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1p36.22 region containing *PGD* gene is frequently gained in human cervical cancer

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Abstract

Aim: To identify commonly occurring DNA copy number alterations in Korean cervical cancers.

Methods: DNA copy number alteration was screened by whole-genome array comparative genomic hybridization (CGH) analysis. For the array CGH discovery, genomic DNA from five cervical cancers and 10 normal cervical tissues were examined. For the independent validation of the most significant chromosomal alteration (1p36.22, *PGD* gene), 40 formalin-fixed paraffin-embedded cervical tissue samples were collected; 10 of them were used for quantitative polymerase chain reaction and the other 30 samples were used for immunohistochemical analysis. Chromosomal segments differently distributed between cancers and normal controls were determined to be recurrently altered regions (RAR).

Results: A total of 13 RAR (11 RAR losses and two RAR gains) were defined in this study. Of the 13 cervical cancer-specific RAR, RAR gain in the 1p36.22 locus where the *PGD* gene is located was the most commonly detected in cancers ($P = 0.004$). In the quantitative polymerase chain reaction replication, copy number gain of the *PGD* gene was consistently identified in cervical cancers but not in the normal tissues ($P = 0.02$). In immunohistochemical analysis, PGD expression was significantly higher in cervical cancers than normal tissues ($P = 0.02$).

Conclusion: Our results will be helpful to understand cervical carcinogenesis, and the *PGD* gene can be a useful biomarker of cervical cancer.

Key words: array comparative genomic hybridization, cervical cancer, copy number alteration, *PGD*, recurrently altered region.

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Introduction

Cervical cancer is the third most common female cancer and the fourth leading cause of cancer-related mortality in women worldwide.[1] Persistent oncogenic human papillomavirus (HPV) infection is a major cause of cervical intraepithelial neoplasia (CIN) and cancer.[2,3] However, most HPV infections do not progress to cancer and there is a need to identify additional factors associated with or predictive of the HPV infections that will progress to high-grade CIN and cancer.

Chromosomal alterations are one of the hallmarks of cancers. The copy number alterations (CNA) observed in diverse solid tumors are thought to be involved in initiation and progression of the cancers by affecting the activities of cancer-related genes in the altered chromosomal regions.[4,5] Thus, genome-wide mapping of CNA in cancers is important to understand tumorigenesis and can facilitate the identification of cancer-driving genes. Microarray-based comparative genomic hybridization (array CGH) has emerged as a powerful tool for identifying and mapping the genomic aberrations genome-wide, which may contain candidate cancer-related genes such as oncogenes or tumor suppressors.[6–8] Using a CGH or array CGH tool, a number of CNA has been reported in cervical cancers.[9–11] For example, gain of chromosome arm 3q was consistently found in cases of advanced stage cervical cancers.[12] Gain of 3q and loss of 11q are reported to be early events in the progression of cervical cancer.[13] However, the resolution of most of the previous reports was not high enough, so it is still difficult to identify the reliable and biologically relevant CNA in cervical cancer. In addition, most of the previous reports were with Caucasians and Asian data is relatively low.

In this study, we aimed to identify commonly occurring CNA regions in Korean cervical cancers using a high-resolution array CGH platform and to validate the significant alteration region by using target-specific genomic quantitative polymerase chain reaction (qPCR) and immunohistochemistry (IHC).

Methods

Patients and tissue specimens

This study was performed under approval from the institutional review boards of the Catholic University of Korea and Kangdong Sacred Heart Hospital of Korea. As a discovery set for the whole genome array CGH analysis, frozen tissues were obtained from five cervical cancer patients and 10 normal cervical tissues from patients who underwent cervical biopsy at St Vincent's Hospital and Kangdong Sacred Heart Hospital, Korea, from January 2011 to February 2012. Normal samples were obtained in patients undergoing hysterectomy for benign disease.

For the independent validation of the CNA identified by array CGH, 40 formalin-fixed paraffin-embedded (FFPE) cervical tissue samples were obtained from the same hospitals described above; 10 of them were used for qPCR and the other 30 samples were used for IHC analysis. Tumor stage was determined according to the International Federation of Gynecology and Obstetrics stage. Clinicopathological characteristics of the study subjects are summarized in Table 1. For array CGH analysis, 10-μm-thick frozen sections of tumor cell-rich areas (>60%) were microdissected. Genomic DNA was extracted from these sections using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). For genomic real-time qPCR analysis, 10-μm-thick paraffin sections of tumor cell-rich areas (>60%) in the replication set were microdissected. After paraffin removal, genomic DNA was extracted using a DNeasy Blood & Tissue Kit (Qiagen). Genomic DNA from a healthy female individual was used as the normal reference. Genomic DNA extracted from the blood of a Korean female individual without cervical cancer was used as universal normal reference for all the array CGH experiments.

Array CGH and image analysis

We used a 3x720K Nimblegene human CGH whole-genome array (Roche, Penzberg, Germany) for array CGH analysis. All the array CGH procedures including DNA labeling, pre-hybridization and hybridization using an MAUI hybridization station (BioMicro Systems, Salt Lake City, UT, USA) were performed as described elsewhere.[14] In brief, 0.5 μg of genomic DNA from the target sample and reference sample were labeled with Cy3- and Cy5-deoxycytidine triphosphate, respectively, and co-precipitated with 50 μg human Cot I DNA (HybMasker, ConnectaGen, Seoul, Korea). After co-hybridization onto the oligoarray and washing, the array was scanned using the GenePix 4100B scanner (Axon Instruments, Union City, CA, USA) and the images were processed using the Nimble scan software. After image processing, each probe was mapped according to its genomic location in the UCSC Genome Browser (human NCBI36/hg18 from March 2006).

Table 1 Clinicopathological characteristics of 45 cervical cancer patients

Characteristics	Cervical cancer (discovery set) ($n = 5$)	Cervical cancer FFPE (replication set) ($n = 10$)	Cervical cancer FFPE (IHC) ($n = 30$)
Age, median (range) (years)	46 (46–72)	51 (36–85)	48 (38–76)
Parity, median (range)	2 (0–3)	2 (0–4)	2 (0–4)
Stage, *n* (%)			
I	4 (80%)	9 (90%)	24 (80%)
II	1 (20%)	1 (10%)	6 (20%)
Tumor grade, *n* (%)			
1	1 (20%)	4 (40%)	15 (50%)
2	4 (80%)	5 (50%)	13 (43%)
3	0	1 (10%)	2 (7%)
Histology, *n* (%)			
Squamous cell carcinoma	4 (80%)	8 (80%)	21 (70%)
Adenocarcinoma	1 (20%)	2 (20%)	9 (30%)
Lymph node metastasis, *n* (%)			
Yes	0	0	0
Tumor size, median (range) (cm)	2 (0–3.5)	2 (0–5)	2 (1–4)

FFPE, formalin-fixed, paraffin-embedded; IHC, immunohistochemistry.

Detection of recurrent CNA

Copy number alterations of each sample were defined by the Rank Segmentation statistical algorithm in NEXUS ver. 3.1 software (BioDiscovery, El Segundo, CA, USA). Our parameters for defining CNA were as follows: significance threshold, 10^{-6}; maximum contiguous probe spacing, 1000 kbp; minimum number of contiguous probes per CNA segment, 5; and threshold of signal intensity ratio, more than 0.5 in $\log_2$ scale for gains and 0.5 or less in $\log_2$ scale for losses. For the visualization, Agilent Genomic Workbench ver. 7.0 was also used. After the CNA profiling of each sample, chromosomal segments differently distributed between cancers and normal controls ($P < 0.05$) were determined to be recurrently altered regions (RAR) using Fisher's exact test in the CNVRuler program.[15]

Genomic qPCR analysis

To validate the cervical cancer-specific copy number gain in the *PGD* gene, we designed four genomic qPCR primers in the *PGD* gene. All four qPCR primers were designed in the exons of the *PGD* gene. Details of the primer information for targets and diploid control locus are available in Table S1. We performed qPCR validation of the significant RAR using the genomic DNA extracted from 15 samples from the discovery set (five cancers and 10 normal cervical tissues) and 10 independent FFPE samples from the replication set. For the qPCR validation, a genomic region which showed no genomic alteration on array CGH data (13q32.1) was used as internal control. Genomic qPCR was performed using Mx3000P qPCR system (Stratagene, La Jolla, CA, USA) as described elsewhere.[16] In brief, 10 μL real-time qPCR mixture contained 20 ng of genomic DNA, SYBR Premix Ex Taq TM II (TaKaRa Bio, Japan), 1 × ROX and 5 pmol of primers. Thermal cycling conditions consisted of one cycle of 30 s at 95°C, followed by 45 cycles of 5 s at 95°C, 10 s at 55–61°C and 20 s at 72°C. All the qPCR experiments were repeated two times. Copy number of each target was defined as 2-ΔΔCT, where ΔCt is the difference in threshold cycles for the sample in question normalized against the reference gene (13q32.1) and expressed relative to the value obtained by calibrator DNA (individual/mxDNA) and relative quantification was performed by the ΔΔCt method. Then, the ratio data was converted to the nearest integer as described elsewhere.[17] Our quality control criterion of qPCR was standard deviation of less than 0.3 of Ct values. In this study, all the qPCR assays passed our quality control criterion.

IHC

Immunohistochemical analysis was performed on tissue samples of the 30 independent FFPE cervical tissue samples. Fifteen normal cervical samples were used as controls. Recipient blocks were made from purified agar in $3.8 \times 2.2 \times 0.5\ cm^3$ frames and were prepared after a thorough evaluation of the hematoxylin–eosin-stained slides consecutive 4-μm-thick sections were cut from each tissue block using an adhesive-coated slide system (Instrumedics,

Hackensack, NJ, USA). The 4-μm-thick sections were placed on silane-coated slides, deparaffinized, immersed in phosphate-buffered saline (PBS) containing 0.3% (v/v) hydrogen peroxide, and then processed in a microwave oven (in 10 mmol/L sodium citrate buffer, pH 6.0) for 15 min at 700 W. After blocking with 1% (w/v) bovine serum albumin in PBS containing 0.05% (v/v) Tween-20, for 30 min, the slides were incubated overnight at 4°C with a PGD-specific antibody (mouse monoclonal, 3 ug/mL; Abcam, Cambridge, MA, USA). Strong cytoplasmic staining was considered a positive result. PGD expression was measured from the 30 cervical cancer tissues and 15 normal cervical tissue controls. Each sample was scored on signal intensity and proportion, and a score greater than or equal to 4 was considered 'positive'. Expression scores were assigned semiquantitatively according to the percentage of cells stained (0, 0%; 1, 1–25%; 2, 25–75%; and 3, >75%) and staining intensity (0, none; 1, weak; 2, moderate; and 3, strong). The two individual scores were then multiplied to yield a final score that takes into account both the number of PGD positive cells and staining intensity. The product of the two scores was 3 or less and PGD expression was considered negative.

Statistical analysis

Fisher's exact test was used for association analysis of CNA (gains and losses) throughout the genome between the cancer and normal group, where $P < 0.05$ was considered significant. For validation qPCR, independent Student's *t*-test was utilized to compare the gene doses of sample genes and control genes: $P < 0.05$ was considered significant. Statistical analyses were done using SPSS ver. 18.0 (SPSS, Chicago, IL, USA).

Results

General characteristics of CNA in cervical cancer

On average, 659 CNA were detected across the whole chromosomes in the 15 samples. The average number of CNA identified in the cervical cancer group (751 per case, ranging 410–1002) were higher than that in the normal controls (613 per case, ranging 381–1140), but not statistically significant. In addition to the small-sized CNA, some of the CNA were entire arm changes or Mb-sized. Figure 1 illustrates the whole genome CNA plot of a cervical cancer which contains copy number gains of 3q, 17q25.1 and 19q13, and loss of 11q as an example.

Recurrently altered chromosomal regions in cervical cancers

To delineate the commonly altered consensus chromosomal regions in the cervical cancers examined, we defined the RAR (RAR-G for gains and RAR-L for

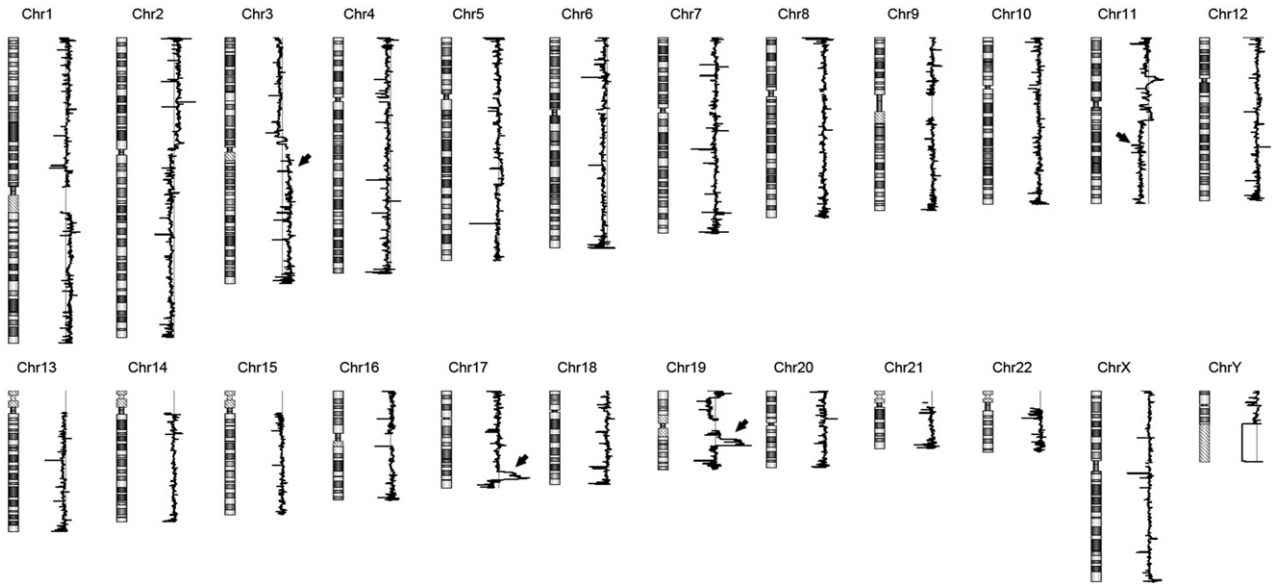

Figure 1 Genome-wide array comparative genomic hybridization (CGH) profile of a primary cervical cancer used for array CGH discovery. Log_2 signal intensity ratios are plotted using Agilent Genomic Workbench software. Arrowheads indicate the copy number gains of 3q, 17q25.1 and 19q13, and loss of 11q. The cytoband image of each chromosome is illustrated to the left of each signal intensity plot.

losses, respectively). A total of 2438 RAR were defined and among them, 13 RAR (11 RAR-L and two RAR-G) were found to be significantly more frequent in the cancers than normal controls in this study (Table 2). Of the 13 cancer-specific RAR, RAR-G in the 1p36.22 locus was the most commonly detected in cancers. Four out of the five cervical cancers (80%) showed the copy number gain of this region, while none of the 10 normal controls had the copy number gain in this region ($P = 0.004$). The phosphogluconate dehydrogenase (*PGD*) gene is located in this region. In addition to RAR-G in 1p36.22, some of cancer-related genes are located in the RARs. For example, polyamine-modulated factor 1 (*PMF1*), a potential tumor suppressor gene, is located in the common deletion region in 1q22 and another candidate tumor suppressor gene, IKAROS family zinc finger 1 (*IKZF1*), is located in the RAR-L in 7p12.2 (Table 2).

Validation of CNA by genomic qPCR

As described above, RAR-G in 1p36.22, where the *PGD* gene is located, was the most commonly detected in cervical cancers but was not detected in normal control tissue. However, the estimated RAR-G covers not the whole *PGD* gene but the distal part of the gene (Fig. 2a). We validated the RAR-G by two qPCR. The first qPCR (PGD-1) targets the estimated RAR and the second qPCR (PGD-2) targets outside the RAR in *PGD* gene. The qPCR validation strategy is illustrated in Figure 2(a). In the validation of the 15 array CGH discovery samples (five cancers and 10 normal tissues), all the RAR-G identified by array CGH were consistently defined with one exception. In brief, three out of the five cervical cancers (60%) consistently showed gain of *PGD* gene in both qPCR validations, while none of the 10 normal samples showed gain in both qPCR ($P = 0.02$) (Fig. 2b). In the independent replication, five of the 10 cervical cancers showed copy number gain in both qPCR validations.

We further explored whether the copy number gain events identified by array CGH covers just the distal part or the whole *PGD* gene by adding two more qPCR (PGD-3 and -4) targeting the proximal end of the *PGD* gene (Fig. 2a). If the two qPCR forming the distal end were gain-positives, and more than one of the two qPCR in the proximal end of the *PGD* gene were consistently gain-positives, our interpretation was that the RAR-G covers the whole *PGD* gene. As a result, all three cancers in the discovery set and the five cancers in the replication set consistently showed copy number gains at both proximal and distal ends of the *PGD* gene;

Table 2 Recurrently altered regions in cervical cancers

Chromosome	Start†	End†	Cytoband	Size (bp)	Frequency		Type	*P*-value‡	Genes	DGV§
					Control ($n = 10$)	Case ($n = 5$)				
1	10 401 579	10 406 258	1p36.22	4 680	0	4	Gain	0.004	*PGD*	Yes
11	9 280 254	9 280 877	11p15.4	624	1	4	Loss	0.017	*TMEM41B*	Yes
1	1 173 239	1 173 752	1p36.33	514	0	3	Loss	0.022	—	Yes
1	154 453 666	154 456 830	1q22	3 165	0	3	Loss	0.022	*PMF1*	No
3	147 867 679	147 873 101	3q24	5 423	0	3	Gain	0.022	—	Yes
7	50 311 346	50 318 808	7p12.2	7 463	0	3	Loss	0.022	*ZNFN1A1, IKZF1*	Yes
10	111 130 699	111 138 379	10q25.1	7 681	0	3	Loss	0.022	—	Yes
11	129 445 509	129 471 639	11q24.3	26 131	0	3	Loss	0.022	*APLP2*	Yes
13	49 972 381	49 973 092	13q14.3	712	0	3	Loss	0.022	*XTP6*	Yes
14	100 986 698	101 001 143	14q32.31	14 446	0	3	Loss	0.022	—	Yes
16	56 280 047	56 289 181	16q13	9 135	0	3	Loss	0.022	*GPR97, CCDC135*	Yes
17	78 520 954	78 524 069	17q25.3	3 116	0	3	Loss	0.022	*B3GNTL1*	Yes
19	35 553 471	35 561 096	19q12	7 626	0	3	Loss	0.022	*ZNF536*	No

†UCSC genome browser (Human NCBI36/hg18 from March 2006). ‡Thirteen recurrently altered regions showing significantly different ($P < 0.05$) distribution between cervical cancer and normal cervical tissues are illustrated. §Overlapping status with DGV (http://projects.tcag.ca/variation/) entries.

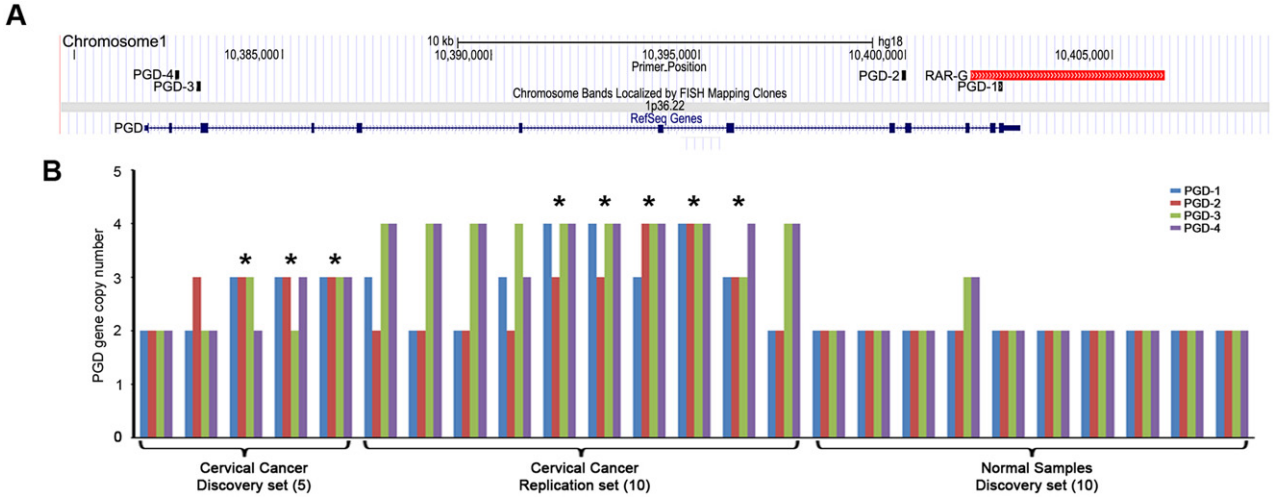

Figure 2 Validation of the recurrently altered region on 1p36.22. (a) Strategy of the genomic quantitative polymerase chain reaction (qPCR) of the *PGD* gene. The 4.7-kb sized recurrently altered region gain (RAR-G) was mapped in the distal end of the *PGD* gene on 1p36.22 (red bar). To validate the RAR-G by genomic qPCR, we designed two primer sets. The first primer (PGD-1) was designed in the estimated RAR and the second primer (PGD-2) was designed outside the RAR in the *PGD* gene. To explore whether the copy number gain events identified by array comparative genomic hybridization (CGH) covers just the distal part or the whole *PGD* gene, we designed two more qPCR (PGD-3 and -4) targeting the proximal end of the *PGD* gene. All four primers were designed in the exons. (b) Interpretation of the genomic qPCR result of the *PGD* gene. If the two qPCR at the distal end were copy number gain-positives, and more than one of the two qPCR at the proximal end of the *PGD* gene were consistently gain-positives, we interpreted that as the copy number gain of the whole *PGD* gene. Genomic qPCR assay was performed with 15 discovery sets and 10 cervical cancers for independent replication. Asterisk represents true copy number gain of the whole *PGD* gene in our criteria. Blue, red, green and purple bars denote the result of PGD-1, -2, -3 and -4 qPCR, respectively. *X*-axis, individual samples; *Y*-axis, estimated *PGD* gene copy number.

therefore, our interpretation was that they had whole *PGD* gain (Fig. 2b). However, none of the normal samples had *PGD* gain (Fig. 2b).

IHC analysis of PGD

To further validate whether PGD protein expression elevated in the cervical cancer tissues compare with normal cervical tissue, we performed IHC analysis for the PGD with 30 independent cervical cancers and 15 normal cervical tissues (Fig. 3). All 30 cervical cancer samples expressed PGD protein in the cytoplasm. Forty percent (6/15) of normal samples express PGD protein, but the staining intensity was weak to moderate. IHC results showed PGD expression is relatively higher in cervical cancer and lower in the normal cervical tissue ($P = 0.02$). However, there was no significant association with tumor grade, stage and PGD staining intensity (data not shown).

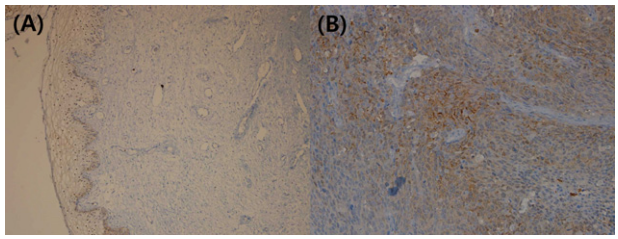

Figure 3 Immunohistochemical staining of PGD protein expression in cervical tissues. (a) Normal cervical tissue. (b) Cervical cancer tissue.

Discussion

In this study, we investigated genomic CNA in patients with cervical cancer and normal control via whole-genome array CGH. We identified 13 RAR which were significantly more common in cervical cancers than normal tissues across the diverse chromosomes: recurrent copy number gain on 1p36.22, 1p36.33 and 3q24; recurrent copy number loss on 1q22, 7p12.2, 10q25.1, 11p15.4, 11q24.3, 13q14.3, 14q32.31, 16q13, 17q25.3 and 19q12. Deletions on 2p, 2q34-q36, 3p14, 4 and 6p, and gains on 1, 3q, 5p15.2 and 5p13, have been reported in cervical cancers.[9–11] Some of the CNA identified in this study such as gains of 1p, 3q and loss of 11q are

coherent with the previous reports.[12,13] These results suggest the reliability of our array CGH analysis. Due to the higher resolution of our array CGH platform, we could delineate the CNA regions more precisely than previous reports and also we identified the recurrent chromosomal regions which were not reported previously, such as copy number gain in 1p36.22 and copy number losses in 7p12.2 and 13q14.3. Some CNA were suggested to be stage-specific or to be associated with clinical outcomes in cervical cancers.[12,13,18–20] However, over 600 copy number changes identified in the normal controls suggest that a substantial number of the CNA identified in this study can be copy number variations (CNV). So, only the validated RAR in this study is a candidate target which we can suggest as cervical cancer-specific CNA. In this study, due to the limited number of study samples, we could not observe the proper clinical correlation of the CNA identified from the five cervical cancers. Further study with a larger cervical cancer cohort group will be required to assess the detailed correlation of each RAR and clinical characteristics.

The most significant and interesting finding in this study was recurrent copy number gain on 1p36.22 in cervical cancer patients, where the *PGD* gene is located. However, the estimated RAR-G just mapped the distal part of the *PGD* gene. To verify whether the copy number gain identified by array CGH covers the whole *PGD* gene, we designed two primer sets targeting the distal end of the gene where RAR-G is located and also two more primer sets targeting the proximal end of the gene. If the qPCR at both loci consistently showed the copy number gains, we can interpret that those individuals have gain of *PGD*. Through this validation, we successfully validated that the RAR-G identified by array CGH is true and covers the whole *PGD* gene. This result is coherent with the result that PGD expression is relatively higher in cervical cancer and lower in the normal cervical tissue. Although qPCR still has limitations in the sensitivity for detecting the copy number differences, this assay is now the state-of-art technology for quantitative measurement.[21] Therefore, the copy number gain of the *PGD* gene dominantly identified in the cervical cancers seemed reliable.

Chromosome 1p is reported to be commonly deleted or duplicated depending on the cancer.[22] Gain of 1p36 was reported in several cancers such as uterine leiomyosarcoma, esophageal squamous cell carcinoma and diffuse large B-cell lymphoma.[23–25] Especially, amplification of 1p36 region has been recurrently reported in uterine cervical cancers.[26–28] These reports suggest that copy number gain in 1p36 may contribute to cervical tumorigenesis; however, a specific target gene in this region has not been suggested yet. The *PGD* gene on 1p36 is the second dehydrogenase in the pentose phosphate pathway.[29,30] It has been well known that pentose phosphate activity is elevated in actively proliferating cells[30] and indeed, 6PGD expression was elevated in the uterine cervical cancer cells in the histochemical studies.[30,31] PGD is also known to be crucial in protection against oxidative stress in mouse embryonic stem cells.[32,33] These findings show that the diversion of glycolytic flux into a specific alternate pathway can be selected during tumor development and may contribute to the pathogenesis of human cancer. In our observation of PGD protein expression in cervical cancers by IHC, consistent with the copy number status, PGD expression was significantly elevated in the cervical cancers compared with the normal controls. Taken together, copy number gain of the *PGD* gene in 1p36.22 may affect elevated expression of PGD which are likely to play an important role in the tumorigenesis of cervical cancer.

There are several candidate cancer-related genes in the RAR identified in this study. *PMF1*, a potential tumor suppressor gene, is located in the common deletion region in 1q22. Polyamine is one of the important regulators of cell growth and PMF1 is known to be involved in the polyamine homeostasis.[34] In human bladder cancer, *PMF1* was reported to be hypermethylated.[34] *IKZF1* located in the RAR-L in 7p12.2 was also frequently deleted in this study. *IKZF1* deletion has been commonly observed in acute lymphocytic leukemia (ALL) and its alteration is suggested as a hallmark of ALL.[35] Our data and previous reports suggest that *PMF1* and *IKZF1* may play a tumor suppressive role in cervical cancer. Further large-scale array CGH study to verify the deletion of these genes in cervical cancers and also further functional studies exploring their role in cervical cancer tumorigenesis will be required.

Our study has limitations. First, due to relatively small sample size, we could not examine the association between the significant RAR and clinical characteristics. Although we performed independent validation with a relatively larger replication set to complement the limitation, it is too early to conclude that the *PGD* alteration is a causative factor for cervical tumorigenesis. For the future large-scale target-specific replication and clinical association study, FFPE samples can be considerable. Second, although we used a well-designed high-resolution array, there is a possibility of false discovery. In this study, we only

validate the most significant RAR. A further large-scale qPCR experiment targeting the candidate loci will be required to validate our whole-genome array analysis. Third, the reference DNA for array CGH analysis was not from the same patient but a normal DNA pool without cervical cancer, therefore, we cannot rule out that the CNA identified in this study can be copy number variations. Although some CNA identified in this study such as gains of 1p, 3q and loss of 11q are coherent with the previous reports, over 600 copy number changes identified in this study suggest that some of them can be CNV. Fourth, we did not examine the functional effect of the genetic alterations in this study.

In conclusion, through whole-genome array CGH analysis, we identified that 13 RAR more commonly occur in the cervical cancers than normal tissues. Among them, we validate the cervical cancer dominant *PGD* gene amplification by genomic qPCR and IHC in the independent cervical cancer group. All array CGH screening, qPCR validation and IHC validation result consistently showed that *PGD* is commonly gained and overexpressed in cervical cancers. Our results will be helpful to understand the genetic tumorigenesis of cervical cancer and *PGD* can be useful as a biomarker of cervical cancer.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1 Primers for PGD genomic quantitative polymerase chain reaction