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Increased expression of mdig predicts poorer survival of the breast cancer patients

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ABSTRACT

Breast cancer is the most common cancer and the second leading cause of cancer death among women of all races and Hispanic origin populations in the United States. In the present study, we reported that the survival time of the breast cancer patients is influenced by the expression level of mdig, a previously identified lung cancer-associated oncogene encoding a JmjC-domain protein. By checking the expression levels of mRNA and protein of mdig through both RT-PCR and immunohistochemistry in samples from 204 patients, we noticed that about 30% of breast cancer samples showed increased expression of mdig. Correlation of the mdig expression levels with the survival time of the breast cancer patients indicated a clear inverse relationship between mdig expression and patient survival, including poorer overall survival, distant metastasis free survival, relapse free survival, and post-progression survival. Taken together, these data suggest that an increased expression of mdig is an important prognostic factor for poorer survival time of the breast cancer patients.

1. Introduction

Breast cancer is the most frequently diagnosed cancer among women in the US in the past several decades. In 2013, an estimation of 232,340 new cases of invasive breast cancer is expected (Siegel et al., 2013). A significant decrease in the incidence rate of breast cancer was noted since 2002, which was largely attributed to the reduced use of hormone replacement therapy (HRT) for the post-menopausal women and increased detection of early stage tumors through breast cancer screening. Based on the molecular features that represent distinct subtypes of the breast epithelial cells, breast cancers can be categorized into basal cell carcinoma, luminal carcinoma and $Her2^{+}$ carcinoma. The current 5-year survival rate of breast cancer is around 90%. Many factors affect patient survival after diagnosis and curative tumor resection, such as overweight, cigarette smoke, alcoholism, and the molecular subtypes of the tumor.

Inherited germline mutations of DNA repair genes BRCA1 and BRCA2 predispose risks of developing breast cancer, which accounted about 5–10% of breast cancer patients with Caucasian origination (Maxwell and Domchek, 2012). A most recent retrospective study also revealed that women with breast cancer and are carriers of brca1 mutations have increased mortality compared with non-carriers (Boyle, 2012). In addition to BRCA1 and BRCA2, a number of studies have also identified other gene signatures that are associated with poorer prognosis or therapeutic responses of the breast cancer patients (Marchionni et al., 2013). However, scanty and contradictory data exist on the survival-associated gene signatures. Such an inconsistence is further complicated by the history of adjuvant therapy and differences of bioinformatics platforms used in each study.

The mineral dust-induced gene (mdig) was first identified from alveolar macrophages obtained from people with occupational lung diseases (Lu et al., 2009; Zhang et al., 2005). This gene was independently characterized in cell lines with an overexpression of c-myc oncogene and named as myc-induced nuclear antigen 53 (mina53) (Tsuneoka et al., 2002) or nucleolar protein 52 (NO52) (Eilbracht et al., 2005). The mdig protein contains a conserved JmjC domain and is indicated in cell growth regulation, possibly through its effect on trimethylation of lysine 9 on histone H3 (H3K9me3) and hydroxylase activity on ribosomal proteins (Ge et al., 2012; Lu et al., 2009). Further studies demonstrated a strong association of mdig expression with human lung cancer (Lu et al., 2009), colon cancer (Teye et al., 2004), esophageal squamous cell carcinoma (Tsuneoka et al., 2004), neuroblastoma (Fukahori et al., 2007), renal cell carcinoma (Ishizaki et al., 2007), gastric carcinoma (Zhang et al., 2008), hepatocellular carcinoma (Ogasawara et al., 2010), and cholangiocarcinoma (Tan et al., 2012). It is unclear whether mdig is overexpressed in human breast cancer and if so, whether such an overexpression contributes to the prognostic

Abbreviations: ACC, adenoid cystic carcinoma; BRCA1, breast cancer 1 gene; DCIS, ductal carcinoma in situ; DMFS, distant metastasis free survival; HRT, hormone replacement therapy; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; Mdig, mineral dust-induced gene; OS, overall survival; PPS, post-progression survival; RFS, relapse free survival.

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outcomes of the breast cancer patients. In this report, we show that about 30% of breast cancers exhibited higher level of mdig expression and increased mdig expression predicts poorer survival of the breast cancer patients, including poorer overall survival (OS), distant metastasis free survival (DMFS), relapse free survival (RFS), and post-progression survival (PPS).

2. Materials and methods

2.1. Breast cancer tissue samples

A panel of cDNAs (TSCE101) derived from total RNAs of the individual breast cancer tissues was purchased from Origene (Rockville, MD). A tissue microarray containing 192 breast cancer tissue samples was purchased from US Biomax, Inc. (Rockville, MD).

2.2. PCR

The expression level of mdig and actin was determined by PCR using One-step RT-PCR kit (Promega, Madison, WI) by omitting the reverse transcription reaction. The PCR primers for mdig are: 5′-TCA TGT CGG GCC TAA GAG AC-3′ and *mdig*-b: 5′-GGC ATT TGA TTC TGC AAA GG-3′, which amplifies a 1510 bp DNA fragment covering the whole coding region of the mdig gene. PCR primers for β-actin are: 5′-TTC TAC AAT GAG CTG CGT GTG-3′ and 5′-GGG GTG TTG AAG GTC TCA AA-3′.

2.3. Immunohistochemistry

The tissue microarray slide BR2086 was purchased from US Biomax, Inc. (Rockville, MD) and deparaffinized with Xylene and hydrated in series of alcohol. To quench the endogenous peroxidase activity, slides were incubated with 1.5% H_2O_2 for 20 min at room temperature. Heat-mediated antigen retrieval was performed by boiling the slides in Citrate buffer, pH 6 for 20 min. To block the non-specific binding of immunoglobulin, slides were incubated with a solution consisting of 5% goat serum and 0.2% triton-X 100 in PBS for 2 h at room temperature, followed by incubation with monoclonal antibody against Mdig/Mina 53 (mouse anti-Mina 53, Invitrogen) in 1:100 dilution overnight at 4 °C. Next day goat anti-mouse biotinylated secondary antibody was applied at 1:200 dilution and incubated for 2 h at room temperature. Slides were then incubated with an ABC reagent (Vectastain Elite ABC kit) for 45 min at room temperature and the chromogen was developed with diaminobenzidine (DAB). The slides were counterstained with hematoxylin and mounted with entellan. All incubation steps were carried out in a humidified chamber and all washing steps were performed with 1× PBS. The images were captured under the bright field optics of the Nikon Eclipse Ti-S Inverted microscope (Mager Scientific, Dexter MI, USA).

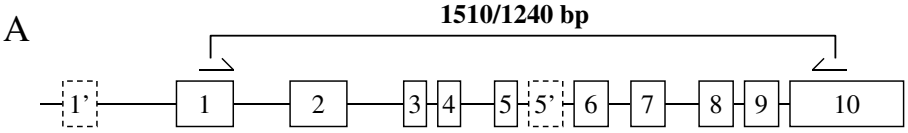
2.4. Kaplan–Meier survival analysis

A Kaplan–Meier survival database that contains survival information of 2880 breast cancer patients and gene expression data obtained by using three different versions of Affymetrix HG-U133 microarrays was used (Gyorffy et al., 2010). Two different mdig probe sets, probes 213188_s_at and 213189_at, are presented in this database. Probe set 213188_s_at was excluded because it detects the far end of the 3′-UTR of mdig mRNA and the antisense of 3′-UTR of the β-γ-crystallin domain containing 3 (CRYBG) mRNA. The probe set 213189_at that detects the open-reading frame (ORF) of mdig mRNA was used in this survival analysis. Survival curves resulting in p values of <0.05 between mdig higher ($mdig^{high}$) and mdig lower ($mdig^{low}$) groups were considered significantly different.

3. Results

3.1. Increased expression of mdig in breast cancer

An overexpression of mdig has been observed in a number of human cancers, implying its important role in the pathogenesis of human malignancies. To investigate whether mdig is also overexpressed in human breast cancer, the level of mdig expression was determined using a panel of cDNA derived from human breast cancer tissues by PCR. In previous studies using human lung cancer cell lines, we had detected multiple alternatively spliced isoforms of mdig mRNA, which rendered difficulties for quantitative real-time PCR. Accordingly, we used a traditional PCR procedure and primers that amplify a 1510 bp fragment covering the entire coding region of the mdig mRNA (Fig. 1A). As exhibited in Fig. 1B, mdig expression was detected in 4 out of 12 breast cancer samples, among which sample 10 showed a size-reduced fragment due to alternative splicing. Further analyses of the



C

	Type*	Grade	ER	PR
1	ILC	5	7	233
2	IDC	9	14	99
3	**IDC**	**6**	**142**	**528**
4	IDC	7	20	9
5	IDC	-	18	7
6	IDC	6	65	30
7	IDC	5	30	32
8	IDC	6	9	0.5
9	**ACC**	-	22	14
10	**IDC**	**5**	**3**	**0**
11	**DCIS**	**-**	**19**	**13**
12	**IDC**	**8**	**6**	**26**

*ILC: invasive lobular carcinoma
IDC: invasive ductal carcinoma
ACC: adenoid cystic carcinoma
DCIS: ductal carcinoma in situ
ER/PR values: fmol/mg

Fig. 1. Detection of mdig mRNA in breast cancer samples. A. Schematic indication of the mdig gene structure. Dashed boxes indicate alternative exons identified in some alternatively spliced mdig mRNAs. Half arrows denote the corresponding regions of the PCR primers. B. Expression of mdig in the indicated breast cancer samples. The expression levels of mdig were calibrated by β-actin. C. Tumor and patient information of B.