

## RESEARCH COMMUNICATION

# Loss of DBC2 Expression is an Early and Progressive Event in the Development of Lung Adenocarcinoma

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### Abstract

**Purpose:** DBC2 (Deleted in Breast Cancer 2) has been indicated to be a tumor suppressor gene in many cancers including lung adenocarcinoma recently. In this study, we aimed to explore the expression status of DBC2 in different subtypes of lung adenocarcinoma (from pre-invasive to invasive lesions), and to determine if downregulation becomes more marked with pathological progression. **Methods:** We collected 172 tissue samples from different subtypes of lung adenocarcinoma and investigated the frequency of DBC2 loss by immunohistochemistry. **Results:** Our results indicated that DBC2 downregulation is a relatively frequent event in lung adenocarcinoma. Moreover, as the adenocarcinoma subtype turns to be more invasive, more downregulation occurred. **Conclusion:** We conclude that loss of DBC2 expression is an early and progressive event in the pathogenesis of lung adenocarcinoma. Positive DBC2 immunohistochemistry may become an indicator for early stage disease and better prognosis of lung adenocarcinomas.

**Keywords:** Lung adenocarcinoma - DBC2 - invasive subtypes - tumor suppressor gene

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### Introduction

Lung cancer is currently the leading cause of cancer-related death in most Asian countries and the world (Merel Kimman et al., 2012), of which adenocarcinoma accounts for more than 50% of all cases (Pao and Girard, 2011). Lung adenocarcinoma is of diverse subtypes, the new classification includes pre-invasive lesions, minimally invasive adenocarcinoma, invasive adenocarcinoma and some variants (Travis et al., 2011). A growing group of research supports a multistep process model in lung adenocarcinoma development, during which genetic mutations are sequentially accumulated (Raz et al., 2006). For some tumor suppressor genes, like P53, of which the incidence of mutation varies with the histological types, there was a trend that more mutation occurred as the adenocarcinoma turns to be more invasive (Koga et al., 2001).

Recently, DBC2 is isolated as a tumor suppressor gene from human chromosome 8p21, and it is the best candidate tumor suppressor gene from this region (Hamaguchi et al., 2002). Incidence of DBC2 losses has been detected in various carcinomas including prostate, breast, lung, colon, rectum, bladder, liver and larynx carcinoma (Emi et al., 1992; Lundgren et al., 1992; Bova et al., 1993; Fujiwara et al., 1993; Sunwoo et al., 1996). In lung carcinoma, there was a report that the allelic losses at chromosome 8p21-23 were over 80% (17 of 21) (Wistuba et al., 1999). Accumulating evidence suggested that DBC2 may play an important part in the carcinogenic process by down

regulation or loss of function (Siripurapu et al., 2005; Collado et al., 2007; Yoshihara et al., 2007). However, the downregulation of DBC2 in different subtypes of lung adenocarcinoma, and the relationship between incidence rates and pathologic subtypes remain unclear. To explore these questions, we collected 172 tissue samples of different subtypes of lung adenocarcinoma and examined the expression status of DBC2 by immunohistochemistry (IHC) in this study.

### Materials and Methods

Neutral formalin fixed and paraffin embedded tissue samples of different subtypes of resectable adenocarcinoma from 172 patients who were admitted and operated from 2006 to 2010 at Department of Thoracic Surgery of Provincial Hospital Affiliated to Shandong University were collected. Totally, we collected 47 pre-invasive lesions tissue samples (bronchioloalveolar carcinoma (BAC) in previous classification of lung adenocarcinoma), 65 minimally-invasive adenocarcinoma (BAC with invasive component in old classification) and 60 invasive carcinoma tissue samples. Tissue sections of 4-um thick were cut and deparaffinized and rehydrated through a graded alcohol series. Endogenous peroxidases activity was quenched by 3% hydrogen peroxide incubation for 15 min, after 15-min rinses in three changes of PBS (Phosphate Buffered Saline), sections were blocked with normal goat serum to suppress nonspecific background staining. The rabbit anti-human DBC2 antibody (N15)

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(Delta Biolabs, CA, USA, dilution 1:50) was applied to the sections at 4 °C overnight. Then the sections were incubated with biotinylated goat anti-rabbit IgG for 30 min at room temperature and processed according to the SP kit (Santa Cruz, Biotechnology, CA, USA) protocol. Sections of normal lung tissue were used as a positive control to prove the specificity of the antibodies. In the negative control, the primary antibody was replaced by PBS instead.

Sections were examined and scored for immunoreactivity by two observers who were unaware of the histological diagnosis or clinical features. Specific classification and gradation were recorded as described elsewhere (Hou et al., 2010): -, no immunopositive cells; +, <30% of tumor cells are immunopositive; ++, 30%-60% of tumor cells are immunopositive; +++, > 60% of tumor cells are immunopositive. The scores represented the percentage of immunopositive cancer cells in the tumor area of one section, and the average count of 5 random high-power fields (×400) on one section was used. For the samples with different results the two observers performed a second review to achieve a conclusive judgment (Wang et al., 2011).

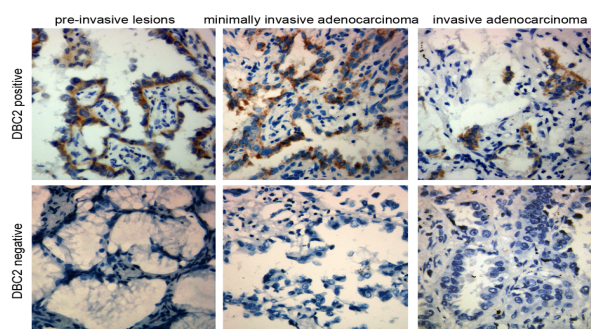
Spearman rank correlation analysis (SPSS statistical software, version 17.0) was used in the analysis of correlation between the DBC2 expression and clinical features. And the Kruskal-Wallis test was used to see whether DBC2 was differentially expressed between different histological types of lung adenocarcinoma. The results were reported to be statistically significant if correlated P value was less than 0.05.

## Results

DBC2 expression in lung adenocarcinoma of different subtypes is shown in Figure 1, and its expression status in patients of different sex, age, degrees of differentiation and histological subtypes is shown in Table 1. Results demonstrated that DBC2 downregulation existed in 55%

**Table 1. The Expression Status of DBC2 in Patients of Different Sex, Age, Degrees of Differentiation and Subtypes of Lung Adenocarcinoma**

	Degrees of expression				Total	Rs	P
	-	+	++	+++			
Sex							
Male	51	6	6	14	77	-0.063	0.408
Female	69	5	6	15	95		
Age							
<50	29	6	6	5	46	-0.048	0.553
≥50	91	5	6	24	126		
Differentiation							
Low	7	2	1	1	11	-0.054	0.482
Media	89	7	9	24	129		
High	24	2	2	4	32		
Subtypes							
pre-invasive lesions	26	4	7	10	47	-0.209	0.006
minimally invasive adenocarcinoma	45	4	5	11	65		
invasive adenocarcinoma	49	3	0	8	60		



**Figure 1. Expression and Downregulation of DBC2 in Pre-invasive Lesions, Minimally Invasive and Invasive Adenocarcinoma. (×400) (DBC2, Deleted in Breast Cancer 2)**

(26/47) of pre-invasive lesions, 69% (45/65) of minimally invasive adenocarcinoma and 82% (49/60) of invasive adenocarcinoma, and totally 70% (121/172) of all these subtypes of lung adenocarcinoma.

The result of Kruskal-Wallis test showed that there was significant difference in the DBC2 downregulation between different histological subtypes ( $\chi^2=8.598$ ,  $P=0.003$ ). And there was statistical significance in the correlation of DBC2 expression degrees and different histological subtypes ( $R_s=-0.209$ ,  $P=0.006$ ), which means as the adenocarcinoma turns to be more invasive, more DBC2 losses occurred. However, the expression status of DBC2 had no significant correlations with different sex, age of patients, and degrees of differentiations of tumor tissues.

## Discussion

Usually, in the course of carcinogenesis, cancer cells acquire a number of critical genetic changes: oncogenes activated in function and tumor suppressor genes downregulated or ablated. Discovery and analysis of these genetic changes have contributed to a better understanding of the molecular basis of cancer development (Mao et al., 2010). Many mutations, especially those involving recessive oncogenes, have been described in invasive lung carcinomas (Akita, 2004). Hamaguchi et al have found a novel tumor suppression gene named Deleted in Breast Cancer 2 (DBC2) in human chromosome 8p21, which belonged to the RhoBTB family (Hamaguchi et al., 2002). Recent researches indicate that DBC2 participates in diverse cellular activities, significantly influences cell-cycle, apoptosis, cytoskeleton and membrane-trafficking pathways (Siripurapu et al., 2005; Chang et al., 2006). DBC2 really plays as an important tumor suppressor gene by inhibiting proliferation, preventing colony formation and promoting the apoptosis of tumor cells (Mao et al., 2011). Successive studies showed that the downregulation of DBC2 was found in 58% (11/19) of breast, 50% (7/14) of lung, and 75% (9/12) of bladder tumor cell lines (Knowles et al., 2005). In our study, we detected the expression level of DBC2 in resectable lung adenocarcinoma by IHC. An overall 70% (121/172) downregulation rate suggested that DBC2 losses were frequent events in pathologic progression of lung adenocarcinoma.

Neoplastic transformation is considered to be the result of a multistep accumulation of genetic abnormalities, including either activation of oncogenes or inactivation of tumor suppressor genes (Koga et al., 2001), and sometimes there are genetic variants between different stages or subtypes (Xiao et al., 2011). In lung adenocarcinoma, tumor carcinogenesis performed as a stepwise progression from atypical adenomatous hyperplasia (AAH) through bronchioloalveolar carcinoma to invasive lung adenocarcinoma (Nakano et al., 2008). However, whether the downregulation of DBC2 in the pathologic progress of lung adenocarcinoma was a stepwise event remains unclear. To explore the possible relationship between DBC2 downregulation and carcinogenesis of lung adenocarcinoma, we examined the DBC2 expression in three different histological subtypes of adenocarcinoma in this study. As we predicted, DBC2 downregulation varied significantly in different histological subtypes. Moreover, as the adenocarcinoma turns to be more invasive, more DBC2 losses occurred, which also indirectly proved the development of lung adenocarcinoma as a multi-step progression disease. And also, expression of DBC2 in lung adenocarcinoma patients may become an indicator for earlier stage of the disease and better prognosis.

To our knowledge, this is the first molecular evidence related to DBC2 expression properties in the different histological subtypes of human lung adenocarcinoma. Although further studies on the mechanism of DBC2 downregulation or silencing, and its specific function in carcinogenesis should be taken, our results suggest that the frequent loss of DBC2 expression is an early and progressive event in the pathogenesis of lung adenocarcinoma.

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