RESEARCH COLLECTION OF VARIANTS OF NORMAL AND FATTY DISEASE HUMAN LIVERS

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Abstract

XenoTech has developed a Research Biobank that is a collection of normal, steatosis and steatohepatitis tissue samples gathered and characterized to facilitate the study of human liver disease with an emphasis on the progression of fatty liver disease. A portion of each of the human livers, which were harvested with the intent of transplantation but subsequently rejected for this purpose, and were obtained through partnerships with non-profit Organ Procurement Organizations (OPO) which are members of the United Network for Organ Sharing (UNOS), is saved in the Research Biobank. Donor-specific data provided by the organ procurement organizations includes demographics, cause of death, BMI, and alcohol and diabetes history. Pathologist's review of the H&E slides includes classification of the samples into normal, steatosis or steatohepatitis categories and quantification of macrovesicular fat, inflammation, ballooning hepatocytes and fibrosis. Presence of fibrosis is confirmed with Masson's Trichrome staining. Tissues deposited in the bank are flash frozen in liquid nitrogen and stored at -80°C. Cells isolated from multiple tissues deposited in the bank are available as cryopreserved hepatocytes for culture in suspension or as an attached cell monolayer. These cells are suitable for an array of studies including *in vivo*/*in vitro* correlation of drug metabolism and biomarker expression that characterize fatty liver disease. The bank contains normal, steatosis and steatohepatitis specimens, with and without a history of alcohol use. Photomicrographs of H&E slides of each tissue can be viewed at the www.xenotech.com. The levels of CYP2A6, CYP2C19 and CYP3A4 mRNA expression in normal (10), steatosis (19) and steatohepatitis (11) specimens were analyzed by RT-PCR. The relative quantification of each of the enzymes was based on the $\Delta\Delta C_T$ with GAPDH serving as an endogenous control. The relative quantification of the mRNAs was not affected by tissue pathology (ANOVA). In conclusion, we have established a Research Biobank, derived from transplantation-rejected organs, that is focused on alcoholic and non-alcoholic fatty liver disease.

Introduction & Objectives

It is generally believed that chronic liver disease affects expression of hepatic xenobiotic metabolizing enzymes and may increase risk of adverse drug effects. Effects of fatty liver disease, with or without significant history of alcohol consumption (nonalcoholic fatty liver disease, NAFLD) on levels of mRNA, protein or enzymatic activity of major CYP enzymes has been a subject of multiple, but often contradictory, studies. It appears that the type of liver disease and its severity modulate expression of the enzymes in a gene-specific manner. Difficulty in conclusive interpretation of these studies may be related to small number of samples representing individual types of liver pathology, diagnostic criteria applied, lack of information on donors' smoking, alcohol consumption and genotype of polymorphically expressed enzymes, and integrity of the mRNA or protein samples used in some of the investigations.

Currently rising prevalence of NAFLD and its progression to nonalcoholic steatohepatitis (NASH), with increased probability of patients developing even more serious liver diseases, emphasizes a need to firmly establish the risks of adverse drug interaction associated with chronic liver disease, and more broadly, to characterize illness-specific regulation of xenobiotic transformation. Collections of relevant human liver specimens are needed to facilitate study in this area. XenoTech's objective of this work has been to create such a research tool by gathering and characterizing normal, steatosis and steatohepatitis tissues in the Research Biobank.

The Research Biobank collection of tissues can support investigation of the effects of liver disease on drug metabolism and provides a well-characterized *in vitro* tool to study pathology-dependent biotransformation of new molecular entities.

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Materials & Methods

Human livers, which were harvested with the intent of transplantation but subsequently rejected for this purpose due to lack of proper match, were obtained through partnerships with non-profit Organ Procurement Organizations (OPO) which are members of the United Network for Organ Sharing (UNOS). Upon arrival of the organ at XenoTech on wet ice, a portion of the liver is rapidly frozen in liquid nitrogen while the bulk of the tissue is utilized for the isolation of hepatocytes. Another small portion of the tissue is fixed in phosphate-buffered formalin for subsequent embedding in paraffin and preparation of hematoxylin and eosin (H&E) slides. Tissues are stored at -80ºC, paraffin blocks at 4ºC. Donor-specific data provided by the organ procurement organizations includes demographics, cause of death, BMI, and alcohol and diabetes history. Often, additional non-identifying data related to donors' hospitalization are available. Pathologist's review of the H&E slides, in addition to classifying the samples into normal, steatosis or steatohepatitis categories, quantifies macrovesicular fat, inflammation, presence of ballooning hepatocytes and fibrosis. Presence of fibrosis is confirmed with Masson's Trichrome staining. Hepatocytes isolated from multiple tissues deposited in the bank are available cryopreserved for culture in suspension or as an attached cell monolayer. These cells are suitable for studies including in vivo/in vitro correlation of drug metabolism and fatty liver disease biomarkers.

Details of hepatocyte isolation and RT-PCR procedures were described elsewhere (Madan et al., 2003; Paris et al., 2009; Czerwinski et al., 2014).

Results

XenoTech's Research Biobank has current inventory of normal (28), steatosis (23) and steatohepatitis (11) specimens and it is expected to increase by about 30 specimens per year. **Fig. 1** represents typical histological H&E staining of (**A**) normal liver tissue (macrovesicular fat <5%), (**B**) steatosis (macrovesicular fat >5%), (**C**) steatohepatitis (lobular inflammation, ballooning necrosis), and (**D**) Masson's Trichrome staining confirmation of bridging fibrosis (same donor as in C).

Most of the tissues in the bank are from adult donors, although 14 pediatric tissues, age 1 month to 4 years old were also collected. NAFLD has high prevalence in obese pediatric cohorts (Pacifico et al., 2010). **Fig. 2** compares (**A**) normal pediatric donor tissue (17 year old, female, Caucasian) and (**B**) one with early micro- and macrovesicular fat deposits (7 years old, female, Caucasian).

Nonalcoholic steatohepatitis (NASH) patients are very rare among intended organ donors. **Table 1** summarizes characteristics of these donors deposited in the bank. Information about alcohol use by these donors was provided by the next of kin.

Table 1. Categories of data characterizing tissues deposited in the XenoTech Research Biobank

Donor	Pathology Comments	Steatosis %	Age	Gender	Ethnicity	BMI	Diabetes	Alcohol
H1027	Steatohepatitis, scattered ballooned hepatocytes, centriolobular steatosis	50	63	F	Caucasian	43	Yes	No
H1028	Steatohepatitis, scattered ballooned hepatocytes	70	51	F	African American	40	Yes	No
H1060	Steatohepatitis, scattered ballooned hepatocytes	50	49	M	Hispanic	44.9	Yes	No
H1069	Steatohepatitis, scattered ballooned hepatocytes	25	39	M	Caucasian	62.7	No	No

Isolated and cryopreserved hepatocytes from some of the donors are available. In rare cases cryopreserved hepatocytes from donors with steatosis are plateable (H1235, BMI 31.9, macrovesicular fat 30-40%; H1249, BMI 28, macro- and microvesicular fat 5-10%).

Intact total RNA can be isolated from the tissues deposited in the bank within the past six years. **Fig. 3** illustrates intactness of the ribosomal RNA bands in the representative group of samples. In a preliminary study, levels of CYP2A6, CYP2C19 and CYP3A4 mRNA expression in normal (10), steatosis (19) and steatohepatitis (11) specimens were analyzed by RT-PCR. Relative quantification of each of the enzymes was based on the $\Delta\Delta C_T$ with GAPDH serving as an endogenous control. The mRNA levels of expression were not affected by tissue pathology (ANOVA, data not shown).

Figure 1. Histological representation of normal (A), steatosis (B) and steatohepatitis (C, D) donor organs

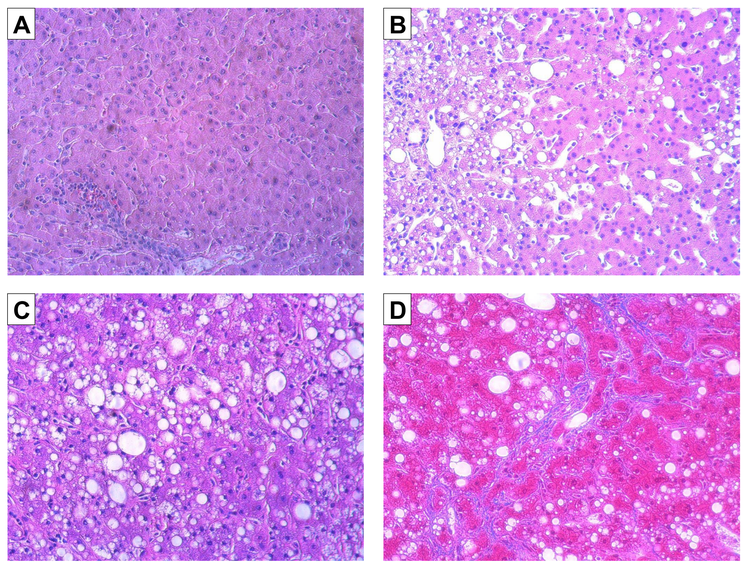


Figure 2. Histology of normal and macrovesicular fat content in pediatric donor livers.

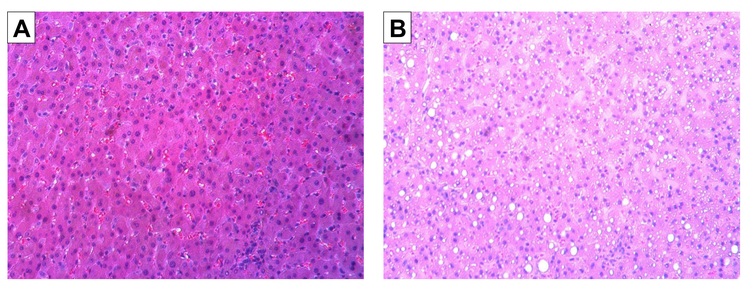
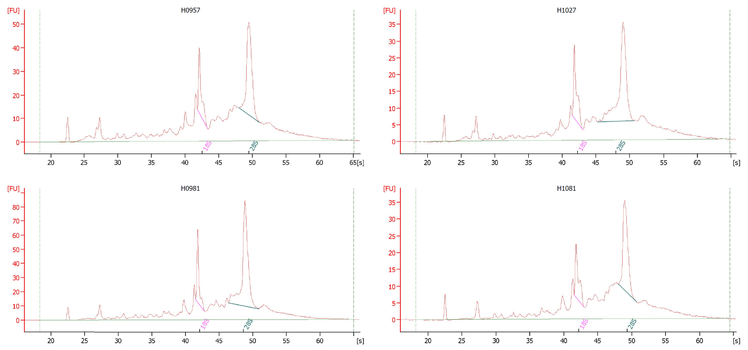


Figure 3. Demonstration of intact 18S and 28S ribosomal RNA in tissues stored up to five years in the Biobank.



Conclusions

XenoTech has established the Research Biobank of normal, steatosis and steatohepatitis liver tissues.

The Research Biobank collection of tissues constitutes a well-characterized *in vitro* tool for investigation of the effects of fatty liver disease on drug metabolism of established drugs and of new molecular entities. Additionally the tissues can be utilized in studies of biomarker expression that characterize progression of fatty liver disease.

References

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