Exploiting DNA repair defects for novel cancer therapies

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ABSTRACT Most human tumors accumulate a multitude of genetic changes due to defects in the DNA damage response. Recently, small-molecule inhibitors have been developed that target cells with specific DNA repair defects, providing hope for precision treatment of such tumors. Here we discuss the rationale behind these therapies and how an important bottleneck—patient selection—can be approached.

DNA DAMAGE RESPONSE AND TUMORIGENESIS

The DNA damage response (DDR) safeguards genetic information stored in DNA. This cellular defense mechanism against DNA injury is of utmost importance for the avoidance of cancer and aging symptoms (Hoeijmakers, 2001; Vermeij et al., 2016). The DDR consists of a complex network of cell cycle–progression checkpoint pathways, as well as multiple DNA repair pathways. In the context of cancer, defects in the DDR are seen as a hallmark of tumor cells (Hanahan and Weinberg, 2011). A defective DDR not only promotes the initiation of cancer, but it also allows the tumor cells to quickly acquire (advantageous) mutations during their evolution. Here we will concentrate on defects in DNA repair mechanisms and DNA metabolism and how these defects can be exploited for precision treatment of selected tumors.

Several cancer predisposition syndromes have highlighted the importance of DNA repair for cancer prevention (Knoch et al., 2012). A classic example is xeroderma pigmentosum, in which defective nucleotide excision repair causes ultraviolet (UV)-induced skin cancer. This recessive genetic disease allows accumulation of a large numbers of point mutation in UV-exposed skin cells of patients, leading to multiple tumors at a very young age.

The etiology of hereditary mammary and ovarian cancer was unraveled more recently (Prakash et al., 2015). Defects in the BRCA1 or BRCA2 gene result in a very high chance of developing breast or ovarian cancer. Both BRCA proteins are required for efficient DNA double-strand break (DSB) repair by homologous recombination (HR). In this case, the inheritance pattern is dominant. The disease-causing mutation is present in heterozygous form in all cells, whereas the tumor cells lose the remaining functional allele.

DNA DSB REPAIR

DSBs are among the most toxic types of DNA damage. If left unrepaired, they can result in loss of chromosome fragments during mitosis and possible loss of heterozygosity for essential tumor suppressor genes. Incorrect repair of DNA ends, on the other hand, can result in chromosomal translocations, which are the driving mutagenic events in many tumors (Janssen and Medema, 2013; Iliakis et al., 2015). However, efficient DSB repair pathways have evolved (Chapman et al., 2012; Brandsma and Gent, 2012; Jeggo and Löbrich, 2015). The two major pathways are mechanistically distinct. The simplest and most widely used pathway is nonhomologous end joining (NHEJ), which couples both DNA ends by direct ligation. Classic NHEJ depends on the DNA-dependent protein kinase and ligase IV complexes, but alternative, more error-prone mechanisms also exist. HR, on the other hand, is a quite intricate mechanism that uses the homologous sequence on the sister chromatid for precise repair of the DSB. This DNA repair pathway is mainly active in the S and G2 phases of the cell cycle, as it needs a sister chromatid as repair template.

In addition to direct DSBs, DNA breaks can also appear indirectly during replication of a damaged DNA template or as intermediates in repair of interstrand cross-links, a covalent linkage between bases

Abbreviations used: DDR, DNA damage response; dNTPs, deoxynucleoside triphosphates; DSB, DNA double-strand break; HR, homologous recombination; MTH1, MutT homologue 1; NHEJ, nonhomologous end joining; PARP 1, poly-[ADP-ribose]-polymerase 1; ROS, reactive oxygen species.

in opposite DNA strands (Zeman and Cimprich, 2014; Zhang and Walter, 2014). In these cases, NHEJ cannot properly repair the lesion. Replication-associated DSBs arise, for example, when the replication fork encounters a single-strand break, resulting in a single DNA end, which cannot be repaired by NHEJ because of the lack of a second DNA end. Therefore this DNA end needs to be reinserted by HR into the DNA double helix that is being replicated in order to restart the replication fork.

HR is initiated by resection of the DNA end, which is carried out by nucleases such that 3'-ended single-strand DNA tails are formed (Symington, 2014; Cejka, 2015). Formation of these protrusions also requires active BRCA1 protein, explaining the HR defect caused by mutation of this gene (Sartori et al., 2007; Jasin and Rothstein, 2013). RPA protein accumulates on the single-stranded DNA, which is subsequently replaced by the RAD51 protein. The accumulation of RAD51 protein at DSBs depends on BRCA2 and can be visualized as RAD51 protein foci by immunofluorescence microscopy (Yuan et al., 1999). The RAD51–single-strand DNA complex recognizes homology on the intact sister chromatid, which it uses to copy lost DNA fragments or restart a replication fork.

EXPLOITING *BRCA* GENE DEFECTS IN BREAST AND OVARIAN CANCER

The selective inactivation of HR capacity in hereditary breast and ovarian cancer not only provides an explanation for the chromosomal instability of these tumors, but it may also be the Achilles heel of the tumor cells (O'Connor, 2015). The HR defect makes these cells very sensitive to treatments that increase the number of single-strand breaks encountered by replication forks. This can be done by inhibition of one of the enzymes involved in single-strand break repair, the poly-[ADP-ribose]-polymerase 1 (PARP1) protein. Several small-molecule inhibitors kill BRCA1 and BRCA2 defective cells very efficiently (Bryant et al., 2005; Farmer et al., 2005). In vitro, BRCA-deficient cells were ~1000-fold more sensitive to PARP inhibitors than their proficient counterparts. This so-called synthetic lethality approach was quickly taken to animal experiments and early clinical studies. The effects for *BRCA*-mutated ovarian tumors were so clear that the phase II clinical study resulted in a clear progression-free survival benefit with relatively mild side effects (Fong et al., 2009). This led to approval of the PARP inhibitor olaparib for the European and American markets. Results for breast cancer were less clear and await completion of phase III trials.

In addition to germline *BRCA* gene mutations in ovarian cancers (14% of the cases), somatic mutations in these genes have been found in 6% of ovarian tumors (De Picciotto et al., 2016). Furthermore, gene inactivation can also be epigenetic; *BRCA1* promoter methylation has been found in another 11% of ovarian cancers. It is not clear whether these methylation events are present in the entire tumor and whether they are sufficiently stable for effective therapeutic use. However, it is clear that a sizeable fraction of these tumors will be eligible for PARP inhibitor treatment.

EXTENDING THE USE OF PARP INHIBITORS

From the rationale behind the effectiveness of PARP inhibitors, it follows that not only should *BRCA*-deficient tumors be PARP inhibitor sensitive, but so should also tumors with a defect in HR, irrespective of *BRCA* inactivation. A number of HR genes are known, such as RAD51 and the gene encoding the BRCA2-interacting protein, PALB2 (Liu et al., 2014). However, rather than analyzing individual genes with genomics techniques, one would rather functionally test for HR deficiency and/or PARP inhibitor sensitivity. Recent developments in ex vivo functional assays on primary tumor material could

bridge this diagnostic gap. Conditions for culturing thin tumor slices for several days without loss of viability or proliferative capacity have been developed, which allow direct drug sensitivity testing on fresh tumor material (Naipal et al., 2014, 2016). The predictive power of this approach needs to be established, but most technical hurdles for direct testing on tumor biopsies have been removed. In combination with new predictive biomarkers, these functional tests pave the way for selection of the optimal treatment regimen for each individual patient.

In addition to genetic or epigenetic changes in the tumor, HR deficiency can also be induced locally. Mild hyperthermia (41–42°C) induces BRCA2 protein degradation, resulting in severely reduced HR capacity in cells and tissues (Krawczyk et al., 2011). Because heat can be applied locally in the patient (Issels et al., 2016; van den Tempel et al., 2016), PARP inhibitor sensitivity can be increased in all tumors that can be heated in a controlled way.

In general, PARP inhibition is seen as a promising new targeted anticancer treatment. This is clear from the extensive number of ongoing clinical trials, ranging from phase I to phase III, involving a number of PARP inhibitors, such as niraparib (MK4827), olaparib (AZD2281), rucaparib (AG014699), talazoparib (BMN 673), and veliparib (ABT-888; Donawho et al. 2007; Evers et al. 2008; Plummer et al. 2008; Jones et al. 2009; Shen et al. 2013).. The trials test PARP inhibitors either as monotherapy or in combination with chemotherapy or radiotherapy and are not limited to *BRCA* mutant patients or to breast and ovarian cancer sites (O'Connor, 2015).

OTHER TREATMENTS TARGETING THE DNA DAMAGE RESPONSE

Although originally considered a collection of linear pathways, the DDR is now seen as a complex interconnected and dynamic network of numerous pathways capable of shuttling repair intermediates between different pathways (Wyman and Kanaar, 2006; Al-Ejeh et al., 2010). This ability provides the rationale for why PARP inhibitors of single-strand break repair are synthetic lethal in HR-defective cells. Indeed, PARP inhibition defined the concept of synthetic lethality in the context of the DDR (Lord and Ashworth, 2008). Given that the DDR consists of multiple pathways, other examples of synthetic lethality involving tumor-specific DDR defects are to be expected in the near future. Their rational design would require mechanistic insight into the interplay and interdependences among DDR pathways.

More recently, MutT homologue 1 (MTH1) inhibition has been explored as a precision therapy for cancer (Gad et al., 2014; Huber et al., 2014). This approach does not directly focus on DNA but on deoxynucleoside triphosphates (dNTPs), the building block of DNA. Just as a defective DDR is one of the hallmarks of cancer, so is deregulation of cellular metabolism, including redox regulation. Indeed, the level of reactive oxygen species (ROS) is generally increased in cancer cells, not only resulting in more direct DNA lesions but also contributing indirectly to DNA damage by incorporation of damaged dNTPs. Oxidized dNTPs form a substantial threat to DNA integrity, as the dNTP pool is much more susceptible to oxidation compared with bases already incorporated in DNA (Topal and Baker, 1982). The enzyme MTH1 removes the high-energy phosphate bond from the damaged dNTPs such that they can no longer be incorporated in DNA by a DNA polymerase (Sakumi et al., 1993). Given the difference in ROS levels between normal and cancer cells, the latter may depend much more strongly on MTH1 to prevent deleterious DNA damage. Therefore inhibitors of MTH1 are expected to preferentially affect cancer cells, a prediction that is supported in some experimental settings (Gad et al., 2014) but does

require further careful experimentation for validation (Kettle *et al.*, 2016).

Besides the enzymes directly governing cell cycle check points and DNA repair, a number of sensor and transducer kinases form a pivotal part of the DNA damage response (Velic *et al.*, 2015). Several small-molecule inhibitors of these kinases are being tested in early-stage clinical trials for a wide variety of cancers (O'Connor, 2015). The major challenge will be to find the optimal inhibitors and match them for efficacy with specific DDR defects in tumors.

THE DNA DAMAGE RESPONSE AS PROMISING SOURCE FOR PATIENT SELECTION METHODS AND PRECISION CANCER THERAPIES

Concomitant with the development of precision cancer therapies based on the DDR, including those described earlier, diagnostic tools will be indispensable for proper therapy selection of individual patients. Because simple robust and reliable biomarkers are often difficult to find, direct functional testing on fresh biopsy material or cancer organoid cultures may be the way to go for many novel therapies. The added advantage of a functional test over a DNA diagnostic test is that it reports on the combined activity of many reaction steps in a pathway, which is expected to enable detection of many more patients eligible for specific precision therapy. To realize this form of testing, ex vivo culture methods should be optimized (Davies *et al.*, 2015), miniaturized, and automated in order to allow multiple functional assays on the limited amount of material available in a cancer-on-chip approach (van de Stolpe A and den Toonder, 2013; Hickman *et al.*, 2014; Benam *et al.*, 2015). It is clear that precision medicine will require precision diagnosis, which is best done by testing for biological function. Successful implementation of these diagnostic tools will depend not only on the predictive value of the test, but also on the practicability, cost, and speed of the assay.

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