



## Forensic evaluation of tetranucleotide STR instability in lung cancer

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### Abstract

The incidence of genetic instability affecting a set of STRs commonly used in forensic DNA analyses was assessed by performing a comparative study on 24 lung carcinomas with paired normal tissue samples. Out of 24 samples, 20 (83%) showed allele drop-out (ADO) in at least one STR locus. Allelic imbalance was detected at all the STR loci analysed. A small-cell carcinoma sample showed loss of heterozygosity (LOH), with complete deletion of one allele, at the D5S818 and D13S317 loci.

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### 1. Introduction

The evaluation of STR stability in human tissues is an important topic in forensic medicine, for a reliable interpretation of the genetic profiles. Tetranucleotide STR loci are, in fact, routinely used in forensic DNA studies and currently employed for individual identification analyses and paternity cases. STR markers are also commonly used to study genetic instability in many types of cancers. Microsatellite instability (MSI), characterised by contraction or expansion in STR length, and loss of heterozygosity (LOH) defined by mutation of one allele followed by deletion of the remaining one, have been detected at different incidence in many different cancers such as colorectal [1], gastric [2,3] and lung [4,5] carcinomas, using mono-, di-, tri-, tetra- and pentanucleotide STR markers. It is possible that, in particular situations, a tumour biopsy may become the reference sample in

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a forensic individual identification analysis or in a paternity testing. In that condition, any relevant information on genetic instabilities observed for the reference cancer tissue should be considered, in order to avoid obtaining incorrect DNA typing results. To evaluate the incidence of instability affecting a set of STRs commonly used in forensic DNA analyses, we decided to perform a comparative study testing 24 lung carcinomas with paired normal tissue samples. Automated fluorochrome-based DNA sequencer was employed to detect STR instability in DNA samples; this methodology allowed simultaneous allele scoring for different STRs, high resolution and sensitivity, and clear interpretation of data.

## **2. Materials and methods**

Twenty-four lung cancer specimens were investigated and subdivided according to the following diagnosis: 13 adenocarcinomas (AdC), 10 squamous-cell carcinomas (SqC) and 1 small-cell carcinoma (SCC). Neoplastic tissues were selected on formalin-fixed paraffin-embedded sections under microscopic control on HE stained paired sections to avoid the contamination of normal cells. The paired normal tissue derived from lymph node sections of the same patient. Ten micrometer sections of each paired normal and tumoral tissue were cut, transferred into an Eppendorf tube and incubated o.n. at 58 °C in a lysis solution containing Tris/HCl pH8.3, 50 mM KCl, 25 mM MgCl<sub>2</sub>, 0.1 mg/ml gelatin, 0.45% Tween 20, 0.45% NP40 and proteinase K which was subsequently inactivated at 95 °C for 10 min. Samples were then vortexed for 10–15 s and centrifuged at 12,000 rpm for 3 min. Aliquots of the extracts were added to the PCR tubes and amplified for the nine STRs (D3S1358, VWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820) and the sex determination marker Amelogenin contained in the AmpFISTR Profiler Plus amplification Kit (Applied Biosystems, CA) according to the manufacturer's specifications. The PCR products were processed by capillary electrophoresis through an ABI Prism 310 Genetic Analyser (Applied Biosystems, CA) and alleles were scored using the Genotyper Software ver 1.1 (Applied Biosystems). Selected cancer specimens were reanalysed in duplicate experiments, to assess the reproducibility of the DNA typing results.

## **3. Results and discussion**

Genetic instability was observed in most of the cancer specimens we analysed; 20 out of 24 (83%) samples showed allele drop-out (ADO) in at least one STR locus. ADO results from the preferential amplification of one of a pair of heterozygous alleles, in which the other allele is under represented. ADO is usually a random event, with both alleles being equally susceptible to drop-out. Allelic imbalance was detected at all the STR loci analysed, with D3S1358, D5S818, D13S317 and D18S51 loci preferentially involved. In 5 out of 24 samples, allele drop-out was seen simultaneously at more than four STR loci. No microsatellite instability (MSI), with new alleles of different size, was observed for all the cancer specimens we investigated. The small-cell carcinoma sample showed loss of heterozygosity, with complete deletion of one allele, at the D5S818 and D13S317 loci

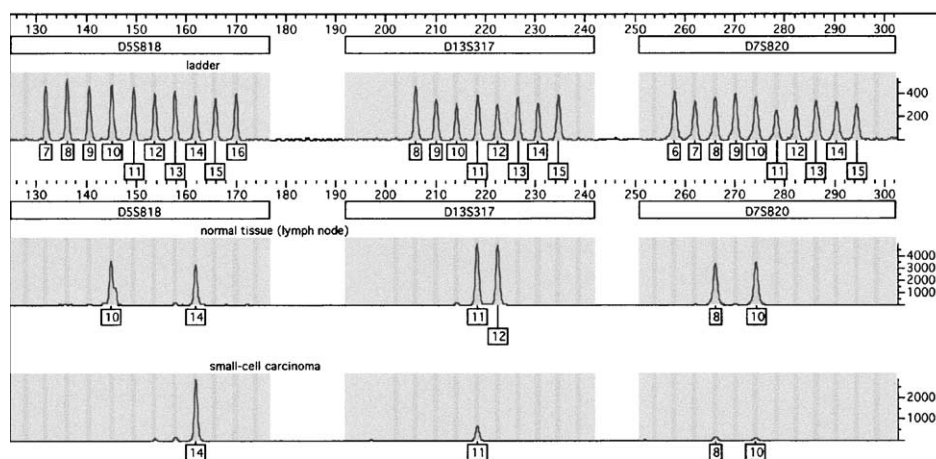


Fig. 1. Electropherogram showing loss of heterozygosity (LOH) at the D5S818 and D13S317 loci. Upper panel: allelic ladders; middle panel: paired normal tissue showing a profile 10/14 and 11/12 (D5S818 and D13S317); lower panel: small cell carcinoma biopsy showing loss of heterozygosity at alleles 10 and 12, respectively.

(Fig. 1) when compared with the paired normal sample. These results seem to confirm previous data on LOH at STR loci on chromosomes 3, 5, 13 and 17 [4] in small cell carcinomas. LOH and allelic imbalance for STR markers on chromosomes 3, 5, 9, 13 and 17 were also detected, in previous studies, in adenocarcinomas and squamous cell carcinomas [5–7]. Microsatellite instability for six STR markers used in forensic analyses was already reported in gastric cancer specimens [3] and DNA recovered from a bladder cancer biopsy showed LOH at the D13S317 locus [8]. The data we show suggest that great care should be taken in the evaluation of the DNA typing results obtained from clinical specimens such as tissue biopsies, in particular when no other reference samples containing normal tissue are available. Genetic instability has been shown to be a very common event observed in many different tumours and STR loci used in forensic medicine for individual identification could sometimes be affected.

## References

- [1] C.R. Boland, S.N. Thibodeau, S.R. Hamilton, D. Sidransky, J.R. Eshleman, et al., *Cancer Res.* 58 (22) (1998) 5248–5257.
- [2] M.G. Rhyu, W.S. Park, S.J. Meltzer, *Oncogene* 9 (1) (1994) 29–32.
- [3] F. Silva, L. Gusmao, C. Alves, et al., *Electrophoresis* 18 (9) (1997) 1633–1636.
- [4] J. Yokota, M. Wada, Y. Shimosato, et al., *PNAS* 84 (24) (1987) 9252–9256.
- [5] P. Mendes da Silva, A. Moreira, et al., *Mol. Pathol.* 53 (4) (2000) 184–187.
- [6] I.I. Wistuba, C. Behrens, S. Milchgrub, et al., *Oncogene* 18 (3) (1999) 643–650.
- [7] V.G. Gorgoulis, A. Kotsinas, P. Zacharatos, et al., *Cancer* 89 (9) (2000) 1933–1945.
- [8] R.J. Rubocki, K.J. Duffy, K.L. Shepard, et al., *J. Forensic Sci.* 45 (5) (2000) 1087–1089.