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Loss of heterozygosity and microsatellite instability of forensically used STR markers in human cervical carcinoma

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Abstract. Analysing tumor materials and normal tissues from 27 patients with carcinoma of the uterine cervix for loss of heterozygosity (LOH) and microsatellite instability (MSI) on 11 chromosome X loci (DXS6800, DXS6807, DXS8377, DXS101, DXS7424, GATA172D05, DXS10011, DXS7130, DXS6803, HPRTB, and ARA), we found the highest frequency of LOH for DXS10011 (20%) and ARA (17%). MSI was seen up to 11% for DXS10011. Mostly, X-chromosomal LOH and MSI were observed at Xq28 and Xq12. Testing 14 autosomal loci (D3S1358, vWA, D16S539, D2S1338, D8S1179, D21S11, D18S51, D19S433, TH01, FGA, SE33, D3S1768, D3S2456, and D17S1537), 4–40% of the informative cases represented LOH. The highest frequency was seen for markers in 3p (21–40%), suggesting putative tumor suppressor gene at this region.

Keywords: Cervical carcinoma; Loss of heterozygosity; X-chromosome

1. Introduction

The mutation rates of polymorphic microsatellites are well known, which makes the risk of mistakes for forensic application calculable. The stability of STR alleles, however, is not realised, for example, in well-known trinucleotide expansions and many carcinomas.

The prevalence of human papillomavirus (HPV) infection in cervical carcinoma (CX) is high, but only 2% of infected women develop high dysplasia or invasive carcinoma. Thus there must exist additional factors needed for tumor development resulting in genomic instability. Common forms of genomic instability are loss of heterozygosity (LOH) and microsatellite instability (MSI). Among chromosomes involving LOH or MSI, the X-

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chromosome may be affected in ovarian carcinomas [1], but limited information exists for CX [2,3].

2. Materials and methods

DNA was extracted from fresh, snap-frozen materials ($n = 17$), tissue blocks containing formalin-fixed and paraffin-embedded tumor materials ($n = 10$), and normal tissues from patients ($n = 27$) with carcinoma of the uterine cervix, surgically staged as pT1b1 to pT2b. We used the QIAamp tissue kit and the nonorganic Chelex 100 extraction protocol recently published [4]. LOH was assessed by polymerase chain reaction (PCR) analysis of the matched pairs of normal and tumor DNA with polymorphic microsatellite markers. Resulting PCR products were detected by capillary electrophoresis (ABI 310). Loss of one allele of a polymorphic marker or a very strong decrease in signal intensity of one allele in tumor DNA was considered as evidence for LOH. MSI was identified when novel alleles of microsatellite markers appeared in tumor DNA compared to the matching germ line DNA [5].

3. Results and discussion

Genetic alterations could be detected in 19 of 27 patients (70%) in at least one of the investigated STR loci; nine patients represented LOH as well as MSI, nine patients represented either LOH or MSI, and one patient represented only MSI. For chromosome X loci, we found LOH up to 20% of informative cases (Table 1). The highest frequency of LOH could be detected for DXS10011, ARA, and DXS8377. X-chromosomal MSI was seen mainly for DXS10011 and DXS8377. Thus the most observed X-chromosomal alterations were detected in the region at Xq28 and Xq12. Testing of 14 autosomal loci of different chromosomal location up to 40% of informative cases represented LOH (Table 2). The highest frequency was seen for markers in 3p (D3S1768: 40%; D3S1358: 24%; D3S2456: 21%), 4q28 (FGA: 24%), 11p (TH01: 23%), and 19q (D19S433: 20%). Genetic alterations for D19S433 (19q12–

Table 1
Chromosome X marker testing (27 patients)

Marker	Number of cases (LOH positive/informative)	LOH (%)	MSI (%)
DXS6800	1/20	5.0	3.7
DXS6807	2/14	14.3	0
DXS8377	4/26	15.4	7.4
DXS101	3/24	12.5	0
DXS7424	1/26	3.8	0
GATA172D05	1/22	4.5	3.7
DXS10011	5/25	20.0	11.1
DXS7130	2/21	9.5	0
HPRTB	1/19	5.3	3.7
DXS6803	1/21	4.8	0
ARA	4/23	17.4	0

Table 2
Autosomal STR marker testing (27 patients)

Marker	Number of cases (LOH positive/informative)	LOH (%)	MSI (%)
SE33	0/25	0	7.4
D3S1358	5/21	23.8	3.7
D3S1768	8/20	40.0	0
D3S2456	4/19	21.0	0
D17S1537	2/21	9.5	0
VWA	1/24	4.2	3.7
D16S539	1/20	5.0	0
D2S1338	3/24	12.5	0
D8S1179	1/24	4.2	0
D21S11	0/23	0	3.7
D18S51	2/25	8.0	0
D19S433	4/20	20.0	3.7
TH01	6/26	23.1	0
FGA	6/25	24.0	0

13.1), coupled with LOH for the FGA locus (4q28) and DXS10011 (Xq28), were found in four patients (15%).

4. Conclusion

LOH in CX on several genetic loci is a common phenomenon, even in CIN lesions, suggesting the existence of tumor suppressor genes in these regions. Further studies are necessary to identify chromosomal regions, which are associated with tumor progression and metastatic diseases, or in alteration of the susceptibility of the host for HPV infection. Furthermore, great care should be taken in the evaluation of typing results obtained from clinical tissue specimens, in particular when no reference samples are available, because genetic instability is a very common event observed in different tumors and the STRs used for individual identification could sometimes be affected.

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