RNA stability in the human liver tissue and the ileum mucosa

Celine Schelcher¹, Serene Lee², Stefanie Schreiber¹, Wolfgang Thasler¹²

1. Tissue Bank under the authority of Human Tissue and Cell Research Foundation (HTCR), Grosshadern Hospital, Ludwig Maximilians University, Munich, Germany
2. Department of General, Visceral, Transplantation, Vascular and Thoracic Surgery, Grosshadern Hospital, Ludwig Maximilians University, Munich, Germany

Introduction

The usage of remnant tissues obtained from consented patients represents a valuable resource for biomedical research. For research purposes, it is of a great importance to acquire and determine whether tissues with longer processing times or different methods of storage are still of a sufficient quality for scientific research and can be included within a tissue bank.

Methods

For each tissue piece:
- RNA extraction
- Ribogreen assay
- RNA integrity (Bioanalyzer)
- cDNA synthesis
- Real time Q-PCR analysis

Results (1): RNA quality in the liver

RNA integrity has been determined by the Agilent Bioanalyzer. The RIN number obtained for each condition is shown above. Even after 24h of leaving the tissue at room temperature, a RIN number of 8 was obtained, which shows that the quality of the RNA is still suitable for gene expression studies.

Results (2): RNA quality in the ileum mucosa

Effect of the ischemia time on the RNA quality in the mucosa of the ileum.

Following tissue collection, RNA was extracted from 30 mg of either snap-frozen (blue) or RNAlater (red) stored tissues. RNA samples (200 ng) were loaded onto the RNA 6000 Nano chip (Agilent technology) and RNA integrity was assessed by the Agilent Bioanalyzer through the production of a RIN number. RNA extracted from tissues with warm ischemia up to 90 min (A) and cold ischemia up to 6h (B) remains of a very high quality (RIN number 8 and above). The storage of tissues in RNAlater resulted in a slight improvement in RNA quality in comparison to snap-frozen tissues. This experiment was performed with 5 different donors.

*p<0.05 significantly different between + RNA later.

The gene expression profiling of the ileum tissues is currently under investigation.

Conclusion

The take home message from this study is that even though the RNA is an easily degradable molecule, if longer processing times are required for biobanking tissues (up to 24h for liver and 6h for ileum tissues), it does not significantly influence the isolation of intact RNA from the liver and the ileum mucosa. The gene expression profile for the ileum is still under investigation but for now we know that longer processing times and different methods of storage of liver tissue did not affect gene expression and such tissues can still be used for scientific research. Snap-frozen tissue or tissues stored in RNAlater are both adequate for biobanking of liver and ileum mucosa.