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ORIGINAL ARTICLE

Mitomycin C modulates DNA-double strand break repair genes in cervical carcinoma cells

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Abstract In a previous study, we elucidated the apoptotic mechanism mediated via Fas/FasL-dependent pathway in mitomycin C-treated cervical carcinoma cells. In this study, 2-D and MALDI-TOF analyses were performed in order to search mitomycin C-induced modulators in cervical carcinoma cells. Some protein spots down- or up-regulated by mitomycin C were separately selected from the 2-D gels. Twenty protein spots were identified from the 2-D gels. Among the 20 spots, 11 spots were down-regulated, whereas 9 spots were up-regulated in SiHa/pRSV-luc cells by mitomycin C. Three spots have not been identified in the database. Ku70-binding protein (KUB3), MHC class I antigen, MHC class I chain-related protein A or multi-PDZ domain protein 1, MAGUK P55 subfamily member 3 or lamda/iota protein kinase C-interacting protein, and GL014 or Sad1/unc-84 protein-like 1 were suppressed by mitomycin C treatment. Heat shock 60 kDa protein 1 (chaperonin), similar to heat shock protein 90 kDa protein alpha or ninein centrosomal protein isoform C, NADP-dependent malic enzyme, mitochondrial precursor, GRB10 adaptor protein, glycogenin-interacting protein 1, cystathionine gamma-lyase, G_2/mitotic-specific cyclin B2 or heat shock 90 kDa protein 1 alpha, peptidyl-prolyl *cis–trans* isomerase B, and PARP-2 (fragment) were induced by mitomycin C. KUB3, Brca1, and E6 gene expressions were down-regulated by mitomycin C in HPV-positive cervical cancer cells, SiHa/pRSV-luc and SiHa. In these studies, we suggest that MMC down-regulated the expression levels of the upstream molecules of DNA-double strand break repair system, non-homologous end joining or homologous recombination, resulting in the suppression of cervical cancer cell growth.

Keywords Mitomycin C · DSB repair genes · Ku70-binding protein · Cervical carcinoma cells

Introduction

Cervical cancer is one of the leading causes of female death from cancer worldwide. Human papillomaviruses (HPVs) have been recognized as the primary cause of cervical cancer. Among them, specific types of HPV (16, 18 and several others) have been identified as causative agents of at least 90% of cervical cancers, and are also linked to more than 50% of other anogenital cancers (zur Hausen and de Villiers 1994). HPVs have circular, double-stranded DNA genomes that are approximately 8 kb in size and encode eight genes, of which E5, E6 and E7 have transforming properties. These proteins have pleiotropic functions, such as transmembrane signaling, regulation of the cell cycle, transformation of established cell lines, immortalization of primary cell lines and regulation

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of chromosomal stability (Filatov et al. 1998; Syrjanen and Syrjanen 1999; Zwerschke and Jansen-Durr 2000). Viral oncoproteins E6 and E7 are selectively retained and expressed in carcinoma cells infected with HPV type 16 and cooperate in immortalization and transformation of primary keratinocyte. E6 and E7 oncoproteins interact and interfere with the functions of tumor suppressor proteins p53 and retinoblastoma protein (pRb), respectively (Dyson et al. 1989; Scheffner et al. 1993, 1990). The HPV 16 E7 protein can form a specific complex with phosphorylated pRb (Bates et al. 1998). The release of E2F influences expression of the genes involved in mitosis and cell cycle control (Scheffner et al. 1994). Therefore, a complex formation between the products of oncogenes and the tumor suppressor genes is believed to be important in the cellular transformation that leads to the disruption of the normal physiological functions of the specific tumor suppressor gene products (Lee et al. 1998).

Two-dimensional gel electrophoresis (2-DE) is a useful technique for visualizing a large set of proteins. Analysis by 2-DE and MALDI/TOF mass spectrometry has become a powerful combination to identify proteins. These technologies can provide potential clues to answer biological questions regarding mechanisms involved in the pathogenesis of cancer. Although protein databases of proteome approaches for several cancer researches are available and profiling analyses of proteins and genes in cervical cancers induced by HPV E7 oncogene were recently reported by previous studies (Lee et al. 2004, 2005), no database exists for cervical cancer modulated by an anti-cancer agent, mitomycin C. In the previous study (Kang et al. 2003), we investigated E6-induced transcriptional activities of several viral promoter–reporters and their applications using viral promoter–reporter gene systems could be available for the development of potential drugs against HPV infection. It was also revealed that cervical cancer drugs consistently reduced luciferase activity in RSV-luc-transfected SiHa cells (SiHa/pRSV-luc, KCTC 0427BP). Thus, RSV-luc promoter analysis system could be useful not only for understanding the role of transcription activity of E6 related to cervical cancer and also for screening drugs against cervical cancers caused by HPV infection. In order to study whether essential genes related to DNA repair or carcinogenesis would be modulated by mitomycin C in cervical carcinoma cells, we selected SiHa/pRSV-luc cells. Using 2-DE, we examined whether mitomycin C could affect the modulating factors involved in apoptosis of HPV-positive cervical carcinoma cells. We identified several DNA-double strand break (DSB) repair proteins, cell cycle regulatory factors, heat shock proteins, cell signaling factors, and novel factors which are modulated by mitomycin C.

Materials and methods

Cell culture

HPV-negative cervical cancer cell line C-33A with mutated p53 which is not functional (Srivastava et al. 1992) and HPV 16 containing cervical cancer cell line SiHa were purchased from American Type Culture Collection (Rockville, MD), and maintained in DMEM supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 25 ng/ml amphotericin B, and 10% fetal bovine serum (FBS) (Hyclone, South Logan, UT) at 37°C in a humidified incubator with 5% CO_2. SiHa/pRSV-luc cells were prepared and maintained as previously described (Kang et al. 2003, 2006).

Sample preparation for 2-DE

Cells from 100 mm dishes were harvested after treatment of mitomycin C, and then washed with PBS. Sample preparation was performed by lysing cells in a lysis buffer (25 mM Tris–phosphate, 2 mM DTT, 2 mM 1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid, 10% glycerol, 1% Triton X-100, pH 7.8) (Promega, USA) containing aprotinin (10 μg/ml) and 0.5 mM phenylmethylsulfonyl fluoride. The cell lysates were obtained by centrifugation at 12,000*g* for 30 min after incubation on ice for 30 min, and dialyzed against 20 mM Tris–HCl, pH 8.0 for 20 h. The protein concentration of the cell lysate was determined by Bradford method.

2-DE

For the first dimension, pH 3–10 immobilized pH gradient (IPG) gel strips (13 cm; Amersham Pharmacia Biotech, USA) which were linear were rehydrated overnight in a rehydration solution containing 250 μg of protein sample in an IPGphor strip holder covered with cover fluid. Isoelectric focusing was conducted at 20°C using an IPGphor Isoelectric Focusing System (Amersham Pharmacia Biotech). A three-phase program was used for the analytical gel. The first phase was set at 1,000 V for 1 h, the second phase was set at 2,000 V for 2 h, and the third phase was a linear gradient spanning from 2,000 to 8,000 V for 14 h. Prior to the 2-DE, the IPG gel strips were equilibrated for 15 min in a SDS equilibration solution (50 mM Tris–Cl, pH 8.8, 6 M Urea, 30% glycerol, 2% SDS, 0.002% bromophenol blue, 60 mM dithiothreitol). The second-dimensional separation was carried out on a 12% SDS-PAGE (polyacrylamide gel electrophoresis) gel (16 × 20 cm) without stacking gel at 4°C. The IPG strips were embedded on top of the gel with 1% agarose. Electrophoresis was carried out at 60 mA/gel for 6 h until the

bromophenol blue reached the bottom of the gel. The gel was fixed and then stained with silver according to the manufacturer's procedure (Amersham Pharmacia Biotech). The gel was stained with silver for 2 h and then destained as described below and previously (Hanash et al. 1991).

Destaining

Silvers were removed from the gel using chemical reducers as described elsewhere (Gharahdaghi et al. 1999). Briefly, a working solution was prepared by mixing a 1:1 ratio of 30 mM potassium ferricyanide and 100 mM sodium thiosulfate. 30–50 μl of working solution was added to cover the gel for 20 min and then discarded. Subsequently, the gel was cut into small pieces, washed with water, and dehydrated repeatedly with changes of acetonitrile until the gel pieces turned opaque white. The gel pieces were then dried in a vacuum centrifuge for 30 min. Trypsin digestion of proteins in the gels was performed as described elsewhere (Shevchenko et al. 1996).

MALDI-TOF mass spectrometric analysis and database search

All mass spectrometric analyses were performed using a PerSeptive Biosystems MALDI-TOF Voyager DE-RP mass spectrometer (Framingham, MA) operated in the delayed extraction and reflector mode. Peptide mixtures were analyzed using a saturated solution of alpha-cyano-4-hydroxycinnamic acid in 50% acetonitrile/0.1% trifluoroacetic acid (Gharahdaghi et al. 1996). PEPTIDENT of the program ExPASY was used for the database search (http://cn.expasy.org/tools/peptident.html).

Reverse transcription-polymerase chain reaction (RT-PCR)

RT-PCR was carried out to identify expression of KUB3 and E6 from total RNA isolated from SiHa/pRSV-luc cells, SiHa, and C-33A cervical carcinoma cells after treatment with carboplatin, mitomycin C, and cisplatin. The cDNA was synthesized from total RNA in 50 μl reaction volume containing 5 μl 10× first-strand buffer, 1 μl of RNase block ribonuclease inhibitor (40 U/μl), 2 μl of 100 mM dNTPs and 1 μl of MMLV-RT (50 U/μl), and the mixture was gently incubated at 37°C for 1 h. The resulting cDNA was amplified under the following PCR conditions (30 cycles: 1 min at 95°C, 1 min at 57°C and 1 min 30 s at 72°C). The PCR products were analyzed on 1% agarose gel. The primers for KUB3 were 5′-CGG AAT TCG GAG GTT ACC TTT CCC AG-3′ (sense) and 5′-CCG CTC GAG TGA TGA TGG AAG CTC TGT-3′ (antisense). The primers for E6 were 5′-GCG GCC GCC ACC ATG TTT CAG GAC CAC AG-3′ (sense) and 5′-CTG CGG CCG CGA TTA CAG CTG GGT TTT CTCT-3′ (antisense). The primer sequences for β-actin as an internal standard were 5′-GTG GGG CGC CCC AGG CAC CA-3′ (sense) and 5′-CTC CTT AAT GTC ACG CAC GAT TTC-3′ (antisense).

Western blot analysis

Western blot analysis was carried out to identify expression of Ku70 and Brca-1 protein in SiHa/pRSV-luc cells, SiHa, and C-33A cervical carcinoma cells after treatment with carboplatin, mitomycin C, and cisplatin. The levels of expressed Ku70 and Brca-1 protein were determined by SDS-PAGE (12% acrylamide) and Western blot analysis. Gels were transferred to an Immobilon-P membrane (Millipore, Bedford, MA) at 50 V for 1.5 h at room temperature and were blocked by soaking into methanol for 5 min and drying at room temperature. The membrane was probed with rabbit anti-Ku70 and Brca-1 polyclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:1,000 in 5% skimmed milk, followed by a horseradish peroxidase-conjugated anti-rabbit antibody (Sigma), and visualization was achieved with ECL kit (iNtRON biotechnology, Daejeon, Korea).

Results

2-D electrophoresis patterns of proteins from SiHa/pRSV-luc cells after treatment of mitomycin C and identification of MMC-modulating proteins by mass spectrometry

Protein profiling was conducted to identify proteins modulated by MMC using 2-D gel electrophoresis and silver staining. In order to elucidate the specific effect of mitomycin C, we compared the differential patterns of 2-D between medium and mitomycin C-treated cells. The overall 2-D patterns of control and mitomycin C-treated cells were apparently similar (Fig. 1a). However, some down- or up-regulated protein spots by mitomycin C were separately selected from the 2-D gels. Twenty protein spots were identified from the 2-D gels. Among the 20 spots, 11 spots were down-regulated, whereas 9 spots were up-regulated by mitomycin C (Fig. 1b). The protein spots were excised from the gels, digested with trypsin, and then analyzed by MALDI-TOF. Peptide mass fingerprints from 17 proteins of the 20 spots were obtained by MALDI-TOF mass analysis. The resulting spectra were used to identify the proteins with the Peptident search program. The up- or down-regulated proteins by mitomycin C are listed in Tables 1 and 2. Nine proteins were down-regulated in SiHa/pRSV-luc cells compared to MMC-treated cells. Two

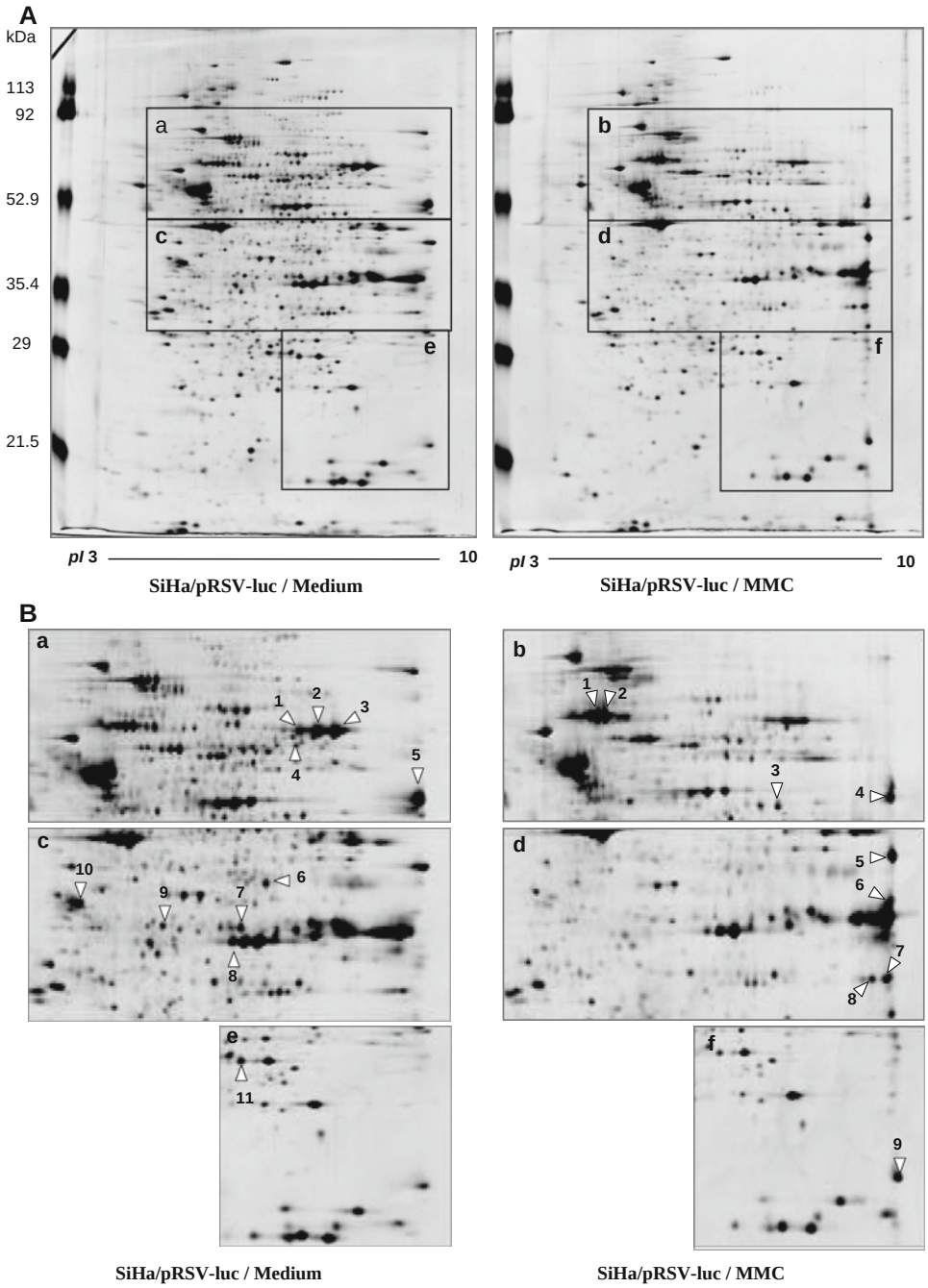

Fig. 1 Two-dimensional electrophoresis patterns of proteins from SiHa/pRSV-luc cells after treatment of MMC. Proteins were separated by 2-DE, and stained with silver nitrate (**a**). Identified protein spots are indicated by *numbers*. Proteins up-regulated by MMC are indicated in the MMC-treated cells (*b*, *d*, *f*) whereas down-regulated proteins indicated in the medium control (*a*, *c*, *e*) (**b**)

spots have not been identified in the database (Table 1). Notably, Ku70-binding protein (KUB3), MHC class I antigen, MHC class I chain-related protein A, and MAG-UK P55 subfamily member 3 or lamda/iota protein kinase C-interacting protein were suppressed by mitomycin C treatment. Compared to non-treated control cells, 8 proteins were up-regulated in SiHa/pRSV-luc/MMC cells. Heat shock 60 kDa protein 1 (chaperonin), similar to heat shock protein 90 kDa protein alpha, mitochondrial precursor, GRB10 adaptor protein, glycogenin-interacting protein 1, cystathionine gamma-lyase, G_2/mitotic-specific cyclin B2 or heat shock 90 kDa protein 1 alpha, peptidyl-prolyl *cis–trans* isomerase B, and PARP-2 (fragment) were up-regulated by mitomycin C (Table 2).

The effect of anti-cancer agents on KUB3 and E6 gene expressions in cervical carcinoma cells

In order to investigate the effect of anti-cancer agents on expressions of KUB3 and E6 genes, SiHa, SiHa/pRSV-luc, and C-33A cervical carcinoma cells were treated with carboplatin, mitomycin C, and cisplatin. Expression levels

Table 1 Identification of MMC-modulated protein spots in SiHa/pRSV-luc cells by mass spectrometry

Spot	Score	No. of peptide matches	Accession number	Description	p*I*	MW	Arbitrary intensity
1	0.2	5	O75138	Hypothetical protein KIAA0643 (fragment)	Undefined	Undefined	++
	0.2	5	Q9H7J2	FLJ00087 protein (fragment)	Undefined	Undefined	
	0.16	4	P51114	Fragile X mental retardation syndrome-related protein 1	5.91	69,691.75	
	0.16	4	Q14622	Chain 1: integrin beta-1	5.31	86,241.6	
	0.16	4	O14689	CEV14 (fragment)	Undefined	Undefined	
	0.16	4	Q92555	Hypothetical protein KIAA0266	6.7	87,187.83	
	0.16	4	Q96D84	Hypothetical protein (fragment)	Undefined	Undefined	
	0.16	4	Q96FN2	Similar to outer dense fiber of sperm tails 2	6.12	88,578.36	
	0.16	4	Q9H4M1	Chain 1: glycosylphosphatidylinositol-specific	5.7	87,795.16	
	0.16	4	Q9UQ37	RNA binding protein (fragment)	Undefined	Undefined	
	0.16	4	O94819	Hypothetical protein KIAA0711	5.78	65,719.85	
2	0.23	5	Q92565	Hypothetical protein KIAA0277	5.89	67,733.18	+++
	0.18	4	P09913	Interferon-induced 54 kDa protein (IFI-54K)	6.32	54,632.21	
	0.18	4	O95914	DJ134E15.1 (Blimp-1) (fragment)	Undefined	Undefined	
	0.18	4	P14868	Aspartyl-tRNA synthetase (EC 6.1.1.12)	6.11	57,136.22	
3	0.14	4	Q13368	MAGUK P55 subfamily member 3 (MPP3 protein)	6.27	66,168.23	++++
	0.14	4	O75341	BRCA1-associated protein 2	5.85	68,408.83	
4	0.15	4	Q96ID6	Hypothetical protein (fragment)	Undefined	Undefined	+
	0.15	4	Q99756	68 kDa type I phosphatidylinositol-4-phosphate 5-kinase alpha (EC 2.7.1.68)	7.22	56,053.03	
5	0.2	5	Q9HCK7	Hypothetical protein KIAA1565 (fragment)	Undefined	Undefined	+++
	0.16	4	Q9NYL5	Cytochrome P450 39A1 (EC 1.14.13.-)	8.92	54,129.84	
6	0.17	6	O97987	MHC class I chain-related protein A (fragment	Undefined	Undefined	+
	0.14	5	P05787	Keratin, type II cytoskeletal 8 (cytokeratin 8)	5.52	53,543.03	
	0.14	5	O95269	Neuralized binding protein (HOMER-2 protein, HOMER-2A splicing form)	6.19	39,467.15	
	0.14	5	Q9NTG1	Chain 1: polycystic kidney disease and receptor	Undefined	Undefined	
7	0.1	7	P04083	Annexin I (lipocortin I) (calpactin II) (chromobindin 9) (P35) (phospholipase A2 inhibitory protein)	6.64	38,583.05	+
	0.1	7	Q9Y6H3	Ku70-binding protein (fragment)	Undefined	Undefined	
	0.09	6	O95958	DJ142L7.3 [connective tissue growth factor (NOV, GIG) like protein] (fragment)	Undefined	Undefined	
	0.07	5	Q96LE5	GC109 (fragment)	Undefined	Undefined	
10	0.17	5	Q13696	ACF7 protein (fragment)	Undefined	Undefined	++++
	0.13	4	Q9UJK7	DJ283E3.3.1 [cell division cycle 2-like 2 (PITSLRE, p58/GTA, galactosyltransferase associated protein kinase)] (isoform beta 1)	5.49	49,623.58	
	0.13	4	O43493	Chain 1: *trans*-golgi network integral membrane	5.46	48,887.04	
11	0.08	5	Q8TEQ5	FLJ00138 protein (fragment)	Undefined	Undefined	+
	0.08	5	Q9Y6H3	Ku70-binding protein (fragment)	Undefined	Undefined	
8, 9				No SWISS-PROT and TrEMBL entries have been found			

Down-regulated proteins by MMC are described. The peptide profiles of the protein spots treated with trypsin were analyzed by MALDI-TOF–MS, and by using the Exp\PASY PEPTIDENT search program

of E6 and KUB3 were decreased in mitomycin C-treated HPV-positive cervical cancer cells, SiHa/pRSV-luc and SiHa compared to the non-treated HPV-positive cervical cancer cells (Fig. 2). E6 gene was not detected in HPV-negative C33A cervical cancer cells as expected (Fig. 2). KUB3 protein could not be detected because a specific anti-Ku70-binding protein (KUB3) antibody was not commercially available.

Table 2 Identification of MMC-modulated protein spots in SiHa/pRSV-luc cells by mass spectrometry

Spot	Score	No. of peptide matches	Accession number	Description	p*I*	MW	Arbitrary intensity
1	0.26	8	P10809	Chain 1: 60 kDa heat shock protein	5.24	57,962.86	+++
	0.26	8	Q96FZ6	Heat shock 60 kDa protein 1 (chaperonin)	5.5	59,812.09	
	0.23	7	Q96PX3	Hypothetical protein KIAA1915 (fragment)	Undefined	Undefined	
	0.13	4	Q07065	P63 protein	5.63	66,022.47	
2	0.17	4	Q9BSM2	Hypothetical protein (fragment)	Undefined	Undefined	+++
	0.17	4	P51157	Ras-related protein Rab-28 (RAB-26)	5.7	24,871.33	
3	0.09	5	P17342	Chain 1: atrial natriuretic peptide clearance	5.94	57,837.44	+
	0.09	5	Q13517	Protein kinase C-binding protein RACK7 (fragment)	Undefined	Undefined	
	0.09	5	Q96HW6	Similar to kinesin family member C3 (fragment)	Undefined	Undefined	
	0.09	5	Q96JA0	NYD-SP28	6.74	57,327.86	
	0.09	5	Q9UNJ5	Cysteine sulfinic acid decarboxylase-related protein 4	6.06	55,007.2	
	0.07	4	Q96HX7	Similar to heat shock 90 kDa protein 1, alpha (fragment)	Undefined	Undefined	
4	0.19	4	O75530	Embryonic ECTODERM development protein (fragment)	Undefined	Undefined	++
	0.19	4	Q9UGN5	Poly (ADP-ribose) polymerase-2 (EC 2.4.2.30) (PARP-2) (NAD(+) ADP-ribosyltransferase-2) (Poly[ADP-ribose] synthetase-2) (pADPRT-2) (hPARP-2)	9.02	66,205.76	
	0.19	4	Q9UNY7	WAIT-1	6.93	48,779.19	
5	0.14	5	Q96AG6	Hypothetical protein (fragment)	Undefined	Undefined	+++
	0.11	4	P00505	Chain 1: aspartate amino transferase	8.98	44,695.28	
	0.11	4	Q00653 (Isoform)	Splice isoform P49 of nuclear factor NF-kappa-B p100/p49 subunits (H2TF1) (oncogene Lyt-10) (Lyt10) (contains: nuclear factor NF-kappa-B p52 subunit)	8.94	49,123.69	
	0.11	4	O15264	Mitogen-activated protein kinase 13 (EC 2.7.1.) (stress-activated protein kinase-4) (mitogen-activated protein kinase p38 delta) (MAP kinase p38 delta)	8.48	42,089.59	
6	0.09	4	O95780	BC39498_1	9.22	58,361.2	++
	0.09	4	Q8TB66	Nucleolar protein interacting with the FHA domain of pKi-67	9.88	34,253.22	
	0.09	4	Q96HX7	Similar to heat shock 90 kDa protein 1, alpha (fragment)	Undefined	Undefined	
7	0.14	4	O95067	G2/mitotic-specific cyclin B2	9	45,281.55	++
	0.14	4	Q96HX7	Similar to heat shock 90 kDa protein 1, alpha (fragment)	Undefined	Undefined	
9	0.14	4	P23284	Chain 1: peptidyl-prolyl *cis–trans* isomerase B	9.25	20,289.29	++
8				No SWISS-PROT and TrEMBL entries have been found			

Up-regulated proteins by MMC are described

The effect of anti-cancer agents on expressions of KU70 and Brca-1 in SiHa, SiHa/pRSV-luc, C33A cervical cancer cells

In order to elucidate whether anti-cancer agents would affect DNA-DSB repair genes such as Ku70, KUB3, and Brca-1, we detected Ku70 and Brca-1 protein expression levels using specific antibodies. There were no changes in expression levels of Ku70 and Brca-1 protein in HPV non-containing C-33A cervical cancer cells (Fig. 3). However, Brca-1 expression was suppressed in mitomycin C-treated SiHa/pRSV-luc and SiHa cells compared to the non-treated HPV-positive cervical cancer cells while Ku70 expression was not affected by any anti-cancer agents in both HPV-positive and HPV-negative cervical cancer cells (Fig. 3). In these results, we suggest that the mitomycin C down-regulates an upstream molecule of DNA-DSB repair system, non-homologous end joining (NHEJ) or homologous recombination (HR), through a suppression of repair gene binding partner for DNA break repair system, such as Ku70-binding protein (KUB3) and Brca1-associated protein.

Discussion

In our proteomic study, several proteins including Ku70-binding protein (KUB3) and Brca1-associated protein

(Brca1AP) were down-regulated, whereas HSP60 and HSP90.1 alpha were up-regulated by mitomycin C. These results reminded our previous study (Lee et al. 2004), where KUB3 and HSP 60 were up-regulated by HPV 16 E7 oncoprotein. In these present results, E7-induced KUB3 was decreased by mitomycin C while E7-induced HSP 60 was synergistically increased by mitomycin C, assumed that HSP60 is a stress-dependent protein induced by virus infection, drug treatment, and other stresses. Brca-1 expression was suppressed in mitomycin C-treated SiHa/pRSV-luc and SiHa cells while Ku70 expression was not affected by any anti-cancer agents in both HPV-positive and HPV-negative cervical cancer cells (Fig. 3).

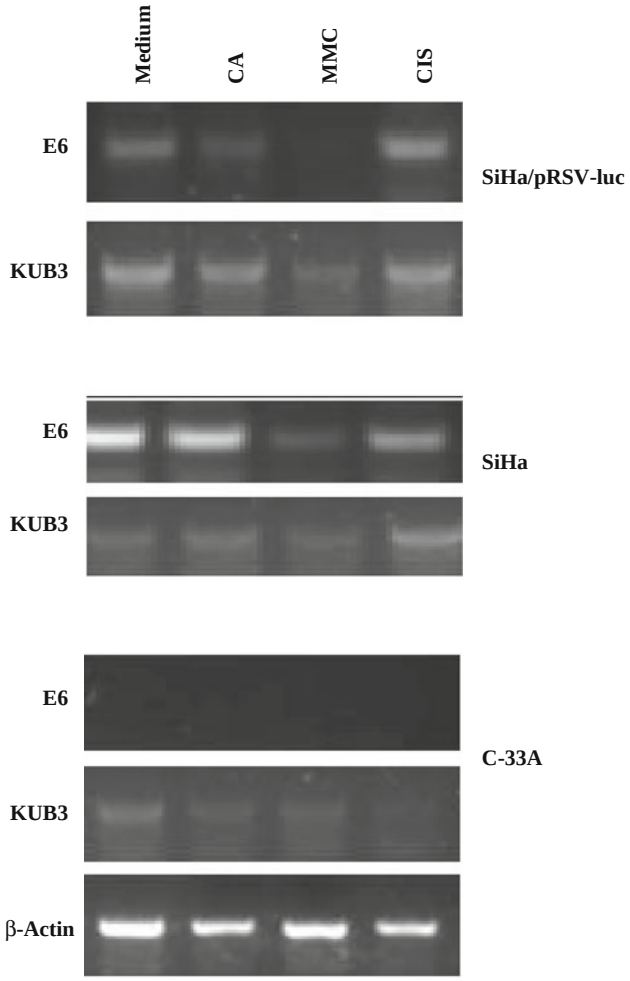

Fig. 2 Identification of KUB3 (Ku70BP) gene expression in cervical carcinoma cells after treatment of anti-cancer agents. E6 and KUB3 gene expression were analyzed by RT-PCR. RT-PCR analysis for E6 and KUB3 was performed on three kinds of cervical cancer cell lines such as SiHa/pRSV-luc, SiHa, and C-33A. cDNA was synthesized from mRNA from three cell lines after treatment of anti-cancer agents, which was used as a template for reverse transcription. β-actin was used as the loading control

It has been well known that at least two distinct processes are responsible for the diverse biological effects exhibited by the mitomycins: DNA alkylation, and the generation of reactive free radicals such as superoxide and hydroxyl radicals, thereby inducing DNA strand scission (Doroshow 1981; Lee et al. 2006). The relative cytotoxicity inherent in these processes is contingent on the extent of DNA damage induced by the given drug, and on the ability of the cell to repair DNA damage. Cells have great difficulty in repairing damage caused by the cross-linking of drugs with DNA. However, modulation of DNA repair proteins by MMC is not clear.

In this study, in order to understand the modulation of DNA repair proteins by mitomycin C, 2-D and MALDI-TOF analyses were performed in cervical carcinoma cells. Among the many factors which were modulated by MMC, we selected and focused on the modulators which are related to the DNA repair. Using Western blotting and RT-PCR, we evaluated the expression levels of Ku70, Ku70-binding protein (KUB3), Brca-1, and E6. We could not detect the protein levels of KUB3 and E7 because an anti-KUB3 antibody was not commercially available and E6 oncoprotein is slightly expressed and easily degraded in the HPV-positive cervical cancer cells. Hence, RT-PCR was performed to detect the expression of KUB3 and E6 gene, and Western blot analysis to confirm the different expression of Ku70 and Brca-1 protein. Collectively, taken together these results, KUB3, Brca1, and E6 gene expressions were down-regulated by mitomycin C in HPV-positive cervical cancer cells.

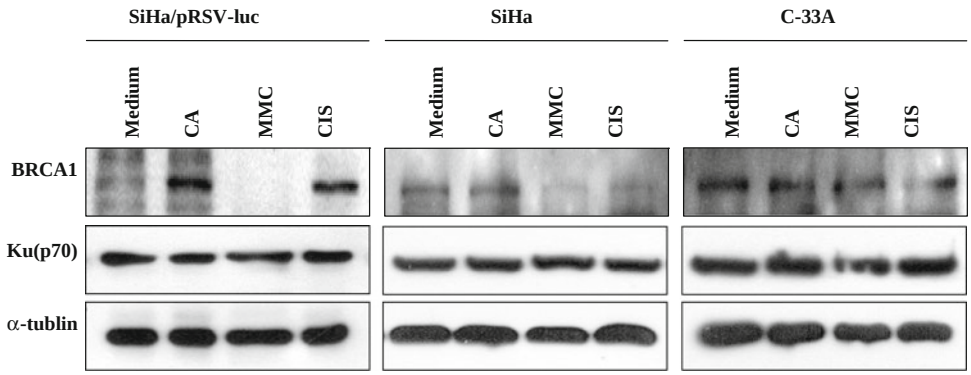

Fig. 3 The effect of MMC on the expression of Ku70 and Brca1 in SiHa/pRSV-luc cells after treatment with MMC. Western blot analysis for both Brca1 and Ku70 was performed on three kinds of cell lines, SiHa/pRSV-luc, SiHa, and C-33A, after treatment of anti-cancer agents such as cisplatin (*CIS*), carboplatin (*CA*), and mitomycin C (*MMC*), respectively. α-Tublin was used as the loading control

In these results, we suggest that mitomycin C effectively renders powerless two kind of DNA-DSB repair system, NHEJ and HR, through down-regulation of repair genes such as Brca1 and their binding or associated proteins such as Ku70-binding protein (KUB3) and Brca1-associated protein (Brca1AP), resulting in cell death during DNA-DSB of cell cycle check point period. Of the many types of DNA damages that exist within the cell, probably the most dangerous is the DSB repair system. These result from exogenous agents such as ionizing radiation (IR) and certain chemotherapeutic drugs, from endogenously generated reactive oxygen species and from mechanical stress on the chromosomes. They can also be produced when DNA replication forks encounter DNA single-strand breaks or other types of lesion. They can occur at the termini of chromosomes due to defective metabolism of chromosome ends (telomeres).

In order to elucidate the apoptotic effect of mitomycin C on cervical cancer cells, down-stream molecule of DNA-DSB repair system should be analyzed in a further study.

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