

## **Metabolomics analysis of human pancreatic cancer tissue and paired adjacent tissue samples.**

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### **Abstract**

**Background/aims:** This study aimed to apply <sup>1</sup>H spectrum Nuclear Magnetic Resonance spectroscopy (1H-NMR) to detect the metabolites in pancreatic cancer tissue and paired adjacent tissue samples.

**Methods:** 48 samples were taken from cancer tissues of 24 pancreatic cancer patients and paired adjacent tissues. The samples were subjected to <sup>1</sup>H-NMR and principle component analysis to compare endogenous small-molecule metabolites.

**Results:** Leucine and isoleucine levels were significantly higher in pancreatic cancer tissues than in control tissues. Lactate, betaine, choline, α-glucose and α-hydroxybutyrate levels were significantly lower in pancreatic cancer tissues than in control tissues.

**Conclusions:** <sup>1</sup>H-NMR-based metabolomics is an effective method to identify small-molecule metabolites in pancreatic cancer tissues. These metabolites can be used as the metabolic markers of pancreatic cancer for early diagnosis.

**Keywords:** Metabolomics, 1H-NMR, Extracted liquid, Pancreatic cancer, Metabolic markers.

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### **Introduction**

Pancreatic cancer is one of the most serious malignant tumors with low radical operation rate and high mortality. For pancreatic cancer patients, the median survival is only 3 to 6 months and five year survival rate is less than 5% [1-4]. In United States 48,960 people were estimated to be diagnosed with pancreatic cancer, and 40,560 people were estimated to die of pancreatic cancer in 2015 [5]. Although the incidence of pancreatic cancer is lower in China than Western countries, it increases rapidly in recent years. 34,509 men and 23,226 women died of pancreatic cancer in 2010 [6-8].

The etiology of pancreatic cancer is unknown and it is difficult for early diagnosis of pancreatic cancer. In this study, we used <sup>1</sup>H-NMR to identify low molecular metabolites of pancreatic cancer tissues and paired adjacent tissues from patients with pancreatic cancer. Furthermore, we performed principal component analysis to explore the characteristic metabolites of pancreatic cancer which will provide a new approach for early diagnosis of pancreatic cancer.

### **Materials and Methods**

#### **Clinical samples**

Tissue samples were collected from pancreatic cancer patients who visited Department of General Surgery, including 24 samples of pancreatic cancer tissues and paired 24 samples of tumor adjacent non-tumor pancreatic tissues. Intraoperative

rapid frozen pathology confirmed tumor tissues and adjacent tumor free pancreatic tissues. All pancreatic cancer patients were diagnosed by pathological examination.

#### **NMR and principal component analysis**

Tissue extracts were prepared as described previously [9]. The samples were lyophilized or nitrogen dry. For NMR analysis, water soluble extract samples were kept at 25°C on a Bruker avance DRX 600 NMR spectrometer (Bruker, Germany), using one-dimensional pre-saturation 1D NOESY spectra (NoesyPr1d). The spectrometer was equipped with three resonance probe and Z axis pulse gradient field, the proton resonance frequency was 600.13 MHz. Spectrum data were analysed using principal component analysis.

### **Results**

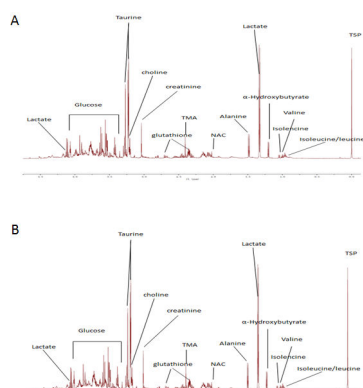
<sup>1</sup>H NMR spectroscopy analysis of endogenous metabolites of pancreatic cancer and paired adjacent tissue samples were shown in Figure 1. Table 1 listed the metabolites that showed significant changes between pancreatic cancer and paired adjacent tissue samples.

**Table 1.** List of the metabolites with significant changes between pancreatic cancer and paired adjacent tissue samples.

Metabolites	Main characteristic peak chemical shift	Cancer vs. adjacent to cancer
Leucine	0.942-0.961	↑

Isoleucine	0.950-0.982	↑
Lactate	1.311-1.332	↓
Betaine	3.684	↓
Choline	4.079	↓↓
A-glucose	3.406-5.243	↓
$\alpha$ -Hydroxybutyrate	1.195-1.215	↓↓

↑, ↓ p<0.05; ↓↓ p<0.01.



**Figure 1.** 600 MHz hydrogen spectrum NMR spectroscopy. (A) Pancreatic cancer tumor samples. (B) Paired adjacent tissue samples.

## Discussion

Because of unnoticeable symptoms and deep location, pancreatic cancer often develops silently without symptoms at early stage. At present, the diagnosis methods of pancreatic cancer include: (1) Tumor markers: current markers lack sensitivity and specificity for early lesions. (2) Gene detection: current clinical trials have not yet found a specific gene for pancreatic cancer. (3) Imaging diagnosis: ultrasonic gastroscopy and Endoscopic Ultrasonography guided Fine Needle Aspiration biopsy (EUS guided FNA), Positron Emission Computed Tomography (PET-CT) and Multislice Spiral Computed Tomography (MSCT) and Magnetic Resonance Imaging (MRI). Imaging technologies lack the sensitivity and specificity of the tumor with a diameter smaller than 2 cm.

Hydrogen spectrum based nuclear magnetic resonance is a new approach for qualitative and quantitative analysis of metabolomics of the organism or cell. The present study was to explore the use of hydrogen spectrum nuclear magnetic resonance spectroscopy to identify low molecular weight metabolites in pancreatic cancer tissue. Using principal component analysis, we found that leucine and isoleucine levels were significantly higher while lactate, betaine, choline, alpha-glucose, alpha-hydroxybutyrate levels were significantly lower in pancreatic cancer tissues compared to adjacent non-tumor pancreatic tissues.

Leucine and isoleucine are essential amino acids. Leucine and isoleucine play an important role in the metabolism of glucose in the liver by downregulating gluconeogenesis and inhibiting the expression of rate limiting enzyme for gluconeogenesis [10]. Pancreatic carcinoma tissues had high levels of leucine and isoleucine compared with adjacent tissues which had active glucose metabolism. Furthermore, alpha-glucose level was lower in tumor tissues than the paired adjacent tissues, consistent with the results of leucine and isoleucine.

Several studies have suggested the link of betaine intake and reduced risk of nasopharyngeal carcinoma and colorectal cancer [11,12]. This is in agreement with our results that the level of betaine in pancreatic cancer tissue was significantly lower than that in the adjacent tissues.

Serum alpha hydroxy acid levels increased in diabetes. Varvel et al. found that the potential cause of the rapid increase in the serum levels of alpha hydroxy acid was the damage of insulin secretion [13]. Many studies have found a correlation between pancreatitis, pancreatic cancer and diabetes. In this study, the level of alpha hydroxy acid in pancreatic cancer tissue was significantly lower than that in the adjacent tissues.

Lactic acid is the product of anaerobic fermentation. Tumor tissue is prone to hypoxia, ischemia and blood supply deficiency, and the level of lactic acid increased significantly in many tumor tissues [14,15]. However, in the present study we found that the level of lactate in the extract of pancreatic cancer tissue did not increase. Further studies are needed to understand the underlying mechanism.

In conclusion, this study showed that endogenous small molecule metabolites of pancreatic cancer tissue and paired adjacent tissue samples were significantly different. Application of hydrogen spectrum NMR metabolic group method to explore the characteristics of low molecular metabolites in the tissue extracts of pancreatic cancer patients with pancreatic carcinoma tissues and paired adjacent tissue can provide a new approach for early diagnosis of pancreatic cancer.

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