Chapter - 7

Conclusion
7.1 SUMMARY

Pancreatic cancer is one of the most lethal forms of cancer and patients die with in few months after diagnosis. This is mostly due to the lack of effective diagnostic tools and identification of the disease at very late stages. Early detection of pancreatic cancer is one of the major challenges in clinical pancreatology. Availability of effective biomarkers and sensitive diagnostic assays will greatly help the treatment of PDAC patients. In the present study we proposed to develop and validate novel diagnostic assays for potential biomarker targets, which include LAD1, LAMB3, JUP, ITGB6, CHI3L1 and DKK1, in PDAC. Efforts were also made to verify if any of these biomarkers has the potential to serve as tumor markers in the early detection of PDAC. As a proof of principle, swELISA was chosen as a diagnostic tool to verify the biomarker protein expression in pancreatic patient sera.

Chapter 1 provided a brief introduction of the current study. Problems that lead to the inception of present study were first discussed. Hypothesis was then generated to address these problems. Based on this hypothesis, objectives were framed out to perform the study. At the same time it is also important to consider how other fields are dealing with the challenges involved with the biomarker studies. Therefore, all possible opportunities were explored to achieve the goals of the current study. A thorough experimental design was provided as per the study objectives. Based on the overall experimental approach, an outline of the study design was laid out. Finally the study limitations were discussed at the end of the chapter.
The second chapter was principally focused on the work that has been done so far in the field of biomarker studies. This chapter presented a brief introduction to the cancer biomarkers followed by the milestones in the cancer biomarker field. The role of advanced technologies that include proteomics and genomics in the identification of novel biomarkers has been discussed. In fact, the list of protein markers used in the present study was generated based on proteomic analyses of PDAC patient sera by various researches. Various types of biomarkers employed in the cancer diagnosis and treatment and their characteristics were then discussed. At the end, a brief outlook was given on currently available diagnostic tools for PDAC.

Development of a diagnostic assay requires proper control reagents to verify the quality of the assay. Whole cell lysates that over-expressed the target proteins were used as controls in this study. Chapter 3 is mainly focused on the generation of these screening reagents that were later used to validate the antibodies and swELISA assays. Gateway cloning technology was employed to over-express the biomarker proteins that contained N-terminal FLAG, c-Myc and C-terminal HA and V5 epitope tags. A novel destination vector, pDS_LPCX_5’ FTM-3’ HAV5_XB that facilitated the generation of fusion proteins with both N- and C-terminal tags was developed. In this study, expression vectors for all biomarker targets were developed and their expression in HEK 293T cells was successfully verified by WB and swELISA assays.

Chapter 4 discussed mouse mAb generation for LAD1 and LAMB3. BALB/c mice were immunized with target-specific peptides and spleens from immunized mice were
fused with mouse myeloma cells. Resulting hybridomas were evaluated for target-specific antibodies by three independent assays that included indirect ELISA, WB and swELISA. Antibodies were then purified using FPLC technique. Purified mAbs were validated by assays other than swELISA to determine if they can be used for any other assays such as IP and WB.

Chapter 5 principally focused on the development and validation of ELISA assays for biomarker targets. Using mouse mAbs generated at our Monoclonal Antibody Core or purchased from vendors, swELISA assays were developed for LAD1, LAMB3, JUP and ITGB6. Since commercial ELISA kits were available for DKK1 and CHI3L1, these pre-made assays were just validated. To demonstrate that the ELISAs recognized the recombinant proteins, all assays were successfully tested on whole cell lysates over-expressing the target protein. Specificities of the assays were demonstrated using RNAi experiments. Assays were then validated on cell lysates from PDAC cell lines to prove that they can detect endogenous proteins. Finally the assays were validated on control serum samples.

Expression of biomarker targets was finally evaluated in serum samples from normal healthy donors, patients with PDAC and patients with chronic pancreatitis. This was discussed in Chapter 6. Since CP is a non-cancerous condition but behaves very closely with PDAC, expression of the targets was verified in CP for differential diagnosis. In order to verify the baseline expression of the targets, their expression was
evaluated in the serum samples from normal healthy donors. Extensive statistical data analysis was performed using Graphpad Prism software.

7.2 CONCLUSIONS

- In this study novel destination vector, pDS_LPCX_5’ FTM-3’ HAV5_XB was developed that can be used to generate fusion proteins with both N- and C-terminal epitope tags by Gateway cloning technology. Using this destination vector, expression vectors for LAD1, LAMB3, JUP, ITGB6, DKK1 and CHI3L1 were developed.

- Mouse mAbs were generated for LAD1 and LAMB3. LAD1 antibodies can be used for swELISA and immunoprecipitation and LAMB3 antibody can be used for swELISA and WB assay.

- In the present study a unique swELISA assay was developed for the early screening of hybridomas. As this assay was performed using parental hybridomas, it served as a powerful tool for early screening of mAbs.

- From the results presented in Chapter 3, we can conclude that the development of swELISA assays for LAD1, LAMB3, ITGB6 and JUP and validation of DKK1 and CHI3L1 ELISA kits was successful.

- Serum evaluation of the biomarker targets confirmed that all targets were over-expressed in pathological conditions. Maximum levels of LAD1, DKK1, and LAMB3 were found in CP. Where as, the same for CHI3L1 was found in PDAC. In case of JUP, the levels were same in both PDAC and CP.
7.3 SIGNIFICANCE OF THE STUDY

One of the major goals of the present study was to develop novel diagnostic assays to detect potential serum biomarkers in pancreatic cancer. The results strongly suggest that this goal has been achieved. We strongly believe that the novel swELISA assays could serve as valuable tools for various clinical and epidemiological studies that might lead to early detection of pancreatic cancer. Furthermore, a novel Gateway destination vector was developed that could be used for various validation studies. The expression clones developed for various targets in this study might be useful to investigate the biological role of these proteins in pancreatic cancer. Novel monoclonal antibodies for LAD1 and LAMB3 that were developed in this study could serve as valuable tools for various other assays such as IP and WB. Sandwich ELISA data on the serum samples is strongly suggestive of elevated levels of these targets in PDAC patients indicating that they have some potential to serve as serum tumor markers in PDAC. Our assay validation approach could serve as an excellent model for several other similar studies.

7.4 FUTURE DIRECTIONS

The past three years of my graduate student life has provided invaluable experience in the field of biomarker study. We strongly believe that the limitations of the study have opened new pathways for further investigation of the target proteins. With a Human Proteome Project looming on the horizon, it is time for the research community to critically review the options available for the discovery of novel biomarker targets that
might play a key role in the early detection of dreadful diseases such as pancreatic cancer.

Although the clinical findings in this study are preliminary, to clearly determine the role of these targets in the early detection of PDAC, further studies with well designed protocols, detailed clinical information and analyzing larger cohorts with early stage PDAC will be required. Regardless of the actual structure of the project, a high level of coordination and clearly defined goals will be central to the success of any future large-scale projects in biomarker study. The results in this study were strongly suggestive of using these biomarker targets in combinations for PDAC diagnosis at an early stage. This was based on the fact that 3-4 patient serum samples showed relatively higher expression of more than two same targets. Biomarker combination studies can be performed by conjugating the antibodies with various types of fluorescent probes. Since many of the antibodies used in this study work for WB assay, it would be interesting to test them by Immunohistochemistry. This could provide valuable information and open gates for various epidemiological studies where tissue sections are used for IHC.

Another interesting area that requires further exploration is the biological role of the tumor markers that were discussed in the present study. Although the role of integrins and catenins has been well investigated in the cancer pathogenesis, biological role of some other targets including LAD1 has not been studied well. The current study has clearly indicated that LAD1, LAMB3 and CHI3L1 were up-regulated in pathological
conditions. Therefore, it would be very interesting to investigate their role in the PDAC pathogenesis. Furthermore, investigation of these targets at the molecular level using advanced techniques such as SNP analysis might provide valuable insights regarding possible genetic alterations in these targets.

Finally, in the light of the rapidly emerging novel technologies such as bioinformatics, proteomics and genomics, the field of biomarker study is undergoing a paradigm shift.