Role of circular RNAs in pancreatic cancer

Principal investigator: Sönke Detlefsen (e-mail: sde@dadlnet.dk. Phone: 6541 4806)
Professor, Senior consultant, Ph.D., Department of Pathology, Odense Pancreas Center (OPAC), Odense University Hospital (OUH)
Dept. of Clinical Research, Faculty of Health Sciences, University of Southern Denmark (SDU)

Background

***Background*:**
In the pancreas research group at the Dept. of Pathology, OUH, we are currently offering a research project focusing on the role of circular RNAs (**circRNAs**) in non-resectable pancreatic cancer. The candidate should be either a Biomedicine student (**master thesis** (Danish: **kandidatspeciale)**) or a Medicine student ("**prægraduat forskningsår**").

Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer. Around 1.000 new cases of PDAC are diagnosed in Denmark each year. Median overall survival is only 8 months. Surgical resection is the only option for long-term survival, but can be offered to only 20%, and even after surgery the median survival is 24 months. Subtyping of PDAC is currently not part of patient management. No cllinically relevant predictive biomarkers are in clinical use. A new method to separate PDAC into two major **microscopic subtypes**, called "gland-forming" and "non-gland forming" subtypes, have been published[1] **(Fig. 1)**. Findings from our group and others showed that the two microscopic subtypes of PDAC are related to median survival[1,2]. We recently found that immune related genes are upregulated in the gland-forming subtype of PDAC[2] **(Fig. 2)**. However, new prognostic and predictive biomarkers in PDAC are urgently needed.

In recent years, **circular RNAs (circRNAs)** have emerged as a class of non-coding transcripts generated by an alternative splicing event, which links a splice-donor site to an upstream splice-acceptor site. The circRNAs have diverse functions related to the binding of other molecules, including microRNAs (miRNAs) and proteins. Many circRNAs may function as **competitive endogenous RNAs (ceRNAs)** by sponging miRNA molecules and thereby relieving the corresponding miRNA target genes from post-transcriptional repression. We recently performed the first genome-wide circRNA profiling study of surgically treated PDAC from Western patients and identified two **new circRNAs that hold independent prognostic value** (**Fig. 3**)[3]. However, the prognostic role of circRNAs in unresectable PDAC (the majority of patients, ~80%) is still largely unknown.

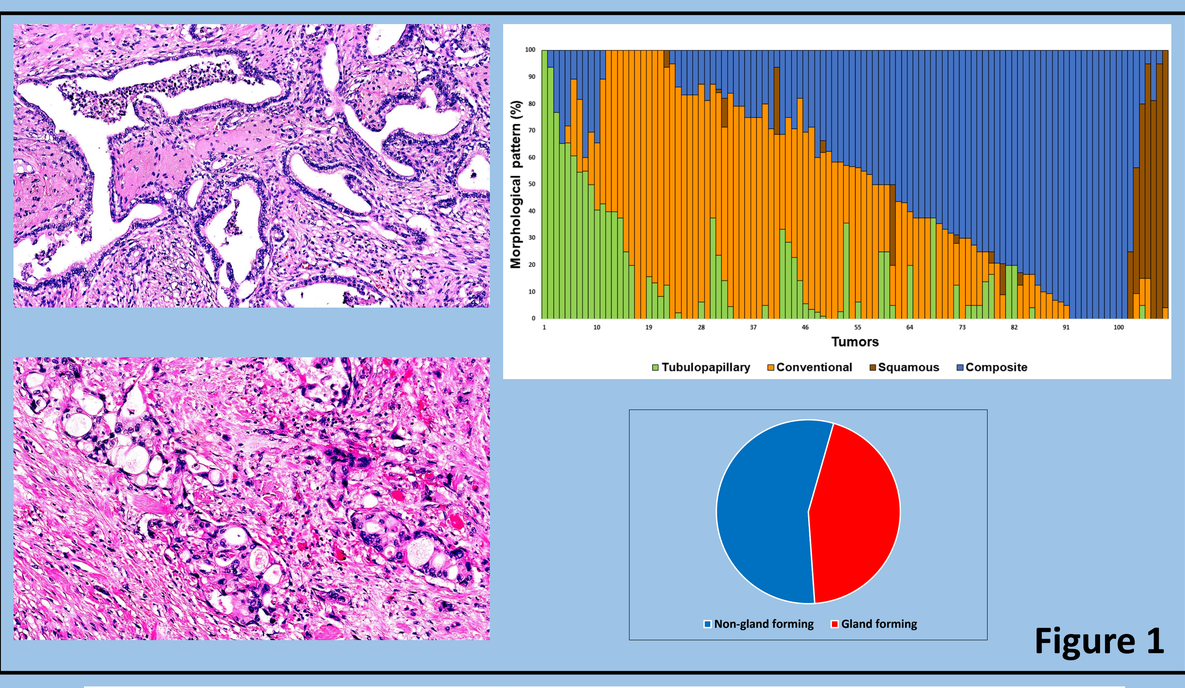
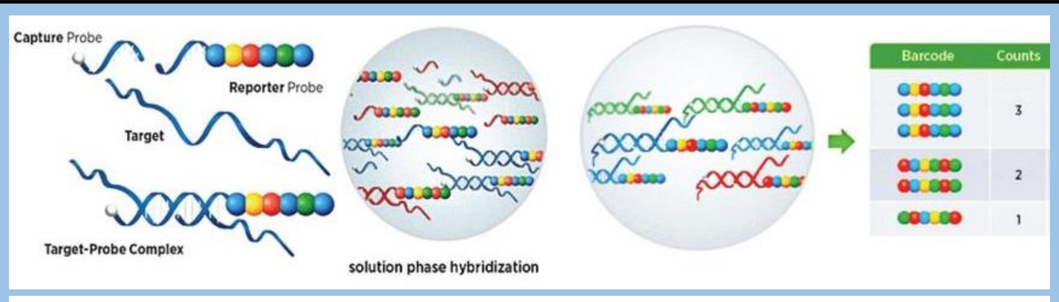


Figure 1



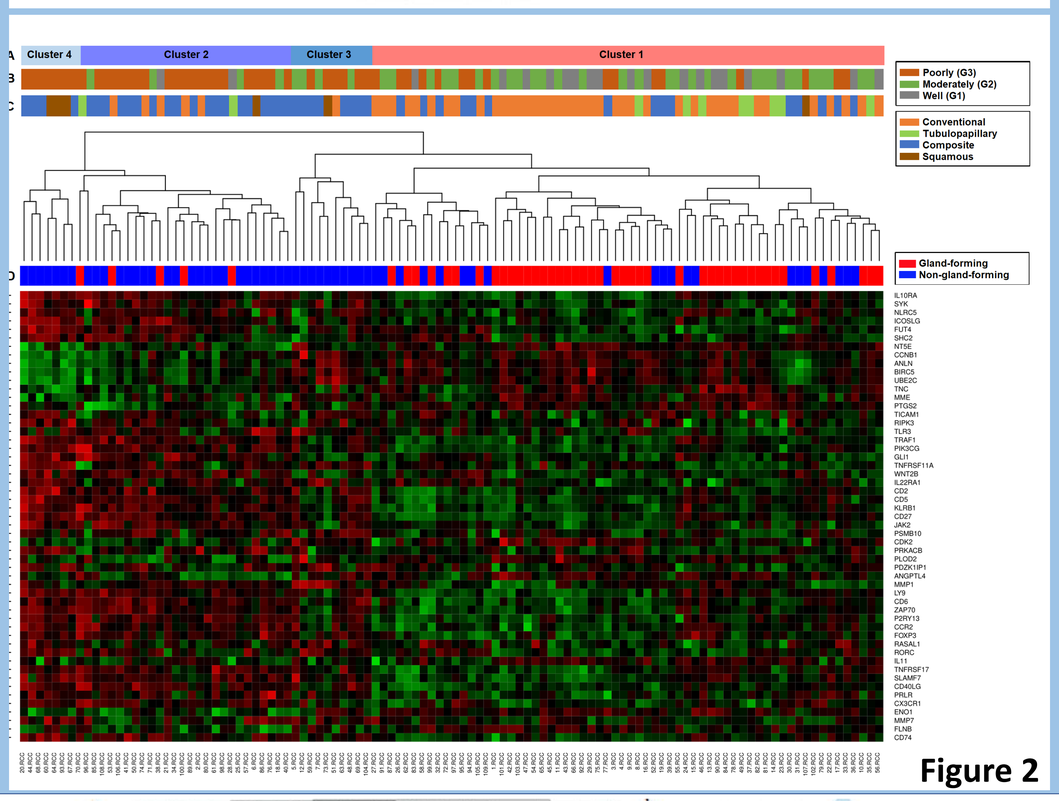


Figure 2

Planned research project

Aims:
With this project, we aim to answer the following questions regarding PDAC:

1) Which key circRNAs hold prognostic value in unresectable PDAC?
2) What is the spatial expression of key circRNAs in PDAC (*i.e.*, cancer cells vs. stromal cells)?
3) What is the biological function of selected circRNAs identified by genow-wide profiling of PDAC?

***Materials*:**
Around 100 consecutive pancreatic biopsies with non-resectable PDAC will be included. Theses biopsies are well-characterized[4] and we have previously shown their utility for mutation profiling (DNA)[5] and mRNA profiling[2].

***Methods*:**

- Characterization of **biopsies** with non-resectable PDAC (n ~ 100)
- **Digital expression profiling of circRNAs** in biopsies with non-resectable PDAC
- BaseScope Chromogenic in-situ hybridization (**CISH**) to identify the cellular location of selected circRNAs
- Kaplan-Meier (w. log-rank) and multivariable cox regression analysis for **survival analyses** regarding the prognostic role of selected circRNAs in non-resectable PDAC
- Cell culture studies examining **functional role of selected circRNAs** using transfection of antisense RNAs

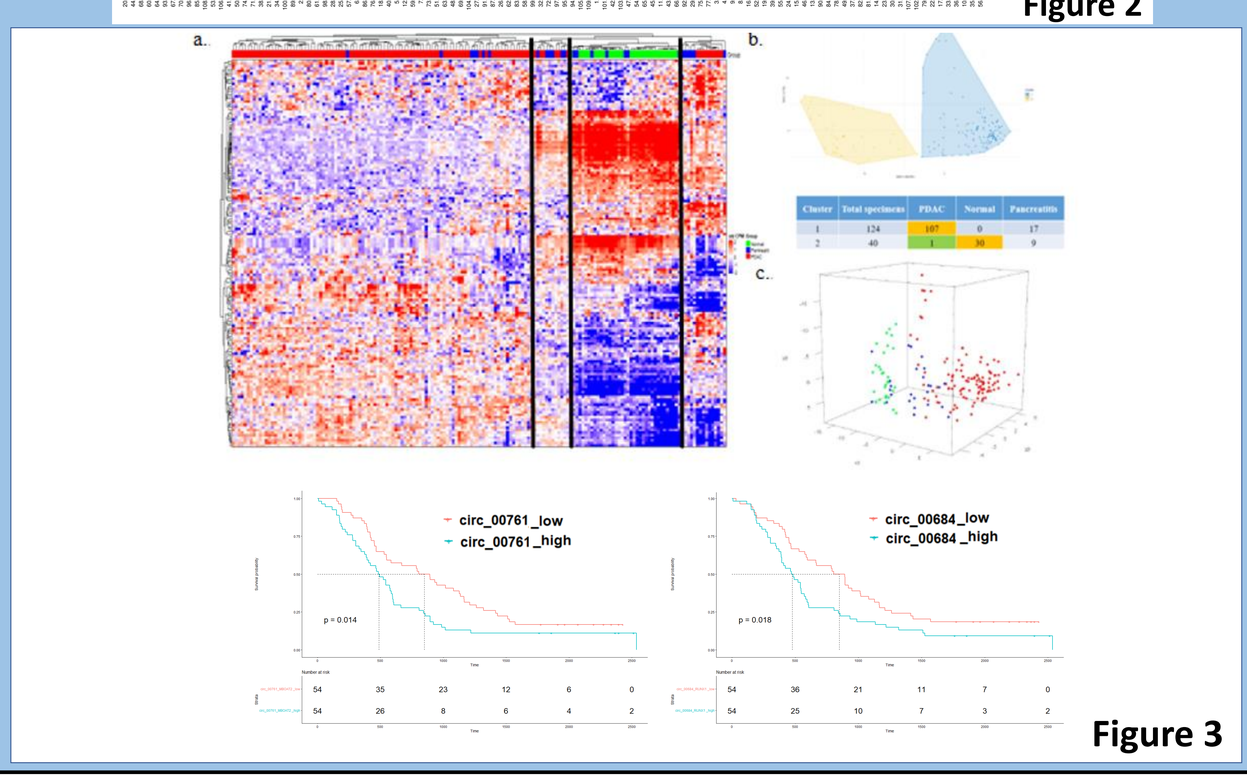


Figure 3

References:
1) Kalimuthu SN, Wilson GW, et al. Morphological classification of pancreatic ductal adenocarcinoma that predicts molecular subtypes and correlates with clinical outcome. *Gut* 2020;69:317-328
2) Rasmussen LG, Verbeke CS, …., Detlefsen S. Gene expression profiling of morphologic subtypes of pancreatic ductal adenocarcinoma using surgical and EUS-FNB specimens. *Pancreatology* 2021; 21(3):530-543
3) Ørbeck SV, …., Kristensen LS, Detlefsen S. Prognostic value of circular RNAs in pancreatic cancer using genome-wide expression profiling. *In preparation*
4) Thomsen MM, …., Detlefsen S. Accuracy and clinical outcomes of pancreatic EUS-guided fine-needle biopsy in a consecutive series of 852 specimens. *Endosc Ultrasound* 2022;11(4):306-18
5) Buchberg, de Stricker, ……., Detlefsen. Targeted next-generation sequencing of pancreatic cancer using EUS-guided fine-needle biopsies. *In preparation*

Contact: Sönke Detlefsen (e-mail: sde@dadlnet.dk. Phone: 6541 4806)