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Alterations in histone deacetylase 8 lead to cell migration and poor prognosis in breast cancer

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ABSTRACT

Aims: Alterations in histone proteins can lead to breast tumorigenesis. Selective histone deacetylase 8 (HDAC8) inhibitors with fewer adverse effects have been developed. A more comprehensive study of alterations and its mechanisms in *HDAC8* is required. In this study, we investigated mechanisms of dysregulation of HDAC8 expression and its biological role and pathways in breast cancer.
Main methods: Alterations in *HDAC8* were analyzed in Taiwanese breast cancer patients; and in tissue samples from The Cancer Genome Atlas (TCGA) data set that were derived from Western countries. Knockdown by si-HDAC8, treatment with the HDAC8-specific inhibitor PCI-34051, SRB assays, wound healing, Transwell migration assays, Illumina BeadArray™ arrays and Ingenuity Pathway Analysis (IPA) were performed in breast cancer cells.
Key findings: HDAC8 mRNA expression was upregulated in paired breast cancer tissue from Taiwanese patients and in paired breast cancer tissues from the TCGA data set. Hypomethylation of promoter regions was significantly correlated with HDAC8 mRNA overexpression in 588 breast cancer patients from the TCGA data set and was associated with poor prognosis in early-stage breast cancer. HDAC8 mRNA overexpression was associated with late stages and tumor progression. Wound healing and Transwell migration assays revealed that knockdown by si-HDAC8 or PCI-34051 treatment significantly inhibited breast cancer cell migration. Knockdown by si-HDAC8, Illumina BeadArray™ arrays and IPA found that ID3 and PTP4A2 pathways were regulated by HDAC8 in cancer cell migration.
Significance: Hypomethylation of the *HDAC8* promoter is correlated with HDAC8 overexpression and breast cancer progression and is a potential prognosis marker and drug target.
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1. Introduction

Breast cancer is the most common noncutaneous cancer in women and is second only to lung cancer as a leading cause of cancer-related mortality in the United States and Taiwan [1–3]. The initiation and progression of cancer, conventionally seen as a genetic disease, has been determined to involve epigenetic abnormalities in addition to genetic alterations [4]. The epigenetic machinery of cancer includes DNA methylation, histone modification, nucleosome positioning, and noncoding RNA expression, specifically microRNA expression [4]. Recently, studies have discovered that reversible alterations in histone proteins can lead to breast tumorigenesis [4–6]. Histone acetylation–deacetylation is considered the most well-understood posttranslational modification of core histones, and histones are acetylated and deacetylated by the opposing action of histone acetylases and histone deacetylases (HDACs) [7]. Recently, 18 HDAC enzymes have been identified in humans [8]. Differences in the HDAC family are not only limited to protein size and subcellular localization but also involve substrate specificity, enzymatic activity, and the tissue expression pattern [9]. Currently, much attention has been paid to the use of the isotype HDAC8 as a drug target [10]. HDAC8 is a zinc-dependent class I HDAC containing 377 amino acids

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(aa, 42 kDa) [11,12]. The forced overexpression of HDAC1, 6, or 8 or their knockdown by specific short interfering RNAs (siRNAs) revealed the involvement of these HDACs in cancer cell invasion and matrix metallopeptidase 9 (MMP-9) expression in MCF-7 and MDA-MB-231 breast cancer cells [13]. Among 20 breast cancer patients, 17 (85%) showed positive immunoreactivity for HDAC8 [14]. Currently, major efforts are focused on developing specific and selective HDAC isoform inhibitors and investigating combination therapies, with the aim of increasing potency against specific cancer types and overcoming drug resistance [9,15]. Studies have also evaluated the therapeutic effect of the HDAC8-specific inhibitor PCI-34051 on malignant peripheral nerve sheath tumors and T-cell lymphomas [16,17]. Developing potent selective inhibitors that specifically target HDAC8 with fewer adverse effects compared with those of pan-HDAC inhibitors is a current challenge [10]. Therefore, a more comprehensive study of alterations and its mechanisms in *HDAC8* is required for future clinical application of such inhibitors. In addition, it is unclear whether an HDAC8-specific inhibitor can provide therapeutic benefits for breast cancer. In this study, we evaluated whether the *HDAC8* gene is overexpressed in breast cancer. We also investigated mechanisms of dysregulation of HDAC8 expression and the biological role of HDAC8 in breast cancer.

2. Materials and methods

2.1. Patients and tissue collection

All human breast cancer samples ($n = 37$) were anonymous specimens obtained from Taipei Medical University Joint Biobank, Taipei Medical University Hospital and Cathay General Hospital, Taipei according to a protocol approved by a joint institutional review board. Before clinical data and sample collection, written informed consent was obtained from all patients. Histological evaluation revealed that all patient samples were composed of >80% tumor tissue. Tissue samples were immediately frozen and stored in liquid nitrogen. Sections of cancerous tissue and corresponding normal tissues were examined by a senior pathologist. Clinical data, including age, sex, personal and family medical history, tumor–node–metastasis (TNM) stage, differentiation grade, and ER and PR status, were prospectively collected.

2.2. RNA extraction

Matched pairs of primary tumors and adjacent normal breast tissues of the same patient were frozen immediately after surgical resection and stored at −80 °C. Total mRNA was extracted from these tissues using the RNeasy Plus Mini kit (QIAGEN, Bonn, Germany). After quantification of RNA, the purity was verified by measuring the A260/A280 ratio (which ranged from 1.8 to 2.0). The cDNA was synthesized using the iScript cDNA Synthesis kit (Bio-Rad Laboratories) according to of the manufacturer's protocols.

2.3. Real-time reverse transcription polymerase chain reaction

The expression of HDAC1 and HDAC8 mRNA was measured by real-time reverse transcription polymerase chain reaction (RT-PCR). The specific designed primers, corresponding Universal Probe Library probe (Roche Applied Science) and LightCycler 480 Probe Master kit (Roche Applied Science, Mannheim, Germany) were used to perform real-time RT-PCR according to the manufacturer's protocol. Relative

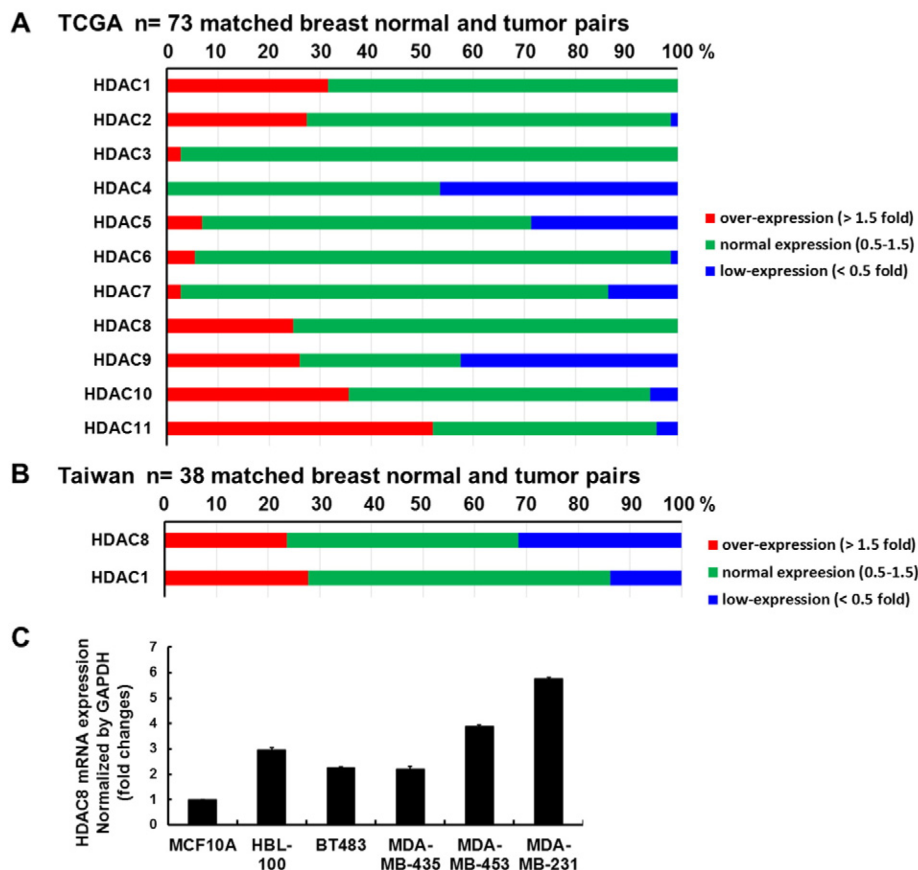

Fig. 1. The mRNA expression of HDACs in breast cancer. A, mRNA levels of HDAC1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 were analyzed in 73 matched normal and cancerous breast tissues from the TCGA data set. B, HDAC1 and HDAC8 mRNA levels were analyzed in 38 matched normal and cancerous breast tissues from Taiwan. C, HDAC8 mRNA expression was analyzed in normal and cancerous breast cell lines.

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