Liquid Biopsy Transcriptomics of Extracellular Vesicles (EV) in Plasma of Pancreatic Ductal Adenocarcinoma (PDAC)

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Introduction

Pancreatic ductal adenocarcinoma (PDAC), the most common and aggressive pancreatic cancer, has a poor prognosis with a 5-year survival rate of 4% because of difficult early diagnosis and lack of response to treatment. Subtyping PDAC based on molecular characteristics is potentially the key to individualized patient stratification, providing more accurate prognostication and optimization of therapeutic approaches over time. Two RNA-derived molecular subtypes of PDAC have emerged as 'classical' or 'basal-like' [1]. In addition, recent findings have shown intratumoral heterogeneity of PDAC through transcriptomic profiling, having a mix of the molecular subtypes within the same tissue. Tumor biopsy through EUS-FNA (endoscopic ultrasound-guided fine-needle aspiration biopsy) is currently the golden standard to confirm diagnosis in PDAC patients. However, this procedure is invasive and often provides limited information due to tumor heterogeneity. On the other hand, liquid biopsy is a minimally invasive method for the real-time monitoring of cancer-derived biomarkers. Among liquid biopsy biomarkers, extracellular vesicles (EVs) have a unique potential because they possess nucleic acids [2]. Therefore, using (EVs) could represent an alternative approach to characterize cancer molecular profiles of PDAC patients. In this study, we aim to perform a molecular classification of 7 PDAC tumors through EV transcriptome analysis.

Materials & Methods

- Plasma EVs from seven PDAC patients and seven sex/age matched healthy individuals were isolated through ultracentrifugation.
- RNA was extracted using the Invitrogen Total Exosome RNA and Protein Isolation Kit. Takara SMART-Seq® v4 Ultra® Low Input RNA Kit was used for cDNA synthesis.
- Libraries were prepared using Illumina Nextera XT DNA Library Prep Kit and sequenced by Illumina NextSeq 550.
- Raw reads were demultiplexed and coding reads were used for the deconvolution by DeMixT [3].
- Deep Cancer subtype classification (Deepcc) framework, based on artificial neural networks (ANN), was used to classify molecular subtypes of plasma EV samples based on a TCGA-ANN model [4]. CMScaller with Moffitt template was used to assign a molecular subtype for each TCGA sample.

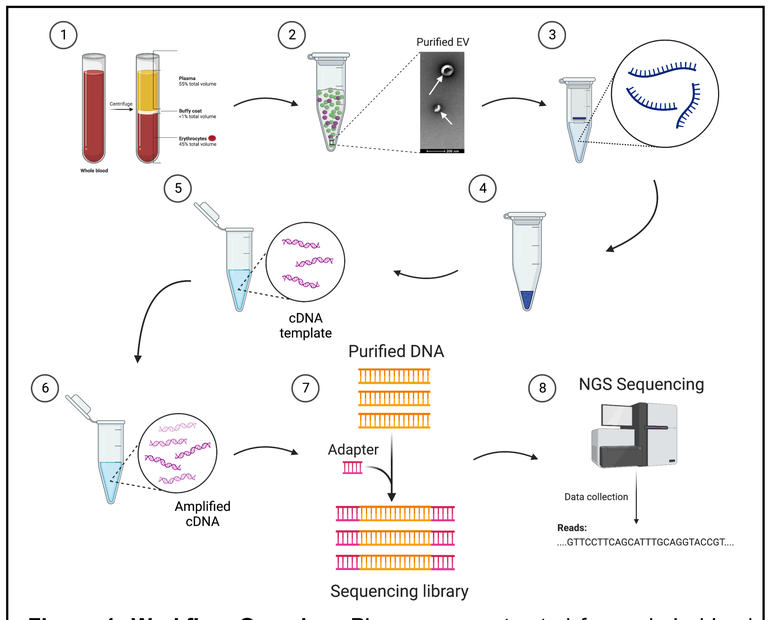

Figure 1. Workflow Overview. Plasma was extracted from whole blood samples of seven PDAC patients. Next, plasma RNA was isolated, washed, and converted to cDNA for amplification. Finally, the cDNA library was prepared for next generation sequencing.

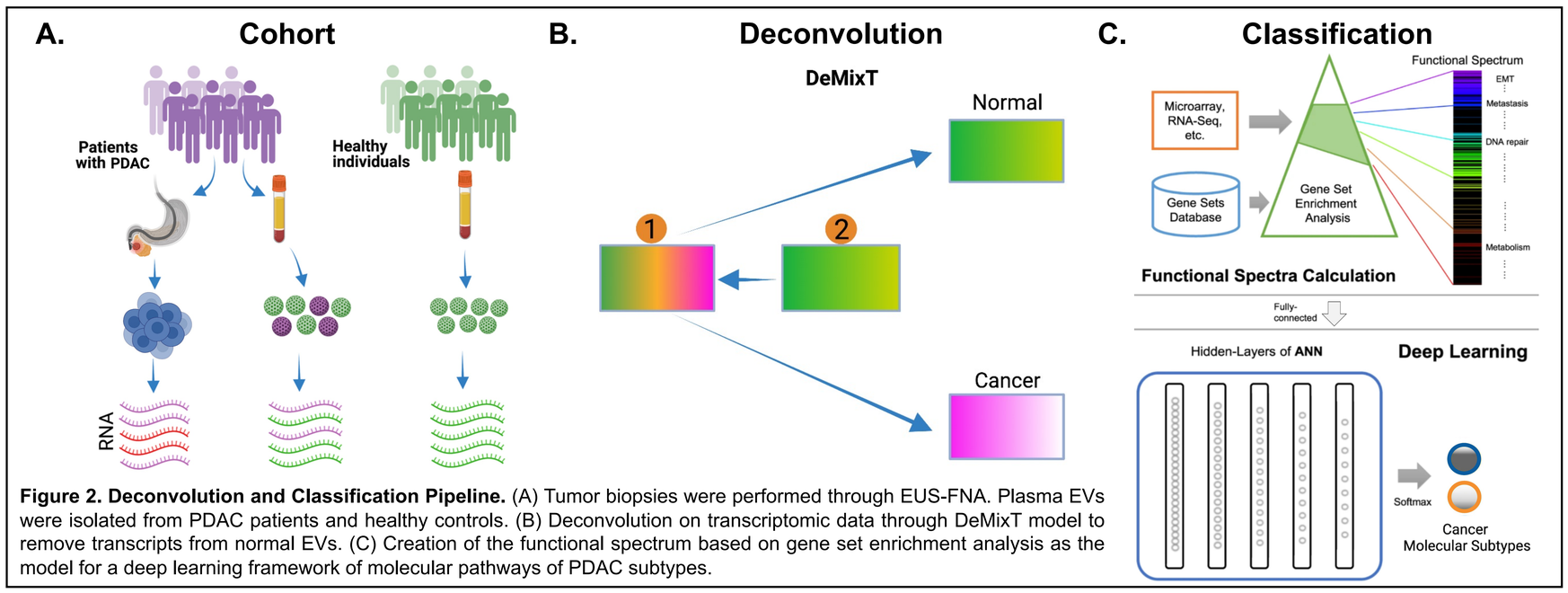

Figure 2. Deconvolution and Classification Pipeline. (A) Tumor biopsies were performed through EUS-FNA. Plasma EVs were isolated from PDAC patients and healthy controls. (B) Deconvolution on transcriptomic data through DeMixT model to remove transcripts from normal EVs. (C) Creation of the functional spectrum based on gene set enrichment analysis as the model for a deep learning framework of molecular pathways of PDAC subtypes.

Results

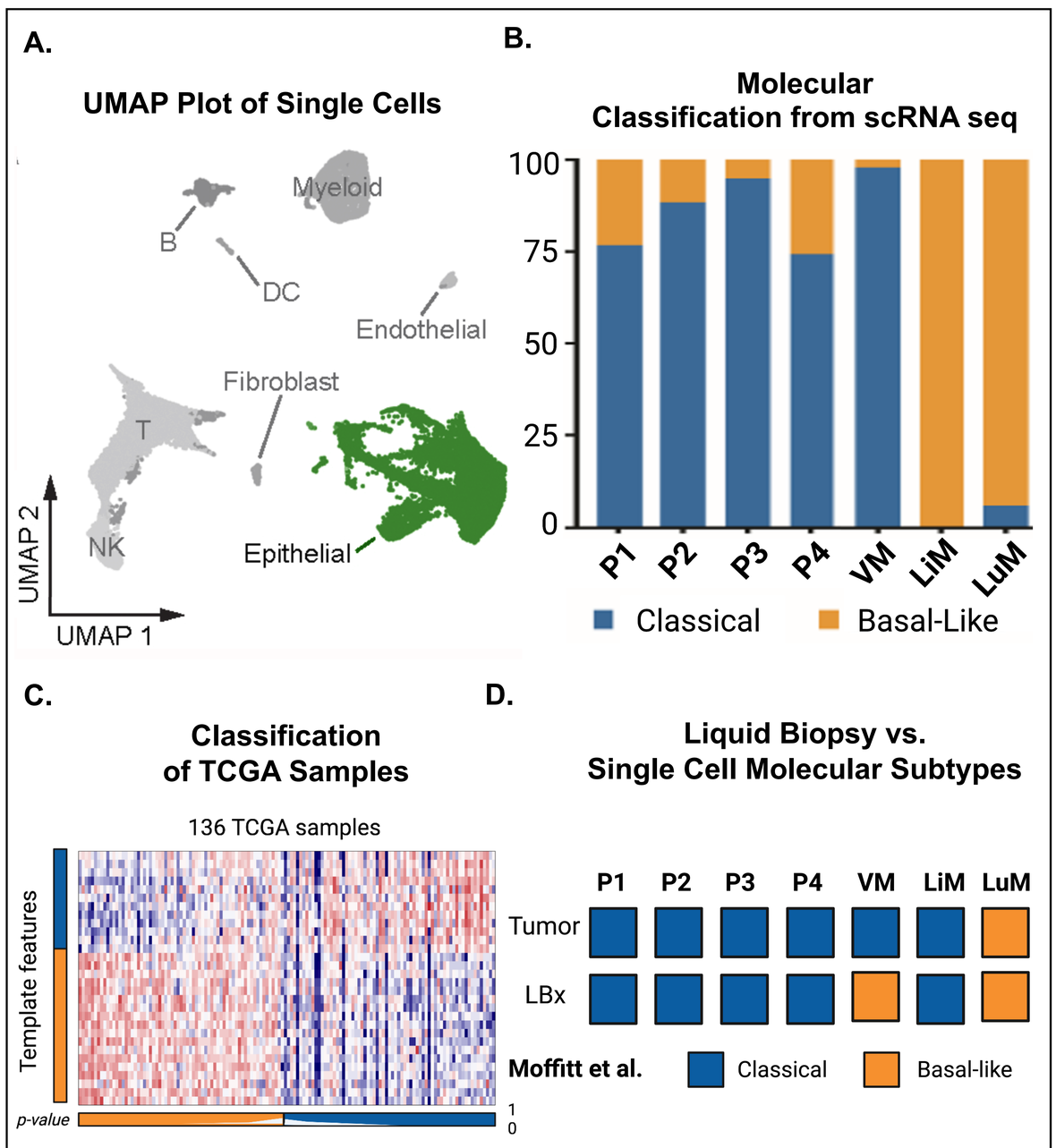

Figure 3. Molecular classifications of PDAC single cells and liquid biopsy. (A) UMAP plot of single cells from biopsy samples. (B) Classical and basal-like molecular subtypes identified in the epithelial sub cluster from 3A. (C) Clustered heatmap of 136 TCGA primary tumor samples, with samples sorted horizontally by classification. (D) Comparison of molecular subtypes between liquid biopsies and paired tissue. An 86% (6/7) was detected between both compartments.

Conclusions

- In this study, we utilized ANN to predict the molecular subtypes present in PDAC patients derived from bulk transcriptome of liquid biopsies.
- Using a small cohort of 7 samples, we were able to show that the RNA encapsulated in plasma extracellular vesicles can effectively recapitulate PDAC molecular subtypes identified in tissue, which are based on the molecular subtype framework by Moffit et al.
- Moving forward, we will expand these studies to a larger cohort and compare the transcriptome of plasma EVs with bulk RNAseq from matching resected tissues of PDAC patients.
- By identifying the tumor molecular landscape from liquid biopsies, we hope to evaluate treatment response and the characterization of the cancer molecular profiles in a minimally invasive manner.

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References

1. Moffitt, R., et al. "Virtual microdissection identifies distinct tumor and stroma-specific subtypes of pancreatic ductal adenocarcinoma." Nat Genet, 47: 1168–1178 (2015).
2. San Lucas, F.A., et al. "Minimally invasive genomic and transcriptomic profiling of visceral cancers by next-generation sequencing of circulating exosomes" Annals of oncology 27(4): 635–641 (2015).
3. Wang, Z., et al. "Transcriptome Deconvolution of Heterogeneous Tumor Samples with Immune Infiltration." iScience, 9: 451-460 (2018).
4. Gao, F., et al. "DeepCC: a novel deep learning-based framework for cancer molecular subtype classification." Oncogenesis 8(44), (2019).
5. Lee, J., et al. "Elucidation of Tumor-Stromal Heterogeneity and the Ligand-Receptor Interactome by Single-Cell Transcriptomics in Real-world Pancreatic Cancer Biopsies." Clin Cancer Res, 27 (21): 5912–5921 (2021).
6. Bailey, P., et al. "Genomic analyses identify molecular subtypes of pancreatic cancer." Nature 531: 47–52 (2016).
7. Collisson, E., et al. "Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy." Nat Med 17: 500–503 (2011).