Chapter 14
Targeting Tumour Hypoxia with PARP Inhibitors: Contextual Synthetic Lethality

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Abstract As previously discussed in Chap. 13 the concept of synthetic lethality is not novel and has been extensively used to dissect yeast-signalling pathways. More recently, this concept has been embraced as a more personalised approach to cancer therapy, exploiting the fact that a tumour with a defect in pathway A will show increased sensitivity to an agent-targeting pathway B. Contextual synthetic lethality refers to a situation where one of the two pathways is lost as a result of the cellular or microenvironmental context and is rendered sensitive to loss of a second pathway. The first example of contextual synthetic lethality to be described was the use of a PARP inhibitor in hypoxic tumour cells. In this chapter we will first discuss how tumour hypoxia arises and then most importantly the effect of hypoxia on the DNA repair pathways. Finally, we will review how reduced levels of homologous recombination lead to an increased sensitivity to PARP inhibitors in hypoxic tumours.

Keywords Contextual Synthetic Lethality · Hypoxia · PARP (poly(ADP-ribose) polymerase) · Homologous Recombination Repair (HRR) · Replication

14.1 The Tumour Microenvironment

The tumour microenvironment refers to a diverse mixture of cells, extracellular matrix and extracellular molecules. Important cellular elements in addition to tumour cells include fibroblasts, infiltrating inflammatory cells as well as endothelial and perivascular cells, which form blood and lymphatic vessels [1, 2]. The extracellular macromolecules, which provide structural support in the tumour tissue

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include collagen, fibronectin, fibrin, proteoglycans and hyaluronan [1, 3]. The stromal elements play an important role in promoting tumour growth and progression and as such have become potential therapeutic targets [4]. Insufficient oxygen levels (hypoxia), lack of glucose, high interstitial fluid pressure, acidic pH, and increased extracellular lactate are all critical features of the tumour microenvironment [5, 6].

14.2 The Origins of Tumour Hypoxia

Tumour hypoxia is a characteristic feature of most solid tumours and occurs as a result of imbalance between the oxygen supply to tumour cells and its consumption rate [6–8]. The principal reason for this is poorly developed vasculature, which is structurally and functionally inefficient, and the highly proliferative tumour cells. During tumour angiogenesis the blood vessels develop chaotically, are poorly differentiated, tortuous and aberrantly branched/twisted. This leads to unstable blood flow, which is additionally perturbed by increased permeability and leakiness of the tumour vasculature [9]. Arterio-venous shunts (abnormal connections between arterioles and venules) and "vascular mimicry" in which tumour cells attempt to mimic normal endothelial cells also contribute to the abnormal tumour vascular architecture [10]. As a consequence, poor blood flow through these vessels leads to inadequate oxygen supply to the tumour cells and perfusion-related hypoxic regions. Chronic hypoxia develops in tumour cells lying beyond the diffusion distance of oxygen (70–200 μm from blood vessels). Depending upon tumour cell proliferation and the resulting transit time through hypoxic gradients, these cells can be exposed to low oxygen levels for 24–96 h [6]. Figure 14.1 shows hypoxic regions in a tumour xenograft, in which at the limits of diffusion is necrotic tissue. A further contributing factor to the development of tumour hypoxia arises from the low concentrations of haemoglobin in the tumour vasculature, which decreases the oxygen-carrying capacity of the blood leading to anaemic hypoxia [11]. Together these morphological and functional changes, in concert with a high metabolic demand for oxygen in rapidly growing tumours, result in inefficient oxygen delivery to the tumour cells and the formation of multiple and dynamic hypoxic regions.

Oxygen concentration in normal tissues is relatively stable and, depending on the tissue type, ranges from 50 to 80 mmHg (or 7–10% O_2). This is in contrast with hypoxic regions in solid tumours where the oxygen concentrations between 0−30 mmHg (0–3% O_2) have been observed [12, 13]. The severity of hypoxia can be classified based on the ranges of oxygen tension with acute hypoxia (also known as extreme or severe) dropping to oxygen concentration below 0.1%, moderate hypoxia with oxygen concentration between 0.1 and 1%, and mild hypoxia with oxygen concentration between 1 and 3% [14]. Oxygen tensions in tumours fluctuate dynamically, with reoxygenation events taking place due to temporarily improved blood flow [15]. The spontaneous closing and opening of chaotic and distorted

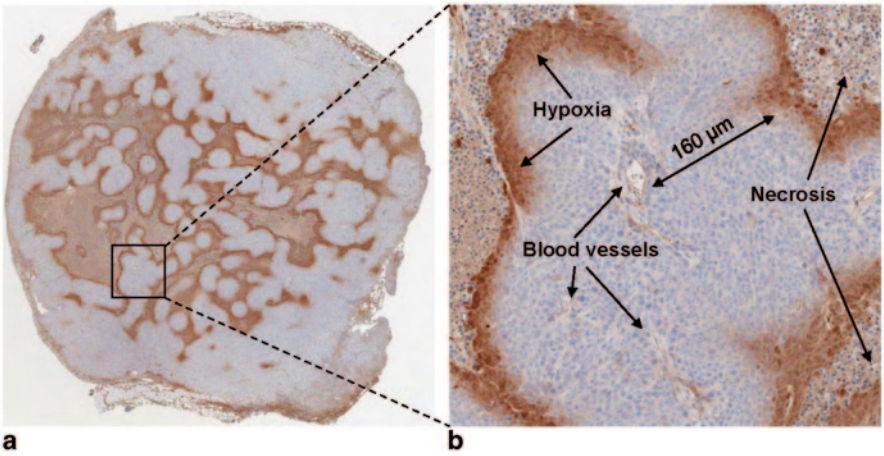

Fig. 14.1 Tumour hypoxia. **a** HCT116 cells (human, colorectal) were grown as a tumour xenograft to an approximate diameter of 800 mμ^3. Prior to sacrifice animals were injected with 60 mg/kg pimonidazole. The hypoxic regions were then visualised by immunohistochemical staining of pimonidazole (*brown*). Nuclei were counterstained with hematoxylin. **b** An enlarged tumour region from **a** showing a hypoxic area and blood vessels (~70–200 μm). Necrotic regions were also identified beyond the hypoxic regions

blood vessels in the tumour leads to cycles of acute hypoxia or anoxia (a complete lack of oxygen) followed by rapid reoxygenation. This phenomenon is known as a transient or "cycling" hypoxia [16]. Together, these oxygen fluctuations lead to the formation of substantial gradients of oxygen and therefore a wide range of oxygen levels within solid tumours.

14.3 Clinical Impact of Tumour Hypoxia

Tumour hypoxia is of significant interest to the field of cancer research as hypoxic cells are aggressive, metastatic and therapy resistant [17–20]. Hypoxia is associated with an adverse clinical prognosis including, decreased local tumour control and lower rates of disease-free and overall survival [11, 17, 21]. There are multiple factors, which contribute to the therapy resistance of hypoxic cells [22]. This includes the finding that some drugs require oxygen to be fully functional, as in the case of doxorubicin, a widely used chemotherapeutic drug which intercalates into DNA [12]. A common feature of hypoxic tumours is increased acidosis, which develops as a result of accumulating lactic acid and can affect activity of alkylating agents and antimetabolites [23, 24]. Some anticancer agents are cell cycle dependent and efficacy is therefore decreased in poorly proliferating hypoxic cells. In addition, hypoxic cells have increased genomic instability and therefore undergo genetic aberrations including gene amplifications potentially increasing resistance

to particular agents [25, 26]. An additional factor contributing to the chemotherapy resistance of hypoxic cells is the poor bio-distribution of drugs to the tumour area due to functionally inefficient blood vessels [27]. In these cases the hypoxic regions of the tumour are effectively shielded from the chemotherapeutic agent. Most notably, hypoxic cells are significantly more resistant to radiotherapy [17, 19]. In the presence of oxygen, ionising radiation (IR) induces free radicals, which damage DNA and lead to cell death. In hypoxia, therefore, this mechanism of IR-induced DNA damage is significantly decreased due to the lack of oxygen [28]. It has been shown that severely hypoxic cancer cells (with oxygen levels < 10 mmHg) require approximately 2–3 times higher dose of radiation in order to give the same effect as cells at normal oxygen levels. This ratio in radiation dose between hypoxic and normal cells is known as the oxygen enhancement ratio (OER) [19, 29]. Hypoxic tumour cells that are deficient in homologous recombination (described below) may have lower OERs (e.g. 1.5).

14.4 The Biological Consequences of Tumour Hypoxia

There are numerous oxygen dependent biological consequences of tumour hypoxia. The hypoxia-inducible factor 1 (HIF-1) is considered the main driver of the transcriptional response [30]. HIF achieves this by regulating the expression of hundreds of target genes, which for the most part aid cancer cells in adapting to the challenging condition of insufficient oxygen. The transcriptional targets of HIF-1 include vascular endothelial growth factor (VEGF, involved in angiogenesis), glucose transporter 1 (GLUT1, involved in metabolism), carbonic anyhydrase IX (CAIX, a regulator of cellular pH) and lysyl oxidase (LOX, in metastasis) [31, 32]. Most recently, a role for HIF-1 in glutamine metabolism has been described in hypoxic conditions [33]. HIF-1 can also control the expression of some micro-RNAs, in particular it transactivates the expression of miR-210, which is implicated in a plethora of pathophysiological processes including cell migration, adhesion differentiation and angiogenesis [34–36]. Although HIF-1 can be stabilised in a range of low oxygen levels including anoxia, its main activity occurs at mild and moderate oxygen concentration [37].

In hypoxia, energy and oxygen consumption rates slow due to a global down regulation of the protein synthesis pathways [14, 38]. Both severe (< 0.1 % O_2) and moderate (> 0.1–1 % O_2) hypoxia induce phosphorylation of eukaryotic initiation factor 2α (EIF2α) which is dependent on the endoplasmic reticulum kinase PERK, and results in the inhibition of mRNA translation [39–41]. Severe and more prolonged hypoxia can disrupt the mRNA cap-binding complex, eIF4F, which then results in inhibition of the transcript recruitment step of mRNA translation [14]. A range of hypoxic conditions also inhibit protein translation through repression of mammalian target or rapamycin (mTOR) signalling and dephosphorylation of eIF4E-binding protein (4E-BP1) [42].

Although there is no detectable DNA damage in hypoxic conditions, severely hypoxic conditions (<0.1% O_2) trigger a DNA damage response (DDR) [43]. Severe hypoxia induces replication stress, which is associated with a rapid drop in nucleotide levels (dNTPs), this is thought to be the DDR initiating signal in hypoxia [44–46]. Hypoxia-induced replication stress is characterised by the presence of RPA foci and pan-nuclear γH2AX in S-phase cells. DDR signalling in hypoxia includes the activation of both Ataxia-telangiectasia mutated (ATM) and Ataxia-telangiectasia and Rad3-related (ATR) kinases [43]. An important biological consequence of DDR signalling in severe hypoxia is stabilisation and activation of the p53 tumour suppressor and subsequent induction of apoptosis. Hypoxia, therefore, exerts a selection pressure to eliminate cancer cells with a high apoptotic potential and promote expansion of cells with mutated p53 and disrupted apoptosis [47, 48].

14.5 Hypoxia-Mediated Repression of DNA Repair

The biological consequence of hypoxia most significant to this review is the repression of the DNA repair pathways, which occurs in a wide range of oxygen tensions [25, 49]. Many proteins critical for the DNA repair pathways are repressed through multiple mechanisms in response to hypoxia and these are summarised in Table 14.1. It is unclear exactly why one of the cellular responses to hypoxia is to turn off DNA repair. One speculation is that it is a means of conserving energy and support for this comes again from the lack of detectable DNA damage in hypoxia, perhaps there is a lower demand for repair pathways. Most notably, hypoxia-mediated repression of DNA repair occurs in response to a wide range of oxygen levels. The significance of this is that if we were able to exploit this therapeutically we would be able to target the most aggressive and therapy resistant regions of solid tumours.

The wide repression of DNA repair proteins in hypoxia occurs both in a HIF-dependent and HIF-independent manner. For example, in severe hypoxia (<0.1% O_2), the expression of NBS1, a component of the MRN (MRE11–RAD50–NBS1) complex, which recognises DNA DSBs, is down regulated and this occurs in a HIF-1α dependent manner, which requires the threonine phosphorylation of the PASB (Per–ARNT–Sim B) domain of the HIF-1α protein [50]. In contrast, the expression levels of RAD51 and BRCA1, both critical to HRR, are repressed in hypoxia in a HIF-independent manner [51, 52]. The levels of these proteins are reduced due to the repressive action of E2F4/p130 complexes on the BRCA1 and RAD51 promoters [49, 53] and/or decreased translation of genes under hypoxic conditions [54]. In addition, both BRCA1 and RAD51 have been shown to be repressed through the accumulation of repressive chromatin marks including H3K4me3, H3K9me3, and H3K9 deacetylation. The histone demethylase LSD1 was found to mediate H3K4 demethylation, a key histone modification at the BRCA1 and RAD51 promoters in response to hypoxia [55]. These data demonstrate that multiple mechanisms are employed to repress the same molecules, which again raises the bigger question of

Table 14.1 Repression of genes in DNA repair pathways by hypoxia

Gene/protein	Repair pathway	Proposed mechanism	Reference
RAD23B	NER	HIF-1 induction of miR373 leading to decreased expression	[56]
RAD52	HRR	HIF-1 induction of miR373 and miR210	[56]
RAD54	HRR	mRNA expression down regulated	[57]
MLHI	MMR	Repressed by DEC1/2 and decreased binding to E-box like motifs in the *mlh1* promoter. HIF-independent shift from c-MYC/MAX to MAD1/MAX and MNT/MAX	[58, 59]
MSH2	MMR	HIF-1 mediated displacement of c-MYC from the *msh2* promoter HIF-independent shift from c-MYC/MAX to MAD1/MAX and MNT/MAX	[60, 61]
MSH6	MMR	HIF displacement of Myc to repress gene transcription in a p53-dependent manner	[61]
RAD51	HRR	Independent of HIF-l and cell cycle Changes in E2F mediated transcriptional transactivation and transrepression and translation	[51, 54]
RAD51 C	HRR	Down regulated at the translational level	[54]
BRCA1	HRR	Independent of HIF-l and cell cycle. Changes in E2F mediated transcriptional transactivation and transrepression and translation	[53]
BRCA2	HRR	Down regulated at the translational level HRR shown to be decreased in hypoxia and cells sensitive to cross-linking agents	[54]
NBS1	DSB	HIF-l dependent and requires phosphorylation of the PASB domain in HIF-1	[50]
XRCC3	NHEJ	Down regulated at the transcriptional level	[54]
XRCC4	NHEJ	Down regulated at mRNA level	[57]
Ku70/80	NHEJ	Down regulated expression in cervical carcinoma	[62]
Ku70/80	NHEJ	Also shown to be up regulated in hypoxia (1 % O_2)	[63]
DNA-PKs	NHEJ	mRNA changes but not protein levels	[57]
DNA-PKs	NHEJ	Up regulated along with DNA-PK activity	[63]
Ligase IV	NHEJ	Down regulated at mRNA level	[57]
UBE2T	FA	Independent of HIF-l and cell cycle. Repressed at the transcriptional and translational levels	[64]
XPC, XPD	NER	Induced through HIF binding to HRE sequences in gene promoters	[65]
MYH	BER	Decreased protein synthesis	[66]
OGG1	BER	Decreased protein synthesis	[66]
POLB	BER	Decreased protein synthesis	[66]

what the biological benefit is to DNA repair repression in hypoxia. Within the HRR pathway, XRCC3, RAD52, RAD54 and BRCA2 are also repressed in response to hypoxia (see Table 14.1). Most importantly, when we used the DR-GFP assay to determine HRR function in hypoxic cells we found that hypoxic cells (0.2 % O_2) were significantly less able to carry out HRR compared to those in normoxic conditions. These data demonstrate that the repression of key components of HRR has a functional consequence i.e. decreased HRR. Equally importantly, the levels of RAD51 have been shown to be low in the hypoxic regions of tumours demonstrating that this is not solely an *in vitro* phenomenon [52, 54, 67] (see Fig. 14.2).

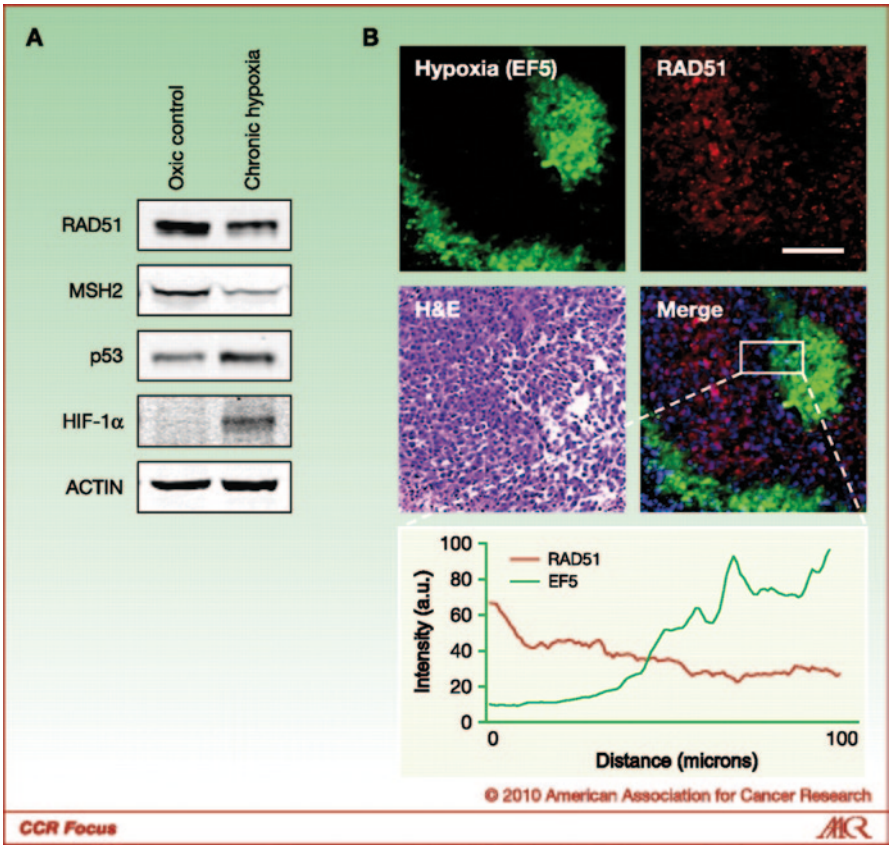

Fig. 14.2 Hypoxia decreases DNA-repair protein expression *in vitro* and *in vivo*. **a** Western blot of RKO colorectal cancer cells showing decreased expression of the HRR DSB repair protein RAD51, and the DNA MMR protein MSH2, under hypoxic conditions *in vitro* (e.g., 72 h exposure at 0.2 % O_2). Hypoxia can also stabilize the p53 protein as shown. HIF-1α is shown as a positive control for hypoxia. **b** RKO xenograft costained *in situ* for hypoxia (EF5, *green*) and RAD51 (*red*). Line intensity profile across the EF5-avid gradient shows inverse association between the hypoxic marker EF5 and RAD51 *in vivo*. Scale bar represents 100 μm. (Reprinted by permission from the American Association for Cancer Research: [68])

Interestingly, non-homologous end joining (NHEJ)-mediated repair was initially thought to be functional in hypoxia despite some of the pathway components being repressed. It was proposed that under hypoxic conditions, cells would favour this error-prone repair pathway because the more accurate HRR repair pathway was compromised. This would have contributed to genomic instability and potentiated the aggressive phenotype of hypoxic tumour cells. However, a recent study demonstrated that due to repression of NHEJ in G_0/G_1 an increased number of unrepaired DNA-double-strand breaks accumulated in hypoxic fibroblasts in this phase of the cell cycle following DNA damage. This observation suggested that the NHEJ pathway is also compromised in hypoxic cells and can give rise to chromosomal instability [69].

14.6 Targeting Tumour Hypoxia Through PARP Inhibition

The role of PARP has been described extensively elsewhere in this book (Chaps. 2–6). Of relevance here are the data demonstrating that inhibition of PARP leads to an accumulation of single strand breaks (SSBs), which when encountered by an on-going replication fork are converted to DSBs [70, 71]. Once formed, these replication fork associated DSBs require efficient HRR for repair and to restart replication [72]. Recently, a role for PARP was described in slowing replication forks in response to DNA damage and most importantly that this was HRR dependent [73]. As previously mentioned cells exposed to severe levels of hypoxia (<0.1 % O_2) experience replication stress although this does not lead to fork collapse and DSBs [74]. Together, these data suggested that cells exposed to severe levels of hypoxia (<0.1 % O_2) might be sensitive to PARP inhibition and particularly so during reoxygenation-induced replication restart.

To test this hypothesis we firstly investigated the cellular localisation of PARP activity (by staining for PAR) in hypoxic cells. This analysis demonstrated that, in response to levels of hypoxia which induce replication stress (<0.1 % O_2) nuclear PAR foci can be detected which colocalise with RPA specifically in S-phase cells (Fig. 14.3). In addition, PARP inhibition led to a decrease in replication rates during reoxygenation-induced restart. Although hypoxia itself does not lead to the accumulation of DNA damage, significant levels of ROS-induced damage are induced by reoxygenation [75]. Therefore when replication restart occurs in response to reoxygenation it does so in the presence of DNA damage, which is potentially deleterious to the cells [46]. By inhibiting PARP in this situation replication is allowed to resume before DNA repair (HRR) has taken place. This is possibly exacerbated by our finding that in some cell lines Rad51 levels do not return to normal for up to eight hours after reoxygenation [76]. We then used colony survival assays to demonstrate that PARP inhibition in hypoxic conditions (<0.1 % O_2) led to a significant loss of viability [52]. Although these data completely supported our hypothesis we appreciated that the clinical utility of using PARP inhibitors to target cells at this extreme

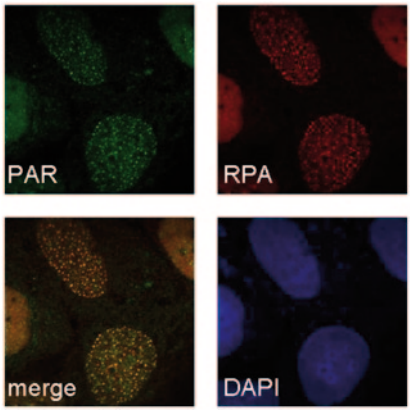

Fig. 14.3 Co-localisation of hypoxia-induced PAR foci with RPA foci. Immunofluorescent staining of PAR (*green*) and RPA (*red*) foci in U20S cells exposed to hypoxia (<0.1 % O_2) for 12 h

level of hypoxia might be limited. Although this level of hypoxia does represent the most radioresistant fraction of a tumour it maybe a small fraction of the tumour and is also the most inaccessible region. Therefore, we extended our study to include milder levels of hypoxia at which proliferation continued normally and all cell cycle checkpoints were bypassed (0.2 % O_2). At this level of hypoxia the DDR is not induced and replication continues normally.

However, normal replication includes sporadic mistakes, which require functional DNA repair pathways for resolution [77, 78]. As a reminder, the hypoxia-mediated repression of the DNA repair pathways is not restricted to severe levels of hypoxia but also occurs in these milder conditions. We hypothesised that proliferating cells in hypoxia might also be sensitive to PARP inhibition due to an inability to repair the errors which accumulate during normal replication. Again, colony survival assays confirmed this hypothesis although it should be noted that the effect was less significant than observed at the lower oxygen level. This is perhaps not surprising given the role of PARP in the response to replication stress [79]. However, our data demonstrating that proliferating hypoxic cells are sensitive to PARP inhibition are extremely promising clinically, as these cells are more abundant and more accessible in a solid tumour. To summarise our *in vitro* data we demonstrated that a variety of cell lines exposed to hypoxic conditions (<0.1–0.2 % O_2) were sensitive to PARP inhibition and that this correlated with decreased HRR [52]. These data raise the possibility of using PARP inhibitors as a means to treat tumours with significant hypoxic fractions in addition to those with known HRR mutations.

Single-agent activity of PARP inhibitors (for example, ABT-888) has been demonstrated in lung cancer models and most importantly independently of BRCA1/2 mutations [80]. To extend these studies and with focus on the hypoxic areas of the tumour we also carried out a xenograft study, using the colorectal RKO cell line. Once grown the RKO tumours were treated twice daily with PARP inhibitor (ABT-888) or vehicle for a period of 5 days and assayed for the presence of DNA damage in the hypoxic regions. DNA damage and apoptosis was measured using the

presence of γH2AX foci and cleaved caspase 3 respectively. Prior to sacrifice the animals were injected with EF5, which is reduced and trapped specifically in hypoxic regions allowing visualisation by immunofluorescence or immunohistochemistry [81]. As observed in the *in vitro* assays, treatment with PARP inhibitor lead to an increase in DNA damage and this was significantly elevated in the hypoxic regions [52]. Interestingly, there was little or no γH2AX staining in the vehicle treated tumour, the relevance of this is that while the tumours were clearly hypoxic (EF5-avid) they were probably not hypoxic enough to induce the DDR. This suggests that the increased DNA damage and apoptosis observed was in hypoxic cells still capable of proliferation i.e. more similar to our *in vitro* studies at 0.2 % O_2. As a result of these promising data, we carried out a study to determine if PARP inhibition *in vivo* selectively kills hypoxic tumour cells.

Mice bearing RKO tumours were treated for 5 days with ABT-888 and then were irradiated 24 hours after the last dose of ABT-888 to make sure that the drug was out of the system at the time of radiation. Waiting for drug washout was important as PARP inhibitors are known to radiosensitise normoxic cells, so had the drug been present at the time of radiation this would have impacted the results (Chap. 11) [82]. To assess the effect on cell viability and specifically in the hypoxic regions an *ex vivo* clonogenic assay was carried out. This is an accepted means of assessing viability of the hypoxic fraction as the oxygenated cells are preferentially killed leaving the hypoxic fraction [83]. Our hypothesis was that the hypoxic fraction in the PARP inhibitor treated cells would be diminished therefore leading to decreased survival in the clonogenic assay. In support of this an increased cell kill was seen in ABT-888 pre-treated and irradiated tumours but not in the irradiated tumours that did not receive the PARP inhibitor [52]. Importantly, the combination of PARP inhibition with radiation was not associated with damage to the normal tissue or loss of viability in a gut clonogenic assay. These encouraging pre-clinical data suggest a wide therapeutic window as the hypoxic tumour cells are sensitive to PARP inhibitor plus radiation whilst the normal cells are undamaged [26, 68, 84].

Interestingly, a recent study by Drew et al. described increased PARP inhibitor (AG014699) sensitivity in cancer cells and tumour xenografts not only in the presence of BRCA1/2 mutation, but also when these genes where epigenetically silenced [85]. This lends additional support to the rationale for PARP inhibitors being a promising therapy for patients with highly hypoxic tumours, as hypoxia was reported to epigenetically silence BRCA1 and 2 [55]. It is also worth noting that while resistance to PARP inhibitors has been described in patients this is, at least in part, due to genetic reversions in the HRR genes and is therefore likely to be of little significance when treating hypoxic tumours (Chaps. 18 and 19).

To fully evaluate the possibility of using PARP inhibitors to target tumour hypoxia a clinical study and subsequent analysis will be required. Most importantly, for this to be informative knowledge of the level of tumour hypoxia, both pre and post treatment will be required. Fortunately, this is rapidly becoming more of a reality as more and more strategies for determining the levels of tumours hypoxia that can be used as part of a clinical study are available (recently reviewed, [13]). Our prediction is that patients with high hypoxic fractions will benefit most from

the combination of PARP inhibitor with their standard therapy. Although, as PARP inhibitors are known to increase sensitivity to a number of agents, including radiation (Chaps. 9–11), this is likely to be less significant than for example when testing a more traditional hypoxia-targeting agent (Chaps. 11 and 12) [86]. Delivering drugs to the hypoxic regions of tumours will always be challenging and therefore it is essential that suitable biomarkers are developed which inform on drug activity in these regions. In our study the DNA damage marker, γH2AX, provided evidence of PARP inhibition in the hypoxic regions of a xenograft tumour. An alternative would be 53BP1, which like γH2AX forms nuclear foci in response to DNA damage. The potential advantage to using 53BP1 is that foci do not form in response to hypoxia alone [44].

14.7 Increasing Drug Delivery to Hypoxic Regions

In addition to the potential of contextual synthetic lethality, PARP inhibitors show promise for combatting tumour hypoxia by targeting other aspects of the tumour microenvironment. Nicotinamide was the first PARP inhibitor used and structurally it served as a model for the subsequent generations of PARP inhibitors [87, 88]. Nicotinamide is well known to act as a vasodilator [89], suggesting that other PARP inhibitors, which are structurally similar to nicotinamide could also improve vessel perfusion and potentially increase drug delivery to adjacent tumour tissues. Recent studies have demonstrated that PARP inhibitors (AG014699 and Olaparib) significantly increase the cytotoxic effect of radiotherapy *in vivo* and *in vitro* [90, 91] (Chap. 12). Through vasodilation, these PARP inhibitors were able to increase blood flow and improve drug accumulation within the tumour. While AG014699 was shown to enhance vessel perfusion in breast (MDA-MB-231) and colorectal (SW620, LoVo), Olaparib was shown to do so in lung (Calu-6) xenograft models *in vitro* and *in vivo* [90, 91]. Therefore, PARP inhibition is a promising therapy to consider in cancers with high hypoxic content resulting from inadequate vasculature. This raises the exciting possibility of using PARP inhibitors to increase delivery of other chemotherapies. This was recently tested by combining AG014699 with doxorubicin [90]. While AG014699 improved tumour perfusion and cardiotoxicity, it did not enhance the efficacy of doxorubicin [90]. Therefore the combination of particular chemotherapeutic agents together with PARP inhibitors as a vasodilating agents needs to be investigated in future studies.

14.8 Further Examples of Context Synthetic Lethality

Studies by Ramaekers and colleagues have revealed that the expression of the ubiquitin-conjugating enzyme UBE2T, which operates in the fanconi anemia (FA) pathway, is repressed under hypoxia due to decreased promoter activity [64]. Therefore,

hypoxic inhibition of the FA pathway could be a novel mechanism underlying increased genetic instability in cancer. Importantly, this down-regulation has been found to correlate with hypersensitivity to mitomycin C-induced DNA crosslinks under hypoxia [64]. This suggests an opportunity for the use of synthetically lethal approaches to selectively target hypoxic cells with crosslinking agents that are known to sensitise cells with defective FA pathway. In a previous study, FANCD2-deficient fibroblasts were shown to be sensitised to radiation under hypoxia but not in normoxia [92]. This sensitivity to radiation in hypoxia was due to increased apoptosis supporting the idea that hypoxic cells with compromised FA pathway might be more sensitive to other treatments, e.g. radiotherapy. In addition, a synthetic lethal phenotype was seen in the mismatch repair (MMR) pathway by inhibition of POLB in MSH2-deficient and POLG in MLH1-deficient cells [93]. Since hypoxia represses both MSH2 and MLH1 MMR proteins, it is likely that inhibition of either POLB or POLG DNA polymerases in hypoxia could lead to a strong synthetic lethality approach. This, however, would need to be tested in the future studies, once clinically useful inhibitors of POLB and/or POLG become available.

Finally, DNA base excision repair (BER) can also be compromised due to hypoxia-mediated suppression of BER protein synthesis (Chan et al. [66] Mol Can Res; In Press, 2014). In multiple colorectal cancer cell lines, functional BER was also impaired as determined by MYH- and 8-oxoguanine (OGG1)-specific glycosylase assays. This was associated with decreased clonogenic survival was observed following exposure to the DNA base damaging agents, H_2O_2 and MMS. Thus, a persistent down-regulation of BER components by the microenvironment modifies and facilitates a BER-associated mutator phenotype, further supporting hypoxia as a driver of genetic instability and cancer progression [26].

14.9 Conclusions

The tumour cell microenvironment is an important consideration when designing novel anti-cancer therapies. Often tumour hypoxia presents a barrier to successful cancer therapy and so any hypoxia-induced Achilles heel provides an ideal opportunity to improve patient outcome. Here, we have exploited hypoxia-mediated repression of DNA repair and demonstrated contextual synthetic lethality. In this case the loss of functional HRR in hypoxic cells renders them sensitive to PARP inhibition. However, as DNA repair is generally repressed in hypoxia, it is hoped that further examples of contextual synthetic lethality will be identified. This approach opens the door to the use of PARP inhibitors for the treatment of hypoxic tumours or perhaps in combination with agents which induce hypoxia (anti-angiogenics for example) in addition to their use in patents with HRR mutations.

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