Ischemia / Fixation Trial Tissue Microarray

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Introduction
Since its introduction over 10 years ago, tissue microarray (TMA) technology has become an indispensable tool in biomedical research as TMAs facilitate high-volume, standardized and quality-assured testing of biomarkers. Although there are clear benefits to their use in biomarker discovery, TMAs have some significant weaknesses:

- Dependent on good quality tissue: TMAs are constructed from large cohorts of archival material that have undergone a series of essential treatments to achieve optimal preservation of morphological structure.
- Dependent on standardized laboratory techniques: histological treatments (grossing, processing and paraffin-embedding) are well defined in literature, however information about pre-analytical parameters are most often unrecorded, introduce significant variability beyond the control of investigators, and constitute a major potential source of bias, whose magnitude is poorly understood.

The most critical variables implicated in expression alterations are ischemia and fixation.

Hypothesis & Aims
Delayed intervals to fixation and fixation length affect the properties of the tissues analyzed in downstream molecular applications.

To better understand the impact of fixation and ischemia on the final IHC result, the Prostate Cancer Biorepository Network (PCBN) has conducted a trial using radical prostatectomy specimens with known ischemic and fixation intervals. This trial includes two arms:

1) Fixation Length: analyses the impact on degree of fixation between samples immersed in fixative for different intervals controlling rate of formalin penetration and biological inactivation by grossing fresh sample before fixation.

2) Delayed Interval to Fixation: compares the impact of differing ischemic intervals by delaying immersion of samples into fixative, controlling for length of fixation.

Various lengths of and delayed intervals to fixation will be evaluated on a single radical prostatectomy specimen (n=42).

Method

Fixation Length (n=27)

- 0hr sample is immersed in 10% NBF and placed in PBS until processing
- 4hr sample is removed from 10% NBF and placed in PBS until processing
- 8hr sample is removed from 10% NBF and placed in PBS until processing
- 12hr sample is removed from 10% NBF and placed in PBS until processing
- 24hr sample is removed from 10% NBF and placed in PBS until processing

Imagery

Immunohistochemical Analyses

The TMA will be assessed with known markers of ischemia, fixation and tissue quality (p27 and phospho-antibodies).

Conclusion

Tissue quality, consequently pre-analytical variables remains the greatest obstruction in biomarker discovery. To understand the impact of pre-analytical variation, biomarkers need to be studied of a controlled range of variability in key parameters. This TMA will be available to researchers accessing PCBN for validating antibodies, determining the significance and magnitude of bias introduced by these variables.

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