The Fanconi anemia/BRCA2 pathway in pancreatic cancer
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Recent years have brought an impressive accumulation of knowledge about the genetic basis of pancreatic cancer, but so far it has not been possible to translate this improved understanding into significant advancements in clinical treatment. In 2002, Howlett et al. discovered that biallelic \( BRCA2 \) mutations were present in a subset of Fanconi anemia (FA) patients. A small percentage of pancreatic cancer cells harbors mutations in the \( BRCA2 \) gene. Other members of the FA pathway had not been assessed in pancreatic cancer.

In this work, we studied 70 pancreatic tumors for genetic defects in \( FANCC \) and 72 tumors for \( FANCG \) mutations. Two somatic truncating mutations were found in \( FANCC \): one germline truncating mutation was found in \( FANCG \). The finding of somatic alterations in the \( FANCC \) gene in pancreatic cancer cells, in combination with the known association of \( BRCA2 \) mutations with pancreatic cancer, implies a role for defects in the FA/BRCA2 pathway in the development of a subset (5-10%) of pancreatic cancers.

The vast majority of pancreatic cancers are aneuploid, reflecting an increased rate of chromosomal instability. Although mutations in the FA/BRCA2 pathway could, in combination with certain checkpoint defects, lead to an increased chromosomal instability, the role of these mutations in pancreatic cancer is not clear yet. Most aneuploid tumors are not defective in a member of the FA/BRCA2 pathway; these defects are therefore unlikely to be a general cause of aneuploidy in cancer.

Alternatively, defects in the FA/BRCA2 pathway could serve a unique role at a certain stage of carcinogenesis, in a subset of tumors, providing a very specific type of genetic instability to the developing neoplasm, to overcome very specific genetic barriers. Although significant progress has recently been made, more work is needed to understand the role of defects in the FA/BRCA2 pathway in carcinogenesis.

Mutations in the proximal FA pathway are present at a substantial frequency in the general population (approximately 1 in 500), however, germline mutations in these genes do not seem to be abundant in pancreatic (or other) cancer patients. No pathogenic \( FANCC \) or \( FANCG \)
germline mutations were found in individuals with a strong family history of pancreatic cancer. In a separate study, Couch et al. assayed blood samples taken from 421 pancreatic cancer patients for mutations in the FANCC and FANCG genes (this study was not designed to detect somatic mutations). Two truncating FANCC mutations were found, both accompanied by loss of heterozygosity in the corresponding original tumors, suggesting a weak association of germline mutations in the FANCC gene with pancreatic cancer. No truncating mutations were identified in germline DNA of 658 control individuals.

While pancreatic cancer remains the only form of cancer (in non-FA patients) known to harbor 'upstream' FA mutations to date, mutations in this pathway are unlikely to be restricted to cancers of the pancreas. In a study of 35 cancer cell lines, we found one breast and one head and neck cancer to be defective in the upstream FA pathway, as assayed by Fancd2 immunoblot. Two ovarian cancer cell lines were recently shown to be defective in the FA pathway, which was attributed to FANCF-methylation. Epidemiological studies so far have not found relatives of FA patients to be at an increased risk for cancer. This could be explained by a low penetrance of mutations in the upstream FA pathway. Also, an increased cancer risk for individuals with mutations in one of the FA genes could be missed due to heterogeneity among the patient populations studied. FANCA is the most commonly mutated gene in the general population. Therefore, low-penetrance mutations in FANCC and FANCG, contributing to the development of less common forms of cancer, such as cancer of the pancreas, could be missed in epidemiological studies. More extensive studies of cancer incidence in FA mutation carriers are needed. These studies should aim to look at carriers of mutations in different complementation groups separately, and with adequate numbers to achieve statistical power.

Mutations in the FANCC and FANCG genes in pancreatic cancer lead to a functional defect in the FA pathway as assayed by Fancd2 monoubiquitination and nuclear focus formation. Additionally, pancreatic carcinomas defective in the FA/BRCA2 pathway are remarkably sensitive to DNA-interstrand crosslinking agents, both in culture and as xenografts in mice. This increased sensitivity at low doses of crosslinking agents is effectuated by a G2/M cell cycle arrest, apoptosis and necrosis. Several lines of evidence suggest the use of combinations of chemotherapy containing mitomycin C (MMC) and other crosslinking agents to be beneficial for pancreatic cancer patients. Although a significant increase
in survival is usually not found. occasional complete and long-term remissions are reported. These reports have not incorporated the genetic testing of these patients, but a gene defect in BRCA2, FANCC, FANCG or another gene in the FA/BRA2 pathway could in theory cause a therapeutically useful hypersensitivity, providing an "Achilles' heel" in a subset of pancreatic cancers. The preclinical findings presented in this work suggest that DNA-interstrand crosslinking agents, particularly MMC or cisplatin, could be selectively used on patients with cancers harboring mutations in one of the members of the FA/BRA2 pathway. Treatment options are not restricted to MMC or cisplatin and include other effective crosslinkers that may be tolerated better, such as oxaliplatin. Also, other groups of therapies may be selectively toxic to cells with defects in the FA/BRA2 pathway. In two recent studies, BRCA2 deficient cells were shown to be hypersensitive to inhibitors of PARP (poly(ADP-ribose) polymerase), an enzyme involved in the repair of DNA single-strand breaks, providing another means to target carcinomas with defects in the FA/BRA2 pathway. However, new studies suggest that these results cannot be directly extended to BRCA2-defective pancreatic cancers cells (Gallmeier E, Kern SE; personal communication). Patients could be screened for germline mutations in BRCA2 and, if an appropriate test could be developed, tumor specimens could be screened for somatic mutations in FANCC or other FA pathway defects. To provide preliminary evidence supporting this principle, six anonymous coded blood samples of pancreatic cancer patients that had experienced a good clinical response to a combination regimen of MMC and 5-FU were analyzed for BRCA2 mutations or deletions: one deleterious mutation was found. Because tumor tissue was not available, we were unable to screen for somatic mutations in FANCC, FANCG or BRCA2 in these patients' tumors. Although finding one mutation in a patient's tumor is not a statistically significant result, BRCA2 mutations are rare in sporadic tumors. Therefore, finding a BRCA2 mutation in a small number of sporadic cases solely selected based upon treatment response is another encouragement to continue investigating the possibility to treat pancreatic cancer patients based upon their FA/BRA2 status. One could envision that in the treatment of pancreatic cancer, after surgical excision (which most readily permits the full genetic analysis envisioned), MMC (or another crosslinking agent) could be a curatively intended adjuvant treatment used selectively in the instance of tumor...
defects in the FA/BRC.A2 pathway. In the meantime, the known germline origin of most pancreatic cancer BRCA2 defects and the availability of reliable and rapid BRCA2 testing allows for the design of directed studies in either resected or unresectable cases of pancreatic cancer. In order to increase the number of patients who could benefit from the rational use of interstrand crosslinking agents targeting a specific genetic defect, future studies should include the development of a test for upstream FA defects in patients' tumors.