Genetic inactivation of the Fanconi anemia gene FANCC identified in the hepatocellular carcinoma cell line HuH-7 confers sensitivity towards DNA-interstrand crosslinking agents.

Inactivation of the Fanconi anemia (FA) pathway through defects in one of 13 FA genes occurs at low frequency in various solid cancer entities among the general population. As FA pathway inactivation confers a distinct hypersensitivity towards DNA interstrand-crosslinking (ICL)-agents, FA defects represent rational targets for individualized therapeutic strategies. Except for pancreatic cancer, however, the prevalence of FA defects in gastrointestinal (GI) tumors has not yet been systematically explored. A panel of GI cancer cell lines was screened for FA pathway inactivation applying FANCD2 monoubiquitination and FANCD2/RAD51 nuclear focus formation and a newly identified FA pathway-deficient cell line was functionally characterized. The hepatocellular carcinoma (HCC) line HuH-7 was defective in FANCD2 monoubiquitination and FANCD2 nuclear focus formation but proficient in RAD51 focus formation. Gene complementation studies revealed that this proximal FA pathway inactivation was attributable to defective FANCC function in HuH-7 cells. Accordingly, a homozygous inactivating FANCC nonsense mutation (c.553C>T, p.R185X) was identified in HuH-7, resulting in partial transcriptional
skipping of exon 6 and leading to the classic cellular FA hypersensitivity phenotype; HuH-7 cells exhibited a strongly reduced proliferation rate and a pronounced G2 cell cycle arrest at distinctly lower concentrations of ICL-agents than a panel of non-isogenic, FA pathway-proficient HCC cell lines. Upon retroviral transduction of HuH-7 cells with FANCC cDNA, FA pathway functions were restored and ICL-hypersensitivity abrogated. Analyses of 18 surgical HCC specimens yielded no further examples for genetic or epigenetic inactivation of FANCC, FANCF, or FANCG in HCC, suggesting a low prevalence of proximal FA pathway inactivation in this tumor type. As the majority of HCC are chemoresistant, assessment of FA pathway function in HCC could identify small subpopulations of patients expected to predictably benefit from individualized treatment protocols using ICL-agents.