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Research Paper

Investigation of single nucleotide polymorphism loci susceptible to degradation by ultraviolet light

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ABSTRACT

DNA in biological fluids is often degraded by environmental factors. Given that single nucleotide polymorphism (SNP) analyses require shorter amplicons than short tandem repeat (STR) analyses do, their use in human identification using degraded samples has recently attracted attention. Although various SNP loci are used to analyze degraded samples, it is unclear which ones are more appropriate. To characterize and identify SNP loci that are susceptible or resistant to degradation, we artificially degraded DNA, obtained from buccal swabs from 11 volunteers, by exposure to ultraviolet (UV) light for different durations (254 nm for 5, 15, 30, 60, or 120 min) and analyzed the resulting SNP loci. DNA degradation was assessed using gel electrophoresis, STR, and SNP profiling. DNA fragmentation occurred within 5 min of UV irradiation, and successful STR and SNP profiling decreased with increasing duration. However, 73% of SNP loci were still detected correctly in DNA samples irradiated for 120 min, a dose that rendered STR loci undetectable. The unsuccessful SNP typing and the base call failure of nucleotides neighboring the SNPs were traced to rs1031825, and we found that this SNP was susceptible to UV light. When comparing the detection efficiencies of STR and SNP loci, SNP typing was more successful than STR typing, making it effective when using degraded DNA. However, it is important to use rs1031825 with caution when interpreting SNP analyses of degraded DNA.

1. Introduction

DNA evidence from biological fluids at a crime scene can be damaged by environmental factors such as temperature, humidity, ultraviolet (UV) radiation, and microbial degradation.[1] Most forensic DNA samples undergo environmental degradation, hydrolysis, or oxidation, causing polymerase chain reaction (PCR) amplification to fail. The quality of DNA profiling depends on the degradation processes to which the sample has been exposed. Nucleases, oxidants, and byproducts of PCR can degrade DNA, cause conformational changes to its structure, and affect genotyping success.[2] Degradation accumulates with time because of ever-changing environmental conditions.[2] The variety of degradation processes affecting forensic samples[2] challenges investigators to increase their repertoire of DNA markers.

Short tandem repeat (STR) analysis is a well-established DNA-typing method that is widely used in forensics. Given that autosomal STR markers have relatively long amplicons,[3] it is often difficult to use them when analyzing degraded DNA samples. Several short-amplicon genotyping approaches have been developed, such as mini-STRs,[4] mitochondrial DNA typing,[5] and single nucleotide polymorphism (SNP) typing.[6]

Recently, the use of SNP assays for human identification in forensics has been increasing. Because SNP marker sets usually differ across studies, the performance of SNP assays has been validated against frequently used STR assays, and SNP assays have been examined for applicability to casework samples. A range from 18 to 169 SNP markers is used in human identification assays.[7,8] Relative to STR analyses, some SNP marker sets exhibit poor performance,[9,10] whereas others exhibit relatively good performance, particularly for artificially degraded or casework samples.[3,7,11,12] Thus, the best set of SNP markers remains controversial. When individually addressed, successful typing ratio of some SNPs decreased with increasing level of DNA degradation.[7] This result suggests that the level of degradation affects typing success of individual SNPs. However, the differences in the susceptibility or resistance to degradation of each SNP remain unclear. Determining

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the specific SNPs that are resistant to degradation may help increase DNA-typing accuracy for degraded samples.

In this study, we attempted to characterize SNP loci that are detectable in degraded DNA samples. After artificially degrading DNA by exposure to UV-C light for various time periods, we assessed DNA damage using gel electrophoresis, STR analysis, and SNP analysis. UV-C light has higher energy than UV-A and UV-B light does, enabling it to cause extensive degradation of DNA. In a previous study, UV-C damage induced loss of STR profiles with increasing duration of irradiation due to formation of single- or double-strand breaks, photoproducts, and crosslinks.[13]

2. Materials and methods

2.1. DNA samples and DNA extraction

After obtaining informed consent, buccal swabs were collected from 11 healthy Japanese volunteers using sterile Omniswabs (WB100035; GE Healthcare, Waukesha, WI, USA). DNA samples were prepared for UV irradiation by removing any potential protective effects from the cells. Genomic DNA was extracted from the swabs, using a QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The extracted DNA was quantified using a NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The DNA concentrations ranged from 6.5 ng/μL to 32.2 ng/μL.

2.2. UV irradiation of genomic DNA

Using sterilized water, genomic DNA samples were diluted to 0.5 ng/μL in 1.5-mL microcentrifuge tubes (SSI, Lodi, CA, USA). Fifty microliters of each DNA sample was then exposed to UV-C light (254 nm) for various times (5, 15, 30, 60, or 120 min) by laying the samples on top of a 3UV transilluminator (UVP, Upland, CA, USA). This apparatus emits UV light at 5.7 mW/cm^2. Thus, a UV exposure dose (mJ/cm^2) was calculated as the UV exposure intensity (5.7 mW/cm^2) multiplied by the exposure time (s). The dose of UV radiation for 5 min was calculated as 1.7 J/cm^2. Similarly, 15, 30, 60, and 120 min of UV irradiation were 5.1, 10.3, 20.5, and 41.0 J/cm^2, respectively.

2.3. STR profiling using capillary electrophoresis

DNA samples (0.5 ng) were amplified using an AmpFlSTR Identifiler kit (Life Technologies, Gaithersburg, MD, USA) in a GeneAmp 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's protocol. The DNA samples were separated by capillary electrophoresis (POP4 polymer; ABI Prism 310 Genetic Analyzer, Life Technologies) and analyzed using GeneScan 3.1.2 software (Life Technologies). A signal peak height of 50 relative fluorescence units (RFU) was set as the analytical threshold for peak detection. Peak heights of 75 and 150 RFU for heterozygous and homozygous loci, respectively, were used as the threshold for detectable alleles.[14] Experiments were performed in triplicate.

2.4. PCR amplification of SNP loci

In a previous study, Asari and colleagues selected 18 SNPs for human identification in the Japanese population. The minor allele frequency (MAF) for these SNPs ranged from 0.36 to 0.5, and these SNPs were used to analyze artificially degraded DNA samples.[7] In our study, we chose SNP loci for which the MAF ranged from 0.3 to 0.5 and the amplicon size ranged from 59 to 115 bp. Twenty-six loci were selected from a group of Japanese individuals using the

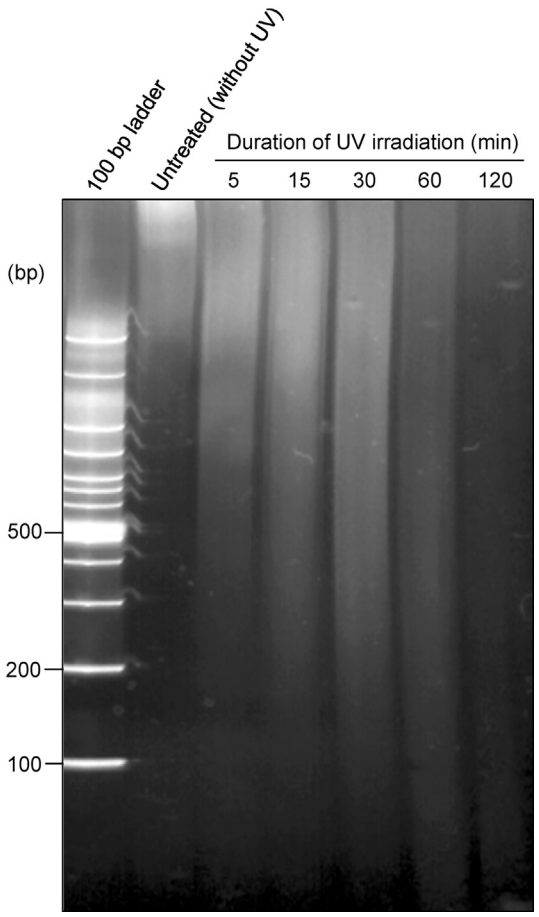

Fig. 1. Fragmentation of genomic DNA was examined using 10% acrylamide gel electrophoresis after UV irradiation.

SNPforID browser. The rs1490413, rs2046361, rs1015250, rs1454361, rs1493232, rs722098, rs1382387, rs1413212, rs1979255, rs891700, rs1528460, rs876724, rs1029047, rs917118, rs901398, rs2016276, rs1031825, rs2111980, rs1463729, rs2076848, rs354439, rs2040411, rs914165, rs1360288, rs1005533, and rs1335873 were used for the SNP assay. The information of assay conditions and primer sequences of these SNP loci are described in the literature.[12]

The PCR amplification protocol described by Sanchez and colleagues[12] and Freire-Aradas and colleagues[15] was used, with slight modifications. Each PCR vessel contained 0.5 ng of DNA in 12.5 μL of 1 × PCR buffer, which comprised 1.5 mM $MgCl_2$, 0.8 mM dNTP mix, 0.5 μM of each primer, and 2 U AmpliTaq Gold DNA polymerase (Life Technologies). Thermal cycling was performed using a GeneAmp 9700 (Life Technologies) running the following program: denaturation at 95 °C for 10 min; 35 cycles of 95 °C for 30 s, 60 °C for 30 s, and 65 °C for 30 s; and a final extension at 65 °C for 7 min. Excess primers and PCR reagents were removed using an ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) by adding 0.5 μL ExoSAP-IT (1 U/μL) per 5 μL PCR product volume. These byproducts were incubated at 37 °C for 30 min and 80 °C for 15 min. These experiments were performed in triplicate.

2.5. SNP profiling using capillary electrophoresis

All PCR products were sequenced using a BigDye Terminator v1.1 cycle sequencing kit (Life Technologies). We used this kit with 26 primers as previously reported[12] and in accordance with the manufacturer's instructions. The DNA samples were analyzed using