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# Mechanisms of Oncogene Activation

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## Abstract

The main modifications that characterize cancer are represented by alterations in oncogenes, tumor-suppressor genes, and non-coding RNA genes. Most of these alterations are somatic and the process is a multistep one. Tumors often arise from an initial transformed cell, and after subsequent genetic alterations different cytogenetically clones lead to tumor heterogeneity.

Oncogenes encode proteins that control cell processes such as proliferation and apoptosis. Among these proteins are transcription factors, chromatin remodelers, growth factors, growth factor receptors, signal transducers, and apoptosis regulators. Oncogenes activation by structural alteration (chromosomal rearrangement, gene fusion, mutation, and gene amplification) or epigenetic modification (gene promoter hypomethylation, microRNA expression pattern) confers an increased or a deregulated expression. Therefore, cells with such alterations possess a growth advantage or an increased survival rate. Given the fact that expression profiling of these alterations determines specific signatures associated with tumor classification, diagnosis, staging, prognosis, and response to treatment, it highlights the importance of studying oncogenes activation mechanisms and the great potential that they hold as therapeutic tools in the near future.

**Keywords:** Oncogenes, genomic instability, epigenetic modification

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## 1. Introduction

The main modifications that characterize cancer are represented by alterations in oncogenes, tumor-suppressor genes, and non-coding RNA genes. Most of these alterations are somatic and the process is a multistep one, although germ-line mutations can predispose a person to heritable or familial cancer.

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Tumors often arise from an initial transformed cell, and after subsequent genetic alterations different cytogenetically clones lead to tumor heterogeneity. Tumor heterogeneity determines different clinical phenotypes, leading to an individual response to treatment for tumors with the same diagnostic type.

Oncogenes encode proteins that control cell processes such as proliferation and apoptosis. Among these proteins are transcription factors, chromatin remodelers, growth factors, growth factor receptors, signal transducers, and apoptosis regulators. Activation of oncogenes by structural alterations (chromosomal rearrangement, gene fusion, mutation, and gene amplification) or epigenetic modification (gene promoter hypomethylation) confers an increased or a deregulated expression. Therefore, cells with such alterations possess a growth advantage or an increased survival rate. Translocations and mutations occur early on in tumor progression, whereas amplification usually occurs during late tumor stages.

A proto-oncogene is a normal gene that presents a potential to become an oncogene after a genetic alteration (mutation), leading to an increased expression. Usually, proto-oncogenes code for proteins that control cell growth and differentiation through signal transduction and execution of mitogenic signals. Upon activation, a proto-oncogene (or its product onco-protein) becomes a tumor-inducing agent. Most known examples of proto-oncogenes include *RAS*, *WNT*, *MYC*, *ERK*, and *TRK*. Another oncogene is the *BCR-ABL* gene found on the Philadelphia chromosome, a piece of genetic material seen in chronic myelogenous leukemia caused by the translocation of pieces from chromosomes 9 and 22 t(9; 22).

Oncogene products can comprise a variety of molecules such as transcription factors, chromatin remodelers, growth factors, growth factor receptors, signal transducers, and apoptosis regulators, each playing an important role in neoplastic transformation. For example, studies have shown that in prostate carcinomas the fusion between the *TMPR52* gene and two transcription factors *ERG1* or *ETV1* creates a fusion protein that increases proliferation and inhibits apoptosis of cells in the prostate gland, thereby facilitating their transformation into cancer cells [1]. Another example is represented by chromatin remodeler factors such as the *MLL* gene that plays a critical role in acute lymphocytic leukemia and acute myelogenous leukemia [2]. Also, an essential role in cancer development is played by apoptosis regulators such as the *BCL2* gene, which is involved in the initiation of almost all follicular lymphomas and some diffuse large B-cell lymphomas [3].

## 2. Mutations

Mutations in an oncogene may lead to a change in the structure of encoded protein, enhancing its transforming activity. Oncogenes are activated by point mutations (substitutions) and may either enhance or degrade the function of a protein. Table 1 shows the occurrences of mutations in each oncogene among some tissues [4].

CANCER TYPE	ONCOGENES		TYPE
T cell lymphoma	<i>KIT</i>	<i>KRAS</i>	Co-occurred
Acute lymphoblastic leukemia	<i>KRAS</i>	<i>NRAS</i>	Co-occurred
Acute myeloid leukemia	<i>KRAS</i>	<i>NRAS</i>	Co-occurred
	<i>FLT3</i>	<i>KIT</i>	Mutually exclusive
Lung adenocarcinoma	<i>BRAF</i>	<i>KRAS</i>	Mutually exclusive
	<i>KRAS</i>	<i>NRAS</i>	Mutually exclusive
	<i>EGFR3</i>	<i>KRAS</i>	Mutually exclusive
Pancreatic cancer	<i>B-CATENIN</i>	<i>KRAS</i>	Mutually exclusive
Biliary tract cancer	<i>BRAF</i>	<i>KRAS</i>	Mutually exclusive
Colorectal cancer	<i>BRAF</i>	<i>KRAS</i>	Mutually exclusive
	<i>KRAS</i>	<i>PIK3CA</i>	Co-occurred
	<i>B-CATENIN</i>	<i>KRAS</i>	Co-occurred
	<i>KRAS</i>	<i>NRAS</i>	Mutually exclusive
Thyroid cancer	<i>BRAF</i>	<i>NRAS</i>	Mutually exclusive
	<i>HRAS</i>	<i>NRAS</i>	Mutually exclusive
	<i>BRAF</i>	<i>RET</i>	Mutually exclusive
	<i>NRAS</i>	<i>RET</i>	Mutually exclusive
Melanoma	<i>BRAF</i>	<i>NRAS</i>	Mutually exclusive
	<i>BRAF</i>	<i>HRAS</i>	Mutually exclusive
	<i>BRAF</i>	<i>KRAS</i>	Mutually exclusive
	<i>KRAS</i>	<i>BRAS</i>	Co-occurred
Prostate cancer	<i>HRAS</i>	<i>NRAS</i>	Mutually exclusive
	<i>KRAS</i>	<i>NRAS</i>	Mutually exclusive
Kidney cancer	<i>B-CATENIN</i>	<i>WT1</i>	Co-occurred
Cervical cancer	<i>PIK3CA</i>	<i>KRAS</i>	Mutually exclusive
	<i>PIK3CA</i>	<i>BRAF</i>	Mutually exclusive

**Table 1.** Frequently mutated oncogenes in various type of cancers

In cancer, mutations occur in many oncogenes, most notable being *RAS* and *BRAF*. The *RAS* family represents the upstream component of the *RAS/RAF/MAPK* pathway and mutations in *RAS* are one of the most common activating events in most of cancers. Mutated *RAS* oncogene (*KRAS*, *HRAS*, and *NRAS*) encodes for a protein that remains in the active state and transduces signals for continuous cell growth. *KRAS* mutations are common in carcinomas of the lung, colon, and pancreas [5], whereas mutations of *NRAS* occur in acute myelogenous leukemia and the myelodysplastic syndrome [6].

BRAF is a protein member of the *RAF* family (*RAF1*, *BRAF*, *ARAF*), also regulated by *RAS* binding. Mutated *BRAF* oncogene encodes for a protein with a modified kinase domain, resulting in a constitutively active protein that uncontrollably stimulates the MAP kinase cascade [7].

## 2.1. Melanoma

*BRAF* mutations are the most common somatic mutations in cutaneous melanoma and are extremely rare in mucosal melanoma. There are found in 48% of metastatic biopsy specimens and can precede neoplastic transformation [8, 9]. Over 90% of the identified mutations in *BRAF* are in codon 600. The most common is *BRAFV600E*, resulting in substitution of glutamic acid for valine (*BRAFV600E*: nucleotide 1799 T>A; codon GTG>GAG). The second most common mutation is *BRAFV600K* (5–6%) substituting lysine for valine, (GTG>AAG), followed by *BRAFV600R* (GTG>AGG), *BRAFV600'E2'* (GTG>GAA). Less common *BRAF* mutations found in cutaneous melanoma are *BRAF V600D* (GTG>GAT) and *L597R* [10–12].

There were identified mutations in hotspot codons (12, 13, and 61) of different *RAS* genes (*HRAS*, *NRAS*, or *KRAS*), but the most prevalent were *HRAS* substitutions that occurred preponderant at codon 61 (*HRAS Q61L* mutation), with fewer mutations at codon 12 and codon 13 [13]. Mutations in *N-RAS* appear to be significant in melanoma even earlier than the discovery of *BRAF* mutations [14]. The base change at position 61 seems to be important in the activation of *N-RAS* genes, transforming activity being detected only when mutant codon 61 was present. *BRAFV600E* mutations are more common in younger persons and in tumors arising from intermittently sun-exposed skin, exclusive with *N-RAS* [15].

C-KIT gene encodes a receptor tyrosine kinase (KIT). All the mutations were founded in exon 11, 13, and 17. The most common is V559A mutation that results in an amino acid substitution at position 559 in KIT, from a valine (V) to an alanine (A) [16]. While *BRAF* and *NRAS* mutations are common and significant in cutaneous melanomas, C-KIT mutations were detected in acral melanomas, mucosal melanomas, conjunctival melanomas, and cutaneous melanomas [17]

## 2.2. Colorectal cancer

The development of colorectal cancer (CRC) is a multistep process that occurs due to the accumulation of several genetic alterations, which are associated with oncogenes and tumor suppressor genes, as well as genes involved in DNA damage recognition and repair.

Most of the *BRAF* mutations associated with CRC are located in exons 11 and 15, coding for the kinase domain. The hotspot mutation at 1796 nucleotide is the T-to-A transversion that corresponds to the *V600E* mutation (7–15%) [18].

In colorectal cancer, *RAS* gene mutations have been reported in 40–50% and the frequency of *KRAS* mutations varies between 24–50%. *KRAS* mutation occurs most commonly in codon 12 and 13 rather than in codon 61, with the most frequent mutations: *G12D*, *G12V*, *G12C*, *G13D*, *Q61H* [19]. *KRAS* mutations exist in the presence of a vast majority of wild-type *KRAS* cells,

which is why they are not detected in initial disease. Thirty-eight percent of patients whose tumors were initially *KRAS* wild-type developed *KRAS* mutations that were detectable in their sera after 5–6 months of treatment [20]. *KRAS* is mutated much more frequently than *NRAS*. *KRAS* mutations were studied to determine their role in the predictability of response to chemotherapy treatment.

### 2.3. Thyroid cancer

*RAS* mutations in thyroid cancer occur in both benign tumors and thyroid cancers (both papillary thyroid carcinoma (PTC) and follicular thyroid cancer (FTC)), with variable frequency in anaplastic thyroid cancers. PTCs with *RAS* mutations tend to display a lower rate of lymph node metastasis [21]. PTCs with *RAS* mutations often present a follicular architecture and a follicular variant of papillary thyroid carcinoma (FVPTC). There are two most common *RAS* mutations associated with thyroid cancer: mutations of *H-RAS* codon 61 and *N-RAS* codon 61 [22].

*BRAF* mutations were first detected missense mutations in thyroid cancer [23], which occurs in exon 15, due to the substitution of the amino acid valine for glutamic acid at residue position 600 [24]. This mutation is the most frequent genetic change in PTC [25], being found in 36–69% of PTC cases. *BRAF* mutation is responsible for the suppression of the sodium/iodide symporter (NIS), which is involved in iodine metabolism [26]. The *V600E* mutation comprises more than 90% of observed *BRAF* mutations, with the highest rate (77%) in the tall cell variant of papillary cancer, and the lowest percentage (12%) in the FVPTC. In PTC, *BRAF* mutation is more frequent in older patients, associated with extrathyroidal invasion [27]. By contrast, others have found that the *BRAF* mutation is not associated with age, gender, multicentricity, recurrence rate, lymphovascular invasion, or distant metastasis [28].

Activating point mutations of *RET* oncogenes are associated with hereditary cancer syndrome (multiple endocrine neoplasia type 2 -MEN 2). *RET* mutations are mostly missense and located in exons 10, 11 (extracellular domain of RET), 13, 14, 15, and 16 (in the TK domain) [29–31]. Mutation of the extracellular cysteine in codon 634 in exon 11 of RET causes ligand-independent dimerization of receptor molecules and enhances phosphorylation of intracellular substrates and cell transformation. Mutation of the intracellular TK (codon 918) results in cellular transformation [32].

There is a high correlation between the position of the point mutation and the phenotype of the disease. Three subtypes based on clinical presentation are defined: MEN 2A, MEN 2B, and FMTC. *RET* mutations are observed in 98% of MEN2A, 95% of MEN 2B, and 88% of familial medullary thyroid carcinoma (FMTC) [33]. Activating mutations of *RET* involving exons 10, 11, 13, 14, and 15 (encoding the highly conserved cysteine-rich domain) have been proven to cause MEN2A. [34]. The mutations for MEN2A are mostly located in exon 10 (10–15%), including codons 609, 611, 618, and 620, and exon 11 (80–85%), as well as codons 630 and 634 [35]. The mutations characteristic of FMTC occur in exons 10 and 11. However, non-cysteine point mutations also have been found in exon 8 (codons 532 and 533), exon 13 (codons 768, 790, and 791), exon 14 (codons 804 and 844), exon 15 (codon 891), and exon 16 (codon 912) [35–

37]. About 95% of MEN2B patients carry a *M918T* mutation within exon 16 and 5% have an *A883F* mutation in exon 15. Mutation in codon 918 gives a more aggressive phenotype [38].

#### 2.4. Hepatocellular Carcinomas (HCC)

In HCC, only one mutation (*KRAS* codon 13; Gly to Asp) was detected among patients and no mutations were found in codons 12 and 61 of *KRAS* or codons 12, 13, and 61 of the *NRAS* and *HRAS* genes. So, the activation of *RAS* oncogenes by point mutations does not play a major role in hepatocellular carcinogenesis [39]. Activating mutations in the *BRAF* oncogene have been found in a small fraction of hepatocellular carcinomas. *KRAS* and *BRAF* mutations are rare events in HCC and therefore not a key event in hepatocarcinogenesis [40].

#### 2.5. Pancreatic cancer

The highest incidence of *KRAS* mutations are found in adenocarcinomas of the pancreas (90%), with activating point mutations in codon 12 of the *KRAS* protein, leading to a glycine (G) to aspartic acid (D) or valine (V) substitution [41]. Single amino acid substitutions at G12, 13, or Q61 lead to the formation of mutated *KRAS* that are insensitive to GAP stimulation. This leads to the accumulation of persistently GTP-bound and active *KRAS*, which leads to pancreatic cancer formation [42].

#### 2.6. Cervical cancer

Cervical cancer harbors high rates of potentially targetable oncogenic mutations. *KRAS* mutations were identified in low percentage (17%) exclusively in cervical adenocarcinomas. Most mutations were missense mutations of codon G12, well-described activating mutations, which have been associated with a worse prognosis in the metastatic process [43].

*EGFR* mutations were identified in 7.5% of cervical squamous cell carcinomas; a missense mutation in exon 15 of the *EGFR* gene produces an alternate spliced transcript (isoform D). Its presence in both tumor and adjacent normal tissue suggests that *EGFR* S703F may be a germline mutation [44, 45].

*PIK3CA* mutations are present in both squamous cell carcinomas and adenocarcinomas (31%). The *PIK3CA* mutations were located in the exon 9 helical domain in two hotspot mutations (*E545K* and *E542K*), which result in the constitutive activation of cellular signaling [46]. *PIK3CA* mutations may impart a more aggressive and treatment-resistant phenotype and decreased survival among patients with these mutations in early stage cancers [47].

### 3. Gene amplification and chromosomal translocations

The interest regarding the role of genomic context in promoting amplification was intensely investigated, but is still under debate. An important interest remains to establish the tendency of some genomic region to be subject to amplification. Past researches showed that different

regions of the genome were more subjected to be amplified than others, but the molecular substrate was unknown [48]. At present, several mechanisms and models have been proposed to explain gene amplification in oncogenesis.

### 3.1. Gene amplification

Besides point mutations resulting in amino acid substitutions, a proto-oncogene may be activated by chromosomal alterations. Among the most important chromosomal abnormalities is gene amplification, which is an increase of the copy number for a specific chromosomal region. The consequence of chromosome fragment amplification is associated with overexpression of the amplified gene(s) and is a characteristic of cancer [49]. Amplified genes (hundreds of copies of normally diploid genes) may be organized as extrachromosomal elements (double minute chromosomes) as repeated units at a single locus or scattered throughout the genome.

At this moment, the relationships between the two forms of gene amplification found in tumors, the intrachromosomal homogeneously staining regions (HSRs) and the extrachromosomal DNA molecules, double minutes (dmins), are not well understood [50].

Several models for initiation of amplification have been described involving defects in DNA replication or telomere dysfunction and chromosomal fragile sites. Regarding the DNA replication initial proposals, based on extra rounds of replication due to replication origins misfiring appear to be incorrect modification of models invoking replication of extrachromosomal DNA [51]. Another theory involves the double-strand DNA breaks (frequent in replicating cells) generated by the collapse of replication forks that are unable to progress due to DNA structure lesions, therefore providing an opportunity to initiate the amplification process [49].

Telomeres are repetitive nucleotide sequences, with the role to prevent the loss of DNA sequences, resulted as a consequence of the incomplete DNA replication at the chromosome ends. Telomere shortening can block cell division; this mechanism appears to prevent genomic instability and development of cancer in aged cells by limiting the number of cell divisions [52]. Telomerase is responsible for telomere replication and is inactive in most somatic cells. With every cell division, the DNA telomere sequence is shortened by 40–50 bp. Telomere shortening in humans can induce replicative senescence, which blocks cell division. When telomeres are short to a critical length (replicative limit), cellular senescence is induced and normal cells cease to proliferate. This mechanism appears to prevent genomic instability and development of cancer in aged cells by limiting the number of cell divisions. In cancer, tumor cells escape replicative limit and acquire the capability to maintain telomere length through cell divisions by telomerase reactivation, or by using a recombination-based mechanism and alternate lengthening of telomeres (ALT) [53, 54]. An experimental murine model (lacking the RNA component of telomerase-TercK/K mice) for telomere dysfunction demonstrated the promotion of gene amplification. Tumor genomes arising in mutant mice contain chromosomal rearrangements, amplifications, and deletions commonly associated with human tumors [55, 56]. Despite the established correlation between telomerase reactivation and telomeres lengthening in cancer, recent literature review and analysis [52] suggest this is unlikely,



because shorter telomeres and telomerase inactivation is more often associated with increased cancer rates, and the mortality from cancer occurs late in life.

In humans, shorter telomeres were associated with poorer health and aging and were also observed in preneoplastic stages, supporting a role for this mechanism in generating genomic aberrations in oncogenesis [57–60]. The model for gene amplification due to telomere abnormalities and the break at fragile sites (discussed below) was first described in maize and results from the breakage/fusion/bridge (B/F/B) cycles [61]. B/F/B cycles are initiated when broken ends of chromosomes fuse, resulting in a dicentric chromosome. During anaphase, the two centromeres are pulled in opposite directions and the dicentric chromosome generates a chromosome with an inverted duplication of terminal sequences to break. The B/F/B cycle continues in the next cell cycle because this chromosome also has broken ends. The B/F/B cycles were observed like primary mechanism for gene amplification in hamster cells [62].

In human cancer, evidence of B/F/B cycles was provided by the high frequency of anaphase bridges in early passage tumor cells and tumors [63, 64]. On the other hand, it was proven that human tumor cells in culture presenting gene amplification contain DM chromosomes, and the clones with low-copy amplification contained structures related to B/F/B cycles [65, 66]. There are evidences that B/F/B cycles may generate amplicons. These results were obtained by cytogenetic analyses of HSRs in tumor cell lines and in model systems with amplifications following drug treatments [67, 68]. The model explains that loss of the DNA sequences distal to the gene under selection or their translocation to another chromosome is also possible.

HSR may arise from the integration or fusion of double minute with a chromosome [50]. Currently, the data available suggests that fusion and reintegration constitute a pathway for the evolution of extrachromosomal elements, but the site of HSR insertion has never been characterized at a nucleotide resolution [50].

### 3.2. Fragile sites

Fragile sites are part of normal chromosome structures existing in each individual and represent chromosome regions that are late in replicating and prone to breakage under conditions of replication stress. Fragile sites occur after partial inhibition of DNA synthesis and are constituted in regions presenting site-specific gaps and breaks on metaphase chromosomes. Common fragile sites are normally stable in somatic cells, but it was observed that following treatment of cultured cells with replication inhibitors, fragile sites display gaps, breaks, rearrangements [69, 70]. Fragile sites extend over large regions of high DNA flexibility and are associated with genes.

The molecular nature and mechanisms involved in fragile site instability was unknown till recently. In many cancer cells, fragile sites and associated genes suffer frequent deletions and/or rearrangement, demonstrating their role in genome instability during the oncogenesis process. As a group, fragile sites are heterogeneous and seem to extend over broad regions 0.3–9-Mb long. The regions comprising fragile site are particularly associated with a high frequency of recombinogenic events, including co-localization with chromosome aberrations sites related to various cancers [69].

Accordingly to several studies, there are around 127 known fragile sites in the human genome, defined as "common" or "rare" based on their frequency [71, 72]. Common fragile sites (CFSs) are a normal part of the human genome and are typically replicative stable [73]. CFSs are not the result of nucleotide repeat expansion mutations. The majority of breakages at CFSs are further distinguished depending on their sensitivity to the drugs used to induce their expression (e.g., low doses of the antibiotic aphidocilin (APH)) [74].

The breakage effect of APH may be reduced by using a co-treatment with low concentrations of the topoisomerase I inhibitor and camptothecin (CPT) [75]. CFS regions are highly conserved in vertebrate species, including mouse and primates [76–78]. CFSs initiate proper replication but slow to complete it, introducing breaks from unreplicated regions of DNA [79]. The mechanism proposed for CFS instability resides in this late replication. Late replication may occur due to formation of non-B DNA structures such as hairpins and toroids that block the replication fork in AT rich regions [80].

Rare fragile sites (RFSs) are classified into two sub-groups based on the compounds that induce breakage, folate-sensitive groups and nonfolate-sensitive groups, which are sensitive at bromodeoxyuridine (BrdU) or distamycin A, an antibiotic that binds to AT-pairs of the DNA sequence. The folate-sensitive group is characterized by an expansion of CGG repeats, while the nonfolate-sensitive group contains many AT-rich minisatellite repeats [81–83]. The genome instability mechanism of CGG and AT-rich repeats characteristic for RFSs can form DNA structure (hairpins and other non-B DNA) replication forks, leading to breakage [84, 85]. On the other hand, it was demonstrated that DNA polymerase stops at CTG and CGG triplet repeat sequences, which can result in continuous DNA synthesis via slippage [79].

Fragile site regions are stable in normal cells and become unstable in tumor cells. The breakage of the fragile sites may be caused by mutations leading to a blockage of replication, or by a cell cycle perturbation and gene involved in the DNA repair process deregulation [86]. Several reports developed the concepts that underlie the mechanisms leading to fragile site expression and chromosomal rearrangements at fragile sites in tumors. The analysis of DNA damage response in various tumor types, including bladder, breast, colorectal, and lung tumors, found that early stages of cancer development are associated with an active DNA damage response, including phosphorylated ATR (ataxia telangiectasia and Rad3-related protein), ATM (ataxia telangiectasia mutated), CHK1 (checkpoint kinases), CHK2 kinases, phosphorylated histone H2AX, and p53 [87-88].

These events are linked to a high frequency of LOH (loss of heterozygosity) at known fragile site regions. The explained mechanisms sustained that in precancerous lesions, the blockage or collapsed replication leads to ATR activation and with subsequent DNA double strand breaks. Tumor cells that escape apoptosis or cell cycle arrest will exhibit allelic imbalances, especially at target fragile sites because of replication sensitivity. Further, the model sustains the necessity of p53 mutation and/or other genes involved in checkpoints control, leading therefore to cancer progression. Lesions at common fragile sites are indicators of replication stress during early stages of tumorigenesis [70].

Fragile sites regions are targets for the initiation of the amplification process due to breakage. Several studies showed that boundaries of some amplicons generated through the amplification process mapped to common chromosomal fragile sites in hamster cells [89–90]. Evidences of the role of fragile sites in human cancer regarding gene amplification are scarce. One example of cell line model is for the *MET* amplicons in the esophageal adenocarcinoma map within the fragile site FRA7G [91].

Aphidicolin-sensitive fragile sites FRA5D, FRA5F, and FRA5C, which map distal to dihydrofolate reductase gene (*DHFR*) on 5q, are infrequently expressed and are less likely to contribute to the amplification process. In order for the gene amplification process to take effect the target gene must be in the close proximity of fragile sites, similar to the *MET* amplicon. The breakage at specific genomic sites may not contribute to the amplification process, and no evidence of recurrent amplicon boundaries was found using array CGH in a human cell culture system [92].

### 3.3. Amplified genes in cancer

The amplification process is important for deciphering oncogenesis molecular biology, prognosis, and targeted therapies. A good example of gene amplification is dihydrofolate reductase gene (*DHFR*), which usually occurs during progression of methotrexate-resistant acute lymphoblastic leukemia [93]. In cancer, the most amplified genes are members of four different oncogene families: *MYC*, cyclin D1 (or *CCND1*), *EGFR*, and *RAS*. The amplified DNA segment usually involves several hundred kilobases and can contain many genes.

In breast cancer, *MYC*, *ERBB2*, *CCND1*, *EGFR*, or *MDM2* were found to be amplified concomitantly [94]. Moreover, it has been reported that there is a direct correlation between the number of amplifications and an advanced breast cancer and poor survival [95]. *MYC* oncogene is amplified in many types of cancer such as small-cell lung cancer, breast cancer, esophageal cancer, cervical cancer, ovarian cancer, and head and neck cancer [96].

Among the best-known oncogenes that are amplified in cancer cells is *N-MYC*. This gene codes for a transcription factor that plays a physiologic role in stimulating cellular proliferation and is commonly amplified in neuroblastoma where patients have poor clinical prognosis. Amplification of *N-MYC* in neuroblastoma has a valuable prognostic significance, and is correlated with an advanced tumor stage [97], along with *MYC* and *ERBB2* in breast cancer [94].

In malignant thyroid tumors, *C-MYC* gene overexpression and amplification has also been correlated with tumor aggressiveness [98]. Overexpression of cyclins is also an important element in thyroid oncogenesis, playing a crucial role in PTC pathogenesis [98]. *CCND1*, a cell cycle key regulator of G1/S transition, is a frequent target of mutagenesis in many tumors; amplification and rearrangement of its gene can lead to the over-production of this cell cycle regulatory protein. *CCND1* amplification also occurs in breast, esophageal, hepatocellular, and head and neck cancer [99].

*EGFR* (*ERBB1*) is amplified in glioblastoma and head and neck cancer. Amplification of *ERBB2* (also called *HER2/neu*) in breast cancer correlates with a poor prognosis. A monoclonal

antibody against the product of this oncogene (trastuzumab) is effective in breast cancers that overexpress HER2/neu.

New data were acquired using array-CGH technique, bringing to knowledge the complex aspect of oncogene amplification in cancer. Using array-CGH analysis on identification of an 8p12 amplicon in anaplastic thyroid carcinoma (ATC) cell lines, six genes were found to be amplified, *DUSP26*, *MET*, *MYC*, *PVT1*, *YAP1* and *CIAP1* [100]. *DUSP26* effectively dephosphorylates *p38* and formed a physical complex with *p38*, promoting the survival of ATC cells by inhibiting *p38*-mediated apoptosis.

The *AIB1* oncogene is located on chromosome 20q, a region frequently amplified and overexpressed in breast cancer [101–102]. High levels of *AIB1* mRNA or protein predict significantly worse prognosis and overall survival in breast cancer patients [103]. *AIB1* is a transcriptional co-activator that promotes the transcriptional activity of multiple nuclear receptors such as the estrogen and progesterone receptors [104].

In cervical cancer, the array-CGH technique revealed that the 3q26.3 amplification was the most consistent chromosomal aberration in primary tissues of cervical carcinoma, and an increased copy number of *PIK3CA* gene was identified [105]. *PIK3CA* is known to be involved in the PI 3-kinase/AKT signaling pathway, which plays an important role in regulating cell growth and apoptosis.

In pancreatic cancer, chromosome 19q13 was found amplified containing *PAK4* gene [106]. *PAK* proteins are critical effectors that link *Rho GTPases* to cytoskeleton reorganization and nuclear signaling. *PAK4* interacts specifically with the GTP-bound form of *Cdc42Hs* and weakly activates the *JNK* family of *MAP* kinases. *PAK4* gene is not in a mutated oncogenic form but the activation of the *PAK4* gene promotes *KRAS2* gene mutation, a very frequent event in pancreatic cancer [106].

DNA amplification represents an important mechanism during human multistep hepatocarcinogenesis. Several genes were found to be amplified within 1q21 amplicon in hepatocellular carcinoma: *CREB3L4* (cyclic AMP responsive element binding protein3-like 4); *JTB* (Jumping Translocation Breakpoint) is a transmembrane protein that suffers an unbalanced translocation in various types of cancers [107]; *INTS3* and *SNAPAP*, whose role in oncogenesis remains to be defined; *SHC1* is involved in signal transduction from receptor tyrosine kinases to downstream signal to *RAS* [108–109]; *CKS1B* (CDC28 protein kinase regulatory subunit 1B), *Cks1* expression was closely associated with poor differentiation and also negatively associated with p27<sup>kip1</sup> in hepatocellular carcinoma [110]; *CHD1L* (Chromodomain Helicase/ATPase DNA Binding Protein 1-Like, also known as Amplified in Liver Cancer 1, *ALC1*) whose increased expression was associated with clinicopathological features such as microsatellite tumor formation, venous infiltration, and advanced tumor stage, overall survival time, and the disease-free survival rate [111]. Moreover, *Glyoxalase 1* (*Glo1*) gene aberrations are associated with tumorigenesis and progression in numerous cancers. Hepatocarcinoma cells with genetic amplified *Glo1* gene express higher levels of *Glo1* and are more sensitive to cell killing effects if *Glo1* expression is down-regulated [112]. The study supports the potential of *Glo1* as therapeutic target in patients with hepatocellular carcinoma and genetic *Glo1* amplification.

Human oncogene *JUN* gene amplification/overexpression was found in highly aggressive sarcomas and in hepatocellular carcinomas, along with amplification/overexpression of *MAP3K5*. *JUN* overexpression could interfere with adipocytic differentiation and promote angiogenesis [113, 114].

Amplification of the *FGFR2* gene was identified in a subset of Chinese and Caucasian patients with gastric cancer. Fibroblast growth factor receptor family members (*FGFR1–4*) belong to the *RTK* superfamily. Through interaction with FGF ligands, the receptors are involved in diverse cellular functions including regulation of development processes, mediation of cell proliferation, and differentiation, as well as angiogenesis and tissue regeneration [115, 116]. FGF ligand binding leads to kinase activation and downstream signaling to phosphoinositide 3-kinase (PI3K)-AKT and mitogen-activated protein kinase–extracellular signal–regulated kinase (MAPK-ERK) pathways [117]. Genetic modifications or overexpression of FGFRs have been associated with tumorigenesis and progression in breast, prostate, stomach, and hematologic malignancies [118, 119]. *FGFR2* amplification leads to constitutive activation of the *FGFR2* signaling pathway in gastric cancer, and furthermore inhibition of this pathway using a well-tolerated, potent, and selective inhibitor can lead to rapid and durable tumor regressions in *FGFR2*-amplified gastric cancer xenograft models, representing an important treatment target [120].

### 3.4. Chromosomal translocations

Chromosomal translocations (CTs) are very common in human cancer, and the molecular mechanisms involved are complex and poorly understood. CTs are involved in several types of cancer, particularly in hematopoietic and lymphoid tumors [121]. This type of chromosomal abnormality seems to provide a selective growth advantage for some stem or progenitor cells, which may further initiate the development of some malignant tumors. In case of oncogenes, CTs may change the original locations of proto-oncogenes, generating effects on the gene products through two major ways [122, 123]. One is to generate oncogenic fusion proteins and the other way is that proto-oncogenes are brought into proximity with regulatory elements, causing the overexpression of proto-oncogene.

The first specific chromosomal translocation identified in human cancer was the Philadelphia chromosome [t(9;22)], which underlies chronic myeloid leukemia (CML). The fusion of chromosomes 9 and 22 leads to the joining of two unrelated genes, the *C-ABL* gene, which encodes a tyrosine kinase and is located on chromosome 9, and the gene *BCR* (for breakpoint recombination) located on chromosome 22.10 [124]. A chimeric protein (BCR-ABL) with novel transforming properties is formed from this specific chromosomal rearrangement. BCR-ABL oncoprotein has an abnormal tyrosine kinase activity and is associated with the tumorigenesis of CML and acute lymphoblastic leukemia (ALL) [124]. Duplication of the Philadelphia chromosome leads to accelerated CML blast phase, suggesting that increased copies of this aberrant gene confer a dose-dependent transforming effect [125]. Similar to t(9;22) in acute promyelocytic leukemia (APL), a chromosomal rearrangement joins a novel gene t(15;17), resulting in the formation of promyelocytic leukemia-retinoic acid receptor  $\alpha$  (*PML-RAR $\alpha$* ) fusion oncoprotein [126].

*PML-RAR* function is unknown, but this translocation underlies the response of this leukemia type to treatment with trans-retinoic acid. Another intergenic, CT t(12;21), leads to a novel chromosomal translocation product, *TEL-AML1*, which requires a specific treatment for pediatric acute lymphoblastic leukemia [127].

A classic example is the overexpression of proto-oncogene *c-MYC* in Burkitt lymphoma due to t(8;14) that results in *c-MYC* gene juxtaposed to immunoglobulin heavy chain (IGH) regulatory elements [128, 129]. Further expression of the gene is directed by the strong immunoglobulin heavy-chain enhancer, which is constitutively active in B lymphocytes. Thus, *c-MYC* overexpression is a potent force driving cellular proliferation.

The t(11;14) translocation juxtaposes *CCND1* and immunoglobulin enhancer elements and is characteristic of mantle-cell lymphoma. The t(11;14) translocation juxtaposes *CCND1* and immunoglobulin enhancer elements [130].

The ability to grow leukemic cells in culture long enough to allow cytogenetic analysis has facilitated the characterization of chromosomal translocations in leukemia. However, specific chromosomal translocations have also been observed in solid tumors. Aside from interchromosomal translocations, intrachromosomal translocations are also associated with cancer. Around 60–70% of PTCs have a characteristic inv(10)(q11.2q21). The breakpoint is represented by *RET* gene locus (10q11.2), which is relegated to the opposite breakpoint of the *H4* (D10S170) or *NCOA4* (ELE1) gene (10q21) in the same chromosome [131]. The H4 protein is widely expressed in the nucleus and cytoplasm and its function is unknown [132]. In PTC, many types of rearrangement loci (11 rearranged forms) were noted and PTC1(H4, *CCDC6*)-*RET* and PTC3(*NCOA4*)-*RET* are the most common [133]. PTC2-*RET* is a less common type of PTC-*RET* [134]. These rearrangements can lead to constitutively ligand-independent *RET* activity involved in thyroid carcinogenesis. The hypothesis sustain that the distances between *RET* and H4 loci are 18 Mb, therefore chromosome folding may close the two loci to each other in thyroid cells, increasing the probability of recombination between them in the interphase nuclei. This chromosomal folding is specific for thyroid cells, and this may explain why inv(10)(q11.2q21) is frequently seen in PTC [135].

It has been shown that in prostate carcinomas, the fusion between *TMPR552* gene and two transcription factors *ERG1* or *ETV1* creates a fusion protein that increases proliferation and inhibits apoptosis of cells in the prostate gland, thereby facilitating their transformation into cancer cells [1].

The translocations of ETS are often found in human cancer, such as Ewing sarcoma [136–137], leukemia [138–139], prostate cancer [140], and breast cancer [141]. These once disparate tumors are now defined by a chromosomal translocation fusing the *EWS* gene to a number of transcription factors of the ETS gene family (the most common chimeric protein is *EWS-FLI1*) [142]. This chimeric product presumably acts directly on target promoters to direct the expression of genes that induce cellular proliferation. Identification of *EWS* translocations allowed the molecular grouping of a class of tumors whose proliferation is driven by similar genetic alterations and that respond to similar chemotherapeutic regimens.

## 4. Oncogene hypomethylation

The first epigenetic modification observed in human cancer was the loss of DNA methylation at the 5' cytosine level (m5C residues replaced by unmethylated C residues), reported in 1983 [143]. This discovery was often regarded as an unwelcome complication, and all of the attention was focused on the opposite effect hypermethylation of promoters of genes that are silenced in cancers (e.g., tumor-suppressor genes). Global hypomethylation of DNA in cancer was found associated especially with repeated DNA elements; this modification did not represent a research direction for many years [144]. However, changes in the pattern of DNA methylation have been a consistent modification in cancer cells. Both hypo- and hypermethylation were observed at various loci, but at this moment it is clear that DNA methylation plays an important role in carcinogenesis.

New deep sequencing methylome analyses have shown much more cancer-linked hypomethylation of unique gene sequences and hypermethylation of repeated sequences than previously found [145–148]. Targeting DNA repetitive sequence, DNA hypomethylation may induce genomic instability and mutation events in cancer genomes [149–152] by altering the intranuclear positioning of chromatin enhancing recombination [153–155] and activating retroviral elements [156]. Promoter hypomethylation of some genes may be associated with the development of cancer by regulating the activity of genes [157].

### 4.1. Genomic hypomethylation profiles in cancer

DNA methylation principally occurs at 5' cytosine from dinucleotide CpG sites [158, 159]. CpG dinucleotides are found in C+G-rich regions in the genome termed CpG islands, localized frequently at promoter or gene regulatory level. However, the vast majority of CpG dinucleotides are localized within the intergenic and intronic regions of the DNA, particularly within repeat sequences and transposable elements. Unmethylated CpG islands at gene level are associated with gene transcription. In normal somatic cells, between 70% and 90% of CpG dinucleotides are methylated, which constitute approximately 0.75–1% of the total number of bases in the genome, while most CpG islands are unmethylated [160]. A part of genes promoter region are methylated as part of normal developmental processes or tissue specific (e.g., germline specific genes-MAGE genes) [161]. In X chromosomes in female dosage compensation (imprinted genes of X chromosomes in females), where only one of two copies is active, methylation of regulatory regions is involved in the repression of the expression of the silent loci [162].

Recently, high-resolution genome-wide analyses of DNA methylation changed the idea that considers oncogenesis being characterized predominantly of hypomethylated DNA repeats and hypermethylated gene regions [163–164]. The hallmark for cancer is represented by global losses of DNA methylation with local hypermethylation and hypomethylation of specific genes [165–167].

Evaluation of the majority of cancers showed that a major contributor to global DNA hypomethylation is hypomethylation of tandem and interspersed DNA repeats [165, 168]. Several

studies using CpG methylation-sensitive restriction endonucleases or sodium bisulfite reported that hypomethylation was often found at gene sequence level (including metastasis-associated genes) [157, 165].

#### **4.2. Hypomethylation of DNA repeats sequence in cancer**

Repeat sequences are represented by transposable elements found interspersed throughout the genome, or simple repeat sequences, such as DNA satellites, found in pericentromeric or subtelomeric region of chromosomes. These are normally methylated within the healthy genome [169].

In cancer, hypomethylation of DNA repeats is a result of the demethylation process rather than the preexisting hypomethylation in a cancer stem cell [170]. The frequency of cancer-associated hypomethylation of DNA repeats is dependent with disease progression (tumor grade, stage) [171, 172]. Hypomethylation is also seen in tumor adjacent tissues and in benign tumors (breast fibroadenomas and ovarian cystadenomas), but at a lower level than cancer [145, 165, 173, 174].

Hypomethylation may affect transcription and hypomethylation of interspersed DNA repeats within promoter modifies the chromatin boundaries resulting in transcription activation of nearby genes [175, 176]. Along with the effects upon transcription, hypomethylation can affect alternative splicing and hypomethylation of a minor portion of interspersed DNA repeats may occasionally cause induction of retroviral element transcription [156]. Several studies reported numerous evidences for the causal relationships between DNA hypomethylation and increased transcription as well as hypomethylation and cancer [177–179].

Regions of cancer-associated changes in DNA methylation are found in short interspersed or clustered regions, as well as in long blocks [180–182]. Dante et al. described hypomethylation of LINE-1 (a highly repeated interspersed repeat) in mononuclear cells from patients with chronic lymphocytic leukemia [183]. Along with hypomethylation of LINE-1, Alu repeats were also subsequently observed hypomethylated in many other types of cancers [183–186]. In breast adenocarcinomas, ovarian epithelial cancers, and Wilms tumors, a hypomethylation of centromeric and juxtacentromeric satellite DNA was noted [173, 174, 187]. Moreover, another classes of tandem repeats (macrosatellite DNAs) and segmental duplications were found hypomethylated in various cancers [188–190]. The loss of DNA methylation in cancer varies according to the tumor type and subclasses of DNA repeat [191–193].

Gathering the result of the presented studies, we may conclude that in many types of cancer, hypomethylation of DNA repeats represents a highly informative prognostic marker and/or predictor of survival [194–197].

#### **4.3. Hypomethylation of DNA gene enhancer sequence in cancer**

Gene expression levels may be further modulated by DNA methylation levels at upstream enhancer sites [198], which can affect the binding of transcription factors at (CpG) islands [199]. In normal cells, DNA demethylation at enhancer's level is correlated with upregulation of expression of the associated gene. It was shown that the binding of FoxA1/FOXA1 transcrip-



tional factors to enhancers is inhibited by DNA methylation at the respective binding site [200]. In this case, modification of DNA methylation status (demethylation) at the enhancer level may lead to an open chromatin state allowing the access of transcription factors at the active enhancer [201–202]. Following DNA demethylation, FoxD3 transcription factor binds at the enhancer level, allowing the recruitment of FoxA1 and conversion of the enhancer to a state that is set for activity. Local DNA demethylation leads also to changes in histone H3K27 or H3K9 methylation [200]. FOXA1 is an important factor for oncogenesis being involved in various types of cancer [203]. Thus, DNA hypomethylation from transcription regulatory regions may cause changes in expression [204].

#### 4.4. Genomic hypomethylation in promoters and within gene bodies

Hypomethylation of transcription regulatory regions is less frequent than hypermethylation of CpG island promoters in cancer. Some of the gene regions (including transcription control sequences) were associated with loss of DNA methylation. Currently, there are data that sustain that promoter hypomethylation of some genes may be associated with the development of cancer, regulating the activity of genes [157]. For example, promoter hypomethylation of specific immunity-related genes (e.g., cytokine IL-10) may activate the specific gene expression to inhibit the immune response in breast cancer [205], and the promoter hypomethylation of SPAN-Xb, an immunogenic antigen, can induce de novo B-cell response in myeloma cells [206]. However, the biological significance of promoter hypomethylation in cancer is still poorly understood [144]. Hypomethylation of gene promoters must cooperate with other key activators such as transcriptional factors to control gene expression [207, 208].

Promoters may overlap tissue-specific (T-DMR) or cancer-specific (C-DMR) differentially methylated DNA regions [209]. Most of the non-imprinted, autosomal T-DMR promoters are not the main type of vertebrate DNA promoters, and the genes presenting T-DMR promoters become activated after experimentally induced demethylation 5-deoxyazacytidine [209].

Intragenic epigenetic marks have been also involved in normal gene expression regulation and inverse relationships between imprinted gene expression and DNA methylation level was observed [210]. T-DMR regions were found not only inside many genes, but also in downstream promoters, flanking certain subsets of genes [211, 212]. Moreover, besides first exon, T-DMRs are also present at exonic and intronic sequences, insulators, intragenic ncRNA genes, and 3' terminal regions [213, 214].

The role of these regions is to connect DNA and chromatin, inducing tissue-specific chromatin epigenetic marks inside genes [215, 216]. This relationship between DNA and chromatin modification at gene level may help determine alternative promoter usage, modulate the rate of transcription initiation or elongation, and direct the choice of alternative splice sites [217, 218]. For moderately expressed genes, DNA methylation level in the middle of the gene is correlated with higher transcription rates, being related to nucleosome positioning [219]. In genes with CpG-poor promoters, methylated sequences located downstream binds Polycomb repressor complexes [212], which are being associated with repression of promoters [220].

On the other hand, certain histone modifications may direct the choice of splice junction through direct interactions with proteins that mark exon–intron junctions, altering rates of transcription and nucleosome positioning [221, 222]. As we mentioned before, DNA methylation may also be involved in regulating alternative splicing, intron–exon junctions being enriched in sharp transitions in DNA methylation levels [223] (e.g., malignant prostate cancer cells have enrichment of DNA hypermethylation at exon–intron junctions [224]). Therefore, these findings highlight the involvement of DNA methylation levels in determining alternative splicing in tumor cells, suggesting that cancer-associated DNA hypomethylation in intronic and exonic sequences can modulate the amount and type of gene products and thereby contribute to tumor formation or progression.

*TGFB2* gene contains an intronic Alu repeat that was found hypomethylated in some cancer cell lines. Their hypomethylation at this site might be related to the significant upregulation of *TGFB2* gene, being an example of cancer-associated hypomethylation and a target chromatin associated epigenetic changes [225]. *PRDM16* presents gene-body hypomethylation (overlapping an exon) in some of the cancer cell lines, whereas *NOTCH2* also showed gene-body hypomethylation (in a subregion of repetitive DNA).

Gene encoding the protease urokinase (*PLAU/uPA*) is overexpressed and was found hypomethylated along with tumor progression in breast cancers and prostate cancers [157]. Also, other genes were observed to display hypomethylation and transcriptional activation in cancer, *S100A4*, mesothelin, claudin4, trefoil factor 2, maspin, *PGP9.5*, *POMC*, and the heparinase gene [144].

DNA hypomethylation is closely associated with morphological dedifferentiation in thyroid cancers. Four oncogenes (*INSL4*, *DPPA2*, *TCL1B* and *NOTCH4*) were frequently regulated by hypomethylation in anaplastic and medullary carcinoma [226].

*Hematopoietin*, *TNF*, *IL1*, *IL10*, and *IL17* families of cytokines had a significant tendency to be hypomethylated in five cancer types (colon, kidney, stomach, lung, and breast) [227].

Hypomethylation and increased expression in cancer has been shown for *R-RAS* [228]. A strong association of *CDH3* promoter demethylation and *P-cadherin* expression evident with histological grade and invasiveness in breast cancer was observed [229]. In Stage III and IV gastric cancer *cyclin D2* activation is associated with promoter demethylation, activation of *synuclein γ* is associated with progression and metastatic potential in a range of solid tumors, and *maspin* expression in colorectal cancer is associated with microsatellite unstable tumors [230–232].

Hypomethylation and overexpression of some imprinted genes, including the *IGF-II* and *H19* genes, are implicated in carcinogenesis [233–235].

The putative oncogene, *ELMO3*, is overexpressed in non-small cell lung cancer in combination with hypomethylation of its promoter and these cancer-specific events are associated with the formation of metastases [236].

Aberrant hypomethylation and overexpression of *WNT5A* may be functionally important in the progression of prostate cancer. Along with *WNT5A*, *S100P*, and *CRIP1*, which have been

previously implicated in cancer progression, are also regulated at the transcriptional level in prostate cancer by hypomethylation [237].

Evidence is accumulating for the biological significance and clinical relevance of DNA hypomethylation in cancer and for cancer-linked demethylation, and those seem to be highly dynamic processes.

## 5. MicroRNA genes

At present, a special consideration is given to small non-coding RNA molecules (microRNA) to their functions and involvement in human diseases. There are an extensive number of studies that link microRNA alterations to cancer pathogenesis. MicroRNA genes encode for a single RNA strand of about 21 to 23 nucleotides, which regulate gene expression by specifically targeting certain mRNAs in order to prevent them from coding for a specific protein. Some microRNA genes are mapped in chromosomal regions that undergo rearrangements, deletions, and amplifications in cancer. A growing amount of data demonstrates that microRNA genes display a different pattern of expression in various malignancies; they are found up-regulated or down-regulated and therefore can function either as oncogenes activating the malignant transformation (by down-regulating tumor-suppressor genes), or as tumor-suppressor genes blocking the malignant transformation (by down-regulating oncogenes). In numerous types of cancers, many different microRNA have been shown to act as oncogenes, their expression profiling presenting specific signatures associated with malignant transformation. Cancer-associated microRNA molecules are also called oncomir (oncomiR).

The first microRNA that has been proven to act as oncogene in human cancer was *miR-17/92* polycistronic cluster known as *OncomiR-1*, which comprises six microRNAs: *miR-17*, *miR-18a*, *miR-19a*, *miR-20a*, *miR-19b-1*, and *miR-92a-1* [238]. The *miR-17/92* cluster is located in the locus of intron 3 of *C13orf25* gene at 13q31.3, in a region frequently amplified in several types of lymphomas and solid tumor. It has been shown that the locus is amplified and overexpressed in human B cell lymphomas, malignant lymphoma cell lines and in lung cancers especially with small-cell lung cancer histology [239, 240]. Insertional mutagenesis studies using retroviruses indicates that *miR-17/92* acts as an oncogene in T cell lymphomas; it was shown that soon after SL3-3 murine leukemia virus infection, mice developed tumors if provirus integrates into the proximity of the gene encoding *miR-17/92* cistron [241]. Moreover, other studies uncovered that *C-MYC* and *E2F3* gene products may induce *miR-17/92* polycistronic expression through direct binding to the cluster promoter. Two microRNAs belonging to the cluster, *miR-17-5p* and *miR-20a* negatively regulate *E2F1* activity, which confirms that the *miR-17/92* can promote cell proliferation through the exchange of *E2F1* to *E2F3* pro-apoptotic proliferative [242]. Thus, *miR-17/92* represents an anti-apoptotic oncogene and *miR-20a* inhibition using antisense oligonucleotides can induce apoptosis after treatment with doxorubicin [243].

Along with the *miR-17/92* cluster from the *miR-17* family, two other paralogue miRNA gene clusters are produced, *miR-106b/25* and *miR-106a/363*, which possess oncogenic potential and

are known to be involved in wide types of cancers. The *miR-106b/25* cluster is located in intron 13 of the minichromosome maintenance complex component 7 (*MCM7*) oncogene at 7q22.1 and it contains the following three miRNAs: *miR-106b*, *miR-93*, and *miR-25*. Recently, findings sustain the oncogenic potential of this cluster and reports correlate *miR-106b/25* member expression levels with processes such as tumor growth, cell survival, and angiogenesis [244, 245]. The oncogenic potential of the *miR-106b/25* cluster in malignant transformation is achieved by targeting and down-regulation of several tumor-suppressor genes such as *p21*, *E2F1*, and *PTEN* [246–248]. Furthermore, other recent work suggests that in breast cancer cells, *miR-106b/25* cluster overexpression leads to overcoming doxorubicin-induced senescence and cells become drug resistant through a mechanism that involves targeting *E-cadherin* transcriptional activators *EP300* [249]. The second cluster, *miR-106a/363*, is located on chromosome X (Xq26.2) and comprises of six miRNAs: *miR-106a*, *miR-18b*, *miR-20b*, *miR-19b-2*, *miR-92a-2*, and *miR-363*. A series of reports indicates an oncogenic potential for members of the cluster, for example *miR-106a* and *miR-92-2* were found overexpressed in colon and prostate cancer and also in leukemia and Ewing Sarcoma [250–252].

Another important oncomir is represented by *miR-155* found overexpressed in several malignancies: chronic lymphocytic leukemia (CLL), B cell lymphoma, Hodgkin's lymphoma or Burkitt's type, and breast cancer. Some reports have shown that clinical isolates from B cell lymphomas, including those with large cells, contain a number of copies of *miR-155*, about 30 times higher than normal B cells [253–256]. Also, results suggest that the pancreatic ductal adenocarcinomas overexpression of *miR-155* determined decreased levels of *TP53INP1* leading to apoptosis elusion and cell growth development [257].

A promising oncomir is also *miR-21*, one of the most common miRNA associated with human cancers. *MicroRNA-21* high expression has been found in a variety of cancers including breast cancer, brain malignant tumors, glioblastomas, pancreatic, colorectal, liver, gastric, lung, skin, thyroid, ovarian, esophagus, prostate, cervical, and different lymphatic and hematopoietic cancers [250, 258–263]. Elevated *miR-21* levels have been linked to cell proliferation, apoptosis reduction, and cell migration in neoplastic transformation; it has been found that this oncomir targets and down-regulates a number of tumor-suppressor genes including *PTEN*, *PDCD4*, *BCL2*, *RECK*, *JAG1*, *HNRPK*, *BTG2*, *TGFBR1*, and thus sustaining cancer's invasion and metastasis [264, 265]. Moreover, experiments using transfection of MCF-7 cell lines with anti-*miR-21* oligonucleotide conducted to cell growth suppression in vitro and tumor growth in vivo had an increase of programmed cell death rate [266].

Altogether, these studies illustrate a major role for microRNA genes in cancer pathogenesis (Table 2); many of them have oncogenic activity and could represent valuable biomarkers very useful for cancer screening or assessment of the therapeutic effects of anti-cancer treatments.

MicroRNA	Cancer type	miRNA function	Potential targets	Reference
<i>miR-21</i>	Glioblastoma	Increase cell growth	<i>HNRNPK</i>   <i>TP63</i>	[267]
		Inhibit apoptosis		
		Promote cell cycle		

MicroRNA	Cancer type	miRNA function	Potential targets	Reference
		-	<i>RECK1/TIMP3</i>	[268]
	Breast	Increase cell growth Increase metastasis	<i>PTEN</i>	[269, 270]
		Promote cell growth Promote cell migration Promote tumor growth	<i>ANKRD46</i>	[271]
		-	<i>PDCD4</i> <i>PTEN</i> <i>BCL2</i>	[272]
		Promote cell invasion Promote metastasis	<i>TPM1</i> <i>PDCD4</i> <i>SERPINB5</i>	[265]
		Cervix	Increase cell Proliferation Inhibit apoptosis Increase cell migration	<i>CCL20</i>
	Promote cell proliferation		<i>PDCD4</i>	[274]
	Prostate	Promote epithelial- mesenchymal transition	<i>BTG2</i>	[275]
		Induce tumor angiogenesis Activate AKT/ERK signaling		[276]
		Increase docetaxel resistance	<i>PDCD4</i>	[277]
		Promote cell proliferation Promote tumor growth		[278]
	Blood	Inhibit apoptosis Promote cell motility Promote cell invasion	<i>MARCKS</i>	[279]
		Promote cell migration Reduce apoptosis Promote cell growth	<i>PDCD4</i>	[280, 281]
	Prostate	Promote cell cycle G1/S transition Increase colony formation	<i>CDKN1B</i>	[282]
<i>miR-221 and 222</i>	Breast	Increase cell migration Increase cell invasion epithelial-mesenchymal transition	<i>TRPS1</i>	[283]

MicroRNA	Cancer type	miRNA function	Potential targets	Reference	
	Glioblastoma	Increase tamoxifen resistance	<i>CDKN1B</i>	[284]	
		Increase cell invasion	<i>TIMP3</i>	[285]	
	Thyroid	Promote cell proliferation Promote cell invasion inhibit apoptosis	<i>GJA1</i>	[286]	
		Promote cell transition to cell cycle S phase	<i>CDKN1B</i>	[287]	
		Lung	Induce TRAIL resistance	<i>PTEN</i>	[288]
	Increase cell migration		<i>TIMP3</i>		
	<i>miR-155</i>	Blood	Increase cell proliferation Increase clonogenicity Inhibit apoptosis	<i>HDAC4</i>	[289]
			Promote PI3K-AKT signaling	<i>PIK3R1</i>	[290]
			Increase cell proliferation	<i>BCL2</i>	[291]
		Breast	Reduce cell cycle arrest	<i>SMAD5</i>	[292]
Promote cell survival Induce chemoresistance Inhibit apoptosis			<i>FOXO3</i>	[293]	
Promote cell proliferation Promote tumor development			<i>SOCS1</i>	[294]	
Blood			Increase cell growth Increase cell cycle G1/S transition	<i>CDKN1A</i>	[295]
		Increase cell proliferation Increase imatinib - induced cell death		[296]	
		Suppress apoptosis	<i>PTEN</i>	[297, 298]	
<i>miR-17/92 cluster</i>		Retinoblastoma	Promote cell proliferation	<i>p21 and p57</i>	[299]
	Colon	Promote angiogenesis	<i>TSP-1</i> <i>CTGF</i>	[300]	
	Breast		<i>ERa</i>	[301]	
	Lung	Promote cell growth		[302]	
		Reduce reactive oxygen species (ROS) generation	<i>HIF-1a</i>		

MicroRNA	Cancer type	miRNA function	Potential targets	Reference
			<i>HIF-1a, PTEN, BCL2L11, CDKNA and TSP-1.</i>	[303,304]
<i>miR-106b-25 cluster</i>	Breast	Activate TGF-beta signaling Induce epithelial mesenchymal transition Induce a tumor initiating cell phenotype	<i>SMAD7</i>	[305]
	Gastric		<i>E2F1</i>	[306]
<i>miR-191</i>	Liver	Promote epithelial-mesenchymal transition Increase cell migration Increase cell invasion	<i>TIMP3</i>	[307]
		Promote cell proliferation Inhibit apoptosis Promote tumor growth		[308]
	Gastric		<i>NDST1</i>	[309]

(Source: OncomiRDB: <http://bioinfo.au.tsinghua.edu.cn/member/jgu/oncomirdb/>) [310].

**Table 2.** OncomiRs in human cancer

## 6. Concluding remarks

Oncogene activation by structural alteration (chromosomal rearrangement, gene fusion, mutation, and gene amplification) or epigenetic modification (gene promoter hypomethylation, microRNA expression pattern) confers an increased or a deregulated expression. Therefore, cells with such alterations possess a growth advantage or an increased survival rate. Given the fact that expression profiling of these alterations determines specific signatures associated with tumor classification, diagnosis, staging, prognosis and response to treatment, it highlights the importance of studying oncogenes activation mechanisms and the great potential that they hold as therapeutic tools in the near future.

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