BRIEF COMMUNICATION

Human Papillomavirus Type and Clinical Manifestation in Seven Cases of Large-cell Neuroendocrine Cervical Carcinoma

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Large-cell neuroendocrine carcinoma (LCNEC) of the uterine cervix is a very rare malignancy. We aimed to investigate the role of human papillomavirus (HPV) on the survival of patients, and its correlation with clinical parameters of HPV status or survival outcomes. Only seven cases of LCNEC were retrospectively collected among 8018 (0.087%) invasive cervical carcinomas from the cancer registry systems at Mackay Memorial Hospital and Veterans General Hospital over a period of 17 years. The median survival time was 17.2 months, including only one long-term survivor (> 5 years). The 2-year and 5-year survival rates after diagnosis were 42% and 30%, respectively. The results indicated that the majority of LCNEC cases were dominated by high-risk HPV-18. No clinical parameters appeared to be associated with HPV-18 or survival outcomes of LCNEC patients. Pelvic lymph node metastasis positivity could also be considered as a prognostic factor for this disease. [*J Formos Med Assoc* 2009; 108(5):428–432]

Key Words: cervical carcinoma, HPV, large cell neuroendocrine carcinoma

Unlike the more commonly encountered small-cell neuroendocrine carcinoma (SCNEC) of neuroendocrine cervical carcinoma (NECC), large-cell neuroendocrine carcinoma (LCNEC) of the uterine cervix is a very rare malignancy. A literature search reveals no more than 30 cases of LCNEC reported worldwide. We retrieved individual medical information of all cases with LCNEC from Mackay Memorial Hospital (MMH) and Veterans General Hospital (VGH). After careful review and classification, we present our experience with seven cases with regard to diagnosis, treatment, and prognosis of this disease type. This is the largest published series of LCNEC with reported HPV status to date. The importance of human papillomavirus (HPV) infection is now widely recognized, and is considered a necessary cause for over 99% of cervical carcinogenesis.[1,2] The purpose of this study was to investigate the role of HPV subtype(s) on the survival of patients, and its correlation with clinical parameters of HPV status or survival outcomes.

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Methods

Individual subject data were retrospectively collected from the cancer registry systems at MMH and VGH between January 1, 1991 and October 31, 2007. A total of 8018 patients were identified with cervical cancer during this same period in these hospitals. All patients identified as NECC or LCNEC in the original pathology reports were at first retrieved from the cancer registry systems. Patients identified as "NECC of the uterine cervix" were then further classified into one of the four histopathologic types as proposed by Albores-Saavedra et al[3] in 1997: typical carcinoid tumor, atypical carcinoid tumor, SCNEC and LCNEC. The selection and reassignment of patients was performed by gynecologic pathologists of respective medical institutions. Additional criteria used for diagnosing NECC tumors were based on immunohistochemical staining results of three neuroendocrine markers (neuron specific enolase, chromogranin, and synaptophysin). Cases other than LCNEC disease type were excluded from the selected cases. All the paraffin-embedded tissue blocks and their corresponding hematoxylin and eosin-stained sections were collected.

Clinical histories on the patients were carefully reviewed. Relevant clinical data included all past HPV-related events (e.g. Pap test, biopsies, or HPV tests), and any test results related to these medical events were extracted directly from chart review of records of clinic visits as well as correspondence with patients and physicians. Overall survival was defined as the time from initial diagnosis to the time of death or last follow-up. All surviving patients were followed-up until December 31, 2007. The study was approved by the respective institutional review boards and ethics committees of the participating hospitals.

The genomic DNA for HPV typing was extracted from paraffin-embedded tissue blocks using the commercially available DNeasy Tissue Kit (Qiagen, Hilden, Germany). Sections were deparaffinized and screened for HPV DNA by L1 consensus PCR (primers MY11/GP61) and HPV 16/18 DNA by PCR amplification, using HPV 16 and 18 specific primers.[4,5]

Significance levels for association between categorical variables in different groups were assessed using Pearson's χ^2 or Fisher's exact tests as appropriate. Survival analysis of patients with LCNEC was evaluated using the Kaplan-Meier method. All statistical tests were performed with SPSS version R13 (SPSS Inc., Chicago, IL, USA). Means are presented with their standard deviations. All significance levels (p values) corresponded to two-sided tests ($\alpha = 0.05$).

Results

The clinical characteristics, treatment modalities and outcome data of the seven patients with LCNEC diseases are listed in the Table. All patients were female with a mean age of 42.3 ± 10.9 years (median, 41 years; range, 28–62 years) at initial diagnosis. Mean tumor diameter was 3.07 ± 1.17 cm. Tumors were clinically staged as FIGO (International Federation of Gynecology and Obstetrics) stage IA2 (1/7, 14%), stage IB1 (4/7, 57%), and stage IB2 (2/7, 29%). Most patients were treated initially with radical hysterectomy (RH) (6/7, 86%), with bilateral pelvic lymph node dissection (BPLD) and para-aortic lymphadenectomy (PALD). Two patients received only RH (2/7), but the other patients (4/7) had postoperative adjuvant treatments: chemotherapy (CT) (2/7); and CT plus radiotherapy (RT) (2/7). Only one patient (1/7) received non-RH surgery, bilateral salpingo-oophorectomy with PALD and BPLD, followed by postoperative adjuvant CT + RT. Adjuvant CT included carboplatin, VEP (etoposide, epirubicin, cisplatin), EP (epirubicin, cisplatin), and VP (etoposide, cisplatin).

HPV DNA was detected in 6/7 paraffin tissues of examined LCNEC patients, where HPV-18 was found as a single infection. Four patients exhibited pure-type histologic pattern (4/7), and three exhibited mixed-type histologic pattern (3/7). Three patients were confirmed to be positive for pelvic lymph node (LN_P) metastasis (3/7), and

Table. Clinical characteristics, treatment modalities and outcome data of LCNEC patients ($n = 7$)

Case	Age (yr)	FIGO stage	Primary treatment	Tumor size (cm)	HPV type	Adjuvant therapy	Site of recurrence	Follow-up (mo)
1	37	IA2	RH	3	18	EP	–	NED (35.2)
2	28	IB1	RH	3.5	18	VEP	Lung, bone	DOD (17.2)
3	35	IB1	RH	3	18	RT, EP	Bone, pancreas	DOD (39.0)
4	45	IB1	RH	3	18	RT, EP	Bone, brain, lungs, skin, pancreas	DOD (3.0)
5	48	IB1	RH	3	18	–	–	NED (114.3)
6	41	IB2	RH	1	18	–	–	DOD (7.0)
7	62	IB2	BSO	5	–	RT, VP	Lung	DOD (11.8)

LCNEC = large-cell neuroendocrine carcinoma; FIGO = International Federation of Gynecology and Obstetrics; HPV = human papillomavirus; RH = radical hysterectomy; BSO = bilateral salpingo-oophorectomy; EP = epirubicin + cisplatin; VEP = etoposide + epirubicin + cisplatin; RT = radiotherapy; VP = etoposide + cisplatin; NED = no evidence of disease; DOD = died of disease.

two of these patients were also confirmed to be positive for para-aortic lymph node LN_{PA} metastasis (2/7).

Treatment responses and long-term survival for patients with LCNEC were disappointing. The mean survival times were 43.8 months (median, 17.2 months; range, 3–114 months), 15.6 months (median, 11.8 months; range, 3–39 months), and 49.1 months (median, 17.2 months; range, 3–114 months) for all patients, expired patients, and patients who underwent RH, respectively. Only two of these patients remain alive (2/7, 29%). These survivors were diagnosed as FIGO stages IA2 and IB1. These two patients were both diagnosed with HPV-18 and confirmed to be negative for both LN_P and LN_{PA} metastases (2/7), exhibiting mixed-type histologic pattern without evidence of recurrence. One patient was a long-term survivor (> 5 years) with a survival time of 114 months. All patients with confirmed LN_{PA} metastases were also associated with confirmed LN_P metastases. The 2-year and 5-year survival rates after diagnosis were 42% and 30% for patients with LCNEC diseases (Figure).

The presence of HPV-18 was not associated with any clinicopathologic parameters: age groups, clinical stage, tumor histology, surgical methods, lymph node status, and chemotherapeutic regimens. Additional analyses, however, reveal no statistical significance for LCNEC patients with HPV-18 and pure-type histologic pattern, alone or in combination.

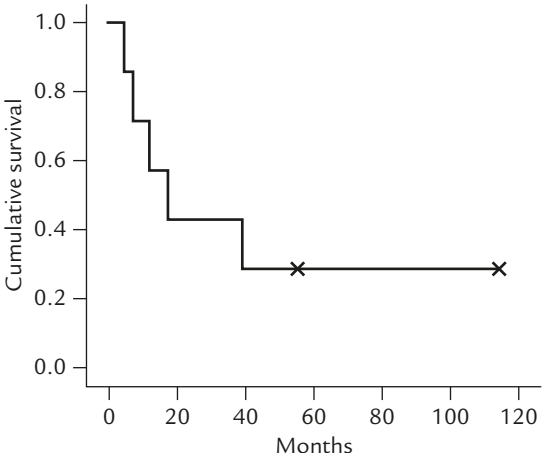

Figure. Kaplan-Meier survival curves for patients with large-cell neuroendocrine carcinoma diseases ($n = 7$).

Discussion

The analyses described in this study extend the scope of a recent study.[6] Only seven clinical cases with LCNEC disease were found among 8018 (0.087%) invasive cervical carcinoma at MMH and VGH over a period of 17 years, which demonstrates the extreme rarity of this histologic type. In this study, our experience shows that the majority of cases are dominated by high-risk HPV-18 subtype, which is not associated with any

clinicopathologic parameters. Unlike primary tumor size, pelvic lymph node metastasis positivity can be considered a prognostic factor for LCNEC disease.

The presence of lymph node metastasis has been reported to be an adverse prognostic factor at time of surgery in early-stage LCNEC patients, which is consistent with our results.[7] Frequent extrapelvic spread of LCNEC tumor has been reported to render very poor treatment outcome, despite aggressive therapy.[8] LCNEC patients without confirmed pelvic LN_P metastasis had better survival outcome with a mean survival time of 67.7 months (median, 39 months; range, 3–114 months); whereas the mean survival time of those with confirmed LN_P metastasis was approximately one-fourth of those without it—a mean survival time of 12 months (median, 12 months; range, 7–17 months).

In our study, primary tumor size had no impact on the survival of LCNEC patients. According to the work by Bermudez et al,[9] no recurrence would occur when the tumor sizes of NECC were less than 4 cm in diameter, and tumors exhibiting mixed-type histologic pattern were over 4 cm in diameter in all cases, which did not agree with our observations on LCNEC. Three out of four recurrences (3/4) in the current study had tumor sizes less than 4 cm in diameter; and only one recurrence had tumor size of 5 cm in diameter. Moreover, no patient had mixed-type tumors of over 4 cm in diameter with a mean size of 2.3 cm; on the contrary, only one patient with pure-type tumors (1/7) had tumor of over 4 cm in diameter with a mean size of 3.3 cm.

Our data supports the presence of a single HPV infection (HPV-18) in LCNEC disease, which is inconsistent with the results reported by Grayson et al,[10] Powell and McKinney,[11] and Yun et al.[12] These authors concluded that HPV-16 was the major subtype associated with LCNEC disease. However, in our series, six LCNEC patients had HPV viral infection (6/7, 86%), whereas Grayson et al[10] detected a slightly lower percentage of 75% (9/12). Our findings agree with those of many researchers in Taiwan.[13–15] At present, we are unable to provide explanation for the discrepancy between the predomination of the HPV-18 subtype in Taiwan and the rest of the world, except to attribute it to regional-specific distribution of HPV. This controversy will remain an ongoing topic of investigation for us.

In conclusion, this study confirms the presence of high-risk HPV-18 in patients with LCNEC disease. Confirmed LN_P status can be considered a prognostic factor for this disease. Primary tumor size, age groups, surgical methods, chemotherapeutic regimens, and LN_{PA} involvement do not appear to be associated with survival outcomes of LCNEC patients. We hope that our seven-case experience on the diagnosis, treatment, and prognosis of LCNEC diseases may contribute to improving clinical decision making for patients with this rare disease.

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Contributions to Authorship

KL Wang and TY Wang conceived the study, obtained funding, and designed the study. KL Wang, TY Wang, YC Huang, JCY Lai, TC Chang, and MS Yen were responsible for data acquisition. KL Wang and TY Wang analyzed and interpreted the data. KL Wang and TY Wang drafted the article and all authors contributed substantially to its revision. KL Wang and TY Wang were responsible

for statistical analysis. YC Huang, JCY Lai, TC Chang, and MS Yen provided administrative, technical, or material support. KL Wang and TY Wang were responsible for overall study supervision; and had full access to all the data and took responsibility for the integrity of the data and the accuracy of the data analysis. KL Wang and TY Wang took responsibility for the paper as a whole.

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