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Survival or Death: The Redox Paradox

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The role of reactive oxygen species (ROS) in the regulation of transduction of extracellular signals is widely acknowledged (32). More or less all the different species belonging to ROS were first identified and studied as mediators of damage produced in different cellular compartments. This damage may be both professional, as in the case of inflammatory cells, or a collateral effect of more complex processes. The latter include oxidative damage correlated with diseases such as cancer, diabetes, or neurodegenerative disorders (3, 4, 17, 27). In any case, oxidant molecules are effectively death tools, through their NONSELECTIVE activity of destruction of biomolecules belonging to the different classes of lipids, proteins, and nucleic acids. *Vice versa*, as usual for any second messenger, reactive oxygen species may have a controlled production from both a kinetic and quantitative point of view. The events controlling ROS production allow a SELECTIVE activity of oxidants against their targets, in particular proteins, widely recognized as the molecular "sensors" of oxidative signals. This oxidative signal, in contrast to oxidative damage, leads to temporary regulation of redox-sensitive events, including cell proliferation, adhesion, and survival. In this latter phenomenon, the difference between oxidative damage and signal may be considered a real paradox. Indeed, if oxidative damage leads invariably to death *exitus*, the redox-based signal regularly drives the cells towards survival. This paradoxical role in the regulation of cell survival has been described in several cellular systems and is mainly mediated by hydrogen peroxide and nitric oxide (17, 32, 37). This is an additional difference between oxidative damage and signaling. Indeed, oxidative damage is mainly produced by highly oxidizing species, as superoxide ion, peroxinitrite, and several radicals including hydroxyl radical. Conversely, during oxidative signaling, the oxidants produced by intracellular regulated sources, very often superoxide ion, is rapidly dismutated into hydrogen peroxide, to which protein thiol groups behave as molecular sensors. It should be noted that oxidant sources are not different between oxidative damage and signaling. NADPH oxidase, widely described by Groeger (15) for both its role in professional macrophages and in the regulation of cell survival pathways, plays indeed a primary role. This source has been involved in regulation of survival of normal and transformed cells, as well as in cardiac preconditioning or in endothelial response to hypoxia (1, 15, 34). More surprisingly, the mitochondrion, a ROS source acknowledged to be the main responsible for redox-mediated cell stress and death, is emerging as an oxidant source that is tightly controlled by both extracellular or intracellular signals and is mandatory for regulation of several processes including cell survival (25, 27). The role of mitochondrial oxidants is particularly important during exposure to hypoxic environment (33). Indeed, it has been widely reported that in mild hypoxia the decrease in oxygen concentration leads to deregulation of complex III of the electron transport chain and to production of superoxide ion in the intermembrane space, rapidly dismutated into hydrogen peroxide released in the cytosolic milieu. These hypoxia-mediated ROS are correlated to cell survival through stabilization of the hypoxia inducible factor 1-α (HIF-1α) and activation of its antiapoptotic transcriptional programme both in healthy and in neoplastic cells (33) and Pani *et al*., (28). In this context, ROS, by their action on prolyl hydroxylase leading to a Fenton reaction with the catalytic Fe++ resulting in enzymatic inactivation, represent an additional mechanism of activation of HIF-1-mediated transcriptional programme (19). Activation of HIF-1 is thereby mandatory to activate the survival pathway, acting on inhibition of pro-apoptotic routes such as Bad and Bid, and activation of secretion of pro-survival factors as vascular endothelial factor, Bcl-2, and Myeloid Cell leukemia-1 (7. 33). This HIF-1-mediated pro-survival pathway elicited by the decrease in oxygen concentration is regulated through a redox-based mechanism only during mild hypoxia (1–3% oxygen) and is actually a compulsory protection system from an incremental danger (*i.e.*, the decrease of O_2). Cells react to this menace with three responses: first they activate a metabolic shift toward a more reducing catabolism involving glycolysis and excluding the strongly oxidizing mitochondria; second, they activate the antiapoptotic pathway to survive in the aggressive environment; and last they react with activation of the escape programme from the hostile hypoxic site and moving away (activation of the metastatic escape programme). Several proofs of the redox regulation of these three programmes are given in the reviews of Chandel and Pani (28, 33).

The further decrease of oxygen concentration, leading gradually from hypoxia to anoxia, activates other interesting cell behaviors. Deep hypoxia has been correlated to cell stemness, both in normal and in neoplastic cells. This correlation between redox cell state and stem cell phenotype, reviewed by Pervaiz (29), opens a new perspective in redox biology of cell survival. Indeed the concept of stemness is in strict correlation with the meaning of survival as the Latin word *stamen* means "fate". Cell stemness is an epigenetic plan

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leading to enlargement of the gene expression pattern, allowing cells to embrace a wide set of differentiation programmes and to regenerate an array of tissues. It has recently been shown that central nervous system stem cells and hematopoietic stem cells, as well as their early progenitors, contain lower levels of ROS than their more mature progeny (10, 16). In addition, these differences in ROS contents are critical for maintaining stem cell function. More recently, epithelial tissue stem cells and their cancer stem cell counterparts have also been reported to share this property. Indeed, lower ROS levels in cancer stem cells are associated with increased expression of free radical scavenging systems, and depletion of these scavengers markedly decreases their clonogenicity and results in radiosensitization of tumor cells (9, 10). In this light, ROS content, which is inversely proportional to stemness hierarchy, may be proposed as a survival control system, not of the single cell, but of a whole hierarchy of populations originating from this cell (29).

A further extension of the concept of cell survival in which ROS act as key factors is the runaway and resistance to hostile environment strategies, adopted by aggressive cancer cells within the primary tumor context. Beside the already mentioned contribution of the hypoxic environment, deregulated intrinsic production of ROS is often correlated with aggressiveness of several cancers (26, 36). Besides, exposure to exogenous oxidative stress or intrinsic deregulated ROS production activates a programme of resistance to *anoikis* in aggressive cancers, thereby allowing survival in nonadherent conditions of metastatic cancers. This mechanism, involving redox deregulation of Src tyrosine kinase activation and leading to sustained pro-survival signals, allows achievement of anchorage independence of several aggressive cancers, thus sustaining their survival when circulating in blood or lymphatic vessels without anchorage and allowing their metastatic spreading (13, 28). In addition, both cancer cells and the stromal cells of the tumor microenvironment are exposed to a constitutive oxidative stress (21). Very often this oxidative stress leads tumor cells to develop several defense mechanisms against chemo/radiotherapy or hypoxia, whose molecular basis is deeply shared (14, 21). Indeed oxidative stress activates a common programme of resistance to anticancer drugs, to apoptotic death due to lack of adhesion developing a real anchorage independence, and to hypoxia shifting towards glycolytic metabolism. In this manner, the cell develops a general survival adaptation, spanning from the natural inclination to limit oxidant production from the mitochondrion, to regulation of its antioxidant properties, to finish with the escapement from the pro-oxidant environment. Several lines of evidence suggest that aggressive tumors develop an increased antioxidant ability, mainly due to thyoredoxin/thyoredoxin reductase couple, which are now acknowledged targets for cancer therapy (2, 30). Of note, thyoredoxin is an important factor in conferring resistance to chemotherapy and for expression of HIF-1 (8). In keeping, during mild hypoxia the regulation of mitochondrial GSH acts as a defense against oxidative stress in hepatoma, neuroblastoma, and colon carcinoma cells (22).

In agreement with resistance to apoptosis or drugs, the escape programme is again activated through an epigenetic redox-based programme, the epithelial mesenchymal transition (EMT), and the achievement of a motile phenotype (28). Definitely, both the epigenetic programme of EMT and its main molecular players as metalloproteases and the proto-oncogene Met, have been reported to be redox-dependent (11, 12, 31).

It is noted that both survival strategies adopted by cancer cells, motility and resistance to apoptosis, have been recently correlated with stemness. Indeed, cells undergoing epithelial–mesenchymal transition (EMT) in both mammary epithelial and tumor cells display a CD44high/CD24low phenotype, an acknowledged stemness feature (18, 23, 28). In addition, the subpopulations of colon carcinoma cells displaying the highest resistance to drugs to radiotherapy and to oxidative stress are cancer stem cells (10, 21). It has been recently reported that Snail and Slug, the main transcription factors involved in EMT, mediate radio- and chemo-resistance by antagonizing p53-mediated apoptosis and acquiring a stem-like phenotype in ovarian cancer cells (20). This evidence allows a redox-based correlation of virtually all key features of cancer progression, including stemness of cancer regenerating populations, achievement of motile and invasive phenotype through EMT, resistance to chemotherapic drugs, and development of anchorage independence.

Beside cancer cells, adjustments in enzymatic and nonenzymatic antioxidants have been reported to play key roles for cell survival in different diseases, including myocardial infarction, neurodegenerative diseases, and liver steatosis. For all these diseases, wide damages have been ascribed to deregulated ROS production, and upregulation of both glutaredoxins and thioredoxins have been reported to be involved in survival of cardiomyocytes [Ahsan *et al.* (1)] and nervous cells (5) exposed to oxidative stress. In this view, the specific pool of mitochondrial antioxidants, and in particular GSH, has been strictly correlated to survival of cancer cells induced by hypoxia and its correlated oxidative stress (22). In addition, mitochondrial GSH is a key factor in granting survival of liver cells to steatosis-linked oxidative stress (24). Indeed, in nutritional and genetic models of hepatic steatosis in which hepatocytes are sensitized to oxidative stress, mitochondrial GSH depletion occurs owing to the enhancement of free cholesterol, resulting in decreased mitochondrial membrane fluidity and commitment to apoptosis. In addition to mitochondrial GSH, Landriscina and colleagues described a role of a mitochondrial chaperone TNF-receptor associated protein-1 (TRAP) in survival to chemotherapeutic drugs in models of thyroid and ovarian carcinomas (21). It is noted that these antioxidant protections are activated through a mechanisms directly elicited by oxidants. Again, the activation of a survival programme by the same molecules from which protection is needed, appears a paradox. But again, the correct reading is in the level of oxidants, different between damage and signaling. Indeed, it has been reported that myocardial protection is granted by a redox and NADPH oxidase-mediated system, commonly called "pre-conditioning." This habituation is a sort of sensitization of cardiomyocytes to undergo and tolerate a future oxidative stress, mainly regulated through an oxidant-induced activation of an antioxidant survival strategy based on glutaredoxin/thioredoxin enhanced activity (1). As far as nervous tissue is concerned, a pleiotropic role is played by nitric oxide, virtually belonging to nitrogen reactive species, but endowed with a similar oxidative power. Again, its role is dual and its levels are decisive for eliciting survival or death (5). In fact, physiological amounts of this gaseous molecule, involved in the regulation

of the cardiovascular, immune, and nervous systems, are neuroprotective, whereas higher concentrations are clearly neurotoxic. Nitric oxide confers neuroprotection to brain cells by *S*-nitrosylation and inactivation of caspase-3 and by blocking Ca flux by the *N*-methyl-D-aspartate receptor, leading to a decrease in cell death, as well as by activation of protein kinase G-mediated Akt phosphorylation, two proteins that are mainly involved in neuroprotection (6). Conversely, excess nitric oxide, produced by activation of inducible nitric oxide synthase owing to the pro-inflammatory response, is a common feature of neurodegenerative disorders. In this perspective, nitric oxide may really be described as a *Janus bifrons* molecule.

Whatever the physiologic setting analyzed, the environment will be similar: tightly controlled oxidant molecules modulate the start of a survival programme, spanning from chemoresistance to activation of a motile phenotype allowed to physically escape from the hostile site, to the regulation of cell stemness. Cancer is a particular example of disease in which cells are able to adapt/resist to and exploit the pro-oxidant environment for dissemination at distance, allowing survival of the cancer stem cells to regenerate the tumor elsewhere. It will be particularly challenging to investigate if these cells, which are oxidant-adapted, highly resistant to radiotherapy/chemotherapy, EMT-driven for motility and metastatic behavior, are the same subpopulations of cancer stem cells.

Conversely, in other diseases, the accumulation of oxidative-damage products, but failure to adapt to ROS stress, may result in excessive cell death, leading to degenerative disorders and aging. In both cancer or degenerative diseases, strategies to modulate cellular redox status, either by oxidants or antioxidants or by affecting selected redox-sensitive signaling pathways, may have major clinical applications. Although we are still far from a comprehensive understanding of the redox cell biology leading cells to decide among survival or death, the rational combinations of pro-oxidant drugs and compounds directed to known redox-sensitive signaling pathways should potentially improve therapeutic efficacy. Further and decisive studies are highly warranted mainly at identifying the molecular sensors affecting redox homeostasis and the final decision whether the cells will survive or die (35). These molecules will be suitable targets for developing effective therapeutic strategies for those diseases, exploiting deregulated ROS production due to either oncogenic activation, mitochondrial dysfunction, or accumulation of oxidative stress, to acquire genetic instability and to attenuate programmed cell death.

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