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Diagnostic and Prognostic Implications of AAGAB Expression in Human Breast Cancer

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Abstract

Background: Alpha and Gamma Adaptin Binding protein p34 (AAGAB) was previously reported as a novel on-treatment biomarker can improve prediction of response to neoadjuvant chemotherapy in breast cancer. However, the expression and prognostic value of AAGAB in breast cancer is unknown, the function of AAGAB remains to be elucidated.

Methods: Herein we investigated the role of AAGAB in human breast cancer from the GEO and TCGA databases, immunohistochemistry, Gene Set Enrichment Analysis (GSEA) and immune infiltration analysis.

Results: The expression of AAGAB in breast cancer was significantly up-regulated relative to normal tissue (all p-values<0.05) in GEO and TCGA databases. Kaplan-Meier survival analysis showed that breast cancer patients with AAGAB-high had a worse prognosis than that with AAGAB-low (p=0.005). Univariate analysis using logistic regression revealed that age, pathological stage, and number of lymph nodes were significantly associated with poor Overall Survival (OS) (all p<0.05). Functional annotations indicated that AAGAB is involved in the most significant signaling pathways including intra Golgi traffic and peroxisomal lipid metabolism pathways.

Conclusions: Our study revealed that elevated AAGAB expression was significantly correlated with aggressive progression, poor survival in patients. AAGAB may serve as a new biomarker and potential treatment target in breast cancer.

Keywords: Breast cancer; AAGAB; Biomarker; Survival analysis; Prognosis

Abbreviations

AAGAB: Alpha and Gamma-Adaptin Binding Protein p34; OS: Overall Survival; IARC: International Agency for Research on Cancer; TCGA: The Cancer Genome Atlas; IHC: Immunohistochemistry; NES: Normalized Enrichment Score; GSEA: Gene Set Enrichment Analysis.

Introduction

Breast cancer is a malignant tumour occurring in mammary epithelial tissue, which proliferating out of control via the action of various carcinogenic factors. According to the latest data from the International Agency for Research on Cancer (IARC) survey in 2018, incidence (24.2%) and mortality (15.0%) of breast cancer occupy the top positions among women cancer worldwide [1]. Over the last few decades, the incidence of breast cancer has been increasing year by year in China. The implementation of neoadjuvant treatment and the popularization of screening can significantly reduce the mortality of breast cancer [2]. However, the mortality rate from breast cancer has not decreased significantly in China, especially in the vast rural areas [3]. So far, scientists have not found the exact cause of breast cancer, but many risk factors have found associated with breast cancer.

As a highly heterogeneous disease, patients' response to treatment and prognosis are different even if the clinical stage and pathological grade are the same [4]. Biomarkers are now widely used for early cancer screening and prognosis assessment. Common biomarkers for breast cancer include Estrogen Receptor (ER), Progesterone Receptor (PR), and Human Epidermal Growth Factor Receptor-2 (Her2) [5]. Despite the advances in screening and diagnosis techniques in recent years, which have greatly improved the survival rate of breast cancer patients, it remains one of the major diseases with the highest female mortality rates [6]. At present, most biomarkers that predict prognosis of breast cancer lack specificity, thus, it is clinically important to discover new biomarkers to enhance prognosis and individualized treatment.

AAGAB encodes the alpha and gamma adaptin binding protein p34, which interacts with the gamma-adaptin and alpha-adaptin subunits of complexes involved in clathrin-coated vesicle trafficking [7, 8]. Previous studies have found that heterozygous loss mutations in AAGAB are associated with palmoplantar keratodermas and Punctate palmoplantar keratoderma type I, which based on hyperproliferation within the punctate lesions [9]. AAGAB maybe involving in endocytic recycling of growth factor receptors such as EGFR, which can result in increasing cell division [10]. There was no close correlation between AAGAB expression and tumor in previous studies. Bownes et al. found that AAGAB is a novel on-treatment biomarker can improve prediction of response to neoadjuvant chemotherapy in breast cancer [11].

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However, the expression and functions of AAGAB in breast cancer are unknown, the role of AAGAB in breast cancer need to be further confirmed.

This study attempts to evaluate the diagnostic and prognostic value of AAGAB expression in human breast cancer. GSEA was performed to gain the biological pathways involved in breast cancer pathogenesis related to AAGAB. According to our informational analysis, we indicated that the significant diagnostic and prognostic value of AAGAB for breast cancer, AAGAB may serve as a new biomarker and potential treatment target for patients with breast cancer.

Materials and Methods

TCGA and GTEx databases

The Cancer Genome Atlas (TCGA), supervised by the National Cancer Institute and the American Human Genome Institute, utilizes high-throughput genomic analysis technology to analyze gene mutations, which have improved the ability to prevent, diagnose and treat cancer [12].

Immunohistochemistry (IHC)

Paraffin-embedded tumour sections of breast cancer (from Tongji Medical College of HUST) were incubated overnight at 4°C with rabbit anti-AAGAB polyclonal antibody (FLJ11506, from Novus) that was diluted 1:200 in TBST containing 1% NGS. After washing, the sections were conjugated with goat anti-rabbit Horseradish Peroxidase (HRP) antibody (from Novus) at 1:5000 dilution for 30 min at room temperature, Subsequently, the sections were stained with 3,3-Diaminobenzidine (DAB) and counterstained with hematoxylin, as well as dehydrated through a graded ethanol series and sealed with neutral gum. Finally, the sections were observed using a light microscope (Olympus).

Immune infiltration analysis

To examine the interactions between tumor infiltrating immune cells and the expression of AAGAB, we performed integrated repository tool for tumor-immune system interactions (TIMER, http://cistrome.shinyapps.io/timer/), which contains 10,897 samples from diverse cancer types available in the TCGA database [13, 14].

Gene set enrichment analysis

GSEA is a computational method to determine whether an a priori defined gene sets is significant between two groups [15, 16]. We used MSigDB database C2 collection to perform GSEA. For the purpose of analysis, we divided breast cancer patients into AAGAB high group and AAGAB low group based on the expression level of AAGAB. Gene set permutations were 1000 times for each evaluation. The nominal p value and Normalized Enrichment Score (NES) were used to sort the enriched pathways of gene sets.

Statistical analysis

All statistical analyses were performed using R. The association between clinical pathologic features and AAGAB levels were analysed with the Wilcoxon signed-rank test and logistic regression. To further assess the prognostic significance of AAGAB in breast cancer, Kaplan-Meier analysis and multivariate Cox regression analysis were performed. The cut-off value of AAGAB expression was determined by its median value.

Results

The expression of AAGAB in breast cancer and its significant up-regulation

We screened the difference in AAGAB gene expression in the relevant cancer datasets from GEO and TCGA databases. The results demonstrated that the expression of AAGAB in GSE10780_GPL570, GSE42568_GPL570, GSE70947_GPL570 databases was significantly higher than that in normal tissues (P<0.05). 1104 breast cancer samples were included in this analysis and 403 normal breast tissues used for comparison. The expression of AAGAB in breast cancer was significantly up-regulated (p<0.001, log2FC=1.163) relative to normal tissue (Figures 1 and 2).

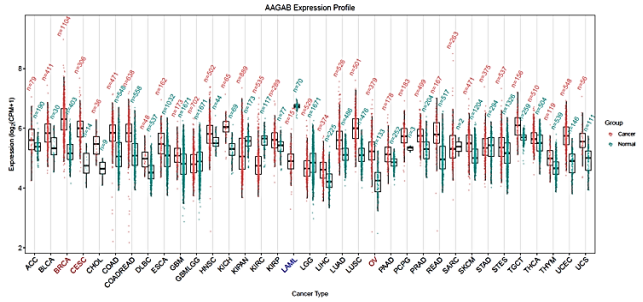


Figure 1: The expression profile of AAGAB in corresponding cancers from TCGA datasets. The cancer names marked in red represent AAGAB is significantly up-regulated, in blue represent AAGAB is significantly down-regulated, in black represent AAGAB is not significantly different. The difference threshold is |log2FC| ≥ 1, P-value ≤ 0.05. **Note:** ( ) Cancer; ( ) Normal.

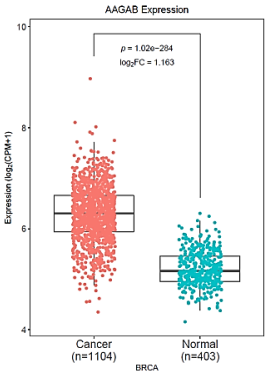


Figure 2: The expression of AAGAB in breast cancer tissues and normal tissues from TCGA database. The expression of AAGAB in breast cancer tissues was significantly up-regulated as compared to normal breast tissues (p<0.001, log2FC=1.163). **Note:** ( ) Cancer; ( ) Normal.

For the purpose of analyzing the association between clinicopathologic variables and AAGAB expression, we divided breast cancer patients into AAGABhigh group and AAGABlow group based on the expression of AAGAB, in which performed chi-square analysis. The pathological information of the patient includes: age, gender, ethnicity, pathological stage, TMN stage, PAM50 typing, typing, tumor purity, number of lymph nodes and histological type. The analysis shows that AAGAB expression was significantly associated with the patient's age, gender, race, ER status, PR status, N-stage, PAM50 classification

and histological type (p<0.05). For N-stage group, which is the case of lymph node metastasis, 43.39% (223/514) of patients of AAGAB high expression were in N0, 56.79% (205/361) of patients were in N1, 51.66% (62/120) of patients were in N2, 52.63% (40/76) of patients were in N3. The results indicate that the expression of AAGAB was relevant to the lymph node metastasis of patients. Regarding age, AAGABhigh group under 60 and over 60 was 43.82% (248/566) and 57.06% (291/510), respectively. These results indicate that the expression of AAGAB may be related to age, AAGAB expression also had a significant relationship with histological type, AAGAB was expressed low in medullary carcinoma and metaplastic carcinoma (Table 1).

Characteristics	High expression (545)	Low expression (546)	X^2	p-value
Years to birth			19.42105	6.06E-05
<60	248	318		
≥60	291	219		
Unknown	6	9		
Pathologic stage			4.639036	0.326378
Stage i	92	89		
Stage ii	294	326		
Stage iii	137	112		
Stage iv	10	10		
Unknown	12	9		
Pathology T stage			8.566171	0.072907
t1	145	134		
t2	308	323		
t3	63	75		
t4	28	12		
Unknown	1	2		
Pathology N stage			20.99004	0.000318
n0	223	291		
n1	205	156		
n2	62	58		
n3	40	36		
Unknown	15	5		
Pathology M stage			0.340587	0.843417
m0	455	453		
m1	12	10		
Unknown	78	83		
gender			4.141734	0.041838
female	535	544		
male	10	2		
race			31.7024	6.05E-07
Asian	35	26		
Black or African American	61	121		
Unknown	63	32		
white	386	367		
PAM50			180.0719	7.20E-38
Basal	13	134		
Her2	26	41		
LumA	268	158		
LumB	133	51		
Unknown	105	162		
ER Status			23.54028	7.73E-06
Negative	4	32		
Positive	39	29		
Unknown	502	485		
PR Status			16.4047	0.000274
Negative	12	39		
Positive	32	22		
Unknown	501	485		
HER2 Status			3.412624	0.181534
Negative	32	45		
Positive	11	16		
Unknown	502	485		
Histological type			23.71073	0.001281
Infiltrating ductal carcinoma	398	382		
Infiltrating lobular carcinoma	94	109		
Medullary carcinoma	0	6		
Metaplastic carcinoma	0	9		
Mixed histology	16	13		
Mucinous carcinoma	13	4		
Other specify	24	21		
Unknown	0	2		

Table 1: AAGAB expression associated with clinical pathological characteristics (Chi-square statistics).

Validation of elevated AAGAB protein level in breast cancer tissues

To assess AAGAB protein level in our paraffin-embedded tumor samples, we performed IHC staining and found that significant elevated AAGAB protein level in terms of density and intensity in breast cancer tissues compared to adjacent normal breast tissues (p=0.001) (Figure 3).

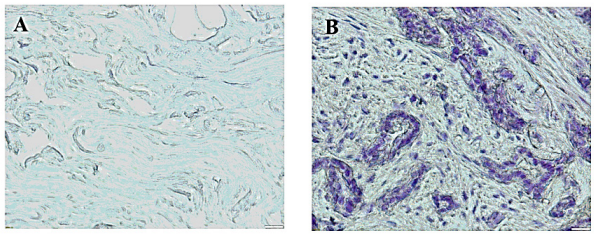


Figure 3: AAGAB IHC staining results. A. Elevated compared to adjacent normal breast tissues; B. AAGAB protein level in breast cancer tissues.

Survival analysis and multivariate analysis

In order to determine the effect of AAGAB gene expression levels on breast cancer prognosis, Cox multiple regression analysis was carried out. According to the expression of AAGAB, the cases were divided into the groups with the high AAGAB expression and the low AAGAB expression, followed by making the survival analysis. The results illustrated that there was no significant difference in disease-free survival between the high and low AAGAB expression groups, but a significant correlation was found for overall survival (p=0.005,

indicated that breast cancer with AAGAB-high had a worse prognosis than that with AAGAB-low. Univariate analysis using logistic regression revealed that age, pathological stage, and number of lymph nodes were significantly associated with poor OS (all p<0.05) (Figures 4 and 5).

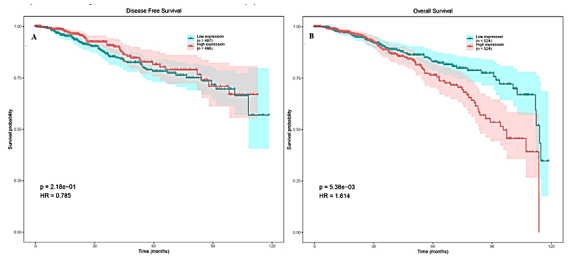
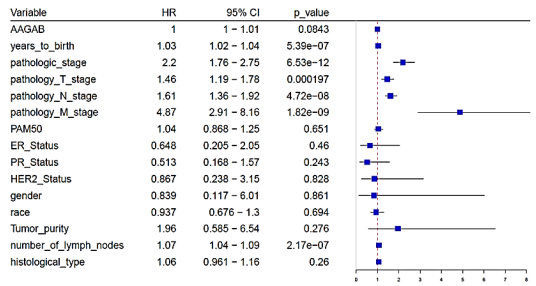


Figure 4: Disease-free survival curve and overall survival curve related to gene expression. There was no significant difference in disease-free survival between the high and low AAGAB expression groups (A), but a significant correlation was found for overall survival (p = 0.005, B). **Note:** A. (—) Low expression (n=487); (—) High expression (n=486); B. (—) Low expression (n=524); (—) High expression (n=524)



Variable	HR	95% CI	p_value
AAGAB	1	1 – 1.01	0.0843
years_to_birth	1.03	1.02 – 1.04	5.39e-07
pathologic_stage	2.2	1.76 – 2.75	6.53e-12
pathology_T_stage	1.46	1.19 – 1.78	0.000197
pathology_N_stage	1.61	1.36 – 1.92	4.72e-08
pathology_M_stage	4.87	2.91 – 8.16	1.82e-09
PAM50	1.04	0.868 – 1.25	0.651
ER_Status	0.648	0.205 – 2.05	0.46
PR_Status	0.513	0.168 – 1.57	0.243
HER2_Status	0.867	0.238 – 3.15	0.828
gender	0.839	0.117 – 6.01	0.861
race	0.937	0.676 – 1.3	0.694
Tumor_purity	1.96	0.585 – 6.54	0.276
number_of_lymph_nodes	1.07	1.04 – 1.09	2.17e-07
histological_type	1.06	0.961 – 1.16	0.26

Figure 5: Univariate regression analysis of clinical information in TCGA database. Univariate analysis using logistic regression revealed that age, pathological stage, and number of lymph nodes were significantly associated with poor OS (all p<0.05).

Association of AAGAB's expression with tumour purity and immune infiltration

The above finding suggested that the expression of AAGAB may have an impact on the prognosis of breast cancer. The relationship between six types of immune infiltrating cells (including B cells, CD4 T cells, CD8 T cells, dendritic cells, macrophages and neutrophils) together with tumour purity and AAGAB expression was analysed to determine whether AAGAB expression was related to the level of immune invasion in cancer. AAGAB expression level was positively significantly correlated with cell purity (Cor=0.326, p=7.9E-28), CD8 T cells (Cor=0.07, p=2.26E-02), and macrophages (Cor=0.104, p=6.3E-04) of infiltration level. In addition, AAGAB expression level in breast cancer was negatively significantly correlated with CD4 T cells (Cor=-202, p=2.18E-11) and dendritic cells (Cor=-0.074, p=1.5E-02). Whereas, the expression of AAGAB in breast cancer was independent of B cells (Cor=0.012, p=7.07E-01) and neutrophils (Cor=-0.033, p=72.76E-01). In breast cancer, AAGAB expression levels were markedly positively correlated with tumour purity, indicating its relative enrichment in tumour cells. These findings indicate that AAGAB can affect the prognosis of breast cancer by interacting with breast cancer immune infiltration (Figure 6).

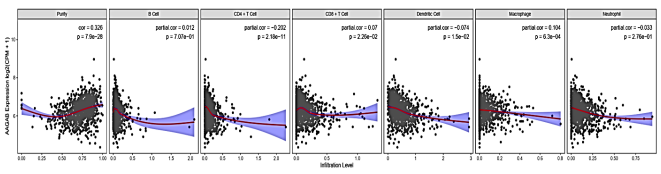


Figure 6: Association of AAGAB's expression with tumor purity and immune infiltration. AAGAB expression level was positively significantly correlated with cell purity, CD8 T cells and macrophages, was positively significantly correlated with CD4 T cells and dendritic cells.

GSEA identifies differentially enriched AAGAB-related signaling pathways

GSEA was conducted between high and low AAGAB expression gene sets to identify differentially enriched signaling pathways in breast cancer. Nom p-val<0.05, FDR q-val<0.25 are considered as significant. Two gene sets are significant at FDR<0.25 in phenotype high, 0 gene set is significant at FDR<0.25 in phenotype low. The Intra Golgi Traffic (p<0.004), Peroxisomal Lipid Metabolism (p<0.006) are differentially activated signaling pathways in AAGAB high expression phenotype (Figure 7).

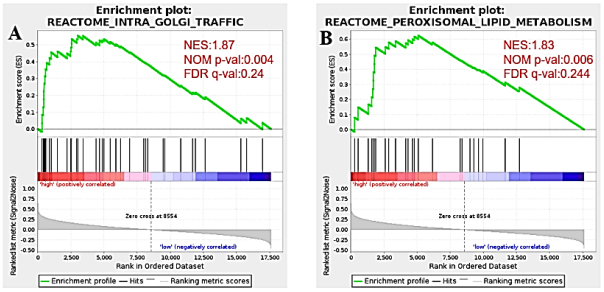


Figure 7: Differentially enriched AAGAB-related signaling pathways identified by GSEA. Intra Golgi Traffic (p<0.004), Peroxisomal Lipid Metabolism (p<0.006) are differentially activated signaling pathways in AAGAB high expression phenotype. **Note:** (—) Enrichment profile; (—)Hits; (—)Ranking metric scores

Discussion

Despite the advances in screening and diagnosis techniques in recent years, which have greatly improved the survival rate of breast cancer patients, it remains one of the major diseases with the highest female mortality rates. At present, most biomarkers that predict prognosis of breast cancer lack specificity, thus, it is clinically important to discover new biomarkers to enhance prognosis and individualized treatment.

AAGAB encodes the alpha- and gamma-adaptin binding protein p34, which interacts with the gamma-adaptin and alpha-adaptin subunits of complexes involved in clathrin-coated vesicle trafficking. Previous studies have found that heterozygous loss mutations in AAGAB are associated with punctate palmoplantar keratoderma, which based on hyperproliferation within the punctate lesions. AAGAB maybe involving in endocytic recycling of growth factor receptors such as EGFR, which can result in increasing cell division. The level of AAGAB was found to be prognostic of response in renal cancers and in thyroid cancers from the TCGA database. AAGAB was

identified as a novel on-treatment biomarker for accurate prediction of pathological Complete Response (pCR) and reaction in patients treated with neoadjuvant chemotherapy. However, the exact role of AAGAB in breast cancer is currently unclear and warrants further investigation.

Herein we investigated the role of AAGAB in human breast cancer from the TCGA database, immunohistochemistry, GSEA and Immune infiltration analysis. Increased AABAB expression in breast cancer was significantly associated with age, gender, race, ER status, PR status, N-stage, PAM50 classification and histological type (all p-values<0.05). Kaplan-Meier survival analysis showed that breast cancer patients with AAGAB-high had a worse prognosis than that with AAGAB-low (p=0.005). Univariate analysis using logistic regression revealed that age, pathological stage, and number of lymph nodes were significantly associated with poor overall survival (OS) (all p<0.05). AAGAB expression level was significantly correlated with cell purity, CD8 T cells, macrophages, CD4 T cells and dendritic cells indicate that AAGAB can affect the prognosis of breast cancer by interacting with breast cancer immune infiltration.

Functional annotations indicated that AAGAB is involved in the most significant signaling pathways including intra Golgi traffic and peroxisomal lipid metabolism pathways. The underlying mechanisms between AAGAB and carcinogenesis of breast cancer remain unclear, elevated AAGAB maybe increase endocytic recycling of EGFR, which induce the signaling and division of breast cancer cells, however, future research is required to explore the detail mechanisms of AAGAB in breast cancer.

Conclusions

Our study revealed that elevated AAGAB expression was significantly correlated with aggressive progression, poor survival in breast cancer patients. AAGAB may serve as a new biomarker and potential treatment target in breast cancer. According to our informational analysis and IHC result, we prove the diagnostic and prognostic value of AAGAB in human breast cancer. GSEA was performed to gain the biological pathways involved in breast cancer pathogenesis related to AAGAB. AAGAB may serve as a new biomarker and potential treatment target for patients with breast cancer.

Compliance and Ethics

The study was approved by HUBU Ethics Committee. All patients have signed an informed consent.

Availability of Data and Materials

The datasets generated and analyzed during the current study are available in the https://xenabrowser.net/datapages/?hub=https://tcga.xenahubs.net:443 and https://www.gtexportal.org/home/.

Competing Interests

The authors declare that they have no competing interests.

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