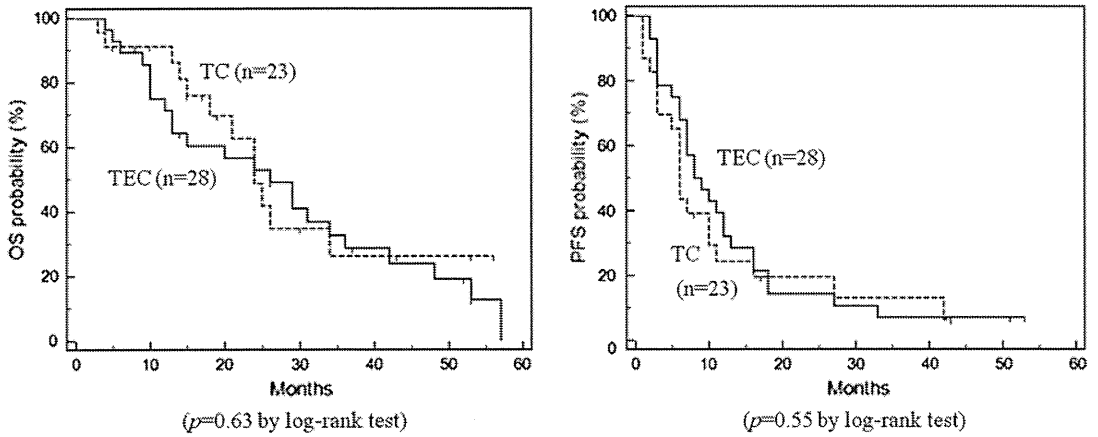

Fig. 1 PFS and OS of TEC and TC groups for unresectable or recurrent diseases. Both PFS and OS were not demonstrated to be significantly different between the TEC and TC groups ($p = 0.63$ and $p = 0.55$ by log-rank test, respectively). *Solid line* TEC group. *Broken line* TC group

Table 3 Clinical characteristics of the completely resected Stages III and IV patients to whom TEC or TC chemotherapy was performed as an adjuvant therapy

Characteristic	TEC ($n = 47$)	TC ($n = 30$)	p value
Age	56 (3–69)	59 (34–70)	0.23
Histology			0.54
Endometrioid	33 (70%)	23 (77%)	
Non-endometrioid	14 (30%)	7 (23%)	
Stage			0.83
IIIa	14 (30%)	8 (27%)	
IIIb	1 (2%)	1 (3%)	
IIIc	26 (55%)	15 (50%)	
IVb	6 (13%)	6 (20%)	

The endometrial carcinomas, confined to the uterus, which were classified in Stage IIIa based on only positive peritoneal cytology, were excluded. Distributions of age, histology and stage did not exhibit any significant differences between the TEC and TC groups

frequently observed in the TEC group than in the TC group ($p = 0.065$); however, a statistical significance was not detected. Non-hematological toxicity was observed to be similar in the TEC and TC groups ($p = 0.49$). The non-accomplishment rates of the scheduled TEC and TC therapies (due to severe toxicities) were not different between the two regimens ($p = 0.81$). Those due to the patients' intermittent desire to stop chemotherapy were also not statistically different between the two regimens ($p = 0.37$).

Conclusions

Adjuvant chemotherapy for endometrial carcinoma is one of the hottest topics in gynecologic oncology. Platinum, anthracycline and taxane derivatives are regarded as critical drugs for treatment of advanced or recurrent endometrial carcinomas [4, 6, 7]. Although combination chemotherapies using these drugs, such as TAP, TEP and TEC, are effective, moderate-to-severe side effects are sometimes observed by Fleming et al. [8], Lissoni et al. [9], Papadimitriou et al. [10] and our previous study (Takata et al. [18]).

TC, which uses only paclitaxel and carboplatin, is the current gold standard therapy for ovarian carcinoma due to its high effectiveness coupled with its high tolerability [17]. TC chemotherapy was somewhat effective for endometrial carcinoma [11–13]. It is, therefore, easy to understand why TC, although not yet an established standard therapy, has been frequently applied worldwide for the treatment of endometrial carcinoma cases. However, until this current work, a comparison of the usefulness of TC to other combination chemotherapies using these three key drugs, such as TAP, TEP and TEC, has not been reported.

A GOG study to compare the combination of TC with TAP (GOG #209) has finished recruitment of the patients; however, analysis of the survival effects of the two regimens may require a few years; a similar JGOG study to compare the combination chemotherapies TC, DP and AP (JGOG #2043) is even less far along; it is still under registration of patients. Thus, our present study to address whether anthracycline is still required in addition to TC chemotherapy for successful treatment of Stage III/IV endometrial carcinoma is quite important.

There are minor drawbacks to our study; it was a retrospective analysis of TC and TEC and thus was not the ideal, which is a prospective randomized study. In addition, the treatment data were from two center hospitals for gynecologic malignancies; the Osaka University Hospital, in which TEC regimen was conducted, and the Osaka Medical Center for Cancer and Cardiovascular Diseases, in which TC was used. Both hospitals shared common practices for

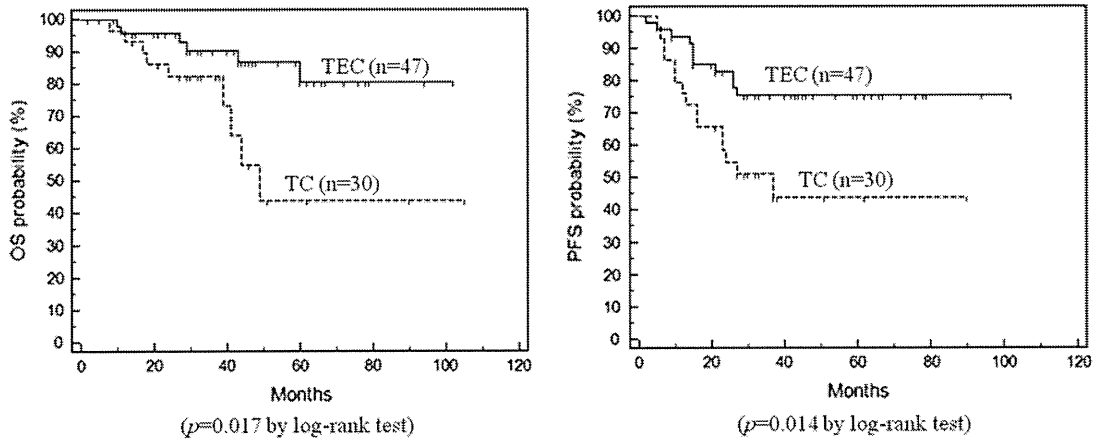

Fig. 2 PFS and OS of TEC and TC groups for adjuvant therapy (Stage III/IV). Both PFS and OS were significantly better in the TEC group than the TC group ($p = 0.017$ and $p = 0.014$ by the log-rank test, respectively). *Solid line* TEC group. *Broken line* TC group

Table 4 Adverse effects of TEC and TC therapies

Toxicities	TEC	TC	*p* value
Hematological (Grade 4)	67/115 (57%)	32/72 (44%)	0.065
Non-hematological (Grades 3, 4)	4/115 (3%)	4/72 (6%)	0.49

Grade of adverse effects was based on the National Cancer Institute's Common Toxicity Criteria (version 2.0)

surgery, chemotherapy indications and treatment procedures. We believe that typical types of bias derived from a retrospective study were minimized as much as possible. The background features of the patients compared in the current study were not significantly different between the TC and TEC groups.

Comparison of the adverse effects of the TEC and TC chemotherapies showed that hematological toxicity, especially neutropenia, tended to be more frequently observed in the TEC group than in the TC group ($p = 0.065$), however the neutropenia was tolerable when given G-CSF support. Non-hematological toxicity was observed similarly in the TEC and TC groups ($p = 0.49$). These toxicities should always be taken into consideration as part of the effectiveness of any chemotherapy.

Our previous study showed that significant survival improvement was not demonstrated using TEC compared to radiation as an adjuvant therapy in Stage I/II cases (submitted), and the positive effects of TEC and TC therapy on survival were not significantly different in the present study, indicating that TC therapy may take the place of an adjuvant therapy for Stage I/II endometrial carcinoma.

Finding similar survival effects for TC and TEC in the most advanced and intractable unresectable cases and recurrent cases ($p = 0.63$ for OS and $p = 0.55$ for PFS, by the log-rank test) implied that TC may be used as a remission induction chemotherapy due to the abjectly poor prognosis of those cases and the lower rate of severe side effects for TC therapy than for TEC. The tendency for improved survival in recurrent cases following TC therapy ($p = 0.32$ and $p = 0.22$ for PFS and OS, respectively) compared to TEC especially suggested that TC may be appropriate for recurrent cases, too. On the other hand, in advanced unresectable cases, TEC therapy tended to be more effective than TC ($p = 0.17$ for PFS). For these cases, although the TEC may improve PFS, it will require G-CSF administration for its associated severe neutropenia. Alternatively, TC may be proposed because, even though it is marginally less effective than TEC, it is more tolerable.

In the completely resected Stage III/IV cases, TEC therapy was demonstrated to provide significantly better PFS than TC ($p = 0.017$ by the log-rank test, Hazard Ratio: 0.3838; 95% CI: 0.1709–0.8623) and OS ($p = 0.014$ by the log-rank test, Hazard Ratio: 0.3108; 95% CI: 0.1048–0.9214) with a median follow-up period of 38 months (2–105 months). The reasons for these epic results are uncertain as yet, although the synergistic or additive effect of a third drug mechanism is anticipated to be the cause. The fact that TEC was much more effective, especially for the completely resected cases in Stage III/IV which were at high risk of recurrence, may imply that the TEC regimen might play a role in not only attacking the carcinoma cells forming the tumor mass but also in killing remaining carcinoma stem cells and metastases elsewhere, such as in the bone marrow, after the complete resection of the tumor mass.

In this study, we proposed to question the apparent increasing application of TC regimen to all forms of endometrial carcinoma with an indication for chemotherapy. Based on our results, TEC should be offered as the preferred adjuvant therapy to Stage III/IV patients, after complete surgical resection of their tumors. TEC may also be effective as a remission induction therapy for patients in

advanced stages with unresectable diseases. On the other hand, the TC regimen may now be considered for listing as a standard treatment useful for recurrent cases as a remission induction therapy having minimal side effects, and for patients in Stage I/II with risk factors requiring an adjuvant therapy. We firmly believe that GOG #209 and JGOG #2043 studies will provide strong evidence supporting our findings and our proposals.

Details of ethics approval All patients provided written informed consent before the treatment commenced. This study was approved by the Institutional Review Board of Osaka Medical Center for Cancer and Cardiovascular Diseases (#1006145029, June 14, 2010) and the Ethics Committee of Osaka University Graduate School of Medicine (#117, July 2, 2001).

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Conflict of interest The authors have no conflicts of interest to declare.

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GYNECOLOGIC ONCOLOGY

Prediction, based on resection margins, of long-term outcome of cervical intraepithelial neoplasia 3 treated by Shimodaira-Taniguchi conization

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Abstract
Purpose The aim of the present study was to analyze the long-term outcome of cervical intraepithelial neoplasia 3 (CIN 3) after treatment with the Shimodaira-Taniguchi conization procedure, based on the status of the resection margins.
Methods In the Osaka University Hospital, conization using the Shimodaira-Taniguchi procedure has been routinely performed for CIN 3. Medical records of patients during the period from 2001 to 2008, whose post-conization diagnosis was CIN 3, were retrospectively analyzed for outcome versus margin status.
Results During the median follow-up period of 565 days (range 34–3,013), CIN disease was again detected in 14 of 243 patients; it was found in 7 patients among 198 margin-negative cases, and in 7 patients among 45 margin-positive cases. There was a significant difference in the reappearance rate demonstrated between the cases with positive and negative margins ($p = 0.0018$). Among the patients whose first follow-up post-conization cytology was normal, recurrence-free probability was significantly higher in margin-negative cases than in margin-positive ones (hazard ratio, 5.19; 95% CI, 1.175–22.994; $p = 0.0041$).
Conclusion For the first time, we demonstrate that after treatment of CIN 3 lesions by Shimodaira-Taniguchi conization the status of the resection margin was a significant predictor for long-term outcome.

Keywords CIN 3 · Shimodaira-Taniguchi conization · Resection margin status · Outcome · Predictor

Abbreviations

AIS	Adenocarcinoma in situ
CIN	Cervical intraepithelial neoplasia
SCC	Squamous cell carcinoma
HSIL	High-grade squamous intraepithelial lesion
HPV	Human papillomavirus
LEEP	Loop electrosurgical excision procedure
LSIL	Low-grade squamous intraepithelial lesion
NILM	Negative for intraepithelial lesion or malignancy

Introduction

Cervical intraepithelial neoplasia (CIN), a non-invasive neoplastic lesion widely regarded as a precursor of squamous cell carcinoma (SCC) of the uterine cervix, has its highest incidence during women's reproductive years. Hysterectomy or radiation therapy is required to treat cases of invasive cervical SCC; however, conization, a fertility-conserving surgery, is often effectively substituted to treat the less dangerous CIN.

Y. Miyoshi and T. Miyatake contributed equally to this work.

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Conization refers to a biopsy of the cervix in which a cone-shaped sample of tissue is removed from the uterine cervix, either for diagnostic reasons, or for therapeutic purposes to remove pre-cancerous cells. A recent study showed that conization and lymphadenectomy may be a potential treatment for small IB1 cervical cancer patients desiring conservative management [1]. Although fertility preserving, the procedure is not without risk; side effects of the treatment may include cervical stenosis and may increase the risk of incompetent cervix.

Our medical indications for performing conization are CIN 3, which includes carcinoma in situ (CIS), and severe dysplasia. According to a review of previous studies, CIN 3 is significantly more likely to persist, or to progress to SCC, than is a CIN 1 lesion; progression from CIN 3 to SCC occurs more than 15% of the time, whereas CIN 1 progresses to SCC only 1% of the time [2].

The standard conization device has evolved from the cold knife to the more conservative, and now widely accepted, 'loop electrosurgical excision procedure' (LEEP) device [3]. There is one study which showed that cold knife conization was less favorable in terms of the appearance of further CIN 3 and cancer risk [4]. However, there is yet another paper from the same period with a different finding; it suggests that conization modalities have a similar efficacy with respect to eliminating CIN and reducing further cancer risk [5]. However, LEEP is also not without its known drawbacks; the cervical tissue is removed as several divided specimens and is accompanied with thermal damage to the resected specimens, making pathological evaluation of the CIN lesions and their margin status difficult.

The Shimodaira-Taniguchi conization procedure was introduced in 1992; it addresses the disadvantages of LEEP by using a high-frequency current and a triangular probe with a 0.25-mm linear excision electrode to extract the tissue as a single informative specimen, without incurring accompanying thermal trauma [3, 6]. Matsumura et al. [3] have recently demonstrated the high utility and reliability of the Shimodaira-Taniguchi conization procedure. However, in comparison to our current study (which looks only at CIN 3 outcomes), their study included many cases of both more advanced microinvasive SCCs, and less advanced CIN 1, CIN 2, and adenocarcinoma in situ (AIS) lesions. In addition, the median follow-up period was not reported in their analysis. The long-term cumulative persistence/recurrence rate, the most important outcome measure of conservative treatment for any pre-malignant lesions, was demonstrated in their report; however, the rate was not compared by the status of their resection margin data, which has been demonstrated elsewhere to be of significant value for the prediction of the outcome of conization procedures for CIN 3 [7, 8]. In our present study, the long-term outcome of CIN 3 treated by Shimodaira-Taniguchi conization in our facility was re-analyzed by the status of their resection margins. The cutting probes for the Shimodaira-Taniguchi conization procedure are illustrated in Fig. 1.

Materials and methods

In the Department of Obstetrics and Gynecology of the Osaka University Hospital in Osaka, Japan, conization using the Shimodaira-Taniguchi procedure was routinely performed for CIN 3, and, in some rare cases, for more advanced microinvasive squamous cell carcinoma of the uterine cervix. Resected cervical tissue by conization was carefully divided into 12 specimens and the pathological diagnosis was made by pathologists in the Department of Pathology of the Osaka University Hospital.

During the 8-year period from 2001 to 2008, a post-conization diagnosis of CIN 3 was made in 243 patients. Medical records, including pathology reports of these patients, were retrospectively analyzed. Patients were routinely followed by cervical cytology conducted every 1–3 months in the first year after conization, every 6 months in the second year, and then annually thereafter. Patients who were consistently 'negative for intraepithelial lesion or malignancy' (NILM) were followed in this manner. However, whenever a case displayed a positive cytological test, a colposcopic observation was performed to biopsy the suspicious lesions for CIN.

For any case that presented with an abnormal cytology anytime within the first 3 months after conization, in which a CIN lesion was confirmed at that time by biopsy under colposcope, the lesion was regarded as a 'persistence' of the earlier CIN, regardless of whether the initial conization

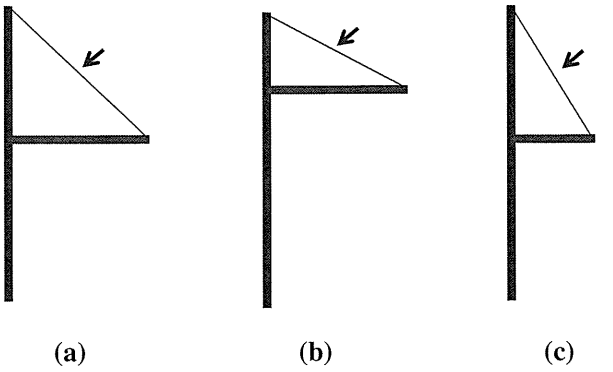

Fig. 1 The cutting probes for the Shimodaira-Taniguchi conization procedure. **a** A triangular probe which has the same base length and height. **b** A triangular probe which has shorter height than base length. **c** A triangular probe which has longer height than base length. *Arrow* excision electrode

pathology report had noted a negative or positive resection margin. For any case in which the first cytology report within the first 3 months after conization was negative, and for which (only at some later time point outside of 3 months) a CIN lesion was later detected by biopsy under colposcope, the case was regarded as a 'recurrence' of CIN. The time to diagnosis of persistent/recurrent disease was calculated from the date of the conization procedure to the day on which the colposcopic biopsy was taken for the CIN diagnosis.

Statistical analysis

MedCalc (MedCalc Software, Mariakerke, Belgium) was used for the statistical analysis. Recurrence-free probability (survival) curves were constructed using the Kaplan–Meier method and evaluated for statistical significance by the log-rank test. Association of the rate of persistence/recurrence of CIN lesions and the status of resection margins was analyzed by Fisher's exact test. Results were considered to be significant when the p value was <0.05.

Results

Clinical characteristics of patients whose post-conization diagnosis was CIN 3

During the study period, a post-conization diagnosis of CIN 3 was made in 243 patients (Table 1). Among them, 132 patients (54%) were pathologically diagnosed as having carcinoma in situ (CIS) and the other 111 patients (46%) were cases of severe dysplasia. The median age of these patients was 37 (21–74) years. A positive resection margin was detected in 45/243 cases (19%); the remaining 198 patients (81%) had a promising negative margin. All the patients were followed after conization by periodic cervical cytology. During the median follow-up period of 565 days (34–3,013), a persistent or recurrent CIN disease was subsequently detected in 14 patients (5.8%); 7 patients had been diagnosed as margin positive and the other 7 had been margin negative (Table 2). The median time to diagnosis of the persistent/recurrent CIN lesions was 316 days (64–2,465). None of these characteristics of the post-conization diagnosis was different between those with CIS versus those with severe dysplasia (data not shown).

Table 1 Clinical characteristics of patients whose post-conization diagnosis was CIN 3

Clinical characteristics	
Total number (cases)	243
Age (years)	21–74 (median 37)
Post-conization diagnosis (cases)	
CIS	132 (54%)
Severe dysplasia	111 (46%)
Status of resection margin (cases)	
Negative	198 (81%)
Positive	45 (19%)

Persistent/recurrent cases after conization

The persistent/recurrent cases are shown in Table 3. Among the 198 patients whose resection margin had been negative, persistence/recurrence was detected in 7 patients: cases 1, 2, 3, 4, 6, 7, and 8 (3.5%) (Tables 2, 3). Pathological diagnosis of the persistent/recurrent lesion was CIN 1 in three cases (cases 2, 6, 7), CIN 2 in one case (case 3), and CIN 3 in three cases (cases 1, 4, 8). Among these margin-negative cases, the first cytology exhibited normal appearance (NILM) and the CIN lesions were detected beyond the initial 3 months of observation (after 299–2,465 days from conization) in three cases (cases 6, 7, 8). These cases were regarded to be recurrences.

In the other four margin-negative cases (cases 1, 2, 3, 4), a low-grade squamous intraepithelial lesion (LSIL) or a high-grade squamous intraepithelial lesion (HSIL) was detected by the first cytology after conization (within the first 3 months), and CIN 1, CIN 2, or CIN 3 was pathologically diagnosed by colposcopic biopsy. These cases were regarded as persistent lesions.

Among the 45 patients whose resection margin had been positive, persistence/recurrence was detected in 7 patients (cases 5, 9, 10, 11, 12, 13, 14) (16%) (Tables 2, 3). The rate of persistence/recurrence was significantly higher in margin-positive cases than in margin-negative ones (3.5 vs. 16%; $p = 0.0018$ by Fisher's exact test) (Table 2). In a

Table 2 Follow-up data of patients whose post-conization diagnosis was CIN 3

Follow-up data		p value
Observation period after conization (days)	34–3,013 (median 565)	–
Persistent/recurrent disease after conization (cases)	14 (5.8%)	0.0018*
Negative margin	7 (3.5%)	
Positive margin	7 (16%)	
Time to diagnosis of persistence/recurrence after conization (days)	64–2,465 (median 316)	–

Time to diagnosis of persistent/recurrent disease was calculated from the date of conization to the day on which CIN was diagnosed by colposcopic biopsy

* The difference in the rates of persistence/recurrence between margin-negative and margin-positive cases was statistically significant ($p = 0.0018$ by Fisher's exact test)

Table 3 Persistent/recurrent cases after conization

Case	Age (years)	Margin status	First cytology after conization	Time to diagnosis of persistence/recurrence (days)	Persistent/recurrent disease diagnosis	Treatment for the persistent/recurrent disease
Persistence						
1	23	−	HSIL	64	CIN 3 (polyp)	Polypectomy
2	57	−	LSIL	93	CIN 1	Observation
3	28	−	LSIL	131	CIN 2	Laser vaporization
4	31	−	HSIL	175	CIN 3	Laser vaporization
5	70	+	HSIL	82	CIN 3	Hysterectomy
Recurrence						
6	37	−	NILM	299	CIN 1	Observation
7	50	−	NILM	821	CIN 1	Observation
8	37	−	NILM	2,465	CIN 3	Re-conization
9	47	+	NILM	252	CIN 2	Re-conization
10	43	+	NILM	333	CIN 3	Unknown[a]
11	36	+	NILM	740	CIN 1	Observation
12	57	+	NILM	1,047	CIN 3	Re-conization
13	34	+	NILM	1,272	CIN 1	Observation
14	35	+	NILM	1,825	CIN 3	Re-conization

Among the cases in which CIN lesion was detected after conization, margin-positive cases with an abnormal first cytology (within 3 months after conization) were regarded as cases of persistence of CIN. Cases of negative first cytology were regarded as recurrence of CIN. Time to diagnosis of persistent/recurrent disease was calculated from the date of conization to the day on which CIN was diagnosed by colposcopic biopsy

NILM negative for intraepithelial lesion or malignancy, *LSIL* low-grade squamous intraepithelial lesion, *HSIL* high-grade squamous intraepithelial lesion

−, Negative resection margin; +, Positive resection margin

[a] The treatment and final outcome for one recurrent CIN 3 case (case 10) was unknown, because the patient moved to another hospital after the diagnosis of recurrence

single case of CIN 3 whose resection margin had been positive (case 5), the result of the first cytological test of the uterine cervix performed in the second month after conization was HSIL (high-grade squamous intraepithelial lesion). The pathological result of a colposcopic biopsy performed 1 month later, in the third month, indicated the lesion was CIN 3; the patient elected to undergo a prophylactic hysterectomy at that time. This case was regarded to be persistent.

In the other six margin-positive patients, the results of the first cytological test were negative (NILM). CIN lesions were first detected after 252–1,825 days from conization. These cases were regarded to be recurrent and not persistent ones.

Long-term outcome of the CIN 3 cases

The long-term outcome of CIN cases treated by Shimodaira-Taniguchi conization was analyzed for persistence/recurrence-free probability, which was found to be significantly higher in margin-negative cases than in margin-positive ones ($p = 0.007$ by log-rank test). The five cases of persistent CIN 3 were set aside from further analysis. Only the nine recurrent cases, whose initial treatment had been successful by the Shimodaira-Taniguchi procedure, as characterized by being negative upon the first cytology follow-up within the first 3 months after conization, and margin-positive cases, which were negative at the first cytology follow-up, were retained. Recurrence-free probability in these cases was significantly higher in the margin-negative cases than in the margin-positive ones (hazard ratio 5.19; 95% CI, 1.175–22.994; $p = 0.0041$ by log-rank test) (Fig. 2).

Discussion

Currently, conization is the standard option for treatment of CIN 3, a precursor of SCC of the uterine cervix. LEEP has largely replaced the cold knife procedure because it overcomes several of the former's drawbacks (including significant blood loss, longer operation times, and a higher rate of post-operative hemorrhage). Both cold knife conization and LEEP have a similar margin-positive rate [9]. Although a high margin cure rate is achieved by LEEP

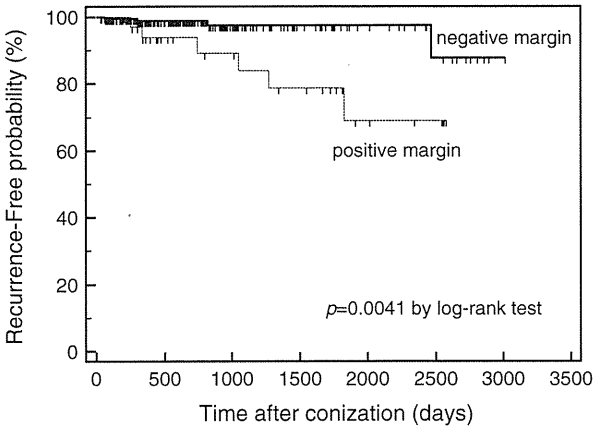

Fig. 2 Long-term outcome of the recurrent cases after conization for CIN 3 by resection margin. Recurrence-free probability was significantly higher in margin-negative cases than in margin-positive ones (hazard ratio 5.19; 95% CI, 1.175–22.994; $p = 0.0041$ by log-rank test)

conization, significant thermal damage is usually observed in the resected tissues [3]. The cervical tissue is also resected into several separated specimens during the LEEP, resulting in later difficulties in determining the critical margin status of the specimens, and the corresponding cervical location of any remaining lesions detected at the margin.

The Shimodaira-Taniguchi conization procedure addresses the disadvantages of LEEP by using a high-frequency current and a triangular probe with a 0.25-mm linear excision electrode to extract the cone of tissue as a single informative specimen, and does so without incurring any undue thermal trauma [3]. Matsumura et al. have demonstrated the benefits of the procedure: the number of the excised specimens was only one per patient in 79% of the case they studied (358 of 455 cases); the mean operation time was 11 min; and the average blood loss was only 9.9 ml.

Matsumura et al. found that persistent disease was observed in 2 (0.7%) of 268 CIN patients soon after the procedure, and, after 6–20 months post-conization, recurrence was detected in only slightly higher numbers (3/268; 1.1%). They also found recurrences within 16–30 months in 4.8% (3/62) of the more advanced microinvasive SCC cases treated with the Shimodaira-Taniguchi conization procedure. Among these six recurrent cases, four cases had been margin positive and the other two were margin negative.

There is one published study which did not find any significant value for using resection margin status in the prediction of long-term outcome of CIN 2 and CIN 3 after conization; that study was done using a CO_2 laser or cold knife [10]. However, others have found that the resection margin of CIN lesions produced by LEEP, cold knife, or laser was demonstrated to be able to aid in predicting the subsequent recurrence of CIN 3 [7, 8]. According to Chen et al., the persistence/recurrence rate of margin-positive cases, 33% (47/141 cases), was significantly higher than that of the margin-negative cases, 2% (21/972 cases) ($p < 0.0001$ by Fisher's exact test).

In our present study, the long-term outcome of CIN 3 cases treated by the Shimodaira-Taniguchi conization procedure was retrospectively analyzed relative to the status of their resection margins. The margin-positive rate for all CIN 3 cases was 19% (45 of 243 cases), and the rate for persistence/recurrence was 5.8% (14 of 243 cases). Our 19% margin-positive rate was lower than the 39% found in the previous study [3], and our 5.8% persistence/recurrence rate was higher than their 1.8%. Although the outcome results in our study were not different between the cases of CIS versus severe dysplasia (defined by the post-conization diagnosis), a previous study had shown that the persistent/recurrent rate was higher in CIS cases than in severe dysplasia cases ($p = 0.007$). The reasons for these discrepancies are currently unclear.

We found, as might be expected, that the probability of being persistence/recurrence-free was significantly higher in the margin-negative cases than in margin-positive ones ($p = 0.007$ by log-rank test), and this result was quite consistent with that of a previous study [7]. When we looked only at CIN cases which had passed their first test for being cured (those that had a normal cervical cytology at some point during the initial 3-month follow-up, regardless of whether they were judged margin negative or margin positive), we again found that the recurrence-free probability was significantly lower in margin-positive cases than in margin-negative cases (hazard ratio 5.19; 95% CI, 1.175–22.994; $p = 0.0041$ by log-rank test). Moreover, these recurrences occurred relatively long after the initial conization (252–1,825 days; median: 894 days).

The late arising lesions, those that were not detected at 'first cytology', possibly indicate very slow growth of a very small number of dysplastic or predisposed cells, which were not detectable by a normal pathological investigation. HPV may be more likely, for some unknown reason, to persist and/or cause progression in the cervix of margin-positive cases, perhaps because of the specific microenvironment of the positive margin; however high-risk type of HPV infections associated with CIN was previously demonstrated to clear gradually after successful conization in most patients showing clear resection margins [11]. The discrepancy of finding persistence of the CIN lesions after receiving a margin-negative status was, in part we feel, due to the presence of minute CIN lesions which had not been detected at the time of conization, or 'skip lesions' that were not detected at the surgical margins even by dividing the specimens of resected cervical tissue into 12 pieces for individual examination.

In our present study, we have re-evaluated the long-term outcome of CIN lesions after treatment with the Shimodaira-Taniguchi conization procedure routinely used in our facility to see if we could gain insight into predicting recurrence of the lesions. A recent study showed unexpected tumor progression after conization for CIS [12]. We found that the positive/negative status of the resection margin was a significant predictor for long-term outcome. Specifically, it was demonstrated that recurrence occurred at a significantly higher rate in margin-positive cases than in margin-negative cases whose post-conization cytology was normal. Further study is still needed to clarify the mechanism of the relatively late recurrences noted in margin-positive cases after Shimodaira-Taniguchi conization.

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Conflict of interest The authors state that there are no conflicts of interest. The authors also declare (1) that the article has not been published elsewhere; (2) it is not being considered for publication elsewhere; and (3) that it has been submitted with the full knowledge and approval of the institution or organization given as the affiliation of the authors.

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Review Article

Two Distinct Pathways to Development of Squamous Cell Carcinoma of the Vulva

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Squamous cell carcinoma (SCC) accounts for approximately 95% of the malignant tumors of the vaginal vulva and is mostly found in elderly women. The future numbers of patients with vulvar SCC is expected to rise, mainly because of the proportional increase in the average age of the general population. Two different pathways for vulvar SCC have been put forth. The first pathway is triggered by infection with a high-risk-type Human Papillomavirus (HPV). Integration of the HPV DNA into the host genome leads to the development of a typical vulvar intraepithelial neoplasia (VIN), accompanied with overexpression of $p14^{ARF}$ and $p16^{INK4A}$. This lesion subsequently forms a warty- or basaloid-type SCC. The HPV vaccine is a promising new tool for prevention of this HPV related SCC of the vulva. The second pathway is HPV-independent. Keratinizing SCC develops within a background of lichen sclerosus (LS) through a differentiated VIN. It has a different set of genetic alterations than those in the first pathway, including p53 mutations, allelic imbalances (AI), and microsatellite instability (MSI). Further clinical and basic research is still required to understand and prevent vulvar SCC. *Capsule.* Two pathway for pathogenesis of squamous cell carcinoma of the value are reviewed.

1. Introduction

Squamous cell carcinoma (SCC) accounts for only 5% of the malignant tumors of the female genital tract, but it represents 95% of vaginal vulvar tumors [1]. The incidence of malignant vulvar tumors in the United States is 1.5 per 100,000 women per year, and this incidence increases with age. The average age at diagnosis is in the 7th to 8th decades of life, with a future rise in absolute numbers of vulvar SCC expected, mainly due to the proportional increase in the average age of the general population [2].

Two different types of vulvar SCC have been delineated, each with their own precursors. The first type is associated with an infection with one of the high-risk types of Human Papillomaviruses (HPV), and it primarily affects younger women. The other type is associated with a lichen sclerosus (LS) condition, and it occurs predominantly in elderly patients independent of any HPV infection [2]. Although the pathogenesis of vulvar SCC has been investigated, it has not been documented nearly as well as the pathogenesis of the more common cervical SCC.

We herein give an overview, focusing on the molecular events of these two distinct HPV-associated and independent pathways for the development of vulvar SCC.

2. Clinical and Pathological Features of SCC and Its Precursors

2.1. SCC. Vulvar SCC accounts for 90% of vulvar cancers and 5% of gynecological cancers. Patients usually present with a vulvar mass, which may be pruritic or painful or be associated with bleeding, and, occasionally, with a groin mass. Clinical factors that have adverse prognostic significance include increased stage, older age, smoking, and fixed or ulcerated groin nodes [3].

The three main histological subtypes of vulvar SCC are: warty, basaloid, and keratinizing (Table 1 and Figure 1). The predominant type, keratinizing, accounts for

65%–80% of vulvar SCCs; the basaloid and warty types of SCC account for the remaining 20%–35% [3]. The keratinizing type usually occurs in postmenopausal women; the warty/basaloid types tend to occur more often in premenopausal or perimenopausal women. The keratinizing type is usually formed by well, or moderately differentiated cells with an absence of koilocytosis. There are usually one or more adjacent epithelial lesions, including LS, squamous cell hyperplasia (SCH), and differentiated vulvar intraepithelial neoplasia (VIN) [3], which will each be described further in the following sections.

The warty or basaloid types of SCC often accompany a normal-type VIN. The basaloid type typically grows in bands, sheets, or nests within a desmoplastic stroma, and focal cytoplasmic maturation and keratinization may be observed. The warty type exhibits invasion as bulbous or irregular jagged nests, often with prominent keratinization. The koilocytotic tumor cells have pleomorphic to bizarre, often multiple, nuclei with irregular contours that vary from hyperchromatic and shrunken to those with clumped or smudged chromatin [3].

Other histological subtypes include verrucous carcinoma, giant cell carcinoma, and acantholytic squamous cell carcinoma. Verrucous carcinoma is highly differentiated squamous carcinoma that has a verrucous pattern and invades with a pushing border in the form of bulbous pegs of neoplastic cells. Squamous cell carcinoma with tumor giant cells is a variant of SCC characterized by multinucleated tumor giant cells, large nuclei with prominent nucleoli, and prominent eosinophilic cytoplasm. This variant is relatively rare and is associated with a poor prognosis. The acantholytic squamous cell carcinoma forms rounded spaces, or pseudoacini, lined with a single layer of squamous cells. Dyskeratotic and acantholytic cells are sometimes present in the central lumen [4].

2.2. VIN. Various terms have been used to define the precursors of vulvar SCC. Bowen first reported on these squamous intraepithelial lesions in 1912, and they are now commonly referred to as Bowen's disease; since then, a myriad of clinical and histopathological terms have been employed to describe these vulvar precancerous lesions [5]. The International Society for the Study of Vulvar Disease (ISSVD) simplified the terminology for carcinoma in situ and vulvar atypia in 1976; in 1986 they adopted the single term of VIN and a 3-grade VIN system based on the terminology from cervical intraepithelial neoplasia (CIN) [5]. In VIN 1, maturation was present in the upper two-thirds of the epithelium. In VIN 2, the dysplasia involves the lower two-thirds of the epithelium, and in VIN 3, the dysplasia extends into the upper third [5]. The terms of warty, basaloid, and differentiated (simplex) are used in the same way as for cervical SCC.

The most recent classifications are shown in Table 2. The World Health Organization (WHO) classifies VIN according to the 3-grade system for both the warty/basaloid types and the simplex type [7]. In 2004, ISSVD modified their VIN terminology and suggested a 2-tier classification: VIN usual type and VIN differentiated type. Moreover, they decided to abolish the term VIN 1. The term of VIN is now applied only to the histologically high-grade squamous lesions that were the former VIN 2 and VIN 3 or differentiated VIN [5]. This revision was made based on the observation that there was neither evidence that the morphologic spectrums of VIN 1, 2, and 3 reflect a biologic continuum nor that VIN 1 was a cancer precursor [2]. In 2005, Medeiros et al. proposed a Bethesda-like grading system of low-grade vulvar intraepithelial lesions (Low-grade VILs) and high-grade vulvar intraepithelial lesions (High-grade VILs) [6]. Low-grade VILs correspond to lesions associated with low-risk HPV infections. Condyloma acuminatum and discrete raised lesions with minimal atypia and lacking the features of dermatosis (VIN 1) were categorized into low-grade VIL [6].

A systematic review of the progression rate from VIN 3 to invasive SCC, after various clinical treatments, was reported to be 3.3% [8]. Nine percent of 88 untreated patients progressed to SCC during 12 to 96 months. Complete regression of usual VIN 3 lesions were observed in 1.2% of 3322 patients, mostly during the first 10 months after diagnosis, 41% of which remission was related to pregnancy. Another study demonstrated that the overall percentage of differentiated VIN lesions with subsequent diagnosis of SCC was 32.8%, and that of usual type VIN was 5.7% [9]. The median time for progression from usual type VIN to SCC was 41.4 months whereas that from differentiated VIN to SCC was significantly shorter: 22.8 months ($P = .005$). Another study demonstrated that the mean time from the incidence of HPV infection to the development of VIN 1–3 was 18.5 months (95% confidence interval, 13.4–23.6) [10].

The typical presentation of usual VIN is a pruritic, burning, or asymptomatic, white, red, or pigmented lesion. The incidence of this form of VIN has almost doubled over the past several decades, with a significant increase in younger women. The lesions of differentiated VIN usually range from 0.5 to 3.5 cm, appearing as single or multiple gray-white areas with a rough surface or ill-defined white plaques or nodules. The lesions usually occur in postmenopausal women [3].

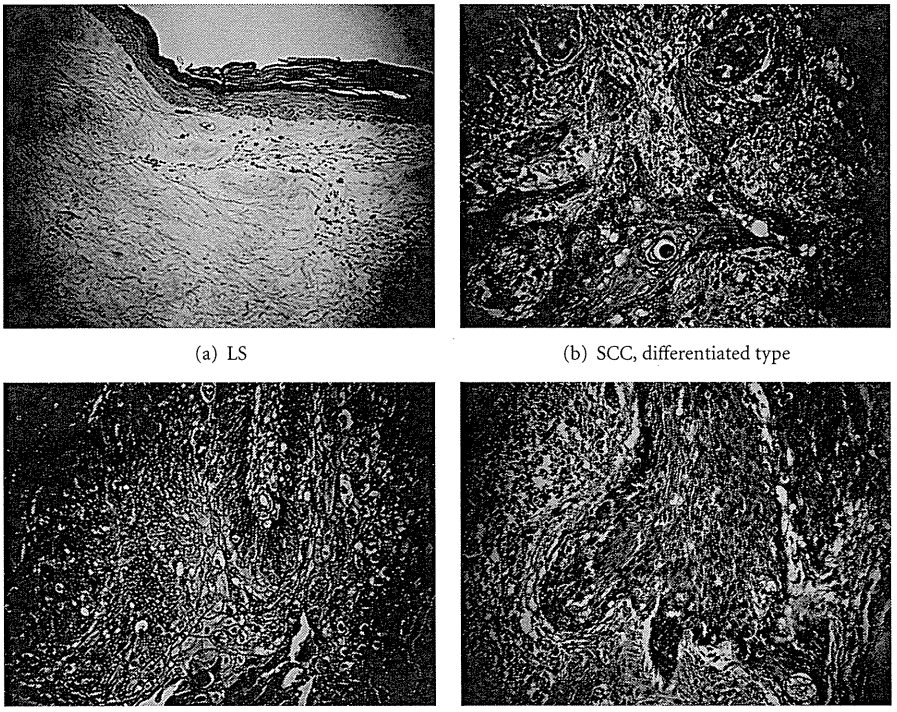
2.3. LS. LS runs a relapsing and remitting course and presenting symptoms include pruritus, soreness, burning, and irritation. Typically, the lesions are white plaques and papules, often with areas of erythema hyperkeratosis, pallor, and ulcer [2].

The histological features of lichen sclerosus (LS) include a thinned epidermis with loss of normal rete pegs, basal layer vacuolar changes and a paucity of melanocytes, and, moreover, a wide band of homogeneous collagen below the dermatoepidermal junction and a band-like lymphocytic infiltrate below the homogenized area are present (Figure 1). The dermis often shows variable degrees of edema [2].

LS most commonly affects the anogenital area (85% to 98%), with extragenital lesions occurring in 15% to 20% of the patients [11]. LS occurs at all ages; however, it has a bimodal peak of incidence in prepubertal girls and postmenopausal women [2]. According to a previous study, 1 out of 30 elderly women suffer from LS [12]. As association of LS with autoimmune disorders has been demonstrated.

TABLE 1: Characteristics of two types of squamous cell carcinoma of the vulva. (Characteristics of the warty/basaloid type and the keratinizing type of SCC of the vulva are shown).

	warty or basaloid type	keratinizing type
Frequency	20%–35%	65%–80%
Age	Younger	Older
	55 (35–65)	77 (55–85)
Precursor	warty or basaloid VIN	Lichen sclerosus differentiated VIN
Molecular characteristics	HPV integration	p53 mutation
	$p14^{ARF}$ · $p16^{INK4a}$ overexpression	Microsatellite instability
Prognosis	better	worse



(a) LS (b) SCC, differentiated type

(c) SCC, warty type (d) SCC, basaloid type

FIGURE 1: Examples of hematoxylin and eosin staining of vulvar lesions (×200) type.

According to previous studies, around 30% of the patients have active autoimmune disease and autoantibodies were detected in about half of the serum of the LS patients [2, 13–16]. LS is considered to occur at sites of injured skin in women with the susceptible immunophenotype who scratch the area because of genital irritation [2].

The risk of development of vulvar SCC in women with LS was shown to be 4% to 5% [11]. A previous review study also estimated a 4.5% frequency of SCC arising in LS with an interval of approximately 10 years (1.67 to 12.5 years) after diagnosis of LS without SCC [17].

3. Mechanisms of Carcinogenesis

3.1. HPV-Related Carcinogenesis. Two distinct pathways, HPV related and HPV independent, were proposed for vulvar carcinogenesis (Figure 2). Warty/basaloid type SCC develops through usual (warty/basaloid) type VIN triggered by infection with high-risk type HPVs, predominantly HPV−16 and −18 [2]. Usual type VIN lesions are observed adjacent to greater than 10%–67% of the vulvar SCC lesions [18]. A previous study showed that 69% to 100% of warty/basaloid type SCC were positive for high-risk type HPVs [19]. High-risk type HPVs were detected in 84% of 45 usual VIN cases [20]. Eighty-seven percent (13 of 15) of the usual high-risk type HPV-positive VIN lesions were shown to express both $p14^{ARF}$ (a cell-cycle regulator which mediates p53 activation) and $p16^{INK4A}$ (a cyclin-dependent kinase inhibitor) [21]. Hoevenaars showed that all 38 usual VIN lesions exhibited positive $p16^{INK4A}$ immunohistochemical staining, and that in all these cases a high MIB1 index was observed. No expression of p53 and $p16^{INK4A}$ was detected in normal epithelium of the vulva [20]. However, so far

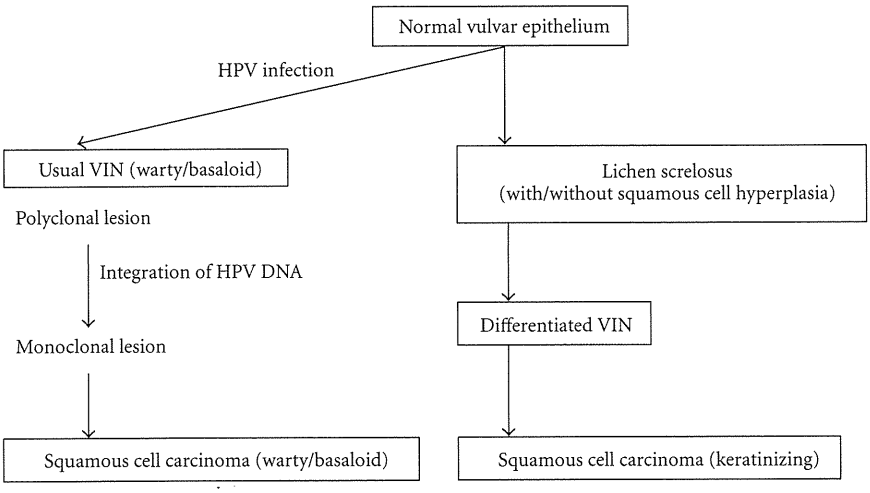


FIGURE 2: Pathogenesis of squamous cell carcinoma of the vulva. (Distinct pathways for carcinogenesis of keratinizing and warty/basaloid types of vulvar SCC from normal epithelium through precursor lesions are demonstrated.)

TABLE 2: Classifications of vulvar intraepithelial neoplasia. (WHO, ISSVD, and Bethesda-like classifications are shown.)

WHO (2003)
VIN 1,2,3 (warty type/basaloid type)
VIN 1,2,3 (simplex type)
ISSVD (2004)*
VIN, usual type (VIN 2,3)
(a) warty type
(b) basaloid type
(c) mixed type
VIN 3, differentiated type (VIN 3)
Bethesda-like system [6]
Low-grade VIL (condyloma NIN 1)
High-grade VIL (VIN 2/VIN 3)

*VIN1: abolished terminology.

little has been found concerning the mechanism of enhanced expression of $p14^{ARF}$ and $p16^{INK4A}$ in the carcinogenesis of vulvar SCC.

The HPV viral gene products E6 and E7 interact with host cell p53 and Rb proteins, resulting in p53 dysfunction and inactivation of Rb, respectively. In cervical carcinogenesis triggered by high-risk type HPV infection, E7 inhibits Rb, resulting in the release of active host E2F-1, which positively regulates host $p14^{ARF}$. E6 inhibits p53 function by binding with E6-AP ubiquitin ligase, and leads to $p14^{ARF}$ upregulation via p53 degradation by negative feedback mechanism [22, 23]. Functional inactivation of Rb by E7 protein also leads to $p16^{INK4A}$ overexpression. Taken together, $p14^{ARF}$ and $p16^{INK4A}$ were over-expressed as a consequence of HPV E6 and E7 expression in cervical carcinomas.

In carcinogenesis of HPV-related SCC, similar mechanisms to cervical carcinogenesis seem to play an important role. Degradation and inactivation of the tumor suppressor genes p53 and Rb leads to absence of cell-cycle arrest and hyperproliferation of tumor cells. Frequent detection of over-expression of $p14^{ARF}$ and $p16^{INK4A}$ in VIN suggests that degradation and inactivation of p53 and Rb are early events in carcinogenesis of HPV-related SCC of the vulva.

3.1.1. Integration of High-Risk Type HPV DNA. In uterine cervical carcinogenesis, integration of the high-risk type HPV DNA into the host genome was demonstrated to be an initial step for monoclonal expansion of dysplastic cells [24]. In the process of the integration, some parts of the E2 open reading frame (ORF), which encode a 48-kd phosphorylated protein involved in the regulation of viral DNA transcription and replication, are usually disrupted or deleted from HPV genome, causing up-regulation of the oncogenic E6 and E7 genes [25]. HPV integration sites were demonstrated to be semirandomly distributed over the whole genome, with a clear predilection for genomic fragile sites, but there was no evidence for targeted disruption or functional alteration of critical cellular genes by the integrated viral sequences. The main function of HPV integration is considered to be for the stabilization of viral oncogene transcription [26, 27].

Similar mechanisms to those for cervical cancer seem to play an important role during the development of HPV-related vulvar SCC triggered by high-risk type HPV infection. Integration of HPV-16 DNA, with deletion of the E2 ORF in both the SCC portion and its adjacent VIN 3 lesions, which were all implied to be formed from a single cell of origin through monoclonal expansion, was first demonstrated in a case of vulvar SCC by Ueda et al. [35]. Monoclonal composition was also demonstrated in 3 of 7 cases of VIN 1/2, and 12 of 13 VIN 3 cases in the study. Later, additional supporting studies have also shown VIN 3 cases associated with infection of HPV in an integrated form [28, 29].

3.1.2. HPV Vaccine. The HPV vaccine is a tremendously promising new tool for the prevention of HPV-related SCC of the vulva, as it has already been for the cervix. The FUTURE I study has demonstrated that a prophylactic quadrivalent HPV-(6/11/16/18) L1 VLP vaccine significantly reduced the incidence of HPV-associated anogenital diseases in young women [30]. The prophylactic efficacy in the study was 100% for vulvar condyloma, VIN 1, and VIN 2/3 in the per-protocol population. Other studies also demonstrated that the prophylactic quadrivalent HPV vaccine completely protected VIN 2/3 [31, 32].

Interestingly, a series of 3-4 vaccinations against a synthetic long-peptide of the HPV-16 oncoproteins E6 and E7 was shown to be therapeutically effective for HPV-16-positive VIN 3 patients [33]. At 3 months after the last vaccination, 5 (25%) of 20 patients had complete remission of the lesion, and HPV-16 was no longer detected in 4 cases (20%). At 12 month of followup, 9 (47%) of 19 patients had a complete response with tolerable adverse effects. The patients who had a complete response at 3 months demonstrated a significantly stronger interferon-γ-associated proliferative CD4+ T-cell response and a broader response of CD8+ interferon-γ T-cells. A phase II clinical trial of the topical immune-modulator imiquimod, followed by therapeutic HPV-16 vaccine using a fusion protein of HPV-16 E6E7L2 on 19 cases with VIN 2/3, demonstrated that complete regression of VIN 2/3 lesions was observed in 63% of the cases (12 of 19) [34].

3.2. HPV-Independent Carcinogenesis. The majority of vulvar SCC are considered to occur in elderly women through differentiated VIN in a background of LS [2]. High-risk type HPVs were detected in none of 75 differentiated VIN cases [20]. They also showed that all 75 differentiated VIN lesions exhibited negative p16 immunohistochemical staining, and a low MIB1 index was observed in 96% (72 of 75 cases) of the cases [20]. No relationship between HPV infection and LS was found in these women [2, 21]. These results strongly suggest that an HPV-independent pathway exists for carcinogenesis of vulvar SCC from LS through differentiated VIN; however, the mechanism of HPV-independent carcinogenesis has not yet been fully elucidated.

We have previously demonstrated that 2 of 6 LS lesions exhibited monoclonality, implying that certain important molecular alterations might occur in some LS lesions well before histologically apparent malignant transformation to differentiated VIN or keratinizing SCC occurs [35]. Rolfe et al. showed that 10 of 12 LS-associated SCCs exhibited a p53 mutation, and in 7 of those 10 cases LS lesions exhibited the p53 mutation at the same codon as in the SCC lesions, suggesting that a p53 mutation is possibly involved early in the HPV-independent pathway of vulvar carcinogenesis [36]. Somatic mutation of PTEN was also demonstrated in some cases of vulvar SCC and VIN, suggesting that PTEN mutation possibly played a role early in the carcinogenesis of vulvar SCC [37]. Pinto et al. found that an allelic imbalance (AI) was present in 67%, 53%, and 43% of usual type VIN, differentiated VIN and LSs, respectively, and that microsatellite instability (MSI) was detected in 3 (20%) of 15 differentiated VIN, and 2 (12%) of 17 LS, but none of usual type VIN, implying that these molecular alterations are also possibly early events in vulvar carcinogenesis, and that MSI may play a critical role for malignant potential of LS [38].

A recent study demonstrated more frequent hypermethylation of RASSF2A, MGMT, and TSP-1 genes in SCC associated with LS than in SCC not associated with LS, suggesting a possible role of these genes in HPV-independent carcinogenesis [39].

A fraction of squamous cell hyperplasia (SCH) lesions were shown to be monoclonal in composition [35] and p53 mutation, AI, and MSI were observed in 22%, 50% and 20%, respectively, of SCH cases [36, 38]. SCH with atypia might be a precursor of SCC; however, SCH without atypia is, currently, not regarded as a direct precursor of SCC. Relationship between SCH and keratinizing SCC is still to be determined [2, 38].

4. Conclusions

Two distinct pathways leading to vulvar SCC have been suggested. One is a pathway primarily linked to infection with high-risk types of HPV; the other is an HPV-independent scenario. Mechanisms similar to those that drive cervical carcinogenesis possibly play an important role in HPV-related carcinogenesis of vulvar SCC. HPV prophylactic and therapeutic vaccines are both promising to prevent HPV infection and prevent development of warty/basaloid type SCC from its precursor, the usual type VIN. On the other hand, keratinizing type vulvar SCC, which by far represents the majority of vulvar SCC, occurs independently from HPV infection in a background of LS. The mechanism of carcinogenic progression forward from LS in this second pathway has not fully delineated, and it is not yet clear whether medical treatments of LS prevent malignant transformation. Further clinical and basic research into these important areas is still required.

Abbreviations

AI: Allelic imbalance
CIN: Cervical intraepithelial neoplasia
HPV: Human Papillomavirus
ISSVD: The International Society for the Study of Vulvar Disease
LS: Lichen sclerosus
MSI: Microsatellite instability
SCC: Squamous cell carcinoma
SCH: Squamous cell hyperplasia
VIL: Vulvar intraepithelial lesions
VIN: Vulvar intraepithelial neoplasia
WHO: World Health Organisation.

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