Gene methylation and levels of circulating tumor DNA as diagnostic/prognostic biomarkers in Pancreatic Cancer: biosignatures built via automated Machine Learning

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Background & objectives

Background. Pancreatic cancer (PnCa) is the fourth cancer leading cause of death worldwide and its incidence is increasing in parallel to diabetes and obesity, presenting strong risk factors. Circulating tumor DNA (ctDNA) may offer a valuable liquid biopsy biomaterial for the timely diagnosis and prognosis to improve its management. **Objectives.** In order to unfold this potential, we investigated ctDNA levels and the methylation status of a panel of cancer-related genes to reveal novel biomarkers for PnCa. Ad-hoc Automated Machine Learning (autoML) was introduced into our analysis, to fully exploit the experimental findings to clinically relevant results.

Methods

ctDNA from 92 PnCa cancer patients (47 grade I-III so-called *early* and 45 *metastatic*) along with 30 age- and gender-matched controls was isolated and quantified by ALU247 element measured by quantitative PCR (qPCR). The methylation status of *BRCA1, BRCA2*, *RASSF1A* and *SOX17* was analyzed by methylation specific qPCR.Standard statistical analysis against clinically relevant end-points was followed by multivariate autoML (JADBio.com).

Results

Levels of ctDNA were significantly higher in PnCa than in health ($p<0.001$) and higher in metastatic than in early PnCa patients ($p=0.022$). *SOX17, RASSF1A, BRCA1* and *BRCA 2* methylation was significantly more frequent in PnCa than in health ($p<0.001$), whereas *RASSF1A* methylation was more frequent in early than metastatic patients ($p=0.025$). No correlation was found between ctDNA levels and methylation to clinicopathological characteristics and response in metastatic patients. In early PnCa, both *BRCA1* and *BRCA2*

methylation was reversely correlated to survival (p=0.012 and 0.001 respectively). AutoML multivariate classification analysis built a 4 feature model via Support Vector Machines, including ctDNA levels, *SOX17, BRCA1* and *BRCA2* methylation, showing high efficiency in discriminating PnCa from healthy individuals (AUC 0.959 [0.912,0.994] precision 0.957 [0.911, 0.994]). A second 5 feature model via Support Vector Machines including also *RASSFF1A* methylation, could discriminate healthy, early and metastatic groups by AUC 0.797 [0.752, 0.841] and precision 0.863 [0.834, 0.890].

Conclusion

Our data support the value of ccfDNA as a liquid-biopsy biomaterial carrying important clinical information for PnCa diagnosis and prognosis. AutoML enabled full exploitation of our results building methylation-based PnCa-specific biosignatures, to be implemented in liquid biopsy diagnostics upon further clinical validation.