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# Subcellular localization of β-arrestin1 and its prognostic value in lung adenocarcinoma

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## Abstract

β-Arrestins play important roles in cancer progression, and the subcellular localization of β-arrestin1 has been receiving increasingly more attention. Intriguingly, several studies, including some of our previous work, showed that the effects of β-arrestin1 on outcomes of cancer patients were controversial.

Specimens were obtained from 133 patients with lung adenocarcinoma. Immunohistochemistry was used to detect the expression of β-arrestin1 and p300 in the collected tissues. The Kaplan-Meier analysis and Cox proportional hazards regression were used to examine the relationship between β-arrestin1 and patient survival.

We found no significant association between β-arrestin1 and clinicopathological variables. The Kaplan-Meier plot showed that patients with high expression of β-arrestin1 (especially in the nucleus) had a poorer overall survival (OS) and shorter disease-free survival (DFS) ($P = .026$, $P = .015$). Additionally, high p300 expression also resulted in worse OS ($P = .039$). Following the univariate analysis, high expressions of nuclear β-arrestin1 and p300 were classed as poor prognostic factors for both OS ($P = .016$) and DFS ($P = .025$).

The expression of β-arrestin1 in the nucleus is associated with increased malignant tendency of lung adenocarcinoma, and the predictive value of β-arrestin1 may be optimized by combining information about the expression of p300 acetyltransferase.

**Abbreviations:** AKT = protein kinase B, DFS = disease-free survival, GPCR = G protein-coupled receptors, GTP = guanosine triphosphate, MAPK = mitogen-activated protein kinase, NSCLC = non-small cell lung cancer, OS = overall survival, PI3K = phosphatidylinositol 3 kinase, PTEN = phosphatase and tensin homolog.

**Keywords:** β-arrestin1, lung adenocarcinoma, p300, prognosis, subcellular localization

## 1. Introduction

Lung adenocarcinoma, with nearly 1.4 million deaths and approximately 1.6 million new cases each year, accounts for almost 50% of all lung cancers, which are the leading cause of cancer-related death worldwide. Even though systemic management, followed by surgery, has progressed during the past decade, tumor recurrence and metastasis are still the most important events that contribute to the high mortality.[1]

β-Arrestins, including β-arrestin1 and β-arrestin2, regulate cellular functions, such as cell migration and apoptotic signaling. β-arrestins were initially appreciated for their capacity to sterically hinder the GPCR (G protein-coupled receptors), ultimately resulting in receptor desensitization and endocytosis. Apart from that, β-arrestins can also function as versatile protein scaffolding platforms within the cytosol, ensuring the transmission of relevant information in space and time, and thus, an appropriate cellular response. This function involves regulating the activities and/or subcellular distribution of their binding partners, via receptor-dependent or receptor-independent processes.[2] Due to various receptors and signaling pathways, β-arrestin1 may play both negative and positive regulatory roles in the neoplasm.

Intriguingly, our recent studies on the prognostic value of β-arrestin1 in lung cancer generated conflicting results. At the focus of the controversy is whether the expression of β-arrestin1 is an independent prognostic marker in operable patients with lung cancer.[3–5]

Recently, an increasing amount of research has been focusing on the subcellular distribution of β-arrestins. Although both β-arrestin1 and β-arrestin2 contain nuclear localization signals, only β-arrestin2 has a nuclear export signal. Consequently, β-arrestin1 is found in both the cytoplasm and nucleus, whereas β-arrestin2 is generally localized in the cytoplasm.[6–8]

About a decade ago, a landmark study brought to light a novel nuclear role of β-arrestin1 in the regulation of gene transcription.[9] Since then, other studies have confirmed this nuclear function and described its contribution to tumor survival, proliferation,

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angiogenesis, and invasion and metastasis, in epithelial ovarian cancer, lung tumors, breast cancer cells, and prostate cancer.[10–13] Upon stimulation of different receptors or in the presence of other conditions (eg, hypoxia), β-arrestin1 undergoes nuclear translocation and acts as a nuclear scaffold, recruiting histone acetyltransferase p300 to the transcription factor or transcription regulator. This results in increased gene expression, through the acetylation of histone and/or non-histone transcription factors. Although there are conflicting reports on whether p300 is a positive or negative regulator of cancer progression, its overexpression is indicative of poor prognosis in most human malignancies, including lung cancer,[14,15] breast cancer,[16] hepatocellular carcinoma,[17] colorectal cancer,[18] prostate cancer,[19] nasopharyngeal carcinoma,[20] and laryngeal cancer.[21]

Based on the aforementioned findings, we wondered whether the different functions of β-arrestin1 in the cytoplasm and nucleus are responsible for the contradictory results. Therefore, we performed a cohort retrospective study to investigate the expression of cytosolic and nuclear β-arrestin1, respectively, and examined their association with clinicopathological characteristics and prognosis. As p300 plays an important role in facilitating chromatin transcription mediated by β-arrestin1, we hypothesized that the combined detection of nuclear β-arrestin1 and p300 could aid in identifying patients with increased risk of cancer recurrence, as well as provide a theoretical basis for further tailored therapy of lung adenocarcinoma.

## 2. Materials and methods

### 2.1. Patients and sample collection

Specimens were obtained from 133 patients with lung adenocarcinoma who had undergone surgical resection of primary tumor, between June 2008 and August 2009, in the Department of Thoracic Surgery, Shandong Provincial Hospital Affiliated to Shandong University. All procedures were in accordance with the ethical standards of the Ethics Committee of the Provincial Hospital Affiliated to Shandong University. The main inclusion criteria were histological diagnosis of lung adenocarcinoma and complete surgical resection. None of the patients had received adjuvant chemotherapy, radiotherapy, or immunotherapy before surgery. Moreover, we also excluded patients with concurrent malignant disease or other previous cancers. Clinical data, including survival, were derived from the Bio-Bank of Shandong Provincial Hospital. Follow-up data were available for all patients.

Clinical information was obtained by reviewing the medical archives. Relevant data available included patient's age, gender, smoking history, tumor size and location, grade of differentiation, lymph-node metastases, and pathologic staging. The pathological diagnosis was confirmed by 2 senior pathologists. Pathologic staging was performed according to the seventh edition of the TNM classification, of the American Joint Committee on Cancer, and histological type was determined according to the World Health Organization classification.[22,23]

Patients were followed up every 3 months for the first year post-surgery and every 6 months for the following years, with a median follow-up period of 42 months (range: 1–73 months). Overall survival (OS) was defined as the time from the date of surgery to the last date of follow-up for patients who remained alive, or to the date of death. Disease-free survival (DFS) was defined as the time from the date of surgery to the date of recurrence, death, or to the end of observation. The REMARK guidelines for reporting tumor marker studies were used.[24]

### 2.2. Immunohistochemistry

Paraffin-embedded tissue samples were collected, cut into 3-μm thick sections and fixed on silicified slides. After deparaffined and rehydrated, the sections were heated in 0.01 mol/L saline citrate buffer at 96°C to 98°C for 15 minutes to unmask antigens, treated with 3% hydrogen peroxide for 15 minutes at room temperature to inactive endogenous peroxidase and incubated with 10% goat serum for 30 minutes at room temperature to block nonspecific binding. Then slides were incubated overnight at 4°C with a rabbit monoclonal to β-arrestin1 antibody (Abcam Biotechnology, ab32099; diluted, 1:200) or a rabbit polyclonal to p300 antibody (Abcam Biotechnology, ab10485; diluted, 1:1000). The subsequent steps were according to the instructions of Zymed (streptavidin-peroxidase method). The sections were then counterstained with hematoxylin before dehydration and mounting. For negative control, we carried out the same steps as described previously while the antibody was replaced by phosphate-buffered saline.

### 2.3. Evaluation of immunostaining

To assess the immunostaining, the intensity reactivity score was used, assessing the staining intensity as negative (= 0), weak (= 1), moderate (= 2), or strong (= 3). Reactivity was determined by the fraction of positive cells in relation to the whole cancer areas, which was scored as 0 (<10%), 1 (11–25%), 2 (26–50%), 3 (51–80%), and 4 (81–100%). At least 10 fields of each specimen were selected. A multiplier of fraction and intensity was calculated for each score, and a mean value of all annotated scores was used in the analyses. The final scores were stratified as low expression (score = 0–6) and high expression (score = 8–12). The immunoreactivity score was determined by 2 independent observers who were blinded to the clinicopathological information of each sample, and discordant cases were discussed using a dual-head microscope, to determine the final score.

### 2.4. Statistical analysis

The chi-square test and Fisher exact test were performed to analyze the group differences. The Kaplan-Meier method and subsequent evaluation by log-rank test were applied for survival analysis. Cox regression proportional hazard's modeling was used to estimate the impact of prognostic factors on OS and DFS. All of the tests were 2-sided, and $P$ values below .05 were considered statistically significant. Statistical analysis was performed using SPSS Statistics version 19.0 (SPSS Inc, Chicago, IL).

## 3. Results

### 3.1. Association of β-arrestin1 and p300 expression with clinicopathological parameters

In total, 41 females and 82 males were enrolled in this study, and the median age was 58 years (range: 20–76 years). Table 1 shows the results of the statistical analysis on the clinicopathological variables. No statistically significant correlation was observed among β-arrestin1(C), β-arrestin1(N), or p300 expression and clinicopathological parameters (age, sex, smoking status, drinking status, degree of differentiation, tumor location, T stage, lymph node involvement, pathological stage, or surgical type).

## Table 1

**Association among β-arrestin1(C), β-arrestin1(N), and p300 expression and clinicopathological variables.**

| Variable | | β-arrestin1(C) | | | β-arrestin1(N) | | | P300 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Low | High | P | Low | High | P | Low | High | P |
| Age | <60 | 30 | 42 | | 39 | 33 | | 35 | 37 | |
| | ≥60 | 24 | 37 | .860 | 32 | 29 | .863 | 23 | 38 | .224 |
| Sex | Female | 22 | 29 | | 26 | 25 | | 20 | 31 | |
| | Male | 32 | 50 | .717 | 45 | 37 | .722 | 38 | 44 | .474 |
| Smoking | No | 28 | 42 | | 35 | 35 | | 26 | 44 | |
| | Yes | 26 | 37 | 1.000 | 36 | 27 | .487 | 32 | 31 | .120 |
| Drinking | No | 38 | 56 | | 49 | 45 | | 40 | 54 | |
| | Yes | 16 | 23 | 1.000 | 22 | 17 | .705 | 18 | 21 | .848 |
| Differentiation | Well | 7 | 7 | | 8 | 6 | | 10 | 4 | |
| | Moderate | 34 | 40 | | 37 | 37 | | 30 | 44 | |
| | Poor | 13 | 32 | .146 | 26 | 19 | .696 | 18 | 27 | .093 |
| Location | Left | 30 | 34 | | 32 | 32 | | 25 | 39 | |
| | Right | 24 | 45 | .163 | 39 | 30 | .490 | 33 | 36 | .382 |
| T stage | 1 + 2 | 49 | 69 | | 65 | 53 | | 55 | 63 | |
| | 3 + 4 | 5 | 10 | .591 | 6 | 9 | .288 | 3 | 12 | .058 |
| Lymph node involvement | Negative | 30 | 36 | | 40 | 26 | | 31 | 35 | |
| | Positive | 24 | 43 | .292 | 31 | 36 | .119 | 27 | 40 | .487 |
| Pathological stage | I + II | 34 | 45 | | 46 | 33 | | 39 | 40 | |
| | III + IV | 20 | 34 | .590 | 25 | 29 | .216 | 19 | 35 | .113 |
| Surgical type | Local resection | 6 | 10 | | 10 | 6 | | 9 | 7 | |
| | Lobectomy | 45 | 66 | | 57 | 54 | | 47 | 64 | |
| | Pneumonectomy | 3 | 3 | .871 | 4 | 2 | .581 | 2 | 4 | .615 |

β-arrestin1(C) = β-arrestin1 expressed in cytoplasm, β-arrestin1(N) = β-arrestin1 expressed in nucleus.

### 3.2. Expression of β-arrestin1 and p300 in lung adenocarcinoma primary tumors

Staining of β-arrestin1 was identified in both the cytoplasm (β-arrestin1(C)) and nucleus (β-arrestin1(N)) of cancer cells, whereas p300 was mostly present in the nucleus (Fig. 1). The expression of β-arrestin1 protein was investigated in 133 cases, of which 79 (59%) showed high cytoplast immunoreactivity and 62 (47%) showed high nuclear immunoreactivity. Regarding p300, 75 (56%) cases showed high expression and 58 (44%) cases showed low expression.

### 3.3. Prognostic value of β-arrestin1 and p300

Figure 2 shows the survival curves of all patients. The Kaplan-Meier plots showed that β-arrestin1(C) had no effect on OS or
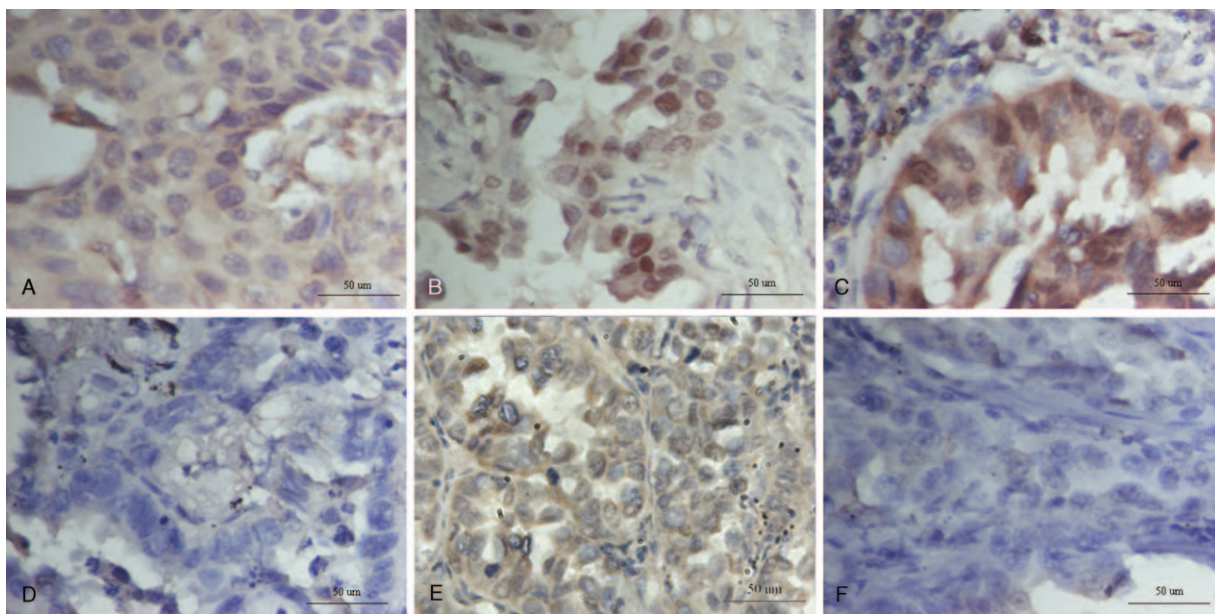


**Figure 1.** Immunohistochemical staining of lung adenocarcinoma showing: (A) β-arrestin1 highly expressed in the cytoplasm, (B) β-arrestin1 highly expressed in the nucleus, (C) β-arrestin1 highly expressed in both cytoplasm and nucleus, (D) negative expression of β-arrestin1 in both cytoplasm and nucleus, (E) high expression of p300 and (F) low expression of p300 (400× magnification).
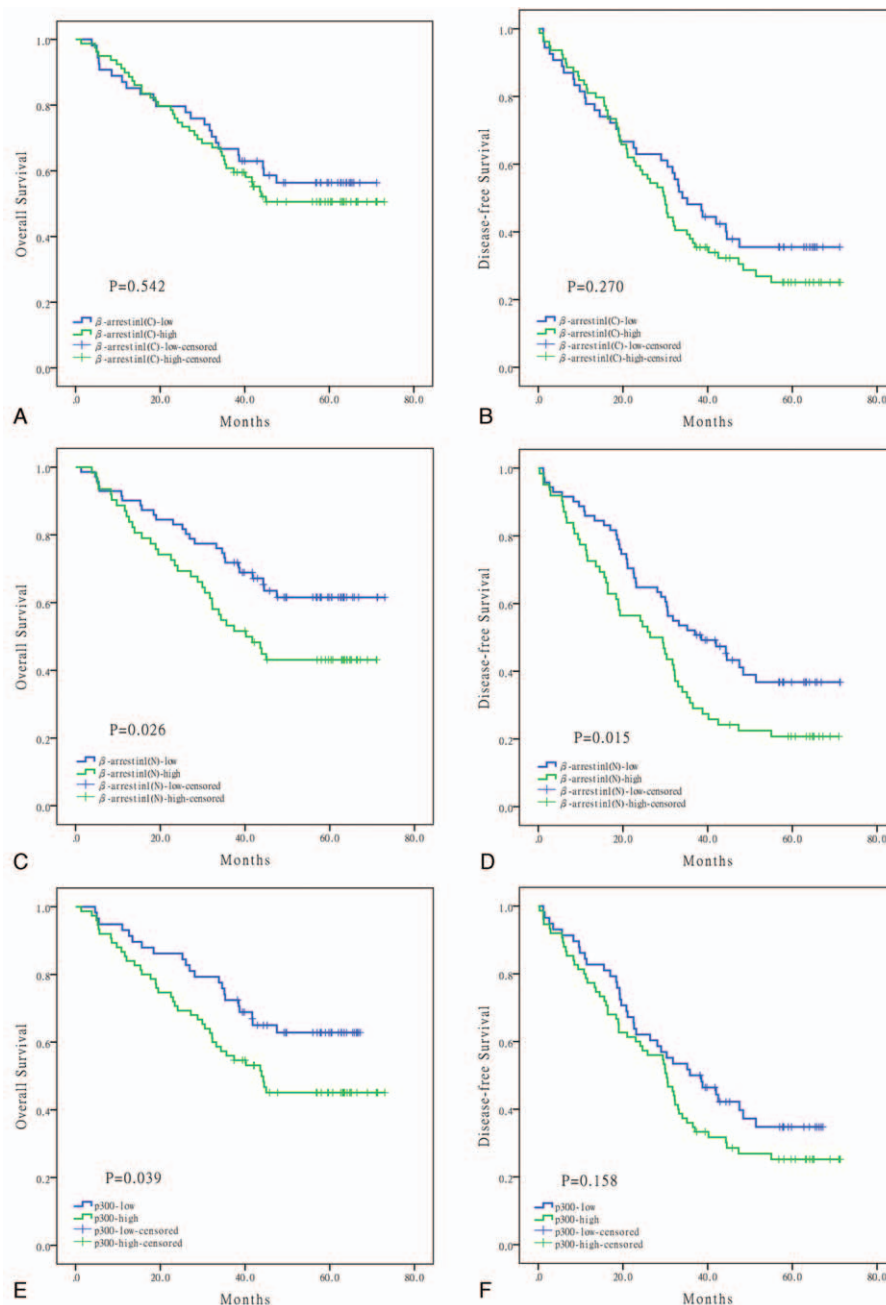
**Figure 2.** Kaplan-Meier cumulative survival analysis for lung adenocarcinoma patients with different expression of β-arrestin1 and p300. (A) Expression of cytoplasmic β-arrestin1 in relation to OS, (B) expression of cytoplasmic β-arrestin1 in relation to DFS, (C) expression of nuclear β-arrestin1 in relation to OS, (D) expression of nuclear β-arrestin1 in relation to DFS, (E) p300 expression in relation to OS, and (F) p300 expression in relation to DFS.

DFS ($P$=.542, $P$=.270), whereas, patients with high expression of β-arrestin1(N) had a poorer OS and DFS than those with low expression of β-arrestin1(N) ($P$=.026, $P$=.015). Survival analyses were also performed by comparing the expression of p300 to both OS and DFS. Results demonstrated that high p300 expression had no impact on DFS ($P$=.158), but resulted in a worse OS ($P$=.039). For the multivariate analysis, the Cox proportional hazards model, involving the expression levels of β-arrestin1(C), β-arrestin1(N), p300 proteins, and various clinical parameters, was utilized (Tables 2 and 3). For OS, only the pathological stage was an independent prognostic factor ($P$<.001). For DFS, both T stage and lymph node involvement

were independent prognostic factors ($P$=.001 and $P$<.001, respectively).

### 3.4. Co-expression of β-arrestin1(N) and p300

Subsequently, we combined β-arrestin1(N) and p300 expression into 4 groups: both β-arrestin1(N) and p300 had low expression, low β-arrestin1(N) and high p300 expression, high β-arrestin1(N) and low p300 expression, and both β-arrestin1(N) and p300 had high expression. Patients with high expression of both β-arrestin1(N) and p300 had lower 5-year survival rates and shorter median survival times than the others (5-year OS, 38% vs

**Table 2**

Univariate and multivariate analyses for the overall survival.

| Variable | | N | Univariate analyses | | | | Multivariable analyses | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | HR | Low CI | High CI | P | HR | Low CI | High CI | P |
| Age | ≥60 versus <60 | 61/72 | 0.81 | 0.49 | 1.34 | .406 | | | | |
| Sex | Male versus female | 82/51 | 1.04 | 0.62 | 1.73 | .895 | | | | |
| Smoking | Yes versus no | 63/70 | 1.00 | 0.60 | 1.66 | .998 | | | | |
| Drinking | Yes versus no | 39/94 | 0.60 | 0.32 | 1.10 | .099 | | | | |
| Differentiation | Poor versus well/moderate | 45/88 | 1.47 | 0.88 | 2.46 | .139 | | | | |
| Location | Right versus left | 69/64 | 0.83 | 0.50 | 1.38 | .471 | | | | |
| T stage | 3/4 versus 1/2 | 15/118 | 3.04 | 1.61 | 5.72 | .001[*] | | | | |
| Lymph node involvement | Positive versus negative | 67/66 | 2.73 | 1.58 | 4.70 | <.001[*] | | | | |
| Pathological stage | III/IV I/II | 54/79 | 3.16 | 1.89 | 5.30 | <.001[*] | 2.97 | 1.76 | 5.00 | <.001[*] |
| β-arrestin1(C) | High/low | 79/64 | 1.17 | 0.70 | 1.97 | .542 | | | | |
| β-arrestin1(N) | High/low | 62/71 | 1.76 | 1.06 | 2.93 | .028[*] | | | | |
| P300 | High/low | 75/58 | 1.73 | 1.02 | 2.94 | .042[*] | | | | |
| Surgical type | Lobectomy/pneumonectomy versus local resection | 117/16 | 0.58 | 0.29 | 1.14 | .112 | | | | |
| Co-expression | Both low | 46 | 1.00 | | | .038[*] | 1.00 | | | .110 |
| | β-arrestin1(N)-low and P300-high | 25 | 1.02 | 0.46 | 2.29 | .962 | 0.95 | 0.42 | 2.12 | .893 |
| | β-arrestin1(N)-high and P300-low | 12 | 0.86 | 0.29 | 2.54 | .778 | 0.84 | 0.28 | 2.49 | .751 |
| | Both high | 50 | 2.07 | 1.14 | 3.74 | .016[*] | 1.80 | 0.99 | 3.27 | .053 |

β-arrestin1(C) = β-arrestin1 expressed in cytoplasm, β-arrestin1(N) = β-arrestin1 expressed in nucleus.
[*] Statistically significant data.

61%; 5-year DFS, 18% vs 36%; median survival times for OS, 34.5 vs 73 months; median survival times for DFS, 24.75 vs 38.48 months).

Notably, Kaplan-Meier plots showed the poorest OS or DFS for the group with high expression of both β-arrestin1(N) and p300 (P=.032, P=.044, respectively; Fig. 3). Interestingly, the survival curves of patients in the low β-arrestin1(N) and high p300 expression group, or of those with high β-arrestin1(N) and low p300 expression, were relatively close to that of patients with tumors exhibiting low expression of both β-arrestin1(N) and p300, but were dramatically discrepant from that of patients with high expression of both β-arrestin1(N) and p300 (Fig. 3). In the univariate Cox proportional hazards regression analysis, these variables also showed insignificant differences in outcomes concerning both OS and DFS. The

multivariate analysis of clinical and pathologic characteristics is summarized in Table 3.

## 4. Discussion

Over the last few decades, the effects and mechanisms of β-arrestin1 on tumorigenesis and progression of malignant tumor has been drawing increasing attention. Elevated expression of β-arrestin1 was described in previous studies of multiple tumor types, including breast cancer, acute lymphoblastic leukemia, and prostate cancer.[12,13,25] In past studies, high expression of β-arrestin1 was associated with unfavorable clinicopathological parameters, such as histological grade, tumor size, lymph node metastases, distant metastases, and so on.[26,27] In the present study, we found that neither β-arrestin1(C) nor

**Table 3**

Univariate and multivariate analyses for the disease-free survival.

| Variable | | N | Univariate analyses | | | | Multivariable analysis | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | HR | Low CI | High CI | P | HR | Low CI | High CI | P |
| Age | ≥60 versus <60 | 61/72 | 0.74 | 0.49 | 1.13 | .163 | | | | |
| Sex | Male versus female | 82/51 | 0.96 | 0.63 | 1.46 | .844 | | | | |
| Smoking | Yes versus no | 63/70 | 0.99 | 0.66 | 1.50 | .965 | | | | |
| Drinking | Yes versus No | 39/94 | 0.73 | 0.45 | 1.17 | .189 | | | | |
| Differentiation | Poor versus well/moderate | 69/64 | 2.02 | 1.33 | 3.08 | .001[*] | | | | |
| Location | Right versus left | 75/58 | 0.88 | 0.58 | 1.33 | .546 | | | | |
| T stage | 3/4 versus 1/2 | 45/88 | 3.23 | 1.83 | 5.70 | <.001[*] | 2.65 | 1.48 | 4.74 | .001[*] |
| Lymph node involvement | Positive versus negative | 15/118 | 3.07 | 1.98 | 4.77 | <.001[*] | 3.00 | 1.91 | 4.70 | <.001[*] |
| Pathological stage | III/IV I/II | 67/66 | 3.04 | 1.99 | 4.63 | <.001[*] | | | | |
| β-arrestin1(C) | High/low | 54/79 | 1.27 | 0.83 | 1.94 | .272 | | | | |
| β-arrestin1(N) | High/low | 79/64 | 1.66 | 1.10 | 2.51 | .016[*] | 1.61 | 0.99 | 2.62 | .056 |
| P300 | High/low | 62/71 | 1.35 | 0.89 | 2.06 | .160 | | | | |
| Surgical type | Lobectomy/pneumonectomy versus local resection | 117/16 | 0.70 | 0.38 | 1.28 | .245 | | | | |
| Co-expression | Both low | 46 | 1.00 | | | .050 | 1.00 | | | .061 |
| | β-arrestin1(N)-low and P300-high | 25 | 0.86 | 0.45 | 1.63 | .644 | 0.79 | 0.41 | 1.51 | .474 |
| | β-arrestin1(N)-high and P300-low | 12 | 1.08 | 0.49 | 2.37 | .848 | 0.90 | 0.40 | 1.98 | .785 |
| | Both high | 50 | 1.73 | 1.07 | 2.81 | .025[*] | 1.61 | 0.99 | 2.62 | .056 |

β-arrestin1(C) = β-arrestin1 expressed in cytoplasm, β-arrestin1(N) = β-arrestin1 expressed in nucleus.
[*] Statistically significant data.
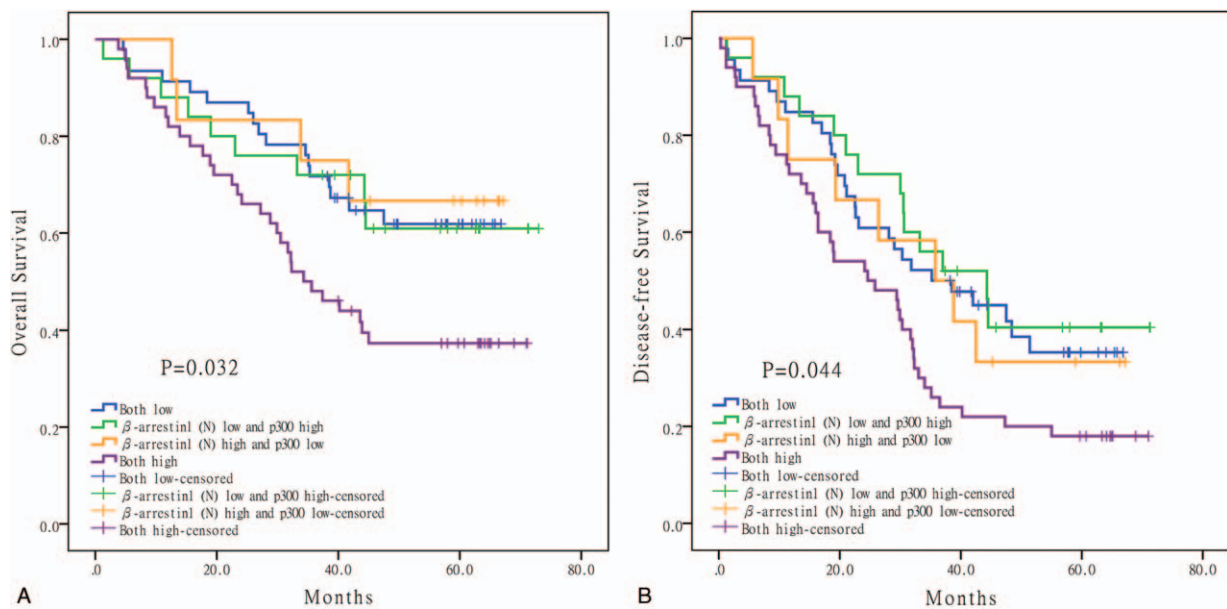
**Figure 3.** Kaplan-Meier cumulative survival analysis for lung adenocarcinoma patients regarding the co-expression of nuclear β-arrestin1 and p300. (A) OS and (B) DFS.

β-arrestin1(N) had a correlation with age, sex, smoking status, drinking status, grade of differentiation, tumor location, T stage, lymph node involvement, pathological stage, or the type of surgical procedure.

Several studies have investigated the effect of β-arrestin1 on patient outcomes, generating conflicting results. For example, Michal et al found that decreased β-arrestin1 was associated with poor clinical outcome in breast cancer,[28] while Lundgren et al showed that high expression of β-arrestin1 markedly increased the risk of recurrence.[26] In addition, Wang et al documented that β-arrestin1 had no relationship with the prognosis of gastric cardia adenocarcinoma.[27] Santulli proposed that, in the adrenal medulla, β-arrestin1 and G-protein-coupled receptor kinases 2 (GRK2) finely regulated the secretion of catecholamines. But in the heart, β-arrestin1 and GRK2 inhibited β-adrenergic receptor (βAR)-mediated inotropic effects, which was detrimental to cardiac function.[29] For non-small cell lung cancer (NSCLC), our previous results were also conflicting. Ma et al found that loss of β-arrestin1 expression predicted unfavorable prognosis for NSCLC patients.[4] In contrast, Qiu et al observed that β-arrestin1 over-expression was associated with an unfavorable prognosis in lung adenocarcinoma.[3,5] So why do we come to opposite conclusions?

Initially, β-arrestin1 was appreciated for acting as a negative regulator of GPCR signaling, through the processes of receptor desensitization and internalization. Different receptors have varied functions in different situations. In the cytoplasm, β-arrestin1 also takes part in cell signaling pathways, including Src, MAPKs, small GTPases, components of the PI3K/AKT signaling cascade, transcription factors, cytoskeletal proteins, etc, by serving as a multifunctional scaffold, downstream of different classes of receptors or even via a receptor-independent mechanisms.[30] β-arrestin1 can play both positive and negative regulatory roles in this signaling axis, depending on upstream inputs and biological contexts. For instance, the β-arrestin1-mediated activation of PI3K results in phosphorylation of AKT, leading to cell growth, survival, and proliferation.[31] However,

β-arrestin1 has also been shown to scaffold the pleckstrin homology domain leucine-rich repeat protein phosphatase 2, with AKT and inactivated AKT.[32] Furthermore, β-arrestin1 can increase PTEN activity and thus inhibit AKT activation.[33]

Although cytoplasmic signaling by β-arrestins has been extensively described, their nuclear signaling has not received attention until very recently. Since Kang et al (2005) provided evidence that β-arrestin1 moved to the nucleus in response to GPCR stimulation, where it regulated gene expression by facilitating histone acetylation at specific gene promoters, nuclear function of β-arrestin1 has become a hot topic for increasingly more researchers.[9] Dasgupta et al found that nicotine induced the binding of β-arrestin1, p300, and Ac-H3 on E2F-regulated genes, then increased the expression of proliferative and survival genes, thereby contributing to the growth and progression of NSCLC.[11] High levels of nuclear β-arrestin1 were also observed in metastatic NSCLC. Shenoy et al later demonstrated that β-arrestin1 in breast cancer robustly interacted with nuclear hypoxia-induced factor 1α that was stabilized during hypoxia, and potentiated HIF-1-dependent transcription of the angiogenic factor VEGF-A.[12] Additionally, Rosano et al also documented that β-arrestin1-β-catenin interactions controlled the expression of β-catenin target genes, by promoting the recruitment of p300 acetyltransferase on these promoter genes, resulting in gene transcription, which were required for cell migration, invasion, and epithelial-to-mesenchymal transition.[10] Recently, another study confirmed that nuclear β-arrestin1 interacted with p300, contributing to the metabolic shift in prostate cancer cells via regulation of HIF1α transcriptional activity, under normoxic conditions.[13] The aforementioned findings indicate that nuclear β-arrestin1 can promote the expression of tumor-related genes, and that this function is largely performed by p300 acetyl-transferase, which enhances the activity of non-histone transcription factors and raises the level of histone acetylation.

In view of the different roles of β-arrestin1 in the cytoplasm and nucleus, we investigated the expression of cytosolic and nuclear β-arrestin1, respectively. Our previous results showed

that the expression of β-arrestin1 was either lost or low in squamous cell lung cancer, whereas the lack of β-arrestin1 expression was rare in adenocarcinoma. To eliminate the effect of tumor type, in this study, expression of β-arrestin1 was investigated in 133 cases of lung adenocarcinoma, of which 59% showed high cytoplast immunoreactivity and 47% showed high nuclear immunoreactivity. Obtained results showed that β-arrestin1(C) had no effect on OS and DFS, whereas patients showing high expression of β-arrestin1(N) had a poorer OS and shorter DFS than patients with low expression of β-arrestin1(N).

P300, a member of the histone acetyltransferase family of transcriptional co-activators, was found to participate in a broad spectrum of cellular processes, such as DNA repair, cell growth, differentiation, apoptosis, and migration.[34] The role of p300 in cancer has long been described. In our present research, p300 expression in lung adenocarcinoma was not associated with clinicopathological characteristics, such as age, sex, smoking status, drinking status, grade of differentiation, tumor location, T stage, N categories, pathological stage, or type of surgical procedure, which was consistent with results presented in a previous study on NSCLC.[15] Numerous studies have demonstrated that p300 overexpression is indicative of poor prognosis in most human malignancies.[14–21] Our study also found that p300 was linked to poor OS in lung adenocarcinoma. However, in the multivariate analysis, individual expression level of p300 was no longer an independent predictor.

Given that β-arrestin1 acts synergistically with p300 in the nucleus by forming a complex, the detection of the co-expression of these 2 factors may have more clinical and prognostic significance than the detection of individual expression. Therefore, we combined β-arrestin1(N) and p300 expression into 4 groups: low expression of both β-arrestin1(N) and p300, low β-arrestin1(N) and high p300 expression, high β-arrestin1(N) and low p300 expression, and high expression of both β-arrestin1(N) and p300. Results showed that patients with high expression of both β-arrestin1(N) and p300 had lower 5-year survival rates and shorter median survival times than the others. Our results could be used as a working model to define a role for β-arrestin1 and p300. However, in the survival analysis, the combination of these 2 factors was not an independent predictor, which should be further investigated.

Taken together, our findings provide a new diagnostic tool for predicting patient outcomes. For lung adenocarcinoma patients, a high expression of both β-arrestin1(N) and p300 is indicative of poor prognosis. Detecting β-arrestin1 in the nucleus, together with p300, can help in identifying patients with increased risk of cancer recurrence. Moreover, these observations and our findings suggest that detecting β-arrestin1(N) and p300 expression could help establish individual treatments for cancer patients. β-arrestin1 and p300 not only play important roles in cellular growth and migration, but also serve as key prognostic factors for lung adenocarcinoma. Based on this semiquantitative study, we consider that more quantitative measures of expression need to be implemented. In addition, the influence of β-arrestin1 and p300 expression levels on the prognosis of lung adenocarcinoma patients deserves further study.

Moreover, there are some limitations to our study. First, selection bias may be present due to the retrospective nature of this study. Second, this is a single-center study, with a limited sample size. More data from other institutions are essential to validate our results. Third, this research is based on semiquantitative measures of expression, which have limitations. More quantitative measures of expression are needed to properly investigate this phenomenon. Our results can only indirectly reflect the prognostic value of the β-arrestin1(N)-p300 complex, because of methodological limitations. Lastly, specific genetic alterations of lung adenocarcinoma show characteristic histology. It would be useful to detect major genetic driver mutations and their respective histological characteristics. We will certainly consider this for future work.

In conclusion, our data suggest that β-arrestin1 and p300 are potentially involved in the progression and prognosis of lung adenocarcinoma. The predictive value of nuclear β-arrestin1 may be optimized by combining information about the expression of p300 acetyltransferase, and co-expression of β-arrestin1(N) and p300 should be considered as a new molecular biomarker for the diagnosis and prognosis of lung adenocarcinoma.

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