Although DNA double-strand breaks (DSBs) are substrates for homologous recombination (HR) repair, it is becoming apparent that DNA lesions produced at replication forks, for instance by many anticancer drugs, are more significant substrates for HR repair. Cells defective in HR are hypersensitive to a wide variety of anticancer drugs, including those that do not produce DSBs. Several cancers have mutations in or epigenetically silenced HR genes, which explain the genetic instability that drives cancer development. There are an increasing number of reports suggesting that mutation or epigenetic silencing of HR genes explains the sensitivity of cancers to current chemotherapy treatments. Furthermore, there are also many examples of re-expression of HR genes in tumours to explain drug resistance. Emerging data suggest that there are several different subpathways of HR, which can compensate for each other. Unravelling the overlapping pathways in HR showed that BRCA1- and BRCA2-defective cells rely on the PARP protein for survival. This synthetic lethal interaction is now being exploited for selective treatment of BRCA1- and BRCA2-defective cancers with PARP inhibitors. Here, I discuss the diversity of HR and how it impacts on cancer with a particular focus on how HR can be exploited in future anticancer strategies.

Introduction

Many anticancer therapies introduce DNA damage to trigger death signals in cancer cells. After surgery, radiotherapy is the most commonly used modality for treating cancer, and cell death caused by ionizing radiation is associated with the introduction of DNA double-strand breaks (DSBs). Several anticancer drugs also cause toxic DSBs (e.g. topoisomerase II inhibitors and some cross linkers such as melphalan) and it is generally accepted that the ability of cancer cells to repair such DSBs influences the therapeutic outcome. Homologous recombination (HR) and non-homologous end joining (NHEJ) repair DSBs either by using a homologous DNA molecule (HR) or by simply ligating the ends together (NHEJ) (Figure 1).

HR repair of DSBs involves repair using preferentially the sister chromatid, which results in error-free repair (3,4). HR is only active in the S and G2 phases of the cell cycle since the sister chromatid is present only after DNA replication (5,6). It is possible that HR could use the homologous chromosome for DSB repair when cells are in the G1 phase of the cell cycle. However, this would invariably lead to loss of heterozygosity and may also result in translocations or other gene rearrangements (4). To avoid such a scenario, HR is not only controlled by DNA damage response signalling pathways (7) but also by the cell cycle and relies on a high cyclin-dependent kinase activity (8,9), present only in the S, G1 and M phases of the cell cycle. HR is suppressed in mitosis, for instance by inactivating phosphorylations on BRCA2 (10). Emerging data also suggest that a subset of DSBs present in the more condensed heterochromatin act as a substrate for HR in the G2 phase of the cell cycle (11).

Although NHEJ was initially believed to be a fairly simple ligation pathway of two ends, recent data suggest that this process is much more complex and is regulated in the DNA damage response (12), in particular in heterochromatin regions (13). Emerging data also show that there are subpathways of NHEJ that involve the usage of microhomology mediated (14) or a different backup NHEJ pathway active in NHEJ-defective cells (15). For more detailed information about NHEJ and HR in DSB repair, there are several recent reviews available (16–19).

The expanding universe of HR proteins

During the past decade, we have seen numerous reports identifying new proteins or pathways with functions in HR (Figure 2). It is clear that both the DNA damage response as well as the cell cycle checkpoints are critical for the onset and regulation of HR (7–10). Chromatin remodelling factors are also critical for efficient HR (20,21), as is the metabolism of HR-related proteins (22), an area that is poorly studied to date. The use of RNA interference screening techniques is rapidly increasing the numbers of proteins and pathways we know to be involved in regulating HR (C.Lundin and T.Helleday, unpublished data). Interestingly, many novel anticancer drugs have recently been shown to target HR, i.e. proteasome inhibitors (22), bcl-abl inhibitor imatinib (23), histone deacetylase inhibitors (24) and the heat shock protein 90 inhibitor 17-AAG (25). The efficiency of these inhibitors in the clinic is possibly related to their ability to inhibit HR as discussed below.

Primary substrates for HR are present at replication forks

The dogma is that HR is primarily involved in repair of two-ended DSBs that arise when cutting dsDNA (Figure 3). This is possibly true in lower eukaryotes and also during meiotic HR, where HR is induced by DSB-inducing proteins, such as Spo11 (33). There is experimental evidence suggesting that DSBs are not the primary substrate for HR in mitotic mammalian cells. Firstly, DSBs in mammalian cells trigger HR repair by gene conversion (34), whereas spontaneous HR, most probably occurring at replication forks, triggers repair via a sister chromatid exchange event (35,36). Support for single-strand breaks (SSBs) underlying spontaneous HR at replication forks is given by the findings of the increase in sister chromatid exchange and HR that are characteristic in SSB repair-defective cells (37–39). Secondly, although NHEJ is the primary repair pathway for DSB repair in mammalian cells, experiments show that knockout mice defective in NHEJ are viable (40). In contrast, mice defective in RAD51 or other major components of HR repair are embryonic lethal (41,42), which is explained by an accumulation of irreparable breaks produced after DNA replication (43). If lack of repair of two-ended DSBs were the cause of lethality in HR-defective animals, one might also expect lethality in NHEJ-defective animals. Thirdly, agents that trigger replication stress (stalled or collapsed replication forks), which may or may not involve DSB formation, are ~10-fold more potent in inducing HR than ionizing radiation (Table 1). Finally, HR-defective cells are highly sensitive to agents that cause replication blocks in spite of several of these blocks not being associated with DSBs. An example of this is the hypersensitivity of HR-defective cells to cisplatin or thymidine, neither producing detectible DSBs (51,54).

With the exception of radiotherapy and topoisomerase II inhibitors, most anticancer drugs produce primary DNA lesions that are not DSBs. These other classes of drugs include cross linkers, alkylators, topoisomerase I inhibitors, antimetabolites and replication inhibitors

**Abbreviations:** BRCA, breast cancer gene; DSB, double-strand break; HR, homologous recombination; NHEJ, non-homologous end-joining; PARP, poly(ADP-ribose) polymerase; SSB, single-strand break.
These drugs kill off cancer cells by producing toxic lesions when the cell attempts to replicate damaged DNA. The ability of these drugs to produce toxic lesions mostly to replicating cancer cells, while sparing non-replicating tissue, is important to explain their therapeutic index. Unfortunately, there is still limited information on the exact nature of the replication lesions formed by these classes of anticancer drugs. Some antimetabolites (e.g. 6-thioguanine) are incorporated into the DNA, methylated and recognized by the mismatch repair machinery in the second round of replication, thus inducing replication-associated DSBs (55). Methylation on the O6-position of guanine (O6meG), caused for instance by temozolomide, is highly toxic and generates a similar mismatch repair-dependent HR lesion (50,56,57).

Topoisomerase I inhibitors poison the topoisomerase I cleavage complex, which traps a SSB that is then converted into a one-ended DSB when a replication fork crashes into it (58). Cross linkers (bifunctional alkylators) cause both oncoming replication forks to stall at the cross link, which can then be collapsed into a DSB (27). Mono-functional alkylators arrest replication forks by causing alkylations on nitrogen residues in purines (P.Groth and T.Helleday, unpublished data).

HR-defective cells are highly sensitive to most DNA-damaging anticancer drugs that cause replication lesions, which is in contrast with NHEJ mutants that appear sensitive primarily to those anticancer drugs that induces DSBs (Table I).

HR in cancer development

Reactive oxygen species produced during a cell’s normal metabolism cause DNA damage that needs to be repaired to maintain genome integrity. It is found that oncogenes produce DNA damage at replication forks (replication stress) (59,60), which emphasizes the need for cancer cells to activate DNA repair. There are several conserved DNA repair pathways, often with overlapping functions, involved in removing DNA damage (61). DNA lesions that are not repaired can cause disruption during DNA replication, if such lesions cannot be bypassed by translesion synthesis. As mentioned earlier, HR is the most important pathway of repair of severe replication lesions at replication forks and has broad substrate specificity. For instance, DNA lesions remaining due to inactivation or loss of base excision repair, nucleotide excision repair or translesion synthesis can be repaired or bypassed by HR during replication. The HR repair pathway can be viewed as a last resort for DNA repair and tumours with mutated HR genes are often characterized by gross gene rearrangements (62). There are several pathways for HR repair that involve differential sets of HR proteins. For instance, HR repair by gene conversion critically depends on both BRCA2 and XRCC2 (63,64). In contrast, transcription-associated recombination depends only on BRCA2 and not XRCC2 (65), whereas spontaneous HR resulting in sister chromatid exchange is independent also of BRCA2 (66,67). There are several proteins involved in HR that are mutated in cancer, e.g. BRCA1 and BRCA2 in breast and ovarian cancer (68,69); RAD54 and CtIP in non-Hodgkin’s lymphoma and colon cancer (70,71); RAD51B in lipoma and uterine leiomyoma (72); RECQL4 in both basal and squamous cell skin carcinomas and osteosarcoma (73) as well as BLM, WRN and Nbs1 in other cancers (73–75). Although many of these mutations are rare, there are >200 proteins involved in different subpathways of HR (C. Lundin and T. Helleday, unpublished data). The genes encoding these proteins may be silenced or mutated in cancer, which probably disrupt a sub-HR pathway, but unlikely disrupt the whole...
HR in cancer development, therapy and drug resistance

For instance, primary ovarian cancers are often highly responsive to platinum-based therapy (e.g. cisplatin). It has been found that the sensitivity to such treatment can be correlated with decreased expression of HR proteins, e.g. BRCA1 or FANCF (78,79), or by mutations in BRCA1 or BRCA2 (80,81). Furthermore, cisplatin resistance in ovarian cancer cells is correlated with re-expression of FANCF (78,79), or by mutations in HR genes are probably to cause genetic instability and inactivate subpathways of HR. This type of genetic instability may be important to drive further genetic defects required in the aetiology of cancer.

**HR affects outcome of cancer treatment and drug resistance**

The exact nature of toxic lesions caused by many anticancer treatments at DNA replication forks is often poorly characterized. Different HR pathways are, however, important to repair a wide variety of toxic lesions at replication forks as discussed above. Inactivation of a HR pathway has a major impact on the survival of a cell and may result in a 300-fold increased sensitivity to an anticancer drug (Table I).

There is often a lack of understanding at the molecular level as to why some anticancer drugs are highly effective in treating certain subgroups of cancer. Considering the high number of HR proteins, it is probable that many cancers will have lost a subpathway of HR, which may result in some genetic instability that can drive further genetic changes required for cancer development. Thus, there is a possibility that the selective toxicity in a subgroup of cancers is linked with a specific HR defect.

For instance, primary ovarian cancers are often highly responsive to platinum-based therapy (e.g. cisplatin). It has been found that the sensitivity to such treatment can be correlated with decreased expression of HR proteins, e.g. BRCA1 or FANCF (78,79), or by mutations in BRCA1 or BRCA2 (80,81). Furthermore, cisplatin resistance in ovarian cancer cells is correlated with re-expression of FANCF (78) or genetic reversion of BRCA1 or BRCA2 mutations (80–82), emphasizing the importance of this pathway not only in response to therapy but also as a resistance mechanism (Figure 4). Similarly, etoposide (VP-16) is often used in treatments of small cell lung cancer, and it was found that high levels of the HR protein RAD51 correlated with increased repair of etoposide-induced damage and resistance in small cell lung cancer cells (86,87).

Several newly developed anticancer drugs are able to sensitize cancers to chemotherapy and improve the overall survival of the patients. Such combination treatments are being rapidly incorporated into clinical practice, often in spite of a lack of information about the detailed mechanism for chemosensitization. Since several of these drugs target HR [i.e. proteasome inhibitors (22), imatinib (23), histone deacetylase inhibitors (24) and heat shock protein 90 inhibitor 17-AAG (25)], there is a possibility that this may provide a molecular explanation for the efficiency of the proteasome inhibitor bortezomib in combination with melphalan and prednisone for treatment of multiple myeloma (85) (Figure 3). Indeed, repair of melphalan-induced cross links is reduced in presence of bortezomib (88).

**Synthetic lethality between HR pathways in cancer treatment**

The RAD51 protein is a key enzyme for HR and absolutely critical for cellular survival (43). However, loss of HR subpathways is compatible with viability, in particular when combined with mutations in genes encoding apoptosis-promoting proteins (e.g. TP53). If there is a mutation in a HR gene, other HR proteins may become more critical for survival in the mutated background, a concept described as synthetic lethality. Selective toxicity to cancer cells can be achieved by targeting such HR backup pathways, leading to a complete inactivation of HR and cell death (see ref. 89 for a comprehensive review on synthetic lethal approaches in cancer treatments). My research group was among

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**Table I. Sensitivity of HR and NHEJ cells to DNA-damaging anticancer treatments**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Relative sensitivity (fold sensitivity to wild-type)</th>
<th>Fold induction of HR in the hprt gene</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR-defective cells</td>
<td>NHEJ-defective cells</td>
<td></td>
</tr>
<tr>
<td>Ionizing radiation</td>
<td>1.5–2</td>
<td>3–4</td>
<td>1.5–2</td>
</tr>
<tr>
<td>Cross linkers (bifunctional alkylators)</td>
<td>50–300</td>
<td>1–5</td>
<td>4–10</td>
</tr>
<tr>
<td>Topoisomerase I inhibitors</td>
<td>10</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>Topoisomerase II inhibitors</td>
<td>2–10</td>
<td>10</td>
<td>10–15</td>
</tr>
<tr>
<td>Monofunctional alkylators</td>
<td>5–20</td>
<td>1–1.5</td>
<td>5–20</td>
</tr>
<tr>
<td>Antimetabolites</td>
<td>10</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Replication inhibitors</td>
<td>5–10</td>
<td>1–2</td>
<td>10–30</td>
</tr>
<tr>
<td>PARP inhibitors</td>
<td>20–1000</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

n.d, not determined.
those that pioneered this novel approach for anticancer therapy, by describing that PARP inhibitors can selectively kill BRCA1- and BRCA2-defective tumours (52,53) and these have recently shown very promising results in clinical phase II treatments (90). The example of PARP inhibitors working in monotherapy to treat BRCA1- and BRCA2-defective tumours is the first example of a successful synthetic lethal approach in the clinic. The critical question is whether the synthetic lethal approach is more widely applicable? To answer this, it is important to understand the molecular mechanism of how PARP inhibitors kill BRCA1/2 tumours. The current hypothesis is that the role of PARP in DNA SSB repair underlies the synthetic lethality. Unrepaired SSBs in PARP inhibited cells may be converted into toxic DSBs during replication, which would not be repaired in the absence of BRCA1 or BRCA2 and result in cell death (52). However, recent data show that PARP1 is involved also in HR repair at replication forks (91). The combined role of PARP1 in HR and SSB repair probably explains the exceptionally strong synthetic lethal interaction between PARP and BRCA1/2. In conclusion, I believe similarly strong synthetic lethal interaction as the one between PARP–BRCA and that can be used as monotherapy are probably present but that these are rare. On a more positive note, I think the concept of synthetic lethality will be more widely applicable in combination therapies either with current chemotherapy or with other selective inhibitors.

**Future directions**

It is clear that HR has a major impact on cancer at several levels, most importantly in determining the efficiency of anticancer therapies. There is an urgent need to understand more about HR subpathways and how they are distinct from one another. Identification of proteins involved in these distinct HR pathways through large-scale RNA interference screens is very important, as is more in-depth analysis of the functions of proteins, to enable development of better HR repair models. The extent of HR defects in cancer need to be determined and new cancer-specific synthetic lethal interactions characterized. Although such synthetic lethal interactions may not be sufficiently strong to work in monotherapy, the increased therapeutic index could significantly improve chemotherapy combination treatments. Finally, we need better and more targeted inhibitors of HR to fully validate HR and its subpathways as relevant cancer targets.

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References

57. Roos, W.P. et al. (2009) Brca2/Xrcc2 dependent HR, but not NHEJ, is required for protection against O(6)-methylguanine triggered apoptosis, DSBs and chromosomal aberrations by a process leading to SCEs. DNA Repair (Amst.), 8, 72–86.

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