Oncogenic splicing of the p53-related tumorsuppressor ASPP2 drives tumor aggressiveness and therapy resistance in pancreatic cancer models

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BACKGROUND

The average patient diagnosed with late-stage pancreatic cancer (PC) will live for about one year – and the five-year survival rate drops down to 1%. Improving the understanding of PC oncogenesis and drug resistance as well as developing novel treatment options is urgently needed. We have identified a novel, oncogenic dominant-negative isoform of the tumor suppressor ASPP2 (ASPP2*kappa* (κ)) with high prevalence in cancer. Physiologically, ASPP2 wt orchestrates cellular homeostasis via directly interacting with key proteins such as p53, RAS, BCL-2 and NFκB. In contrast, ASPP2κ is characterized by loss of important p53-, BCL-2 and NFκB-binding sites, thus impairing major pathways controlling cellular fate.

We here show that ASPP2*kappa* is highly expressed and functionally active in pancreatic cancer.

METHODS/RESULTS

The role of ASPP2*kappa* in PC was studied in patient tumor tissue and isogenic cell models (PSN-1, DAN-G, HUP T4). Proteome arrays, RNA sequencing and Incucyte live cell imaging analyses were performed on these models and a PC NSG mouse model was established. In addition, we tested novel, ASPP2κ-specific siRNAs designed for *in vivo* application.

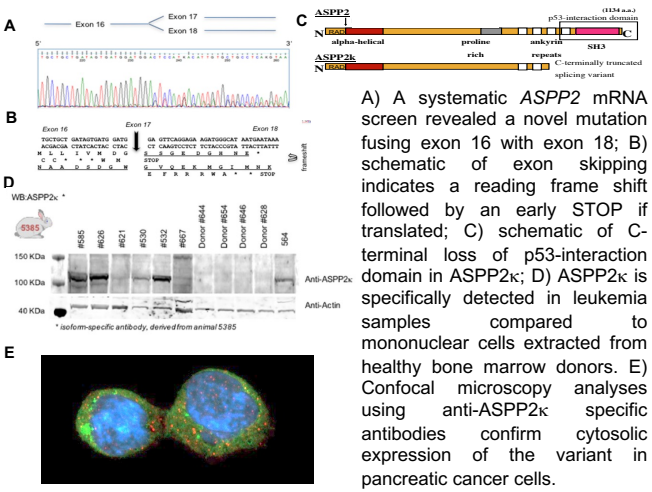
We unravel a functional role of ASPP2κ in PC tumor growth, invasion, metastasis and drug resistance: Silencing of ASPP2κ (KD) resulted in attenuated cell proliferation (doubling times decreased on av. -55%) and increased susceptibility towards CX (av +25% apoptosis for cisplatin and paclitaxel). Additionally, migration was inhibited on av. by 45%, hinting towards a role of ASPP2κ in early metastasis. Vice versa, ASPP2κ-overexpressing cells presented with accelerated proliferation and migration and displayed an even higher apoptotic threshold. Ongoing mouse xenotransplant models confirm significantly longer OS rates and inhibition of metastasis for mice bearing ASPP2κ KD tumors. Most importantly, employing novel, fully chemically modified ASPP2κ-specific siRNAs, we were able to silence ASPP2κ in vivo and again attenuate tumor progression.

CONCLUSIONS

ASPP2κ is highly expressed in PC and associates with a more aggressive tumor biology and drug resistance. We here provide absolutely novel insights into PC tumor biology in dependency of ASPP2κ and demonstrate first successful attempts to silence this novel isoform *in vivo*. Future studies evaluating ASPP2κ as a novel target for therapy are warranted.

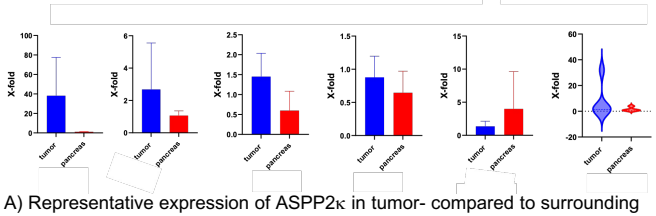
RESULTS

Figure 1. ASPP2κ discovery: sequence and structure



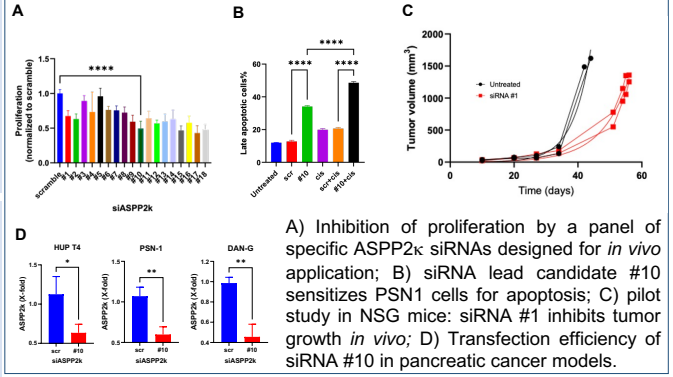
A) A systematic *ASPP2* mRNA screen revealed a novel mutation fusing exon 16 with exon 18; B) schematic of exon skipping indicates a reading frame shift followed by an early STOP if translated; C) schematic of C-terminal loss of p53-interaction domain in ASPP2κ; D) ASPP2κ is specifically detected in leukemia samples compared to mononuclear cells extracted from healthy bone marrow donors. E) Confocal microscopy analyses using anti-ASPP2κ specific antibodies confirm cytosolic expression of the variant in pancreatic cancer cells.

Figure 2. ASPP2κ is frequently expressed in pancreatic cancer



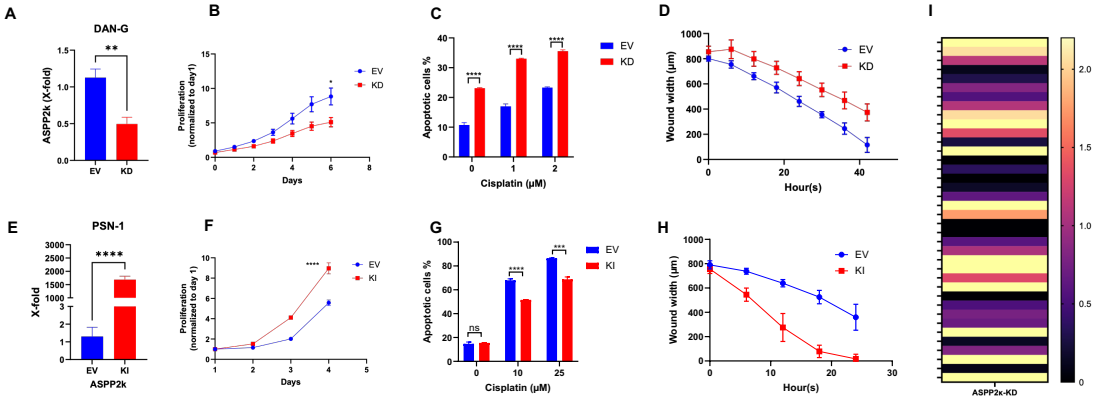
A) Representative expression of ASPP2κ in tumor- compared to surrounding tissue B) 80% of pancreatic tumors significantly overexpress ASPP2κ

Figure 5. Silencing ASPP2κ *in vivo,* employing specific siRNAs



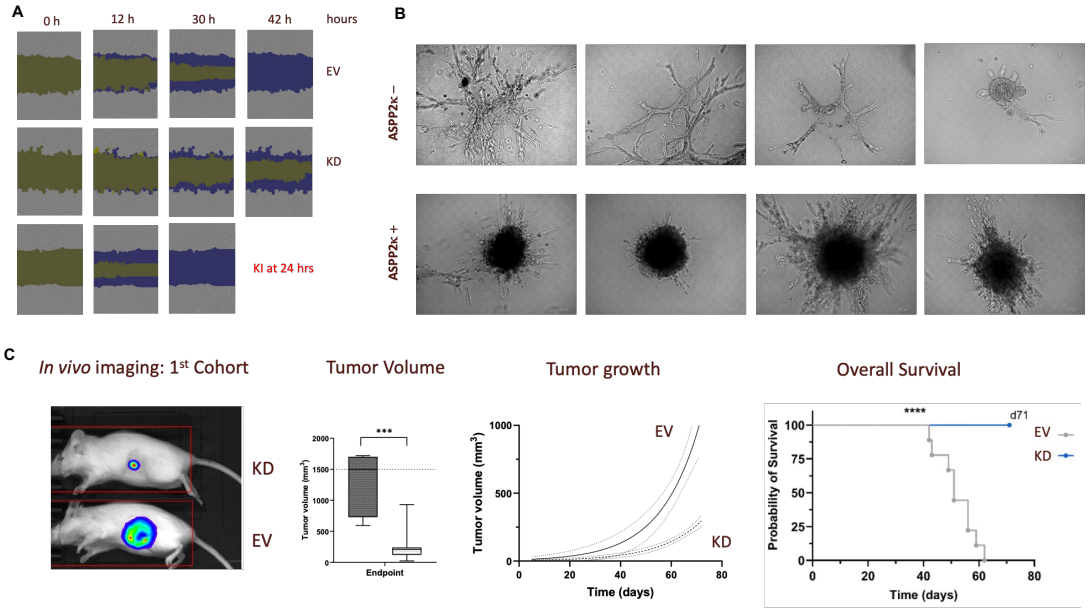
A) Inhibition of proliferation by a panel of specific ASPP2κ siRNAs designed for *in vivo* application; B) siRNA lead candidate #10 sensitizes PSN1 cells for apoptosis; C) pilot study in NSG mice: siRNA #1 inhibits tumor growth *in vivo;* D) Transfection efficiency of siRNA #10 in pancreatic cancer models.

Figure 3. Functional analyses of ASPP2κ in pancreatic cancer cell models in dependency of ASPP2κ



A/E) Transduction efficiency A) ASPP2κ Knock-down, E) Knock-in, B/F) Cell proliferation of pancreatic cancer cell models EV and ASPP2κ KD/KI; C/G) Apoptosis analyses via FACS in depenency of ASPP2κ expresssion, D/H) Migration capacity of pancreatic cancer cell models in dependency of ASPP2κ expresssion, EV and ASPP2κ KD/KI; I) Pathway analyses: expression of target proteins in an ASPP2κ KD model compared to EV controls; here: apoptosis signaling analyses.

Figure 4. Silencing of ASPP2κ inhibits migration, tumoroid formation and tumor growth *in vitro* and *in vivo*



A) Migration capacities analysed via Incucyte live cell imaging over time in DAN-G EV controls, KD and KI cells. B) Tumoroid formation of PSN-1 cells at day 11: silencing of ASPP2κ inhibits tumoroid formatiton. C) Mice injected with ASPP2κ knock-down cells present with significantly inhibited tumor formation, tumor volume and growth as well as longer overall survival.

DISCLOSURES

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