Chapter - 6

**Serum Evaluation of Biomarker Targets in Pancreatic Cancer**

**Attributes:** Validation of all six swELISAs on patient serum samples was performed by Melissa Johnson from Dr. Hanash’s lab, Fred Hutchinson Cancer Center, Seattle, USA.
6.1 INTRODUCTION

Sandwich ELISAs have been developed and validated for all six targets (LAD1, LAMB3, ITGB6, JUP, DKK1 and CHI3L1) used in the current study. To demonstrate that any of the above targets can serve as potential biomarkers in PDAC, their expression levels need to be evaluated in normal healthy donors versus pancreatic cancer patients. Since Chronic Pancreatitis (CP) behaves very closely with PDAC both clinically and morphologically, one of the major challenges for the early detection of pancreatic cancer will be distinguishing it from CP. In most cases PDAC is misdiagnosed as CP or vice versa. Therefore, evaluation of the biomarker targets in CP is also necessary. The present chapter focuses on the serum evaluation of LAD1, LAMB3, ITGB6, JUP, DKK1 and CHI3L1 in three groups of serum samples: normal healthy donors, patients with pancreatic cancer and chronic pancreatitis.

6.2. MATERIALS AND METHODS

6.2.1. Sandwich ELISA

Sandwich ELISA assay was performed as described in the previous chapter (Section 5.2.3). In brief, EIA/RIA plates were coated with target-specific capture antibody overnight at 4°C. Plates were blocked with 1x TBS-tween/0.05% BSA for 1 hour at RT. Serum samples were diluted in blocking buffer 10-25 fold and 100 µL of the diluted samples were added to each well and incubated for 2 hours at RT. Biotinylated detection antibody was then added to the plate and incubated for 1 hour at RT.
Streptavidin-HRP was added to the plate and incubated for 1 hour at RT. Plates were developed with HRP substrate and read at 450nm in a spectrophotometer.

**Patient samples:** Serum samples were obtained from patients with pancreatic cancer (n=30), CP (n=10) and age and sex matched healthy donors (n=20). All sample collected procedures were followed according to the IRB-approved protocols with approved consents from the subjects. Serum samples were diluted 10-25 fold in blocking buffer. All samples were analyzed in duplicate. Results are reported as the mean ± SD.

**Data analysis:** Statistical analysis and generation of graphs were performed using Graph Pad Prism software. Student T tests were used to compare two groups of data sets and one way ANOVA tests were used to analyze the three groups of clinical samples that include normal healthy donors, patients with pancreatitis and those with pancreatic cancer. Data significance (generation of p value where ever mentioned) was evaluated by using the above mentioned tests.

**6.3. RESULTS AND DISCUSSION**

**6.3.1 Serum evaluation of LAD1, DKK1 and CHI3L1**

LAD1, DKK1 and CHI3L1 and were tested on the serum samples to evaluate the expression levels of these three targets. Figure 6.1a shows the determination of serum LAD1 in 30 PDAC patients, 10 patients with CP and other benign pancreatic disorders.
and 20 normal healthy controls. Higher levels of LAD1 were observed in sera from patients with PDAC and CP compared to those from normal healthy donors. One way ANOVA analysis suggested that this observation was significant ($p = 0.0374$). However, it should be noted that serum levels of LAD1 in CP patients were higher than those of PDAC.

For DKK1, mean expression levels of the protein were maximal in pancreatitis patients among the three groups. Average serum levels of DKK1 were higher in PDAC compared to those of the normal individuals although a couple of samples showed relatively higher expression in PDAC. However, none of these data were statistically significant (Figure 6.1b).

Mean serum levels of CHI3L1 were found to be minimal in normal donors followed by patients with CP and then PDAC. Relatively higher expression of CHI3L1 was observed in two PDAC samples (Figure 6.1c). However these data were not statistically significant. Interestingly, these same samples had higher DKK1 and LAD1 expression in PDAC.
Figure 6.1. Serum levels of LAD1 (6.1a), DKK1 (6.1b) and CHI3L1 (6.1c) in PDAC, CP and normal sera. ELISA assays were tested on serum samples from three groups of individuals: normal healthy donors, patients with Chronic CP and patients with PDAC. Each dot in the graph represents one sample. Mean values with standard errors were shown in the graph. Serum samples were diluted 10-fold in blocking buffer.
6.3.2 Serum expression of LAMB3, JUP and ITGB6

Evaluation of serum expression for LAMB3, JUP and ITGB6 was performed in the same way as done for the other three targets. However, the serum levels of the targets could not be quantified due to the unavailability of standard curve. Therefore, the expression levels were compared based on the OD values obtained in the ELISA assay. Mean serum expression of LAMB3 was higher in PDAC and CP compared to that of the normal donors, with expression in CP being the highest (Figure 6.2a). It is hard to draw any conclusion for ITGB6 because the expression levels were too low to compare (Figure 6.2b). On the other hand, expression of JUP was found to be almost similar in PDAC and CP (Figure 6.2c). However, these two groups showed higher expression of JUP compared to that of the normal donors. None of these data were statistically significant. However, interestingly, the same two samples that had higher expression of LAD1, DKK1 and CHI3L1 also showed high expression of LAMB3 and JUP, although some other samples had high expression levels of these targets in PDAC.

These data indicate that the biomarker targets used in this study were up-regulated in pathological conditions such as CP and PDAC compared to normal, healthy conditions.
Figure 6.2. Serum levels of LAMB3 (6.2a), JUP (6.2b) and ITGB6 (6.2c) in PDAC, CP and normal sera. ELISA assays were tested on serum samples from three groups of individuals: normal healthy donors, patients with Chronic CP and patients with PDAC. Each dot in the graph represents one sample. Mean values with standard errors were shown in the graph. Serum samples were diluted 10-fold in blocking buffer.
Our major goal of this study was to evaluate potential biomarker proteins using our ELISA assays on patient serum samples leading to the development of a clinical application for early detection of PDAC. Even though no statistically significant differences were observed among the three groups for many targets, the results were suggestive of elevated levels of these proteins in PDAC compared to that of the normal healthy donors. This data are also indicative of some potential of these targets for early detection of PDAC. Interestingly, 4 patient samples showed higher expression of at least four out of six targets tested. This strongly suggests that the combined evaluation of expression of more than one target could be very helpful in the early detection of PDAC. However, these findings are preliminary. To clearly determine the role of these proteins in the early detection of PDAC, further studies with well designed protocols, detailed clinical information and analysis of a large number of patients with early stage PDAC will be required. In conclusion, novel swELISA assays for LAD1, LAMB3, JUP and ITGB6 were developed and DKK1 and CHI3L1 ELISA kits were validated for the identification of PDAC. These antibodies as well as the ELISA assay could serve as valuable tools for various clinical and epidemiological studies which would allow for early detection of PDAC.