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Interaction between APC and Fen1 during breast carcinogenesis

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Abstract

Aberrant DNA base excision repair (BER) contributes to malignant transformation. However, inter-individual variations in DNA repair capacity plays a key role in modifying breast cancer risk. We review here emerging evidence that two proteins involved in BER – adenomatous polyposis coli (APC) and flap endonuclease 1 (Fen1) – promote the development of breast cancer through novel mechanisms. APC and Fen1 expression and interaction is increased in breast tumors versus normal cells, APC interacts with and blocks Fen1 activity in Pol- β -directed LP-BER, and abrogation of LP-BER is linked with cigarette smoke condensate-induced transformation of normal breast epithelial cells. Carcinogens increase expression of APC and Fen1 in spontaneously immortalized human breast epithelial cells, human colon cancer cells, and mouse embryonic fibroblasts. Since APC and Fen1 are tumor suppressors, an increase in their levels could protect against carcinogenesis; however, this does not seem to be the case. Elevated Fen1 levels in breast and lung cancer cells may reflect the enhanced proliferation of cancer cells or increased DNA damage in cancer cells compared to normal cells. Inactivation of the tumor suppressor functions of APC and Fen1 is due to their interaction, which may act as a susceptibility factor for breast cancer. The increased interaction of APC and Fen1 may occur due to polymorphic and/or mutational variation in these genes. Screening of APC and Fen1 polymorphic and/or mutational variations and APC/Fen1 interaction may permit assessment of individual DNA repair capability and the risk for breast cancer development. Such individuals might lower their breast cancer risk by reducing exposure to carcinogens. Stratifying individuals according to susceptibility would greatly assist

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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epidemiologic studies of the impact of suspected environmental carcinogens. Additionally, a mechanistic understanding of the interaction of APC and Fen1 may provide the basis for developing new and effective targeted chemopreventive and chemotherapeutic agents.

Keywords

Adenomatous polyposis coli; flap endonuclease 1; base excision repair; environmental carcinogens; breast cancer development; mortality

1. Introduction

In 2016, an estimated 249,260 new cases of invasive breast cancer will be diagnosed among women and 40,890 deaths will occur in the United States [1]. Familial inheritance contributes to about 10% of all breast cancers. The systematic epidemiological studies suggest that *BRCA1* and *BRCA2* are important susceptibility genes of familial breast cancer [2–6]; however, population and hospital-based studies indicate that mutations in these two genes account for only 4–6% of total breast cancer cases [7, 8]. Other susceptibility factors that contribute to breast cancer have been identified as being related to exposure to environmental carcinogens (including certain chemicals and ionizing radiation), as well as increasing age, family and reproductive history, obesity, heavy alcohol intake and hormonal replacement therapy [9–19]. There is considerable inter-individual variation in their susceptibility to environmental carcinogens. Environmental carcinogens result in the generation of reactive oxygen species (ROS), oxidative lesions, apurinic/apyrimidinic sites, alkylated and alternative bases, bulky DNA adducts, and DNA strand breaks [20–23]. Carcinogen-induced DNA adducts, which are characteristic of exposure to complex mixtures of aromatic compounds (such as polycyclic aromatic hydrocarbons, PAH) and tobacco smoke, have been detected in breast tumors [24–28]. Several epidemiologic studies report an association between PAH-DNA adducts and breast cancer incidence [29–36]. Most of this damage is repaired by the base excision repair (BER) pathway [37–39]. If the damaged DNA is not efficiently repaired, then the increased accumulation of damage can predispose individuals to breast cancer [40–42]. However, not all individuals that are exposed to DNA-damaging carcinogenic agents develop breast cancer, which suggests the possibility that inter-individual variation in DNA repair capacity may play a significant role in modifying breast cancer risk. Indeed, it has been reported that either BER deficiency or aberrant BER function is associated with a high risk of mutations that may lead to malignant transformation [43–46]. An interaction between APC and Fen1 can block Fen1-mediated BER [47–49]. To understand the susceptibility of individuals to environmental carcinogens and development of breast cancer, which is the most common cancer and the second leading cause of cancer death among American women, we have focused our efforts on evaluating the novel concept that the interaction of APC with Fen1 is a critical risk factor that influences individual breast cancer risk from exposure to endogenous and exogenous DNA-damaging carcinogenic agents. This implies that environmental carcinogens that affect the APC and Fen1 interaction (including certain mutations of the genes that encode the domains involved in the association or polymorphisms that affect these domains) may play a role in breast carcinogenesis.

2. Novel functions of APC in DNA repair and replication

Our hypothesis is based on our recent discovery of a novel function for APC in DNA repair. It is well established that APC plays an important tumor suppressor function in many cellular processes, such as migration, proliferation, differentiation, adhesion, chromosomal stability, mitochondrial transport and apoptosis [50–54]. While mutations in the *APC* gene have been implicated in the impairment of these functions leading to tumorigenesis in colorectal cancer, the potential role of wild-type APC in other tumors, such as breast tumors, is not well-understood. In breast tumors, the transcriptional silencing of the *APC* gene by promoter hypermethylation has been detected in up to 70% of inflammatory human breast tumors and 7% of metaplastic breast carcinomas [55–58]. We have found that APC interacts with PCNA, Pol- β and Fen1 and blocks Pol- β -directed single-nucleotide (SN) and long-patch (LP) BER pathways [47, 48, 59–61] (Fig. 1). The DNA repair inhibitory (DRI)-domain of APC (amino acids 1250-1269), which is the site of interaction with Pol- β and Fen1, is located in the N-terminal region and is spared by mutations in the mutator cluster region (MCR) that result in truncation of the protein (Fig. 2). Thus, most mutant APC proteins (those with an intact DRI-domain), as well as wild-type APC, are capable of modulating BER [48].

It is well established that APC can interact with numerous proteins including CSNK2B, β -catenin, casein kinase 2 α 1, XPO1, AXIN1, TFAP2A, SIAH1, plakoglobin, DLG3, KIFAP3, catenin (cadherin-associated protein) α 1, ARHGEF4, MAPRE2, TUBA4A and BUB1 [[http://en.wikipedia.org/wiki/APC_\(gene\)](http://en.wikipedia.org/wiki/APC_(gene))]. APC also interacts with MUC1, which is involved in regulating β -catenin signaling in breast tumorigenesis [62] and MUC1-CT (MUC1 cytoplasmic tail) translocates with β -catenin to the nucleus and affects transcription [63]. The fact that most of the interactions of APC with other proteins that have been described occur in the cytoplasm and the report that the majority of wild-type and mutant APC resides in the cytoplasm and only wild-type APC has been detected in the nuclei of epithelial cells [64] seemed contrary to our proposal that APC plays a critical role in nuclear function. Recently, however, another nuclear function of APC (amino acids 1000-1326) has been described by other investigators, *i.e.*, its interaction with topoisomerase II α in G₂/M transition. Moreover, in the same study, an interaction of APC with PCNA was also shown [65], which confirmed our previous findings [59]. Our findings have been described and reviewed by peers in their recent publications [66–72] and additional evidence is now accumulating concerning the role of APC in DNA repair. It has been suggested that nuclear APC, through a region (amino acids 1441-2077) that is truncated in the majority of colorectal tumors, cooperates in the recruitment of DNAPKcs to the damaged DNA chromatin and enhances early response to double-strand breaks (DSB) DNA repair by promoting S139 histone γ H2AX phosphorylation [73]. APC also interacts directly with genomic DNA, preferentially with A/T rich sequences [74], implying a role for APC in DNA replication [75]. In these studies, it has been suggested that APC through its C-terminus end (amino acids 2140-2421) interacts with DNA and negatively regulates cell cycle progression through inhibition of DNA replication. From these findings, it can be concluded that different domains of APC are associated with the formation of different protein/protein complexes and mediate different cellular functions, such as β -catenin

regulation, cell cycle control, gene regulation, DNA repair and replication [48, 53, 73–76]. It seems that during breast cancer development, the tumor suppressor function of the wild-type APC is lost due to interaction with Fen1 at amino acids 1250-1269 and this interaction results in blockade of LP-BER. This mechanism would differ from the loss of the tumor suppressor function of APC in colorectal cancer development, which is mostly associated with truncation mutations of APC (amino acids 1441-2077) that blocks DSB DNA repair [73].

3. Role of Fen1 in DNA replication, recombination and repair

Fen1 is a structure-specific 5' nuclease superfamily member that recognizes single-stranded (ss)/double-stranded (ds) DNA junctions and cleaves one nucleotide into the dsDNA [77, 78]. Fen1 is well known for its involvement in Okazaki fragment maturation, recombination and LP-BER [47, 79–83]. When Fen1 interacts with PCNA, Pol- δ , replication protein A (RPA) and DNA ligase I, it removes RNA primer during DNA replication [84]. On the other hand, when Fen1 interacts with Pol- β , APE1 and PCNA, it modulates their activity in LP-BER [85–87]. Other proteins, such as WRN helicase interacts with and stimulates Fen1 activity in LP-BER [88]. A heterodimeric protein complex Rad9-Rad1-Hus1 also interacts with and stimulates the activity of Fen1 in DNA repair [89]. Our recent findings indicate another novel role for Fen1 in LP-BER, i.e., its interaction with APC [47, 48, 53]. The expression of Fen1 is increased in several cancer cells, including breast [90], as well as prostate [91], gastric [92], neuroblastoma [93], pancreatic [94, 95] and lung [96]. Fen1 is a highly versatile protein and performs various cellular functions, depending on its interaction with different proteins [81]. In breast cancer cells, Fen1 is significantly up-regulated upon treatment of chemotherapeutic drugs such as mitomycin C (MMC) and Taxol, which is regulated by transcription factor/repressor YY1 [97]. YY1 down-regulates Fen1 and sensitizes the breast cancer cells to these drugs. Thus, the level of Fen1 can be inversely related to drug-resistance and with survivorship in breast cancer patients.

4. Evidence that APC and Fen1 play a role in breast carcinogenesis

Detailed molecular and biochemical analysis of breast tumor tissues and cell lines have implicated defects in many different molecules, including BRAC1, BRAC2, c-Myc, c-erbB2, PTEN, LKB1, ATM, MSH2/MLH1, Chk2, BACH-1, Ki-67, cyclin D1, p53, estrogen, progesterone and androgen receptors in breast tumorigenesis [98–102]. Although there is less evidence of an association with defects in APC and Fen1 with breast cancer, it should be noted that in most studies these molecules have been analyzed independently and the focus has not been on protein/protein interactions. Genetic mutations of APC or β -catenin are often found in colonic tumors [103–108], but are rarely observed in other tumors, such as breast tumors [109–112]. As described above, however, APC can interact with numerous proteins and the concept that modification of these interactions can play a critical role in the development of breast cancer is now emerging.

A recent study suggests that *Apc*^{Min/+} mice when crossed with mouse mammary tumor virus (MMTV)-Polyoma virus middle T antigen (PyMT) mice showed enhanced mammary tumorigenesis that was mediated by enhancing the focal adhesion kinase (FAK), sarcoma

(Src) tyrosine kinase and c-Jun N-terminal kinase (JNK) signaling [113]. It is also suggested that MMTV-PyMT/*Apc*^{Min/+} mammary tumors express multidrug resistance antigen 1 (MDR1) in an *Apc*-dependent manner, which causes resistance to chemotherapeutic drugs, such as cisplatin and doxorubicin [114]. Since cisplatin and doxorubicin cause oxidative DNA damage, the chemotherapeutic resistance of the MMTV-PyMT/*Apc*^{Min/+} mammary tumors could also be due to increased BER because mutant *Apc* will decrease DNA damage burden and facilitate cell survival. In support of this hypothesis, 5-fluorouracil-mediated anticancer activity in colon cancer cells is mediated through the induction of APC and the inhibition of LP-BER [115]. A distinct role for the interaction of APC with MUC1 has also been shown in breast tumors and metastases as compared to adjacent normal tissues. In these studies, the interaction of APC and MUC1 was dependent on epidermal growth factor receptor (EGFR) kinase activity, which down-regulates β -catenin signaling [62].

Other evidence that APC may play a critical role in breast cancer comes from various mouse models. Mice with a hypomorphic mutant allele of *Apc* (*Apc*^{1572T}), which results in an intermediate level of Wnt/ β -catenin signaling activation, develop multifocal breast adenocarcinomas and subsequent pulmonary metastasis rather than the multifocal intestinal tumors that are associated with other *Apc* mutations [116]. Other models that implicate *Apc* in breast tumorigenesis include a model in which a K14-cre-mediated *Apc* heterozygosity (in which exon 14 of the *Apc* is flanked by loxP sequences) is associated with development of mammary adenocarcinomas [117] and *Apc*^{Min/+} mice. *Apc*^{Min/+}, which are heterozygous for a truncation allele at residue 850, spontaneously develop polyps and tumors of the small intestine and are a widely used model of colorectal cancer. However, *Apc*^{Min/+} mice are highly susceptible to the carcinogen ENU and the tumors that develop in response to ENU exposure are primarily mammary and ovarian tumors rather than tumors of the small intestine [118, 119]. These tumors harbor truncation mutations in a defined region in the remaining wild-type allele of *Apc* that would retain some down-regulating (dosage) effect on β -catenin signaling. In these animal models, it is clear that the expression of one copy of the wild-type *Apc* is present during the early stages of mammary tumor development. In later stages, the remaining wild-type allele of *Apc* is likely inactivated due to frame-shift mutations and in some cases truncation mutations [117]. Notably, these mutations are located beyond amino acid 1500, rather than the MCR region of *Apc* mutations (amino acids 1284-1580) frequently found in mouse gastrointestinal tumors [118, 120–122] and the mutated *Apc* typically retains the DRI-domain (amino acids 1245-1273) capable of interacting with Fen1, whether or not the mutations are frame-shift or truncation mutations.

APC regulation of Wnt/ β -catenin signaling is considered to play a critical role in APC tumor suppressor functions and several studies have suggested that APC/ β -catenin signaling can play an important role in mammary tumorigenesis [110–112, 123]. Most recently, studies of the *Apc*^{1572T} mouse model have been interpreted as indicating that the specific dosage of Wnt/ β -catenin signaling differentially affects tissue homeostasis and thereby initiates tumorigenesis in an organ-specific manner [116]. However, the Wnt/ β -catenin dosage effect does not readily explain the production of mammary tumors in the ENU-treated *Apc*^{Min/+} mice [118, 119]. Rather, this model suggests that an additional event might accelerate the effect of the loss of APC function on mammary tumorigenesis. We hypothesize that this additional event is the APC/Fen1-mediated compromised LP-BER capacity of the mammary

epithelial cells. In this scenario, there would be two events that, in combination, accelerate the tumorigenesis in these tissues: (i) The reduced tumor suppressor functions of the single copy of *Apc*; and (ii) the attenuated LP-BER due to APC/Fen1 interaction. Alternatively, Fen1 after interaction with APC may block the remaining tumor suppressor function of the *Apc*^{+/-} cells and create a dominant negative effect, which in combination with attenuated LP-BER accelerates neoplastic transformation of mammary epithelial cells. This hypothesis will be tested in future studies.

5. *APC* and *Fen1* genes are overexpressed in breast tumors and correlate with enhanced mortality

We have shown that after treatment with environmental carcinogens both Fen1 [124] and APC expression is increased in spontaneously immortalized normal human breast epithelial cells, human colon cancer cells and mouse embryonic fibroblast cells [125–128]. Since both Fen1 and APC are tumor suppressors [50, 52, 70, 83, 129, 130], an increase in their levels would be expected to protect against carcinogenesis; however, this does not seem to be the case in carcinogen-induced breast cancer development. It has been suggested that the increased expression of Fen1 in breast and lung cancer cells may reflect the increased proliferation rate of cancer cells as compared to normal cells or that the increased Fen1 expression may be a response caused by increased DNA damage in cancer cells compared to normal cells [90, 96, 131].

Furthermore, genomic and protein expression analysis suggest Fen1 as a key biomarker in breast and ovarian cancer [132]. Based on our model of the interaction of Fen1 and APC that blocks LP-BER, it is highly tempting to suggest that even though APC and Fen1 are expressed in higher amounts in breast and lung tumors, the tumor suppressor functions of these two important proteins has been lost in breast cancer. The impaired LP-BER may cause accumulation of AP-sites resulting in the neoplastic transformation of mammary epithelial cells.

We used Oncomine's gene search function to locate microarray studies of expression of *APC* and *Fen1* in breast cancer. Oncomine processes and normalizes each dataset used in these analyses independently. For the differential expression analysis, they use t-statistics with false discovery rates as a corrected measure of significance. We sorted the results based on each class of analysis, noted the significant studies produced, and then created a Box-Plot with the results of this analysis. These microarray values were pre-processed for normalization by Oncomine. We compared the data from two different studies and found that both *APC* and *Fen1* gene expression were significantly increased in breast carcinoma as compared to adjacent normal or benign tissues (Fig. 3).

We assessed the prognostic significance of our findings to examine whether the increased levels of APC and Fen1 expression in breast tumors with decreased BER and increased DNA damage will correlate with disease progression and mortality. In fact, the Kaplan-Meier analysis supported our hypothesis and showed significantly worse survival of breast cancer patients with high level mRNA expression of *APC* or *Fen1* as compared to the low level expression of these genes (Fig. 4) (<http://kmplot.com/analysis>). The log-rank test of the

APC and *Fen1* levels of the Kaplan-Meier analysis negatively correlated with patient survival rates ($P = 2e-05$ and $P < 1e-16$, respectively) and showed increased hazard ratios (HR) of 1.28 (range = 1.14–1.44) and 1.68 (range = 1.5–1.89), respectively (Fig. 4). The median follow-up of patients was 250 months. These results may have major implication for the role of *APC* and *Fen1* in breast tumorigenesis and poor survival as the Kaplan-Meier analysis includes all categories of breast cancer irrespective of the status of the estrogen receptor (ER), progesterone receptor (PR), HER2 receptor, lymph node involvement, intrinsic subtype, p53, or grade.

6. Polymorphic variations in *APC* and *Fen1* genes that may affect the interaction of *APC* and *Fen1* proteins, LP-BER, accumulation of AP lesions, and susceptibility to breast cancer

Not all individuals that are exposed to cigarette smoke carcinogens develop breast cancer. It is possible that inter-individual differences in the propensity of *APC* and *Fen1* to interact are associated with susceptibility to cigarette smoke-induced breast carcinogenesis. A number of factors could affect this protein/protein interaction including polymorphisms/mutations of the genes that encode the proteins and post-translational alterations. Polymorphisms in the *APC* gene have been reported [133, 134]. A specific germ-line missense mutation, involving transition of T to A at nucleotide 3920 of the *APC* gene causes the substitution of lysine for isoleucine at codon 1307 [135]. In a recent study, the *APC* I1307K variant has also been associated with an increased risk of CRC among Ashkenazi Jewish, Croatian, and Egyptian subjects [136–142]. While studies indicate that I1307K substitution predisposes to colorectal cancer, it has also been shown to predict the prevalence of breast, lung, urologic, pancreatic, and skin cancers [143, 144]. Several additional genetic changes on the affected allele have also been identified, such as an A insertion in the 1306-9 region; a G deletion at 1314; a G to T change at codon 1309, as well as several other point mutations: 1309 GAA(Glu) to GGAA (stop at 1314), 1306 GAA(Glu) to TAA(stop), 1283 GCT to GC (stop at 1297), 1274 TGT to TG (stop at 1274), and 1270 TGT to TG (stop at 1287). We have examined the NCBI database for single nucleotide polymorphisms, other than those creating stop codons, of the *APC* gene and found some that occur within the DRI-domain: NM_000038.3:c.730-3780A>G, NM_000038.3:c-18-3823G>C, NM_000038.3:c.-19+3825G>T, NM_000038.3:c.730-3838A>G and NM_000038.3:c.646-3844C>T. From these results, it appears that codons 1250-1350 of the *APC* gene are highly dynamic and that mutations in this region may play important roles in the susceptibility of individuals to non-colonic as well as colonic carcinogenesis [145].

Recently, an association of functional FEN1 genetic variants and haplotypes in breast cancer risk has been described [146]. Polymorphisms of the *Fen1* gene have been found in the promoter, coding and 3'-untranslated regions of this gene, and have been implicated in lung carcinogenesis [147, 148]. Some of the mutations of *Fen1* (I39T, L53 insertion, Q112R, P151L, A159V, R245W, S317F and A353V mutant proteins) have been found to be defective in EXO and GEN activities, but retain 5'-flap endonuclease activity [147]. The E160D knock-in mouse cell line shows a deficiency in the nuclease activity of *Fen1*, which is similar to the mutations identified in human lung cancers and linked with autoimmunity

and chronic inflammation [147]. Whether these mutations affect the interaction with APC and whether they might play a role in breast cancer development is not yet known. Some of the known polymorphisms in the APC and/or Fen1 genes may affect the formation or stability of the APC/Fen1 complexes. In support of this idea, although not related to polymorphism, a recent study suggests that the Fen1 heterozygous mice when combined with a mutation in the *Apc* gene developed an increased number of adenocarcinomas of the gastrointestinal tract and decreased survival. The tumors from these mice show microsatellite instability [149].

From a clinical point of view, the repression of Fen1 level by the YY1 transcription factor has been shown to increase the sensitivity of breast cancer cells to MMC and Taxol [97]. Similarly, as we have shown that APC blocks Fen1 activity [47] and targeting Fen1(D181) with a small molecule increases the sensitivity of colon cancer cells to temozolomide (TMZ) [150], it can be hypothesized that APC mediated blockade of Fen1 activity may also increase the sensitization of breast cancer cells to DNA damaging agents.

7. Future perspectives

The APC/Fen1 interaction increases in the highly susceptible individual due to polymorphic and/or mutational variation in the *APC* and/or *Fen1* genes. This interaction results in compromised LP-BER, accumulation of apurinic/aprimidinic (AP)-sites, and subsequently causes neoplastic transformation of normal breast epithelial cells. The altered Fen1 function due to interaction with APC influences Fen1-mediated BER capacity and implies that individuals with polymorphisms or mutations of *APC* and/or *Fen1* that promote this interaction will be at higher risk of developing carcinogen-induced breast cancer than individuals that do not carry these polymorphisms or mutations. The development of a putative “high-risk” profile through the screening of *APC* and *Fen1* polymorphic and/or mutational variations and the APC/Fen1 interaction may provide an important tool in the assessment of individual DNA repair capability and the risk for breast cancer development.

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Abbreviations

AAG/MPG	alkyladenine/methylpurine DNA glycosylase
APC	adenomatous polyposis coli
BER	base excision repair
DRI	DNA-repair inhibitory
EGFR	epidermal growth factor receptor
FAK	focal adhesion kinase

Fen1	flap endonuclease 1
JNK	c-Jun N-terminal kinase
MBD4	methyl-CpG-binding domain-4
MDR1	multi-drug resistant antigen-1
MMC	mitomycin-C
MMTV	mouse mammary tumor virus
PAH	polycyclic aromatic hydrocarbons
PyMT	Polyoma virus middle T antigen
RPA	replication protein-A
SMUG1	single-strand-selective mono-functional uracil DNA glycosylase-1
TDG	thymine DNA glycosylase
TMZ	temozolomide
UDG	uracil DNA glycosylase
UNG	uracil DNA N-glycosylase

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Highlights

- APC and Fen1 are BER proteins involved in breast cancer development.
- APC interacts with Fen1 and blocks Fen1-mediated LP-BER.
- LP-BER block is linked with CSC-induced transformation of breast epithelial cells.
- APC/Fen1 levels in breast tumors correlate with disease progression and mortality.
- APC/Fen1 interaction provides a tool for risk assessment for breast carcinogenesis.

Figure 1. A simplified model for the BER reaction based on *in vitro* studies showing the interaction of APC with Pol-β and Fen1

There are two sub-pathways for BER - single nucleotide (SN) BER and multiple nucleotide or long patch (LP) BER. The first step in BER is the removal of the damaged base, which is accomplished by DNA glycosylases. Based upon their catalytic mechanisms the DNA glycosylases can be either mono- or bifunctional. The mono-functional glycosylases, i.e., uracil DNA glycosylase (UDG) family of proteins: uracil DNA N-glycosylase (UNG), thymine DNA glycosylase (TDG), single-strand-selective mono-functional uracil DNA glycosylase 1 (SMUG1) and methyl-CpG-binding domain 4 (MBD4) and alkyladenine/methylpurine DNA glycosylase (AAG/MPG) that catalyzes the removal of alkylated bases. The bi-functional glycosylases exhibit base excision activity as well as create a single-strand break (SSB) with a non-conventional 3'-terminus to the AP site either by β -elimination (OGG1 and NTH1) or by two consecutive β - and δ -elimination steps (NEIL1/2). Once the base is removed by DNA glycosylases, the AP endonuclease 1 (APE1) incises the DNA backbone 5' of the abasic site and generates a strand break with a generation of a 3'-OH group and a non-conventional 5'-deoxyribose phosphate (dRP), which is removed by DNA polymerase β (Pol- β). Then for SN-BER, Pol- β incorporates the correct base and DNA ligase III α (LIGIII α)/XRCC1 seals the gap. Once the AP site is oxidized or reduced it becomes resistant to the dRP lyase activity of Pol- β and thus proceeds to LP-BER pathway. In LP-BER, Pol- β incorporates more than one nucleotide and creates a single-nucleotide flap which is removed by flap endonuclease 1 (FEN1). Finally, the gap is sealed by LIGI. The interaction of APC with different steps of SN- and LP-BER pathways is shown.

Figure 2. Schematic representation of the structure of APC showing the DNA-repair inhibitory (DRI)-domain

The 2843 amino acid sequence of APC protein displays an armadillo domain near the N-terminus. There are two β-catenin binding domains. The first 15-amino acid repeat can bind β-catenin, but its functional significance is still obscure, while the 20-amino acid repeat can bind β-catenin with a high affinity upon phosphorylation. The DRI-domain, just upstream of MCR, is involved in the regulation of the BER pathway. Asef, APC-stimulated guanine nucleotide exchange factor; DLG, Drosophila discs large; EB1, end-binding protein 1, KAP3A, kinesin super-family-associated protein 3A; NES, nuclear export signal; NLS, nuclear localization signal; PP2-B56α, protein phosphatase 2A B56α subunit.

Figure 3. APC and Fen1 gene expression in normal and breast carcinoma tissues

APC and *Fen1* gene expression levels in normal human and breast cancer samples. Box-Plots are denoted with blue and red colors, respectively. Results are from two independent analyses.

Figure 4. Kaplan-Meier survival curve of all breast cancer patients
Data were analyzed from 1788 and 1766 patients and from 1779 and 1775 patients with low and high levels of *APC* and *Fen1*, respectively (<http://kmplot.com/analysis>). Patients with higher *APC* or *Fen1* mRNA expression showed reduced overall survival.