ATM activation in hypoxia - causes and consequences

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The DNA damage response is a complex signaling cascade that is triggered by cellular stress. This response is essential for the maintenance of genomic integrity and is considered to act as a barrier to the early stages of tumorigenesis. The integral role of ataxia telangiectasia mutated (ATM) kinase in the response to DNA damaging agents is well characterized; however, ATM can also be activated by non-DNA damaging agents. In fact, much has been learnt recently about the mechanism of ATM activation in response to physiologic stresses such as hypoxia that do not induce DNA damage. Regions of low oxygen concentrations that occur in solid tumors are associated with a poor prognostic outcome irrespective of treatment modality. Severe levels of hypoxia induce replication stress and trigger the activation of DNA damage response pathways including ataxia telangiectasia and Rad3-related (ATR)- and ATM-mediated signaling. In this review, we discuss hypoxia-driven ATM signaling and the possible contribution of ATM activation in this context to tumorigenesis.

Introduction

The integrity of the genetic material is constantly being threatened by exogenous and endogenous environmental factors.[1] It is imperative that challenges to DNA integrity are sensed and recognized efficiently in order to maintain genomic stability.[2] To prevent the deleterious, and sometimes lethal, consequences of unrepaired DNA damage, a complex signaling cascade known as the DNA damage response (DDR) has evolved. The DDR is characterized by a series of phosphorylation events that ultimately lead to cell cycle arrest, DNA repair, apoptosis or senescence.[3] Apical PI3K (phosphatidylinositol-3-kinase)-like kinases of this signaling pathway include ataxia telangiectasia mutated (ATM), ataxia telangiectasia and Rad3-related (ATR), and DNA-dependent protein kinase (DNA-PK). ATM is generally considered to be the kinase that orchestrates the response to DNA double-strand breaks (DSBs), whereas ATR recognizes single-strand breaks or areas of single-stranded DNA (ssDNA).[4] It is important to note that although considerable crosstalk exists among these kinases they are not functionally redundant.[5]

Activation of ATM by non-DNA damaging agents, such as hypotonic salt, chloroquine, heat, and oxidative stress, has also been described.[6-8] Hypoxia may now be added to this group of non-DNA damaging stresses that induce ATM activity.[9] Hypoxia describes a situation of insufficient oxygen supply. Hypoxic regions occur in solid tumors primarily because the inefficient tumor vasculature is unable to deliver sufficient oxygen to the rapidly proliferating cancer cells. Hypoxia in tumors occurs as a gradient of oxygen concentrations with levels ranging from 6–0% O_2.[10] In this review the term 'severe hypoxia' refers to < 0.1% O_2, whereas 'mild hypoxia' corresponds to 0.5–2% O_2. It is important to note that both mild and severe hypoxia can occur within the same tumor and that both elicit a hypoxia-inducible factor (HIF) response.[11] Severe hypoxia (also known as radiobiologic hypoxia) would be expected in regions adjacent to necrotic areas or as a result of vessel occlusion.[12] Clinically, tumor hypoxia is associated with a poor prognostic outcome and treatment resistance.[13,14] Importantly, hypoxic regions can become reoxygenated, for example following the reopening of occluded blood vessels.[15] Reoxygenation results in the formation of reactive oxygen species (ROS) and ROS-dependent DNA damage with subsequent classic ATM activation.[16] We recently described the mechanism of ATM activation by hypoxia (without reoxygenation) and identified novel roles for ATM in these conditions.[17] In this report these roles will be summarized and their significance put into context with respect to tumor development and progression.

DNA damage-induced ATM activation

Before discussing hypoxia-induced ATM activation it is important to describe the ATM response to DSBs, and in particular its dependence on specific chromatin modifications. Chromatin is composed of nucleosomes consisting of 146 base pairs of DNA wrapped twice around an octamer of histones, namely two H2A-H2B dimers and an H3-H4 tetramer. Post-translational modifications of histones can lead to either a more relaxed or a more compact chromatin state. Histone modifications such as histone 3 lysine 4 trimethylation (H3K4me3), H3K36me3, H3K79me3, or H3 acetylation are common in more open regions of the genome, typically referred to as euchromatin. In contrast, H3K9me3, H3K27me3, and H4K20me3 histone modifications are associated with chromatin compaction and are frequently found in genomic regions known as heterochromatin.[18] Histone modifications not only play a role in modifying chromatin states but can also have signaling functions, including appropriate coordination of DNA damage signaling and repair.[19] Recently, it has become clear that the chromatin context plays a significant role in ATM activation. Specifically, areas of H3K9me3 surrounding a damaged site are important for the initial activation of the acetyltransferase TIP60. Acetylation of ATM by TIP60 is required for stimulation of ATM kinase activity.[20] Furthermore, c-ABL–dependent TIP60 phosphorylation on tyrosine 44 has recently been shown to enhance binding of TIP60 to H3K9me3 following DNA damage.[20-22] Another important event in ATM activation is the formation of active monomers from inactive ATM dimers, a process aided by autophosphorylation at several serine residues including Serine 367, 1983, 1981, and 2996. ATM phosphorylation is important for its retention at the break but is not essential for initial tethering of ATM to the DSB site.[6,23,24]

A chromatin modification that is notably responsive to DNA damage and particularly associated with coordination of DDR signaling is phosphorylation of histone variant H2AX on serine 139 (γH2AX). ATM, DNA-PK, and ATR can all phosphorylate H2AX in response to DNA damage.[25] This modification is important for the recruitment of mediator of DNA damage checkpoint 1 (MDC1).[26] The interaction between γH2AX and MDC1 initiates a feedback loop that favors recruitment of the MRN complex comprising mitotic recombination 11 (MRE11), RAD50, and Nijmegen breakage syndrome 1 (NBS1) and subsequent ATM activation. The evolution of this complex feedback loop, which allows maximal ATM activation in response to DSBs, probably reflects the importance of full ATM signaling for maintenance of genome integrity.[27-30] It is important to note, however, that despite the central role described for ATM in the response to DNA damage, it is estimated that only 10–15% of DSBs depend on ATM activity for repair.[31] This observation stimulated detailed analysis that demonstrated that ATM is critical for the repair of DSBs generated in heterochromatin areas, which represent 10–15% of the genome.[31] ATM phosphorylates Krüppel-associated box (KRAB) domain-associated protein 1 (KAP-1) in these compact regions of chromatin, leading to relaxation of the chromatin and thereby facilitating repair of DSBs.[32-35]

In addition to its roles in orchestrating the repair of DSB, ATM is important for the appropriate checkpoint responses following DNA damage and defects in G_1, S, and G_2 checkpoints arise in the absence of ATM. Cells from patients with ataxia telangiectasia (AT) caused by mutations in the *ATM* gene or from genetically engineered $ATM^{-/-}$ mice, exhibit radioresistant DNA synthesis (RDS) after exposure to ionizing radiation because they cannot delay S-phase entry.[36,37] ATM-dependent phosphorylation of a number of downstream targets, including, p53, CHK1, and CHK2, is required for checkpoint activation.[36,38] In the case of the S-phase checkpoint, ATM has been shown to inhibit DNA replication in the presence of DSBs predominantly by phosphorylating CHK1 and CHK2, which in turn phosphorylate CDC25A. Degradation of CDC25A after CHK1/CHK2-dependent phosphorylation maintains CDK2 in its inactive phosphorylated state thereby precluding the loading of CDC45 on DNA replication origins.[39,40]

ATM activation in the absence of DNA breaks

As mentioned above, although the best-characterized role for ATM is in the response to DNA damage several non-DNA damaging agents have also been reported to activate ATM. Heat, hypotonic stress, and agents that modify chromatin (e.g., trichostatin A) have all been associated with an increase in ATM activity independent of DNA damage.[6,8,41] In such cases, ATM activation appears to require alternative signaling pathways to those involved in activation in response to DSBs. For example, the nuclear zinc-finger protein ATMIN is necessary for ATM activation after treatment with chloroquine or high salt.[42-45] In response to DNA damaging agents ATM is retained at the sites of DNA damage through its interaction with the MRN complex and this interaction facilitates ATM activation.[46,47] Recently, oxidative stress induced by treatment with hydrogen peroxide has been demonstrated to activate ATM independent of the MRN complex and in the absence of DNA damage. Interestingly, some of the classic ATM targets, such as KAP-1, are not phosphorylated following H_2O_2 treatment. One of the main functions of the MRN complex in ATM activation after DNA damage is sensing the DNA break, so it is perhaps not surprising that this complex appears to be dispensable for several mechanisms of ATM activation in the absence of DNA damage.[41]

Hypoxia is one of the most physiologically relevant stresses known to activate ATM in the absence of DNA damage.[9,48] The activation of DDR pathways by hypoxia involves both ATM- and ATR-mediated signaling. Hypoxia-induced increases in ATR signaling are not unexpected as severe hypoxia induces replication stress.[49] Replication stress is defined as a decrease in the rate of replication fork progression, leading to fork stalling and decreased DNA synthesis. Under these conditions, the MCM helicase continues unwinding DNA despite the decrease in replication fork progression, leading to the accumulation of ssDNA. These ssDNA regions are then coated by the ssDNA binding protein replication protein A (RPA) in order to prevent

processes such as DNA reannealing.[4,50] Typically, replication stress can arise following treatment with ribonucleotide reductase (RNR) inhibitors such as hydroxyurea (HU), or DNA polymerase inhibitors such as aphidicolin. Aberrant oncogene activation has also been shown to induce replication stress as a result of increased origin firing and nucleotide pool depletion.[51] DNA replication rates are significantly slower in cells under severe hypoxia compared with those of cells replicating under normoxic conditions (20% O_2) or even under mildly hypoxic conditions (2% O_2). Nucleotide imbalances have also been demonstrated to occur under severely hypoxic conditions and have been correlated with the slow replication rates. Hypoxia-induced nucleotide imbalance has been proposed to result from the requirement for oxygen for RNR activity.[52,53] Coinciding with the slowing of DNA replication and the formation of areas of ssDNA, severe hypoxia leads to the induction of RPA foci as well as ATR activation. A strong pan-nuclear γH2AX signal in S phase cells is also characteristic of severely hypoxic conditions (**Fig. 1A**). This signal is distinct from the nuclear foci that can be observed following DNA damage (**Fig. 1B**) and occurs in response to conditions of replication stress.[9,54,55] Once active, ATR phosphorylates downstream targets including CHK1 in order to maintain replication fork integrity and prevent aberrant origin firing during hypoxia/reoxygenation. These findings are consistent with the reported role of ATR in response to replication stress.[52,56,57] Interestingly, ATR has been shown to phosphorylate ATM following exposure to UV radiation or HU.[58] It is therefore possible that ATR signaling contributes to ATM activation in hypoxia. Further investigation of this hypothesis is technically challenging because ATM is activated in response to pharmacologic inhibition or genetic knockdown of ATR in hypoxic conditions,[59] most likely as a result of the collapse of stalled replication forks.[54] Nonetheless, in support of this model, ATR inhibition using a specific ATR inhibitor (VE-821) induces DNA damage as detected by the presence of 53BP1 foci (a marker of DNA damage) in hypoxic cells.[52,59]

Consistent with an absence of detectable DNA damage, hypoxia-induced ATM autophosphorylation at serine 1981 is independent of the MRN complex. The mediator protein MDC1, however, does amplify hypoxia-induced ATM activity and is required for maximal phosphorylation of ATM targets such as KAP-1, as in ATM activation in response to DNA damage. However, MDC1 does not recruit DNA repair factors such as BRCA1, RNF8, or 53BP1 into nuclear foci in response to hypoxia, probably because of the lack of DNA breaks. Additional downstream targets of ATM include CHK2 and DNA-PKcs.[9] ATM is a predominantly nuclear protein although some reports indicate that it is also active in the cytoplasm.[60] Under hypoxia, phosphorylated ATM is found in the nucleus but does not appear to be tightly associated with chromatin and does not form nuclear foci. This is consistent with the finding that hypoxia-induced ATM is not activated in response to, or recruited to, sites of DNA breaks. Furthermore, mitochondrial activity appears to be indispensable for ATM activation under severely hypoxic (< 0.1% O_2) conditions.[9]
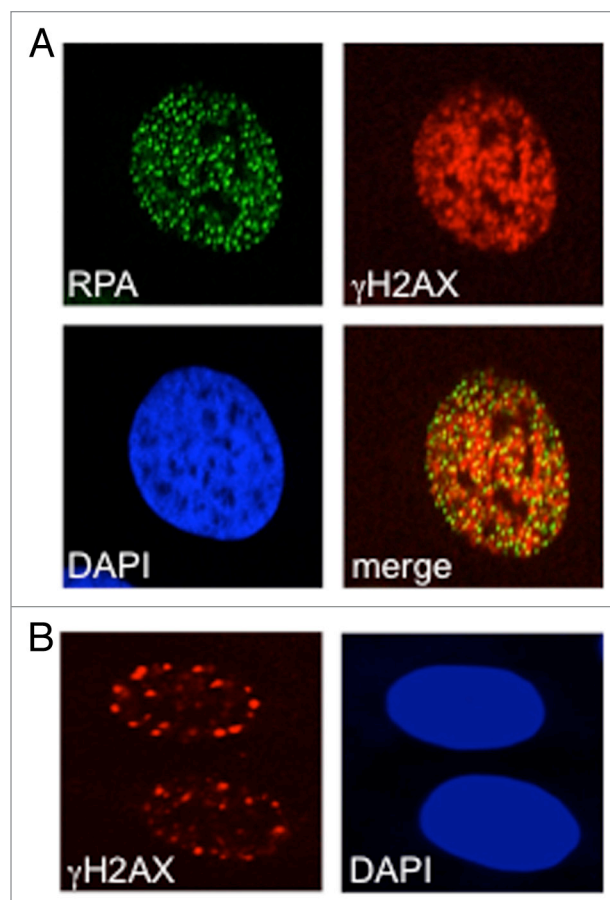

Figure 1. Hypoxia-induced replication stress. (**A**) U2OS osteosarcoma cells were exposed to hypoxia (< 0.1% O_2, 8 h), and then fixed and stained for RPA and γH2AX as indicated. Hypoxic cells were fixed in hypoxic conditions with equilibrated solutions to avoid reoxygenation. (**B**) U2OS cells were exposed to 5 ng/ml neocarzinostatin (NCS) for 3 h and then fixed and stained for γH2AX. In contrast to the hypoxia-treated cells, cells exposed to NCS exhibit distinct discrete nuclear γH2AX foci.

Hypoxia-induced chromatin changes

Although hypoxia-induced ATR signaling is to be expected, it is surprising to observe strong activation of ATM under hypoxic conditions. This raises the question of the nature of the ATM-initiating signal in hypoxia. Our data demonstrating that hypoxia-induced ATM activity only occurs at levels of hypoxia that induce replication stress and only in S phase cells strongly suggest that the initiating signal is replication stress rather than other factors associated with a range of hypoxic conditions.[16] However, although hypoxia-induced replication stress undoubtedly contributes to ATM activation and signaling it does not offer a complete mechanistic explanation. In contrast to hypoxia, the induction of replication stress by agents such as HU within a timeframe when no DNA damage is observed (6 h treatment), fails to activate ATM.[17,34,35] These conflicting observations suggest that replication stress alone is not sufficient to induce ATM activity and leads to the hypothesis that an additional signal must
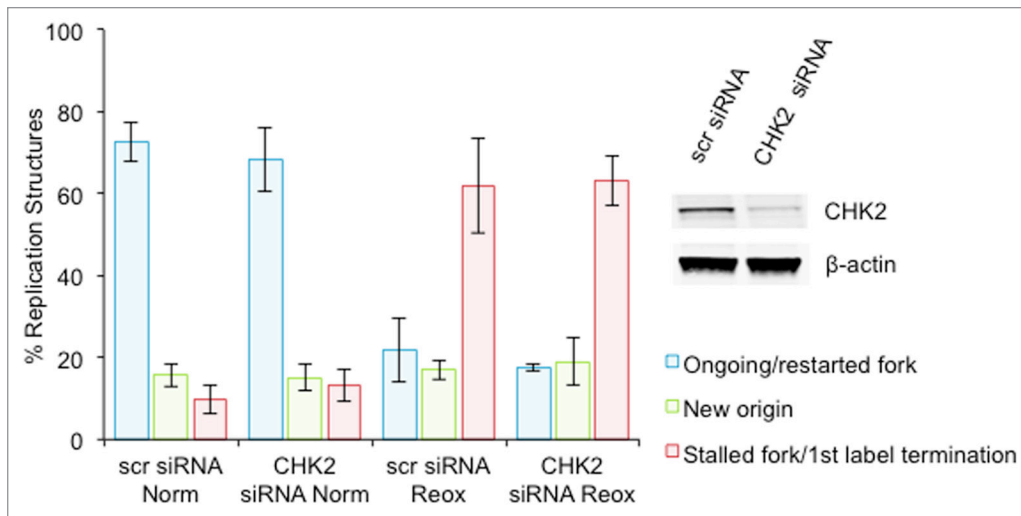
Figure 2. siRNA-mediated knockdown of *CHK2* has no effect on origin firing following reoxygenation-induced replication restart. RKO colon carcinoma cells were treated with scrambled (scr) or *CHK2*-specific siRNA and exposed to hypoxia (< 0.1% O_2, 5 h) followed by reoxygenation (Reox; 20% O_2, 1 h) Norm = 20% O_2. Scrambled siRNA: stealth RNAi negative control (Invitrogen); *CHK2* siRNA: 3′-GAAAUUGCAC UGUCACUAA-5′ (Thermo Scientific). DNA fibers were spread, stained, and scored. Graphs represent the average percentage of total replication structures counted. Western blotting was performed to validate the level of CHK2 knockdown (anti-CHK2 antibody: sc-17747, Santa Cruz Biotechnology). Antibody against β-actin (sc-69879, Santa Cruz Biotechnology) was used as the loading control.

be present in hypoxia that results in ATM signaling. It should be noted that prolonged exposure to HU (e.g., 24 h) does result in ATM activation and this correlates with the induction of DNA damage. This probably reflects the fact that in certain situations disruption of DNA replication can eventually lead to DNA breaks after replication fork stalling and collapse. In contrast, even prolonged exposure to hypoxia does not lead to the generation of detectable DNA damage, despite the presence of persistent replication stress. To date we have only observed accumulation of DNA breaks in hypoxia when DDR components such as ATR/CHK1 or ATM are depleted or inhibited.[17,56] This suggests that although severe hypoxia induces significant replication stress it does not result in complete abrogation of DNA replication and highlights the role that DDR components may play in maintaining replication fork integrity in this context.[17]

The importance of appropriate ATM signaling for the repair of DSBs present in areas of heterochromatin demonstrates the impact that the chromatin environment can have on the requirement for certain DDR components.[31] In addition to activating DDR signaling, hypoxia results in a number of characteristic chromatin changes.[61] Notably, H3K9me3, a chromatin mark associated with heterochromatin and damage-induced ATM signaling, is induced in an oxygen-dependent manner.[20,62] The oxygen-dependent increase in this histone modification probably reflects a balance of increased activity of some of the principal H3K9 methyltransferases and decreased activity of Jumonji C domain-containing histone demethylases in hypoxia. The Jumonji C family of demethylases uses oxygen as a cofactor to catalyze the oxidation of methyl groups.[63,64] Together, these findings prompted us to investigate whether the induction of H3K9me3

by hypoxia combined with replication stress leads to ATM activity.

Hypoxia-induced ATM activation is abrogated when H3K9me3 levels are reduced through loss of the principal methyltransferases Suv39h1/2. Therefore, both replication stress induced in hypoxia and the chromatin context appear to be important for ATM activation under these conditions. A potential mechanism by which Suv39h1/2 could facilitate ATM phosphorylation in hypoxia is through repression of the phosphatase PP2A, which dephosphorylates ATM.[17]

The role of hypoxia-induced ATM

Using the recently described IPOND (Isolation of Proteins On Nascent DNA) technology we have demonstrated that although hypoxia-induced H3K9me3 occurs throughout the nucleus, it is particularly accumulated at replication forks.[65] We hypothesized that this increase in H3K9me3 around the replication forks in hypoxia could further hamper replication and lead to a requirement for ATM-mediated signaling. Indeed, analysis of DNA replication rates under hypoxic conditions following ATM depletion/inhibition indicates that replication is dramatically reduced in the absence of ATM, whereas no effect on DNA replication can be observed under normoxic conditions where baseline replication stress is observed.[17] This suggests a novel stress-specific role for ATM in maintaining replication under hypoxic conditions where increases in heterochromatin and replication stress coexist. These observations suggest that ATM is probably not directly regulating the classic S-phase checkpoint under conditions of hypoxia. ATM is typically involved in activating cell cycle checkpoints to prevent S-phase progression in the presence of DNA damage, whereas in hypoxic conditions it appears to be the lack of ATM that hampers DNA replication completion. These observations further underscore the differences between ATM activity induced by hypoxia and DSBs.[36] Interestingly, ATR and ATM have previously been reported to regulate origin firing during unperturbed replication, with transient ATM activity being detected during ongoing replication in the absence of replication stress in *Xenopus laevis* egg extracts. The authors of this study proposed that regulation of CDK2 or CDC7 by ATR and ATM could regulate origin firing during replication.[66] Differences between egg extracts and human cancer cells provide a possible explanation

for the difference in requirement for baseline ATM activity during replication in the absence of stress. Notably, elegant studies by Gautier and colleagues previously showed that DSBs might be generated during normal DNA replication in *Xenopus laevis* egg extracts. Furthermore, MRE11 phosphorylation following DSB generation during DNA replication has been proposed to be important for repair of the DSBs generated during normal DNA replication in this system.[67] The generation of DSBs in *Xenopus* egg extracts could explain the requirement for ATM activation during normal replication under these conditions.

Interestingly, it has been demonstrated that ATM is also active in milder hypoxic conditions (0.2–1% O_2), where it appears to play a role in increasing HIF-1α stability through phosphorylation at serine 696.[68] ATM-dependent phosphorylation of HIF-1α leads to a reduction in mammalian target of rapamycin complex 1 (mTORC1) signaling in hypoxia through the upregulation of a negative regulator of mTORC1 called regulated in development and DNA damage responses 1 (REDD1).[68] Given the high degree of crosstalk between ATM and other PI3K-related kinase (PIKK) family members it is perhaps not surprising that ATR and DNA-PK have also been shown to regulate HIF-1α levels.[59,69,70] The transient decrease in HIF-1α stability and activity following ATR inhibition is likely due to concomitant induction of ATM, which can also stabilize HIF-1α as described above.[59] The therapeutic use of ATR inhibitors in tumors where loss of ATM has been reported could represent an interesting application of these compounds.

ATM and reoxygenation

After reoxygenation from severely hypoxic conditions (< 0.1% O_2 for < 8 h) the stalled DNA replication forks can restart.[71] Interestingly, reoxygenation also results in a rapid decrease in the levels of H3K9me3, probably reflecting the fact that histone demethylases regain activity in the presence of oxygen. DNA fiber analysis after reoxygenation-induced replication restart showed that loss of ATM activity had no effect on the replication restart, consistent with the fact that replication stress and the heterochromatin context are both relieved under these conditions.[17] Remarkably, however, loss of ATM activity during reoxygenation did increase the percentage of new origin firing, suggesting a role for ATM in maintaining appropriate origin firing control at this time.[17] In response to reoxygenation, ATM maintains the hypoxia-induced phosphorylation of downstream targets, including p53 and CHK2. Reoxygenation-induced G_2 arrest has been attributed to phosphorylation of CHK2 by ATM. Indeed, CHK2 loss results in abrogation of G_2 arrest and increased apoptosis.[72,73] ATM signaling to downstream checkpoint kinases could potentially maintain appropriate regulation of origin firing during reoxygenation. However, analysis of replication structures following siRNA-mediated knockdown of *CHK2* suggests that loss of CHK2 has no effect on the number of new origins being fired (**Fig. 2**).

It is important to note that although hypoxia induces activation of DDR signaling it has also been reported to lead to the

	Replication stress	DNA damage	Increased H3K9me3	ATM activity
Hyp 2% O_2	✗	✗	✓	✗
Hyp <0.1% O_2	✓	✗	✓	✓
Hu	✓	✗	✗	✗
Hu + Hyp 2% O_2	✓	✗	✓	✓

Figure 3. ATM activation in response to replication stress. Various treatments that induce replication stress within a particular chromatin environment induced by hypoxia result in ATM activation even in the absence of DNA damage. Treatment conditions: 1 mM HU (6 h in 20% O_2); hypoxia 2% O_2 (6 h); hypoxia < 0.1% O_2 (6 h), 1 mM HU + 2% O_2 (6 h). In contrast, ATR activity is expected in all situations that induce replication stress. ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related.

repression of a variety of DNA repair pathways (as demonstrated both in vitro and in vivo).[74,75] Repression of DNA repair pathway activity has been linked to the increased genomic instability that is characteristic of hypoxic tumors. Importantly, as mentioned earlier, reoxygenation following periods of hypoxia results in the induction of ROS-induced DNA damage that could further contribute to genomic instability if it occurs in conditions of compromised repair.[75] Furthermore, unscheduled origin firing in response to reoxygenation upon loss of ATM activity may further contribute to increased DNA damage and genomic instability.[17]

Hypoxia-induced ATM activity and tumorigenesis

Our work describing the activation of ATM in response to replication stress in the context of increased heterochromatin associated factors led to the hypothesis that other inducers of replication stress would also result in ATM activation in the absence of DNA breaks provided a "heterochromatin-like" context was present. To test this hypothesis we used milder hypoxic conditions (2% O_2), which result in induction of H3K9me3 but do not cause replication stress. Using this approach we were able to demonstrate that replication stress in mild hypoxia also leads to ATM activation. Specifically, we confirmed this using both HU and careful titration of oncogene overexpression. K-Ras overexpression under normoxic conditions resulted in minimal ATM activation; however, oncogene overexpression under mild hypoxic conditions (2% O_2) resulted in strong ATM activation.[17] These data support the idea that replication stress within a particular chromatin environment induced by hypoxia results in ATM activation even in the absence of DNA damage (summarized in **Figure 3**). These data led us to speculate that during the early stages of tumor development the hypoxia-induced chromatin context may play a role in facilitating the activation of DDR signaling in response to replication stress before DNA
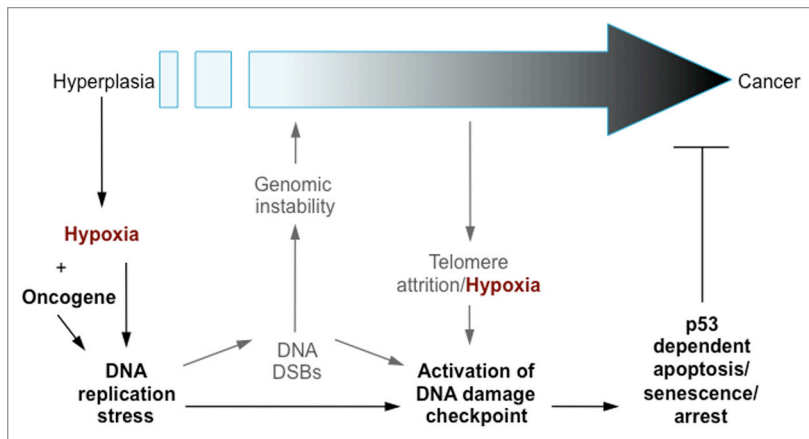
Figure 4. Hypoxia contributes to the barrier to tumorigenesis. DNA replication stress present in preneoplastic lesions as a result of uncontrolled cell proliferation (for example, due to oncogene overexpression) can lead to the generation of DSBs. The presence of unrepaired DSBs can result in genomic instability that further drives tumorigenesis. Activation of the DDR therefore acts as a barrier to tumorigenesis. Hypoxia may contribute to this barrier to tumorigenesis earlier than previously predicted by providing a chromatin context that, together with DNA replication stress, can trigger ATM activation even in the absence of DNA damage. Activation of ATM by this mechanism may also occur in areas of severe hypoxia because levels of oxygen < 0.1% induce replication stress together with a chromatin context associated with heterochromatin. Figure adapted from references 76 and 77 with the permission of *Nature* journal

suggest that certain chromatin changes may favor tumor development, perhaps in the later stages of tumor development and progression. It is tempting to speculate that hypoxia-induced chromatin changes facilitate the barrier to tumorigenesis at the very early stages of neoplasia by stimulating DDR activation, whereas in later stages they become part of the response adopted by the tumor to survive. At these later stages in tumorigenesis, an increase in H3K9me3 might become one of the weapons that are hijacked by the tumor in order to survive and thrive under hypoxic conditions.

Conclusions

Our understanding of the role of ATM in hypoxia/reoxygenation has increased tremendously in recent years; however, it is clear that many questions remain unanswered. Nonetheless, the critical importance of ATM activity under these conditions is exemplified by the fact that ATM knockdown or inhibition causes sensitization to hypoxia/reoxygenation and that ATM appears to have a novel stress-specific role in maintaining DNA replication under these conditions.[17] The search for downstream ATM targets that may be involved in mediating these functions will be an exciting future field of study. This research will not only advance our understanding of how tumors adapt and survive under hypoxic conditions, but might also shed light on potentially useful future therapeutic targets. However, any therapeutic approach aimed at either directly targeting ATM activation or indirectly modulating the chromatin changes that are required for ATM signaling should take into account the fact that ATM activation in the early stages of tumorigenesis may have a protective role. Inhibition of ATM function in such cases may accelerate the development of secondary or incipient malignancies.

damage accumulates. Therefore, cells undergoing hyperproliferation as a result of oncogenic activation in a hypoxic environment would activate ATM. This implies that the barrier to tumorigenesis induced by the DDR in hypoxic pre-neoplastic or early neoplastic lesions could occur earlier than predicted and, most importantly, before the accumulation of damage in response to oncogene activation.[76,77] This hypothesis, however, remains to be formally proven (**Fig.** 4).

Interestingly, chromatin changes characteristic of heterochromatin are frequently found in tumors.[78] H3K9me3 induction upon oncogene overexpression has been proposed to maintain viability of oncogene-expressing cells via protection of the genome through chromatin compaction.[19] It is possible that some of the chromatin changes observed in response to hypoxia originally evolved in an attempt to protect genome integrity. In this case, the protection of hypoxic cells by inducing a heterochromatin-like environment may promote, rather than halt, tumorigenesis. Interestingly, chromatin-modifying enzymes associated with heterochromatin have been shown to be overexpressed in a variety of tumor types.[79,80] Taken together, these observations

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