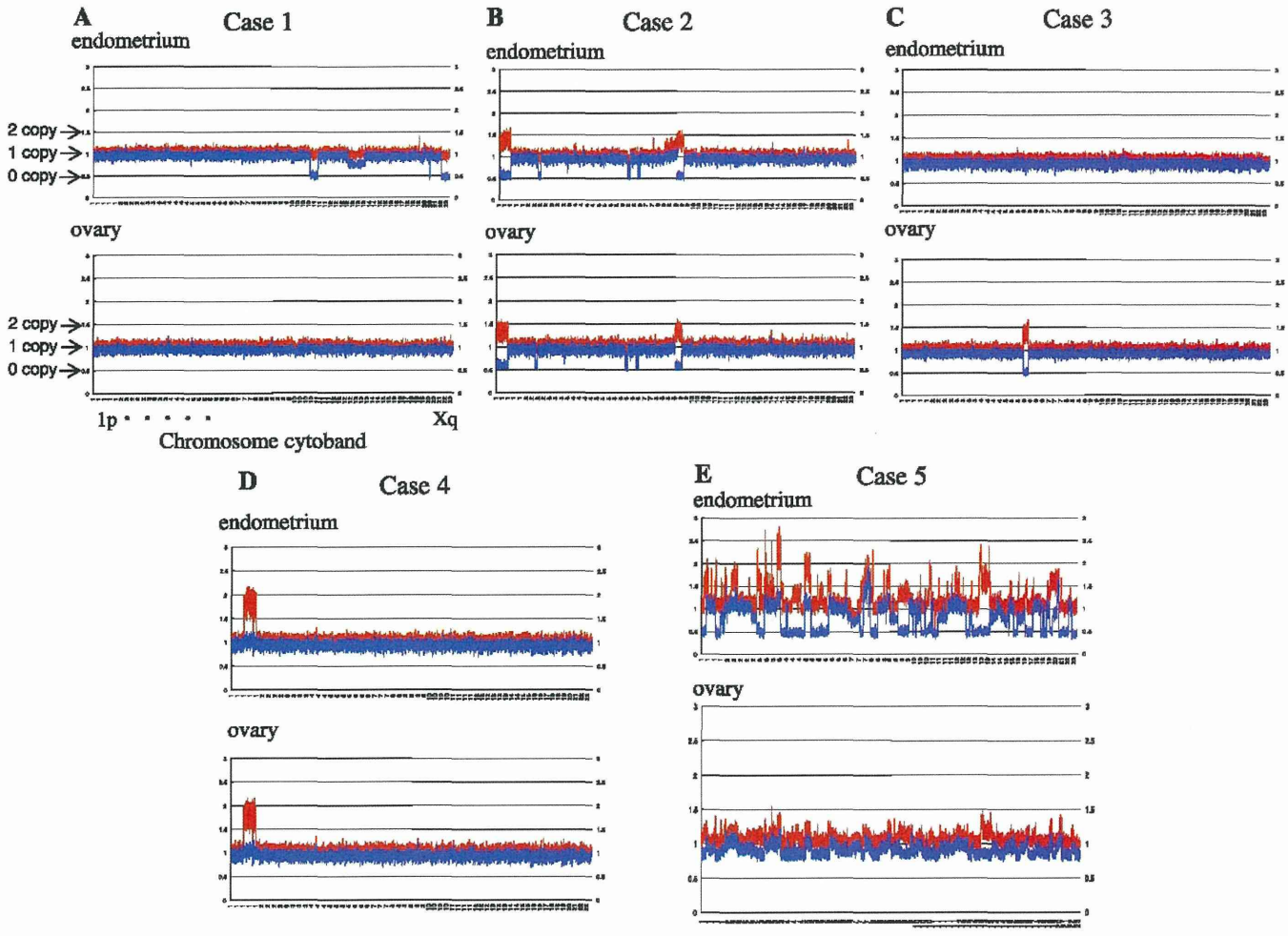

FIGURE 1. Single nucleotide polymorphism array "karyograms" of 10 tumors from 5 synchronous endometrial and ovarian carcinomas. The signal intensity ratio between the raw signal intensity from the cancer and paired normal samples is ordered by position in the genome, beginning at 1p and ending with X. 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	—

Received December 3, 2011, and in revised form January 5, 2012.
Accepted for publication January 23, 2012.

(*Int J Gynecol Cancer* 2012;22: 725–731)

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.

ACKNOWLEDGMENTS

The authors thank Yuriko Uehara, Keiko Shoji, Tomoko Kashiyama, Yuichiro Miyamoto, Michihiro Tanikawa, Hiroko Meguro, Akira Watanabe, Seitaro Nomura, Takahide Arimoto, Akira Tsuchiya, Yoko Matsumoto, Kenbun Sone, Katsuyuki Adachi, Shiho Miura, Ayako Tomio, Kensuke Tomio, Satoko Kojima, and Takayuki Seiki for their support and assistance.

REFERENCES

1. Eisner RF, Nieberg RK, Berek JS. Synchronous primary neoplasms of the female reproductive tract. *Gynecol Oncol.* 1989;33:335 339.
2. Kline RC, Wharton JT, Atkinson EN, et al. Endometrioid carcinoma of the ovary: retrospective review of 145 cases. *Gynecol Oncol.* 1990;39:337 346.
3. Ayhan A, Yalcin OT, Tuncer ZS, et al. Synchronous primary malignancies of the female genital tract. *Eur J Obstet Gynecol Reprod Biol.* 1992;45:63 66.
4. Pearl ML, Johnston CM, Frank TS, et al. Synchronous dual primary ovarian and endometrial carcinomas. *Int J Gynaecol Obstet.* 1993;43:305 312.
5. Piura B, Glezerman M. Synchronous carcinomas of endometrium and ovary. *Gynecol Oncol.* 1989;33:261 264.
6. Zaino RJ. FIGO staging of endometrial adenocarcinoma: a critical review and proposal. *Int J Gynecol Pathol.* 2009;28:1 9.
7. Baak JP, Mutter GL, Robboy S, et al. The molecular genetics and morphometry-based endometrial intraepithelial neoplasia classification system predicts disease progression in endometrial hyperplasia more accurately than the 1994 World Health Organization classification system. *Cancer.* 2005;103:2304 2312.
8. Scully RE, Richardson GS, Barlow JF. The development of malignancy in endometriosis. *Clin Obstet Gynecol.* 1966;9:384 411.
9. Ulbright TM, Roth LM. Metastatic and independent cancers of the endometrium and ovary: a clinicopathologic study of 34 cases. *Hum Pathol.* 1985;16:28 34.
10. Young RH, Scully RE. Metastatic tumors of the ovary. In: Kurman RJ, Ronnett BM, Ellenson LH, eds. *Blaustein's Pathology of the Female Genital Tract.* 5th ed. New York, NY:Springer; 2002;1063 1068.
11. Fujita M, Enomoto T, Wada H, et al. Application of clonal analysis. Differential diagnosis for synchronous primary ovarian and endometrial cancers and metastatic cancer. *Am J Clin Pathol.* 1996;105:350 359.
12. Irving JA, Catasus L, Gallardo A, et al. Synchronous endometrioid carcinomas of the uterine corpus and ovary: alterations in the beta-catenin (*CTNNB1*) pathway are associated with independent primary tumors and favorable prognosis. *Hum Pathol.* 2005;36:605 619.
13. Kaneki E, Oda Y, Ohishi Y, et al. Frequent microsatellite instability in synchronous ovarian and endometrial adenocarcinoma and its usefulness for differential diagnosis. *Hum Pathol.* 2004;35:1484 1493.
14. Ricci R, Komminoth P, Bannwart F, et al. *PTEN* as a molecular marker to distinguish metastatic from primary synchronous endometrioid carcinomas of the ovary and uterus. *Diagn Mol Pathol.* 2003;12:71 78.
15. Shenson DL, Gallion HH, Powell DE, et al. Loss of heterozygosity and genomic instability in synchronous endometrioid tumors of the ovary and endometrium. *Cancer.* 1995;76:650 657.
16. Kennedy GC, Matsuzaki H, Dong S, et al. Large-scale genotyping of complex DNA. *Nat Biotechnol.* 2003;21:1233 1237.
17. Gorringe KL, Jacobs S, Thompson ER, et al. High-resolution single nucleotide polymorphism array analysis of epithelial ovarian cancer reveals numerous microdeletions and amplifications. *Clin Cancer Res.* 2007,13:4731 4739.
18. Murayama-Hosokawa S, Oda K, Nakagawa S, et al. Genome-wide single-nucleotide polymorphism arrays in endometrial carcinomas associate extensive chromosomal instability with poor prognosis and unveil frequent chromosomal imbalances involved in the PI3-kinase pathway. *Oncogene.* 2010;29:1897 1908.
19. Minaguchi T, Nakagawa S, Takazawa Y, et al. Combined phospho-Akt and *PTEN* expressions associated with post-treatment hysterectomy after conservative progestin therapy in complex atypical hyperplasia and stage Ia, G1 adenocarcinoma of the endometrium. *Cancer Lett.* 2007;248:112 122.
20. Ishikawa S, Komura D, Tsuji S, et al. Allelic dosage analysis with genotyping microarrays. *Biochem Biophys Res Commun.* 2005;333:1309 1314.
21. Oda K, Stokoe D, Taketani Y, et al. High frequency of coexistent mutations of *PIK3CA* and *PTEN* genes in endometrial carcinoma. *Cancer Res.* 2005;65:10669 10673.
22. Minaguchi T, Yoshikawa H, Oda K, et al. *PTEN* mutation located only outside exons 5, 6, and 7 is an independent predictor of favorable survival in endometrial carcinomas. *Clin Cancer Res.* 2001;7:2636 2642.
23. Oda K, Okada J, Timmerman L, et al. *PIK3CA* cooperates with other phosphatidylinositol 3′-kinase pathway mutations to effect oncogenic transformation. *Cancer Res.* 2008;68:8127 8136.
24. The Cancer Genome Atlas (TCGA) Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature.* 2008;455: 1061 1068.
25. Shoji K, Oda K, Nakagawa S, et al. The oncogenic mutation in the pleckstrin homology domain of AKT1 in endometrial carcinomas. *Br J Cancer.* 2009;101:145 148.

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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
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Biochemical and Biophysical Research Communications 424 (2012) 604–610

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

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

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ARTICLE INFO

Article history:
Received 25 June 2012
Available online 7 July 2012

Keywords:
SIRT1
E-cadherin
Receptivity
Activity

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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0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.06.160

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

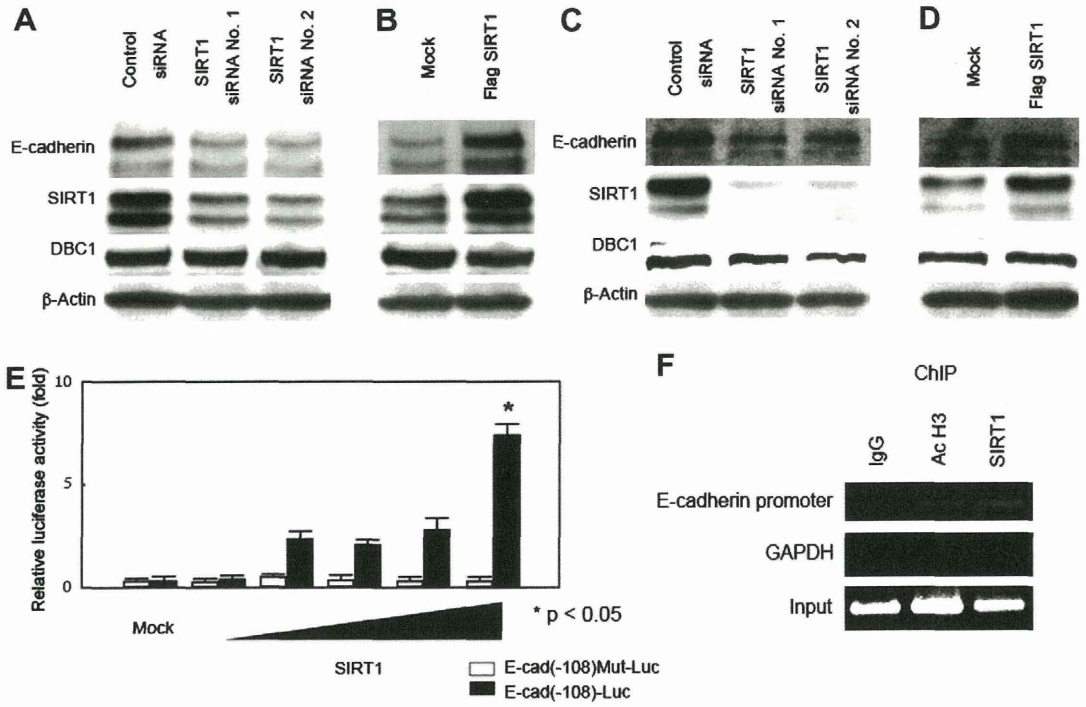

Fig. 1. E-cadherin promoter dependent regulation of E-cadherin expression by SIRT1 deacetylase. Ishikawa (A, B) and RL95-2 (C and D) cells were transfected with expression vector of SIRT1 or indicated siRNA and analyzed by Western blotting. The immunoblotting analysis using anti-SIRT1 antibodies revealed 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.

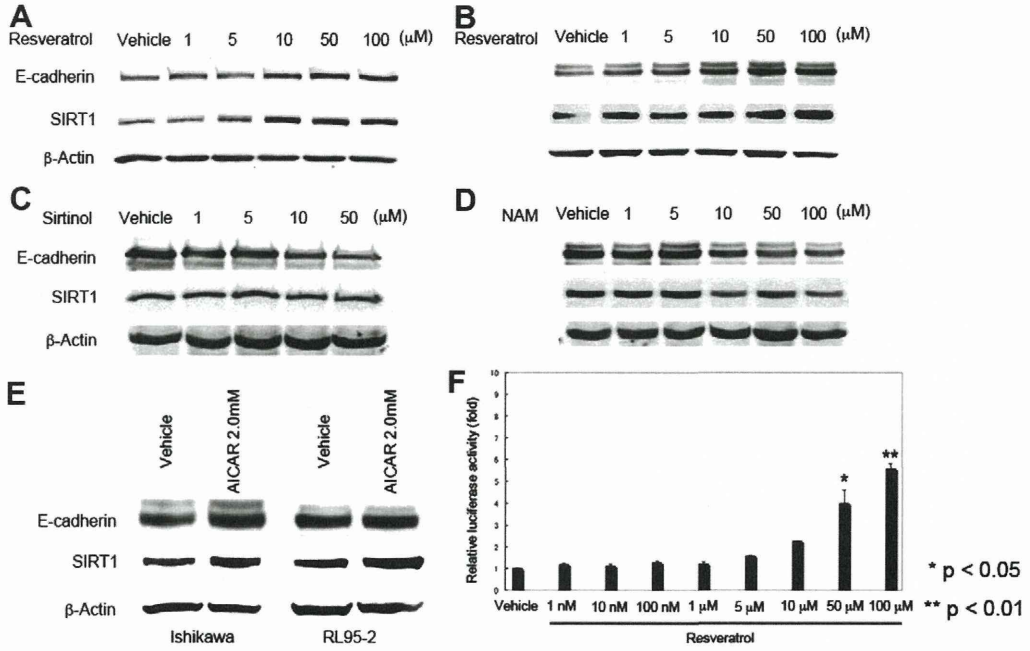

Fig. 2. Activation of SIRT1 using chemical compounds resulted in an increased expression of E-cadherin and SIRT1. Ishikawa (A, C, D, and E left panel) and RL95-2 (B, E right panel) cells were treated with various doses of vehicle, SIRT1 activators (resveratrol and AICAR), and SIRT1 repressors (sirtinol 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

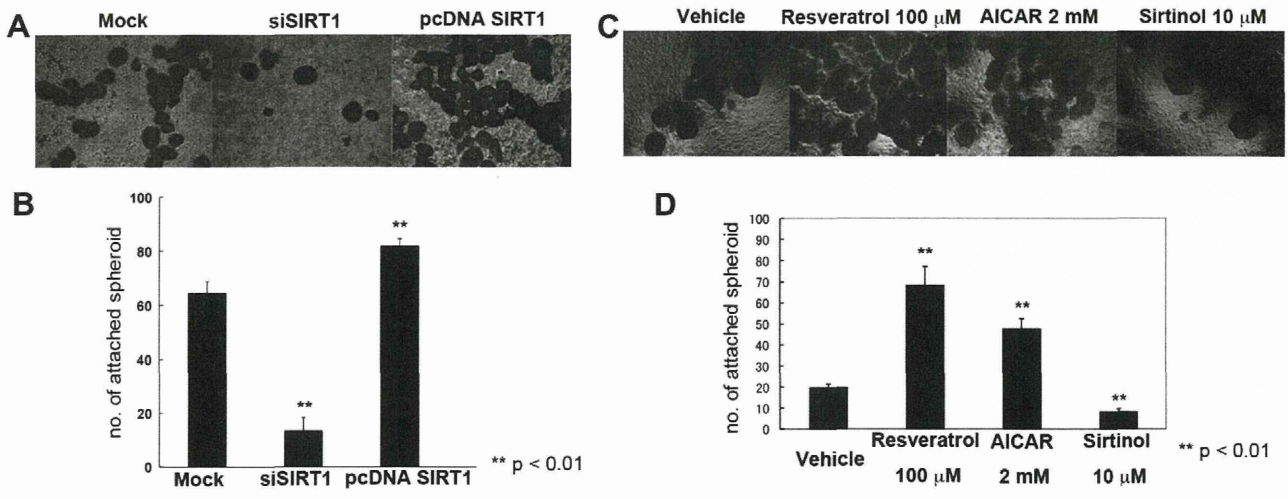

Fig. 3. SIRT1 expression and activity affects uterine receptivity. Spheroid adhesion to Ishikawa cell monolayer was examined by *in vivo* spheroid attachment assay. (A) Representative phase-contrast micrographs of JAR spheroids attached to Ishikawa cell monolayer. (B) Graph showing spheroid adhesion 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.

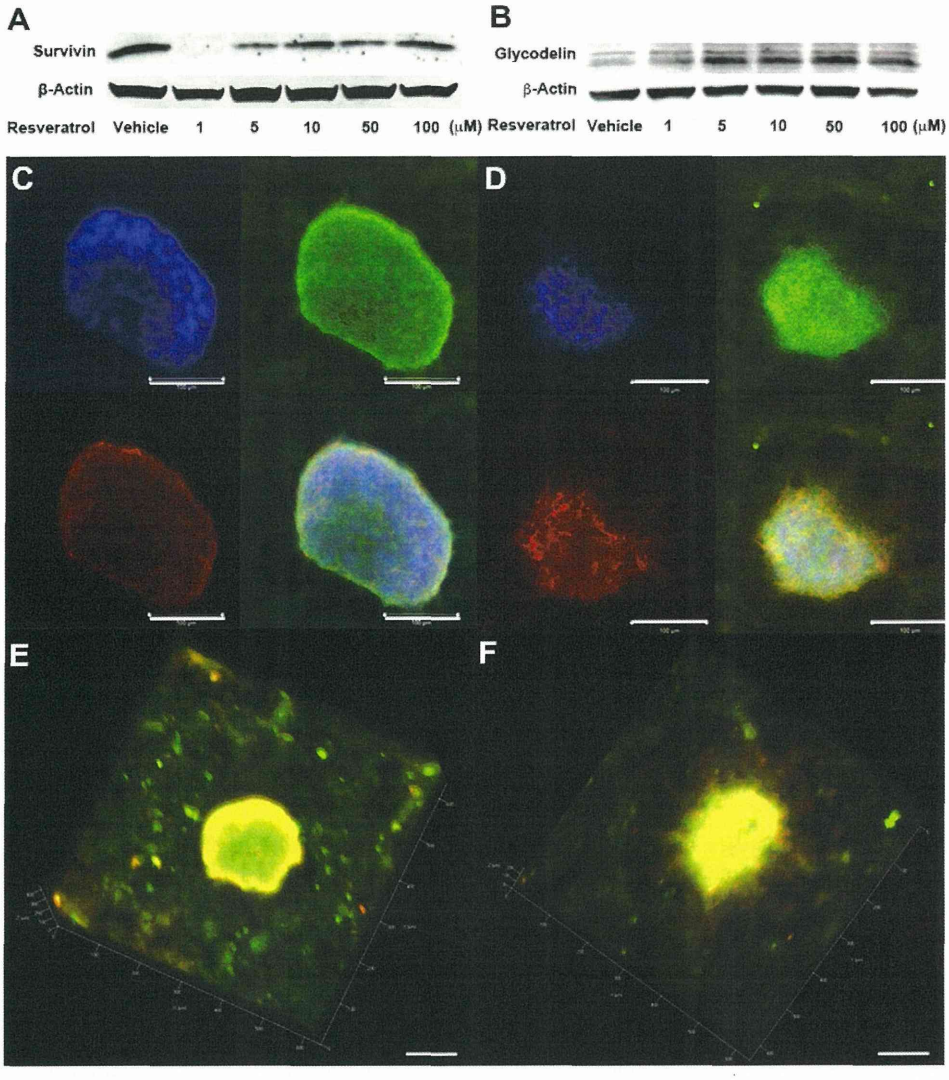

Fig. 4. Expression of implantation-related proteins and colocalization of E-cadherin and Glycodelin in Ishikawa cells. (A, B) Ishikawa cells were treated with various doses of resveratrol and protein expression of Survivin and Glycodelin was analyzed by Western blot. (C–F) In *in vivo* spheroid assay, 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.

Acknowledgments

This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, JMS Bayer Schering Pharma Grant, and Kanzawa Medical Science Foundation, Japan.

References

[1] H.W. Denker, Implantation: a cell biological paradox, J. Exp. Zool. 266 (1993) 541–558.

[2] J.D. Aplin, H. Singh, Bioinformatics and transcriptomics studies of early implantation, Ann. N.Y. Acad. Sci. 1127 (2008) 116–120.

[3] H. Singh, J.D. Aplin, Adhesion molecules in endometrial epithelium: tissue integrity and embryo implantation, J. Anat. 215 (2009) 3–13.

[4] H. Uchida, T. Maruyama, K. Ohta, M. Ono, T. Arase, M. Kagami, H. Oda, T. Kajitani, H. Asada, Y. Yoshimura, Histone deacetylase inhibitor-induced glycodelin enhances the initial step of implantation, Hum. Reprod. 22 (2007) 2615–2622.

[5] F. Rahnama, B. Thompson, M. Steiner, F. Shafiei, P.E. Lobie, M.D. Mitchell, Epigenetic regulation of E-cadherin controls endometrial receptivity, Endocrinology 150 (2009) 1466–1472.

[6] R. Grummer, H.P. Hohn, M.M. Mareel, H.W. Denker, Adhesion and invasion of three human choriocarcinoma cell lines into human endometrium in a three-dimensional organ culture system, Placenta 15 (1994) 411–429.

[7] C. Coutifaris, L.C. Kao, H.M. Sehdev, U. Chin, G.O. Babalola, O.W. Blaschuk, J.F. Strauss 3rd, E-cadherin expression during the differentiation of human trophoblasts, Development 113 (1991) 767–777.

[8] R.K. Jha, S. Titus, D. Saxena, P.G. Kumar, M. Laloraya, Profiling of E-cadherin, beta-catenin and Ca(2+) in embryo-uterine interactions at implantation, FEBS Lett. 580 (2006) 5653–5660.

[9] D. Riethmacher, V. Brinkmann, C. Birchmeier, A targeted mutation in the mouse E-cadherin gene results in defective preimplantation development, Proc. Natl. Acad. Sci. USA 92 (1995) 855–859.

[10] O. Wada-Hiraike, H. Hiraike, H. Okinaga, O. Imamov, R.P. Barros, A. Morani, Y. Omoto, M. Warner, J.A. Gustafsson, Role of estrogen receptor beta in uterine stroma and epithelium: insights from estrogen receptor beta−/− mice, Proc. Natl. Acad. Sci. USA 103 (2006) 18350–18355.

[11] K. Pruitt, R.L. Zinn, J.E. Ohm, K.M. McGarvey, S.H. Kang, D.N. Watkins, J.G. Herman, S.B. Baylin, Inhibition of SIRT1 reactivates silenced cancer genes without loss of promoter DNA hypermethylation, PLoS Genet. 2 (2006) e40.

[12] V. Byles, L. Zhu, J.D. Lovaas, L.K. Chmilewski, J. Wang, D.V. Faller, Y. Dai, SIRT1 induces EMT by cooperating with EMT transcription factors and enhances prostate cancer cell migration and metastasis, Oncogene (2012). http://dx.doi.org/10.1038/onc.2011.612 [Epub ahead of print].

[13] S. Chung, H. Yao, S. Caito, J.W. Hwang, G. Arunachalam, I. Rahman, Regulation of SIRT1 in cellular functions: role of polyphenols, Arch. Biochem. Biophys. 501 (2010) 79–90.

[14] Y. Morita, O. Wada-Hiraike, T. Yano, A. Shirane, M. Hirano, H. Hiraike, S. Koyama, H. Oishi, O. Yoshino, Y. Miyamoto, K. Sone, K. Oda, S. Nakagawa, K. Tsutsui, Y. Taketani, Resveratrol promotes expression of SIRT1 and StAR in rat ovarian granulosa cells: an implicative role of SIRT1 in the ovary, Reprod Biol Endocrinol 10 (2012) 14.

[15] J.E. Kim, J. Chen, Z. Lou, DBC1 is a negative regulator of SIRT1, Nature 451 (2008) 583–586.

[16] W. Zhao, J.P. Kruse, Y. Tang, S.Y. Jung, J. Qin, W. Gu, Negative regulation of the deacetylase SIRT1 by DBC1, Nature 451 (2008) 587–590.

[17] R.H. Wang, Y. Zheng, H.S. Kim, X. Xu, L. Cao, T. Luhasen, M.H. Lee, C. Xiao, A. Vassilopoulos, W. Chen, K. Gardner, Y.G. Man, M.C. Hung, T. Finkel, C.X. Deng, Interplay among BRCA1, SIRT1, and Survivin during BRCA1-associated tumorigenesis, Mol. Cell 32 (2008) 11–20.

[18] X. Hou, S. Xu, K.A. Maitland-Toolan, K. Sato, B. Jiang, Y. Ido, F. Lan, K. Walsh, M. Wierzbicki, T.J. Verbeuren, R.A. Cohen, M. Zang, SIRT1 regulates hepatocyte lipid metabolism through activating AMP-activated protein kinase, J. Biol. Chem. 283 (2008) 20015–20026.

[19] G. Suchankova, L.E. Nelson, Z. Gerhart-Hines, M. Kelly, M.S. Gauthier, A.K. Saha, Y. Ido, P. Puigserver, N.B. Ruderman, Concurrent regulation of AMP-activated protein kinase and SIRT1 in mammalian cells, Biochem. Biophys. Res. Commun. 378 (2009) 836–841.

[20] I. Lekli, D. Ray, D.K. Das, Longevity nutrients resveratrol, wines and grapes, Genes Nutr. 5 (2010) 55–60.

[21] C. Canto, Z. Gerhart-Hines, J.N. Feige, M. Lagouge, L. Noriega, J.C. Milne, P.J. Elliott, P. Puigserver, J. Auwerx, AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity, Nature 458 (2009) 1056–1060.

[22] O. Wada-Hiraike, T. Yano, T. Nei, Y. Matsumoto, K. Nagasaka, S. Takizawa, H. Oishi, T. Arimoto, S. Nakagawa, T. Yasugi, S. Kato, Y. Taketani, The DNA mismatch repair gene hMSH2 is a potent coactivator of oestrogen receptor alpha, Br. J. Cancer 92 (2005) 2286–2291.

[23] K.M. Hajra, D.Y. Chen, E.R. Fearon, The SLUG zinc-finger protein represses E-cadherin in breast cancer, Cancer Res. 62 (2002) 1613–1618.

[24] H. Hiraike, O. Wada-Hiraike, S. Nakagawa, S. Koyama, Y. Miyamoto, K. Sone, M. Tanikawa, T. Tsuruga, K. Nagasaka, Y. Matsumoto, K. Oda, K. Shoji, H. Fukuhara, S. Saji, K. Nakagawa, S. Kato, T. Yano, Y. Taketani, Identification of DBC1 as a transcriptional repressor for BRCA1, Br. J. Cancer 102 (2010) 1061–1067.

[25] S. Fest, N. Brachwitz, A. Schumacher, M.L. Zenclussen, F. Khan, P.O. Wafula, P.A. Casalis, S. Fill, S.D. Costa, G. Mor, H.D. Volk, H.N. Lode, A.C. Zenclussen, Supporting the hypothesis of pregnancy as a tumor: survivin is upregulated in normal pregnant mice and participates in human trophoblast proliferation, Am. J. Reprod. Immunol. 59 (2008) 75–83.

[26] M. Lappas, A. Mitton, R. Lim, G. Barker, C. Riley, M. Permezel, SIRT1 is a novel regulator of key pathways of human labor, Biol. Reprod. 84 (2011) 167–178.

[27] M. Suwa, H. Nakano, Z. Radak, S. Kumagai, Short-term adenosine monophosphate-activated protein kinase activator 5-aminoimidazole-4-carboxamide-1-beta-d-ribofuranoside treatment increases the sirtuin 1 protein expression in skeletal muscle, Metabolism 60 (2011) 394–403.

[28] D. Llobet, J. Pallares, A. Yeramian, M. Santacana, N. Eritja, A. Velasco, X. Dolcet, X. Matias-Guiu, Molecular pathology of endometrial carcinoma: practical aspects from the diagnostic and therapeutic viewpoints, J. Clin. Pathol. 62 (2009) 777–785.

[29] K.T. Howitz, K.J. Bitterman, H.Y. Cohen, D.W. Lamming, S. Lavu, J.G. Wood, R.E. Zipkin, P. Chung, A. Kisielewski, L.L. Zhang, B. Scherer, D.A. Sinclair, Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan, Nature 425 (2003) 191–196.

[30] A.K. Joe, H. Liu, M. Suzui, M.E. Vural, D. Xiao, I.B. Weinstein, Resveratrol induces growth inhibition, S-phase arrest, apoptosis, and changes in biomarker

expression in several human cancer cell lines, Clin. Cancer Res. 8 (2002) 893–903.
[31] D. Delmas, A. Lancon, D. Colin, B. Jannin, N. Latruffe, Resveratrol as a chemopreventive agent: a promising molecule for fighting cancer, Curr. Drug Targets 7 (2006) 423–442.
[32] E. Chatzaki, C.J. Gallagher, R.K. Iles, T.E. Ind, A.M. Nouri, C.M. Bax, J.G. Grudzinskas, Characterisation of the differential expression of marker antigens by normal and malignant endometrial epithelium, Br. J. Cancer 69 (1994) 1010–1014.
[33] V.A. Brown, K.R. Patel, M. Viskaduraki, J.A. Crowell, M. Perloff, T.D. Booth, G. Vasilinin, A. Sen, A.M. Schinas, G. Piccirilli, K. Brown, W.P. Steward, A.J. Gescher, D.E. Brenner, Repeat dose study of the cancer chemopreventive agent resveratrol in healthy volunteers: safety, pharmacokinetics, and effect on the insulin-like growth factor axis, Cancer Res. 70 (2010) 9003–9011.

Original Clinical Research 临床论著

Responsiveness of three subjective report of outcome measures for chronic heart failure

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OBJECTIVE: To compare the responsiveness of a newly designed symptom scale, the Chinese Medical Symptom Rating Scale for Heart Failure (CMSRS-HF), with the Chinese version of Minnesota Living with Heart Failure Questionnaire (MLHFQ) and the Medical Outcomes Study Short-form 36 (SF-36), and provide basis for the selection of subjective outcome measures for clinical evaluation of treatment of chronic heart failure by integrated traditional Chinese and Western medicine.

METHODS: One hundred and fifty-six patients with chronic heart failure were recruited from three clinical centers and were treated with Chinese herbal medicine based on syndrome classification. The patients were classified with standard of New York Heart Association and evaluated with CMSRS-HF, MLHFQ and SF-36. Three techniques for the quantification of responsiveness were utilized: paired *t*-test, effect sizes (ES) and standardized response means (SRM).

RESULTS: a) After 2-week treatment, patients scored significantly lower in CMSRS-HF, while scores of each dimension on MLHFQ and SF-36 increased significantly ($P=0.000$). b) ES of CMSRS-HF was greater than 0.8; ES of physical and emotional dimensions and comprehensive scores of MLHFQ were between 0.37 and 0.61; ES of each dimension, physical and emotional domains, and comprehensive scores were between 0.14 and 0.49. c) SRM of CMSRS-HF was greater than 0.8; SRM of physical and emotional dimensions and comprehensive scores of MLHFQ ranged from 0.53 to 0.92; SRM of each dimension, physical and emotional domains, and comprehensive scores were between 0.23 and 0.83. d) By stratified analysis according to NYHA classification, the acute patients (NYHA Ⅲ, Ⅳ) were more sensitive to subjective outcome measures.

CONCLUSION: Responsiveness of the newly designed CMSRS-HF is high. However, responsiveness of MLHFQ and most dimensions in SF-36 is moderate. When evaluating clinical effects of integrated traditional Chinese and Western medicine on chronic heart failure, different scales can be

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DOI: 10.3736/jcim20121207
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Zhu YB, Origasa H, Luo XX, Wang YY, Di J, Lin L. Responsiveness of three subjective report of outcome measures for chronic heart failure. *J Chin Integr Med*. 2012; 10(12): 1375-1381.
朱燕波，折笠秀树，骆晓霞，王洋洋，邸洁，林琳. 慢性心力衰竭主观报告结局量表的反应度比较研究. 中西医结合学报. 2012; 10(12): 1375-1381.

Received August 15, 2012; accepted August 30, 2012; published online December 15, 2012.
Full-text LinkOut at PubMed. Journal title in PubMed: *Zhong Xi Yi Jie He Xue Bao*.

基金项目：国家自然科学基金资助项目(No. 30873256)
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ISSN 1672-1977. Published by JCIM Press, Shanghai, China.

applied according to actual clinical presentations.

KEYWORDS: heart failure; outcome measures; responsiveness; integrated TCM WM; treatment outcome; evaluation studies as topic

In the field of health evaluation, the measurement parameters must be checked for reliability, validity, and responsiveness with appropriate scientific methods, in order to arrive at accurate and meaningful conclusions. Only reliable and valid measurement standards should be used for evaluation. But in practical work, good reliability and validity are insufficient. Clinical researchers often place most emphasis on objective health status outcomes of a therapeutic intervention, and ignore the other subjective aspects affected by treatment, which are also important for the patient. Therefore, clinical evaluation tools should have the ability to detect small but significant changes in objective and subjective health status over time. This ability, called responsiveness[1-4], can help to determine experimental design by optimizing the study scale, finding the selection priority of outcome measures in clinical evaluation, or reducing the number of outcome measures. In this study, we examined the selection of outcome measurements in clinical evaluation by comparing the responsiveness of three subjective outcome measures in evaluating the clinical efficacy of integrated traditional Chinese and Western medicine for treatment of chronic heart failure.

1 Materials and methods

1.1 Research objects A total of 156 patients with chronic heart failure were recruited from Integrated Traditional Chinese and Western Medicine Center for Cardiovascular Diseases of China-Japan Friendship Hospital and Department of Cardiovascular Internal Medicine of Dongzhimen Hospital and Dongfang Hospital affiliated to Beijing University of Chinese Medicine in Beijing, from September 2009 to August 2011. All patients signed the written informed consent.

1.1.1 Diagnostic criteria and classification standard The patients were diagnosed by the Framingham criteria for chronic heart failure[5] and classification standard of New York Heart Association (NYHA)[6].

1.1.2 Inclusion criteria (1) Signed the written informed consent; (2) NYHA classes Ⅱ to Ⅳ; (3) NYHA stages B, C and D.

1.1.3 Exclusion criteria (1) Congenital heart disease, psychosis, dementia or nerve disorders; (2) acute myocardial infarction within the last one month; (3) unstable angina within the last one month; (4) pregnant patients; (5) severe liver and kidney dysfunction; (6) allergic constitution or multiple medication allergies.

1.2 Investigation method A 2-week longitudinal observational study was conducted to identify responsiveness of the scales. The patients were treated with Chinese herbal medicine based on syndrome differentiation and conventional anti-HF therapy, named integrated traditional Chinese and Western medicine, for two weeks. Two surveys were arranged, one each before and after the 2-week study period.

1.3 Investigation content (1) General condition: gender, age, level of education, family history of heart disease and the course of chronic heart failure.

(2) Classification of chronic heart failure according to NYHA.

(3) Chinese Medical Symptom Rating Scale for Heart Failure (CMSRS-HF): the rating scale includes 16 items, including palpitations, shortness of breath, wheezing, malaise and fatigue, chest pain, edema, sweating, cough, expectoration, oliguria, abdominal distention, chills, anxiety, insomnia, poor appetite and cyanotic lips. Score of each item is 0 to 3, and the total score of CMSRS-HF is 0 to 48[7]. Higher scores reflect worse quality of life (QOL). Two surveys of CMSRS-HF were conducted by the study doctor before and after the course of treatment.

(4) Minnesota Living with Heart Failure Questionnaire (MLHFQ): MLHFQ was developed by Rector *et al*[8] to systematically and comprehensively assess a variety of physical and psychological aspects of patients living with chronic heart failure, which include activities of daily living, economic issues, ability to work, enjoyment of leisure time activities, relations with family and friends, sexual activity, and depression. The MLHFQ is composed of 21 items rated on a score from 0 (no effect) to 5 (very much). Item ratings are summed for a total score that can range from 0 (no effect) to 105 (very much) which include physical domain (0 to 40), emotional domain (0 to 25), and other domain (0 to 40). Higher scores reflect worse QOL. Transformation score of each domain ranges from 0 to 100. Higher transformation scores reflect better QOL. Two surveys of MLHFQ were conducted on the patient before and after the course of treatment. Zhu *et al*[9,10] developed the Chinese version of MLHFQ, and evaluated its applicability in patients with chronic heart failure in China. The Chinese version of MLHFQ was approved by the Original Scale Development Agency, University of Minnesota, USA and Scale Management Agency, Mapi Research Institute, France.

(5) Medical Outcomes Study Short-form 36 (SF-36): SF-36 is composed of 36 questions developed by Medical Outcomes Study in the 1980s[11]. It consists of physical health components (PCS) and mental health components (MCS), which respectively includes physical functioning (PF), role limitation due to physical problems (RP) and bodily pain (BP), general health (GH) and energy/vitality (VT), social functioning (SF), role limitation due to physical problems (RE), and mental health (MH). Scores of each dimension range from 0 to 100. Higher scores reflect better health. Two surveys of SF-36 were conducted on the patient before and after the course of treatment. Gong *et al*[12] evaluated its applicability in the measurement of QOL in patients with chronic heart failure in China.

1.4 Statistical methods Paired *t*-test, effect sizes (ES), and standardized response means (SRM) were adopted to analyze the responsiveness of these measurement tools (CMSRS-HF, MLHFQ, and SF-36). The statistical analysis was completed with SPSS 17.0 and JMP 9.0. The significance level in paired *t*-test was set at *P* value less than 0.05. ES is defined as the score difference between follow-up and baseline divided by the standard deviation of the group's baseline scores. The score difference (between follow-up and baseline) divided by the standard deviation of the group's score differences determines the SRM. The absolute value of ES and SRM around 0.2 indicates low responsiveness, around 0.5 indicates moderate responsiveness, and greater than 0.8 indicates high responsiveness[13].

2 Results

2.1 General condition A total of 156 patients (76 male (48.72%), 80 female (51.28%)) with chronic heart failure participated in this study. Age range was 27 to 83 years, with average age 69.35 (11.90) years. General information of the patients is shown in Table 1.

Table 1 Baseline characteristics of 156 participants

Characteristic	Classification	*n*	Percentage (%)
Experimental center	China-Japan Friendship Hospital	58	37.18
	Dongzhimen Hospital	17	10.90
	Dongfang Hospital	81	51.92
Gender	Male	76	48.72
	Female	80	51.28
Age (years)	≤59	32	20.51
	60-69	29	18.59
	70-79	66	42.31
	≥80	29	18.59
Education	Primary school	44	28.21
	Middle school	89	57.05
	College degree or above	23	14.74
Payment (n=141, 15 cases missing)	Free medical care	28	19.86
	Social medical insurance	104	73.76
	Pay out-of-pocket	6	4.25
	Others	3	2.13
Smoking history	No	101	64.74
	Yes	20	12.82
	Quit	35	22.44
Alcohol history	No	126	80.77
	Yes	15	9.62
	Quit	15	9.62
Family heart diseases history (n=154, two cases missing)	No	120	77.92
	Yes	34	22.08
HF course (n=150, six cases missing)	<1 year	37	24.67
	1-4 years	46	30.67
	5-9 years	26	17.33
	≥10 years	41	27.33
NYHA classification	Ⅱ	48	30.77
	Ⅲ	75	48.08
	Ⅳ	33	21.15

HF: heart failure; NYHA: New York Heart Association.

2.2 The responsiveness of subjective outcome measures After 2 weeks of treatment with integrated traditional Chinese and Western medicine, the results of paired *t*-test showed that score of CMSRS-HF reduced significantly, and the scores of MLHFQ and SF-36 scale increased significantly (P=0.005 for GH, P=0.000 for others). For ES, the CMSRS-HF was more than 0.8; total score, physical domain and emotional domain of MLHFQ ranged from 0.37 to 0.61; the total score, each domain and each dimension in SF-36 ranged from 0.14 to 0.49. With regard to SRM, CMSRS-HF, physical domain and total score of MLHFQ and physical domain of SF-36 were more than 0.8; emotional

domain of MLHFQ was 0.53; total score, MCS of SF-36 were 0.79 and 0.59 respectively; each dimension of SF-36 ranged from 0.23 to 0.58. Evaluation results of responsiveness were different. See Table 2 for details.

2.3 The subjective outcome measures responsiveness of different NYHA classifications After receiving treatment with integrated traditional Chinese and Western medicine, results of paired *t*-test displayed that CMSRS-HF score of NYHA classes Ⅱ, Ⅲ and Ⅳ reduced significantly ($P=0.000$), and scores of MLHFQ and SF-36 improved. The results showed no significantly difference in PF, GH, VT, SF, and MH scores of SF-36 among NYHA Ⅱ patients and in GH of NYHA Ⅳ patients. With regard to CMSRS-HF, ES and SRM in different NYHA classifications scored greater than 0.8, the NYHA Ⅲ and Ⅳ had better score. Regarding MLHFQ and SF-36, ES and SRM of patients with NYHA Ⅲ and Ⅳ were better than those of the NYHA Ⅱ. In the evaluation of integrated traditional Chinese and Western medicine in the treatment of patients with chronic heart failure, we found that patients with NYHA Ⅲ and Ⅳ were more sensitive to subjective outcome measures. See Table 3 to 5 for details.

Table 2 Responsiveness of CMSRS-HF, MLHFQ and SF-36 evaluated in 156 patients with chronic heart failure

Measure	Domain	Score (Mean±standard deviation)			ES	SRM	Paired-*t* test	
		Before	After	Difference			*t* value	*P* value
CMSRS-HF	CMSRS	16.60±8.45	8.10±5.25	−8.51±6.84	1.01	1.24	−15.52	0.000
MLHFQ	Physical health	44.10±30.47	62.56±26.69	18.46±19.97	0.61	0.92	11.54	0.000
	Mental health	64.33±26.64	74.10±22.72	9.77±18.49	0.37	0.53	6.60	0.000
	Comprehensive score	55.89±24.84	68.53±21.95	12.65±14.97	0.51	0.85	10.56	0.000
SF-36	Physical functioning	40.03±27.93	46.67±27.68	6.63±13.56	0.24	0.49	6.11	0.000
	Role-physical	36.94±31.78	49.60±31.33	12.66±24.00	0.40	0.53	6.59	0.000
	Bodily pain	59.98±28.41	73.79±23.62	13.81±23.68	0.49	0.58	7.28	0.000
	General health	41.06±21.82	44.10±21.63	3.04±13.41	0.14	0.23	2.84	0.005
	Energy/vitality	41.07±25.98	50.60±23.51	9.54±19.38	0.37	0.49	6.14	0.000
	Social functioning	58.49±30.02	65.63±26.93	7.13±24.68	0.24	0.29	3.61	0.000
	Role-emotional	64.80±32.00	76.66±26.24	11.86±26.37	0.37	0.45	5.62	0.000
	Mental health	67.69±24.50	73.94±19.42	6.25±15.25	0.26	0.41	5.12	0.000
	Physical health components	44.50±21.11	53.54±18.72	9.04±10.91	0.43	0.83	10.35	0.000
	Mental health components	58.01±22.71	66.71±19.25	8.69±14.67	0.38	0.59	7.40	0.000
	Comprehensive score	51.26±20.92	60.12±17.71	8.87±11.21	0.42	0.79	9.87	0.000

Total score of CMSRS-HF ranges from 0 to 48. Higher scores reflect worse quality of life. Transformation scores of MLHFQ and SF-36 range from 0 to 100. Higher scores reflect worse quality of life. CMSRS-HF: Chinese Medical Symptom Rating Scale for Heart Failure; MLHFQ: Minnesota Living with Heart Failure Questionnaire; SF-36: Medical Outcomes Study Short-form 36; ES: effect sizes; SRM: standardized response means.

Table 3 Responsiveness of CMSRS-HF, MLHFQ and SF-36 in 48 patients with NYHA Ⅱ

Measure	Domain	Score (Mean±standard deviation)			ES	SRM	Paired-*t* test	
		Before	After	Difference			*t* value	*P* value
CMSRS-HF	CMSRS	10.83±5.97	5.27±3.14	−5.56±5.70	0.93	0.98	−6.76	0.000
MLHFQ	Physical health	67.81±26.38	79.53±19.31	11.72±17.80	0.44	0.66	4.56	0.000
	Mental health	77.08±22.23	83.42±17.70	6.33±12.70	0.28	0.50	3.46	0.001
	Comprehensive score	74.38±20.34	82.08±15.30	7.70±11.96	0.38	0.64	4.46	0.000
SF-36	Physical functioning	65.00±24.88	66.88±24.00	1.88±11.37	0.08	0.17	1.14	0.259
	Role-physical	57.68±34.41	68.36±29.69	10.68±31.42	0.31	0.34	2.35	0.023
	Bodily pain	62.63±28.26	75.42±23.00	12.79±25.72	0.45	0.50	3.45	0.001
	General health	47.63±23.75	48.65±22.72	1.02±11.83	0.04	0.09	0.60	0.553
	Energy/vitality	56.25±21.99	60.16±18.93	3.91±18.35	0.18	0.21	1.47	0.147
	Social functioning	72.14±27.68	74.48±23.20	2.34±18.71	0.08	0.13	0.87	0.390
	Role-emotional	74.13±29.59	83.68±22.54	9.55±29.52	0.32	0.32	2.24	0.030
	Mental health	76.35±20.31	79.06±15.39	2.71±13.49	0.13	0.20	1.39	0.171
	Physical health components	58.23±22.02	64.82±17.21	6.59±11.20	0.30	0.59	4.08	0.000
	Mental health components	69.72±21.12	74.34±15.27	4.63±11.25	0.22	0.41	2.85	0.007
	Comprehensive score	63.98±20.36	69.58±15.10	5.61±9.07	0.28	0.62	4.28	0.000

NYHA: New York Heart Association; CMSRS-HF: Chinese Medical Symptom Rating Scale for Heart Failure; MLHFQ: Minnesota Living with Heart Failure Questionnaire; SF-36: Medical Outcomes Study Short-form 36; ES: effect sizes; SRM: standardized response means.

Table 4 Responsiveness of CMSRS-HF, MLHFQ and SF-36 in 76 patients with NYHA Ⅲ

Measure	Domain	Score (Mean±standard deviation)			ES	SRM	Paired-*t* test	
		Before	After	Difference			*t* value	*P* value
CMSRS-HF	CMSRS	17.13±7.72	8.15±4.01	−8.99±6.34	1.16	1.41	−12.27	0.000
MLHFQ	Physical health	37.37±25.83	58.90±23.93	21.53±18.94	0.83	1.14	9.85	0.000
	Mental health	62.88±25.54	72.32±22.54	9.44±19.25	0.37	0.49	4.25	0.000
	Comprehensive score	51.33±21.56	65.58±20.17	14.26±14.94	0.66	0.95	8.27	0.000
SF-36	Physical functioning	31.93±21.10	40.27±22.70	8.33±13.29	0.39	0.63	5.43	0.000
	Role-physical	32.83±27.13	47.92±27.48	15.08±20.88	0.56	0.72	6.25	0.000
	Bodily pain	56.29±27.25	70.72±23.38	14.43±22.02	0.53	0.66	5.67	0.000
	General health	39.00±21.85	43.19±21.66	4.19±15.15	0.19	0.28	2.39	0.019
	Energy/vitality	36.08±25.35	48.00±24.41	11.92±20.01	0.47	0.60	5.16	0.000
	Social functioning	55.67±29.01	64.83±26.69	9.17±26.98	0.32	0.34	2.94	0.004
	Role-emotional	63.11±31.10	77.33±24.49	14.22±23.84	0.46	0.60	5.17	0.000
	Mental health	64.93±25.05	73.67±19.16	8.73±15.86	0.35	0.55	4.77	0.000
	Physical health components	40.02±18.34	50.52±16.70	10.51±10.77	0.57	0.98	8.45	0.000
	Mental health components	54.95±21.04	65.96±18.35	11.01±16.15	0.52	0.68	5.90	0.000
	Comprehensive score	47.48±18.77	58.24±16.11	10.76±12.25	0.57	0.88	7.61	0.000

NYHA: New York Heart Association; CMSRS-HF: Chinese Medical Symptom Rating Scale for Heart Failure; MLHFQ: Minnesota Living with Heart Failure Questionnaire; SF-36: Medical Outcomes Study Short-form 36; ES: effect sizes; SRM: standardized response means.

Table 5 Responsiveness of CMSRS-HF, MLHFQ and SF-36 in 33 patients with NYHA Ⅳ

Measure	Domain	Score (Mean±standard deviation)			ES	SRM	Paired-*t* test	
		Before	After	Difference			*t* value	*P* value
CMSRS-HF	CMSRS	23.79±7.22	12.09±7.31	−11.70±7.88	1.62	1.48	−8.53	0.000
MLHFQ	Physical health	24.92±24.55	46.21±28.97	21.29±23.28	0.87	0.91	5.25	0.000
	Mental health	49.09±26.42	64.61±25.34	15.52±22.58	0.59	0.69	3.95	0.000
	Comprehensive score	39.34±21.47	55.53±24.05	16.19±17.41	0.75	0.93	5.34	0.000
SF-36	Physical functioning	22.12±20.46	31.82±27.12	9.70±15.56	0.47	0.62	3.58	0.001
	Role-physical	16.10±17.82	26.14±24.73	10.04±17.47	0.56	0.57	3.30	0.002
	Bodily pain	64.51±30.92	78.39±24.78	13.88±24.92	0.45	0.56	3.20	0.003
	General health	36.18±16.61	39.58±19.20	3.39±11.16	0.20	0.30	1.75	0.090
	Energy/vitality	30.30±23.44	42.61±23.57	12.31±18.19	0.53	0.68	3.89	0.000
	Social functioning	45.08±28.46	54.55±28.78	9.47±26.52	0.33	0.36	2.05	0.049
	Role-emotional	55.05±34.67	64.90±31.37	9.85±27.36	0.28	0.36	2.07	0.047
	Mental health	61.36±26.08	67.12±23.29	5.76±15.62	0.22	0.37	2.12	0.042
	Physical health components	34.73±15.51	43.98±17.64	9.25±10.46	0.60	0.88	5.08	0.000
	Mental health components	47.95±22.06	57.29±22.21	9.35±14.68	0.42	0.64	3.66	0.000
	Comprehensive score	41.34±17.84	50.64±18.67	9.30±10.80	0.52	0.86	4.95	0.000

CHF: chronic heart failure; NYHA: New York Heart Association; CMSRS-HF: Chinese Medical Symptom Rating Scale for Heart Failure; MLHFQ: Minnesota Living with Heart Failure Questionnaire; SF-36: Medical Outcomes Study Short-form 36; ES: effect sizes; SRM: standardized response means.

3 Discussion

3.1 Subjective outcome measure and responsiveness

QOL is an important consideration for patients with chronic heart failure. These patients and their families already bear the burden of high financial costs and complicated medical management, which are compounded by the emotional stress of the chronic heart failure patients' deterioration in symptoms, physical decline, side effects of treatments, and repeated hospitalizations. Therefore, proper case management of patients with chronic heart failure must focus on not only preventing further deterioration of heart function, and reducing repeated hospitalization rates and mortality, but also helping the patients alleviate suffering, enhancing physical function, and improving general QOL. One way to accurately assess treatment outcomes for patients with chronic heart failure is by using the therapeutic effect outcomes of integrated traditional Chinese and Western medicine, which take into account patient-reported outcomes, doctor-reported outcomes, caregiver-reported outcomes and laboratory indicators reflecting physiological or pathological changes.

Clinical evaluation is placing increasing weight on subjective outcome measures, which are selected based on demonstration of validity and reliability. The health care workers and researchers in a clinical study usually focus more on objective outcomes of specific effective therapeutic interventions. But, to evaluate any given therapy's clinical curative effect, many scholars believe that responsiveness, which takes into account subjective and psychological aspects of patient evaluation, is an equal or even more important feature of measurement. Respon-

siveness is defined as the capability of a given scale to measure longitudinal change over time in a specific area[1,14]. There are at least 25 different definitions and 31 different measurement parameters[15], which are still in dispute within this academic discipline. This study focused on the standard scales of patient-reported outcomes (MLHFQ and SF-36) and doctor-reported outcomes (CMSRS-HF), which reflected the clinical evaluation of integrated traditional Chinese and Western medicine in the treatment of chronic heart failure. Paired *t*-test, ES and SRM were used to calculate various aspects of the responsiveness of MLHFQ, SF-36 and CMSRS-HF.

3.2 Paired *t*-test before and after intervention Paired *t*-test is a commonly used method in the evaluation of efficacy. After 2 weeks of treatment with Chinese herbal medicine and conventional therapy, the total sample score significantly decreased in CMSRS-HF (16.60±8.45 *vs* 8.10±5.25); scores of physical health domain (44.10±30.47 *vs* 62.56±26.69), mental health domain (64.33±26.64 *vs* 74.10±22.72) and comprehensive score (55.89±24.84 *vs* 68.53±21.95) in MLHFQ significantly increased. Scores of PCS (44.50±21.11 *vs* 53.54±18.72), MCS (58.01±22.71 *vs* 66.71±19.25), comprehensive score (51.26±20.92 *vs* 60.12±17.71) and 8 dimensions in SF-36 significantly increased (GH: $P=0.005$; others: $P=0.000$). After 2 weeks of treatment, different NYHA classifications except PF, GH, VT, SF, MH of NYHA Ⅱ and GH dimension of NYHA Ⅳ, as well as others of SF-36 all showed varying degrees of statistical significance.

3.3 ES and SRM The null hypothesis of a paired *t*-test is that the difference before and after a treatment is zero (no difference). Statistically significant results are more likely with increasing sample size, but the *t* values and *P* values of a paired *t*-test cannot be used to evaluate responsiveness. In order to overcome the deficiencies of the paired *t*-test, two evaluating indices of responsiveness (ES and SRM) were utilized in our research. Generally speaking, an absolute value of ES and SRM around 0.2 indicates low responsiveness; around 0.5 indicates moderate responsiveness; greater than 0.8 indicates high responsiveness[13]. ES evaluation results of our study showed that total sample score of CMSRS-HF was greater than 0.8; physical domain and emotional domain of MLHFQ ranged from 0.37 to 0.61; total score, each domain and each dimension in SF-36 ranged from 0.14 to 0.49. Comparing three subjective outcome measures in different NYHA classifications revealed that the ES evaluation results of NYHA Ⅲ and Ⅳ were better than those of NYHA Ⅱ. Evaluation results of SRM indicated that the CMSRS-HF was greater than 0.8; total score, physical domain and emotional domain of MLHFQ ranged from 0.53 to 0.92; PCS, MCS, comprehensive score and 8 dimensions in SF-36 ranged from 0.23 to 0.83. Comparing three subjective outcome measures in different NYHA classifications, the results revealed that the SRM evaluation results of NYHA Ⅲ and Ⅳ were better than those of NYHA Ⅱ.

The results of this study show that responsiveness of CMSRS-HF (doctor-reported outcome) is high, and the responsiveness of MLHFQ (patient-reported outcome) and most dimensions in SF-36 (patient-reported outcome) are moderate. When evaluating clinical effect of integrated traditional Chinese and Western medicine on chronic heart failure, the patients with NYHA class Ⅲ or Ⅳ are more sensitive to these three subjective outcome measures. Selection of appropriate outcome measure should be based on the actual clinical situation

4 Competing interests

The authors declare that they have no competing interests.

REFERENCES

1 Zhu YB. Measurement and evaluation of quality of life. Beijing: People's Melitary Medical Press. 2010: 49-62. Chinese.
朱燕波. 生命质量(QOL)测量与评价. 北京: 人民军医出版社. 2010: 49-62.

2 Xu J, Guo R, Liu YS, Wan P, Huang WY, Chen ZL. The study of responsiveness on Self-rated Health Measurement Scale (the Revised Version 1.0). Zhongguo Wei Sheng Tong Ji. 2003; 20(5): 272-275. Chinese with abstract in English.
许军, 郭榕, 刘永生, 万萍, 黄伟毅, 陈志良. 自测健康评定量表修订版的反应度研究. 中国卫生统计. 2003; 20(5): 272-275.

3 Zhu YB, Li YL, Wang W, Jiang FC. Development of a patient-reported outcome scale for stable chronic obstructive pulmonary disease and its clinical applicability. J Chin Integr Med. 2011; 9(8): 857-865. Chinese with abstract in English.
朱燕波, 李友林, 王伟, 江芳超. 慢性阻塞性肺疾病稳定期患者报告结局量表的研制与临床适用性. 中西医结合学报. 2011; 9(8): 857-865.

4 Chen XL, Liu FB, Guo L, Liu XB. Development of patient-reported outcome scale for myasthenia gravis: a psychometric test. J Chin Integr Med. 2010; 8(2): 121-125. Chinese with abstract in English.
陈新林, 刘凤斌, 郭丽, 刘小斌. 重症肌无力患者报告结局指标量表的研制——计量心理学测评. 中西医结合学报. 2010; 8(2): 121-125.

5 Zheng XY. Guiding principles for clinical research on new drugs of traditional Chinese medicine (trial implementation). Beijing: Chinese Medical Science and Technology Press. 2002: 78. Chinese.
郑筱萸. 中药新药临床研究指导原则(试行). 北京: 中国医药科技出版社. 2002: 78.

6 Ye RG, Lu ZY. Internal medicine. 6th ed. Beijing: People's Medical Publishing House. 2005: 165. Chinese.

叶任高，陆再英. 内科学. 第 6 版. 北京：人民卫生出版社. 2005：165.

7 Luo XX. Study of minimal clinical important difference value of heart failure health status measurements. Beijing: Beijing University of Chinese Medicine. 2010. Chinese with abstract in English.
骆晓霞. 心功能不全健康状况测量工具的临床最小差异值研究. 北京：北京中医药大学. 2010.

8 Rector TS, Kubo SH, Cohn JN. Patient's self-assessment of their congestive heart failure Ⅱ: content, reliability and validity of a new measure, the Minnesota Living with Heart Failure Questionnaire. Heart Fail. 1987; 3: 198-209.

9 Zhu YB, Origasa H, Zheng J, Du JH. Evaluation on reliability and validity of heart failure QOL (LHFQ) Chinese version. Zhongguo Xing Wi Yi Xue Ke Xue. 2004; 13(3): 337-339. Chinese with abstract in English.
朱燕波，折笠秀树，郑洁，杜金行. 心功能不全 QOL 量表中文译本信度效度的初步评价. 中国行为医学科学. 2004; 13(3): 337-339.

10 Zhu YB, Du JH, Lin L, Origasa H, Chen KF, Zheng J, He L, Li CY, Fang F, Luo XX. The translating, editing and testing of the Minnesota Living with Heart Failure Questionnaire of Chinese version. Zhonghua Xing Wei Yi Xue Yu Nao Ke Xue Za Zhi. 2010; 19(2): 178-181. Chinese with abstract in English.
朱燕波，杜金行，林琳，折笠秀树，陈柯帆，郑洁，贺琳，李春岩，方芳，骆晓霞. 明尼苏达心功能不全生命质量量表(MLHFQ)中文版的研制及临床试用. 中华行为医学与脑科学杂志. 2010; 19(2): 178-181.

11 Stewart AL, Ware JE Jr. Measuring functioning and well-being: the medical outcomes study approach. Durham, N. C.: Duke University Press. 1992.

12 Gong KZ, Zhang ZG, Zhu N, Sun HG, Feng YL. Evaluation of reliability and validity of SF-36 in patients with chronic heart failure. Zhongguo Kang Fu Yi Xue Za Zhi. 2004; 19(3): 182-184. Chinese with abstract in English.
龚开政，张振刚，朱宁，孙红光，凤以良. SF-36 在慢性心力衰竭患者中的信度和效度评价. 中国康复医学杂志. 2004; 19(3): 182-184.

13 Crosby RD, Kolotkin RL, Williams GR. Defining clinically meaningful change in health-related quality of life. Clin Epidemiol. 2003; 56(5): 395-407.

14 Wan CH. Quality of life evaluation of cancer patients. Beijing: Publishing House. 2007: 98. Chinese.

15 Terwee CB, Dekker FW, Wiersinga WM, Prummel MF, Bossuyt PM. On assessing responsiveness of health-related quality of life instruments: guidelines for instrument evaluation. Qual Life Res. 2003; 12(4): 349-362.

慢性心力衰竭主观报告结局量表的反应度比较研究

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目的：比较 3 种中西医结合治疗慢性心力衰竭主观报告结局量表，包括心功能不全症状积分表(Chinese Medical Symptom Rating Scale for Heart Failure, CMSRS-HF)、明尼苏达心功能不全问卷(Minnesota Living with Heart Failure Questionnaire, MLHFQ)、简明健康状况问卷(Medical Outcomes Study Short-form 36, SF-36)的反应度，为中西医结合临床疗效评价结局指标的选择提供依据。

方法：对 3 个临床研究中心招募的 156 例慢性心力衰竭患者实施调查，采用治疗前后差异的配对 t 检验、效应量(effect size, ES)和标准反应均数(standardized response means, SRM)评价反应度。

结果：(1)中西医结合干预 2 周后，中医症状评分表得分显著降低，MLHFQ 身体领域、情绪领域及综合得分显著升高；SF-36 的 8 个维度及生理领域、心理领域、总分显著升高($P=0.000$)。(2)中医症状评分表的 ES 大于 0.8，MLHFQ 身体领域、情绪领域及综合得分的 ES 为 0.37～0.61，SF-36 的 8 个维度及生理领域、心理领域、总分的 ES 为 0.14～0.49。(3)标准反应均数 SRM：中医症状评分表大于 0.8，MLHFQ 身体领域、情绪领域及综合得分为 0.53～0.92，SF-36 的 8 个维度及生理领域、心理领域、总分为 0.23～0.83。(4)不同 NYHA 分级分层分析，各主观报告结局量表对中西医结合治疗中、重度(NHYAⅢ、Ⅳ级)慢性心衰更敏感。

结论：中医症状评分表反应度较好，MLHFQ 反应度适中，SF-36 大部分维度反应度适中。慢性心力衰竭中西医结合临床疗效评价时，可根据实际情况选择应用不同的结局评价量表。

关键词：心力衰竭；结局量表；反应度；中西医结合；治疗结果；评价研究(主题)