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## **Tspan8, CD44v6 and alpha6beta4 are the most reliable pancreatic cancer stem cell markers**

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Patients with pancreatic cancer (PaCa) have a very poor prognosis, which is partly due to late stage diagnosis. Thus, a reliable non-invasive screening could possibly be of a great help. Serum derived exosomes could serve as such a diagnostic tool, provided suitable pancreatic cancer stem cell (PaCSC) markers can be identified. To approach to answer this question by enriching PaCSC and defining thereafter the marker profile of the PaCSC enriched population. PaCSC have been enriched from long term and freshly established PaCa lines by spheroid growth or holoclone formation. By repeated cloning, PaCSC were enriched during the first 3 rounds of cloning and remained stable thereafter. Enrichment of PaCSC was controlled in vitro for motility, anoikis, drug-resistance and CSC marker expression. Spheres and holoclones showed increased motility and upregulated CXCR4 expression, an increased rate of anchorage-independent growth, where the latter correlated well with the increase in the capacity to form spheres. Instead, drug resistance was strongly upregulated only in one of seven lines, indicating that drug resistance in PaCa is not restricted to CSC. Most striking has been the enrichment of the PaCSC markers CD44v6 and alpha6beta4 in spheres and holoclones. A similar enrichment has been observed for Tspan8 that probably should be considered as a PaCSC marker. Expression of other CSC markers like CD24, EpCAM and CD133 was not increased and expression of the metastasis suppressor marker CD82 was not affected, whereas CD9 expression was frequently reduced.

These data demonstrate that PaCSC can be enriched by spheroid growth or by holoclone formation, where PaCSC are recovered at a low, but comparable frequency in long term and freshly established PaCa lines. Furthermore, PaCSC become strongly enriched by spheroid growth and holoclone formation, but remain stable at a defined level upon repeated recloning. Instead, paraclones die upon repeated recloning. These

findings indicate that PaCSC are essential for long term PaCa line maintenance. The data also provide evidence that PaCSC either constantly differentiate or remain dependent on support by non-CSC. Last, and importantly, as the selection procedure is based on inherent growth features of PaCSC, my studies allowed for an unbiased evaluation of PaCSC markers, where CD44v6, alpha6beta4 and Tspan8 are dominating.