RESEARCH ARTICLE

Expression of β-arrestin 1 in Gastric Cardiac Adenocarcinoma and its Relation with Progression

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Abstract

Objective: Arrestins act as mediators of G protein-coupled receptor (GPCR) desensitization and trafficking, also actin as a scaffold for many intracellular signaling network. The role that β-arrestin 1 plays in gastric cardiac adenocarcinoma (GCA) and its clinicopathologic significance are untouched. Methods: Fifty patients with gastric cardiac adenocarcinoma were retrospectively enrolled and β-arrestin 1 was detected using immunohistochemistry in tissue samples. Results: Nuclear expression of β-arrestin 1 was observed in 78% of GCA samples (39/50) and cytoplasmic expression in 70% (35/50). β-arrestin 1 could be found in both nucleus and cytoplasm of 54% GCA (27/50) or in either of them in 94% (47/50). β-arrestin 1 protein positivity in well/moderately differentiated carcinomas was significantly higher than that in poorly differentiated carcinomas (P=0.005). We found increased expression of β-arrestin 1 in cytoplasm was correlated with lymph nodal metastasis (P=0.002) and pathological lymph nodal staging (P=0.030). We also found β-arrestin 1 to be over-expressed in glandular epithelia cells of mucinous adenocarcinoma, a tumour type associated with an adverse outcome of gastric cardiac adenocarcinoma (P=0.022). Conclusion: β-arrestin 1 is over-expressed in the nucleus and/or cytoplasm of gastric cardiac adenocarcinoma. However, β-arrestin 1 has no relationship with the prognosis of gastric cardiac adenocarcinoma (P>0.05). Our data imply that β-arrestin 1 in cytoplasm may be involved in differentiation and metastasis of gastric cardiac adenocarcinoma.

Keywords: Gastric cardiac adenocarcinoma - β-arrestin 1- mucinous adenocarcinoma - metastasis

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Introduction

Adenocarcinoma of gastric cardia tract is associated with several different underlying risk factors and originates from the glandular epithelium of gastric mucosa with intestinal metaplasia or gastric/cardiac-type mucosa without intestinal metaplasia (Xiao et al., 2012). It has been reported to establish a specific category distinct from carcinoma of the rest of the stomach (Ohno et al., 1995; Ichikura et al., 2003). Gastric cardiac adenocarcinoma continues to rise in incidence in singulos annos around the world. Many studies have been and are being applied to reveal the genetic basis and molecular mechanisms for gastric cardiac adenocarcinoma formation and progression. However, the causal relationships between them have not been proven, yet.

Arrestins are originally characterized as structural adaptor proteins, which modulate the desensitization and endocytosis of 7-transmembrane receptors (7TMRs) (Sudha et al., 2011). There are four members of the arrestin family. Visual arrestin (arrestin 1) is localized to retinal rods and cones, whereas X-arrestin (arrestin 4) is found exclusively in retinal cones (Kovacs et al., 2009). β-arrestins 1 and 2 (arrestins 2 and 3, respectively) are ubiquitously expressed in combinations of tissues of mammals and have different functions (Buchanan et al., 2006). Furthermore, new insights have been given into the complexity of arrestins for the last few years. β-arrestins translocate from the cytoplasm to the nucleus and associate with transcription cofactors such as p300 and cAMP-response element-binding protein (CREB) at the promoters of target genes to promote transcription (Kang et al., 2005). β-arrestins are also involved in the development (Chen et al., 2004; Wilbanks et al., 2004) and cellular movement (Ge et al., 2004; Hunton et al., 2005; Buchanan et al., 2006) of cancers. Involvement of β-arrestin 1 in cellular movement may be important for mechanisms of cellular migration and metastasis.

This study aims to analyze the expression of β-arrestin 1 in gastric cardiac adenocarcinoma and its relationships with clinicopathologic characteristics and survival status, which might help to investigate the functions of β-arrestin 1 in the differentiation and metastasis progression of gastric cardiac adenocarcinoma.

Materials and Methods

Subjects

Fifty patients with gastric cardiac adenocarcinoma were collected, of whom there were forty-two males

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and eight females with an average age of 63 ± 9 years. The fifty patients with gastric cardiac adenocarcinoma underwent surgery at Provincial Hospital affiliated to Shandong University, Shandong, China, from January 2008 to December 2008. None of these fifty patients had received preoperative adjuvant therapy. This study was approved by the Ethics and Scientific Committees of our institution and had been conducted with written informed consent. All records of patients were derived from the Bio-Bank of Shandong Provincial Hospital.

All removed nodes were classified according to the anatomic subsites as described in the 7th UICC TNM staging system (Sobin et al., 2009). Patients were followed up until death or March 2012.

Tssue processing

All specimens were fixed with formalin and embedded in paraffin, and each block was sectioned at four-micrometer-thick. We made five slides for every individual, one of which was stained with hematoxylin and eosin for histopathological analysis by two pathologists and the others were used for immunostaining.

Immunohistochemical staining

Anti-β-arrestin 1 antibody is a rabbit polyclonal antibody against β-arrestin 1 (Abcam Biotechnology, America). In brief, with slides dewaxed at first, endogenous peroxidase activity was then quenched with 3% H_2O_2. After that, tissue samples were heated in 1 mmol/L Ethylenediaminetetraacetic acid (EDTA) buffer for 15 min in a water bath (96-98 °C) to retrieve antigens, and cross-reactivity was blocked with normal goat serum. The slides were then incubated overnight at 4 °C with primary antibodies (1:500 for anti-β-arrestin-1 antibody). The subsequent steps were according to the producer of Zymed (Streptavidin-Perosidase Method). The primary antibody was replaced by normal serum or phosphate-buffered saline (PBS) as negative controls (Song et al., 2003).

Evaluation of immunostaining

The criterion for a positive reaction was clear cytoplasm and nucleus staining. The samples with more than 10% of the tumor cells stained were considered to be β-arrestin-1-positive carcinomas. For the evaluation of β-arrestin 1 expression, extent of staining was scored as 0 (<10%), 1 (11-25%), 2 (26-50%), 3 (51-75%) and 4 (76-100%).

Statistical analysis

Pearson Chi-Square and Fisher's exact tests were used to evaluate clinicopathologic significance of β-arrestin 1 expression in human gastric cardiac adenocarcinoma. Chi-square tests were used to evaluate correlations between single variable and overall survival. The correlations between multiple variables and 4-year survival were measured by multivariate Cox regression model. Variables entered into the multivariate Cox regression model were obtained from the variables that were statistically significant in univariate analysis. All the statistical analyses were performed by SPSS software (version 18.0). Two-sided P values were calculated and a difference was considered significant if the P value was less than 0.05.

Results

Expression of β-arrestin 1 in human gastric cardiac adenocarcinoma

β-arrestin 1 was observed as nuclear expression in78% samples with GCA (39/50) and cytoplasmic expression in 70% samples with GCA (35/50), and β-arrestin 1 could be found in both nucleus and cytoplasm of 54% GCA (27/50) or in either of them of 94% GCA (47/50) (Figure 1). β-arrestin 1 could be detected in vascular endothelium and smooth muscle cells as well.

Clinicopathologic significance of β-arrestin 1 in human gastric cardiac adenocarcinoma

The relation between β-arrestin 1 expression and clinicopathologic variables in human gastric cardiac adenocarcinoma was examined. There was no significant association found between β-arrestin 1 expression and age, gender, smoking, alcohol consumption, pathological staging or metastasis status. β-arrestin 1 protein positivity

Table 1. Relationship Between β-arrestin 1 Expression and Clinicopathological Variables in Gastric Cardiac Carcinomas

Variable	n	β-arrestin 1 in GCA[2]			β-arrestin 1 in cytoplasm[2]			P value[1]
		Negative	Low	High	Negative	Low	High	
Total	50	3	19	28	15	30	5	
Differentiation								0.005
well/moderately	26	0	6	20				
poorly	24	3	13	8				
Lymph nodal staging								0.004
N_0	12				5	3	4	
N_1	30				5	24	1	
N_2	5				3	2	0	
N_3	3				2	1	0	
Lymph nodal metastasis								0.002
Negative	12				5	3	4	
Positive	38				10	27	1	

[1]Pearson Chi-Square and Fisher's exact tests were used; [2]The expression of β-arrestin 1 was classified into three groups as negative, low and high according to expression scoring; Negative, 0; Low, 1-2; High, 3-4

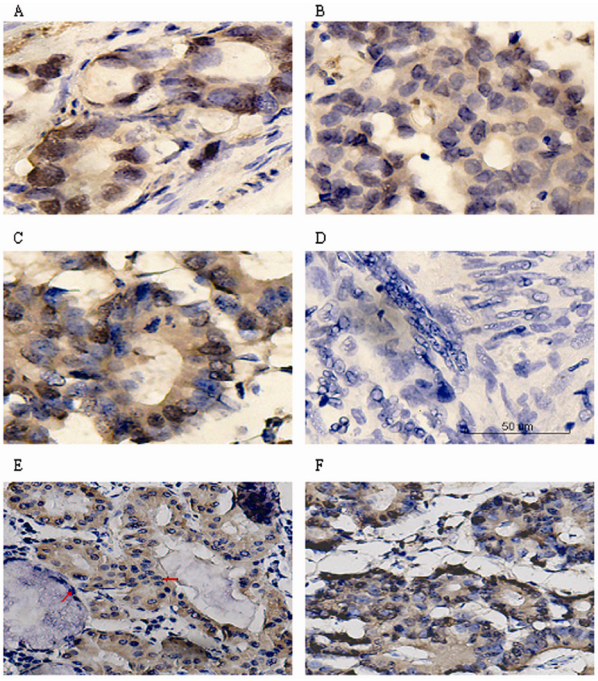

Figure 1. Immunohistochemical Staining of Gastric Cardiac Adenocarcinomas Using an Antibody Against β-arrestin 1. A: β-arrestin 1 predominantly expressed in nucleus; B: β-arrestin1 predominantly expressed in cytoplasm; C: β-arrestin-1-positive in both; D: β-arrestin-1-positive in neither; E: Comparison of the expression of β-arrestin 1 in mature and immature glandular epithelium as arrows pointed to in the picture; F: β-arrestin-1-positve in gastric cardiac carcinomas. E and F are from the same slide

Table 2. Comparison of Mucinous Adenocarcinoma and Overall Survival

Status	Non-mucinous adenocarcinoma	Mucinous adenocarcinoma	P value[1]
Death of disease	13	15	0.022
Survival with GCA	3	19	

[1]Pearson Chi-Square and Fisher's exact tests were used

in well/moderately differentiated carcinomas was significantly higher than that in poorly differentiated carcinomas (P=0.005) (Table 1). β-arrestin 1 was over-expressed in cytoplasm and significantly correlated with lymph nodal metastasis (P=0.002) and lymph nodal staging (P=0.030) (Table 1). The grades of β-arrestin 1 expression were classified into three groups (Negative, Low and High) according to expression scoring (Negative: 0; Low: 1-2; High: 3-4).

Relationship between overall survival and mucinous adenocarcinoma

We found patients with mucinous adenocarcinoma had poor prognosis (P=0.022). Interestingly, smattering mature glandular epithelia cells of mucinous adenocarcinoma showed weak-positive expression of β-arrestin 1, whereas adjacent immaturate glandular epithelia cells showed over-expression of β-arrestin 1 (Figure 2). Tumor cells secreted mucus which was delivered into the interstitium. It seemed that β-arrestin 1 would fade away along with the development of glandular epithelia cells in the mucinous adenocarcinoma of gastric cardiac adenocarcinoma.

Table 3. Univariate Analysis of Clinical Characteristic on 4 Years Survival

Characteristics	Chi-square	P value
Gender Female vs Male	0.336	0.562
Age <60 vs >=60	5.258	0.022
Smoking Index Never vs >=400	4.1041	0.0431
Alcohol consumption Negative vs Positive	1.945	0.163
Tumor differentiation well/moderately vs poorly	6.7682	0.0092
Mucus secretion Negative vs Positive	9.913	0.007
Distant metastasis Negative vs Positive	15.816	<0.001
β-arrestin 1 expression Negative vs Positive	3.921	0.141
Nodal stage N_0N_1 vs N_2N_3	7.842	0.049

[1]Compare of never smokers to smoking index>=400; [2]Compare of well and moderately differentiation to poorly differentiation

β-arrestin 1 disassociated with gastric cardiac adenocarcinoma prognosis

Our data showed that β-arrestin 1 expression was not correlated with gastric cardiac adenocarcinoma prognosis (P=0.141) (Table 3). Since β-arrestin 1 was universally expressed in gastric cardiac adenocarcinoma (either in nucleus or in cytoplasm of 94% samples with GCA), it seemed that β-arrestin 1 could not associate with gastric cardiac adenocarcinoma prognosis.

Survival analysis

Chi-square tests were used to evaluate the correlations between univariate analyses of clincal and pathology characteristics and overall survival. There were no statistical differences observed in gender (χ^2=0.336, P=0.562) or alcohol consumption (χ^2=1.945, P=0.163). However, age (P=0.022), smoking index (P=0.043), tumor differentiation (P=0.009), mucus secretion (P=0.007), lymph node staging (P=0.049) and distant metastasis (P<0.001) were significantly related with prognosis of gastric cardiac adenocarcinoma. In multivariate Cox Regression analysis, only distant metastasis was significantly and independently unfavorable prognostic factor of gastric cardiac adenocarcinoma (P<0.001) (data not shown).

Discussion

AIn current study, we found β-arrestin 1 was ubiquitously expressed in nucleus or/and in cytoplasm of samples with gastric cardiac adenocarcinoma, and there was positive expression in vascular endothelium and smooth muscle cells as well. We also found significant correlations between clinicopathologic characteristics and expression of β-arrestin 1 in cytoplasm. Expression of β-arrestin 1 in cytoplasm was significantly correlated with lymph nodal metastasis and lymph nodal staging,

which indicated that β-arrestin 1 in cytoplasm would be involved in the migration and metastasis of cancers (Buchanan et al., 2006).

Previous reports revealed that β-arrestin 1 not only modulated the desensitization and trafficking of G protein-coupled receptors, but also served as multi-functional adaptor which contributed to the regulation of multiple signaling molecules (Sudha et al., 2011). The abilities of β-arrestins to associate with and activate c-Src/ MAPK were observed, whose family members were involved in the development and progression of cancers (DeFea et al., 2000; Imamura et al., 2001). In addition, it has been shown that, LS-174T cells which constitutively express wild-type β-arrestin 1 metastasize at an extremely high rate (Buchanan et al., 2006). The association of β-arrestin function with c-Src/MAPK activation and metastasis, highlights the possible link of β-arrestin function to the development and progression of cancer.

Our data showed that there were no significant differences between gender or alcohol consumption and prognosis of gastric cardiac adenocarcinoma. However, controversy exits as to whether alcohol consumption associates with gastric adenocarcinoma prognosis. There is a meta analysis which testified no association between alcohol drinking and gastric cardia adenocarcinoma risk, even at higher doses of consumption (Tramacere et al., 2012), whereas other researches would say otherwise (Duell et al., 2011). As Table 3 mentioned, age, smoking index, tumor differentiation, mucus secretion, lymph nodal staging, and distant metastasis had been associated with adverse outcome of gastric cardiac adenocarcinoma. Besides, survival analysis showed median survival of GCA is 23.9 months (95%CI: 6.181-41.819), and survival rate of 4 years overall of GCA is 38% (data not shown).

β-arrestin 1 acts as a nuclear scaffold that recruits histone acetyl-transferase p300 to the transcription factor CREB, which leads to increased gene expression through the acetylation of histone H4 and chromatin reorganization (Wilbanks et al., 2004). Then, β-arrestin 1 potentially regulates Bcl-2 expression, which is critically involved in regulating the apoptosis of both normal and transformed cells (Shi et al., 2007). Furthermore, β-arrestin 1 can also inhibit tumor necrosis factor-induced NF-κB activity, the canonical pathway of which plays a major role in the control of immune response and inflammation. In addition, NF-κB is required to enhance the survival and proliferation of cells (Ghosh et al., 2002; Hayden et al., 2008). There are many other genes transactivited by β-arrestin 1 directly or indirectly, and further researches are urgently demanded to confirm them. β-arrestin 1 going nuclear may have a crucial role in biological behaviors.

β-arrestin 1 is differently expressed between maturate and immaturate glandular epithelia cells, and it is expressed apparently in malignant glandular epithelia cells in nucleus and in cytoplasm alone or in concert within the same specimen synchronously. This phenomenon is of interest, which implied that β-arrestin 1 would fade away along with the development of glandular epithelia cells in the mucinous adenocarcinoma of gastric cardiac adenocarcinoma, other than that β-arrestin 1 entering into nucleus may play a pivotal role in the progression of gastric cardiac adenocarcinoma. Our study may be the first focus on correlations between β-arrestin 1 and clinicopathologic characteristics and mucinous adenocarcinoma of gastric cardiac adenocarcinoma.

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