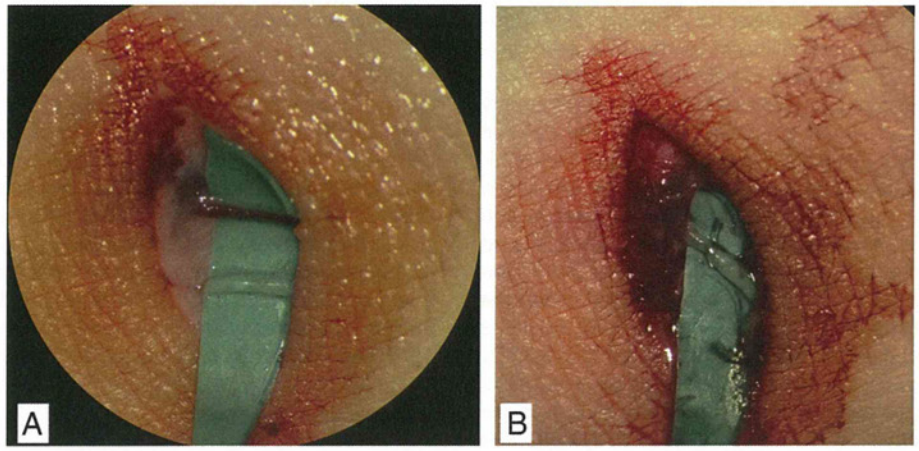

Fig. 1. Lymphaticovenular anastomosis. (A) Pre-anastomosis image. The upper vessel is a vein; the lower image is a lymphatic channel. (B) Post-anastomosis image. The anastomosis was performed with 5 sutures of 11–0 nylon. The left side of the anastomosis looks clear because the lymphatic fluid is under higher pressure than venous blood; therefore, flow from the lymphatic channel on the right washes out blood from inside the vein.

our series suffered any complications of LVA. Specifically, there were no infections and no wound-healing problems.

Representative case

The lymphocele was detected on CT 11 days before LVA (Fig. 3A). The patient had abdominal pain, urinary frequency, and lower-extremity lymphedema. A percutaneous catheter was inserted 3 days before the operation and the daily volume of drained fluid was recorded until the tube was removed (Fig. 3D). The operation site of LVA was noted (Fig. 3B). A CT image taken 3 days after the operation showed that the lymphocele had disappeared (Fig. 3C). The catheter was removed after confirming that the symptoms had disappeared.

Discussion

Since Teruel et al. [21] first reported successful sclerotherapy with povidone iodine for lymphoceles, several types of sclerotherapy with a variety of agents have been reported [1,6,9]. The cure rate for sclerotherapy is reported to be between 77% and 98% [1], and the recurrence rate is 31% [22]. However, the success of this treatment is inversely proportional to the size of the lymphocele [1]—larger lymphoceles are more likely to be symptomatic and cause complications; thus, when the lymphoceles most require treatment, this therapy is likely to be relatively less effective.

Laparoscopic or open surgical fenestration can be used to open a pathway from the lymphocele into the peritoneal cavity, allowing the peritoneum to absorb lymphatic fluid [23]. These techniques enable lymphatic fluid to re-circulate into the venous system. However, they are more invasive than other therapies and have been associated with complications including bladder perforation, ureter transection, and injury of pelvic vessels [6,10]. Recurrence can occur with closure of the fenestrated window in 6–15% of cases [6,24].

The ideal therapy for lymphoceles would be more effective and less invasive than traditional treatment methods (including sclerotherapy and surgical fenestration), with fewer complications and a lower chance of recurrence. Moreover, restoration of lymphatic circulation, broken by lymphadenectomy, is desired.

LVA is emerging as the treatment of choice for lymphedema of the extremities. Before the LVA operation was available, only conservative therapies, such as massage and compression garments, could be used for lymphedema. These techniques do not enable re-establishment of lymphatic fluid circulation into the venous system, but simply release it into the trunk lesion. Therefore, patients are never able to discontinue the therapy if they wish to reduce the edematous lesion. LVA was

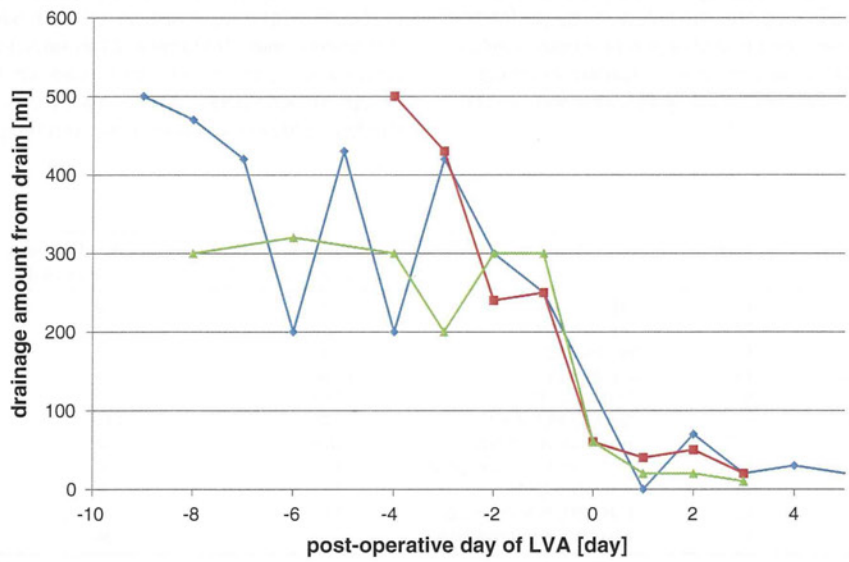

Fig. 2. Daily drainage from 3 patients who had preoperative placement of percutaneous drains. Note the dramatic decrease in drainage following LVA.

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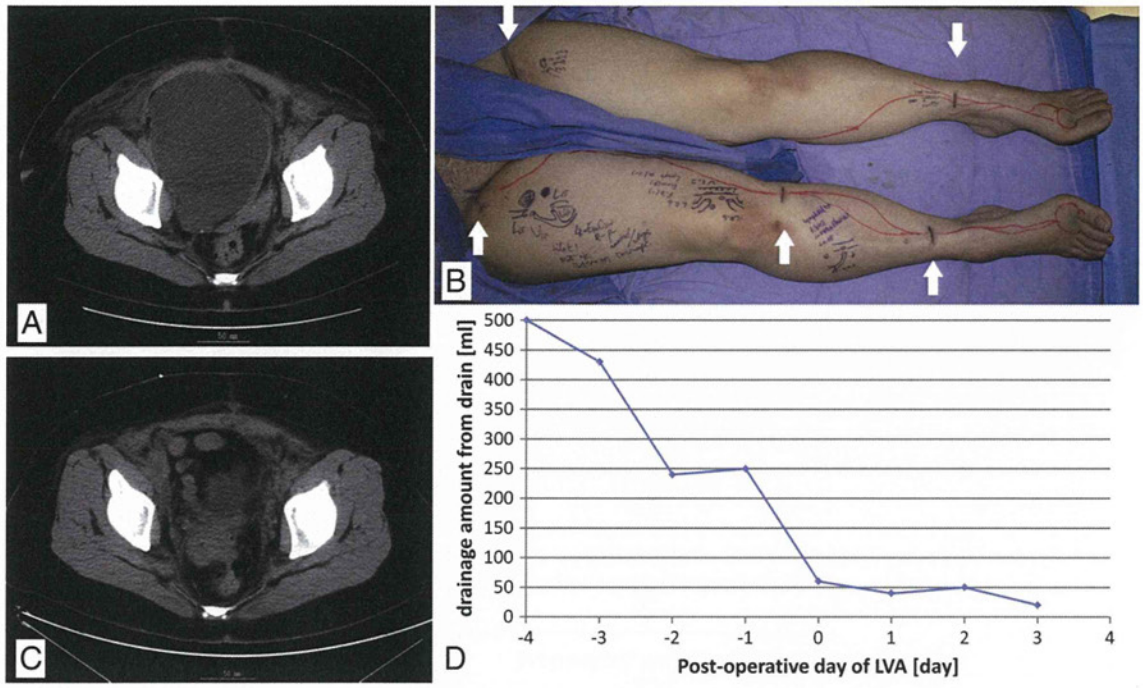

Fig. 3. Resolution of pelvic lymphocele after LVA. (A) CT scan of a large pelvic lymphocele after gynecologic surgery. (B) Immediate postoperative view. Seven anastomoses were performed through five 3-cm incisions under local anesthesia. (C) A CT scan of the same patient 3 days after LVA demonstrates complete resolution of the lymphocele. (D) The drainage chart of the same patient demonstrates large daily drainage volumes before LVA. Following LVA, the volume of fluid drained was dramatically reduced, and the drain was removed on postoperative day 4.

introduced as a new concept for lymphedema therapy [13–15]. The aim is to bypass proximal lymphatic blockages that cause congestion of lymphatic flow and thereby provide an alternative route for lymphatic fluid recirculation. Although the lymphatic channels normally have autokinetic movement because of smooth muscles, when the muscle damage due to lymphedema is irreversible, compression therapy is needed as an adjuvant therapy to direct lymphatic fluid into venulae. However, when the damage is dormant, the muscles react by pushing lymphatic fluid into the venous system. In this case, the patients do not need to receive any further adjuvant therapy.

In lymphoceles, the lymphatic flow from the lower limbs is similarly interrupted at the surgical region, where it flows into the cavity. This is illustrated in Fig. 4, where ICG injected into the dorsum of the feet is seen to escape into the percutaneous drainage catheter of a lymphocele. We reasoned that LVA would enable the lymphatic flow from the limbs to bypass the lymphocele, reducing its volume and preventing lymphatic flow into the lymphocele, thereby allowing spontaneous resolution. Our results supported this hypothesis, with total recovery in 6 of the 11 cases and improvement in the remaining 5 cases.

We believe that LVA has multiple advantages over the other methods currently used to treat lymphoceles. First, LVA is minimally invasive because it can be performed under local anesthesia and requires only 2 or 3 small skin incisions. Second, LVA is effective for

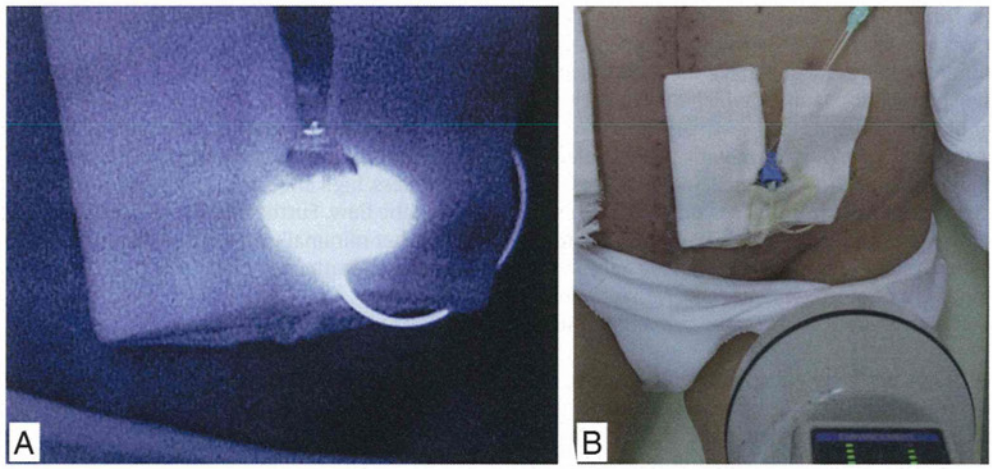

Fig. 4. Demonstration of lymphatic flow from the leg into a pelvic lymphocele. (A) Fluorescence lymphatic image of a percutaneous catheter, 5 min after injecting ICG into the first dorsal web space of the foot, indicates the lymphatic flow from the leg rapidly entering the lymphocele. It also indicates that the fluorescing root is the dominant lymphatic channel pouring into the lymphocele. (B) Conventional photograph of the same area. The Photodynamic Eye camera used to obtain the picture in (A) is seen at the bottom of the picture.

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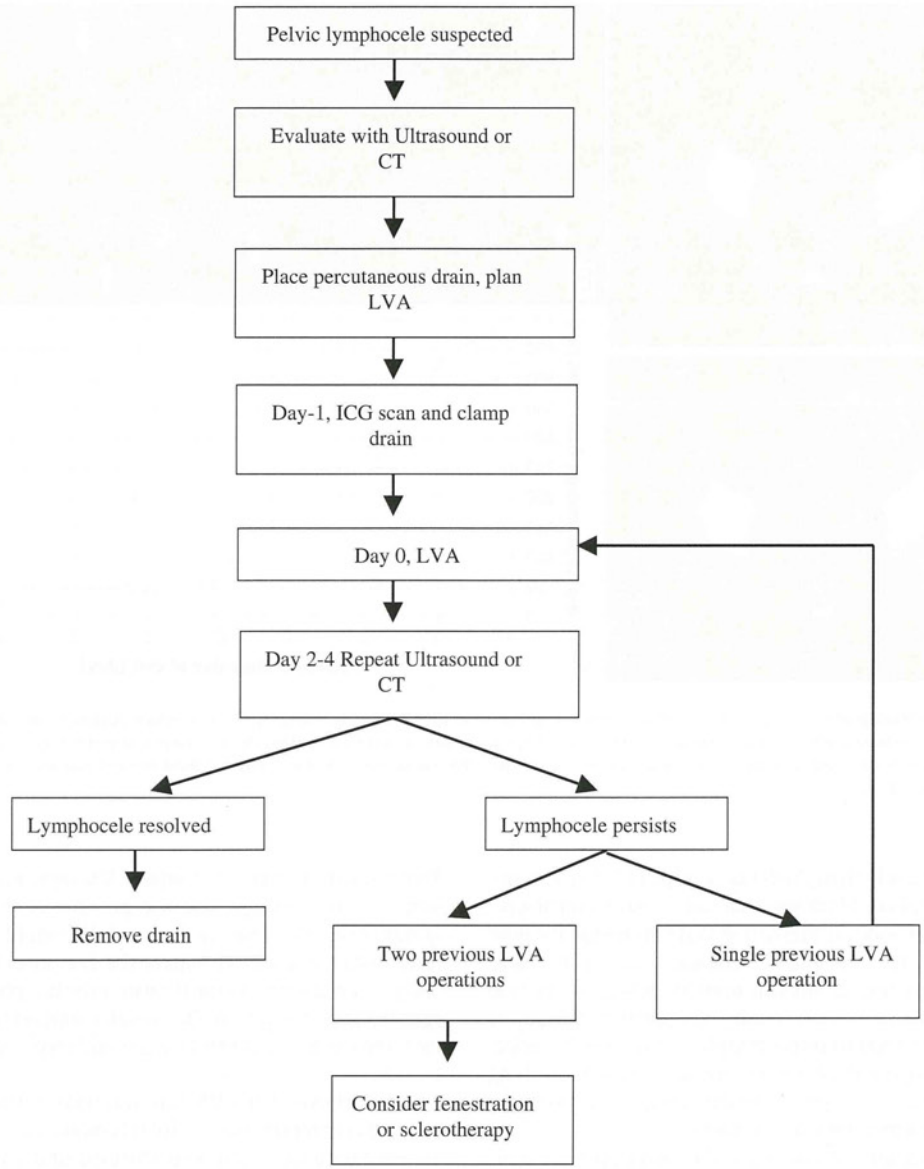

Fig. 5. Algorithm for the management of pelvic lymphocele.

all sizes of lymphocele. Third, LVA can prevent or improve lymphedema, which is a common complication of pelvic lymphadenectomy. This is in contrast to other techniques that resolve the fluid collection by blocking or sclerosing lymphatic channels, which may in itself provoke the development of lymphedema.

Our experience of reverse lymphatic flow with the valvular incompetence of lymphatic channels in lymphedema indicates that lymphatic flow into the lymphocele from places other than the leg may occur in a retrograde pattern into the leg's lymphatic channels and then into the venous system. Competent lymphatic valves may account for the partial failure of our technique, and we recommend that a percutaneous catheter be used to drain the remaining fluid if it is symptomatic.

Unfortunately, LVA is not perfectly effective for all patients. We suggest that the reason for this is that the lymphatic channels used for LVA are sometimes not the dominant lymphatic channels for the lymphoceles. In such cases, the lymphocele could diminish but not vanish. A second LVA might be able to locate the dominant lymphatic channel. Other therapies could also be used: LVA is an indirect approach to the lymphocele whereas other therapies approach lymphoceles directly. We present our algorithm for management of pelvic lymphoceles in Fig. 5.

In conclusion, our technique is minimally invasive and is performed under local anesthesia. It is therefore suitable for patients who have recently undergone major pelvic surgery. LVA should be considered as an initial therapy for lymphoceles because of its low invasiveness, high effectiveness, and ability to re-establish circulation of lymphatic flow. Further studies should be performed to compare LVA with other minimally invasive techniques, such as percutaneous catheter and sclerotherapy.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Genome-Wide Single Nucleotide Polymorphism Arrays as a Diagnostic Tool in Patients With Synchronous Endometrial and Ovarian Cancer

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Objective: Synchronous carcinomas in the endometrium and ovaries can be a single primary tumor with metastasis (SPM) or dual primary tumors (DP). Although the prognosis of DP without any metastases is significantly better than that of SPM, pathological diagnosis is difficult in tumors with similar histological features.

Materials and Methods: In 10 tumors from 5 patients with synchronous endometrial and ovarian carcinomas, 250K single nucleotide polymorphism arrays were performed. The patients were genetically diagnosed according to the pattern of copy number alterations (CNAs), in addition to microsatellite status and mutational analysis of *PIK3CA*, *PTEN*, *K-Ras*, and *CTNNB1*.

Results: Of the 5 patients, 3 exhibited identical CNA patterns, including type, loci, and degree of each alteration in the endometrial and ovarian carcinomas. The other 2 exhibited CNAs only in either endometrial or ovarian carcinoma. All 5 tumors had 1 or more genetic mutations in the genes examined. One patient exhibited mutations both in *PIK3CA* and *PTEN* at discordant sites between endometrial and ovarian carcinomas, whereas the other 4 exhibited concordant mutations. Overall, 4 of the 5 patients were genetically diagnosed with SPM, and the remaining 1 with DP. The pathological diagnosis was not in agreement with the genetic diagnosis in 4 of the 5 patients.

Conclusions: Genome-wide genotyping diagnosis may represent a useful approach for distinguishing between SPM and DP in synchronous endometrial and ovarian carcinomas.

Key Words: Synchronous carcinomas, Endometrial cancer, Ovarian cancer, SNP arrays, Genetic diagnosis

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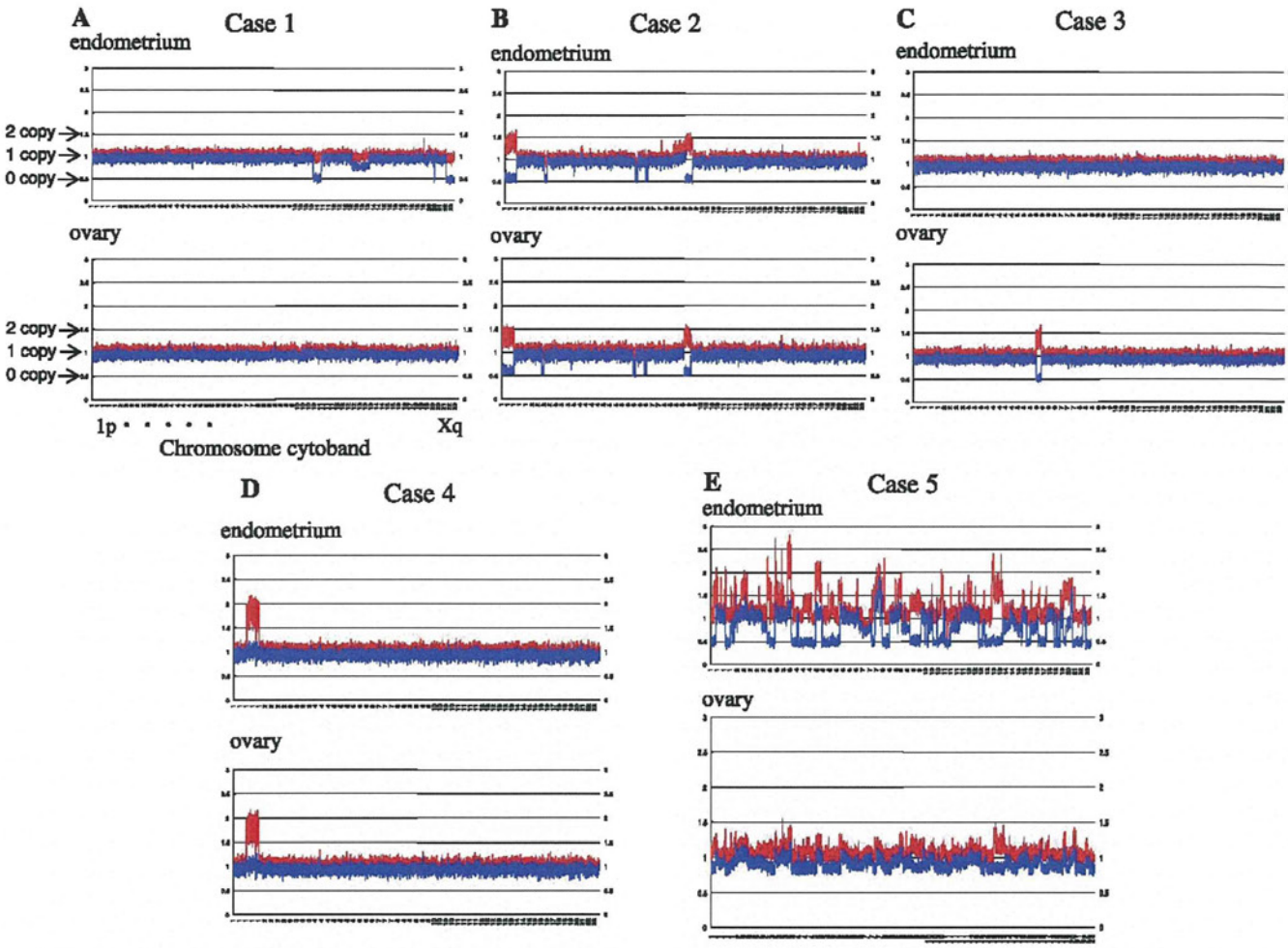

FIGURE 1. Single nucleotide polymorphism array "karyograms" of 10 tumors from 5 synchronous endometrial and ovarian carcinomas. The signal intensity ratio between the raw signal intensity from the cancer and paired normal samples is ordered by position in the genome, beginning at 1p and ending with X. The top panel shows allele-specific copy number in the endometrial carcinoma, and the bottom panel is that in the paired ovarian carcinoma. A, B, C, D, and E correspond to cases 1, 2, 3, 4, and 5, respectively.

Ovarian Cancer								Other Sites of Involvement		Pathological Diagnosis	
Tumor Size, cm	Tumor Site	Endometriosis	LVI	Grade	Presumed Origin	ER	PgR	Lymph Nodes	Peritoneum	Stage (Uterus)	Stage (Ovary)
6 × 5	Inside	−	−	1	Endometrium	−	2+	−	−	IIIa	—
3 × 4	Inside	−	−	1	Ovary	−	2+	−	−	IIb	Ia
15 × 11	Inside	(Adenomyosis)	−	1	Ovary	+	+	−	−	Ia	Ia
4 × 3	Inside	+	−	1	Ovary	−	2+	+	+	IIIc (pT3a N1 M0)	La
5 × 4	Inside + surface	−	+	3	Endometrium	−	−	−	+	IIIa	—

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Synchronous cancers involving the ovaries and the uterine corpus are well-known events in gynecologic malignancies.[1,2] These tumors can be independently derived, nonmetastatic tumors (dual primary tumors [DP]) or a tumor from 1 organ with metastasis to another (single primary tumor with metastasis [SPM]). In most of these synchronous endometrial and ovarian cancers, both tumors are diagnosed histologically as endometrioid adenocarcinomas, which may complicate the distinction between DP and SPM. Among 326 cases of endometrial carcinoma encountered at the University of Tokyo Hospital since 1999, 27 (8.3%) were synchronous endometrial and ovarian cancers. Of these 27 synchronous carcinomas, 9 were histopathologically diagnosed as DP and 18 as SPM.

Correct diagnosis of DP and SPM is clinically very important because the prognosis of DP is significantly better than that of SPM.[3–5] Using the guidelines according to the International Federation of Gynecology and Obstetrics,[6] DP tumors, when confined to the ovary (or ovaries) and uterine corpus alone, would represent 2 stage I cancers. Patients with DP may not require any adjuvant chemotherapy or radiotherapy, depending on the stage of each tumor. On the other hand, a primary endometrial cancer with ovarian metastases would be classified at least as stage IIIa, and a primary ovarian cancer with metastasis to the uterine corpus would be at least stage IIa. These patients with SPM require more aggressive treatment, including adjuvant chemotherapy (and/or radiotherapy). Thus far, the diagnosis of synchronous endometrioid adenocarcinomas has been mainly based on multiple pathological features, including tumor grade, extent of myometrial invasion, vascular invasion, ovarian tumor size, pattern of ovarian involvement, and presence or absence of precursor lesions (such as atypical endometrial hyperplasia and ovarian endometriosis).[7–10] However, similar histology cannot be used as evidence of metastasis from one organ to another. Two tumors may have a similar histological appearance but could still represent independent tumors. Conversely, a metastatic tumor may appear to be dissimilar from the primary tumor owing to morphologic variations in either tumor, such as dedifferentiation and epithelial-mesenchymal transition. Thus, a nondefinitive diagnosis between SPM and DP might result in an inaccurate prognostic evaluation and consequently unsuitable adjuvant treatments (mostly excessive treatments) in a significant proportion of synchronous tumors.

To increase the accuracy of the diagnosis, a number of attempts have been made to characterize synchronous tumors based on their molecular alterations. The approaches include loss of heterozygosity (LOH), X chromosome inactivation, microsatellite instability (MSI), and mutational analysis of *PTEN* and *CTNNB1*.[11–15] However, distinguishing primary cancer from metastatic cancer is still challenging because additional molecular changes in either the primary or the metastatic tumor can obscure the genetic identity. Genome-wide genotyping, such as single nucleotide polymorphism (SNP) arrays, has improved greatly and has revealed chromosomal copy number alterations (CNAs) throughout the genome in a single assay.[16] Allele-specific copy number information by SNP arrays has unveiled copy number–neutral (CNN) LOH (loss of 1 allele and gain of the opposite allele) in various types of tumors, including endometrial and ovarian carcinomas.[17,18]

In this study, we assessed whether SNP array genotyping is useful for diagnosing synchronous endometrioid adenocarcinomas of the uterine corpus and the ovary. In addition, we validated the SNP array genotyping diagnoses by analyzing the microsatellite status and genetic mutations of several cancer-related genes.

TABLE 1. Clinicopathological features and diagnosis

						Endometrial Cancer							
Case	Age, y	Menopause	Gravidity	Parity	BMI, kg/m^2	Tumor Size, cm	Grade	MI	EMH	LVI	Cx	ER	PgR
1	56	54	0	0	14.3	8 × 4	2	>2/3	−	+	+	−	−
2	47	—	1	1	21.7	2 × 1, 4 × 2	1	>1/3, <2/3	−	+	+	−	−
3	52	50	0	0	19.6	3 × 1	1	<1/3	−	−	−	+	2+
4	32	—	0	0	22.9	7 × 5	1	>2/3	−	−	+	−	+
5	51	50	1	1	19.9	4 × 4	3	>1/3, <2/3	−	+	−	2+	3+

BMI, body mass index; MI, myometrial invasion; EMH, endometrial hyperplasia; Cx, cervical involvement.

positive microsatellite markers were detected and diagnosed as microsatellite stable in the other 4 patients.

Among the 5 synchronous endometrial carcinomas, we detected *PIK3CA* mutations in 4 (80%), *PTEN* mutations in 1 (20%), and *CTNNB1* (β-catenin) mutations in 1 patient (20%). No *K-Ras* mutations were detected in these samples. Both endometrial and ovarian carcinomas in case 1 possessed coexistent mutations in *PIK3CA* and *PTEN*; however, no mutation sites were overlapped between the 2 tumors (Table 2). In contrast, the other 4 synchronous tumors harbored identical mutations in the endometrial and ovarian carcinomas (Table 2). We confirmed that all these mutations were somatic by analyzing the corresponding normal DNA (Supplemental Digital Content, Figure 1 and legend, http://links.lww.com/IGC/A87, http://links.lww.com/IGC/A88).

Discrepancy Between Pathological and Genetic Diagnoses

The histopathological diagnosis and all the genetic diagnostic data are summarized in Table 3. Single nucleotide polymorphism array genotyping and genetic mutations were identical in 3 (cases 2, 4, and 5) of the 5 cases, indicating that these synchronous tumors were SPM. In case 3, the 2 point mutations in the *PIK3CA* gene and alterations of microsatellite markers were the same in the endometrial and the ovarian carcinomas. These data suggest that the case was more likely to be SPM and that the CNN LOH detected only in the ovarian carcinoma might have occurred after metastasis to the ovary. Case 1 was genetically diagnosed as DP.

DISCUSSION

In this study, we applied a genome-wide genotyping approach by SNP typing arrays to diagnose whether 2 tumors in the endometrium and ovary in the same patient represent DP or SPM. Among gynecologic synchronous tumors, endometrioid adenocarcinomas of the endometrium and ovary are clinically important for the following reasons: (1) this combination is the most frequent among gynecologic synchronous malignancies, (2) histopathological diagnosis is very difficult owing to the morphologic similarities between the tumors, and (3) the prognosis is much better in DP (with early stage of each tumor) than in SPM (with more advanced stage of the single primary tumor).

Genome-wide genotyping technologies have now become feasible for practical use in cancer therapy and diagnosis.[24] Microarray gene expression profiling (GeneChip; Affymetrix) is used for genome-wide analyses, but the results can be significantly affected by normal cell contamination. In contrast, purity of tumor epithelium of 50% is sufficient for copy number evaluation in SNP arrays.[20] In this study, we demonstrated that allele-specific SNP array genotyping is a useful diagnostic methodology in synchronous endometrial and ovarian carcinomas. The information about CNAs that can be obtained throughout the genome includes (1) the type of alterations (gain, loss, or CNN LOH), (2) the locus and the length (minimal regions) of each alteration, and (3) the degree of each CNA. Thus, the diagnosis would be more definitive with a larger number of CNAs, as seen in cases 2 and 5 in this study. Concordant "karyograms" in the SPM cases suggest that CNAs might occur mainly before metastasis and that subsequent alterations after the metastasis might not be frequent in SPM. However, the limitation of diagnosis by SNP arrays should be considered because secondary changes cannot be excluded, as observed in case 3. Using SNP typing arrays, we previously reported that CIN (CIN-extensive, CNAs with 5 or more loci) is an independent poor prognostic factor in endometrial cancer.[18] Taken together with the present results, this suggests that SNP array genotyping might be useful to predict poor prognostic patients with synchronous endometrial and ovarian carcinomas via the evaluation of clonality and CIN.

Assessment of the status of genetic mutations and microsatellite markers was helpful for the validation of the diagnosis by SNP arrays. We selected 4 genes (*K-Ras*, *PTEN*, *PIK3CA*, and *CTNNB1*) in this study, which are commonly mutated in endometrial carcinomas.[23,25] Among the 5 sets of synchronous endometrial and ovarian carcinomas, discordant mutational patterns were only detected in case 1, which was diagnosed as DP by the SNP arrays. The mutational data should also be applied with careful consideration because identical "hot spot" mutations in these genes might occur independently in each DP tumor.

TABLE 3. Pathological diagnosis and genetic diagnosis

Case	Pathological Diagnosis	Chromosomal Instability	Genetic Mutations (Mutated Genes)	MSI (Ut/Ov)	Genetic Diagnosis	Tumor Stage
1	SPM	Different; Ut; intermediate (4)/Ov; negative	Different (different mutations both in *PTEN* and *PIK3CA*)	Low/low	DP	EM, IIb; OV, Ic
2	DP	Identical; extensive (5)	Identical (*CTNNB1*)	Low/low	SPM	EM, IIIa
3	DP	Different; Ut; negative/Ov; intermediate (1)	Identical (*PIK3CA*)	High/high	SPM	EM, IIIa
4	DP	Identical; intermediate (1)	Identical (*PIK3CA*, *CTNNB1*)	Low/low	SPM	EM, IIIc
5	SPM	Identical; extensive (>20)	Identical (*PIK3CA*)	Low/low	SPM	EM, IIIa

Values in parentheses indicate number of loci of copy number imbalances.
Ut, endometrial cancer; Ov, ovarian cancer.

MATERIALS AND METHODS

Tumor Samples and Genomic DNA

In total, 10 surgical specimens were obtained from 5 patients with synchronous endometrial or ovarian endometrioid adenocarcinomas who underwent resection of their tumors at the University of Tokyo Hospital. All the patients provided informed consent for the collection and use of their samples for research, and the use of tissues for this study was approved by the appropriate institutional ethics committees. The clinicopathological features of the 5 cases are detailed in Table 1. Treatment protocols for endometrial carcinomas were described previously.[18] All of the patients received primary surgery, followed by 6 cycles of platinum-based chemotherapy. The fresh frozen tumors were embedded in optimal cutting temperature compound and were cut into 4-μm tissue sections, which were stained with hematoxylin and eosin. Sections with high content and purity of tumor epithelium were used for DNA extraction. Genomic DNA was isolated from the tumor sections or lymphocyte pellets using a QIAamp DNA Easy Kit (Qiagen, Valencia, CA) according to the manufacturer's specifications.

Immunohistochemistry

Immunohistochemistry for estrogen receptor (ER) and progesterone receptor (PgR) was performed, and the intensity of staining in tumor cells was scored independently (0–3) by 2 investigators as described previously (Y.T. and D.M.).[19]

SNP Array and Genome Imbalance Map

Single nucleotide polymorphism array was performed in the 10 synchronous carcinomas from the 5 patients with paired control DNA. Experimental procedures for GeneChip were performed according to the GeneChip Expression Analysis Technical Manual (Affymetrix, Santa Clara, CA), using a Human Mapping 250K Nsp Array (Affymetrix). The Genome Imbalance Map algorithm was applied to the raw data of endometrial (or ovarian) cancer and peripheral blood obtained from SNP arrays as described previously.[18,20] The purity of tumor epithelium at 50% was previously confirmed to be sufficient for copy number evaluation in SNP arrays.[20] One ovarian cancer specimen (case 5) contained more normal DNA contamination than the others did, and cut-off ratios of greater than 1.15 for gain and less than 0.85 for loss were used in each region. We classified tumors with 5 or more loci of copy alterations as chromosomal instability (CIN)–extensive, those with 1 to 4 loci as CIN-intermediate, and those without any copy alterations as CIN-negative.

Polymerase Chain Reaction and Sequencing

Mutational analysis of *PIK3CA*, *PTEN*, *K-Ras*, and *CTNNB1* was performed. Mutations for *PTEN* (exons 1–8), *K-Ras* (exons 1 and 2), and *PIK3CA* (exons 9 and 20) were analyzed as previously described.[21–23] The primer sequences of exon 3 for the *CTNNB1* gene were as follows: forward, 5′-ATTTGATGGAGTTGGACATGGC-3′ and reverse, 5′-CCAGCTACTTGTTCTTGAG-3′. The polymerase chain reaction products were sequenced using the BigDye

TABLE 2. Genetic mutation status in *PIK3CA, PTEN, K-Ras,* and *CTNNB1* and loci of chromosomal CNAs in dual site carcinomas of the uterus and ovary

Case		Genetic Mutations			
		PIK3CA	*PTEN*	*K-Ras*	*CTNNB1*
1	EM	**E365K**	**D24Y, R142W**	WT	WT
	OV	**E81K**	**R15K, F81C**	WT	WT
2	EM	WT	WT	WT	**S36C**
	OV	WT	WT	WT	**S36C**
3	EM	**N345D, Y1021C**	WT	WT	WT
	OV	**N345D, Y1021C**	WT	WT	WT
4	EM	**H1047R**	WT	WT	**S32C**
	OV	**H1047R**	WT	WT	**S32C**
5	EM	**M10431**	WT	WT	WT
		M10431	WT	WT	WT
	OV	**M10431**	WT	WT	WT

EM, endometrial cancer; OV, ovarian cancer; WT, wild type. Bold values signify the existence of genetic mutations in the tumors.

In conclusion, our data revealed that each copy number imbalance was well preserved between the endometrial and the paired ovarian tumors in SPM, suggesting that genome-wide SNP typing arrays might be a useful method to diagnose synchronous endometrial and ovarian carcinomas comprehensively.

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positive microsatellite markers were detected and diagnosed as microsatellite stable in the other 4 patients.

Among the 5 synchronous endometrial carcinomas, we detected *PIK3CA* mutations in 4 (80%), *PTEN* mutations in 1 (20%), and *CTNNB1* (β-catenin) mutations in 1 patient (20%). No *K-Ras* mutations were detected in these samples. Both endometrial and ovarian carcinomas in case 1 possessed coexistent mutations in *PIK3CA* and *PTEN*; however, no mutation sites were overlapped between the 2 tumors (Table 2). In contrast, the other 4 synchronous tumors harbored identical mutations in the endometrial and ovarian carcinomas (Table 2). We confirmed that all these mutations were somatic by analyzing the corresponding normal DNA (Supplemental Digital Content, Figure 1 and legend, http://links.lww.com/IGC/A87, http://links.lww.com/IGC/A88).

Discrepancy Between Pathological and Genetic Diagnoses

The histopathological diagnosis and all the genetic diagnostic data are summarized in Table 3. Single nucleotide polymorphism array genotyping and genetic mutations were identical in 3 (cases 2, 4, and 5) of the 5 cases, indicating that these synchronous tumors were SPM. In case 3, the 2 point mutations in the *PIK3CA* gene and alterations of microsatellite markers were the same in the endometrial and the ovarian carcinomas. These data suggest that the case was more likely to be SPM and that the CNN LOH detected only in the ovarian carcinoma might have occurred after metastasis to the ovary. Case 1 was genetically diagnosed as DP.

DISCUSSION

In this study, we applied a genome-wide genotyping approach by SNP typing arrays to diagnose whether 2 tumors in the endometrium and ovary in the same patient represent DP or SPM. Among gynecologic synchronous tumors, endometrioid adenocarcinomas of the endometrium and ovary are clinically important for the following reasons: (1) this combination is the most frequent among gynecologic synchronous malignancies, (2) histopathological diagnosis is very difficult owing to the morphologic similarities between the tumors, and (3) the prognosis is much better in DP (with early stage of each tumor) than in SPM (with more advanced stage of the single primary tumor).

Genome-wide genotyping technologies have now become feasible for practical use in cancer therapy and diagnosis.[24] Microarray gene expression profiling (GeneChip; Affymetrix) is used for genome-wide analyses, but the results can be significantly affected by normal cell contamination. In contrast, purity of tumor epithelium of 50% is sufficient for copy number evaluation in SNP arrays.[20] In this study, we demonstrated that allele-specific SNP array genotyping is a useful diagnostic methodology in synchronous endometrial and ovarian carcinomas. The information about CNAs that can be obtained throughout the genome includes (1) the type of alterations (gain, loss, or CNN LOH), (2) the locus and the length (minimal regions) of each alteration, and (3) the degree of each CNA. Thus, the diagnosis would be more definitive with a larger number of CNAs, as seen in cases 2 and 5 in this study. Concordant "karyograms" in the SPM cases suggest that CNAs might occur mainly before metastasis and that subsequent alterations after the metastasis might not be frequent in SPM. However, the limitation of diagnosis by SNP arrays should be considered because secondary changes cannot be excluded, as observed in case 3. Using SNP typing arrays, we previously reported that CIN (CIN-extensive, CNAs with 5 or more loci) is an independent poor prognostic factor in endometrial cancer.[18] Taken together with the present results, this suggests that SNP array genotyping might be useful to predict poor prognostic patients with synchronous endometrial and ovarian carcinomas via the evaluation of clonality and CIN.

Assessment of the status of genetic mutations and microsatellite markers was helpful for the validation of the diagnosis by SNP arrays. We selected 4 genes (*K-Ras*, *PTEN*, *PIK3CA*, and *CTNNB1*) in this study, which are commonly mutated in endometrial carcinomas.[23,25] Among the 5 sets of synchronous endometrial and ovarian carcinomas, discordant mutational patterns were only detected in case 1, which was diagnosed as DP by the SNP arrays. The mutational data should also be applied with careful consideration because identical "hot spot" mutations in these genes might occur independently in each DP tumor.

TABLE 3. Pathological diagnosis and genetic diagnosis

Case	Pathological Diagnosis	Chromosomal Instability	Genetic Mutations (Mutated Genes)	MSI (Ut/Ov)	Genetic Diagnosis	Tumor Stage
1	SPM	Different; Ut; intermediate (4)/Ov; negative	Different (different mutations both in *PTEN* and *PIK3CA*)	Low/low	DP	EM, IIb; OV, Ic
2	DP	Identical; extensive (5)	Identical (*CTNNB1*)	Low/low	SPM	EM, IIIa
3	DP	Different; Ut; negative/Ov; intermediate (1)	Identical (*PIK3CA*)	High/high	SPM	EM, IIIa
4	DP	Identical; intermediate (1)	Identical (*PIK3CA*, *CTNNB1*)	Low/low	SPM	EM, IIIc
5	SPM	Identical; extensive (>20)	Identical (*PIK3CA*)	Low/low	SPM	EM, IIIa

Values in parentheses indicate number of loci of copy number imbalances.
Ut, endometrial cancer; Ov, ovarian cancer.

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Regulation of SIRT1 determines initial step of endometrial receptivity by controlling E-cadherin expression

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ABSTRACT

Sirtuin 1 (SIRT1), originally found as a class III histone deacetylase, is a principal modulator of pathways downstream of calorie restriction, and the activation of SIRT1 ameliorates glucose homeostasis and insulin sensitivity. We examined the role of SIRT1 in the regulation of uterine receptivity using Ishikawa and RL95-2 endometrial carcinoma cell lines. Exogenous expression of SIRT1 significantly enhanced E-cadherin expression, while small interfering RNA-mediated depletion of endogenous SIRT1 resulted in a significant reduction of E-cadherin expression. A SIRT1 activator resveratrol elevated E-cadherin expression in a dose dependent manner, while SIRT1 repressors nicotinamide and sirtinol exhibited a dose dependent reduction of E-cadherin expression. We also showed that both forced expression of SIRT1 and activation of SIRT1 promote E-cadherin-driven reporter gene constructs, and SIRT1 is localized at E-cadherin promoter containing E-box elements in Ishikawa cells. Using an *in vitro* model of embryo implantation, we demonstrate that exogenous expression of SIRT1 and stimulation of SIRT1 activity resulted in the Ishikawa cell line becoming receptive to JAR cell spheroid attachment. Furthermore, resveratrol enhanced E-cadherin and Glycodelin protein expression at sites of intercellular contact, suggesting an additive role of resveratrol in promoting implantation. The initial step of human reproduction depends on the capacity of an embryo to attach and implant into the endometrial wall, and these results revealed the novel mechanism that activation and increased expression of SIRT1 play an important role in uterine receptivity.

1. Introduction

After the blastocyst attaches onto the endometrial glandular epithelium, broad adhesion, transient disruption of the uterine epithelium by degradation of extracellular matrix, and penetration into the uterine stroma occurs. The implantation window is hormonally regulated and is characterized by changes in the molecules expressed by uterine epithelial cells [1]. Although numerous cell surface components including adhesion molecules, cytokines, growth factors, and lipids are postulated to be involved in implantation, only a few genes are essential to this process [2,3]. The study of implantation is both technically and ethically difficult to investigate *in vivo*. *In vitro* study using primary tissues possesses many limitations due to the individual variations. Currently endometrial epithelial carcinoma cell lines such as Ishikawa [4] and RL95-2 [5] cells, and trophoblast cell lines have been used to mimic the receptive state of the uterine epithelium in *in vitro* implantation assays, and the *in vitro* implantation assay is regarded as a useful model for studying mechanisms of human implantation [4–6].

E-cadherin would be involved in the initial attachment of embryos because E-cadherin is found on luminal epithelium and on trophectoderm [7]. E-cadherin in uterine endometrium was known to be hormonally regulated because E-cadherin expression is significantly enriched at the apical membranes of mouse uterine epithelial cells during the preimplantation stage [8]. Embryos lacking functional E-cadherin by targeted disruption exhibit defective preimplantation development and failure to implant [9]. E-cadherin is known to maintain organized architecture and plays a pivotal role in the regulation of epithelial cell proliferation, differentiation, and survival [10]. In addition, genetic or epigenetic alterations of E-cadherin expression have been often associated with various cancers and a class III histone deactylase (HDAC), SIRT1, is linked to the E-cadherin expression [11,12].

Abbreviations: AICAR, 5-aminoimidazole-4-carboxyamide ribonucleoside; AMPK, AMP-activated kinase; HDAC, histone deacetylase; NAM, nicotinamide.

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SIRT1, the mammalian homologue of yeast Sir2 (silent information regulator 2), deacetylates multiple targets in mammalian cells [13]. By regulating various molecules, SIRT1 functions as a master regulator of energy homeostasis, transcriptional regulation, heterochromatin formation, genomic stability, p53 function, and cell survival [14]. SIRT1 is associated with the oncogenic functions because SIRT1 promotes cell survival by inhibiting acetylated-p53 dependent apoptosis [15,16]. SIRT1 has been shown to be involved in the maintenance of gene silencing by associating with CpG island of promoter regions in tumor suppressor genes [11]. However, SIRT1 also possesses anti-oncogenic function because SIRT1 inhibits Survivin expression by changing the epigenetic modification of histone H3, and a phytochemical compound resveratrol (trans-3,5,40-trihydroxystilbene) mimic the inhibitory effects of SIRT1, thus serves as an anti-carcinogenic compound [17]. Polyphenols have been known to activate SIRT1 either directly or indirectly, and the deacetylating activity of SIRT1 can be inhibited by nicotinamide (NAM) [13]. Resveratrol is an indirect activator of SIRT1 and has been shown to activate the expression of nicotinamide phosphoribosyltransferase and AMP-activated kinase (AMPK) [18–20]. In addition, SIRT1 and AMPK mutually affect the functions of each other [18,21]. So far, it is not known whether these chemicals are able to modulate the expression of E-cadherin.

To better understand the functional significance and the transcriptional regulation by SIRT1, we studied the effect of the transcriptional regulation of E-cadherin driven by SIRT1. We demonstrate that E-cadherin expression is regulated by SIRT1 in endometrial carcinoma cells. Either activation of SIRT1 or increased expression of SIRT1 plays a key role in the development of human uterine receptivity via inducing E-cadherin expression. These findings establish a principal biological function of SIRT1 in the modulation of E-cadherin function, and further identify SIRT1 as a possible determinant and potential therapeutic target in implantation failure.

2. Materials and methods

2.1. Cell culture and chemicals

Ishikawa human endometrial adenocarcinoma cell line was kindly provided by Dr. M. Nishida (Tsukuba University, Ibaraki, Japan). RL95-2 cells (CRL-1671, human endometrial adenocarcinoma), 293T cells (CRL-11268, human embryonic kidney cells), and JAR cells (HTB-144, human choriocarcinoma cells) were purchased from the American Type Culture Collection (Manassas, VA, USA). Resveratrol, NAM, and sirtinol were from Sigma–Aldrich (St. Louis, MO, USA). AICAR (5-Aminoimidazole-4-carboxyamide ribonucleoside) was purchased from Cell Signaling Technology (Danvers, MA, USA).

2.2. Western blot

To determine the effect of SIRT1 functions, cells were treated with indicated concentrations of resveratrol, sirtinol, NAM, or AICAR. Western blot analysis and immunostaining were performed as described previously [22].

2.3. RNAi

The ablation of SIRT1 and DBC1 was performed by transfection of the Ishikawa cells and RL95-2 cells with small interfering RNA (siRNA) duplex oligos synthesized by Invitrogen (Carlsbad, CA, USA) and Qiagen (Hilden, Germany). Control siRNA (AllStars Negative Control siRNA, 1027281) and DBC1-specific siRNA [DBC1-RNAi: 5' AAACGGAGCCUACUGAACA 3', which covered mRNA regions of nucleotides 1379–1397 (amino acids 460–466) of DBC1, and KIAA1967-RNAi, SI00461853] were transfected by using HyperFect reagent (Qiagen). Stealth RNAi Duplex (Invitrogen) specific for SIRT1 (Oligo ID: HSS118729, HSS177403 and HSS117404) was transfected by using Lipofectamine RNAi MAX (Invitrogen).

2.4. Luciferase assay

Transfection was performed with Effectene reagent (Qiagen) according to the manufacturer's recommendation. For luciferase assay, indicated expression vectors were cotransfected with E-cad(−108)–Luc or E-cad(−108)Mut–Luc [23]. As an internal control to equalize transfection efficiency, phRL CMV-*Renilla* vector (Promega Corp., Madison, WI, USA) was also transfected in all the experiments. Individual transfections, each consisting of triplicate wells, were repeated at least three times [22].

2.5. In vitro implantation assay

To generate spheroids of JAR cells for use as blastocyst models, the JAR cells were grown in suspension in petri dishes at a density of 2×10^5 cells/ml, Petri dishes were placed on a slow shaker in 37 °C, 5% CO_2 humidified incubator overnight. During this incubation, JAR cells form spheroids of 50–200 μm in diameter through natural aggregation. Ishikawa cells were seeded in quintuplicate in 24-well plates and incubated until they reached subconfluent monolayers. Thereafter, Ishikawa cells were treated with indicated chemical compounds. On the day of the assay, co-culture of Ishikawa cells and JAR spheroids (approximately 100 spheroids/well) for 24 h was performed. After incubation, the monolayers were gently washed twice with PBS to remove unattached spheroids. Cell monolayers were then examined under light microscope for spheroids numbers.

2.6. Fluorescence microscopy

Ishikawa cells were grown on 12 mm BD BioCoat (BD Biosciences, Franklin Lakes, NJ, USA) glass coverslips in 6-well plates. Spheroids of JAR cells seeded on the Ishikawa monolayer cells were fixed with PBS containing 4% paraformaldehyde. After blocking, Ishikawa cell and spheroids were incubated sequentially with anti-E-cadherin (610181, BD Biosciences) and anti-Glycodelin (EP870Y, Novus Biologicals, CO, USA) antibodies. Secondary antibodies were Alexa fluor 488 conjugated donkey anti-mouse IgG (A-21428), and Alexa fluor 555 conjugated goat anti-rabbit IgG (A-21202, Invitrogen). The slides were briefly counter-stained and analyzed under the confocal fluorescence microscope (Carl-Zeiss Micro Imaging Inc., Oberkochen, Germany).

2.7. Chromatin immunoprecipitation assay

Preparation of soluble Ishikawa chromatin for PCR amplification was performed essentially as described [24]. Primers to amplify E-cadherin promoter region containing three E-box domains are as follows; forward, 5'-GTGAACCCTCAGCCAATCAG-3'; reverse, 5'-TCACAGGTGCTTTGCAGTTC-3'.

3. Results

3.1. E-cadherin expression is regulated by SIRT1 deacetylase

To determine the effect on E-cadherin expression, nonpolar Ishikawa and RL95-2 cells were transfected with siRNA oligos. In contrast to the previous study using breast cancer cell line MDA-MB-231 [11] and prostate cancer cell line PC3 and DU145 [12],

RNAi-mediated knockdown of SIRT1 expression resulted in a decreased expression of E-cadherin in endometrial cancer cell lines (Fig. 1A, C). Thus our data demonstrate that SIRT1 has a critical role in regulating the expression level of E-cadherin in endometrial cancer cells. The forced expression of SIRT1 revealed that the increased expression of SIRT1 resulted in an increased expression of E-cadherin (Fig. 1B, D), confirming that E-cadherin expression was paralleled by the SIRT1 expression. DBC1 is a negative regulator of SIRT1 deacetylase function [15,16], and we investigated the possibility that the regulation of E-cadherin expression by SIRT1 is affected by DBC1. However, DBC1 expression remained unchanged by siRNA-mediated knockdown of SIRT1. The result that SIRT1 is able to stimulate E-cadherin expression led us to examine the role of SIRT1 in the activation of E-cadherin promoter. Transient transfection assays were performed using an E-box-wild type luciferase [E-cad(−108)-Luc] or E-box-mutated [E-cad(−108)Mut-Luc] reporter plasmid, carrying minimum promoter region (−108 to + 125 bp) for the E-cadherin expression. The E-box elements (consensus sequence 5'-CANNTG-3') were originally shown to be binding regions of zinc-finger transcription factors such as SLUG and SNAIL that repressed E-cadherin-driven reporter gene constructs and three E-box sites within the promoter have been demonstrated to drive the expression of E-cadherin [23]. Although SIRT1 efficiently elevated the promoter activity of the reporter plasmid in 293T cells in a dose dependent manner, the transactivation function of SIRT1 was not observed by the expression of E-cad(−108)Mut-Luc in luciferase assays (Fig. 1E). To test whether SIRT1 is indeed recruited to E-cadherin promoter, we performed a chromatin immunoprecipitation assay using the E-cadherin gene promoter containing three E-box elements. As expected, clear recruitment of endogenous SIRT1 to the target sequence in the E-cadherin promoter was observed (Fig. 1F). Thus our results suggest that SIRT1 plays a significant role in the E-cadherin expression, and E-box domains were shown to be crucial for the SIRT1-mediated expression of E-cadherin.

3.2. Stimulation of SIRT1 function increases the expression level of SIRT1 and E-cadherin

We next hypothesized that expression of E-cadherin would be regulated by small molecules that govern SIRT1 function. In concordance with the previous report, SIRT1 expression was also stimulated by resveratrol (Fig. 2A, B). Contrary to this, Ishikawa cells exposed to sirtinol (Fig. 2C) and NAM (Fig. 2D) exhibited decreased expression of E-cadherin in a dose-dependent manner. AICAR is an AMPK activator and AMPK enhances SIRT1 activity by increasing cellular NAD^+ levels [21]. We also showed that AICAR stimulated E-cadherin and SIRT1 expression in Ishikawa and RL95-2 cells, but the extent of increase by AICAR was relatively modest compared to that by resveratrol (Fig. 2E). We further examined the ligand-induced transactivation function of E-cadherin promoter, and the resveratrol-induced transactivation was dose-dependent with a roughly estimated concentration value required for one-half maximal activation (EC_{50}) of about 10 μM (Fig. 2F).

3.3. SIRT1 expression and activity affect the spheroid attachment to the Ishikawa cell monolayer

We next examined whether the expression of SIRT1 and its activity affected the implantation capacity of Ishikawa cells using an *in vitro* model of embryo attachment. Forced expression of SIRT1 in Ishikawa cells exhibited a 1.3-fold increased number

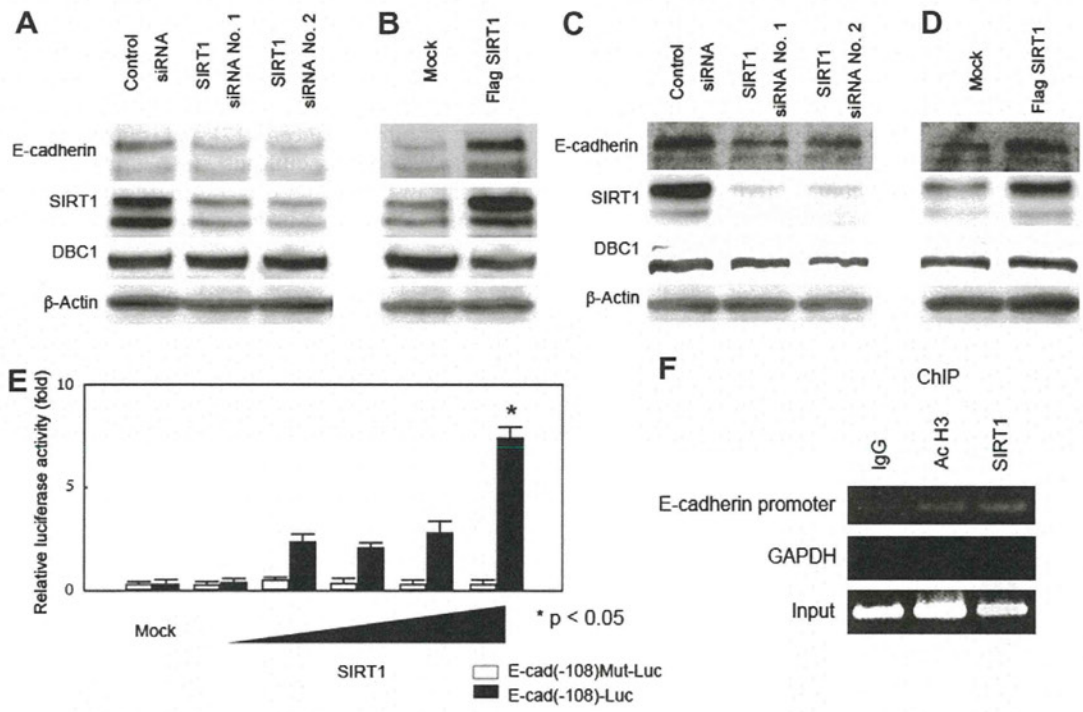

Fig. 1. E-cadherin promoter dependent regulation of E-cadherin expression by SIRT1 deacetylase. Ishikawa (A, B) and RL95-2 (C and D) cells were transfected with expression vector of SIRT1 or indicated siRNA and analyzed by Western blotting. The immunoblotting analysis using anti-SIRT1 antibodies revealed that the expression of SIRT1 paralleled the expression of E-cadherin in cell lysates. (E) 293T cells were transfected with the indicated amounts of SIRT1 expression plasmids (pcDNA Flag SIRT1), and transfected whole cell lysates were assayed for luciferase activity produced from the reporter plasmid [E-cad(−108)-Luc or E-cad(−108) Mut-Luc]. SIRT1 showed a dose-dependent stimulation of the transactivation function of E-cadherin promoter, while E-cadherin promoter possessing mutations within three E-box domains failed to show activation function of SIRT1. (F) Chromatin immunoprecipitation assay was performed to confirm the recruitment of SIRT1 at E-cadherin gene promoter, a region containing three E-box domains. AcH3 denotes acetylated histone H3.

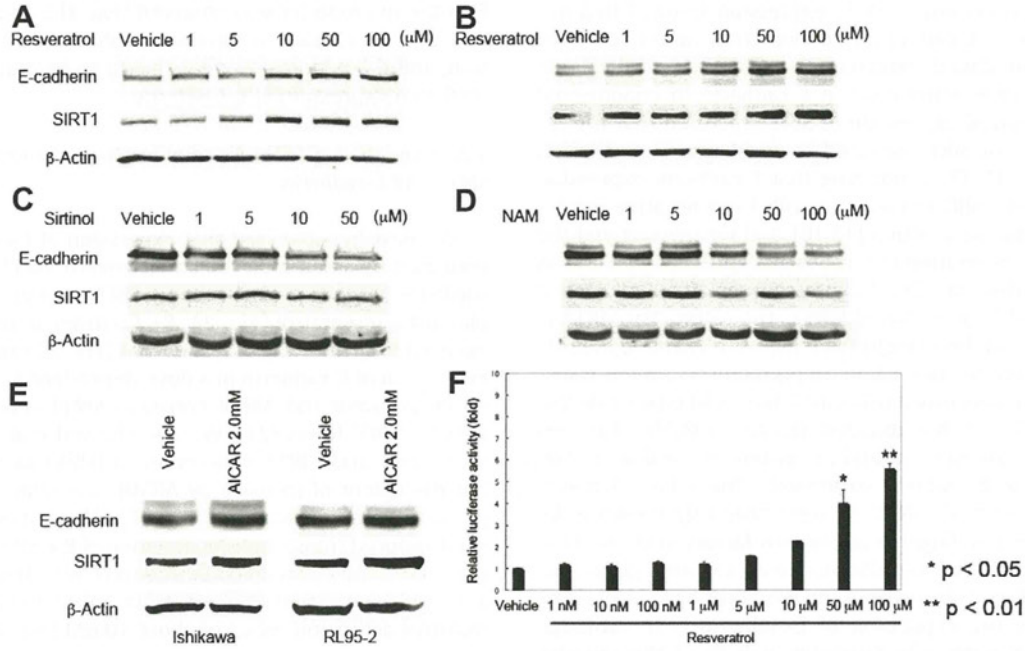

Fig. 2. Activation of SIRT1 using chemical compounds resulted in an increased expression of E-cadherin and SIRT1. Ishikawa (A, C, D, and E left panel) and RL95-2 (B, E right panel) cells were treated with various doses of vehicle, SIRT1 activators (resveratrol and AICAR), and SIRT1 repressors (sirtinol and NAM). Twenty-four hours after treatment, cells were harvested and protein expression of E-cadherin and SIRT1 was analyzed by Western blot. The immunoblotting analysis revealed that SIRT1 activators increased the expression of E-cadherin and SIRT1 in cell lysates, while SIRT1 repressors decreased the expression of E-cadherin and SIRT1 in cell lysates. (F) 293T cells were transfected with E-cad(−108)–Luc plasmids, and treated with various doses of resveratrol. Resveratrol showed a dose-dependent transactivation function of E-cadherin promoter.

of spheroids attached to the cell monolayers, while siRNA-mediated depletion of endogenous SIRT1 resulted in a 4-fold decreased uterine receptivity (Fig. 3B). Then we tested whether activation or inactivation of SIRT1 may modulate the uterine receptivity. Ishikawa cells treated with resveratrol exhibited a 3.5-fold greater number of spheroids attached to cell monolayers compared with cells treated with vehicle alone, while repression of SIRT1 by sirtinol resulted in a 2-fold decrease in spheroid attachment to the Ishikawa cell monolayer (Fig. 3D). Thus, the expression of SIRT1 and its activity influenced on the initial attachment of embryos through the mechanism involved in enhanced human uterine receptivity.

3.4. Localization of E-cadherin and Glycodelin to sites of intercellular contact is essential for initial attachment

To further pursue the role of resveratrol, other implantation molecules such as Glycodelin and Survivin were examined. Glycodelin is a progesterone-induced glycoprotein secreted into uterine luminal cavity by endometrial glands in secretory phase [4]. Survivin is an anti-apoptotic molecule and is overexpressed in the majority of human cancers [17], and is upregulated in early gestation [25]. We found significantly higher protein levels for Glycodelin in Ishikawa cells after resveratrol treatment but not for Survivin (Fig. 4A, B). We further investigated whether resveratrol possesses

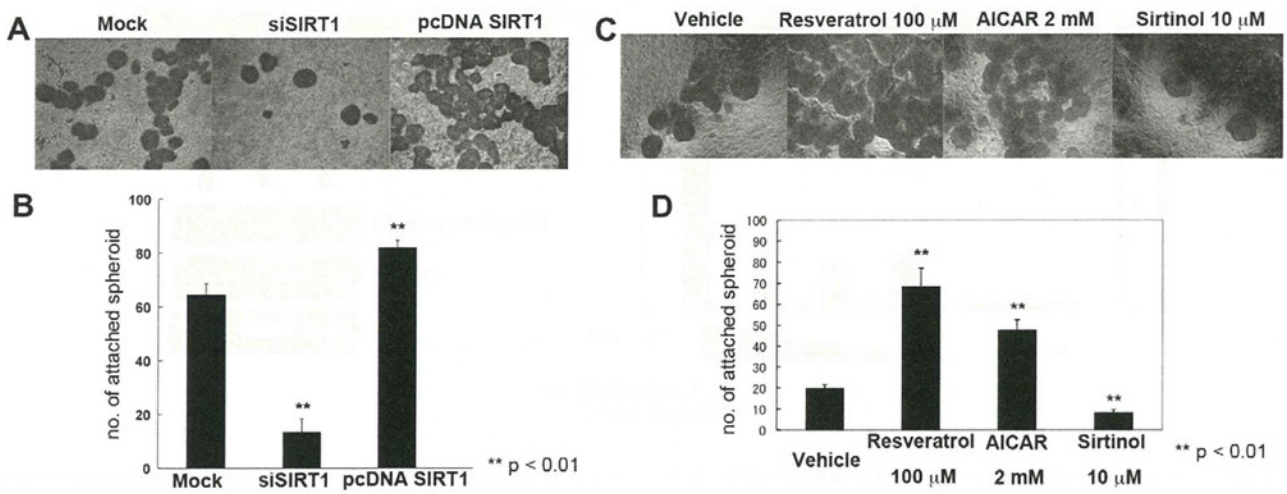

Fig. 3. SIRT1 expression and activity affects uterine receptivity. Spheroid adhesion to Ishikawa cell monolayer was examined by *in vivo* spheroid attachment assay. (A) Representative phase-contrast micrographs of JAR spheroids attached to Ishikawa cell monolayer. (B) Graph showing spheroid adhesion to Ishikawa cell monolayer. Appropriate controls were also analyzed (scramble siRNA and empty pcDNA vector), and no significant difference was observed. (C) Representative phase-contrast micrographs of JAR spheroids attached to Ishikawa cell monolayer. (D) Graph showing spheroid adhesion to Ishikawa cell monolayer after the treatment with chemical compounds.

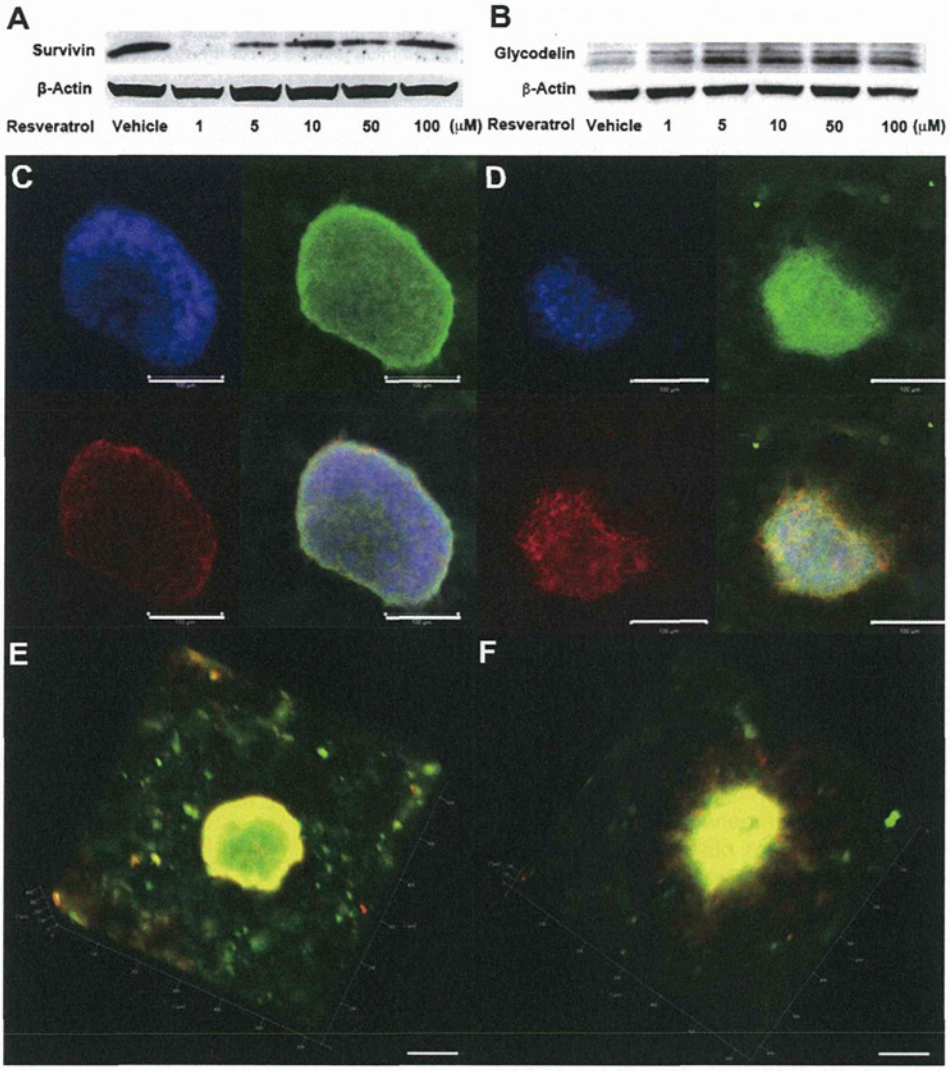

Fig. 4. Expression of implantation-related proteins and colocalization of E-cadherin and Glycodelin in Ishikawa cells. (A, B) Ishikawa cells were treated with various doses of resveratrol and protein expression of Survivin and Glycodelin was analyzed by Western blot. (C–F) In *in vivo* spheroid assay, colocalization of E-cadherin and Glycodelin was examined by immunofluorescence study. (C and D) spheroids attached to the cell monolayer at 1 h (C) and at 24 h (D) are shown. (E, F) Three dimension views of attached spheroids at 1 h (E) and at 24 h (F) are shown. Note that the colocalization signal (yellow; merge) is becoming intense. Bars indicate 100 μm. Results shown are representative photographs.

an additional effect on the uterine receptivity compared with SIRT1 alone. Immunofluoresence study was performed to localize E-cadherin and Glycodelin expression at sites of intercellular contact under the confocal microscopy. Although E-cadherin and Glycodelin were expressed at sites of intercellular contact, colocalization signal was not significant in spheroid cells after 1 h incubation (Fig. 4C). However, both E-cadherin and Glycodelin are intensively expressed and the degree of colocalization signal was significantly elevated in spheroid cells showing dispersing morphological changes after 24 h incubation (Fig. 4D), suggesting that E-cadherin stimulates initial step of spheroid attachment and Glycodelin helps to invade spheroids in endometrial lining. Since both SIRT1 expression and SIRT1 stimulation by chemical compounds increase expression of E-cadherin and Glycodelin, these data confirmed that SIRT1 plays pivotal roles in initial step of implantation.

4. Discussion

The studies of SIRT1 in uterine physiology are limited. A recent study examined the SIRT1 and SIRT2 expression and regulation in human intrauterine tissues [26]. In the present study, we report the possibility that SIRT1 expression and SIRT1 function can regulate human implantation because the stimulation of E-cadherin expression and small molecules that affect SIRT1 activities produced a substantial effect on spheroid attachment ability in an *in vitro* model of endometrial receptivity in nonpolar endometrial cancer cells. Our data was different from the previous observations that SIRT1 is involved in epigenetic silencing of DNA-hypermethylated tumor suppressor genes in breast cancer cells [11], and that SIRT1 serves as a positive regulator of epithelial-mesenchymal transition and metastatic growth of prostate cancer cells [12]. Further investigation should be required to determine the mode of regulation of E-cadherin expression whether this difference could be simply attributed to the difference of cell line.

It is also interesting that resveratrol treatment resulted in an increased expression of SIRT1 protein levels. Our previous study using rat ovarian granulosa cells also demonstrated resveratrol treatment was associated with an increased expression of SIRT1 [14]. The enhancement of SIRT1 expression by resveratrol was considerably potent compared to that by AICAR. This was consistent with the report that the enhancement of SIRT1 expression by AICAR is at best

1.3-fold [27]. Our data provided a novel anti-tumorigenic property of SIRT1 activating chemicals because decreased E-cadherin expression has been identified in a wide variety of malignancies including endometrial cancer [28]. While the underlying mechanism remains elusive, it has been shown that resveratrol has the ability to activate SIRT1 deacetylase activity [29], and resveratrol might have anti-tumorigenic properties. It was reported that resveratrol inhibited the proliferation of a wide variety of human cancer cell lines through the induction of S-phase cell cycle arrest and apoptosis [30] while presenting very low cytotoxicity in animal models. Interestingly, low doses of resveratrol can sensitize cells to low doses of cytotoxic anti-cancer drugs, therefore resveratrol is expected to facilitate the efficacy of anticancer therapy in various human cancers [31].

Suberoylanilide hydroxamic acid (vorinostat), a HDAC inhibitor drug utilized as an anti-cancer drug, has been shown to induce differentiation of endometrial glandular cells and to increase the expression of Glycodelin [4]. Glycodelin was postulated to be secreted from cell that exhibited interactions between spheroids and endometrial lining (Ishikawa cells) because Glycodelin was not found in cultured media from Ishikawa cells [32]. We believe that both Glycodelin protein expression and E-cadherin protein expression synergistically help to improve initial steps of implantation, including attachment, adhesion, and invasion. Considering the teratogenicity of vorinostat in the treatment of infertility, our result is fascinating and may provide a possibility for the practical use of resveratrol because adverse events associated with resveratrol intake were gastrointestinal symptoms alone [33]. The study revealed that repeated administration of high doses of resveratrol resulted in micromolar plasma concentrations. Therefore, our data that elevated E-cadherin expression was observed at a dose of micromolar concentrations of resveratrol can be tolerable and accomplishing dose.

In conclusion, our data indicate that SIRT1 plays an important role in regulating E-cadherin expression. Therefore, SIRT1-activating chemicals including resveratrol and AICAR would be novel therapeutic targets for improvement of initial step of implantation, thereby improvement of assisted reproductive technology success can be expected. However, in view of the difference in the pathophysiology of implantation between cultured cells (*in vitro*) and human (*in vivo*), our data should be interpreted with caution and the present observations should be further verified.

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Retreatment with nedaplatin in patients with recurrent gynecological cancer after the development of hypersensitivity reaction to carboplatin

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Abstract

Aim: Platinum is a milestone drug against gynecologic malignancies. The purpose of this retrospective study was to investigate the feasibility of replacing carboplatin with nedaplatin in patients who had developed a hypersensitivity reaction to carboplatin.

Material and Methods: Fifteen patients with recurrent gynecologic cancer (12 ovarian, 1 fallopian tube, 1 endometrial and 1 cervical cancer) who had experienced a hypersensitivity reaction to carboplatin and a possible clinical indication for continuing treatment with platinum were treated with nedaplatin (80 mg/m^2)-containing regimen.

Results: The total number of nedaplatin cycles given was 137 (range 1–29). Four (27%) patients developed hypersensitivity reactions on the second, second, fourth, and ninth administration, respectively. The severities of all the hypersensitivity reactions were grade 3 or less. The other 11 patients (73%) had no nedaplatin-associated hypersensitivity reactions. The incidence of hypersensitivity reactions in the paclitaxel and nedaplatin group (three of four, 75%) was more frequent than the docetaxel and nedaplatin group (none of seven, $P = 0.024$). The objective response rate in eleven patients with measurable disease was 36% (complete response at 9% and partial response at 27%), and the disease control rate was 73% (stable disease at 36%).

Conclusion: Nedaplatin-associated hypersensitivity reactions are not rare in patients who developed allergic reactions to carboplatin. Retreatment of carboplatin-allergic patients with nedaplatin cannot be recommended without careful consideration of the potential risks and benefits.

Key words: carboplatin, cross-reaction, hypersensitivity reaction, nedaplatin, retreatment.

Introduction

Carboplatin is one of the most effective and well-tolerated chemotherapeutic agents for gynecologic malignancies. In addition to a standard first-line regimen, platinum-containing chemotherapy is repetitively administered to patients with platinum-sensitive recurrence in gynecologic cancer.[1–3] However, the repeated treatment with carboplatin is associated with an increased risk of hypersensitivity reactions (HSR). The incidence of HSR has been reported to be 8–44% in patients receiving retreatment with carboplatin.[4–7] Whereas there are several types of drugs available for treatment of recurrent ovarian cancer, platinum is still regarded as the single most active agent. Safety of rechallenge with cisplatin after the development of HSR to carboplatin is controversial. Although there have been several reports suggesting its safety, two

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deaths due to anaphylaxis following rechallenge with cisplatin have been reported.[8,9] So far, 39 of 46 reported cases (85%) were successfully retreated with cisplatin.[10]

Nedaplatin is one of the platinum analogues with the same carrier ligands of ammine as cisplatin and a five-membered ring structure in which glycolate is bound to the platinum ion as a bidentate ligand. Two phase II studies showed its effectiveness against gynecological cancers. The response rate of the nedaplatin monotherapy against cervical and ovarian cancer was 34–46% and 38%, respectively.[11,12]

Retreatment with nedaplatin in patients with hypersensitivity to carboplatin has not been reported yet. The purpose of this study was to evaluate safety and efficacy of rechallenge with nedaplatin in this population.

Patients and Methods

Fifteen patients (12 ovarian, 1 fallopian tube, 1 endometrial, and 1 cervical cancer) who had experienced a hypersensitivity reaction to carboplatin were treated with nedaplatin between 2004 and 2010 after informed consent regarding the potential risks as well as benefits of treatment. All the patients were platinum-sensitive (progression-free interval more than 6 months) at their primary treatment. All had recurrent disease and had experienced HSRs during receiving carboplatin in retreatment. The patient characteristics are summarized in Table 1. Four patients were administrated with single-agent nedaplatin and the other 11 were treated with combination chemotherapy (four with paclitaxel and seven with docetaxel).

Table 1 Patient characteristics

Median age, years (range)	58 (47–67)
Type of cancer (*n*)	
Ovarian cancer	12
Serous	7
Endometrioid	4
Unclassified adenocarcinoma	1
Fallopian tube cancer	1
Endometrial cancer	1
Cervical cancer	1
Prior chemotherapy (*n*)	
One regimen	3
Two regimens	4
>Three regimens	8
Protocol (*n*)	
Nedaplatin	4
Paclitaxel/nedaplatin	4
Docetaxel/nedaplatin	7

In the single-agent protocol, nedaplatin at a dose of 80 mg/m^2 was infused intravenously in 500 mL of normal saline over 2 h. In the paclitaxel/nedaplatin combination protocol, paclitaxel at a dose of 175 mg/m^2 was infused intravenously in 500 mL of normal saline over 3 h, followed by nedaplatin at a dose of 80 mg/m^2 in 500 mL of normal saline over 2 h. In the docetaxel/nedaplatin combination protocol, docetaxel at a dose of 70 mg/m^2 was infused intravenously in 250 mL of 5% glucose over 60 min, followed by nedaplatin at a dose of 80 mg/m^2 in 500 mL of normal saline over 2 h. In all regimens, the patients received intravenous hydration with 1000 mL of 5% dextrose over 4 h after administration of nedaplatin. No patients were administrated a desensitization protocol.

The severity of allergic reactions and anaphylaxis was graded according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. Tumor response was assessed in those with measurable disease using radiographic and clinical assessment. Response Evaluation Criteria in Solid Tumors (RECIST) were employed for evaluation of measurable disease.[13]

Results

Hypersensitivity reactions

The management of the patients is summarized in Table 2. A total of 137 cycles of nedaplatin were administered. One hundred and thirty-three cycles (97%) were completed without nedaplatin-associated HSRs. All the 15 patients were successfully treated with nedaplatin on the first administration without experiencing any symptoms suggestive of HSRs. Eleven of the 15 patients (73%) had no nedaplatin-associated HSRs during the nedaplatin-containing chemotherapy (HSR-negative group). Ten of the 11 patients continued the treatment until the disease became progressive (range 1–29 courses), and one patient (#14) periodically receives the chemotherapy without progression of disease (total 23 courses). The other four patients stopped the protocol due to HSRs to nedaplatin (HSR-positive group). One patient (#4) experienced HSRs on the ninth cumulative cycle. She received paclitaxel/nedaplatin against peritoneal dissemination for six cycles without HSRs with a partial response. After a 9-month interval, the disease recurred and she was retreated with nedaplatin. On the third cycle, she developed HSRs with rash, edema, nausea, and vomiting (grade 3 of allergic reaction) immediately after

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