Redox Biology 5 (2015) 347–357

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Mini Review

The redox biology network in cancer pathophysiology and therapeutics

Gina Manda [a,*], Gheorghita Isvoranu [a], Maria Victoria Comanescu [a], Adrian Manea [b], Bilge Debelec Butuner [c], Kemal Sami Korkmaz [d]

[a] Cellular and Molecular Medicine Department, Radiobiology Laboratory, "Victor Babes" National Institute of Pathology, Bucharest, Romania
[b] Cellular and Molecular Pharmacology Laboratory, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania
[c] Faculty of Pharmacy, Department of Pharmaceutical Biotechnology, Ege University, Izmir, Turkey
[d] Department of Bioengineering, Cancer Biology Laboratory, Ege University, İzmir, Turkey

ARTICLE INFO

Article history:
Received 19 May 2015
Received in revised form
22 June 2015
Accepted 23 June 2015
Available online 25 June 2015

Keywords:
Cancer
Tumor niche
Redox signaling
Radiotherapy
Photodynamic therapy
Bystander and abscopal effects

ABSTRACT

The review pinpoints operational concepts related to the redox biology network applied to the pathophysiology and therapeutics of solid tumors. A sophisticated network of intrinsic and extrinsic cues, integrated in the tumor niche, drives tumorigenesis and tumor progression. Critical mutations and distorted redox signaling pathways orchestrate pathologic events inside cancer cells, resulting in resistance to stress and death signals, aberrant proliferation and efficient repair mechanisms. Additionally, the complex inter-cellular crosstalk within the tumor niche, mediated by cytokines, redox-sensitive danger signals (HMGB1) and exosomes, under the pressure of multiple stresses (oxidative, inflammatory, metabolic), greatly contributes to the malignant phenotype. The tumor-associated inflammatory stress and its suppressive action on the anti-tumor immune response are highlighted. We further emphasize that ROS may act either as supporter or enemy of cancer cells, depending on the context. Oxidative stress-based therapies, such as radiotherapy and photodynamic therapy, take advantage of the cytotoxic face of ROS for killing tumor cells by a non-physiologically sudden, localized and intense oxidative burst. The type of tumor cell death elicited by these therapies is discussed. Therapy outcome depends on the differential sensitivity to oxidative stress of particular tumor cells, such as cancer stem cells, and therefore co-therapies that transiently down-regulate their intrinsic antioxidant system hold great promise. We draw attention on the consequences of the damage signals delivered by oxidative stress-injured cells to neighboring and distant cells, and emphasize the benefits of therapeutically triggered immunologic cell death in metastatic cancer. An integrative approach should be applied when designing therapeutic strategies in cancer, taking into consideration the mutational, metabolic, inflammatory and oxidative status of tumor cells, cellular heterogeneity and the hypoxia map in the tumor niche, along with the adjoining and systemic effects of oxidative stress-based therapies.

Contents

Abbreviations: BER, base excision repair; CSC, cancer stem cells; DAMPs, danger-associated molecular patterns; DDR, DNA damage response; EGFR, epidermal growth factor receptor; HMGB1, high-mobility Group Box 1; HR, homologous recombination repair; IR, ionizing radiation; LET, linear energy transfer; PDT, photodynamic therapy; PS, photosensitizer; ROS, reactive oxygen species; SNP, single-nucleotide polymorphism; Treg, T regulatory cell

* Correspondence to: Victor Babes" National Institute of Pathology, 99-101 Splaiul Independentei, 050096 Bucharest, Romania. Fax: +40 21 319 45 28;
E-mail address: gina.manda@gmail.com (G. Manda).

http://dx.doi.org/10.1016/j.redox.2015.06.014

1. Introduction

Cancer is one of the main causes of death worldwide (see WHO database at http://www-dep.iarc.fr/WHOdb/WHOdb.htm). It brings a considerable economic and social burden despite intensive research for deciphering its molecular mechanisms, and for developing targeted therapeutic strategies using the personalized medicine concept.

This review aims to summarize currently operational concepts on the critical role of the intrinsic and microenvironmental oxidative stress in sustaining cancer development and spreading. We are particularly highlighting that reactive oxygen species (ROS) may not only act as supporters of tumor cells, but can be turned into their enemy that may be highly efficacious in cancer treatment.

It has been long proven that cancer cells display a pro-oxidative shift [1] generated by: (1) chronic activation of various metabolic sources of ROS, related to NADPH oxidases (NOXs 1–5 and dual oxidases DUOX1/2) [2], alterations of mitochondrial DNA, oxidative phosphorylation and energy metabolism, accompanied by enhanced aerobic glycolysis [3]; (2) a dysfunctional antioxidant response that is unable to counteract sustained production of ROS during tumorigenesis [4]. This intracellular oxidative turmoil is complemented by constant exposure of cancer cells to exogenous ROS derived from anoxia-reoxygenation cycles [5], and from the oxidative activity of tumor-infiltrating monocytes and neutrophils [6].

Starting from the insidious oxidative stress in the tumor microenvironment, the review pinpoints without aiming to be exhaustive key genetic alterations and repair mechanisms, along with critical turning points in redox signaling pathways, that confer a survival advantage to cancer cells. Cancer is not a "all-or-none" process, but integrates various cues into a pathologic network of events and cellular responses in the tumor niche under the pressure of multiple stresses (oxidative and inflammatory).

The therapeutic use of the cytotoxic face of ROS is exemplified by oxidative stress-based therapies, such as the radiotherapy and photodynamic therapy. The mechanisms underlying the resistance to an oxidative attack of particular cancer cells, such as cancer steam cells, are highlighted. Finally, we show that the effects of oxidative stress-based therapies go beyond local cytotoxicity, being propagated in the close vicinity and having even a systemic echo mediated by the immune response.

2. Oxidative stress-induced genetic alterations and repair mechanisms in cancer cells

Depending on its intensity and intracellular localization, oxidative stress can alter mitochondrial and nuclear DNA. DNA damage may include point mutations and single or double DNA strand breaks. When the oxidative error is incorporated into critical genes, such as those involved in cell cycle control, important cellular changes of metabolic rate and/or cellular response occur. Accordingly, point mutations that occur in cancer-associated genes result in defective DNA repair, apoptosis and cell cycle deregulation that sustain the malignant phenotype [7].

The most common forms of DNA alterations mediated by oxidative stress are 8-oxoguanine and/or guanosine, induced by deregulated intracellular metabolism and uncontrolled oxidative stress, as well as by injurious environmental factors, such as ionizing radiation.

mtDNA is more susceptible to oxidative damage than nuclear DNA and basically contains a higher level of base damage, commonly 8-oxoguanine [8]. It has been shown that hydrogen peroxide or menadione-mediated 8-oxoguanine foci do not co-localize with y-H2AX$^{(S139)}$ foci that are a hallmark of DNA strand breaks in the nuclear genome [9]. It is possible that these two types of DNA damage are not inter-connected, or that exposure to hydrogen peroxide or menadione may not always lead to single or double strand breaks. This is consistent with previous studies showing that 8-oxoguanine occurs more frequently in mitochondrial (mtDNA) than in nuclear DNA [7], but both genomes are accumulating 8-oxoguanine with increasing age [10].

Oxidative phosphorylation in mitochondria is an important source of ROS, with up to 4–5% of molecular oxygen picking up electrons directly from the flavin dehydrogenases and ubiquinol to generate superoxide anion. Since mitochondrial DNA is not covered by histones, DNA-associated proteins are directly exposed to ROS. Moreover, as mtDNA is intronless and has high transcription rates, the probability of oxidative modification of the coding region is increased [11–13]. Because mitochondrial respiration and consequent production of ATP are key cellular events, oxidative stress-induced damage of mitochondria and mtDNA may result in reduced energy production, compromised cellular functions and defective repair mechanisms. Therefore, oxidative damage of mtDNA has been linked to the onset of various pathologic conditions, such as neuronal degeneration, cardiovascular disorders, reproductive malfunctioning, cancer and aging.

Divergences in cellular function can cause cycles of oxidative damage that could contribute to cancer-related changes of physiological functions. Genome variation can induce an important shift of cellular responses towards oxidative damage. These pathologic changes are induced by critical single-nucleotide polymorphisms (SNPs) that affect cell susceptibility to defective or malfunctioning of encoded proteins. Most of the available data on SNPs in cancer are provided by follow up studies focused on SNPs that can predict the response or resistance of particular cancers to chemotherapy. Some of these include ERCC polymorphisms in non-small cell lung cancer, BRCA1 in mammary cancer, TMPRSS-ERG in prostate cancer, certain phase II and III ABC transporters, along with polymorphisms of oxidative damage response genes (OGG1, GPX2/3 and SOD2/3) in renal cell carcinoma, lung, mammary and prostate cancers [14,15].

The consequences of toxic and mutagenic stresses are minimized in normal cells by specific repair mechanisms that continuously monitor DNA for maintaining genome integrity. Normal cells respond to intracellular ROS generation by activating specific

molecular pathways, such as the NFκB pathway, to eliminate injured cells and to prevent further oxidative damage. Profound DNA damage, such as DNA breaks, elicits base excision repair (BER), as well as homologous recombination repair (HR). The removal of ROS-induced base damage in mitochondrial and nuclear DNA is mainly mediated by BER [16,17] through activation of damage-specific DNA glycosylases [18]. A well-known example is the AP-endonuclease OGG1 that is frequently mutated in renal cell carcinomas and is responsible for removal of ROS-mediated abasic sites from DNA [19]. If 8-oxoguanine lesions are not removed before cells enter the S phase, they may be converted into lethal single or double strand breaks during replication. Therefore, ubiquitous expression of AP-endonucleases is required for maintaining genome integrity, and is regulated throughout the cell cycle in most eukaryotic cells.

3. Distorted redox signaling networks in cancer cells

ROS are key players in signal transduction, and redox reactions are crucially involved in maintaining cellular homeostasis in aerobic organisms [20]. From all metabolic ROS, hydrogen peroxide seems to have the attributes of a "second messenger" which specifically interacts with effectors in signaling pathways [21]: it is readily diffusible across membranes, and its chemistry, enzymatic production and degradation provide adequate specificity for thiol oxidation in thermodynamically favorable environments [22].

Being produced in discrete subcellular locations, ROS act as local rheostats for intracellular signaling. Simple but highly targeted changes induced in signaling molecules by oxidation–reduction reactions, in conjunction with the interplay of phosphorylation–dephosphorylation, transduce messages from membrane receptors to the nucleus. ROS-triggered formation of cysteine sulfonic acid derivatives, disulfides and glutathionylated proteins results in conformational and functional changes of signaling proteins [23]. Through a self-sustaining process (ROS-induced ROS release) mediated by an inter-mitochondria signaling network [24], the initial redox signal propagates within the cell and coordinates the global signal transduction pattern. The location, intensity and duration of the oxidative burst, along with interconnected redox-sensitive signaling pathways, decide whether death or survival of normal and diseased cells occur in response to physiologic stimuli and stressors. It is possible that cancer cells derive from cells that adapted to a persistently smoldering intra- and extracellular oxidative environment by developing potent survival mechanisms [25]. Such death-resistant cells with accumulating epi- or genetic abnormalities may lead to malignant transformation.

Without aiming to be exhaustive, we present below some critical turning points in the signaling networks that sustain the survival of tumor cells in an oxidative microenvironment by enhancing proliferation, resistance to death signals and ability to repair damages.

3.1. Mitogen-activated protein kinases

The family of mitogen-activated protein kinases (MAPK), comprising extracellular signal-regulated kinases ½ (ERK½), c-Jun N-terminal kinases (JNK), and p38 MAPK [26], is a critical turning point that drives cancer cells towards survival and proliferation, instead of entering death pathways which are physiologically designed to remove abnormal cells [27]. MAPKs are activated by sequential phosphorylation mediated by upstream dual specificity kinases MAPKKK, MAPKK and MAPK, and are inactivated by dual specificity MAPK phosphatases [28]. MAPK pathway components and upstream activators, such as the Ras oncogene, are sensitive to ROS and are decisively involved in redox signaling [29,30].

MAPKs mediate opposing biological effects, depending on the stimulus, type of activated MAPK, duration of kinase activation and its subcellular localization [31]. Whilst uncontrolled activation of ERK½ pathway sustains tumorigenesis, stress-induced activation of JNK and p38 MAPK underlies the efficiency of cancer therapies by controlling the balance of autophagy and apoptosis [32,33]. Activation of ERK½ is generally but not exclusively triggered by receptor tyrosine kinases, resulting in cell proliferation and/or resistance to cell death [32]. For example, the activation of ERK½ by the epithelial growth factor receptor (EGFR) induces ERK½ nuclear translocation and consequent cellular proliferation, while direct activation of ERK½ by hydrogen peroxide leads to its retention in the cytoplasm and mediates cytoprotective responses [34].

The constitutive activation of ERK½ in cancer is partly derived from gene mutations centered around the Ras–Raf axis, and is associated to over-expression and/or mutation-driven activation of receptor tyrosine kinases, along with sustained production of activating ligands, such as mutated K-RAS in lung and colon cancer, and B-RAF in melanoma [35]. Additionally, oxidative stress induced by exogenous hydrogen peroxide can trigger ligand-independent EGFR activation either through phosphorylation of receptor tyrosine kinase or oxidative inactivation of phosphatases [36,37]. Unlike ERK½, JNK normally acts as tumor suppressor that triggers apoptosis in response to various stresses. Therefore, JNK suppression in cancer cells could sustain their survival due to enhanced resistance to apoptotic signals [38].

The tumor suppressor p38 MAPK also opposes growth signals transduced by ERK½, and is required for cancer cells dormancy. Down-regulation of p38 MAPK in various types of cancer promotes survival of tumor cells [39].

Intracellular oxidative stress regulates JNK and p38 activity [40] through the redox-sensitive complex ASK-1-thioredoxin. ASK-1 is a member of the MAPKKK superfamily that is maintained inactive by its binding to reduced thioredoxin in non-stressed cells. If thioredoxin gets oxidized, it disassociates from ASK-1, leading to activation of JNK and p38 through oligomerization of ASK-1 [41].

MAPKs integrate multiple signals and direct them *via* transcription factors towards the nucleus for mounting clear-cut cellular responses in cancer, such as resistance to oxidative stress, proliferation, metastasis or apoptosis. The interaction specificity within the MAPK pathway and the interactions of selectively activated MAPK members with transcription factors is guided by scaffolding proteins as crosstalk integrators [42].

3.2. FOXO transcription factors

Downstream of MAPK signaling pathways, important decision regarding the cell fate is taken at the level of the forkhead box O (FOXO) family of transcription factors (FOXO1, FOXO3, FOXO4 and FOXO6). The ROS-sensitive FOXOs maintain cellular homeostasis and coordinate cell responses for counteracting environmental aggressions (growth factor deprivation, metabolic and oxidative stress), hence acting as tumor suppressors that control the cell cycle [43,44]. FOXOs also confer resistance to moderate oxidative stress through transcription of antioxidant genes, such as those encoding for superoxide dismutase, catalase and peroxyredoxins [43]. In case of aggressive oxidative stress, FOXOs promote apoptosis by inducing the expression of pro-apoptotic factors (FAS ligand, Bim, bNIP3 and Bcl-XL) [45].

In normal cells, antagonistic mechanisms regulate FOXOs activity, depending on the context: (1) insulin and growth factors signaling through the PI3K/Akt pathway inhibits FOXO transcriptional activity by phosphorylation and subsequent cytoplasmic retention of FOXOs through increased binding to their 14–3–3

regulator [46]; (2) JNK-mediated nuclear translocation of FOXO in response to an intense oxidative burst augments its transcriptional activity and tumor suppressor function [47]. Concurrently, JNK inhibits insulin signaling, hence overruling FOXO inhibition by growth factors [43]; (3) down-regulation of FOXO by poly-ubiquitylation in the cytoplasm favors its proteosomal degradation [48].

The PI3K/Akt signaling pathway is critically involved in regulating cell proliferation and survival, glucose metabolism, genome stability, and neo-vascularization [49]. It is over-expressed or highly activated in many types of tumors, and most of components are involved in tumorigenesis, either as oncoproteins or tumor suppressors [50]. Over-expression or constitutive activation of the PI3K/Akt pathway in tumor cells leads to the inhibition of FOXO tumor suppressors by phosphorylation and cytoplasmic sequestration [46]. Meanwhile, FOXO acetylation shifts FOXO-mediated gene expression from an apoptotic to a pro-survival pattern [51].

3.3. The Keap1-Nrf2 system

The Kelch-like ECH-associated protein 1 (Keap1)-nuclear factor E2-related factor 2 (Nrf2) system is critically involved in cell defense against various endogenous and exogenous stresses [52]. Keap1 and to a lesser extent Nrf2 are redox sensors, and the transcription factor Nrf2 transactivates genes that encode antioxidant enzymes. Nrf2 is retained in the cytoplasm through interaction with Keap1, which controls proteosomal degradation of ubiquitylated Nrf2 in a redox-dependent manner. Oxidative stress causes disulfide bond formation between Cys273 and Cys288 in Keap1, leading to Nrf2 release and its nuclear translocation for transcriptional activity. Additionally, multiple external stimuli induce the activation of MAPK and PI3K, which in turn phosphorylate Nrf2 at Ser40 to dissociate from Keap1 [53].

In cancer cells, Keap1 mutations or epigenetic modifications in its promoter region lead to Keap1 inactivation or reduced expression, hence up-regulating Nrf2 activity and consequent transactivation of antioxidant genes [54]. Therefore, cancer cells get shielded against oxidative stress and gain a survival advantage. Alternatively, Nrf2 repression by oncogene-induced activation of the Ras/Raf/ERK pathway may be an adaptive response for certain incipient cancers to acquire a pro-oxidant state that favors cell survival and tumor growth [55]. Accordingly, activation of Nrf2 may be a valuable preventive strategy to avoid tumorigenesis in patients with cancer risk Fig. 1.

4. Inflammation in the tumor niche

4.1. Communication in the tumor niche

Tumor progression is underlined not only by epi- or genetic changes and distorted signal transduction, but also by an active crosstalk of cancer cells with the surrounding stroma [56]. Fibroblasts, immune, endothelial and mesenchymal cells, all immersed in an oriented cellular matrix, build the tumor niche and its particular oxidative, acidic, inflammatory and hypoxic milieu that drives tumors towards a more aggressive phenotype [57].

Autocrine and paracrine communication in the tumor niche is mediated by “soluble” factors, adhesion molecules and gap junction channels. Additionally, exosomes of endolysosomal origin and plasma membrane-derived microvesicles carry a load of bioactive molecules that faithfully reflect the physiological state of the cells

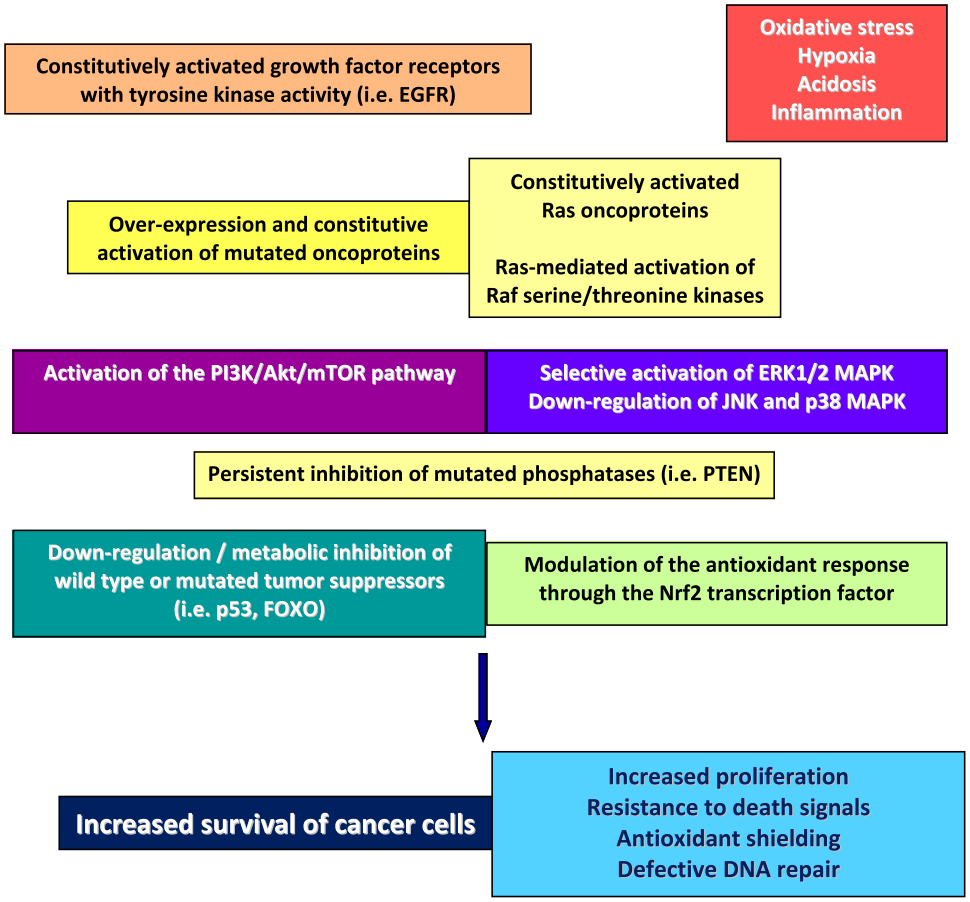

Fig. 1. Critical turning points of signaling pathways in cancer cells.

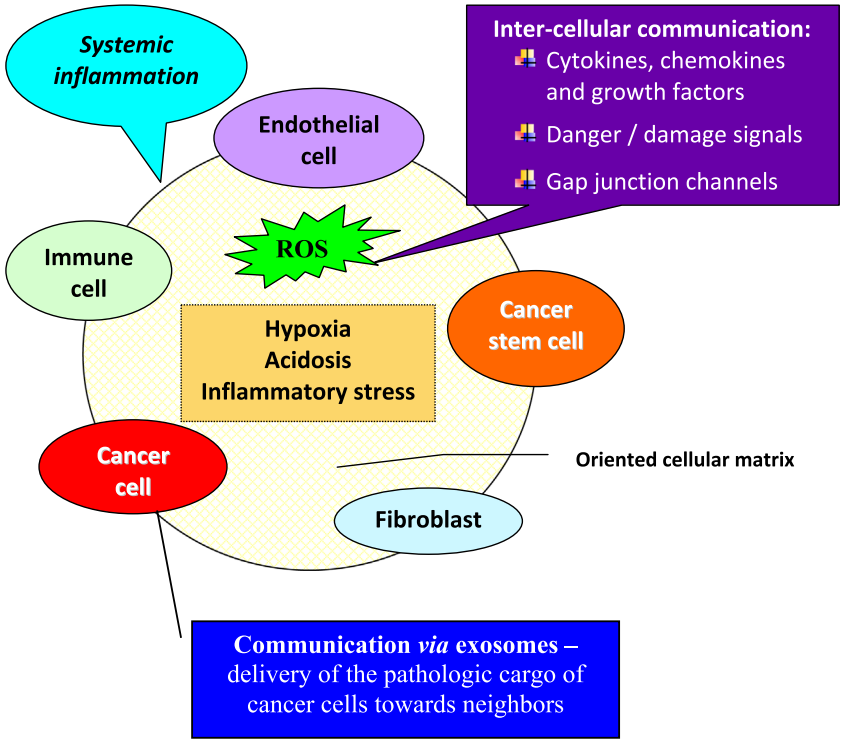

Fig. 2. Inside the tumor niche – cellular communication in stressful conditions.

of origin. In the extracellular environment, exosomal membranes make enclosed molecules to be more stable than the corresponding soluble forms, hence accurately delivering messages over a long distance [58]. Extracellular vesicles released by most malignant and normal cells, possibly due to tumor-specific hypoxia [59] and acidosis [60], have been recently demonstrated to spread within the tumor niche pathologic signals (oncogenes and other pro-tumorigenic factors) [61,62]. These inter-cellular signalosomes trigger secretion of tumor-promoting growth factors, cytokines, and angiopoietic factors by stromal and immune cells, hence sustaining angiogenesis, local inflammation and metastasis [63]. Moreover, exosomes released by cells exposed to oxidative stress have the ability to induce stress tolerance in neighboring cells through mRNA shuttle [64], thus favoring their oncogenic transformation Fig. 2.

4.2. Tumor-associated inflammation

Tumor-associated inflammation is mediated by cytokines, chemokines, growth factors and ROS released by almost all cells in the tumor niche [65,66]. Inflammation can drive tumor progression by increasing mutation rates and by enhancing the survival of mutated cells [67] (Fig. 3). The tumor-associated inflammation resembles to a defective wound healing process, associating long-lasting and self-sustained inflammation with excessive tissue remodeling and loss of tissue architecture.

Stressed and/or damaged cells within the tumor niche release “danger” signals (DAMPs, danger-associated molecular patterns), which elicit an inflammatory response in neighboring cells (the danger theory) [68]. DAMPs are nuclear and cytosolic proteins (HMGB1, shock proteins, the S100 family of calcium-binding proteins, histones, IL-1), nucleotides and their metabolites (uric acid), along with extracellular molecules (hyaluronan, heparin sulfate) [69].

Mitochondria have recently emerged as key source of DAMPs [70] which signals to neighboring cells the local oxidative injury. Some DAMPs, like the nuclear and cytosolic HMGB1, are sensors of

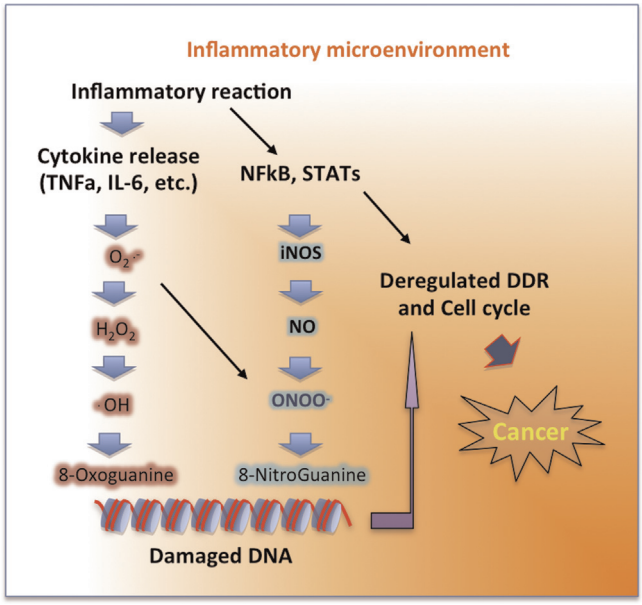

Fig. 3. Players of the oxidative DNA damage response (DDR).

the intracellular oxidative status, being released or exposed following oxidation of critical cysteine residues [71]. The release of HMGB1 from the nucleus leaves DNA unprotected against oxidative stress and fosters genomic instability in the tumor niche [72]. Extracellular HMGB1 interacts with specific receptors on neighboring cells (the receptor for advanced glycation end products, toll-like receptors etc. [73]), and triggers angiogenesis and activation of endothelial cells, recruitment of immune cells, and an overall inflammatory response to environmental stress or damage. Both sided signaling mediated by DAMPs is tightly regulated by the spatial and temporal distribution of ROS [74]. While intracellular DAMPs are activated by oxidative stress, their pro-

inflammatory activity outside the cell is switched-off by an extracellular oxidative milieu [75].

The inflammatory environment in tumors is partly sustained by tumor-resident macrophages, along with newly recruited monocytes and neutrophils [76]. Leukocytes recruitment mediated by chemotactic factors released by cancer and stromal cells is dependent on the local vasculature network which exhibits an activated phenotype [77]. Cancer cells rely on this exogenous contribution for survival, development and spreading. Due to their high plasticity, cancer cells may even trans-differentiate into tumor endothelial cells to sustain vasculo- and lymph-angiogenesis [78]. Neutrophils and macrophages contribute to the enhanced oxidative status in the tumor niche by producing considerable amounts of ROS through the activation of NOX2 in response to danger, damage or inflammatory stimuli [76].

Tumor-associated macrophages are a major source of pro-inflammatory cytokines and chemokines, like TNFα, IL-1, IL-6 and IL-8. The pleiotropic cytokine TNFα plays crucial roles in tumor development and progression by stimulating the growth, proliferation, invasion and metastasis of cancer cells [79–81]. TNFα also induces ROS production *via* NFκB activation by increasing the transcription of various members of the NOX family. In turn, ROS stimulate or inhibit multiple upstream and downstream components of the NFκB pathway, depending on the cell type and the context [82].

DAMPs and inflammatory cytokines trigger the activation of inflammasomes in myeloid and epithelial cells (e.g. NLRP3 and NLRC4 inflammasomes, respectively), resulting in production of the pro-inflammatory cytokines IL-1β and IL-18that are critically involved in various types of cancers [83]. In late stages of tumor progression, inflammasomes may be constitutively activated [84]. Schematically, inflammasome activation is initiated by NFκB-mediated transcription of pro-IL-1β and pro-IL-18 in response to toll like receptors signaling, continued by activation of the cysteine protease caspase-1 [85]. Most of NLRP3 inflammasome activators induce ROS generation, partly through mitochondrial damage [86]. In turn, mitochondrial superoxide anion may prime the NLRP3 inflammasome by de-ubiquitination [87]. The antioxidant system is turned on in response to the oxidative challenge, but can indirectly sustain inflammation, as thioredoxin-interacting protein binds to and activates the NLRP3 inflammasome after dissociating from thioredoxin [88].

We emphasize herein the paradigm shift occurring in the last decade, stating that ROS and cytokine production is not exclusively confined to phagocytes and immune cells, respectively. For example, stromal carcinoma-associated fibroblasts exhibit a particular inflammatory phenotype (myofibroblastic) [89]. ROS produced by fibroblasts carrying mitochondrial dysfunction, or hypoxia-related ROS production in mutated fibroblasts can induce their trans-differentiation into such myofibroblasts [90]. Myofibroblasts release multiple soluble factors, such as the chemokine stromal-derived factor-1 that interacts with CXCR4 and promotes tumor and stromal cell migration through matrix remodeling. CXCR4 also triggers in cancer cells ROS production by NOX2 enzymes [91]. Moreover, fibroblast-derived exosomes can sustain cancer cell dynamics through Wnt signaling [92]. A vicious circle is thus established, in which mutations in carcinoma cells drive alterations in the stroma that in turn facilitate carcinoma progression [93].

Cancer-associated inflammation has a particular profile that favors tumor growth, but inhibits the anti-tumor immune response. For example, myeloid-derived suppressor cells are enriched in melanoma lesions and lymphatic organs during tumor progression and inhibit tumor-reactive T cells [94]. Despite their inflammatory phenotype, tumor-associated macrophages were shown to induce immune suppression mediated by IL-10 and TGFβ [94]. Moreover, they contribute to the polarization of the local immune response towards tolerogenic T regulatory cells (Treg) [95]. Being more resistant to oxidative stress, immunosuppressive Treg have a survival advantage over tumor-specific cytotoxic TCD8+ lymphocytes in the oxidative environment of tumors [96].

5. Using ROS for cancer therapy

ROS sustain tumorigenesis and cancer progression, but are also efficient therapeutic tools to fight cancer. By increasing ROS levels in the tumor niche the damaging face of ROS can be brought to the forefront to overcome the growth-promoting action of metabolic ROS in cancer cells.

5.1. Oxidative stress-based therapies in cancer

Several therapeutic strategies, such as radiotherapy and photodynamic therapy, were specifically designed to increase ROS levels in tumor cells to elicit their death through sudden and intense oxidative stress. By generating a therapy-induced overload of ROS in cancer cells the oxidative threshold separating survival from death could be exceeded. Due to the high intrinsic oxidative activity of cancer cells and their faulty programming, less additional ROS are required compared to normal cells for triggering cell death. Levels of ROS that are cytotoxic for cancer cells induce less drastic effects in normal cells, which have a lower oxidative status and are endowed with efficient tools to repair ROS-induced injuries within certain limits. Nevertheless, precise targeting of oxidative stress-based therapies to the diseased tissue is a priority, aiming to protect normal tissues against deleterious action of "therapeutic" ROS.

Tumor cells have different intrinsic susceptibilities to oxidative stress. A major drawback of oxidative stress-based therapies relies in the outstanding resistance of cancer stem cells (CSCs). These cells have the ability to self-renew, to differentiate into multiple lineages and to initiate tumors, hence being responsible for the uncontrolled growth of tumors, maintenance of minimal residual disease and tumor recurrence following therapy [97]. CSCs resistance relies on enhanced repair mechanisms, up-regulated cell cycle control, over-expression of antioxidant enzymes and efficacious free radical scavenging [98]. Various molecular networks regulate CSCs and their adaptive responses to hypoxia and oxidative stress in the tumor niche. For example, the PI3K/PTEN/AKT/mTOR pathway controls ROS levels in CSCs by regulating the nuclear localization of FOXO and the consequent over-expression of antioxidant enzymes [99]. Additionally, constitutive activation of Nrf2 in CSCs inhibits their differentiation by reinforcing antioxidant shielding [100]. Therefore, Nrf2 inhibitors may sensitize CSCs and cancer cells to the effects of oxidative stress-based therapies by down-regulating their antioxidant response [101]. Delivery of Nrf2 inhibitors should be highly targeted towards the tumor for avoiding down-regulation of the intrinsic antioxidant system of normal cells.

Tumor heterogeneity derives also from the non-uniform spatial distribution of microenvironmental stresses, such as hypoxia, acidosis, oxidative stress and nutrient deprivation [102]. Since oxidative stress-based therapies are particularly dependent on the local supply of molecular oxygen, cancer cells placed in hypoxic regions might not respond to such therapies. Accordingly, *in vivo* imaging of the hypoxia map could guide the therapeutic strategy for eradicating such "hidden" cells.

Radiotherapy is the prototype of oxidative stress-based therapy. Ionizing radiation (IR), electromagnetic or particulate, can directly disrupt atomic structures in cells, resulting in major chemical and biological changes. At therapeutically relevant doses, direct

interaction of IR with nuclei is low and its indirect action through water radiolysis prevails. As described by Azzam et al. (2012) [103], IR energy deposition results in the generation of secondary electrons and unstable species, which further produce radicals and molecular products of radiolysis, distributed in a highly heterogeneous track structure (10^{-12} s). Chemically reactive species further diffuse and react with one another and with biologic structures (10^{-6} s). In an aerobic cellular environment water radiolysis generates superoxide anion, hydroxyl radical, hydrogen peroxide, depending on the linear energy transfer (LET) of the irradiating particles. For example, hydrogen peroxide prevails over hydroxyl radical with increasing LET, while high LET particles mainly produce superoxide anion. Although this spectrum of ROS is similar to that produced by metabolic processes, biologically-relevant differences are to be noticed, mainly because IR generates almost instantaneously high concentrations of localized ROS that lead to clustered lesions and extensive, irreparable oxidative injury.

Photodynamic therapy (PDT) is another strategy to increase ROS to cytotoxic levels within cancer cells [104,105]. Briefly, cells are loaded with a light-sensitive photosensitizer (PS), which is activated by irradiation with light of appropriate wavelength, and generates a localized burst of singlet oxygen. Currently available PSs preferentially accumulate into tumors. Their intake by normal tissues cannot be completely avoided and therefore PSs are designed to have low dark toxicity. Targeting of PDT towards the tumor and sparing of normal tissue is achieved by precise light irradiation of the diseased tissue using flexible fiber-optic devices. The use of 600–800 nm light is recommended for PS activation due to the low tissue-damaging action of red to far red light.

The intracellular localization of PS dictates the distribution of the deleterious light-elicited oxidative stress mediated by singlet oxygen. Available PSs localize mostly in lipid membranes, lysosomes, mitochondria and/or endoplasmic reticulum [104]. Unlike IR, PDT does not target the nucleus and this may represent a therapeutic advantage by avoiding the spreading of genomic instability. The short lifetime of singlet oxygen limits its diffusion to only 10–55 nm [106], hence deciding on the action field of PDT.

For improving PDT efficacy, huge efforts are now focused on real-time monitoring of PDT-associated photoreaction for adjusting the irradiation parameters during the therapeutic procedure. For singlet oxygen dosimetry one may take advantage of the fluorescent light emitted by the excited PS [107] or of the singlet oxygen phosphorescence at 1270 nm [108].

5.2. Types of cell death induced by oxidative stress-based therapies

Excessive oxidative stress can induce directly irreparable cellular lesions, or may commute the signaling machinery from pro-survival to death signals delivery. The lethal action of oxidative stress-based therapies in the tumor niche is dependent on the quality of the elicited oxidative burst (intensity and intracellular localization), target cell susceptibility to oxidative stress (genetic background, oxidative status, repair mechanisms), and the global microenvironment response to the oxidative challenge. A non-physiologically intense and sudden sparkle of intracellular ROS generated by IR generally leads to clustered DNA double strand breaks which fail to be repaired or are misrepaired, either because cells are not prepared to face such an aggression, or their enzymatic repair mechanisms are defective. We highlight several cancer-specific mutations and polymorphic variants of cancer susceptibility genes associated with genomic instability, which support cancer progression, and underlie cellular responses to anti-cancer therapies: (a) ataxia telangiectasia mutated (ATM) which is central to cell cycle checkpoint responses initiated by DNA double-strand breaks by phosphorylating oncogenes like p53 and Chk2 [109]; (b) the Mre11/Rad50/Nbs1 complex which recruits ATM to DNA double strand breaks and mediates non-homologous recombination repair, predominantly during the G1/early S phase [110], (c) the breast cancer predisposition genes BRCA1/2 which are phosphorylated by Chk2 and mediate homologous recombination in the S phase [111]. IR-induced DNA damage triggers mitotic cell death after several cell cycles, and such cancer cells can progress either to apoptosis or necrosis depending on the context [112]. Cancer cells exhibiting deficient apoptotic response may become senescent [113], exit from the cell cycle, but persist in the tumor and release pro-inflammatory cytokines and growth factors. Part of the senescent cells may recover and even acquire a more aggressive malignant phenotype, along with increased resistance to therapy due to genetic instability [114]. Additionally, the crosstalk between senescence and autophagy in cancer cells may contribute to tumor dormancy [115].

Oxidative stress-based therapies may induce apoptosis indirectly in response to oxidative and metabolic stresses. This death mechanism is mostly confined to “normal” cells in the tumor niche, as a common stress response mediated by the activation of JNK and p38 MAPK [116]. JNK1 triggers apoptosis in response to stress, but induces also compensatory proliferation of the neighboring non-apoptotic cells [117]. Unlike normal cells, cancer cells are endowed with anti-apoptotic mechanisms that reinforce their survival in noxious conditions. The synergy between growth-inducing oncogenes, like c-Myc, and over-expression of the cell death inhibitor Bcl-2, along with mutation-induced functional loss of the tumor suppressor p53, all can block the apoptotic machinery in cancer cells, hence supporting malignancy and resistance to therapy [118]. Agents that inhibit apoptosis might be useful in conjunction with oxidative stress-based therapies for reducing dose-limiting side-effects due to apoptosis of normal cells [116].

5.3. Distant effects of oxidative stress-based therapies

The outcome of oxidative stress-based therapies depends not only on the death of directly irradiated cells, but also on the pattern of delayed effects in the neighboring area (bystander effects). Non-irradiated cells in the close vicinity of oxidative stress-damaged cells are induced to exhibit a similar phenotype characterized by DNA strand breaks, point mutations, gene deletions and micronucleation, along with increased levels of ROS and inflammatory reactions [119]. Such delayed cellular changes are transmitted to progenitors for several cellular doublings [120].

Accordingly, therapy-damaged cancer cells may trigger the death of neighboring non-irradiated cell, hence enlarging the therapeutic action area of targeted oxidative stress-based therapy (biologic penumbra). This contributes to tumor eradication more than expected from the initial therapy field. Unfortunately, the bystander effect may also injure neighboring normal tissue, hence increasing therapy side effects. Alternatively, signals delivered by oxidative stress-injured cells may induce adaptive responses in neighboring cells by up-regulation of repair mechanisms, such as those mediated by p53 [121]. Therefore, the bystander effect can limit in certain cases tumor cells response to current and future therapies, and may also support the development of secondary cancers.

The transfer of information between cells is achieved by gap junctions, soluble factors and exosomes in the tumor niche, as mentioned in the section “Inflammation in the tumor niche”. Irradiated cancer cells carrying profound DNA damage and metabolic alteration are signaling to neighboring cells the oxidative damage by releasing ROS, oxidized extracellular DNA, danger signals (HMGB1) and a plethora of pro-inflammatory cytokines (TNFα, IL-1β, IL-6, IL-8, IL-33, TGFβ1) [122]. The exact mechanism underlining the bystander effect is still not known. It is improbable

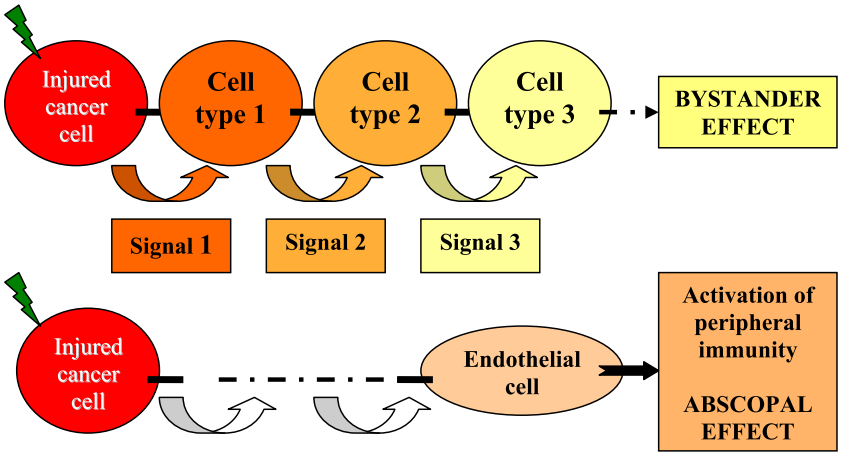

Fig. 4. Distant effects of ROS-based therapies – propagation of signals delivered by oxidative stress-injured cells.

that a unique molecule could spread cancer cell messages in the heterogeneous tumor niche. One could consider that distinct cell types may not use the same panel of molecules for inter-cellular communication. Moreover, soluble factors released by injured cancer cells have a short-ranged action due to interaction with specific receptors on neighboring cells, or attachment to cellular matrix components. Thus, sequential and polarized inter-cellular signaling, mediated by different cell-specific molecules and signal transduction mechanisms, may hypothetically account for distant effects of oxidative stress-based-therapies (Fig. 4).

In particular conditions, oxidative stress-based therapies can trigger the immunogenic cell death of cancer cells: tumor antigens are revealed and become accessible for uptake by dendritic cells, which further elicit antigen-specific cytotoxic T cell responses and production of tumor-specific antibodies [123]. The enhanced tumor-specific immunity and blood cytokines underline the distant effect of oxidative stress-based therapies (abscopal effect), through which regression of distant metastatic cancer may occur [124]. Therefore, combinations of radiotherapy/chemotherapy and immunotherapy are currently under development in clinical settings.

6. Future perspective

Tumor is a web of interconnected genomic and signal transduction alterations in a stressful local environment. Using systems biology for investigating the sophisticated network of events in the tumor niche, along with a theory unifying common molecular pathways underlying the pressure of various stresses, might be a fruitful approach in the endeavor to understand cancer and to design innovative therapeutic strategies to fight against it. Taking advantage of the new “omic” technologies for drawing meaningful molecular maps of pathologic events, in-depth investigations are under development for getting the “big picture” and for identifying new therapeutic targets addressing the distorted redox balance in cancer.

Acknowledgments

Gina Manda, Adrian Manea, Bilge Debelec Butuner and Kemal Sami Korkmaz were supported by the European Cooperation in Science and Technology (COST Action BM1203/EU-ROS); Gheorghita Isvoranu was supported by the Sectorial Operational Program Human Resources Development (SOPHRD), financed by the European Social Fund and the Romanian Government under the Contract no. POSDRU141531; the work of Maria Victoria Comanescu was supported by the Romanian National Agency for Research and Innovation, under the Program Capacities, Romania-CERN (Grant E05/2014).

References

[1] G. Manda, M.T. Nechifor, T.M. Neagu, Reactive oxygen species, cancer and anti-cancer therapies, Curr. Chem. Biol. 3 (2009) 342–366, http://dx.doi.org/10.2174/187231309787158271.
[2] K. Roy, Y. Wu, J.L. Meitzler, A. Juhasz, H. Liu, G. Jiang, J. Lu, S. Antony, J. H. Doroshow, NADPH oxidases and cancer, Clin. Sci. 128 (2015) 863–875.
[3] J. Zheng, Energy metabolism of cancer: glycolysis versus oxidative phosphorylation (Review), Oncol. Lett. 4 (2012) 1151–1157, http://dx.doi.org/10.3892/ol.2012.928.
[4] D.A. Frohlich, M.T. McCabe, R.S. Arnold, M.L. Day, The role of Nrf2 in increased reactive oxygen species and DNA damage in prostate tumorigenesis, Oncogene 27 (2008) 4335–4362, http://dx.doi.org/10.1038/onc.2008.79.
[5] H. Du, W. Yang, L. Chen, B. Shen, C. Peng, H. Li, D.K. Ann, Y. Yen, W. Qiu, Emerging role of autophagy during ischemia-hypoxia and reperfusion in hepatocellular carcinoma, Int. J. Oncol. 40 (2012) 2049–2057, http://dx.doi.org/10.3892/ijo.2012.1415.
[6] T. Lu, D.I. Gabrilovich, Molecular pathways: tumor-infiltrating myeloid cells and reactive oxygen species in regulation of tumor microenvironment, Clin. Cancer Res. 15 (2012) 4877–4882, http://dx.doi.org/10.1158/1078-0432.CCR-11-2939.
[7] L. Khandrika, B. Kumar, S. Koul, P. Maroni, H.K. Koul, Oxidative stress in prostate cancer, Cancer Lett. 282 (2009) 125–136, http://dx.doi.org/10.1016/j.canlet.2008.12.011.
[8] G.L. Dianov, N. Souza-Pino, S.G. Nyaga, T. Thybo, T. Stevsner, V.A. Bohr, Base excision repair in nuclear and mitochondrial DNA, Prog. Nucleic Acid Res. Mol. Biol. 68 (2001) 285–297, http://dx.doi.org/10.1016/S0079-6603(01)68107-8.
[9] D.R. Pilch, O.A. Sedelnikova, C. Redon, A. Celeste, A. Nussenzweig, W. M. Bonner, Characteristics of gamma-H2AX foci at DNA double-strand breaks sites, Biochem. Cell Biol. 81 (2003) 123–129, http://dx.doi.org/10.1139/o03-042.
[10] V.A. Bohr, Repair of oxidative DNA damage in nuclear and mitochondrial DNA, and some changes with aging in mammalian cells, Free Rad. Biol. Med. 32 (2002) 804–812, http://dx.doi.org/10.1016/S0891-5849(02)00787-6.
[11] A. Kaniak- Golik, A. Skoneczna, Mitochondria-nucleus network for genome stability, Free Rad. Biol. Med. 82 (2015) 73–104, http://dx.doi.org/10.1016/j.freeradbiomed.2015.01.013.
[12] M.K. Shigenaga, T.M. Hagen, B.N. Ames, Oxidative damage and mitochondrial decay in aging, Proc. Natl. Acad. Sci. USA 91 (1994) 10771–10778, http://dx.doi.org/10.1073/pnas.91.23.10771.
[13] B. Bandy, A.J. Davison, Mitochondrial mutations may increase oxidative stress: implications for carcinogenesis and aging? Free Rad. Biol. Med. 8 (1990) 523–539, http://dx.doi.org/10.1016/0891-5849(90)90152-9.
[14] P. Rodrigues, G. de Marco, J. Furriol, M.L. Mansego, M. Pineda-Alonso, A. Gonzalez-Neira, J.C. Martin-Escudero, J. Benitez, A. Lluch, F.J. Chaves, P. Eroles, Oxidative stress in susceptibility to breast cancer: study in Spanish population, BMC, Cancer 14 (2014) 1471–2407, http://dx.doi.org/10.1186/1471-2407-14-861.
[15] A.E. Yuzhalin, A.G. Kutikhin, Inherited variations in the SOD and GPX gene families and cancer risk, Free Rad. Biol. Med. 46 (2012) 581–599, http://dx.doi.org/10.3109/10715762.2012.658515.

[16] J.E. Klaunig, L.M. Kamendulis, B.A. Hocevar, Oxidative stress and oxidative damage in carcinogenesis, Toxicol. Pathol. 38 (2010) 96–109, http://dx.doi.org/10.1177/0192623309356453.
[17] A. Barzilai, K. Yamamoto, DNA damage responses to oxidative stress, DNA Repair (Amst) 3 (2004) 1109–1115, http://dx.doi.org/10.1016/j.dnarep.2004.03.002.
[18] S.S. Wallace, Base excision repair: a critical player in many games, DNA Repair (Amst) 19 (2014) 14–26, http://dx.doi.org/10.1016/j.dnarep.2014.03.030.
[19] M. Audebert, S. Chevillard, C. Levalois, G. Gyapay, A. Vieillefond, J. Klijanienko, P. Vielh, A.K. El Naggar, S. Oudard, S. Boiteux, J.P. Radicella, Alterations of the DNA repair gene OGG1 in human clear cell carcinomas of the kidney, Cancer Res. 60 (2000) 4740–4744.
[20] M.A. Briehl, Oxygen in human health from life to death – an approach to teaching redox biology and signaling to graduate and medical students, Redox Biol. 5 (2015) 124–139, http://dx.doi.org/10.1016/j.redox.2015.04.002.
[21] H.S. Marinho, C. Real, L. Cyrne, H. Soares, F. Antunes, Hydrogen peroxide sensing, signaling and regulation of transcription factors, Redox Biol. 2 (2014) 535–562, http://dx.doi.org/10.1016/j.redox.2014.02.006, mailto:fantunes@fc.ul.pt.
[22] D.H.J. Forman, M. Maiorino, F. Ursini, Signaling function of reactive oxygen species, Biochemistry 49 (2010) 835–842, http://dx.doi.org/10.1021/bi9020378.
[23] S. Rehder, C.R. Borges, Cysteine sulfenic acid as an intermediate in disulfide bond formation and nonenzymatic protein folding, Biochemistry 49 (2010) 7748–7755, http://dx.doi.org/10.1021/bi1008694.
[24] J. Park, J. Lee, C. Choi, Mitochondrial network determines intracellular ROS dynamics and sensitivity to oxidative stress through switching inter-mitochondrial messengers, PLoS ONE 6 (2011) e23211, http://dx.doi.org/10.1371/journal.pone.0023211.
[25] M. Landriscina, F. Maddalena, G. Lauderio, F. Esposito, Adaptation to oxidative stress, chemoresistance, and cell survival, Antioxid. Redox Signal. 11 (2009) 2701–2716, http://dx.doi.org/10.1089/ars.2009.2692.
[26] J.S.C. Arthur, S.C. Ley, Mitogen-activated protein kinases in innate immunity, Nat. Rev. Immunol. 13 (2013) 679–692, http://dx.doi.org/10.1038/nri3495.
[27] L. Poillet-Perez, G. Despouy, R. Delage-Mourroux, M. Boyer-Guittaut, Interplay between ROS and autophagy in cancer cells, from tumor initiation to cancer therapy, Redox Biol. 4 (2015) 184–192, http://dx.doi.org/10.1016/j.redox.2014.12.003.
[28] R. Roskoski, ERK½ MAP kinases: structure, function and regulation, Pharmacol. Res. 66 (2012) 105–143, http://dx.doi.org/10.1016/j.phrs.2012.04.005.
[29] L. Mitchell, G.A. Hobbs, A. Aghajanian, S.L. Campbell, Redox regulation of Ras and Rho GTPases: mechanism and function, Antioxid. Redox Signal. 18 (2013) 250–258, http://dx.doi.org/10.1089/ars.2012.4687.
[30] M. Torres, H.J. Forman, Redox signaling and the MAP kinase pathways, BioFactors 17 (2003) 287–296, http://dx.doi.org/10.1002/biof.5520170128.
[31] Y. Mebratu, Y. Tesfaigzi, How ERK½ activation controls cell proliferation and cell death: is subcellular localization the answer? Cell Cycle 8 (2009) 1168–1175, http://dx.doi.org/10.4161/cc.8.8.8147.
[32] M. Raman, W. Chen, M.H. Cobb, Differential regulation and properties of MAPKs, Oncogene 26 (2007) 3100–3112, http://dx.doi.org/10.1038/sj.onc.1210392.
[33] X. Sui, N. Kong, L. Ye, W. Han, J. Zhou, Q. Zhang, C. He, H. Pan, p38 and JNK MAPK pathways control the balance of apoptosis and autophagy in response to chemotherapeutic agents, Cancer Lett. 344 (2014) 174–179, http://dx.doi.org/10.1016/j.canlet.2013.11.019.
[34] C.M. Rosseland, L. Wierød, M.P. Oksvold, H. Werner, A.C. Østvold, G. H. Thoresen, R.E. Paulsen, H.S. Huitfeldt, E. Skarpen, Cytoplasmic retention of peroxide-activated ERK provides survival in primary cultures of rat hepatocytes, Hepatology 42 (2005) 200–207, http://dx.doi.org/10.1002/hep.20762.
[35] A.S. Dhillon, O. Rath, W. Kolch, MAP kinase signalling pathways in cancer, Oncogene 26 (2007) 3279–3290, http://dx.doi.org/10.1038/sj.onc.1210421.
[36] A. Meves, S.N. Stock, A. Beyerle, M.R. Pittelkow, D. Peus, H2O2 mediates oxidative stress-induced epidermal growth factor receptor phosphorylation, Toxicol. Lett. 122 (2001) 205–214, http://dx.doi.org/10.1016/S0378-4274(01)00359-9.
[37] T.H. Truong, K.S. Carroll, Redox regulation of epidermal growth factor receptor signaling through cysteine oxidation, Biochemistry 51 (2012) 9954–9965, http://dx.doi.org/10.1021/bi301441e.
[38] C. Tournier, The 2 Faces of JNK Signaling in, Cancer, Genes Cancer 4 (2013) 397–400, http://dx.doi.org/10.1177/1947601913486349.
[39] C. Bradham, D.R. McClay, P38 MAPK in development and cancer, Cell Cycle 5 (2006) 824–828, http://dx.doi.org/10.4161/cc.5.8.2685.
[40] G.Y. Liou, P. Storz, Reactive oxygen species in cancer, Free Radic. Res. 44 (2010) 479–496, http://dx.doi.org/10.3109/10715761003667554.
[41] H. Nagai, T. Noguchi, K. Takeda, H. Ichijo, Pathophysiological roles of ASK1-MAP kinase signaling pathways, J. Biochem. Mol. Biol. 40 (2007) 1–6, http://dx.doi.org/10.5483/BMBRep.2007.40.1.001.
[42] D.K. Morrison, R.J. Davis, Regulation of MAP kinase signaling modules by scaffold proteins in mammals, Annu. Rev. Cell Dev. Biol. 19 (2003) 91–118, http://dx.doi.org/10.1146/annurev.cellbio.19.111401.091942.
[43] A. Eijkelenboom, B.M. Burgering, FOXOs: signalling integrators for homeostasis maintenance, Nat. Rev. Mol. Cell Biol. 14 (2013) 83–97, http://dx.doi.org/10.1038/nrm3507.
[44] K.E. Van Der Vos, P.J. Coffer, The extending network of FOXO transcriptional target genes, Antioxid. Redox Signa. 14 (2011) 579–592, http://dx.doi.org/10.1089/ars.2010.3419.
[45] I. Ciechomska, B. Pyrzynska, P. Kazmierczak, B. Kaminska, Inhibition of Akt kinase signalling and activation of Forkhead are indispensable for upregulation of FasL expression in apoptosis of glioma cells, Oncogene 22 (2003) 7617–7627, http://dx.doi.org/10.1038/sj.onc.1207137.
[46] A. Brunet, A. Bonni, M.J. Zigmond, M.Z. Lin, P. Juo, L.S. Hu, M.J. Anderson, K. C. Arden, J. Blenis, M.E. Greenberg, Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor, Cell 96 (1999) 857–868, http://dx.doi.org/10.1016/S0092-8674(00)80595-4.
[47] B.M. Burgering, G.J. Kops, Cell cycle and death control: long live Forkheads, Trends Biochem. Sci. 27 (2002) 352–360, http://dx.doi.org/10.1016/S0968-0004(02)02113-8.
[48] Y. Zhao, Y. Wang, W.G. Zhu, Applications of post-translational modifications of FoxO family proteins in biological functions, J. Mol. Cell. Biol. 3 (2011) 276–282, http://dx.doi.org/10.1093/jmcb/mjr013.
[49] A. Bellacosa, C.C. Kuman, A. Di Cristofano, J.R. Testa, Activation of AKT kinases in cancer: implications for therapeutic targeting, Adv. Cancer Res. 94 (2005) 29–86, http://dx.doi.org/10.1016/S0065-230X(05)94002-5.
[50] D.A. Altomare, J.R. Testa, Perturbations of the AKT signaling pathway in human cancer, Oncogene 24 (2005) 7455–7464, http://dx.doi.org/10.1038/sj.onc.1209085.
[51] M.E. Giannakou, L. Partridge, The interaction between FOXO and SIRT1: tipping the balance towards survival, Trends Cell Biol. 14 (2004) 408–412, http://dx.doi.org/10.1016/j.tcb.2004.07.006.
[52] A.I. Rojo, P. Rada, M. Mendiola, A. Ortega-Molina, K. Wojdyla, A. Rogowska-Wrzesinska, D. Hardisson, M. Serrano, A. Cuadrado, The PTEN/NRF2 axis promotes human carcinogenesis, Antioxid. Redox Signal. 21 (2014) 2498–2514, http://dx.doi.org/10.1089/ars.2014.5843.
[53] I. Bellezza, A.L. Mierla, A. Minelli, Nrf2 and NF-κB and their concerted modulation in cancer pathogenesis and progression, Cancers 2 (2010) 483–497, http://dx.doi.org/10.3390/cancers2020483.
[54] E. Kansanen, S.M. Kuosmanen, H. Leinonen, A.L. Levonenn, The Keap1-Nrf2 pathway: mechanisms of activation and dysregulation in cancer, Redox Biol. 1 (2013) 45–49, http://dx.doi.org/10.1016/j.redox.2012.10.001.
[55] J.M. Funes, S. Henderson, R. Kaufman, J.M. Flanagan, M. Robson, B. Pedley, S. Moncada, C. Boshoff, Oncogenic transformation of mesenchymal stem cells decreases Nrf2 expression favoring in vivo tumor growth and poorer survival, Mol. Cancer 13 (2014) 20–36, http://dx.doi.org/10.1186/1476-4598-13-20.
[56] I. Pietras, A. Ostman, Hallmarks of cancer: interactions with the tumor stroma, Exp. Cell Res. 316 (2010) 1324–1331, http://dx.doi.org/10.1016/j.yexcr.2010.02.045.
[57] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell 144 (2011) 646–674, http://dx.doi.org/10.1016/j.cell.2011.02.013.
[58] C. Thery, L. Zitvogel, S. Amigorena, Exosomes: composition, biogenesis and function, Nature Reviews Immunology 2 (2002) 569–579, http://dx.doi.org/10.1038/nri855.
[59] P. Kucharzewska, H.C. Christianson, J.E. Welch, K.J. Svensson, E. Fredlund, M. Ringner, M. Morgelin, E. Bourseau-Guilmain, J. Bengzon, M. Belting, Exosomes reflect the hypoxic status of glioma cells and mediate hypoxia-dependent activation of vascular cells during tumor development, Proc. Natl. Acad. Sci. USA 110 (2013) 7312–7317, http://dx.doi.org/10.1073/pnas.1220998110.
[60] I. Parolini, C. Federici, C. Raggi, L. Lugini, S. Palleschi, A. de Milito, C. Coscia, E. Iessi, M. Logozzi, A. Molinari, M. Colone, M. tatti, M. Sargiacomo, S. Fais, Microenvironmental pH is a key factor for exosome traffic in tumor cells, J. Biol. Chem. 284 (2009) 34211–34222, http://dx.doi.org/10.1074/jbc.M109.041152.
[61] J.A. Tickner, A.J. Urquhart, S.A. Stephenson, D.J. Richard, K.J. O'Byrne, Functions and therapeutic roles of exosomes in cancer, Front. Oncol. 4 (2014) 127, http://dx.doi.org/10.3389/fonc.2014.00127.
[62] A.S. Azmi, B. Bao, F.H. Sarkar, Exosomes in cancer development, metastasis, and drug resistance: a comprehensive review, Cancer Metastasis Rev. 32 (2013) 623–642, http://dx.doi.org/10.1007/s10555-013-9441-9.
[63] C. Roma-Rodrigues, A.R. Fernandes, P.V. Baptista, Exosome in tumour microenvironment: overview of the crosstalk between normal and Cancer Cells, BioMed Res. Int. 2014 (2014) 10, http://dx.doi.org/10.1155/2014/179486.
[64] C. Corrado, S. Raimondo, A. Chiesi, F. Ciccia, G. De Leo, R. Alessandro, Exosomes as intercellular signaling organelles involved in health and disease: basic science and clinical applications, Int. J. Mol. Sci. 14 (2013) 5338–5366, http://dx.doi.org/10.3390/ijms14035338.
[65] D.B. Vendramini-Costa, J.E. Carvalho, Molecular link mechanisms between inflammation and cancer, Curr. Pharm. Des. 18 (2012) 3831–3852, http://dx.doi.org/10.2174/138161212802083707.
[66] F. Mariani, P. Sena, L. Roncucci, Inflammatory pathways in the early steps of colorectal cancer development, World J. Gastroenterol. 20 (2014) 9716–9731, http://dx.doi.org/10.3748/wjg.v20.i29.9716.
[67] S.I. Grivennikov, F.R. Greten, M. Karin, Immunity, inflammation, and cancer, Cell 140 (2010) 883–899, http://dx.doi.org/10.1016/j.cell.2010.01.025.
[68] S. Gallucci, P. Matzinger, Danger signals: SOS to the immune system, Curr. Opin. Immunol. 13 (2001) 114–119, http://dx.doi.org/10.1016/S0952-7915(00)00191-6.
[69] G. Li, D. Tang, M.T. Lotze, Ménage à trois in stress: DAMPs, redox and autophagy, Sem. Cancer Biol. 23 (2013) 380–390, http://dx.doi.org/10.1016/j.semcancer.2013.08.002.
[70] D.V. Krysko, P. Agostinis, O. Krysko, A.D. Garg, C. Bachert, B.N. Lambrecht,

P. Vandenabeele, Emerging role of damage-associated molecular patterns derived from mitochondria in inflammation, Trends Immunol. 32 (2011) 157–164, http://dx.doi.org/10.1016/j.it.2011.01.005.
[71] D. Tang, R. Kang, K.M. Livesey, H.J. Zeh, M.T. Lotze, High mobility group box 1 (HMGB1) activates an autophagic response to oxidative stress, Antioxid. Redox Signal. 15 (2011) 2185–2195, http://dx.doi.org/10.1089/ars.2010.3666.
[72] M. Stros, HMGB proteins: interactions with DNA and chromatin, Biochim. Biophys. Acta 1799 (2010) 101–113, http://dx.doi.org/10.1016/j.bbagrm.2009.09.008.
[73] G. Li, X. Liang, M.T. Lotze, HMGB1: the central cytokine for all lymphoid cells, Front. Immunol. 4 (2013) 68, http://dx.doi.org/10.3389/fimmu.2013.00068.
[74] I. Goitre, B. Pergolizzi, E. Ferro, L. Trabalzini, S.F. Retta, Molecular crosstalk between integrins and cadherins: do reactive oxygen species set the talk? J. Signal Transduct. 2012 (2012) 12.
[75] S. Carta, P. Castellani, L. Delfino, S. Tassi, R. Vene, A. Rubartelli, DAMPs and inflammatory processes: the role of redox in the different outcomes, J. Leukocyte Biol. 86 (2009) 549–555, http://dx.doi.org/10.1189/jlb.1008598.
[76] M.R. Galdiero, E. Bonavita, I. Barajon, C. Garlanda, A. Mantovani, Tumor associated macrophages and neutrophils in cancer, Immunobiology 218 (2013) 1402–1410, http://dx.doi.org/10.1016/j.imbio.2013.06.003.
[77] A.S. Chung, J. Lee, N. Ferrara, Targeting the tumour vasculature: insights from physiological angiogenesis, Nat. Rev. Cancer 10 (2010) 505–514, http://dx.doi.org/10.1038/nrc2868.
[78] Z. Cao, B. Shang, G. Zhang, L. Miele, F.H. Sarkar, Q. Zhou, Tumor cell-mediated neovascularization and lymphangiogenesis contrive tumor progression and cancer metastasis, Biochim. Biophys. Acta 1836 (2013) 273–286, http://dx.doi.org/10.1016/j.bbcan.2013.08.001.
[79] B. Debelec Butuner, C. Alapinar, L. Varisli, B. Erbaykent-tepedelen, S. M. Hamid, C. Gonen-Korkmaz, K.S. Korkmaz, Inflammation-mediated abrogation of androgen signaling: an in vitro model of prostate cell inflammation, Mol. Carcinog. 53 (2014) 85–97, http://dx.doi.org/10.1002/mc.21948.
[80] B.B. Aggarwal, Signalling pathways of the TNF superfamily: a double-edged sword, Nat. Rev. Immunol. 3 (2003) 745–756, http://dx.doi.org/10.1038/nri1184.
[81] B. Debelec Butuner, C. Alapinar, N. Ertunc, C. Gonen-Korkmaz, K. Yorukoglu, K.S. Korkmaz, TNFα-mediated loss of β-catenin/E-cadherin association and subsequent increase in cell migration is partially restored by NKX3.1 expression in prostate cells, PloS One (2014), http://dx.doi.org/10.1371/journal.pone.0109868.
[82] M.J. Morgan, Z.G. Liu, Reactive oxygen species in TNFalpha-induced signaling and cell death, Mol. Cells 30 (2010) 1–12, http://dx.doi.org/10.1007/s10059-010-0105-0.
[83] R. Kolb, G.H. Liu, A.M. Janowski, F.S. Sutterwala, W. Zhang, Inflammasomes in cancer: a double-edged sword, Protein Cell 5 (2014) 12–20, http://dx.doi.org/10.1007/s13238-013-0001-4.
[84] I. Okamoto, W. Liu, Y. Luo, A. Tanaka, X. Cai, D.A. Norris, C.A. Dinarello, M. Fujita, Constitutively active inflammasome in human melanoma cells mediating autoinflammation via caspase-1 processing and secretion of interleukin-1beta, J. Biol. Chem. 285 (2010) 6477–6488, http://dx.doi.org/10.1074/jbc.M109.064907.
[85] E. Latz, T.S. Xiao, A. Stutz, Activation and regulation of the inflammasomes, Nat. Rev. Immunol. 13 (2013) 397–411, http://dx.doi.org/10.1038/nri3452.
[86] C. Nathan, A.C. Bussel, Beyond oxidative stress: an immunologist's guide to reactive oxygen species, Nat. Rev. Immunol. 13 (2013) 349–361, http://dx.doi.org/10.1038/nri3423.
[87] C. Juliana, T. Fernandes-Alnemri, S. Kang, A. Farias, F. Qin, E.S. Alnemri, Non-transcriptional priming and deubiquitination regulate NLRP3 inflammasome activation, J. Biol. Chem. 287 (2012) 36617–36622, http://dx.doi.org/10.1074/jbc.M112.407130.
[88] R. Zhou, A.S. Yazdi, P. Menu, J. Tschopp, A role for mitochondria in NLRP3 inflammasome activation, Nature 469 (2011) 221–225, http://dx.doi.org/10.1038/nature09663.
[89] M.M. Mueller, N.E. Fusenig, Friends or foes – bipolar effects of the tumour stroma in cancer, Nat. Rev. Cancer 4 (2004) 839–849, http://dx.doi.org/10.1038/nrc1477.
[90] M.L. Taddei, E. Giannoni, G. Raugei, S. Scacco, A.M. Sardanelli, S. Papa, P. Chiarugi, Mitochondrial oxidative stress due to complex I dysfunction promotes fibroblast activation and melanoma cell invasiveness, J. Signal Transduct. 2012 (2012) 10, http://dx.doi.org/10.1155/2012/684592.
[91] K.J. Jones, M.A. Chetram, D.A. Bethea, L.K. Bryant, V.O. Marah, C.V. Hinton, Cysteine (C)-X-C receptor 4 regulates NADPH Oxidase-2 during oxidative stress in prostate cancer cells, Cancer Microenviron. 6 (2013) 277–288, http://dx.doi.org/10.1007/s12307-013-0136-0.
[92] V. Luga, L. Zhang, A.M. Viloria-Petit, A.A. Ogunjimi, M.R. Inanlou, E. Chiu, M. Buchanan, A.N. Hosein, M. Basik, J.L. Wrana, Exosomes mediate stromal mobilization of autocrine Wnt-PCP signaling in breast cancer cell migration, Cell 151 (2012) 1542–1556, http://dx.doi.org/10.1016/j.cell.2012.11.024.
[93] G. Kharaishvili, D. Simkova, K. Bouchalova, M. Gachechiladze, N. Narsia, J. Bouchal, The role of cancer-associated fibroblasts, solid stress and other microenvironmental factors in tumor progression and therapy resistance, Cancer Cell Int. 14 (2014) 41, http://dx.doi.org/10.1186/1475-2867-14-41.
[94] J.G. Quatromoni, E. Eruslanov, Tumor-associated macrophages: function, phenotype, and link to prognosis in human lung cancer, Am. J. Transl. Res. 4 (2012) 376–389.
[95] Y.Q. Li, F.F. Liu, X.M. Zhang, X.J. Guo, M.J. Ren, L. Fu, Tumor secretion of CCL22 activates intratumoral Treg infiltration and is independent prognostic predictor of breast cancer, PLoS ONE 8 (2013) e76379, http://dx.doi.org/10.1371/journal.pone.0076379.
[96] D. Mougiakakos, C.C. Johansson, R. Kiessling, Naturally occurring regulatory T cells show reduced sensitivity toward oxidative stress-induced cell death, Blood 13 (2009) 3542–3545, http://dx.doi.org/10.1182/blood-2008-09-181040.
[97] R. Stoyanova, K. Huang, K. Sandler, H. Cho, S. Carlin, P.B. Zanzonico, J. A. Koutcher, E. Ackerstaff, Mapping tumor hypoxia in vivo using pattern recognition of dynamic contrast-enhanced MRI data, Transl. Oncol. 5 (2012) 437–447, http://dx.doi.org/10.1593/tlo.12319.
[98] S.H. Sahlberg, D. Spiegelberg, B. Glimelis, B. Stenerlow, M. Nestor, Evaluation of cancer stem cell markers CD133, CD44, CD24: association with AKT isoforms and radiation resistance in colon cancer cells, PloS ONE 9 (2014) e94621, http://dx.doi.org/10.1371/journal.pone.0094621.
[99] S. Ding , C. Li, N. Cheng, X. Cui, X. Xu, G. Zhou, Redox regulation in cancer stem cells, Oxidative Medicine and Cellular Longevity, 2015, Article ID 750798.
[100] R. Gargini, J.P. Cerliani, M. Escoll, I.M. Antón, F. Wandosell, Cancer stem cell-like phenotype and survival are coordinately regulated by Akt/FoxO/Bim pathway, Stem Cells 33 (2015) 646–660, http://dx.doi.org/10.1002/stem.1904.
[101] J. Verrax, R.C. Pedrosa, R. Beck, N. Dejeans, H. Taper, P.B. Calderon, In situ modulation of oxidative stress: a novel and efficient strategy to kill cancer cells, Curr. Med. Chem. 16 (2009) 1821–1830, http://dx.doi.org/10.2174/092986709788186057.
[102] Y. Mitsuishi, H. Motohashi, M. Yamamoto, The Keap1–Nrf2 system in cancers: stress response and anabolic metabolism, Front. Oncol. 2 (2012), http://dx.doi.org/10.3389/fonc.2012.00200.
[103] E.I. Azzam, J.P. Jay-Gerin, D. Pain, Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury, Cancer Lett. 327 (2012) 48–60, http://dx.doi.org/10.1016/j.canlet.2011.12.012.
[104] P. Agostinis, K. Berg, A. Keith, K.A. Cengel, T.H. Foster, A.W. Girotti, S. O. Gollnick, S.M. Hahn, M.R. Hamblin, A. Juzeniene, D. Kessel, M. Korbelik, J. Moan, P. Mroz, D. Nowis, J. Piette, B.C. Wilson, J. Golab, Photodynamic therapy of cancer: an update, CA: A Cancer J. Clin. 61 (2011) 250–281, http://dx.doi.org/10.3322/caac.20114.
[105] R. Socoteanu, R. Boscencu, A. Hirtopeanu, G. Manda, A. Sousa Oliveira, M. Ilie, L.F. Vieira Ferreira, in: Reza Fazel-Rezai (Ed.), Trends in Interdisciplinary Studies Revealing Porphyrinic Compounds Multivalency Towards Biomedical Application, in Biomedical Engineering – From Theory to Applications, InTech Publishing House, 2011, pp. 355–390 , ISBN: 978-953-307-637-9.
[106] J.S. Dysart, M.S. Patterson, Characterization of Photofrin photobleaching for singlet oxygen dose estimation during photodynamic therapy of MLL cells in vitro, Phys. Med. Biol. 50 (2005) 2597–2616, http://dx.doi.org/10.1088/0031-9155/50/11/011.
[107] M.T. Jarvi, M.S. Patterson, B.C. Wilson, Insights into photodynamic therapy dosimetry: simultaneous singlet oxygen luminescence and photosensitizer photobleaching measurements, Biophys. J. 102 (2012) 661–671, http://dx.doi.org/10.1016/j.bpj.2011.12.043.
[108] M.K. Kuimova, G. Yahioglu, P.R. Ogilby, Singlet oxygen in a cell: spatially dependent lifetimes and quenching rate constants, J. Am. Chem. Soc. 131 (2009) 332–340, http://dx.doi.org/10.1021/ja807484b.
[109] I. Kühne, E. Riballo, N. Rief, K. Rothkamm, P.A. Jeggo, M. Löbrich, A double-strand break repair defect in ATM-deficient cells contributes to radiosensitivity, Cancer Res. 64 (2004) 500–508, http://dx.doi.org/10.1158/0008-5472.CAN-03-2384.
[110] J. Heikkinen, K. Rapakko, S.M. Karppinen, H. Erkko, S. Knuutila, T. Lundán, A. Mannermaa, A.L. Børresen-Dale, A. Borg, R.B. Barkardottir, J. Petrini, R. Winqvist, RAD50 and NBS1 are breast cancer susceptibility genes associated with genomic instability, Carcinogenesis 27 (2006) 1593–1599, http://dx.doi.org/10.1093/carcin/bgi360.
[111] H. Zhang, G. Tombline, B.L. Weber, BRCA1, BRCA2 and DNA damage response: collision or collusion? Cell 92 (1998) 433–436, http://dx.doi.org/10.1016/S0092-8674(00)80936-8.
[112] D. Murray, R. Mirzayans, Role of therapy-induced cellular senescence in tumor cells and its modification in radiotherapy: the good, the bad and the ugly, J. Nucl. Med. Radiat. Therapy (2013), http://dx.doi.org/10.4172/2155-9619.S6-018, S6:018.
[113] S.H. Kaufmann, M.O. Hengartner, Programmed cell death: alive and well in the new millennium, Trends Cell Biol. 11 (2001) 526–534, http://dx.doi.org/10.1016/S0962-8924(01)02173-0.
[114] R. Mirzayans, B. Andrais, A. Scott, Y.W. Wang, D. Murray, Ionizing radiation-induced responses in human cells with differing TP53 status, Int. J. Mol. Sci. 14 (2013) 22409–22435, http://dx.doi.org/10.3390/ijms141122409.
[115] D.A. Gewirtz, Autophagy, senescence and tumor dormancy in cancer therapy, Autophagy 5 (2009) 1232–1234, http://dx.doi.org/10.4161/auto.5.8.9896.
[116] A. Munshi, R. Ramesh, Mitogen-activated protein kinases and their role in radiation response, Genes Cancer 4 (2013) 401–408, http://dx.doi.org/10.1177/1947601913485414.
[117] F. Chen, JNK-induced apoptosis, compensatory growth and cancer stem cells, Cancer Res. 72 (2012) 379–386, http://dx.doi.org/10.1158/0008-5472.CAN-11-1982.
[118] R. Gerl, D.L. Vaux, Apoptosis in the development and treatment of cancer, Carcinogenesis 26 (2005) 263–270, http://dx.doi.org/10.1093/carcin/bgh283.
[119] A. Marin, M. Martin, O. Linan, F. Alveranga, M. Lopez, L. Fernandez, D. Buchser, L. Cerezo, Bystander effect and radiotherapy, Rep. Pract. Oncol. Radiother. 20 (2015) 12–21, http://dx.doi.org/10.1016/j.rpor.2014.08.004.

[120] A.M. Chaudhry, Bystander effect: biological endpoints and microarray analysis, Mutat. Res. 597 (2006) 98–112, http://dx.doi.org/10.1016/j.mrfmmm.2005.04.023.
[121] C. Mothersill, C.B. Seymour, Radiation-induced bystander effects and the DNA paradigm: an "out of field" perspective, Mutat. Res. 597 (2006) 5–10, http://dx.doi.org/10.1016/j.mrfmmm.2005.10.011.
[122] S. Havaki, A. Kotsinas, E. Chronopoulos, D. Kletsas, A. Georgakilas, V. G. Gorgoulis., The role of oxidative DNA damage in radiation-induced bystander effect, Cancer Lett. 356 (2015) 41–51, http://dx.doi.org/10.1016/j.canlet.2014.01.023.
[123] J. Kono, K. Mimura, R. Kiesling, Immunogenic tumor cell death induced by chemoradiotherapy: molecular mechanisms and a clinical translation, Cell Death Dis. 4 (2013) e688, http://dx.doi.org/10.1038/cddis.2013.207.
[124] E.F. Stammel, J.D. Wolchok, S. Gnjatic, N.Y. Lee, I. Brownell, The abscopal effect associated with a systemic anti-melanoma immune response, Int. J. Radiat. Oncol. Biol. Phys. 85 (2013) 293–295, http://dx.doi.org/10.1016/j.ijrobp.2012.03.017.