

## Original Article

# High expression of REGγ is associated with metastasis and poor prognosis of patients with breast cancer

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**Abstract:** REGγ (REGγ) has been recently found in several types of human cancer, however, its clinical significance in metastasis and prognosis of breast cancer remains unknown. In this study, immunohistochemical staining and western blot analysis were performed to evaluate REGγ expression in both mouse and human breast cancer specimens. We found that in MMTV-PyMT mice, 14 out of 20 (70%) mouse mammary carcinomas were REGγ positive, which was significantly higher than control (0/20, 0%,  $P < 0.001$ ) and lower than metastatic lung tumour (20/20, 100%,  $P = 0.027$ ). Further investigation for REGγ expression in 136 human breast cancer tissues with the paired peritumoural normal breast tissues and 140 breast benign disease tissue samples showed that REGγ was undetectable in normal breast tissues and nonmetastatic axillary lymph nodes (ALNs), whereas 111 out of 136 (81.6%) breast cancer tissue samples were REGγ positive, which was significantly higher than breast benign disease tissues (9/140, 6.4%,  $P < 0.001$ ) and lower than metastatic ALNs (116/116, 100%,  $P < 0.001$ ). The 5-year disease-free and overall survivals of patients with negative/low level of REGγ were significantly higher than those of patients with high level of REGγ ( $P < 0.05$ ). Cox regression analyses further indicated that REGγ could serve as a novel independent prognostic factor for breast cancer (OR = 4.369,  $P = 0.008$ ). Our results suggest that the high expression of REGγ might predict metastasis and poor prognosis in breast cancer.

**Keywords:** REGγ, breast cancer, prognosis, survival analysis

## Introduction

Breast cancer is the most frequently diagnosed cancer among women worldwide [1, 2]. While many breast cancer biomarkers have been identified for clinical practice, it is still difficult to match a particular patient with appropriate treatment because of the heterogeneity of cancer cells. Hence, there is an urgent need to identify new indicators for breast cancer diagnosis and prognosis.

Proteasome activator subunit 3, also known as Ki, PA28γ or REGγ (REGγ), was first identified as Ki antigen, which is a nuclear protein targeted by autoantibodies and is found in sera of systemic lupus erythematosus patients [3]. REGγ is a member of 11S proteasome regulator which includes three subunits: alpha, beta and gamma [4-6]. Although the bio-

logical roles of REGγ have not been completely understood, it has been reported that REGγ stimulated the proteolytic activity of 20S proteasome core, whereby protein substrates could diffuse into the environment of proteasomes [7, 8]. The function of REGγ was elucidated by gene-targeting and while REGγ-deficient mice were born without appreciable abnormalities, but growth retardation and cell specific mitotic defects were observed [9]. The REGγ-deficient embryonic fibroblasts showed impeded entry from G to S phase in cell cycle [10]. Recently, it had been reported that REGγ was highly expressed in multiple human cancers, such as breast cancer [11, 12], thyroid cancer [13, 14], hepatocellular carcinoma [15] and colorectal cancer [16-18]. In breast cancer, REGγ enhanced the oncogenicity of breast cancer cells by promoting cell growth, inhibiting cell apoptosis, degrading p21 and suppressing acti-

vation of NK, suggesting that REGy is involved in multiple processes of cancer progression [12]. However, the clinical significance of REGy expression in breast cancer has not been systematically studied. In the present study, we examined the expression of REGy in mouse mammary tumour virus-polyomavirus middle T antigen transgenic mouse model (MMTV-PyMT), as well as in surgical specimens of human breast cancer, axillary lymph node (ALN), breast benign disease and normal breast tissues, and analyzed the relationship between REGy expression and prognosis of breast cancer patients as well as clinical pathological parameters. REGy was highly expressed in breast cancer tissues and metastatic ALNs, but not in normal breast tissues and rarely in breast benign disease tissues. REGy expression was positively correlated with poor clinical parameters and prognosis of patients with breast cancer. These results suggested that REGy could be a novel prognostic indicator for breast cancer.

### Materials and methods

#### *Mouse samples*

MMTV-PyMT mice (FVB/N-Tg MMTV-PyVT 634 Mul/J mice, #002374) and control FVB ones (FVB/NJ, nontransgenic, #001800) were obtained from Jackson Laboratory (Bar Harbor, Maine, USA) and bred in the specific pathogen free level animal facilities. Experimental mice were generated from the same cohort of breeding pairs. All pups were weaned and tail-clipped at age 4 weeks, genotyped for MMTV-PyMT transgene (5'-GGAAAGTCACTAGGAGCAGGG-3' and 5'-GGAAGCAAGTACTTCAAGGG-3'). Animal care and use followed National Research Council guide for the laboratory animals.

#### *Patient specimens*

A total of 136 breast cancer with paired peritumoural (> 3 cm) normal breast tissue and 140 breast benign disease tissue paraffin specimens were obtained from patients who experienced open surgical excision from December 2008 to April 2009 in breast disease center of Southwest Hospital, Third Military Medical University, Chongqing, China. The paired metastatic ALNs were included as well. As a validation of REGy expression pattern as detected by immunohistochemical (IHC) staining, another

set of fresh breast tissue samples (30 breast cancers and paired peritumoural normal breast tissues, ALNs and 30 breast benign disease tissues) were collected for the western blot analysis. All pathological diagnoses were performed by pathology department of Southwest Hospital. All patients enrolled in this study were completely monitored for 5 years. This study was in agreement with the Helsinki Declaration and approved by the ethics committee of Southwest Hospital, the informed consent forms were signed by all the subjects.

#### *Immunohistochemistry and scoring*

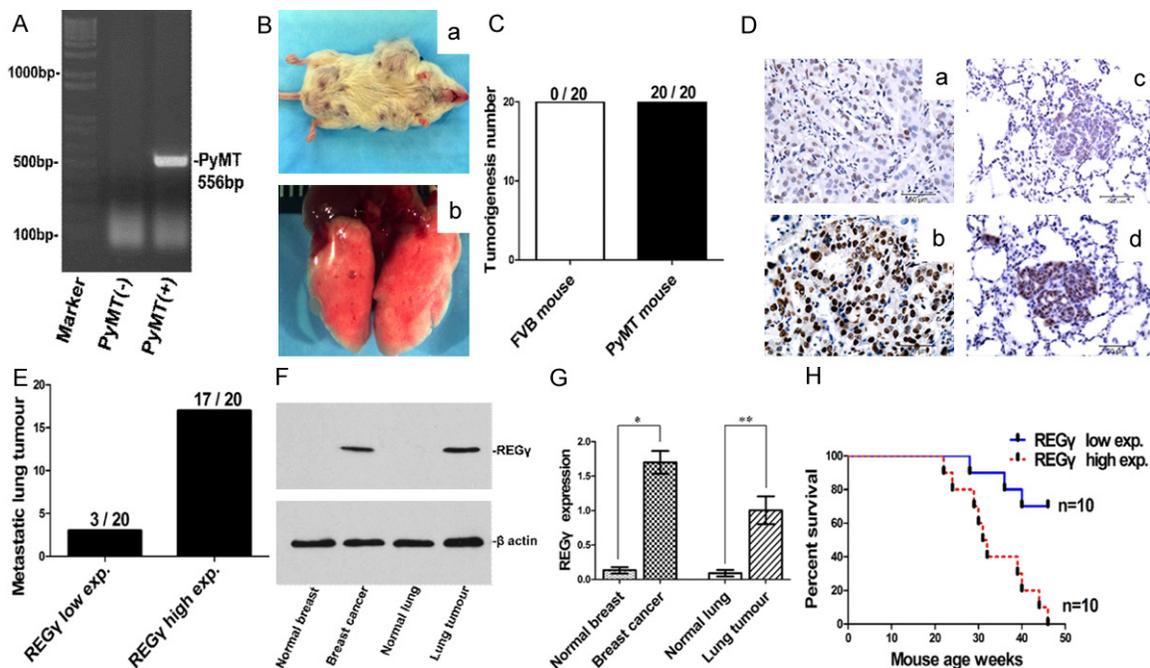
Sections were deparaffinized and rehydrated, antigen retrieval was performed in a pressure cooker with sodium citrate buffer (10 mM/L, pH 6.0). The endogenous peroxidase activity was blocked by 3% hydrogen peroxide in methanol. Sections were blocked with serum-free blocker (DAKO, USA, #X0909), and incubated with anti-REGy monoclonal antibody (Invitrogen, USA, #710800, at 1:600 dilution) for 12 hours at 4°C. Sections were incubated with EnVision + System-HRP antibody (DAKO, USA, #K4010) for 15 minutes at room temperature before DAB (DAKO, USA, #K3467) staining. Finally, sections were counterstained with Mayer's haematoxylin (DAKO, USA, #S3309). A REGy-positive section was used as positive control, and the same concentration of non-immune rabbit IgG was applied as negative control.

Immunoreactivity was scored for the extent and intensity of the nuclear staining. Extent of positivity was scored as follows: 0, no positive cells; 1, < 25% positive cells; 2, 25-50% positive cells; 3, 50-75% positive cells and 4, > 75% positive cells. Intensity was scored as follows: 0, no positive staining; 1, weak staining; 2, moderate staining and 3, strong staining. Multiplying extent by intensity gave the REGy staining scores from negative expression (0), positive low expression (1-6) to positive high expression (> 6) accordingly. The REGy scoring was evaluated independently by two investigators in a blinded fashion, and the scores were thereafter averaged.

#### *Western blot analysis*

For tissue protein extraction, samples were snap-frozen in liquid nitrogen and homogenized in RIPA buffer with proteinase inhibitors. Protein

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**Figure 1.** REGy expression in mouse mammary carcinoma and metastatic lung tumour. A. DNA genotyping showing MMTV-PyMT transgene band at 556 bp. B. Mammary carcinoma (a) and metastatic lung tumour (b) in MMTV-PyMT mice. C. Tumorigenesis was found in all MMTV-PyMT mice (20/20, 100%) whereas no tumour was found in FVB control mice (0/20, 0%). D. Representative REGy IHC staining images for mouse mammary carcinoma and metastatic lung tumour (bar = 50  $\mu$ m). REGy low expression in mammary carcinoma (a) was associated with low level of REGy expression in metastatic lung tumour (c), and REGy high expression in mammary carcinoma (b) was correlated with high level of REGy expression in the metastatic lung tumour (d). E. Statistical analysis showed that all metastatic lung tumours were REGy positive (20/20, 100%): 17 metastatic tumours had high level of REGy expression and 3 tumours had low level of REGy expression. F. Representative Western blot result of REGy expression in mouse fresh tissue. G. Densitometry analysis showing relative expression of REGy as detected by Western blot in fresh samples (20 mice for each group). Statistical analysis showed the significant difference between normal breast tissue and breast cancer tissue ( $P = 0.0051$ ), as well as between normal lung tissue and metastatic lung tumour ( $P = 0.0190$ ). H. Survival analysis in a set of MMTV-PyMT mouse (10 mice for each group) showed that mice with high expression of REGy had significantly poor survival than those with low expression of REGy (Log-rank Mantel-Cox Test,  $P = 0.0013$ , Hazard Ratio = 0.1448, 95% CI of ratio is 0.04474 to 0.4686).

was quantified by ELISA plate reader (BIO-TEK Synergy HT, USA). Total protein lysate (30  $\mu$ g) was separated in 10% SDS-PAGE and transferred to nitrocellulose membranes (BIO-RAD, USA, #162-0115). Membranes were blocked with Blotting-Grade Blocker (BIO-RAD, USA, #170-6404) and incubated with anti-REGy monoclonal antibody (Invitrogen, USA, #710800, at 1:1000 dilution). Blots were developed by using the enhanced chemiluminescence method (Thermo Scientific, USA, #1856135/6). To verify the equal loading, blots were stripped and re-probed for  $\beta$ -actin.

### Statistical analysis

Correlations between REGy expression and clinicopathological parameters were studied with the chi-square test. Survival rates were estimated by Kaplan-Meier method, survival

curves were compared with the Log-rank (Mantel-Cox) test. The Cox proportional hazards regression analysis was applied for patient prognosis. A value of  $P < 0.05$  was considered as a significant difference. GraphPad Prism 5.01 (GraphPad Software, Inc., La Jolla, CA, USA) and SPSS statistics 20 (IBM Corp., Armonk, NY, USA) were used for analyses.

### Results

#### *REGy is highly expressed in MMTV-PyMT mouse mammary carcinomas and metastatic lung tumours*

REGy expression was investigated in MMTV-PyMT mouse mammary carcinoma samples. DNA genotyping was performed to confirm mice genetic backgrounds (**Figure 1A**). As shown in **Figure 1B**, all MMTV-PyMT virgin female mice

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**Table 1.** REGy expression in mouse mammary gland, mammary carcinoma and lung tumour

	Case	REGy expression			Positive (%)	P-value
		Neg.	low	high		
Normal mammary gland	20	20	0	0	0/20 (0%)	< 0.001
Mammary carcinoma	20	6	5	9	14/20 (70%)	
Metastatic lung tumour	20	0	3	17	20/20 (100%)	0.027

The expression of REGy in mouse mammary carcinoma was significantly higher than normal mammary gland tissue ( $P < 0.001$ ) and lower than metastatic lung tumour ( $P = 0.027$ ).

(20/20, 100%) developed mammary gland carcinoma (a) and metastatic lung tumour (b) at age 18 weeks, whereas no tumour was found in the control FVB normal mice (Figure 1C). Figure 1D showed MMTV-PyMT mice sample IHC staining results, low level of REGy expression in mammary carcinoma (a) was associated with weak REGy expression in metastatic lung tumour (c) whereas the high level of REGy expression in mammary carcinoma (b) was correlated with strong REGy expression in metastatic lung tumour (d). Statistics showed that all MMTV-PyMT mice metastatic lung tumours were REGy positive, and most of the metastatic tumours (17/20, 85%) had high level of REGy expression (Figure 1E). The IHC staining showed that mammary gland tissues in all FVB control mice were REGy negative (0/20), while REGy positive expression was detected in 14 out of 20 (70%) MMTV-PyMT mouse mammary carcinomas and all the metastatic lung tumours (20/20, 100%). REGy expression in MMTV-PyMT mouse mammary carcinoma was significantly higher than FVB normal mouse mammary gland tissue ( $P < 0.001$ ) and lower than metastatic lung tumour ( $P = 0.027$ ) (Table 1). REGy protein expression level was next validated by western blot. As shown in Figure 1F and 1G, there was no obvious REGy protein expression in FVB mouse normal breast tissue and normal lung tissue, but a significant high level of REGy expression was detected in MMTV-PyMT mouse breast cancer tissue ( $P = 0.0051$ ) and metastatic lung tumour ( $P = 0.0190$ ). In a set of MMTV-PyMT mice overall survival analysis (10 mice for each group), mice with the high expression level of REGy showed lower percent of survival than that of mice with the low expression of REGy (Figure 1H). These data suggested that MMTV-PyMT was an ideal mouse model to further investigate the biological functions of REGy in breast cancer research. Moreover,

these results further indicated a prognostic role of REGy in MMTV-PyMT mouse mammary gland carcinoma progression.

*REGy is highly expressed in human breast cancer tissue*

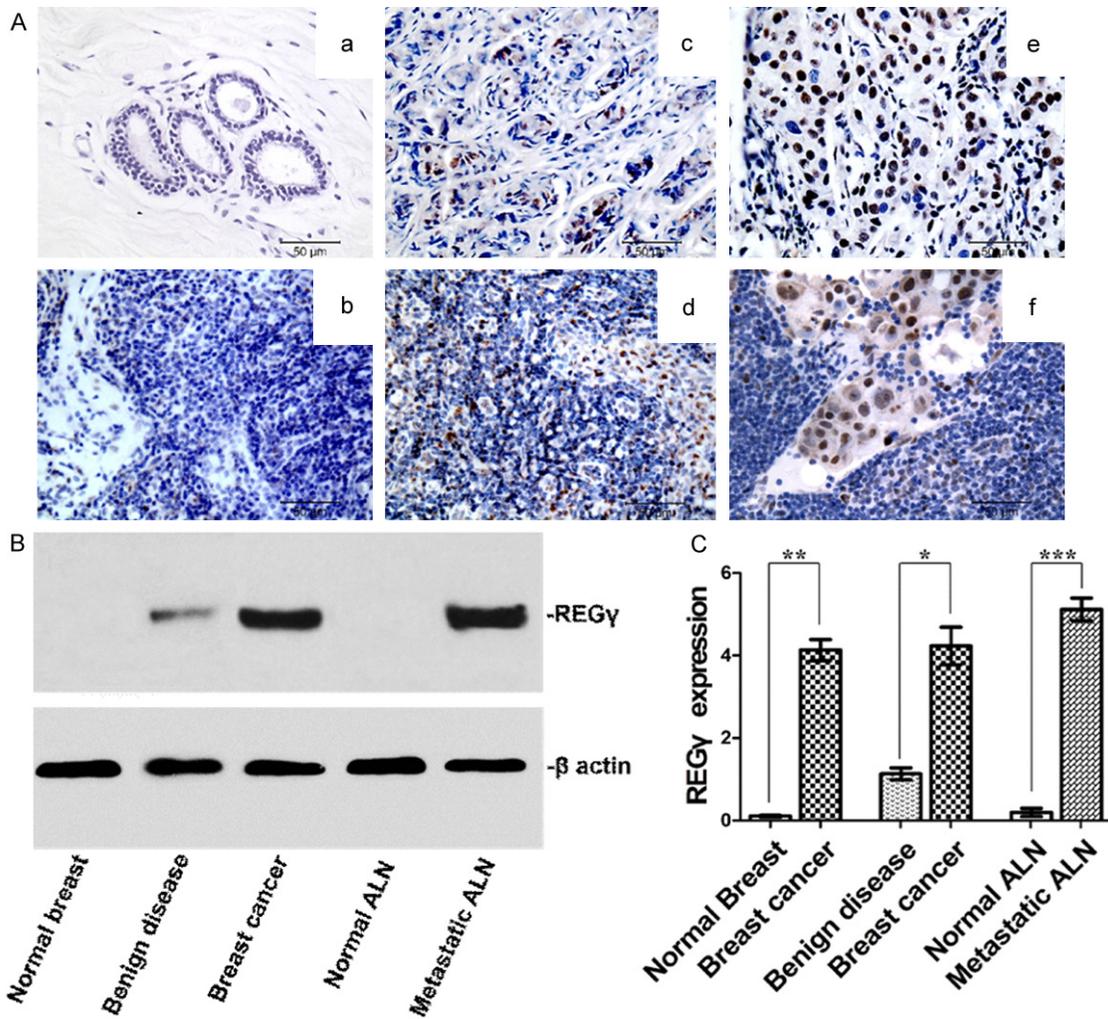
Out of the 136 breast cancer samples evaluated by IHC, 25

specimens (18.4%) were REGy negative while 111 specimens (81.6%) were REGy positive. REGy was undetectable in normal breast tissues and normal ALN (Figure 2Aa and 2Ab). Breast cancer tissue with low expression level of REGy (c) was associated with low level of REGy expression in ALN (d), whereas breast cancer tissue with high expression level of REGy (e) was correlated with high level of REGy expression in ALN (f). The positive rate of REGy expression in breast cancer tissue (111/136, 81.6%) was significantly higher than normal breast tissue (0/136, 0%) as well as breast benign disease tissue (9/140, 6.4%) ( $P < 0.001$ ), and lower than metastatic ALN (116/116, 100%) ( $P < 0.001$ ) (Table 2). Consistent with the IHC data, western blot analysis in human fresh tissue samples did not detect REGy protein expression in normal breast tissue and normal ALN. In contrast to control normal tissues, a significant higher level of REGy expression was detected in breast cancer tissue ( $P = 0.0024$ ) as well as in metastatic ALN ( $P = 0.0007$ ) (Figure 2B, 2C). The expression of REGy in human breast tissue was in accordance with its expression pattern in metastatic ALN, which suggested a potential prognostic role of REGy in human breast cancer.

*REGy expression is positively correlated with poor clinicopathological features*

Using chi-square test, REGy expression level did not correlate with breast cancer patient age (mean age = 50) ( $P = 0.702$ ), patient menopausal state ( $P = 0.171$ ), progesterone receptor (PR) status ( $P = 0.211$ ) or human epidermal growth factor receptor-2 (HER-2) status ( $P = 0.557$ ). However, REGy expression was positively correlated with breast tumour size ( $P < 0.001$ ), ALN metastasis state ( $P < 0.001$ ), ALN

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**Figure 2.** REGy expression in human normal breast tissue, normal axillary lymph node (ALN), breast cancer tissue and metastatic ALN. A. Representative REGy IHC staining images (bar = 50  $\mu$ m). No REGy expression was found in normal breast tissue (a) and normal ALN (b). Breast cancer tissue with low expression level of REGy (c) was associated with low level of REGy expression in ALN (d). Breast cancer tissue with high expression level of REGy (e) was correlated with high level of REGy expression in ALN (f). B. Representative western blot result of REGy expression in a different set of human fresh tissue. C. Densitometry analysis showing relative expression of REGy as detected by western blot (30 for each). Statistical analysis showed significant difference between normal breast and breast cancer ( $P = 0.0012$ ), breast benign disease and breast cancer ( $P = 0.0024$ ), as well as between normal ALN and metastatic ALN ( $P = 0.0007$ ).

metastasis number ( $P < 0.001$ ), tumour TNM stage ( $P = 0.038$ ), histological differentiation ( $P = 0.001$ ) and estrogen receptor- $\alpha$  (ER $\alpha$ ) status ( $P < 0.001$ ). Our data indicated a significant positive correlation between REGy expression and the poor clinicopathological features in patients with breast cancer (Table 3).

*REGy expression is correlated with poor clinical prognosis*

At the last follow up point (April 30, 2014), there were 101 breast cancer patients alive while 35

deceased. None of breast benign disease patients experienced recurrence or progression to invasive cancer. The Kaplan-Meier survival curves demonstrated 5-year survival rate of breast cancer patients. The period covered range from 0 to 64 months after the initial surgery. Significant difference in breast cancer patient clinical outcome was found between REGy negative expression and REGy low expression groups (OS, Log-rank Mantel-Cox Test,  $P = 0.0406$ . Hazard Ratio = 0.2913, 95% CI of ratio is 0.08157 to 1.040. DFS, Log-rank Mantel-Cox

**Table 2.** REGy expression in human breast tissue and meta-static axillary lymph node

	Case	REGy expression			Positive %	P-value
		Neg.	Low	High		
Benign disease	140	131	9	0	6.4%	0.008
Normal breast	136	136	0	0	0%	
Breast cancer	136	25	52	59	81.6%	< 0.001
Metastatic ALN	116	0	29	87	100%	< 0.001

ALN, axillary lymph node. REGy expression in breast benign disease tissue was significantly higher than normal breast ( $P = 0.008$ ). REGy expression in breast cancer was significantly higher than normal breast tissues ( $P < 0.001$ ) and lower than metastatic ALN ( $P < 0.001$ ).

Test,  $P = 0.0405$ . Hazard Ratio = 2.432, 95% CI of ratio is 0.9813 to 6.027) as well as between REGy low expression and REGy high expression groups (OS, Log-rank Mantel-Cox Test,  $P = 0.0351$ . Hazard Ratio = 0.4949, 95% CI of ratio is 0.2516 to 0.9736. DFS, Log-rank Mantel-Cox Test,  $P < 0.0001$ . The median survival time in REGy low expression group is 57 months, and 14 months in REGy high expression group, Hazard Ratio = 4.071, 95% CI of ratio is 3.505 to 4.638) (Figure 3A, 3B). In breast cancer patient, the Cox proportional hazards regression analysis data indicated that the expression level of REGy could be an independent prognostic factor for breast cancer ( $P = 0.008$ , OR = 4.369, 95% CI of ratio is 1.482 to 12.880). ER $\alpha$  positive was a protective factor (OR = 0.152, 95% CI of ratio is 0.073 to 0.312) and ALN metastasis was a risk factor (OR = 1.648, 95% CI of ratio is 1.141 to 2.382) for breast cancer patients in this study (Figure 3C-E). Collectively, based on the current survival analysis results, a significant positive correlation was established between REGy positive expression and breast cancer patient poor clinical outcomes, which suggesting an important prognostic significance of REGy expression in breast cancer.

**Discussion**

As a proteasomal regulator, REGy has been reported to be associated with the degradation of both oncogenic and tumour suppressing proteins such as HCV core protein [19, 20], SRC-3 [21], p16, p19, p21 [22, 23], and p53 [24-26]. Despite the recent progress in REGy-related

studies, the biological function and tissue distribution of this protein remained unclear, especially in breast cancer research. In the present study, we showed direct proof that REGy is a candidate biomarker to distinguish malignant and normal breast tissues in both human and MMTV-PyMT mouse samples. As shown by western blot analyses, REGy protein was highly expressed in human breast cancer tissue, metastatic ALN, MMTV-PyMT mouse mammary gland carcinoma as well as in metastatic lung tumour, which is highly consistent with the

results from IHC staining. The capability of REGy expression level to be used as biomarker to distinguish breast cancers from normal breast and breast benign disease tissues suggested that REGy has potential to be used as a new indicator for breast cancer. Our findings that REGy was highly expressed in human breast cancer tissue and metastatic tumour were in accordance with the previous REGy-related studies [11, 12]. Compared with the reports in which polyclonal PA28y antibody was used, we had improved IHC technique to detect REGy expression by using anti-REGy monoclonal antibody, which we have shown to yield more accurate and credible results. So far, we did not find the correlation between REGy expression and HER-2 expression status, which is different with the previous report [11]. Considering that both studies only used a small sample size in research, the further larger sample size investigation is still needed for better understating the relationship between REGy expression and HER-2 status. Interestingly, our data showed a significant high positive rate of REGy expression in ER $\alpha$  positive human breast cancer tissue samples (65/77, 84.4%) compared to ER $\alpha$  negative ones (26/59, 44.1%) (Table 3), which may shed light on the therapeutic significance of REGy in breast cancer endocrine therapy, and we will further investigate the possible mechanisms in our future intensive studies.

We also examined the expression pattern of REGy in MMTV-PyMT transgenic mouse, which is a long-term used mammary gland carcinoma

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**Table 3.** Relationship between REGy expression and cancer patient clinicopathological features

Characteristics	REGy expression			P-value
	Case (%)	Negative (%)	Positive (%)	
Age				0.702
≤ 50	76 (55.9%)	23 (30.3%)	53 (69.7%)	
> 50	60 (44.1%)	20 (33.3%)	40 (66.7%)	
Menopausal state				0.171
No	80 (58.8%)	42 (52.5%)	38 (47.5%)	
Yes	56 (41.2%)	36 (64.3%)	20 (35.7%)	
Tumour size				< 0.001
T1 + T2	84 (61.8%)	58 (69.0%)	26 (31.0%)	
T3 + T4	52 (38.2%)	17 (32.7%)	35 (67.3%)	
ALN metastasis state				< 0.001
No	20 (14.7%)	20 (100%)	0 (0%)	
Yes	116 (85.3%)	0 (0%)	116 (100%)	
ALN metastasis number				< 0.001
< 4	66 (48.5%)	46 (69.7%)	20 (30.3%)	
≥ 4	70 (51.5%)	16 (22.9%)	54 (77.1%)	
TNM Stage				0.038
0 + I	79 (58.1%)	39 (49.4%)	40 (50.6%)	
II + III	57 (41.9%)	18 (31.6%)	39 (68.4%)	
Histological differentiation				0.001
well	67 (49.3%)	36 (53.7%)	31 (46.3%)	
Poor + moderate	69 (50.7%)	18 (26.1%)	51 (73.9%)	
Estrogen receptor-α				< 0.001
Negative	59 (43.4%)	33 (55.9%)	26 (44.1%)	
Positive	77 (56.6%)	12 (15.6%)	65 (84.4%)	
Progesterone receptor				0.211
Negative	58 (42.6%)	27 (46.6%)	31 (53.4%)	
Positive	78 (57.4%)	28 (35.9%)	50 (64.1%)	
HER-2				0.557
Negative	92 (67.6%)	34 (37.0%)	58 (63.0%)	
Positive	44 (32.4%)	14 (31.9%)	30 (68.1%)	

ALN, axillary lymph node. HER-2, Human epidermal growth factor receptor-2. P = Statistical significance by chi-square test. A two-sided  $P < 0.05$  was considered to be statistically significant.

mouse model. Mammary gland specific expression of PyMT under the control of MMTV promoter/enhancer resulted in widespread transformation of mouse mammary epithelium and the development of multifocal mammary adenocarcinomas as well as the metastatic lesions in lungs [27-29]. MMTV-PyMT mouse has short tumorigenesis latency, high penetrance and high incidence of metastatic lung tumour occurring independently of pregnancy and with reproducible kinetics of progression [30-35]. In our study, the expression level of REGy in MMTV-

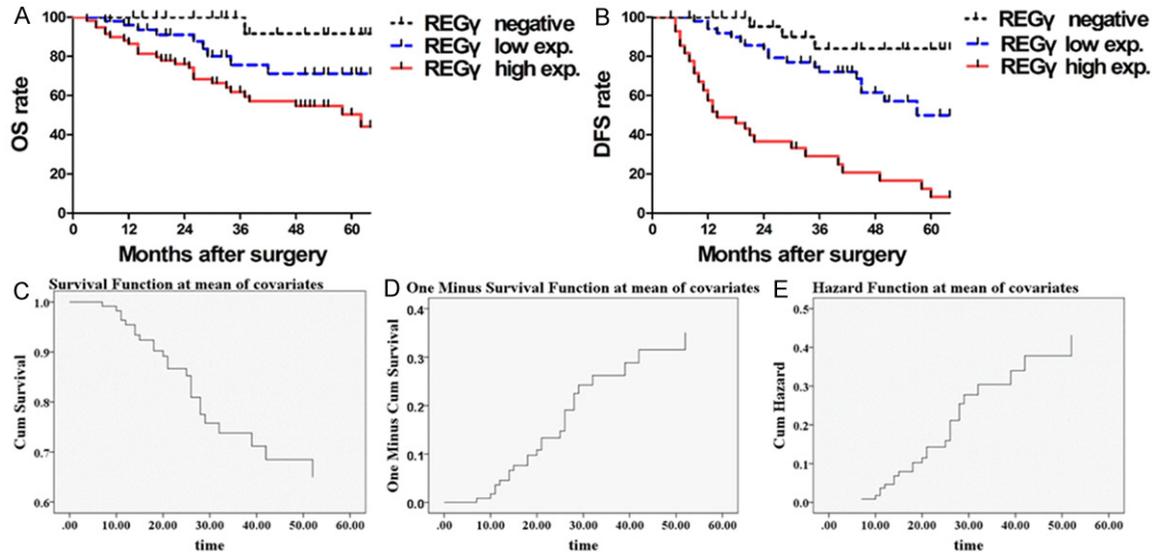
PyMT mouse mammary gland carcinoma and metastatic lung tumour were comparable to that in human breast cancer tissue and the metastatic ALN. REGy expression in MMTV-PyMT mouse was exact mimic of human breast cancer tumorigenesis and progression, as well it implied a potential role of REGy to be a new indicator for mammary gland carcinomas in this model. These results further implied that MMTV-PyMT mouse will be a relevant model to further investigate the biological functions of REGy in breast cancer pathogenesis and progression.

REGy expression level was positively correlated with the poor clinicopathological features, such as bigger breast tumour size, higher TNM stage, poor histological differentiation and more number of metastatic ALNs in breast cancer patients. In addition, patients with REGy high level expression exhibited lower DFS and OS rate compared with REGy negative/low level expression breast cancer patients. In comparison

to earlier REGy-related investigations, our study is the first report which demonstrates that high level of REGy expression is positively correlated with poor clinical outcomes in breast cancer patients with complete 5-year follow up data. These findings not only improved current knowledge of REGy biological functions, and will also shed light on therapeutic potential of REGy for the future investigations.

In view of our results, this study is consistent with previous reports that REGy is highly

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**Figure 3.** The relationship between REGy expression and prognoses of patients with breast cancer. A. The Kaplan-Meier graph for overall survival (OS) rate for breast cancer patients: patients with low expression of REGy had longer OS than those with high expression of REGy (Log-rank Mantel-Cox Test,  $P = 0.0351$ . Hazard Ratio = 0.4949, 95% CI of ratio is 0.2516 to 0.9736) and poor OS rate compare to REGy negative patients (Log-rank Mantel-Cox Test,  $P = 0.0406$ . Hazard Ratio = 0.2913, 95% CI of ratio is 0.08157 to 1.040). B. Kaplan-Meier graph for disease-free survival (DFS) for patients: patients with low expression of REGy had longer DFS than those with high expression of REGy (Log-rank Mantel-Cox Test,  $P < 0.0001$ . The median survival time in REGy low expression group was 57 months, and 14 months in REGy high expression group, Hazard Ratio = 4.071, 95% CI of ratio is 3.505 to 4.638), however, has shorter DFS as compared to those with negative expression of REGy (Log-rank Mantel-Cox Test,  $P = 0.0405$ . Hazard Ratio = 2.432, 95% CI of ratio is 0.9813 to 6.027). As showed by survival function diagram (C), one minus survival function diagram (D) and hazard function diagram (E), REGy expression played role as an independent prognostic factor for the breast cancer (Cox Regression analyses,  $P = 0.008$ , OR = 4.369, 95% CI is 1.482 to 12.880).

expressed in human breast cancers. We subsequently addressed the significance of REGy expression in breast cancer as a new biological marker for the prognostic application.

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### Disclosure of conflict of interest

None.

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