DNA Repair 42 (2016) 26–32

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

Mismatch repair enhances convergent transcription-induced cell death at trinucleotide repeats by activating ATR

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ARTICLE INFO

Article history:
Received 17 February 2016
Received in revised form 15 March 2016
Accepted 16 March 2016
Available online 16 April 2016

Keywords:
Mismatch repair
Convergent transcription
ATR DNA damage response
Trinucleotide repeat instability
Neurodegenerative diseases
Cell death

ABSTRACT

Trinucleotide repeat (TNR) expansion beyond a certain threshold results in some 20 incurable neurodegenerative disorders where disease anticipation positively correlates with repeat length. Long TNRs typically display a bias toward further expansion during germinal transmission from parents to offspring, and then are highly unstable in somatic tissues of affected individuals. Understanding mechanisms of TNR instability will provide insights into disease pathogenesis. Previously, we showed that enhanced convergent transcription at long CAG repeat tracks induces TNR instability and cell death via ATR activation. Components of TC-NER (transcription-coupled nucleotide excision repair) and RNaseH enzymes that resolve RNA/DNA hybrids oppose cell death, whereas the MSH2 component of MMR (mismatch repair) enhances cell death. The exact role of the MMR pathway during convergent transcription-induced cell death at CAG repeats is not well understood. In this study, we show that siRNA knockdowns of MMR components—MSH2, MSH3, MLHI, PMS2, and PCNA—reduce DNA toxicity. Furthermore, knockdown of MSH2, MLH1, and PMS2 significantly reduces the frequency of ATR foci formation. These observations suggest that MMR proteins activate DNA toxicity by modulating ATR foci formation during convergent transcription.

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1. Introduction

Trinucleotide repeats (TNRs) are a type of microsatellite sequence distributed in both coding and noncoding regions of the genome [1,2]. TNRs are hypermutable, gaining or loosing repeat units at a high frequency. Variations in TNR tract length have been proposed to fine-tune gene expression and positively impact evolution [3,4]. These important evolutionary benefits are balanced by negative effects that occur when repeats cross a length threshold beyond which they are biased towards expansion.

In humans, expanded repeats are the cause of neurodegenerative disorders [2,5]. As many as 13 TNR diseases are caused by expansion of CAG repeats, as exemplified in Huntington disease (HD), myotonic dystrophy type 1 (DM1), dentatorubralpallidolusian atrophy (DRPLA), and seven different spinocerebellar ataxias (SCAs) [6,7]. Repeat instability occurs not only in the germlines of affected families but also in many somatic tissues, where they exacerbate disease symptoms [6,8–10]. By their ability to form intramolecular secondary structures, expanded repeats evoke deleterious DNA damage responses via DNA metabolic pathways. Thus understanding mechanisms of repeat instability at expanded repeats is critical to any program of therapeutic intervention.

Studies of TNR instability in model organisms—bacteria, yeast, mammals, and human cells—shed light on the spectrum of DNA metabolic processes at play in expanded repeats, including replication, transcription, DNA repair, genome wide demethylation, and rereplication [11–19]. We previously showed that transcription across long CAG repeats induces repeat instability in human cells and defined the modulating role of DNA repair factors in this process [14,20]. When we sought to study the role of convergent transcription in inducing repeat instability, we discovered a novel phenomenon, 'DNA toxicity'—a synergistic increase in cell death following convergent transcription at expanded repeats [21,22]. DNA toxicity could be a potential source of neuronal cell death seen in neurodegenerative patients. Interestingly, a high fraction of the human genome undergoes convergent transcription, indicative of the genome-wide impact of this process [23]. Additionally, convergent transcription-induced cell death is not restricted to CAG repeats. We showed that this process also inhibits cell growth and induces DNA toxicity across other expanded TNRs such as GAA,

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http://dx.doi.org/10.1016/j.dnarep.2016.03.016
1568-7864/Published by Elsevier B.V.

CGG, and CCTG, suggesting that convergent transcription could be a common cell death mechanism among TNR disorders [24].

Using small interfering RNA (siRNA), we previously showed during convergent transcription that ATR (ataxia-telangiectasia mutated [ATM] and Rad3 related) acts as the key mediator in the DNA damage response via its phosphorylated serine-428 residue [21,24]. The mechanism of ATR recruitment and its biological role at expanded repeats during convergent transcription is not known. Additionally, we showed that DNA repair components—TC-NER, MMR, and R-loop resolution enzymes—are also important in modulating effects of convergent transcription-induced cell death [25]. In these studies, siRNA knockdown of single MMR component, MSH2, decreased the frequency of cell death, suggesting that the MMR pathway may play a role in inducing cell death during convergent transcription.

Ordinarily, MMR—a highly conserved pathway—corrects DNA mismatches or small insertions and deletions (indels) in the DNA [26–28]. In human and mammalian cells, the damage recognition step—of a mismatched base or an indel—is facilitated by two main complexes: MutSα (MSH2/MSH6) and MutSβ (MSH2/MSH3), which display some overlapping affinity to either substrate [28,29]. After binding to DNA, the MutS complex recruits the MutL complex. MutLα (MLH1/PMS2) by means of its endonuclease activity is known to cleave the mismatch-containing strand, which is followed by strand extension and joining [29]. The importance of this process is exemplified by the occurrence of debilitating hereditary and sporadic cancers in humans, especially the microsatellite instability (MSI) disorders, where defective MMR fails to correct replication errors at repetitive sequences [26,30].

Interestingly, recent studies indicate MMR-independent roles for both MutS and MutL proteins, in which ATR kinase is activated and recruited to methylation based DNA damage sites [31]. Also, MSH2 is shown to specifically bind and recruit ATR to nuclear foci during cisplatin treatment in human cells, which is independent of RPA, Rad17, and Mus8 proteins [32,33]. Based on these observations, we hypothesized that the MMR proteins are involved in inducing cell death via ATR during convergent transcription. In this study, we show that knockdown of MSH2, MSH3, MLH1, PMS2, and PCNA during convergent transcription across CAG repeats caused less cell death. Additionally, knockdown of MSH2, MSH3, and MLH1 reduced the ATR foci formation at the expanded repeat, suggesting that the MMR pathway is involved in inducing cell death via ATR signaling. This is the first report showing an association of MMR proteins with ATR foci formation at CAG repeats during convergent transcription.

2. Materials and methods

2.1. Cell lines and culture

DIT7 cells were derived from HT1080 cells via the intermediate cell line RS11, as described previously [21]. Briefly, RS11 cells express the rtTA protein under a pTRE-CMV^{mini} promoter when induced with doxycycline. The rtTA protein is a fusion construct of reverse tetracycline repressor and HSV VP16 transcription activation domain. RS11 cells also express genes for RheoReceptor-1 and RheoActivator, under the control of the pNERB-X1 promoter when induced with RSL1. DIT7 cells are derivatives of RS11 cells that each carry a single integrated copy of an *HPRT* minigene with a CAG_{95} tract within its intron. Promoters pTRE-CMV^{mini} and pNERB-X1 flank the minigene and drive sense and antisense transcription, respectively, across the HPRT minigene (Fig. 1). An additional cell line, DIT7-R103, was derived from DIT7 by contraction of the repeat to 15 units. The DIT7 and DIT7-R103 cell lines were grown at 37 °C with 5% CO_2 in DMEM/F-12 medium (Gibco) supplemented with 10% fetal bovine serum (Hyclone, Thermo Scientific) and 1% MEM nonessential amino acids (Gibco). Cells were trypsinized using 0.25% trypsin-EDTA (Gibco) for passaging and further analysis.

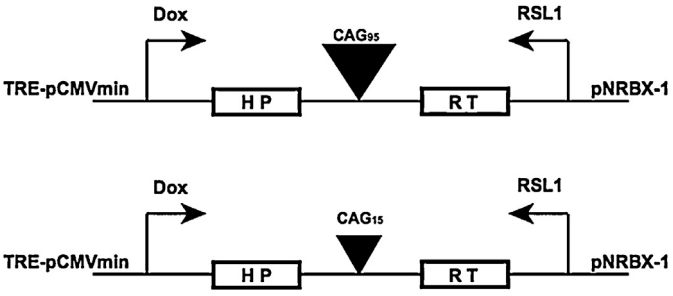

Fig. 1. Schematic of DIT7 and DIT7-R103 cell lines used to study the effects of convergent transcription-induced cell death. DIT7 cells carry 95 units of CAG in its repeat tract and DIT7-R103 carries 15 units. Both CAG_{95} and CAG_{15} tracts were integrated within the center of the 2.1 kb long intron, as shown. The *HPRT* minigene was modified to contain promoters pTRE-pCMV^{mini} and pNEBR-X1 at its ends. Expression of the modified HPRT minigene is regulatable in the sense direction by doxycycline and in the antisense direction by RSL1.

Table 1
Table shows sequences of siRNAs used in knocking down genes.

Gene	siRNA sequence
Vimentin	GAAUGGUACAAAUCCAAGU
MSH2-1	UCUGCAGAGUGUUGUGCUU
MSH2-2	GGAGGUAAAUCAACAUAUA
MSH3-1	AUACGCCGCUAGAAUUACA
MSH3-2	GCAAGGAGUUAUGGAUUAA
MSH6-1	GAAUACGAGUUGAAAUCUA
MSH6-2	CGCCAUUGUUCGAGAUUUA
MLH1-1	AUCAGGCAGGUUAGCAAGCUG
PMS2-1	ACUGAUUUCCUUGCCAACUAGUAAA
PMS2-2	GGCCAACCAUGAGACACAUCGCCAA
PCNA-1	AAGCACCAAACCAGGAGAAAG

2.2. Experimental outline and siRNA treatments

For a typical experiment to test convergent transcription-induced effects on long repeats, on day −1, 100,000 DIT7 or DIT7-R103 cells were plated in each well of a 6-well plate. After 24 h (Day 0), inducers—doxycycline and RSL1—were added to the media at a concentration of 2 μg/ml and 500 nM, respectively. Because the half-life of doxycycline is 24 h, from day 1 through 4, doxycycline was added daily at a concentration of 1 μg/ml without any additional RSL1. Viable and dead cells were counted on day 5.

For siRNA treatments, on day −3, 100,000 DIT7 or DIT7-R103 cells were plated in each well of a 6-well plate. On day −2, siRNAs at a final concentration of 200 nM were transfected into cells using Oligofectamine (Invitrogen), per the manufacturer's protocol. For single gene knockdowns, 100 nM target specific siRNA and 100 nM vimentin control siRNA were used for transfection. For double knockdowns, 100 nM of each siRNA was used to knockdown both target genes. In all cases, total siRNAs concentrations were 200 nM. siRNA sequences (Dharmacon Thermo Scientific) used in this study are listed in Table 1. After 48 h (Day 0), a second round of siRNA treatment was administered, with the addition of inducers—doxycycline and RSL1—at the same concentrations stated above. Additional doxycycline was added to media from day 1 through day 4 at a concentration of 1 μg/ml. Knockdown efficiencies of target genes were evaluated on day 1, by isolating RNA and measuring percent knockdown by real time RT-PCR, as shown before [20]. In each case, at least 70% gene expression was lowered by targeted siRNA treatment.