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Hereditary Common Cancers: Molecular and Clinical Genetics

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Abstract. *This review focuses on the functional role and structural features of the genes involved in common hereditary cancers. Most of these tumors are sporadic and the genetic alterations responsible for their genesis take place over several cell generations; nevertheless, 5 to 10% of the human tumors are hereditary, with a rapid development. Cancer susceptibility genes have been classified as "gatekeepers" (e.g. RB1, ki-ras) and "caretakers" (e.g. hMLH1 and hMSH2, BRCA1). The first step in identifying individuals at high risk of developing a specific inherited form of cancer, and who should therefore undergo genetic tests, is the detailed construction of family history (an accurate cancer family history that includes at least three generation pedigrees, an appropriate cancer risk assessment and an effective genetic counseling). At present, the most useful methods of risk assessment are those performed on the following genes: BRCA1 and BRCA2 especially for hereditary breast and ovarian cancer, hMLH1 and hMSH2 for hereditary non polyposis colorectal cancer, APC for familial adenomatous polyposis, ret for medullary thyroid carcinoma, p53 for the Li-Fraumeni syndrome, p16 for melanoma and RB1 for retinoblastoma. In conclusion, the development of new diagnostic tests will permit a more accurate assessment of risk in individuals who have not so far shown any sign or symptom of the disease.*

All tumors are the result of a series of genetic events leading to uncontrolled cell growth and consequent hyperproliferation. Most of them are sporadic and the genetic alterations responsible for their genesis take place over several cell generations; nevertheless, 5 to 10% of the human tumors are hereditary (1).

There are basically two classes of genes responsible for the neoplastic process: oncogenes and tumor suppressor genes. The former act dominantly on cell cycle control and carcinogenesis will therefore require only one mutation, while the latter have a recessive effect so that in the tumoral cell both copies must be inactivated. Mutations in tumor suppressor genes cause familial and hereditary tumors, with the exception of one oncogene ret, whose germ-line mutations lead to multiple endocrine neoplasia type 2 (1). The term familial is used in situations where several members of the same family are affected by the same type of neoplasia; often the genetic and environmental factors causing it are unknown (2). The term hereditary is used when the susceptibility to develop a certain type of tumor is inherited in a Mendelian fashion (Figure 1). The mutation is constitutionally present in all the cells of certain family members and is transmitted by means of the germ-line to following generations. Individuals who inherit it will be more likely to develop the tumor since only one more mutation is needed at the somatic level for the second copy to be altered, thus resulting in complete loss of function of the gene. Although inherited mutations in these genes predispose the individuals to the development of a neoplasia, further mutations in other genes are needed in order to transform the cell involved into a neoplastic cell.

Gatekeeper and caretaker genes. Cancer susceptibility genes have further been classified as "gatekeepers" (e.g. RB1, ki-ras) and "caretakers" (e.g. hMLH1 and hMSH2, BRCA1) (3). The former directly regulate the cell cycle by inhibiting cell growth and division or by promoting cell death while the latter play a role in detecting or repairing damaged DNA ("DNA damage response genes"). Gatekeeper genes tend frequently to be mutated both in sporadic tumors, at the somatic level and in hereditary tumors at the germ-line level. In the gatekeeper pathway, after the first mutation in one of the two alleles, only one other mutation in the second allele is required. Mutations in caretaker genes are rarely found in sporadic tumors, but frequently occur in the germ-line, where they cause genetic instability leading to further mutations in other important genes involved in cell cycle control. In the caretaker pathway, after a first mutation in one of the two alleles, three following mutations are generally required: in the second allele of the

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caretaker gene, in one of the alleles of a gatekeeper gene and in the second allele of the gatekeeper gene (Figure 2).

The importance of diagnostic tests. The research for new genes involved in tumor initiation and progression may improve the development of diagnostic tests permitting an accurate assessment of risk in individuals who have not so far shown any sign or symptom of the disease (1). The first step in identifying individuals at high risk of developing a specific inherited form of cancer and who should therefore undergo genetic tests, is the detailed reconstruction of family history (an accurate cancer family history that includes at least three generation pedigrees, an appropriate cancer risk assessment and effective genetic counseling). These tests are normally performed on the DNA extracted from whole blood or from tissue. At the present time, the most useful methods of risk assessment are those performed on the following genes: BRCA1 and BRCA2 especially for hereditary breast and ovarian cancer, hMLH1 and hMSH2 for hereditary non polyposis colorectal cancer, APC for familial adenomatous polyposis, RET for medullary thyroid carcinoma, p53 for the Li-Fraumeni syndrome, p16 for melanoma and RB1 for retinoblastoma (1). Major inherited cancer syndromes with genetic alterations in gatekeeper and caretaker genes are reported in Table I.

For the identification and assessment of gene mutations, an indirect study by means of linkage analysis may be performed, although such cases require large families where several members are affected, which does not often occur. For this reason, a direct study of mutation is generally performed. There are at present several direct methods available, for example, Single Strand Conformation Polymorphism (SSCP) Analysis, Heteroduplex Analysis (HA), Denaturing Gradient Gel Electrophoresis (DGGE), Protein Truncation Test (PTT), Microsatellite Analysis and DNA Sequencing. The choice of the mutation detection method depends on several factors, such as sensitivity, specificity and cost (2).

Breast and ovarian carcinoma

Breast Carcinoma (BC) is the most common form of neoplasia in women of the Western world, with a mean incidence of 10%. It is a fairly heterogeneous disease caused by a large number of environmental, socio-economic, hormonal and, of course, genetic factors (4). Although this tumor generally appears in a sporadic form, between 5 and 10% of cases can be considered familial, attributable to inherited autosomal dominant mutations in several susceptibility genes (4, 5). Up to now, two such genes have been identified as responsible for about 90% of inherited BC: BRCA1 on chromosome 17 (17q21) and BRCA2 on chromosome 13 (13q12) (6, 7).

BRCA1 and BRCA2 are two high penetrance BC susceptibility genes. BRCA mutation carriers, present a 70-90% risk of developing the neoplasia from seventy years of age onwards (8, 9). Recent studies have identified a possible locus of a third gene, BRCA3, on the short arm of chromosome 8 (10). Linkage studies suggest that BRCA1 may be involved in about 45% of familial BC and in most cases an ovarian tumor is also present (11). In fact, women with BRCA1 mutations present a 30% increase in the risk of developing ovarian cancer compared to the basic risk. Moreover, individuals with such mutations who have already developed a BC have a 64% risk of developing a contralateral BC after the age of 70 and a 44% risk of developing an ovarian tumor. On the contrary, BRCA2 is probably responsible for about 35% of familial cases including those families where men are affected (12). The risk of developing ovarian cancer appears to be less in the presence of this gene. BRCA-associated BCs exhibit an increased risk of developing several other types of tumors, mainly cancer of the colon, pancreas and prostate (13). BC cases with negative family history rarely show BRCA1 and BRCA2 mutations, although a role may be played by a reduced expression of BRCA1 (14). BRCA1 promoter may be repressed by the Brn-3b POU family trascription factor (15).

Other genes are also involved in the development of BC in 1% of the cases; one with high penetrance is the p53 gene, which is mutated in the Li-Fraumeni Syndrome (individuals affected by this syndrome develop tumors of the soft tissues, including early-onset BC) (16); another gene is AR which encodes for the androgen receptor (17). Germ-line mutations of the PTEN gene, leading to the Cowden Syndrome which predisposes to the breast fibrocystic dysplasia, bring a 30% risk of developing BC (18, 19). Since mutations in low penetrance genes, are more frequent than high penetrance mutations, they may contribute to a high percentage of all the cases of BC. These mutations include those of the ATM gene, responsible for the neurodegenerative disease, ataxia telangiectasia, which possibly justifies about 10% of BC (20).

The presence of rare allelic variants in the minisatellite HRAS1, VNTR locus, at germ-line level, confers a risk of developing several tumors, such as BC (9% of all cases), bladder cancer, colorectal cancer and acute leukemia (21).

Genetic features of BRCA genes. Both BRCA genes present very long coding sequences and an extremely complex genetic structure (Figure 3). The large size of these two proteins might explain the existence of several functional domains which permit them to act at several levels. BRCA1 consists of 24 exons (5529 nucleotides), 22 of which encode for a 1863 aminoacids nuclear phosphoprotein (22) with several domains (BARD1 binding domain, 3 NLS, Rad51 binding domain, granins homology domain, BRCT domain). BRCA2, consists of 27 exons (10443 nucleotides), 26 of which encode for a 3418 aminoacids nuclear phosphoprotein. It contains eight copies of a repeated motif known as BRC and no other previously defined functional domains (7). Some studies suggest that the domain encoded by the exon 23 may be important for BRCA2 function (23). In both genes exon 11

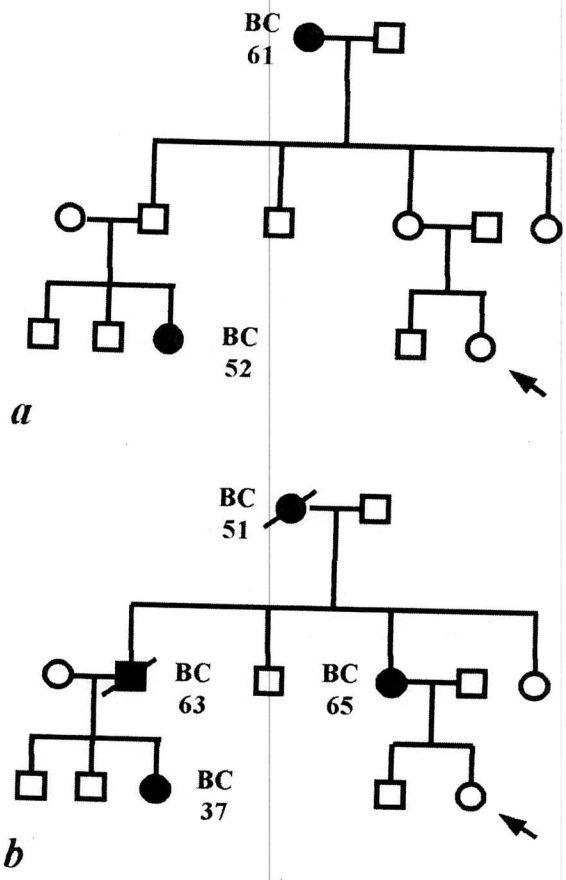

Figure 1. *Pedigrees of familial breast cancer (A) and of inherited breast cancer (B). In the genealogical tree (A) two BC cases in the same family have been observed. Enviromental factors rather than genetics factors may be responsible. On the contrary in the family of pedigree (B) a large number of individuals have been affected, which means that the susceptibility of developing BC was inherited in a Mendelian manner. The presence of an affected male suggests a BRCA2 linked hereditary transmission.*

Table I. *Major inherited cancer syndromes with genetic alterations in gatekeeper and caretaker genes.*

Inherited cancer syndromes	Incidence *	Susceptibility gene	Mutation detection rate * (%)
1. Gatekeeper genes-associated syndromes			
Familial Adenomatous Polyposis (FAP)	1:4000-10000	APC	70-90
Cutaneous Melanoma	1:5000	p16, CDK4	60
Multiple Endocrine Neoplasia type 1 (MEN1)	1:100000	MEN1	70-90
Multiple Endocrine Neoplasia type 2 (MEN2)	1:500000	RET	>95
Retinoblastoma	1:14000-34000	RB1	>95
Li-Fraumeni Syndrome	1:50000	p53	70-75
2. Caretaker genes-associated syndromes			
Breast and ovarian cancer	1: 400-4,000	BRCA1 (80%) BRCA2 (20%)	50-65 35
Hereditary Non Polyposis Colorectal Cancer (HNPCC)	1:1,000-2000	MLH1 (30%), MSH2 (60%), PMS2, PMS1, MSH6	50-80

• For incidence and mutation frequency of inherited cancer syndromes see Reference (2, 39, 59, 84, 103, 105, 108).

makes up more than half of the encoding sequence and presents the majority of the mutations.

BRCA genes function. Although the exact role of BRCA genes in tumor suppression is still controversial, convincing evidence suggests that BRCA genes may be caretakers (3, 32). Thus, BRCA gene mutations may lead to genetic instabiliy and an increased mutation rate of gatekeeper genes. A great many studies have attempted to identify the localization and the function of the BRCA1 and BRCA2 proteins; this has not been easy because of their partially homology with other proteins.

BRCA1 presents a Ring finger domain at the N-terminal position, which is typical of trascriptional factors, where a sequence made up of seven cysteines and one histidine binds two zinc ions (24). In 1996, Wu *et al.* identified a BARD 1 protein (BRCA1-associated Ring domain protein 1) which also possessed a Ring finger domain at the N-terminal position and was able to interact with the BRCA1 Ring finger domain. The two molecules work together in the sequence-specific recognition of DNA (25). In the C-terminal position, however, BRCA1 presents a domain named BRCT (BRCA1 C-terminal) preserved during evolution and made up of proteins involved in the cell cycle checkpoints and in the DNA repair mechanisms (26, 27). The importance of this domain is confirmed by the fact that most of the mutations with a susceptibility to BCs are to be found in this region.

Two variants of BRCA1, BRCA1a/p110 and BRCA1b/p100, obtained by alternative splicing have been

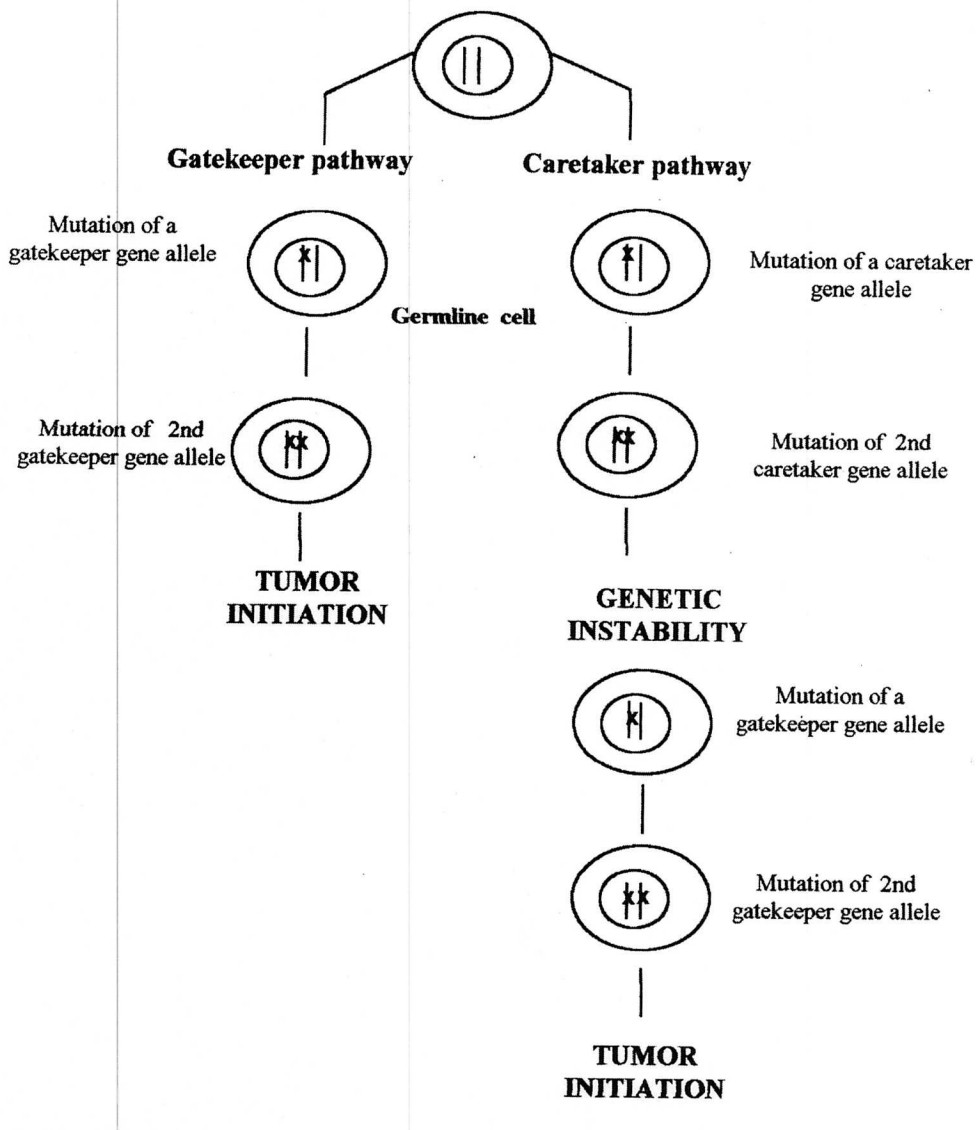

Figure 2. *Gatekeepers and caretakers pathways [modified from Kinzler and Vogelstein (3)].*

identified; both of these, however, present the same BRCT domain in the C-terminal position, whereas only BRCA1a presents another transactivation domain (BNT) in the N-terminal position (28). It is quite likely that these proteins are also involved in cell cycle control, since they are able to bind E2F, cyclins and kinases.

BRCA and cell cycle. It has recently been shown that BRCA1 is involved in cell cycle checkpoints and in the maintenance of genomic stability and, in fact, it has been discovered in mitotic cells associated with one of the components of centrosome, γ-tubulin, which is also what happens for other proteins such as cyclins A and B, Rb and p53 (29). In embryonic mouse fibroblasts with BRCA1 exon 11 deficiency there are defects in G2/M transition, chromosome anomalies and an unequal chromosome segregation with resulting aneuploidy (30). Further data also report a role played by the BRCA1 protein in processes requiring remodelling of chromatin as for example in DNA transcription and repair; it has also been seen that it interacts with Rb and with two other proteins of the histone deacetylase complex and also with the CBP of the RNA polymerase and with the Rad51 protein, the eukaryotic homologue of the bacterial Rec A protein, involved in meiotic recombination processes and chromosome repair (31-33).

DNA damage also interferes with the nuclear localization and phosphorylation level of BRCA1 (32). During the S-G2 phase, BRCA1 is found in nuclear dots, in a complex in association with Rad51 and BARD1. After exposure to UV or γ-rays hyperphosphorylation of BRCA1 has been observed, together with a relocalization of the complex into

macrostructures, which may include DNA at the replication phase and PCNA. It has been shown in the mouse that there is also an interaction between Brca2 and Rad51, so it may be possible that BRCA2 is also part of the complex (34).

Mouse embryonic stem cells which are nullizygous for Brca1 have deficient repair coupled with transcription mechanisms and are more sensitive to ionizing radiation and oxidation damage (35). Knock out mice for Brca1 and Brca2 show a similar effect to Rad 51 nullizygous mice and death during early embryogenesis which may confirm that BRCA1 and BRCA2 might be part of the same Rad 51 pathway (36-38).

These experimental observations support the idea that the BRCA genes are actively involved in DNA repair, which means that BRCA1 and BRCA2 mutations might cause genetic instability, leading to the accumulation of mutations in other genes probably involved in cell cycle regulation, with consequent development of the tumor (3). A model has been proposed where there are 2 different monitoring levels for genomic integrity; the first is controlled by BRCA1 and BRCA2; the second by p53, with a transcription increase of p21 and possible apoptosis (39). A direct interaction has been observed between BRCA1 and p53, which might therefore become activated; at this point BRCA1 and p53 could cooperatively induce apoptosis of cancer cells (40). An involvement of BRCA1 in apoptosis, not p53-dependent, has been reported (41). BRCA1 expression *in vitro* induces activation of GADD 45 and JNK/SAPK. It is still not clear how BRCA1 leads to a p21 transcription increase. Several data, in fact, report that in BRCA1 nullizygous mice there is an increase in p21 expression, which is also found in a cell-line with BRCA1 overexpression (38, 42). In both genes there is a region which may act as a transactivation domain (43, 44); furthermore BRCA1 interacts with the C-terminal domain of c-myc inhibiting its transcription activity in mammalian cells (45).

BRCA1 and BRCA2 mutations. About 90% of BRCA1 mutations described up to now are frameshift (small insertions or deletions), nonsense or splicing site mutations, which are distributed along the whole gene without forming hot-spots (46). These alterations cause a change in the reading frame, thus producing the appearance of a premature stop codon and resulting in the formation of a truncated protein; they might be at the basis of breast tumorigenesis, since it is highly probable that they may be involved in the loss of protein function. Missense modifications are instead less frequent. Some recurring mutations, the so-called "founder" mutations, have been identified with unusually high frequency in certain populations, where they are responsible for the majority of breast and ovarian hereditary cancers. For example, in the Ashkenazi Jewish population three mutations prevalently occur: 185delAG and 5382insC into BRCA1, 6174delT into BRCA2 (47). The 185delAG is considered the most frequent in the gene BRCA1 and its presence in non-

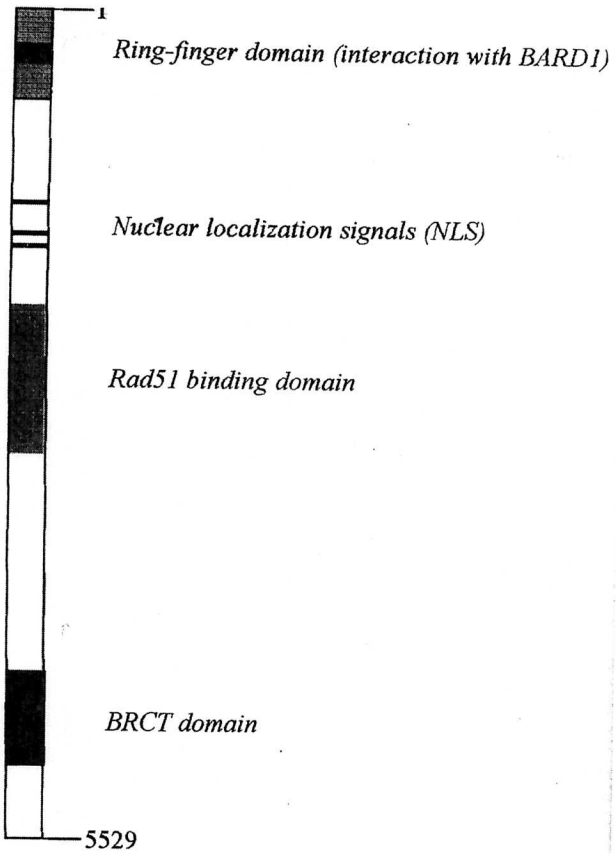

Figure 3. *Structure and functional domains of BRCA1.*

Ashkenazi Jewish carriers suggests that it had already appeared before the Diaspora about two thousand years ago (48).

"*Genotype-phenotype correlations*". No data are available to confirm a BRCA1 and BRCA2 "genotype-phenotype correlation ". It would seem that BRCA1 mutations at the 5' terminal and BRCA2 mutations in the region of the exon 11, between nucleotides 3035 and 6629, may be more commonly associated with an increased risk of developing ovarian tumors (49, 50).

BC and prognosis: significance of BRCA1 and BRCA2 mutations. The difference found between BRCA1 and BRCA2 associated BC and sporadic BC has led to the assumption that the two types of neoplasia may involve different phenotypes and prognoses and therefore may require a different therapeutic approach. It appears, in fact, that they present not only differences from the histological point of view, but that there is also a different mutation frequency among genes playing an important role in cell cycle control (51, 52). A large number of studies have shown, for example, that BRCA1-associated BC is more often at a high histological grade (G3) and less frequently presents estrogen

and progesterone receptors, which normally leads to a worse prognosis (53, 54). On the contrary, other authors have reported that these are medullary or atypical medullary histotypes; they show high lymphocyte infiltration which gives a consistent immune response, resulting in a better clinical outcome (54). Studies based on BC associated with BRCA2 mutations have not shown any phenotype differences.

Mutations in the p53 gene might be important in the pathogenesis of BRCA-associated BC; in fact, some studies have shown an increase in p53 mutations in individuals with BRCA1 mutations (51, 52), whereas no association between mutations in BRCA2 and those in p53 have been demonstrated (52, 55). There is also very little clear information available at present regarding the amplification and overexpression of erbB-2 in BC associated with BRCA mutations. (53, 56). Studies conducted on animals or in vitro have shown that BRCA1 and BRCA2 associated BC show a better response to therapeutic agents leading to DNA rupture; clinical evidence, however, is still insufficient to suggest different therapeutic approaches in these patients (57, 58).

Colorectal carcinoma

Colorectal carcinoma (CRC) is extremely common both in the United States and in Western Europe, with an incidence peak at the age of 70. Although most of these tumors are sporadic, there are hereditary syndromes which have been particularly useful, not only for the early diagnosis of individuals in such families, but at least also for the discovery of the biomolecular mechanisms behind CRC tumorigenesis (59). Hereditary CRC can be divided at least into two main groups: a) those with multiple adenomas (the most common syndrome studied is faminal adenomatous polyposis; b) those without polyposis (hereditary non-polyposis colorectal carcinoma) (59, 60).

a) Familial Adenomatous Polyposis (FAP). This form accounts for 1.1% of all CRC and its pathogenesis is relevant to sporadic colorectal tumorigenesis. It is an autosomal dominant inherited disorder with about 100% penetrance and results in development of hundreds to thousands of adenomatous polyps between the second and third decade of life (59, 60). Patients affected by FAP also present a higher risk of tumor development in other sites, such as the thyroid, small intestine, stomach and brain (61, 62). A variant of FAP is AAPC (Attenuated Adenomatous Polyposis Coli), also known as HFAS (Hereditary Flat Adenoma Syndrome), which presents fewer adenomatous polyps (<100) and develops about fifteen years later than classical FAP and ten years earlier than sporadic tumors of the colon (63).

Advanced molecular biological studies have led to the identification of the gatekeeper gene responsible for this hereditary syndrome, APC, which is localized on chromosome 5q (64).

Genetic features of APC gene. The APC gene consists of 15 exons encoding a 2843 aminoacids protein organized in several domains, each having a different function: a) the oligomerization domain (codons 1-171) (65); b) the β-catenin binding domain (codons 1020-1169) (66, 67); c) the second β-catenin binding domain and the GSK phosphorylation site (codons 1324-2075) (68, 69); d) the microtubules binding domain (codons 2130- 2843) (70); e) the binding domain to the protein EB-1 of unknown function (the codons 2560-2843) (71); and f) the DLG protein binding domain (codons 2771- 2843) (72).

APC protein function. Although so far the role of the APC protein has not been clearly understood, what is certain is that it is involved in cell adhesion and in the signal transduction pathway by means of the diffusible factor Wnt/Wingless and in assembling microtubules (73, 74). In normal conditions, the APC protein, toghether, with GSK-3b responsible for its phosphorylation can bind and promote the degradation of the β-catenin protein (74). β-catenin protein together with α-catenin, are involved in cell adhesion, since they act as a link between the transmembrane protein caderine and the actinic cytoskeleton (69, 75).

The Wnt/Wingless signal inhibits the GSK-3b activity and then APC function, so that the β-catenin turnover is no longer regulated (74). As a consequence, β-catenin increases in concentration and associates in the nucleus in heterodimers with members of the Lef/Tcf family transcription factors (76), thus promoting the transcription of genes involved in cell cycle control. The alteration of this pathway may lead to hyperproliferation and development of an adenomatous polyp (74, 77).

It is thought that the APC protein associated with the microtubules also promotes cell migration (73). This gene has also been associated with high levels of prostaglandins H synthase-2 (known as cyclooxygenase-2). Cyclooxygenase enzymes (COXs) are involved in the conversion of arachidonic acid into prostaglandins. COX 1 presents constant levels in the normal epithelium of the colon, while the levels of COX 2 are often higher in adenomas and carcinomas of the colon, which may be due to growth factors, oncogenes and tumor promotors (78). The COX 2 overexpression would appear to be an initial, fundamental event in colorectal carcinogenesis, responsible for the development of several adenomas (79). The proposed sequence of events is, first of all, functional failure of both the APC alleles and formation of an adenomatous polyp, and then an increase in COX 2 levels, which would lead to the development of further adenomas. The eventual progression to CRC would require further mutations in other genes (78).

APC mutations. Mutations of this gene have been identified in 80% of both benign and malignant tumors. Almost all of these (over 75%), whether sporadic or germ-line, are

frameshift mutations found mainly in the 5' region of the gene, resulting in the formation of a truncated protein (73, 80) which can bind to β-catenin but cannot regulate it (66, 67). The immediate result is that there is an increased concentration of cytoplasmic β-catenin, which may trigger off the chain of events leading to the formation of an adenoma (77).

"*Genotype-phenotype correlations*". There is evidence of a "genotype-phenotype correlation" which associates different mutations of the APC gene with particular forms of FAP (73). Gardner's Syndrome, presenting with osteomas, dental anomalies and congenital hypertrophy of the pigmented epithelium of the retina (CHRPE), is associated with mutations in the residues between codons 463 and 1387 (81); the attenuated form of AAPC presents mutations above residue 157 (63, 82). A particularly aggressive, early onset form of FAP, with a great many colorectal adenomas, is associated with mutations between codons 1250 and 1464 (especially in codon 1309) (83); cases with desmoid tumors and mandibular osteomas show mutations between codons 1403 and 1578 (84). In Turcot's Syndrome, which presents tumors in the central nervous system, particularly medulloblastoma, together with familial propensity for CRC, no correlation has been found with any mutation site.

Genetic model for colorectal tumorigenesis (Vogelstein's Theory) (85). Biomolecular studies regarding CRC at various stages have produced a theory about colorectal tumorigenesis which suggests that there is a progressive accumulation of mutations within specific genes (multistep carcinogenesis), together with the development of a type of cell behavior which becomes more and more proliferative and invasive. CRC, in fact, gradually processes through a series of clinical and histopathological stages ranging from alterations in the single glandular crypt (aberrant crypt foci), to the formation of small adenomatous polyps, up to the development of carcinomas.

Mutations which inactivate the APC gene (inherited in patients affected by FAP) would seem to be the first step of carcinogenesis: they can already be identified during the development of small adenomas (59, 86). Mutations activating the ki-ras oncogene occur slightly later (87); they are rarely found in early adenomas but are frequent in larger ones, which show alterations in cell differentiation and tissue organization (88). DCC and p53 mutations take place even later; they are rare in adenomas but common in carcinomas developing from them. It is thought that the loss of p53 function induces not only cell hyperproliferation but also, as the cell cycle progresses, a very rapid accumulation of other genetic mutations (85). This has been a useful model for 80% of sporadic CRC as well as for those due to FAP.

b) Hereditary Non-Polyposis Colorectal Cancer (HNPCC). There is also another mechanism leading to the development of CRC, discovered during the study of another hereditary form known as HNPCC, which, unlike FAP, does not present multiple polyps. HNPCC, (also known as Lynch's Syndrome I) is an autosomal dominant disease, with high penetrance (about 80%), representing 5-10% of all cases of CRC and is diagnosed in individuals of about 40 years of age (60, 89). Although they generally present an incidence of adenomas similar to that of the general population, the adenoma-carcinoma sequence appears to be more rapid in these patients (89). Where, apart from these features, patients present carcinomas in other sites, such as the stomach, the biliopancreatic system and the urinary tract, they are said to be affected by Lynch's Syndrome II (90). It is now understood that both these syndromes show mutations affecting the same genes known as MMR (DNA Mismatch Repair). These are caretaker genes discovered during the observation of tumors in patients with a particular form of genomic instability

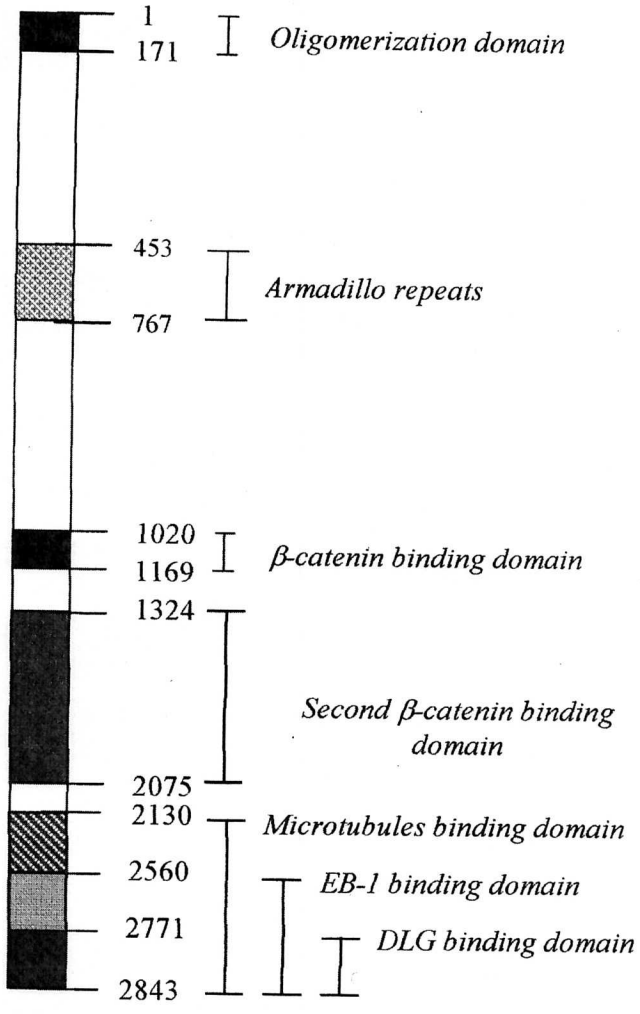

Figure 4. *Structure and functional domains of APC.*

leading to alterations in the microsatellites number (small, extremely polymorphic DNA sequences made up of 1-6 nucleotides repeated over and over again in the genome, especially in the non-coding sequences). It has been observed that this mutation pattern is similar to that observed in bacteria and yeasts and is associated with mutations in genes involved in the DNA repair: MUT H, L, S (91-94).

General features of MMR genes. Human genes homologous of the MUT H, L, S genes have been cloned. So far, 8 genes which are homologous of MUT S have been found; the most studied of these are the hMSH2 gene, the hMSH6 gene (GTBP/p160) and 3 genes homologous of MUT L known as hMLH1, hPMS1 and hPMS2 (93-96).

MMR genes function. When an error is present, the unpaired couple or the loop deform the double helix of the DNA, thus creating a physical aberration. The deformed DNA is recognized and bound either by a particular complex made up of a heterodimer of hMSH2 and hMSH3, which recognizes loops formed by means of deletion/insertion of 2 or more nucleotids, or by a heterodimer of hMSH2 and hMSH6 which recognizes smaller loops or single-base mispairs. This complex, known as hMUTS-α, must then recognize the newly synthesized strand to be repaired and this task is performed by another heterodimer made up of hMLH1 and hPMS2, called the hMUTL-α complex, which binds to the first complex and identifies the newly synthesized DNA (84). The system removes the strand from the recognition point, as far as the error and then resynthesizes it by means of the intervention of several other enzymes, such as helicase, DNA-polymerase, DNA-ligase, exonuclease and SSBPs (Single Strand Binding Proteins) (97).

MMR genes mutations. Mutations in any of the MMR genes lead to 80% risk of developing a CRC and in women a 60% risk of developing an endometrial tumor (98). Mutations of the hMSH2 gene have been found in 45% of HNPCC cases. This gene is expressed in the intestinal tract in the replication compartment of the crypt only and regulated by the cell cycle; the peak is reached during mitosis (94). hMLH1 and hMSH2 gene mutations have been identified in about 50% of HNPCC cases; in the remaining 6%, other genes are involved. Seventy percent of the mutations lead to truncated proteins, while no hot-spots have been observed. More than 90% of HNPCC tumors show microsatellite instability, also found in 15% of sporadic CRC; both these groups are poorly-differentiated, mucinous, found in the proximal colon and present mutations above all in the hMLH1 and hMSH2 genes (99).

HNPCC genetics. The genetics of HNPCC are complex but follow the pattern of other tumor suppressor genes. HNPCC is inherited as a dominant autosomal trait when hMSH2 or other genes of the mismatch repair are inactivated by a germ-line mutation. The phenotype is normal until the loss or the mutation of the other allele in certain tissues produces a "hypermutable phenotype" described as MIN (microsatellite instability) or RER (replication error) (99). The "hypermutable cell" is therefore predisposed to extremely rapid mutation accumulation, which may lead to clonal expansion and a neoplastic phenotype. MIN is found and causes inactivation, for example, of genes presenting repetitions of mononucleotides such as hMSH3 (A_8), GTBP/160 (C_8) or the BAX (G_8) gene or even in other genes like APC, Ki-ras or p53 (100). It is still not fully understood why only certain specific organs are at high risk of tumor development. After mutation of the second gene, further somatic mutations are required in other genes before tumorigenesis can be triggered off. Although the role played by the APC, Ki-ras or p53 genes does not seem very clear in the cases described so far, recent studies have suggested an alternative pathway. In fact, inactivating mechanisms have been reported in the gene encoding the TGF-β receptor, an important cell cycle regulator which promotes the expression of cyclin-dependent kinase inhibitors (CDKI), thus leading to arrest of the cell cycle (101).

Thyroid carcinoma

Thyroid carcinoma (TC) may develop either in the C cells (MTC) or in the follicular cells (papillary-type thyroid carcinoma - PTC). Twenty to 25% of MTC is hereditary and is made up of three different forms with a common genetic cause: familial medullary thyroid carcinoma (FMTC), multiple endocrine neoplasia 2a (MEN2a) and multiple endocrine neoplasia 2b (MEN2b) (1). The first of these is not associated with any other type of neoplasia, the second is a rare dominant autosomal form, with MTC, parathyroid hyperplasia and adrenalin gland tumor, the third is similar to the second but much rarer, more aggressive and not associated with parathyroid hyperplasia.

Mutations in the ret gene (re-arranged during transfection) are behind the development of all three of these hereditary forms. This gene is on chromosome 10q11.2 and consists of 21 exons distributed throughout a genomic region of about 55 kb. It encodes for a membrane glycoprotein with three isoforms which functions as a cell surface tyrosine kinase receptor. The binding of the soluble ligand GDNF (glial-derivative neurotrophic factor) to the receptor RET sets off its activation, its signal transduction and then proliferation (102). Individuals found to be affected by germ-line mutations of the *ret* oncogene have been advised to undergo prophylactic thyroidectomy (1).

In the majority of MEN2a (98%) and FMTC (88%) missense point mutations have been observed in one of the 5 cysteines of the extracellular domain of the receptor near the transmembrane domain (codons 609, 611,618, 620, in exon 10, codons 634 in exon 11) (103, 104). In particular in MEN2a the more frequent mutation involves cysteine 634 and in FMTC also cysteine 618. Furthermore, other mutations have been

identified: replacement of Tyr791Phe (exon 15) and an in-frame duplication of 12 bases (exon 11) in MEN2a cases and missense point mutations within codons 768, 790, 791 (exon 13), 804 (exon 14), 891(exon 15) in FMTC cases. In almost all cases of MEN2b (95%) there is a single missense point mutation (replacement methionine→threonine) in codon 918 within the ret tyrosine-kinase domain (exon 16). This methionine is essential for the catalytic core of the tyrosine-kinase receptor. More rarely a mutation in exon 15 (codon 883) has been reported in MEN2b cases.

"*Genotype-phenotype correlations*". Specific mutations which probably activate the protoncogene ret (by means of a change in the substrate specificity of the tyrosine-kinase domain) are associated with the MEN2b phenotype. Instead, mutations in specific cysteine residues (involved in the formation of intramolecular disulfide bridges) seem to be associated with MEN2a and FMTC phenotypes. These mutations induce ret activation independently of the ligand. Furthermore, MEN2a families with pheochromocytoma and parathyroid involvement, present almost all the mutations in codon 634 (103, 104).

Melanoma

Melanomas originate from the melanocytes and show rapid growth, local tissue invasion and high metastatic tendency. Environmental factors, skin coloring and melanin composition may be implicated in melanoma risk. Furthermore 5-10% of the cases are inherited in an autosomal-dominant manner. This hereditary form involves multiple primary tumors and dysplasiae. In sporadic cases, the disease appears between 40 and 50 years of age, while the hereditary form has an early onset (20-30 years) (105). Fifty percent of the latter result from mutations of the CDKN2A/MTS1gene, which encodes for a protein, p16, able to prevent progression in the cell cycle, especially entry into the G1 phase, by blocking the cyclin complex D-CDK4/CDK6 (106). Mutations of the CDKN2A gene also seem to lead to a predisposition for pancreatic and oropharyngeal carcinomas.

Retinoblastoma

This is a malignant tumor of the retina, which, in the USA, affects 1 out of 20000 children below the age of 4 years; if not diagnosed in time it may lead to blindness or even death (1). In 40% of cases, retinoblastoma is hereditary; in any case it develops as a result of the inactivation of the tumor suppressor gene RB1, on chromosome 13q14.1, consisting of 27 exons distributed in a genomic region above the 180 kb with two large introns of 30 and 70 kb. Rb1 is a high penetrance (about 90%) retinoblastoma susceptibility gene. The overall function of this protein is still not clearly understood; it appears to interact with transcriptional factors of the E2F family, thus negatively regulating their ability to activate a set of genes required before entry into the S-phase. In about 40% of familial cases, about 20% of bilateral sporadic cases and in about 10% of monolateral sporadic cases, the mutations include cytogenetic rearrangements and deletions and point mutations (insertions of fewer bases, nonsense and missense mutations, at the splicing sites and at the intronic level) (107, 108). Early diagnosis permits focal treatment with laser cryotherapy, whereas later phases require surgery, radiotherapy and chemotherapy in order to save the patient's sight and avoid the development of metastases.

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