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# A "CROSS-TALK" BETWEEN OXIDATIVE STRESS AND REDOX CELL SIGNALING

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**Summary**. *Cells are sensitive to a variety of signals in their environment, which they respond to and integrate. Signal transduction at the cellular level refers to the movement of signals from outside the cell to inside. A "cross-talk" between heterologous cell signaling systems gives a complex communication of molecules in various tissues a new dimension and specifies the type of the obtained biological response. A biochemical basis for transducing extracellular signals into an intracellular event has long been the subject of enormous interest. The mechanisms underlying ROS-mediated signal transduction in oxidative stress response involve a direct alteration in transcription factors and kinases and indirect modulation of cysteine-rich redox-sensitive signaling proteins. In a given signaling protein, oxidative attack induces either a loss of function, a gain of function, or a switch to a different function. Identifying the target proteins for redox modification is the key to understanding how oxidants mediate pathological processes such as tumor promotion, diabetes, atherosclerosis, aging, etc. Antioxidants and kinase inhibitors may provide an additional therapeutic effect and act synergistically with current treatments.*

**Key words**: *Redox cell signaling, ROS, PCK, MAP kinases*

## Introduction

Signal transduction at the cellular level refers to the movement of signals from outside the cell to inside. This process is possible due to highly specialized cell systems that integrate, transmit and amplify extracellular information contained in chemical signals. In most cases, signaling molecules are natural cell products (endocrine hormones, neurotransmitters, paracrines, autocrine hormones, and growth factors) or synthetic products. Co-action of and interaction between a variety of membrane and cytosol proteins makes it possible for molecular signals to be identified, signal transmission to be regulated, and the effects to be amplified. All signaling pathways at particular levels of information transmission utilize non-covalent protein-protein interaction or covalent protein modification (phosphorylation, nitrosylation, glycoxidation, and oxidation). Post-translational protein modification can be thus considered a key event in cell signaling. Intracellular signaling in general and hormone action in particular comprise the following steps: 1) synthesis of the signaling molecule; 2) release of a biologically active molecule from the cells; 3) transportation to the target cells; 4) detection of the signaling molecule by specific receptors; 5) response within the cell; and 6) termination of the signal by destruction of the signaling molecule.

Signaling molecules form a specific complex with their receptors, which makes this ligand-receptor interaction similar to the enzyme-substrate interaction. Upon binding the signaling molecule to the active receptor site (termed the binding site), the information is transmitted through a regulatory cascade that comprises diverse membrane and intracellular proteins. By sequential interaction of these proteins, the obtained information is transmitted onto the second messenger, which, in most cases, initiates cascade phosphorylation of cytosol proteins, many of which act as transcription factors responsible for the regulation of gene expression.

## Mechanisms of cell signaling mediated by free radicals

A biochemical basis for transduction of extracellular signals into an intracellular event has long been the subject of enormous interest. Recently, much attention has been paid to reactive oxygen species [ROS], which play a significant role of mediators in signaling processes (1-3).

Being initiators, transmitters, or modifiers of cellular response, free radicals occupy a significant place in the complex system of transmitting information along the cell to the target sensor. The effects of most extracellular signals are promoted via receptor ligation on either cell surface or cytoplasmic receptors. However, some low-molecular-weight signaling molecules, such as ROS and nitric oxide [NO], are able to penetrate the plasma membrane and directly modulate the activity of catalytic domain of transmembrane receptors or cyto-

plasmic signal transducing enzymes, thus leading to abnormal activation of transcription factors. By the initiation of gene expression and the consequent synthesis of responding functional and structural proteins, reactive free radicals allow for adaptation and survival of the cell or, depending on the intensity and duration of the signal, activate the processes responsible for the cell damage or death (4,5). In a given signaling protein, oxidative attack induces either a loss of function or a gain of function or a switch to a different function. The effect of free radicals on the process of cell signaling is promoted through a number of simultaneous mechanisms and, most commonly, by activating an extensive network of various interactive intracellular signal transduction pathways.

However, a great number of signaling pathways, especially those that can be modified by free radicals and their oxidized products, have not been fully explained, nor is it much known about the exact cell sensors for redox stress in eukaryotes.

The ability of oxidants to act as second messengers is a significant aspect of their physiological activity. The incorporation of free radicals into a complex cascade of transducing the signal to the effectors modifies and alters the order of events: numerous second messengers acquire the properties of third messengers, while intermediaries of free radical activity often function in both initiating and terminating signal transduction. These sequential events ultimately lead to either normal cell proliferation or development of cancer inflammatory conditions, aging, and two common age-related diseases – diabetes mellitus and atherosclerosis (6-9).

The effect of free radicals upon the process of cell signaling is commonly promoted via multiple and associated signal transduction pathways. Unlike prokaryotes with clearly defined "sensor" proteins on oxidants (10,11), in eukaryotes many proteins that participate in cell signaling are in addition to transcription factors sensitive to a change in the redox status of the cell (12). However, the sensor sites and the cellular response have not been to date completely defined.

## Regulation of transcription of oxidative stress-inducible genes – direct activation of transcription factors by oxidants

Due to a distant process of transcription and translation, eukaryotes, unlike prokaryotes, possess mechanisms for controlling cell signaling in the initiation of stress-inducible gene expression that are significantly more complex. The finding that free radicals occupy an important place in the cell signaling of eukaryotes has contributed to a more accurate characterization of target signaling molecules activated by radicals such as: activator protein-1 [AP-1], activator protein-2 [AP-2], nuclear transcription factor [NF-κB], and p21ras. These factors of transcription play a crucial role in controlling cell proliferation, cell differentiation, and morphogenesis.

### 1. Activation of AP-1 transcription factor by oxidative stress

The mechanism for activating AP-1 transcription factor by free radicals is one of the best-explained mechanisms in eukaryotes. AP-1 is a heterodimer comprising proteins of products of c-fos and c-jun proto-oncogenes, or a homodimer comprising products of c-jun gene (13). Protein products of these genes (FOS and JUN proteins) exhibit co-operability in inducing specific genes responsible for a change in cell phenotype, cell differentiation, cell regeneration and/or apoptosis (14). AP-1 controls the expression of numerous genes, including those that determine the synthesis of collagenase, thrombocyte growth factor [TGF-1b] and cytokines, and binds to the promoter of these genes. The expression of c-jun and c-fos genes is significantly induced in the presence of mitogen and phorbol esters such as 12-O-tetradecanoyl phorbol-13acetate [TPA]. Growth factors, tumour necrosis factor [TNF], $H_2O_2$, ultraviolet and ionizing radiation activate this transcription factor as well (13,15-17).

The AP-1 activity is regulated not only at the genetic level (regulation of transcription) but also at both post-transcriptional and post-translational levels (18,19). The exposure of HeLa cells to hydrogen peroxide or UV radiation leads to a significant increase in DNK-binding activity of AP-1, irrespective of FOS and JUN protein synthesis. Under the conditions, AP-1 is activated by phosphorylation of specific residues of AP-1 subunits. For example, the activation of cascade phosphorylation of MAP kinase family (c-Jun N-terminal kinase [JNK] i.e. stress-activated protein kinase [SAPK]) (19) leads to the phosphorylation of two serine residues (Ser-63 and Ser-73) in the JUN subunit of AP-1 to promote the activation of this subunit. Oxidative stress, too, can be a factor that mediates promotion of ligand effects exerted at the post-translational level of AP-1 activity regulation, by activating signaling via JNK protein kinases (20). FOS protein in AP-1 is also activated, by the phosphorylation of threonine residue (Thr-232) due to fos-regulatory kinase activated by $p21^{ras}$ protein (21). On the other hand, the phosphorylation of JUN protein (Thr-231, Ser-243, and Ser-149) by constitutive protein kinases, casein kinases II, and DNK-dependent protein kinase (22) results in inhibiting the binding of AP-1 to DNK. Dephosphorylation of threonine and serine residues of JUN protein increases the affinity of AP-1 active transcription factor for binding to DNK. This transcription factor is activated due to PKC that initiates dephosphorylation of the JUN subunit of AP-1 protein following activation of phosphatases. $P21^{ras}$ itself is a signaling target of radicals generated by $H_2O_2$ and nitric oxide. Their overexpression is also responsible for the activation of PKC and dephosphorylation of serine and threonine residues in DNA-binding domain of c-Jun (23). Dimer complex (FOS and JUN products) interacts with DNA regulatory element known as activator protein-1 [AP-1] binding site or with cAMP-responsible element [CRE]. These elements are present in the regulatory domain of AP-1 inducible genes.

The nuclear factor REF-1 (a.k.a. HAP-1 and APEX), which stimulates DNA binding activity of FOS/JUN proteins, was recently identified (24). The activity of this factor may be inhibited by a high concentration of reducing agents in the cell. It has been determined that the nuclear factor associated with thioredoxin participates in regulating the redox status of key cysteine residues responsible for DNA binding activity of AP-1 in FOS (Cys-154) and JUN (Cys-272) dimers. Intensified oxidative stress stimulates the activity of the nuclear factor and the binding of FOS and JUN heterodimers to DNK. HeLa cells, in which REF-1 expression is blocked by the presence of REF-1 antisense oligo-nucleotides, exert a high degree of sensitivity to oxidants, paraquat, hypoxia, hyperoxia, and inhibitors of glutathione synthesis (24,25). These results suggest that REF-1 could have a key role in protecting cells against the effects of various stress factors, including free radicals and a change in the oxygen partial pressure.

### 2. Activation of NF-κB transcription factor by oxidative stress

Since its discovery in 1986, NF-κB transcription factor has aroused a wide interest in its unusual regulation, diverse stimuli that activate it, and its apparent involvement in a variety of human diseases, including atherosclerosis, asthma, diabetes, cancer, arthritis, AIDS, inflammatory diseases, etc.

NF-κB belongs to the Rel-family of pluriprotein transcription activators. It is a regulatory protein that controls the expression of numerous inducible and tissue-specific NF-κB responsible genes (26) and participates in the regulation of pro-inflammatory and immune cellular responses, the regulation of cell proliferation, and apoptosis (27,28). Many dimeric forms of NF-κB have been detected, but the prototypic activated form of NF-κB is a heterodimer consisting of p65/RelA and p50 subunits (26). The genes activated by NF-κB transcription factor encode the synthesis of various cytokines (IFN-β, TNF-α, IL-1, IL-2, IL-6, IL-8, and monocyte chemoatractant protein-1), receptors for cytokines, cell adhesion molecules (including vascular adhesion molecule-1 [VCAM-1], intercellular adhesion molecule-1 [ICAM-1], and E-selectin) endothelin-1, hematopoietic growth factors, and reactants of the acute phase (29). It has been found that inducible nitric oxide synthase [iNOS] gene promoter possesses an NF-κB binding site. Early genetic expression of some of the viruses (HIV-1, cytomegaloviruses, and adenoviruses) is also regulated by NF-κB transcription factor. This factor is activated by signaling molecules, such as TNF-α, endotoxin, interleukin 1β, mitogens, lipopolysaccharide, viruses, agents promoting oxidative stress, lectin, Ca ionophores, and UV radiation (30, 31). Free oxygen radicals, which are formed on the respiratory chain of mitochondria to act as second messengers, mediate NF-κB activation promoted by TNF-α and interleukin 1β. In the absence of cell stimulation, NF-κB is present in cytoplasm, in the complex with its inhibitor (IkB). NF-κB can be activated by a variety of stimuli, including inflammatory cytokines such as TNF-α and IL-1, T-cell activation signals, growth factors, and oxidative stress inducers. The binding of stimuli to cell receptors is followed by phosphorylation of IkB at position Ser-32 and Ser-36 catalyzed by IkB kinase (32). This mode of post-translational covalent modification of inhibitors allows for dissociation of NF-κB from the complex, degradation of inhibitors, and rapid translocation of NF-κB into the nucleus where it binds to target DNA elements and positively regulates transcription of the genes involved in immune and inflammatory responses, cell growth control, and apoptosis.

NF-κB activation is stimulated by pro-oxidative cell status, especially by an increased presence of $H_2O_2$. The exact signaling cascade seems to be due to the activation of MAP kinase pathway. On the other side, the activation of NF-κB is blocked by thiol components such as N-acetyl-L-cysteine (glutathione precursor) and antioxidants. It has been demonstrated that a low concentration of thiol compounds in the cell, primarily glutathione as the most widespread thiol compound, plays a key role in positive regulation of NF-κB activity (28,29,31,32). Therefore, the mechanisms that regulate and control the level of glutathione in the cell indirectly participate in regulating the expression of the genes with an NF-κB binding site in the promoter. It is postulated that ROS regulate NF-κB activity and modify some of the links in a complex activating cascade of NF-κB transcription factor: 1) oxidation of key sites in enzymes (kinases) that phosphorylate and activate IkB kinase which, due to the phosphorylation of serine residues of inhibitors, activates NF-κB complex; 2) redox modulation of IkB kinase activity; and 3) modulation of transport of the activated NF-κB from the cytoplasm into the nucleus.

Since NF-κB has a ubiquitous role in controlling cytokine activity and immunoregulatory genes, the inhibition of NF-κB activity by steroid hormones, antioxidants, non-steroid anti-inflammatory drugs, and protease inhibitors represents a pharmacological basis for the intervening adjuvant therapy in numerous diseases, including cancer, diabetes mellitus, AIDS, and diverse inflammatory disorders (33).

## Redox sensitive regulation of gene expression mediated by protein kinases

It is now recognized that ROS can modify cell signaling proteins and that these modifications have functional consequences. Considering the fact that one of the most widespread modes of signal transduction along the cell is target protein phosphorylation controlled by a joined kinase-phosphatase activity, the effects of oxidative stress upon the activity of these enzymes represent key sites for signal transduction modulation by oxidative stress. ROS and the altered redox potential can be considered primary intracellular changes that regulate protein kinases and thereby serve as an important cellu-

lar component linking external stimuli with signal transduction in stress response.

**a) Oxidative stress and modulation of PKC activity**

Protein kinase C [PCK] is a family of structurally and functionally related proteins derived from multiple genes and the alternative splicing of single RNA transcripts. A total of 12 PKC isozymes have been cloned and characterized. Activation and translocation of PKC from cytosol to plasma membrane occurs in response to a transient increase in diacylglycerols [DAGs] or exposure to exogenous tumor-promoting agents known as phorbol esters (34,35).

The first observations referring to modulation of PKC activity by redox sensitive modulators date as early as ten years ago. It is certainly not by accident that changes in PKC activity related to redox sensitive regulation dominate in diabetes mellitus (36,37). The origin of this disease, its course and development of complications are closely associated with a misbalance in pro/anti-oxidative cell status and a change in redox potential (8,38,39). Extracellulary, glucose has been demonstrated to interact non-enzymatically with primary amines of proteins, thus forming glycated compounds or oxidants. Although precise mechanisms for PKC activation by free radicals and oxidation products – advanced glycation end products [AGE] - have not yet been fully examined, it is postulated that PKC activity modulation is promoted indirectly, i.e., it is mediated by DAG whose production grows as a consequence of phospholipase C activation after exposing cells to the effect of oxidants (40). It is for this reason that the activated DAG-PKC pathway of signal transduction in numerous tissues, particularly in vascular endothelium, is most often referred to as a molecular mechanism responsible for developing diabetic complications. However, direct modulation of SH group in the regulatory, i.e., "activation segment" of PKC by redox factors should not either be excluded as a mechanism likely to activate protein kinase C. That DAG-PKC pathway of signal transduction is amplified by oxidants is supported by the fact that PKC activity decreases by adding E vitamin, resulting in a decreased DAG level (41). The mechanism of this effect involves the stimulation of DAG kinase and an increased conversion of DAG to phosphatidic acid (42). At the same time, E vitamin does not affect the activity of purified PKC isoforms. Polyamines, by decreasing PKC activity, may affect the development of complications in diabetes (43).

PKC modulation by oxidants represents the critical event in developing vascular complications, especially in diabetes. The intensified DAG-PKC signaling pathway in endothelial cells is responsible for a disturbance in the regulation of permeability, contraction, extracellular matrix deposit, cell growth, angiogenesis, cytokine activation, and synthesis of adhesive molecules. These disturbances, as basic mechanisms for development of vascular complications such as atherogenesis and hypertension, allow for several target sites of PKC activity, some of which are the following (38,44-46):

- increased expression of endothelin-1;
- increased expression of inducible nitric oxide synthase [iNOS];
- initiation of NF-kB and AP-1 transcription factor expression;
- initiation of arachidonic acid cascade by stimulation of A2 phospholipase;
- stimulation of NADH/NADPH oxidase activity of macrophage and endothelium cells;
- increased synthesis of vascular endothelium growth factor [VEGF].

**b) Oxidative stress and modulation of proteins with intrinsic tyrosine kinase activity**

The term "protein tyrosine kinase" is a generic term used for a large super-family of enzymes consisting of both transmembrane-spanning receptors with intrinsic tyrosine kinase activity in their cytoplasmic domains and a wide range of subfamilies of cytoplasmic tyrosine kinases, such as Src, Abl, or Janus kinase [JAK] families.

A large body of evidence suggests that tyrosine kinase is implicated in promoting the effects of growth factors, cytokines, and hormones (47-49). One of the ways of regulating the activity of proteins with tyrosine kinase activity is redox sensitive regulation. This regulation is based on a change in the redox status of the cell, which is determined by the content of thiol compounds in the cell, primarily by the content of glutathione as the most widespread thiol compound in the cell.

The fact that hydrogen peroxide induces the phosphorylation of tyrosine residues of numerous cell proteins is supportive of the observation that modulation of the activity of tyrosine kinase pathway in cell signaling is the most frequent target site of the redox modulator effect in physiological control of signal transduction in the cell. The exposure of cells to hydrogen peroxide simulates numerous effects that are due to the activity of various extracellular ligands. For example, $H_2O_2$ simulates insulin effects, by employing some of the signaling pathways: by the activation of tyrosine kinases responsible for the phosphorylation of receptor subunits and other intracellular proteins whose phosphorylation intensifies when affected by insulin; by PIK3 activation; by p38 MAPK activation (50). Some of these pathways differ from those being activated by insulin. The exposure of lymphocytes to hydrogen peroxide also leads to the activation of a specific $p56^{lck}$ tyrosine kinase to initiate its auto-phosphorylation at Tyr-394 position. Lck tyrosine kinases belong to the S*rc* family of cytoplasmic tyrosine kinases that play a significant role in the activation of T lymphocytes.

In a complex cascade phosphorylation of various cell proteins to the end effectors (transcription factors), p21 protein, in addition to tyrosine kinases, represents a common target sensor protein for free radicals and redox stress. The activation of this protein by nitric monoxide and/or redox-modifying agents initiates GDP/GTP sub-

stitution on the protein. The pre-treating of the cells with L-buthionine-(S,R)-sulfoximine, by a specific inhibitor of γ-glutamylcysteine synthetase (regulatory enzyme in glutathione synthesis), results in glutathione decrease and up to 100 times higher sensitivity of p21 protein to oxidants. Redox modification of the amino acid residue of cysteine (Cys118) on the monomer G-protein Ras (p21) is a key moment for the activation of this protein by oxidative stress and nitric monoxide. Mutant and activated forms of p21 protein found in various types of cancer in humans and a high sensitivity of this signaling protein to free radicals and redox status allow for additional mechanisms for the promotion of oncogenesis by free radicals (51,52).

Due to the activation, induced conformational changes in Ras protein are responsible for its interaction with various signaling proteins termed Ras protein effectors. Ras can interact with various effectors and, depending on the stimulus type, one or more effectors can be engaged in the further cascade propagation of the signal. Some of the effectors of Ras protein are: phosphatidylinositol 3'kinase [PI3K], Raf-1, protein kinase C, diacylglycerol kinase, and MAP-kinase-kinase-kinases. In the transmission of signals via activated Ras protein, phosphatidylinositol 3'kinase [PI3K] participates in the regulation of many cellular responses such as: cell differentiation, cell division, phagocytosis, glucose transport. There are observations that Ras protein activation by redox modifiers is a "trigger" for a selective interaction of this protein with PI3K (53).

The oxidized products can initiate cell signaling by activating some of the redox sensors. The products of non-enzymatic glycation of serum proteins and lipids, so called advanced glycation end products [AGE], which develop independently in diabetes and aging, activate signal transduction pathways. These products bind to specific receptors (RAGE) on the surface of the cells of various tissues (49,54). Receptors accept glycosylated proteins that are further metabolized in the cell. However, in case of intensified oxidative stress, a long-term stimulation of receptors is responsible for the activation of Ras-MAP kinase pathway. RAGE activation induces the formation of free radicals, but the way in which they are formed is not fully clear. Radicals, having been formed, further activate Ras-MAP signaling pathway, which leads to a misbalance in vascular homeostasis and to the development of vascular complications in diabetes. In Alzheimer's disease, the binding of amyloid-peptides to RAGE and its deposition in plaques correlates to the neuron dysfunction resulting in dementia. The exact effectors and the effects of the activated MAP kinases via RAGE complex are not known. Those that have been suggested include a change in cell phenotype and cell proliferation The binding of the ligands to RAGE activates the translocation of NF-κB to the nucleus, but proximal factors in the RAGE pathway of cell signaling have not been precisely determined (55).

Available contemporary studies in the field are consistent with the observations that free radicals are active second messengers formed during controlled reactions of cell signaling initiation, and that their rapid production may result from the altered cell phenotype, cell damage, or cell death. In the process of signal transduction via lactosylceramides [LacCer] (which stimulate p44MAPK activation and the expression of c-fos transcription factor that regulates genes essential to cell proliferation), free radicals act as second, that is, third messengers. It has been confirmed that LacCer, by activating NADPH oxidase in human smooth muscle cells of aorta, produce a superoxide radical – a redox stress signaling molecule responsible for the activation of MAP-kinase cascade resulting in cell proliferation. This cascade undergoes down-regulation mediated by N-acetyl-L-cysteine and reduced glutathione (56,57).

## Conclusion

Free radicals and their metabolites most probably act as intracellular and intercellular mediators, transforming the initial signal (receptor stimulation) into a biochemical cellular response. Their general properties (pretty small, highly reactive and diffusible molecules) support the hypothesis that free radicals and redox stress actively participate in cell signalling as second messengers that activate transcription factors and induce gene expression. If this is the fact, then new prospects are offered for applying antioxidant therapy in diverse pathological conditions, such as hypertension, diabetes, atherosclerosis, and Alzheimer's disease, in which redox cell signaling plays a crucial role.

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## References

1. Goldschmidt-Clermont PJ. Moldovan L. Stress, superoxide, and signal transduction. Gene Expresion 1999; 7: 255-260.
2. Demple B. Radical ideas: genetic responses to oxidative stress. Clin Exp PharmacolPhysiol 1999; 26:64-68.
3. Duranteau J. Chandel NS, Kulisz A, Shao Z, Schumacker PT. Intracellular signaling by ractive oxygen species during hypoxia in cardiomyocytes. J Biol Chem 1998; 273 (19): 11619-11624
4. Eizirik DL. Manndrup-Poulsen T. A chice of death - the signal-transduction of immuneimediated beta-cell apoptosis Diabetologia 2001; 44: 2115-2133.
5. Buttke TM, Sandstrom PA. Oxidative stress as mediator of apoptosis. Immunol Today 1994; 15: 1-4.
6. Niki E. Free Radicals in the 1900's: from in vitro to in vivo. Free Radic Res 2001(6): 693-704.
7. Borek C. Free radical processes in multistage carcinogenesis. Free Radical Res Commun 1991; 12: 745-750.
8. Pavlovic D, R. Kocic, G. Kocic, T. Jevtovic, S. Radenkovic, D. Mikic, M. Stojanovic, PB Djordjevic. Effect of a four week metformin treatment on plasma and erythrocyte antioxidative

defense enzymes in newly diagnosed obese NIDDM PATIENTES. Diabetes, Obesity&Metabol. 2000; 2: 251-256.
9. Diaz MN, Frei B, Vita JA, Keaney JF. Antioxidants and atherosclerotic heart disease. N Engl J Med 1997; 337: 408-416.
10. Toledano MB, Kullik i, Trinh F, Baird PT, SchneiderbTD, Storz G. Redox-dependent shift of OxyR-DNA contacts along an extended DNA-binding site: a mechanism for differential promoter selection. Cell 1994; 78: 897-909.
11. Amabile-Cuevas CF, Demple B. Molecular characterization of the soxRS genes of Escherichia coli: two genes control a superoxide stress regulon. Nucleic Acids Res 1991; 19: 4479-4484.
12. Irani K Goldshmidt-Clermont PJ. Ras, superoxide and signal transduction. Biochem Pharmacol 1998. 55: 1339-1346.
13. Ng L, Forrest D, Curran T. Differential roles for Fos and Jun in DNA-binding: redox-dependent and independent functions. Nucleic Acids Res 1993; 21(25): 5831-5837
14. Angel P, Karin M.The role of Jun, Fos and the AP-1complexin cellproliferation and transformation. Biochim Biophys Acta 1991; 1072: 129-157.
15. Ding M, Li JJ, Leonard SS, Ye JP, Shi X, Colburn NH, Castranova V, Vallyathan V. Vanadate-induced activation of activator protein-1: role of reactive oxygen species. Carcinogenesis 1999; 20: 663-668.
16. Janssen YMW, Van Houten B, Borm PJA, Mossman BT.Cell and tissue responses to oxidative damage Lab Invest 1993; 69: 261-274.
17. Abate C, Patel L, Rauschher FJ, Curran T. Redox regulation of fos and jun DNA-binding activity in vitro. Science 1990; 249:1157-1161.
18. Hunter T, Karin M. The regulation of transcription by phosphprylation Cell 1992; 70: 375-387.
19. Karin M. The regulation of AP-1activity by mitogen-activated protein kinases.J Biol Chem 1995; 270: 16483-16486.
20. Go YM, PatelRP, Maland MC, Park H, Beckman JS, Darley-Usmar VM, Jo H. Evidence for peroxynitrite as a signaling molecule in flow-dependent activation of c-Jun NH(2)-terminal kinase. Am J Physiol. 1999; H1647-1653.
21. Deng T, Karin M. c-Fos transcriptional activity stimulated by H-ras-activated protein kinase distinct from JNK and ERK. Nature 1994; 371: 171-175.
22. Lin A, Frost J, Deng T, Smeal T, Al-Alawi N. Casein kinase II is negative regulator of c-Jun DNA binding and Ap-1 activity. Cell 1992; 70: 777-789.
23. Lander HM, Ogiste JS, Teng KK, Novogrodsky A. p21 ras as a common signaling target of reactive free radicals ans cellular redox stress. J Biol Chem 1995; 270: 21195-21198.
24. Nakamura H, Nakamura K, Yodoi J. Redox regulation of cellular activation. Ann Rev Immunol 1997; 15: 351-369.
25. Walker LJ, Craig RB, Harris AL, Hickson ID. A role for thebhuman DNA repair enzyme HAP1 in cellular protection against DNA damaging agents and hypoxic stress. Nucleic Acida Res 1994; 22:4884-4889.
26. Ghosh S, May m, Kopp E. NF-kB and Rel proteins: evolutionaril conserved mediators of immune respones. Annu Rev Immunol 1998; 16: 225-260.
27. Baeuerle PA, Henkel T. Function and activation of NF-kappa B in the immune system. Annu Rev Immunol 1994; 12:141-179.
28. Yamamoto Y, Gaynor RB. Role of NF-kB pathway in the pathogenesois of human disease states. Curr. Mol Med. 2001, 1: 287-296.
29. Collins T, Cybulsky MI. NF-kB: pivotal mediator or innocent bystander in atherogenesis? J Clin Invest 2001; 107(3): 255-264.
30. Dalton PT, Shertzer HG, Puga A. Regulation of gene expression by reactive oxygen. Annu Rev Pharmacol Toxicol 1999; 39: 67-101.
31. Shi X, Dong Z, Huang C, Ma W, Liu K, Ye J, Chen F, Leonard SS, Ding M, Castranova V, Vallyathan V. The role of hydroxyl radical as a messenger in the activation of nuclear transcription factor NF-kappaB. Mol Cell Biochem 1999; 194(1-2): 63-70.
32. Traenckner EMB, Pahl HL, Schmidt KN, Wilk S, Baeuerle PA. Phosphorylation of human IκB on serine 32 and 26 controls IκB-α proteolysis and NF-κB activation in response to diverse stimuli. EMBO J 1995; 14: 2876-2883.
33. Yamamoto Y Gaynor RB. Therapeutic potential of inhibition of NF-κB pathway in the treatment of inflammation and cancer. J Clin Invest 2001; 107; 135-142.
34. Nishizuka Y. The molecular heterogeneity of protein kinase C and its implications for cellular regulation. Nature 1988; 334: 661-665.
35. Derubertis FR, Craven PA. Activation of protein kinase C in glomerular cells in diabetes: mechanisms and potential link to the patogenesis of diabetic glomerulopathy. Diabetes 1994; 43: 1-8.
36. Craven PA, De Rubertis FR. Protein kinase C is activated in glomeruli from streptozotocin diabetes rats. J Clin Invest 1989; 83: 1667-1675.
37. Lee T-S, Saltsman KA, Ohashi H, King GL. Activacion of protein kinase C by elevation of glucose concentration: Proposal fer a mechanism in the development of diabetic vascular complications. Proc Natl Acad Sci USA 1989; 86: 5141-4145
38. Baynes JW Role of oxidative stress in development of complications in diabetes. Diabetes 1991; 40: 405-412.
39. Ceriello A, Mercuri F, Quagliaro L, Assaloni R, Motz E, Tonutti L, Taboga C. Detection of nitrotyrosine in the diabetic plasma: evidence of oxidative stress. Diabetologia 2001; 44: 834-838.
40. Avdonin PR. Receptor-dependent regulation of (Ca2+)i and phospholipase C in vascular endothelial cells. J Recept Signal Transduct Res 2000; 20(4): 235-254.
41. Kunisaki M, Bursell S-E, Umeda F, Nawata H, King GL.Normalization of diacylglycerol-protein kinase C activation by vitamin E in aorta of diabetic rats and cultured rat smooth muscle cells exposed to elevated glucose levels. Diabetes 1994; 43:1372-1377.
42. Tran K, Proulx PR, Chan AC. Vitamin E suppresses diacylglycerol (DAG) level in thrombin-stimulated endothelial cells through an increase of DAG kinase activity. Biochim Biophys Acta 1994; 1212: 193-202.
43. Pavlovic D, Bjelakovic G. A possible link between polyamines and thiol redox signaling patways in diabetic liver. J Hepatology 2001; 34 (suppl.1): 94
44. Fukuchi M, giaid A. Endothelial expression of endothelial nitric oxide synthase and endothelin-1 in human coronary artery disease. Lab Invest 1999; 79: 659-670.
45. Park CW, Kim JH, Lee JE, Kim YS, Ahn HJ, Shin YS, Kim SY, Choi EJ, Chang YS, Bang BK. High glucose intercellular adhesion molecule (ICAM)-1) exspression through an osmotic effect in rat mesangial cells is PKC-NF-kB-dependent. Diabetologia 2000; 43:1544-155
46. Williams B, Schrier RW. Glucose-induced protein kinase C activity regulates arachidonic acid release and eicosanoid production by cultured glomerular mesangial cell. J Clin Invest 1993; 92: 2889-2896.
47. Arbabi S, Maier RV. Mitogen-activated protein kinases. Crit Care Med 2002; 30(1 Suppl): S74-S79.
48. Velarde V, Jenkins AJ, Christopher J, Lyons TJ, Jaffa AA. Activation of MAPK by modified low-density lipoproteins in vascular smooth muscle cells. J Appl Physiol 2001 91(3): 1412-1420.
49. Huang JS, Guh JY, Chen HC, Hung WC, Lai YH, Chuang LY. Role of receptor for advanced glycation end-product (RAGE) and the JAK/STAT-signaling pathway in AGE-induced collagen production in NRK-49F cells. J Cell Biochem 2001; 81(1):102-113.
50. Koshio O, Akanuma Y, Kasuga M.Hydrogen peroxide stimulates tyrosine phosphorilation of the insulin receptor and its tyrosine kinase activity in intact cells. Biochem J 1988; 250: 95-101.
51. Lander HM, Ogiste JS, Teng KK, Novogrodsky A. p21 as a common signaling target of reactive free radicals and cellular redox stress. Am Soc Biochem Mol Biol 1995; 279(36): 21195-21198.
52. Kopnin BP. Targets of oncogenes and tumor suppressors: key for understsanding basic mechanisms of cancerogenesis. Biochemistry 2000; 65: 2-27.
53. Bernstein KE, Ali MS, Sayeski PP, Semeniuk D, Marrero MB. New insights into the cellular signaling of seven transmem-

brane receptors: the role of tyrosine phosphorylation. Lab Invest 1998; 78:3-7.
54. Yan SD, Schmidt AM, Anderson GM, Zhang J, Beett J, Zou YS, Pinsky D, Stern D. Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. J Biol Chem 1994; 269: 9889-9897.
55. Thornalley PJ. Cellactivation by glycated proteins. AGE receptors, receptor recognition factors and functional classification of AGEs. Cell Mol Neurobiol 1998; 44: 1013-1023.
56. Bhunia AK, Han H, Snowden A, Chatterjee S Redox-regulated signaling by lactosylceramide in the proliferation of human aortic smooth muscle cells. J Biol Chem 1997; 272(25): 15642-15649.
57. Kolesnick R, Golde DW. Cell 1994; 77: 325-328. The sphingomyelin signal transduction pathway mediates apoptosis for tumor necrosis factor, Fas, and ionizing radiation. Biochem Cell Biol 1994; 72(11-12): 471-474.

# INTERAKCIJA IZMEĐU OKSIDACIONOG STRESA I REDOKS ĆELIJSKE SIGNALIZACIJE

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Kratak sadržaj: *Ćelije su osetljive na različite signale u svojoj okolini, na koje reaguju i koje integrišu. Signalna transdukcija na ćelijskom nivou podrazumeva kretanje signala kroz ćeliju od spolja ka unutra. Interakcija između heterolognih sistema ćelijske signalizacije daje kompleksnoj komunikaciji molekula u različitim tkivima novu dimenziju i određuje specifični tip dobijenog biološkog odgovora. Biohemijska osnova za pretvaranje vanćeliskih signala u unutarćelijski događaj je dugo predmet interesovanja. Mehanizmi koji su u osnovi ROS posredovane signalne transdukcije na prisustvo oksidacionog stresa uključuju direknu promenu faktora transkripcije i kinaza i indirektnu modulaciju signalnih proteina bogatih cisteinom i osetljivih na redoks ćelijsku signalizaciju. U datom signalnom proteinu, oksidacioni atak indukuje ili gubitak funkcije ili sticanje funkcije ili promenu funckije. Identifikacija target proteina za redoks modifikaciju je ključ za razumevanje načina na koji oksidansi posreduju u patološlim procesima, kakvi su pojava tumora, dijabetes, ateroskleroza, starenje, itd. Antioksidansi i inhibitori kinaza mogu obezbediti dodatni terapeutski efekat agenasa i delovati sinergistučki sa tekućim tretmanima*.

Ključne reči: *Redoks ćelijska signalizacija, ROS, PKC, MAP kinaze*