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Crosstalk between the DNA damage response, histone modifications and neovascularisation

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Abstract

Neovascularisation is critical in several malignant and inflammatory conditions, as well as in the course of eye disorders. During new vessel formation, endothelial cell functions, such as proliferation and sprouting are very important and are regulated by a variety of growth factors. The DNA damage response machinery as well as factors regulating histone modifications, such as histone deacetylases, regulate cell fate as well as gene expression. Recent evidence has pointed to potential interactions among BRCA1, H2AX and SIRT1 in these intracellular pathways and neovascularisation, which will be reviewed here.

Keywords

γ-H2AX; BRCA1; Sirtuins; Angiogenesis

1. Angiogenesis

While the generation of blood vessels is integral to embryonic organ formation, vessel growth after birth takes place under both physiologic and pathophysiologic conditions. In particular, neovascularisation is important in the wound healing response or under pathologic conditions, such as in malignant diseases, in inflammatory disorders, as well as in proliferative retinopathies, such as diabetic retinopathy or retinopathy of prematurity (ROP)(Arjamaa and Nikinmaa, 2006; Carmeliet, 2005; Fraisl, 2009; Simmons, 2005). Angiogenesis, which is the formation of new capillaries from other vessels e.g. venules involves endothelial cell proliferation, migration and sprouting, as well as endothelial cell interactions with pericytes and mural cells(Carmeliet, 2005; Fraisl, 2009; Simmons, 2005).

In tumor angiogenesis or in vasoproliferative retinopathies, neovascularisation is regulated by the response to tissue ischemia and hypoxia, which is coordinated by the transcription factor hypoxia-inducible factor (HIF). HIFs form heterodimers; three HIFα isoforms exist that form a complex with the HIFβ subunit(Fraisl et al., 2009). HIF function is regulated by the prolyl hydroxylase domain (PHD) proteins and the von Hippel Lindau E3 ubiquitin ligase complex (Kaelin and Ratcliffe, 2008). Under hypoxic conditions, the degradation of HIF, which is

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facilitated by PHDs, is decreased, resulting in HIF protein stabilization. This activation of HIF in hypoxic tissues increases the expression of multiple growth factors involved in neovascularisation(Maxwell and Ratcliffe, 2002; Semenza, 2003). The vascular endothelial growth factor (VEGF) family(Ferrara et al., 2003) is an important target of HIF1α in the context of neovascularisation. VEGF-A is a prominent member of this family. VEGF-A and its receptors Flt-1 and FLK-1 as well as the co-receptors of the neuropilin family are crucial for developmental and postnatal angiogenesis(Tirziu and Simons, 2009). Further factors important in angiogenesis are angiopoietins-1 and -2 and their receptor Tie-2 that predominantly control vessel maturation by regulating interactions between the endothelium and pericytes(Thomas and Augustin, 2009; Tirziu and Simons, 2009). The reader is referred to detailed reviews on angiogenesis(Carmeliet, 2005; Tirziu and Simons, 2009; Simmons, 2005)

2. A role for histone H2AX in postnatal angiogenesis

Induction of DNA damage in cells results in activation of a complex cellular signalling response that regulates cell cycle progression, DNA repair and apoptosis. The major kinases that orchestrate the DNA damage response belong to the phosphatidylinositol-3 kinase-like family, and include the ataxia teleangiectasia mutated (ATM), the ATM-and Rad3-related (ATR) and the DNA dependent protein kinase (DNA-PK)(Fernandez-Capetillo et al., 2004; Hurley and Bunz, 2007; Zhang et al., 2007; McGowan and Russell, 2004; Bartek and Lucas, 2007; Kastan and Bartek, 2004). ATM plays a predominant role in the response to DNA double strand breaks (DSB), e.g. induced by ionizing radiation, whereas ATR is activated upon replication-associated stress, e.g. at stalled replication forks(Kastan and Bartek, 2004). The Mre11-Rad50-NBS1 (MRN) complex functions to sense DSBs and plays a crucial role in the recruitment and activation of ATM(Bartek and Lukas, 2007; Falck et al., 2005). On the other hand the recruitment of ATR during replication stress is mediated by the ATR interacting protein (**ATRIP**)(Kastan and Bartek, 2004) and replication protein A (RPA) that localizes to single-stranded DNA breaks(Zou and Elledge, 2003). Histone H2AX is an adaptor molecule of the DNA repair machinery. H2AX is phosphorylated at its C-terminus (designated γ-H2AX) thereby being a marker of DNA lesions(Fernandez-Capetillo et al., 2004). At the same time H2AX can mediate DNA repair by promoting the maintenance of repair factors close to the DNA lesion(Bassing and Alt, 2004; Bonner et al., 2008; Celeste et al., 2003).

Interestingly, hypoxia, the major trigger of postnatal pathologic angiogenesis, is a replication stress and can activate a DNA damage response (DDR) in cells that may involve the activation of either ATR or ATM(Bencokova et al., 2004; Hammond et al., 2003, 2004). Phosphorylation of H2AX has been found as a component of a DDR to different levels of hypoxia especially in proliferating cells(Economopoulou et al., 2009; Hammond et al., 2004). It is not clear whether hypoxia really induces DNA lesions in cells; in fact it is more likely that the low degree of DNA damage that takes place during replication is simply enhanced and thereby accrues in the presence of hypoxic conditions(Hammond et al., 2007). On the other hand, hypoxia may negatively regulate the expression of DNA repair factors, e.g. the Nijmegen breakage syndrome protein(NBS1), in a HIF-dependent manner, thereby indeed resulting in DNA damage (To et al., 2006).

The hypoxia-induced phosphorylation of H2AX in proliferating endothelial cells was important for the cells to keep up their proliferation under hypoxia and thereby for postnatal hypoxia-driven pathological neovascularisation(Economopoulou et al., 2009). In particular, hypoxia-induced γ-H2AX generation was observed predominantly in proliferating vascular endothelial cells *in vitro* as well as *in vivo* in the course of hypoxia-induced retina angiogenesis in the model of retinopathy of prematurity (ROP)(Economopoulou et al., 2009). In H2AX deficient mice as well as in endothelial-specific H2AX deficient mice pathologic retina neovascularisation in the ROP model was impaired, which was accompanied by reduced

endothelial cell proliferation and increased endothelial cell apoptosis. In addition, H2AX deficiency resulted in reduced new vessel formation in tumor angiogenesis as well as in the model of hind limb ischemia induced by ligation of the femoral artery(Economopoulou et al., 2009). It is not clear whether there is a crosstalk between the function of H2AX and the HIF-dependent response to hypoxia, since HIF-1α and HIF-2α may regulate cell survival and proliferation, which may be associated with modulation of H2AX phosphorylation(Gordan et al., 2007, 2008; Huang, 2008). Although the exact mechanism underlying this function of H2AX requires further investigation, H2AX and possibly an efficient DDR may support endothelial cells to maintain their proliferation in the course of pathologic hypoxia-driven angiogenesis. In fact, other factors involved in DNA repair, such as the breast cancer associated gene 1 (BRCA1) and sirtuin 1 also named SIRT1(Bonner et al., 2008; Deng, 2006; Finkel et al., 2009), which are reviewed below, have also been implicated in angiogenesis.

3. BRCA1 in the DDR and neovascularisation

BRCA1, a well known tumor suppressor whose mutations predispose women to familial breast and ovary cancers(Miki et al., 1994) plays an important role in the DDR. BRCA1 is recruited to ionizing radiation (IR)-induced foci (IRIF) and binds to DSBs. The formation of BRCA1 IRIF at DSBs is facilitated by the interaction between BRCA1 and many proteins involved in DNA damage sensing and repairing, although the specific relationship between BRCA1 and each of these proteins remains elusive(Wang et al., 2000; Yan and Jetten, 2008). For instance, targeted disruption of H2AX impaired the BRCA1 IRIF formation(Celeste et al., 2002). On the other hand, BRCA1 interacts with γ-H2AX during spermatogenesis and BRCA1 deficiency resulted in aberrant localization of γ-H2AX on sex chromosomes, leading to the failure of meiotic sex chromosome inactivation(Turner et al., 2004). Numerous studies have revealed that BRCA1 plays essential roles in the major types of DNA damage repair and in maintaining normal cell cycle progression through its role in centrosome duplication, the G2/M and the spindle checkpoint(Deng 2006; Deng and Wang, 2003). Consistently, loss of BRCA1 in mice results in accumulation of DNA damage and genetic instability, which activates DDR signaling, leading to embryonic lethality that is accompanied by profound proliferation defects and widespread apoptosis(Deng, 2002). The activation of the DDR triggered by BRCA1 deficiency is primarily mediated by p53, ATM, the kinase CHK2 and p53-binding protein 1 (53BP1) signaling. In particular, deletion of p53 or its downstream mediator p21 partially repressed lethality of BRCA1-null embryos(Hakem et al., 1997; Shen et al., 1998). In a mutant mouse strain carrying a targeted deletion of exon 11 and thereby of the full length BRCA1, embryonic lethality could be rescued by deletion of p53, ATM, CHK2 or more recently 53BP1 (Xu et al., 2001; Cao et al., 2009).

Activation of the DDR by BRCA1 deficiency serves as a double edged sword. On one hand it prevents malignant transformation of the mutant cells. At the same time, it induces cell cycle arrest, apoptosis and stem cell depletion, which compromise organism survival leading to premature aging of BRCA1 mutant mice(Cao et al., 2003). In addition, the persistent unrepaired DNA damage causes genomic instability. The impaired DDR in turn allows BRCA1 mutant cells to overcome apoptosis, senescence and cell cycle arrest, thereby leading to tumorigenesis (Deng, 2001; Xu et al., 1999). In conclusion, BRCA1 associated tumorigenesis is secondary to DNA damage repair defects and genetic instability(Deng, 2001).

Recent evidence has also pointed to a role of BRCA1 in neovascularization. In men, BRCA1-associated breast cancers were found to have higher levels of glomeruloid microvascular proliferation, which is associated with poorer prognosis(Goffin et al., 2003). Microarray analysis revealed that breast cancers from BRCA1 mutation carriers exhibited higher expression levels of genes involved in angiogenesis, such as type IV alpha collagen and endothelial cell growth factor 1(van 't Veer et al., 2002). BRCA1 also serves as a negative

regulator for VEGF transcription and secretion in breast cancer cells through interacting with estrogen receptor alpha (ER-α)(Kawai et al., 2002). When a wild type BRCA1, but not three mutated forms of BRCA1 (A1708E, M1775R and Y1853X) that are identified in familial breast cancers, was introduced into a BRCA1 mutant breast cancer cell line, HCC-1937, transcription of VEGF and its secretion were significantly inhibited(Kawai et al., 2002). Mammary tumors derived from BRCA1 mutant mice also exhibited extensive enlargement of the vasculature (Furuta et al., 2006). In these tumors, the proangiogenic factor angiopoietin-1 was upregulated. BRCA1 worked together with two other transcription factors, CtBP-interacting protein (CtIP), and zinc finger and BRCA1-interacting protein with a KRAB domain (ZBRK1), to form a complex that binds to a ZBRK1 recognition site in the angiopoietin-1 promoter and represses its expression. The upregulated angiopoietin-1 stabilized endothelial cells in a capillary-like network structure. Consistently, mammary tumors developed in $Brca1^{\Delta 11/\Delta 11};p53^{\Delta 5-6/\Delta 5-6}$ mice displayed higher levels of angiopoietin-1 than tumors derived in $p53^{\Delta 5-6/\Delta 5-6}$ mice, and exhibited more aggressive proliferation and pronounced neovascularisation(Furuta et al., 2006). Altogether, these results suggest that, besides its role in DDR, and maintaining genomic stability, BRCA1 also regulates transcription of some angiogenic factors to modulate tumor microenvironment.

4. Sirtuins in DNA damage and angiogenesis

Histone deacetylases (HDACs) oppose the activity of histone acetyltransferases by removing the acetyl groups from lysine residues within specific promoters leading to gene silencing (Marks et al., 2001). In addition, many non-histone proteins have been identified as substrates of HDACs implying acetylation as a post-translational modification which affects various aspects of cell physiology(Yang and Seto, 2008). HDACs can be divided into class I, II and IV based on their subcellular localization and class III, which includes the sirtuin family of proteins that require the co-factor NAD for the deacetylase activity(Glozak and Seto, 2007; Imai et al., 2000). Sirtuins are categorized in mammals according to their localization to the nucleus (SIRT6, SIRT7), mitochondria (SIRT3, SIRT4 and SIRT5) or both nucleus and cytoplasm (SIRT1, SIRT2)(Michan and Sinclair, 2007). They play a role in lifespan extension (Kaeberlein et al., 1999; Kennedy et al., 1995) and have been implicated in processes such as DNA repair, cell fate, metabolic regulation, apoptosis, cell survival, tumorigenesis and last but not least aging(Deng, 2009; Finkel et al., 2009; Michan and Sinclair, 2007).

The first insights into the role of mammalian sirtuins in DDR came from the observation that SIRT6 knockout mice exhibited impaired base excision repair and accumulated chromosomal abnormalities(Mostoslavsky et al., 2006). Moreover, SIRT6 can maintain normal telomere function, since hyperacetylation of telomeres leads to end-to-end chromosomal fusion and premature cellular senescence(Michishita et al., 2008). The predicted role of SIRT1 in DNA repair, given its structural and functional homology to yeast Sir2, was first described by showing that the DNA damage sensing factor NBS1 is a substrate for SIRT1, and that the SIRT1-mediated NBS1 deacetylation is a prerequisite for the subsequent NBS1 Ser343 phosphorylation induced by ionizing radiation(Yuan et al., 2007). The role of SIRT1 in genomic stability was further established by a recent study where SIRT1−/− embryos exhibited chromosome abnormalities, aneuploidy, cell-cycle abnormalities as well as impaired DNA double strand repair(Wang et al., 2008). Furthermore, upon oxidative stress, SIRT1 is recruited to DNA breaks and H_2O_2 treatment causes a significant increase in chromosomal aberrations specifically in SIRT1-deficient cells(Oberdoerffer et al., 2008). In contrast, little is known about the involvement of other members of the sirtuin family in the DDR. SIRT3 translocates from nucleus to mitochondria upon etoposide treatment or UV-irradiation(Scher et al., 2007). In the mitochondrion, it deacetylates many proteins and plays a role in regulating energy homeostasis(Ahn et al., 2008; Jacobs et al., 2008; Lombard et al., 2008). SIRT2 is implicated

in the deacetylation of H3K56 indicating that SIRT2 may also be related to DDR(Das et al., 2009).

Although several lines of evidence exist for the role of class I, II and IV HDACs mainly in tumor angiogenesis with promising clinical applications(Ellis et al., 2009), limited information is available about the function of sirtuins in vascular biology and neovascularisation. Resveratrol, an activator of SIRT1, either in combination with the HMG-CoA reductase inhibitors (statins) or alone, increased the activation of endothelial nitric oxide synthase (eNOS) resulting in better recovery after myocardial infarction(Penumathsa et al., 2007, 2008), whereas endothelium-specific overexpression of SIRT1 was atheroprotective by improving both survival and function of endothelial cells(Zhang et al., 2008). Interestingly, eNOS was found to be a substrate for SIRT1 and deacetylation increases its activity leading to production of nitric oxide(Mattagajasingh et al., 2007) a mechanism that mediates the beneficial effects of caloric restriction or resveratrol treatment in the myocardium.

Less information is available about the role of NAD-dependent deacetylation in angiogenesis. It was shown that SIRT1 deacetylase activity is critical for the angiogenic activity of endothelial cells. Specifically, inhibition of SIRT1 function blocked endothelial migration, sprout formation, and the assembly of a primitive vascular network *in vitro* (Potente et al., 2007). More interestingly, SIRT1 mutant mice exhibited an impaired ability to form new vessels in response to an ischemic stress, while SIRT1-deficient zebrafish showed vascular patterning defects and hemorrhages due to dysregulated endothelial spouting and vessel navigation (Potente et al., 2007). The function of SIRT1 in angiogenesis is related to its property to inhibit the activity of the transcription factor Foxo1, which is an important negative regulator of blood vessel formation(Potente et al., 2007). Whether inhibition of sirtuins may be effective in blocking hypoxia-induced tumor angiogenesis according to the results described previously remains to be investigated. Interestingly, SIRT1 can inhibit the hypoxia-induced acetylation of HIF-2α thereby increasing HIF-2α activity, a finding that may also have implications for angiogenesis(Dioum et al., 2009).

5. Discussion-Outlook

Increasing evidence points to a role of factors and pathways involved in the DDR in angiogenesis and there are some potential points of convergence of these pathways (Fig. 1). For instance, BRCA1 may regulate the expression of angiogenic growth factors, such as VEGF and angiopoietin-1(Furuta et al., 2006;Kawai et al., 2002) (Table 1). In a mouse model for BRCA1 associated breast cancer, it was demonstrated that BRCA1 elicits its tumor suppressor function, at least in part, through positively regulating SIRT1 transcription(Wang et al., 2008a, 2008b). BRCA1 also interacts with γ-H2AX during spermatogenesis and is responsible for the proper localization of γ-H2AX on sex chromosomes(Turner et al., 2004). On the other hand, histone H2AX may help endothelial cells maintain their proliferation in the course of pathologic angiogenesis(Economopoulou et al., 2009). In addition, HIF-2α may also regulate H2AX phosphorylation(Gordan et al., 2008) and can be deacetylated by SIRT1, which results in enhanced HIF-2α activity and signaling(Dioum et al., 2009). At the same time SIRT1 also plays an important role in postnatal angiogenesis(Potente et al., 2007) can regulate the acetylation of NBS1 and thereby the DDR(Yuan et al., 2007). Postnatal angiogenesis has enormous importance for situations, where vascular repair is essential, such as in wound healing or in ischemic diseases, as well as is crucial determinant in pathologies, such as proliferative retinopathies and tumors. Thus, the mounting evidence about the crosstalk between the DDR, histone modifications and neovascularisation may have important implications for the above mentioned pathophysiologic conditions, although further studies are required to address the underlying mechanism and biologic relevance of this interaction.

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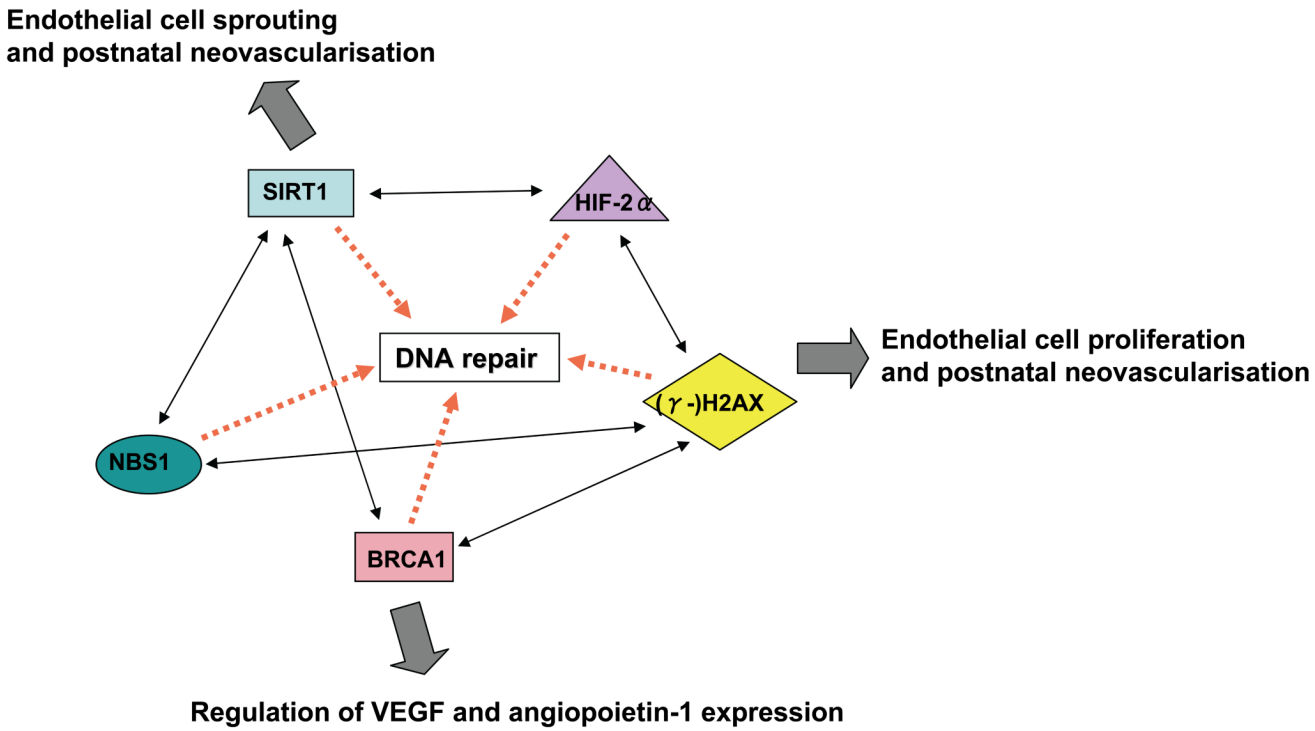

Fig. 1.

A hypothesis for potential cellular pathways related to the DDR that may participate in angiogenesis. H2AX is important in endothelial cells for supporting endothelial cell proliferation under hypoxic conditions. H2AX is thereby involved in pathological angiogenesis. H2AX interacts with BRCA1 that has been implicated in the regulation of the expression of angiogenic growth factors, as well as in the expression of SIRT1. H2AX phosphorylation may be modulated by HIF-2α, which in turn is deacetylated and regulated by SIRT1. Moreover, SIRT1 regulates the activity of NBS1, a DNA repair factor that interacts with H2AX too. SIRT1 has been shown as a regulator of endothelial cell function and postnatal angiogenesis. Further experimental studies are required to address these hypotheses.

Table 1

Roles of H2AX, BRCA1 and SIRT1 in DNA repair and angiogenesis*.

	DNA repair	Angiogenesis
H2AX	- γ-H2AX marker of DNA lesions - promotes DNA repair	- promotes endothelial cell proliferation and pathologic angiogenesis
BRCA1	- binds to DSBs - important for cell cycle progression - deficiency is associated with unrepaired DNA damage and genomic instability	- negative regulation of VEGF and angiopoietin-1 expression
SIRT1	- deacetylates NBS1 - deficiency associated with impaired DNA repair	- promotes endothelial migration and sprouting and postnatal angiogenesis via inhibition of Foxol transcription factor

* References for these functions of H2AX, BRCA1 and SIRT1 are found in the corresponding text.