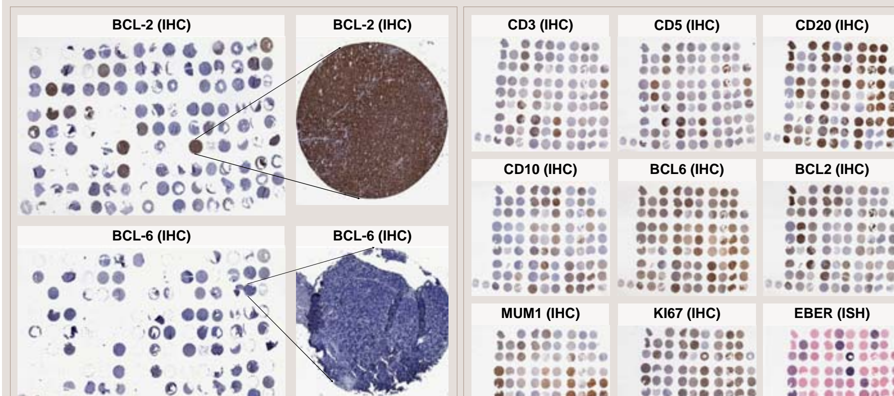

Figure 3: TMA and tissue cores from hard and brittle donor paraffin blocks. Note the thickness of the core sections, a tendency to break and fold, and partial or complete loss of tissue core section from the TMA section. These block and core section conditions are likely from poor fixation and excessive processing times. Images scanned with ScanScope XT (Aperio, Vista, CA, USA).

Figure 4: Images of TMA sections stained with antibodies (IHC-Dako) or *in situ* hybridization (ISH) within suitable tissue with adherent cores. All biomarkers were positive within at least some of the tissue core sections.

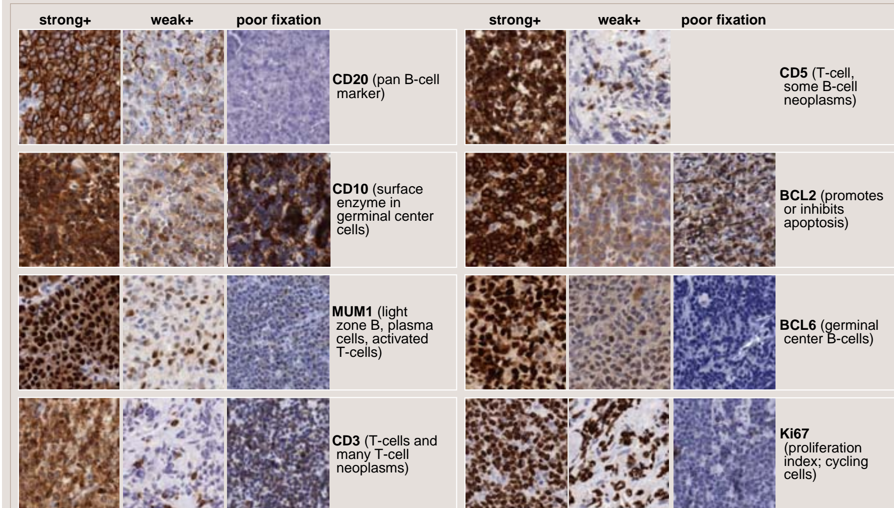

Figure 5: Antibody (IHC) stain results. The technical quality of CD10 and Bcl-6 were most adversely affected by tissue fixation/processing. Occasional CD20 biomarkers were faint due to necrosis or tissue preservation. Biomarker positive cells in crushed tissues were distorted but still evaluable.

Conclusions

- TMA technology offers a rapid and economical method for evaluation of biomarkers in large tissue collections but tissue quality remains the greatest obstruction to accurate international comparative evaluations of tumor phenotypes using this method.
- The use of un-buffered formalin may account for some differences in immunoreactivity due to tissue acidity but does not appear to be the only factor.
- Paraffin blocks that are hard and too brittle to core without the use of a paraffin softening agent (Nair®) are also not able to be cut at 4-5μ and thicker, brittle core sections tend not to adhere well to glass slides – likely due to excessive processing times in poorly fixed tissues.
- Existing archived tissues are only adequate for immunophenotyping and biomarker surveys if tissues are properly fixed and have controlled processing times at the time of accessioning because without buffer the tissue become acidic and the paraffin hard and too brittle for TMA cores.

Acknowledgements

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