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Enhanced DNA repair of bleomycin-induced 3′-phosphoglycolate termini at the transcription start sites of actively transcribed genes in human cells

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ABSTRACT

The anti-tumour agent, bleomycin, cleaves DNA to give 3′-phosphoglycolate and 5′-phosphate termini. The removal of 3′-phosphoglycolate to give 3′-OH ends is a very important step in the DNA repair of these lesions. In this study, next-generation DNA sequencing was utilised to investigate the repair of these 3′-phosphoglycolate termini at the transcription start sites (TSSs) of genes in HeLa cells. The 143,600 identified human TSSs in HeLa cells comprised 82,596 non-transcribed genes and 61,004 transcribed genes; and the transcribed genes were divided into quintiles of 12,201 genes comprising the top 20%, 20–40%, 40–60%, 60–80%, 80–100% of expressed genes. Repair of bleomycin-induced 3′-phosphoglycolate termini was enhanced at actively transcribed genes. The top 20% and 20–40% quintiles had a very similar level of enhanced repair, the 40–60% quintile was intermediate, while the 60–80% and 80–100% quintiles were close to the low level of enhancement found in non-transcribed genes. There were also interesting differences regarding bleomycin repair on the sense and antisense strands of DNA at TSSs. The sense strand had highly enhanced repair between 0 and 250 bp relative to the TSS, while for the antisense strand highly enhanced repair was between 150 and 450 bp. Repair of DNA damage is a major mechanism of resistance to anti-tumour drugs and this study provides an insight into this process in human tumour cells.

1. Introduction

Bleomycin is a glycopeptide antibiotic that is used in the treatment of testicular cancer, Hodgkin's lymphoma and squamous cell carcinoma [1–3]. Bleomycin cleaves cellular DNA to give single-strand and double-strand breaks as well as producing other DNA damage lesions. Double-strand breaks are regarded as being the most important for the cancer chemotherapeutic activity of bleomycin [4,5].

The reaction of bleomycin with DNA involves a divalent cation, usually Fe^{2+}. After intercalation of the bithiazole moiety into DNA, a hydrogen atom is abstracted from the C4′ of the deoxyribose sugar in DNA to produce a free radical at C4′ [6]. On reaction with O_2, a series of reactions leads to the cleavage of the DNA phosphodiester backbone and production of 3′-phosphoglycolate and 5′-phosphate ends and a pyrimidine propenal [7–12]. Bleomycin also produces abasic DNA damage where a base is removed from DNA.

The cell employs several DNA repair processes to respond to bleomycin damage to cellular DNA. Abasic bleomycin DNA damage is repaired by the base excision repair pathway using apurinic/apyrimidinic endonuclease 1 (APE1) and DNA polymerase [13]. However, APE1, and several other potential 3′-terminal processing enzymes, are very poor at repairing sites containing 3′-phosphoglycolate [12,14,15]. It is probable that the enzyme tyrosyl-DNA phosphodiesterase 1 (TDP1) is the endogenous enzyme that converts the 3′-phosphoglycolate to 3′-phosphate ends and then polynucleotide kinase 3′-phosphatase (PNKP) converts the 3′-phosphate to 3′-OH ends [12,15–17]. Alternatively Artemis and/or Metnase enzymes remove 3′-phosphoglycolate in overhanging single-stranded regions of double-stranded DNA [18]. Double-strand breaks produced by bleomycin are further repaired by non-homologous end joining and homologous recombination repair [19,20].

These repair processes are important in the treatment of cancer since elevation of DNA repair activity is an important mechanism

Abbreviations: TCR, transcription coupled repair; TSS, transcription start site; TDP1, tyrosyl-DNA phosphodiesterase 1; PNKP, polynucleotide kinase 3′-phosphatase; APE1, apurinic/apyrimidinic endonuclease 1.

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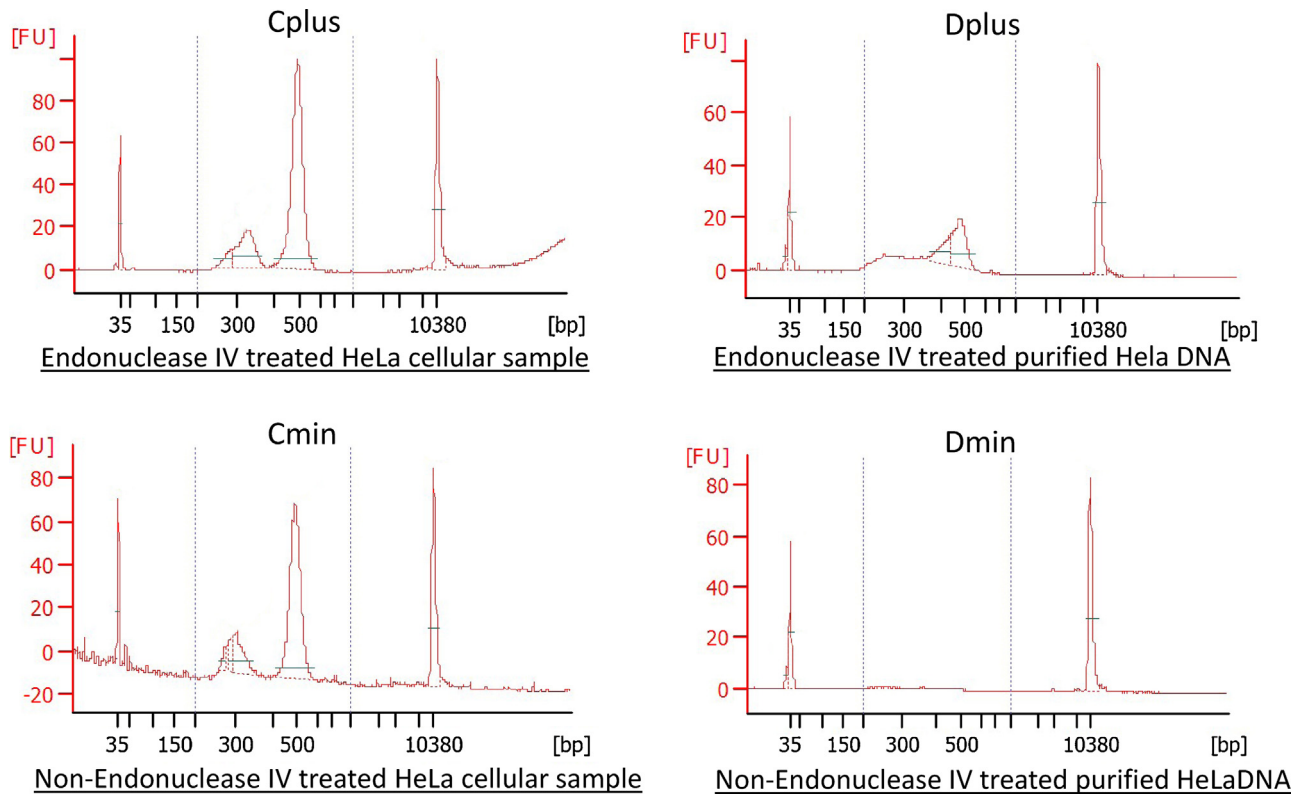

Fig. 1. DNA size profiles on the Agilent Technologies 2100 expert high sensitivity DNA assay system. The Agilent Technologies 2100 expert high sensitivity DNA assay system was used to examine the DNA size profile of four DNA samples after bleomycin treatment, linker ligation and amplification. Cellular DNA with endonuclease IV treatment (Cplus); cellular DNA with no endonuclease IV treatment (Cmin); purified genomic DNA with endonuclease IV treatment (Dplus); and purified genomic DNA with no endonuclease IV treatment (Dmin) are shown in the indicated panels. The peaks at 35 and 10,380 bp are size standards.

of cellular resistance to anti-tumour drugs, including bleomycin, in cancer cells [12,21]. Hence it is clear that an important first step in the repair of bleomycin damage is the conversion of 3′-phosphoglycolate to 3′-OH ends because 3′→5′ exonucleases, DNA ligases and DNA polymerases, cannot use 3′-phosphoglycolate ends as substrates and they must be converted to 3′-OH ends before these enzymes can act.

Next-generation DNA sequencing has been employed in many areas of molecular and cellular biology including genome sequencing, mapping of micrococcal nuclease and DNase I hypersensitive sites, the positioning of nucleosomes and post-translational histone modifications [22–35]. The Illumina Hi-Seq 2000 can be utilised to investigate these processes and is capable of generating more than 2 billion short DNA sequence reads in a single session.

Previously, using next-generation DNA sequencing techniques, we have demonstrated that bleomycin preferentially cleaves actively transcribed genes compared with non-transcribed genes [36]. In this study we examined the DNA repair of bleomycin-induced 3′-phosphoglycolate termini at transcription start sites (TSSs) in human cells using next-generation sequencing techniques. This enabled features such as the level of transcription at TSSs and chromatin structure [36] to be correlated with the sites of bleomycin repair. This study represents the first occasion that the repair of DNA lesions caused by an anti-tumour agent has been mapped at high resolution for the entire human genome.

2. Materials and methods

Bleomycin was from Bristol Laboratories and Endonuclease IV was obtained from New England Biolabs. Bleomycin treatment was as previously described [36] where 2×10^7 human HeLa cells were incubated with 0.5 mM bleomycin for 30 min at 37 °C in phosphate buffered saline. During this 30 min incubation, cellular repair of 3′-phosphoglycolate to 3′-OH ends rapidly occurred due to endogenous cellular enzymes. The cells were washed twice with phosphate buffered saline, suspended in 50 mM Tris–HCl, pH 7.5, 20 mM EDTA, lysed with sodium dodecyl sulfate, incubated with proteinase K, phenol extracted, ethanol precipitated and the purified DNA was suspended in 10 mM Tris–HCl, pH 7.9, 0.1 mM EDTA [37]. Purified HeLa DNA (10 μg) was also treated with 0.33 mM bleomycin and ethanol precipitated prior to analysis [37].

The purified bleomycin-treated cellular DNA was treated with endonuclease IV to give the Cplus sample. In addition, a cellular sample, Cmin, was prepared that was not treated with endonuclease IV. Similarly, the bleomycin-treated purified DNA was treated with and without endonuclease IV (Dplus and Dmin, respectively). These bleomycin-treated DNA samples were processed by the Illumina preparation procedure that included 3′-A addition and ligation of the Illumina linker oligonucleotides. These processed DNA samples were then electrophoresed on an Agilent Technologies 2100 expert high sensitivity DNA assay system (Fig. 1). DNA fragments that were between 200 and 1000 bp in size were purified and placed in a flow cell for DNA sequencing on the Illumina Hi-Seq 2000 at the Ramaciotti Centre for Gene Function Analysis, University of NSW.

The 51 bp Illumina sequence reads were analysed using techniques described in a previous paper [36]. The DNA sequence reads were mapped to the hg19 repeat masked human genome sequence and processed using Bowtie [38] and Samtools [39]. HTSeq (http://www-huber.embl.de/users/anders/HTSeq/doc/tss.html#tss) was utilised to map bleomycin cleavage sites onto HeLa cell TSSs.

The HeLa RNA expression data were obtained from the file "J80–J81_TSSGencv7.gff" accessed from "http://genome.ucsc.edu/cgi-bin/hgFileUi?db=hg19&g=wgEncodeRikenCage" and give