MHC class I chain-related gene A (MICA) polymorphism and the different histological types of cervical cancer*

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Cervical cancer has been one of the most important gynecologic cancer in Taiwan with incidence of 24/100,000 and mortality of 8.7/100,000 annually. About 70–80% are squamous cell carcinoma; the remainder are composed of various types of adenocarcinoma, adenosquamous carcinoma and undifferentiated carcinoma. The Major Histocompatibility Complex (MHC) class I chain-related gene A (MICA) is expressed by keratinocytes and epithelial cells and interacts with gamma-delta T cells. Although MICA was not associated with cervical cancer in the study of Northern Sweden, there are no further studies about the association of MICA polymorphism and the different histological types of cervical cancer.

We analyzed the MICA polymorphism in 110 cervical cancer cases (88 squamous cell carcinoma, 12 adenocarcinoma and 10 adenosquamous carcinoma) and 82 randomly selected unrelated controls from 1994 to 2000 in the Mackay Memorial Hospital, Taipei, Taiwan. DNA was extracted part from leukocytes of peripheral blood, part from tumor tissue and 5 polymorphic microsatellite alleles (A4,A5,A5.1,A6,A9) of MICA were identified by a polymerase chain reaction-based (PCR) technique using ABI Prism 377-18 DNA sequencer (Applied Biosystems, Foster City, CA, USA).

The phenotypes, alleles and genotypes of MICA gene were calculated. There was no association with cervical cancer patients and non-cervical cancer patients (p=0.337, 0.356 and 0.414). After dividing the cervical cancer patients into 3 major histological types (squamous cell carcinoma, adenocarcinoma and adenosquamous carcinoma), the result was still the same (p=0.598, 0.172 and 0.617) in our study.

We found no association between MICA gene polymorphism and cervical cancer in Taiwan. Different histological types of cervical cancer also have no significant correlation with MICA gene polymorphism. It demonstrates that polymorphism of MICA gene bears no relation to cervical cancer and the different histological types of cervical cancer in Taiwan. We need further studies for identifying the factors causing the differentiation of cancer cells of the uterine cervix.

Key words: cervical cancer, MICA gene, polymorphism

Cervical cancer is the most common cancer among women in Taiwan, with an incidence of 17.35 cases per 100,000. More than half of those effected die from this disease [33]. About 70–80% are squamous cell carcinoma; the remainder are composed of adenocarcinoma, adenosquamous carcinoma and undifferentiated carcinoma. Adenocarcinoma comprises approximately 5–20% of cervical cancers [7]. Adenosquamous and small cell carcinomas are relatively rare [3] and patients with these histologic types usually have poorer outcome even if detected in early-staged disease [17]. The major cause of cervical cancer is considered to be infection with high-risk types of human papillomaviruses (HPV) 16 and 18 [27, 31, 34]. The transient HPV infections and regression of cervical intraepithelial neoplasia (CIN) lesions to normal epithelia, suggest that immunological and genetic co-factors are involved in the cervical carcinogenesis.

The human leukocyte antigen (HLA) has been implicated in the development of squamous cell carcinoma of head and neck [15, 16, 29]. Certain HLA class II genotypes are found

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more frequently in CIN patients than in healthy populations [9, 26]. The Major Histocompatibility Complex (MHC) class I chain-related gene A (MICA) gene is located near HLA-B on chromosome 6, and is by far the most divergent mammalian MHC class I gene known [20, 33]. It has triplet repeat microsatellite polymorphism $(GCT)_n$ in the transmembrane region. This polymorphism consists of five alleles, with 4, 5, 6 and 9 repetitions of GCT or 5 repetitions of GCT with 1 additional nucleotide insertion (G), designated as A4, A5, A6, A9 and A5.1, respectively. The alleles vary among individuals [23].

MICA encodes molecules similar to MHC class I antigens and may share the capacity to bind peptides or other short ligands [16]. Recently, it was shown that this polymorphic molecule is mainly expressed by epithelial cells [14] and interacted with the gamma-delta (γ/δ) T cells. Expression of MICA by epithelium and its recognition by γ/δ T cells suggest that it may play role in immune surveillance and direct induction of mucosal immunity [5]. Investigation of a possible correlation of MICA polymorphism with genetic predisposition in the histological types of uterine cervical cancer might clarify more about the molecular events involved in the pathogenesis of this disease. In this study, we tried to find the association of MICA polymorphism with the cervical cancer in Taiwan and with the three major histological types of uterine cervical cancer including squamous cell carcinoma, adenocarcinoma and adenosquamous carcinoma.

Material and methods

Subjects. From 1994 to 2000, one hundred and ten patients with pathologically proven uterine cervical cancer were enrolled. We selected 82 control subjects from women who underwent hysterectomy without malignant pathological evidence. Those with autoimmune disorders, blood disease and previous malignancy were excluded. All of these cases were Chinese women. This study was approved by the Institutional Review Board (IRB) Human Research Review Committee of the Mackay Memorial Hospital.

Polymorphism analysis of the MICA. The method was according to previous report [2, 4, 18, 20, 28]. A PCR-based polymorphism assay was used in this study.

Statistical analysis. Evaluation of the Hardy-Weinberg equilibrium was performed by comparing observed and expected heterozygotes and homozygotes, as well as observed and expected genotypes, using Chi-square test analysis. Because the various alleles are expressed codominantly, heterozygotes were counted as having the allelic phenotype. Phenotype or gene frequencies of various types of cervical cancer and controls were compared by Chi-square test analysis with Yates' correction where appropriate (one expected number). Cervical cancer patients and controls positive for a factor were compared by the same test using SPSS^R 10.0 for Windows^R (SPSS, Inc.). Statistical significance was defined as $p<0.05$.

Results

The age of cervical cancer group was 47.45±10.45 (mean ±SD) years and that of control group was 47.88±8.69 years. Phenotype frequencies of the MICA polymorphism in patients with cervical cancer are A4(30.9%), A5(43.6%), A5.1(17.3%), A6(3.6%) and A9(4.5%), while in controls are A4(22.0%), A5(56.1%), A5.1(11.0%), A6(4.1%) and A9(6.1%) (Tab. 1). Allele frequencies of the MICA polymorphism in patients with cervical cancer are A4(18.2%), A5(33.6), A5.1(24.1%), A6(5.9%) and A9(18.2%), while in controls are A4(11.0%), A5(39.0%), A5.1(25.0%), A6(4.5%) and A9(20.1%) (Tab. 2). No significant difference was found between patients with cervical cancer and controls in phenotype (p=0.337), allele (p=0.356), or genotype (p=0.414) frequencies of the MICA polymorphism. We subdivided the cervical cancer group into cervical squamous cell carcinoma (n=88), adenocarcinoma (n=12), adenosquamous carcinoma (n=10) and benign condition (n=82) to see if any of the analyzed are related to the histological type of cervical cancer. There were also no differences among patients with different histological types of cervical cancer in phenotype (p=0.598), allele (p=0.172), or genotype (p=0.617) frequencies of the MICA polymorphism (Tab. 1, 2, 3).

Discussion

MHC class I molecules are important in the efferent limb of immunity, which is designed to destroy cells bearing foreign antigens. Foreign peptides present within the cell are deposited in the binding groove of MHC class I molecules and expressed on the cell surface. Cytotoxic T cells recognize it and destroy the cell [20]. MICA is located near HLA-B on chromosome 6 and is by far the most divergent mammalian MHC class I gene known. The MICA gene has recently been found to be significantly associated with susceptibility to certain diseases linked with the HLA-B locus like type 1 diabetes [19, 24], and Graves' disease [21].

Another intriguing question is the relation between cervical cancer and natural killer cells. NKG2D, a C-type lectin encoded by a member of the NK receptor gene complex, was reported to be the MICA receptor [25]. In humans, NKG2D is expressed on most natural killer cells, γ/δ T-cells, and CD 8αβ T-cells [13]. Ligands of NKG2D include the MHC class I homologues MICA and MICB, which function as signals of cellular stress [6, 11]. These molecules are absent from most cells and tissues but can be induced by viral and bacterial infections and are frequently expressed in epithelial tumors [30]. MIC engagement of NKG2D triggers natural killer cells and co-stimulates antigen-specific effector T-cells [12]. GROH et al recently showed that binding of MIC induces endocytosis and degradation of NKG2D [14]. After bacterial or viral infection, immune deficiency is associated with circulating tumor-derived soluble MICA, causing down-regulation of NKG2D and, in turn, severe impairment of tu-

Table 1. Phenotype frequencies of the MICA gene among different kinds of cervical carcinoma and controls

MICA	Cervical Cancer						Total		Controls	
	SCC[1]		AdenoCA[2]		AdenosquaCA[3]					
	n	%	n	%	n	%	n	%	n	%
A4	25	28.4	6	50.0	3	33.3	34	30.9	18	22.0
A5	37	42.0	5	41.7	6	60.0	48	43.6	46	56.1
A5.1	17	19.3	1	8.3	1	16.7	19	17.3	9	11.0
phaA6	4	4.5	0	0	0	0	4	3.6	4	4.9
A9	5	5.7	0	0	0	0	5	4.5	5	6.1
Total	88	100.0	12	100.0	10	100.0	110	100.0	82	100.0
P value			0.598					0.337		

SCC[1] – squamous cell carcinoma, AdenoCA[2] – adenocarcinoma, AdenosquaCA[3] – adenosquamous carcinoma

Table 2. Allele frequencies of the MICA gene among different kinds of cervical carcinoma and controls

MICA	Cervical Cancer						Total		Controls	
	SCC[1]		AdenoCA[2]		AdenosquaCA[3]					
	n	%	n	%	n	%	n	%	n	%
A4	29	16.5	8	33.3	3	15.0	40	18.2	18	11.0
A5	55	31.3	11	45.8	8	40.0	74	33.6	64	39.0
A5.1	46	26.1	4	16.7	3	15.0	53	24.1	41	25.0
lphaA6	12	6.8	0	0	1	5.0	13	5.9	8	4.9
A9	34	19.3	1	4.2	5	25.0	40	18.2	33	20.1
Total	176	100.0	24	100.0	20	100.0	220	100.0	164	100.0
P value			0.172					0.356		

SCC[1] – squamous cell carcinoma, AdenoCA[2] – adenocarcinoma, AdenosquaCA[3] – adenosquamous carcinoma

Table 3. Genotype frequencies of the MICA gene among different kinds of cervical carcinoma and controls

MICA	TYPE						Cervical cancer total		Control	
	Cervical Cancer									
	SCC[1]		AdenoCA[2]		AdenosquaCA[3]					
	n	%	n	%	n	%	n	%	n	%
A4-A4	4	22.0	2	1.7	0	0	6	5.5	0	0
A5-A4	8	9.1	3	25.0	0	0	11	10.0	9	11.0
A5-A5	10	11.4	3	25.0	2	20.0	15	13.6	9	11.0
A5.1-A4	7	8.0	0	0	0	0	7	6.4	5	6.1
A5.1-A5	13	14.8	2	16.7	2	20.0	17	15.5	22	26.8
A5.1-A5.1	9	10.2	1	8.3	0	0	10	9.1	5	6.1
A6-A5	5	5.7	0	0	1	10.0	6	5.5	2	2.4
A6-A5.1	2	2.3	0	0	0	0	2	1.8	1	1.2
A6-A6	1	1.1	0	0	0	0	1	0.9	1	1.2
A9-A4	6	6.8	1	8.3	3	30.0	10	9.1	4	4.9
A9-A5	9	1.0	0	0	1	10.0	10	9.1	13	1.6
A9-A5.1	6	6.8	0	0	1	10.0	7	6.4	3	3.7
A9-A6	3	3.4	0	0	0	0	3	2.7	3	3.7
A9-A9	5	5.7	0	0	0	0	5	4.5	5	6.1
Total	88	100.0	12	100.0	10	100.0	110	100.0	82	100.0
P value			0.617					0.414		

SCC[1] – squamous cell carcinoma, AdenoCA[2] – adenocarcinoma, AdenosquaCA[3] – adenosquamous carcinoma

mor-antigen-specific effector T-cell responsiveness. This mode of T-cell silencing may promote tumor evasion and, by inference, compromise host resistance to infection [30]. Cervical cancer has been one of the most important gynecologic cancers in Taiwan. The evidence implicating specific human papillomavirus (HPV) in the etiology of cervical cancer is well established. The discrepancy between the high rate of HPV infection, the less common incidence of CIN and the relatively low rate of cervical cancer suggest the contribution of cofactors or difference of susceptibility to cervical carcinogenesis. We had previously published the effect of p53 polymorphism on the susceptibility of cervical cancer in Taiwan in which we found that p53 polymorphism may have a role in the development of adenocarcinoma but not in that of squamous cell carcinoma [32]. In addition, it was demonstrated that HLA class II antigens are expressed in CIN, whereas not in normal cervical epithelium [10].

There appears to be a great diversity in the phenotype frequencies of MICA gene among different populations [5]. GHADERI et al underwent a cohort study for determining the possible role of MICA polymorphism in pathogenesis of CIN and cervical cancer [8]. They found no association of MICA polymorphism with these pre-cancer and cancer diseases in Sweden. In our study, we were interested in MICA gene polymorphism and the pathogenesis of different histological types of cervical cancer in Taiwan. We investigated the allelic distribution of microsatellite polymorphism in the transmembrane region of the MICA gene among cervical cancer patients. We found that there is no significant difference in patients of cervical cancer in comparison with normal individuals. Furthermore, there is no significant difference in patients with different histological types of the cervical cancer.

In conclusion, we found no association between the MICA gene polymorphism and cervical cancer as well

as different histological types of cervical cancer in Taiwan. Because cervical cancer is associated with the infection of HPV at the transitional zone of the uterine cervix, the local immune status may aeffect the HPV status over here. But we still needs further studies for finding more possible relationship and pathways of the differentiation of cancer cells and immune regulation of the uterine cervix.

References

[1] APPLE RJ, ERLICH HA, KLITZ W, MANOS MM, BECKER TM, WHEELER CM. HLA-DR-DQ associations with cervical cancer show paillomavirus-type specificity. Nat Genet 1994; 6: 157.

[2] BAHRAM S, BRESNAHAN N, GERAGHTY DE, SPIES T. A second lineage of mammalian major histocompatibility complex class I genes. Proc Natl Acad Sci USA 1994; 91: 6259–6263.

[3] BORUTA DM, SCHORGE JO, DUSKA LA, CRUM CP, CASTRILLON DH, SHEETS EE. Multimodality therapy in early-stage neuroendocrine carcinoma of the uterine cervix. Gynecol Oncol 2001; 81: 82–87.

[4] BUFFON GJ, DARLINGTON GJ. Isolation of DNA from biological specimens without extraction with phenol. Clin Chem 1985; 31: 164–165.

[5] CHRISTMAS SE, BREW R, DENIZ J, TAYLOR JJ. T cell receptor heterogeneity of gamma delta T cell clones from human female reproductive tissues. Immunology 1993; 78: 436–442.

[6] DAS H, GROH V, KUIJL C. MICA engagement by human Vgamma2 Vdelta2 T cells enhances their antigen-dependent effector function. Immunity 2001; 15: 83–93.

[7] FU YS, REAGAN JW. Pathology of the uterine cervix, vagina and vulva. Philadelphia: W.B. Saunders Co., 1989: 228–335.

[8] GHADERI M, HJELMSTROM P, HALLMANS G, WIKLUND F, LENNER P et al. MICA gene polymorphism and the risk to develop cervical intraepithelial neoplasia. Hum Immunol 1999; 60: 970–973.

[9] GHADERI M, NIKITINA ZAKE L, WALLIN K, WIKLUND F et al. Tumor necrosis factor A and MHC class I chain related gene A (MIC-A) polymorphisms in Swedish patients with cervical cancer. Hum Immunol 2001; 62: 1153–1158.

[10] GLEW SS, DUGGAN KM, CABRERA T, STERN PL. HLA class II antigen expression in human papillomavirus-associated cervical cancer. Cancer Res 1992; 52: 4009.

[11] GROH V, BAHRAM S, BAUER S, HERMAN A, BEAUCHAMP M, SPIES T. Cell stress-regulated human major histocompatibility complex class I gene expressed in gastro-intestinal epithelium. Proc Natl Acad Sci USA 1996; 93: 12445–12450.

[12] GROH V, RHINEHART R, RANDOLPH-HABECKER J, TOPP MS, RIDDELL SR, SPIES T. Costimulation of CD8alphabeta T cells by NKG2D via engagement by MIC induced on virus-infected cells. Nat Immunol 2001; 2: 255–260.

[13] GROH V, STEINLE A, BAUER S, SPIES T. Recognition of stress-induced MHC molecules by intestinal epithelial gamma delta T-cells. Science 1998; 279: 1737–1740.

[14] GROH V, WU H, YEE C, SPIES T. Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. Nature 2002; 419: 734–738.

[15] HOUCK JR, ROMANO PJ, BARTHOLOMEW M, SMITH PJ, KLOSZEWSKI F, VESELL ES. Do histocompatibility antigens influence the risk of head and neck carcinoma? Cancer 1992; 69: 2327–2332.

[16] KOLDOVSKY P, HAAS I, BEIR H, GANZER U. Immunohistochemical examination of 11 cell lines derived from human head and neck squamous cell carcinomas, their recurrence or metastases. Europ Archi Oto-Rhino-Laryngo 1995; 252: 359–365.

[17] LEA JS, COLEMAN RL, GARNER EO, DUSKA LR, MILLER DS, SCHORGE JO. Adenosquamous histology predicts poor outcome in low-risk stage IB1 cervical adenocarcinoma. Gynecol Oncol 2003; 91: 558–562.

[18] LEE HH, CHAO HT, NG HT, CHOO KB. Direct molecular diagnosis of CYP21 mutations in congenital adrenal hyperplasia. J Med Genet 1996; 33: 371–375.

[19] LEE YJ, HUANG FY, WANG CH, LO FS, TSAN KW et al. Polymorphism in the transmembrane region of the MICA gene and type 1 diabetes. J Pediatr Endocrinol Metab 2000; 13: 489–496.

[20] LIU CJ, LEE YJ, LIU HF, DANG CW, CHANG CS et al. The increase in the frequency of MICA gene A6 allele in oral squamous cell carcinoma. J Oral Pathol Med. 2002; 31: 323–328.

[21] LO FS, LEE YJ, HUANG CY, LIN CH, DANG CW, LIU HF. Polymorphism in the transmembrane region of the major histocompatibility class I chain-related gene: an association of five GCT repetitions with Graves' disease in children. Thyroid 2003; 13: 839–842.

[22] MIZUKI N, OTA M, KIMURA M, OHNO S, ANDO H et al. Triplet repeat polymorphism in the transmembrane region of the MICA gene: A strong association of six GCT repetitions with Bechet disease. Proc Natl Acad Sci USA 1997; 94: 1298–1303.

[23] OTA M, KATSUYAMA Y, MIZUKI N, ANDO H, FURIHATA K et al. Trinucleotide repeat polymorphism within exon 5 of the MICA gene (MHC class I chain-related Gene A): Allele frequency data in the nine population group Japanese, Northern Han, Hui, Uygur, Kazakhstan, Iranian, Saudi Arabian, Greek and Italian. Tissue Antigens 1997; 49: 448–454.

[24] PARK Y, LEE H, SANJEEVI CB, EISENBARTH GS. MICA polymorphism is associated with type 1 diabetes in the Korean population. Diabetes Care 2001; 24: 33–38.

[25] PETERSDORF EW, SHULER KB, LONGTON GM, SPIES T, HANSEN JA. Population study of allelic diversity in the human MHC class I-related MICA gene. Immunogenetics 1999; 49: 605–612.

[26] SANJEEVI CB, HJELMSTROM P, HALLMANS G, WIKLUND F, LENNER P et al. Different HLA-DR-DQ halotypes are associated with cervical intraepithelial neoplasia among human papillomavirus type-16 seropositive and seronegative Sweden women. Int J Cancer 1996; 68: 409.

[27] SCHIFFMAN M, HERRERO R, HILDESHEIM A, SCHERMAN ME, BRATTI M et al. HPV DNA testing in cervical cancer screening: result from women in a high risk province of Costra Rica. JAMA 2000; 283: 87.

[28] SHIH SC, LEE YJ, LIU HF, DANG CW, CHAN SC et al. Polymorphism in transmembrane region of MICA gene and cholelithiasis. World J Gastroenterol 2003; 9: 1541–1544.

[29] SLOOTWEG PJ, DE PAGTER M, DE WEGER RA, DE WILDE PC. Lymphocytes at tumor margins in patients with head and neck cancer. Int J Oral Maxillofac Surg 1994; 23: 286–289.

[30] TIENG V, LE BOUGUENEC C, DU MERLE L. Binding of Escherichia coli adhesin AfaE to CD55 triggers cell-surface expression of the MHC class I-related molecule MICA. Proc Natl Acad Sci USA 2002; 99: 2977–2982.

[31] WALLIN KL, WIKLUND F, ANGSTROM T, BERGMAN F, STENDAHL U et al. Type-specific persistence of human papillomavirus DNA before the development of invasive cervical cancer. N Engl J Med 1999; 341(22): 1633–1638.

[32] YANG YC, CHANG CL, CHEN ML. Effect of p53 polymorphism on the susceptibility of cervical cancer. Gynecol Obstet Invest 2001; 51: 197–201.

[33] YANG YC, SHYONG WS, CHANG MS, CHEN YJ, LIN CH et al. Frequent gain of copy number on the long arm of chromosome 3 in human cervical adenocarcinoma. Cancer Genet Cytogenet 2001; 131: 48–53.

[34] ZUR HAUSEN H. Papillomaviruses in human cancers. Proc Assoc AM Physicians 1999; 111(6): 581.