Title: GATA6-positive lung adenocarcinomas are associated with invasive mucinous adenocarcinoma morphology, HNF4α expression, and *KRAS* mutations

Short running title: GATA6 expression in lung adenocarcinoma

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Conflicts of interest and source of funding: None of the authors have any conflicts of interest to disclose. The present study did not receive any funding.

Word counts: 2343

ABSTRACT

Aims: GATA6 is known to play a role in lung development. However, its role in the carcinogenesis of lung cancer is not well studied. The aim of this study was to analyze GATA6 expression in lung adenocarcinomas (LA) by immunohistochemistry (IHC) to define its association with clinicopathological characteristics.

Methods and results: IHC analysis of GATA6 was performed using tissue microarray slides containing 348 LAs. The association between GATA6 expression and clinicopathological parameters was evaluated. GATA6 expression in epithelial tumors other than lung cancer was also evaluated. GATA6-positivity occurred in 47 LAs (13.5%). This was observed more frequently in younger patients ($p = 0.005$), was associated with the absence of lymph node metastasis ($p = 0.024$), well to moderately-differentiated tumors ($p < 0.001$), the absence of lymphatic invasion ($p = 0.020$), and the absence of vascular invasion ($p = 0.011$). GATA6 expression was associated with mucin production ($p < 0.001$), the invasive mucinous adenocarcinoma subtype ($p < 0.001$), *KRAS* mutations ($p = 0.026$), positive expression of MUC2 ($p < 0.001$), CDX2 ($p = 0.049$), MUC5AC ($p < 0.001$), and negative expression of TTF-1 ($p = 0.002$). GATA6 expression was also associated with HNF4α expression ($p < 0.001$). Positive expression of GATA6 tended to indicate better prognoses, whereas patients

with HNF4α expression had significantly worse prognoses ($p = 0.033$). Of 270 tumors other than lung cancer, 110 expressed GATA6.

Conclusions: These findings suggested that GATA6 might interact with HNF4α and contribute to the development of mucinous type LAs.

Key words: Lung adenocarcinoma, GATA6, immunohistochemistry, hepatocyte nuclear factor 4 alpha, invasive mucinous carcinoma

Introduction

GATA6 is a member of zinc finger transcription factor that is expressed during the development of several organs, including the lungs.[1, 2] During fetal development, GATA6 plays a crucial role in the branching morphogenesis and epithelial cell differentiation of the lung.[3-6] In the mature lung, this factor activates the transcription of surfactant proteins A and C [7, 8] and regulates progenitor cell numbers during airway regeneration.[9] Thus, some studies have been conducted focusing on its role in the development of lung cancer, which is a leading cause of cancer-related death worldwide.[10-12] However, the clinicopathological characteristics of lung cancer as they relate to GATA6 expression have not been well studied.

The aim of this study was to analyze GATA6 expression in resected lung adenocarcinoma, which is the most common histologic type of primary lung cancer,[13] and compare this to prognosis and gene alterations to define clinicopathological associations.

Materials and Methods

Patient cohort

Between January 2001 and December 2009, consecutive patients with lung

adenocarcinomas who underwent pulmonary resections at Kyoto University Hospital were enrolled. Patients were excluded if they had multiple primary lung cancers, had undergone chemo- or radiotherapy before surgery, had incomplete resection, or lacked complete follow-up data in the Thoracic Surgical Database of the Department of Thoracic Surgery. Clinical data, including sex, age, and smoking status, were obtained from the database. Patients provided informed consent at the time of surgery. Approval for this retrospective study was granted by the institutional ethics committee (R0044-1).

Histologic evaluation

All resected specimens were fixed in formalin, sectioned, and stained with hematoxylin and eosin in the conventional manner. Elastic stains were also performed to detect invasion of the pleura or vessels. Slides of lung adenocarcinoma were reviewed by two pathologists (A.Y. and S.S.), who were blinded to patient outcomes.[14] All lung adenocarcinoma cases were classified according to 2015 World Health Organization criteria.[13] In addition, tumor staging was performed according to the 7th edition of the International Union Against Cancer tumor, node, and metastasis classification.[15] Lymphatic invasion, vascular invasion, pleural invasion, and/or tumor grade were further assessed according to the International Association for the Study of Lung

Cancer/American Thoracic Society/European Respiratory Society classification.[16]

Tissue microarray (TMA) construction

We prepared TMA slides of lung adenocarcinomas that we had used in previous lung cancer studies.[17-19] Briefly, tissue cores were punched from each donor tumor block using thin-walled 2-mm stainless steel needles (Azumaya Medical Instruments, Tokyo, Japan), and cores were arrayed in a recipient paraffin block. Non-neoplastic tissue cores from selected patients were arrayed in the same block. All subsequent tests, including immunohistochemistry and fluorescence in-situ hybridization, were performed on serially-cut, 4-μm, paraffin-embedded tissue sections.

To study the expression of GATA6 in other tumors, we prepared TMA slides containing 23 ovarian epithelial tumors, 16 endometrial adenocarcinomas, four uterine cervical adenocarcinomas, 51 pancreatic adenocarcinomas, 53 colorectal adenocarcinomas, and 123 bile duct adenocarcinomas.

Immunohistochemistry (IHC) studies

IHC was performed on the TMA slides. Staining of GATA6 was manually performed. Sections were incubated with primary anti-GATA6 antibody (sc-9055, rabbit polyclonal,

Santa Cruz Biotechnology, Santa Cruz, CA, USA) and successively reacted with Novolink Polymer (Leica Microsystems, Wetzlar, Germany). The sections were visualized using diaminobenzidine counterstained with hematoxylin. Scoring IHC for GATA6 was based on the distribution and intensity of staining (H score). Only nuclear staining was considered to contribute to GATA6 positivity (Figs. 1C,D), whereas cytoplasmic staining was considered non-specific, as shown in previous studies using the same antibodies.[20, 21] A positive case was indicated by an H score of 50–300 and a negative case by an H score of 0–49, according to previous reports.[20, 21] At this time, we confirmed that normal lung tissue exhibits the absence of or extremely low GATA6 expression.

We attempted to evaluate the association between GATA6 expression and the expression of the following proteins using specific antibodies, indicated as follows: CDX2 (clone AMT24, Leica Microsystems), hepatocyte nuclear factor 4 alpha (HNF4α; clone H1415, Perseus Proteomics Microsystems Inc., Tokyo, Japan), MUC2 (clone Ccp58, Leica Microsystems), MUC5AC (clone CLH2, Leica Microsystems), MUC5B (clone H-300, Santa Cruz Biotechnology), TTF-1 (clone SPT24, Novocastra, Newcastle upon Tyne, UK). IHC using antibodies except for GATA6 was performed with an automatic immunostainer (Benchmark, Ventana Medical Systems, Tucson, AZ, USA), according

to the manufacturer's instructions.[19]

Detection of genetic alterations in EGFR, KRAS, BRAF, HER2, ALK, and ROS1

The association between GATA6 expression and gene alterations in *EGFR*, *KRAS*, *HER2*, *BRAF*, *ALK*, and *ROS1* was evaluated. Mutation analysis was reported in our previous studies.[17, 22-27]

Statistical analysis

Data were analyzed using JMP® Pro 12.2.0. Comparisons between two groups were performed using the chi-squared test or Fisher's exact test to analyze categorical variables. Kaplan–Meier analysis was used to assess overall survival (OS) and disease-free survival (DFS), and the differences were analyzed by performing a log-rank test. Statistical significance was indicated at $p < 0.05$. Correction for multiple comparisons was not performed.

Results

GATA6 expression in lung adenocarcinoma and association with clinicopathological features

The clinicopathological features of cases as they related to GATA6 expression are shown in Tables 1 and 2. Of 348 cases, GATA6 expression was positive in 47 lung adenocarcinomas (13.5%) (Figs. 1C,D). GATA6 expression was observed more frequently in younger patients (65 or younger) than in older patients (66 or older) ($p = 0.005$), but was not associated with gender or smoking status. Interestingly, it was also associated with the absence of lymph node metastasis ($p = 0.024$). Regarding its relationship with histological features, GATA6 expression was associated with well to moderately differentiated tumors ($p < 0.001$), the absence of vascular invasion ($p = 0.011$), and the absence of lymphatic invasion ($p = 0.020$), although it was not associated with tumor size, tumor stage, or pleural invasion. Moreover, GATA6 expression was associated with presence of a lepidic component ($p < 0.001$), invasive mucinous adenocarcinoma (IMA) ($p < 0.001$), and mucin production ($p < 0.001$). It was also associated with the absence of a micropapillary component ($p = 0.022$) and a solid component ($p < 0.001$).

Association between GATA6 expression and other immunohistochemical parameters

The association between GATA6 expression and other markers is shown in Table 3. GATA6 expression was associated with negative expression of TTF-1 ($p = 0.002$) and

positive expression of MUC5AC ($p < 0.001$), MUC2 ($p < 0.001$), CDX2 ($p = 0.049$), and HNF4α ($p < 0.001$). We showed that most GATA6-expressing tumors were classified as well to moderately differentiated (n = 39, 82.9%) (Table 2), of which about one third were the mucinous type associated with HNF4α (n = 15, 38.4%) and about two thirds were non-mucinous type tumors not associated with HNF4α (n = 24, 61.5%) (Table 4).

Association between GATA6 expression and gene alterations

The association between GATA6 expression in lung adenocarcinoma and gene alterations is shown in Table 5. GATA6-positive tumors tended to harbor *KRAS* mutations ($p = 0.026$), whereas mutations in *EGFR*, *HER2*, and *BRAF*, amplification of *HER2*, and fusion of *ALK* or *ROS1* were not associated with GATA6 expression.

GATA6 expression and patient survival

Patients with GATA6 expression tended to have better prognoses with an 85.1% OS rate at 5 years; however, this was not statistically significant (OS, $p = 0.27$; DFS, $p = 0.16$) (Figs. 2A,B). In contrast, patients with HNF4α expression, which was associated with GATA6 expression, had significantly worse prognoses (OS, $p = 0.033$; DFS, $p= 0.20$)

(Figs. 2C,D). Since GATA6 expression was strongly associated with IMA morphology and HNF4α expression, we explored survival based on the expression of GATA6 and HNF4α. We divided the cases into four groups based on expression patterns and compared the survival curves among the groups (Figs. 2E,F). The OS rate in GATA6-negative and HNF4α-positive cases was significantly lower than that in GATA6-positive and HNF4α-negative cases ($p = 0.011$) and GATA6-negative and HNF4α-negative cases ($p = 0.018$). The DFS rate was significantly lower in GATA6-negative and HNF4α-positive cases than in GATA6-positive and HNF4α-negative cases ($p = 0.026$). The subset of patients with GATA6 and HNF4α coexpression appeared to have an intermediate survival prognosis (not statistically significant). We performed multivariate analysis to evaluate the prognostic role of GATA6, which had no significant influence on the OS or DFS (data not shown).

GATA6 expression in other epithelial tumors

GATA6 expression in tumors other than lung adenocarcinoma was evaluated using TMA slides (Table 6). Of 23 ovarian epithelial tumors, five tumors (21.7%) were positive for GATA6. Of these, four were of the mucinous subtype, including mucinous cystadenoma, mucinous borderline tumor, and mucinous carcinoma. Of 16 endometrial

adenocarcinomas, only one tumor (6.3%), which was of the mucinous subtype, was positive for GATA6. No uterine cervical adenocarcinoma expressed GATA6, although no tumors of the mucinous subtype were included in this cohort. Forty-two (82.4%) of 51 pancreatic adenocarcinomas, 28 (52.8%) of 53 colorectal adenocarcinomas, and 34 (27.6%) of 123 bile duct adenocarcinomas were positive for GATA6.

Discussion

GATA6, which belongs to a family of zinc finger transcription factors, has been known to play important regulatory roles in the development of tissues, including the lung.[1, 2, 28] Thus, many researchers have speculated that this role is recapitulated during lung cancer initiation,[11, 12, 29, 30] and thus its role in lung cancer development has been studied. In the current study, we demonstrated that lung adenocarcinoma with GATA6 expression represents a minor subset (13.5%), and expression was strongly associated with well to moderately differentiated tumors, lepidic morphology, the absence of lymph-vascular invasion, and the absence of lymph node metastasis. In addition, cases with GATA6 expression tended to be associated with better prognoses. These results were similar to those of previous studies.[11, 12] In contrast, our results revealed that GATA6 expression was associated with mucin-producing tumors, IMA subtype, *KRAS* mutations, positive

expression of MUC2, CDX2, MUC5AC, and HNF4α, and negative expression of TTF-1. These results, for the first time, demonstrated that GATA6 could play a role in the development of the mucinous type of lung adenocarcinoma.

GATA6 has been implicated in aspects of lung development [1, 2, 28, 31] and the pathophysiology of lung cancer.[11, 12] Some studies have been conducted to assess the potential role of GATA6 in lung cancer. In 2008, Zhang et al. reported a marked loss in airway epithelial differentiation in lung epithelium-specific Gata6-deficient mice and concluded that GATA6 may be involved in differentiation of regional stem cells.[9] Moreover, other groups demonstrated that the expression of GATA6 is associated with more differentiated and a favorable histologic pattern, such as lepidic-predominant adenocarcinoma.[11, 12] These reports may support our results that indicate lung adenocarcinoma with GATA6 expression is a subtype of disease associated with lepidic morphology and better prognosis. On the other hand, we also showed that GATA6 expression is strongly associated with the expression of HNF4α as well as mucinous type tumors and *KRAS* mutations. These results might indicate that GATA6 interacts with HNF4α and contributes to the development of mucinous type adenocarcinoma. Morrisey et al. reported that *HNF4* gene expression is absent in *GATA6*−/− ES cells and GATA6-deficient embryos and that forced expression of GATA6 activates the *HNF4*

promoter.[32] Jonckheere et al. reported that *Muc5B*, which is a target gene of GATA6, is involved in lung differentiation during development and carcinogenesis.[33] Moreover, this group also reported that GATA6 and HNF4α are transactivators of *Muc5ac* promoter activity and mRNA expression.[34] We previously demonstrated that MUC5B and/or MUC5AC are representative markers of non-terminal respiratory unit (non-TRU) type lung adenocarcinoma, including mucinous type adenocarcinoma.[19] Recently, Chia et al. showed that HNF4α, which is a direct target of KLF5/GATA4/GATA6, is required for gastric cancer proliferation.[35] Taken together, GATA6 might promote the expression of HNF4α, and this interaction could have a role in the carcinogenesis of mucinous type lung adenocarcinoma. However, the current study demonstrated differences in the prognostic significance of GATA6 and HNF4α expression; specifically, GATA6-positive cases tended to have relatively better prognoses, which may seem contradictory. We speculate that GATA6-positive tumors contain two distinct subgroups; one subgroup depends on HNF4α, and the other subgroup is independent of HNF4α (Fig. 3). Our study demonstrated that the subset of patients with HNF4α and GATA6 coexpression appear to have an intermediate survival prognosis (not statistically significant). The result may support our speculation, although further studies are needed.

Several researchers have reported that GATA6 expression is observed in cancers of

multiple organs including gastrointestinal, ovarian, and pancreatobiliary carcinomas.[36-40] In our study, most mucinous-type tumors derived from different organs expressed GATA6. Therefore, this marker, as well as HNF4α, could not be used to distinguish between primary lung cancer and metastases from other organs. However, GATA6 was expressed in lung adenocarcinoma cancer cells, whereas it was not expressed in normal lung tissue. We often observed goblet-rich epithelium or glandular metaplasia in conditions of idiopathic interstitial pneumonias (IIPs). Because it is difficult to distinguish between this type of epithelium and IMA using small biopsy specimens, GATA6, as well as HNF4α, could be helpful to distinguish these tissue types.[41] Although we did not examine GATA6 expression in epithelia with IIPs, we plan to evaluate this in the future.

There were limitations in this study. First, TMA slides were used for the evaluation of protein expression in our study; thus, the effects of histologic heterogeneity on the results cannot be disregarded. Based on these concerns, some researchers have examined discrepancies in protein expression between TMA sections and whole sections and have obtained equally informative results.[42, 43] Therefore, we believe that our study using a 2-mm core was accurate, representative, and efficient for evaluating IHC status. Second, this study enrolled Japanese patients who had high rates of *EGFR*

mutations and low rates of *KRAS* mutations. This raises the possibility that the *KRAS* associations may not be applicable to other populations, and the association seen in this study may not be reproducible across other cohorts. Future studies using cohorts with high rates of *KRAS* mutations are needed.

In conclusion, GATA6 expression in lung adenocarcinoma was associated with mucinous histology, IMA subtype, HNF4α expression, and *KRAS* mutations. These findings suggested that GATA6 might play an important role in the development of mucinous type lung adenocarcinoma. In addition, GATA6 expression might direct mucinous tumors to more differentiated or less-invasive status.

Acknowledgments

We would like to thank Editage (www.editage.jp) for English language editing.

Conflicts of interest

The authors declare no conflicts of interest.

Author contributions

Naoki Nakajima and Akihiko Yoshizawa wrote the paper. Naoki Nakajima, Akihiko Yoshizawa, Mariyo Rokutan-Kurata, and Hironori Haga designed the study. Tomoyuki

Nakajima, Masahiro Hirata, and Ayako Furuhata provided technical guidance for the immunohistochemistry and fluorescence in-situ hybridization. Naoki Nakajima, Akihiko Yoshizawa, and Shinji Sumiyoshi analyzed the pathological data. Makoto Sonobe, Toshi Menju, Ei Miyamoto, Toyofumi F. Chen-Yoshikawa, and Hiroshi Date analyzed the clinical data.

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Figure legends

Figure 1. Representative images of GATA6-positive lung adenocarcinoma; invasive mucinous adenocarcinoma (IMA) (A, C, E) and non-mucinous lepidic growth adenocarcinoma (B, D, F). (A, B) Hematoxylin and eosin staining. (C, D) Immunohistochemical images of GATA6. Tumor cells in IMA sample (C) and in non-mucinous lepidic growth adenocarcinoma sample (D) showed nuclear staining (inset, high magnification). (E, F) Immunohistochemical images of HNF4α. Tumor cells in IMA samples showed nuclear staining (E), whereas no expression of HNF4α was seen in tumor cells of non-mucinous lepidic growth adenocarcinoma (F) (inset, high magnification). Bar indicates 100 μm.

Figure 2. Overall survival (OS) and disease-free survival (DFS) curves for lung adenocarcinoma based on GATA6 and HNF4α expression. (A, B) Cases with GATA6-positive tumors tended to be associated with better prognosis; however, this difference was not statistically significant (OS, $p = 0.27$; DFS, $p = 0.16$). (C, D) Cases with HNF4α-positive tumors were associated with significantly worse prognosis (OS, $p = 0.033$; DFS, $p = 0.20$). (E, F) OS rate for GATA6-negative and HNF4α-positive cases was significantly lower than that for GATA6-positive and HNF4α-negative cases ($p = 0.011$) and GATA6-negative and HNF4α-negative cases ($p = 0.018$). DFS was

significantly lower for cases with GATA6-negative and HNF4α-positive tumors than those with GATA6-positive and HNF4α-negative tumors ($p = 0.026$).

Figure 3. Visual schematic of possible relationships between GATA6 and other factors in lung cancer. Either directly or indirectly through promotion of transcription of HNF4α, GATA6 may promote the transcription of MUC5AC and/or MUC5B, contributing to the development of mucinous type adenocarcinoma (Ref. 32-35). Conversely, GATA6 may inhibit development of mucinous type adenocarcinoma through activation of transcription of TTF-1, which represses HNF4α and MUC5B expression (Ref . 31, 44). Thus, GATA6-positive lung adenocarcinoma may be considered a heterogenous group. Abbreviations: Ref. , reference; TRU, terminal respiratory unit

Table 1. Association between GATA6 expression and clinical characteristics of lung adenocarcinoma

Characteristics		Expression of GATA6		
		Positive	Negative	*p*-Value
Total		47	301	
Age	≤65	31	133	0.005
	>65	16	168	
Gender	Male	19	164	0.073
	Female	28	137	
Smoking	Never	22	135	0.80
	Ever	25	166	
Stage	IA	29	160	0.070
	IB	11	61	
	IIA	1	27	
	IIB	0	9	
	IIIA	3	32	
	IIIB	1	0	
	IV	2	12	
T factor	T1a	21	115	0.81
	T1b	10	73	
	T2a	12	89	
	T2b	1	5	
	T3	1	13	
	T4	2	6	
N factor	N0	44	242	0.024*
	N1-3	3	55	

* Fisher's exact test

Table 2. Association between GATA6 expression and histological characteristics

		Expression of GATA6		
Characteristics		Positive	Negative	*p*-Value
Total		47	301	
Grade	Well to Moderately diff.	39	154	<0.001
	Poorly diff.	8	147	
Tumor size	≤30 mm	34	220	0.91
	>30 mm	13	81	
Pleural invasion	Negative	40	220	0.078
	Positive	7	81	
Vascular invasion	Absence	41	209	0.011
	Presence	6	92	
Lymphatic invasion	Absence	44	239	0.020
	Presence	3	62	
WHO classification	AIS non-mucinous	3	6	<0.001
	AIS mucinous	1	0	
	MIA non-mucinous	2	15	
	Lepidic	2	25	
	Acinar	10	38	
	Papillary	17	131	
	Solid	1	71	
	Micropapillary	0	14	
	IMA	11	1	
Lepidic component	Absence	10	158	<0.001
	Presence	37	143	
IMA or not	IMA	11	1	<0.001*
	non-IMA	36	300	
Solid component	Absence	37	149	<0.001
	Presence	10	152	
MP component	Absence	40	207	0.022
	Presence	7	94	
Mucin production	Absence	31	293	<0.001*
	Presence	16	8	

* Fisher's exact test

Abbreviations: AIS, adenocarcinoma in situ; MIA, minimally invasive adenocarcinoma; IMA, invasive mucinous adenocarcinoma; diff., differentiated; MP, micropapillary

Table 3. Association between GATA6 expression and immunohistological results of lung adenocarcinoma staining

		Expression of GATA6		
antibody		Positive	Negative	*p*-Value
TTF-1	Positive	35	267	0.002
	Negative	12	30	
MUC5B	Positive	20	90	0.094
	Negative	27	207	
MUC5AC	Positive	17	19	<0.001
	Negative	30	281	
MUC2	Positive	9	4	<0.001*
	Negative	38	197	
CDX2	Positive	2	1	0.049*
	Negative	45	300	
HNF4α	Positive	25	29	<0.001
	Negative	22	272	

* Fisher's exact test

Table 4. Histological characteristics of 47 GATA6-positive lung adenocarcinomas and association between HNF4α expression and histological subtypes.

		mucinous subtype		non-mucinous subtype	
		Well to moderately diff.	poorly diff.	Well to moderately diff.	poorly diff.
HNF4α	Positive	15	1	6	3
	Negative	0	0	18	4

Abbreviation: diff., differentiated

Table 5. Association between GATA6 expression and gene alterations in lung adenocarcinoma

		Expression of GATA6		
Gene		Positive	Negative	*p*-Value
EGFR mutations	Wild type	17	108	0.28
	mutated	12	99	
KRAS mutations	Wild type	22	187	0.026*
	mutated	7	19	
HER2 mutations	Wild type	17	127	1.0*
	mutated	0	6	
BRAF mutations	Wild type	31	205	1.0*
	mutated	0	3	
HER2 FISH	not amplified	30	206	1.0*
	Amplified	1	11	
ALK rearrangement	Negative	47	294	0.59*
	Positive	0	7	
ROS1 rearrangement	Negative	47	299	1.0*
	Positive	0	2	

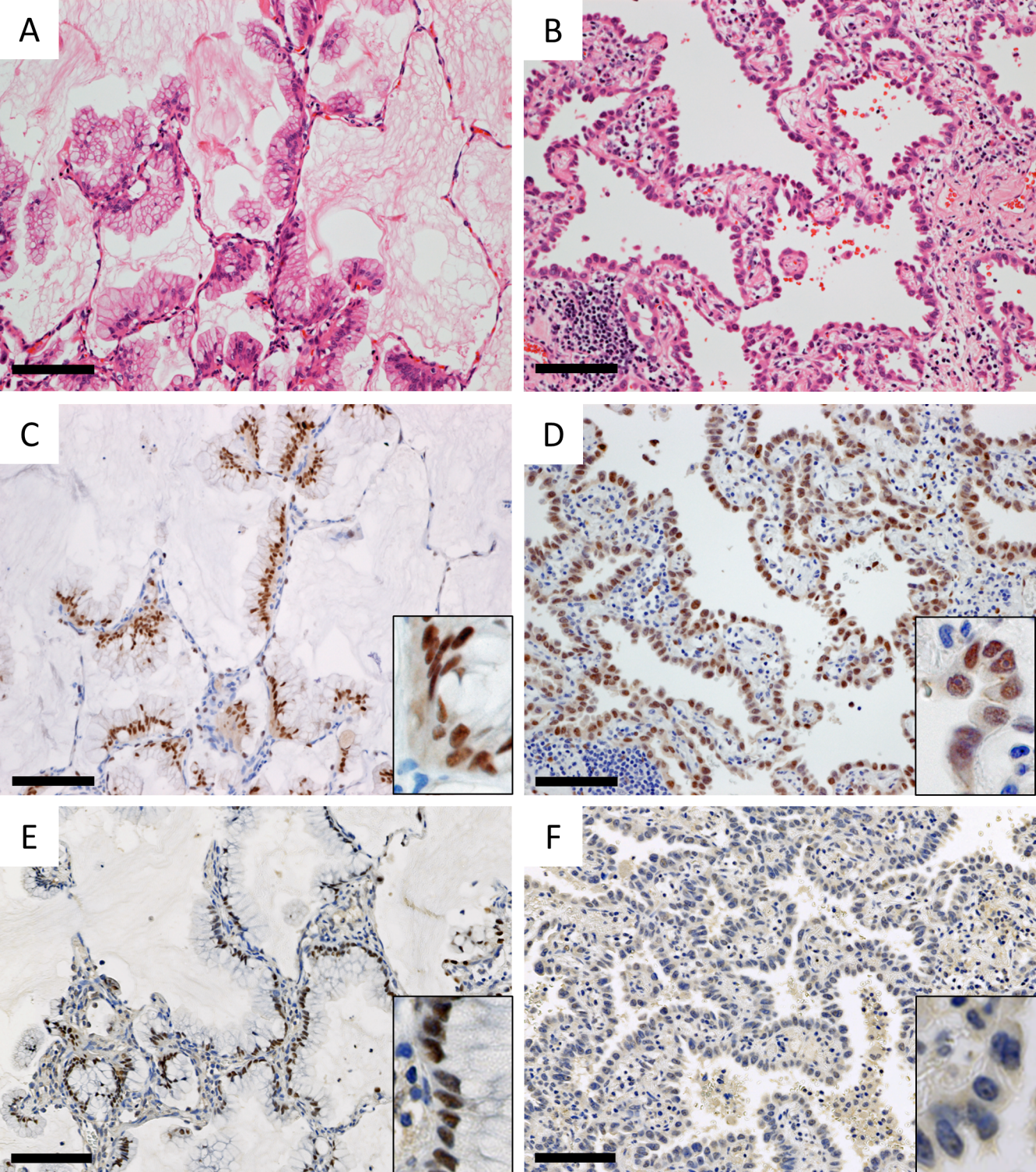
* Fisher's exact test

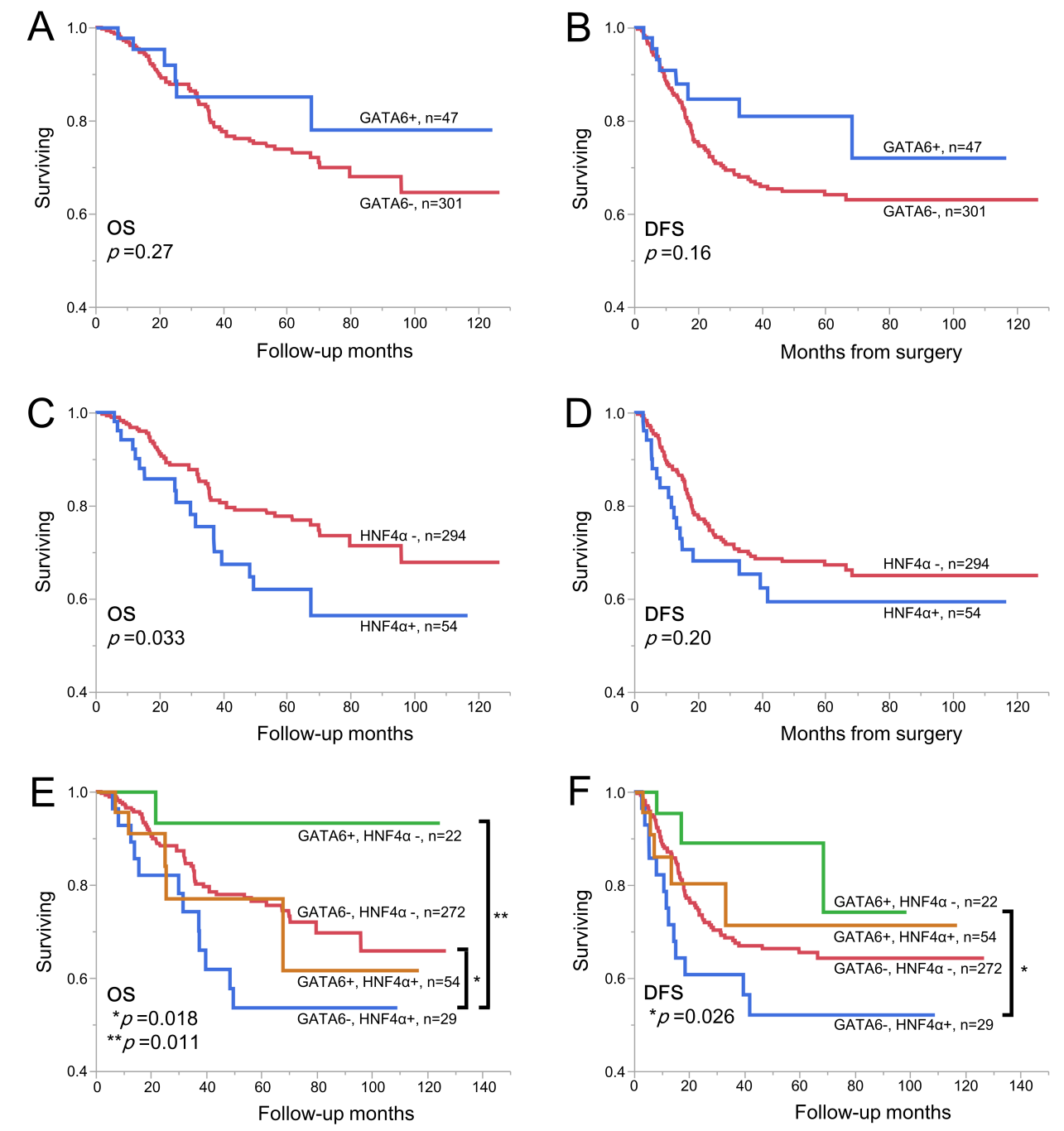
Table 6. Expression of GATA6 in epithelial tumors other than lung adenocarcinoma

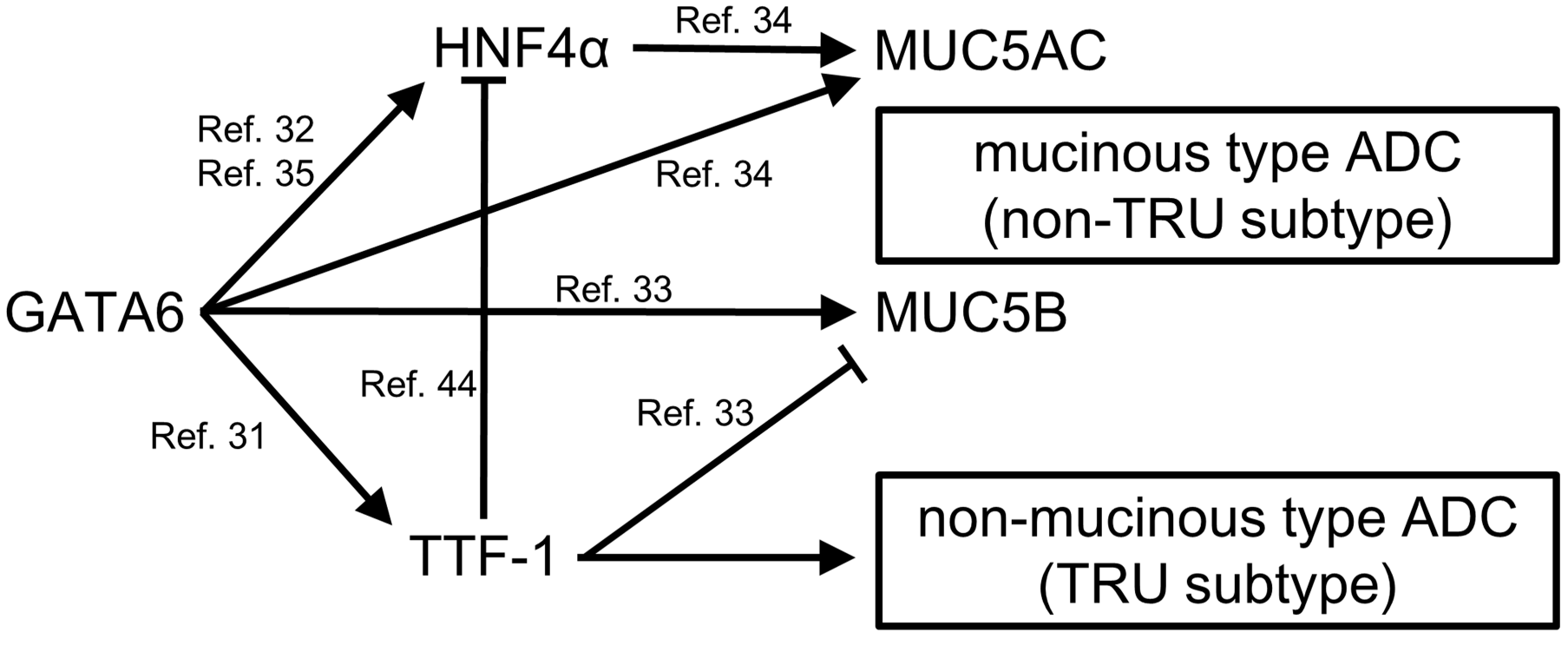
			Expression of GATA6		
Organs	subtype	Total (n)	Positive	Negative	*p*-value
Lung ADC		348	47	301	
	non-mucinous	324	31	293	<0.001
	mucinous	24	16	8	
Ovarian epithelial tumor		23	5	18	
	non-mucinous	18	1	17	0.002*
	mucinous	5	4	1	
Endometrial ADC		16	1	15	
	non-mucinous	15	0	15	0.06*
	mucinous	1	1	0	
Uterine cervical ADC		4	0	4	
	non-mucinous	4	0	4	NA
	mucinous	0	0	0	
Pancreatic ADC		51	42	9	
	non-mucinous	19	17	2	0.45*
	mucinous	32	25	7	
Colorectal ADC		53	28	25	
	non-mucinous	45	22	23	0.25*
	mucinous	8	6	2	
Bile duct ADC		123	34	89	
	non-mucinous	95	20	75	0.002
	mucinous	28	14	14	

* Fisher's exact test

Abbreviations: ADC, adenocarcinoma; NA, not available


A
B
C
D
E
F


A
Surviving
1.0
0.8
0.6
0.4
GATA6+, n=47
GATA6-, n=301
OS
p =0.27
0
20
40
60
80
100
120
Follow-up months
B
Surviving
GATA6+, n=47
GATA6-, n=301
DFS
p =0.16
Months from surgery
C
Surviving
HNF4α -, n=294
HNF4α+, n=54
OS
p =0.033
Follow-up months
D
Surviving
HNF4α -, n=294
HNF4α+, n=54
DFS
p =0.20
Months from surgery
E
Surviving
GATA6+, HNF4α -, n=22
GATA6-, HNF4α -, n=272
GATA6+, HNF4α+, n=54
GATA6-, HNF4α+, n=29
**
*
OS
*p =0.018
**p =0.011
140
Follow-up months
F
Surviving
GATA6+, HNF4α -, n=22
GATA6+, HNF4α+, n=54
GATA6-, HNF4α -, n=272
GATA6-, HNF4α+, n=29
*
DFS
*p =0.026
Follow-up months


HNF4α
Ref. 34
MUC5AC
Ref. 32
Ref. 35
Ref. 34
mucinous type ADC
(non-TRU subtype)
GATA6
Ref. 33
MUC5B
Ref. 44
Ref. 31
Ref. 33
TTF-1
non-mucinous type ADC
(TRU subtype)