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ANALYSIS OF THE STRUCTURE AND EXPRESSION OF THE C-MYC ONCOGENE IN CERVICAL TUMOR AND IN CERVICAL TUMOR-DERIVED CELL LINES

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The structure of the c-myc oncogene in 17 cervical tumors and patient-matched nontumor tissues from Chinese patients residing in Taiwan was analysed. In contrast to recent reports on Mexican patients, none of the samples showed rearrangements and sequence amplification in the c-myc gene. The discrepancy may be explained by different carcinogenesis mechanisms being in operation in different geographic regions. Although no structural alterations in the c-myc gene were found in seven cervical carcinoma cell lines analysed, Northern blot analysis indicated different levels of c-myc gene expression which may be related to the presence of human papilloma-virus (HPV) sequences in the cell and suggests a possible c-myc-HPV interaction in some stages of the transformation process.

Activation of cellular oncogenes has been implicated in carcinogenesis. Cellular oncogenes may be activated by over-expression, transposition or by structural alterations within the gene sequences. Collaboration of more than one oncogene from different classes has also been shown to be essential for transformation of primary cell cultures (1, 2).

Recent reports have described over-expression, sequence amplification and structural alterations of the c-myc proto-oncogene in cervical tumors of Mexican patients (3-5). Human papillomavirus (HPV) types 16 and 18 are also associated with most cases of cervical tumors (6). Cell lines derived from this tumor frequently contain HPV16 or HPV18 (7-12). There is strong evidence to indicate

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Abbreviations: HPV, human papillomavirus; kb, kilobases.

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that some of the open reading frames of HPV16 code for transforming genes (13). In this study, we analysed the c-myc gene structure in cervical tumor samples taken from Chinese patients in Taiwan. Histologically normal tissues of the same patient were also analysed. We further examined the possible relationship between the presence of HPV and c-myc gene expression in cervical carcinoma cell lines.

MATERIALS AND METHODS

Tissues and cell lines. Cervical squamous cell tumor and the patient-matched nontumor tissues were obtained from the Department of Obstetrics and Gynecology, Mackay Memorial Hospital, Taipei. The nontumor tissues were histologically proven. All the cervical tumor cell lines have been described (see Table 1). For control, a histologically normal tissue of the uterine cervix was obtained from a surgical specimen of a patient with uterine myoma.

Nucleic acids preparation and molecular hybridization. Cellular DNAs and RNAs were prepared from the tissues or cell lines and Southern blot hybridization was performed as previously described (6, 7, 14, 15). For Northern blot analysis, 20 μg of total RNA was used in each sample for electrophoresis in 1.0% agarose gel after denaturation by glyoxal treatment (15). RNA blotting and hybridization were carried out as for Southern blots using 32P-labelled probes prepared by nick translation (15). C-myc probe was a 1.8 kb HindIII-EcoRI fragment containing exon III sequences of the c-myc structural gene excised from plasmid pMC41-3RC (16). The β-globin gene probe was prepared from plasmid JW102 (17).

RESULTS

Seventeen matched pairs of cervical tumors and histopathologically proven nontumor tissues adjacent to the carcinoma were collected. A DNA fragment containing exon III sequence of the c-myc oncogene was used as hybidization probe in Southern blot analysis of EcoRI or HindIII-digested cellular DNAs prepared from the tissue samples. For quantitation purposes, a DNA probe containing the human β-globin gene sequences were also included in the hybridization. Fig. 1 shows some representative results of the experiment. In the blot, cellular DNA prepared from the white blood cells of a healthy laboratory individual was included as control. In the DNA samples of all the 17 pairs of tumor and nontumor tissues, only the 14-kb EcoRI and the 11.5-kb HindIII bands of the germ-line c-myc

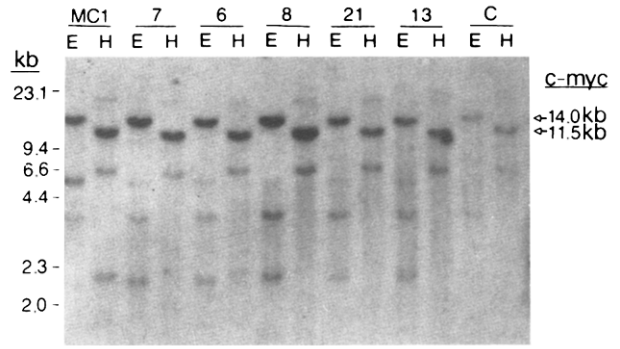

Fig. 1. Southern blot analysis of the c-myc gene structure in cervical tumors. The tumor DNAs were either digested with EcoRI (E) or HindIII (H) before electrophoresis and blotting. In the hybridization reaction, a mixture of c-myc and β-globin gene probes was used. The control (C) DNA was prepared from white blood cells of a healthy individual. The molecular weight markers are shown on the left of the panel. The germ-line c-myc bands are indicated by arrows on the right with the expected molecular weights.

gene (16) were detected. Using the hybridized bands of the β-globin gene (18) as signal-intensity reference and adjusting for experimental variations, the c-myc bands in all the DNA samples analysed showed similar signal intensity. Thus, the c-myc structural gene sequences were unaltered and were not amplified in the cellular DNA of the cervical tumor and the paired nontumor tissues in Taiwan.

The c-myc oncogene can be activated in tumor cells by over-expression of the gene. This has been reported for cervical tumor biopsies (5). Since not enough samples were available in this study for both DNA and RNA analysis in most cases, we examined c-myc gene expression in seven cell lines derived from this tumor (Table 1). In these cell lines, C-33A and HT-3 are negative for HPV, Siha, CaSki and CC7T/VGH contain HPV16 while KB and ME180 are HPV18-positive lines (7-12). The KB cell line was originally described as nasopharyngeal carcinoma cells but was later shown to represent HeLa cells which were cervical carcinoma-derived (19). On Southern blot analysis of DNAs from these cell lines using the c-myc probe as for the tumor cases shown above, again no sequence alterations and gene amplification were observed (data not shown).

Table 1. Relative transcription levels of c-myc gene in cervical tumor cell lines in relation to HPV type and copy number

Cell line	HPV type	HPV copy no.	Reference	Relative c-myc levels[1]
CC7T/VGH	16	300-400	7	4 +
CaSki	16	600	9,11,12	3 +
Siha	16	1-2	9,11,12	+
ME180	18	<1	9,12	3 +
KB (HeLa)	18	50	9,11,12	3 +
HT-3	None	None	12	+
C-33A	None	None	9,12	+
Normal cx	None	None	-	+

[1] "+" to "4+" indicate 1- to 5-fold, 6- to 10-fold, 11- to 20-fold and over 20-fold of the normal level, respectively, as determined by scanning of the autoradiographs.

To determine if the c-myc gene expression was altered in these cell lines, Northern blot analysis was performed. In these experiments, RNA prepared from a normal cervical tissue obtained from a patient who had undergone total hysterectomy because of cervical myoma was used as control. The results showed a 2.4-kb c-myc transcript in all the cell lines analysed (Fig. 2). However, the cell lines contained different levels of c-myc transcripts.

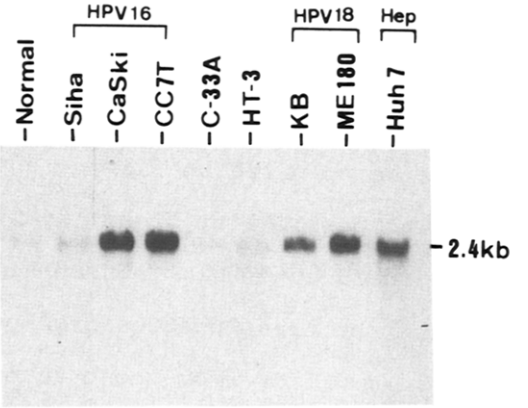

Fig. 2. Northern blot analysis of total RNA prepared from cervical carcinoma cell lines for the presence of the c-myc transcripts. In each lane, 20 µg of total RNA was loaded and the filter was probed with a DNA sequence containing the exon III region of the c-myc gene. In each cell line, the HPV type present was also indicated. The control contained RNA prepared from a histologically normal cervix removed from a patient with myoma. Huh7 was liver cancer cell line used here as a positive control for elevated c-myc transcription (T.-S. Su, personal communication).

Expression of the c-myc gene in the HPV-free C-33A and HT-3 and in Siha which contains only 1-2 copies of integrated HPV16 genome appeared to be similar to that in the control cells. Marked increases in the c-myc mRNA levels were found in four of the five HPV-containing cell lines (CaSki, CC7T/VGH, KB and ME180). With the exception of ME180 which contains one copy or less of HPV18 genome, three other cell lines contain multiple copies of HPV16 or HPV18 sequences. The relative levels of c-myc transcript are shown in Table 1. For positive control, we used RNA prepared from a hepatocellular carcinoma cell line (Huh7) in which increased c-myc had previously been demonstrated (T.-S. Su, personal communication).

DISCUSSION

In this study, we showed that none of the 17 cervical tumors and their matched nontumor DNAs of Chinese patients contained sequence rearrangements and amplification in the c-myc oncogene, in contradiction with recent reports on Mexican patients (3, 4). To explain this discrepancy, we suggest that in the carcinogenesis process, one or more of the steps involve a co-factor which affects the structure of the c-myc gene and this co-factor is different in various geographic regions. This co-factor may further be proposed to be genetical in nature originated from racial differences, or chemical in nature related to different living environments, eating and social habits. The possibility of an involvement of a second viral factor cannot be ruled out (20). In Burkitt lymphomas, the structural changes and the translocation breakpoints of the c-myc gene in the endemic form differ from those found in the sporadic form (21). Endemic Burkitt lymphomas are found mainly in the equatorial Africa and in Papua New Guinea and are assocaited with Epstein-Barr virus infection in almost all the cases but the sporadic form is distibuted worldwide and is only occasionally associated with EBV (22).

Rious et al. (5) have described over-expression of the c-myc oncogene in cervical tumors but no data on the HPV status of the samples analysed were presented. On the analysis of seven cervical carcinoma cell lines, we observed a possible correlation between enhanced c-myc expression and the presence of HPV genomes in the cell lines. In a study by Gariglio et al. (3), the presence of high copy number of HPV sequences was also correlated with increased c-myc structural alterations in some of the tumors analysed although the expression of c-myc gene was not analysed. Thus, it is tempting to speculate that some of the HPV gene products may be involved in the over-expression of the c-myc gene either by enhanced transcription, or by stabilization of the c-myc transcripts. On the other hand, evidence which supports a cis-acting role for HPV on c-myc gene expression is found in the integration of HPV genome adjacent to the 5' region of the c-myc gene (23).

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