Abstract: Since access to high quality human biospecimens has been identified as a critical resource to support genomic- and proteomic-based studies, the NCI established the Office of Biospecimens and Biospecimen Research (OBBR) to coordinate biospecimen-related policies and practices for NCI-supported biospecimen resources. Few studies have been published demonstrating the relationship between specimen handling, quality, and reproducibility of data in cancer research. The NCI Biospecimen Research Network (BRRN), organized by the OBBR, is conducting biomolecular studies for the development of appropriate data-driven, evidence-based practices and protocols for specific specimen types and molecular analysis platforms.

The present study examines the influence of warm ischemic time on RNA quantity, quality, and gene expression profiles in colon cancer tissue. Twenty-eight cases of matched frozen colon-normal and cancer tissue samples with four cases per five-minute interval from 20 to 50 minutes were microdissected using laser capture microdissection (LCM) and RNA purified. RNA was quantified by Nanodrop and quality determined by Bioanalyzer. After RNA amplification and labeling, gene expression was assessed for cancer samples using Affymetrix Human U133 Plus 2.0 GeneChips. Differential expression was compared using the shortest ischemic time with other ischemic times were performed using principal component analysis, hierarchical clustering, and ANOVA. There was no correlation between ischemic time and RNA quantity and RNA quality. All cancer samples showed good to excellent RNA quality with minimal RNA degradation. The study demonstrated that warm ischemic time in colon cancer may give rise to artifactual changes in expression of genes that are significant in colon cancer.

Conclusions and Future Experiments:
- No correlation between RNA quality and ischemic time in colon cancer.
- Colon cancer showed good RNA quality compared to ischemic times.
- Normal colon tissue showed excellent RNA quality.
- Further validation using Signature analysis to find overlap candidate genes.

Figure 1: LCM of colon tissue (A) Cancer and (B) Normal.
Figure 2: Recovery and quality of RNA from microdissected (A) colon cancer and (B) normal colonic epithelium. (C) Quantity and quality for both normal and cancer. (D) Percent probe sets called present for cancer samples.
Figure 3: Gene expression analysis using Z-transformation and Quantile Normalization to find overlap candidate genes.
Figure 4: Gene expression profile, biological gene ontology and canonical pathways of 128 candidate genes for colon cancer.
Figure 5: Gene expression profile and gene ontology of 128 candidate genes for colon cancer in different ischemic times.
Figure 6: Gene expression profile and gene ontology of 31 candidate genes found in patient 1.
Figure 7: Gene expression profile and gene ontology of 31 candidate genes found in patient 2.

Table: Statistically significant genes of clinical significance that are affected by ischemic times.

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>TP53</td>
<td>Tumor protein p53</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit A</td>
</tr>
<tr>
<td>PTEN</td>
<td>Phosphatase and tensin homolog</td>
</tr>
<tr>
<td>RB1</td>
<td>Retinoblastoma 1</td>
</tr>
<tr>
<td>CDK4</td>
<td>Cyclin-dependent kinase 4</td>
</tr>
</tbody>
</table>

Materials and Methods:
- Microdissection and RNA isolation:
  - Total 56 colon specimens (28 matched normal/cancer).
  - Tissue sections cut at 8μm thick sections; Hematoxylin and Eosin stained.
  - Microdissected tumor and normal epithelium with 3000 shots (approximately 15,000 cells) in less than 45 minutes.
  - Isolated total RNA using PicoPure Kit with 15 minute DNase digestion.
- RNA Quality and Quantity:
  - Bioanalyzer for quality. RNA Integrity Numbers (RIN) 25, 28/18S ratio ≥ 8.8
  - Nanodrop for concentration.

Different morphological and biomolecule analysis techniques require different methods of biospecimen preservation.