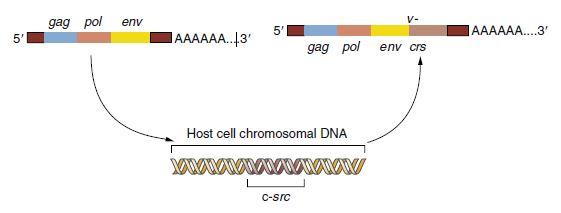
**ONCOGENES**

Oncogenes are evolutionarily highly conserved genes essential for cell survival. They encode proteins involved in signaling pathways that stimulate cell proliferation. These proteins are classified into several groups: growth factors, growth factor receptors, proteins involved in signal transduction, and transcription factors.

One of the most significant contributions to the discovery of oncogenes was made by the study of Bishop and colleagues on the **Rose sarcoma virus** (**RSV**). Their initial research was related to understanding the replication of this virus. However, the attention of these researchers was then focused on the transformation of a cell infected with this virus in the direction of a malignant cell. Studies by other researchers that preceded this one established the presence of the viral gene **src** and its importance in malignant potential. This was definitely confirmed by the study of Bishop and colleagues, the effect of different retroviruses on normal cells. Only the virus carrying the **src gene** triggered the malignant transformation of the cell. In addition, this group found the same gene in normal chicken cells and designated it as **c-src (cellular) versus v-src (viral).**



***Figure 1.*** *Cellular src gene*

This s-src gene is called a **proto-oncogene**, and it indicates an independent potential to be activated into an oncogene, that is, a gene responsible for participating in the malignant transformation of cells. This initial research was followed by many that showed the presence of a larger number of proto-oncogenes in normal cells that are in an inactive form, not only in birds, but also in vertebrates and finally in mammals. In this research, the discovery that a cell can be transformed if its genome is put under the control of a virus is very significant. However, the question remains whether proto-oncogenes can be activated without the presence of a virus.

Thus, a proto-oncogene is a normal gene, which encodes proteins important for the regulation of proliferation. It becomes an oncogene after mutation or increased expression (due to a virus). Oncogenes encode proteins important for the transformation of a normal cell into a tumor and increase in the malignancy of a tumor cell.

**Multistage tumorigenesis**

The discovery of mutated genes, tumor-associated genes-oncogenes in human tumors, simplified the model of oncogenesis. The mutated gene of a tumor cell, the incorporation of this gene into the cell of the target tissue, the mutation of the proto-oncogene, can be represented as a simplified model of carcinogenesis. The growth of cells changed in this way over time forms a mass that can be seen macroscopically, i.e. a visible tumor is formed. On the basis of histopathological analyzes of such a tumor, it was established that the formation of a tumor is a multistage process in which an initially normal cell passes through a series of intermediate states on its way to a potentially malignant one. At each of these steps there are different changes in the cells that make them different from the previous and the next. These changes are mainly related to changes in the phenotype of the cells. This multistage genetic model of tumor progression differs significantly from the primary theory of the single-hit model in the transformation of a normal cell into a malignant one. This dilemma was clarified in 1983 when it was shown that the mutation of only one gene could not induce the development of a malignant cell. According to this research, it was shown that two, sometimes even more, oncogenes are needed to transform a normal cell into a malignant one. This experiment demonstrated that human tumor cells carry two or more mutated oncogenes that influence each other in the formation of aberrant phenotypes characteristic of malignant cells.

**Proto-oncogenes**

Proto-oncogenes play a key role in cell proliferation and differentiation, as well as in the growth and development of the organism, and their expression is precisely regulated. Cell proliferation depends on an external signal. There are many cell-specific factors that circulate and signal to certain types of cells whether or not to divide. After engaging the receptor on the surface of the membrane with a suitable ligand, the signal from this receptor is transmitted from the membrane to the cytoplasm and finally to the nucleus, where DNA synthesis begins. Proto-oncogenes have been found to play a role in every step of this signaling pathway. This means that proto-oncogene products are all types of molecules involved in cell signaling: secretory proteins, transmembrane receptors, GTP-binding proteins, protein kinases, growth factors, receptors for growth factors, as well as proteins that regulate gene activity, etc.

In normal cells, proto-oncogenes act as controller genes, i.e. "guards of order" and can be transformed into active oncogenes, important in tumorigenesis, through various structural or regulatory changes. The srs protein of Rose sarcoma virus is the first investigated oncogenic product. A mutated form of the src gene was detected in advanced colon cancer cells.

The **ras** proto-oncogene family encodes **p21 proteins** in the cytoplasm that have the structural and functional properties of G proteins and act as mediators between receptors on the cell membrane and adenylate cyclase in the transmission of external physical and chemical signals, i.e. participate in signal transduction. Mutations of the ras gene are frequent abnormalities in a large number of malignant tumors. Ras oncogene proteins are involved in about 25% of human cancers. N-ras was detected by transduction in human bladder cancer cells, while K-ras was detected by the same method in human colon cancer cells.

When a growth factor binds to a receptor on a target cell, a cascade of phosphorylation is initiated from the receptor to the Ras protein and **mitogen-activated protein kinase (MAP).** These kinases phosphorylate enzymes that enter the nucleus and phosphorylate transcription factors. Another signaling pathway activates the **myc gene**. This pathway goes through the src protein, also a tyrosine kinase. Both signaling pathways are necessary for the cell cycle and the G1/S transition. Both signaling pathways are thought to trigger the synthesis of cyclin D. This cyclin initiates the cell into the S phase of the cell cycle. In this way, mitogens (hormones, paracrine growth factors) induce the cell to proliferate.

Strictly controlled growth and differentiation of cells in the organism involves numerous control mechanisms, including physical parameters and interaction with the extracellular matrix and adhesive molecules. A key role, however, is played by soluble factors known as growth factors. In addition to growth factors, growth factor receptors and transducing molecules also play an important role. Growth factors perform their function by binding to membrane receptors and thus start a signaling cascade. After growth factor binding, most receptors display tyrosine kinase activity that enables phosphorylation of cytoplasmic proteins.

Growth Factors:

granulocyte colony stimulating factor (G-CSF)

granulocytic monocytic colony stimulating factor (GM-CSF)

nerve growth factor (NGF)

neurotrophins

platelet-derived growth factor (PDGF)

erythropoietin (EPO)

thrombopoietin (TPO)

myostatin (GDF-8)

fibroblast growth factors (FGF)

epidermal growth factor (EGF)

hepatocyte growth factor (HGF)

vascular endothelial growth factor (VEGF)

Platelet-derived growth factor (**PDGF**) is a mitogen and chemoattractant for mesenchymal cells, including fibroblasts and vascular smooth muscle cells. It plays an important role in embryonic development, cell proliferation, cell migration and blood vessel formation (angiogenesis). Receptor engagement initiates signaling pathways through Ras, PI3K, and phospholipase C. PDGF receptors are expressed in numerous types of tumors and are almost always expressed in colorectal, lung, and breast cancers. It is involved in evading the cell cycle checkpoint at the G1 phase checkpoint. It has a significant role in mitosis of fibroblasts and proliferation of oligodendrocyte stem cells.

The **VEGF** (vascular endothelial growth factor) family consists of 6 proteins, of which VEGF-A has the most potent pro-angiogenic activity. To date, 3 VEGF receptors have been identified. VEGFR-2 is expressed on endothelial cells and is thought to play the most important role in angiogenesis, migration and cell survival. The binding of VEGF-A to VEGFR-2 induces the migration and proliferation of endothelial cells and affects the increased permeability of blood vessels. Engagement of VEGFR-2 activates the downstream signaling pathways PI3K/AKT, ERK1/2 and MAPK. Activation of some subgroups of receptors of this growth factor conditions angiogenesis in embryonic development and affects lymphogenesis.

Some of the oncogenes are found in the DNA genomes of oncogenic viruses, while others arise from the alteration of normal cellular proto-oncogenes. One of the causes of disturbances in the regulation of cell proliferation is hyperactivity of oncogenes. Several mechanisms responsible for the disruption in the expression of these genes have been described, such as DNA amplification, point mutations, reciprocal translocations (interruption of two chromosomes and reciprocal exchange of genetic material), deletions, duplications, inversions, insertion of viral DNA or RNA into the cell genome. Inadequate expression of these genes results in forms that differ from normal forms only in inadequate expression during the cell cycle or in inadequate tissue expression, thus contributing to the development of malignancy. Their products, i.e. oncoproteins, also structurally do not differ from normal forms, but are characterized by increased production. The consequence of excessive activation of oncogenes is: increased amount and activity of growth factors, increased number of receptors for growth factors, increased number of proteins involved in various signaling pathways and increased creation of transcription factors. Such changes induce disturbances in the cell cycle. They can stop the cell cycle and then they are not critical. Then the cells exit the cell cycle. If they die, they will most likely be replaced by new ones. A much more common outcome of the mentioned changes is continuous cell proliferation, which prevents differentiation and enables the creation of a larger number of progenitor cells.

Important oncogenes that mediate malignant cell transformation are Ras genes. The Ras gene products are G-proteins that bind guanine nucleotides and activate different sets of signaling cascades. These proteins play an important role in the activation and control of various signaling pathways, which participate in the processes of maintaining cell integrity, proliferation, cell adhesion, apoptosis and cell migration. When bound to **GDP (guanosine diphosphate)** the Ras protein is in an inactive form, but with the help of **SOS proteins (Son-Of-Sevenless)** that replace GDP with **GTP (guanosine triphosphate)** it undergoes conformational changes and becomes active form. There are three members of this gene family: K-ras, H-ras, N-ras.

Activated Ras proteins trigger many signaling pathways, and one of the important ones is the MAP kinase pathway (mitogen-activated protein). The signal is then transmitted downstream to the nucleus where it activates various transcription factors including c-myc. The ultimate outcome of this signaling cascade is cell proliferation, cytoskeleton reorganization and cell survival (**Figure 2**).



***Figure 2****. Signaling pathways induced by Ras activation. The growth factor binding to its receptor induces the mobilization of numerous adapter proteins responsible for the conversion of Ras-GDP (inactive form) to Ras-GTP (active form). The result of Ras activation is the initiation of various signaling pathways, the ultimate outcome of which is cell proliferation, cytoskeleton reorganization, survival, vesicle transport, and calcium release.*

Ras proteins are ubiquitously expressed. The frequency of mutations of these oncogenes has been proven in different types of tumors: bladder, kidney, thyroid, lung, pancreas, colon, malignant melanoma, hepatocellular carcinoma and hematological malignancies. Mutation of the Ras gene produces oncoproteins that stimulate cell division independently of growth factors. The resulting modified proteins cannot hydrolyze GTP. These mutated Ras proteins are therefore "locked" in a GTP-bound, active form that continuously activates the MAPK signaling pathway and drives cell proliferation.

They are considered to have an important role in angiogenesis and tumor metastasis. Ras gene mutation induces increased production of pro-angiogenic vascular endothelial growth factor (VEGF), while reducing the synthesis of TSP-1 (thrombospondin-1) as an anti-angiogenic factor.

Oncogene cooperation is an important molecular concept of oncogenesis. The best example of such cooperation is Ras and the c-myc oncogene. Unlike c-myc, which is responsible for increased cell sensitivity to apoptosis, Ras is thought to decrease their sensitivity. However, in cells in which both of these oncogenes are simultaneously activated, signal transduction interacts to stop terminal differentiation and such cells enter proliferation and become resistant to apoptosis. c-myc is a transcription factor, an important component in the control of cell growth.

The c-myc oncogene was discovered for the first time in patients with Burkitt's lymphoma showing frequent translocations on chromosome 8. A mutated or over-transcribed c-myc oncogene becomes an oncogene that increases cell proliferation. A tumor is thought to develop when the c-myc oncogene is translocated to the immunoglobulin gene promoter region (on chromosomes 2, 14, and 22). The promoters activate the c-myc oncogene instead of immunoglobulin genes and induce continuous lymphocyte proliferation.

c-myc participates in various cellular processes including proliferation, differentiation and apoptosis. In response to growth signals, c-myc gene expression is tightly regulated in normal cells. In general, in cells that are at rest and not dividing, its expression is unmeasurable, while after mitogenic stimulation it increases, and in proliferating cells it remains expressed at a very low level.

Numerous studies hypothesize that its activation is sufficient to induce various types of tumors and that it is necessary for the maintenance of malignant cell transformation. Overexpression of the c-myc gene has been detected in various tumor types. Given the ability of the c-myc gene to stimulate cell proliferation, it is thought that its ectopic expression may induce disruption of DNA synthesis and genetic instability. Another mechanism by which c-myc contributes to carcinogenesis is that it favors angiogenesis, and by suppressing adhesive molecules (LFA-1 from Leukocyte Function-associated Antigen-1 and α3β1), malignant cells lose contact inhibition and continuously proliferate. The results of certain studies indicate that c-myc plays an important role in cell immortalization by enhancing the activity of telomerase, the enzyme that synthesizes telomeres. Many authors point out that the amplification of the myc gene family is not the primary event in carcinogenesis, but rather occurs in already transformed cells, most often as a consequence of the effects of cytostatic therapy.

**HER2/neu**

The HER2/neu gene encodes a receptor with tyrosine kinase activity. HER2 activates downstream signaling pathways, including MAPK and PI3K. This gene is overexpressed in about 25% of breast cancers. Breast cancers with hyperexpression of HER2/neu are clinically much more aggressive. HER2/neu hyperexpression is due to gene amplification. Multiple copies of genes accumulate in tumor cells. An increased number of HER2/neu receptors on tumor cells results in increased sensitivity of the ras-MAP kinase pathway and intensification of proliferation. The discovery of HER2/neu amplification in breast cancer led to the development of the monoclonal antibody Trastuzmab-herceptin, which was approved for clinical use (for breast tumors).

Herceptin binds to HER2/neu on the cell surface, blocks MAPK and PI3K signaling pathways and inhibits the growth of HER2-expressing cells. It also induces tumor cell killing by the host's immune system. Adding a monoclonal anti-HER2 antibody to chemotherapy reduces the degree of disease recurrence by 50%. This monoclonal antibody inhibits tumor growth in at least 3 ways: (1) prevents the interaction of growth factors with HER2; (2) activates NK cells involved in antibody-dependent cytotoxicity (ADCC) that kill tumor cells; (3) reduces angiogenesis, by reducing the migration of endothelial cells.

**Cyclin D**

Cyclin D is involved in cell cycle regulation. Synthesis of cyclin D begins during the G1 phase and enables the G1/S transition in the cell cycle. Cyclin D interacts with cyclin-dependent kinases 2, 4, 5 and 6. In proliferating cells, the accumulation of the cyclin D-CDK4/6 complex enables cell cycle progression. This complex phosphorylates and thus inactivates the Rb anti-oncogene protein. Inhibition of Rb induces the expression of some genes (eg cyclin E) important for the transition of the cell cycle to the S phase. Activation of the MAR kinase pathway in the cell activates the transcription factors Myc and AP-1, which consequently drive the transcription of the genes for Cdk4, Cdk6 and cyclin D.

In a normal cell, increased expression of cyclin D shortens the duration of the G1 phase of the cell cycle. Increased concentration of cyclin D and cyclin D-CDK4 complex can induce the transition of cells from G0 to S phase of the cell cycle even in the absence of growth factors. Cyclin D is often overexpressed in cancer, reducing the activity of Rb. In breast cancers, this gene is often hyperactive. Cyclin D has a role in the development of parathyroid adenomas, too.

**Bcl-2 family**

This protein family contains more than 15 members divided into 3 classes based on function and the number of Bcl-2 homologous domains. The anti-apoptotic members (Bcl-2, Mcl-1 and Bcl-xl) contain four BH domains (BH1 to BH4). Pro-apoptotic Bcl-2 is divided into effector proteins (BAX and BAK) and they also contain four BH domains. BID and BIM pro-apoptotic proteins contain only the BH3 domain. Proteins in all three classes have the ability to form homo- and hetero-dimers with each other and play different roles in the regulation of mitochondrial membrane permeability. The link between Bcl-2 and tumors has been demonstrated before. The Bcl-2 gene locus has been identified to be translocated in several different tumor types, including follicular lymphoma and chronic lymphocytic leukemia. Other oncogenes, such as Ras, can also stimulate Bcl-2 expression. The frequency of Mcl-1 and Bcl-X in tumors is increased and overexpression of anti-apoptotic members as well as down-regulation or inactivation of pro-apoptotic proteins have been found in several human malignant tumors.

**Oncogenes in colorectal cancer**

K-ras, H-ras, N-ras are frequently altered by somatic mutations in human tumors. K-ras gene mutation can be detected in 40% of colorectal cancers. In precancerous lesions, adenomas, the frequency of K-ras mutations depends on the size of the adenoma. Thus, only 10% of adenomas below 1 cm in diameter have K-ras mutations, while the same mutations were found in over 50% of adenomas larger than 1 cm. Ras proteins can also activate the phosphatidyl-inositol-3-phosphate (PIP3) signaling pathway, which results in the subsequent activation of protein kinase B/AKT and subsequent activation of antiapoptotic factors and the mTOR signaling pathway, which directly controls cell growth. Mutations of other oncogenes are not as common in colorectal cancers. Less frequent mutations were noted for HER2/neu, c-myc, cyclin D, cyclin E.

**Oncogenes in breast cancer**

Amplification of the HER2 gene and subsequent increased expression of the HER2 protein (in 20-30% of invasive breast cancers) is associated with accelerated cell growth and aggressiveness of the disease. At the molecular level, HER2 amplification is associated with deregulation of the G1/S phase of the cell cycle, increased activity of cyclin D1, E and cdk6, and degradation of some anti-oncogene products.

In the 19th century, Beatson showed that estrogen plays a significant role in the growth of breast cancer. Estrogen is a steroid hormone that has a strong proliferative effect on the mammary epithelium through the activation of ER-α, a nuclear hormone receptor. ER-α is overexpressed in more than 70% of breast cancers. Activated ER-α induces the transcription of several other oncogenes: cyclin D and myc. Amplification of the ER-α gene is a significant factor in breast tumor carcinogenesis. Today, ER-α is an important biological target for breast tumor therapy and antiestrogens are included in the recommended therapy for all ER-α expressing tumors. Tamoxifen is a selective inhibitor of the ER signaling pathway, which binds to ER-α and prevents transcriptional activation. However, resistance to tamoxifen often develops.

**Oncogenes in lung cancer**

c/kit belongs to the PDGF/c-kit receptor family that activates JAK-STAT, PI3K and MAPK signaling pathways in the cell and plays an important role in proliferation and differentiation. Together with the SCF (stem cell factor) ligand, it is expressed in many lung cancers (primarily SCLCs).

HER2 is intensively expressed in one third of NSCLCs, especially adenocarcinomas. Increased expression of HER2 is a poor prognostic factor for lung cancer.

Activation of the nuclear product of oncogenes, such as those encoded by the myc family of genes (MYC, MYCN…), is often the final link of the signaling cascade. Activated MYC functions as a transcription factor very important for cell proliferation, differentiation and apoptosis. Amplifications and transcriptional disturbances of the myc gene are frequently encountered in SCLC, and much less frequently in NSCLC.