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Environmental Stress Induces Trinucleotide Repeat Mutagenesis In Human Cells By Alt-Nonhomologous End Joining Repair

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Abstract

Multiple pathways modulate the dynamic mutability of trinucleotide repeats (TNRs), which are implicated in neurodegenerative disease and evolution. Recently, we reported that environmental stresses induce TNR mutagenesis via stress responses and rereplication, with more than 50% of mutants carrying deletions or insertions—molecular signatures of DNA double-strand break repair. We now show that knockdown of alt-nonhomologous end joining (alt-NHEJ) components—XRCC1, LIG3, and PARP1—suppress stress-induced TNR mutagenesis, in contrast to components of homologous recombination and NHEJ, which have no effect. Thus, alt-NHEJ, which contributes to genetic mutability in cancer cells, also plays a novel role in environmental stress-induced TNR mutagenesis.

Graphical Abstract

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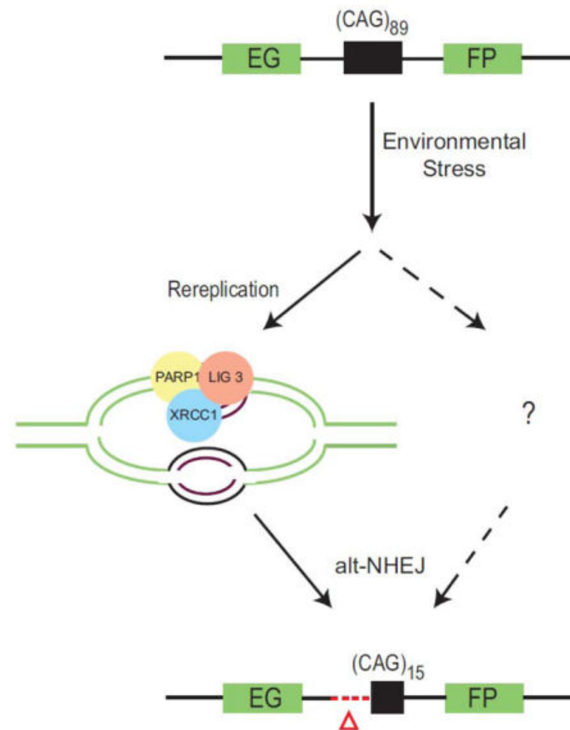

Keywords

Environmental Stress; Trinucleotide Repeats; Mutagenesis; Double-Strand Break Repair (DSBR); Alt-Nonhomologous End Joining (Alt-NHEJ)

Previously, we showed that four different environmental stresses—cold, heat, hypoxic and oxidative—induce trinucleotide repeat (TNR) mutagenesis in human cells via a pathway that involves stress response factors (SRF's) and DNA rereplication [1]. Other pathways known to modulate TNR instability—transcription, mismatch repair, nucleotide excision repair, and base excision repair—are not involved in environmental stress-induced TNR mutagenesis, consistent with the known repression of these processes during stress [1–5]. The mechanism by which the stress response and DNA rereplication bring about TNR mutagenesis remains unclear. Because about 50% of the CAG tracts in these stress-induced mutants harbored indels—the molecular signature of repair of double-strand breaks (DSBs)—we asked whether a DSB repair (DSBR) pathway might process repeats during environmental stress [1].

DNA DSBs in mammalian genomes are genotoxic lesions that are repaired by two major DSBR pathways: error-free homologous recombination (HR) and error-prone nonhomologous end joining (NHEJ) [6, 7]. Recent studies have established two pathways for NHEJ: the classic NHEJ (c-NHEJ) pathway that joins DNA ends together after minimal processing; and the so-called alternative NHEJ (alt-NHEJ) pathway that depends on end resection to expose short sequence homologies. Each of these DSBR pathways is defined genetically by the requirement for a specific set of genes [6–8].

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To test which of these DSBR pathway might play a role in environmental stress-induced TNR mutagenesis, we knocked down key components of each pathway and measured the effects on stress-induced mutagenesis of a CAG repeat tract in a chromosomal copy of a GFP gene (Figure S1). To test the HR pathway, we knocked down BRCA1 and RAD51, which promotes the essential step of strand pairing. To test c-NHEJ, we knocked down XRCC4 and LIG4, which form a complex that is essential for ligating the DNA ends together in this pathway. To test alt-NHEJ, we knocked down PARP1, XRCC1, and LIG3, three essential components of this pathway. We also knocked down RAD50, which as part of the MRN complex is required for end resection, an essential step for both HR and alt-NHEJ [9]. To knock down gene expression, we used the siRNAs listed in Table S1; each gave more than 75% mRNA knockdown. We then measured the frequency of GFP+ cells at day 3 of recovery from cold, heat, and hypoxic stress by flow cytometry [1] (Supplement). Knockdown of all the components for alt-NHEJ—PARP1, XRCC1, LIG3, and RAD50—significantly reduced the frequency of GFP+ mutants in stressed cells (Figure 1).

These results suggest that alt-NHEJ is the cell's principal way of processing DSBs that arise near TNR repeats during environmental stress. The source of the DNA DSBs may be DNA rereplication, which we previously implicated in the pathway of stress-induced CAG repeat mutagenesis [1], but that remains to be determined. The involvement of alt-NHEJ in stress-induced TNR mutagenesis is consistent with previous observations that hypoxic stress represses HR and c-NHEJ [3]. Interestingly, the key changes observed during stress-induced repeat mutagenesis in human cells—overexpression of stress response factors, DNA rereplication, and alt-NHEJ—are also seen in the tumor microenvironment, which drives the mutagenesis and metastatic evolution of human cancers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Research Highlights

- Environmental stresses induce TNR mutagenesis by rereplication in human cells
- siRNA knockdown was used to define repair pathways for the assumed DSB intermediate
- Knockdown of Rad51 and BRCA1 had no effect, ruling out homologous recombination
- XRCC4 and LIG4 knockdown had no effect, excluding classic nonhomologous end joining
- PARP1, XRCC1, and LIG3 knockdowns link alt-NHEJ to stress-induced TNR mutagenesis

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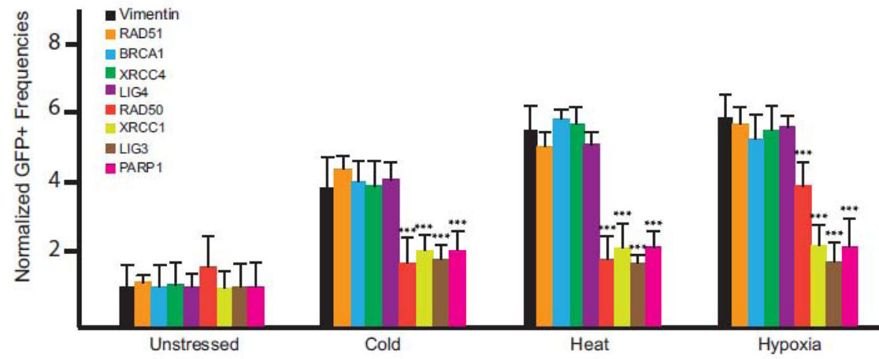

Figure 1. Effects of siRNA knockdown of DSBR pathway components on stress-induced GFP+ cells

HEK293(CAG)$_{89}$ cells were plated two days before stress, and then transfected with siRNA one day before stress (Supplement). At the end of the stress treatment, the medium was changed, and cells were analyzed by flow cytometry after three-day of recovery at 37°C. GFP+ frequencies in all stressed conditions were normalized to unstressed control cells transfected with an ineffective vimentin-siRNA. Results were measured in two independent experiments, each done with four biological replicates. Error bars represent standard deviations. ***$P < 0.001$ versus control, based on Student's two-tailed t test.