Chapter 18
"Synthetic Lethality": Molecular Co-targeting to Restore the DNA Repair Mechanisms in Prostate Cancer Cells

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Abstract Resistance to anticancer radiation treatment has a strong negative impact upon morbidity and mortality related to prostate cancer (Liu et al., Radiother Oncol 88(2):258–268, 2008).

This justifies the great interest in the advancing efforts toward the design of new molecularly-targeted agents which could improve the therapeutic ratio for aggressive prostate cancers via tumor radio-sensitization (Fan et al., Cancer Res 64(23):8526–8533, 2004).

Tumor progression of prostate cancer is associated, as in most of human malignancies, with the sequential loss of function of genes that normally protect against DNA damage.

Malignant prostate cells respond to both endogenous and exogenous DNA damage through complex signaling responses. Due to a specific genetic background, or in an acquired manner during tumor progression, PC cell clones show defect in either DNA single-strand break (SSB) and/or double-strand break (DSB) repair, and/or base damage repair (Stewart et al., Biochem Pharmacol 81(2):203–210, 2011), DSBs are the principal responsible for cell killing due to ionizing radiation (Ward 1988).

A defective DNA double-strand break repair increases genetic instability of PC cells, could be considered as part of their "mutator" phenotype (Tyson et al., Prostate 67:1601–1613, 2007).

During the last decades, it has emerged the concept of "synthetic lethality" (Chalmers et al., Semin Radiat Oncol 20(4):274–281, 2010).

This concept derives from the observation that the use of a single inhibitor of a DNA repair enzyme leads to the selective killing of tumor cells, bearing a

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second DNA repair defect (Bryant et al., Nature 434(7035):913–917, 2005; Jones and Plummer, Br J Radiol 81(Spec No 1):S2–S5, 2008; Fong et al., N Engl J Med 361:123–134, 2009).

To this end, PARP inhibitors are the well-known class of drugs that have recently been proposed to reach synthetic lethality in DNA repair-defective, radio-resistant prostate tumors.

This chapter aims to provide a framework for understanding the recent therapeutic trends designed to overcome radioresistance in prostate cancer via synthetic lethality, we review what it is actually known about the structures and functions of the members of the PARP family of enzymes, outlining a series of open questions that should be addressed in the short time to better guide the development (and the safe clinical use) of PARP inhibitors as new anticancer agents for prostate cancer (Cybulski et al., Cancer Res 64:1215–1219, 2004; Stewart et al., Biochem Pharmacol 81(2):203–210, 2011).

Radiotherapy, either in the form of external beam radiotherapy or brachytherapy, still represents a key therapeutic option for localized or locally advanced prostate cancers.

The effectiveness of radiotherapy is strongly influenced by the occurrence of adverse effects on surrounding normal tissues, ranging from radiation-induced cystitis and/or proctitis, up to erectile dysfunction (Stewart et al. 2011; Barreto-Andrade et al. 2011).

This acute and chronic by-stander toxicity has been greatly reduced with the introduction of the intensity-modulated radiation therapy (IMRT), which allows a more specific targeting of the tumor area.

Despite the advances in radiotherapy techniques (Esgueva et al. 2012; Meng et al. 2005) up to 30 % of radio-treated (Pollack et al. 2003) (intermediate and high risk Zelefsky et al. 2006; Bill-Axelson et al. 2005) prostate cancer patients experience a very aggressive, metastatic disease (Esgueva et al. 2012).

Radioresistance of PC cells is thought to be due to complex inter-relationships between intrinsic genetic and micro-environmental factors (Bristow and Hill 1998; Bristow et al. 2007).

This scenario is further complicated by the significant variability in normal tissue reactions to the radiation-induced DNA damage among prostate cancer patients.

Ionizing radiation kills eukaryotic cells mainly through the induction of DNA-double-strand breaks (DNA-DSBs) (Ward 1988) and, with a lesser extent, (Bristow et al. 2007) *via* DNA single-strand breaks (DNA-SSBs), alteration/loss of DNA bases or DNA-DNA/DNA-protein cross-links (Chalmers et al. 2010).

The ratio of SSBs and DSBs generated by therapeutic ionizing radiation is about 25:1, but DNA double-strand breaks (DSBs) are by far the most potent inducers of cancer cell death (Chalmers et al. 2010).

DNA damage detection and repair require several well-characterized epigenetic events, represented, in first instance, by the relaxation of chromatin and phosphorylation of histone H2AX on the chromatin area lining the DNA lesions,

followed either by methylation/acetylation, depending on the specific damaged residue (Escargueil et al. 2008).

DSBs result from the collision of base damage or SSBs with the advancing replication fork, and represent the most cytotoxic lesions (Curtin 2012).

They are usually repaired through two interacting pathways: the homologous recombination (HR) and the non-homologous end joining (NHEJ)-one.

HR utilizes the undamaged sister chromatid (or chromosomal homologue) as a template (Sonoda et al. 2006). This means that HR can take place only in S and G2 phases (Bertrand and Saintigny 2004) operating then during DNA replication (Bernstein et al. 2002; Hansen and Kelly 2000; Hoeijmakers 2001).

NHEJ rapidly binds directly to broken DNA ends during all phases of the cell cycle (Weterings and van Gent 2004; Collis et al. 2005; Riballo et al. 2004; Fan et al. 2004; Rothkamm et al. 2003; Willers et al. 2004) but it lacks the ability to restore any DNA that is lost during the breakage event or subsequent processing, thus resulting in error prone (Sonoda et al. 2006; Chalmers et al. 2010).

These two DNA repair pathways are hyper-activated in normal cells in response to radiation-induced DNA damage (Bromfield et al. 2003).

The non-repair or mis-repair of radiotherapy-induced DNA-DSBs, due to the inhibition of HR or NHEJ, leads to chromosomal deletions, translocations and rearrangements (Bertrand et al. 2004; Bindra and Glazer 2005; Guirouilh-Barbat et al. 2004; Richardson et al. 2004), favouring the onset of genetic instability (Collis et al. 2005; Weterings and van Gent 2004) DNA-DSBs repair has been found to be defective in prostate cancer cell lines (Yuan et al. 1999; Collis et al. 2002; Trzeciak et al. 2004; Fan et al. 2004).

Furthermore, models of prostate carcinogenesis have shown the association with increased levels of chromosomal aberrations and instability can drive the progression from high-grade PIN to PC (Elliott and Jasin 2002; Pihan et al. 2001; Vukovic et al. 2003). Accumulating evidences indicate that the defective DNA double-strand break repair could be considered as part of the "mutator" phenotype of PC cells (Loeb et al. 2003; Bristow et al. 2007).

This has particular relevance, if we consider that the fractionated prostate radiotherapy protocols lead to the generation of a huge number of DNA-DSBs.

During the last few years, in order to overcome PC aggressiveness and radioresistance (Overgaard 2007; Wouters et al. 2002), in fact a positive trend toward multiple promising kinds of "combined" therapeutic approaches has been registered.

Intriguing therapeutic approaches to radiosensitize hypoxic, metastasizing and highly lethal PC cells are focusing on the concept of "synthetic lethality". This definition refers to a situation where the simultaneous presence of two genes mutation results in cell death, whereas each mutation *per se* does not impair cell viability (Curtin 2012).

This phenomenon has inspired new fascinating chances for cancer treatment.

The most promising clinical translations of synthetic lethality concern cancers with specific defects in the HR-mediated repair of double-strand breaks (Antonarakis and Armstrong 2011), as the tumor suppressors BRCA1 and BRCA2 mutant, hereditary breast or ovarian cancers (Venkitaraman 2002).

These tumors represent the first successful examples of treatments based on the use of a single inhibitor of a DNA repair enzyme to selectively kill tumor cells with a second complementary DNA repair pathway defect (Fong et al. 2009; Bryant et al. 2005).

The absolute requirement of HR for DSB repair results in an extreme dependency of BRCA-mutated tumors on PARP-1 action and BER to maintain genomic integrity (Chalmers et al. 2010; Saleh-Gohari et al. 2005).

PARP-1 is the prototypical member of the "poly(ADP-ribose) polymerases (PARPs) superfamily", highly active in protecting cells from endogenous and/or therapeutically induced DNA damage (Curtin 2012).

This large family of enzymes is characterized by the "PARP signature" (GenBank XP_037275 residues 796–1014 de Murcia and Ménissier de Murcia 1994): a 50-amino acid sequence within the enzymatic domain, which catalyzes the cleavage of NAD + into nicotinamide and ADP-ribose. This latter is used to synthesize long, branching, negatively charged polymers, which are then covalently attached to a variety of partner nuclear proteins, as core histones, linker histone H1 (Giner et al. 1992; Grube et al. 1991), HMG proteins, topoisomerases I and II, DNA helicases, single-strandbreak repair (SSBR) and base-excision repair (BER) factors, various transcription factors and PARP-1 itself, involved in DNA damage signalling and repair (Pleschke et al. 2000; Ruf et al. 1996; Oliver et al. 2004), proximally to the DNA breaks.

This poly(ADP-ribosyl)ation leads to the loosening of chromatin structure (Schreiber et al. 2006) allowing the spatial organization of DNA repair through the exposure to the cellular DNA repair machinery (Grube et al. 1991).

PARP-1 is *a 113-kDa* nuclear protein that accounts for at least 80 % of human cellular PARP activity. It is a highly conserved, multifunctional enzyme (Schreiber et al. 2006), with a modular structure (Pfieffer et al. 1999). Under normal conditions, PARP-1 is found associated with histones, DNA and other chromatin associated factors.

In response to DNA damage, it acts as a molecular sensor for DNA-breaks through two zinc-finger motifs, referred to as zf-PARP (Tulin et al. 2002; Menissier et al. 1997), undergoing conformational change and becoming activated.

The binding to DNA breaks, either single-strand break (SSB) and double-strand break (DSB), rapidly stimulated its catalytic activity more than 500-fold. PARP1, as well as his isoenzyme PARP2, acts in SSBs repair mostly by activating base-excision repair (BER).

PARP-1–deficient (or inhibited) cells show, in fact, reduced BER activity (Dantzer et al. 2000) and hypersensitivity to SSB-inducing agents (Horton and Wilson 2007).

If PARP-1 fails to promote SSBs repair, replication forks collapse, converting the DNA damage into replication-associated DSBs, which PARP-1 and PARP-2 attempt to repair either via HR and NHEJ (Chalmers et al. 2010).

The success of PARPs action is strictly dependent upon the extent of the DNA damage. This is due to the transient action of PARP-1 and 2, caused by the rapid degradation of poly-ADP chains due to the poly(ADP-ribose) glycohydrolase (D'Amours et al. 1999).

The half-life of polyADPribose ranges from seconds to minutes, and the hyperactivation of PARP1 consumes the cell pool of NAD + to generate pADPr, lowering cellular energy. So, low-to-moderate DNA damage triggers polyADPribose-dependent DNA repair (Rouleau et al. 2010).

As a complementary effect, pADPr diminishes the affinity for DNA of PARP1, which is then removed from DNA, favouring the post-repair chromatin compaction (Timinszky et al. 2009; Ogata et al. 1980).

In case of excessive DNA damage, PARP1 hyperactivation leads to the excessive NAD + consumption (Juarez-Salinas et al. 1979; Berger et al. 1986; Carson et al. 1988), inducing the catastrophic events that trigger cell death through mechanisms ranging from parthanatos (David et al. 2009; Andrabi et al. 2006), which is directly driven by the longest pADPr chains, to necrosis (Berger et al. 1986; Carson et al. 1988; Zong et al. 2004) or to the establishment of an autophagic state (Huang et al. 2009; Huang and Shen 2009; Munoz-Gamez et al. 2009) of damaged cells.

Due to its fundamental role in DNA-repair, PARP-1 has been identified as the ideal therapeutic target to either specifically kill cancer cells lacking HRR function, and increase the efficacy of radio/chemotherapy in terms of selective tumor cytotoxicity (Farmer et al. 2005; Bryant et al. 2005).

Consistently with these postulates, PARP inhibition in (Bryant et al. 2005) BRCA1 and/or BRCA2-mutated cancer cell (Antonarakis and Armstrong 2011) leads to accumulation of single-strand DNA breaks and (Chalmers et al. 2010) impairs the efficient resolution of collapsed replication forks, impeding the release of PARP molecules from damaged sites, leading to double-strand DNA breaks at replication forks (Antonarakis and Armstrong 2011).

The result is chromosomal instability, cell cycle arrest and subsequent apoptosis caused by the persistence of DNA lesions (Bryant et al. 2005). In other words, the synthetic lethality has been reached.

This synthetic lethality approach has been validated in a multitude of preclinical models, *in vitro* and *in vivo* (Bryant et al. 2005; Farmer et al. 2005), and several PARP inhibitors (olaparib-AZD2281, 3-AB, ISQ, NU1025, KU0058684 or AG14361) have shown promising results, when used as single-agents against *BRCA1*- or *BRCA2*-mutant tumors in clinical testing (Carnell et al. 2006), and promises, as radio-sensitizers in these tumors (Bristow et al. 2007). However, BRCA2 and BRCA1 germ-line mutation carriers have a higher risk to develop PC respect to the normal population. Respectively, prostate cancer relative risk ranges from 2.5 to 7.5 in BRCA2-mutated and <2.0 in BRCA1-carriers; a data particularly significant in tumors diagnosed in younger patients (between ages 40 and 45) (Dong 2006; Levy-Lahad and Friedman 2007).

However, these subsets of PC are relatively poorly differentiated, with poor prognosis (Horsburgh et al. 2005). Additionally, a new BRCA2-interacting protein, PALB2, has been found to be associated with an increased risk of prostate cancer (Erkko et al. 2007).

Olaparib was the first PARP inhibitor to reach human clinical testing in patients with *BRCA1/2*-mutated tumors. In a phase I study, oral olaparib allowed a >50 % PSA drop with resolution of bone metastases in a man with *BRCA2*-related CRPC

(Antonarakis and Armstrong 2011). Nevertheless, BRCA1 and BRCA2 mutations are not considered a major cause of familial or sporadic prostate cancer. A number of other mutations that decrease HR and NHEJ DNA repair responses, that can also sensitize PC cells for synthetic lethality induced by PARP inhibitors, are in fact being increasingly detected (Barreto-Andrade et al. 2011; Plummer et al. 2008; Miknyoczki et al. 2003; Calabrese et al. 2003). They include the phosphatase and tensin homolog gene (PTEN) that is located on chromosome 10, frequently deleted in human cancers and commonly inactivated in prostate cancer (Delaney et al. 2000).

PTEN is a tumor suppressor which, besides inactivating the P13-K/AKT pathway, controls chromosomal integrity and regulates the expression of the repair protein Rad51, reducing the incidence of spontaneous double strand breaks (Shen et al. 2007).

PTEN-deficient tumors exhibit genomic instability due to the down-regulation of Rad51 and the impaired homologous recombination, and result extremely sensitive to PARP inhibitors (Antonarakis and Armstrong 2011).

This sounds of even particular interest, if we consider that, during PC progression the impairment of DNA repair processes mediated by tumor hypoxia greatly contributes to the increase of the genetic instability of prostate cancer (Fan et al. 2004).

Hypoxia, in fact, occurs in 30–90 % of prostate tumors (Chan et al. 2007; Stewart et al. 2010).

Chronic hypoxia, down-regulates the expression and function of many of the DNA-dsb-associated genes, as RAD51 decreasing homologous recombination and DNA double-strand break repair (Vaupel and Mayer 2007).

Thus contributing to the overall genetic instability and aggressiveness of prostate cancer cells.

Tumor hypoxia is indeed progressively emerging as a common feature of prostate tumors associated with poor prognosis.

In-line with these findings, hypoxia-induced metastatic lesions are characterized by gene amplification, point mutation, hyper-mutagenesis and a large amount of DNA strand breaks (Tannock et al. 2005).

Thus, it is becoming increasingly clear that multiple approaches may be hypothesized to overcome radio-resistance of PC cells.

Targeting the hypoxic response has been shown to sensitize PC cells to ionizing radiation *in vitro* (Russell et al. 2003; Slupianek et al. 2001) and may be effective as a complement to radiotherapy of prostate cancer patients. The first attempts of RAD51 expression inhibition by imatinib mesylate (Gleevec) have provided encouraging results (Bristow et al. 2007).

As well, treatment with ABT-888 (Veliparib) has shown some efficacy in PTEN defective PC-3 prostate cancer cells (Barreto-Andrade et al. 2011; Mendes-Pereira et al. 2009).

In addition, ABT-888 enhanced the antitumor activity of TMZ in orthotopic human breast and prostate xenografts.

Radiosensitization *in vivo* has also been demonstrated using several PARPi: AG14361, GPI 15427, ABT-88 and E7016; all showed good radio-sensitization against colon, head and neck, lung and prostate cancer xenografts (Donawho et al. 2007; Calabrese et al. 2004a, b; Palma et al. 2009; Barreto-Andrade et al. 2011).

It could be then hypothesized that PARPi could be successfully used as monotherapy to achieve tumor control, and/or as radio-sensitizers of PTEN-deficient prostate tumors (Barreto-Andrade et al. 2011; Curtin 2012).

A further, extremely interesting, finding derives from a recent report indicating that PARPi can increase the vascular perfusion of tumors through direct vasoactive effects, thus increasing their oxygenation and radio-sensitivity (Liu et al. 2008), leading us to conclude that it may be possible that some PARP inhibitors could induce short-term, vasodilatory effects by virtue of their structural similarities to nicotinamide.

Nicotinamide, a weak PARPi, inhibits contraction of vascular smooth muscle, and its utility in combination with carbogen is being tested in radiotherapy clinical trials (Horsman 1995).

Recently, the AG14361 PARPi was shown to improve intra-tumoral perfusion and possibly reduce tumor hypoxia in mouse xenografts (Calabrese et al. 2004b). Additionally, pharmacological inhibition of PARP has been recently demonstrated to impair HIF-1a induction and angiogenesis (Martin-Oliva et al. 2006; Rajesh et al. 2006a, b).

ABT-888 has been shown to inhibit endothelial tubule formation as well as decreased tumor vascular density (Albert et al. 2007).

This could enhance tumor growth delay after radiotherapy by increasing tumor blood flow, enhancing drug penetration, and increasing oxygen concentrations to offset hypoxic cell radio-resistance. Vasoactive properties and/or anti-endothelial effects have also been documented for AG14361 and ABT888 (Albert et al. 2007; Calabrese et al. 2004a, b; Ali et al. 2009).

Accumulating clinical evidence indicates, in addition, that the short-term use of PARP inhibitors would be extremely well tolerated, even in patients who have undergone multiple previous cytotoxic therapies (Curtin 2012).

Several small-molecule PARP1 and PARP2 inhibitors are currently in preclinical and clinical trials, alone or in combination with DNA-damaging agents (Rodon et al. 2009; Rouleau et al. 2010).

The use of radio-sensitizers to target recognition and repair of DNA damage is becoming an emerging strategy to improve the efficacy of radiotherapy at lower IR doses (Ljungman 2009).

PARP is activated by ionizing radiation (IR) and chemotherapy agents, and this has provided the rationale to examine the combined effects of PARP inhibitors and genotoxic therapy in tumor models and in clinical trials (Donawho et al. 2007; Plummer et al. 2008; Powell et al. 2010).

Several aspects concerning the use of PARPi as radiosensitizers for PC are still to be better elucidated. Here we will briefly examine the most debated ones.

1. PARP inhibition has been shown to radiosensitize mainly replicating cells through the increase of unrepaired DSB (Noel et al. 2006; Dungey et al. 2008).

   This favors the increase of the therapeutic index of radiation therapy in highly replicating tumors (Kastan and Bartek 2004) as recently demonstrated *in vivo,* in colon, head and neck, lung and prostate cancer xenografts using several PARPi, as ABT-88, AG14361, E7016, GPI 15427 (Donawho et al. 2007; Calabrese et al. 2004a, b; Palma et al. 2009; Barreto-Andrade et al. 2011).

   However, the long-term success of radiation therapy of PC depends upon the eradication of mostly non-replicating tumor stem cells, that constitute up to 1 % of total tumor cells (Wong and Hill 1998).

   As it is well-known, cancer stem cells typically reside in hypoxic niches.

   Some PARPi, such as ABT-888, have shown the ability to radio-sensitize *in vivo* also hypoxic tumor clonogens.

   It seems, then, that at least some PARPi could improve the therapeutic ratio of clinical radiotherapy by overcoming both oxic and hypoxic radioresistance.

   These findings are still to be confirmed on large case–control studies. Nevertheless, they look very promising. Moreover, these PARPi would be used also as a complement for new biological imaging-guided hypoxic tumor regions-targeted, high doses-radiotherapy ("dose painting") (Liu et al. 2008) and, some evidences, show that radiotherapy may induce prostate cancer cell death also through a terminal growth arrest (Schwarze et al. 2001). As indicated by the overexpression of markers of senescence, such as p21WAF1/Cip1 and p16INK4a (Stein et al. 1999), therapy-induced senescence is increasingly being reported as an alternative mode of cell death.

   It can result from several inducers, including accumulation of unrepaired DNA damage and is proposed to contribute to tumor control following treatment with cytotoxic agents (Roninson 2003; Lleonart et al. 2009). Some results in PC-3 cells and tumors have suggested that accelerated senescence may be a factor in the therapeutic response of some human tumors to IR combined with PARP inhibition (Efimova et al. 2010).

   A terminal growth arrest should probably be considered an adjunctive end-point and novel therapeutic approach for radiotherapy of prostate carcinoma. PARP inhibitors are among the favorite candidates for inducing this purpose.

   However, this point still deserves consideration in clinical trials and, mainly due to the lack of reliable senescence-inducing agents, this area constitutes an open field for further research (Barreto-Andrade et al. 2011).
2. First generation PARP inhibitors have produced defects in lymphocytes and muscle cells differentiation in several cases. This side-effect may be due to the need of inhibit >90 % of PARP activity to produce a therapeutic impair of DNA repair (Satoh et al. 1994; Farzaneh et al. 1982; Johnstone and Williams 1982).

   However, the third generation of highly potent and specific PARP inhibitors has not produced these adverse effects, suggesting that they might have been only a result of an off-target effect specific for the first type of inhibitors.

However, given the high potency of the new generation of PARPi, the systemic effects of near-complete PARP inhibition should be tested with additional studies on animal model (105. Konishi et al. 1986; Takahashi et al. 1984).

As an example, PARP1 is required either for the protection of the cardiovascular system and for the development of memory (Pacher and Szabo 2007; Goldberg et al. 2009).

As well, long-term PARP1 inhibition could also lead to secondary malignancies, particularly when inhibitors are administered with DNA damaging agents. This hypothesis is supported by several reports. Recently, a high incidence of cancers in mice knocked-out for Parp1 has been reported (Morrison et al. 1997; Tong et al. 2002, 2003).

Reasonably, in each case the risk of occurrence of secondary tumors should be challenged against the chance of improving the therapeutic ratio of currently lethal cancers (Bristow et al. 2007).

The better understanding of both acute and late effects of therapeutic DNA repair inhibition may allow oncologists to focus on the possible way to prevent second malignancies in PARPi-treated patients, by chemopreventive strategies or alternative pathway activation.

Studies on large collections of tumor specimens will be essential to evaluate, in situ, new potential targets for complementary therapies (Antonarakis and Armstrong 2011).

Lastly, it will be interesting to see how durable will be the response rates of patients treated with PARPi.

It appears worrisome, in fact, that resistance to PARP inhibitors has been described in *BRCA1*- or *BRCA2*-deficient cancer cells, following to the reactivation of these genes by secondary mutations (Ashworth 2008; Sakai et al. 2008; Edwards et al. 2008).

3. Little is actually known about the effects of inhibiting PARPs other than PARP-1 and 2 (Rouleau et al. 2010).

   Among the 17 members of the 'PARP superfamily' identified to date, only PARP3, V-PARP and Tankyrase-1 and -2 (TNKS-1 and -2) have the ADP-ribose polymerizing activity (Hakame et al. 2008) PARP-3 co-operate with PARP-1 in the response to DNA double strand breaks (Boehler et al. 2011).

   Tankyrases (TNKS) 1 and 2 are involved in telomere maintenance (Midorikawa et al. 2006) and V-PARP is associated with large ribonuclear protein structures (cytoplasmic vaults), which are amplified in some drug resistance models (Cohen-Armon et al. 2004; Kickhoefer et al. 1999; Fang et al. 2006).

   A potentially specific tankyrase inhibitor, XAV-939, has been identified, raising the possibility that *BRCA1*- or *BRCA2*-mutant tumors might be successfully targeted without inhibiting PARP1 (Ju et al. 2004).

   To date, however, it is unclear to what extent the inhibition of other PARPs contributes to the cellular effects of PARP inhibitors. Only a few studies exist. Moreover, specific inhibitors of all the different PARP-family members are still incompletely available (Curtin 2005).

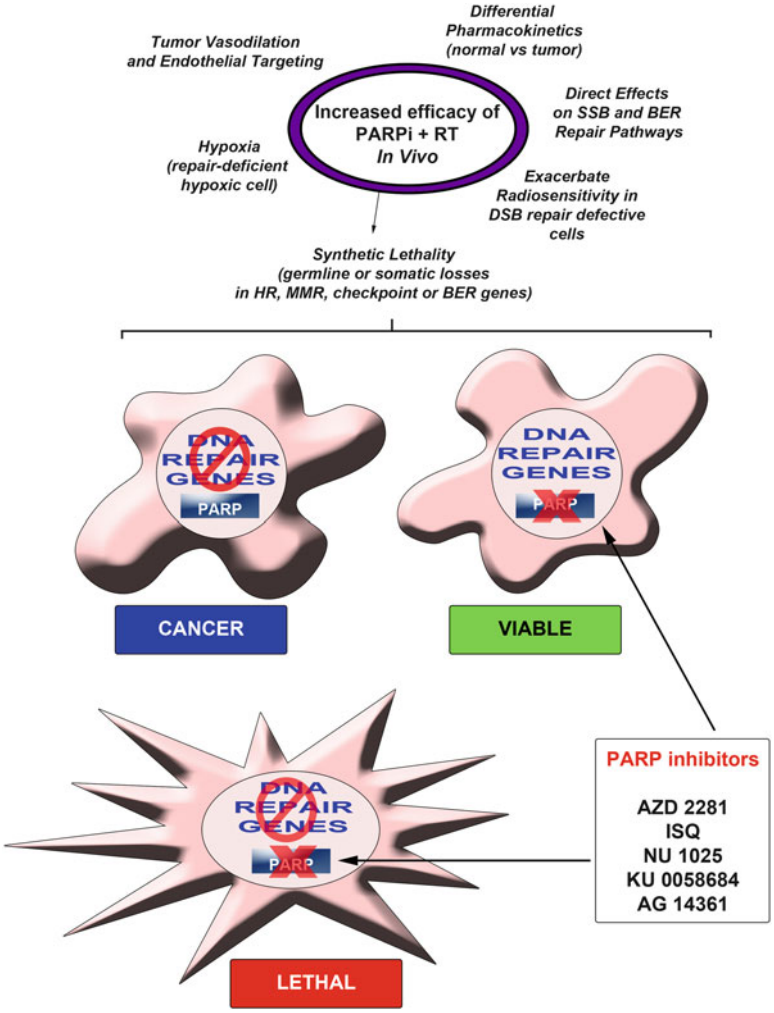

Fig. 18.1 "Synthetic lethality" in the therapy of PCa. Ionizing radiation kills eukaryotic cells through the induction of DNA- double-strand breaks (DNA-dsbs) that represent the most cytotoxic lesions. They are usually repaired through the homologous recombination (HR) and the non-homologous end joining (NHEJ) pathways that, in fact, result hyper-activated in normal cells in response to radiation-induced DNA damage. The non-repair of radiotherapy-induced DNA-dsbs causes genomic instability. The term "synthetic lethality" refers to a condition of simultaneous presence of mutation of two genes resulting in cell death. The most promising clinical translations of synthetic lethality concern cancers with specific defects in the HR-mediated repair of double-strand breaks that results in an extreme dependency of tumors on PARP1 action and BER to maintain genomic integrity. Basing on its fundamental role in DNA-repair, PARP-1 represents the ideal therapeutic target to kill cancer cells with loss of HRR function and to increase the efficacy of radio/chemotherapy. PARP inhibition leads to chromosomal instability, cell cycle arrest and apoptosis caused by the persistence of DNA lesions

This topic, in any case, will certainly be a matter of intense investigation in the near future.

At present, the prediction of radio-responsiveness of prostate cancer is based upon the pre-treatment PSA level/doubling time, Gleason score and T-stage (Nichol et al. 2005).

Novel therapeutic approaches, differentially targeting HR and/or NHEJ DNA-dsb repair, could necessitate of new identifiers of DNA repair (i.e., single nucleotide polymorphisms (SNPs), protein expression, functional assays for DNA damage sensing and repair) related to normal and tumor radio-sensitivity, to drive for individual prostate cancer therapy (Bristow et al. 2007).

These tests may result useful as biomarkers of the genetic instability, malignant progression and aggressiveness of tumor (Choudhury et al. 2006).

This approach may protect normal tissues, allowing the delivery of high doses of radiation and DNA repair inhibitors exclusively on tumor areas targeted by hypoxic signals from MRI, CT or PET-based imaging.

This is an exciting time for oncologists, radio-therapists and pathologist now able to surf over the mounting data concerning the molecular interactions responsible for DNA repair, to discover and apply new therapies based upon a direct collaboration between basic science, industry, academia, and regulatory agencies.

The chances to achieve a new integrative and interdisciplinary approach to prostate cancer patient care, based upon translational oncology, are indeed rapidly becoming reality.

We are now almost ready to take on the challenge to apply next-generation discovered biomarkers able to drive a successful control of previously untreatable, radio-resistant prostate cancers (Fig. 18.1).

References

Albert JM, Cao C, Kim KW et al (2007) Inhibition of poly(ADPRibose) polymerase enhances cell death and improves tumor growth delay in irradiated lung cancer models. Clin Cancer Res 13:3033–3042

Ali M, Telfer BA, McCrudden C et al (2009) Vasoactivity of AG014699, a clinically active small molecule inhibitor of poly(ADP-ribose) polymerase: a contributory factor to chemopotentiation *in vivo*? Clin Cancer Res 15:6106–6112

Andrabi SA et al (2006) Poly(ADP-ribose) (PAR) polymer is a death signal. Proc Natl Acad Sci USA 103:18308–18313

Antonarakis ES, Armstrong AJ (2011) Emerging therapeutic approaches in the management of metastatic castration-resistant prostate cancer. Prostate Cancer Prostatic Dis 14(3):206–218, Review

Ashworth A (2008) Drug resistance caused by reversion mutation. Cancer Res 68:10021–10023

Barreto-Andrade JC, Efimova EV, Mauceri HJ, Beckett MA, Sutton HG, Darga TE, Vokes EE, Posner MC, Kron SJ, Weichselbaum RR (2011) Response of human prostate cancer cells and tumors to combining PARP inhibition with ionizing radiation. Mol Cancer Ther 10(7):1185–1193

Berger SJ, Sudar DC, Berger NA (1986) Metabolic consequences of DNA damage: DNA damage induces alterations in glucose metabolism by activation of poly (ADP-ribose) polymerase. Biochem Biophys Res Commun 134:227–232

Bernstein C, Bernstein H, Payne CM, Garewal H (2002) DNA repair/pro-apoptotic dual-role proteins in five major DNA repair pathways: fail-safe protection against carcinogenesis. Mutat Res 511(2):145–178

Bertrand P, Saintigny Y et al (2004) p53's double life: transactivation-independent repression of homologous recombination. Trends Genet 20:235–243

Bill-Axelson A, Holmberg L, Ruutu M, Haggman M, Andersson SO, Bratell S et al (2005) Radical prostatectomy versus watchful waiting in early prostate cancer. N Engl J Med 352:1977–1984

Bindra RS, Glazer PM (2005) Genetic instability and the tumor microenvironment: towards the concept of microenvironment-induced mutagenesis. Mutat Res 569:75–85

Boehler C et al (2011) Poly(ADP-ribose) polymerase 3 (PARP3), a newcomer in cellular response to DNA damage and mitotic progression. Proc Natl Acad Sci U S A 108:2783–2788

Bristow RG, Hill P (1998) Molecular and cellular basis of radiotherapy. In: Tannock IF, Hill RP (eds) The basic science of oncology. McGraw-Hill, Toronto, pp 295–321

Bristow RG, Ozcelik H, Jalali F, Chan N, Vesprini D (2007) Homologous recombination and prostate cancer: a model for novel DNA repair targets and therapies. Radiother Oncol 83:220–230

Bromfield GP, Meng A, Warde P, Bristow RG (2003) Cell death in irradiated prostate epithelial cells: role of apoptotic and clonogenic cell kill. Prostate Cancer Prostatic Dis 6:73–85

Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, Kyle S, Meuth M, Curtin NJ, Helleday T (2005) Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature 434(7035):913–917

Calabrese CR et al (2003) Identification of potent non-toxic poly(ADPribose) polymerase-1 (PARP-1) inhibitors: chemopotentiation and pharmacological studies. Clin Cancer Res 9:2711–2718

Calabrese CR, Almassy R, Barton S et al (2004a) Anticancer chemosensitization and radiosensitization by the novel poly(ADPribose) polymerase-1 inhibitor AG14361. J Natl Cancer Inst 96:56–67

Calabrese CR et al (2004b) Preclinical evaluation of a novel poly(ADPribose) polymerase-1 (PARP-1) inhibitor, AG14361, with significant anticancer chemo- and radio-sensitization activity. J Natl Cancer Inst 96:56–67

Carnell DM, Smith RE, Daley FM, Saunders MI, Bentzen SM, Hoskin PJ (2006) An immunohistochemical assessment of hypoxia in prostate carcinoma using pimonidazole: implications for radioresistance. Int J Radiat Oncol Biol Phys 65:91–99

Carson DA, Carrera CJ, Wasson DB, Yamanaka H (1988) Programmed cell death and adenine deoxynucleotide metabolism in human lymphocytes. Adv Enzyme Regul 27:395–404

Chalmers AJ, Lakshman M, Chan N, Bristow RG (2010) Poly(ADP-ribose) polymerase inhibition as a model for synthetic lethality in developing radiation oncology targets. Semin Radiat Oncol 20(4):274–281

Chan N, Milosevic M, Bristow RG (2007) Tumor hypoxia, DNA repair and prostate cancer progression: new targets and new therapies. Future Oncol 3:329–341

Choudhury A, Cuddihy A, Bristow RG (2006) Radiation and new molecular agents part I: targeting ATM-ATR checkpoints, DNA repair, and the proteasome. Semin Radiat Oncol 16:51–58

Cohen-Armon M et al (2004) Long-term memory requires polyADP-ribosylation. Science 304:1820–1822

Collis SJ, Sangar VK, Tighe A et al (2002) Development of a novel rapid assay to assess the fidelity of DNA double-strand-break repair in human tumour cells. Nucleic Acids Res 30:E1

Collis SJ, DeWeese TL et al (2005) The life and death of DNA-PK. Oncogene 24:949–961

Curtin NJ (2005) PARP inhibitors for cancer therapy. Expert Rev Mol Med 7:1–20. Together with reference 15, excellent reviews describing the therapeutic promise of PARP Identification of a PAR-binding motif that mediates selective interaction between PAR and protein partners. inhibitors in cancer treatment or in inflammatory diseases

Curtin NJ (2012) Poly(ADP-ribose) polymerase (PARP) and PARP inhibitors. Drug Discov Today Dis Mod Target DNA Repair 9(2):e51–e58
Cybulski C, Gorski B, Debniak T et al (2004) NBS1 is a prostate cancer susceptibility gene. Cancer Res 64:1215–1219
D'Amours D, Desnoyers S, D'Silva I et al (1999) Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. Biochem J 342:249–268
Dantzer F, de la Rubia G, Menissier-De Murcia J et al (2000) Base excision repair is impaired in mammalian cells lacking poly(ADP-ribose) polymerase-1. Biochemistry 39:7559–7569
David KK, Andrabi SA, Dawson TM, Dawson VL (2009) Parthanatos, a messenger of death. Front Biosci 14:1116–1128
de Murcia G, Ménissier de Murcia J (1994) Poly(ADP-ribose) polymerase: a molecular nick-sensor. Trends Biochem Sci 19:172–176
Delaney CA et al (2000) Potentiation of temozolomide and topotecan growth inhibition and cytotoxicity by novel poly (adenosine diphosphoribose) polymerase inhibitors in a panel of human tumor cell lines. Clin Cancer Res 6:2860–2867
Donawho CK, Luo Y, Luo Y, Penning TD, Bauch JL, Bouska JJ et al (2007) ABT-888, an orally active poly (ADP-ribose) polymerase inhibitor that potentiates DNA-damaging agents in preclinical tumor models. Clin Cancer Res 13:2728–2737
Dong JT (2006) Prevalent mutations in prostate cancer. J Cell Biochem 97:433–447
Dungey FA, Loser DA, Chalmers AJ (2008) Replication-dependent radiosensitization of human glioma cells by inhibition of poly(ADP-ribose) polymerase: mechanisms and Therapeutic potential. Int J Radiat Oncol Biol Phys 72:1188–1197
Edwards SL et al (2008) Resistance to therapy caused by intragenic deletion in BRCA2. Nature 451:1111–1115
Efimova EV, Mauceri HJ, Golden DW, Labay E, Bindokas VP, Darga TE et al (2010) Poly(ADP-ribose) polymerase inhibitor induces accelerated senescence in irradiated breast cancer cells and tumors. Cancer Res 70:6277–6282
Elliott B, Jasin M (2002) Double-strand breaks and translocations in cancer. Cell Mol Life Sci 59:373–385
Erkko H, Xia B, Nikkila J et al (2007) A recurrent mutation in PALB2 in Finnish cancer families. Nature 446:316–319
Escargueil AE, Soares DG, Salvador M, Larsen AK, Henriques JA (2008) What histone code for DNA repair? Mutat Res 658(3):259–270
Esgueva R, Park K, Kim R, Kitabayashi N, Barbieri CE, Dorsey PJ Jr, Abraham C, Banerjee S, Leung RA, Tewari AK, Terry S, Shevchuk MM, Rickman DS, Rubin MA, Weill Cornell Medical College (2012) Next-generation prostate cancer biobanking: toward a processing protocol amenable for the International Cancer Genome Consortium. Diagn Mol Pathol 21(2):61–68
Fan R, Kumaravel TS, Jalali F, Marrano P, Squire JA, Bristow RG (2004) Defective DNA strand break repair after DNA damage in prostate cancer cells: implications for genetic instability and prostate cancer progression. Cancer Res 64(23):8526–8533
Fang Y et al (2006) BubR1 is involved in regulation of DNA damage responses. Oncogene 25:3598–3605. doi:10.1038/sj.onc.1209392
Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB et al (2005) Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 434:917–921
Farzaneh F, Zalin R, Brill D, Shall S (1982) DNA strand breaks and ADP-ribosyl transferase activation during cell differentiation. Nature 300:362–366
Fong PC, Boss DS, Yap TA et al (2009) Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med 361:123–134
Giner H et al (1992) Overproduction and large-scale purification of the human poly(ADP-ribose) polymerase using a baculovirus expression system. Gene 114:279–283
Goldberg S, Visochek L, Giladi E, Gozes I, Cohen-Armon M (2009) PolyADP-ribosylation is required for long-term memory formation in mammals. J Neurochem 111:72–79

Grube K et al (1991) Direct stimulation of poly(ADP-ribose) polymerase in permeabilised cells by double-stranded DNA oligomers. Anal Biochem 193:236–239
Guirouilh-Barbat J, Huck S et al (2004) Impact of the KU80 pathway on NHEJ-induced genome rearrangements in mammalian cells. Mol Cell 14:611–623
Hakame A et al (2008) The expanding field of poly(ADP-ribosyl)ation reactions. EMBO Rep 9:1094–1100
Hansen K, Kelly M (2000) Review of mammalian DNA repair and translational implications. J Pharmacol Exp Ther 295(1):1–9
Hoeijmakers JH (2001) Genome maintenance mechanisms for preventing cancer. Nature 411:360–374
Horsburgh S, Matthew A, Bristow RG, Trachtenberg J (2005) Male BRCA1 and BRCA2 mutation carriers: a pilot study investigating medical characteristics of patients participating in a prostate cancer prevention clinic. Prostate 65:124–129
Horsman MR (1995) Nicotinamide and other benzamide analogs as agents for overcoming hypoxic cell radiation resistance in tumours. A review. Acta Oncol 34:571–587
Horton JK, Wilson SH (2007) Hypersensitivity phenotypes associated with genetic and synthetic inhibitor-induced base excision repair deficiency. DNA Repair (Amst) 6:530–543
Huang Q, Shen HM (2009) To die or to live: the dual role of poly(ADP-ribose) polymerase-1 in autophagy and necrosis under oxidative stress and DNA damage. Autophagy 5:273–276
Huang Q, Wu YT, Tan HL, Ong CN, Shen HM (2009) A novel function of poly(ADP-ribose) polymerase-1 in modulation of autophagy and necrosis under oxidative stress. Cell Death Differ 16:264–277
Johnstone AP, Williams GT (1982) Role of DNA breaks and ADP-ribosyl transferase activity in eukaryotic differentiation demonstrated in human lymphocytes. Nature 300:368–370
Jones C, Plummer ER (2008) PARP inhibitors and cancer therapy - early results and potential applications. Br J Radiol 81(Spec No 1):S2–S5
Ju BG et al (2004) Activating the PARP-1 sensor component of the groucho–TLE1 corepressor complex mediates a CaMKinase IIδ-dependent neurogenic gene activation pathway. Cell 119:815–829
Juarez-Salinas H, Sims JL, Jacobson MK (1979) Poly(ADP-ribose) levels in carcinogen-treated cells. Nature 282:740–741
Kastan MB, Bartek J (2004) Cell-cycle checkpoints and cancer. Nature 432:316–323
Kickhoefer VA, Siva AC, Kedersha NL, Inman EM, Ruland C, Streuli M, Rome LH (1999) The 193-kD vault protein, VPARP, is a novel poly(ADP-ribose) polymerase. J Cell Biol 146(5):917–928
Konishi Y et al (1986) Possible model of liver carcinogenesis using inhibitors of NAD + ADP ribosyl transferase in rats. Toxicol Pathol 14:483–488
Levy-Lahad E, Friedman E (2007) Cancer risks among BRCA1 and BRCA2 mutation carriers. Br J Cancer 96:11–15
Liu SK, Coackley C, Krause M, Jalali F, Chan N, Bristow RG (2008) A novel poly(ADP-ribose) polymerase inhibitor, ABT-888, radiosensitizes malignant human cell lines under hypoxia. Radiother Oncol 88(2):258–268
Ljungman M (2009) Targeting the DNA damage response in cancer. Chem Rev 109:2929–2950
Lleonart ME, Artero-Castro A, Kondoh H (2009) Senescence induction; a possible cancer therapy. Mol Cancer 8:3
Loeb LA, Loeb KR, Anderson JP (2003) Multiple mutations and cancer. Proc Natl Acad Sci USA 100:776–781
Martin-Oliva D, Aguilar-Quesada R, O'Valle F et al (2006) Inhibition of poly(ADP-ribose) polymerase modulates tumor-related gene expression, including hypoxia-inducible factor-1 activation, during skin carcinogenesis. Cancer Res 66:5744–5756
Mendes-Pereira AM, Martin SA, Brough R, McCarthy A, Taylor JR, Kim JS et al (2009) Synthetic lethal targeting of PTEN mutant cells with PARP inhibitors. EMBO Mol Med 1:315–322

Meng AX, Jalalia F, Cuddihya A, Chan N, Bindrab RS, Glazerb PM, Robert G (2005) Bristow Hypoxia down-regulates DNA double strand break repair gene expression in prostate cancer cells. Radiother Oncol 76:168–176

Menissier de Murcia J et al (1997) Requirement of poly(ADP-ribose) polymerase in recovery from DNA damage in mice and in cells. Proc Natl Acad Sci USA 94:7303–7307

Midorikawa R, Takei Y, Hirokawa N (2006) KIF4 motor regulates activity-dependent neuronal survival by suppressing PARP-1 enzymatic activity. Cell 125:371–383

Miknyoczki SJ et al (2003) Chemopotentiation of temozolomide, irinotecan, and cisplatin activity by CEP-6800, a poly(ADP-ribose) polymerase inhibitor. Mol Cancer Ther 2:371–382

Morrison C et al (1997) Genetic interaction between PARP and DNA-PK in V(D)J. Recombination and tumorigenesis. Nat Genet 17:479–482

Munoz-Gamez JA et al (2009) PARP-1 is involved in autophagy induced by DNA damage. Autophagy 5:61–74

Nichol AM, Warde P, Bristow RG (2005) Optimal treatment of intermediate-risk prostate carcinoma with radiotherapy: clinical and translational issues. Cancer 104:891–905

Noel G, Godon C, Fernet M et al (2006) Radiosensitization by the poly(ADPribose) polymerase inhibitor 4-amino-1,8-naphthalimide is specific of the S phase of the cell cycle and involves arrest of DNA synthesis. Mol Cancer Ther 5:564–574

Ogata N, Ueda K, Kagamiyama H, Hayaishi O (1980) ADP-ribosylation of histone H1. Identification of glutamic acid residues 2, 14, and the COOH-terminal lysine residue as modification sites. J Biol Chem 255:7616–7620

Oliver AW et al (2004) Crystal structure of the catalytic fragment of murine poly(ADP-ribose) polymerase-2. Nucleic Acids Res 32:456–464

Overgaard J (2007) Hypoxic radiosensitization: adored and ignored. J Clin Oncol 25:4066–4074

Pacher P, Szabo C (2007) Role of poly(ADP-ribose) polymerase 1 (PARP-1) in cardiovascular diseases: the therapeutic potential of PARP inhibitors. Cardiovasc Drug Rev 25:235–260

Palma JP et al (2009) ABT-888 confers broad *in vivo* activity in combination with temozolomide in diverse tumours. Clin Cancer Res 15:7277–7290

Pfieffer R et al (1999) Quantitative nonisotopic immuno-dot-blot method for the assessment cellular poly(ADP-ribosyl)ation capacity. Anal Biochem 275:118–122

Pihan GA, Purohit A, Wallace J, Malhotra R, Liotta L, Doxsey SJ (2001) Centrosome defects can account for cellular and genetic changes that characterize prostate cancer progression. Cancer Res 61:2212–2219

Pleschke JM, Kleczkowska HE, Strohm M, Althaus FR (2000) Poly(ADP-ribose) binds to specific domains in DNA damage checkpoint proteins. J Biol Chem 275:40974–40980

Plummer R, Jones C, Middleton M, Wilson R, Evans J, Olsen A et al (2008) Phase I study of the poly(ADP-ribose) polymerase inhibitor, AG014699, in combination with temozolomide in patients with advanced solid tumors. Clin Cancer Res 14:7917–7923

Pollack A, Hanlon A et al (2003) Radiation therapy dose escalation for prostate cancer: a rationale for IMRT. World J Urol 21:200–208

Powell C, Mikropoulos C, Kaye SB, Nutting CM, Bhide SA, Newbold K et al (2010) Pre-clinical and clinical evaluation of PARP inhibitors as tumor-specific radiosensitizers. Cancer Treat Rev 36:566–575

Rajesh M, Mukhopadhyay P, Batkai S et al (2006a) Pharmacological inhibition of poly(ADP-ribose) polymerase inhibits angiogenesis. Biochem Biophys Res Commun 350:352–357

Rajesh M, Mukhopadhyay P, Godlewski G et al (2006b) Poly(ADPribose) polymerase inhibition decreases angiogenesis. Biochem Biophys Res Commun 350:1056–1062

Riballo E, Kuhne M et al (2004) A pathway of double-strand break rejoining dependent upon ATM, Artemis, and proteins locating to gamma-H2AX foci. Mol Cell 16:715–724

Richardson C, Stark JM et al (2004) Rad51 overexpression promotes alternative double-strand break repair pathways and genome instability. Oncogene 23:546–553

Rodon J, Iniesta MD, Papadopoulos K (2009) Development of PARP inhibitors in oncology. Expert Opin Investig Drugs 18:31–43

Roninson IB (2003) Tumor cell senescence in cancer treatment. Cancer Res 63:2705–2715

Rothkamm K, Kruger I et al (2003) Pathways of DNA double-strand break repair during the mammalian cell cycle. Mol Cell Biol 23:5706–5715

Rouleau M, Patel A, Hendzel MJ, Kaufmann SH, Poirier GG (2010) PARP inhibition: PARP1 and beyond. Nat Rev Cancer 10(4):293–301, Review

Ruf A, Mennissier de Murcia J, de Murcia G, Schulz GE (1996) Structure of the catalytic fragment of poly(AD-ribose) polymerase from chicken. Proc Natl Acad Sci USA 93:7481–7485

Russell JS, Brady K, Burgan WE et al (2003) Gleevec-mediated inhibition of Rad51 expression and enhancement of tumor cell radiosensitivity. Cancer Res 63:7377–7383

Sakai W et al (2008) Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers. Nature 451:1116–1120

Saleh-Gohari N et al (2005) Spontaneous homologous recombination is induced by collapsed replication forks that are caused by endogenous DNA single-strand breaks. Mol Cell Biol 25:7158–7169

Satoh MS, Poirier GG, Lindahl T (1994) Dual function for poly(ADP-ribose) synthesis in response to DNA strand breakage. Biochemistry 33:7099–7106

Schreiber V, Dantzer F, Ame JC, de Murcia G (2006) Poly(ADP-ribose): novel functions for an old molecule. Nat Rev Mol Cell Biol 7(7):517–528, Review

Schwarze SR et al (2001) Role of cyclin-dependent kinase inhibitors in the growth arrest at senescence in human prostate epithelial and uroepithelial cells. Oncogene 20:8184–8192

Shen WH, Balajee AS, Wang J, Wu H, Eng C, Pandolfi PP et al (2007) Essential role for nuclear PTEN in maintaining chromosomal integrity. Cell 128:157–170

Slupianek A, Schmutte C, Tombline G et al (2001) BCR/ABL regulates mammalian RecA homologs, resulting in drug resistance. Mol Cell 8:795–806

Sonoda E, Hochegger H, Saberi A, Taniguchi Y, Takeda S (2006) Differential usage of non-homologous end-joining and homologous recombination in double strand break repair. DNA Repair (Amst) 5:1021–1029

Stein GH, Drullinger LF, Soulard A, Dulic V (1999) Differential roles for cyclin-dependent kinase inhibitors p21 and p16 in the mechanisms of senescence and differentiation in human fibroblasts. Mol Cell Biol 19:2109–2117

Stewart GD, Ross JA, McLaren DB, Parker CC, Habib FK, Riddick AC (2010) The relevance of a hypoxic tumour microenvironment in prostate cancer. BJU Int 105:8–13

Stewart GD, Nanda J, Katz E, Bowman KJ, Christie JG, Brown DJ, McLaren DB, Riddick AC, Ross JA, Jones GD, Habib FK (2011) DNA strand breaks and hypoxia response inhibition mediate the radiosensitisation effect of nitric oxide donors on prostate cancer under varying oxygen conditions. Biochem Pharmacol 81(2):203–210

Takahashi S et al (1984) Enhancement of DEN initiation of liver carcinogenesis by inhibitors of NAD + ADP ribosyl transferase in rats. Carcinogenesis 5:901–906

Tannock IF, Hill RP, Bristow RG, Harrington L (2005) The basic science of oncology. McGraw-Hill Professional, New York

Timinszky G et al (2009) A macrodomain-containing histone rearranges chromatin upon sensing PARP1 activation. Nat Struct Mol Biol 16:923–929

Tong WM et al (2002) Synergistic role of Ku80 and poly(ADP-ribose) polymerase in suppressing chromosomal aberrations and liver cancer formation. Cancer Res 62:6990–6996

Tong WM et al (2003) Null mutation of DNA strand break-binding molecule poly(ADP-ribose) polymerase causes medulloblastomas in p53−/− mice. Am J Pathol 162:343–352

Trzeciak AR, Nyaga SG, Jaruga P, Lohani A, Dizdaroglu M, Evans MK (2004) Cellular repair of oxidatively induced DNA base lesions is defective in prostate cancer cell lines, PC-3 and DU-145. Carcinogenesis 25:1359–1370

Tulin A, Stewart D, Spradling AC (2002) The *Drosophila* heterochromatic gene encoding poly(ADP-ribose) polymerase (PARP) is required to modulate chromatin structure during development. Genes Dev 16:2108–2119

Tyson DR, Inokuchi J, Tsunoda T, Lau A, Ornstein DK (2007) Culture requirements of prostatic epithelial cell lines for acinar morphogenesis and lumen formation *in vitro*: role of extracellular calcium. Prostate 67:1601–1613

Vaupel P, Mayer A (2007) Hypoxia in cancer: significance and impact on clinical outcome. Cancer Metastasis Rev 26:225–239

Venkitaraman AR (2002) Cancer susceptibility and the functions of BRCA1 and BRCA2. Cell 108:171–182

Vukovic B, Park PC, Al-Maghrabi J et al (2003) Evidence of multifocality of telomere erosion in high-grade prostatic intraepithelial neoplasia (HPIN) and concurrent carcinoma. Oncogene 22:1978–1987

Ward JF (1988) DNA damage produced by ionizing radiation in mammalian cells: identities, mechanisms of formation, and reparability. Prog Nucleic Acid Res Mol Biol 35:95–125

Weterings E, van Gent DC (2004) The mechanism of non-homologous end-joining: a synopsis of synapsis. DNA Repair (Amst) 3:1425–1435

Willers H, Dahm-Daphi J et al (2004) Repair of radiation damage to DNA. Br J Cancer 90: 1297–1301

Wong CS, Hill RP (1998) Experimental radiotherapy. In: Tannock IF, Hill RP (eds) The basic science of oncology, 3rd edn. McGraw-Hill, Toronto, pp 322–349

Wouters BG, Weppler SA, Koritzinsky M, Landuyt W, Nuyts S, Theys J, Chiu RK, Lambin P (2002) Hypoxia as a target for combined modality treatments. Eur J Cancer 38:240–257

Yuan R, Fan S, Wang JA et al (1999) Coordinate alterations in the expression of BRCA1, BRCA2, p300, and Rad51 in response to genotoxic and other stresses in human prostate cancer cells. Prostate 40:37–49

Zelefsky MJ, Chan H, Hunt M, Yamada Y, Shippy AM, Amols H (2006) Longterm outcome of high dose intensity modulated radiation therapy for patients with clinically localized prostate cancer. J Urol 176:1415–1419

Zong WX, Ditsworth D, Bauer DE, Wang ZQ, Thompson CB (2004) Alkylating DNA damage stimulates a regulated form of necrotic cell death. Genes Dev 18:1272–1282