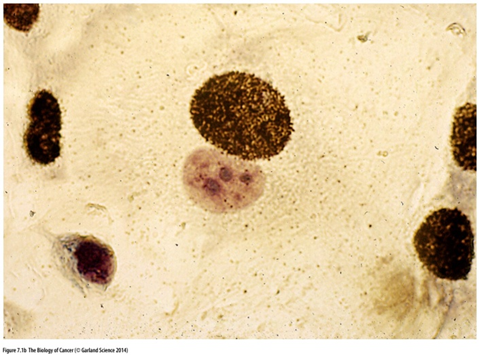
**TUMOR SUPPRESSOR GENES 1**

**Tumor phenotype**

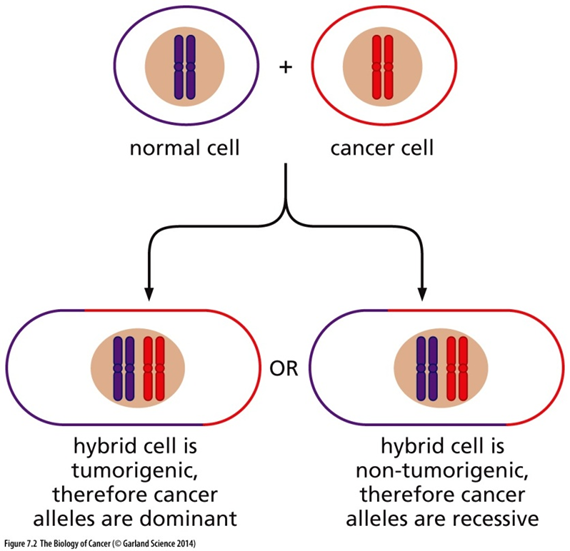
By examining oncogenic viruses in the 70s of the last century, it was established that these infectious agents carry numerous viral oncogenes, which behave dominantly if the viral genomes are integrated into normal cells, resulting in cellular transformation. This response of the cell means that the viral oncogene has the ability to dictate the behavior of the cell despite the function of "opposing" genes, which have a role to ensure normal cell proliferation. However, in the 1980s, it was suspected that most human cancers do not arise as a result of a viral infection.

If two cells are close to each other, and their plasma membranes are in contact, as a result of treatment with a fusion agent, one large cell will be formed, with one cytoplasm and two nuclei, which is called a syncytium (*Figure 1*).



*Figure 1. Syncytium*

If cells of different origin are combined, the resulting hybrid cell is called a heterokaryon, to emphasize the fact that it contains two genetically different nuclei. To everyone's surprise, in numerous experiments in which tumor cells were fused with normal, the initially formed tetraploid cell lost its oncogenic potential when injected into a suitable host. This meant, quite unexpectedly, that the malignant cell phenotype was recessive in relation to the phenotype of normal, wild type growth. In fact, when cancer cells originate mainly from non-virus-induced human tumors (or from chemically induced rodent tumors), the hybrid cells are non-tumorigenic. In contrast, when the cancer cells derive from virus-induced tumors, the hybrids are mostly tumorigenic (*Figure 2*).



*Figure 2: Dominance and recessiveness of the tumor phenotype*

It was hypothesized that a normal cell carries genes that suppress its proliferation. During tumor development, cancer cells lose completely or functionally one or more of these genes. Once the growth-suppressing genes are lost, the cancer cells are no longer under their control, and their proliferation accelerates. As long as there is no function of these genes in the cancer cell, its "malignant" proliferation continues. However, at the moment when the wild type genes, as an intact version of these genes, start functioning again within the cancer cell (since they were introduced by the cell fusion technique), the proliferation of the cancer cells stops.

Since the wild type of these hypothetical genes antagonizes the cancer cell phenotype, these genes are called tumor suppressor genes (TSGs). Their existence was supported by the fact that it was much easier to inactivate a gene by various mutational mechanisms than to hyperactivate it by means of mutations. For example, the ras proto-oncogene can be (hyper)activated only by a point mutation affecting the 12th, 13th, and 61st codons. In contrast, a tumor suppressor gene, or any other gene, can easily be inactivated by point mutations that affect many sites in its protein-coding sequence, or by random deletions that cut blocks of nucleotides out of it.

An important insight into the function and importance of tumor suppressor genes was provided by research on a rare childhood eye tumor, retinoblastoma. This tumor of the retina, which arises from precursors of photoreceptor cells, occurs in 1 in 20,000 children. The tumor is diagnosed from birth to 6-8 years of age, rarely later. This "tumor syndrome" occurs in 2 forms. Some children (those born into families without a family history of retinoblastoma) have one tumor in one eye. If the tumor is removed, with radiation or surgery, these children have no further risk of retinoblastoma or another tumor. Since the tumor occurs in children without a family history of this disease, it is considered a sporadic form of the disease, and since it occurs in one eye, it is also called unilateral retinoblastoma.

The familial form of retinoblastoma occurs in children whose parents were also affected and cured in childhood. In this case, multiple tumors are registered in both eyes (that's why it's called bilateral retinoblastoma). Curing eye tumors, with radiation or surgical intervention, does not protect children from a high risk (500 times greater than normal children) of bone tumors (osteosarcoma) during adolescence nor from an increased susceptibility to developing many other tumors during life.

This familial form of retinoblastoma is passed from generation to generation, as a trait carried by a dominant allele.

It was then assumed that there is a gene, called Rb, whose mutation is responsible for the development of childhood retinoblastoma. Mutations of the Rb gene make it inactive - recessive.

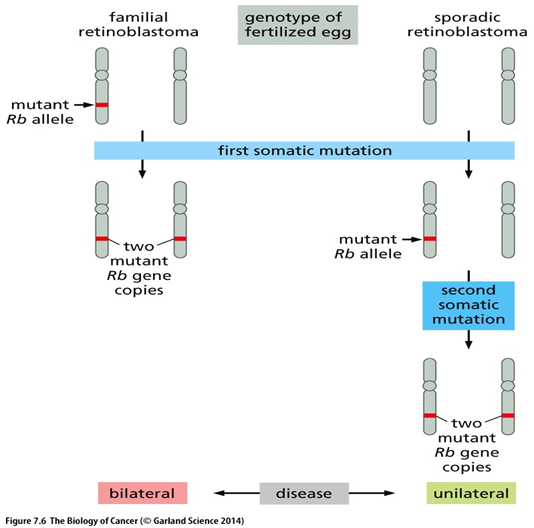
"On the inheritance of tumors... If tumors can arise in this way, homologous elements on both chromosomes must be damaged in the same way"

Theodore Boveri, pathologist in 1914.

The importance of Rb-1 and p53 genes in the genesis of tumors has been proven by the identification of mutations of the same genes in people with tumor predisposition syndromes, such as congenital retinoblastoma (Rb-1) and Li Fraumeni multicancer syndrome (p53). Inactivation or mutation of one allele of a tumor suppressor gene is not sufficient for tumor development, a change in both loci is required - loss of heterozygosity. The oncogenic DNA virus, the human papillomavirus, the causative agent of most cervical and perianal region tumors, inhibits the action of both mentioned tumor-suppressor genes.

Studying the kinetics of the appearance of both forms of retinal tumors in children, Alfred Knudson calculated in 1971 that bilateral tumors arise from the kinetics of "one hit", while unilateral tumors are the result of the kinetics of "two hits". Each hit implies a somatic mutation.

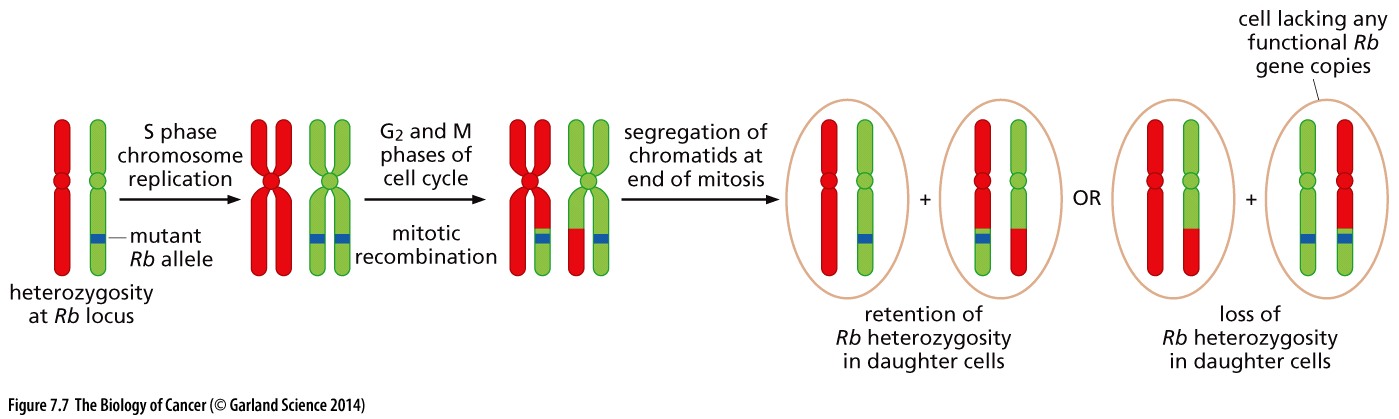
The two affected genes, according to Knudson's predictions, are both copies of the Rb gene located on chromosome 13. In familial retinoblastoma, during embryogenesis, the zygote carries one defective copy of the Rb gene, so that all retinal cells of the child carry only one functional copy of the Rb gene. If this copy of the Rb gene is eliminated by a somatic mutation, the cell will lack a functional Rb gene, and it will proliferate rapidly. In sporadic retinoblastoma, both Rb genes are wild type, and therefore two consecutive somatic mutations (affecting both copies of the Rb gene) are required for the development of retinoblastoma. Because only one somatic mutation is required to eliminate Rb gene function in familial cases, many cells in both eyes are affected. Considering that the occurrence of two somatic mutations (each of which is a rare event) on one cell is mathematically unlikely, this type of tumor occurs more often in a unilateral form (*Figure 3*).



*Figure 3. Dynamics of retinoblastoma formation*

Discoveries related to the Rb gene indicated the necessity of eliminating both copies of this tumor-suppressor gene as a prerequisite for the occurrence of sporadic retinoblastomas.

They hypothesized that the first of the two copies of the Rb gene was indeed inactivated by a mutation that occurred at a frequency similar to that of most other mutational events—about 106 per cell generation. A cell that has undergone this mutation will be heterozygous, and will have one wild type copy of the gene and one defective copy - Rb +/-. Since the Rb allele is recessive, this heterozygous cell will continue to exhibit a wild type phenotype. But what if the second, still-intact genetic copy of the Rb gene is inactivated by some mechanism other than an independent mutation? Perhaps there is some other kind of exchange of genetic information between paired homologous chromosomes, one of which carries the wild type and the other the mutated, defective type of Rb allele. Normally, recombination between chromosomes occurs only during meiosis, so in this case the question was asked what would happen if recombination occurred between one of the chromatid arms carrying the wild type Rb allele and the chromatid carrying the mutated allele (*Figure 4*).



*Figure 4. Elimination of the wild type gene copy*

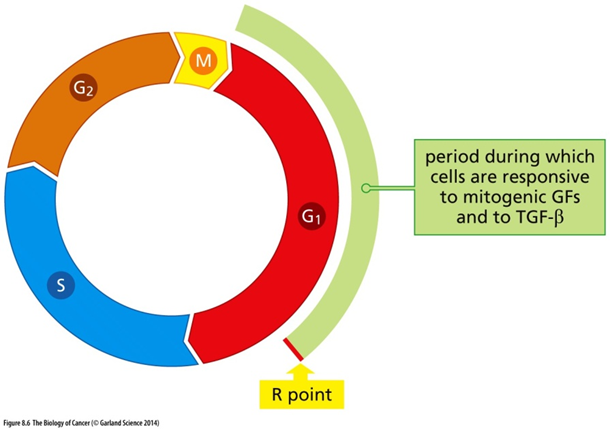
Such recombination is thought to occur during active cell proliferation and is therefore called mitotic recombination, to distinguish it from recombination in meiosis, which combines chromosome arms primarily during sperm and egg formation.

Tumor suppressor genes and the proteins they encode block cancer development by various mechanisms. By careful examination of the list of cloned tumor suppressor genes, it was shown that some of them function by directly suppressing cell proliferation by various signals that inhibit growth and induce differentiation.

The first two tumor suppressor genes that have been intensively studied are the Rb gene and the p53 gene, also called Trp53 or TP53. They play a major role in the pathogenesis of human cancers. The protein encoded by the Rb gene directs the progression of various cells through the cycle of division and growth. P53 and its protein also play a central role in cancer development.

The products of other tumor suppressor genes are translocated to different sites within the cell where they suppress cell proliferation in different ways. These proteins "occupy" all the mechanisms of cell cycle control, which govern cell proliferation and survival. Examples of well-studied tumor suppressor genes are NF1, APC and VHL.

Except for early embryonic cells, all other normal cells in the body require the presence of an external signal to start the process of growth and division. They respond to extracellular mitogens and inhibitory factors (such as TGF-β) only for a short period of time, from the onset of G1 phase until 1-2 hours before the transition from G1 to S phase. The end of this time frame is marked as the restriction point (R point), which means that it is the moment when the cell must make a decision whether to continue progressing through the cell cycle towards the M phase, remain in G1, or give up the active cell cycle. and return to the G0 phase (Figure 5).

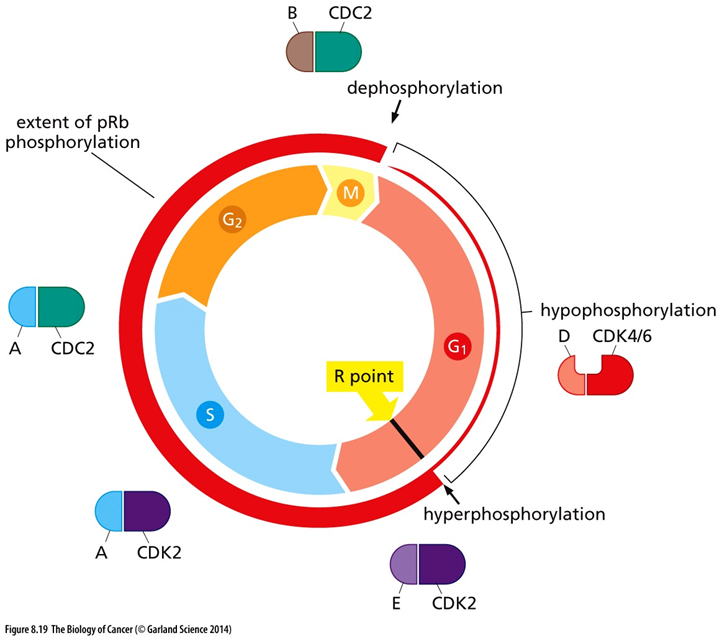


*Figure 5. Response to extracellular signals during the cell cycle*

If the cell decides at the R point to continue progressing through the cycle of growth and division, it commits to the transition from G1 to S phase and thus completes a rigid programmed series of events (entry into S, G2, and M), allowing it to divide into two daughter cells. This decision will be respected even if growth factors were not present in the extracellular space during the remaining phases of the cell cycle. The S, G2 and M phases follow a fixed pattern.

The isolation of the Rb tumor suppressor gene, whose defective versions are involved in the pathogenesis of retinoblastoma, sarcoma, and small cell lung cancer, as well as other tumors, contributed to the elucidation of the molecular mechanism by which the cell manages to pass through the R-point of the cell cycle. It encodes a nuclear phosphoprotein with a molecular weight of about 105kD, designated as pRb or RB, and it has been proven to be absent, or present in a defective form, in the cells of many of the tumor types listed above.

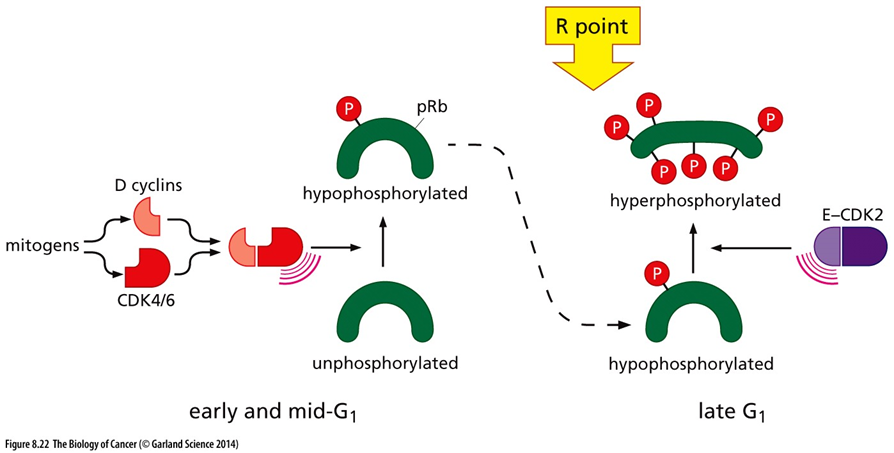
It was also found that the phosphorylated state of pRb is closely related to cell cycle progression. When the cell goes through the M/G1 transition, all existing phosphate groups are removed from pRb, leaving it in an unphosphorylated configuration. As the cell progresses through G1, one or two phosphate groups bind to some of the 14 different phosphorylation sites (by the cyclin CDK4/6 complex), inducing hypophosphorylation of pRb. However, when the cell passes through the R restriction site, cyclin E/CDK2 complexes phosphorylate pRb at at least 12 remaining phosphorylation sites, converting this protein into a hyperphosphorylated state. Then this hyperphosphorylated state is maintained until the cell enters the M phase (Figure 6). After the cell exits mitosis, phosphate groups are removed from pRb by an enzyme called protein phosphatase type 1 (PP1). By this "dephosphorylation" of pRb, it is possible to start the next cell cycle, and thus a new cycle of pRb phosphorylation.



*Figure 6. Cell cycle progression depends on pRb phosphorylation*

The fact that pRb phosphorylation occurs synchronously with the passage through the R point indicated that this protein is a molecular controller of the transition through the R point.

Since pRb is the final arbiter in the growth process, it is clear that its phosphorylation must be tightly controlled. It is, of course, controlled by the components of the cell cycle clock. In the early and middle G1 phase, D-CDK4/6 complexes are responsible for the initiation of pRb phosphorylation, leading to its hypophosphorylation. Since cyclin D levels are mainly controlled by extracellular signals, ie. mitogenic growth factors, this means that both the cell cycle transition and passage through the R point are also controlled by these same factors (Figure 7). Then, complete control over this process is taken over by cyclin E-CDK2 complexes, which increase the level of pRb phosphorylation more than 10 times, resulting in its hyperphosphorylation and complete functional inactivation. pRb molecules that have not previously undergone hyperphosphorylation mediated by D-CDK4/6 complexes are not good substrates for completing phosphorylation by E-CDK2 complexes.



*Figure 7. Control of transition through the R point by mitogens*

Once a cell passes through the R point, the continued hyperphosphorylation and functional inactivation of pRb is largely complete, and cell cycle control is taken over by the cyclin E-, cyclin A-, and cyclin B- CDK complexes, none of which respond to extracellular signals and guarantee the execution of strictly programmed cell transitions through S, G2 and M phase.

This scheme recalls why pRb is a key player in the regulation of cell proliferation. If its functions in the cell are lost (due to mutations of the chromosomal copies of the pRb gene, methylation of the promoter of the pRb gene, or due to the activity of the DNA oncoprotein of oncogenic viruses), then this protein can no longer serve as a gatekeeper of the R point. In addition, in some cancer cells the phosphorylation of Rb is deregulated, resulting in inadequate phosphorylation and functional inactivation of pRb. There is evidence that in some cancer cells the dephosphorylation (and consequent inactivation) of pRb, which normally occurs during the M/G1 transition by PP1 phosphatase activity, never occurs, leaving pRb in a hyperphosphorylated, inactive state throughout the growth and division cycle. Without pRb on guard, the cell moves from G1 to S phase without control by mechanisms that ensure that the transition to the next phase of the cell cycle occurs only after the previous phase has been completed.

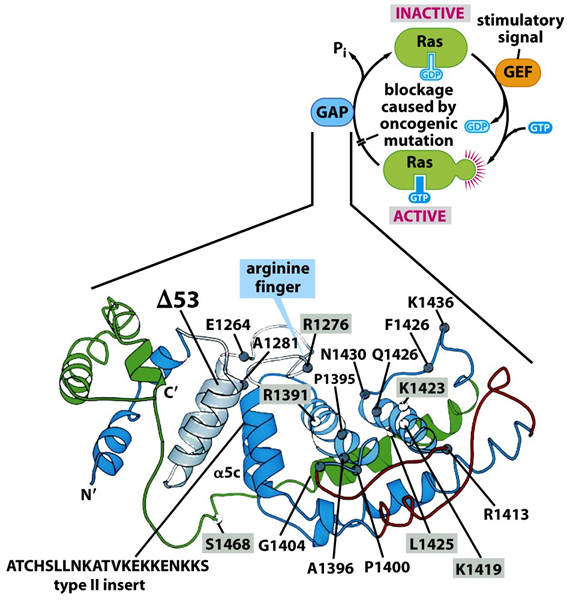
**NF1 protein as a negative regulator of the Ras signaling pathway**

Neurofibromatosis was first described by Friedrich von Recklinghausen in 1862. Today we know that neurofibromatosis type 1 is a relatively common familial tumor syndrome, with an incidence of 1 in 3,000 people. The main feature of the disease is the development of benign tumors of the cells covering neurons, the peripheral nervous system. Sometimes, some neurofibromas progress to neurofibrosarcomas, malignant tumors. Patients with neurofibromatosis type 1 have an increased risk of glioblastoma (tumor of astrocytes in the CNS), pheochromocytoma, and myeloblastic leukemia.

Familial tumor syndrome neurofibromatosis type 1 has a genetic background, a mutated allele of a gene called NF1. Genetic changes in NF1 are thought to correspond to changes in the Rb gene. A mutated, inactivated allele of the NF1 gene creates the characteristic phenotype of this syndrome. At the cellular level, this means that the originally heterozygous conformation of the NF1+/- gene is converted to the homozygous NF1-/- state in tumor cells, by loss of heterozygosity. Since half of patients with neurofibromatosis have no family history of the disease, mutations of both NF1 alleles arise de novo.

The NF1 protein has a lot of similarities with the IRA protein and functions as a GTPase activating GAP protein for the Ras molecule. In a growing cell (proliferation), Ras proteins regulate important metabolic and proliferative processes. By inducing GTPase activity, IRA converts Ras from an activated to an inactive state, GDP-Ras.

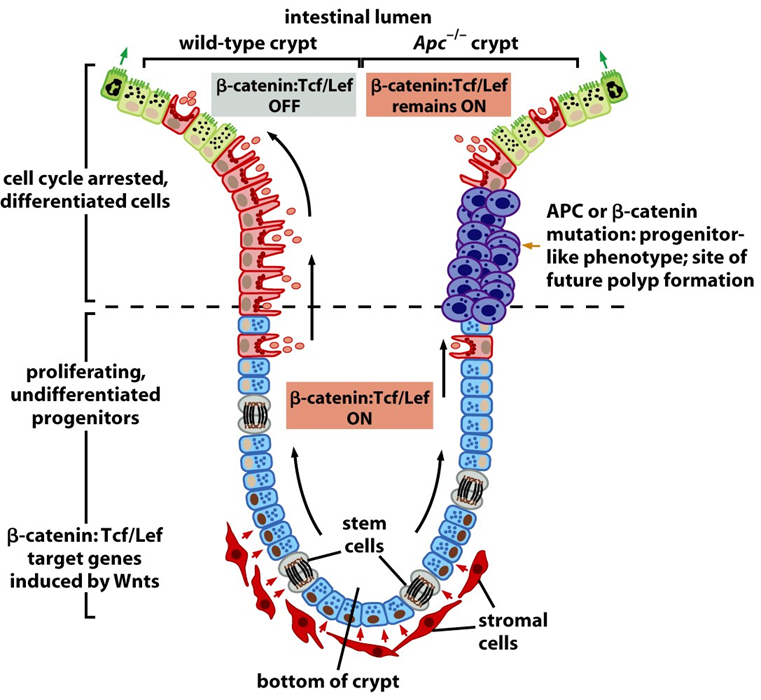
NF1 is expressed in many cells, especially in cells of the peripheral and central nervous system. After growth factor stimulation, NF1 is degraded in the cell, which enables activation of the Ras signaling pathway. However, after 60-90 minutes, the level of cellular NF1 returns to normal and blocks the Ras signaling pathway, in a negative feedback loop. In neuroectodermal cells in which NF1 is not functional, the Ras protein is in the active GTP-Ras form significantly longer than in normal cells.



*Figure 8. Ras signaling pathway*

**Apc**

The epithelium of the duodenum and colon is organized like many other epithelia in the body. The division of relatively undifferentiated stem cells produces two different daughter cells. One daughter cell remains a stem cell and thus allows the total number of stem cells to remain constant in each tissue. Another daughter cell and its progeny differentiate. In the small intestine, these differentiated cells participate in the absorption of food from the lumen of the intestine and the transport (of nutrients) into the circulation. They absorb water from the intestinal lumen into the colon.



*Figure 9. Stem cells in the GIT*

The localization of stem cells and cells in differentiation is shown in figure 9. Stem cells are located and protected at the bottom of depressions called crypts. While some of the progenitor cells remain at the bottom of the crypts where they maintain a constant number of stem cells, most of them separate and migrate from the crypts to the surface of the intestinal lumen, where they form the intestinal wall, perform their function, then die by apoptosis and separate into the lumen of the colon. The whole process from emergence, through migration, effector function and death take 3-4 days.

There are effective defense mechanisms against colon tumors. The cells die within a few days of their formation. It is clear that the mutations that lead to the formation of tumors are those that block the migration of epithelial cells from the crypts and cell death. Enterocytes whose mutations have enabled them to remain in the crypts will live significantly longer.

Molecular mechanisms controlling the migration of enterocytes from the crypts. This process depends on the expression of intracellular β-catenin. Enterocyte stem cells have high levels of intracellular β-catenins. These cells maintain the "stem cell state" by maintaining the concentration of intracellular β-catenin. When one of the progenitor cells begins to migrate, the amount of β-catenin decreases. These cells then lose their stem cell phenotype, exit the cell cycle and differentiate into functional enterocytes.

Apc is the product of the adenomatous polyposis coli (Apc) gene and is responsible for the negative control of β-catenin levels in the cytosol. In cells at the base of normal crypts, Apc genes are not expressed and β-catenin is present in large amounts. As the cell migrates out of the crypt, the level of Apc gene expression increases, and the Apc protein decreases the level of β-catenin.

Accumulation of β-catenin is certainly the most significant consequence of Apc gene inactivation, and is noted in 90% of all sporadic colon cancers.

When β-catenin accumulate in enterocyte precursors as a consequence of Apc gene inactivation or by some other mechanism, these cells retain a stem cell-like phenotype, which keeps them in the crypts. The first of the mutations is the inactivation of Apc. Such a cell is "trapped" in the crypt and accumulates mutations of other genes, such as K-ras, which enables faster growth. This causes the accumulation of large numbers of relatively undifferentiated cells in the crypts of the colon, which sometimes form adenomatous polyps. Mutations can accumulate in these cells that allow them to form more "aggressive" polyps or cancers.

In addition to the known role in β-catenin expression, Apc molecules bind to microtubules that form the dividing spindle and are responsible for chromatid separation during anaphase and telophase of mitosis. Cells without functional Apc are characterized by significantly increased chromosomal instability, accompanied by an increased or decreased number of chromosomes. The resulting aneuploidy changes the relative number of critical tumor-promoting and tumor-inhibiting genes.

**DNA repair system**

Another group of tumor suppressors consists of the BRCA1 and BRCA2 genes. These genes also belong to the DNA repair system.

BRCA1 (breast cancer 1) is expressed in the cells of the mammary gland but also in other tissues where it helps to repair damaged DNA or to destroy the cell if the DNA cannot be repaired. Repairs DNA double helix breaks. Most often, DNA strand breaks are single (only one of the 2 strands), but sometimes the break is complete (both strands). BRCA1 forms part of a protein complex that repairs DNA when both strands are broken. When the DNA is completely broken it is difficult to insert the appropriate nucleotides (no missing sequence is recorded). Repair by the BRCA1 protein involves homologous recombination. The repair system uses the sequence of nucleotides from the sister chromatid (homologous chromosome). Although the structures of the BRCA1 and BRCA2 genes are different, the functions of the proteins they encode are similar - repairing damaged DNA.

The ATM protein detects damage and sends a signal to the BRCA 1/2 proteins, which are phosphorylated under the action of ATM-kinase, then interact with the p53 protein, which induces the formation of the p21 protein, resulting in cell cycle arrest. BRCA 1/2 also interact with estrogen receptors (suppressing their function) as well as with the promoter of the c-Mys oncogene (suppressing the expression of this oncogene).

Dysfunctions of these genes increase the possibility of breast and ovarian cancer. About 50-65% of women born with a BRCA 1/2 gene mutation will develop breast cancer by age 70, and 35-46% will develop ovarian cancer. Breast cancers associated with BRCA gene dysfunction are much more aggressive than normal breast cancers. They are most often negative for hormone receptors and appear on average 20 years earlier than other forms of breast cancer. In these genes, apart from loss of heterozygosity, the most frequently found change in DNA is methylation. Non-functional BRCA 1 gene in a significantly higher percentage were found in hereditary forms of breast cancer and ovarian cancer, compared to BRCA 2 genes, found in sporadic forms of breast cancer. People with non-functional BRCA 1/2 genes often also have p53 mutation, HER2/neu oncogene amplification and more aggressive tumor forms.