BRIEF REPORTS

A Porcine Model of Familial Adenomatous Polyposis

TATIANA FLISIKOWSKA,* CLAUDIA MERKL,* MARTINA LANDMANN,* STEFAN ESER,‡ NOUSIN REZAEI,* XINXIN CUI,* MAYUKO KUROME,§ VALERI ZAKHARTCHENKO,§ BARBARA KESSLER,§ HAGEN WIELAND,* OSWALD ROTTMANN,* ROLAND M. SCHMID,‡ GÜNTER SCHNEIDER,‡ ALEXANDER KIND,* ECKHARD WOLF,§ DIETER SAUR,‡ and ANGELIKA SCHNIEKE*

*Chair of Livestock Biotechnology, Technische Universität München, Freising, Germany; ‡Klinikum Rechts der Isar II, Technische Universität München, Munich, Germany; §Molecular Animal Breeding and Biotechnology, Ludwig-Maximilians-Universität München, Oberschleissheim, Germany

See Covering the Cover synopsis on page 1125; see editorial on page 1133.

We created gene-targeted pigs with mutations in the *adenomatous polyposis coli* (*APC*) gene (*APC*) that are orthologous to those responsible for human familial adenomatous polyposis (FAP). One-year-old pigs with the APC^{1311} mutation (orthologous to human APC^{1309}) have aberrant crypt foci and low- and high-grade dysplastic adenomas in the large intestine, similar to the precancerous lesions that develop in patients with FAP. Dysplastic adenomas accumulate β-catenin and lose heterozygosity of *APC*. This large-animal, genetic model of FAP will be useful in the development of diagnostics and therapeutics for colorectal cancer. DNA sequence data: NCBI accession number GU951771.

Keywords: Colon Cancer; Model System; Tumor Development; Genetic Predisposition.

Familial adenomatous polyposis (FAP) is characterized by foci of dysplastic growth in the colon and rectum that develop to adenomatous polyps and adenocarcinoma.[1] Germline mutations in the adenomatous polyposis coli (*APC*) tumor-suppressor gene are responsible for FAP, and somatic *APC* mutations are found in most sporadic colorectal tumors at the earliest stages.[2] A representative, human-scale animal model of colorectal cancer will aid in the development of novel drug, endoscopic or surgical interventions, and investigations of dietary components and gut flora. No confirmed cases of polyps or spontaneous colon cancer in wild-type pigs have been reported.[3]

We generated gene-targeted cloned pigs carrying translational stop signals in the *APC* gene at codons 1061 and 1311, orthologous to common germline mutations (APC^{1061} and APC^{1309}) in human FAP[4] (Supplementary Figure 1). Mutation at human codon 1309 is associated with a particularly severe phenotype with early onset and prolific polyposis; mutation at codon 1061 causes less severe polyposis.[5]

The gastrointestinal tracts of a wild-type, an $APC^{1061/+}$ mutant, and an $APC^{1311/+}$ mutant pig were examined at 1 year. The wild-type animal was normal. The $APC^{1061/+}$ founder pig also showed no evidence of polyposis, which was unsurprising at this young age given the human phenotype. Older $APC^{1061/+}$ animals will be investigated for later onset of polyposis. Examination of the $APC^{1311/+}$ pig, however, revealed more than 100 macroscopically visible lesions, including more than 60 sessile polyps, in the colon and rectum (Figure 1*A* and *B*, Supplementary Figure 2*A*–*C*). Polyps ranged from barely visible mucosal nodules of 1–2 mm, to flat polyps up to 1 cm. They were scattered along the whole large bowel, with most in the proximal colon (Figure 1*D*). As in young human FAP patients, no gastric polyps or duodenal adenomas were observed. In humans, colonic adenomas develop during childhood, but gastric polyps and duodenal adenomas occur later in adulthood. Again, examination of older animals will reveal whether the pig phenotype replicates this aspect of human FAP. This contrasts with murine *Apc* mutants such as *Min* (multiple intestinal neoplasia), in which polyps form almost exclusively in the duodenum and small bowel.[6]

To show that the porcine FAP model is suitable for evaluation of diagnostic procedures using human-sized equipment, we performed high-resolution magnification chromoendoscopic imaging of the small and large intestines. Methylene blue staining revealed more than 30 additional colonic lesions that were not visible by white light (Figure 2*A*). Some showed classic features of aberrant crypt foci (ACF).[7] Lesions were classified according to pit pattern[8] as non-neoplastic (pit pattern types I and II), or neoplastic microadenomas (pit pattern type III/IV) (Figure 2*A*). Histologic evaluation of more than 30 such lesions confirmed endoscopic classification in approximately 80% of cases, consistent with the accuracy of human chromoendoscopy.[8] Most ACF showed features of

Abbreviations used in this paper: ACF, aberrant crypt foci; APC, adenomatous polyposis coli; FAP, familial adenomatous polyposis; Ki67, antigen detected by monoclonal antibody Kiel-67.

0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2012.07.110

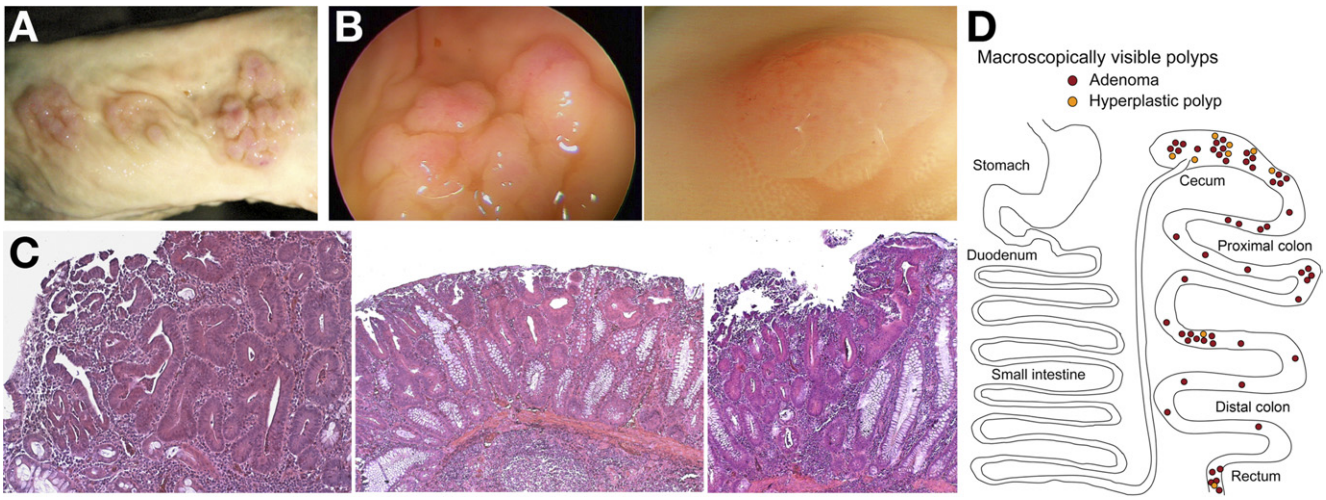

Figure 1. (*A*) Macroscopic and (*B*) endoscopic view of colonic lesions in the *APC*$^{1311/+}$ mutant pig. Morphology is typical of sessile polyps. (*C*) H&E-stained sections of polyps in *panels A* and *B* indicate tubular adenomas with dysplasia. (*D*) Overview of macroscopically visible intestinal lesions. *Red circle:* polyp with dysplasia (adenomas); *yellow circle:* hyperplastic polyp without dysplasia.

dysplastic crypts (unicryptal adenomas) and, similar to most pit pattern type III lesions, showed elongated and crowded cells with hyperchromatic nuclei that recapitulated organized glandular structures (Figure 2*B*). These were classified as adenomas with low-grade intraepithelial neoplasia.[6] Adenomas in human FAP also arise from a single dysplastic crypt.[9]

Pit pattern type I and II lesions (Figure 2*A*) showed serrated morphology without dysplasia, reminiscent of human hyperplastic polyps (Supplementary Figure 3*A–D*). Such lesions are common in humans, but not in *Apc* mutant mice.[6] Once considered benign,[10] they now are thought to be associated with increased risk of colorectal cancer.[11,12] Our observations suggest this may apply in pigs.

Some larger adenomas showed focal features of more advanced tumors: pronounced nuclear atypia and pleomorphism with aberrant chromatin pattern, large nuclei with loss of polarity with respect to the basement membrane, large nucleoli, and cribriform gland architecture. These were classified as adenomas with focal high-grade intraepithelial neoplasia[6] (Figure 2*B*). Porcine tumors appeared identical to human colonic adenomas with respect to surface involvement, with dysplastic cells on the superficial mucosal surface (Figures 1*C* and 2*B* and *D*, and Supplementary Figures 2*A* and 4). This contrasts with murine *Apc* mutant adenomas, which have a surface layer of nondysplastic epithelium (Supplementary Figure 2*D*).[6]

Wnt pathway activation caused by inactivation of the second (wild-type) *APC* allele is a hallmark of human FAP and sporadic disease.[13] We assessed the wild-type *APC* allele in 5 porcine adenomas (0.4–1 cm), 3 with low-grade and 2 with low and focal high-grade dysplasia. The wild-type *APC* allele was lost in each case (Figure 2*C*). ACF and colonic tumors also showed strong nuclear and cytoplasmic β-catenin staining outside the proliferative zone, suggesting that tumor initiation and progression occurs through Wnt pathway activation (Figure 2*D* and Supplementary Figure 4). Consistent with human FAP, there was no increase in epithelial proliferation of histologically normal intestinal mucosa, as assessed by Ki67 immunohistochemistry (Figure 2*D* and Supplementary Figure 4). However, adenomatous epithelium was highly proliferative, with almost all dysplastic cells Ki67 positive. Adenomas expressed cytokeratin-19 and CDX2 (caudal type homeobox2) markers of colonic epithelium, validating their origin (Supplementary Figure 5). Adenomas also showed strong expression of the β-catenin target c-MYC, and frequent phosphorylation of extracellular signal-related kinase (ERK) 1 and 2, markers of mitogen-activated protein kinase (MAPK) pathway activation, a known driver of intestinal tumorigenesis[14] (Supplementary Figure 5).

These data confirm that in pigs, unlike mice, a single heterozygous germline *APC* mutation is sufficient to initiate the well-characterized precancer sequence leading to adenomas in the large intestine, replicating early stage human FAP.

F1 generation *APC*$^{1311/+}$ piglets were born recently, enabling further characterization of the FAP model including progression to cancer. The molecular pathogenesis of colorectal cancer is marked by multiple genetic alterations of proto-oncogenes and tumor-suppressor genes. As with

Figure 2. (*A*) Detection of aberrant crypt foci and microadenomas by high-magnification chromoendoscopy. Lesions were classified by pit pattern. *Left to right*: aberrant crypt foci (pit pattern type III S; histology: unicryptal adenomas with low-grade dysplasia); pit pattern type III S lesion (adenoma with low-grade dysplasia); and pit pattern type I lesion (hyperplastic polyp without dysplasia). (*B*) H&E-stained colonic sections showing normal crypts, unicryptal adenomas with low-grade (LG) intraepithelial neoplasia (IEN), adenoma with LG IEN, and adenoma with focal high-grade (HG) IEN. (*C*) Polymerase chain reaction analysis showing loss of the wild-type *APC* allele in laser-microdissected adenomas. (*D*) Wnt pathway activation and proliferation of *APC*^{1311} mutant colonic adenomas. β-catenin and Ki67 staining of colonic adenoma with low-grade intraepithelial neoplasia (*left*) and normal mucosa (*right*).