Background: Human tissue is subjected to pre-analytical conditions before preservation that impact tissue quality. Some conditions are known to affect gene expression levels, i.e. duration of warm ischemia, duration of autolysis, high protein pre-operative diet. Developing a metric to assess preservation of RNA, DNA, protein could help to both select out for experiments low quality samples and develop a tissue preservation fingerprint for normalizing gene expression levels.

Materials and methods: We assessed multiple metrics of tissue quality in snap frozen samples from 50 prostatectomy patients (time interval of vessel ligation to receipt in the lab, time interval to freezing, and Bioanalyzer RIN) and from 15 rapid autopsies (following death from metastatic prostate cancer) - histologic preservation, percent of necrosis, time interval between death and freezing, and Bioanalyzer RIN. We also evaluated immunohistochemically defined expression of beta-actin and beta-2-microglobulin by 20 primary prostate cancers and of PSA and PSAP in 8 prostate tissues where tissue aliquots were let autolyze in a humid atmosphere at either 37°C or 5°C for 8 time intervals of between 1 and 56 hours.

Results: RIN had virtually no correlation with time to freezing of prostatectomy tissue (range: 20 to 70 minutes) or of rapid autopsy samples (range: 1.5 to 6 hours; r < 0.9). Extent of necrosis of autopsy samples (range: 0 to 20% necrosis) did not predict RIN. Main intensity of immunoreactive PSA and PSAP was variable, decreasing by 3 hours in tissue kept at 37°C and by 4 hours in tissue kept at 5°C. However, some samples kept at 5°C for 32 hours were indistinguishable from 1 hour samples. Finally, immunoreactivity for beta-actin and beta-2-microglobulin was highly heterogeneous within a sample. Conclusion: Better metrics of tissue quality, of nucleic acid and protein integrity, and reference housekeeping genes are urgently needed.

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Abstract

RNA integrity number (RIN): A measure of RNA integrity. Higher RIN values indicate better RNA quality.

Histology: A microscopic examination of tissue samples. It can show the presence of necrosis or other pathological changes.

Immunohistochemistry: The use of antibodies to detect the presence of specific proteins or cells in tissue samples. It can help assess the preservation of immunoreactivity.

Results

Correlation between RIN number and ratio of 5' vs. 3' amplified housekeeping gene beta-actin, using RNA extracted from prostate tissue samples. The ratio of 5' vs. 3' beta-actin is a potential indicator of quality of RNA in tissue samples. Note: The poor correlation between RIN and the extent of necrosis (right).

Discussion

Based on our studies, we think that (1) better metrics for quality of tissue macromolecules need to be developed, and (2) identification of the steps in tissue handling that most affect quality of macromolecules need to be undertaken. These steps span the range from post-macromolecular steps (slow degradation of mRNA) to the function of a single step (normal protein degradation).

These “Biopspecimen Lifecycle” schematizations were adapted from earlier work and have been modified by the creation of Dr. Helen Moore.