ABSTRACT

Genes of the Fanconi complementation groups [Fanconi anemia (FA) genes] 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 [N. G. Howlett et al., Science (Wash. DC), 297: 606–609, 2002]. Here we report inherited and somatic mutations of FANCC and FANCG present in young-onset pancreatic cancer. This may imply a general involvement of Fanconi cells to mitomycin and other therapeutic agents [M. S. Sasaki, Cancer CA62924.]

INTRODUCTION

FA is an inherited autosomal recessive syndrome, characterized cellurally by hypersensitivity to cross-linking agents such as mitomycin C. 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 mitomycin C (1, 2). Among human tumors, pancreatic cancers harbor the highest prevalence of BRCA2 mutations, which are present in 7–10% of “sporadic” pancreatic cancers (those with a family history negative for cancer) and 17% of families with a strong history of the disease [kindred with ≥3 family members affected, including at least 2 first-degree relatives (3–6)]. 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 genes mutations might occur as homoyzgous mutations in pancreatic cancers.

MATERIALS AND 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 (7). 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 and 11 unselected pancreatic cancer cell lines (BxPC3, AsPC1, CAPAN1, CAPAN2, MiaPaCa2, Panc-1, Hs766T, CFPC1, Su86.86, PL9, and PL45) obtained from the American Type Culture Collection (Manassas, VA) and European Collection of Animal Cell Cultures (Salisbury, United Kingdom). FANC 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 germ-line in origin. FANC exons were amplified using the following PCR primers: (a) 5′-AGAGCCTTTTGAAGATGTCCT and 5′-CCAGAGTCAGAAGATATTT-GTT, exon 1; (b) 5′-CCCTTATAGGGTGAGG and 5′-ATCATATGCTAGTGAAGGTTTT, exon 3; (d) 5′-TCTCTTCTCATTG and 5′-GGCCACTACCACTTAAAAC, exon 4; (e) 5′-AACTACTTCTCTCTGAGGAAAG and 5′-AACATCATTCTCATT-GAATT, exon 5; (f) 5′-GTGTCTAGCATGTTTG and 5′-TGTCG-TAGCCTTCCA, exon 6; (g) 5′-GATGAAAGTGCTCTACAAATT and 5′-ATTATATATAAGGTTTCCAATGT, exon 7; (h) 5′-AGGATGTA-CAGGAAATAAG and 5′-ACTCTAATTTCCCCCATGAT, exon 8; (i) 5′-TCACCAAGACTGAAAATCTG and 5′-AAGGTGTCCTCCTGCA-AATAC, exon 9; (j) 5′-TGTTGGCAGCTTATTGCTAC and 5′-AATGCTCT-TCCAAGGAAATC, exon 10; (k) 5′-TCTCAAGGCACAAGATG and 5′-TGTTGCCCAGCGAATGTT, exon 11; and (l) 5′-CTGGTGTAAG-CACAATTTAGG and 5′-GGCAAGTTGGCATGACAT, exon 12. The following PCR primers were used for FANC: (a) 5′-CGTGGCGGCGGCTG-CAAGA and 5′-CCCGATATTTATTCGACT, exons 1 and 2; (b) 5′-GGGTGTGTCTTTATTTGAG and 5′-AGCACTACGCTTCACTA-GA, exons 3 and 4; (c) 5′-GTCTGAGCCAGTATG and 5′-AGTG-CTCTTCTGAGGTT, exons 5 and 6; (d) 5′-GGAAACACAAACACAT-TAG and 5′-GAGGATGTCGCGCCTGAT, exons 7 and 8; (e) 5′-ATC-CATGATGCAAAAATG and 5′-CATGTCTTCTTGGATTCA, exons 9; (f) 5′-TGGTGTTCCCCCTTATAATG and 5′-AGGGTTAAGGTTACAT-GAACAT, exon 10; (g) 5′-GGTTGCGGAGGATGTTATCC, exon 11; and (h) 5′-GCTTGAAG-CATGCTAATG and 5′-AGCAACATAGCAGTCTAAG, exons 13 and 14.

RESULTS AND DISCUSSION

Disease-inducing intragenic BRCA2 mutations in pancreatic cancer are uniformly accompanied by loss of the wild-type allele (4); we therefore studied conventional (“sporadic”) ductal pancreatic adenocarcinomas that had LOH at 9q22.3 (FANCC) and 9p13 (FANCG). Three homozygous variants were observed: one in FANCC, resulting in a frameshift alteration. Upon comparison with the nonneoplastic DNA of this patient, this mutation was found to be somatic. The other FANCC mutation was a germ-line missense mutation in exon 6: 839 A>T, D195V. This mutation has been described previously in a FA patient and was associated with a milder clinical presentation (8). The FANCG mutation of the Hs766T cell line is identical to a mutation common in FA patients with a German ancestry and leads (in its homozygous form)
to a relatively early onset of the disorder (9, 10). In addition to these homozygous mutations, one heterozygous mutation was observed in 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 is not reported in FA patients or as a normal polymorphism.

These findings echo prior studies of BRCA2 suggesting that low-penetrance mutant alleles, ones that are not uncommon among the unsuspecting general population, might also serve as a widespread

<table>
<thead>
<tr>
<th>Gene and setting</th>
<th>Mutations</th>
<th>LOH</th>
<th>Origin</th>
<th>Age (yrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BRCA2</strong> (familial) (reference population)</td>
<td>17%</td>
<td>--</td>
<td>Inherited</td>
<td>66.0</td>
</tr>
<tr>
<td><strong>BRCA2</strong> (sporadic) (reference population)</td>
<td>7–10%</td>
<td>LOH</td>
<td>Usually inherited</td>
<td>71.5</td>
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<tr>
<td><strong>FANCC</strong> (sporadic)</td>
<td></td>
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<tr>
<td>PX19 tumor</td>
<td>D195V (GAT to GTT)</td>
<td>LOH</td>
<td>Inherited</td>
<td>44</td>
</tr>
<tr>
<td>PX102 tumor</td>
<td>Frameshift (CCTTAAA to CA)</td>
<td>LOH</td>
<td>Somatic</td>
<td>47</td>
</tr>
<tr>
<td>CAPAN2 cell line</td>
<td>E521K (GAG to AAG)(^{c})</td>
<td>No LOH</td>
<td>Unknown</td>
<td>56</td>
</tr>
<tr>
<td><strong>FANC</strong> (sporadic)</td>
<td></td>
<td></td>
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<tr>
<td>Hs766T cell line</td>
<td>E105ter (GAG to TAG)</td>
<td>LOH</td>
<td>Probably inherited</td>
<td>46</td>
</tr>
</tbody>
</table>

\(^{a}\) BRCA2 data are from Refs. 4–6. Average ages for BRCA2 mutations include only inherited mutations. PX19 and PX102 had no family history of cancer by chart review. Variants present in the constitutional DNA were considered inherited.

\(^{b}\) LOH in tumors resulting in homozygosity for the mutation.

\(^{c}\) Functional significance unknown. --, not studied.

Fig. 1. Chromatograms of mutations identified in pancreatic cancers. All sequences depicted are from genomic DNA except for the CAPAN2 repeat, which represents cDNA sequence.
cause of an inherited risk of cancer among a population. “Sporadic” cancer is not always sporadic, an inconvenient but instructive terminological problem (4). The BRCA2 example is now extended by the tumor-associated mutations of FANCC and FANCG genes. The carrier rate of FA gene mutations has not been directly assessed but is estimated at 1 in 300 individuals in the general population (11), with higher rates in identifiable subpopulations.

Unlike BRCA2 mutations, which may not convey a decreased age of onset of pancreatic cancer (4, 6), the inherited FA gene mutations of HS766T and tumor PX19 involved patients that are unusually young for pancreatic cancer (Table 1). Interestingly, the FANCC homozygous mutation in PX102, although of somatic origin, also affected a relatively young patient. The mutations here affected a third of the patients less than 50 years of age (three of nine patients) in our study population (samples of the remaining four young patients that lacked LOH were subsequently sequenced; no mutations were found).

Both FANCC–mutant tumors were from nonsmokers. All four cases with sequence variants were otherwise histologically and genetically typical; they were ductal adenocarcinomas with aneuploidy and multiple genetic abnormalities known from prior studies (12). In the CDKN2A, KRAA2, TP53, and MADH4 genes, for example, the following changes are seen, respectively: in PX19, homozygous deletion, mutation, mutation, and homozygous deletion; in PX102, homozygous deletion, mutation, mutation, and mutation; in CAPAN2, mutation, mutation, wild-type, and wild-type; and in HS766T, mutation, mutation, wild-type, and homozygous deletion.

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 a scaffold for assembly of the RAD51 filament (14, 15). Mouse gene expression analysis using Northern blotting indicates that expression of Fancd2 is up-regulated in response to DNA damage. Subsequently, Fancd2 is targeted to nuclear foci, the formation of which may involve Brca1 protein (13). BRCA1−/− and BRCA2−/− cells share mitomycin C hypersensitivity with all FA cells, and wild-type BRCA2 restores resistance in BRCA2−/− cells (1). 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 assembly of the RAD51 filament (14, 15). 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 (16).

Breeds produced during the repair of mitomycin interstrand crosslinks may accumulate in the absence of an intact homologous repair system in cells deficient in members of the FA complex. Recently, HS766T has indeed been found to be especially sensitive to mitomycin, occasionally complete reversions of pancreatic cancer have been reported (20–24), although the BRCA2 and FA gene status of such occasional patients has not been reported. A wide spectrum of hematological and nonhematological cancers has been reported in FA patients. However, FA patients have apparently not been observed to have an increased rate of pancreatic cancer (25, 26). Although other nonhematological malignancies are reported in such patients, most FA patients may not survive to an age highly susceptible to pancreatic cancer. It also remains possible that due to random variance in observations, the association could be missed in any general characterization of the Fanconi population but could be more easily seen in a focused study of pancreatic cancers. The relationship of pancreatic cancer to BRCA2 mutations, for example, is best seen in studies of pancreatic cancer families (6, 27). As another example, the high rate of pancreatic cancer in Peutz-Jeghers syndrome largely escaped notice until recently (28).

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 (3).

Note Added in Proof

We recently identified additional homozygous variants in cell lines Su86.86 (FANCCM350V, ATG to GTG, 57 y.o.) and CAPAN1 (FANCG, STF, TCT to TTT, 40 y.o.). Neither variant is reported in Fanconi anemia patients.

REFERENCES


Fanconi Anemia Gene Mutations in Young-onset Pancreatic Cancer

Michiel S. van der Heijden, Charles J. Yeo, Ralph H. Hruban, et al.