

Amplification at 9p in cervical carcinoma by comparative genomic hybridization

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DNA copy number changes were studied by comparative genomic hybridization on 10 tumor specimens of squamous cell carcinoma of cervix obtained from Korean patients. DNA was extracted from paraffin-embedded sections after removal of non-malignant cells by microdissection technique. Copy number changes were found in 8/10 tumors. The most frequent changes were chromosome 19 gains ($n = 6$) and losses on chromosomes 4 ($n = 4$), 5 ($n = 3$), and 3p ($n = 3$). A novel finding was amplification in chromosome arm 9p21-pter in 2 cases. Gains in 1, 3q, 5p, 6p, 8q, 16p, 17, and 20q and losses at 2q, 6q, 8p, 9q, 10p, 11, 13, 16q, and 18q were observed in at least one of the cases.

Keywords: Cervical cancer, CGH, amplification at 9p21-pter

1. Introduction

Carcinoma of the uterine cervix is the second most common female malignancy world-wide that causes high morbidity and mortality rate in women and ranks first in incidence among malignant tumors of Korean women according to the data from Korean Cancer Registry. Specific types of human papilloma virus (HPV)

are known to be the principal etiologic agents for cervical cancer [1–3]. Cytogenetic studies of primary cervical tumors have indicated nonrandom structural changes, particularly on chromosomes 1, 3, 4 or 5, 8, 11 and 17 [4,5]. Comparative genomic hybridization (CGH) in cervical cancer has shown recurrent DNA copy number gains at 1q, 3q, 5p, 8q, 15q, and 20, and losses at 3p, 4p, 6q, 11q, and 13q [1,6–9]. We used CGH to analyze primary tumors in 10 Korean cases of squamous cell carcinoma of cervix (SCC). Among the numerous DNA sequence copy number changes we detected, recurrent high-level amplification at 9p stands out as a significant novel finding.

2. Materials and methods

2.1. Specimens

All of our samples were collected in Korea. Ten paraffin-embedded cervical tumor tissue specimens obtained from radical hysterectomies were analyzed using CGH. The tumor grade and stage were identified according to the International Federation of Gynecology and Obstetrics (FIGO) criteria. The diagnoses were made by the pathologist (K.R. Kim) in the Department of Pathology, ASAN Medical Center, Seoul, Korea. Table 1 shows clinical data of all cases.

2.2. DNA extraction

High molecular weight DNA was extracted from archival tissue samples by microdissection as described by Abeln et al. [10]. Briefly, tumor cells were collected after removal of stroma by scraping with a clean scalpel from three unstained slide sections of 10 μm thickness. The tumor cells were transferred to a microcentrifuge tube containing digestion buffer, which was incubated overnight at 45°C. After addition of proteinase K, the samples were incubated in water bath at 100°C for 10 minutes and centrifuged. After centrifuge the supernatant was transferred to a sterile tube.

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Table 1
Clinical data and comparative genomic hybridization results from 10 patients with cervical squamous cell carcinoma

Case	Age	Stage	CGH	
			Gain	Loss
1	48	Ib	–	–
2	40	Ib	19	13q21-32
3	36	Ib1	1q11-qter, 6p11-pter, 9p21-pter, 19	3p11-pter, 4, 5q, 11p12-pter, 11q14-qter
4	65	Ib	5p, 8q24-qter, 9p21-pter, 16p12-pter, 19, 20q11-12, X	8p12-pter, 16q
5	44	Ib2	19, 20q	3p, 4q, 5q13-31, 6q11-24
6	43	Ib	1p32-pter, 8q22-qter, 9q33-qter, 11q13, 16p11-pter, 17, 19	2q14-22, 3p, 4q26-31, 6q12-16, 9p12-pter, 10p11-pter, 11q21-qter, 13, 16q, 18q, Xp, Xq11-26
7	41	Ib1	3q, 19	4q21-28
8	61	Ib	–	–
9	66	Ib	3q26-qter	2q34-qter, 5
10	67	Ib	3q23-qter	–

–, normal.

2.3. Comparative genomic hybridization

CGH was performed as described previously [11]. Normal DNA was extracted from peripheral blood of healthy donors and labeled with Texas red dCTP and dUTP (DuPont, Boston, MA) in a standard nick-translation reaction. The tumor DNA was labeled with a mixture of fluorescein dCTP and dUTP (DuPont). Equal amounts of the labeled tumor and reference DNA were hybridized onto normal metaphase slides. Before hybridization, the DNA was denatured for 5 minutes at 75°C and metaphase slides were treated in 70, 80, and 100% ethanol series, and denatured at 65°C for 2 minutes in formamide solution. Hybridization was performed in a moisture chamber at 37°C for 48 hours and washing was performed in formamide solutions. Then the slides were mounted with antifading medium containing a counterstain (Vector Laboratories, Burlingame, CA) and analyzed using the ISIS digital image analysis system (MetaSystems GmbH, Altussheim, Germany). The average ratio profile was calculated as a mean value obtained from 10 to 15 metaphase spreads. Based on previous experience, we used 1.17 and 0.85 as cut-off levels for gains and losses, respectively. The region was considered as highly amplified when the ratio exceeded 1.5.

3. Results

Eight of the 10 specimens (Table 1, Fig.1) showed DNA copy number changes with a mean of 5.1 aberrations per specimen (range: 0–18). The most common gains were found in 19 (6 cases), 3q (2), 8q (2), 9p (2), 16p (2), and 20q (2). High-level amplifications were seen at 5p14-ter (1 case), 20q11 (2), and 9p21-ter (2). The most common losses were observed at 4q (4 cases), 3p (3), and 5 (3). Figure 2 shows high-level amplifications at 9p (green-to-red ratio higher than 2.5) in 2 cases (nos 3 and 4).

4. Discussion

Our novel finding is a recurrent high-level amplification in the short arm of chromosome 9. In 2 out of 12 cases the level of this amplification was higher than that in any other of the detected gains.

In all types of tumor, 9p is one the most common areas of DNA copy number losses [12], whereas DNA copy number amplifications are relatively rare [13], online access <http://www.helsinki.fi/~lgl/www/CMG.html>. In previous studies, gains at 9p have not been considered to be a significant recurrent change in SCC, even though this gain (not high-level amplifica-

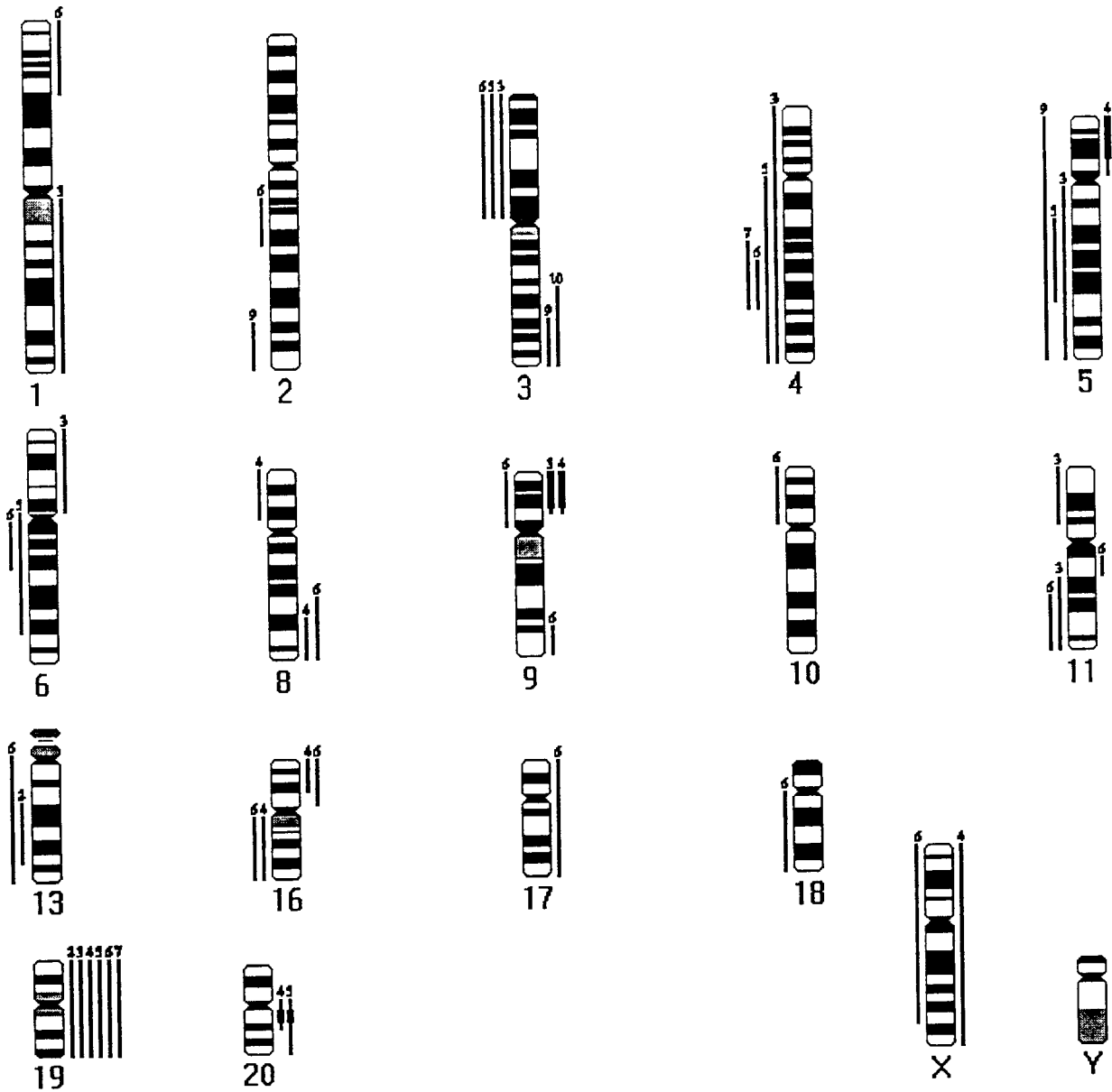


Fig. 1. Summary of DNA gains and losses detected by comparative genomic hybridization in cervical squamous cell carcinomas (stage Ib). Each chromosome is schematically depicted with areas of DNA loss represented by bars to the left and areas of DNA gain represented by bars to the right of the chromosome ideogram. Thick bars represent areas of high-level DNA amplification.

tion) has been detected in about 5–7% of cases [1,6,8]. Other recurrent gains (3q, 8q, 16p, 19, and 20q) and losses (4q, 3p, 5, 6q, 11q, 13, and 16q) in SCC have been reported also by others [1,6,8].

So far no genes located at 9p have been implicated in SCC. Some of the genes, such as *TEK* (9p21, endothelial cell surface receptor tyrosine kinase) and *BAG1* (9p12, BCL2-associated Anthano Gene 1) encode proteins that have oncogenic or anti-apoptotic functions

[14]. Although CGH showed the DNA sequence amplification, it cannot be ruled out that the amplicon includes also lost gene sequences from, for instance, suppressor genes *CDKN2A* and *CDKN2B*. In the characterization of DNA copy number amplifications by PCR with polymorphic markers, Wolf et al. [15] demonstrated that, in addition to highly amplified sequences, the 12q amplicon in sarcomas contains non-amplified and even lost sequences. Consequently, the aim of

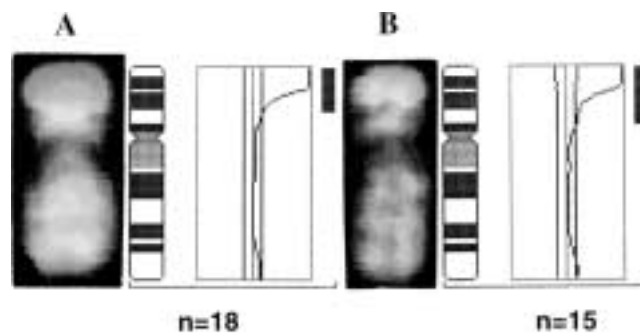


Fig. 2. High-level amplification at chromosome arm 9p21-pter in 2 cases. The line in the middle of the profile indicates the base line ratio (1.0), the lines to the left and right indicate ratio values 0.5 and 1.5, respectively. A, case 3; B, case 4; n , number of chromosomes counted to provide the mean.

molecular characterization of 9p amplicon should be widened from identification of amplified target genes to detection of lost genes.

Human papilloma virus (HPV) infection is considered to be the initial event in cervical carcinoma [2,3,16]. Given that we studied only Korean patients, it is relevant to question whether ethnic differences between HPV subtypes could account for the divergent findings we and others report regarding 9p amplification. Incidence studies around the world indicate that HPV16 is the the most frequent subtype (in 5% of the tumors), followed by HPV18. It should be noted that in Korean population HPV33 is more prevalent than HPV18 and there are also specific variants of HPV16 [16,17]. Further studies are needed to determine whether these differences are associated with the presence of 9p amplification.

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