Introduction

Biochemical basis for the translation of extracellular signals into intracellular events is the result of a complex communication of molecules in signal transduction cascade, which, depending on the tissue, and after signal detection, amplification and integration, determines the specific type of biologic response. Cell signaling mediated by oxidative stress (redox signaling) significantly influences growth, differentiation and cell death, and dysregulation of these processes can lead to disorders such as cancer and metabolic dysbalances. Redox modification of nuclear proteins (transcription factors, kinase and phosphatase enzymes) alters the affinity of the proteins for an appropriate place in the gene promoter, which induces altered gene expression. Free radicals are important as actuators, transmitters or modifiers of cellular response in the complex system of information transfer through the cell. Free radical action on the process of cell signaling is most commonly effectuated through multiple and joint pathways of signal transduction, which have not yet been fully elucidated in eukaryotes, as well as sensory places and cell response. Antioxidants and modulators of redox cell signaling can provide additional therapeutic effect and act in synergy with ongoing treatments of the diseases and conditions having redox stress as the common denominator.

Cellular signaling in physiologic processes

Thus far the knowledge on the signal transmission through the cell to the target places includes the well studied receptor structure and function of secondary messengers; however, structure and function
of the proteins providing specific cellular response interacting with each other and with secondary messengers is still insufficiently studied. Regardless of the nature of signal molecules, all signaling pathways at particular transmission levels use non-covalent protein-protein interaction or covalent modification (most commonly phosphorylation). Therefore, posttranslational protein modification can be regarded as a key event in cell signaling, and systematic analysis of the proteomics (all protein products of gene expression) and defining of the protein-protein as well as protein-DNA/RNA interaction will enable identification of new disease markers and application of proteins as specific therapeutic target molecules.

In the close future, further development of technology in the field of gene expression analysis will be redirected from the studies of structure and sequence of genes (genomics) to the technology of analysis of gene expression products – mRNA and proteins (transcriptomics and proteomics). Approximately 35.000 genes in the human genome are translated into 200.000 different proteins produced on account of alternative splicing. Further protein amplification occurs through their posttranslational modification, so that the total number of proteins in the human cell is over 1.000.000. In contrast to the genomics structure which is to a large extent unchanged, the content and type of proteins within each cell undergo significant changes depending on the gene activity and specific processes of posttranslational protein modification. Due to the fact that different messages can be generated from the same gene, the new gene definition: one gene – multiple related proteins (polypeptide chains), i.e. multiple protein isoforms, more clearly reflects the functional relationship genome – transcriptome – proteome.

Signaling generally follows the sequence (1):
• synthesis and secretion of signal molecule (messenger) as a response to the stimulus;
• transport of chemical messenger by blood or other extra cellular fluids to target cells;
• specific binding of messenger to the target cell (a plasma membrane receptor or intracellular receptor);
• signal reading i.e. detection and cell response;
• signal termination.

The means of signal termination is an exceedingly important aspect of cell signaling, and failure of a message contributes to a number of diseases, such as cancer.

The actions of chemical messengers are often classified as endocrine, paracrine, or autocrine. Endocrine signaling molecules, hormones above all, are secreted by endocrine cells and these messengers are transported by blood to specific target cells at a distance from the endocrine glands. In contrast to endocrine hormones, paracrine actions are those performed on nearby cells, and the location of the cells plays a role in the specificity of response. Autocrine actions involve a messenger acting on the cell from which it is secreted, or on nearby cells of the same type as the secreting cells (2). Three major signaling systems in the body employ chemical messengers: the nervous system, the endocrine system, and the immune system. The immune system messengers are called cytokines. The different classes of cytokines (interleukins, tumor necrosis factors, interferon, and colony-stimulating factors) are secreted by the immune system cells and usually alter the behavior of other cells in the immune system activating the transcription of genes for proteins involved in the immune response.

The eicosanoides (including prostaglandins, PG, thromboxanes, and leukotrienes) control cellular function in response to injury. These compounds are all derived from arachidonic acid that is usually present in the cells as part of the membrane lipid phosphatidylycholine. The eicosanoids act principally in paracrine and autocrine functions.

Growth factors are polypeptides that function through the stimulation of cellular proliferation. Some growth factors are considered hormones, and some have been called cytokines. Each of the chemical messengers has its own specific receptor, which will usually bind no other messenger.

Most receptors fall into two broad categories: intracellular receptors or plasma membrane receptors. Most signaling molecules (chemical messengers) due to their hydrophilic nature (protein hormones, growth factors, mediators of cellular proinflammatory and immune response, neurotransmitters) cannot pass the cell membrane, but indirectly regulate the activity of intracellular proteins binding to the cell surface receptors. Other liposoluble signaling molecules (steroid hormones, thyroid hormone, retinoic acid, vitamin D, NO, H2O2) easily pass the cell membrane and directly interact with cytosolic or nuclear target proteins. Most of the intracellular receptors for lipophilic messengers are gene-specific transcription factors. A transcription factor is a protein that binds to a specific site on DNA and regulates the rate of transcription of a gene (i.e. synthesis of the mRNK). Lipophilic messengers use intracellular gene-specific transcription factors primarily in the nucleus, although some are found in the cytoplasm. When the activating ligand binds to the receptor, the receptor undergoes a conformational change and the complex (including bound ligand) translocates to the nucleus, where it binds to a portion of the DNA called the hormone response element.

Polar chemical messengers such as peptide hormones, cytokines, catecholamines, growth factors, cannot rapidly cross the plasma membrane and must bind to a plasma membrane receptor. Based on
the plasma membrane receptor’s general structure and means of signal transduction, they are grouped into the categories of ion channel receptors, receptors that are kinases or bind kinases, and receptors that work through second messengers. The pathways of signal transduction for plasma membrane receptors have two major types of effects on the cell: (1) rapid effects on cellular ion levels or activation/inhibition of enzymes and/or (2) slower changes in the rate of gene expression for a specific set of proteins. Many of the messengers produce both kinds of effects.

**Redox state and carcinogenesis**

Recent literature data (3–5) indicate that over expression and activation of a certain pathway of signal transduction through the cell represent the generally accepted cancerogenesis theory, regardless of whether genotoxic or epigenetic (nongenotoxic) agents are the primary acting ones. Genotoxic agents usually refer to chemicals that directly damage genomic DNA, which in turn can result in mutation and/or clastogenic changes. Nongenotoxic genetic compounds appear to function through non-DNA reactive or indirect DNA reactive mechanisms. Although much less is known about the exact mechanism of action of nongenotoxic carcinogens, they modulate cell growth and cell death. Malignantly altered cells, losing the control of reception, processing and transmission of signals, represent genetically unstable clones in which the factors determining specific and unspecific antitumor activity are ineffectively functioning. Stimulation of neovascularization provides nourishment to the malignant cells and enables their survival. Malignant cells have the following characteristics (6):

- **clonal origin** – all cancer cells derive from one original tumor cell which by proliferation produces malignant cell clones;
- **autonomy** – growth of malignant cells escapes the control of regulatory mechanisms of adjacent cells;
- **anaplasia** – loss of co-ordinated cell differentiation;
- **metastasizing** – propensity for continual growth and multiplication, as well as widespread dissemination.

Experimental evidence supports an important role for reactive oxygen species in the cancer process. Oxidative stress occurs in the situations of imbalance between reactive oxygen species (ROS) and cellular antioxidants, as the consequence of either increased ROS production or reduced antioxidants, or in both instances. Abundant evidence indicates that ROS can provoke not only proto-oncogene/suppressor gene mutations, but can also have an impact on cellular proliferation, differentiation and apoptosis (7, 8, 9, and 10). The signaling pathways controlling cell growth and differentiation are almost invariably altered in cancer. The imbalance between production and elimination of radicals leading into oxidative stress may contain the

![Figure 1](https://via.placeholder.com/150)

**Figure 1** Initiation, promotion and progression of cancerogenesis

answer to the question how some of the physical, chemical and even biologic agents (with oxidative stress induction as a common denominator) can influence the mechanisms leading to cancerogenesis. Most tumors in their development follow the multistep pattern: initiation, promotion and progression. Induction of mutations and clonal growth of premalignant and malignant cells is a critical event in the above mentioned steps.

Initiation is the process in which one somatic cell becomes immortal but inherits the mutation. DNA modification has to be strong enough to escape effective reparation processes, but not too strong to induce death. Most genetic lesions induced by oxidative stress are very dangerous. However, deletions or rearrangements of the promoter or part of the enhancer can lead to gene dysregulation which induces its activation (activation of gene-proto-oncogene into oncogenes) or inactivation of antioncogenes i.e. tumor-suppressor genes. Damage induced by oxidative stress can result in inactivation or complete loss of certain tumor-suppressor genes, which inevitably leads into initiation or progression of cancerogenesis. Inactivation of tumor-suppressor genes can also be the consequence of point mutation. Up to the present, around 100 potential oncogenes (cellular and viral) have been identified, as well as 12 tumor-suppressor genes. The most potent suppressor regulatory gene is p53, the so-called «genome protector», activated in cases of DNA damage, hypoxia, as well as under permanent mitogenic stimulation (11). The most common p53 gene mutation is transversion G:C-T:A. This substitution can be induced by 8-hydroxyguanine (8oxoG) the production of which is induced by hydroxyl radical (12). In normal circumstances, p53 gene stimulates the expression of specific PIG gene, the products of which activate caspases and initiate apoptosis. Dysregulation of this
gene induces the loss of apoptosis, which introduces the cell into continual proliferation.

Promotion is the process in which the initiating cell is exposed to tumor promoters which induce phenotypic clonal expansion. Tumor promoters can be internal or external stimuli. Only the initiating cells are stimulated to grow (13). Many antioxidants and oxidant scavengers have been demonstrated to slow down the promotion of cancerogenesis. Associated initiation and promotion lead to relatively benign growth. Only in the third step the growth acquires malignant characteristics. Hence, the phenomenon of tumor progression, which in vivo advances as a series of structural changes, occurs gradually through accumulation and amplification of gene lesions. The mechanism of cancerogenesis is a complex and long-lasting one. Oncogene collaboration implies synergistic and cumulative damage to the genome as a critical prerequisite of cancerogenesis.

In the progression phase, due to genetic alteration, cellular growth becomes totally dysregulated, progressing being the most complex phase of cancerogenesis. It requires both genotypic and phenotypic changes which induce accelerated cellular growth. Tumor promoters selectively modify gene expression in initiated cells, thus translating benign into malignant cells. Experimentally treated papilloma (benign proliferation) can be transformed into cancer by an agent producing free radicals. This can ensue as the result of direct modification of the bases or transposition of genetic material. The second, hereditary genetic lesion is thus created in one or more benign tumor cells, which results in irreversible transformation and altered cellular phenotype (14).

The effects of reactive oxygen species and oxidative stress within cell appear to be cell-specific and dependent upon the form as well as the intracellular concentration of ROS.

Oxidative stress and gene expression

Many xenobiotics, by increasing the cellular levels of oxidants, alter gene expression through a host of signaling pathways including calcium-calmodulin pathways, cAMP-mediated cascades, and intracellular signal transducers such as nitric oxide, resulting in either cell proliferation or selective cell death. An unstable radical such as *OH reacts with all components of a DNA molecule and induces direct damage, but can also induce DNA damage through other mechanisms, i.e. by the stimulation of increase of intracellular calcium, which stimulates endonucleases to degrade the DNA molecule. Calcium has long been recognized as a signaling factor involved in the regulation of a wide range of processes including cell proliferation, differentiation and apoptosis (15). Release of calcium from intracellular stores by ROS results in the activation of kinases, such as protein kinase C (PKC) (16). PKC can also be activated by H2O2 and redox cycling quinones. Similarly, H2O2 leads to the activation of protein kinase B (PKB/Akt).

In a given cell, it is estimated that 105 oxidative lesions are formed daily (17). Over 100 oxidative DNA adducts have been identified (18–20). Reactive oxygen species can directly produce single- or double-stranded DNA breaks, purine, pyrimidine, or deoxyribose modifications, and DNA cross-links. In in vitro conditions, both copper and iron can induce DNA damage by ROS generation. The use of metal chelators blocks *OH generation and thus DNA damage, mutations and malignant transformations. Oxidative stress in the mitochondria induces mutations responsible for many malignant diseases. The consequence is reduced ATP production on account of which the normal cell cycle is arrested. The fragments of the degraded mitochondrial DNA can be incorporated into nuclear DNA, by which oncogenes can be activated.

Among the first kinases to be identified and linked to tumorgenesis were receptor tyrosine kinases. Two major intracellular signaling cascades that are activated by tyrosine kinase receptors and co-opted in tumor cell are Ras-mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-OH kinase PI(3)K-AKT-mTOR (mammalian target of rapamycin) pathways. MAPK are serine/threonine kinases which, after stimulation, phosphorylate their substrata in the regions of serine and threonine residues, regulating proliferation, differentiation and apoptosis. MAPK consists of three distinct families: (1) extracellular signal-regulated kinases (ERK 1 and 2), (2) c-Jun N-terminal kinases (JNK 1, 2, and 3) and (3) p38 kinases. MAPK modulate gene expression by phosphorylation of various transcription factors. Out of the three sub-families, the ERK pathway is most commonly associated with the regulation of cell proliferation. Cellular redox state alteration activates ERK, JNK and p38 sub-families. The balance between ERK activation and JNK inhibition is the key determinant of cell survival, while reduced ERK expression and JNK induction are the prerequisites for apoptosis initiation. MAPK activation directly activates AP-1, which binds to gene promoter cyclin D1. Activation of this promoter leads to activation of cyclin-dependent kinase and cell entry into the cycle. c-Jun also stimulates cell cycle progression, either through cyclin D1 induction or suppression of p21waf, the protein which inhibits cell cycle progression. It has recently been demonstrated that MAPK pathway activation is responsible for tumor neo-vascularization as the prerequisite of its progression and metastasizing. K-ras and c-mos (oncoproteins triggering MAPK hyperexpression) alteration is directly correlated with the degree of tumor angiogenesis and with the synthesis of vascular endothelial growth factor A (VEGF-A), a key factor in vascularization/angiogenesis (22).
NF-κB is an inducible and ubiquitously expressed transcription factor for genes involved in cell survival, differentiation, inflammation and growth. This transcription factor is associated with cancerogenesis because of its role in inflammation, differentiation and cell growth processes. NF-κB regulates several genes involved in cellular transformation, proliferation and angiogenesis. Carcinogens and tumor promoters, such as UV irradiation, phorbol esters, asbestos, alcohol and benzo(a)pyrens, are external NF-κB stimulators. Expression of several genes regulated by NF-κB (bcl-2, bclXL, TRAF1, TRAF2, SOD and A20) promotes cell survival partly through apoptosis inhibition. The importance of reactive oxygen species on NF-κB activation is further supported by the studies demonstrating that activation of NF-κB by nearly all stimuli can be blocked by antioxidants, including l-cysteine, NAC, thiols, green tea polyphenols, and vitamin E (23, 24).

Redox-sensitive signaling cascade involves cytoplasmatic (thioredoxin), nuclear signaling factors such as Ref-1 (Redox factor-1), and transcription factors (AP-1, NF-κB, Nfr-1, Egr-1). Cytoplasmatic SH protein, such as thioredoxin, is the key signaling protein regulating intracellular processes (DNA synthesis, cell growth etc.). Signaling cascades triggering ROS lead to activation c-Jun and c-Fos subunits of the active nuclear transcription factor AP-1 (activator protein-1), which activates genes involved in cell proliferation. Redox-sensitive signaling factors regulate different multiple processes among which are cell proliferation and cell cycle; they act anti-apoptotically. Inhibition of thioredoxin inhibits several transcription factors such as Egr-1, AP-1 and NF-κB, which results in a G1 phase arrest (25).

Cell signaling – a novel option and strategy in cancer treatment

Many other agents, such as TNF-α, IL-1 and bacterial or viral proteins, also induce oxidative stress. Although biologic cancerogenesis has been the subject of discussions for the last hundred years, only in the last ten sufficient data and material evidence has been collected linking viruses, bacteria and even parasites to the onset and development of cancer. Most common cancer causes are DNA viruses. Liver cancer is thus associated with hepatitis B virus (HBV), T cell leukemia with HTLV-1 virus, uterine cancer with papilloma virus (HPV), whereas infections with Helicobacter pylori are associated with most gastric cancers. All HPV types have similar genomes composed of double-stranded DNA, 7.9 Kb in length. Regions of E1-E7 gene code for the proteins involved in the viral preservation and survival, while E7 creates the complex with Rb gene product, a negative regulator of cell growth. The loss of Rb gene activity results in malignant transformation (26). E6 protein creates the complex with another suppressor protein – p53 gene product. Significantly reduced amount of p53 in the cells containing active viral protein precedes malignant transformation (27). Inhibition of the region E6/E7 of HPV with oligosense nucleotides can restore transformed cell phenotype of the cancer cells infected with HPV into the normal phenotype (28).

Activation of NF-κB and other transcription factors involved in immune and/or inflammatory response can upregulate the expression of many genes, the products of which promote and support the malignant phenotype. Supporting this is the fact that leukemia cells, similar to other malignant cells, have constitutively activated expression of NF-κB (25). Given these considerations, NF-κB inhibitors are more likely to be of use in cancer therapy. Many of the non-specific inhibitors of NF-κB belong to antioxidants, including l-cysteine, NAC, green tea polyphenols, thiols and vitamin E (23). The induction of c-jun and c-fos expression from RAO generated by ionizing radiation can be inhibited by NAC (29).

Iron, since it catalyzes the generation of the most reactive OH radical, participates in cancer initiation. It has been proven that iron increase in the stores and high level of ferritin are associated with increased incidence of primary liver cancer and adenocarcinoma of the colon (30, 31). Knowledge of the mechanisms by which iron initiates cancerogenesis is the basis of cancer treatment. Two possibilities exist:

Table 1   Targets of approved cancer drugs

<table>
<thead>
<tr>
<th>Modulators of redox cell signaling (cancer drug)</th>
<th>Genomic and proteomic targets (DNA changes or protein expression)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D, IFG</td>
<td>Receptors of growth factors</td>
</tr>
<tr>
<td>Genestine, Selen</td>
<td>Cell cycle</td>
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<tr>
<td>Genestine, Selen, Sodium-butyric acid</td>
<td>Apoptosis</td>
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<tr>
<td>Retinoic acid, dehydroepiandrosterone</td>
<td>Differentiation</td>
</tr>
<tr>
<td>Selen, indometacine</td>
<td>COX-2, apoptosis, adhesion properties</td>
</tr>
<tr>
<td>Endostatin, 2-metoxyestradiol</td>
<td>Angiogenesis</td>
</tr>
<tr>
<td>Difluoromethylominitinem</td>
<td>Cell proliferation</td>
</tr>
<tr>
<td>NSAID</td>
<td>PPAR-γ, apoptosis, proliferation</td>
</tr>
<tr>
<td>Avastin</td>
<td>VEGF receptor, proliferation, apoptosis</td>
</tr>
<tr>
<td>Herceptin</td>
<td>ERBB2, growth inhibition, apoptosis</td>
</tr>
</tbody>
</table>
(1) reduction of iron content effectuated by the administration of an iron chelator – desferoxamine or the use of specific monoclonal antibodies against transferrin receptors (Mo Ab 42/6), and (2) use of the transferrin receptors as the target loci for drug entry into the malignantly altered cell, since lots of malignant cells synthesize transferrin or a transferrin-like protein [32, 33].

Development of drugs specifically directed at the activity of oncoproteins can make tumor cells sensitive to immunologic and genetic surveillance, which is a novel cancer treatment strategy. Pharmacologic inhibitors of protein kinases are of special interest. Highly selective kinase inhibitor to be approved was the monoclonal antibody trastuzumab (Herceptin), which targets the ErbB2 (HER-2/neu) receptor. This drug is an important component of therapy for HER-2-positive breast cancer. The monoclonal antibodies cetuximab (Erbitux) and bevacizumab (Avastin), targeting epidermal growth factor receptor (EGFR) and VEGF respectively, have also gained regulatory approval [34, 35].

The use of non-steroid antiinflammatory drugs (NSAID) in human cancer treatment is based on the inhibition of MAPK (ERK2) pathway, the target place in cancer proliferation and growth inhibition [36]. Activated ERK2 is translocated across the nuclear membrane, resulting in the activation of numerous transcription factors, including estrogen receptor, Myc, c-Fos, peroxisome proliferator-activated receptor-γ (PPAR-γ), STAT1 and STAT3. Sulindac, an agent from the same group, inhibits Ras/Raf dependent transactivation binding non-covalently to p21 Ras, thus preventing the interaction of p21 Ras protein with p21 Ras binding site on the Raf protein, interrupting further cascade signal transfer to the MEK and MAP kinase pathway [32].

Since free radicals occupy a special place in cancer genesis, the efforts to define therapeutic indications of antioxidants in many immunologically and inflammation-driven cancers are by no way surprising. Having in mind that redox cell signaling is of fundamental importance in signal transduction through the complex IC communication network, clear definition of modulators acting as inhibitors of redox cell signaling can be the pharmacologic basis of interventional adjuvant therapy of cancer, but other diseases as well, including diabetes, ischemic-reperfusion tissue damage, cirrhosis, rheumatoid arthritis etc.

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MODULATORI TARGET MESTA GENOMSKE I PROTEOMSKE REDOKS ĆELIJSKE SIGNALIZACIJE U KANCEROGENEZI: NOVE DIJAGNOSTIČKE I TERAPIJSKE MOGUĆNOSTI

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Kratak sadržaj: U složenom putu prenosa signala kroz čeliju delovanjem faktora spoljašnje sredine, pokreće se transmisija signala kroz specifičnu komunikaciju, interakciju protein-protein (fosforilacija, defosforilacija, asocijacija, disocijacija, oksidacija, nitrozilacija, proteoliza) koja određuju biološki odgovor čelije. Mnogi putevi prenosa signala kroz čeliju kao što su JAK-STAT, MAP, PKC-kinazna kaslada, NF-κB put transdukcije signala, podležu down regulaciji posredovanoj N-acetil-cisteinem i redukovanim glutatijonem, dok opadanje koncentracije GSH i prisutan redoks stres iniciraju njihovu aktivaciju. Aktivirani redoks signalni putevi su medijatori mitogenih efekata u kancerogenezi. Dve vrste gena, koji inače čine samo jedan mali deo genom-a humane čelije, ostvaruju ključnu ulogu u kancerogenezi. Disfunkcija protoonkogena i tumor supresor gena dovodi do poremećaja u regulaciji puteva prenosa signala koji kontrolisu čelijak ciklus, apoptozu, stabilnost genoma, diferencijaciju i morfogenezu. Promene u ovim važnim fiziološkim procesima odgovorne su ne samo za inicijaciju i promociju maligne transformacije čelije već i za dalju progresiju tumorja. Slobodni radikali i njihovi oksidativno preuređeni proizvodi dovode do aktiviranja kritičnih senzornih mesta u proteomu signalnih puteva koji kontrolisu čeljsku proliferaciju, apoptozu i promenu čelijakog fenotipa. Stoga je veoma važno definisanje terapijskih indikacija primene antioksidanata i drugih modulatora target mesta redoks čelijak signalizacije genomike i proteomike kao složene mreže multifaktorijalne onkogene kolaboracije u procesu kancerogeneze.

Ključne reči: redoks čelijak signalizacija, kancerogeneza, tretman kancerne
References


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