Background

Pancreatic cancer is one of the highest mortality rate cancers worldwide with 5-year relative survival of 10.8%. Lack of screening methods for early-stage detection as well as proper patient sub-characterization, make it a fatal cancer due to poor diagnoses and insufficient treatment.

Methylation flags are kept on tumorigenesis, akin to the tissue-specific traces that are acquired and maintained throughout development. Methylomes have been explored allowing to track tumor clonal evolution, besides enabling to unearth the tumor cell type of origin.

Our aim was to investigate a methylation signature link to clonal evolution in pancreatic tumors that allows us to stratify cellular differences on the origin of the tumor and predict patient prognosis and clinical outcome.

Method

Using a combination of DNA methylation, transcriptome, and mutation data from TCGA Pancreatic Adenocarcinoma (TCGA-PAAD) set, a total of 195 patients were studied retrospectively. Epigenetic classes were defined by identifying patterns in the differential methylation of sub-genomic components and differential gene expression of transcription factors (TFs) and performing unsupervised hierarchical clustering.

The methylation clusters were correlated with variables such as age, gender, mutation state, anatomical location and tumor stage. Gene ontology (GOs) and Biological Pathway Analysis (BPA) were performed, and survival analysis was done to assess the capability of methylation markers to predict survival prognosis.

Results

PAAD hierarchical clustering showed four patient groups with well differentiated methylation levels, a highly methylated cluster (C1), hypomethylated cluster (C2) and progressive hypomethylation on clusters C3 and C4.

In C1, significant enrichment in driver mutations of KRAS (P<.001) and TP53 (P<.001) were found, while in C1 and C4 the enrichment of SMAD4 (*P*=.002, P= .01 respectively) and CDKN2A (P=.002 and P=.02 respectively) was found. C2 showed significant enrichment of Stage 1 patients (P=.012) and lack of mutations in driver genes. Cancer in tail or body was significantly enriched in C1 (P<.001).

Analysis of GOs related to morphogenesis, development and regulation of transcription and BPA analysis in Transcriptional regulatory network in Embryonic Stem Cells and Wnt/catenin signaling showed that methylation markers contributing to cluster separation were linked to cell fate and differentiation.

Kaplan-Meier analysis indicated that patients in C2 group had significantly (P=.0072) better 5- year survival post- treatment; patients in hypermethylated cluster, C1, had the lowest survival probability up

to 3- years but cumulative survival rate up to 5-years remained lowest for clusters C3 and C4 at probabilities of P=.0094 and P=.002, respectively.

Other parameters such as age, gender, race and presence/absence of prior pancreatitis diagnosis had no significant (P>.05) link to the clusters and outcomes.

Conclusion

Methylation based clustering shows clear molecular sub-groups in the pancreatic cancer patient cohort, with clear connection to biological pathways. Further on the clusters were significantly linked to patient survival outcomes. This indicates that utilization of methylation markers could help to better stratify the patients, which could aid in choosing more appropriate treatment strategies based on tumor biology itself. Understanding heterogeneity of the patient group could also aid in further developing options for early detection, based on methylation signals.