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Chapter 10

Apoptosis, Free Radicals and Antioxidant Defense in Antitumor Therapy

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Additional information is available at the end of the chapter

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1. Introduction

Tumor markers are measurable biochemicals that are associated with a malignancy. They are either produced by tumor cells (tumor-derived) or by the body in response to tumor cells (tumor-associated). They are usually substances that are released into the circulation and then measured in the blood sample. There are a few exceptions to this, such as tissue-bound receptors that must be measured in a biopsy from the solid tumor or proteins that are secreted into the urine. Despite the fact that tumor markers are hardly ever specific enough to be used alone to diagnose cancer, they do have a number of clinical applications. They can be used to stage cancer, to indicate a prognosis, to monitor treatment, or in follow-up to watch for cancer recurrence. Changes in some tumor markers have been sensitive enough to be used as targets in clinical trials. Tumor markers for diagnosis are used in combination with other clinical parameters such as biopsy and radiological findings. Although there are a multitude of tumor markers, very few of them have found their way into clinical practice because of their lack of specificity. However, some of these non-specific markers have found a place in monitoring cancer treatment rather than in diagnosis.

Tumor marker discovering is focuses currently much research and attention. Their final clinical usage is directed by approval from the Food and Drug Administration (FDA) and guidelines established by organizations such as the American Society of Clinical Oncology and the American Cancer Society. Not all tumor receptor marker tests are widely available nor are they widely accepted.

In the current review we attempt to propose and bring closer some new "cancer markers" connected to oxidative stress and cell death. In recent times, therapeutic approaches take advantages from determination of oxidative stress markers. These markers have gained importance in the evaluation of cancer treatment and prognosis. In this chapter we try to

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explain the beneficial application of oxidative stress and apoptotic markers for medical requirements [1].

2. Apoptosis

Apoptosis is the process of programmed cell death which is very important when cells harmful for organism appear. In this way organism destroys cells that endanger the homeostasis, are malign, , mutated, cells that ignore the signals of cell cycle regulation, lose the ability to undergo apoptosis, and cannot communicate with neighboring cells. In case of cancer process of programmed cell death is inhibited and tumor cells are allowed to tolerate apoptotic signals. Defective Apoptosis has been recognized as a fundamental factor in the development and progression of cancer. Restore of appropriately induce apoptosis may establish antitumor therapy based on o triggering selective death of cancer cell [2,3].

Large varieties of different stimuli are able to initiate programmed cell death by apoptosis signaling pathways. Thus there is the extrinsic pathway that depends on triggering of death receptors expressed on the cell surface, the intrinsic pathway mediated by molecules released from the mitochondria and the third pathway activated by granzmes [4,5]. These pathways lead to activation of the specific proteinase caspases and result in DNA fragmentation, degradation of cytoskeletal and nuclear proteins, cross-linking of proteins, formation of apoptotic bodies, expression of ligands for phagocytic cell receptors and finally uptake by phagocytic cell [6].

2.1. Regulation and inhibition of apoptosis in cancer treatment

The cells homeostasis is regulated by different mechanism included proliferation, growth arrest, and apoptosis [7]. The disorder in the balance between cell growth and death often leads to the carcinogenesis. The cells proliferation is regulated by cell cycle, which is an involved sequence of grow and cells replication [8]. It is now accepted that cancer is more accurately described as being the product of malfunctions within the regulation of the cell cycle, such that injured or mutated cells which are normally killed, are allowed to progress through the cell cycle, accumulating mutations. During this process the cells are tending to genetic lesions. However, well-organized control mechanisms are shown to exist, which detects damage. It may lead to malignancy or the early stages of carcinogenesis [9]. There are a few control spots of cell cycle. The restrictive points lead to repair damage in cells or eliminated cells in different ways: necrosis, senescence (permanent arrest) or apoptosis [10-13]. Problem of selective direct selected cells on apoptotic pathway is still unclear. Many genes and their proteins products play a dual role in the cell division and apoptosis including p53, pRb, Bcl-2 family. Subsequent stimulation these molecules may induce cell proliferation, cell cycle arrest or cell elimination [8]. The different result is dependent on different factors, which respectively inhibit or support apoptotic cell death. In many types of cancer the mutation of gene responsible for check points are observed [14]. Apoptosis play a central role in the pathogenesis of human disease especially in malignance while the factors controlling the apoptotic progression are suppressed, overexpressed or modified their

function (mutation, phosphorylation, acetylation) [15,16]. Defects in its pathways be able to promote cancer cell survival and also confer resistance to antineoplastic drugs. The study into apoptosis is going at a fast pace and this has led to the possibility of new therapeutic approaches to some human diseases [11].

2.2. Signaling pathways of apoptosis

Mitochondria play a crucial role in apoptosis. Their function is essential for the process of programmed cell death.

In case of the intrinsic pathway several stimuli, including reactive oxygen species and other cytotoxic elements, lack of growth factors, kinase inhibitors initiate this pathway. As a result, activated the pro-apoptotic proteins permeabilize the outer mitochondrial membrane to trigger the release of Smac/DIABLO and cytochrome c to cytosol [17]. The former mentioned, Smac/DIABLO, directly binds to cytosolic IAP (inhibitor of apoptosis protein) and removes it from active caspases, and thus allows the caspases to cleave their substrates.

During organization of these proteins complex cytochrome c, released into the cytosol, promote formation of the apoptosome. Cytochrome c binds apoptotic protease activating factor-1(Apaf-1) using the energy provided by ATP and procaspase 9 is activated into its active form [18,19]. This event results in the activation of caspases 3 and 7 as the down-stream effector caspases. Some authors indicated that the release of cytochrome c is tightly regulated by the pro- and anti-apoptotic members of the Bcl-2 family [5].

The extrinsic pathway of programmed cell death involves interaction between ligand and plasma membrane receptor (Fas/CD95, TNFα, TRAIL) [20]. Consequently caspase-8 recruitment and activation occurs. This caspase cleaves and activates Bid, which releases cytochrome c from the mitochondria to activate the apoptosome, and apoptosis events. Caspase-8 may bypass the mitochondria and induce apoptosis by directly activating caspase-3.

Perforin/granzyme-induced apoptosis is the main pathway activated by cytotoxic T lymphocytes to eliminate virus-infected or transformed cells. Granzymes are a different family of serine proteases and the granule protein, perforin, supports granzyme (A or B) release to the target cell cytosol and, on entry [21,22]. Granzyme B can operate by specific cleavage of Bid and induction of cytochrome c release, by activation of initiator caspase-10 or by direct activation of caspase-3 [23]. Sutton et al. show that granzyme B triggers the mitochondrial apoptotic pathway in mouse myeloid cells through direct cleavage of Bid; however, cleavage of procaspases was stalled when mitochondrial disruption was blocked by Bcl-2 [24]. In case of granzyme A caspase-independent cell death can occur via initiation of DNA cleavage. Apart from granzyme A pathway caspases are essential during apoptosis. They are aspartate-specific cysteine proteases that are present in healthy cells as zymogens, which are usually activated by proteolytic cleave to form a fully functional active site [19]. The group of initiator caspases (2, 8, 9 and 10) triggers cleaves inactive proenzymes of effector caspases. Effector caspases (3, 6 and 7) in turn cleave other protein substrates within

the cell, to initiate irreversible events of the apoptotic process [18]. Important factors involved in the regulation of apoptosis are inhibitors of apoptosis proteins (IAPs) that can block caspase cascade, but only some of them directly interact with caspases [25].

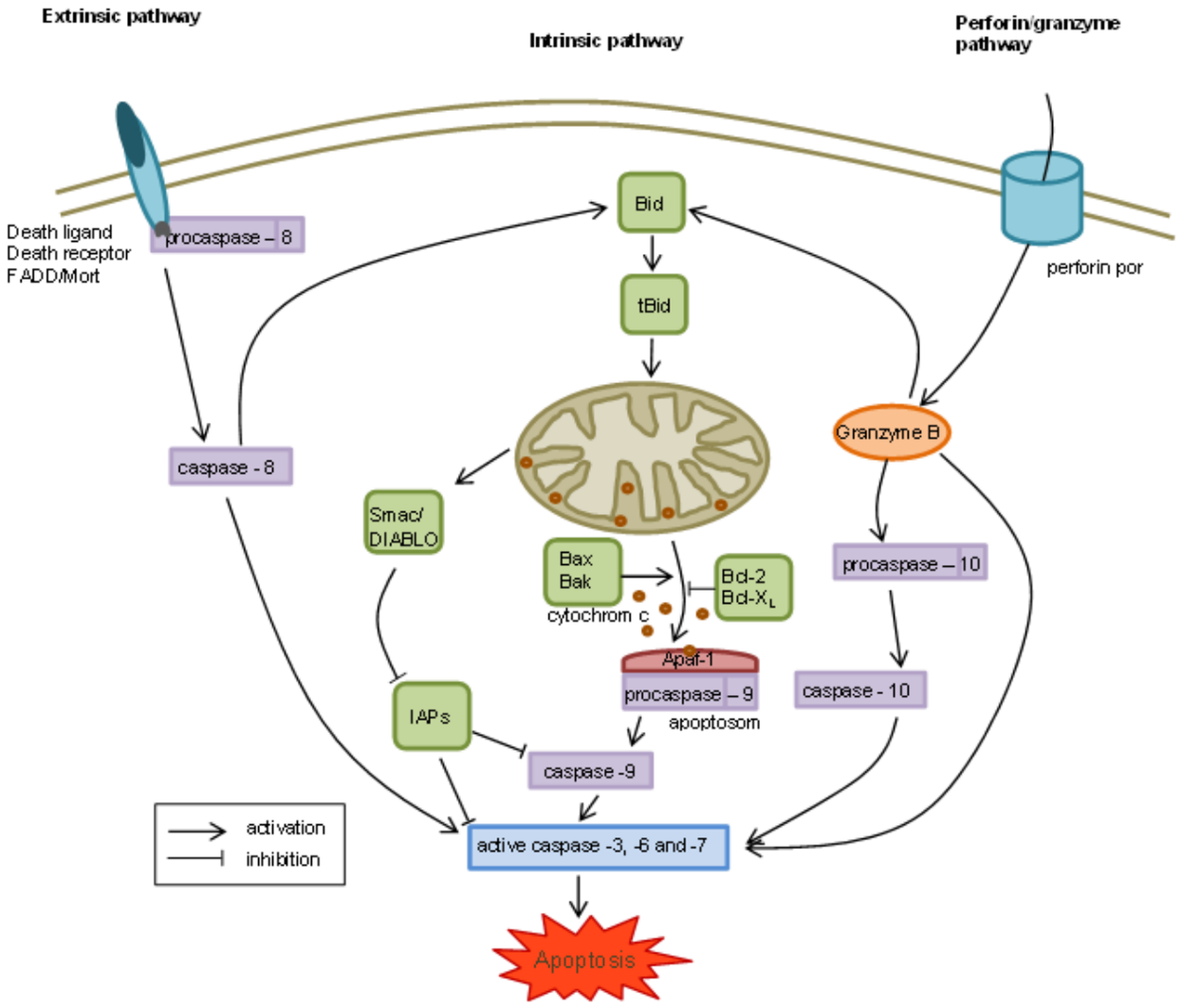

Figure 1. Interactions and the diversity of apoptotosis signaling pathways. Caspase cascade can be activated by the apoptosome, death receptors orgranzyme B. Initiator caspases -8, -9 or -10 directly or by with the participation of specific protein trigger effector caspases -3, -6 or -7 which results in cell death.

The ability of chemotherapeutic agents to initiate caspase activation appears to be a crucial element of drug efficacy. Consequently, defects in caspase activation often results in chemoresistance.

The normal epithelial cells of gastrointestinal tract colonic epithelium strongly express caspases 1 [26], 3, 7, 8, and 9 [27]. In case of colon cancer it was shown abnormal caspase expression. Downregulation of caspase 1 expression was observed in colon cancer samples [26]. Palmerini *et al.* [27] found the downregulation of caspase 7 in most colonic carcinoma tissues. The colon cancer response to immune attack and chemoradioresistance induced

apoptosis. Death receptor (extrinsic) pathway of apoptosis and mitochondrial (intrinsic) pathway of apoptosis were investigated in SW480 and SW620 colon cancer cells. The investigation study revealed that SW620 cell lines appeared more resistant to apoptosis induced by CH-11, cisplatin and ionizing radiation then SW480 and that Fas receptor and Apaf-1 was decreased in comparison to SW480 cells [28]. Mese et al. (2000) noticed that caspase-3 may mediate apoptosis induced by Cisplatin derivative in human epidermoid carcinoma cell line A431 [29] .

The protease activating factor Apaf-1, which was identified as the molecular core of the apoptosome executes mitochondria dependent apoptosis. The relevant levels of Apaf-1 are crucial in the inhibition of tumor progression and for preserving the sensitivity of cancer cells to apoptosis [30]. Melanoma cells avoid apoptosis by inhibiting the expression of the gene encoding Apaf-1. Suppression of release of SMAC/DIABLO from mitochondria was reported in melanoma cells [31]. Allelic loss and subsequent absence of Apaf-1 expression in melanoma cells is associated with chemoresistance [32]. Treating melanoma cells with the methylation inhibitor 5-aza-29-deoxyctyidine increased Apaf-1 expression and chemosensitized melanoma cells [32]. Shinoura et al. transduced Apaf-1 and caspase-9 into U-373MG glioma cells and observed increases in chemosensitivity of study cells [33]. In the other study Shinoura et al showed A-172 cells did not undergo apoptosis after p53 transduction, whereas U251 cells were markedly sensitive to p53-mediated apoptosis. A-172 cells showed higher endogenous expression of Bcl-X(L) than U251, and transduction of Bcl-X(L) repressed p53-mediated apoptosis in U251 cells, suggesting that high endogenous expression of Bcl-X(L) renders A-172 cells, at least in part, resistant to p53-mediated apoptosis. In the next step researchers transduced A-172 cells and U251 cells with the Apaf-1 or caspase-9 genes; both are downstream components of p53-mediated apoptosis and found that A-172 cells were highly sensitive to Apaf-1- and caspase-9-mediated apoptosis [34].

The presence of the specific antigen on the surface of the cell makes it the ideal aim for the antibodies, which could be used to the therapy of given kind of cancer. CD20 is a B-cell surface antigen that is an effective target for immunotherapy of B-cell malignancies using unmodified or radiolabeled murine monoclonal anti-CD20 antibodies. This cell surface phosphoprotein is involved in cellular signaling events including proliferation, activation, differentiation, and apoptosis. Shan et al. show that murine antiCD20 monoclonal antibodies inhibit B-cell proliferation, induce nuclear DNA fragmentation, and leads to cell death by apoptosis [35].

The monoclonal antibody against the protein CD20 selectively down-regulated the expression of antiapoptotic Bcl-xL and up-regulated the expression of proapoptotic Apaf-1 in Ramos cells [36].

Jazirehi et al. showed that anti-CD20 antibody in ARL cell line diminishes the activity of the p38MAPK signaling pathway resulting in inhibition of the interleukin (IL)-10 leading to the inhibition of constitutive STAT-3 activity and subsequent downregulation of Bcl-2 expression leading to chemosensitization [37]. They also observed upregulation of Raf-1 kinase inhibitor protein (RKIP) expression in non-ARL cell line.

Immunotherapy with specific antibody could be applied alone or in combination with chemotherapy. In the Jurkat clone J16, CD95 stimulation as well as anti-cancer agents' etoposide induces apoptosis. Etoposide was also found to induce caspase-8 processing and apoptosis in a CD95-independent fashion because blocking of CD95 receptor function with a specific antibody does not inhibit etoposide-induced apoptosis [38].

Jin et al. 2007 showed cancer cell lines transfected with chemokine-like factor CMTM8 submit to apoptosis. Caspase-dependent and independent mediated apoptosis, induced by CMTM8 overexpression, was facilitated by the mitochondria and inhibited by knockdown of Bad or overexpression of Bcl-xL [39].

An apoptosis promotion involves signaling through members of the tumor necrosis factors (TNF). On binding to their proper receptors, some members of the TNF family can initiate caspase activation, resulting in apoptosis. There was also observed that TNF can induce apoptosis in a limited number of tumor cell lines [40]. The effect of TNF induction with anticancer agent OK-432 on the survival rate of colorectal cancer patient was investigated. Patients in the TNF- producing group proved a better prognosis than those of the nonproducing group [41]. Ito et al. showed that endogenous TNF production peaked after stimulation with OK-432 (Ito et al. 1996). The apoptosis-signaling pathways stimulated by TNFs, require further explanation of the physiological role of these ligands in the potential application for cancer therapy and prevention [41].

2.3. The role of p53 and Bcl-2 family in apoptosis

Oxidative stress oncogene activation and arrest of cell growth lead to activation of tumor suppressor gene p53, which activates apoptosis or senescence [42-44]. The p53 gene has been called "guardian of the genome", due to crucial role in protecting the genome against the proliferation of mutated cells [45]. The gene p53 is a 53-kDa nuclear phospho-protein that binds to DNA to act as a transcription factor, and controls cell proliferation and DNA repair. p53 gene encoded the p53 proteins. In physiological conditions p53 occurs inactive form on the low level in the cells. However tumor suppressor protein p53 is induced in response to stress such as DNA damage, oncogene activation and hypoxia. Under the influence of DNA damaging agents the level of p53 protein increased and stopped the cell cycle in order to repair or cell death [46]. p53 protein interacts with other proteins, whose job is to protect and preserve DNA stability of the genes. One such protein is a polymerase poly-ADP-ribose (PARP, PARP-1, EC 2.4.2.30) [47]. DNA damage in the course of therapy cancer increases the expression of PARP and increases the amount of poly-ADP-ribose (PAR) in tumor cells, which positively correlates with the severity of the reaction of proapoptotic [48]. Mutations of p53 have been observed in over 50% of human cancers (e.g. ovarian, colon carcinoma), the mutations are connecting with resistance to radio- or chemotherapy treatment [49,50].

This fact supports that p53 plays an important role in the prevention of tumor development. The decisive function of p53 regulating the verdict of a cell to live or die makes it an attractive target for anticancer therapeutics [51]. The role of p53 in cell's reply to chemotherapy remains unclear. Moreover, there are many conflicting studies and

approaches which would be the main therapeutic strategy to cancer therapeutics. Previous study was based on the idea that activation of p53 can induce apoptosis in the tumor. Other are based on the observation that cells with defective p53 are more sensitive to combinations of chemotherapeutic drugs [52].There are numerous investigations where the cells with defective p53 undergo apoptosis. The p53 protein can mediate apoptosis in response to DNA damage caused by chemotherapy but the inducing cell cycle arrest and favoring DNA repair might increase resistance by allowing cells to live after DNA has been damaged by chemotherapeutic treatment [53]. Loss of p53 and Bcl-2 family take part in a decisive role in apoptosis. This date create the question: is defective p53 the Achilles heel of the tumor?

Mutations of p53 have been observed in over 50% of human cancers (e.g. ovarian, colon carcinoma), the mutations are connecting with resistance to radio- or chemotherapy treatment [48,49]. This fact supports that p53 plays an important role in the prevention of tumor development. The decisive function of p53 regulating the verdict of a cell to live or die makes it an attractive target for anticancer therapeutics [50]. The role of p53 in cell's reply to chemotherapy remains unclear. Moreover, there are many conflicting studies and approaches which would be the main therapeutic strategy to cancer therapeutics. Previous study was based on the idea that activation of p53 can induce apoptosis in the tumor. Other is based on the observation that cells with defective p53 are more sensitive to combinations of chemotherapeutic drugs [52]. There are numerous investigations where the cells with defective p53 undergo apoptosis. The p53 protein can mediate apoptosis in response to DNA damage caused by chemotherapy but the inducing cell cycle arrest and favoring DNA repair might increase resistance by allowing cells to live after DNA has been damaged by chemotherapeutic treatment [53]. It is common known that malignant cells undergoing apoptosis p-53 dependent or p-53 independent. Many cases showed that p53 is needed if cells are to submit to chemotherapy. However many examination conducted in recent years have resulted in the discovery of drugs which have been used successfully to treat patients with p53- defective tumors. Additionally, radiation therapy is usually applied patients independent of their p53 status [54]. The crucial investigation supporting the significance of p53 in mediating DNA damage and induced apoptosis in cells derived from p53 knockout mice. The authors showed that p53 is required for radiation-induced apoptosis in mouse thymocytes. The cells isolated from this mouse were totally resistant to γ-irradiation and died [55]. Similar the fibroblast isolated from the same mice were also resistant to radiotherapy and chemotherapy with adriamycin [56]. Other scientific reports indicated that radiation of T cell lymphoma derived from the same mice with knockout p53 was able to its killing. There are many studies in which the effect of p53 on the cellular response to chemotherapy and radiotherapy is controversial. Some reports suggesting that p53 wild-type are more sensitive to many of anticancer drugs, but there are many investigation, which demonstrated that p53-defective are or not sensitive to chemotherapy. Similar results obtained after photodynamic treatment in different cancer cells. Some examination designate that p53 is necessary for executor caspase 3 activation, suggesting that can play a decisive role in PDT- induced early apoptosis in malignant tissue [57]. Other study examined the outcome of photodynamic reaction with Photofrin (Ph-PDT) on clear human ovarian carcinoma OvBH-1 with "silent mutation" in the *p53* gene. They suggest that this

mutation may inhibit apoptosis in these cells [58]. The modification of this method by chemotherapeutic drug 2-methoxyestradiol leads to apoptotic pathway induction in these cells [59]. However, additional studies demonstrate that PDT can induce apoptosis in cancer cells by pathway independent of p53 [60].

Differential cells sensitivity on chemo- and other anticancer therapy is probably dependent not only on p53 status but other genes and their products, which control cancer cells responding (c-Myc, protein kinase A, protein kinase C, cyclins). Moreover p53 work together with different tumor suppressor family. The big influence on anticancer therapy effectiveness is also individual dependent on type of cancer and their environment [61,62]. Recent studies have shown that important role in the effectiveness on anticancer therapy plays a modification by its phosphorylation [63].

One of approach, which leads to regulation of p53 function, depends on its post-translational modifications. Some of amino acid in p53 proteins is phosphorylated. One of the most important issues is the phosphorylation of p53 at position Ser 15. It is common known that chemotherapy resulting in an increased stabilization of this protein [64,65]. The cells with phosphorylated p53 protein most often were observed in serine 392 and N-terminal and in serine 20 et C-terminal end observed in cells [66]. This process enhanced the stability form of p53 protein. Recent investigation have shown that p53 phosphorylation et serine 15 and 20 was necessary to induce apoptosis in ovarian cancer cells after chemotherapy with cisplatin. The p53 protein is also change in lung cancer, but there was no strong correlation between changes in expression of this protein (both mutant and native) and course of disease [67,68]. There was, however, significant improvement in patients undergoing therapy combined, in which the course of exacerbation p53 protein expression [67]. It is known that phosphorylation area of p53 protein binding MDM2 inhibits degradation of p53, a C-terminal phosphorylation of Ser392 alters the cell cycle [69,70]. In addition the new examination have shown that stimulation or inhibition of tumor growth might be due to changes in proteins modify p53. The in vitro and in vivo studies demonstrate that Sir2 protein is involved in this procedure. They cause p53 deacethylation which inhibits its activity. These proteins blocks apoptosis induced by “guardian of the genome” in response to stress, which may promote tumor growth The use of Sir2 inhibitors, that prevent p53 deacethylation along with its promoters allow the development of new anticancer strategy based on the maximization of action of this protein [71, 72]. It is obvious that only p53-tageted therapeutic strategy is not enough for the treatment of all type of malignant tissue. Ideally anticancer strategy will be therapy adapted to patients based on the p53 status, checkpoint proteins and gene controlling and oncogenic changes [73].

Bcl-2 family is the other important proteins which near and with p53 take part in a decisive role in apoptosis [74]. About twenty five members of the Bcl-2 family of proteins have been identified [75]. The products of Bcl-2 gene family are divisible into two main groups: antiapoptotic Bcl-2, Bcl-Xl, and Bclw, and proapoptotic Bax, Bak, Bad, and Bim, which respectively inhibit or support the effecting of apoptotic cell death [76,77]. The other researcher divided this proteins into three subfamilies based on structural and functional features: pro-survival, whose members are most structurally similar to Bcl-2; proapototic

Bax and Bak and antagonize their prosurvival functions BH3-only proteins [78,79]. Thus families of proteins control mitochondrial stability by maintaining the balance between proapoptotic proteins that translocate to the mitochondria and antiapoptotic ones that exist in the mitochondrial membrane [76,77]. The Bcl-2 gene product is located in the membranes of the endoplasmic reticulum, nuclear envelope and the external membranes of the mitochondria [76]. The fact that key Bcl-2 family genes are p53 targets including pro- and antiapoptotic [80]. Bak and especially Bax were the groups induced by p53 mainly in response to stress [81,82]. P53 plays a crucial role in regulation of proapoptotic Bcl-2 proteins. Bax induced the mitochondrial pathway by outflow of apoptogenic proteins, such as cytochrome c. However, in different studies the involvement of Bax and p53 in different anticancer therapy mediated apoptosis was observed [83,84].

Bax gene encoding the protein contains on the promotor sequence the binding location for p53. The requirement of Bax and Bak in p53 –activated apoptosis occurs to be cancer cell-type dependent. Moreover, p53 can also independently activate Bax present in the cytoplasm and this protein forms a homodimer and releases cytochrome c from the mitochondria [85]. Bax protein takes part in apoptotic response of the developing nervous system to γ irradiation and leads to sensitivity fibroblast cells with E1A-expressing to chemotherapy [86,87]. Additional studies showed that the level of Bax protein acts not crucial role in inducing apoptosis or growth arrest in other cancer cells. In epithelial colon carcinoma undergoing apoptosis in response to radiotherapy, Bax did not appear to be main inductor of cell death [88,89]. The explanation of enigmatic function of Bax in apoptosis has recently been examined in the context of PUMA. *PUMA* gene is as well as Bax activating by p53 especially in response to DNA damage. This gene encodes to BH3-domain-conteinig protein: PUMA α and PUMA β [90,91]. A fundamental balance between PUMA and p21 which controlled cell cycle determine growth arrest by senescence or death by apoptosis in cooperation with p53. This date was obtained from colorectal cancer cells where the growth arrest through of p21 is the normal rescue response to p53 expression in these cells. The defect of p21 induced in these cells apoptosis pathway, whereas PUMA is damage the apoptosis is prevented. These results suggest that Bax is absolutely necessary for *PUMA* – induced apoptosis [92]. Probably *PUMA* expression promotes mitochondrial translocation and multimineralization of Bax and in consequence inducing apoptosis. Bax takes part in the apoptotic death response indirectly target of p53 through *PUMA* [92]. The pro-apoptotic member of Bcl-2 protein Bid acts crucial function in connecting between the extrinsic and intrinsic apoptotic pathway. It has unique ability to connect two different types of apoptosis. Activation of Bid involves cleavage of cytoplasmic Bid by activator caspase 8 and induces post-translational changes. This process leads to Bid translocation into the mitochondrial membrane and activates pro-apoptotic Bax and Bak proteins which initiate apoptosome formation. *Bid* gene succumb p53 regulation in response to chemo- and radiotherapy in many type of cancer. Cellular sensitivity to chemotherapy with adriamycin or 5-fluoroacyl is dependent on wild-type p53 and Bid. These results suggest that p53 can regulate the intrinsic and extrinsic pathway through *Bid* regulation [93]. It is common that inhibition of apoptosis can lead to cancer. In the large Bcl-2 family we can find also inhibitors of cell death or growth arrest. Bcl-2 (Bcl-2 its self) residue in the outer mitochondrial membrane

and mainly plays an anti-apoptotic function [94]. The Bcl-2 anti-apoptotic protein inhibits apoptosis in cancer cells and promotes cell survival. In many malignancies especially in hematologic the overexpression of Bcl-2 family was found. Recent studies showed that increasing levels of Bcl-2 and Bcl-X_L have associated with a more aggressive malignant phenotype often connected with drug resistance to various type of chemotherapy not only in hematologic but also solid tumors [95,96]. As an example in primary prostate cancer, high Bcl-2 level is con were connected with high Gleason scores and an increase rate of cancer recurrence after radical prostatectomy. Also the high expression of BCL-XL in the NCI 60 cell line is strongly correlated with resistance to most chemotherapy agents. There are many investigations determining the levels of expression of cell death inhibitors in various types of cancer. These studies afford correlative evidence, but also are designed to search new possibilities of tumor destruction [52]. Many in vitro experiments confirm the preventing role of Bcl-2 in apoptosis activating in different type of cancer [97].

This fact decides about therapeutic targets through inhibition of this protein and arrest malignance process [98]. Wild-type p53 can establish complex with Bcl-2 and Bcl-X_L and suppress there anti-apoptotic function. However, in 50% of cancers the *p53* gene is disrupted and losses its ability to bind to these proteins. Hence research is continuing on the use of synthetic inhibitors in preclinical and clinical study (Table 1.) [97]. The most of them determined the direction of future clinical development and are promising.

Agents	Target proteins	Sponsor	Stage
Apogosypol	Bcl-2	Mcl-1 Brnham (NCI)	Preclinical
HA14	Bcl-2	Maybrige Chem	Preclinical
Antimycin A	Bcl-2, Bcl-X_L	U of Washington	Preclinical
Oblimersen sodium	Bcl-2	Genta	Phase III
Gossypol (AT-101)	Bcl-2, Bcl-X_L, Bcl-w,	Mcl-1(NCI)	Phase I/II
ABT-737(ABT 263)	Bcl-2, Bcl-X_L, Bcl-w	Abbott	Phase I
BH31s	Bcl-X_L	Harvard U	Preclinical
GX15-070	Bcl-2, Bcl-X_L, Bcl-w	Mcl-1 Gemin X	Phase I

Abbreviations: BH3Is, BH3 inhibitors; NCI, National Cancer Institute; Maybridge Chem, Maybridge Chemical Co. Ltd.; U, University. Acc. to Kang and all [97].

Table 1. Agents targeting anti-apoptotic Bcl-2 family proteins.

Apoptosis is perturbed in many cancers. It is the major barrier leads to destruction of cancers. The p53 and Bcl-2 pro-apoptotic protein are one of the many proteins that induce the intrinsic signaling pathway. Previous and present studies yield new information about various factors which regulate apoptosis. Among them are proteins that inhibit apoptosis. Protein plays the crucial role in regulatory cell development, cell cycle, cell growth and apoptosis. The intracellular proteins are selective stabilized or eliminated by ubiquitin-depenedent pathways. This procedure leads to correcting the regulation of many metabolic

processes in cells. Ubiquitin is a protein complex composed of the activating enzyme (E1), a coniugating enzyme (E2) and protein ligase (E3) [100-103]. Ubiquitin targets the protein substrate for damage via the 26S proteosome. The free ubiquitin is recycled. This process plays a crucial role of many significant signaling pathways and important role in many cellular pathways including apoptosis. Many proteins which can regulate apoptotic pathways have been recognized as target substrates for ubiquitination [103]. Elements of the cell apoptosis mechanism are often altered in cancer. The resistance to apoptosis is one of the major problems in the anticancer therapy. The ubiquitin –proteosome protein damage can inhibit apoptosis by degradation proapoptotic controller. From this studies appear that proteosome inhibitors can apply as antitumor therapies through enhancing apoptosis [103]. Apoptosis regulatory molecules have been recognized as substrates and degraded in proteosome. The degradation leads to apoptosis resistance in cancer cells. To these we can include inter alia members of Bcl-2 family and IAP [104-106]. The heat shock proteins can play an important role in recognition and degradation of damaged proteins by ubiquitination [107]. The heat shock proteins (HSPs) are a highly conserved class of proteins whose expression in increased in cells exposed to different kinds of stress. HSPs are a family which limit the consequences of damage and facilitate cellular recovery [108,109]. When there are damaged proteins, HSP binds injured molecules. This results in dissociation of HSF (heat shock factor), then migrates to the nucleus, where it binds with HSE (heat shock rudiments) leading to HSP overexpression [110]. The basis for the classification on these proteins was their chaperone activity and molecular weight. HSPs can be divided into three subfamilies: large (HSP100, 90), intermediate HSP 70, 60 and 40) and small (sHSP less than 40 kDa). In addition to many function the HSPs protein plays a crucial role in inhibition of apoptotic process. Anti-apoptotic role of sHSP proteins makes it encourages the development of tumor progression and metastasis. sHSP in the receptor pathway of apoptosis blocks DAXX protein and through AKT kinase inhibits activation and translocation of Bax to mitochondria [111]. There are several reports according to which the use of antisense oligonucleotides directed against HSP27 can be basis of anti-cancer therapy. The overexpression HSP27 is often finds in malignant cells for example in ovarian and breast cancer. Blocking apoptosis by the sHSP lead to interfere with some cytostatics and decrease the effectiveness of chemotherapy. It is often connected with poor prognosis of cancer [112-114]. HSP90 inhibits formation of an active apoptosome whereas HSP70 prevents the recruitment of procaspase-9 to the apoptosome complex [60]. Previous investigation involved both HSP70 and HSP27 of Bid dependent apoptosis. Bid is the protein which associated two apoptotic pathways intrinsic and extrinsic. HSP-mediated regulation of apoptosis through inhibition of major pro-apoptotic proteins is involved in this process [115,116].

3. Enzymatic antioxidants

Defense against oxidative stress is provided by a system of antioxidants enzymes and non-enzymatic antioxidant substances capable of neutralizing free radicals and preventing an excess production of reactive oxidative species (ROS) [117]. The first line of cellular defense

against oxidative stress enzymes are: the family of superoxide dismutases (SODs), glutathione peroxidases (GPXs), and catalase (CAT) enzymes. They are the main free radical-scavenging enzymes which decomposing superoxide radicals and H_2O_2. Also glutathione transferase (GST) plays an important role in the protective mechanisms. It plays an important role in catalyzing the conjugation of reactive electrophilic agent to glutathione (GSH) [118]. Antioxidant enzymes drive chemical reactions to convert ROS into non-toxic molecules:

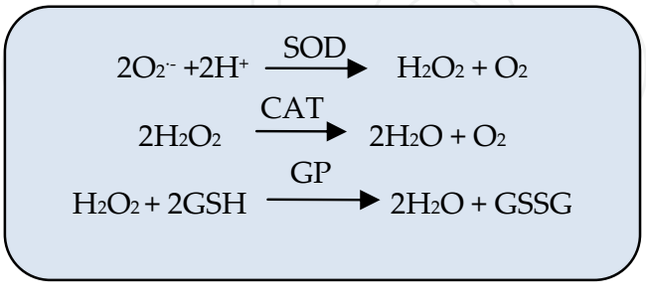

Figure 2. The chemical reactions involving ROS and antioxidant enzymes.

This enzymatic system is complex and highly integrated [119-121]. SOD is an essential antioxidant enzyme that defends cells against potentially damaging superoxide radicals. There are three known human isoforms of SOD, which defends cells against potentially damaging superoxide radicals:

- SOD1 (CuZnSOD) is found in the cytoplasm and nucleus in the form of a dimer;
- SOD2 (MnSOD) is a tetrameric protein that functions in the mitochondria, more than 95% of cellular oxygen is metabolized in the mitochondria during oxidative phosphorylation;
- SOD3 (CuZnSOD) is a tetrameric, extracellular form of the enzyme while each enzyme performs a critical function, SOD2 is particularly important due to its location within the mitochondria [122-124].

Enyzmes of the GPX family are selenoproteins, which catalyze the reduction of hydroperoxides to water and the respective alcohols, while oxidizing GSH to GSSG [122]. Glutathione sulfide reductase is responsible for converting GSSG to GSH. These two compounds serve as the major redox couple within the cell, which determinant the total cellular antioxidant capacity [125, 126]. There has been identified four distinct isoforms of GPX in humans. GPX1, which is localized in the cytoplasm and mitochondria in the liver, kidney, lung, and red blood cells, catalyzes the reduction of H_2O_2 and some organic peroxides [127]. GPX2, localized mainly in the liver and gastrointestinal tract, protects against lipid hydroperoxides. GPX3 is has the same function, but it is highly detected in plasma. GPX4, expressed in the testis, is capable of reducing phospholipid hydroperoxide, including lipid peroxides derived from cholesterol [128,129].

Catalase is an antioxidant enzyme which catalyzes the conversion of H_2O_2 to water and oxygen. It is concentrated mainly in peroxisomes [130].

Persistent oxidative stress is a major initiator to progress cancer [119,121,131]. Reactive oxidative species (ROS) may cause irreparable damage, therein: base modification, DNA

strand breaks, DNA-protein cross-links [132]. Following cellular damage initiated the deregulation of cell signaling pathways, tumor suppressors and an inhibition of apoptosis [121]. A variety of studies involving antioxidant enzyme levels and cancer development have been performed. Tumor cells nearly always show a decrease in SOD1 and SOD2 expression. Glutathione peroxidase activities have been found to be changeable, while catalase activity is generally lower in tumor cells than in healthy tissue [133-135].

The changes in enzymatic antioxidants status [118], the level of lipid oxidation [136] and an increase of DNA breaks number in tumor cells and leukocytes of blood indicate the process of malignancy [137]. Oxidative DNA damage in blood and other tissues were detected in various types human carcinogenesis [118,138,139,140]. The GST is involved in detoxification of carcinogens. Its activity increased significantly in cancer patients [141]. In smokers the role of GST is crucial in modulating susceptibility to smoking-related lung cancer, oral cancer and chronic obstructive pulmonary disease [118,142,143]. It is also observed that the GPx and SOD activities decrease in the group of cancer patients during cancer development [118,144]. Burlakova et al. noted that the absence of a response these enzymes indicate a weakening of antioxidant enzymes system [118]. They found no change in the malondialdehyde (MDA) level, which is consistent with the previous work in patients with oral cancer [117,145,146,147]. In initial period its level is increased, but later it decreased [118]. It was observed that SOD, GPx and GSH levels in the erythrocyte and plasma was significantly lower in cervical cancer patients, as well as Vitamin E, Vitamin C and GST level. These results suggest possible use of antioxidant supplementation as prophylactic agents for prevention and treatment of this cancer [148].

Carcinogenesis process is accompanied by weakening of the antioxidant enzyme system, but also by high expression ROS-generated enzymes [149]. The NADPH oxidases (Nox enzymes) share the capacity to transport electrons across the plasma membrane and to generate superoxide and other reactive oxygen species (ROS) [150,151]. The physiological functions of Nox enzymes include: cell differentiation, host defense, posttranlational processing of proteins, cellular signaling, and regulation of gene expression. Those enzymes could also induce a wide range of pathological processes, including the process of carcinogenesis [150]. NOX1 homolog of the NADPH oxidase is highly expressed in the colon [152,153] and it might contribute to development of colon cancer through at least two mechanisms: ROS-dependent DNA damage and ROS-dependent enhancement of cell proliferation [150]. NOX4 homolog of the NADPH oxidase is suggested to promote cell growth in melanoma cells [153]. Drugs directly inhibiting the NADPH oxidases activation could successfully inhibit oxidative stress and inflammation caused by this enzymes [149]. Apocynin (4-hydroxy-3-methoxyacetophenone) is now used indiscriminately as a NOX4 [154] and as a NOX5 inhibitor [155].

In some situations ROS are used in anticancer therapies. Photodynamic therapy (PDT), a promising therapy for solid tumors, based on the photochemical reaction produces singlet oxygen and other forms of reactive oxygen, such as superoxide ion, hydrogen peroxide, hydroxyl radical [156-160]. Tumor cells can respond to photodynamic damage by apoptosis or necrosis [161-163]. Singlet oxygen and superoxide anion have been demonstrated to play

a main role in the cytotoxic effects induced by PDT [164, 165]. SOD1 and SOD2 scavenged cells from singlet oxygen and significant extent the antitumor efficacy of PDT [156, 166, 167]. Combinations of SOD inhibitor with PDT might result in significant increase in the efficiency of anticancer treatment [154]. Overexpression of SOD2 suppresses apoptosis, negatively correlates with the sensitivity of tumor cells to radiation therapy and anticancer drugs [168, 169]. 2-methoxyestradiol (2-$MeOE_2$) was shown to selectively inhibit the activity of superoxide dismutases [170]. PDT with 2-$MeOE_2$ selectively enhance free radical generation and suppress antioxidant defenses, which significantly increases the effectiveness of therapy [156].

PDT is also antagonized by other cellular antioxidant defense mechanisms: catalase, lipoamide dehydrogenase, the glutathione system, heme oxygenase-1 (HO-1) [171-173]. HO-1 catalyses the rate-limiting step in the oxidative degradation of heme. Products of the reaction catalyzed by this enzyme are CO and biliverdin which is rapidly converted to bilirubin. Biliverdin and bilirubin are potent antioxidants capable of scavenging peroxy radicals and inhibiting lipid peroxidation [174-176]. Induction of HO-1 protects against the cytotoxicity of oxidative stress, which seems to play a protective role against PDT-induced cell death [173, 177].

Administration of HO-1 inhibitors might be an effective way to potentiate antitumor effectiveness of PDT. Zinc (II) propoporphyrin IX, and HO-1 inhibitor, markedly augmented PDT-mediated cytotoxicity towards colon adenocarcinoma C-26 and human ovarian carcinoma MDAH2774 cells [173]. Kocanova et al showed that treatment of HeLa (human cervix carcinoma cells) and T24 cells (human transitional cell carcinoma of the urinary bladder) with hypericin-PDT dramatically induced of HO-1 expression. This HO-1 stimulation is governed by the p38MAPK (p38 mitogen-activated protein kinase) and PI3K (phosphatidylinositol 3-kinase pathways). Blocking these signaling pathways by p38MAPK inhibitors or small interfering RNA (siRNA) for p38MAPK suppress HO-1 increases, raising the propensity of the cells to undergo PDT-induced apoptosis [178].

4. Non-enzymatic antioxidants

Among the non-enzymatic antioxidants can be distinguished based compounds, both endogenous (glutathione, melatonin, estrogen, albumin) and exogenous (carotenoids, vitamin C, vitamin E, flavonoids), which must be delivered to the body with food because the body is not able to produce them himself.

Carotenoids are natural antioxidants present in the chloroplasts and chromatopfores, giving the plants the color yellow, red and orange, visible especially in autumn. Their function is to stabilize the lipid peroxide radicals as well as provide protection against damage from sunlight by absorbing energy or redirecting it to other processes in the cell. Carotenoids ingested with food (beta-carotene) are precursors of retinoids (vitamin A). Vitamin A is fat-soluble antioxidant.

Some studies have shown that supplementation with high doses of β-carotene or carotenoid in smokers, as well as in laboratory animals exposed to tobacco smoke increases the risk of

lung cancer [179-181]. A study have shown that administering both vitamin A and vitamin C to the cell culture of human breast cancer cells was three times more effective than the administration of these vitamins separately [184]. β-carotene it normally functions as an antioxidant, at high concentration it exhibits prooxidant effects especially at high oxygen tension [185, 186]. Carotenoids diets have demonstrated some anticarcinogenic activity in animal experiments [187-190].

Ascorbic acid (vitamin C) is antioxidant that works in aqueous environments of the body. Humans cannot synthesize vitamin C, it must be provided exogenously in the diet and transported intracellularly. Prolonged absence of vitamin C in the diet leads to the development of scurvy. Vitamin C has important roles in vascular and connective tissue integrity, leukocyte function, and defense against microorganisms. Vitamin C is considered as a most powerful ROS scavenger because of its ability to donate electrons in a number of non-enzymatic and enzymatic reactions. Some authors demonstrated ability to neutralize free radicals produced by exposure to light to compounds of lower toxicity [191,192]. Vitamin C plays an important role in the detoxification of substances such as tobacco smoke, ozone and nitrogen dioxide [193]. Ascorbic acid reduces tocopheryl radical formed by the reaction of vitamin E with lipid radicals, protects membranes against oxidation, and prevents lipid peroxidation and affect the regeneration of vitamin E [194,195].

The data reported here suggest that the dose of vitamin C supplement used may induce additional defenses against oxidative damage, through an increase in lymphocyte SOD and CAT activity [196]. Experimental data suggest that these antioxidants such as carotenoids, vitamin C and vitamin E can interact synergistically; they protect each other from degradation and/or promote their regeneration [197-200]. Low serum levels of Vitamin C in high risk population may contribute to the increased risk of chronic gastritis or gastric metaplasia, which are both precancerous lesions [201]. The positive effect of Vitamin C has also been found in lung and colorectal cancer [202]. Vitamin C has proven to be beneficial as a factor in preventing cancer of the lungs, larynx, mouth, esophagus, stomach, colon, rectum, pancreas, bladder, cervix, endometrium, breast, and malignant brain tumor. Vitamin C is effective in the defense against oxidative stress-induced damage [203].

***α*-tocopherol** Vitamin E is a fat-soluble antioxidant that stops the production of reactive oxygen species formed when fat undergoes oxidation[204]. The most active form of vitamin E in humans is *α*-tocopherol and there is considered as a major antioxidant in biomembranes.

It has been noticed that in colorectal cancer patients the incidence decrease vitamin E [205-207] and the intake of Vitamin E [200 IU] reduced the incidence of colorectal cancer by triggered apoptosis of cancer cells [208]. Colorectal carcinogenesis may be reflected by greater elevation of MDA and decrease level of vitamin E and vitamin C in the serum [209]. Other study reported negative results for Vitamin E in combination with Vitamin C and beta carotene to prevent colorectal cancer adenoma [210, 211]. Since Vitamin C regenerates Vitamin E, it has been proposed that addition of Vitamin E hinders the protective effect of Vitamin C against oxidative damage.

Flavonoids are polyphenolic compounds that are ubiquitous in nature. Over 4,000 flavonoids have been identified, many of which occur in fruits, vegetables and beverages (tea, coffee, beer, wine and citrus fruits, grapes, soy products). The flavonoids have been reported to have antiviral, anti-allergic, anti-inflammatory, and antitumor and antioxidant activities. Protective effect, preventing lipid peroxidation, is also responsible for maintaining the appropriate level of glutathione in the cells. For the flavonoids and their derivatives with the strongest antioxidant potential include: delfinina, epicatechin, kaempferol, quercetin, luteolin. Quercetin, the most abundant dietary flavonol, is a potent antioxidant because it has all structural features for free radical scavenging activity.

Flavonoids are most commonly known for their antioxidant activity in vitro. At high experimental concentrations that would not exist in vivo, the antioxidant abilities of flavonoids in vitro may be stronger than those of vitamin C and E, depending on concentrations tested [212]. Epidemiological studies have shown that regular consumption of fruits and vegetables is associated with reduced risk of chronic diseases such as cancer and cardiovascular disease [213,214]. It has been reported that fresh apples have potent antioxidant activity inhibit the growth of colon and liver cancer cells in vitro [215]. Apples are commonly consumed and are the major contributors of phytochemicals in human diets. Some studies have demonstrated that whole apple extracts prevent mammary cancer in rat models in a dose-dependent manner at doses comparable to human consumption of one, three, and six apples a day. Consumption of apples may be an effective strategy for cancer chemoprevention. Fresh fruits could be more effective than a dietary supplement [216]. The inhibitory effect of black tea polyphenols on aromatase activities has been investigated. Black tea polyphenols, TF-1, TF-2, and TF-3, significantly inhibited rat ovarian and human placental aromatase activities. In in vivo models, these black tea polyphenols also inhibited the proliferation in MCF-7 cells [217].

Glutathione (GSH) is the most important non-enzymatic cytosolic antioxidant. This tripeptide is produced by the body from three amino acids: cysteine, glutamic acid and glycine [218]. In addition to neutralize free radicals, glutathione is responsible for maintaining the antioxidant activity of other antioxidants, stabilizing its reduced form. One of the basic functions of glutathione is to maintain the sulfhydryl groups of proteins in the reduced state and inhibition of oxidation by hydrogen peroxide [191, 193, 219]. Glutathione together with glutathione peroxidase (GSH-Px) reduces hydrogen peroxide H_2O_2 and lipid peroxides, which is accompanied by the formation of glutathione disulfide, which is reduced by NADPH in a reaction catalyzed by glutathione reductase [220]. Equally effective could lead hydroxyl radical HO, the most dangerous of free radicals to form water. It is able to regenerate vitamin E and vitamin C back to their active forms.

Preliminary results indicate glutathione changes the level of reactive oxygen species in isolated cells grown in a laboratory, which may reduce cancer development [221, 222]. Glutathione supplementation increases mean survival time treated mice [223]. Others study demonstrates that in colorectal carcinoma patients, a very highly significant decrease in total plasma thiols and intracellular glutathione [224].

Selenium is a trace element that is essential in the human diet. The antioxidant properties of selenoproteins help prevent cellular damage from free radicals. Many studies confirm that selenium reduces the risk of all cancers especially cancer of the liver, prostate, colorectal and lung cancer [225, 226]. The results showed that Selenium could significantly inhibit tumor growth as well as extend the median survival time of tumor-bearing mice [227]. Selenium significantly inhibits the proliferation cancer cells in vitro [228]. Selenium deficiency is associated with an increased risk of cancer and cancer death [229, 230].

Non-enzymatic antioxidants are relatively ineffective in comparison with the action of antioxidant enzymes. Only together with enzymes is effective line of defense against oxidative stress [187].

5. ROS and RNS

Nitric oxide (NO) is a diffusible, short-lived, diatomic free radical ubiquitously produced by mammalian cells, and it is a multifunctional signaling molecule that regulates complex cellular processes. L-arginine derived NO production is mediated by activation of nitric oxide synthase (NOS). There are three isoforms: endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS). It has been detailed that iNOS gene transcription and promoter activity are increased by oxidative stress and it regulates chromatin modification leading to cellular injury. NO has been used for various diseases as a screening marker, such as cerebral strokes, asthma and chronic obstructive pulmonary diseases [231-235]. Consequently, measurement of NO might be a reliable biomarker to predict earlier oxidative stress mediated cellular response including injury and specific differentiation of stem cells.

The excess of ROS/RNS (reactive oxygen species/reactive nitric species) generated from endogenous sources, for example mitochondria in response to inflammatory conditions, upregulation of enzymes (NADPH oxidase, hemoxygenase-1, xanthine oxidase, nitric oxide synthases), or from the environment (smoking, radiation, industrial pollution) may damage macromolecules such as lipids, proteins and DNA and induce neurological disorders, atherosclerosis or aging. The identification of valid biomarkers of stress is involved with previous characterizing the event of stress and for early identification of the disease development which might follow [236]

5.1. Lipid peroxidation

Lipid peroxidation is a normal metabolic process extending under regular conditions. It can proceed into three steps: initiation, propagation and termination. The initiation phase is connected to the activation of oxygen and is rate limiting. Polyunsaturated fatty acids (the main component of membrane lipids) are receptive to peroxidation. The process of lipid peroxidation is one of the most investigated consequences of reactive oxygen species (ROS) actions on membrane structure and function. Production of oxygen radicals increases with clinical progression of disease. It is also involved with the increase of lipid peroxidation products and resulting membrane degeneration [237]. Peroxidation of cell membranes, which contain a high concentration of polyunsaturated fatty acids, is a critical mechanism

leading to growth inhibition and cell death. The cell death can occur by necrosis; however lipid peroxidation can induce also apoptosis, activating the intrinsic suicide pathway present within all cells [238]. This type of cell death eliminates precancerous and cancerous, virus-infected and otherwise damaged cells that threaten our health. Some authors also demonstrated that lipid hydroperoxides and oxygenated products of lipid peroxidation degradation as well as lipid peroxidation initiators (ROS) can be involved in the cascade of signal transduction the control of cell proliferation, and the induction of differentiation, maturation, and apoptosis. It has been shown that lipid peroxidation and ROS are triggers and essential mediators of apoptosis [238, 239].

Saintot et al. suggested that lipid peroxidation could be verified to be a prediagnostic marker for breast cancer. Lipid peroxidation levels in breast ductal cells may become a promising cancer biomarker to detect, through non-invasive methods such as nipple fluid aspirate sampling, for example, women at high risk for breast cancer. In addition, a better understanding of the relationship between breast cancer risk factors and oxidative stress/lipid peroxidation-related biomarkers and genes may prove useful in identifying the dietary or non-dietary exposure, genotype combinations that put women at the lowest risk. In addition, lipid peroxidation markers could also be applied in prognosis. Decreased concentration of malondialdehyde (MDA) in plasma, another lipid peroxidation product, has been found to be significantly related with severity of prognosis factors for breast cancer. MDA concentration was significantly lower in the plasma of patients with large tumors or in whom nodes and/or metastasis was observed [239-241]. There was also observed increased concentration of MDA (malonodialdehyde) in colorectal carcinoma patients. Authors suggest ROS production in gut due to phagocytes, which are accumulated in mucus of patients with bowel disease [237,242, 243]. Bahat et al. also demonstrated thet colorectal carcinogenesis may be associated with greater MDA concentration and decreased level of vitamin C in the patients' serum [243]. Other authors hypothesize that lipid peroxidation can be a principal mechanism in rodent renal carcinogenesis. Saczko et al. demonstrated that MDA marker and concentration of –SH groups can be a validate marker for efficiency in PhII mediated photodynamic therapy (PDT) in lung carcinoma cells (A549). Authors proved that the level of lipid peroxidation was significantly higher for cells after PDT, comparing to control cells. They observed much lower concentrations of -SH groups in A549 cells after PDT treatment, in comparison with respective values in control cells [244].

5.2. Protein damage

Proteins contained by cells undergo oxidative stress in the presence of various reactive oxygen species (ROS). The consequential damage of proteins may take the form of nitration or oxidation of various residues, depending on the presence of ROS. ROS can also induce the formation of advanced oxidation protein products (AOPP) or advanced glycation end products (AGE), both of which are stable markers of oxidative stress. Increased AOPP, malondialdehyde levels, and decreased thiol and nitric oxide concentrations, may imply that patients are under oxidative stress. Proteins damage can provoke reduced cell-specific functional ability and may then allow other mutations to produce signaling components

which will then go unconstrained aiding tumourigenesis. Many studies use oxidative protein damage markers for determination of stages in cancer patients' and disease progression [245].

Protein oxidation by ROS is related with the formation of many different kinds of protein cross-linkages, including those formed by addition of lysine amino groups to the carbonyl group of an oxidized protein; by interaction of two carbon-centered radicals obtained by the hydroxyl radical-driven abstraction of hydrogens from the polypeptide backbone; by the oxidation of sulphydryl groups of cysteine residues to form –S–S– crosslinks, and the oxidation of tyrosine residues to form –tyr–tyr– cross-links. Protein damage is repairable and is a known non-lethal event for a cell. There was reported that two mitochondrial proteins: aconitase and adenine nucleotide – translocase can be significant targets of long-term oxidative destruction. It has been presented that the hydroxyl radical represents the major species responsible for the oxidation of proteins [121,246]. Low concentrations of superoxide radical and hydrogen peroxide may stimulate proliferation and enhance survival in a different cell types. In consequence ROS can play a very important physiological role as secondary messengers [121].

ROS and RNS induce modification in protein structure and function. These changes observed in protein concentration and structure modification and may be monitored and regarded as biomarkers. There are some widely used protein tumor markers listed in Table 2. These indicators are associated with many types of cancer; others, with as few as one. However there are many not widely applied proteins that may help in cancer treatment and diagnosis; only several of them are described below.

5.2.1. Filamin-A

Recent studies indicated the possibility that filamin-A (cytoskeleton protein) may play a role in cancer response to DNA damage based chemotherapy reagents. This protein can be served as a biomarker to predict cancer prognosis for chemotherapy, or as an inhibition target to sensitize filamin-A positive cancer to therapeutic DNA damage. Yue et al. [247] proved that lack of filamin-A expression sensitizes cells to chemotherapy reagents, such as bleomycin and cisplatin, and a wide range of DNA repair activities require filamin-A. They presented that the level of filamin-A in melanoma cells correlates with their sensitivity to bleomycin and cisplatin. Authors also presented that inhibition of filamin-A sensitizes xenograft tumors to bleomycin and cisplatin treatment. These results suggest that filamin-A status may be used as a biomarker for prognosis after treatments. However this protein marker could also be used as a target to sensitize filamin-A positive cells to therapeutic DNA damage [247]. Thus, filamin-A status in cancer would be a novel marker for prognosis assessment and optimization of individualized treatment planning. Second, as shown in, even an incomplete inhibition of filamin-A expression in C8161 cells can confer a sensitivity to bleomycin and cisplatin treatment in mouse xenograft model. Thus, filamin-A may be used as an effective therapeutic target for these cancers with high or normal level of filamin-A expression. Filamin-A despite of being a cytoskeleton protein, plays a role in the repair of

multiple forms of DNA damage. Furthermore, filamin-A can be used as a biomarker to predict cancer sensitivity to therapeutic DNA damage, and as an inhibition target to improve therapy efficacy for filamin-A positive cancers [247].

Tumor marker	Application
AFP (Alpha-fetoprotein)	liver, testicular, and ovarian cancer
Her-2/neu	stage IV breast cancer
Bladder Tumor Antigen	urothelial carcinoma
Thyro-globulin	Thyroid cancer metastasis
PSA	Prostate cancer
Leptin, prolactin, osteoponin and IGF-II	Ovarian cancer
CD98, fascin, sPIgR[4] and 14-3-3 eta	Lung cancer
Troponin I	Myocardial infraction
B-type natriuretic peptide	Congestive heart failure
Beta-HCG (Beta-human chorionic gonadotropin)	testicular cancer and tumors, such as choriocarcinoma and molar pregnancies, that begin in placental cells called trophoblasts
CA 125 (Cancer antigen 125)	ovarian cancer, non-small cell lung cancer
CA 15-3 (Cancer antigen 15-3	breast cancer
CA 19-9 (Cancer antigen 19-9)	pancreatic cancer
CA 27-29 (Breast carcinoma-associated antigen)	breast cancer
CEA (Carcinoembryonic antygen)	many cancers, malignant pleural effusion, peritoneal cancer dissemination especially liver, intestinal, and pancreatic.

Table 2. Commonly applied FDA (Food and Drug Administration) tumor markers [1, 248].

5.2.2. Troponin I

TNI is a protein present exclusively in heart cells. The TNI concentration measured in blood is a well-established marker of heart muscle injury that's widely used to diagnose and treat heart attacks and other acute coronary syndromes. However Cardinale at al. indicate TNI as a protein marker for prediction of possible heart damage after chemotherapy. The increased levels of troponin I (TNI) protein in the blood helps identify possible heart damage after cancer treatment [232]. Authors also suggest that tracking TNI levels can help form a heart disease prevention plan for some chemotherapy patients. TNI categorizes heart disease risk early, long before impairment in heart function and symptoms develop, and when many preventive treatments would probably help prevent long-term health effects. However TNI can be assessed and monitored for the safety and effectiveness of different treatments [232]

5.2.3. Caveolin- 1

Caveolin-1 (Cav-1) plays an important role in cell transformation and the process of tumorigenesis. Moreover, Cav-1 is involved in metastatic processes. It has also been shown that Cav-1 expression is induced under oxidative stress conditions. It was demonstrated that Cav-1 can be a prognostic markers of aggressive (high-grade) forms of prostate cancer [249, 250]. Authors found that in patients with high serum Cav-1 the antioxidant capacity of the body was reduced. These results signify that Cav-1 may be an interesting biomarker for the prediction of disease burden [249]. Mercier et al. indicated Cav-1 as a new therapeutic target for the treatment of breast cancer. They described Cav-1multiple functions as a controller of estrogen signaling and kinase activity and its lately found role as an important factor monitoring the dynamic relationship between cancer epithelia and stroma position [251].

6. Conclusions

According the current review we tried to assume oxidative stress related markers in Table 3. The association of free radicals, antioxidant enzymes and oxidants at different steps of the malignant transformation and in cancer therapeutic applications is evident. Many details regarding the detailed role of apoptosis, free radicals and antioxidant markers in multifactor diseases such as cancer are still discovered.

Molecular biomarker	Process involved in oxidative stress
iNOS, eNOS, nNOS (inducible/endothelial/neuronal nitric oxide synthase)	ROS and NO
NO	
Singlet oxygen	
Malondialdehyde (MDA)	Lipid peroxidation
4-hydroxynonenal (HNE)	
Hydroxypropanodeoxyguanosines (HO-PdGs)	
Exocyclic etheno DNA adducts (etheno-dA,-dC,-dG)	
Isoprostanes	
Bityrosine cross-links	Protein oxidation
Filamin A	
Oxidative scissions	
Amino acid radicals (i.e. proline, histidine, arginine, lysine, cysteine)	
paraoxonase-1	
Carbonyl and thiol groups	
GSTpi, Caspases, catalase, superoxide dismutase	
Caveolin-1	

Table 3. Molecular biomarkers of lipid and protein oxidation [249, 252-254].

To determine with confidence which type and what level of oxidative damage can be really a applicable biomarker for cancer, needs measuring the DNA of healthy patients during a few decades to map the individuals who can develop cancer [121].

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