TECHNICAL NOTE

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Loss of Heterozygosity Detected in a Short Tandem Repeat (STR) Locus Commonly Used for Human DNA Identification

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ABSTRACT: Short tandem repeat (STR) markers are commonly used in basic genetic research and in human identification testing. Clinically, STRs can be used to study genetic alterations in tumors. A genetic deletion common to many types of cancer is referred to as the loss of heterozygosity (LOH). Numerous examples of LOH in cancer have been described and some have been mapped to areas located in close proximity to markers employed in human identity testing. Despite this fact, LOH has rarely been observed for STR loci commonly employed in forensic testing. Recently, for medico-legal purposes, we were asked to determine whether a tissue biopsy originated from a particular individual. For a reference source we assessed two specimens, one from normal tissue and one from cancerous tissue. When both reference specimens were used to generate DNA profiles, we observed LOH at one STR locus, D13S317. As demonstrated in other cancers only the cancerous biopsy demonstrated LOH. The forensic community should be cognizant of these unusual circumstances because, as identification of human DNA continues to be used more extensively, certain instances will arise in which reference material will not be readily available. In these situations, archived specimens may be employed as a reference source. Clinical specimens such as tissue biopsies should be used with caution if they have not been confirmed to contain normal tissue.

KEYWORDS: forensic science, short tandem repeat, loss of heterozygosity, LOH, polymerase chain reaction, DNA

In humans, thousands of DNA loci that are composed of tandem repetitive sequences have been characterized. Many of these loci demonstrate levels of variability among individuals in a population making them ideal tools for human identity testing. These repetitive elements are classified according to size of the repeat region. An example is microsatellites, or short tandem repeats (STR), that have been mapped throughout the human genome (1,2). STR loci that display high levels of heterozygosity and polymorphism as well as robust amplification have become generally accepted by the scientific community for use in DNA profiling (1–10). The polymorphic nature of these loci allows for the inclusion of an individual as a potential donor of a biological specimen. The capacity to discriminate among potential sources may be strengthened when multiple loci are analyzed. This allows the vast majority of falsely accused individuals to be excluded as possible specimen sources. With current methodologies, the STR loci are amplified and analyzed simultaneously (multiplexed). The detected alleles, which may differ in length by a single base pair, can be accurately identified and assigned allelic designations, thus, allowing results to be easily compared among laboratories.

Linkage analysis employing markers, such as STRs, can be used to map genetic elements to specific chromosomal locations. In addition to genetic mapping, STR loci may be used to study genetic instability. One example of a genetic alteration observed in many types of cancers is the loss of an allele at a heterozygous locus. This imbalance in allele expression is referred to as loss of heterozygosity (LOH). Numerous examples of LOH in cancer have been described (11–19). Interestingly, some deletions have been mapped to areas located in close proximity to markers employed in human identity testing. One example is the deletion of 13q21–22 in prostate cancer (13). While LOH can be readily detected by quantitative fluorescent detection systems, it has been very rarely observed for STR loci commonly employed in forensic testing.

Recently, for medico-legal purposes, we assessed whether separate tissue biopsies taken at another medical center originated from the same individual. We observed an allelic imbalance at the D13S317 locus that is routinely used for forensic identification purposes. The D13S317 locus, composed of TATC repeats (GenBank designation), has been characterized and mapped to chromosome location 13q22–q31 (20–23).

Materials and Methods

Specimens

Testing was performed on paraffin-embedded formalin fixed specimens from a bladder cancer biopsy and a normal gastric tissue biopsy from the same patient. The bladder tumor was a grade ¾ transitional cell carcinoma. No staging information concerning the patient was available. The tissues were taken at time of surgery and

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fixed in 10% buffered formalin for several hours prior to processing. The tissues were then embedded in paraffin blocks and stored at room temperature until time of testing.

DNA Extraction

Small portions of the tissue were excised from the paraffin blocks. The paraffin was removed from the tissue and the DNA extracted by a non-organic extraction method (24). Briefly, the tissue was incubated overnight at 37°C in 500 μL Rapid Lysis Buffer [50 mM Tris (pH 8.5), 1 mM EDTA, 1% NP-40] and 10 μL of proteinase K (20 mg/mL). The extracts were heated for 8 min at 100°C to inactivate the proteinase K and 1–10 μL was used as a source of DNA for PCR amplification.

PCR Amplification

Specimens were analyzed by PCR amplification for polymorphic markers composed of short tandem repeats (STR). The cancer specimen was amplified a total of three times. Nine STR markers including D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317, D7S820, and the gender marker, Amelogenin, were amplified (AmpF1STR Profiler PCR Amplification Kit; Perkin Elmer-Applied Biosystems, Foster City, CA). The specimen DNA was amplified for 28 cycles (94°C for 1 min, 59°C for 1 min, 72°C for 1 min) based on the manufacturer's recommendations. The quantity of amplified product obtained was estimated from a 2.5% wide-range/standard 3:1 agarose gel (Sigma, St. Louis, MO) prior to analysis by capillary electrophoresis on a ABI Prism™ 310 Genetic analyzer. This provides information for setting up dilutions of amplified product if necessary. The alleles were analyzed using Genotyper™ software and assigned specific allele designations (Perkin Elmer Applied Biosystems, Foster City, CA).

Results/Discussion

STR analysis is a powerful method to determine, with high scientific certainty, the likely source of a biological specimen. Normally, reference material is readily available and only the evidence presents potential problems. However, occasionally reference material can not be easily obtained and secondary sources need to be located. One such source may involve the use of historical specimens (i.e., pathology tissue blocks) which can be used for both clinical and forensic testing. Recently, we obtained interesting results using an historical specimen as reference material. For medico-legal purposes, our lab was asked to determine whether a tissue biopsy originated from a specific individual. For a reference source we assessed two specimens, one from normal gastric tissue and one from cancerous bladder tissue. When both reference specimens were used to generate DNA profiles, we observed LOH at one STR locus, D13S317. The results demonstrated the same genetic aberration, loss of heterozygosity, that has been demonstrated previously in a number of clinical conditions. Of interest to the forensic community is that we detected LOH using an STR locus commonly employed in human identification studies. The results of the STR analysis are shown in Table 1. Eight of the nine STR loci gave identical results when the cancerous specimen profile was compared with that of the normal specimen. The probability of an unrelated individual matching the eight locus profile from these specimens is extremely rare, confirming that these two specimens originated from the same individual. At the ninth locus, an imbalance in the strength of the detected alleles was noted. The allele demonstrating LOH (allele 12) is not completely absent, this most likely represents the small quantity of benign stromal tissue in the bladder biopsy. The results demonstrating the loss of heterozygosity are shown in Fig. 1. While the quantity of PCR products obtained varied slightly from the two specimens they were both within the linear range of the detection system (<8100 relative fluorescent units). The loss of an allelic marker (LOH) is most commonly the result of a deletion within the chromosome studied. This has been described for LOH in several other tumor studies (11–19). The particular LOH we observed may result from a point mutation in the target template DNA of the tumor or more likely from a deletion of one of the two chromosome 13 homologues. If a large deletion occurred, additional studies by cytogenetic methods could confirm the extent of the deletion. However, this is not routine and beyond the scope of the question this paper is addressing.

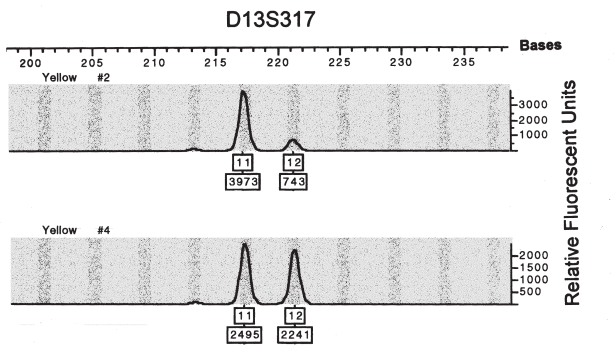

FIG. 1—*Electropherogram from the tissue biopsies analyzed by STR amplification. Shown is only the D13S317 locus. Upper panel: Results obtained from the cancerous bladder tissue demonstrating the loss of heterozygosity at allele "12." Lower panel: Results obtained from normal gastric tissue demonstrating the normal balance of signals generally obtained when testing heterozygous loci. The vertical scale is the quantity in relative fluorescent units (RFU) and the horizontal scale is the length of the amplified fragments in nucleotide bases. The numbers under the peaks refer to the allelic designation (e.g., #11 or #12) and the peak height in RFU.*

TABLE 1—*Results of STR analysis on tissue specimens. Data are reported as phenotypes.*

	Genetic Locus									
Specimen	D3S1358	vWA	FGA	Amel.	TH01	TPOX	CSF1PO	D5S818	D13S317*	D7S820
Normal tissue biopsy	16, 18	15, 19	20, 23	XY	9, 10	9, 11	11, 12	11, 12	11, 12	10
Cancer tissue biopsy	16, 18	15, 19	20, 23	XY	9, 10	9, 11	11, 12	11, 12	11, (12)	10

* Locus demonstrating loss of heterozygosity; (12) = weakly detected allele 12;
XY indicates male gender; X indicates female gender.

These results are consistent with other cancer studies in that the LOH was noted only from DNA extracted from the cancerous tissue. We point out this fact because, as identification of human DNA continues to be used more extensively, certain occasions will arise in which reference material will not be readily available. In these situations, archived specimens may be employed as references. Clinical specimens such as tissue biopsies should be used with caution if they have not been confirmed to contain normal tissue. When generating DNA profiles from archived cancer specimens genetic anomalies may occasionally be observed and discretion is needed in their evaluation. Similar caution should be used if references are obtained from certain transplant patients, for example, individuals transplanted with allogeneic hematopoietic stem cells. In this situation, if a blood sample is used as a reference specimen, the stem cell recipient will not match the other somatic tissues from this same individual (e.g., buccal swab; unpublished observation).

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