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*Chapter 2*

# The Classification, Mechanisms of Activation and Roles in Cancer Development of Oncogenes

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## Abstract

Cancer is an uncontrolled cell growth caused by accumulation of genetic and epigenetic mutations in genes that normally play a role in the regulation of cell proliferation, survival, apoptosis and cell cycle. Mutations are occurring mainly in oncogenes, tumor-suppressor genes, microRNA genes, or as DNA repair defects and aberrant DNA methylation. Oncogenes encode proteins that control cell proliferation and/or apoptosis. Products of oncogenes can be classified into 6 groups by its biological activity: transcription factors, growth factors, growth factor receptors, signal transducers, chromatin remodeling and apoptotic

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regulators. The main changes related to oncogene activation are chromosomal translocations and mutations that can occur as early events or during tumor progression; whereas amplification usually occurs during the tumor progression. These alterations are usually somatic events, although germ-line mutations can also predispose to familial cancer. A single genetic change is rarely insufficient for developing a malignant tumor. Most evidences point to a multistep process of sequential alterations in a wide number of oncogenes, tumor-suppressor genes, or microRNA genes related with cancer.

Recently, other mechanisms as the inflammation or alterations in the cellular metabolism have been associated with cancer. This chapter describes some of the key molecular mechanisms involved in the development and progression of cancer.

## Introduction

During already long time, it has been accepted that the cancer is caused by the accumulation of genetic and epigenetic changes that lead to abnormal regulation of the cell growth control [1]. Discovery that abnormal genes, named oncogenes, play a crucial role in developing the malignant disease has been decisive in understanding the genetic nature of cancer. More recently, six biological capabilities acquired during the multistep development of human tumors were established as the principal hallmarks of cancer. These features rationalize the complexities of the neoplastic disease and comprise: sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis.

In addition, two essential components of the tumorigenic disease, which guides the tumor progress, are: genome instability, which generates the genetic diversity that accelerates the hallmarks acquisition, and inflammation, which promotes multiple hallmark functions. Two other emerging tumor features have been added recently: reprogramming of energy metabolism and the microRNA genes that can initiate tumorigenesis, enhance its progression and evade the immune destruction. Finally, neoplastic cells exhibit another type of complexity: a repertoire of recruited and apparently normal cells that contribute to create a “tumor microenvironment” [2-5]. Only the whole understanding of this biological complexity will permit us to develop more efficient cancer treatments. In this chapter we intend review the most

important features related with the oncogenes, their activation mechanisms and roles in cancer, and the malignant metabolic reprogramming.

## Oncogenes

Are altered forms of normal cellular genes called proto-oncogenes. In human cancers, proto-oncogenes are frequently located adjacent to chromosomal breakpoints and are targets for mutation.

Products of proto-oncogenes are highly conserved in evolution and serve to regulate the cascade of events that maintains the ordered progression through the cell cycle, reproduction, and differentiation. In cancer cells, this ordered progression is partially lost when one or more of the components of this pathway are altered [6].

Our understanding of the molecular mechanisms leading to cancer has considerably advanced from the study of oncogenes. The application of techniques from many cancer research disciplines has led to the discovery of both dominantly acting transforming genes and of tumor suppressor genes [6].

Discovery of oncogenes. The majority of oncogenes were initially isolated as altered forms of proto-oncogenes acquired (transduced) by RNA tumor viruses (*v-onc*). In 1990, Payton Rous [7] discovered that transplantable sarcomas in chickens could be induced by a cell free agent. The transforming agent was a retrovirus that had transduced part of a normal cellular gene called *src* (sarcoma).

The virally transduced *src* gene (v-*src*) was altered by mutation compared with its normal cellular counterpart (c-*src*), rendering it constitutively activated. This discovery demonstrated that our cells harbor genes that, when abnormally activated, are capable of inducing tumorigenesis [8, 9].

Over 70 proto-oncogenes activated by proviral insertion of a non-transforming retrovirus have been identified. This number includes some genes first identified as viral oncogenes [10-12].

Detection of oncogenes in human tumors. Evidence for a genetic role in cancer comes from multiple sources. Many of the cancer prone syndromes, such as Fanconi syndrome, Bloom syndrome, and ataxia telangiectasia, show greatly increased chromosome instability [13]. Studies of colon cancer demonstrate that many cancers have accumulated multiple chromosome deletions and mutations [14].

Identification that the bacterial *mut*S and *mut*L DNA mismatch repair genes as genetic lesions that predispose individuals to colon cancer further supports the role of mutation in the generation of cancer [15, 16].

Tumoral transformation. Transforming events in cancer development includes three stages: initiation, promotion and progression [17].

**Table 1. Chromosome translocation in human cancers [1]**

| Affected gene | Rearrangements | Disease | Protein type |
|---|---|---|---|
| Oncogenes juxtaposed with IG loci | | | |
| c-MYC | t(8:14) (q24:q32)<br>t(2:8) (p12:q24)<br>t(8:22) (q24:q11) | Burkitt Lymphoma; BL-ALL | HLH domain |
| BCL1 (Cyclin D1) | t(11:14) (q13:q32) | B-cell chronic lymphocyte leukemia | PRADI-G1 Cyclin D1 |
| BCL-2 | t(14:18) (q32:q21) | Follicular Lymphoma | Inner mitochondrial membrane |
| BCL-3 | t(14:19) (q32:q13.1) | Chronic B cell leukemia | CDC10 motif |
| IL-3 | t(5:14) (q31:q32) | Acute pre-B cell leukemia | Growth factor |
| Oncogenes juxtaposed with TRC loci | | | |
| c-MYC | t(8:14) (q24;q11) | Acute T cell leukemia | HLH domain |
| LYLA | t(7:19) (q35;p13) | Acute T cell leukemia | HLH domain |
| TALI/SCL/TCL-5 | t(1:14) (q32;q11) | Acute T cell leukemia | HLH domain |
| TAL-2 | t(7:9) (q35;q34) | Acute T cell leukemia | HLH domain |
| Rhombotin 1/ttg-1 | t(11:14) (p15;q11) | Acute T cell leukemia | HLH domain |
| Rhombotin 2/ttg-2 | t(11:14)(p13;q11)<br>t(7:11) (q35;p13) | Acute T cell leukemia | HLH domain |
| HOX11 | t(10:14) (q24;q11) | Acute T cell leukemia | Homeodomain |
| TAN-1 | t(7:9)(q34;q34.3) | Acute T cell leukemia | Notch homologue |
| TCL-1 | t(14:14) (q11;q32.1),<br>t(7q35-14q32.1) or<br>inv14(q11;q32.1) | T- cell prolymphocytic leukemia | |
| Hematopoietic tumor | | | |
| Gene fusion | | | |
| c-ABL (9q34)<br>BCR (22q11) | t(9:22) (q34;q11) | Chronic and acute myelogenous leukemia | Tyrosine Kinase activated by BCR |
| PBX-1 (1q23) | t(1:19) (q23;p13.3) | Acute pre-B cell leukemia | Homeodomain |
| E2A (19p13.3) | | | HLH |
| PML(15q21) | t(15:27) (q21;q11-22) | Acute promyelocytic leukemia | Zn finger |
| RAR (17q21) | | | |
| CAN (6-23) | T(6:9) (p23;q34) | Acute myeloid leukemia | No homology |
| DEK (9q34) | | | |
| REL | Ins (2:12) (p11.2-14) | Non- Hodgkin lymphoma | NF-KB family |
| ALL1(MLL)* | 11q23 | ALL and AML | Chromatin modifiers |

| Affected gene | Rearrangements | Disease | Protein type |
|---|---|---|---|
| Solid tumors | | | |
| Gene fusions in sarcomas | | | |
| FL11, EWS | t(11:22) (q24; q12) | Ewing´s sarcoma | Ets transcription factor family |
| ERG, EWS | t(21:22) (q22;q12) | Ewing´s sarcoma | Ets transcription factor family |
| ATV1, EWS | t(7:21) (q22;q12) | Ewing´s sarcoma | Ets transcription factor family |
| ATF1, EWS | t(12:22) (q13;q12) | Soft-tissue clear cell sarcoma | Transcription factor |
| CHN, EWS | t(9:22) (q22.31;q12) | Myxoid chondrosarcoma | Steroid receptor family |
| WT1,EWS | t(11:22) (p13;q12) | Desmoplastic small round cell tumor | Wilm´s tumor gene |
| SSX1, SSX2, SYT | t(X:18) (p11.2;q11.2) | Synovial sarcoma | HLH domain |
| PAX3, FKHR | t(1:13) (q36;q14) | Alveolar | Homeobox homologue |
| PAX7, FKHR | t(1:13) (q36;q14) | Rhabdomyosarcoma | Homeobox homologue |
| CHOP, TLS | t(12:16) (q13;p11) | Myxoid liposarcoma | Transcription factor |
| var, HMG1-C | t(var:12) (var:q13-15) | Lipomas | HMG DNA binding protein |
| HMG2-C | t(12:14) (q13;q15) | Leiomyomas | HMG DNA binding protein |
| Gene fusions in thyroid carcinomas | | | |
| RET/ptc1 | Inv(10) (q11.2;q2.1) | Papillary thyroid carcinomas | Tyrosine kinase activated by H4 |
| Ret/ptc2 | t(1:17) (q11.2;q23) | Papillary thyroid carcinomas | Tyrosine kinase activated by RIa (Pka) |
| RET/ptc3 | Inv(10) (q11.2) | Papillary thyroid carcinomas | Tyrosine kinase activated by ELE1 |
| TRK | Inv(1) (q31;q22-23) | Papillary thyroid carcinomas | Tyrosine kinase activated by TPM3 |
| TRK-t1 (T2) | Inv(1) (q31;q25) | Papillary thyroid carcinomas | Tyrosine kinase activated by TPR |
| TRK-T3 | T(1q31;3) | Papillary thyroid carcinomas | Tyrosine kinase activated by TFG |
| Gene fusions in prostate cancer | | | |
| TMPR552 | ERG | T(21q22;21q22.3) | Ets transcription factor family activated by TMPR552 |
| | ETV1 | 7p21.2 | |
| Deregulation of oncogenes | | | |
| Parathyroid tumors | | | |
| PTH deregulates PRAD1 Cyclin D1 | Inv(11) (p15q; q13) | Parathyroid adenoma | PRAD1/Cyclin D1 |
| *ALL1 can fuse with more than 50 different genes. More frequently it fuses with AF4 in ALL and AF9 in AML. | | | |

Oncogenes encode proteins that control cell proliferation, apoptosis or both and chromatin modification [1, 18]. They can be activated by chromosomal alterations resulting from mutation or gene fusion, by juxtaposition to enhancer elements, or by amplification. Genomic changes as chromosomal rearrangements, point mutations, deletions and gene amplification, activation of proto-oncogenes and inactivation of tumor suppressor genes are required for cancer initiation [19]. Amplification usually occurs during tumor progression [1, 20, 21].

Tissue specific mutations. Oncogene activation in human tumors is specific for some tissues; for example, gene amplification of *N-myc* occurs in neuroblastoma and small cell lung cancer but is extremely rare in other tissues; bcr-abl translocation is observed in most patients with chronic leukemia; mutations in the *RAS* gene are found in a high percentage of pancreatic, colorectal and lung cancers [22]. The activation of oncogenes by chromosomal rearrangements, mutations and gene amplification confers to the cells, carrying these alterations, greater growth and survival [1, 23].

Cytogenetic rearrangements. Chromosome abnormalities commonly found in cancer cells are inversions and translocations. In hematopoietic cancers and solid tumors, translocations and inversions deregulating the oncogenic transcription are present. In prostate cancer, the mechanism of oncogenic activation is a fusion between a gene with a very active promoter as *TMPRSS2*, and other with oncogenic activity as *ERG1* [24]. In cancers of T and B cells, the most common alterations giving *MYC* gene deregulation are translocations; while gene fusion is more common in myeloid cancers and soft tissue sarcomas [1]. Table 1 summarizes the most important chromosome abnormalities giving human cancer.

Oncogene response. After the oncogenic activation by mutations, the structure of the encoded protein enhances its transforming activity [25]. An example is the RAS oncogenes family: *KRAS, HRAS* and *NRAS*. Mutations in *RAS* genes has been associated with exposure to environmental carcinogens; when mutation occurs in codon 12, 13 or 61, the *RAS* gene encodes a protein which remains in the active state, so that induces continuous cell growth. *KRAS* mutations are common in carcinomas of the lung, colon and pancreas, whereas *NRAS* mutations occur primarily in acute myelogenous leukemia and myelodysplastic syndrome [1, 26].

Activating mutations in the *BRAF* gene occur in 59% of melanomas, 18% of colorectal cancers, 14% of hepatocellular carcinomas and 11% of gliomas [27]. Most *BRAF* mutations occur in the protein kinase domain by changing the valine residue to glutamic acid at position 599 (V599E), producing a

protein with uncontrolled constitutive activity that stimulates the MAP kinase cascade, allowing proliferation, differentiation and cell survival [1, 27, 28]. Table 2 summarizes some oncogenes and tumor suppressors with their metabolic changes and related diseases.

**Table 2. Biochemical functions of oncogenes and tumor suppressor genes [29]**

| Gene | Function | Disease |
|---|---|---|
| Oncogenes | | |
| *PI3K* | Activates Akt (via PIP3); reduces β-oxidation (via Akt) enzyme carnitine palmitoyltransferase 1A (CPT1A) | Ovarian and gastrointestinal cancer |
| *Akt* | Upregulates fatty acid synthase (FASN); activates mTOR complex 1 | Breast and ovarian cancer |
| *Her2* | Increases, through activation of PI3K, Akt, and mTOR expression of FASN and acetyl- CoA carboxylase α (ACCα) at the translational level | Mammary carcinoma |
| Tyrosine kinases | Generate phosphotyrosines that can bind to pyruvate kinase isoform PKM2, converting it from a tetramer to a less active dimer | Multiple cancers |
| E7 from HPV16 | Binds PKM2, converting it from a tetramer to a less active dimer | Cervical carcinoma |
| Tumor Suppressor Genes | | |
| *p53* | Required for expression of SCO2 and hence optimal OXPHOS; enhances the expression of TIGAR; as glycolysis inhibitor, reduces the expression of the glycolytic enzyme phosphoglyceromutase | Multiple cancers |
| *VHL* | Ubiquitin ligase required for degradation of HIF-1α | Clear cell renal carcinoma |
| *TSC1* (hamartin) and *TSC2* (tuberin) | Negative regulators of Rheb (Which inhibit mTOR) | Tuberous sclerosis and lymphangioleiomyomatosis |
| *PTEN* | Negative regulator of class 1 PI3K | Cowden syndrome and prostate cancer |
| *LKB1* | Required for activation of AMPK | Peutz-Jeghers and sporadic lung adenocarcinoma |
| *NF1* | Negative regulator of RAS and PI3K-Akt pathway | Neurofibromatosis |
| *PML* | Negative regulator of mTOR complex 1 | Promyelocytic leukemia and lung cancer |
| Succinate dehydrogenase (SDH). Subunits B,C and D. | Accumulated succinate competitively inhibits HIF-1α prolylhydroxylases (PHDs) | Paraganglioma and pheochromocytoma. |
| Fumarate hydratase (fumarase) | Accumulated fumarate inhibits PHDs | Leiomyomatosis and papillary renal carcinoma |

## Oncogene Classification

The products of oncogenes can be classified into six broad groups: growth factors, growth factor receptors, transcription factors, chromatin remodelers, signal transducers, and apoptosis regulators (Table 3).

**Table 3. Classification of oncogenes [1]**

| Oncogene | Chromosome | Neoplasm | Mechanism of Activation |
|---|---|---|---|
| Transcription factors | | | |
| *v-myc* | 8q24.1 (MYC) | Carcinoma myelocytomatosis | Deregulated activity |
| *N-MYC* | 2p24 | Neuroblastoma: lung carcinoma | Deregulated activity |
| *L-MYC* | 1p32 | Carcinoma of lung | Deregulated activity |
| *v-myb* | 6q22-24 | Myeloblastosis | Deregulated activity |
| *v-fos* | 14q21-22 | Osteosarcoma | Deregulated activity |
| *v-jun* | 1p32-p31 | Sarcoma | Deregulated activity |
| *v-ski* | 1q22-24 | Carcinoma | Deregulated activity |
| *v-rel* | 2p12-14 | Lymphatic leukemia | Mutant NFkappa B |
| *v-est-1* | 11p23-24 | Erythroblastosis | Deregulated activity |
| *v-erbA1* | 17p11-21 | Erythroblastosis | T3 transcription factors |
| *v-erbA2* | 3p22-24.1 | Erythroblastosis | T3 transcription factors |
| Related to apoptosis and others | | | |
| *BCL2* | 18q21.3 | B cell lymphomas | Antiapoptotic protein |
| *MDM2* | 12q14 | Sarcomas | Complexes with p53 |
| Chromatin remodelers | | | |
| *ALL1 (MLL)* | 11q23 | Chromosome translocation | Chromatin modifier |
| Growth factors | | | |
| *v-sis* | 22q12.3-13.1 | Glioma/fibrosarcoma | B chain PDGF |
| *Int2* | 11q13 | Mammary carcinoma | Member of FGF family |
| *KS3* | 11q13.3 | Kaposi´s sarcoma | Member of FGF family |
| *HST* | 11q13.3 | Stomach carcinoma | Member of FGF family |
| Growth factor receptors<br>Tyrosine kinases: integral membrane protein | | | |
| *EGFR* | 7p1.1-1.3 | Squamous cell carcinoma | EFG receptor |
| *v-fms* | 5q33-34 | Sarcoma | CSF1 receptor |
| *v-KIT* | 4q11-21 | Sarcoma/GIST | Stem cell factor receptor |
| *v-ros* | 6q22 | Sarcoma | ? |
| *MET* | 7p31 | MNNG-treated human osteosarcoma cell line | HGF/SF receptor |
| *TRK* | 1q21-q22 | Colon/thyroid carcinomas | NGF receptor |
| *NEU* | 17q11.2-12 | Neuroblastoma/breast | ? |
| *RET* | 10q11.2 | Carcinomas of thyroid Men 2A Men 2B | GFNG/NTT/ART/PSP receptor activation/ fusion proteins |
| Receptors lacking protein kinase activity | | | |
| *Mas* | 6q24-27 | Epidermoid carcinoma | Angiotensin receptor |

| Oncogene | Chromosome | Neoplasm | Mechanism of Activation |
|---|---|---|---|
| Signal transducers<br>Cytoplasmic tyrosine kinases | | | |
| *SRC* | 20q12-q13 | Colon carcinoma | Protein tyrosine Kinase |
| *v-yes* | 18q21.3 | Sarcoma | Protein tyrosine Kinase |
| *v-fgr* | 1p36.1-36.2 | Sarcoma | Protein tyrosine Kinase |
| *v-fes* | 15q25-26 | Sarcoma | Protein tyrosine Kinase |
| *ABL* | 9q34.1 | CML | Protein tyrosine Kinase |
| Membrane-associated G proteins | | | |
| *H-RAS* | 11p15.5 | Colon, lung, pancreas carcinomas | GTPase |
| *K-RAS* | 12p11.1-12.1 | AML, thyroid carcinoma | GTPase |
| *N-RAS* | 1p11-13 | Carcinoma, melanoma | GTPase |
| *BRAF* | 7q34 | Melanoma, thyroid, colon ovary | |
| *Gsp* | 20q13.3 | Adenomas of thyroid | Gs alpha |
| *Gip* | 17q21.3-q22 | Ovary, adrenal carcinoma | Gi alpha |
| GTPase exchange factor (GEF) | | | |
| *Dbl* | Xq27 | Diffuse B cell lymphoma | GEF for Rho and Cdc42Hs |
| *Vav* | 19p13.2 | Hematopoietic cells | GEF for Ras ? |
| Serine/threonine kinases: cytoplasmic | | | |
| *v-mos* | 8q11 | Sarcoma | Protein Kinase (ser/thr) |
| *v-raf* | 3p25 | Sarcoma | Protein Kinase (ser/thr) |
| *Pim-1* | 6p21 | T-cell lymphoma | Protein Kinase (ser/thr) |
| Cytoplasmic regulators | | | |
| *v-crk* | 17p13.3 | | SH-2/SH-3 adaptor |

ALL: Acute lymphoblastic leukemia; AML: acute myeloid leukemia; CML: chronic myelogenous leukemia; GTPase: guanosine triphosphatase; PDGF: Platelet-derived growth factor.

## Growth Factors and Growth Factor Receptors

Activation of a single growth factor gene can result in malignant transformation. Platelet-derived growth factor (PDGF) is released from platelets during coagulation, induce proliferation of various cell types and stimulate fibroblasts to participate in wound healing [1, 30]. Over-expression of PDGF induces the *in vitro* transformation of fibroblasts containing PDGF receptors, but it does not influence fibroblasts lacking these receptors [1]. This autocrine loop entails over-expression of *PDGF-β,* an antibody against its receptor, or small molecules that block the receptor and inhibit growth of the transformed fibroblasts.

Another example is the WNT glycoprotein family that inhibits the phosphorylation of *β*-catenin, which participate in cell-cell adhesion and

activation of several signal transduction pathways [31-33]. The APC protein actively participates controlling the activity of $\beta$-catenin. In familial adenomatous polyposis, inactivating mutations of *APC* block the degradation of $\beta$-catenin by inhibiting its phosphorylation. As a result, free $\beta$-catenin in the cytoplasm translocates into the nucleus, where activates genes involved in cell proliferation and invasion [33] (Figure 1). Growth factor receptors are altered in several cancer types (figure 2) [1, 34]. In many tumors, a deletion of the ligand-binding domain of epidermal growth factor receptor (EGFR), a transmembrane protein with tyrosine kinase activity, causes constitutive activation of the receptor in absence of ligand binding. The activated receptor phosphorylates tyrosines in the intracellular domain of the receptor, providing interactions sites for cytoplasmic proteins containing the SRC homology domain and other binding domains. These interactions deregulate signaling in several pathways. Activating mutations occur in three other members of the EGFR family (*ERBB2*, *ERBB3* and *ERBB4*) and within the kinase domains of the HER2/neu and KIT signaling receptors [35].

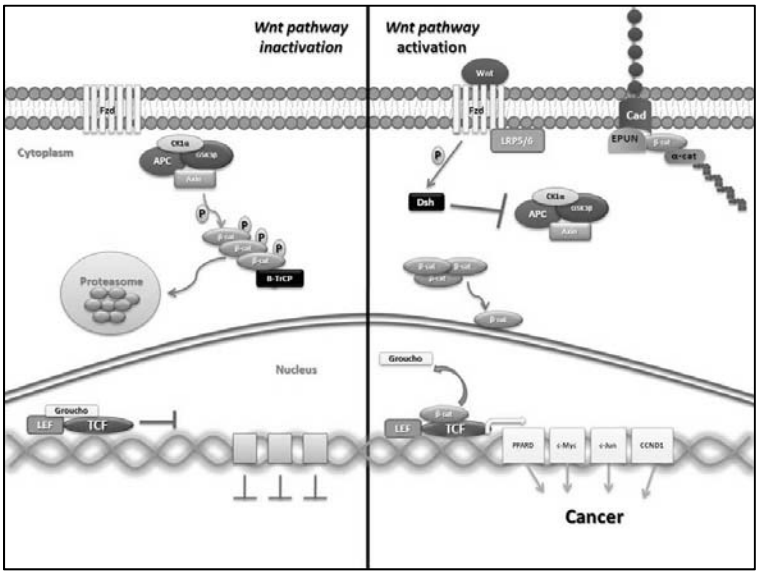


Figure 1. Dual functions of $\beta$ catenina in cell adhesion and transcription activation. $\beta$ catenin is in a destructive cytoplasmic complex composed of activated protein C (APC), axin, glycogen synthase kinase 3 beta (GSK-3β), and casein kinase (CK1).

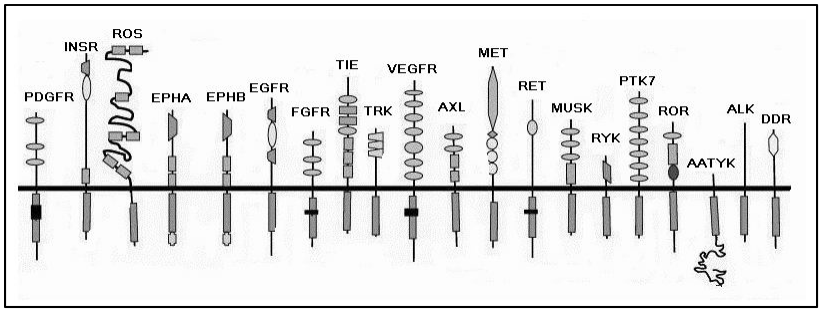


Figure 2. Examples of receptor tyrosine kinases. The epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) receptors have been found to be involved in a variety of human cancers. Modified to [36].

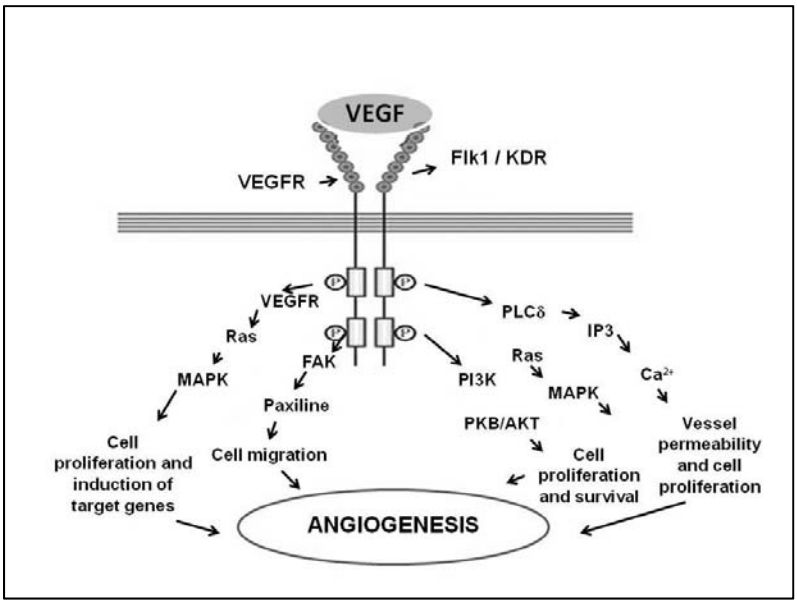


Figure 3. Role of VEGF-VEGFR interaction in angiogenesis. Several pathways are activated by the interaction of vascular endothelial growth factor (VEGF) and VEGF receptor (VEGFR) FAK denotes fatal adhesion kinase, Flk fetal liver kinase, IP3 inositol triphosphate, KDR kinase insert domain containing receptor, MAPK mitogen-activated protein kinase, PI3K phosphoinositol 3 kinase, PKB protein kinase B, and PLC phospholipase C. (Modified to [1].

Such mutations occur in lung and breast cancer and gastrointestinal stromal tumors. Two classes of clinically active anti-EGFR agents have been

developed: a monoclonal antibody against the extracellular domain of the receptor (e.g., cetuximab) and competitive inhibitors of the tyrosine kinase activity of the receptor (e.g., erlotinib and gefitinib) [35].

Vascular endothelial growth factor (VEGF) regulates hypoxia-dependent control of gene transcription (Figure 3). The activity of VEGF is mediated by three receptor tyrosine kinases: VEGFR1 (FLT1), VEGFR2 (FLK1-KDR) and VEGFR3 (FLT4). VEGF stimulates angiogenesis in a variety of cancers, and inhibitors of VEGF and VEGFRs have been developed. Bevacizumab is a monoclonal anti-VEGF antibody, and SU5412, a small molecule, binds the receptor tyrosine kinases of the VEGFR1 and VEGFR2 as well as the kinases of the PDGF receptor and KIT. In addition to inhibiting the ABL kinase, imatinib also inhibits the PDGF and KIT receptor kinases. Gastrointestinal stromal tumors that carry activating mutations of KIT respond to imatinib or other inhibitors of these receptor kinases [37, 38].

## Transcription Factors

Usually, transcription factors are members of multigene families sharing common structural domains. Many transcription factors require interaction with other proteins; for example, the Fos transcription protein dimerizes the Jun transcription factor to form the AP1 transcription factor, which increases the expression of several genes that control the cell division [39].

Chromosomal translocations often activate transcription factor genes in lymphoid cancers and sometimes in solid tumors (e. g., prostate cancer, see table 2). In certain sarcomas, chromosomal translocations resulting in fused proteins occur consistently; in Ewing´s sarcoma, for example, the *EWS* gene is fused with one of a number of partner genes, resulting in aberrant transcriptional activity of the fused proteins.

The EWS protein is an RNA binding molecule with a domain that, when fused to a heterologous DNA binding domain, can greatly stimulate gene transcription. Prostate carcinomas carry translocations of the *TMPR552* gene that fuse with and activate *ERG1* or *ETV1*. These genes are members of the Ets (E-26*)* family of transcription regulators, which can activate or repress genes involved in cellular proliferation, differentiation, and apoptosis.

The Ets family of transcription factors characterized by an evolutionarily-conserved DNA-binding domain regulates expression of a variety of viral and cellular genes by binding to a purine-rich GGAA/T core sequence in cooperation with other transcriptional factors and co-factors.

Most Ets family proteins are nuclear targets for activation of Ras-MAP kinase signaling pathway and some of them affect proliferation of cells by regulating the immediate early response genes and other growth-related genes. Some of them also regulate apoptosis-related genes.

Several Ets family proteins are preferentially expressed in specific cell lineages and are involved in their development and differentiation by increasing the enhancer or promoter activities of the genes encoding growth factor receptors and integrin families specific for the cell lineages. The fusion of TMPR552, which has androgen-responsive promoter elements, with an ETS related gene creates a fusion protein that increases proliferation and inhibits apoptosis of cells in the prostate gland, thereby facilitating their transformation into cancer cells [1, 40, 41].

## Chromatin Remodelers

Chromatin compaction plays a critical role in the control of gene expression, replication and repair, and chromosome segregation. Two kinds of enzymes participate in the remodeling of chromatin: 1. ATP dependent enzymes that move the positions of nucleosomes, the repeating subunits of histones in chromatin around which DNA winds, and 2. Enzymes that modify the N-terminal tails of histones [42, 43].

The pattern of histone modifications constitutes an epigenetic code that determines the interaction between nucleosomes and chromatin associated proteins. These interactions, in turn, determine the structure of chromatin and its transcriptional capacity [44].

In acute lymphocytic leukemia and acute myelogenous leukemia, the *ALL*1 (also named *MLL*) gene can fuse with 1 of more than 50 genes. ALL1 is part of a very large, stable multiprotein complex. Most of the proteins in the complex are components of transcription complexes; others are involved in histone methylation and RNA processing [45].

The entire complex remodels, acetylates, and methylates nucleosomes and free histones. The fusion of ALL1 with 1 of more than 50 proteins results in the formation of the chimeric proteins that underlie acute lymphoblastic leukemia and acute myelogenous leukemia. ALL1 fusion proteins deregulate homeobox genes (which encode transcriptions factors) the *EPHA7* gene (which encodes a receptor tyrosine kinase), and microRNA genes such as *miR191* [45].

## Signal Transducers

Binding of receptor tyrosine kinases to the appropriate ligand causes reorganization of the receptors and autophosphorylation of tyrosines in the intracellular portion of the molecules [46]. Autophosphorylation enhances the kinase activity of the receptor or promotes the interaction of the receptor with domains of cytoplasmic proteins that are effectors and regulators of intracellular signaling [47]. In humans, there are approximately 120 SRC homology 2 domains in 100 different proteins that mediate responses to signals initiated by phosphorylated tyrosines. Some of these proteins share domains with enzymatic activity, whereas others link activated receptors to downstream targets. Many oncogenes encode members of signal transduction pathways. They fall into two main groups: nonreceptor protein kinases and guanosine-triphosphate-binding proteins. The nonreceptor proteins kinases are of two types: tyrosine kinases (e.g., ABL, LCK, and SRC) and serine and threonine kinases (e.g., AKT, RAF1, MOS and PIM1). Proteins involved in signal transduction become oncogenic if they bear activating mutations. An important example is PI3K and some of its downstream targets, such as AKT and SGK, which are critical in tyrosine kinase signaling and can be mutated in cancer cells [48, 49].

## Apoptosis Regulators

The *BCL2* gene, which is involved in the initiation of roughly all follicular lymphomas and some diffuse large B-cell lymphomas [50, 51], encode a cytoplasmic protein that localizes in mitochondria and increases cell survival by inhibiting apoptosis. The *BCL2* family members inhibit apoptosis and are up-regulated in several other cancer types as chronic lymphocytic leukemia and lung cancer [52]. Two main pathways lead to apoptosis: the stress pathway and the death-receptor pathway (Figure 4). The stress pathway is triggered by proteins that contain the BCL2 homology 3-domain, which inactivates BCL2 and BCL-XL and inhibits apoptosis. Drugs that mimic the BCL2 homology 3-domain and can bind to BCL-XL or BCL2 (peptides or small organic molecules that bind in a groove of these proteins) are under development. The death-receptor pathway is activated by binding of Fas ligand, TRAIL, and tumor necrosis factor $\alpha$, to their corresponding (death) receptors on the cell surface. Activation of death receptors activates caspases that cause cell death [53, 54] (Figure 4).

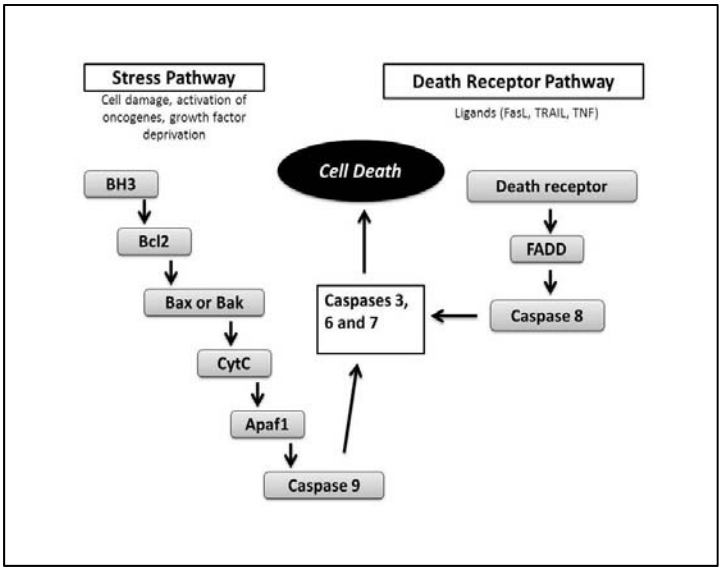


Figure 4. The two main pathways to programmed cell death, or apoptosis. The effectors of cell death are the downstream caspases, proteolytic enzymes activated by caspases 8 and 9, which are capable of clearing many of the cellular proteins causing cell death. FADD denotes Fas-associated death domain. Modified to [1].

Gene amplification usually occurs before the tumor progression; an example is the *DHFR* amplification in methotrexate-resistant acute lymphoblastic leukemia [55]. Amplification of *DHFR* is accompanied by cytogenetic abnormalities that reflect the oncogene amplification [56, 57]. The amplified DNA segment contains many genes and usually involves hundreds of kilobases. Amplification phenomenon is frequently involved in oncogenes families as *MYC, CCND1, EGFR* and *RAS.* In several cancer types as small-cell lung, breast, esophageal, cervical, ovarian and head and neck, *NMYC* amplification correlates with advanced tumor stage [58]. *CCND1* amplification occurs in breast, esophageal, hepatocellular, and head and neck cancer. *EGFR* is amplified in glioblastoma and head and neck cancer. *ERBB2* amplification in breast cancer correlates with a poor prognosis [1, 59].

## Chronic Inflammation as Activation Mechanism

Inflammation is an important mechanism that can remove the agent responsible for the injury and initiate tissue repair and regeneration by a

coordinated release of immune response [19]. The mechanism inflammatory involves innate and adaptive immune response. After elimination of the pathogen and wound healing, inflammation decreases [60].

However, an unsolved inflammatory process can disrupt cellular microenvironment, which leads to alterations in genes related to cancer and posttranslational modification of key proteins in the cell cycle, DNA repair and apoptosis [60].

In early stages of development and progression of tumor, mononuclear cells, macrophages, mast cells and neutrophils are present through up-regulation of pro-inflammatory cytokines such as interferon-γ, tumor necrosis factor (TNF), interleukin (IL)-1α / β or IL-6. Also the activated nuclear factor-kB (NF-kB) is a transcription factor that relates inflammation and tumorigenesis, allowing pre-neoplastic and malignant cells evade apoptosis. Therefore, all these factors may act as initiators and promoters of carcinogenesis [60].

The role of chronic inflammation in carcinogenesis has been evaluated in several epidemiological studies, where pro-inflammatory and anti-inflammatory cytokines, viral infections and genetic markers involved in the inflammatory response were analyzed [60, 61]. It is estimated that the underlying infections and inflammatory reactions are related to 15-25% of all cancer cases [60-62].

Association between inflammatory processes and cancer are well known in the case of intestinal disease and colorectal cancer, virus hepatitis B (or C) and alcoholic liver cirrhosis or hepatocellular carcinoma, chronic esophageal reflux resulting in Barrett's esophagus and esophageal carcinoma, infection by human papilloma virus and cervical cancer, prostatitis and prostate cancer and infection by helicobacter pylori with gastric cancer [60, 63, 64]. In other cases, cancer is related with chronic irritation caused by long exposure to cigarette, silica and asbestos [63, 64].

The mechanism by which inflammation predispose cancer depends on whether it is secondary to chronic irritation or infection. In the case of HPV malignant transformation is mediated by E6 and E7 oncoproteins [65]. In other cases, the oncogenic activation occurs by the classical mode; for example, H. pylori contain protein factors that affect host cell signaling [66].

The chronic inflammatory response can lead to genomic injury and initiation of malignant transformation. A defense mechanism is the production of free radicals such as reactive oxygen intermediated (ROI), hydroxyl radical (•OH) and superoxide (O2-•) and reactive nitrogen intermediates (RNI), nitric oxide (NO•) and peroxynitrite (ONOO-), which are formed by the enzymatic

reaction in the host (myeloperoxidase, NADPH oxidase and nitric oxide) governed by different signaling pathways [67].

Intrinsic cellular mechanisms to prevent the unregulated proliferation or mutations in DNA are diverse, among them tumor suppressors involved in DNA repair, cell cycle arrest, apoptosis and senescence [19]. During the face of massive cell death, by infectious or noninfectious injury, cell repopulation occurs from the undifferentiated precursor cells, for which two sequential steps are required: first, some cells must survive, and second, must be a clonal expansion of this undifferentiated precursor cells in order to maintain the tissue functioning. The development of new cells is regulated by inflammatory pathways [68, 69] as part of the repair process and in defense of the infection. In initiated cells, the inflammatory response, providing cell survival and proliferation, possibly is leading to tumor promotion [19].

Evident association between tumorigenesis and host defense and / or tissue repair have been reported. Most of the studies described are based in tissue injuries and wounds that support tumor growth and neoplastic progression. For example, injection of the Rous sarcoma virus causes sarcoma in the injection site [70], probably mediated by transforming growth factor-β (TGF-β) and fibroblast growth factors (FGFs) [71]. Some metabolic signaling pathways are involved; among them the Wnt-β-catenin pathway [72] and molecules COX-1 and 2 [73].

Other studies have focused on the role NF-kB (transcription factors) in tumorigenesis. Inactivation of the classical NF-kB in colonic epithelial cells due to deletion of the IKB protein kinase β (IKKβ) decreases the frequency of visible tumors [19, 74]. So in the colonic epithelium injured and that was added as a mutagen azoxymethane (AOM), the NF-B provides a survival signal for cells initiated [75]. Otherwise, the IKKβ acts protection injury infectious or non-infectious and host defense in intervening in survival of colon epithelial cells [76, 77].

Many inflammatory mediators such as cytokines, chemokines and eicosanoids stimulate the proliferation of normal and transformed cells [63]. After the administration of the phorbol ester TPA (12-O-tetradecanoylphorbol-13-acetato) and the mutagen DMBA (7, 12-dimethylbenza-anthracene) in TNF-deficient mice, fewer skin tumors were observed. Based on these experiments, the authors suggest that inflammatory mediators act as tumor promoters [78].

In the presence of tumor initiation and tissue injury and apoptosis, activation of inflammation depend tissue repair/compensatory proliferation leading to tumor promotion [19].

## Reprogramming of Metabolic Pathways in Cancer

Cancer cells require greater amounts of nutrients from the bioenergy reserves, for which different metabolic pathways are activated or modified. The signals that stimulate cell proliferation are also involved in the reorganization of the metabolic activities that allow the resting cells, initiate proliferation. The new conditions of the metabolism of cells differ from normal cells metabolism mainly by the high rate of glycolysis, lactate production, biosynthesis of lipids and other macromolecules [79].

The first observations on metabolic alterations in tumors were reported in 1920, highlighting that tumor cells of ascites in conditions of rapid proliferation consume large amount of glucose, and excrete most of the carbon as lactate, rather than oxidize it completely as do the normal cells. This phenomenon is called "Warburg effect" [80]. Warburg proposed that tumor cells use the increase of the glycolytic flow as protection before permanent damage of oxidative metabolism [79, 81]. The Warburg effect is not observed in the process of cell proliferation from all tumors [29]. This increase in the consumption of glucose, which increases the production of ATP and anabolic reactions, offers some advantages for tumor growth. *First,* for obtaining ATP, tumor cells use glucose as the most abundant extracellular nutrient in anaerobic glycolysis, and although the production of ATP per glucose is low, the high glycolytic flow generated exceeds the production of ATP from oxidative phosphorylation (OXPHOS) [29, 81]. *Second,* degradation of glucose provides the necessary intermediate compounds for different anabolic reactions, thus: ribose for synthesis of nucleotides; glucose-6-phosphate to form glycogen and ribose-5-phosphate; dihydroxyacetone phosphate for synthesis of triglycerides and phospholipids; pyruvate for synthesis of alanine and malate; and, through oxidative pentose phosphate pathway (PPP) produce nicotinamide adenine dinucleotide phosphate (NADPH) [29, 79]. *Third*, the cancer cells produce lactic and bicarbonic acid; lactate is the main end product of anaerobic glycolysis. The acidic conditions create a favorable environment for the tumor, inhibiting autoimmune effects of anti-cancer. In this atmosphere the anaerobic components (cancer cells) and aerobic (non-transformed stromal cells) are involved in metabolic pathways, complementary, recycling products of anaerobic metabolism to maintain the survival and growth of cancer cells. *Fourth,* tumors can metabolize glucose by the pentose phosphate pathway to

generate NADPH and thus ensure antioxidant defenses against a hostile environment with chemotherapeutic agents [29].

In this way, the entire metabolism is reorganized to increase anabolic processes that enable growth and cell proliferation [29]. We will summarize some of the mechanisms modified in cellular signaling and key aspects of the metabolism of both normal physiological and tumorigenic proliferation. Likewise, the PI3K/Akt/mTOR pathway and the *MYC* and *HIF-1a* transcription factors, which appear to regulate complementary aspects of cell metabolism, will be analyzed [79].

## Metabolic Activity in Cell Proliferation

Normal mammalian cells do not proliferate in an autonomous way, entering the cell cycle by indication of growth factors and signaling pathways that influence gene expression and cellular physiology. The proliferating cells depend on growth factors to generate this metabolic flux and enhance the uptake of nutrients from the extracellular space [79]. In the absence of growth factors, mammalian cells quickly lose expression of transporters of nutrients and cannot keep cell autonomy for the synthesis of basal bioenergetics and macromolecular replacement. In this case, the cells carry out autophagy, which provides a limited supply of substrates generated from the macromolecular to maintain the production of ATP for cell survival [82].

The transduction of signals for cell proliferation has direct effects on the metabolic flux [79]. The mechanisms that integrate the signal transduction and cell metabolism are highly conserved between normal cells and tumor cells. The biggest difference is that in normal cells, the initiation of signaling requires extracellular stimulation, while cancer cells present mutations chronically favoring these routes, maintaining metabolic biosynthesis phenotype, regardless of the physiological limitations of the normal cell. In other words, the cancer cells achieve an increase in metabolic autonomy. For example, in cell proliferation the enzyme lactate dehydrogenase (LDH-A) is induced by oncogenes like *HER2/neu* and *MYC* promoting cell proliferation [79]. Other enzymes highly expressed in tumors are the embryonic isoforms of pyruvate kinase (PKM2), fatty acids synthase (FASN), choline kinase (ChoK) [29, 83], and the lipogenic enzymes ATP citrate lyase and fatty acid synthase [79, 81, 84].

Rarely, tricarboxylic acid (TCA) cycle enzymes as succinate dehydrogenase (SDH) and fumarate hydratase (FH) behave as tumor

suppressor; thus, mutations in the SDHB, SDHC and SDHD subunits can cause familial paraganglioma or pheochromocytoma [85], mutations in FH produce a dominant syndrome of uterine fibrosis, leiomyomata and renal carcinoma of papillary cells [79, 86]. Proliferating cells use intermediate compounds derived from the TCA cycle to synthesize lipids, proteins and nucleic acids, so use this cycle as a center of biosynthesis [79].

## PI3K/Akt/mTOR Signaling Pathway

The activation of the PI3K/Akt/mTOR pathway both in growth factors dependent cells as in tumour cells, improves many of the metabolic activities that keep the cell biosynthesis by several processes. *First*, the cells increase expression of transporters and the consumption of glucose, amino acids and other nutrients [87, 88].

Second, Akt (v-akt murine thymoma viral oncogene) increases glycolysis and the production of lactate, which is sufficient to induce the Warburg effect in normal and cancer cells [89]. Third, PI3K and Akt stimulate the synthesis of lipids in many cell types, mTOR (mechanistic target of rapamycin) is key in regulating the protein translation [81, 90, 91].

In normal cells, the activation of the PI3K system is controlled by the phosphatase PTEN (phosphatase and tensin homolog). In malignant cells, the pathway can be triggered by mutations that activate PI3K or increase the stimuli of the system (BCR-ABL, HER2/neu amplification, etc.) or eliminate negative regulators of the same PTEN [79] (Table 4).

**Table 4. Key mutations involved in PI3K pathway [79]**

| Gene | Mutation | Cancer | Frequency |
|---|---|---|---|
| *PIK3CA* | Gene activation | Breast | 25% |
| | | Colon | >30% |
| | Gene amplification | Head and neck | >35% |
| *Akt2* | Gene amplification | Ovary | 12% |
| *PTEN* | Loss of heterozygosity | Glioma | ≤40% |
| *BCR-ABL* | Fusion by chromosomal translocation | Chronic myelogenous leukemia | >90% |
| | | Acute lymphocytic leukemia | 20% |
| *HER2/neu* | Gene amplification | Breast | 25% |
| *EGFR* | Gene amplification | Lung (non-small cell) | >50 |

## Regulation of Glycolysis by HIF-1

One of the most important mechanisms involved in the aerobic glycolysis is the activation of hypoxia inducible factor (*HIF*); it is a transcription factor induced by hypoxic and oxidative stress, as well as oncogenic, inflammatory and metabolic factors [81, 92]. HIF-1 is a heterodimer composed of a stable β subunit and a unstable α subunit, both synthesized in normoxia by sequential action of the enzymes prolyl hydroxylase dependent of oxygen (PHDs) and ubiquitin ligase VHL [29, 81]. The active form of HIF-1 (HIF-1a subunit) is expressed under the control of growth factor signaling pathways, mainly PI3K/Akt/mTOR [93]. In hypoxia, prolyl hydroxylation is inhibited by reactive oxygen species (ROS) from mitochondria, resulting in stable transcriptional activity of HIF-1 complex [79]. In tumor cells, stimulation by growth factors is required to express HIF-1a, necessary for regulating the intracellular fate of carbon derived from glucose [79] (Figure 5).

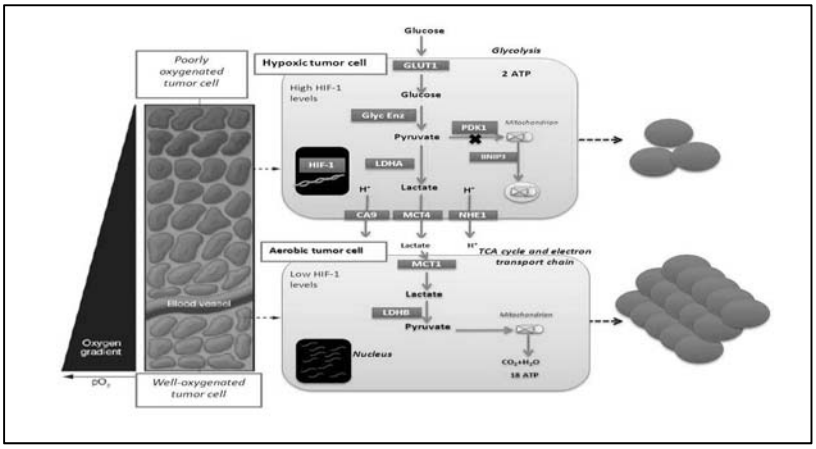


Figure 5. Tumor cells near to blood vessels are well oxygenated, whereas more distant tumor cells are poorly oxygenated and express high levels of HIF-1, which induces the expression of proteins that increase: uptake of glucose (glucose transporter 1 (GLUT1); conversion of glucose to pyruvate (glycolytic enzymes [Glyc. Enz.]); generation of lactate and H+ (LDHA); and efflux of these molecules out of the cell (carbonic anhydrase IX (CA9), sodium-hydrogen exchanger 1 (NHE1), encoding the transporter 4 monocarboxylate (MCT4). In these hypoxic cells, two moles of lactate are produced for each mole of glucose, associated with a reduced substrate delivery to the mitochondria through the action of pyruvate dehydrogenase kinase 1 (PDK1). Hypoxic cells generate 2 mol of ATP and 2 mol of lactate for each mol of glucose consumed, whereas aerobic cells generate 36 mol of ATP per 2 mol of lactate consumed. Modified to [94].

HIF-1 stimulates the conversion of glucose to pyruvate and lactate by means of glucose transporter type 1 (GLUT1), hexokinase (HK2 and HK1), LDHA and monocarboxilate transporter type 4 (MCT4). In addition, HIF-1 reduces the conversion of pyruvate to acetyl-CoA by action of the pyruvate dehydrogenase (PDH) [79, 94]. HIF-1 cooperates with the proto-oncogene *MYC* to aerobic glycolysis promotion by induction of *HK2* and pyruvate dehydrogenase kinase 1 (*PDK1*) [29, 79].

Tumor cells show cyclical changes in phases of oxygenation, which involves a dynamic regulation of metabolic symbiosis, where cells can change from a state of lactate production to one of consumption of the same. Intratumoral hypoxia is associated with increased risk of metastasis and mortality [94] (Figure 5).

## Metabolic Changes and Cancer Cells

Cancer cells differ from normal cells by a number of changes in their physiological processes, including autonomous proliferation, angiogenesis, apoptosis evasion, limitless replicative potential, tissue invasion or metastasis and immune response (Figure 6) [5, 29].

- Autonomous proliferation: Growth factors regulate two transduction signals in the Ras/Raf/MAP kinase pathway: ERK and PI3K. Both activate mTOR to stimulate cell growth. Most cancers have mutations in the main regulators of this pathway: K-Ras, H-Ras, N-Ras, B-Raf, subunit p110a of PI3K, receptor tyrosine kinase (RTKs) or its effectors downstream (Akt and PDK1), or inactivating mutations of negative regulators of these proteins [95]. Over activation of the PI3K/Akt system in autonomous cells provides increase in the flow of glucose and amino acids that may be attributable to the activation of HIF-1a. Akt stimulates the expression of GLUT1 and translocation to the plasma membrane of GLUT4. In addition, Akt stimulates glycolysis by activation of 6-fosfofructo-2-kinase (PFK2) and synthesis of fatty acids by phosphorylation of ATP citrate lyase [29].
- Apoptosis evasion: Alterations in the OXPHOS can induce resistance to apoptosis. Likewise, inhibition of the respiratory chain can inhibit the activation of proteins pro-apoptotic Bcl-2, Bax and Bak [96]. In studies published by Bonnet et al. [97], the pharmacological inhibition of PDK1, an enzyme that catalyzes the phosphorylation and

inactivation of PDH, produces the reactivation of PDH, induces apoptosis in cancer cells, which is an interesting example of reversal of resistance to apoptosis and metabolic reprogramming [29].

- Continuous replication: To immortalize the replicative power, tumor cells often mutate or inactivate inducers of senescence as the tumor suppressor p53 [98]. In glycolysis, enzyme phosphoglycerate mutase (PGM), which is negatively regulated by p53, catalyzes the conversion of 3-phosphoglycerate (3PG) to 2-phosphoglycerate (2PG) [99].
- Angiogenesis: In response to hypoxia and HIF-1, many tumors over-express the vascular endothelial growth factor (VEGF) by activation of the ERK and PI3K signaling pathways [29, 100]. On the other hand, mitochondrial protein F1F0-ATPase, whose function is to provide the necessary protons for aerobic glycolysis, is inhibited by angiostatin, which prevents the endogenous angiogenesis by intracellular acidification [29]. Chi et al. [101], reported an antibody similar to angiostatin with angiogenesis inhibitor effect.
- Tissue invasion and metastasis: E-cadherin is involved in the intercellular union [29]. Activation of HIF-1a causes loss of E-cadherin and expression of proto-oncogenes *met* and *TWIST* that induce metastasis through the chemokines receptor (CXCR4) and lysyl oxidase (LOX) [102, 103]. Thus the activation of HIF-1 can lead to metabolic reprogramming and tissue invasion and metastasis [29].
- Immune response: The antitumor effects of cytotoxic T lymphocytes (CTLs) can be inhibited by the metabolic micro-environment of the tumor cells [29]. The macrophages associated with tumors are markers of poor prognosis: facilitate angiogenesis as well as promotion and migration of tumor cells [104]. In advanced stage cancer, the production of lactate inhibits the production of cytokines, the CTLs cytolytic activity and the cell proliferation. Alternative therapies modifying the tumor metabolism have been proposed inhibiting the protons exportation and the production of lactate or indolamine 2, 3-dioxygenase, which could restore the defective antitumor immune response [29].

The metabolic inhibitors used as therapeutic targets in cancer, are in early stages; only the antagonists of mTOR and large AMPK activators have shown to reduce the incidence of cancer [105, 106] (Table 5).

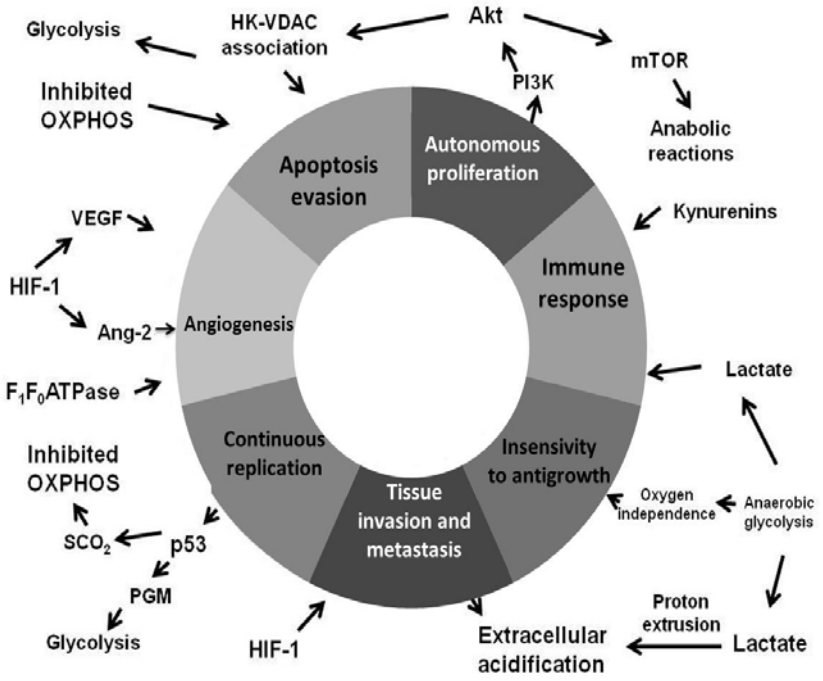


Figure 6. Cancer cell changes and their links to tumor metabolism. Hypothetical links between different metabolic alterations and the non-metabolic characteristics of neoplasia [circle] are depicted. Centripetal arrows indicate how the changes in cancer cells can impinge on metabolism. Centrifugal arrows illustrate how neoplasia-associated metabolic reprogramming can contribute to the acquisition of cancer changes. Ang-2: angiopoietin-2; GLUT: glucose transporter; HIF: hypoxia-inducible factor; HK: hexokinase; OXPHOS: oxidative phosphorylation; PGM: phosphoglycerate mutase; PI3K: phosphatidylinositol 3-kinase; SCO2: synthesis of cytochrome c oxidase 2; VDAC: voltage-dependent anion channel; VEGF: vascular endothelial growth factor. Modified to [29].

**Table 5. Metabolic pathways in cancer as therapeutic target [29]**

| Target | Desired Effects | Examples of Compounds |
|---|---|---|
| Glycolysis | | |
| Glucose uptake | Glucose transport or initial glycolysis inhibition. | 2-Deoxyglucose has radiosensitizing and chemosensitizing effects |
| Hexokinase [HK1 and HK2] | Inhibition of enzymatic activity and dissociation from mitochondria | 3-Bromopyruvate has potent antitumor effects in vitro and in vivo |

| Target | Desired Effects | Examples of Compounds |
|---|---|---|
| Pyruvate dehydrogenase kinase 1 [PDK1] | Inhibition of PDK1 for deinhibition of pyruvate dehydrogenase | Dichioroacetate [DCA] |
| Lactate dehydrogenase A [LDHA] | Inhibition | SiRNA |
| Pyruvate kinase [PK] isoenzyme PKM2 | Translocation of PKM2 to the nucleus for induction of apoptosis | Somatostatin and its derivative TT-232 [in vitro] |
| Fatty Acid Synthesis | | |
| ATP citrate lyase [ACL] | Inhibition | SB-2049990 inhibits pancreatic cancer growth in nude mice |
| Acetyl-CoA Carboxylase [ACC] | Inhibition | Soraphen A induces apoptosis or autophagy in vitro |
| Fatty acid Synthase [FASN] | Inhibition | Cerulenin and its derivative C57 inhibit human ovarian cancer |
| Choline kinase [ChoK] | Inhibition | MN58b reduces phosphomonoesters in human cancer xenografts |
| HIF | | |
| HIF-1 α prolylhydroxylases [PHDs] | Activation of PHDs for inhibition of HIF | Cell-permeating α-ketoglutarate derivatives reverse HIV activation in SDH- or FH-deficient cells |
| Hypoxia-inducible factor 1 [HIF-1] | Inhibition of DNA binding | Echinomycin |
| Reactive oxygen species [ROS] | Antioxidants neutralize ROS and reduce HIF-1 function via PDHs and VHL | N-acetylcysteine [NAC]; vitamin C |
| Hypoxia | Cytotoxic effects of components enriched in hypoxic cells | Tirapazamine [TPZ], a hypoxia activated prodrug. |
| Proton Extrusion | | |
| $Na^+/H^+$ exchanger | Inhibition | Cariporide |
| Bicarbonate/$Cl^-$ exchanger | Inhibition | S3705 |
| MCT1 lactate /$H^+$ symporter | Inhibition | α-cyano-4-OH-cinnamate |
| Carbonic anhydrases 9 and 12 [CA9 - CA12] | Inhibition | Sulfonamide indisulam |
| $F_1F_0$ ATP synthase | Inhibition | Angiostatin; antibodies |
| Other | | |
| AMPK | Activation | Biguanides and thiazolidinediones activate AMPK through inhibition of OXPHOS, reducing the risk of cancer in diabetic patients |
| eIF4E | Inhibition of translation initiation by eIF4E | Antisense oligonucleotide inhibits growth of human breast cancer |
| L-type amino acid transporter 1 [LAT1] | Inhibition to reduce amino acid transport | 2-aminobicyclo [2.2.1]-heptane 2-carboxylic acid inhibits tumor growth in a xenograft model |

## Cancer Initiation and Progression

Cancer is a very heterogeneous disease. Tumors that arise in the same tissue can even exhibit an array of cellular pathologies, ranging from benign hyperplasia to highly invasive malignancies. On the other hand, cancer is a complex disease which involves the deregulation of multiple pathways. Microarray analysis has identified thousands of genes that are transcriptionally deregulated in cancer [107]; however, it remains unclear which deregulated genes play a causative role in tumor initiation and maintenance and which ones represent passerby with no selective advantage. Regardless of the role that specific genes may play in cancer progression, when a tumor is histological or clinically identified, a large number of molecular lesions have been accumulated [108].

A literature survey on all published cancer genes identified 291 genes for which there were molecularly characterized mutations and evidence of a causative role in tumorigenesis; these genes represent more than 1 percent of the human genome [109]. The large number of mutations found in tumor samples raises the question of whether there exists a common thread that underlies most, if not all, human malignancies, or biological rules that govern cancer initiation and progression. Despite the heterogeneity observed in cancer, most tumors share certain characteristics: self-sufficiency in growth signals, insensitivity to anti-growth signals, evasion of apoptosis, acquisition of a limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis [5, 108]. The similarity in the cellular processes in all cancer cells, regardless of their tissue of origin, likely indicates common tumor-initiation mechanisms.

When chronic myelogenous leukemia converts to acute leukemia, the malignant clone acquires an additional t(9;22) translocation, an isochromosome 17, or trisomy of chromosome 8. Likewise, when follicular lymphoma becomes aggressive, the lymphoma cells often bear a t(8;14) translocation in addition to the original t(14;18) translocation [1]. These findings support the hypothesis that most hematopoietic tumors and soft-tissue sarcomas are initiated by the activation of an oncogene, followed by alterations in tumor-suppressor genes and other oncogenes. In contrast, most carcinomas are initiated by the loss of function of a tumor-suppressor gene, followed by alterations in oncogenes and additional tumor-suppressor genes [110].

Comparative genomic hybridization has revealed a number of genes that can be amplified or deleted in cancer. Breast cancer genome analyses indicate

that there are only a few genes that are frequently mutated but many that are rarely mutated, providing an explanation for the heterogeneity in cancer. Complex somatic DNA rearrangements, mostly intra-chromosomal duplications, have been found in the breast cancer genomes [111].

Genome sequences of primary breast cancer and cancer metastasis show restricted de novo mutations in metastasis, but significantly shared mutations in the primary tumor. These studies provide information on tumor evolution and identify pathways critical to breast cancer metastasis [112].

The *ErbB2*, *PI3KCA*, *MYC*, and *CCND1* oncogenes are frequently deregulated in breast cancer. Loss-of-function mutations of *RB* in breast cancer cell lines and primary tumors were reported since 1988 [113]. At least ten tumor suppressor genes, all of which are involved in the regulation of genomic integrity, have been associated with hereditary breast cancer [114].

Cellular transformation is thought to take place through the accumulation of mutations, as well as epigenetic changes, that activate oncogenes or down regulate tumor-suppressor genes and lead to uncontrolled clonal expansion. Oncogenes originally were identified as the transforming agents of tumor viruses; later oncogenes were recognized as mutated versions of normal cellular genes, or proto-oncogenes, which had been incorporated into the viral genome by recombination [115].

The positive control of cellular growth is associated with mutations in oncogenes, which are generally dominant. In contrast, tumor suppressor genes function as negative regulators of cellular growth and are generally recessive. Thus, inactivation of both copies of a tumor-suppressor gene is usually necessary for tumor development. Several lines of evidence indicate that mutations in a single oncogene or tumor suppressor are insufficient to give rise to cancer [115]. First, most cancers develop late in life and the incidence of disease increases dramatically with age. Statistical analysis of epidemiological data shows that a cell needs to accumulate four to five sequential genetic lesions in key regulatory pathways in order to become malignant [116]. Second, *in vitro* experiments using cell lines, as well as *in vivo* models of cancer, confirm the multiple-hit hypothesis (retinoblastoma and certain types of leukemia are exceptions). Experiments using retroviruses containing activated versions of growth-controlling genes could lead to transformation of rodent cells in culture [117, 118]. However, the cells used in these initial cancer studies were immortal and could therefore proliferate indefinitely. When these experiments were repeated with primary cell lines, it was found that activation of at least one pair of oncogenes was required for transformation [119].

Tumors are histologically classified as presenting with different "grades," which correspond to a set of physiological markers as loss of differentiation, abnormal ploidy, and morphology, and correlate with patient outcome. Higher grade tumors have a more negative prognosis, while low-grade tumors are often considered early lesions and may progress to more invasive, high-grade disease. These observations have led to the hypothesis that cancer progression can be dissected into a small number of crucial steps whose sequential deregulation is critical for the clinical progression from low- to high-grade cancer. At least four pathways must be altered in order for tumor progression to occur: maintenance of telomere length (achieved by expressing human telomerase), deregulated cell-cycle entry (inactivation of *Rb*), deregulated cell growth arrest and apoptosis (inactivation of *p53*), and growth-factor independence (by oncogenic *Ras* overexpression). It remains to be explored how different oncogenes and tumor-suppressors found in tumor samples contribute to these cancer pathways and how they interact with each other to reinforce their tumorigenic potential [120].

Multistep process observed in human cancer has also been found in mouse models carrying activated oncogenes or inactivated tumor-suppressor genes, in which the duration and aggressiveness of the disease can be changed by introducing into the mouse genome the same sequential genetic alterations observed in human tumors.

## Oncogenes as Therapeutic Targets

Cancer mouse models have been used to test the hypothesis that, if tumor growth remains dependent on their original transforming oncogenic mutations, oncogene inactivation could lead to tumor regression [121]. Several studies evaluating the over expression of the *MYC* oncogene in lymphoid and epidermal tissues showed that the inactivation of *MYC* led to sustained tumor regression with concomitant promotion of either differentiation or apoptosis [121-123]. However, in other models, a fraction of tumor cells were refractory to *MYC* inactivation; these cells presumably had acquired new mutations that allowed Myc-independent growth [124-126].

The main cause of treatment failure for cancer patients are the metastasis. According to the model of metastatic progression only a small subset of cells from the primary tumor acquire the requisite mutations to metastasize to distant sites, where new mutations are accumulated as a response to the different selective pressures of a novel environment [127]. However, recent

data suggest that most cells in primary tumors with metastatic potential already contain the lesions necessary for metastasis and, possibly, for survival in a foreign environment. Microarray analysis compared patterns of gene expression in breast cancer patients with their known five-year survival and recurrence rates. Seventy genes were identified that could predict clinical outcome with a combined 83 percent accuracy [128]. Moreover, solid tumors of different origin shared the same metastatic signature, implying there is a common set of molecules regulating metastasis in a variety of primary tumors [129, 130].

Oncogenic proteins in cancer cells can be targeted by monoclonal antibodies when expressed on the cell surface, or by small molecules acting on specific molecules in particular metabolic ways. For example, imatinib targets the initial step of the multistep process in chronic myelogenous leukemia [131]. The same drug can affect the KIT and PDGFR receptor kinases. [132, 133]. Of particular interest are inhibitors of the BCL2 family, which can induce the apoptotic death of cancer cells. In acute promyelocytic leukemia, which is initiated by a t(15;17) chromosome translocation that fuses the *PML* gene to *RARα* (a nuclear receptor for retinoic acid), addition of retinoic acid can induce terminal differentiation and death of APL cells. This modality is called differentiation therapy [134, 135].

## MicroRNA Genes

Recent findings in molecular biology show that the participation of small regulatory non-coding RNA is required for cellular diversity in developing and mature organisms. These molecules act as sequence-specific post-transcriptional regulators in the expression of other RNA transcripts [136]. Small regulatory non-coding RNA molecules include microRNAs (miRNAs), small interfering RNAs (siRNAs) and repeat-associated siRNAs (rasiRNAs), which are unified by their association with the argonaute (AGO) family of proteins and by their function. All these RNAs direct the binding of protein complexes to specific nucleic acid sequences [137- 139].

Unlike other genes involved in cancer, miRNAs do not encode proteins. Their products are a single RNA strand of about 21 to 23 nucleotides implicated in to regulate the gene expression. A miRNA molecule can anneal to a messenger RNA (mRNA) containing a complementary nucleotide sequence and blocking the protein translation or causing degradation of the mRNA [140]. Mapping of numerous miRNA genes has shown that many of

them occur in chromosomal regions that undergo rearrangements, deletions, and amplifications in cancer cells. The genome regions that are consistently involved in chromosomal rearrangements in cancer cells but that lack oncogenes or tumor-suppressor genes appear to harbor miRNA genes [141].

Recent studies have shown that the RNA Pol III drives miRNA transcription from dense human clusters interspersed among repetitive Alu elements. These gene clusters are transcribed as polycistronic primary transcripts and subsequently cleaved into multiple miRNAs, or from intergenic regions as independent transcriptional units, or from intronic sequences of protein-coding or non-coding transcription units or exonic sequences of non-coding genes [142] (Figure 7).

Primary miRNAs transiently receive a 7-methylguanosine (7mGpppG) cap and a poly(A) tail and is processed into a precursor miRNA (pre-miRNA) by the nuclear RNase III enzyme Drosha and its partner DiGeorge syndrome critical region gene 8 (DGCR8). Exportin-5 transports premiRNA into the cytosol, where it is processed by the Dicer RNaseIII enzyme into a mature double strand miRNAs. The RNA strand is recruited into the RNA-induced silencing (RISC) effector complex and assembled through processes that are dependent on Dicer and other double strand RNA binding domain proteins, as well as on members of the argonaute family. MiRNAs guide the RISC complex to the 30 untranslated regions (30-UTR) of the complementary messenger RNA (mRNA) targets for repress their expression by several mechanisms: repression of mRNA translation, destabilization of mRNA transcripts through cleavage, deadenylation, and localization in the processing body (P-body), where the miRNA-targeted mRNA can be sequestered from the translational machinery and degraded or stored for subsequent use. Nuclear localization of mature miRNAs has been described as a novel mechanism of action for miRNAs. Scissors indicate the cleavage on pri-miRNA or mRNA [143].

Expression profiling of miRNA genes has revealed signatures associated with tumor classification, diagnosis, staging, and progression, as well as prognosis and response to treatment. For example, miRNA expression profiling can distinguish between indolent and aggressive forms of chronic lymphocytic leukemia, and expression of a small panel of miRNA genes correlates with prognosis in lung cancer [144- 146].

Some miRNA genes that are deregulated in chronic lymphocytic leukemia have germ-line or somatic mutations in a miRNA precursor that affect the processing of short single-stranded miRNA molecules [144]. MiRNA genes can be up-regulated or down-regulated in cancer cells. The up-regulated

miRNA genes function as oncogenes by down-regulating tumor-suppressor genes, whereas the down-regulated miRNA genes function as tumor-suppressor genes by down-regulating oncogenes [147]. Table 6 display a number of examples of up or down regulated miRNAs in cancer.

The function of miRNA genes depends on their targets in a specific tissue. A miRNA gene can be a tumor suppressor if its critical target is an oncogene and it can be an oncogene if its target is a tumor-suppressor gene. Up-regulation of miRNA genes can be due to amplification, deregulation of a transcription factor, or demethylation of CpG islands in the promoter regions of the gene. For example, the ALL1 (MLL) fusion proteins of acute lymphoblastic leukemia or acute myeloblastic leukemia carrying chromosome 11q23 translocations target the Drosha nuclease complex to specific miRNA genes, including *miR191*, thereby enhancing the processing of their miRNA precursors [148]. The *miR191* gene is also up-regulated in numerous types of solid cancers [149], suggesting that it is the downstream target of signal-translocation pathways involved in cancer. MiRNA genes functioning as tumor suppressors can be down-regulated because of deletions, epigenetic silencing, or loss of the expression of one or more transcription factors [150].

The *miR155* gene is over expressed in diffuse large B-cell lymphoma, the aggressive form of chronic lymphocytic leukemia, and in breast, lung, and colon cancers [151]. In transgenic mice under control of the Eμ enhancer of the immunoglobulin genes, over expression of *miR155* causes acute lymphoblastic leukemia or high-grade lymphoma, indicating that deregulation of a single miRNA gene can cause malignant transformation. Since it takes several months for the tumors in these mice to become aggressive, it is likely that additional genetic alterations are needed for the development of truthful neoplasia [152].

**Table 6. MiRNAs aberrantly expressed in cancers [146]**

| Cancer type | Up regulated | Down regulated |
|---|---|---|
| Breast cancer | miR-10b, miR-21, miR-22, miR-27a, miR-155, miR-210, miR-221, miR-222, miR-328, miR-373, miR-520c | let-7, miR-7, miR-9-1, miR-17/miR-20, miR-31, miR-125a, miR-125b, miR-146, miR-200 family, miR-205, miR-206, miR-335 |
| Chronic lymphocytic leukemia | miR-21,miR-155 | miR-15, miR-16, miR-29b, miR-29c, miR-34a, miR-143, miR-145, miR-181b, miR-223 |

**Table 6. (Continued)**

| Cancer type | Up regulated | Down regulated |
|---|---|---|
| Lung cancer | miR-17-92 cluster, miR-21, miR-106a, miR-155 | miR-1, let-7 family, miR-7, miR-15a/miR-16, miR-29 family |
| Lymphoma | miR-17-92 cluster, miR-155 | miR-143, miR-145 |
| Prostate cancer | miR-221, miR-222 | miR-15a-miR-16-1 cluster, miR-101, miR-127, miR-449[a] |
| Glioblastoma | miR-21, miR-221, miR-222 | miR-7 |
| Hepatocellular carcinoma | miR-17-92 cluster, miR-21, miR-143, miR-224 | miR-1, miR-101, miR-122a |
| Colorectal cancer | miR-17-92 cluster, miR-21 | miR-34a, miR-34b/c, miR-127, miR-143, miR-145, miR-342 |
| Gastric cancer | miR-21, miR-27[a] | miR-143, miR-145 |
| Ovarian cancer | miR-214 | miR-34b/c, miR-200 family |
| Melanoma | miR-221, miR-222 | let-7[a], miR-34[a] |
| Head and neck squamous cell carcinoma | miR-21 | let-7d, miR-138, miR-205 |

The *LET7* miRNA family, which are deleted or under expressed in lung cancer, target *RAS*; loss of *LET7* results in overexpression of *RAS* [153]. *MiR15a* and *miR-16-1*, the microRNAs those are deleted or down-regulated in chronic lymphocytic leukemia, cause overexpression of *BCL2*, which protects cells from apoptosis [154].

The expression of a set of 21 miRNAs is altered in at least three types of solid tumors [149]. Among these, *miR21* is of particular interest because inhibits expression of the tumor suppressor PTEN, which encodes a phosphatase involved in the PI3K kinase signaling pathway and is mutated in advanced breast, lung, gastric, and prostate cancers [155].

Primary miRNAs (pri-miRNAs) receive a 7-methylguanosine (7mGpppG) cap and a poly(A) tail. The pri-miRNA is processed into a precursor miRNA (pre-miRNA) and exported by the protein exportin-5 into cytosol, where is processed by the Dicer RNaseIII enzyme, into 22 nt-long double strand miRNAs. Then, miRNAs guide the RISC complex to the untranslated regions of the mRNA targets for repressing their expression.

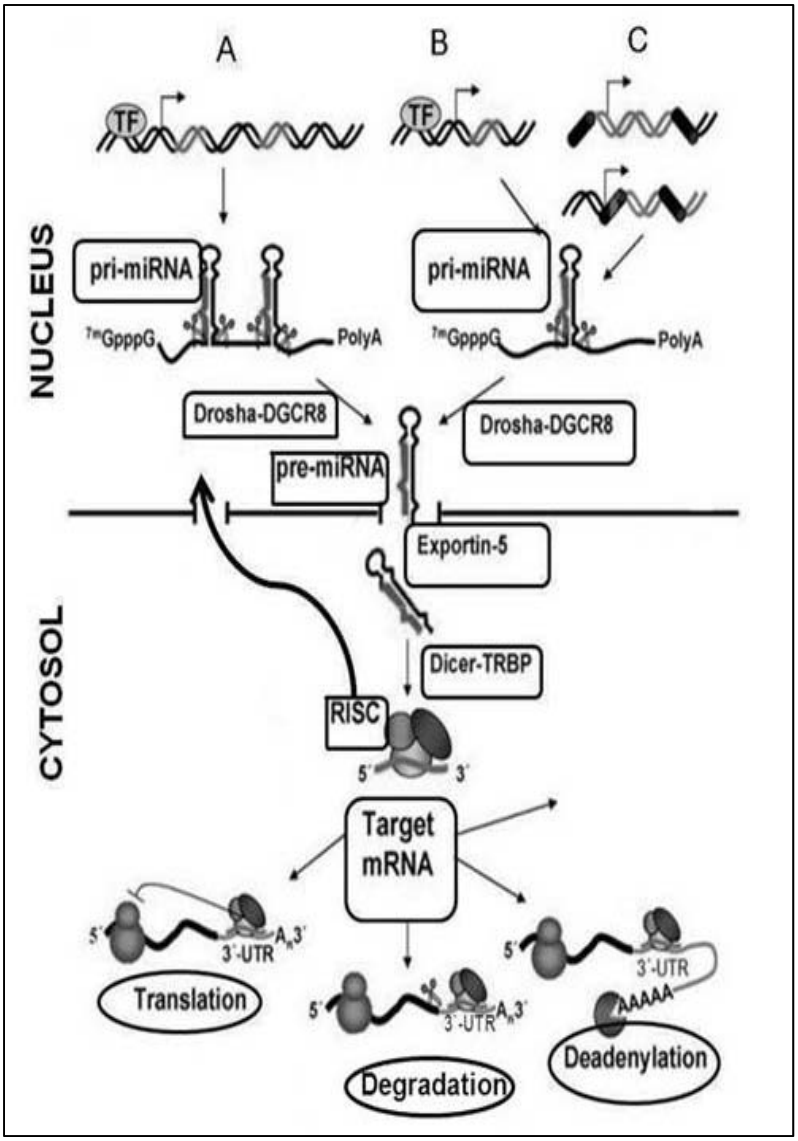


Figure 7. On the top line: (A) Gene clusters giving polycistronic primary transcripts and cleaved into multiple miRNAs; (B) intergenic regions transcribed as independent transcriptional units; (C) intronic sequences (in grey) of non-coding transcription units or exonic sequences (black cylinders) of non-coding genes. TF: transcription factor.

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