Transcriptional analysis of CXCR4, DNMT3A, DNMT3B and DNMT1 gene expression in primary advanced uterine cervical carcinoma

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Abstract. The development of cervical cancer requires genetic and epigenetic factors which result in the persistence of a malignant phenotype. Cervical cancer exhibits also some unique differences from other solid tumors. Normal cervical stratified epithelia have characteristics of hypoxic tissue with over-expression of HIF-1 (hypoxia-inducible factor-1) transcription factor, which targets the transcription of over 70 genes involved in many aspects of cancer biology. One of the genes, which could be induced by HIF-1 is chemokine (C-X-C motif) receptor 4 (CXCR4). CXCR4 could also be epigenetically regulated by methylation of CpG dinucleotides located in the promoter region. Here, we examined the CXCR4, DNMT3A, DNMT3B and DNMT1 transcript levels in cancer tissue (n=30) and non-cancer, normal uterine cervical tissue (n=30) from a Polish cohort. We also compared the methylation status of CXCR4 promoter region in cancer and normal tissue samples. Our result showed significantly higher levels of CXCR4, DNMT3A, DNMT3B and DNMT1 transcript (p=0.0058, 0.0163, 0.0003 and <0.0001, respectively) levels in cancer tissue as compared to normal samples. We did not observe DNA methylation in the CXCR4 promoter region in either control or cancer tissue samples. CXCR4 has a functional hypoxia response element (HRE) in the promoter region, located -1.3 kb from the transcription start site. Our work shows for the first time that HIF-1A could promote the induction of CXCR4 gene expression (Spearman’s correlation coefficient = 0.515, p=0.003) in patients with primary advanced uterine cervical carcinoma.

Introduction

Worldwide, cervical cancer is the second most common cancer in women (after breast cancer), with more than half a million new cases diagnosed in 2005 (1). The primary etiological factor associated with cervical cancer development is exposure to the human papillomavirus (HPV), particularly types 16 and 18 (2). The development of cervical cancer requires additional genetic and epigenetic factors which result in the persistence of a malignant phenotype (3). One of these genetic factors are mutations in the germ line or changes in DNA sequences arising in somatic tissues during life (4). These mutations may abnormally enhance the function of proto-oncogenes, or erase effects of the tumor suppressor gene products (5,6). One of the most important epigenetic factors associated with cancer development is DNA methylation. DNA methylation occurs primarily in CpG dinucleotides, called ‘CpG islands’, located in 5’ upstream sequences of gene promoters. The methylation of mammalian genomic DNA appears to be established by a complex interplay of at least three independently encoded DNA methyltransferases (DNMTs), including de novo (DNMT3A and DNMT3B) and maintenance (DNMT1) enzymes (7-9). Most promoter regions in animal genomes are methylated, while CpG islands located in housekeeping genes are constitutively demethylated (10). Global genome demethylation (hypomethylation) is usually associated with cellular differentiation during early development (11,12). Furthermore, malignant cells also exhibit hypomethylation of various DNA regions (13), which activates the transcription of proto-oncogenes, retrotransposons, and genes encoding proteins associated with genomic instability, invasion and metastasis (4). Moreover, hypermethylation of various tumor suppressor genes causes their transcriptional silencing and removes a barrier of normal proliferation, which may result in malignant transformation. Most interestingly, overexpression of DNMT1 and DNMT3B has been found to be common in human tumors (14-17), and this overexpression could also be associated with HPV infections (18-20).

Under low O₂ (hypoxic) conditions, cells activate a number of adaptive responses to match O₂ supply with metabolic, bioenergetic, and redox demands. Hypoxia induces hypoxia-
inducible factor-1 (HIF-1), which has been identified as an important transcription factor that mediates the cellular response to hypoxia. HIF-1 is a heterodimer composed of one of three α subunits (HIF-1α, HIF-2α or HIF-3α) and one HIF-1β subunit. HIF-1β is constitutively expressed and identical to the aryl hydrocarbon receptor, known as AhR nuclear translocator (ARNT) (21). In well-oxygenated environments, HIF-1α subunits are hydroxylated at conserved proline Pro402 and Pro564 residues. These modifications are mediated by proline hydroxylases (PHDs), whose activity is regulated by O₂ availability (22). Hydroxylated HIF-1α forms hydrogen bonds with pVHL (von Hippel Lindau protein) side chains, which promote polyubiquitination of HIF-1α, followed by 26S-proteasomal degradation (23).

Under hypoxic stress, PHD activity is diminished. Stabilized HIF-1α is involved in the activation of numerous cellular processes (24,25), and this stabilization of HIF-1α is observed in a vast number of solid tumors (26). Adaptation to hypoxic stress via HIF-1 transcriptional factor is highly complex and depends on the presence of hypoxia response element (HRE) in the promoter region of genes supporting anaerobic metabolism. One of the genes with a functional HRE in its promoter region is chemokine (C-X-C motif) receptor 4 (CXCR4). The presence of HRE suggests that this gene could be regulated by hypoxia via HIF-1 transcriptional factor.

Here, we studied the transcript levels of the CXCR4, DNMT3A, DNMT3B and DNMT1 genes in cancer and non-cancer (control) uterine cervical tissue from a Polish cohort. We also analyzed CXCR4 promoter methylation in cancer and control tissue samples.

**Materials and methods**

**Tissue samples.** Cancer and non-cancer (control) tissue samples were collected from Caucasian women of the Wielkopolska district of Poland and represent the female Polish population. Samples were collected from patients undergoing gynecological procedures at the Department of Radiotherapy, Greater Poland Cancer Center in Poznań, Poland. Normal uterine cervical sample were obtained from women undergoing uterine surgical resection in Department of Surgical Gynecology, Poznań University of Medical Sciences, Poland. Cancer (n=30) and control specimens (n=30) were collected following approval by the Local Ethics Committee of Poznań University of Medical Sciences. Written informed consent was obtained from all participating patients.

All specimens were subjected to initial H&E staining (27), followed by review by an experienced pathologist. Cervical sections with cancer cells were used as cancer samples. The same criteria were applied to identify non-cancer, normal cervical tissue samples. After surgical removal, tissue samples were frozen in liquid nitrogen and stored at -80°C until used. All 30 studied patients had squamous cell carcinoma (SCC). Among 30 patients with SCC, 1 patient was classified as stage II, 28 as stage III and 1 as stage IV, based on the International Federation of Gynecology and Obstetrics (FIGO) classification system (Table I).

**Reverse-transcription and real-time quantitative PCR (RQ-PCR) analysis of CXCR4, DNMT3A, DNMT3B and DNMT1 transcripts.** Total RNA from control and cancer tissue samples was isolated using TRI Reagent (Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) according to the method of Chomczynski and Sacchi (28). RNA integrity was confirmed by denaturing agarose gel electrophoresis, and the concentration was quantified by measuring the optical density (OD) at 260 nm using a BioPhotometer (Eppendorf AG, Hamburg, Germany). RNA samples were treated with DNase I and reverse-transcribed into cDNA using oligo-dT primers. Reverse transcription was performed using M-MLV Reverse Transcriptase (Invitrogen, Carlsbad, CA) as described in the manufacturer's protocol.

The quantitative real-time RT-PCR assay was performed using the CFX96 real-time PCR detection system (Bio-Rad Laboratories, CA, USA) using iQ Sybr Green as detection dye. Target cDNA was quantified by relative quantification method using a calibrator. For the calibrator, 1 µl of cDNA from all samples were mixed together. To create a standard curve, five 2-fold serial dilutions of the cDNA were used. The cycle threshold was recorded and plotted as a function of the dilution to generate a straight line with a slope that was related to the doubling efficiency (10^{-1/slope}). The efficiency raised to the value of 95% of the intercept of the line at no dilution is a measure of the relative amount of cDNA for each gene in the tissue samples. The quantity of CXCR4, DNMT3A, DNMT3B and DNMT1 transcripts in each sample was standardized by PBGD transcript level. For amplification, 5 µl of iQ Sybr Green Supermix (Bio-Rad Laboratories), 1 µl of primers (Oligo, Warsaw, Poland) (Table II), 3 µl of water (Sigma-Aldrich Chemie) and 1 µl of cDNA solution were mixed together. One RNA sample of each preparation was processed without RT-reaction to provide a negative control in subsequent PCR. Sample amplification included a hot start (95°C, 3 min) followed by 50 cycles of denaturation at 95°C for 10 sec, annealing at 60°C for 10 sec, and extension at 72°C for 8 sec. After amplification Melt Curve analysis was performed to analyze product melting temperature. The amplification products were resolved by 3% agarose gel electrophoresis and visualized by ethidium bromide staining. One sample of each primer pair was also used for commercial sequencing analysis.

**Sodium bisulfite DNA sequencing of CXCR4 promoter region.** Genomic DNA from control and cancer tissues was extracted
using the salting-out method. Extracted DNA was treated using EZ DNA Methylation Kit™ (Zymo Research Corp., Orange, CA), according to the manufacturer’s protocol. The location of CpG islands in the promoter regions of the CXCR4 gene was determined by a program found at http://www.ebi.ac.uk/emboss/cpgplot/ (Fig. 2). The promoter regions containing CpG islands were amplified by the primer pairs (Table II) complementary to the modified DNA. For amplification, 1.5 µl of 10X concentrated PCR buffer (Roche, Mannheim, Germany), 2.4 µl of 25 mM MgCl₂ (Roche), 1.2 µl of 2.5 mM dNTPs (Novazym, Poznan, Poland), 0.6 µl of each 10.0 µM primers (Oligo), 0.2 µl of FastStart Taq DNA Polymerase (5 U/µl) (Roche, Mannheim, Germany), 6.5 µl of water (Sigma-Aldrich Chemie) and 2 µl of modified DNA were used. One PCR reaction of each preparation was processed without a DNA template to provide a negative control in subsequent PCR. Sample amplification included a hot start (95°C, 5 min) followed by 49 cycles of denaturation at 95°C for 35 sec, annealing at 56°C for 35 sec, extension at 72°C for 45 sec and final extension at 72°C for 7 min. The amplification products were resolved by 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining. The PCR products were purified using Agarose Gel DNA Extraction Kit Roche (Mannheim, Germany) and cloned into pGEM-T Easy Vector System I Promega (Madison, WI) following transformation into TOPO10 E. coli strain cells. Plasmid DNA extracted with Pure Yield Plasmid Miniprep System (Promega, Madison, WI) from ten positive bacterial clones was used for commercial sequencing of the cloned fragments of DNA.

Table II. Primer pairs used for DNA amplification of studied gene fragments.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence (5'-3' direction)</th>
<th>Position</th>
<th>ENST no.</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXCR4</td>
<td>AAGATTGGTAGGTGAAGTG</td>
<td>-1198 -1179</td>
<td>00000241393</td>
<td>401</td>
</tr>
<tr>
<td></td>
<td>TTTCAATTCACTCCCTCTCC</td>
<td>-817 -798</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CXCR4</td>
<td>TTCTAACTGGCATTTGTTGG</td>
<td>+157 -176</td>
<td>00000241393</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>GAAGCGTGTAGCAGAAGAGG</td>
<td>+267 -286</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNMT3A</td>
<td>GGTGCCTGTCTCTCTTTGATG</td>
<td>+2243 -2262</td>
<td>00000264709</td>
<td>178</td>
</tr>
<tr>
<td></td>
<td>ATGCTTTCTGTGTGACGCTG</td>
<td>+2402 -2420</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNMT3B</td>
<td>GGAAGGAGTTTGGGAATAGG</td>
<td>+980 -999</td>
<td>00000328111</td>
<td>183</td>
</tr>
<tr>
<td></td>
<td>CCAGTGACCAGGTGTTGTC</td>
<td>+1144 -1162</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNMT1</td>
<td>GATGAGAAGAAAGCAGAAGT</td>
<td>+1128 -1148</td>
<td>00000359526</td>
<td>149</td>
</tr>
<tr>
<td></td>
<td>TCATTGGGGGCTGTTTGGCG</td>
<td>+1257 -1276</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBGD</td>
<td>GCCAAGCACCAGGCACCATC</td>
<td>+833 -850</td>
<td>00000278715</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>TCAGGTACAGGTGCCCATC</td>
<td>+974 -992</td>
<td></td>
<td></td>
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</tbody>
</table>

Statistical analysis. Data groups for cell lines were assessed by ANOVA to evaluate if there was significance (p<0.05) between the groups. For all experimental groups, which fulfilled the initial criterion, individual comparisons were performed by post hoc Tukey test with the assumption of two-tailed distribution and two samples with equal variance at the P<0.05 level. The normality of the observed patient data distribution was assessed by Shapiro-Wilk test and unpaired, two-tailed t-test or Mann-Whitney test was used to compare the mean values. P<0.05 was considered statistically significant. Spearman’s rank correlation was used to determine whether a correlation between the analyzed gene expression was positive or negative. Positive correlation is taken to mean that the expression of one gene increases when the expression of the gene it is being compared to also increases. Negative correlation is taken to mean that the expression of a gene decreases when the expression of the gene it is being compared to increases.

Results

Increased CXCR4 expression in primary advanced uterine cervical carcinoma may be induced by hypoxia and CXCR4 overexpression is not associated with epigenetic regulation due to overexpression of DNMTs. RQ-PCR was used to compare CXCR4, DNMT3A, DNMT3B and DNMT1 transcript levels in control and cancer tissues. Our results show significantly higher levels of CXCR4, DNMT3A, DNMT3B and DNMT1 transcript (p=0.0058, 0.0163, 0.0003 and <0.0001, respectively) levels in cancer tissue samples compared to the control tissue samples (Fig. 1 and Table III). We did not find significant differences between transcript levels in comparing patient groups with different tumor stage and histological grade. We also did not observe changes in the DNA methylation pattern in the CXCR4 promoter region in either control or cancer tissue samples (data not shown). In our previous report we found significantly increased HIF-1A transcript and protein levels in tumor tissue samples compared to their normal, non-cancer counterpart (29). A statistically significant association was found between HIF-1A
and CXCR4 expression (Spearman’s correlation coefficient = 0.445, p=0.01).

Discussion

Cervical cancer is a gynecological malignancy with one of the highest mortality rates of women worldwide. Cervical cancer exhibits some unique characteristics that are different from other solid tumors. Healthy cervical cells have characteristics of chronically hypoxic tissue (30,31) with partial stabilization of the HIF-1α protein. This stabilization increases during carcinogenesis and the progression of cervical cancer. Increased vascularity, as a result of stabilized HIF-1α protein, is observed in low grade cervical cancer, while such stabilization is a late event in the progression of many other solid cancers (31,32). In non-cancer, well-oxygenated tissue (normoxia), HIF-1α subunits are continuously hydroxylated at conserved proline residues, located in the oxygen-dependent degradation domain, by proline hydroxylases (PHDs), whose activities are O₂ dependent (22). The inhibition of PHDs activity is critical for HIF-1α stabilization and escape from proteosome degradation during hypoxia (33-35). In addition to hypoxia, other factors independent of hypoxia can promote HIF-1α protein stabilization and accumulation via translational or post-translational mechanisms (36). HIF-1A as a transcription factor can target the transcription of over 70 genes (36) involved in the cellular response to hypoxia (25,26), such as induction of proliferation, migration, and blood vessel formation by endothelial cells (37). Transcription of hypoxia-inducible genes is regulated by one or more hypoxia response elements (HREs) located in either the promoter or enhancer regions (38). The majority of hypoxia induced gene HRE contains a HIF-1 ancillary sequence (HAS), which is located 8-9 nt down- or upstream of hypoxia binding sites. This sequence is necessary for HIF-1A mediated transcription activation (38). Furthermore, efficient gene activation requires recruiting of additional transcriptional factors, such as ATF-1/CREB-1, AP-1 and HNF-4 factor (39), which are not hypoxia dependent. Single nucleotide mutations or epigenetic alternation may remove binding sites for transcription factors, which are required for hypoxia HIF-1 induced gene expression (39). Nucleotides located close to HRE in the promoter sequence can also affect HIF-1 gene activation (36). Therefore, mutations or CpG methylation present in the promoter region of a gene could perturb the gene activation via HIF-1.

The CXCR4 receptor binds the chemokine CXCL12 and activates intracellular signaling pathways that promote survival, proliferation and migration of cancer cells (40). CXCR4 is expressed in a wide variety of non-cancer tissues such as bone marrow, blood, spleen, thymus, lymph nodes, and the pituitary
and adrenal glands (40), as well as in ovarian, breast, lung, prostate, stomach, colon, kidney, brain, thyroid, liver, pancreatic, esophageal, cervical, and oral cancers, and melanoma and leukemia (40). Expression of CXCR4 could also be epigenetically regulated by methylation of CpG dinucleotides located in the promoter region. Computational analysis demonstrated that CpG islands are located in the promoter region of the CXCR4 gene (Fig. 2). Therefore, we also analyzed the expression levels of those DNMTs that could regulate the expression of the CXCR4 gene epigenetically.

The expression of CXCR4 has been investigated in different tumor types. Chick and Szyf (41) and Ateeq et al (42) demonstrated that the demethylating agent 5'-aza-2'-deoxycytidine induced prometastatic CXCR4 gene expression by the demethylation of its promoter in MCF-7 and ZR-75-1 breast cancer cell lines. By contrast, the results presented by Dejeux et al (43) in a group of 6 normal breast tissue samples and 163 unselected samples from locally advanced breast cancer did not demonstrate methylation of CpGs located in the promoter region of the CXCR4 gene either the non-cancer samples nor in the breast cancer samples. Przybylski et al (44) and Sato et al (45) analyzed the methylation status of the CXCR4 gene in pancreatic cell lines. Przybylski et al (44) employed knockdown of the DNMT1 and DNMT3B genes in AsPC1 pancreatic cancer cell line and demonstrated that depletion of both of these genes was associated with CXCR4 promoter demethylation and the production of CXCR4 transcript and protein. Sato et al (45) demonstrated that CpG islands of the CXCR4 gene were unmethylated in normal pancreatic ductal epithelial samples, whereas promoter hypermethylation was detected in pancreatic cancer cell lines and in primary pancreatic adenocarcinomas (45). Promoter methylation of the CXCR4 gene was also reported in estrogen receptor positive Ishikawa endometrial adenocarcinoma (46), melanoma cells (47) and in patients with primary myelofibrosis (48). The levels of CXCR4 have also been evaluated in cervical cancer but using only an immunohistochemical method. Kodama et al (49) examined the immunostaining profiles of CXCR4 in 174 cervical cancer patients presenting with stage IB-IIb cervical cancers. The authors concluded that the expression of CXCR4 may be associated with lymph node metastasis and also provide evidence that CXCR4 expression can serve as an indicator of poor prognosis in patients with cervical cancer. Similar results were presented by Zhang et al (50). Immunohistochemical detection of CXCR4 in 35 analyzed squamous cell carcinoma tissue samples revealed that this gene probably participates in lymph node metastasis in cervical cancer (50).

Also, Yang et al (51) demonstrated that immunohistochemically detected CXCR4 expression was associated with cervical adenocarcinoma cell migration and proliferation. Moreover, cancer cells expressing CXCR4 were significantly more likely to metastasize to pelvic lymph nodes (51). Interestingly, Rein et al (52) used purified primary cervical cancer cells from three patients, normal control cervical cells from healthy women, as well as various established cervical cancer cell lines, and demonstrated that the CXCR4 promoter was active only in the cancer cervical tissues and cancer cell lines but its activity was low.

Staller et al (40) showed that the HRE located -1.3 kb in the CXCR4 promoter is critical for the hypoxia HIF-1 inducible activity of this gene, and this HRE is located in the region of CpG islands of the CXCR4 gene (Fig. 2). Tang et al (53) were the first to report the expression of HIF-1A target CXCR4 gene expression in HIF-1A-transfected SiHa cells. Overexpression of HIF-1A affects the proliferation, apoptosis, and migration of SiHa cells in part by regulating the expression of the target gene, CXCR4.

Our experiments clearly show that the CXCR4 gene is not epigenetically regulated in primary advanced cervical carcinoma. We did not observe methylation in the promoter region of the CXCR4 gene in either the control tissue samples or the cancer tissue samples (data not shown). Moreover, we observed an overexpression of the CXCR4 gene in tumor tissue samples compared to their normal, non-cancer counterparts, which could be associated with increased levels of HIF-1A (29); we found a statistically significant, positive Spearman's rank correlation, between HIF-1A and CXCR4 expression. Thus, our results show for the first time that HIF-1A could promote the induction of CXCR4 gene expression in patients with primary advanced uterine cervical carcinoma. Our results are the first to confirm, on a patient level, the results presented by Tang et al (53).

DNMT3A is thought to function in de novo methylation and its expression is developmentally regulated by its different isoforms (54-56). DNMT3A -/- knock-out mice appear to be normally developed, but die shortly after birth (57). However, the relationship between DNMT3A and tumorigenesis is still largely unknown. Deng et al (58) reported that

### Table III. CXCR4, DNMT3A, DNMT3B and DNMT1 transcript levels in advanced uterine cervical carcinoma and corresponding control tissues.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Control tissue median (range)</th>
<th>Control tissue mean (± SD)</th>
<th>Cancer tissue median (range)</th>
<th>Cancer tissue mean (± SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXCR4</td>
<td>0.68 (0.13-2.35)</td>
<td>0.82±0.56</td>
<td>1.35 (0.11-3.95)</td>
<td>1.39±0.93</td>
<td>0.0058a</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>0.65 (0.01-2.26)</td>
<td>0.76±0.57</td>
<td>0.87 (0.26-4.81)</td>
<td>1.26±1.10</td>
<td>0.0163b</td>
</tr>
<tr>
<td>DNMT3B</td>
<td>0.22 (0.07-1.45)</td>
<td>0.29±0.28</td>
<td>0.80 (0.07-5.07)</td>
<td>1.28±1.38</td>
<td>0.0003a</td>
</tr>
<tr>
<td>DNMT1</td>
<td>0.56 (0.13-2.32)</td>
<td>0.68±0.63</td>
<td>1.16 (0.46-3.97)</td>
<td>1.48±0.94</td>
<td>0.0001b</td>
</tr>
</tbody>
</table>

The target mRNA levels were corrected to the amount of PBGD cDNA and expressed as multiplicity of these cDNA copies in the calibrator. *Unpaired, two-tailed t-test. **Mann-Whitney test.*
inhibition of DNMT3A expression, by stable transfection of a DNMT3A-RNA interference construct, dramatically inhibited melanoma growth and metastasis in mouse melanoma models. Ng et al (59) described an upregulation of DNMT3A in primary colorectal cancer tissues compared with their paired non-cancer colonic tissues. Also, Daniel et al (60), using an immunohistochemical method, reported overexpression of DNMT3A in oral squamous cell carcinoma (grade I-III) specimens compared to control tissues. Similar results were presented by Qu et al (61) in patients with retinoblastoma. Zhao et al (62) were the first who provided evidence that DNMT3A could be involved in promoter hypermethylation during carcinogenesis. The authors observed increased DNMT3A expression in hepatocellular carcinoma cell lines and tissue samples. Knockdown of DNMT3A in the SMMC-7721 cell line revealed that 153 genes were upregulated and 91 of them contained CpG islands in their 5' promoter region. Moreover, 13 of them were crucial tumor suppressor genes involved in the cell cycle and cell proliferation (62). The most recent data on preferential target sites for human DNA methyltransferases, including distinct isoforms of DNMT3A, is reported by Choi et al (63).

DNMT1 (64) and DNMT3B (65) were both shown to be important for cancer cell survival and tumorigenesis, and the overexpression of these genes has been described in many different tumor types (17). Only Sawada et al (66) analyzed the protein levels of DNMT1 in histologically normal squamous epithelium, low-grade (CIN1) and higher-grade (CIN2 and CIN3) cervical intraepithelial neoplasia, and samples of squamous cell carcinoma of the uterine cervix using an immunohistochemical examination. These authors observed progressively increased levels of DNMT1 protein with increasing cancer grades and a plateau in microinvasive carcinoma. These data suggest that expression of the DNMT1 protein is associated with an early step of multistage cervical carcinogenesis. DNMT1 protein overexpression is a very early event during multistage cervical carcinogenesis because DNMT1 may be activated by the HPV-16 E7 protein (66). The HPV-16 E7 protein has been reported to directly associate with DNMT1 and stimulate the methyltransferase activity of DNMT1 in vitro (67). Au Yeung et al (18) reported that HPV-16 E6 could also up-regulate DNMT1 expression in human cervical cancer cell lines. Our research is the first to show a complete transcriptional analysis of the DNMT1, DNMT3B and DNMT3A methyltransferases in primary advanced uterine cervical carcinoma. The expression of all of the analyzed DNMTs was significantly increased in primary advanced cervical carcinoma compared to non-cancer counterparts.

In conclusion, we are the first to demonstrate increased CXXCR4, DNMT3A, DNMT3B and DNMT1 transcript levels in advanced uterine cervical carcinoma. We also observe that the increase in CXXCR4 expression could be HIF-1A dependent and the CXXCR4 gene is not epigenetically regulated by overexpressed methyltransferases.

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References


