Crosslinks and crosstalk: Human cancer syndromes and DNA repair defects

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A subset of human cancer syndromes result from inherited defects in genes responsible for DNA repair. During the past few years, discoveries concerning the intersection of certain DNA repair processes have increased our understanding of how the disruption of specific DNA repair mechanisms leads to genomic instability and tumorigenesis. This review focuses on the human genes *MUTYH*, *BRCA2/FANCD1*, and *BLM*.

Human DNA repair mechanisms

The genome is continuously assaulted by reactive species within cells (reactive oxygen species and various metabolites) and by environmental agents from without (UV light, ionizing radiation, and toxins). It is not surprising, therefore, that the human genome encodes a large number of proteins, at least 130, that function in DNA repair processes (Wood et al., 2001).

Several types of DNA repair processes are tailored to deal with various types of damage, although some are overlapping (reviewed in Hoeijmakers, 2001). For example, three types of excision repair have been described: base excision repair (BER), nucleotide excision repair (NER), and mismatch repair (MMR). Small chemical alterations of bases (usually a result of endogenous oxidation or alkylation) are generally repaired by BER. Lesion-specific glycosylases cleave the damaged base(s) from the sugar-phosphate backbone, and the damaged strand is incised by an endonuclease at the abasic site. Local DNA synthesis and ligation are employed to complete the repair, either through the more commonly used short-patch BER pathway or the more complex long-patch BER pathway. NER removes bulky adducts such as pyrimidine dimers resulting from exposure to various environmental agents. Multiprotein complexes recognize and bind to the damage site, unwind the duplex DNA, incise on either side of the damage, and remove the damaged segment. DNA is then synthesized to fill the gap, using the undamaged strand as a template, and the ends are ligated. MMR corrects nucleotide mismatches that occur during replication or recombination. Mismatches are recognized, multiple factors are recruited to the site, the newly synthesized strand is identified and degraded past the mismatch, and the degraded stretch is resynthesized.

Two additional types of DNA repair, homologous recombination (HR) and nonhomologous end-joining (NHEJ), are employed when the most serious type of DNA damage, a double-strand break, occurs. HR is considered an error-free pathway since it uses a copy (usually the sister chromatid available during the S and G_2 phases of the cell cycle) of the damaged segment as a template to guide repair. NHEJ is considered an error-prone pathway, since free ends are joined without the use of a template or through use of very small microhomologous repeats and, consequently, there often may be an associated loss of nucleotides or translocation.

During the past few years, considerable progress has been made in understanding how these repair processes might interact and how specific disruptions of these mechanisms lead to genomic instability and tumorigenesis. This review focuses on three of the major findings in this regard: the identification of biallelic germline mutations in *MUTYH* (previously called *MYH*) in individuals with a predisposition to multiple colorectal adenomas and carcinomas, the identification of the DNA repair protein BRCA2 as a Fanconi anemia protein, and the recognition of a potential key role of recQ-like helicases in the coordination of DNA repair pathways due to their interaction with and recruitment of numerous types of DNA repair proteins to sites of damage.

The BER gene *MUTYH* and colorectal cancer

Although oxidative DNA damage has been implicated in cancer etiology, as of two years ago, there were no known human genetic disorders attributed to BER defects. *MUTYH*, the human homolog of the *E. coli mutY* gene encoding a DNA glycosylase (Slupska et al., 1996), is the first BER gene associated with a human cancer syndrome, as biallelic germline mutations in *MUTYH* have been identified in individuals with a predisposition to multiple colorectal adenomas and carcinomas (Al-Tassan et al., 2002).

Isolated adenomatous polyps are a common finding in the colon, especially with advancing age; adenomatous polyps then undergo malignant transformation to become carcinomas over time. When multiple polyps are present, the likelihood of developing a carcinoma is directly related to their number, although there are additional modifying factors (Debinski et al., 1996). Germline truncating mutation of the *APC* tumor suppressor gene is associated with an autosomal dominant colon cancer predisposition syndrome known as familial adenomatous polyposis coli, or FAP. FAP is characterized by the presence of a hundred to thousands of colorectal adenomas, early onset of colorectal carcinoma, and variable extracolonic manifestations (congenital hypertrophy of the retinal pigment epithelium, upper GI tract polyposis, or desmoid tumors). Tumors develop after the wild-type *APC* gene undergoes somatic mutation. The key role of *APC* mutation in early tumor development is also indicated by the fact that somatic mutations of *APC* are found in the great majority of sporadic colorectal tumors (Powell et al., 1992). A major role of APC in growth control is due to its function in the WNT signaling pathway and its effects on β-catenin degradation (reviewed in Goss and Groden, 2000).

Other individuals present with fewer colorectal adenomas (less than 100), develop colorectal carcinoma at an older age

Table 1. Major mutational pathways in colorectal cancer

Type of genomic instability	CIN chromosomal instability	MSI microsatellite instability	CIN–, MSI–
Karyotype	aneuploid/ polyploid	near-diploid	near-diploid
Common somatic gene mutation/inactivation	*APC*, *KRAS*, *SMAD4*, *TP53*	*TGFβRII, IGFFIIR, BAX, BRAF, MSH3, MSH6, E2F4;* fewer *APC, KRAS, SMAD4,* and *TP53* mutations	*APC, KRAS*
Familial colorectal cancer syndrome associated with pathway	FAP (via *APC* defect)	HNPCC (primarily via *MSH2* or *MLH1* defect; rarely *MSH6, PMS2,* or *PMS1* defect/MMR defect)	MAP (via *MUTYH* defect/ BER defect)

than those with FAP, and carry germline mutations in *APC* that lie in the 5′ and 3′ regions outside the common regions of mutation; these individuals usually have a family history of colorectal adenoma/carcinoma and are described as having attenuated polyposis (AAPC). Still other individuals present with less than 100 colorectal adenomas, generally without a family history of colorectal adenoma/carcinoma, and are described as having "multiple colorectal adenomas."

Al-Tassan et al. (2002) studied a family with multiple colorectal adenomas and carcinoma predisposition, but no germline *APC* defect, and found that tumors from family members demonstrated a high incidence of somatic mutations in *APC* that involved G:C → T:A transversions. These types of *APC* mutations are uncommon in tumors from classic FAP individuals or in sporadic colorectal tumors. Oxidative DNA damage leads to the formation of a particularly stable product, 8-oxoguanine (8-oxoG), which can pair with cytosine or mispair with adenine. If repair does not occur, 8-oxoG:A base pairs can be converted to T:A base pairs during subsequent replication. The mutT homolog MTH removes oxidized nucleotide precursors from the nucleotide pool prior to incorporation into DNA, 8-oxoG glycosylase (OGG) excises 8-oxoG from 8-oxoG:C base pairs, and MUTYH excises adenine from 8-oxoG:A base pairs. Consequently, Al-Tassan et al. searched for germline mutations in *MTH*, *OGG1*, and *MUTYH* and found inherited biallelic mutations in *MUTYH*. Functional analysis of the most common *MUTYH* variants, Y165C and G382D, was performed by mutating the corresponding conserved residues in *E. coli mutY*; the protein products of these alleles demonstrated a significant reduction in adenine glycosylase activity.

Additional studies confirmed the association of mutated *MUTYH* with some cases of multiple colorectal adenoma and some rare FAP cases and verified the recessive transmission of *MUTYH*-associated polyposis (MAP) (Jones et al., 2002; Sampson et al., 2003; Sieber et al., 2003). Heterozygous germline *MUTYH* mutations have been detected in individuals with colorectal cancer, but the larger scale studies required for determining whether there is an increased colorectal cancer risk in individuals heterozygous for *MUTYH* mutation have yet to be performed (Halford et al., 2003; Sampson et al., 2003; Sieber et al., 2003). Somatic mutation of *MUTYH* has not been observed in sporadic colorectal cancers (Halford et al., 2003); however, *MUTYH* maps to 1p32-34 (Slupska et al., 1996), a genomic region where loss of heterozygosity (LOH) is a fairly common event in colorectal tumorigenesis. It has recently been observed that tumors from individuals heterozygous for *MUTYH* mutations may have a higher than expected frequency of 1p LOH (Kambara et al., 2004).

Genetic pathways in colorectal cancer

As colorectal carcinoma generally develops over the course of years, usually through the readily identifiable precancerous adenoma, this tumor type has provided a valuable paradigm for the study of tumor progression. The identification of *MUTYH* mutation in association with familial susceptibility to colorectal adenoma and carcinoma and the role of MUTYH in BER have amended the way in which we think of gastrointestinal tumor progression.

Colorectal carcinomas develop according to specific genetic pathways (Chung, 2000). A common feature of these pathways is the development of genomic instability, an important event in multistep carcinogenesis since it provides for the accumulation of genetic mutations. The most common pathway for colorectal carcinoma development is the pathway characterized by mutation of the *APC*, *KRAS*, *SMAD4*, and *TP53* genes and an aneuploid/polyploid karyotype. Some term this pathway "CIN" to represent this type of variability in chromosome number. Other colorectal cancers develop through the "microsatellite instability" (MSI) pathway. Microsatellites are short repetitive sequences distributed throughout the human genome in which frequent alterations of length correlate with mismatch repair defects (Dietmaier et al., 1997). Tumors that develop through this pathway have lower mutation frequencies of *APC*, *KRAS*, *SMAD4*, and *TP53*, higher mutation frequencies of *TGFβRII*, *IGFFIIR*, *BAX*, *BRAF*, and *E2F4*, and a near-diploid karyotype. An additional subset of tumors demonstrates neither an excess of major chromosomal abnormalities nor microsatellite instability (Georgiades et al., 1999; Yao et al., 1999). This has suggested the existence of at least one additional pathway to tumorigenesis (CIN–, MSI–).

In FAP, tumor formation generally (but not always) occurs by the CIN pathway (Konishi et al., 1996), presumably at least partially due to a role of APC in chromosome segregation that is independent of its role in signal transduction (Fodde et al., 2001; Kaplan et al., 2001). Other acquired mutations in oncogenes and tumor suppressor genes are likely to work in synergy with *APC* mutation in creating chromosomal instability (Fodde et al., 2001). In hereditary nonpolyposis colorectal cancer (HNPCC), which results from germline mutations in genes encoding mismatch repair proteins, usually *MSH2* or *MLH1*, but also *MSH6*, *PMS2*, or *PMS1* (Narayan and Roy, 2003), tumors generally develop via the MSI pathway (Konishi et al., 1996; Muller and Fishel, 2002). Lipton et al. (2003) examined MAP tumors and determined that the MAP pathway is distinct from the CIN and MSI pathways but shares some features with each of the other pathways. Consistent with a BER defect, G:C → T:A transversions were found in *APC* and *KRAS*; p53 overexpression was common. *SMAD4*, *TGFBIIR*, and *BRAF* were not mutated, and the karyotype was near-diploid. Thus, the MAP pathway can be characterized as CIN–, MSI– (Table 1).

Although colorectal tumors can develop instability via the CIN pathway, the MSI pathway, or a BER defect (CIN–, MSI–

pathway), they tend to develop a single type of instability. This may be due to a lack of selective pressure for additional sources of instability or to the compromise of cell survival by "too much" instability (Cahill et al., 1999). Numerous mutations and epigenetic effects are likely to modify the progression of cells along each of these pathways. For a more in-depth discussion of genetic instability and pathways to tumorigenesis in colon cancer, the reader is referred to Ilyas et al. (1999).

MUTYH and connections among DNA repair pathways

The association of human MUTYH with apurinic/apyrimidinic endonuclease (APE1), proliferating cell nuclear antigen (PCNA), and replication protein A (RPA) suggests a role for MUTYH in long-patch BER (Parker et al., 2001). In addition, the cell cycle-dependent expression of MUTYH and its colocalization with replication foci suggest that its repair function is coupled to replication (Boldogh et al., 2001). The recent findings that MUTYH physically interacts with the MSH2/MSH6 heterodimer (the human MutSα homolog involved in MMR) via MSH6 and that the MSH2/MSH6 heterodimer stimulates the DNA binding and glycosylase activities of MUTYH in the presence of an 8-oxoG:A mismatch suggest cooperation between BER and MMR in the repair of this type of oxidative damage (Gu et al., 2002). Since both MUTYH and MSH2/MSH6 interact with PCNA, PCNA may have a role in coordinating the BER and MMR pathways to accomplish the repair of these lesions.

Identification of the DNA repair protein *BRCA2* as a Fanconi anemia protein

Another recent finding provides new information about connections among repair pathways. The discovery that the *BRCA2* gene is the Fanconi anemia gene *D1* (*FANCD1)* has linked the genes responsible for seemingly unrelated cancer predisposition syndromes to a common pathway (Howlett et al., 2002).

Fanconi anemia (FA) is a rare multigenic autosomal recessive cancer susceptibility syndrome characterized by progressive bone marrow failure, congenital abnormalities, hypersensitivity to crosslinking agents (such as mitomycin C and cisplatin), a more modest hypersensitivity to ionizing radiation (IR) and oxygen radicals, and the development of hematologic and solid tumors. At the cellular level, it is characterized by spontaneous chromosomal breakage. Somatic cell genetics and complementation analysis first identified at least eight distinct FA genes (A, B, C, D1, D2, E, F, G); six were subsequently cloned (A, C, D2, E, F, G). The protein products of five of these genes (A, C, E, F, and G) form a multisubunit nuclear core complex (reviewed in D'Andrea and Grompe, 2003). Recently, a sixth component of the nuclear core complex was identified as the ubiquitin ligase PHF9 or FANCL (Meetei et al., 2003a), and a seventh component was identified as Fanconi anemia-associated polypeptide 95 (FAAP95) or FANCB (Meetei et al., 2004). There is also new evidence for the existence of additional Fanconi anemia genes (Levitus et al., 2004).

During S phase or in response to DNA damage, the FA nuclear core complex mediates the monoubiquitination of the downstream FANCD2 protein and targets it to nuclear foci, where it becomes colocalized with BRCA1 (Garcia-Higuera et al., 2001). To investigate the associations between the breast cancer susceptibility genes, *BRCA1* and *BRCA2*, and the FA genes, Howlett et al. (2002) sequenced *BRCA1* and *BRCA2* in cells derived from individuals with FA subtype B (FA-B) and FA subtype D1 (FA-D1), the two subtypes for which the corresponding genes had not yet been identified. They failed to detect mutations in *BRCA1* but detected biallelic truncating mutations in *BRCA2* in both FA-B and FA-D1 cell lines. When full-length BRCA2 was expressed in a FA-D1 cell line, a correction of mitomycin C sensitivity was observed, confirming that BRCA2 is a Fanconi anemia protein.

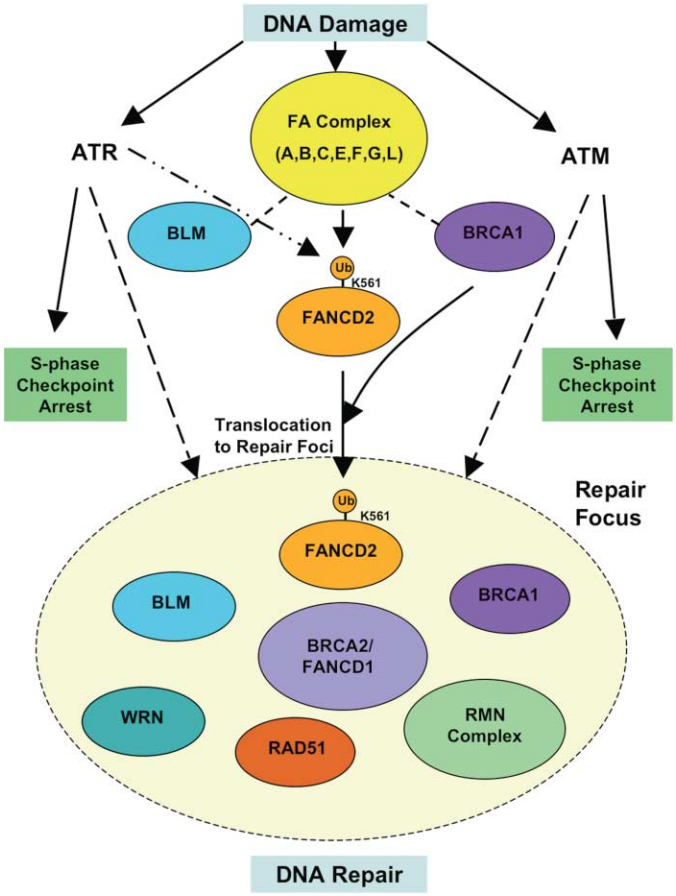

Figure 1. Simplified model of the FA/BRCA pathway in DNA repair and checkpoint control

Several FA proteins (including A, B, C, E, F, G, and L) form a core complex in the nucleus of human cells. Other DNA repair proteins, including BLM and BRCA1, associate with the FA nuclear core complex. The FA/BRCA pathway is most likely activated by double-strand breaks formed directly by ionizing radiation (IR) or indirectly by the initiation of interstrand crosslink repair (Rothfuss and Grompe, 2004). The FA nuclear core complex mediates the monoubiquitination (Ub) of FANCD2 at lysine 561 (K561), probably by the monoubiquitin ligase FANCL; this modification is associated with the translocation of FANCD2 to DNA repair foci. Efficient FANCD2 monoubiquitination may also be dependent on the ATR kinase (represented by the arrow of dots and dashes). Although BRCA1 has monoubiquitin ligase activity when bound to BARD1, it is not required for the monoubiquitination of FANCD2 in vivo; BRCA1 is required for the translocation of FANCD2 to DNA repair foci (Vandenberg et al., 2003). Numerous DNA repair proteins accumulate in these foci, including those represented in this schematic (BRCA1, BRCA2/FANCD1, RAD51, RMN complex, BLM, and WRN). Numerous associations among proteins localized to repair foci have been reported, but the exact composition of repair complexes is unknown. The FA/BRCA pathway is regulated by the ATR and ATM kinases. Phosphorylations of a number of substrates, including CHK kinases, the NBS1 subunit of the RMN complex, BRCA1, and FANCD2 are involved in activation of the S phase checkpoint. ATR and ATM also phosphorylate several of the proteins that localize to DNA repair foci and presumably play a role in the repair process (represented by dashed arrows to repair focus). The functional implications of these modifications are largely unknown.

Role for the FA/BRCA pathway in DNA repair and checkpoint control

The FA and BRCA proteins most likely function in a common pathway that regulates several aspects of the cellular response to DNA damage (Figure 1). After DNA damage, the FA nuclear core complex activates the monoubiquitination of FANCD2 at lysine 561. This modification mediates the targeting of FANCD2 to DNA repair foci in which it colocalizes with BRCA1 (Garcia-Higuera et al., 2001) and RAD51 (Taniguchi et al., 2002a), the protein that facilitates pairing and strand exchange during HR. Interestingly, the monoubiquitination and relocalization of FANCD2 occurs in response to DNA damage initiated by mitomycin C, IR, or UV light (Garcia-Higuera et al., 2001), types of damage that require different but overlapping repair mechanisms. In this context, it should be noted that BRCA1 is present in large protein complexes in association with a variety of DNA repair proteins, including: the MMR proteins MSH2, MSH6, and MLH1; ATM (ataxia telangiectasia mutated), a serine-threonine kinase which phosphorylates a number of S phase checkpoint and DNA repair proteins; BLM, a recQ-like helicase involved in repair via HR; the RAD50-MRE11-NBS1 (RMN) complex, which functions in both HR and NHEJ; and replication factor C (RFC), which facilitates the association of PCNA with DNA (Wang et al., 2000).

BRCA2/FANCD1 is present in DNA repair foci containing activated FANCD2, BRCA1, and RAD51, and binds both to DNA and RAD51 (Davies et al., 2001). Recent studies demonstrate a direct interaction between BRCA2/FANCD1 and FANCD2 and indicate that monoubiquitinated FANCD2 promotes BRCA2 recruitment to DNA-damage inducible foci (Hussain et al., 2004; Wang et al., 2004). BRCA2/FANCD1 may be involved in the assembly of RAD51 into repair foci (Digweed et al., 2002). Studies in Brca2-deficient ES cells (Tutt et al., 2001) and in FA-D1 cells (Digweed et al., 2002) suggest that mutations in *BRCA2/FANCD1* result in the use of the less accurate, RAD51-independent HR pathway, single strand annealing, rather than the more accurate, RAD51-dependent HR pathway, gene conversion. This is consistent with the chromosomal instability observed in Fanconi anemia and *BRCA2*$^{-/-}$ cells. It is also consistent with the sensitivity to DNA crosslinking agents observed in these cells, since interstrand crosslink repair requires both NER and HR (de Silva et al., 2000). Daniels et al. (2004) have recently described abnormal cytokinesis in BRCA2-deficient cells, suggesting that such a defect could be an additional cause for chromosomal instability in these cells.

Although numerous details remain to be worked out, the FA/BRCA pathway is apparently regulated by both ATR (ATM- and Rad3-related) and ATM kinases. After IR treatment, FANCD2 is phosphorylated in an ATM-dependent manner (Taniguchi et al., 2002b), while after treatment with crosslinking agents, it is phosphorylated in an ATR-dependent manner (Pichierri and Rosselli, 2004). Recently, two ATR-dependent pathways induced by interstrand crosslinks and which cooperatively result in full S phase checkpoint activation have been described (Pichierri and Rosselli, 2004). Activation of the ATR-CHK1 pathway depends upon the phosphorylation of CHK1 by ATR. Activation of the ATR-NBS1-FANCD2 pathway depends upon the FA nuclear complex-dependent assembly of the RMN complex and the translocation of FANCD2 to nuclear foci, the phosphorylation of NBS1 by ATR, and the NBS1-dependent phosphorylation of FANCD2 by ATR. Another recent study suggests that ATR may be required for the efficient monoubiquitination of FANCD2 in response to various types of DNA damage (Andreassen et al., 2004). Although FANCD2 is a substrate of ATR in vitro, the mechanism of ATR-dependent activation of FANCD2 by monoubiquitination is unknown.

After exposure to IR, ATM phosphorylates FANCD2 on serine 222, and this phosphorylation is required for activation of the S phase checkpoint (Taniguchi et al., 2002b). ATM also phosphorylates other substrates involved in the S phase checkpoint, including NBS1, CHK2, and BRCA1; the relative and/or cooperative contributions of these protein modifications have yet to be determined.

Not surprisingly, biallelic disruption of *FANCD2* results in an S phase checkpoint defect and hypersensitivity to both crosslinking agents and IR. Future studies of proteins that interact with activated FANCD2 should be helpful in gaining an understanding of how S phase checkpoints and DNA repair are accomplished at the molecular level.

The recQ-like helicase *BLM* functions in pathways that respond to various types of DNA damage

Recent studies indicate that the recQ-like helicases (reviewed in Bachrati and Hickson, 2003; Risinger and Groden, 2004), another family of DNA repair proteins which have a major role in the maintenance of genomic stability, are also likely to function in a common pathway that responds to various types of DNA damage. The recQ-like helicases are named for the similarity of family members to the prototype *E. coli* recQ helicase that participates in the bacterial recF genetic recombination pathway. They are ATP- and Mg^{2+}-dependent 3′-5′ helicases, i.e., they translocate along the DNA strand to which they bind in the 3′-5′ direction as they unwind duplex DNA. RecQ-like helicases are unusual among helicases in their DNA substrate preferences. They are generally unable to unwind blunt-ended duplex DNA unless a bubble is present, but they efficiently unwind such non-Watson-Crick DNA structures as G quadruplexes (extremely stable DNA secondary structures that can form in guanine-rich regions of DNA) and Holliday junctions (four-way crossover structures formed by DNA strand exchange during HR or in the resolution of stalled replication forks). They also efficiently unwind D loops (intermediates formed during the strand invasion step of recombination).

In humans, there are 5 recQ-like helicases, encoded by the *BLM*, *RECQL* (also referred to as *RECQ1*), *RECQL4*, *RECQL5*, and *WRN* genes. Defects in three of the human recQ-like helicases result in chromosome breakage syndromes (autosomal recessive human disorders characterized by spontaneous chromosome breakage); germline defects in *BLM*, *RECQL4*, and *WRN* are responsible for Bloom syndrome, Rothmund-Thomson syndrome, and Werner syndrome, respectively. In addition to chromosome instability at the cellular level, these rare diseases are characterized by striking cancer susceptibility, although each to a unique tumor spectrum. Due to space limitations, this review will focus on the recQ-like helicase BLM. For recent discussions of recQ-like helicases and their roles in maintaining genomic stability and suppressing tumorigenesis that also include RECQL4 and WRN, the reader is referred to Bachrati and Hickson (2003) and Risinger and Groden (2004).

Individuals with Bloom syndrome (BS) are proportional dwarfs with sun-sensitive facial erythema, male infertility, immune deficiency, and an unusually high incidence of nearly all forms of cancer at an early age (average 24 years). The hallmark of BS cells, and a feature which is considered diagnostic

of the disease, is a multifold increase in the frequency of sister chromatid exchange, the reciprocal exchange of DNA between sister chromatids by homologous recombination. An abnormal profile of DNA replication intermediates is also characteristic of BS cells.

BLM is expressed in all tissues in which there is active cell proliferation. It is barely detectable in cells during the G_1 phase of the cell cycle, but dramatically increases in amount during the S and G_2 phases. BLM generally has a punctate nuclear distribution that partially overlaps with PML bodies, named for the presence of promyelocytic leukemia tumor suppressor protein. After treatment of cells with DNA replication inhibitors or DNA damaging agents, BLM associates with sites of DNA damage/repair, defined by the presence of phosphorylated histone H2AX or RAD51 and thought to be sites of stalled replication forks.

A number of other BLM-protein associations have been described, many of which result in alterations in the activity of BLM or the associated protein and provide explanations for some of the proposed functions of BLM in replication, recombination, and repair. RPA, a single-stranded DNA binding protein involved in replication, recombination, and repair, physically interacts with BLM and stimulates its helicase activity (Brosh et al., 2000). BLM binds topoisomerase IIIα (TOPO IIIα) and stimulates its DNA strand passage activity (Wu and Hickson, 2002, 2003). BLM also interacts with 5′-flap endonuclease/5′-3′ exonuclease (FEN-1; involved in Okazaki fragment processing and in primarily BER and NHEJ DNA repair pathways) and stimulates both the endonuclease and the exonuclease activities of FEN-1 on a variety of DNA substrates that represent intermediates in DNA replication and repair (Sharma et al., 2004).

The RAD51 paralog RAD51D binds to BLM, and the RAD51D-XRCC2 complex stimulates the ability of BLM to process synthetic Holliday junctions in vitro (Braybrooke et al., 2003). In contrast, the tumor suppressor p53 binds to BLM and attenuates its ability to unwind synthetic Holliday junctions in vitro (Yang et al., 2002). BLM associates with the MMR proteins MLH1 (Langland et al., 2001) and MSH6; the MSH2/MSH6 heterodimer regulates the ability of BLM to process synthetic Holliday junctions in vitro (Yang et al., 2004). BLM-deficient cells, however, do not demonstrate a MMR defect (Langland et al., 2001), suggesting that these MMR proteins may play a role in HR repair. BLM-deficient cells do demonstrate defects in NHEJ (Langland et al., 2002).

BLM is also likely to have a role(s) in the FA/BRCA pathway (Figure 1). BLM has been isolated from human HeLa cell extracts in a complex containing the FA nuclear core complex, RPA, and TOPO IIIα; this complex has DNA unwinding activity that is dependent on the presence of BLM (Meetei et al., 2003b). Pichierri et al. (2004) have recently shown that the FA nuclear core complex is necessary for BLM phosphorylation and recruitment to nuclear foci in response to DNA crosslinking agents. Monoubiquitination of FANCD2 occurs normally in BS cells; however, BLM and monoubiquitinated FANCD2 colocalize and coimmunoprecipitate after replication arrest or treatment with crosslinking agents. As other proteins that function in the FA/BRCA pathway, BLM associates with, and is phosphorylated by, the ATM (Beamish et al., 2002) and ATR kinases (Franchitto and Pichierri, 2002; Davies et al., 2004) after specific types of DNA damage. BLM is one of the first proteins to relocalize to sites of DNA damage/repair and binds directly to RAD51 (Wu et al., 2001). BLM may subsequently recruit p53 (Sengupta et al., 2003), BRCA1 (Davalos and Campisi, 2003), and RMN (Franchitto and Pichierri, 2002; Davalos and Campisi, 2003) to sites of stalled replication forks/DNA repair.

In general, loss of recQ-like helicase function results in abnormal DNA replication and increased recombination. These helicases have unusual substrate preferences that include G quadruplexes, structures that resemble Holliday junction recombination intermediates and D loops. They associate with numerous proteins implicated in replication, recombination, and repair, and associate with stalled replication forks after replication has been interrupted or DNA damage has occurred. These data suggest that the major function of recQ-like helicases is to restart stalled replication forks and thereby maintain genomic stability. Mechanistically, recQ-like helicases may reset replication forks stalled after encountering DNA damage, and/or they may have a role in repair of stalled forks by HR, while suppressing the production of "nonparental" recombinants. They may also resolve DNA secondary structures such as G quadruplexes ahead of the replication fork.

Interestingly, the recQ helicases BLM and WRN interact with one another; BLM can inhibit the exonuclease activity of WRN (von Kobbe et al., 2002). The synergistic increase in hypersensitivity to DNA damaging agents in *BLM*$^{-/-}$/*WRN*$^{-/-}$ chicken DT40 cells (Imamura et al., 2002), the major differences in the human syndromes, and the unique patterns of somatic cell chromosome instability suggest that BLM and WRN have largely complementary rather than redundant roles in DNA repair. One can argue that there may be some overlap in function, as loss of function of a single recQ helicase is compatible with survival in humans. As BLM and WRN interact with and recruit numerous types DNA repair proteins to sites of damage and both undergo numerous posttranslational modifications, they may indeed be involved in coordinating DNA repair pathways.

Future perspectives

The next few years hold great promise for the study of human cancer syndromes associated with DNA repair defects. Identifying interactions of DNA repair proteins and the effects of these interactions on enzymatic function and signaling will no doubt continue to be an exciting avenue of research. Little is currently known about the in vivo processing of specific DNA structures created by different types of damage and the processing of specific repair intermediates. How one repair mechanism is chosen over another and how repair mechanisms might be utilized in sequence are also unknown. Understanding the links between repair mechanisms, cell cycle regulation, and apoptotic mechanisms will require further investigation. Recent studies and those performed nearly thirty years ago suggest that heterozygosity for loss of function mutations in DNA repair genes may be associated with increased cancer risk (Goss et al., 2002; Gruber et al., 2002; and Swift et al., 1976). Consequently, validation of these studies in large populations and the study of how more subtle DNA repair protein sequence variants affect human disease risk in the general population are indicated.

Recent advances in imaging, mouse modeling, microarray analysis, and proteomic technologies will make it possible to study gene and protein expression in individual tumors and provide additional perspectives on the role of DNA repair proteins in tumor initiation and progression. This, in turn, will assist in the

design of new types of therapeutic agents. More exciting is the possibility that understanding the DNA repair capacity of an individual or the capacity of a specific tumor may enable the personalized choice of effective therapeutic agents, most of which function by producing specific types of DNA damage in cancer cells. The great gifts of nature's experiments in human variation have given us a wonderful beginning to understand how genomic integrity is maintained.

Acknowledgments

This work is dedicated to James German to celebrate the fortieth anniversary of the discovery of genetically determined chromosome instability (German, 1964). Regrettably, numerous contributions to the fields of genomic instability and DNA repair could not be directly cited due to space limitations.

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