JBUON 2021; 26(5): 1723-1734
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
Email: editorial_office@jbuon.com

REVIEW ARTICLE

Basic principles of molecular biology of cancer cell-Molecular cancer indicators

Emmanuel N. Kontomanolis[1], Antonios Koutras[2], Athanasios Syllaios[3], Dimitrios Schizas[3], Sofia Kalagasidou[4], Athanasios Pagkalos[5], Dimitra Alatzidou[6], Pelagia Kantari[1], Thomas Ntounis[2], Zacharias Fasoulakis[2]

[1]Department of Obstetrics and Gynecology, Democritus University of Thrace, Alexandroupolis, Greece. [2]Department of Obstetrics and Gynecology, Alexandra Maternity Hospital, National and Kapodistrian University of Athens, Athens 11524, Greece. [3]First Department of Surgery, National and Kapodistrian University of Athens, Laikon General Hospital, Athens 11527, Greece. [4]Department of Obstetrics and Gynecology, General Hospital Mamatsio of Kozani Greece. [5]Department of Obstetrics and Gynecology, General Hospital of Komotini, Greece. [6]Department of Obstetrics and Gynecology, General Hospital of Giannitsa, Greece.

Summary

Molecular biology of cancer cell is a domain of medical science that is rapidly growing in our days. Knowing the ways and paths that cancer cells follow is crucial to the prevention of cancer itself. Central role to these paths, concerning the cell cycle and the process of apoptosis, has the protein p53. The whole mechanism of the cell cycle is activated by the action of various mitogens, such as growth factors, hormones and cytokines. Carcinogenesis involves alterations of genes (proto-oncogenes and tumor suppressor genes), which encode proteins of the signal transduction. Many of the damages that lead to carcinogenesis may be due to the lack of repressive signals for cell division, but also to the absence of the sensitivity of cells to repressive signals. The cell has mechanisms of receiving apoptotic-antitumor signals and mechanisms of execution of these instructions. A percentage of cancers (4-8%) are etiologically linked to germ (stem) cells mutations and occur at an increased frequency in families (hereditary cancers). Substantial progress in understanding the mechanisms of carcinogenesis, filtration and metastasis of cancer has highlighted the key role of specific genes, primarily oncogenes and tumor suppressor genes.

Key words: *molecular biology, cancer cells, molecular cancer indicators, principles, cancer*

Introduction

Carcinogenesis is a multistage process that etiologically involves mutations in a series of genes that play a role in maintaining the balance between cell proliferation and apoptosis, i.e. maintaining a stable cell mass (number) and also in regulating complex metabolic pathways, which ensure functional and structural integrity of cells and tissues [1]. Involved disorders in the genes could lead to uncontrolled cell growth, breakdown of cellular tissue, invasion of cancer cells into adjacent tissues and, finally, metastasis. Identification of these genes and their corresponding gene products, i.e. proteins, is of fundamental importance in order to elucidate the causative pathogenesis of malignant transformation, provides additional important information and is used in the diagnosis and prognosis of cancer and of the potential success of the therapeutic treatment [2-4]

Corresponding author: Athanasios Syllaios, MD, MSc. First Department of Surgery, National and Kapodistrian University of Athens, Laikon General Hospital, Ag. Thoma 17 street, Athens 11527, Greece.
Tel: +30 6972374280; Email: nh_reas@hotmail.com
Received: 30/09/2020; Accepted: 17/11/2020

The cell cycle and its regulation

Most differentiated cells are in a state of rest (G0), in which they are metabolically active, but do not duplicate their genetic material nor divide. However, if they are affected by a mitogenic stimulus, the cell cycle is activated and the cells go through a series of phases, each of which ultimately serves to replicate the genetic material and ultimately to form two identical daughter cells. These phases are G_1 (Gap1 phase, subdivided into G_{1a}, G_{1b}, G_{1c} and G_{1d}), S phase (DNA synthesis phase), G_2 phase (Gap2) and finally the mitosis phase (M). As long as the mitogen continues to act on the daughter cells, the cycle is repeated, but if not, the cell will enter in the resting phase G0 and remain there until the mitogen affects it again. The transition from one phase of the cycle to another is regulated by key molecules, which must 'remove' any barriers that inhibit this transition (Figure 1) [5,6].

Such a central key molecule is the phosphoprotein RB, a product of the Rb gene [7]. The protein in its non-phosphorylated form suppresses the transition from G1 to S phase, which is why the corresponding gene is classified in the major class of tumor suppressor genes (Table 1). The repressive effect of the RB is due to the fact that its non-phosphorylated form binds to and inactivates transcription factors (E2-E1, DP1). These factors are involved in the transcription of genes that encode proteins either involved in DNA replication, or are structural components of the cell, or are transcription factors that activate other genes in the cell cycle. Upon phosphorylation of RB, it breaks down its complex with the transcription factors E2-F1 and DP1 and after being released it could activate transcription. Phosphorylation of RB is mediated by a specific phosphokinase, which is activated after binding to a specific protein, cyclin (which is why phosphokinase is called cdk, cyclin dependent kinase). There are various cdk kinases and various cyclins (A-E and H). In the specific case of RB activation, phosphorylation is initially encoded by the cdk4 / 6 complex with cyclin D and then the cdk2 complex with cyclin E (Figure 1) [6,8,9].

Important for the transition from G2 phase to M phase is the involvement of MPF (Maturation Promotion Factor), which represents a complex of cdk1 and cyclin B. This phosphorylates various proteins, such as histone H1, lamins and proteins of the mitotic spindle. The affection of the protein CAK (cdk-activating kinase) is necessary for the complete activation of cdk phosphokinases. Cyclins are involved in other functions such as meiosis, differentiation and apoptosis [8,10].

Cell cycle regulation also involves cdk kinase inhibitors, the CKI (cyclin dependent kinase inhibitors) belonging to two families, the Cip / Kip family (proteins p21, p27, p57) and the Ink4 family (proteins p15, p16, p18 and p19). These inhibitors act by forming complexes with kinases [9,11].

Central to both regulation of the cell cycle and apoptosis is protein p53, a product of the p53 tumor suppressor gene. This tumor suppressor protein acts in suppressing the transition from G1 phase to S phase. This is achieved indirectly by acting as a transcriptional activator of the genes, which encode the repressive proteins of the cycle (such as the p21WAF-1 protein that inactivates cdk kinases

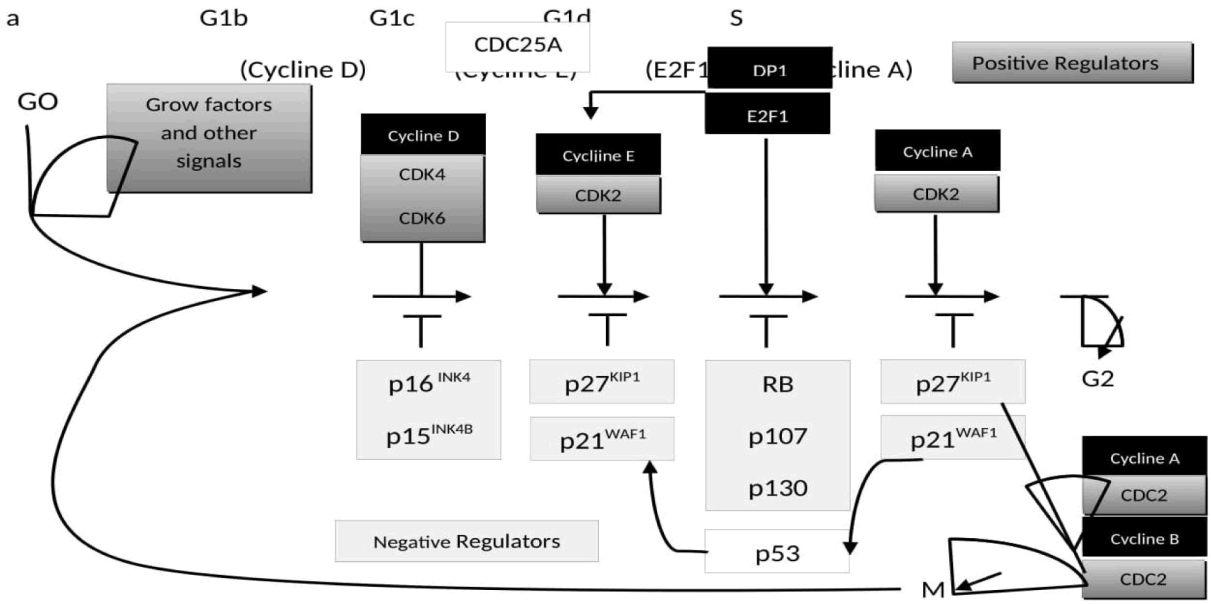

Figure 1. Cell cycle and its regulation.

and the MDM2 protein that binds to p53 and inactivates it) and Bax and Fas proteins, which have an apoptotic role, and proteins involved in DNA repair. DNA damage, hypoxia, viral cell infiltration and damage due to RB inactivation, lead to increased p53 levels, resulting in the inhibition of the cell cycle. The purpose of this inhibition is to give the cell time to repair DNA damage. If this is not achieved, the cell enters in the process of apoptosis (programmed cell death). P53 probably also suppresses the transition from G2 to M.

Activation of the cell cycle

The whole mechanism of the cell cycle is activated by the action of various mitogens, such as growth factors (PDGF, EGF, FGF, etc. which are proto-oncogene expression products) [10] hormones and cytokines. Mitogenic signals also originate from constituents of the matrix and from intracellular contact molecules (integrins) [12-14].

Proto-oncogene expression products, such as various transcription factors and cyclins transduct mitogenic stimulus from the membrane to the nucleus (Table 2). These genes in their mutated form (oncogenes) are involved in the process of carcinogenesis. Growth factors trigger the mechanism of cellular proliferation through interaction with membrane receptors, which represent transmembrane proteins, with an extracellular domain that binds to the growth factor, a transmembrane domain and an intracellular domain, which is involved in the signal transduction through the phosphorylation of a series of proteins. The receptors may either have tyrosine phosphokinase activity themselves or indirectly activate phosphokinases coupled with G-proteins, tyrosine or serine-threonine kinases. Whatever the type of receptor is, the mitogenic stimulus is transmitted inside the cell and reaches the nucleus through a ''cascade'' of protein phosphorylations, many of which are themselves phosphokinases. Eventually, specific phosphokinases enter the nucleus, phosphorylate and activate a number of proteins involved in the cell cycle (such as cyclins and transcription factors) [10,15,16].

Such an example is the signal transduction of growth factors TGFa and PDGF, in which are involved not only the receptors of these factors, but also SOS-Ras-Raf-MEK and MAPK (cytoplasmic) proteins and fos-jun-myc transcription factors (of the core). Signal transduction could also be initiated by the extracellular matrix, through integrins and finally through the previously mentioned Ras - MAPK cascade. There are many such signal transduction systems that are activated by different growth factors and interact [17].

In addition to the stimulatory signals of cell division, the cell receives several repressive signals. One of the most important is the TGF-β factor, which through activation of its receptor and Smad4 cytoplasmic proteins, leads to the activation of gene transcription and synthesis of the corresponding p15 and p21 proteins. Thus, it inhibits the formation of complexes between cyclins and cdk, hence the phosphorylation of RB. Additionally, TGF-β suppresses the expression of the c-myc gene, a transcription factor that is positively involved in the regulation of the G1-phase of the cycle [18].

Disorders of the cell cycle activation-inactivation mechanisms and of the cycle key components leading to uncontrolled hyperactivity

Carcinogenesis involves alterations of genes (proto-oncogenes and tumor suppressor genes), which encode proteins of the signal transduction and the cell cycle.

Table 1. The most important tumor suppressor genes/proteins

Genes	*Protein*
p53	53kDa phosphoprotein, transcription factor, induces p21 protein, (cdks suppressor)
Rb (retinoblastoma)	105kDa phosphoprotein, inactivates (in the non-phosphorylated form) the transcription factor E_2-F1
Wt (Wilm's tumor)	35kDa protein, contains 4 Zn fingers, transcription factor
BRCA1 (Breast cancer gene 1)	220kDa protein, transcription factor, co-stimulator of p53
BRCA2 (Breast cancer gene 2)	Protein 2329 of amino acids, DNA correction
APC (adenomatosis-polyposis colon genes)	300kDa protein, inhibits the formation of a β-catenin complex with transcription factor Tcf-4 and thus its induction to c-myc
DCC (deleted in colon cancer gene)	190kDa transmembrane protein
NF-1 (neurofibromatosis gene)	250kDa protein, homology with protein which activates GTPase

Table 2. The most important oncogenes/oncoproteins

Gene	*Protein*
a) Growth factors	
C-sis	PDGF platelet derived growth factor, b-chain
Int-2	fibroblast growth factor (FGF-3)
hst	fibroblast growth factor FGF-4)
b) Growth factor receptors, membrane	
c-erbB	epidermal growth factor receptor (EGF-R)
HER2/neu (crbB2)	receptor structure with tyrosine phosphokinase activity
RET	amputated receptor, tyrosine phosphokinase
ros	receptor structure
fms	mutated receptor CSF-1
c) Phosphokinases, non-receptors, membrane-bound	
src	
yes	
fgr	
lck	
d) G-proteins, membrane-bound	
H-ras	GTPase
K-ras	GTPase
N-ras	GTPase
e) serine-threonine phosphokinases, cytoplasmic	
raf/mil	
pim-1	
cot	
mos	
f) transcription factors	
myc	
fos	
jun	

(Diamandis, 1992, with variations)

Various gene alterations include point mutations (such as change of codon 12 [GGC] of the H-ras gene into GTC in bladder cancer), total or partial deletion of the gene (loss of heterozygosity, in case of mutation), the insertion of sequences that can inactivate or activate genes depending on the insertion site (within the gene or its regulatory parts), gene translocation (such as that of c-myc next to immunoglobulin genes, resulting in overexpression) and gene amplification ie. increase in the number of copies of a gene (such as the c-erbB-2 in breast and ovary cancer) (Table 3) [15,19-21].

These alterations are partially reversible thanks to the existence of a DNA repair mechanism involving the hMS1, hMS2, hPMS1 and hPMS2 repair genes and their corresponding repair enzymes that they encode. However, these genes can also mutate and be inactivated, thus

Table 3. Various genes / proteins involved in the etiopathogenesis of carcinogenesis, cancerous growth, invasion and metastasis (many of which are controlled as biological indicators)

Intercellular communication proteins (Cadherins, Type I and II, catenin α and β, integrins), fibronectins
Angiogenic Endothelial Agents (such as the vascular endothelial growth factor, VEGF), and its Receptor (VEGF-R), FGF and EGF
Kallikrein family (gene KLK_3 encodes PSA)
Other proteases (MMP-II, MMP-9, MMP-2, which break down the matrix and collagen), cathepsins
Telomerase
Genes encoding repair enzymes (mismatch repair genes) $hMSH_1$, $hMSH_2$, $hPMS_1$, $hPMSH_2$.
Genes and Proteins Positive or Negative in Apoptosis (Positive: Bcl, BclXL, MC-1. Negative: Bax, Bad, BcX5)

removing the repair capacity of the cell and promoting carcinogenesis [22].

Mutations in oncogenes and tumor suppressor genes make cell proliferation being independent of extracellular mitogenic signals. Some cells (glioblastomas and sarcomas) produce growth factors, such as PDGF and TGFa, by themselves, thus creating a permanent autocrine cycle [23,24].

Another way of self-regulation is the disruption of growth factor receptors, due to either overexpression or mutations. In the first case, the cell becomes sensitive to concentrations of growth factors that would not normally lead to mitosis: if the production of receptors is excessive, it could lead to mitosis and absence of growth factors. In the latter case, the receptor is transformed into a constitutively active molecule, activating the signal transduction process in the absence of growth factor. Spontaneous mitogenic stimuli may also result from disorders of cellular interconnection molecules, such as integrins and beta-catenin [25].

Many of the molecules involved in signal transduction are involved in the carcinogenic process in their mutated form. Typical examples are the Ras mutant protein, which activates the next steps of the signal transduction cascade without itself being previously stimulated by mitogens. It is possible that some of the disorders leading to the induction of spontaneous mitogenic signals originate from the layer's cells [26].

Many of the damages that lead to carcinogenesis may be due to the lack of repressive signals for cell division, but also to the absence of the sensitivity of cells to repressive signals. Damages related to TGFβ, which normally suppresses cell division, are well-studied and involve either its downregulation, or its mutation leading to function loss [27]. Moreover, the absence of functionality of some integrins, which normally also send repressive signals, contributes to the cancer phenotype. The mutation of proteins, that transmit repressive stimuli from the cell membrane to the nucleus, has a similar effect (eg mutation of the Smad4 protein) [28].

Disorders in cell cycle components, which are located in the cell nucleus, such as RB protein, cyclins, cdk kinases and transcription factors are significant and well-studied. Mutations in the Rb gene, observed in many cancers (colon, small cell lung, esophagus, breast, prostate) (Table 3), abolish the ability of the molecule to form a complex with the transcription factor E2F-1, which is free to act constitutively [7,29].

Mutations of CDK-4[1] in its interaction with the p15 INK4B suppressor protein may render it unable to accept repressive signals. Many mutations in transcription factor genes (fos, myc, etc.) that lead to activation of these oncoproteins are often observed in various types of cancers [30,31].

The role of telomeres and telomerase in carcinogenesis

Hayflick's work has shown that cells in culture have limited cell division capacity, ranging from 60-70 divisions for most cell types. It seems that the telomeres-telomerase system plays a role in limiting the reproductive process [24].

The ends of chromosomes consist of thousands of repeated sequences of 5-6 nucleotides, the telomeres, which exert protective action on the chromosomes. At each cell division, 50-100 nucleotide sequences are lost from the ends of these telomeres. Thus, after a certain number of cell divisions, the telomeres lose their ability to exert protective action on the ends of chromosomes, which are then closely coalesced, causing karyotype disorders and eventually the cell death [32]. Cancer cells show increased expression of the telomerase enzyme which adds repetitive hexanucleotides to the ends of the DNA and restores telomere length and cell division ability. The importance of telomeres and telomerase is reinforced by the fact that expression of telomerase in cell transfection experiments renders them immortal. Similar results have been obtained with transgenic animals [33].

Apoptosis

As stated in the Introduction, the rate of apoptosis (programmed cell death) plays a key role in maintaining a stable cell number. The cell has mechanisms of receiving apoptotic-antitumor signals and mechanisms of execution of these instructions. Insulin-like growth factors IGF-R and IL-3R act in an anti-apoptotic way through the IGF-R and IL-3R receptors, respectively, whereas FAS and TNFα act in an apoptotic way through the FAS TNF-R1 receptors, respectively [5]. Intracellular mechanisms determine whether or not the cell is functioning properly and, in the event of DNA damage, oncogenic activity, survival factors deficiency or hypoxia, activate the apoptotic pathway by releasing the mitochondrial cytochrome C, which activates a series of proteases, the caspases, which eventually, selectively disrupt subcellular structures, organelles and genome [34,35]. Many proteins act on apoptosis via mitochondria and the release of cytochrome C, such as Bax, Bak, Bid, Bim (apoptotic) and Bcl-2, B cl-XL, Ccl-W (anti-apoptotic). In the event of DNA damage and its non-repair, p53 promotes apoptosis by enhancing Bax expression. In contrast, overexpression of bcl-2, as in the translocation of the gene to a strong transcription site, acts antagonistically and positively on oncogenesis [36].

Resistance to apoptosis is achieved in several ways. One common way is to mutate p53 and its corresponding protein (observed in 50% of cancers). The anti-apoptotic mechanism could also be activated by extracellular factors (IGF-1/2, IL-3), by signals from Ras, or by loss of the pTEN tumor suppressor gene. Another mode of anti-apoptotic action is through a mutant FAS death factor receptor, such as in colon and lung cancer, which in the mutant form does not understand the apoptotic messages of FAS. It is very likely that all cancer cells have mutations that allow them to bypass apoptosis [37,38].

Genes involved in tumor perfusion, invasive process and cancer metastasis

The first phase of carcinogenesis is followed by an increase in cell mass, tumor cell invasion into adjacent tissues and vessels and metastasis (migration-installation into distant tissues and organs). Cell migration is based on invasion of the cytoplasmic membrane on the guiding side of the cell, formation of new extracellular adhesion sites on the guiding part of the cell, release of adhesion sites at the back, and contraction of the cytoskeletal elements [1].

A number of genes are activated to accomplish these processes, encoding, inter alia, autocrine motility factors, angiogenesis factors and their receptors (e.g. VEGF, VEGF-R), proteases (collagenases, kallikreins), cell adhesion and interconnection molecules and growth factors (Table 4). The importance of these genes in the above processes is demonstrated in their neutralization experiments and their expression products (with antisense RNAs, with antibodies, etc.), and from pilot clinic applications in various types of cancers, leading to reduced tumor growth and shrinkage [24].

Carcinogenesis as a multistage process

Many of the aforementioned genes are involved in the causative pathogenesis of malignant transformation and the manifestation of the cancer phenotype. In some cancers, it appears that specific mutated genes are involved in a defined timing. The case of colon cancer is characteristic, where the conversion of normal intestinal epithelium, through intermediate stages (adenomas), into metastatic tumor cell involves activation of oncogenes and inactivation of tumor suppressor genes, in a particular sequence (FAP - Ras - DCC - p53) [39]. Likewise, in the case of endometrial cancer, the conversion of normal cell, through a stage of hyperplasia, into cancer and cancer-metastatic cell involves sequential activation of the ras oncogene, inactivation of the p53, DCC and Rb oncogene-suppressor genes and finally activation of oncogenes of erbB2, myc and Sms. In other cases, it appears that gene alteration is accidental and only the cumulative sum of the gene lesions, irrespective of their timing, is significant. This explains the high incidence of cancer in old age [40,41].

The utilisation of genes and their expression products involved in the etiology of cancer as biological cancer indicators

Progress in elucidating the mechanisms of carcinogenesis and identifying the involvement of many genes (such as oncogenes and tumor suppressor genes) and their expression products in the carcinogenic process, as well as the introduction of sensitive molecular detection techniques of slight

Table 4. Frequency (%) of oncogene activation or tumor suppressor gene inactivation in adult cancers

Cancer	*Oncogenes*			*Tumor suppressor genes*			
	Her2/neu	*ras*	*myc*	*p53*	*Rb*	*APC*	*DCC*
Exocrine pancreas cancer	10-20	75-90	40	20	30	50	
Colon, colorectal cancer	10-20	65	5-65	70-75	35	70-80	70-80
Small cell lung cancer	rarely	0	25	99-100	95-100	-	-
NSCLC*	55-60	40(adeno)	48	50	10-20	30	15
Ovarian cancer	30	20-25	1-40	50	10	rarely (LOH)**	35
Breast cancer	25-40	rarely	20-30	25-50	20	5-10 (LOH)**	30
Prostate cancer	70-80	0-5	50	10-20	25	20	25
Oesophageal cancer	20	rarely	5-10	33-50	35-50	5-15	5-10
Gastric cancer	20	20	5-20	20-50	rarely	20	50

*NSCLC = non small cell lung cancer, **LOH = loss of heterozygosity, (from Lyerly and Sullenger, 1994)

quantities of DNA, RNA or protein, have been determinants for the utilization of the aforementioned biological cancer indicators genes / proteins [24]. An ideal cancer indicator is one that ensures early diagnosis, has predictive value for disease progression and detection of recurrence, has predictive value for treatment, is suitable for population control, is readily measurable and easy to use and is not costly. None of the indicators used today have all of these properties, a disadvantage bypassed with the utilization of more than one indicator. It should be emphasized that genes/proteins may be defective in different types of cancer, but damage to specific genes is characteristic of some forms of cancer [15,42].

The methodology for detecting biological indicators allows the detection of molecular damage at the level of DNA, RNA and protein. The method of qualitative and quantitative polymerase chain reaction is important (PCR), combined with mutation detection techniques (such as single stranded spatial polymorphism (SSCP), restriction fragment length polymorphism (RFLP), etc.) and DNA sequencing. At the detection level of the protein, immunochemical methodology using polyclonal or monoclonal antibodies in the form of western blots provides significant sensitivity. These techniques could also be applied at the cell/histological level, on fine-needle biopsies or even on circulating cellular elements [43,44].

According to Wang et al microRNAs are endogenous single-stranded non-coding small RNA molecules that can be secreted into the circulation and exist stably. They usually exhibit aberrant expression under different physiological and pathological conditions [45].

As of late, there were different types of microRNAs described as potential biomarkers used for screening cancer (Figure 2). This means that from the moment that the levels of distinct microRNAs are abnormal, they can be observed at the beginning of cancer, during its progression and, also, after metastasis. Moreover, they are less or non-invasive and can be obtained by liquid biopsies (i.e. via urine, saliva, semen, or breast milk) without causing severe damage and pain to people [46]. Additionally, when the pattern of circulating microRNAs becomes dynamically expressed, it can be associated with tumor progression and aggressiveness.

On the other hand, the differences of the potential microRNA markers between healthy people and cancer patients are, usually, tiny. For instance, in blood sampling methods there should be a careful microRNA consideration, as their levels do not differ significantly between artery and vein. Generally, microRNAs can be either clearly related to cancer (oncomiR) – in a certain cancer type – or a suppressor in another [45].

To be more specific, for instance regarding breast cancer, there are two miRNAs miR-155 and miR-195 that were reported in studies as potential biomarkers, but even them are not still importantly altered in order to be fully used in an early detection of breast cancer; they are indicative, though [45,47,48]. Moreover, as far as the ovarian cancer is concerned, the miRNAs highly expressed in patients suffering from ovarian cancer are serum miR-21, miR-29b, miR-92, miR-93 and miR-126, while miR-21, miR-92 and miR-93 have been indicated before CA-125 (the only diagnostic index so far indicating ovarian cancer) started to increase; this showcased that those

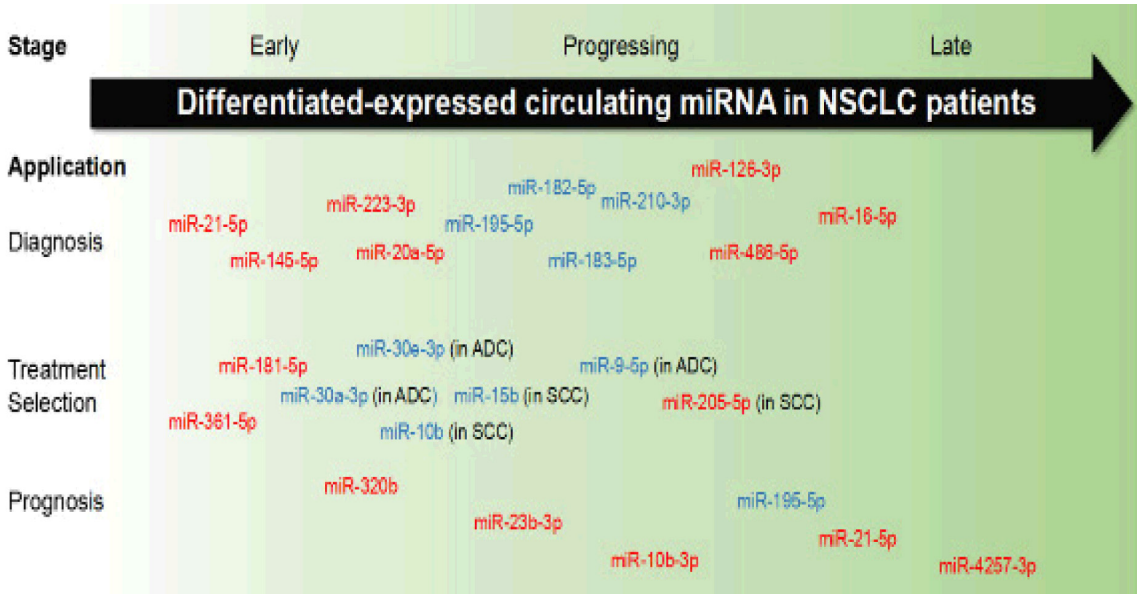

Figure 2. Differentiated-expressed circulating miRNA in NSCLC patients.

three miRNA types could be used as biomarkers for an early ovarian cancer diagnosis [49-51].

All in all, it is important to implement microarrays, which allow a simultaneous evaluation of the expression of thousands of genes and, on this basis, a possible classification of tumors into categories for their aggressiveness or response to treatment. Proteomic, or simultaneous analysis of many proteins with potential prognostic or predictive importance, will have a wide application [52]. Of the many genes / proteins studied, only a few have proven to be important biological indicators in sporadic cancers. On the contrary, the contribution of specific genes as indicators in cases of hereditary cancers is valuable (see the section 'Biological Indicators in Hereditary Cancer'). Table 3 shows the frequency of activation of oncogenes and inactivation of tumor suppressor genes in certain adult cancers [24].

In lung adenocarcinoma, K-ras mutation is a prognostic indicator of malignancy. In almost all cases of small cell carcinomas, inactivation of p53 and Rb tumor suppressor genes is observed [53]. In 27% of non-small cell cancers (NSCLC), overexpression of the mos oncogene (encoding threonine-serine phosphokinase) is observed. The expression is greater in stages II and III (34%) than in stage I (17%), without expansion of the gene. There is a correlation between increased gene expression and decreased apoptosis. Lesions of p53 were found in 86% of tumors [54].

Pancreatic cancer has a high incidence of ras oncogene activation and APC tumor suppressor gene inactivation [55].

In colon cancer, inactivation of p53, APC and DCC tumor suppressor genes and activation of ras and myc oncogenes are characteristic [56].

In ovarian cancer, increased expression of EGFR and expansion and overexpression of Her-2 / neu (c-erbB2) are poor prognostic indicators. In cases of c-erbB2 overexpression, the oncoprotein is also detected in the serum in equivalent quantities with CAI25 indicator. Colony-activating factor (CSF-1) is also detected in patient serum and the tumor overproduces mutant c-fms (CSF-1 receptor). Overexpression of K1-ras and expansion and overexpression of c-myc is also observed in about 25% of the cases [57,58].

Molecular biological indicators have proved very useful in both sporadic and hereditary breast cancers. The proto-oncogene HER-2 / neu and the corresponding oncoprotein have been found in non-invasive breast cancer, at 5-55% (average 26%). There is gene expansion in 1/3 of cancers, which could be detected with Southern blot, with PCR, as well as with *in situ* hybridization with fluorescence techniques (FISH) [53,59].

In invasive porcine cancer, the HER-2 / neu gene is overexpressed in 15% of the cases, and in invasive non porcine (*in situ*) in 56%. Gene overexpression is associated with age, grade of differentiation (higher expression in low-grade differentiation of cancer and lower expression in high-grade), absence of estrogen and progesterone receptors, increased expression of cathepsin D, p53 overexpression, tumor size, lymph node invasion and ploidy (higher expression in tetraploid cells compared to diploid ones). Also, overexpression of HER-2 / neu is associated with a poor response to endocrine therapy. The results are contradictory as far as chemotherapy is concerned [60,61]. The therapeutic potential of HER-2 / neu overexpression, using monoclonal antibodies instead of protein (Herceptin), is promising.

C-myc expansion is observed in approximately 1/5 of breast cancers, whereas in patients with metastases the expansion is found in 1/3 of the cases and has been associated with early relapses and a short survival period, especially in tumors negative for estrogen receptors without lymph node invasion. The myc gene is an independent prognostic indicator of reduced survival and characterises expansive tumors. Its predictive value for endocrine therapy is insignificant, whereas a better response to chemotherapy has been observed in tumors without gene expansion than in those with expansion [62-64].

P53 is the most frequently mutated gene (50% of the cases) in breast cancer. The detection of oncoprotein in the nucleus is almost always evidence of a mutant gene leading to an increase in the half-life of the protein. However, some mutations do not affect the half-life of the protein, so analysis at the gene level is necessary. Mutations may concern one or both alleles, with or without loss of heterozygosity. Mutations of p53, mainly at some conserved sites, are associated with a poor prognosis and are an independent prognostic indicator and go along with the absence of estrogen / progesterone receptors, a greater grade of differentiation (III> I) and increased cell division. In terms of their predictive importance, endocrine therapy (tamoxifen administration) is less valuable in patients with mutations in p53, than in those without mutations. In contrast, chemotherapy resulted in increased survival in p53-positive patients, although this observation is also disputed [65,66].

Overexpression of bcl-2 has been associated with a good prognosis, whereas low expression of bax with a poor one.

In prostate cancer, many gene disorders are observed, probably in a specific order, which characterizes the stage of the carcinogenic process. Thus, cells in the pre-cancerous stage (carcinogenic initiation stage) show c-myc expansion, overexpression

Table 5. Genes involved in hereditary cancer

Gene	*Disease*
Rb	Retinoblasoma
APC	Familial adenomatous polyphagia (FAP)
$hMSH_2$, $hPMS_2$	Hereditary nonpolyposis colorectal cancer (HNPCC)
BRCAI, II	Breast cancer, Ovarian cancer
RET	Multiple endocrine type 2

of bcl-2, c-erbB2 and EGFR and, in 25% of the cases, telomerase activation. Activation of telomerase (in 75% of cases), ras activation and loss of E-cadherin are observed in cancer cells at the promotion stage, whereas invasive metastatic cells overexpress the mutant tumor suppressor gene p53 and the angiogenic factor FGF. Overexpression of p53 is associated with aggression, risk of relapse and a high degree of Gleason [67-69].

The expansion of N-myc into the neuroblastoma, the fourth most common pediatric tumor, is characteristic. The degree of the expansion is associated with tumor aggression and is an indication for a more aggressive treatment [70,71].

To sum up, all cancer types have their own expression and mechanism and their own biomarkers that can be used as tools to an early diagnosis and monitoring of the disease course. Here is where the need for better proteomics understanding emerges as it could be extremely helpful to the whole issue of cancer comprehension and treatment.

Proteomics is the use of quantitative protein-level measurements of gene expression to characterize biological processes (e.g., disease processes and drug effects) and decipher the mechanisms of gene expression control [52,72].

Proteomics – or in this case – oncoproteomics are proteins related to cancer cell pathogenesis understanding. They help clinical practice, including diagnosis as well as screening via the mechanism of proteins in order to assist early cancer prognosis [52].

Biological indicators in hereditary cancer

A percentage of cancers (4-8%) are etiologically linked to germ (stem) cells mutations and occur at an increased frequency in families (hereditary cancers). Sufferers inherit a mutated tumor suppressor gene: however, the tumor suppressor protein produced by the healthy allele prevails and, thus, the phenotype appears normal. However, during the life of a person, the loss of the other allele occurs (LOH, loss of heterozygocity) and consequently the loss of its normal product expression, which results in the development of cancer [23,53,73].

The most important hereditary cancers include breast cancer, retinoblastoma, Wilms tumor, thyroid cancer and hereditary non-polyposis colorectal cancer (Lynch syndrome, HNPCC). Table 5 lists the genes whose mutations characterize specific hereditary cancers. Detection of the mutations in these cases is crucial in monitoring individuals and taking precautions and early therapeutic measures (such as breast or thyroid removal, etc.) [29,62].

Hereditary breast cancer accounts for 1/4 of all breast cancer cases diagnosed before the age of thirty [31]. In 45% of hereditary cancers, there is a mutation in the BRCA1 tumor suppressor gene and in the other 45% there is a mutation of the BRCA2 gene [20,74,75]. Both genes are stimulated by estrogens. Their expression products (Table 1) act as transcription factors, and BRCAI suppresses estrogen-induced transcription. Mutations, which affect different areas of the genes, lead to non-functional proteins or proteins that inhibit the action of the corresponding physiological proteins (dominant mechanism of action). The predisposition to breast cancer is caused by the existence of the mutated allele, while an upcoming loss of the normal allele (loss of heterozygosity) leads to carcinogenesis [75,76].

Conclusion

Substantial progress in understanding the mechanisms of carcinogenesis, filtration and metastasis of cancer has highlighted the key role of specific genes, primarily oncogenes and tumor suppressor genes and their expression products in these processes and led to the effort of using them as biological indicators.

In the case of hereditary cancers, the clinical use of molecular biological indicators has proven to be important and in many cases life-saving. In sporadic cancers their benefits mainly focus on their prognostic and predictive importance. It is important to apply the microarrays method in this field, where thanks to the sequencing of the human genome and its consequent ability to simultaneously analyze the expression of thousands of genes, it would be possible to classify cancers in different behavioral groups based on their gene expression patterns and the association of these patterns with possible prognosis and prediction. Significant developments are also expected regarding the ability of gene analysis in individual circulating (pre) cancer cells to timely detect a potential cancer process.

Conflict of interests

The authors declare no conflict of interests.

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