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The role of cancer susceptibility candidate 5 (CASC5) in pancreatic cancer

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Cancer Susceptibility Candidate 5 (CASC5) regulates formation of the kinetochores and spindle assembly checkpoint complexes. CASC5 is one of the few cancer/testis antigens of potential prognostic or therapeutic relevance. The aim of this study was to explore role of CASC5 in pancreatic cancer. CASC5 mRNA expression was measured by qRT-PCR in normal cells (n=5) and tissues (n=22), pancreatic cancer cell lines (n=9), and tissues derived from patients with inflammatory, benign and malignant pancreatic diseases (n=162). Cellular and subcellular protein location was determined with immunohistochemistry and Immunofluorescence. The significance of CASC5 was deduced from clinic-pathological correlations and from morphological and functional changes caused by the siRNA-based knockdown in pancreatic cancer cells.

We found that CASC5 was specifically overexpressed in malignant pancreatic lesions. Morphological type (intraductal vs. ductal carcinoma) but none of other clinic-pathological parameters – including survival - correlated with CASC5 levels in malignancies. Restricted to the nucleus of cancer cells, diffuse CASC5 clustered on centromeres (kinetochores) during mitosis. CASC5-depleted cultures showed 1) suppressed proliferation of the fast-cycling cells without significant cytotoxicity, whereby the growth arrest was irrevocable and consequently abolished clonogenicity; 2) enlarged multinucleated cells with unequally distributed chromosomes; 3) an accumulation of mostly tetraploid, phosphoHistH3-negative/ γ H2AX-positive cells unable to enter mitosis in response to serum after STLC-induced synchronization at G2/M-border but 4)

overcoming this block upon exposure to UCN01 or caffeine known to abrogate DNA damage response (DDR)-induced arrest by inhibiting CHK1 and ATM kinases, respectively. That mitotic re-entry was, however, lethal.

Conclusion and Discussion: in PDAC, overexpression of CASC5 is required for efficient proliferation of malignant cells by controlling chromosomal reorganization during mitosis. Its loss causes nuclear disorganization which consequently activates DDR. Although the possibility of directly damaged DNA activating pre-mitotic DDR/G2 checkpoint cannot be completely excluded, simultaneous failure of both known CASC5 functions is most likely to occur. That means that disrupted chromosomal attachment to kinetochores and faulty spindle assembly checkpoint supposing to correct mitotic spindle's anomalies will generate cells with chromosomal misalignments, which might undergo apoptosis, unequal division/senescence or – preferentially - indefinite post-mitotic DDR-mediated interphase arrest as a result of mitotic 'slippage'. Thus, combination of the CASC5-targeted therapy with DDR-abrogation may represent the most effective approach to drive 'frozen' cells to the death.