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"Optimization of Dual-Color Immunofluorescence Protocols for Formalin-Fixed, Paraffin-Embedded Archival Tissues"

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Study Findings

We developed a protocol for producing high-contrast, high-quality immunofluorescence images from formalin-fixed paraffin-embedded (FFPE) thymus blocks prepared up to 60 years ago. This dual-color immunofluorescence protocol enables state-of-the-art archival-tissue image analyses. The protocol also enables pathological analyses using smaller sample quantities. It will thus be instrumental in studies that use precious specimens obtained from A-bomb survivors.

Explanation

Archival FFPE tissues are generally unsuited for immunofluorescence staining because of tissue quality degradation and background noise from autofluorescence. This was particularly true for FFPE tissues from A-bomb survivors stored at RERF for as long as 60 years. Dr. Junko Kajimura, research scientist, RERF Department of Molecular Biosciences, et al. recently developed a combination of tissue processing and staining techniques that minimize noise from autofluorescence[*] and maximize fluorescence signals from cells under study. We also developed a dual-color immunofluorescence protocol that, with the latest digital image processing techniques, provides images of high contrast and quality.

* Autofluorescence: Intrinsic fluorescence from cells and tissues and sometimes difficult to distinguish from fluorescence of stained cells under study.

1. Objectives

The objective was to develop a dual-color immunofluorescence protocol suitable for analyzing the FFPE tissue specimens of A-bomb survivors stored at RERF.

2. Methods

Using 10 FFPE archival thymus tissue specimens from A-bomb survivors in Hiroshima prepared between 1954 and 1970, we employed the dual-color immunofluorescence protocol to stain thymic epithelial cells with anti-CK14 antibody* and cortical thymocytes with anti-CD1a antibody.* Attempting to improve the staining and image analysis techniques, we (1) included multiple independent steps to enhance the specificity of immunostaining; (2) used chemical processing procedures to reduce noise from autofluorescence without losing fluorescence signals in tissues; and (3) produced high-quality, high-contrast fluorescent images provided by the fluorescence microscope's digital image processing functionality.

*Anti-CK14 antibody: An antibody that reacts with type I keratin expressed in the cytoplasm of thymic epithelial cells (CK14)

*Anti-CD1a antibody: An antibody that reacts with a membrane glycoprotein expressed on cortical thymocytes (CD1a)

3. Results

We obtained high-quality, high-contrast, low-noise dual-color fluorescent images of

thymic epithelial cells and cortical thymocytes from old archival FFPE thymus tissues through (1) the inclusion of multiple independent steps for tissue processing and staining; (2) treatment with NH_3/EtOH* and removal of autofluorescence with Sudan Black B*; and (3) enhancement of the signal-to-noise ratio using digital image processing.

*Treatment with NH_3/EtOH: Tissue sections are treated with a mixture of ammonia (NH_3) and ethanol (EtOH) to remove cross-links (stable bonds of proteins) formed by formalin fixation, expose amino acids comprising antigenic determinants, and consequently increase their reactivity with antibodies.

*Removal of autofluorescence with Sudan Black B: Staining FFPE tissue sections with 0.3% Sudan Black B reduces autofluorescence (noise) attributable to blood cells or elastin fibers in FFPE tissue sections without affecting fluorescence signals from fluorescent-labeled antibodies.

Significance of the Study

The improved protocol we developed provided high-quality dual-color fluorescent images using FFPE tissue specimens (thymic epithelial cells and cortical thymocytes) stored up to 60 years in the past. This protocol is considered to be suitable also for immunofluorescence staining of other types of archival specimens besides thymus tissues.

The Radiation Effects Research Foundation has studied A-bomb survivors and their offspring in Hiroshima and Nagasaki for more than 60 years. RERF's research achievements are considered the principal scientific basis for radiation risk assessment by the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) and for recommendations regarding radiation protection standards by the International Commission on Radiological Protection (ICRP). RERF expresses its profound gratitude to the A-bomb survivors and survivors' offspring for their cooperation in our studies.

§***Journal of Histochemistry & Cytochemistry*** is a peer-reviewed scientific journal of cell biology established in 1953. It covers research into the structure and function of cells, tissues, and organs as well as components of development, differentiation, and disease, in addition to microscopy and imaging techniques. (Impact factor for 2014: 1.959)