Alternative pathways of non-homologous end joining (NHEJ) in genomic instability and cancer

Rositsa Dueva, George Iliakis

Institute of Medical Radiation Biology, University of Duisburg-Essen Medical School, Essen, Germany

Corresponding to: George Iliakis. Institute of Medical Radiation Biology, University of Duisburg-Essen Medical School, Essen, Germany. Email: georg.iliakis@uk-essen.de.

Abstract: An incompletely defined alternative non-homologous end joining pathway (A-NHEJ) frequently functioning as backup has recently been shown to process DNA double strand breaks (DSBs) when canonical cellular DSB repair activities are somehow compromised. While A-NHEJ offers survival advantage for the damaged cell, its propensity for large sequence alterations at the junction and the relatively frequent joining of unrelated DNA ends generates alterations in the genome that can have severe biological consequences. Indeed, evidence accumulates that A-NHEJ is a driver of the chromosomal rearrangements and the overall genomic instability that underpin the development of certain forms of cancer. Features of A-NHEJ causally related to these unfavorable outcomes are the apparent promiscuity in utilized factors and the delayed repair kinetics. The purpose of this review is to briefly summarize recent advances in our understanding regarding the function of A-NHEJ and to review emerging evidence for its role in oncogenic transformation.

Key Words: DNA repair; non-homologous end joining (NHEJ); alternative non-homologous end joining pathway (A-NHEJ); homologous recombination repair; double strand breaks; genomic instability; cancer

Background on DSB repair

DNA double-strand breaks (DSB) are generated in a random manner in the genome by exogenous agents, such as ionizing radiation (IR) or radiomimetic drugs. Endogenous events leading to accidental DSBs also include oxidative damage, replication fork collapse and telomere erosion. DSBs also occur as programmed events during meiosis, as well as during V(D)J recombination and class switch recombination (CSR) required for immunoglobulin diversity and function. Accidental or programmed DSBs, if left unrepaired or if repaired in an erroneous manner, can have severe adverse consequences for the genome including the generation of mutations and chromosomal aberrations. Both forms of genomic alterations are implicated in cell death (1,2), as well as in genomic instability leading to the development of cancer (3).

To maintain genomic integrity, cells have evolved several pathways to process DSBs and mitigate their adverse consequences. The two key DSB repair pathways engaged to this task are non-homologous end joining (NHEJ) and homologous recombination repair (HRR) (4-7).

The classical or canonical form of NHEJ is a very fast process operating with half times of 10-30 min. It functions by simply joining the DNA ends and has no build-in potential of restoring the original sequence in the vicinity of the DSB (Figure 1). Essential components of NHEJ are the three subunits of the DNA-PK complex (Ku70, Ku80, DNA-PKcs), and the LIG4/XRCC4/XLF complex (8-12). As this pathway relies on the evolutionarily new DNA-PKcs, it is sometimes termed as D-NHEJ. However, the term classical or canonical NHEJ (C-NHEJ) is more frequently used and is also adopted here [reviewed in (7,13,14)].

C-NHEJ is error-prone on two counts: first, it has no build-in mechanisms ensuring the restoration of the original DNA sequence in the vicinity of the DSB. As a result, it
is associated with sequence alterations including random exchanges of nucleotides, as well as the addition or deletion of several base pairs at the junction. These events are more likely to occur during repair of radiation-induced DSBs, due to the end-processing requirement for the generation of ligatable DNA ends. Second, C-NHEJ has no built-in mechanisms ensuring the restoration of the original DNA molecule and can in principle join any DNA ends irrespective of molecular origin. As a result, the generation of new sequence-combinations is possible, which in higher eukaryotes can manifest as chromosomal translocations. Possibly as a direct consequence of its operational speed, C-NHEJ is associated with more limited sequence alterations at the junction and has a lower probability of translocation formation than end-joining pathways operating with slower kinetics (see below). As a result, C-NHEJ is considered a guardian of genomic stability and suppressor of carcinogenesis (15-17). The molecular determinants underpinning the high speed of C-NHEJ remain uncharacterized.

In contrast to C-NHEJ, HRR operates with slower kinetics and requires a homologous template to not only repair the DSB, but to also restore the sequence around the break. Higher eukaryotes use the sister chromatid as a homologous template, and as a direct consequence HRR is restricted to the S and G2 phases of the cell cycle. Initial step in HRR is DNA nucleolytic end resection by the MRN complex (comprising Mre11, Rad50, Nbs1) along with other accessory proteins such as CtIP and the tumor suppressor protein BRCA1. An eminent role for the long-range resection have the Bloom helicase (BLM), Exonuclease 1 (Exo1) and Dna2 helicase/nuclease (Figure 2) (23-25). Thereby, terminal nucleotides in the 5’ ends are removed generating long 3’ single-stranded DNA (ssDNA) overhangs on both sides of the break. These 3’-ssDNA tails, representing the substrate for HR repair machinery, are coated and stabilized by the Replication protein A (RPA) complex, which subsequently becomes displaced by Rad51 recombinase generating Rad51 nucleoprotein filament. After homology search, strand invasion with the donor DNA and D-loop formation, a polymerase catalyzes DNA synthesis until finally the Holliday junctions become resolved, resulting in a crossover or non-crossover product (for a detailed description of this process and the participating proteins see in Figure 2) (22).

In addition to C-NHEJ and HRR, recent studies demonstrate the operation of a third pathway of DSB processing, functioning on simple end-joining principles, but repairing DSBs slower (t_{50} 30 min to 20 h) than C-NHEJ (9,16,26-28). This repair pathway is considered to be an alternative form of NHEJ and is frequently abbreviated as A-NHEJ, or simply A-EJ (29). Since A-EJ is suppressed by C-NHEJ, and possibly also by HRR, and gains functional relevance when these standard repair processes fail, globally or locally, it is also considered to be a backup pathway and has been abbreviated as B-NHEJ (6,7). Throughout this review we will use the term A-EJ to refer to this repair pathway.

Although A-EJ does not require homology for function, as for example HRR does, it is occasionally facilitated by microhomologies fortuitously found at the DNA ends, particularly when resection and the generation of single stranded DNA regions precedes end-joining. The form of
The mechanism of homologous recombination repair (HRR). HRR requires a larger set of proteins than C-NHEJ. Once a DSB is shunted into HRR, end resection is initiated by the MRN complex and other accessory proteins including CtIP, BRCA1, BLM and Exo1. In this step, terminal nucleotides in the 5’ ends are digested, resulting in 3’ single-stranded DNA (ssDNA) overhangs, which are then covered by the Replication Protein A (RPA) trimer. RPA not only stabilizes ssDNA by preventing secondary structure formation, but also protects it from nucleolytic cleavage. RPA mediates the formation of Rad51 nucleoprotein filament, which forms the presynaptic complex with the aid of additional factors such as the tumor suppressor BRCA2, the recombination mediator Rad52 and the Rad51 paralogs (Rad51-B, Rad51-C, Rad51-D, XRCC2 and XRCC3) (18). Rad51 catalyzes homology-search, strand-pairing and strand-exchange, supported by Rad54, which has branch-migration activity. In this later step of HRR DNA synthesis begins, with the donor sequence serving as a template and Rad51 being removed from the DNA. Two models of DSB repair by HRR are shown: Synthesis-Dependent Strand Annealing (SDSA) and Double-Strand Break Repair (DSBR). During SDSA, one 3’-ssDNA participates in the formation of a single Holliday junction. In contrast, DSBR engages both 3’-overhangs resulting in a double Holliday junction. Finally, enzymes termed resolvases, such as GEN1, MUS81/EME1, SLX1-SLX4, complete the process by resolving the Holliday junctions (19-21) and the DNA sequence around the DSB is restored with or without cross-over (22).
DSB processing utilizing microhomologies is frequently termed microhomology-mediated end-joining (MMEJ). The occasional use of microhomologies in this repair pathway makes A-EJ a term semantically preferable to A-NHEJ. In the following discussion we consider MMEJ as a subset of A-EJ and refer explicitly to it only if it is relevant to the discussed context [reviewed in (7,29-32)].

A-EJ, as C-NHEJ, is error prone on two counts: it has no build-in mechanisms for restoring the DNA sequence in the vicinity of the DSB, and can catalyze the joining of unrelated DNA molecules, leading thus to the formation of translocations (7,16,32-34). On both counts, A-EJ is more error prone than C-NHEJ, i.e., sequence alterations at the junctions are more frequent and more extensive and the probability of translocation formation is much higher. As a result, and in contrast to C-NHEJ that is considered as a guardian of genomic stability, A-EJ is considered a major source of genomic instability (17,35-37).

The above outlined basic features of C-NHEJ, A-EJ and HRR indicate distinct functional characteristics, distinct possible outcomes during normal operation, and widely different risks for errors. These large and fundamental differences make the question of repair pathway choice for the processing of each individual DSB difficult to answer. Correct repair is only afforded by HRR, but is only available in a fraction of the cell cycle. C-NHEJ functions throughout the cell cycle and removes DSBs with high efficiency at the cost of small sequence alterations and a low probability for translocations. Finally, A-EJ functions throughout the cell cycle but is clearly functionally enhanced in S and G2 (38-42); it has the highest probability for generating translocations, as well as large deletions and other sequence alterations at the junction. Such dramatic differences in features, outcomes and risks make simple competition an “unwise” mechanism for pathway selection and suggest that undefined parameters underpin repair pathway choice. Such candidate parameters are presently hotly debated, but the issue remains for the most part unresolved.

In the following sections, we review the known enzymatic requirements of A-EJ and discuss emerging evidence for the involvement of this pathway in the generation of genomic instability and the development of cancer.

### Core components of A-EJ

Several factors have been implicated in A-EJ (Figure 3) and their functional diversity has led to the postulate that there are several sub-pathways in operation, engaging prospectively at each DSB on the basis of as of yet undefined parameters in competition with other repair pathways (Figure 4A). A different model emerges by regarding all functions of A-EJ as backup operations that are initiated at DSBs only after C-NHEJ or HRR have engaged but failed to successfully complete processing (Figure 4B). According to the former model, the recruitment at the ends of factors of a given repair pathway will determine the processing of the DSB by this pathway. However, according to the second model, A-EJ will engage at DSBs where either C-NHEJ or HRR have attempted processing but somehow failed. Thus, at each DSB where A-EJ engages, factors of either C-NHEJ or HRR have attempted processing but somehow failed. Thus, at each DSB where A-EJ engages, factors of either C-NHEJ or HRR, particularly those involved at early steps, will be present when A-EJ takes DSB processing over. Also, it is possible, and even likely, that these factors have already operated at DNA ends and have carried out one or more of the initial steps of C-NHEJ or HRR, which of course alters the state of the substrate presented to A-EJ. Furthermore, the presence of C-NHEJ and HRR factors at the DNA ends may either facilitate or compromise A-EJ. In the following paragraphs, we review the function of A-EJ and the factors implicated from this mechanistic/operational perspective.

When the engagement of A-EJ follows failure of...
C-NHEJ, several of the early C-NHEJ factors may be present at the junction, but the process must be abrogated prior to ligation by LIG4. Available evidence suggests that under these conditions, end ligation is possible with one of the remaining ligases, LIG3 and LIG1 (43-48). While LIG3 is likely to be more effective, LIG1 is also remarkably fit for this function (48). Since LIG3 operates in other repair pathways together with XRCC1 and PARP1, its involvement in A-EJ implicates these factors in A-EJ as well. PARP1 is a sensor for DNA discontinuities, originally shown to operate in base excision and single-strand break repair (49). Previous work implicated PARP1 also in repair by A-EJ (46,50-53). There is even evidence for competition between Ku and PARP1 for DSBs (50,54,55) raising the possibility that pre-existing C-NHEJ factors at the DSB compromise A-EJ.

DNA end stabilization provided in C-NHEJ by Ku may be provided in A-EJ by histone H1 (56). However, it should be emphasized that to date the evidence for a role of histone H1 in A-EJ is of purely biochemical nature. Backup of C-NHEJ by A-EJ is likely to be associated with increased processing at the ends, and to result in the more frequent use of microhomologies. This form of backup function can occur in all phases of the cell cycle, because C-NHEJ is active throughout the cell cycle.

However, it is also possible that A-EJ backs up failures of HRR. Such events are possible only when HRR is active, i.e., in the S- and G2-phase of the cell cycle. It will also pertain only for the subset of DSBs that are processed by this repair pathway, and which according to current estimates lies between 10-20% of radiation induced DSBs (57,58). Under these conditions A-EJ may operate on resected ends that must be generated in order to inactivate C-NHEJ and allow end resection to prepare for HRR. This may explain the observed dependence of A-EJ on the MRN complex as well as CtIP, BRCA1 et al (45,59-65). Also, as a result of the processed ends likely to be present, it is possible that microhomologies fortuitously present are used as means of intermolecular stabilization of the two DNA ends to efficiently complete rejoining (29). However, end joining using microhomologies was also reported in vitro in reactions setup with cell-free extracts (66), and has also been described between repetitive elements in the human genome through single-strand annealing leading to genome rearrangements (67).

A-EJ is considered to be a mechanistically distinct repair pathway, and has been shown to be active throughout the cell cycle (7,16,31,33,68). Notably though, it is markedly enhanced in the G2 as compared to G1 phase, and is compromised in stationary-phase cells tested either in the G1 or G2 phase of the cell cycle (31,41,42,69). There are speculations that the latter response may be regulated by phosphorylation of BRCA1 at S988 through Chk2, where in its phosphorylated form BRCA1 promotes error-free NHEJ and suppresses mutagenic A-EJ. Therewith, it reduces the size of deletions at the breakpoint junction (65,70-72). However, this dependency is more likely in G2 than in G1 cells as BRCA1/CtIP/MRN initiates DSB resection during S/G2 phases (73), and therefore alternative mechanisms should be explored.

**A-EJ and the development of leukemias**

Chromosomal translocations are a type of rearrangements, where parts of two different non-homologous chromosomes fuse together. Such types of chromosomal abnormalities have been described in cancer, mainly in leukemia and many types of lymphoma (34,74-77). Several lines of evidence implicate A-EJ to chromosome translocation formation that underlie leukemia and lymphoma (36,37,78,79). Since
these cancers frequently originate from erroneous repair of programmed DSBs generated during V(D)J and CSR recombination, we briefly review these processes before summarizing the available evidence for role of A-EJ in their formation.

\( \text{V(D)J recombination, also known as somatic recombination, and class switch recombination (CSR) are fundamental physiological mechanisms for the generation of the immune repertoire in mammals and for the development and survival of B and T cells. In both processes DNA DSBs occur as programmed events and are necessary for triggering recombination.} \)

\( \text{V(D)J recombination aims the generation of different combinations of pre-existing gene segments: variable (V), diversity (D) and joining (J). The first step is cleavage within specific recombination signal sequences (RSSs) by the lymphocyte-specific endonucleases RAG1 and RAG2, operating as a complex called RAG. These segments are rearranged next to each other during the resolution of these DSBs that are rejoined almost exclusively by C-NHEJ resulting in a V(D)J segment [for a review see (80-85)].} \)

\( \text{CSR is a process occurring in antigen-stimulated mature B cells, which changes the antibody production from one immunoglobulin (Ig) class or subclass (isotype) to another— from IgM to IgG, IgE or IgA. The constant regions of different Ig isotypes are encoded by distinct C\text{\textsubscript{H}} exon clusters in the immunoglobulin heavy chain locus. CSR is initiated by activation-induced cytidine deaminase (AID), which deaminates cytidines to uridines within switch (S) regions. The ensuing cascade of repair reactions leads to the generation of DSBs. Deleting sequences between the S regions leads to expression of a new constant region, and thus to the production of an antibody of different class. Hence, during CSR the heavy chains are altered but the variable regions remain unchanged and the antibody retains its antigen specificity [for a detailed review see (86,87)].} \)

\( \text{Several NHEJ factors have already been implicated in V(D)J recombination (88-92). However, evidence exists that the essential component of C-NHEJ, Ku70, is dispensable for T cell antigen receptor (TCR) V(D)J recombination, suggesting the involvement of other repair pathways (93).} \)

\( \text{Deficiencies in the immune system development, particularly lymphocytes, lead to severe combined immunodeficiency syndrome (SCID), which is often associated with mutations in DNA repair proteins (94-99). Notably, the SCID phenotype is observed in mice deficient in any of C-NHEJ proteins (100,101), as well as in mice lacking one of the RAG proteins (81,102,103). These mice also frequently develop tumors with translocations involving the Ig locus that are now known to be generated by A-EJ (see below). A model was proposed, where RAG1/2 proteins together with C-NHEJ factors suppress A-EJ mediated genomic instability during V(D)J recombination (104,105). This result could also be reproduced in a plasmid model system (106).} \)

\( \text{It is relevant to point out here that RAG post-cleavage complex shunts the broken DNA ends to C-NHEJ, thus suppressing aberrant recombination events. In compromised V(D)J recombination, through mutations destabilizing the post-cleavage complex, the ends are free to participate in HRR or A-EJ repair leading to CSR but also to genomic instability (104,105,107-109).} \)

\( \text{Although RAG-mediated DSBs during V(D)J recombination are predominantly rejoined by the C-NHEJ pathway, repair of DSBs in the switch (S) regions during CSR is not largely compromised in C-NHEJ deficient cells and a direct shift of processing to A-EJ is observed (110-112). CSR is not affected by the absence of DNA-PKcs (113,114), and is likely to use Lig1 or Lig3 (44). Taking into account the requirement of Ku for CSR (115,116), these findings strongly suggest the use of A-EJ during CSR.} \)

\( \text{As mentioned above, A-EJ frequently joins IgH locus breaks to breaks in genes such as c-myc in other chromosomes generating translocations and causing leukemias. This has been observed in the combined absence of Ku70 and Lig4, as well as in the absence of XRCC4 (36,110). Besides normal CSR frequency, B cells heterozygous for XRCC1 also show reduced IgH/c-myc translocations during CSR implicating XRCC1 in A-EJ (117). An interesting observation is that this proposed factor for A-EJ, XRCC1, which acts in a complex with Lig3, is not required for A-EJ during CSR and its absence even slightly increases CSR efficiency (118,119). Similar results were also obtained with mouse cells deleted of different forms of LIG3 (120). Also PARP1 and PARP2 do not seem to be required for CSR. However, PARP1 was shown to favor A-EJ and PARP2 to suppress translocations during CSR (121). Interestingly, in L29µB cell lymphoma and splenic B cells, PARP inhibitors lead to increased antibody class switching (122). It appears therefore that the context of the DSB determine the requirements for components such as PARP1 and XRCC1. In this regard, DSB repair by PARP1-dependent/Ku-independent EJ is more efficient in the presence of microhomology termini containing G:C base pairs (123,124).} \)

\( \text{An A-EJ pathway using microhomologies has been invoked in a general model of formation of oncogenic} \)
complex translocations (complicons). Pro-B lymphomas in mice lacking both p53 and classical NHEJ contain complicons that co-amplify c-myc sequence in chromosome 15 and IgH in chromosome 12 associated with microhomology at the translocation junctions (125). Furthermore, deficiency of C-NHEJ component XLF/Cernunnos is also associated with human B cell malignancies, where CSR junctions are also characterized by long microhomologies (126).

The expression of oncogenic BCR-ABL gene fusion is a result of reciprocal translocation t(9;22) and is predominantly associated with chronic myelogenous leukemia (CML). BCR-ABL tyrosine kinase facilitates cell division and results in increased reactive oxygen species (ROS), which in turn lead to increased DNA damage including DSBs (127,128). Moreover, BCR-ABL-positive CML is associated with up-regulated A-EJ (129).

The BCR-ABL translocation is often associated with microhomologies at the junctions and with interspersed repeats (130). Furthermore, in BCR-ABL-positive CML cells key proteins of C-NHEJ, Artemis and Lig4, are down-regulated. In contrast, the levels of proteins involved in A-EJ, Lig3$\alpha$ and WRN, are elevated. Additionally, depletion of either Lig3$\alpha$ or WRN results in decreased end-joining efficiency. The authors suggest therefore that A-EJ enables CML cells to repair ROS-induced DSBs and survive. Since A-EJ is error-prone, the survival is associated with increased genomic instability and disease progression (131).

The most common mutations in acute myeloid leukemia (AML) are internal tandem duplications (ITD) of FMS—like tyrosine kinase-3 (FLT3) receptor, known as FLT3-ITD. Cells expressing FLT3-ITD and bone marrow mononuclear cells from FLT3-ITD knock-in mice utilize microhomology-mediated A-EJ during translocation formation (130). Additionally, the level of Lig3$\alpha$ in FLT3-ITD-expressing cells is up-regulated and the protein level of the C-NHEJ component Ku is decreased, indicating that the FLT3 signaling pathway shifts DSB repair toward A-EJ (132).

But how is C-NHEJ suppressing chromosome translocations? In a model for DSB rejoining of correct ends, DNA-PKcs has a key role: together with Ku, DNA-PKcs improves the interactions between the participating proteins generating thus some form of molecular rejoining machine (16,133,134). Additionally, the local chromatin structure is altered and the correct ends are rapidly captured within this machine. In contrast, the evolutionarily older and slower A-EJ is using proteins that fail to undergo efficient intermolecular interactions, reducing thus efficiency and speed of repair and increasing the probability of end resection and exchange formation (16).

This model is supported by the observation that the C-NHEJ protein Ku80 ensures positional stability of broken DNA ends (135) and suppresses chromosomal aberrations in mouse cells (136). Along this line, cells deficient in Ku70 display increase in reciprocal translocations (137).

Moreover, a detailed work by Jasin et al. implicates A-EJ as a main pathway for chromosomal translocations in mammalian cells. The C-NHEJ component XRCC4-Lig4 suppresses A-EJ-mediated translocation formation in wild-type cells. The authors found longer microhomologies at the junctions in XRCC4$^{+/+}$ cells, and that DSB repair causing translocations does not rely on XRCC4 (37). These observations are further supported by the requirement of Lig3 for microhomology-mediated EJ during translocation formation (44). Finally, there are speculations about a second alternative of EJ, which acts independently of microhomologies and utilizes Lig1 (44). An interesting observation is that the high level of genomic instability of bladder cancer is a consequence of microhomology-mediated end-joining of DSBs, which is likely a main repair pathway in bladder cancer cells (138).

Thus, A-EJ is more translocation prone, probably due to its mechanistic basis and its slower kinetics, and therewith responsible for genomic instability and cancer development.

The function of A-EJ in telomere maintenance

If not protected, chromosome ends (telomeres) are likely to be recognized as broken DNA ends and to elicit DNA damage response (DDR) (139,140). As a direct consequence of the initiated DDR, different chromosomes can fuse end-to-end by NHEJ and less frequently by homology-mediated repair, resulting in dicentric chromosomes, which are unstable during mitosis. Similar unfavorable effects are initiated when the chromatids of one chromosome join to form a sister union that can cause anaphase bridges leading to genomic instabilities or cell death.

To escape this problem, the ends of eukaryotic chromosomes are protected by telomeres, which also circumvent the sequence loss problem associated with semi-conservative DNA replication. Telomeres are nucleoprotein structures, consisting of long stretches of TTAGGG repeats in humans and the telomere-specific protein complex, shelterin. The key enzyme in telomere maintenance, which adds telomeric repeats to the 3' end of DNA strands, is the telomerase. Moreover, end protection benefits from the unique structure of the telomere,
A novel and promising therapeutic strategy is the use of PARP1 inhibitors to improve the therapy of cancers with BRCAness (152). Similarly, PARP1 inhibitors could be implicated in the therapy of cancers associated with increase in the use of A-EJ pathway for DSB repair.

Attractive therapeutic targets are also the DNA ligases, which complete the process by rejoining the DNA ends (153). As already described above, the levels of two proteins involved in A-EJ, Lig3α and Werner syndrome helicase (WRN), are up-regulated in BCR-ABL-positive CML cells (131). Since BCR-ABL-positive CML is treated by the tyrosine-kinase inhibitor Imatinib (Gleevec), the inhibition of A-EJ factors reveals a novel and more effective therapeutic approach. A recent study from Tobin and colleagues reported that BCR-ABL-positive CML cells resistant to Imatinib were hypersensitive to the combined treatment of Ligase and PARP inhibitors, correlating with hyperactive A-EJ. Furthermore, elevated levels of Lig3α and PARP1 in CML patients were proposed to be biomarkers for therapies targeting A-EJ components when treatment with tyrosine kinase inhibitors is ineffective (154). This novel therapeutic approach could also be applied to therapy-resistant breast cancer cell lines, which were shown to be sensitive to DNA ligase and PARP inhibitors (155). Similarly, new therapeutic strategies for AML associated with FLT3 mutations may also include Lig3α or PARP1 inhibitors (132).

Concluding remarks

The involvement of A-EJ in genome instability and cancer development is indisputable. However, the mechanisms balancing C-NHEJ and A-EJ in healthy cells remain unknown and require in-depth study.

Parameters and proteins having positive and negative effects on A-EJ are depicted in Figure 5. As already outlined above, Ku ensures positional stability of broken DNA ends (135) and competes with PARP1 for DSB repair (50,54,55). In addition, Ku is considered the main determinant of the choice between C-NHEJ and A-EJ in human somatic cells, preferring to assist C-NHEJ (156). Another DNA damage response factor, 53BP1, also favors CSR through C-NHEJ by preventing DNA end resection and A-EJ (157,158).

<table>
<thead>
<tr>
<th>Parameters with positive effects on A-EJ</th>
<th>Parameters with negative effects on A-EJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microhomologies</td>
<td>general factors</td>
</tr>
<tr>
<td>Ligase1 / Ligase3</td>
<td>53BP1, H2AX</td>
</tr>
<tr>
<td>Histone H1</td>
<td>PARP2, Ku, DNA-PKcs</td>
</tr>
<tr>
<td>PARP1</td>
<td>XRCC4-Ligase4</td>
</tr>
<tr>
<td>BRCA1/CtIP/MRN</td>
<td>specific factors</td>
</tr>
<tr>
<td>WRN</td>
<td>RAG1/2</td>
</tr>
<tr>
<td></td>
<td>TRF2</td>
</tr>
</tbody>
</table>

A-EJ factors as targets for cancer therapy

The presence of these proteins in the telomeric regions suggests that in case of telomere erosion, end-joining by C-NHEJ may ensue. However, using a telomerase-deficient mouse model, Maser and colleges reported that fusion of critically shortened telomeres does not depend on the C-NHEJ components DNA-PKcs and Lig4, and suggested that A-EJ has a major role (149). Other reports suggest that dysfunctional telomeres may be processed for joining by both NHEJ pathways, although A-EJ may be preferentially functional on naturally shortened telomeres (150).

Attractive therapeutic targets are also the DNA ligases, which complete the process by rejoining the DNA ends (153). As already described above, the levels of two proteins involved in A-EJ, Lig3α and Werner syndrome helicase (WRN), are up-regulated in BCR-ABL-positive CML cells (131). Since BCR-ABL-positive CML is treated by the tyrosine-kinase inhibitor Imatinib (Gleevec), the inhibition of A-EJ factors reveals a novel and more effective therapeutic approach. A recent study from Tobin and colleagues reported that BCR-ABL-positive CML cells resistant to Imatinib were hypersensitive to the combined treatment of Ligase and PARP inhibitors, correlating with hyperactive A-EJ. Furthermore, elevated levels of Lig3α and PARP1 in CML patients were proposed to be biomarkers for therapies targeting A-EJ components when treatment with tyrosine kinase inhibitors is ineffective (154). This novel therapeutic approach could also be applied to therapy-resistant breast cancer cell lines, which were shown to be sensitive to DNA ligase and PARP inhibitors (155). Similarly, new therapeutic strategies for AML associated with FLT3 mutations may also include Lig3α or PARP1 inhibitors (132).
Besides proper DNA repair, the maintenance of genome integrity requires checkpoint activation. In this regard, ATM was shown to prevent prolonged presence of DSBs and chromosomal translocations in lymphocytes (162). ATM is also directly involved in RAG-induced DSB repair ensuring stable DSB complexes and preventing aberrant rearrangements (163). Possible connections between checkpoint proteins and A-EJ regulation remain to be established.

In the front of cancer treatment, the possibility of combining inhibitors of A-EJ with other treatment modalities to improve the outcome, at least in tumors with enhanced A-EJ, has strong rationale and is likely to see application in the future. Finally, the intriguing possibility of protecting organisms from carcinogenesis by limiting the function of A-EJ should be considered and tested.

Acknowledgements

Work supported by grants from the DFG (GRK1739), the “Bundesministerium für Bildung und Forschung” (BMBF: 02NUK005C and 03NUK001B) and the “Bundesministerium für Wirtschaft und Technologie” (BMWi: ESA-AO-08-IBER, 50WB1229).

Disclosure: The authors declare no conflict of interest.

References

15. Burma S, Chen BP, Chen DJ. Role of non-homologous end joining (NHEJ) in maintaining genomic integrity. DNA Repair (Amst) 2006;5:1042–8.
76. Elliott B, Jasin M. Double-strand breaks and translocations...


129. Sallmyr A, Tomkinson AE, Rassool FV. Up-regulation


