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Evaluation of reliability of STR typing in different types of cancerous tissues used for identification purpose

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Abstract. In this study we screened 48 gastrointestinal carcinomas, 13 urogenital and 7 oral carcinomas in parallel with control samples for the 15 STR loci of the AmpFlSTR Identifiler Kit to provide further data useful to evaluate the applicability on cancerous tissues of STRs used in forensic field. A total of 37 cancerous tissues (54.4%) showed allelic alterations when compared with the corresponding normal tissues: 29 (78.4%) gastrointestinal tumors, 4 (10.8%) urogenital tumors and 4 (10.8%) oral tumors. The loci most frequently affected by allelic alterations were VWA, FGA and D18S51. These results suggest that great care should be taken in the evaluation of the DNA typing results obtained from clinical cancerous specimens when no other reference samples containing normal tissue are available. © 2005 Elsevier B.V. All rights reserved.

Keywords: Cancerous tissue; Short tandem repeat (STR); Loss of heterozygosity (LOH); Microsatellite instability (MSI)

1. Introduction

Aberrations in coding or non-coding region of genome have been reported in cancerous tissues also for those short tandem repeats (STRs) widely used in forensic analysis. Loss of heterozygosity (LOH) and microsatellite instability (MSI) were described in gastrointestinal, esophageal, lung, oral, renal cell carcinomas, melanomas, head and neck squamous cancers

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with variable frequencies [1–5]. To provide further data about the applicability of STR markers on cancerous tissues for forensic applications, we analysed 15 STR loci of the AmpFlSTR Identifiler Kit in 68 primary tumor samples from 48 gastrointestinal carcinomas, 13 urogenital carcinomas and 7 oral carcinomas in parallel with control non-cancerous samples.

2. Materials and methods

Cancerous and non-cancerous control tissues were obtained at surgical resection from 68 patients. Forty-two of the samples were from males and twenty-six from females. The tumors included 48 gastrointestinal carcinomas, 13 urogenital carcinomas and 7 oral carcinomas. The samples were stored at -20 °C until analysis. DNA extraction was carried out by Nucleo Spin Tissue (Macherey and Nagel, Duren, Germany) according to the manufacturer's instructions on tissues previously minced into 1-mm pieces; the DNA extracted was visualized by electrophoresis in 1% agarose gel containing ethidium bromide. Fluorescent multiplex polymerase chain reaction (PCR) was performed to amplify the 15 STR loci included in AmpFlSTR Identifiler PCR amplification Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's recommendations. The amplified products were separated by ABI PRISM 310 Genetic Analyzer (Applied Biosystems) and results were analysed using GeneScan Analysis software 3.7. For uniformity, we classified allelic alterations according to Vauhkonen et al. [1].

3. Results and discussion

All 68 cancerous and control tissues were successfully amplified and compared. Stringent criteria to classify MSI were followed as suggested by Sobrido et al. [6] and only frozen tissues

Table 1
Genetic profile alterations in 68 cancerous samples

	n	%
Genetic profiles in the gastrointestinal cancer		
MSI-H	2	4.2
MSI-L	3	6.3
LOH-H	0	0
LOH-H with MSI-L	2	4.2
LOH-L	15	31.2
LOH-L with MSI-H	2	4.2
LOH-L with MSI-L	5	10.3
MSS	19	39.6
Genetic profiles in the urogenital cancer		
MSI-L	1	7.7
LOH-L	3	23.1
MSS	9	69.2
Genetic profiles in the oral cancer		
MSI-L	1	14.2
LOH-L	3	42.9
MSS	3	42.9

Table 2
Amel and STR alterations per locus

Locus	STR altered	MSI	LOH
D8S1179	10 (14.7%)	9 (13.2%)	1 (1.5%)
D21S11	10 (14.7%)	5 (7.4%)	5 (7.4%)
D7S820	5 (7.4%)	3 (4.4%)	2 (2.9%)
CSF1PO	10 (14.7%)	5 (7.4%)	5 (7.4%)
D3S1358	7 (10.3%)	3 (4.4%)	4 (5.9%)
TH01	3 (4.4%)	1 (1.5%)	2 (2.9%)
D13S317	2 (2.9%)	1 (1.5%)	1 (1.5%)
D16S539	11 (16.2%)	4 (5.9%)	7 (10.3%)
D2S1338	7 (10.3%)	4 (5.9%)	3 (4.4%)
D19S433	7 (10.3%)	4 (5.9%)	3 (4.4%)
VWA	13 (19.1%)	5 (7.4%)	8 (11.8%)
TPOX	7 (10.3%)	6 (8.8%)	1 (1.5%)
D18S51	12 (17.6%)	4 (5.9%)	8 (11.8%)
D5S818	10 (14.7%)	3 (4.4%)	7 (10.3%)
FGA	13 (19.1%)	4 (5.9%)	9 (13.2%)
Amel	7 (16.7%)	/	7 (16.7%)
Total	134	61	73

were submitted to the study. A total of 37 cancerous tissues (54.4%) showed allelic alterations when compared with the corresponding normal tissues: 29 (78.4%) gastrointestinal tumors, 4 (10.8%) urogenital tumors and 4 (10.8%) oral tumors. Table 1 illustrates the genetic profile alterations and Table 2 the STRs alterations per locus in 68 total samples. No samples showed simultaneous loss of both loci. A genomic instability at all the STRs analysed and no occurrence of new alleles instead of those found in normal tissue were observed, being difficult to interpret the simultaneous presence of LOH and MSI in 9 homozygous samples. In MSI cases the size of new alleles ranged from -3 to $+3$ repeat units, even if contraction or expansion of one repeat unit was more often observed. Our results on gastrointestinal carcinomas with 60.4% of microsatellites alterations are in line with Vauhkonen et al. [1]. In the limited sample of 7 oral carcinomas we found microsatellites alterations in 57.1%, a high percentage compared to data from oral cancers by Pai et al. [5]. Of 13 urogenital cancers 4 (30.8%) presented genomic alterations, nevertheless, available data on the same loci are not enough to perform a significant comparison. The loci most frequently affected by allelic alterations were VWA, FGA and D18S51. About LOH, high percentages for D18S51 (11.8%) could be due to allelic size of this marker and consequently more prone to DNA degradation effects. The mutation rates in parentage analysis agree with the instability of VWA and FGA loci. These results suggest that great care should be taken in the evaluation of the DNA typing results obtained from clinical cancerous specimens, in particular when no other reference samples containing normal tissue are available.

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