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Multiplex STR and mitochondrial DNA testing for paraffin embedded specimen of healthy and malignant tissue: Interpretation issues

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Abstract. STR typing results for 70 pairs of healthy vs. malignant tissues demonstrate the occurrence of microsatellite instability (MSI) and loss of heterozygosity (LOH) in the tumor samples. In order to not misinterpret degradation effects as LOH, this study recommends to only interpret alleles ≤350 bp with a peak intensity >300 RFU. mtDNA data for the same samples did not reveal increased mutation rates but for many cases were affected by DNA contamination. The mtDNA assay can be valuable in confirming an inclusion. Overall these sample types can still be used for identification purposes since any observed discrepancies can be explained. © 2005 Elsevier B.V. All rights reserved.

Keywords: STR; mtDNA; Malignant tumors; Allelic instability; Loss of heterozygosity; Human identification

1. Introduction

DNA-based short tandem repeat (STR) typing is a powerful tool for the confirmation of suspected sample mix-ups or the presence of contamination in histology material [1]. Histology specimens also have potential as reference samples in body identification efforts. But microsatellite analysis of tumor DNA has shown signs of allelic alterations in

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the form of microsatellite instability (MSI) and/or loss of heterozygosity (LOH) [2,3]. The aim of this study was to further evaluate the STR approach to determine whether the biological variability influences the validity of the human identity testing and to investigate the usefulness of mitochondrial DNA testing as a supplemental genetic test.

2. Material and methods

70 blocks of paraffin embedded malignant tissues (11 different types) vs. healthy tissue from the same anonymous individuals were examined. All 140 slides were set up in a double blind manner where the researchers did not know which tissue was healthy or cancerous. DNA extraction was performed using a standard organic method.

2.1. Nuclear DNA–STR analysis

Extracts were amplified using the fluorescent labeled primers of the PowerPlex®16 multiplex STR system (Promega Corp., Madison, WI), in the GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA), following manufacturer's recommendations and 32 cycles. If available, 1 ng of DNA was used. The amplified products were analyzed using capillary electrophoresis. Heterozygote ratio was determined using the formula: $HR = A_{low}/A_{high}$, where $[A]$ is allelic intensity in RFU.

2.2. mtDNA–SNP detection in the HVI/HVII regions

Mitochondrial DNA testing was performed using an immobilized sequence-specific oligonucleotide probe strip typing system—Linear Array Mitochondrial DNA HVI/HVII Region-Sequence Typing Kit (Roche Applied Science, Indianapolis, IN) according to the manufacturer's protocol. 0.1 ng of DNA was the target amount for the amplification and roughly 75 ng of amplified product was used for the hybridization to the immobilized SSO probes.

3. Results and discussion

3.1. STR analysis and interpretation rules

Out of the 70 tested healthy samples and 1050 expected loci retrieved, 690 (65.70%) yielded profiles. Among them, 89.42% are loci ≤350 bp and 10.58% >350 bp. Out of the 70 tested tumor samples and 1050 expected loci retrieved, 722 (68.76%) yielded profiles. Among them, 88.36% are loci ≤350 bp and 11.64% >350 bp. These results are concordant with similar levels of degradation in both normal and tumor tissue. Tissue fixation and paraffin embedding have been shown to negatively affect DNA quality and degradation and allelic drop out can be accounted for in the interpretation. In addition to the degradation effects the tumor tissue shows signs of allelic alterations

Table 1
STR interpretation rules

RFU>300	≤350 bp	>350 bp
Normal tissue	Even degraded samples should show full types and an HR≥0.7	LOH can cause peak imbalance and allelic drop out
Tumor tissue	LOH can cause peak imbalance and allelic drop out	LOH cannot be distinguished from degradation effects

in the form of suspected MSI and/or LOH. In this study, normal tissue showed 21 loci (3.04%) with HR $\leq$ 0.7 (3 with HR $\leq$ 0.45), while tumor tissue showed 122 loci (16.90%) with HR $\leq$ 0.7 (36 with HR $\leq$ 0.45). Most of these instances were at loci $<$350 bp. This difference demonstrates LOH and not just a degradation effect. The occurrence of MSI/LOH can be recognized and explained and thus does not lead to exclusions (Table 1).

3.2. mtDNA analysis and interpretation rules

The hybridization assay questions 10 polymorphic sites of two hypervariable regions on the mitochondrial d-loop. Due to the increased sensitivity of mtDNA testing, linear array (LA) tests had a higher success rate but were also more prone to contamination, which creates mixtures. It is not always possible to distinguish mixtures from heteroplasmy. The best option for proper interpretation is to reanalyze questionable samples starting with “clean microtome cuts” if possible. The presence of mtDNA heteroplasmy is a known fact for tumor as well as healthy tissue [4]. Therefore, only one discrepancy in the mtDNA LA type is inconclusive. Two or more differences are required for an exclusion. The mtDNA type can be useful to confirm an inclusion.

4. Conclusions

The use of the described identity testing systems is a valuable method for determining the origin of malignant tissue or the identity of the tissue donor, despite tumor DNA instability. Distinction of DNA degradation effects from true LOH can be challenging at times. Thus interpretation for this purpose should consider only loci $\leq$350 bp, with peak intensity $>$300 RFU. For mtDNA testing mutation and heteroplasmy issues will be difficult to establish unless histology specimen can be processed under ultraclean conditions.

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