HHS Public Access

Author manuscript

Radiat Res. Author manuscript; available in PMC 2015 May 23.

Published in final edited form as:

Radiat Res. 2015 May ; 183(5): 487–496. doi:10.1667/RR13959.1.

Cellular and Molecular Mechanisms Underlying Oxygen-Dependent Radiosensitivity

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Abstract

Molecular oxygen has long been recognized as a powerful radiosensitizer that enhances the cell-killing efficiency of ionizing radiation. Radiosensitization by oxygen occurs at very low concentrations with the half-maximum radiosensitization at approximately 3 mmHg. However, robust hypoxia-induced signal transduction can be induced at <15 mmHg and can elicit a wide range of cellular responses that will affect therapy response as well as malignant progression. Great strides have been made, especially since the 1990s, toward identification and characterization of the oxygen-regulated molecular pathways that affect tumor response to ionizing radiation. In this review, we will discuss the current advances in our understanding of oxygen-dependent molecular modification and cellular signal transduction and their impact on tumor response to therapy. We will specifically address mechanistic distinctions between radiobiological hypoxia (0–3 mmHg) and pathological hypoxia (3–15 mmHg). We also propose a paradigm that hypoxia increases radioresistance by maintaining the cancer stem cell phenotype.

Introduction

A solid tumor grows autonomously as an independent tissue that defies or evades normal physiological controls of the host tissue or organ. Abnormalities in angiogenesis, vascular formation, cellular compositions and tissue structures are commonly observed in solid tumors. This unique tumor microenvironment can induce adaptive responses in tumor cells that will eventually affect tumor cell sensitivity to chemotherapy and/or radiation therapy.

In addition to intrinsic (genetic and epigenetic) factors, tumor response to therapy is also heavily influenced by a plethora of extrinsic factors. Hypoxia or insufficient oxygenation is arguably the most prominent feature of tumor microenvironment and can be found in a wide range of solid tumors. Hypoxia develops as a consequence of insufficient oxygen (O_2) supply to meet the demands of the metabolically active tumor cells. Several excellent review articles offer detailed discussion on the potential causes of tumor hypoxia (1–4). Data suggest that intratumoral O_2 transport is often retarded due to structurally defective blood vessel formation, fluctuating red blood cell flux and limited arteriolar blood supply. It is

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important to note that tumor hypoxia is highly dynamic in terms of both duration and degree of hypoxia. The intermittent or transient nature of tumor hypoxia was first reported by Dr. J. Martin Brown in 1979 (5). Dewhirst and colleagues further observed that many tumor types exhibited periodically fluctuating hypoxia, which led them to define “acute” or “intermittent” hypoxia, which is now more appropriately called “cycling hypoxia” (1, 6). It is worth noting that hypoxic areas are heterogeneously distributed throughout a solid tumor proper and both chronically stable hypoxia and cycling hypoxia can be found in the same tumor.

In the classic study of human lung cancer specimens, Thomlinson and Gray (7) found that tumor cords with >200 μm in diameter inevitably developed central necrosis and the sheath of viable tumor cells surrounding the necrotic center never exceeded 180 μm. Using mathematic models, they analyzed the supply and consumption as well as regional distribution of oxygen in these tumors, which led to the prediction of tumor hypoxia. However, the presence of hypoxia in human tumors was not directly demonstrated until 1985 when Gatenby *et al*. measured oxygen tensions in 16 cancer patients using CT-guided pO_2 needle electrodes (8). In a subsequent study examining 31 fixed lymph node metastases from squamous cell carcinoma of the head and neck, Gatenby *et al*. found that tumors containing >26% tumor volume with $pO_2 \leq 8$ mmHg responded poorly to radiotherapy (9). It should be noted however that the Clark-type pO_2 electrodes used by Gatenby *et al*. consume large quantities of pO_2 during the measurement process and therefore the reported intratumoral pO_2 is likely to be lower than the actual values. Using a computerized pO_2 measurement system with a programmed pattern of electrode movement through tissue to improve measurement accuracy, Vaupel *et al*. later found that human breast tumors contained regions of hypoxia with a significant proportion of pO_2 readings between 0 and 10 mmHg compared to normal breasts ($pO_2 > 12.5$ mmHg and median $pO_2 = 65$ mmHg) (10). Subsequently, tumor hypoxia has been observed in many other human tumors including tumors of uterine cervix, prostate, pancreas, lung, brain, soft tissue sarcoma, melanoma, non-Hodgkin lymphoma, liver, kidney and rectum (11–13). In cancers of the uterine cervix, head and neck and breast, it has been found that the overall median tumor pO_2 was 10 mmHg (11). Importantly, $pO_2 \leq 10$ mmHg predicts poor outcomes for these patients (14–19). It should be noted that different oxygenation parameters (i.e. pO_2 values) have been used to determine the prognostic significance of tumor hypoxia by different research groups and/or in different tumor types [extensively reviewed by Vaupel *et al*. (11)]. The lack of a generally accepted pO_2 threshold to categorize tumor hypoxia in the clinic might reflect differences in patient- or tumor-dependent response/adaptation to hypoxia or different approaches to tumor sampling by different investigators. It is also worth noting that, despite the prevailing evidence showing tumor hypoxia as an independent prognostic parameter for aggressive tumor behavior, Nordsmark *et al*. found no significant correlation between pO_2 and response to radiotherapy from a prospective multicenter study of 120 patients with primary cervical cancer (20). Although the reason for this discrepancy remains to be determined, it is possible that these results could be affected by the type (cycling or chronic), heterogeneity and extent (hypoxic volume) of hypoxia in different studies. Nonetheless, a preponderant majority of the clinical evidence has shown that tumor hypoxia predicts poor overall survival and disease-free survival of cancer patients independently of tumor grade,

nodal involvement and other commonly used prognostic factors. In this review, we discuss molecular mechanisms underlying oxygen-dependent regulation of tumor response to ionizing radiation.

Radiobiological Hypoxia Versus Pathological Hypoxia

Radiobiological Hypoxia and Oxygen Fixation Hypothesis

The potential impact of oxygen on radiosensitivity was first implicated in the 1900s by work showing that compression of skin by the applicator reduced the skin reaction to X rays [see reviews (21, 22)]. Fast forward to the 1950s, Gray and colleagues conducted a series of experiments investigating the effects of oxygen on radiation response of microbes, plants, cells and animals (7, 23–25). This body of classic work defined oxygen effects on cellular sensitivity to X-ray radiation. When X irradiated under anoxia, i.e., the absence of oxygen, mammalian cells are approximately 2.5–3 times more resistant to radiation-induced clonal cell death than the same cells irradiated at physiological or higher levels of oxygen concentration (22, 26). The ratio of X-ray doses between anoxia (pO_2 = 0 mmHg) and a given pO_2 that are required to kill the same numbers of clonogenic tumor cells is commonly referred to as the oxygen enhancement ratio (OER). In essence, the OER is a quantitative representation of the relative radiosensitivity of mammalian cells with radiosensitivity at anoxia (pO_2 = 0 mmHg) set as 1 (22, 26). O_2-dependent radiosensitivity rises sharply when pO_2 increases from 0 to 10 mmHg. The half-maximum radiosensitivity occurs at approximately pO_2 =; 3 mmHg (21, 27). Once pO_2 rises into the physiological range (>30 mmHg) and even up to 100% pure O_2, radiosensitivity increases only gradually (22, 26), suggesting that mammalian cells are by and large fully sensitized by molecular oxygen to ionizing radiation under most physiological conditions. For the purposes of discussion herein, we define the radiobiological hypoxia as pO_2 <3 mmHg or 0.4% O_2 where mammalian cells are the most radioresistant.

The ability of molecular oxygen as a potent radiosensitizer lies in its chemical properties as a highly reactive electrophile. The customarily accepted mechanism for O_2-dependent enhancement of ionizing radiation-induced damage is known as the oxygen fixation hypothesis (OFH), developed from the work of Alexander and Charlesby (28) as well as that of Johansen and Howard-Flanders (29). As shown in Fig. 1, X rays do not directly cause significant damages to cellular macromolecules including DNA. Instead, the ionizing radiation produces hydroxyl free radicals upon encountering the abundant water molecules in biological systems. Subsequent interaction between hydroxyl free radicals and DNA strands results in the formation of DNA-derived free radicals. In the absence of O_2, the DNA free radicals can be relatively easily restored through chemical reduction by sulfhydryl compounds and/or other reducing molecules. However, upon interaction with O_2, the DNA-derived free radicals can be converted to peroxides that cannot be structurally restored by chemical reduction, which leads to “fixation” of DNA damages. However, the OFH has its own limitations. It does not consider lesions in DNA-associated proteins or chemical alterations in chromatin. Furthermore, work by David Ewing (30) suggests that DNA repair pathways may also affect O_2-dependent radiosensitization.

Pathological Hypoxia and Activation of the Hypoxia-Inducible Factor Pathway

Mammalian cells respond to O_2 deprivation by mobilizing multiple intracellular signal transduction pathways. Hypoxia-induced signal transduction is primarily mediated by the hypoxia-inducible factor (HIF)-1 and HIF-2 by directly binding the hypoxia-responsive enhancer element (HRE, 5′-ACGTG-3′) in hypoxia-induced genes (31, 32). These heterodimeric transcription factors consist of an O_2-regulated alpha subunit (primarily HIF-1α or -2α) and the common O_2-insensitive HIF-1β (33). Under most physiological conditions with $pO_2 \geq 20$ mmHg, HIF-α subunits are unstable due to O_2-dependent hydroxylation of the two proline residues located in the O_2-dependent degradation (ODD) domain (34, 35). The hydroxylated HIF-α is ubiquitinated via interaction with the von Hippel-Lindau (VHL) protein in the E3 ubiquitin ligase complex and eventually degraded by proteasomes (36, 37). Both HIF-1α or -2α proteins become stable when environmental pO_2 decreases to or below 2% or 15 mmHg (38, 39). To draw contrast to the radiobiological hypoxia (pO_2 = 0–3 mmHg), we will define the range of moderate hypoxia (approximately pO_2 = 3–15 mmHg) as pathological hypoxia where robust HIF-α stabilization and HIF-dependent signal transduction occur. Radiosensitivity of mammalian cells is likely to be affected more strongly by hypoxia-dependent signaling mechanisms than by the OFH under such moderate or pathological hypoxia.

Hypoxia-Induced Molecular and Cellular Events Affecting Radiosensitivity

Hypoxia and Selection for Apoptosis-Resistant Clones

The distribution of hypoxia and cell proliferation in patients' tumors has been investigated using immunohistochemical approaches (40–42). Tumor hypoxia has been detected by the hypoxia marker pimonidazole hydrochloride (42) or EF5 (40, 41), both of which are derivatives of 2-nitroimidazole. Nuclear antigen Ki-67 (MIB-1) and/or proliferating cell nuclear antigen (PCNA) were used to identify proliferating cells. These clinical studies found an inverse correlation between binding of hypoxia markers and expression of the proliferation markers. Similar observations were also made in xenografts of several human tumor cell lines (43). According to the Law of Bergonié and Tribondeau (44), hypoxia is capable of reducing radiosensitivity of tumor cells by suppressing cell proliferation.

In an elegant study using mixed populations of transformed cells either proficient or deficient for the tumor suppressor gene p53, the Giaccia group showed that the p53-deficient cells out-survived their p53-proficient counterparts when treated with severe hypoxia *in vitro* (45). Furthermore, the p53-deficient cells also resisted hypoxia-induced apoptosis in xenografts (45). This seminal work clearly demonstrates that the hypoxic microenvironment favors the survival and expansion of apoptosis-resistant clones. The enrichment of these apoptosis-refractive cells will likely contribute to increased tumor cell survival in response to ionizing radiation.

Hypoxia and Cancer Stem Cells

Clonogenic and tumor-initiating cells are likely to be subpopulations of the cancer stem cell (CSC) repertoire. Recent work has shown that CSCs have much improved DNA repair capability, which confers CSCs with robust survival and also makes them a likely source of

relapse after radiotherapy. Elevated DNA repair potentials have been observed in CSCs of several tumor types. Using the mouse mammary tumor virus (MMTV)-Wnt1 spontaneous breast tumor model, Diehn *et al*. have shown that the $Thy1^{+}/CD24^{+}/Lin^{-}$ CSCs develop fewer γH2AX-positive foci, an often used surrogate marker of DNA double-strand breaks, after ionizing radiation in comparison to their non-CSC counterparts (46). Interestingly, the CSC population is nearly doubled in MMTV-Wnt1 mammary tumors after *in vivo* X irradiation (46). Consistently, ionizing radiation dramatically increased the $Lin^{-}CD24^{+}CD29^{+}$ population of CSCs in MCF7 human breast cancer cells (47).

The remarkable radioresistance exhibited by CSCs can potentially be attributed to increased DNA damage checkpoint signal transduction and/or expression of DNA repair genes. Using a syngeneic p53-null mouse mammary tumor model, Zhang *et al*. have shown that the CSC-like $Lin^{-}CD29^{hi}CD24^{hi}$ cells have increased expression of a set of genes involved in DNA damage response and repair (48). Bao *et al*. observed that glioma cells expressing the surface antigen CD133, a commonly used CSC marker, are capable of repairing ionizing radiation-induced DNA damage more efficiently than $CD133^{-}$ glioma cells (49). The enhanced repair activity in these CSC-like glioma cells is attributed to robust activation of the ATM-CHK2 and ATR-CHK1 pathways in response to ionizing radiation (49). The ATM-CHK2 and ATR-CHK1 pathways are the critical checkpoints activated in response to DNA double-strand and single-strand breaks, respectively. Consistent with this observation, DNA damage checkpoint activation was also involved in radioresistance of the $CD133^{+}/CD44^{+}$ prostate CSCs (50) and $CD44^{+}/CD24^{-/low}$ breast CSCs (51).

A rapidly growing body of literature has shown that hypoxia can play a significant role in cell fate decisions and stem cell maintenance (52, 53). Hypoxic tumor cells tend to be poorly differentiated *in vivo* and often show elevated expression of stem cell-associated genes (54, 55). Hypoxic tumor cells exhibit enhanced clonogenicity *in vitro* (56–58). Tumor cells pretreated *in vitro* by hypoxia also have enhanced tumorigenic potential *in vivo* (55). Furthermore, the metastatic potential of established xenograft tumors is increased when tumor-bearing mice are acutely exposed to atmospheric hypoxia (59, 60). Histological examinations have demonstrated that poorly differentiated primary pancreatic cancer cells show strong nuclear accumulation of HIF-1α protein (61). The CSC-like cells of neuroblastomas (62, 63) or gliomas (64) tend to have increased levels of HIF-1α and/or HIF-2α. Downregulation of HIF-1α by RNA interference results in reduced clonogenic growth of glioma CSCs (64) and chronic myeloid leukemia CSCs (65). Mathieu *et al*. have shown that hypoxia can activate an embryonic stem cell-like transcription program in a HIF-dependent manner (66). These results suggest that HIF-1 and/or HIF-2 facilitate the maintenance of cancer stem cells.

Indeed, the HIF pathway can induce the expression of specific genes associated with stem cell maintenance. The prominent pluripotency gene POU5F1 or Oct3/4 is capable of promoting tumorigenesis (67). Several types of human cancers have elevated levels of *POU5F1* (68–71). Covello et al. have shown that HIF-2α, but not HIF-1α, regulates the transcription of *POU5F1* by directly binding to its promoter/enhancer in mouse embryonic stem cells (72). However, it has yet to be determined whether hypoxia also increases *POU5F1* expression in common types of tumors.

Delta-like 1 homolog (*Drosophila*) or DLK1, an emerging stem cell gene, is expressed primarily in embryonic tissues and immature cells (73). Overexpression of DLK1 has been observed in a wide range of tumors (74–79). DLK1 is expressed in undifferentiated neuroblastoma cells only and is required to sustain both clonogenicity and tumorigenicity (57, 80). Hypoxia strongly increases DLK1 expression mediated by direct binding of HIF-1α and HIF-2α to *DLK1* promoter/enhancer (57). In addition, the DLK1-positve neuroblastoma cells are also preferentially localized in the pimonidazole-positive hypoxic region (80).

The penta-span transmembrane glycoprotein prominin-1 (CD133), a widely used CSC marker (81), is upregulated in hypoxia (1% O_2)-treated human glioma cells and can promote the expansion of CSCs (82–84). Knocking down either HIF-1α (84) or HIF-2α (83) reduces the hypoxia-induced *CD133* expression in glioma cells, suggesting that both HIF-1α and HIF-2α are involved in the hypoxia-induced *CD133* expression. However, it is worth noting that severe hypoxia (0.1% O_2) downregulates *CD133* expression in gastric, colorectal and lung cancer cell lines (85). It is possible that CD133 is involved in CSC maintenance under moderate (1% O_2) but not severe (0.1% O_2) hypoxia. These observations suggest that stem cell pathways are differentially regulated at different levels of hypoxia or that different subpopulations of CSCs are preferentially selected at different levels of hypoxia. Collectively, these studies demonstrate the ability of hypoxia to create a favorable niche where CSCs proliferate while maintaining their undifferentiated state and sustaining self-renewal (52, 53).

Hypoxia and DNA Repair and Genomic Integrity

Hypoxia has been well recognized as a potent environmental stress on genomic integrity (86, 87). Severe hypoxia or anoxia is capable of inducing DNA over-replication (88) and point mutations (89). Interestingly, increased mutation rates were also found in tumor allografts containing large areas with 0–2 mmHg pO_2 (89). Furthermore, hypoxia strongly induces fragile site formation that leads to genomic rearrangements, fusion of double minutes (DMs) and generation of homogeneously staining regions (HSRs) (90). Mechanistically, hypoxia-induced genomic instability can be attributed to diminished repair under hypoxia (91).

The homology-directed repair (HDR) is suppressed by hypoxia (92, 93). The expression of several key HDR genes including *RAD51* and *BRCA1* is repressed under hypoxic conditions (92, 93). Consistently, reduced expression of RAD51 protein was also observed in hypoxic regions of xenograft tumors in mice (93). Mechanistically, hypoxia increases the binding of E2F4, a transcription inhibitor of the E2F transcription factor family, to the promoter/enhancer regions of *RAD51* and *BRCA1* genes while decreasing the binding by the transcription activator E2F1 (92). On the other hand, epigenetic mechanisms may also contribute to hypoxia-induced gene silencing (94). Under chronic hypoxic conditions, downregulation of RAD51 and BRCA1 protein expression can potentially be caused by hypoxia-induced selective repression of mRNA translation (95). In contrast, the expression of NBS1, a component of the MRE11A-RAD50-NBS1 (MRN) complex, is also downregulated by hypoxia, but through a HIF-1-specific mechanism (96). These

observations demonstrate that expression of HDR-associated genes under hypoxia is subject to complex regulations at multiple levels including chromatin remodeling, transcription factors and protein synthesis.

The impact of hypoxia on mismatch repair (MMR) has been extensively investigated. When exposed to severe hypoxia (<0.01% O_2), both human and mouse tumor cells downregulated the expression of *MLH1* with minimal effects on other members of the MMR genes (97). The rate of mutagenesis induced by severe hypoxia was inversely correlated with MLH1 levels (97). *MLH1* expression was also reduced in several human tumor cell lines exposed to 1% O_2, and the repression was mediated by the transcription repressors DEC1 and DEC2, both of which were induced by hypoxia (98). However, epigenetic mechanisms were also implicated in hypoxia-dependent suppression of MLH1 (97). Very recently, it has been shown that the lysine-specific demethylase LSD1, a member of the histone demethylases, forms a co-repressor complex in the promoter/enhancer region of the *MLH1* gene to epigenetically silence *MLH1* expression under hypoxia (99). As for other members of the MMR genes, Koshiji *et al*. reported that expression of *MSH2* and *MSH6* was decreased in both normal and human tumor cells upon exposure to 1% O_2 (100). Mechanistically, HIF-1α mediated the repression by displacing the transcription factor Myc from the gene promoters (100). This association between HIF-1 and reduced expression of MSH2 protein is more pronounced in human colorectal tumors with undetectable tumor suppressor p53 (100).

In addition to the HDR and MMR pathways, hypoxia can also negatively regulate the expression of genes involved in other repair pathways, including non-homologous end joining (NHEJ), nucleotide excision repair (NER) and Fanconi anemia pathway (101). Collectively, these studies clearly indicate that hypoxia has the potential to affect all the major DNA repair pathways by multiple mechanisms. These studies have also painted a complex picture of transcriptional repression mechanisms from direct action of specific transcription factors to chromatin modifications. It is likely that the actual mechanisms deployed will depend on both the cell-intrinsic properties and the severity of hypoxia.

Hypoxia-Regulated MicroRNAs

In addition to transcriptional regulation of protein-coding genes, hypoxia has been found to regulate the expression of non-coding genes. As an important class of non-coding genes, microRNAs or miRNAs are single-stranded oligoribonucleic acids consisting of approximately 22 ribonucleotides that primarily function as inhibitors of mRNA translation (102). Typically, each miRNA can have hundreds of mRNA targets. Conversely, each mRNA often contains target sites for multiple miRNAs. The expression, processing and target recognition of miRNA have been extensively reviewed in the literature. Herein, we will focus on hypoxia-regulated miRNAs and their roles in modulating radiosensitivity.

Studies during the past decade have shown that hypoxia can have a profound effect on the entire miRNA genome with both induction and suppression of miRNA expression, likely via complex mechanisms (103–105). However, with the exception of miR-210, there is little consensus among hypoxia-dependent miRNA profiles in different cell lines. These discrepancies may result from cell type-dependent genomic landscapes, different hypoxia conditions and various technology platforms of miRNA expression analysis.

Interestingly, the archetype of hypoxia-induced miRNA appears to be miR-210 (106–109). It is robustly induced by hypoxia in a wide range of cells (103). The hypoxic induction of miR-210 is transcriptionally regulated by HIF-1 (106–109) and/or HIF-2 (110). The promoter/enhancer region of the *miR-210* gene contains a functional hypoxia-responsive element (HRE) that is conserved between mice and humans and is directly bound by HIF-1 (108, 111).

It has been shown that miR-210 has the potential to play an important role in the regulation of radiosensitivity by increasing radioresistance. Ectopic overexpression of miR-210 can significantly increase the clonal survival of the A549 human lung cancer cells X irradiated (0–10 Gy) in ambient air to the level of control-treated A549 cells irradiated at 1% O_2 (112). The clonal survival of miR-210-expressing cells is further enhanced under hypoxia after exposure to ionizing radiation (112). On the other hand, downregulation of miR-210 expression in human hepatoma cell lines using a lentivirus-based miR-210 antisense gene results in decreased cell viability under hypoxia (1% O_2) and elevated radiosensitivity *in vitro* (113). Because miR-210 has a multitude of targets that are involved in a wide range of cell functions including DNA repair and genomic maintenance, it remains to be determined how miR-210 regulates cellular response to ionizing radiation.

The expression of miR-21 is also significantly increased by hypoxia at 1% O_2 in colon cancer cell lines (114) and in pancreatic cancer cell lines (115). The hypoxic induction of miR-21 is also mediated transcriptionally by HIF-1 (115). Antisense-mediated downregulation of miR-21 increases apoptosis and reduces proliferation of pancreatic cancer cells (115). Others have shown that miR-21 promotes radioresistance of breast cancer cells perhaps by activating the G2/M checkpoint (116). The radioresistance conferred by miR-21 may also be attributed to its involvement in the negative regulation of CDC25A phosphatase (114).

Babar *et al*. found that human lung cancer cell lines significantly increase miR-155 expression when exposed to severe hypoxia (117). Ectopic expression of miR-155 enhances clonogenic survival after exposure to ionizing radiation (117). It is possible that miR-155 promotes cell survival by targeting the pro-apoptosis gene FOXO3 (118) whose expression is downregulated under hypoxia (117). Another potential mechanism involves WEE1 kinase, an important G_2/M-checkpoint regulator whose expression is often downregulated in malignant tumors (119). Studies have shown that miR-155 suppresses the expression of WEE1 protein (120, 121). However, the exact mechanism by which miR-155 interacts with *WEE1* mRNA remains to be delineated.

Collectively, these observations suggest that the miRNA transcriptome is differentially regulated in different cell types under hypoxic conditions. Nonetheless, it can be reasonably extrapolated that different sets of miRNAs are involved in the regulation of radiosensitivity in different tumor cells exposed to different levels of hypoxia.

Summary

Undoubtedly, hypoxic cells tend to be more radioresistant than nonhypoxic cells. Nonetheless, it is also true that genes of the major repair pathways are negatively regulated

by hypoxia. Thus, the unresolved question is why hypoxic cells exhibit elevated radioresistance despite the overall down-regulation of various DNA repair genes. In Fig. 2, we propose a model to reconcile these discrepancies. Central to this model is the idea that nonlethal pathological hypoxia facilitates the maintenance and expansion of CSCs. Compared to nonstem cells, CSCs may have higher basal levels of activation of the DNA damage checkpoint pathways, including the ATR-CHK1 and ATM-CHK2 pathways. The enhanced DNA damage checkpoint activities can counterbalance or compensate for the decreased levels of DNA repair genes, leading to improved efficiency of the overall DNA damage response. In addition, hypoxia-induced miRNAs such as miR-210, miR-21 and miR-155 desensitize the response of hypoxic cells to apoptosis. Several other mechanisms, including hypoxia-dependent defense against reactive oxygen species (122, 123) and hypoxia-induced autophagy (122, 124–126), may also contribute to hypoxia-dependent regulation of cellular radiosensitivity. Nevertheless, these mechanisms will likely work in synergy to increase the survival and expansion of CSCs or clonogenic tumor-initiating cells in a hypoxic microenvironment after ionizing radiation.

Acknowledgments

The authors thank members of the Yun Laboratory for insightful discussions. CL is supported by a scholarship from Xiangya Hospital, Central South University, China. ZY is supported by grants from the NIH-NCI (CA178254 and CA148996).

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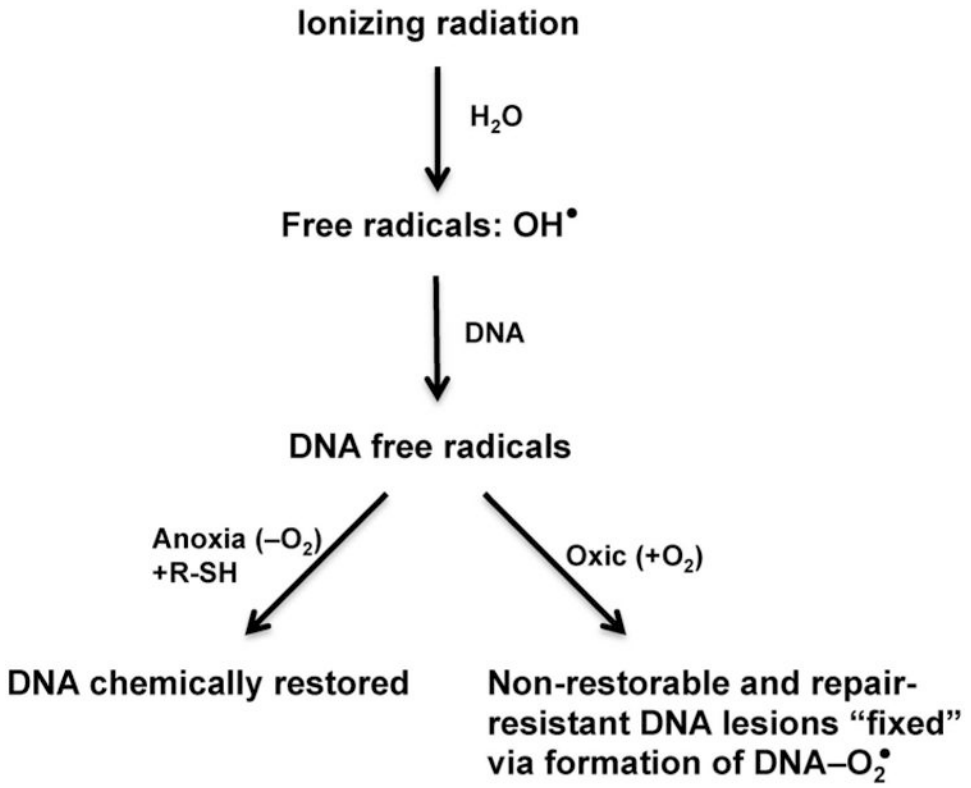

Fig. 1.

Schematic representation of the oxygen fixation hypothesis (OFH). When living cells are irradiated, X rays interact primarily with H_2O to generate the hydroxyl free radicals. These highly reactive free radicals then interact with DNA macromolecules to generate initial DNA free radicals. In the absence of O_2, these highly unstable DNA radicals can be chemically restored upon reduction by sulfhydryl compounds (R-SH) and/or other reducing molecules. Under oxic conditions, O_2 reacts with DNA radicals and converts them into chemically nonrestorable oxidized derivatives, as if these DNA lesions are "fixed". These "fixed" lesions are also resistant to enzyme-mediated repair.

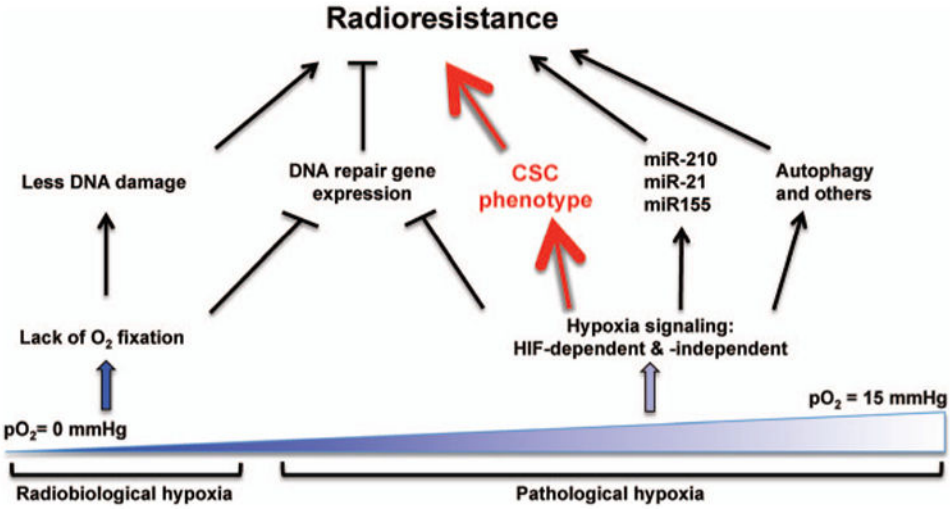

Fig. 2.

Mechanisms of radioresistance at different pO_2. Under the radiobiological or severe hypoxia (pO_2 < 3 mmHg), the oxygen fixation hypothesis (OFH) is likely the main mechanism of radioresistance even though expression of DNA repair genes is strongly reduced. In contrast, hypoxia-activated pathways, both HIF-dependent and independent, will be actively operative under the pathological hypoxia (pO_2 = 3–15 mmHg) to elicit a multitude of molecular and cellular changes. Chief among them, the cancer stem cell (CSC) phenotype has the potential to play a prominent role in conferring radioresistance.