Int J Clin Exp Pathol 2014;7(8):5071-5082
www.ijcep.com /ISSN:1936-2625/IJCEP0000683

Original Article

Genome-wide screening of DNA copy number alterations in cervical carcinoma patients with CGH+SNP microarrays and HPV-FISH

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Received May 1, 2014; Accepted June 24, 2014; Epub July 15, 2014; Published August 1, 2014

Abstract: Alterations in the genome that lead to changes in DNA sequence copy number are characteristic features of solid tumors. We used CGH+SNP microarray and HPV-FISH techniques for detailed screening of copy number alterations (CNAs) in a cohort of 26 patients with cervical carcinoma (CC). This approach identified CNAs in 96.2% (25/26) of tumors. Array-CGH discovered CNAs in 73.1% (19/26) of samples, HPV-FISH experiments revealed CNAs in additional 23.1% (6/26) of samples. Common gains of genetic sequences were observed in 3q (50.0%), 1q (42.4%), 19q (23.1%), while losses were frequently found in 11q (30.8%), 4q (23.1%) and 13q (19.2%). Chromosomal regions involved in loss of heterozygosity were observed in 15.4% of samples in 8q21, 11q23, 14q21 and 18q12.2. Incidence of gain 3q was associated with HPV 16 and HPV 18 positive samples and simultaneous presence of gain 1q ($P = 0.033$). We did not found a correlation between incidence of CNAs identified by array-CGH and HPV strain infection and incidence of lymph node metastases. Subsequently, HPV-FISH was used for validation of array-CGH results in 23 patients for incidence of *hTERC* (3q26) and MYC (8q24) amplification. Using HPV-FISH, we found chromosomal lesions of *hTERC* in 87.0% and *MYC* in 65.2% of specimens. Our findings confirmed the important role of HPV infection and specific genomic alterations in the development of invasive cervical cancer. This study also indicates that CGH+SNP microarrays allow detecting genome-wide CNAs and copy-neutral loss of heterozygosity more precisely, however, it may be less sensitive than FISH in samples with low level clonal CNAs.

Keywords: Cervical carcinoma, whole-genome profiling, CGH+SNP microarrays, HPV-FISH, copy number alterations

Introduction

Carcinoma of the uterine cervix (CC) is the second most common form of cancer in women worldwide. The overall incidence rate is 10.6/100 000, and mortality is 7/100 000 women in Europe; however, the numbers differ in various regions [1]. Despite the preventive and screening programs, the incidence and mortality has not decreased over the last 20 years [2]. There are two main types of cervical cancer: 85% to 90% of cases are squamous cell carcinoma, while adenocarcinoma represents 10% to 15%. Other types, such as endometrioid adenocarcinoma, clear cell adenocarcinoma etc., are rare [3, 4].

Several premalignant stages, known as cervical intraepithelial neoplasia (CIN) or cervical dysplasia, are related to the development of invasive carcinoma. It is known that the human papillomavirus (HPV) infection plays an initial role in the development of cervical lesions [5]. However, the high-grade lesions (CIN II - III) and cervical cancer develop only in a part of infected patients despite the high incidence of HPV infection in dysplasia samples. Therefore, the infection alone is not able to activate the malignant transformation [6, 7]. Similarly to other malignant diseases, extensive screening of cervical carcinoma firmly confirm that specific genomic alterations play an important role in the origin and development of cervical intraepi-

Table 1. Clinical characteristics of cohort of 26 patients with cervical carcinoma

Clinical feature	Parameter	Incidence
Histological type	Squamous carcinoma	19 (73.1%)
	Adenocarcinoma	7 (26.9%)
Tumor diameter	T1a1	1 (3.8%)
	T1a2	2 (7.7%)
	T1b1	17 (65.4%)
	T1b2	4 (15.4%)
	T2b	1 (3.8%)
	T4a	1 (3.8%)
FIGO stage	IA1	1 (3.8%)
	IA2	2 (7.7%)
	IB1	12 (46.2%)
	IB2	3 (11.5%)
	IIIB	7 (26.9%)
	IVA	1 (3.8%)
Histological grading	Well-differentiated	4 (15.4%)
	Moderately-differentiated	9 (34.6%)
	Poorly-differentiated	13 (50%)
Lymph node metastases	No	16 (61.5%)
	Yes	7 (26.9%)
	Unknown	3 (11.5%)
Vaso-invasion	No	13 (50%)
	Yes	13 (50%)
High risk HPV	Negative	4 (15.4%)
	Positive	18 (69.2%)
	Unknown	4 (15.4%)
HPV type	HPV-16	6 (33.3%)
	HPV-18	3 (16.6%)
	HPV-45	2 (11.1%)
	Unknown	7 (38.9%)

thelial neoplasia and influence progression to cervical cancer [8, 9]. FISH analyses using targeted DNA probes have identified several specific structural chromosome changes associated with cervical cancer. The most frequent genetic alteration, detected in approximately 70% of cervical carcinoma, is gain/amplification of the long arm of chromosome 3 [10, 11]. The region 3q26 contains the gene for RNA subunit of human telomerase (*hTERC*). Many studies show that gain of *hTERC* correlates with progression of CIN lesions to carcinoma; thus, it can be a predictive factor of malignant transformation [12, 13]. Another common chromosomal aberration in CC is gain/amplification in 8q24 area, affecting the locus for avian myelocytomatosis viral oncogene homolog (*MYC),* a well-known protooncogene in the process of cancerogenesis. There is consensus that gain of *MYC* gene is not important only in progression of the tumor, but even in the cell transformation during pre-invasive stages [14]. Previous studies have also shown that the increased copy number of *MYC* gene is associated with more advanced stages of cervical cancer [15]. Using large-scale genomic technologies, such as chromosomal (CGH) and array comparative genomic hybridization (array-CGH), other recurrent unbalanced copy number alterations (CNAs) have been repeatedly identified in cancer genome, including loss in chromosome arms 3p, 4p, 6q, 11q, 13q and gain of genetic material in chromosome regions 1q, 5p, 3q, 8q, 15q, 17q and Xq, which have also been suggested to be relevant in the development and progression of cervical cancer [16]. Some of these CNAs such as 17q gain have been associated in CC patients with histological subtype (adenocarcinoma), poor prognosis and metastatic behavior (gain 5p, loss 9p and 18q) [17, 18].

Recently, new platforms of high resolution DNA microarrays have been used as a powerful genome-wide screening tool allowing simultaneous evaluation of copy number aberrations and copy number neutral regions of loss of heterozygosity (cnLOH). In this pilot study, we used Agilent SurePrint G3 Human CGH+SNP 4x180K microarray platform to accurately identify the chromosomal regions most frequently gained and lost in cervical carcinoma specimens.

Our study has three principal aims: 1) to analyze genome-wide profile in 26 cervical tumors obtained from Czech patients with high density CGH+SNP microarray and to identify recurrent unbalanced DNA copy number alterations and regions with loss of heterozygosity associated with malignant phenotype and progression of cervical carcinoma, 2) to evaluate genome-wide profiles and differences in chromosome rearrangements in relation to HPV infection

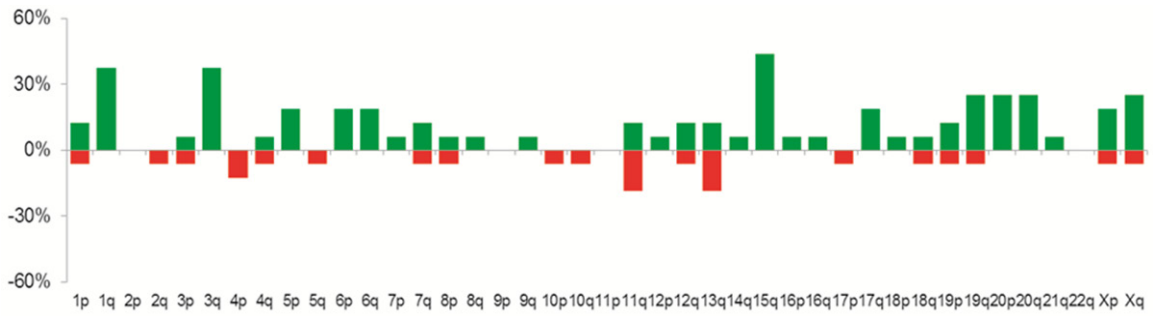

Figure 1. Graphical summary of all gains and losses of DNA sequences obtained by array-CGH technique in cohort of 26 cervical carcinoma patients. Green bars represent areas of gains; red corresponds with areas of loss of genetic material.

Table 2. The total number of CNAs/patient (chromosome losses and gains) detected by array-CGH and in relation to regional lymph node metastases status

	With metastasis (N = 7 patients)	Without metastasis (N = 16 patients)	*P*-value[2]
Losses[1]	0 (0; 10)	2 (0; 9)	.871
Gains[1]	3 (0; 29)	6 (0; 20)	.922
Total number of CNAs[1]	12 (0; 29)	9 (0; 22)	.452

[1]described by median (min; max) losses or gains. [2]*p*-value of the Mann-Whitney test.

and metastatic behavior of tumors, 3) to compare the array-CGH sensitivity in detecting the copy number changes of *hTERC* and *MYC* genes with results obtained from PAP smears using HPV Cervical FISH Probe Kit.

Material and methods

Cervical samples

Cervical cancer tumors from 26 patients (median of age 42.5 years; range 33 - 68) were obtained between 2009 and 2013 in the Masaryk Memorial Cancer Institute (MMCI), Brno, Czech Republic. All samples were obtained only after the patients signed the informed consent approved by the Ethical committee of the MMCI and were immediately frozen in liquid nitrogen. Patients after surgical procedures or any adjuvant treatment were monitored in regular intervals according to onco-gynecological guidelines [19]. The follow-up period was 6 - 36 months, and overall survival was not reached as all patients in our cohort are still alive without any sign of recurrence of the tumor disease.

Seven clinical parameters known to have a prognostic value were chosen to be investigated in this study: histological type, tumor diameter, FIGO stage, histological grading, pelvic lymph node status, vaso-invasion and HPV status (**Table 1**).

Histological typing

Squamous cell carcinoma or adenocarcinoma was based on histochemical staining with H&E, periodic acid-Schiff (PAS) reagent and Alcian blue for mucin detection and all the samples were reviewed by pathologist.

Human papillomavirus (HPV) detection and genotyping

PCR was used with consensus primers MY09 and MY11 for the L1 region of the viral genome. After 5 min. denaturation at 94°C, 100 ng of DNA were subjected to 35 amplification cycles with the following parameters: 94°C for 1 min., 55°C for 2 min. and 73°C for 3 min., with a final extension step of 7 min. at 72°C. The amplicon was labeled using the Big Dye sequencing kit and sequenced on an ABI371 sequencer (Applied Biosystems, Foster City, CA, USA). BLAST http://www.ncbi.nlm.nih.gov/BLAST/ sequence comparison was used in order to define the viral type.

Table 3. The association of the HPV type with the total number of CNAs/patients and with the chromosomal gains at 3q26 and 8q24 (N = 9 patients with described HPV type status)

	array-CGH Total number of CNAs			HPV-FISH/array-CGH	
Patients	Losses	Gains	Losses or gains	3q26 gain	8q24 gain
HPV type 16	Median: 2	Median: 8	Median: 10		
Patient id 89	0	8	8	yes	yes
Patient id 94	10	7	17	yes	yes
Patient id 96	0	0	0	yes	yes
Patient id 97	0	0	0	yes	yes
Patient id 108	3	8	11	yes	yes
Patient id 126	9	16	25	yes	yes
HPV type 18	Median: 3	Median: 8	Median: 10		
Patient id 91	3	7	10	yes	no
Patient id 124	1	8	9	yes	no
Patient id 130	9	9	18	yes	no

Array-comparative genomic hybridization

Tumor samples were stored at -70°C until DNA isolation. Genomic DNA (gDNA) for array-CGH experiments was isolated using standard phenol extraction. Simultaneous whole-genome analysis of unbalanced chromosomal changes (CNAs) and copy-neutral regions of loss of heterozygosity (cnLOH) in cervical tumors was done using oligonucleotide-based or SurePrint G3 CGH+SNP Array 180K platform (Agilent Technologies, Santa Clara, CA, USA), as described elsewhere [20]. Briefly, 1 - 1.5 μg of reference DNA (Agilent Euro Female) and patient DNA were digested with *Alu1* and *Rsa1* (Promega, Madison, WI, USA) for 2 hours at 37°C. Fluorescent labeling was done by the SureTag DNA Labeling Kit (Agilent Technologies). Purified and differentially labeled sample and reference DNA were co-hybridized at 65°C for 24 hours to the array. Microarrays were scanned with Agilent SureScan C Scanner with 3 μm resolution, features were extracted using Feature Extraction software (v11.1) and normalized data were analyzed and visualized by Agilent Genomic Workbench v. 7.0.1.4 (Agilent Technologies). The aberration detection module 2 (ADM-2) with threshold 6 was used for calculating CNAs. Five-probe 0.20_log2 filter was used for aberration evaluation, given an average genomic resolution of 25.3 Kb. Data were manually curated, and the DGV database (hg19) was used for elimination of the common CNV regions from the dataset. Microarray data are available in the Array Express database (www.ebi.ac.uk/arrayexpress) under accession number E-MTAB-2293.

Fluorescence in situ hybridization

Vysis Cervical FISH Probe Kit was used for identification of human papillomavirus (HPV) infected cells and to evaluate chromosomal lesions in 3q26 (*hTERC*) and 8q24 (*MYC*) loci via fluorescence *in situ* hybridization (HPV-FISH). This kit enables the identification of high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51-53, 56, 58, 59, 66 and 68) using biotin labeling and tyramide signal amplification assay. The *hTERC* probe was labeled with SpectrumGold fluorescent label covering approximately 495 kb region; the *MYC* probe was labeled with SpectrumRed fluorescent label covering approximately 821 kb region. Evaluation of chromosomal aberration in *hTERC* and *MYC* loci was done using standard FISH technique as previously described [21].

Slide analysis

Slides were analyzed under the fluorescent microscope BX-61 (Olympus) using DAPI, green, gold and red single band pass filter sets. The entire hybridized surface area was analyzed in all cases. Cells were evaluated according to Cervical FISH Probe Kit directions.

HPV staining was visualized with a green filter and localized to the nucleus as confirmed by DAPI staining. HPV staining pattern was classified as diffuse, mixed and punctate, as described previously [22]. The sample was considered positive for HPV infection if at least one HPV-positive cell was found.

All HPV-positive cells and their pattern were recorded; number of *hTERC* or *MYC* signals was determined for each HPV-positive cell. According to manufacturer's recommendation, the case was considered positive for chromo-

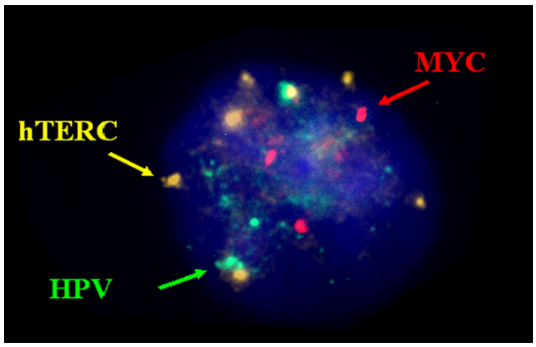

Figure 2. Interphase HPV-FISH showing HPV-positive cell (green signals) with amplification in 3q26 (yellow signals) and 8q24 (red signals).

somal aberration if 4 or more HPV-positive cells demonstrated copy number gain (more than 2 fluorescent signals) of at least one chromosome locus (*hTERC* or *MYC*). Otherwise, case was considered HPV positive but chromosome negative.

In the absence of HPV-positive cells, slide was evaluated for the presence of cells with amplified *hTERC* and/or *MYC* genes. The sample was considered positive for chromosomal aberration if more than 5,8% (cut-off value from negative controls at the 95% confidence limit) of cells demonstrated copy number gain (more than 2 fluorescent signals) of at least one chromosome locus (*hTERC* or *MYC*). Otherwise, the case was considered HPV and chromosome negative.

Statistical analyses

A pairwise analysis of interactions of genetic aberrations between different chromosomes was performed with Fisher's exact test. Bonferroni correction was used for multiple comparisons, for number of analyzed chromosomal arms.

Fisher's exact test was applied when two categorical variables were compared. Mann-Whitney test was used for testing differences in continuous parameters between groups. McNemar's test with continuity correction was used for comparison of results obtained from FISH and array-CGH experiments.

Statistical analyses were performed using the software IBM® SPSS® Statistics 21.

Results

Genomic profiling and copy number measurements in tumor cells using Agilent Sureprint G3 CGH+SNP 4x180K microarray platform

Genome-wide screening showed large genomic heterogeneity in cervical tumors and revealed copy number alterations in 73.1% (19/26) of samples.

Overall, we found 213 CNAs (141 regions of gain and 72 regions of loss of genetic material) with median of incidence 5 gains and 3 losses per sample (range 0 - 25 for gains; 0 - 10 for losses).

Structural CNAs were most common genetic lesions in our dataset (81.7%; 174/213). The most common chromosomal regions of segmental copy number gain were observed in 3q (50.0%; 13/26), 1q (42.4%; 11/26), 19q (23.1%; 6/26), 5p and 8q (both 15.4%; 4/26), whereas most frequent regions affected with loss of genetic sequences were found in 11q (30.8%; 8/26), 4p (23.1%; 6/26), 13q and 2q (both 19.2%; 5/26). The size of the segmental CNAs ranged from 0.174 to 138 Mbp, with median size 28.4 Mbp for losses and 24.7 Mbp for gains. Overall, we observed 106 segmental gains (median size = 25.2 Mbp; min - max 0.17 - 137.2 Mbp) and 68 segmental losses (median size = 28.1 Mbp; min - max 0.18 - 128.3 Mbp). The incidence of structural CNAs was most frequent in chromosome 11, which was affected in 73.1% (19/26) of samples.

Chromosomal aneuploidies were presented in 18.3% of all CNAs (39/213). Monosomy of chromosome 13, 19, X and 10 was found in single cases, whereas trisomies were more frequent (16.4%; 35/213). The most common was trisomy of chromosome 15 and 20 (both 23.1%; 6/26) and chromosome X (11.5%; 3/26). Graphical overview of incidence of CNAs in the cohort of 26 patients with cervical cancer is shown in **Figure 1**.

The incidence of cnLOH was observed in 15.4% (4/26) of cases. In our cohort, cnLOH regions were observed in 8q21, 11q23, 14q21 and 18q12.2. The median of size was 12.1 Mbp (range 10.2 - 15.4 Mbp), and all cnLOH were observed in different patients.

Table 4. The incidence of increased copy numbers of *hTERC* (3q26) and *MYC* (8q24) in 23 patients with cervical carcinomas analysed by HPV-FISH and array-CGH

	HPV-FISH	array-CGH
3q26 gains	20 (87.0%)	10 (43.5%)
8q26 gains	15 (65.2%)	4 (17.4%)

[1]described by absolute count and relative frequencies - N (%).

The Fisher's exact test was performed to find pairwise associations of CNAs on different chromosomes. In our cohort, statistically significant associations were found for simultaneous presence of gain 1q and 3q (9/26 gain positive vs. 13/26 gain negative; P_{corr} = 0.031).

We also compared genomic profiles between patients with adenocarcinoma (n = 7) and spinocellular cases (n = 19). Although the number of samples was low, in both cohorts we observed similar genomic profile with frequent gains in 1q, 3q and 8q, while most common deletions were observed in 11q. On the other hand, we found increased number of patients without CNAs in the adenocarcinoma cohort in comparison to the spinocellular cases (4/7 vs. 3/19; P = 0.057).

Genomic profiling of patients with lymph nodes positive or negative for metastases

Presence of regional lymph node metastases is an important prognostic factor in cervical carcinoma. In order to identify the recurrent regions of genomic alterations that are specific for invasive disease spreads to regional lymph nodes, the incidence and distribution of CNAs in patients with lymph nodes positive for metastases (MP) and negative for metastases (MN) were evaluated. Even though we observed a higher number of total CNAs per patient in MP subgroup (**Table 2**), both subgroups showed high similarity in both array-CGH profiles and in the frequency of high-risk genetic alterations. Gains in 1q, 3q26 and 8q24 were observed in MP group in 28.6% (2/7), 57.1% (4/7) and 14.3% (1/7) of samples, while in MN group, the incidence of both gain 1q and gain 3q26 was 43.1% (7/16) and 12.5% (2/16) for 8q24. We observed a higher incidence of gains in 11q, 17q and 19q in MP group, while in MN group gains in 15q and 20p were more frequent. Single cases in MP group also carried monosomy of chromosome 10 and trisomy of chromosomes 7, 12 and 22, which were not seen in MN group. On the contrary, single cases in MN group were affected by the incidence of trisomy 6 and gain of 18q. Although this data could lead to better understanding of cervical carcinoma biology, the impact of these observations needs to be verified on a larger cohort of patients.

Genomic profiling of patients in relation to HPV strain positivity

It is well established that HPV infection is the main cause of cervical cancer. In our study, overall 69.2% (18/26) of samples were HPV positive and in 61.1% of them (11/18) HPV typing was obtained. To analyze correlation between HPV type infection and CNAs, we tried to compare genomic profiles in two groups of samples with confirmed high risk HPV status (HPV 16 and HPV 18). Although this study was limited by low level of samples, both subgroups showed similarity in the total number of CNAs/patients. The most common regions of gains were observed in 1q21 - 1q44, 3q and 19q11 - q13.43, whereas frequent losses of genetic material were observed in 11q13.4 - q25. Each of HPV 18 positive cases had at least one aneuploidy (trisomy 3, 6, 15, and 20); while in all HPV 16 cases we observed only one trisomy X. Furthermore, HPV-FISH evaluation showed that increased copy number of the 3q26 region is common for both HPV 16 and HPV 18 positive cases (**Table 3**).

Incidence of copy numbers in 3q26 (hTERC) and 8q24 (MYC) loci using HPV-FISH: concordance with array-CGH assay

Array-CGH technique using high density microarrays is a powerful tool for analysis of genomic alterations in tumors. However, it is known that failure of array-CGH in detecting the aberrations can be caused by low level of cells with chromosomal aberrations in samples.

In order to validate the data obtained by array-CGH platform, we evaluated samples from 23 patients by HPV-FISH and compared those results array-CGH. This HPV-FISH technique allows simultaneous identification of HPV positive cells and copy number alterations of *hTERC* (3q26) and *MYC* (8q24) genes using targeted DNA probes (**Figure 2**).

HPV-FISH evaluation showed amplification of *hTERC* in 87.0% (20/23) and *MYC* genes in 65.2% (15/23) of cervical cancer specimens, while array-CGH analyses revealed gain of *hTERC* in 43.5% (10/23) and *MYC* in 17.4% (4/23) of samples. Thus, array-CGH was not able to detect copy number alterations in 3q26 and 8q24 region in 10/23 and 11/23 cases, respectively, when compared to results obtained by Cervical FISH Probe Kit (**Table 4**). In addition, the HPV-FISH evaluation revealed amplification of *hTERC* and *MYC* genes in 6 of 7 array-CGH negative samples. When the CC samples were analyzed using both HPV-FISH and array CGH methods, the copy number alterations were identified overall in 96.2% (25/26) of tumors.

Discussion

Reliable diagnostics and classification of malignancies on the molecular level are currently the essential factors for effective treatment in many oncological diseases. In this study, we explored genomic alterations in 26 cervical cancer samples obtained by array-CGH technique based on novel Agilent Human G3 CGH+SNP 4x180K microarray platform allowing simultaneous detection of unbalanced genetic lesions and regions of copy-neutral loss of heterozygosity in single microarray. The results obtained by genomic profiling from our cohort of patients confirmed that cervical cancer samples show genetic heterogeneity involving both aneuploidies and complex structural aberrations. Overall, we found 213 CNAs in our datatset with the use of whole genome screeing by means of array-CGH technique. The detailed analysis showed that gain of DNA sequences represent predominant genomic alterations in CC. In agreement with previous results, the most common regions of DNA gain were observed in 1q, 3q, 5p, 8q and 19q, while loss of genetic material was typical for regions 4p, 11q and 13q [23, 24]. The recurrent gain in 3q26 area carrying *hTERC* was the most frequently affected region in our cohort (50% of patients). In 26.9% of cases (7/26), we found minimal region of gain of genetic material (MGR) of 48.3 Mbp between 3q25.1-3qter, with amplification (> 5 copies) *hTERC* loci in 16.6% (4/26) of samples. In addition, whole 3q duplication was detected in 19.2% (5/26) of cases; in 1 case we observed trisomy of chromosome 3. In 4 cases, we found loss of whole 3p. Similar incidence of CNAs in 3q region detected by microarrays was reported recently [25]. This study confirmed that this alteration plays an important role in precancerous as well as in later stages of the disease and are associated with HPV-mediated carcinogenesis [26, 27]. Frequent genetic lesions were also observed in chromosome 1. Incidence of gain/amplification 1q in cervical carcinoma is known to be associated with infection of high-risk HPV types [28, 29], and it is considered as a potential hallmark of CIN2/3 lesions with a high short-term risk of progression [30, 31]. We also showed that gain of genetic sequences in 3q was significantly associated with gain of chromosome 1q arm, which suggests that these regions might be relevant for faster progression of cervical carcinoma. Loss of genetic material was found in 1p13.3 - 1p13.11, where we defined 6.7 Mbp minimal deleted region (MDR) carrying 75 genes between *CSF1 - IGSF3.*

Another chromosomal aberration with prognostic impact in cervical carcinoma is gain 5p. While the incidence of duplication/gain 5p in CC patients and cell lines ranges from 22-63% [32, 33], in our cohort of patients, the occurrence of gains in 5p was somewhat lower (15.4%; 4/26). Nevertheless, it seems that gain of genetic material in 5p arm gives an important contribution to the selective advantage in cervical neoplastic progression. Functional analyses showed that upregulated transcription is associated with oncogenic activation of miRNA processor *DROSHA* leading to over expression of cancer-associated microRNAs and thus they have the potential of deregulation of numerous protein-coding genes [34, 35]. Most common area of loss of genetic material in our study was 11q (30.8%; 8/26), which is in good agreement with previous observations [36]. Previous analyses suggested that deletions in 11q arm are common in both precancerous and advanced stages of the disease; however, the effect on prognosis remains unclear [37, 38]. On the other hand, gain/amplification in 11q was also common in our dataset (23.1%; 6/26) with 1 case of high-level amplification in region 11q22.3, including apoptosis inhibitors *BIRC2* and *BIRC3*. Incidence of gain/amplification in 11q23 may have a function in the development/progression of cervical cancer and could be a novel predictive marker for resistance to radiotherapy [39, 40]. Using CGH+SNP microarrays we

observed regions of LOH in 11q23, 14q21, 18q12.2 and 8q21, overall in 15.3% of the patients. Recently, Tillart *et al.* reported similar results (cnLOH detected in > 10% of cases in dataset) in larger cohort of CC patient with the use of SNP microarrays [41].

Whole-genome screening results did not show significant chromosomal signatures associated with histopathological type of tumors. Although we found higher number of CNAs in spinocellular specimens similarly to study made by Wilting *et al.* [31], this observation is limited by relatively small sample cohort obtained for analysis.

Several studies also showed that incidence of gains of genetic material in 7p, 7q, 9p and 17q are typical for cervical adenocarcinoma [17, 41]. In our cohort, gains in 17q arm were more common in squamous CC (9/16 vs. 1/9; P = 0.190), while other CNAs occurred in similar manner. In both subgroups, gain in 7p and 7q was observed in 1 case, and we did not found gain in 9q area in any of the samples. Recently, whole-exome sequencing revealed genomic differences between spinocellular carcinoma and adenocarcinoma that may explain the observed clinical differences [42].

Metastases in pelvic lymph nodes are considered as a negative prognostic factor for patients with CC. Previous studies showed positive correlation between number of the lymph nodes affected with metastasis and portion of patients who reached 3-year disease-free interval (62% for 1 lymph node affected vs. 20% for 3-4 lymph nodes affected with metastases) [43, 44]. Comparison of whole-genome profiles between in our patients with and without lymph node metastases showed equivalent incidence of high-risk genetic features, such as gain in 3q or 8q. Furthermore, previous studies showed association of loss in 9p and 11q with incidence of metastasis in cervical carcinoma with use of metaphase CGH [45, 46]. In our dataset, we did not observe deletion in 9p in any case, and we found no statistical difference between the incidence of 11q loss in MP and MN subgroup (3/7 vs. 5/16; P = 0.998). Similarly, no difference was found when we compared the total number of all CNAs or gains and losses alone (P = 0.452; P = 0.992; P = 0.871, respectively). Even though these associations did not reach statistical significance (probably due to low number of patients in our cohort), connection between number of CNAs and effect on prognosis was observed in other solid tumors, such as breast or colorectal cancer [47, 48].

We also tried to analyze genomic alterations in relation to high risk HPV types (16 and 18). There was no correlation between HPV type and total CNAs/patients detected by array-CGH. Thomas *et al.* showed that gains in 3q are more common in HPV 16 samples [25]. In our dataset, occurrence of copy number gains in 3q region was 100% in both HPV 16 and HPV 18 positive cases. However this result can be caused by relatively small number of patients in our cohort.

Despite the development of whole-genome screening techniques, HPV-FISH technique is still considered as a gold standard for investigation of specific high-risk chromosomal alterations, such as amplification of *hTERC* or *MYC* genes in patients with cervical dysplasia and cervical cancer [1]. Using a combination of array-CGH and HPV-FISH we were able to detect clinically significant genomic imbalances in 96.2% of the CC cases. Comparison of HPV-FISH and array-CGH techniques showed that whole-genome profiling was unaffected by false positivity; however, using array-CGH, we were not able to detect gain in 3q26 and 8q24 in almost 50% of cases which were positive by HPV-FISH. In addition, using HPV-FISH evaluation we were able to detect genomic imbalances in 6 of 7 array-CGH negative specimens which suggest that the prevalence of copy number changes of *hTERC* and *MYC* is higher in CC compared to the rates presented by array-CGH. These discrepancies arise from approaches in evaluation of biological material for both techniques. The FISH analysis using probes for *hTERC* and *MYC* genes enables to assess the copy number alterations of these genes on a cell-by-cell basis and thus provides information about the heterogeneity in the tumors. For these reasons, HPV-FISH is much more sensitive in detection of *hTERC* and *MYC* amplifications presented in small clones compared to microarrays. Furthermore, our previous studies focused on cell sorting in hematologic malignancies showed that array-CGH technique is unable to detect unbalanced genetic lesions when CNAs are presented in less than 25%, which is in good correlation with manufacturer's specifications for cancer samples [49, 50].

In conclusion, our findings confirmed the important role of HPV infection and specific genomic alterations, especially 3q26 and 8q24 copy gains in the development of invasive cervical cancers. This study also indicates that CGH+SNP microarrays are suitable for simultaneous detection of unbalanced copy number aberrations and copy-neutral loss of heterozygosity associated with the development of CC despite of the lower sensitivity when compared with HPV-FISH evaluations in samples with low level CNAs. Thus, the combination of both techniques has been proven to be beneficial in detecting genomic imbalances in premalignant lesions and cervical cancer patients. This approach could lead to better understanding of cervical carcinoma biology, finding new genetic markers with impact on patients' prognosis and therefore improve our set of diagnostic tools.

Acknowledgements

This study was supported by grant IGA MZ NT11089-4/2010 and OP VK CZ.1.07/2.3.00/20.0183 project.

Disclosure of conflict of interest

None.

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