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The *p53* Tumor Suppressor Gene

The *p53* tumor suppressor gene plays a central role in cell cycle control, apoptosis, and maintenance of genetic stability. It encodes a 53-kDa nuclear phosphoprotein translated from a 2.8 kb mRNA. The gene spans about 20 kb on the short arm of human chromosome 17 (17p13). The p53 protein binds to specific DNA sequences and controls the expression of different regulator genes involved in growth. It interacts with other proteins in response to DNA damage and mediates apoptosis (cell death) of the cell when the damage is beyond repair. Its basic function is to control entry of the cell into the S phase. Somatic mutations in the *p53* gene occur in about half of all tumors. Mutations in the gene for p53 also cause tumors; in more than 90% of human cutaneous squamous cell carcinomas (skin cancers) and about 50% of all other human cancers, *p53* is defective. Those very rare individuals who *inherit* one defective copy of *p53* commonly have the Li-Fraumeni cancer syndrome, in which multiple cancers (of the breast, brain, bone, blood, lung, and skin) occur at high frequency and at an early age.

Genomic Instability Diseases

Ataxia-telangiectasia, Fanconi anemia, and Bloom syndrome are important examples of hereditary diseases resulting from mutations in genes that contribute to genome stability. Different patterns of chromosomal breaks and rearrangements are visible by light microscopy of metaphase cells. The underlying genetic defects predispose affected individuals to different types of cancer.

A. Ataxia-telangiectasia (A-T)

A-T is a variable disease due to autosomal recessive mutations in the *ATM* gene at gene map locus 11q23. Affected individuals are highly sensitive to irradiation and are prone to develop lymphomas and leukemias. The *ATM* gene has 66 exons spanning 150 kb of genomic DNA. A 3056- amino acid (350kD) protein kinase, ATM, is translated from its alternatively spliced 13-kb transcript. ATM is activated in response to double-strand DNA breaks. It has a central role in a network of proteins that regulate cellular responses to DNA damage and recombination.

B. Fanconi anemia (FA)

FA is a heterogeneous group of autosomal recessive and X chromosomal diseases manifest in early childhood as growth deficiency. About eight FA genes form a complementation group (see table in appendix). The proteins encoded by these genes form the FA complex. Together with other proteins, they detect DNA damage or errors

in replication. The most prevalent mutation is of *FA-A* (also called *FANCA*), in about 65% of patients. FA cells are hypersensitive to DNA-crosslinking agents, such as diepoxybutane (DEB), which induces chromosomal breaks.

C. Bloom syndrome (BLM)

BLM is a prenatal and postnatal growth deficiency disease (birth weight 2000g, birth length 40 cm, adult height 150 cm) with a distinct phenotype including a narrow face, sunlight-induced facial erythema, variable immune deficiency, and a greatly increased risk of different malignancies (about 1in 5 patients). Chemotherapy is very poorly tolerated. The hallmark is a tenfold increase in the spontaneous rate of sister chromatid exchanges. Breaks in one or both chromatids and exchanges between homologous chromosomes occur in about 1–2% of metaphase cells. BLM results from autosomal recessive mutations in the *BLM* gene at gene map locus 15q26.1, encoding a member of the RecQ family of DNA helicases. The 1417-amino acid BLM protein interacts with the FA complex and is involved in meiotic recombination. It is homologous to yeast Sgs1 (slow growth suppressor) and the human WRN protein (Werner syndrome). Mainly protein-truncating nonsense mutations are distributed fairly evenly along the gene, but some missense mutations exist. Most distinct is a founder mutation in populations of Ashkenazi Jewish origin, consisting of a 6-bp deletion/7-bp insertion at nucleotide 2281. Homozygosity for mutations in the *BLM* gene results in an increased rate of somatic mutations.

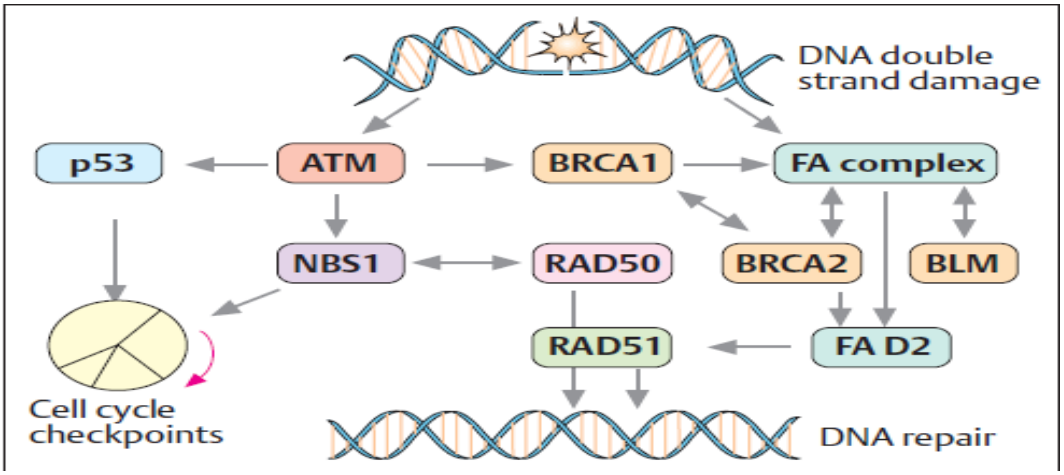

Figure: Relation of ATM to other proteins Maintaining genomic stability

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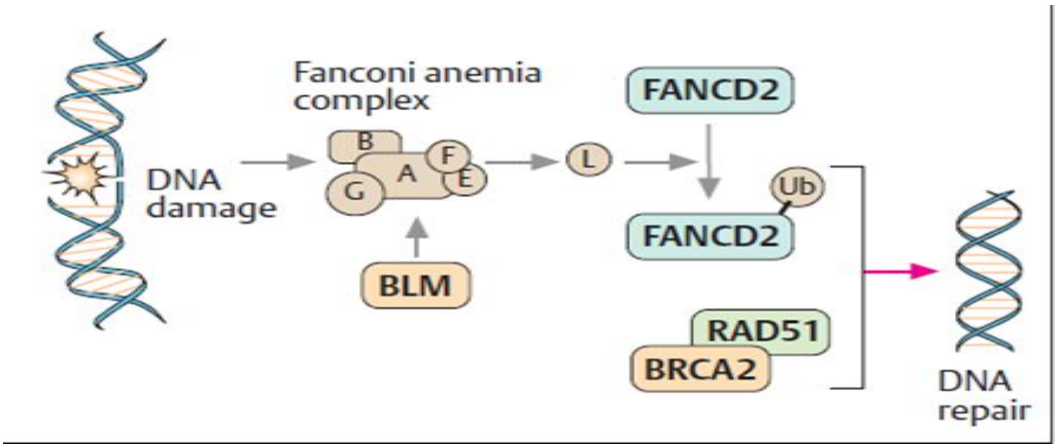

figure: Fanconi anemia-associated proteins

Chromosomes and cancer cells

Two prominent features of cancer cells are abnormal numbers of chromosomes **(aneuploidy)** and large-scale structural rearrangements of chromosomes.

These chromosome aberrations are caused by genomic instabilities inherent to most cancers. **Aneuploidy arises through chromosomal instability (CIN**) by the persistent loss and gain of whole chromosomes.

Chromosomal rearrangements occur through **chromosome structure instability (CSI**) as a consequence of improper repair of DNA damage. The mechanisms that cause CIN and CSI differ.

Both CIN and CSI are associated with advanced stage tumors with increased invasiveness and resistance to chemotherapy, indicating that targeted inhibition of these instabilities might slow tumor growth. Here, we review recent efforts that define the mechanisms and consequences of CIN and CSI.

Breast Cancer Susceptibility Genes

Breast cancer is one of the most common forms of cancer, accounting for 32%of all cancers in the Western world. Two genes confer susceptibility to breast and ovarian cancer when mutated, the breast cancer genes *BRCA1* and *BRCA2*. Both encode multifunctional proteins that play important roles in genomic stability, homologous recombination, and double-stranded and transcription- coupled DNA repair. The BRCA1 and BRCA2 proteins interact and participate in cell cycle control .

A. The breast cancer susceptibility gene *BRCA1*

The *BRCA1* gene on chromosome 17 at q21.1 accounts for 20–30% of inherited, autosomal dominant forms of breast cancer. This gene has 24 exons spanning 80 kb of

genomic DNA. Somatic mutations in breast tissue and germline mutations observed in unrelated patients are evenly distributed throughout the gene. About 55% of all mutations occur in the large (3.4-kb) exon 11. A deletion of an adenine (A) and a guanine (G) in nucleotide position 185 (185delAG) and an insertion of a cytosine in position 5382 (5382insC) are the most frequent, each accounting for about 10% of mutations.

B. The breast cancer susceptibility gene *BRCA2*

Mutations in the *BRCA2* gene, at 13q12, occur throughout the gene. A deletion of thymine at nucleotide position 6174 (6174delT) is relatively frequent (1%) in the Ashkenazi Jewish population. The BRCA2 protein has distinct functional domains. A large central domain consists of eight copies of a 30–80-amino acid repeat, which are conserved in all mammalian BRCA2 proteins (BRC repeats).

Telomerase

It is known that eukaryotic DNA is shortened in somatic cells from division to division because of the characteristics of replication. This occurs in the subtelomeric and telomeric repetitive sequences of chromosomes, and following approx. 50-70 divisions it leads to cell senescence, arrest of cell division and aging. In germ line cells the telomerase enzyme, which comprises a reverse transcriptase, and a telomeric DNA complementary RNA can restore the length of the telomere. It's crucial in the transmission of the same sized genome from generation to generation. However, telomerase activity is also linked to cancer cells. They can restore the telomeres either by up-regulating telomerase enzyme or by recombination based alternative telomere lengthening. If a cell - due to different mutations - avoids cell death caused by the extreme short telomeres, its genome becomes unstable, leading to the oncogenic transformation of the cell through the aforementioned mutations (amplifications, translocations).

pharmacogenomics

This deals with the influence of genetic variations on drug response in patients by correlating gene expression or single-nucleotide polymorphisms with a drug's efficacy or toxicity. By doing so, pharmacogenomics aims to develop rational means to optimize drug therapy, with respect to the patients' genotype, to ensure maximum efficacy with minimal adverse effects. Pharmacogenomics is the whole genome application of pharmacogenetics, which examines the single gene interactions with

drugs. These types of studies are at least as significant as the discovery of new drug targets. Genomic differences between people can result in significant differences in their responses to the drugs. E.g. in 30% of people the β-blockers used against hypertension are ineffective, while the antidepressants are ineffective in 50% of the treated persons. The situation is similar in the cases of most drugs

Goals of Pharmacogenomics has two main goals. One of them is to search for new drugs and drug targets with the help of genomic methods. It has great significance, because current existing therapies only hit about 400 different drug targets compared to the 20-22 thousand protein coding genes coding for about 2 million different proteins (because of e.g. posttranslational modifications, splice variants). In addition, DNA or RNA sequences can also be regarded as potential targets, and we know from the ENCODE project (http://www.nature.com/encode/#/threads) and the amount of conserved regions that about 10% of the human genome has some functions, and the number of cell specific enhancer regions is about 400,000 . Naturally, only a fraction of these are really drug targets, but according to estimations there are at least 10 times more drug targets than presently exists.

Genomic background of adverse effects One of the main questions of pharmacogenomics is that, what the mechanism is, with which the genetic variants influence the drug-response. **There are three main possible mechanisms** :

Pharmacokinetic: Genetic variations, which influence the mechanisms of absorption and distribution of the administered drug, the chemical changes of the substance in the body, and the effects and routes of excretion of the metabolites of the drug .

Pharmacodynamic: Genetic variants, which are in the genes of the drug targets or in their associated pathways. Pharmacodynamics is often summarized as the study of what a drug does to the body, whereas pharmacokinetics is the study of what the body does to a drug.

Idiosyncratic: Genetic variations in genes coding for proteins, which are not in the drug target or pharmacokinetic pathways, but could influence the drug response. The adverse effects could be caused by e.g. an enzymopathy, so that the triggering substance cannot be processed properly in the organism and causes symptoms by accumulating or blocking other substances to be processed.

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Difficulties of the pharmacogenomic researches

1- that the main development of the high throughput genomic, bioinformatic and other methods have been carried out only in the last few years, and there was not enough time (10-15 years) for the marketing of the drugs developed by the new methods.
2- Often environmental factors can cause similar effects as the genetic variants, which is called phenocopy. From a statistical point of view it can cause great difficulties in the evaluation.
3- Another disturbing factor is gene-gene interactions
4- There is also a less ethical explanation to why the pharmacogenetics results are so few today. In some cases the pharmaceutical companies are not interested that their drugs may be used only on people where the drugs are really effective, because it can result in less user and less profit.