The Fanconi anemia/BRCA2 pathway in pancreatic cancer
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Citation for published version (APA):

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CHAPTER 5

FANCONI ANEMIA GENE MUTATIONS IN PANCREATIC CANCER

Van der Heijden MS, Yeo CJ, Hruban RH, Kern SE

Cancer Research 2003; 63 (10): 2585-2588
Abstract

Genes of the Fanconi anemia (FA) complementation groups are suggested to be involved in homologous DNA recombination and produce FA when two allelic mutations are inherited. BRCA2 is an FA gene and additionally conveys an inherited risk for breast, ovarian, and pancreatic cancer for individuals carrying a single mutated allele. Here we report inherited and somatic mutations of FANCC and FANCG present in pancreatic cancer. This may imply a general involvement of FA genes with an inherited risk of cancer. The known hypersensitivity of FA cells to mitomycin C and other therapeutic agents suggests a therapeutic utility for a more complete characterization of the DNA repair defects and their causative genetic mutations in pancreatic cancer.

Introduction

Fanconi anemia (FA) is an inherited autosomal recessive syndrome, characterizedcellularly by hypersensitivity to crosslinking agents such as mitomycin C (MMC). Patients often present with congenital bone deformities and bone marrow failure and are highly susceptible to the occurrence of hematological tumors (especially acute myelogenous leukemia) and squamous cell tumors of the head and neck, gynecological system, and other organs. Recently, BRCA2 mutations have been shown to be responsible for a subset of FA patients: complementation group D1. Cells from these patients were recently reported to be complex heterozygous, containing both hypomorphic and nonfunctional alleles of BRCA2: complementation of such cells with wild-type BRCA2 cDNA restored resistance to MMC. Among human tumors, pancreatic cancers harbor the highest prevalence of BRCA2 mutations, which are present in approximately 4-7% of the “sporadic” pancreatic cancers and 17% of families with a strong history of the disease (kindred with >3 family members affected, including a least 2 first-degree relatives). As is BRCA2, the FANCC and FANCG genes are the sites of additional FA gene mutations carried in the general population. To help understand the potential inherited basis of pancreatic cancer, we determined whether inactivating FANCC and FANCG gene mutations might occur as homozygous mutations in pancreatic cancers.
**Methods**

Cancers of the pancreas and distal common bile duct resected at the Johns Hopkins Hospital between 1992 and 1997 were expanded as xenografts in immunodeficient mice as described previously\(^1\). At the time of surgery, resected normal duodenum was frozen and stored at -80°C.

**FANCC** was sequenced using automated capillary sequencing of PCR products in 22 tumors selected for LOH at 9q22.3\(^2\) and 11 unselected pancreatic cancer cell lines (BxPC3, AsPC1, CAPAN1, CAPAN2, MiaPaCa2, Panc-1, Hs766T, CFPAC1, Su86.86, PL9 and PL45) obtained from the ATCC (American Type Culture Collection, Manassas, VA) and ECACC (European Collection of Animal Cell Cultures, Salisbury, UK).

**FANCG** was sequenced in 22 tumors selected for LOH at 9p13 and the same 11 cell lines used for **FANCC**.

All exons were amplified and sequenced from genomic DNA. Mutations were confirmed by sequencing of independent PCR products and confirmed in case PX19 by analysis of two xenografts derived independently from the same primary tumor. Constitutional DNA, where available, was sequenced to determine whether the alterations were somatic or germline in origin.

**FANCC** exons were amplified using the following PCR primers: (a) 5'-AGA GCC TTT TAG AAA TGC TTC and 5'-CCT GAA GTC AGA AAA TAA TTT C. Exon 1; (b) 5'-CCC ATT TAA GGA TGA AGT and 5'-CAT ACA TGG ACA ACA GTA TAG. exon 2; (c) 5'-ATG TTA TAT TCA GGG ATA CTT G and 5'-TAA CAG TGA AGG GTA TGT TTG. exon 3; (d) 5'-TAG GTA AAG CAC TGC TCA TTT and 5'-TGG CAC ATT CAG CAT TAA AC, exon 4; (e) 5'-ACAGAGTGAAACATGAGAAG and 5'-AAC ATC CAT TTC CTA TGA ATT, exon 5; (f) 5'-TGT TCA TCG TCG GTG TTA GAG and 5'-TGT CGT ACA GTC TTT CCA A. exon 6; (g) 5'-GAT GAG AAC TCT CAC AAA TTG and 5'-ATT ATA TAT AAA GGT TCC AAT TG, exon 7; (h) 5'-AGG AGT ATA CAG AGG AAT AAG and 5'-ACT CTA ATT TCC CCA TGA TAC, exon 8; (i) 5'-TCA CAC AAG GAC TGA AAT CTG and 5'-AAG TGG TCT TGT CCA AAA TAC, exon 9; (j) 5'-TGT TCT GAC CAT GTT AGT AC and 5'-AAT CTT CCT CCC AGG AAA TC, exon 10; (k) 5'-TCC GTG AAC CAG AAC TAA AG and 5'-TGG TCC CAG ACC AGT AAT G. exon 11; (l) 5'-CAG TGG ATA AGT ACA ATT TAA G and 5'-CCA GGT TGC CAT GAC ATA TG, exon 12. The following PCR primers were used for **FANCG**: (a) 5'-CTC GGC GGG GTG CAG AA and 5'-CCC GAG TAA TTA TAT CGA TC, exons 1 and 2; (b) 5'-GGG TGG GTT CTT TAT TGT AG and 5'-AGA CAA CTA.
GCA CTC AAC TAG, exons 3 and 4; (c) 5'-GGT CTA GCC AGG ATA GAT G and 5'-AGT GCT CTC TGT GGA TTT C, exons 5 and 6; (d) 5'-GGG AAA CCA CAA GCA TTA TG and 5'-GAG GAG TGG CGA CCT ATG, exons 7 and 8; (e) 5'-ATC CAT ACT GAG CCA AAA TTG and 5'-CAG TCT TGC TGT ATT TCA AAG, exon 9; (f) 5'-TGG TGT CCC CTT TGA AAT AC and 5'-AGG GGA ACT AGG TCA ACA TG, exon 10; (g) 5'-GGT GTG GAG GGA TTA TTC T and 5'-AAC ACC ACT CCT ACA CTT AC, exons 11 + 12; (h) 5'-GCC TAA GAC TAT GTC AAG TTC and 5'-AGA CAA CTA GCA CTC AAC TAG, exons 13 and 14.

Results and Discussion

Disease-inducing intragenic \textit{BRCA2} mutations in pancreatic cancer are uniformly accompanied by loss of the wild-type allele\cite{58}; we therefore studied conventional ("sporadic") ductal pancreatic adenocarcinomas that had LOH at 9q22.3 (\textit{FANCC}) and 9p13 (\textit{FANCG}). Five homozygous variants were observed: two in \textit{FANCG} and three in \textit{FANCC} (Table 5.1). Specifically, a deletion of 5 bp (nucleotides 1903-1907; National Center for Biotechnology Information) was observed in exon 14 of \textit{FANCC}, resulting in a frameshift alteration. Upon comparison with the nonneoplastic DNA of this patient, this mutation was found to be somatic. The other germline \textit{FANCC} variants were D195V and M350V. The missense variant D195V has been described for the first time in a FA patient with a mild clinical presentation\cite{101}. This variant was later shown by Lo Ten Foe \textit{et al.}\cite{152} to be able to correct the FA phenotype. Although the D195V variant could still lead to a reduced activity of the Fanc c protein, it does not lead to a null-phenotype. The M350V variant has not been reported before. The \textit{FANCG} mutation of the cell line Hs766T is identical to a mutation common in Fanconi anemia patients with a German ancestry, and leads (in its homozygous form) to a relatively early onset of the disorder\cite{153,154}. The cell line CAPAN1 contained another homozygous variant, S7F, in \textit{FANCG}, which has been described as a polymorphic variant\cite{154}. In addition to these homozygous mutations, one heterozygous mutation was observed in \textit{FANCC} exon 14, 1813G>A, E521K in cell line CAPAN2. This allele was confirmed to be expressed upon analysis of cDNA. No mutation was found in the other allele. The functional significance has not yet been determined: the mutation has not been reported in FA patients or as a normal polymorphism. These findings echo prior studies of \textit{BRCA2} suggesting that low-pene-
Table 5.1 Fanconi anemia gene sequence changes in pancreatic cancer

<table>
<thead>
<tr>
<th>Gene and setting</th>
<th>Mutations</th>
<th>Origin</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BRCA2</strong> (familial)</td>
<td>17%</td>
<td>Inherited</td>
<td>66.0</td>
</tr>
<tr>
<td>(reference population)</td>
<td></td>
<td></td>
<td>66.7</td>
</tr>
<tr>
<td><strong>BRCA2</strong> (&quot;sporadic&quot;)</td>
<td>4-7%</td>
<td>Usually inherited</td>
<td>71.5</td>
</tr>
<tr>
<td>(reference population)</td>
<td></td>
<td></td>
<td>64.3</td>
</tr>
<tr>
<td><strong>FANCC</strong> (&quot;sporadic&quot;)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PX102 tumor</td>
<td>frameshift (CCTAAA to CA)</td>
<td>Somatic</td>
<td>47</td>
</tr>
<tr>
<td>PX19 tumor</td>
<td>D195V (GAT to GTT)</td>
<td>Inherited</td>
<td>44</td>
</tr>
<tr>
<td>Su86.86 cell line</td>
<td>M350V (ATG to GTG)</td>
<td>Probably inherited</td>
<td>57</td>
</tr>
<tr>
<td><strong>FANCG</strong> (&quot;sporadic&quot;)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hs766T cell line</td>
<td>E105ter (GAG to TAG)</td>
<td>Probably inherited</td>
<td>46</td>
</tr>
<tr>
<td>CAPAN1 cell line</td>
<td>S7F (TCT to TTT)</td>
<td>Probably inherited</td>
<td>40</td>
</tr>
</tbody>
</table>

Average ages for BRCA2 mutations includes only inherited mutations. PX19 and PX102 had no family history of cancer by chart review. Variants present in the constitutional DNA were considered inherited. 1Probable synonymous change.

trance mutant alleles, ones that are not uncommon among the unsuspecting general population, might also be associated with somatic cancer. "Sporadic" cancer is not always sporadic, an inconvenient but instructive terminological problem. The BRCA2 example is now extended by the tumor-associated mutations of FANCC and FANCG. The carrier rate of FA gene mutations has not been directly assessed, but is estimated at 1 in 300 individuals in the general population, with higher rates in identifiable subpopulations.

FA proteins appear to be ubiquitously expressed among proliferating normal cells in culture. Fancc (558 amino acids, M, 63,000) and Fancg (622 amino acids, M, 68,000) proteins assemble together with Fanca. Fance and Fancf proteins in a nuclear complex, which is believed to
mediate the monoubiquitination of Fancl2 protein in response to DNA-damage. Subsequently, Fancl2 is targeted to nuclear foci, the formation of which may require Brca1 protein\cite{154}. BRCA1-/- and BRCA2-/- cells share MMC-hypersensitivity with all FA cells, and wildtype BRCA2 restores resistance in BRCA2-/- cells\cite{12}. The precise role of BRCA2 in the FA pathway has not yet been elucidated, but its BRC repeats are likely to serve as a scaffold for the assembly of RAD51 filament\cite{129,128}. Mouse gene knockout models and the human disease of FA, however, indicate a potential difference between BRCA2 and other FA genes. Whereas the homozygous null state for most FA genes is not incompatible with live birth and viability, BRCA2-deficient cells appear to require retention of at least one conditional or hypomorphic allele to retain viability\cite{46}. Breaks produced during the repair of MMC DNA-interstrand crosslinks accumulate in the absence of an intact homologous repair system in cells deficient in members of the FA complex. It has been suggested that BRCA2-null tumors may offer an especially wide therapeutic window for chemotherapeutic agents that require homologous recombination for their repair\cite{129,139,140}. Using therapies that included MMC, occasional complete remissions of pancreatic cancer have been reported\cite{4-6,141,142}, although the BRCA2 and FA gene status of such occasional patients has not been reported.

A wide spectrum of hematological and nonhematological malignancies has been reported in FA patients. However, FA patients have apparently not been observed to have an increased rate of pancreatic cancer\cite{45,143}. Although other nonhematological cancers are reported in such patients, many patients may not survive to an age highly susceptible to pancreatic cancer. It also re-mains possible that due to random variance in observations, the association could be missed in any general characterization of the FA population but could be more easily seen in a focused study of pancreatic cancer families\cite{59,144}. As another example, the high rate of pancreatic cancer in Peutz-Jeghers syndrome largely escaped notice until recently\cite{92}.

Additional study of FA genes is needed to determine possible mutations in other FA genes in pancreatic cancer or mutations in these genes among other tumor types. The stage at which FA mutations play a role in the initiation and progression of pancreatic cancer warrants investigation. Future clinical and preclinical studies should attempt to identify through genetic testing and optimize the therapeutic dosing, of the subgroup of patients whose tumors contain FA defects.