Fixation Delay Adversely Impacts Androgen Receptor Staining

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Abstract

Background: Autopsy studies (AS) can be of utility in assessing protein expression and the results can shape clinical medicine. For example, the only method to obtain large numbers of metastatic prostatic adenocarcinoma (CaP) tissues is to perform an autopsy. Analysis of Immunohistochemical Stains

The results can shape clinical medicine. For example, the only method to obtain large numbers of metastatic prostatic adenocarcinoma (CaP) tissues is to perform an autopsy. Immunohistochemical staining was analyzed using FRIDA (Framework for Image Dataset Analysis), our custom open source image analysis software (Fig. 2).

• The delay before fixation and the fixative of choice must be considered when interpreting IHC stains, especially in autopsy material, and tests should be run to determine whether the specific analyte in question is stable to delayed fixation.

• In formalin-fixed tissues, AR-1, AR-2, C-MYC, and NKX 3.1 stains showed a stepwise decrease in both the percentage of cells stained and the staining intensity (Fig. 1, 3 and 4).

• In contrast, staining with AMACR and H2AX showed a stepwise increase with delayed fixation (Fig. 3 and 4).

• C9, and Filibrarin staining was stable throughout fixation delay, and interestingly, Phospho-S6 staining also remained remarkably stable for the first 3 hours (Fig. 1 and 3).

• In contrast to formalin fixation, there was little or no staining with AR-1, AR-2, C-MYC and H2AX in ETOH-fixed tissues, while CK-8, Filibrarin, NKX 3.1 and Phospho-S6 staining remained comparable to formalin-fixed tissues.

Materials and Methods

• 5 mycotic nude mice were xenografted with LNCaP and LAPC-4 prostatic adenocarcinoma cells.

• After 2 months, the mice were sacrificed and subjected to either formal or 95% ethanol (ECHO) fixation after a delay of 0, 1, 3, 24 or 48 hours.

• Tissues were stained immunohistochemically with two different anti-androgen receptor (AR) antibodies, AMACR, C9, C-MYC, Filibrarin, H2AX, NKX 3.1 and Phospho-S6. A mouse Homozygous-Tmrt (HETmrt) strain was also performed.

• Immunohistochemical staining was first manually assessed, followed by computerized image analysis of selected stains.

• Staining percentage was manually assessed as the percentage of cells showing any staining.