CHAPTER III

RESULTS

3.1 Determination of urinary protein by 2-DE analysis

3.1.1 The expression profiles of pooled urinary proteins

To search for potential biomarkers of lung cancer, urinary proteins secreted from lung cancer patients and healthy controls were systematically analyzed. First, the protein expression pattern of pooled urine samples from healthy controls and patients were separated using 2-DE analysis with a narrow pH range of 4-7. Six proteins were up-regulated and three proteins were down-regulated, especially GM2AP protein spot (**Figure 3.1**).

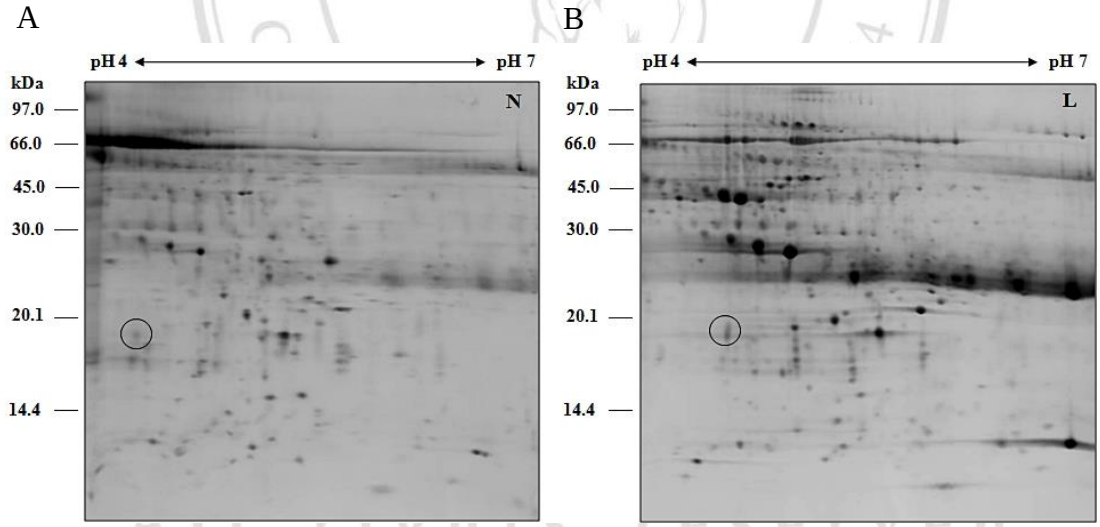
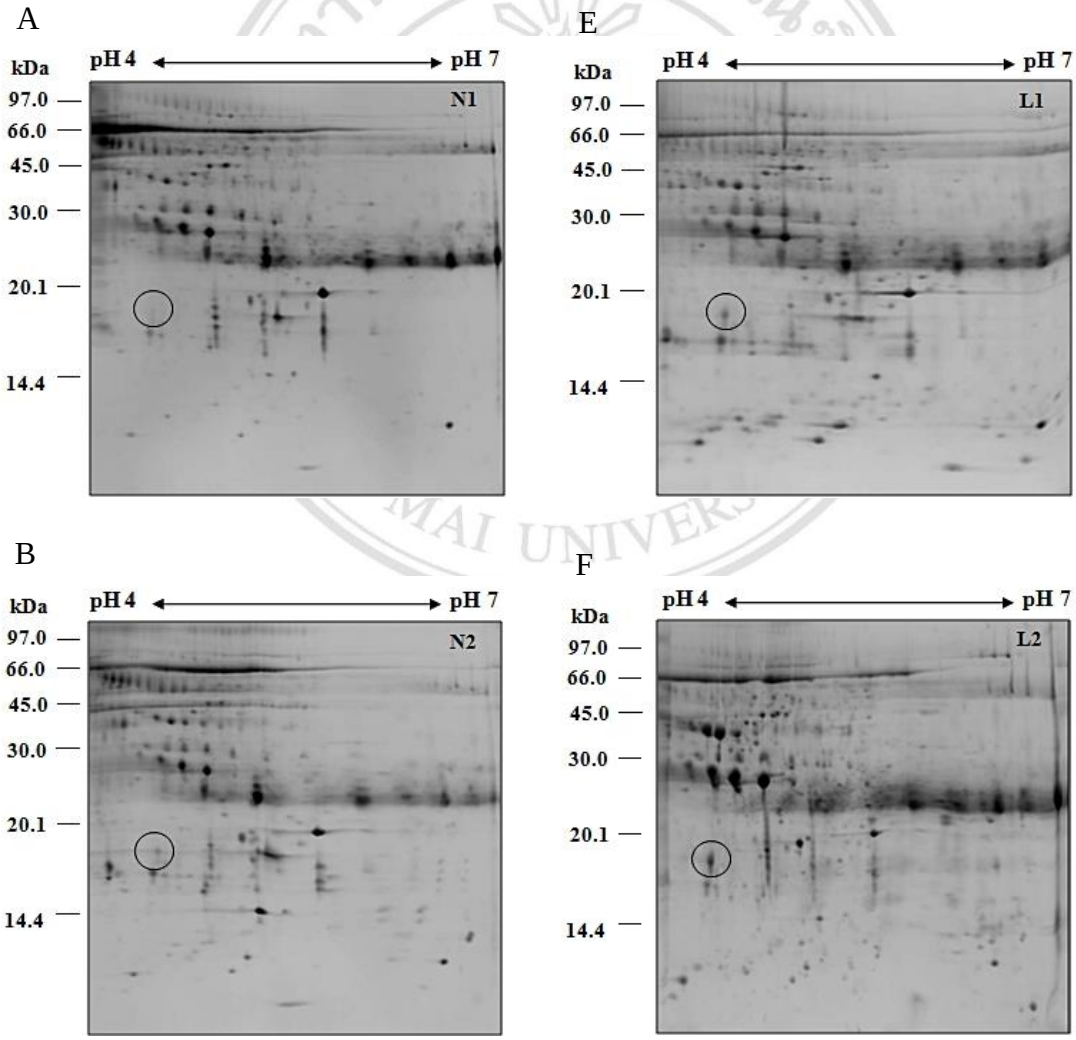


Figure 3.1 2-DE images of pooled urinary proteins. A total of 200 µg of urine samples from healthy donors (A) and lung cancer patients (B) were applied to a pI 4-7 IPG strip for the first dimension and separated on 15% SDS-PAGE gel for the second dimension in the 2-DE. The gels were strained using Sypro® Ruby. The black circles indicate the position of GM2AP with approximate pI of 4.3 and molecular weight of 18.6 kDa.

3.1.2 The expression profiles of individuals urinary proteins

Due to the differentially expressed levels of pooled urinary proteins, the protein of interest was identified as GM2AP marked in the 2-DE map. Then, the urine samples from healthy individuals and patients with different subtype of lung cancer were used for 2-DE analysis to confirm the GM2AP expression. The result revealed that GM2AP level in each subtype of lung cancer patients (**Figure 3.2 E-H**) were also greatly over-expressed compared to those of healthy controls (**Figure 3.2 A-D**).



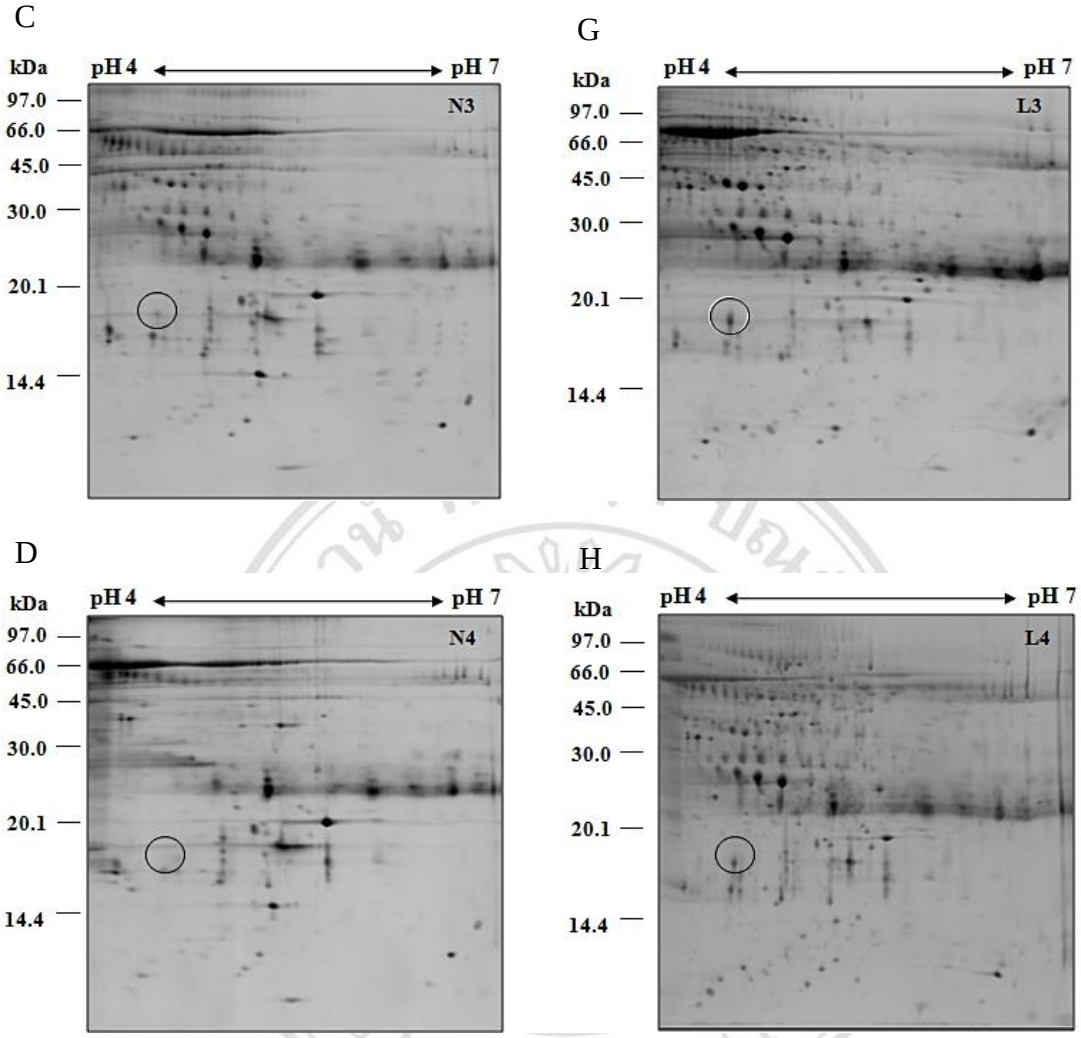


Figure 3.2 2-DE images of individual urinary proteins. Urine samples of healthy controls (A-D) and patients with different subtypes of lung cancer (E-H), N: Healthy control. L: Lung cancer patient (L1: Adenocarcinoma, L2: Small cell lung cancer, L3: Squamous cell carcioma, L4: Other types of carcinoma). The black circles indicate the position of GM2AP.

3.1.3 Identification of GM2AP spot from 2-DE gels

GM2AP spots from 2-DE gel were excised and subjected to in-gel enzymatic digestion using trypsin and chymotrypsin, followed by the nanoLC-MS/MS analysis as previously described in experimental procedure. The database searches were performed against Swiss-Prot database using Mascot software with fixed modification as carbamidomethyl (C). The protein spot was identified as GM2AP (**Figure 3.3**).

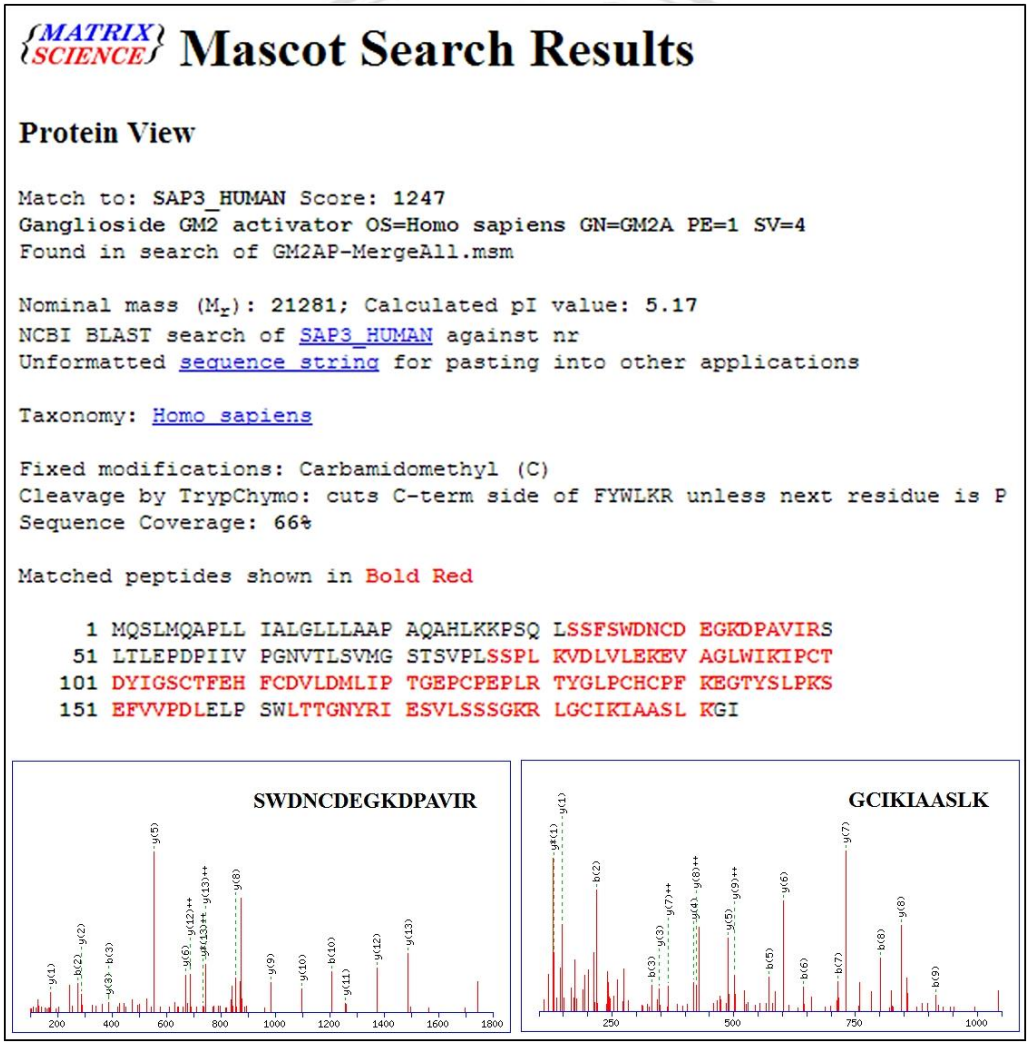


Figure 3.3 Mascot searches of GM2AP spot from Swiss-Prot database. Mascot search result of GM2AP on MS and MS/MS peptides and ion masses I and ion masses II.

3.2 Identification of GM2AP as a biomarker for lung cancer

3.2.1 Confirm of GM2AP spot on 2-DE gels

To verify the cancer selective character of candidate proteins identified by mass spectrometry, the antibody-binding capacity of spot corresponding to GM2AP protein was investigated by 2-DE Western blot analysis of healthy controls and lung cancer patients to confirm the identity of the protein spot matched after 2-DE. The region containing GM2AP in 2-DE gel was picked and then the protein was electrically transferred to the PVDF membrane prior to detecting with antibodies GM2AP, which was shown in the **Figure 3.4**. Data suggested that GM2AP was over-expressed in lung cancer patients, consistent with the increase in protein spot intensity observed in the 2-DE gel.

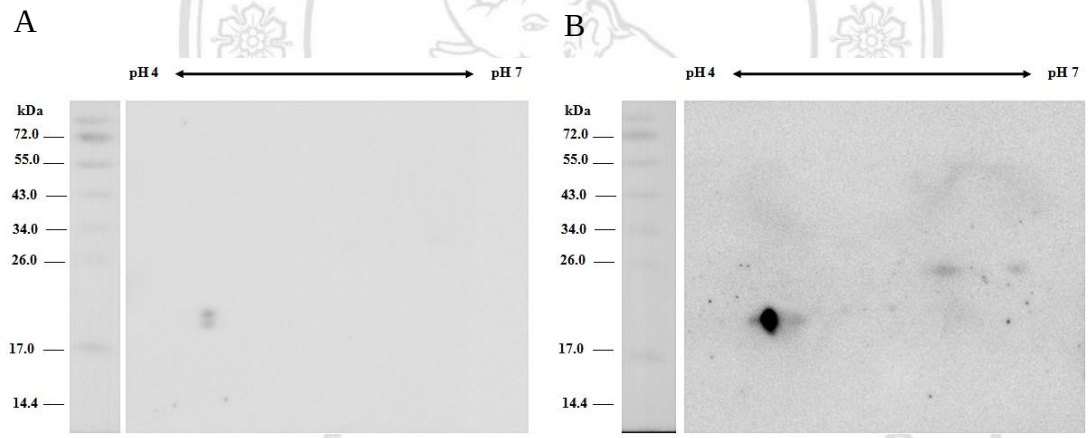


Figure 3.4 Detection of GM2AP on 2-DE gel using antibodies against GM2AP. The GM2AP spot was transferred to PVDF membrane prior GM2AP specific antibody. (A) Urine sample of healthy control. (B) Urine sample of lung cancer patient.

3.2.2 Investigation of the kidney function by Western blot analysis

3.2.2.1 Patients with anticancer treatments

3.2.2.1.1 Urine samples

First, we investigated the presence of markers of acute renal failure such as kidney injury molecule 1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) in lung cancer patients and healthy controls in urine samples to confirm the kidney failure. Because both of KIM-1 and NGAL has been reported as markers of kidney failure. The result found that the KIM-1 and NGAL were not present in lung cancer patients and healthy controls. While the expression of GM2AP level was found in only lung cancer patients. Thus, we confirmed that the increased of urinary GM2AP was not the result of kidney failure.

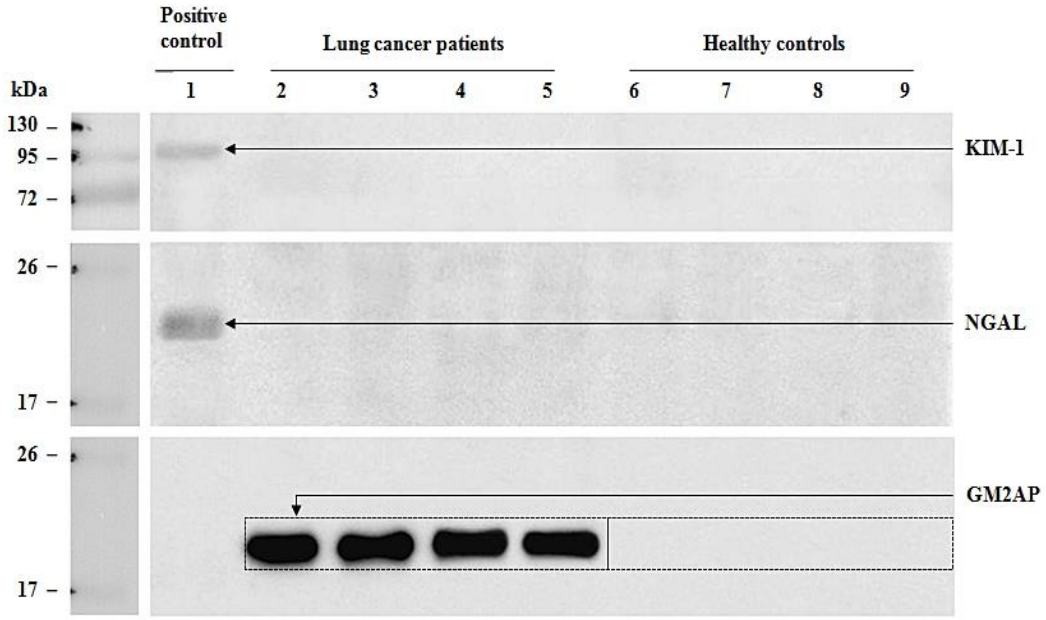


Figure 3.5 Investigation of the kidney failure in urine samples of lung cancer patients with anticancer treatments and healthy controls. The proteins on SDS-PAGE were transferred to PVDF membrane and blotted with KIM-1, NGAL and GM2AP specific antibody. Positive control was T293 kidney cell line.

3.2.3 Validation of GM2AP level by Western blot analysis

3.2.3.1 Patients with anticancer treatments

3.2.3.1.1 Urine samples

The expression level of GM2AP in lung cancer patient urine samples (n = 48) were validated by SDS-PAGE following Western blot analysis to compare their GM2AP levels with those of healthy controls (n = 44). **Figure 3.6A** is a representative panel of urinary GM2AP profile from the four subtypes of lung cancer patients and healthy controls. The expression level of GM2AP was significantly increased in the each subtype of lung cancer patients when compared to the mean of healthy controls ($P < 0.05$) (**Figure 3.6B**). These results suggest that the urine levels of GM2AP are correlated with lung cancer.

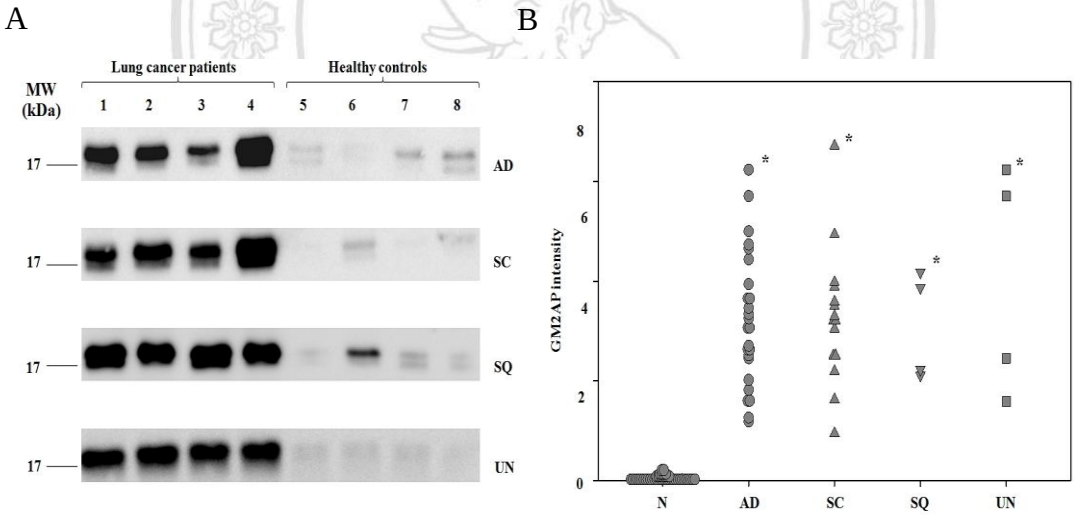


Figure 3.6 Validation of GM2AP level in urine samples of patients with anticancer treatments. (A) Four representative lung cancer patients in four subtypes are shown (lanes 1-4) and from a representative of healthy donors (lanes 5-8). (B) Scatter plot of the relative intensities of GM2AP; N: Healthy control, AD: Adenocarcinoma, SC: Small cell lung cancer, SQ: Squamous cell carcinoma, UN: Other type of carcinoma. *: *P* value < 0.05 compared to the value for the healthy urine samples.

3.2.3.2 Patients without anticancer treatments

3.2.3.2.1 Urine samples

The expression level of GM2AP in lung cancer urine samples (n = 4) was validated by SDS-PAGE following Western blot analysis, using those from healthy donors (n = 4) as controls. The result showed the blots of urinary GM2AP from patients and healthy controls (**Figure 3.7**). The expression levels of GM2AP in urine samples were greatly increased in patients when compared to those from healthy controls ($P < 0.05$).

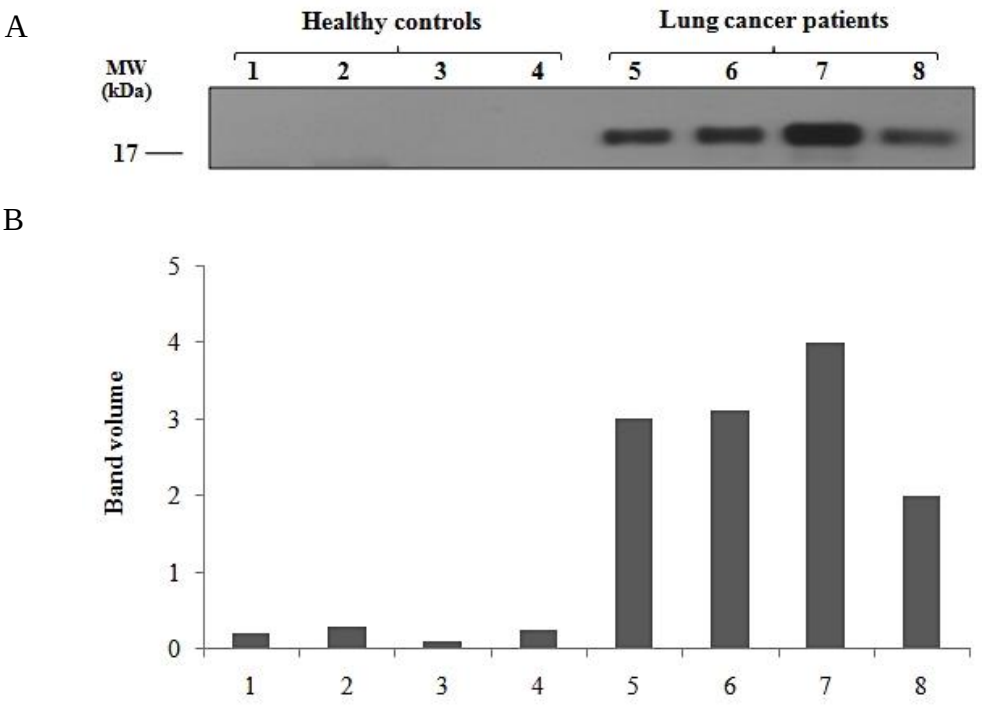


Figure 3.7 Validation of GM2AP level in urine samples of patients without anticancer treatments. (A) Four healthy controls are in lane 1-4, and 4 lung cancer patients are in lanes 5–8. (B) Band volume of urinary GM2AP intensities.

3.2.3.2.2 Serum samples

The serum samples of lung cancer patients (n = 4) were examined using SDS-PAGE and followed by Western blot analysis. Those of healthy donors (n = 4) were used as controls to compared with the expression of GM2AP level in samples from the patients. The result showed that the blots of serum GM2AP from patients and healthy controls (**Figure 3.8**). The levels of GM2AP in serum samples were greatly increased in patients when compared to those from healthy controls ($P < 0.05$).

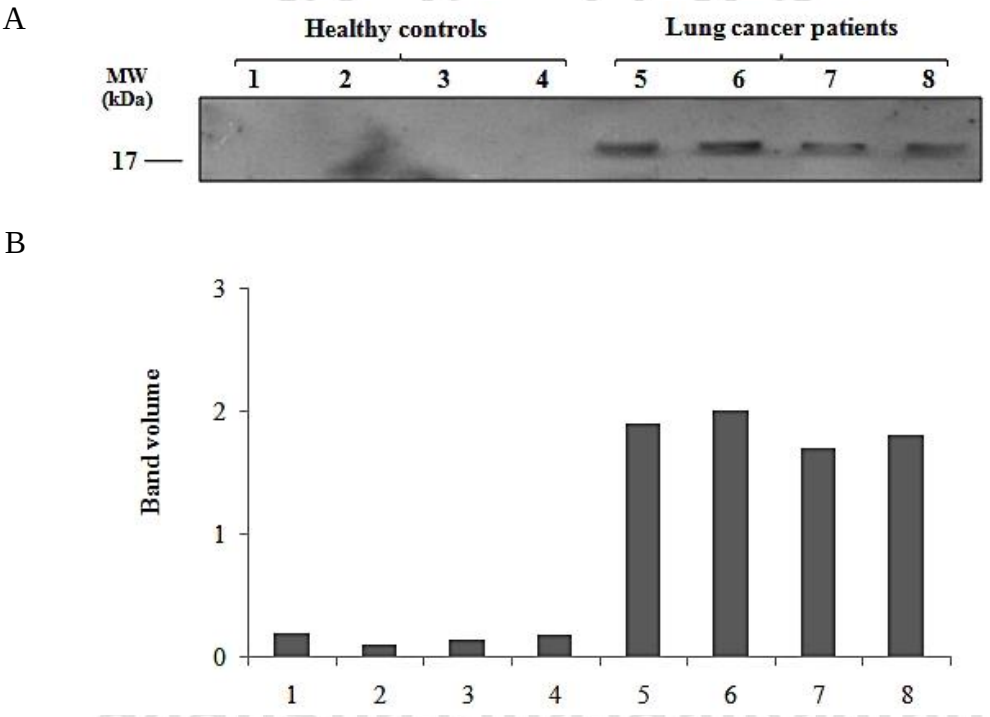


Figure 3.8 Validation of GM2AP level in serum samples of patients without anticancer treatments. (A) Four healthy controls are in lane 1-4, and 4 lung cancer patients are in lanes 5–8. (B) Band volume of serum GM2AP intensities.

3.2.4 Quantification of the GM2AP level by ELISA

3.2.4.1 Patients with anticancer treatments in Thailand samples

3.2.4.1.1 Urine samples

The expression level of GM2AP in urine of healthy controls (n = 44) and lung cancer patients (n = 48) were quantified using ELISA. The patients included 33 male and 15 female individuals. The mean age of the patients was 53.3 years (range, 28-74). The mean age of healthy donors was 42.1 years (range, 25-74). The mean of GM2AP level in all of lung cancer patients was calculated to be 1.60 ± 1.21 ng/mL, whereas the mean of GM2AP level for healthy controls was 0.21 ± 0.14 ng/mL. There was a significant increase in the GM2AP level for patients compared to healthy controls ($P < 0.05$), which is about 7.62 ± 1.06 fold increase on the median. The data of expression level presented above was used to plot the ROC curves (**Figure 3.9**).

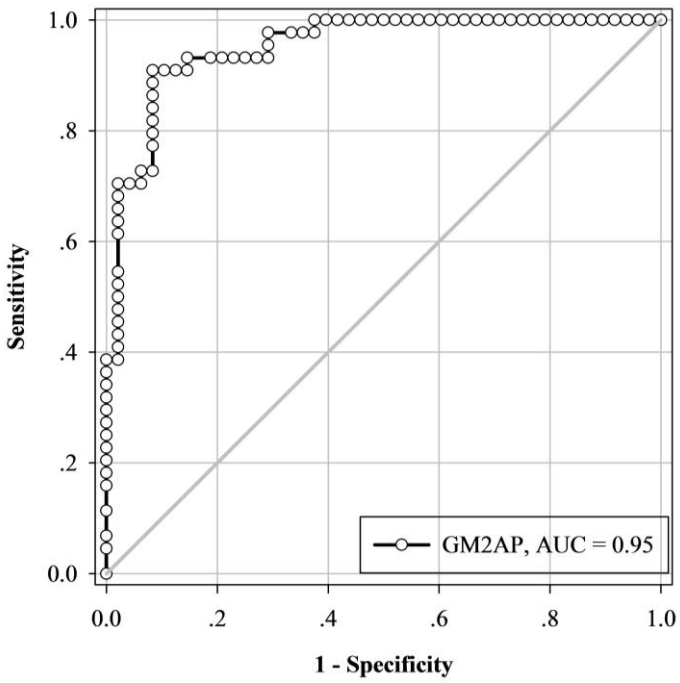


Figure 3.9 ROC curves for expression level of GM2AP in urine samples from patients with anticancer treatments. AUC is showed in Insert.

The area under the ROC curve for the GM2AP was 0.95 (95% CI, 0.91-0.99) with a sensitivity of 90.91% and a specificity of 91.67% at optimal cutoff < 0.387 (**Figure 3.10**).

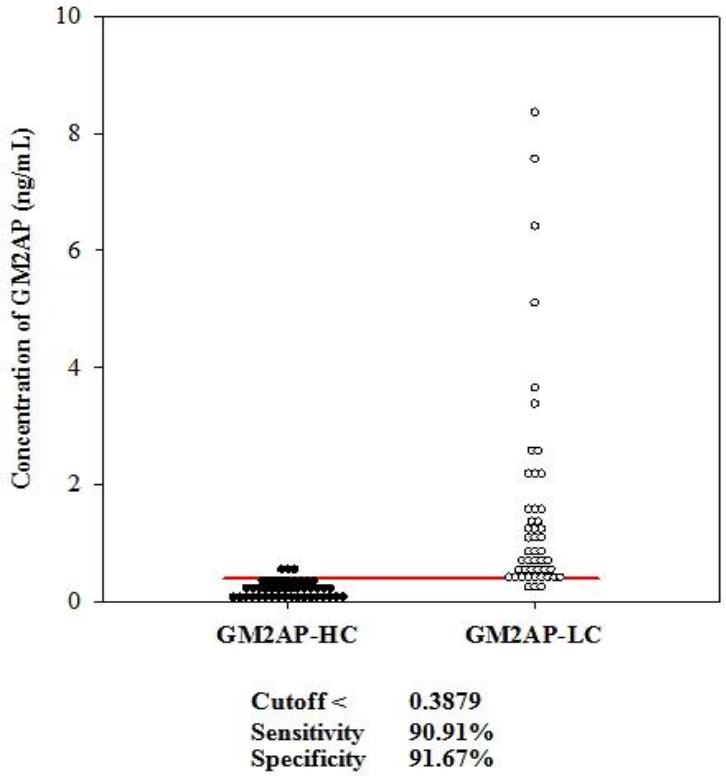


Figure 3.10 Dot histogram pair for expression level of GM2AP in urine samples from patients with anticancer treatments. The horizontal lines and the below graph show the optimal cutoff values determined from the pre-test-probability and cost ratio. GM2AP-HC = Healthy controls; GM2AP-LC = Lung cancer patients

Moreover, the urinary GM2AP level measured in the male patients (1.16 ± 1.07 ng/mL) was higher than that measured in the female patients (1.13 ± 1.05 ng/mL). According to histologic type, the urinary GM2AP level measured in patients with adenocarcinoma, small cell lung cancer and squamous cell carcinoma were 1.25 ± 1.12, 1.48 ± 1.35 and 2.27 ± 2.20 ng/mL, respectively. The urinary GM2AP level was measured to be 1.69 ± 1.54 and 0.63 ± 0.38 ng/mL in patients with stage III and IV, respectively. The expression levels of GM2AP of all the patients were included in the

statistical analysis and significant correlation ($P < 0.05$) was found with histology cancer types, whereas gender, and pathologic stage were not correlated (**Table 3.1**).

Table 3.1 Clinicopathological features and distribution of GM2AP levels in urine samples

Parameters	n	GM2AP (ng/mL)	
		Urine	
		Mean ± SD	*P* value
All healthy donors	44	0.21 ± 0.14	
Gender			
Male	23	0.16 ± 1.03	0.364
Female	21	0.12 ± 1.05	
All patients	48	1.60 ± 1.21	
Gender			
Male	33	1.16 ± 1.07	0.358
Female	15	1.13 ± 1.05	
Histology			
Adenocarcinoma	25	1.25 ± 1.22	0.009
Small cell lung cancer	15	1.48 ± 1.35	
Squamous cell carcinoma	4	2.27 ± 2.20	
Other types of carcinoma	4	2.99 ± 2.63	
Pathologic stage			
I	-	-	0.312
II	-	-	
III	7	1.69 ± 1.54	
IV	41	0.63 ± 0.38	

3.2.4.2 Patients without anticancer treatments in Taiwan samples

3.2.4.2.1 Urine and serum samples

The mean urinary GM2AP level in all of lung cancer patients was 1.46 ± 1.55 ng/mL whereas the mean of GM2AP level in healthy controls was 0.18 ± 0.19 ng/mL. There was an 8.03 ± 1.36 fold increase of GM2AP level in urine, and a 5.41 ± 0.73 fold increase in the serum compared to those obtained from healthy controls. ROC curve analysis showed that the urinary GM2AP predicts the diagnosis of lung cancer with an AUC of 0.89 (95% CI, 0.83-0.95) at a cut-off point of 0.234. This cut-off point provides 88.46% sensitivity and 85.71% specificity as shown in **Figure 3.11A**. A dot histogram plot demonstrates the distribution of GM2AP levels in urine samples of healthy controls (**Figure 3.12A**).

A B

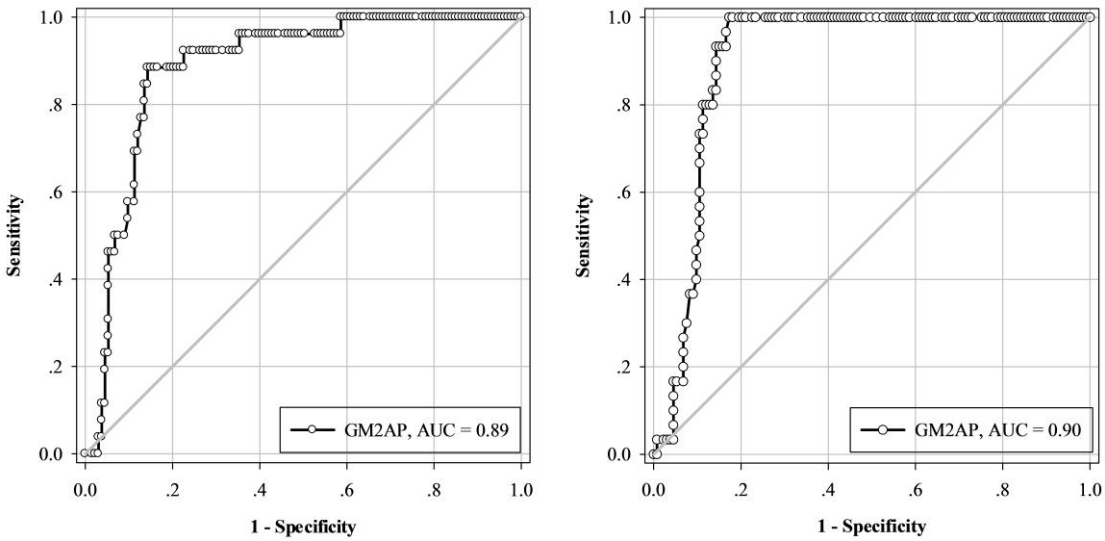


Figure 3.11 ROC curves for expression level of GM2AP in urine and serum samples from patients with anticancer treatments. (A) The expression level of GM2AP in urine samples. (B) The expression level of GM2AP in serum samples. AUC is showed in Insert.

The mean serum GM2AP level in lung cancer patients was calculated to be 0.92 ± 0.27 ng/mL whereas the mean of GM2AP level in healthy controls was calculated to be 0.17 ± 0.07 ng/mL. The ROC curve showed an AUC of 0.90 (95% CI, 0.85-0.95) at a cut-off point of 0.342, with 100% sensitivity and 82.71% specificity in predicting lung cancer (**Figure 3.11B**). The dot histogram plot was shown in **Figure 3.12B**. The results suggested that a significant increase of GM2AP levels in urine and serum in lung cancer patients ($P < 0.0001$), and demonstrates a high accuracy as a potential diagnostic marker for lung cancer.

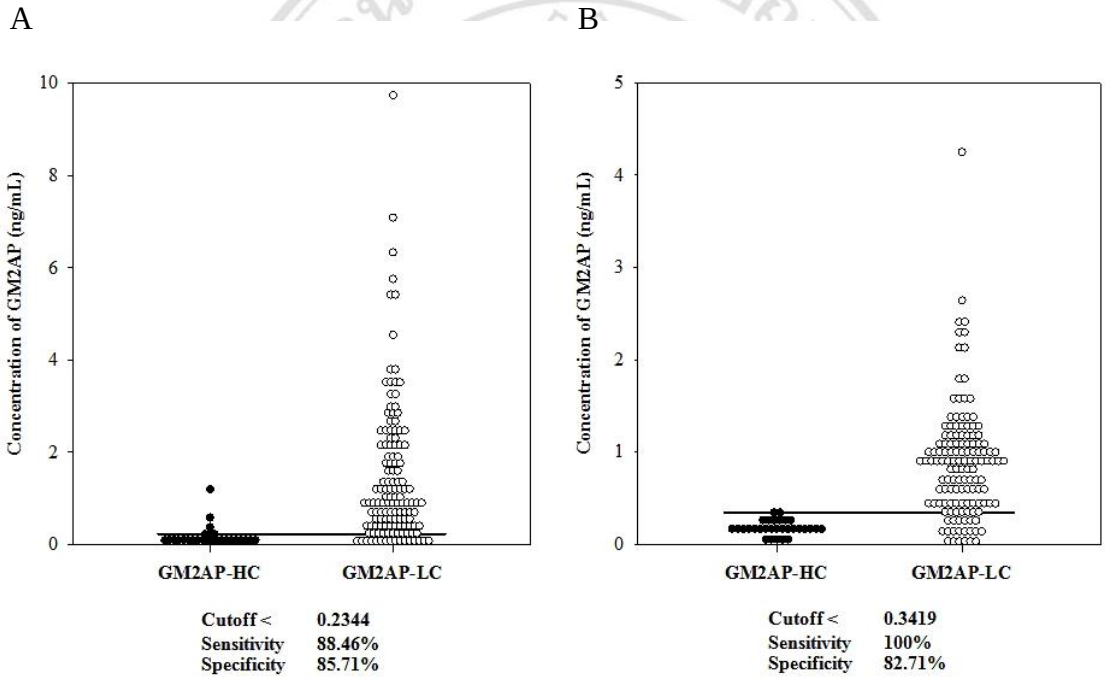


Figure 3.12 Dot histogram pair for expression level of GM2AP in urine and serum samples from patients with anticancer treatments. (A) Dot histogram pair for expression level of GM2AP in urine samples. (B) Dot histogram pair for expression level of GM2AP in serum samples. The horizontal lines and the below graph show the optimal cutoff values determined from the pre-test-probability and cost ratio. GM2AP-HC = Healthy controls; GM2AP-LC = Lung cancer patients

3.2.4.3 Patients with other types of cancer without anticancer treatments in Taiwan samples

3.2.4.3.1 Serum samples

We then perform a further study to assess the relationship between GM2AP expression and other types of cancers such as colon cancer and liver cancer to confirm GM2AP specific biomarker in lung cancer using ELISA assay. We found that GM2AP expression in serum samples was highly increased in patients with lung cancer ($P < 0.05$). The mean serum GM2AP level in lung cancer patients, colon cancer and liver cancer were calculated to be 0.92 ± 0.27, 0.17 ± 0.05, 0.17 ± 0.02, 0.17 ± 0.07 ng/mL, respectively (**Tables 3.2**).

Table 3.2 The expression level of GM2AP in serum from healthy controls and other types of cancers

Types of cancer	n	GM2AP (ng/mL)
		Mean ± SD
Healthy donors	26	0.177 ± 0.07
Colon cancer	50	0.179 ± 0.02
Liver cancer	42	0.178 ± 0.05
Lung cancer	133	0.920 ± 0.27

The mean of GM2AP level in healthy controls was calculated to be 0.17 ± 0.07 ng/mL. The levels of GM2AP in serum samples from patient with colon cancer and liver cancer were not different when compared to those from healthy controls **(Figure 3.13)**.

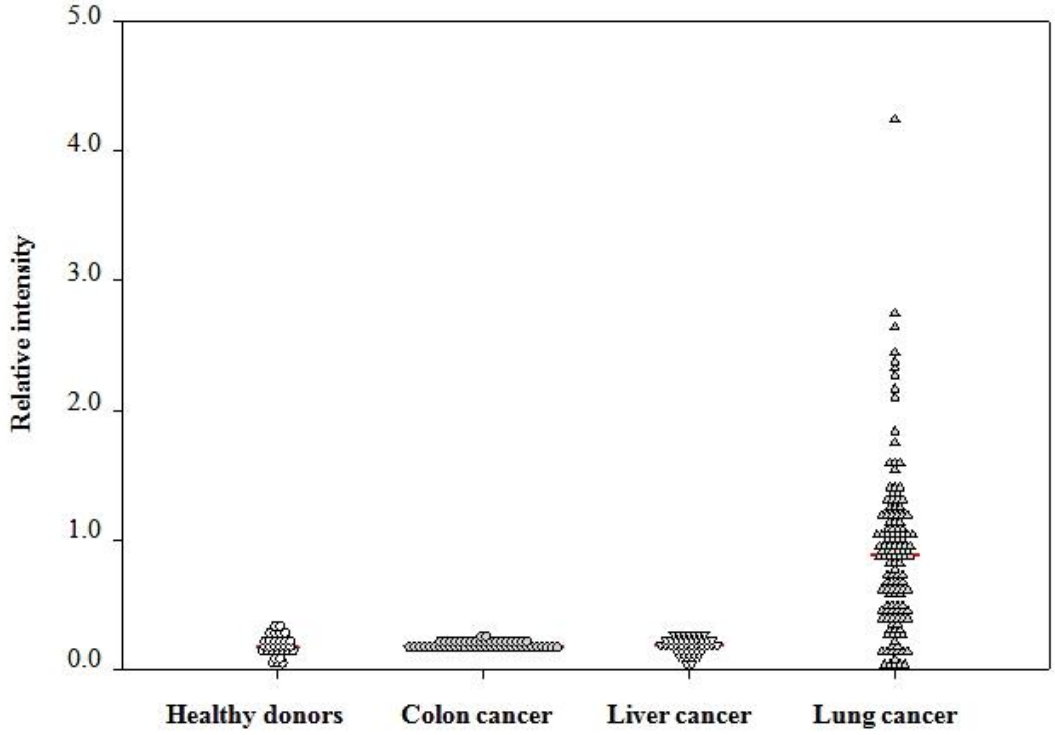


Figure 3.13 A dot histogram plot of other types of cancer. These were demonstrated the distribution of GM2AP levels in serum of healthy controls and other types of cancers.

3.2.5 The expression of GM2AP in urine and serum of lung cancer and clinicopathological features in Taiwan samples

The GM2AP levels in urine and serum samples from ELISA were correlated with the clinicopathological features of lung cancer patients. The urine level of GM2AP was similar in both genders: male (1.65 ± 1.86 ng/mL) and female (1.31 ± 1.25 ng/mL). There was no difference in the urinary GM2AP levels between the non-smokers (1.36 ± 1.54 ng/mL) and smoker (1.64 ± 1.58 ng/mL). The levels of urinary GM2AP was also similar among those with activating EGFR mutation (1.47 ± 1.40 ng/mL), wild type EGFR (1.28 ± 1.39 mg/mL) and unknown (2.56 ± 2.93 ng/mL). There was no difference in urinary GM2AP levels among different histology subtypes, and were 1.97 ± 1.45, 1.09 ± 0.85, 0.57 ± 0.48 and 1.95 ± 3.09 ng/mL for adenocarcinoma, small cell lung cancer, squamous cell carcinoma and other types of carcinoma, respectively. Earlier stages had higher urinary GM2AP levels, which were 2.31 ± 1.39, 2.53 ± 3.65, 1.57 ± 1.40 and 1.17 ± 1.31 ng/mL for stage I, II, III and IV, respectively (P=0.009, **Table 3.3**).

The serum levels of GM2AP were also similar in both genders: male (1.03 ± 0.91 ng/mL) and female (0.83 ± 0.56 ng/mL). There was no difference related to smoking status: nonsmokers (0.88 ± 0.77 ng/mL) vs. smokers (0.99 ± 0.69 ng/mL), and EGFR mutation status: activating EGFR mutations (0.91 ± 0.60 ng/mL), wild type EGFR (0.75 ± 0.39 ng/mL) vs unknown (2.11 ± 1.82 ng/mL). There was no difference in serum GM2AP levels among histology subtypes, and were 0.90 ± 0.63, 0.78 ± 0.63, 1.10 ± 0.21 and 1.26 ± 1.72 ng/mL for adenocarcinoma, small cell lung cancer, squamous cell carcinoma and other types of carcinoma, respectively. Earlier stages had higher serum GM2AP level: 1.61 ± 0.93, 1.47 ± 2.00, 0.98 ± 0.52 and 0.71 ± 0.43 ng/mL for stage I, II, III and IV, respectively (P<0.0001, **Table 3.3**).

In brief, no difference was shown in urinary or serum GM2AP levels when stratified by gender, smoking status, EGFR status or histology subtypes. However, we demonstrated a significant difference in urinary and serum GM2AP level among pathology stages (P = 0.009 for urine levels and P < 0.0001 for serum levels, respectively).

Table 3.3 Clinicopathological features and distribution in urine and serum levels of GM2AP

Parameters	GM2AP (ng/mL)					
	Urine			Serum		
	n	Mean ± SD	*P* value	n	Mean ± SD	*P* value
Healthy volunteers						
Age						
Median, y 60 (range 27-78)	26	0.18 ± 0.19				
Median, y 64 (range 30-73)				30	0.17 ± 0.07	
Gender						
Male	15	0.23 ± 0.19	0.219	13	0.19 ± 0.08	0.408
Female	11	0.11 ± 0.09		17	0.16 ± 0.07	
Patients with lung cancer						
Age						
Median, y 62 (range 30-80)	133	1.46 ± 1.55		133	0.92 ± 0.74	
Gender						
Male	60	1.65 ± 1.86	0.244	60	1.03 ± 0.91	0.119
Female	73	1.31 ± 1.25		73	0.83 ± 0.56	
Smoking						
No	85	1.36 ± 1.54	0.337	85	0.88 ± 0.77	0.4
Yes	48	1.64 ± 1.58		48	0.99 ± 0.69	
EGFR status						
Wild type	58	1.28 ± 1.39	0.462	58	0.75 ± 0.39	0.102
Activating mutation	66	1.47 ± 1.40		66	0.91 ± 0.60	
Unknown	9	2.56 ± 2.93		9	2.11 ± 1.82	
Histology						
Adenocarcinoma	109	1.97 ± 1.45	0.415	109	0.90 ± 0.63	0.475
Small cell lung cancer	4	1.09 ± 0.85		4	0.78 ± 0.63	
Squamous cell carcinoma	11	0.57 ± 0.48		11	1.11 ± 0.21	
Other types of carcinoma	9	1.95 ± 3.09		9	1.26 ± 1.72	
Phatologic stage						
I	18	1.17 ± 1.31	0.009	18	1.61 ± 0.93	< 0.0001
II	6	2.31 ± 1.39		6	1.47 ± 2.0	
III	25	2.53 ± 3.65		25	0.98 ± 0.52	
IV	84	1.57 ± 1.40		84	0.71 ± 0.43	

3.2.6 The expression of GM2AP level in NSCLC tissues

The expression of GM2AP was detectable by IHC in lung tissue samples from 122 out of 143 patients with NSCLC (90 of 106 invasive adenocarcinoma, 22 of 26 squamous cell carcinoma and 10 of 11 adenocarcinoma in situ (previously named bronchioloalveolar carcinoma), which makes a 83.9% positive rate of tissue expression of GM2AP in NSCLC and the majority were observed in the cytoplasm as brown granules via staining (**Figure 3.14**). The distribution of IHC score was 21 patients in score 0, 92 patients in score 1 and 30 patients in score 2.

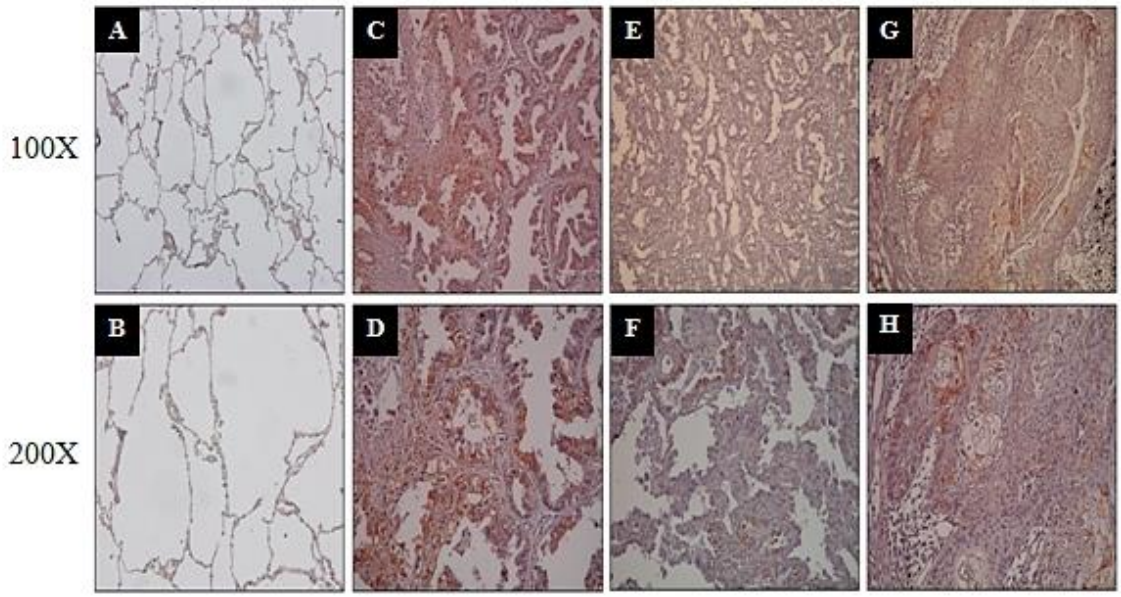


Figure 3.14 Immunohistochemical staining of GM2AP in NSCLC; (A, B), non-tumor lung tissue (A, Magnification ×100; B, Magnification ×200) (C, D), Positive expression of the GM2AP in invasive adenocarcinoma (C, Magnification ×100; D, Magnification ×200) (E, F), in bronchioloalveolar carcinoma (E, Magnification ×100; F, Magnification ×200; (G, H) and in squamous cell carcinoma (G, Magnification ×100; H, Magnification ×200)

3.2.7 The expression of GM2AP in NSCLC tissues and clinicopathological feathers

There was no difference among different histology subtypes in GM2AP expression. But there was a significant correlation between GM2AP expression and pathologic stages (P = 0.001). The positive rate of GM2AP was 75.0% (45 out of 60) in stage I, 95.1% (58 out of 61) in stage II and 95.0% (19/20) in stage III, but 0% in stage IV (0 out of 2). There were no statistical correlations between GM2AP expression and the remaining clinicopathologic features, such as age and gender (**Table 3.4**).

Table 3.4 Relationship between the GM2AP expression and clinicopathologic features in NSCLS lung tissues

IHC Score	GM2AP expression			*P* Value
	0 (n = 21)	1 (n = 92)	2 (n = 30)	
Age				
Median (range)	64 (38-76)	64 (33-81)	68 (41-82)	0.425
Gender				
Male	11	52	16	0.916
Female	10	40	14	
Histology				
Adenocarcinoma	16	72	18	0.234
Squamous cell carcinoma	4	15	7	
Bronchioloalveolar carcinoma	1	5	5	
Pathologic stage				
I	15	30	15	0.001
II	3	48	10	
III	1	14	5	
IV	2	0	0	

3.2.8 The expression of GM2AP in NSCLC tissues and survival

The association between tissue GM2AP expression in NSCLC and the survival of 143 NSCLC patients was analyzed with Kaplan-Meier survival analysis. Patients with a high GM2AP expression (Score 2) were likely to have a significantly shorter overall survival (P = 0.045) (**Figure 3.15A**) and disease-free survival (P = 0.049) (**Figure 3.15B**). Since GM2AP expression was significantly correlated with pathologic stage, the impact of GM2AP IHC score and pathology stage on the survival of NSCLC patients were further determined.

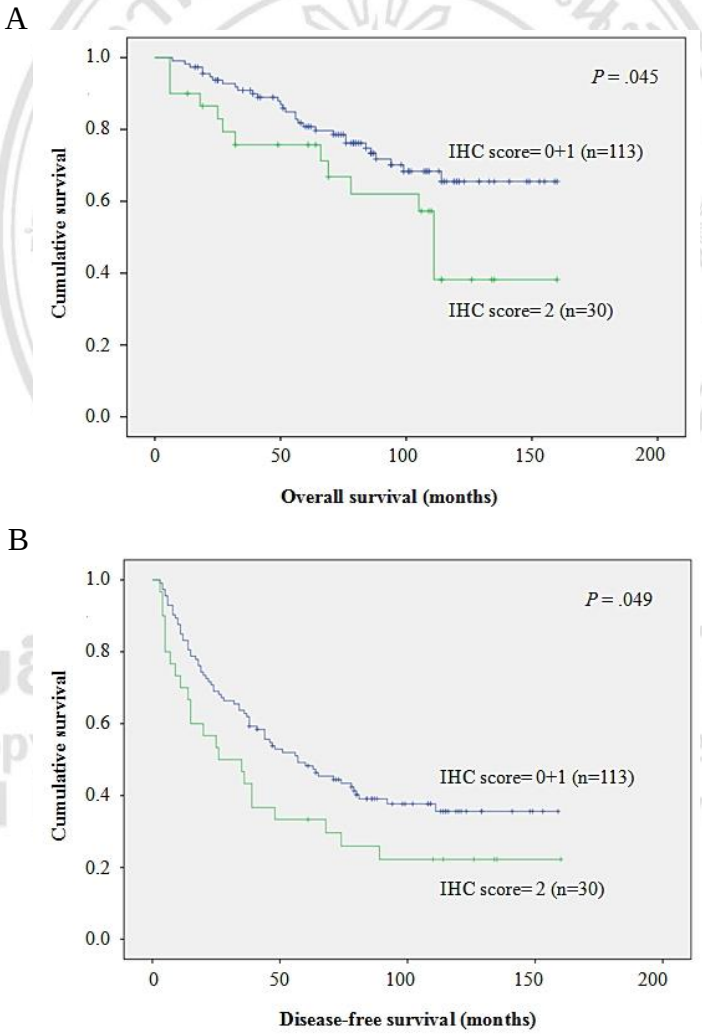


Figure 3.15 Kaplan-Meier analyses for GM2AP expression in NSCLC. (A) overall survival and (B) disease-free survival

The multivariate analysis of disease-free survival revealed that in addition to the stage (stage I/II vs III/IV; odds ratio [OR] 0.364 [95% CI, 0.216-0.612]; $P < 0.001$), the IHC score (0/1 vs 2; OR 0.563 [95% CI, 0.349-0.910]; $P = 0.024$) was also an independent predictor of disease recurrence (**Table 3.5**). Similar results were also shown in overall survival, in which the stage; I/II vs III/IV (OR 0.335 [95% CI, 0.163-0.689]; $P = 0.003$) and the IHC score; 0/1 vs 2 (OR 0.475 [95% CI, 0.250-0.906]; $P = 0.024$) was both independent predictors (**Table 3.5**).

Table 3.5 Multivariate analysis of the GM2AP expression in lung cancer patients

Variable	Hazard ratio (95% CI)	*P* value
Disease-free survival		
Stage I+II vs stage III+IV	0.364 (0.216-0.612)	< 0.001
IHC score 0+1 vs 2	0.563 (0.349-0.910	0.019
Overall survival		
Stage I+II vs stage III+IV	0.335 (0.163-0.689)	0.003
IHC score 0+1 vs 2	0.475 (0.250-0.906)	0.024

3.3 Detection of intact protein mass of GM2AP in lung cancer

To confirm the molecular mass of GM2AP in lung cancer patients, the GM2AP spot in 2-DE gels were excised and applied to electro-elution for MALDI-TOF/MS as described in experimental procedure. The protein mass spectrum of GM2AP appeared as one major peak at 18663 Da for urine samples obtained from lung cancer patients. This was significantly greater than the predicted GM2AP mass of 17589 Da (**Figure 3.16**), thus suggesting post-translational modifications (PTM).

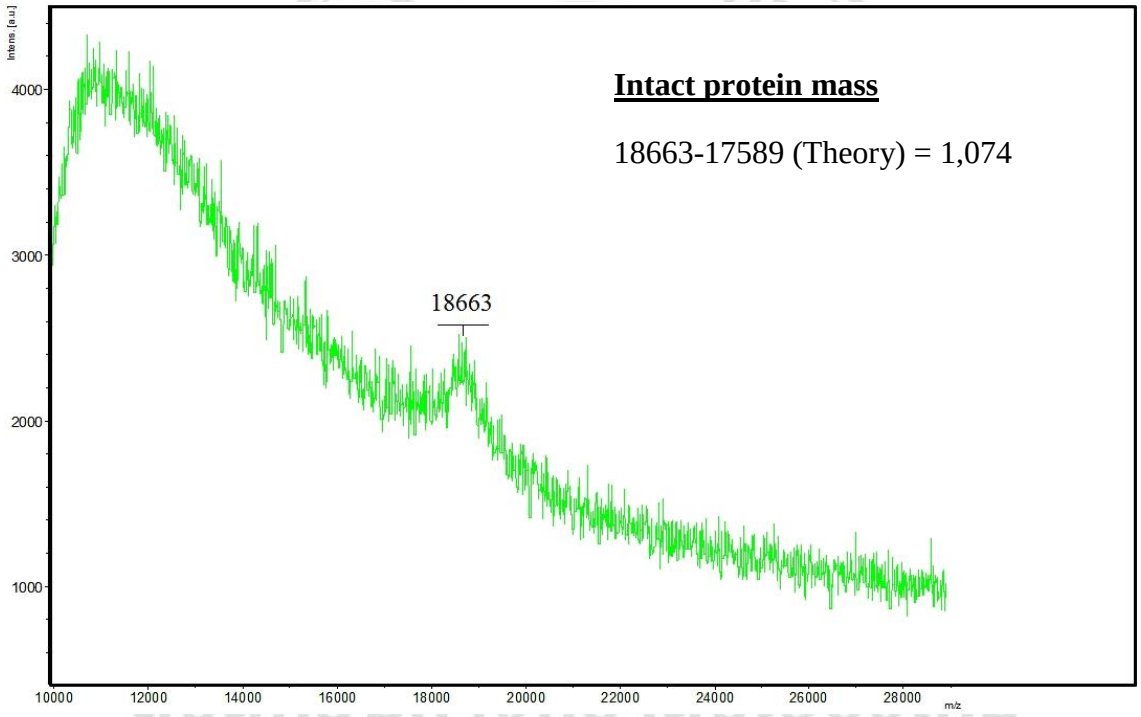


Figure 3.16 MALDI-TOF mass spectra of GM2AP. One major peak at 18663 Da was identified as GM2AP.

The PTM of GM2AP has been identified as glycosylation. We then investigated the difference of urinary GM2AP glycan moiety in healthy control and cancerous samples. The region containing GM2AP in 2-DE gel was picked and then the protein was electrically transferred to the PVDF membrane prior to detecting with lectin staining. AAL lectin signaling was increased fucosylated urinary GM2AP in lung cancer patient compared to that of healthy control (**Figure 3.17**).

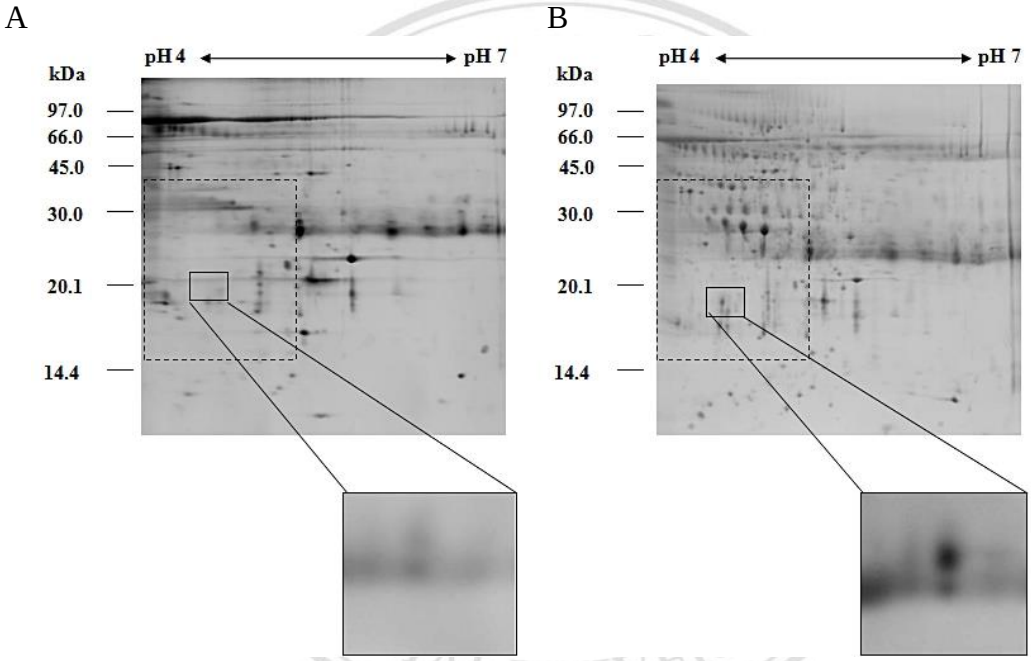


Figure 3.17 Detection of fucosylation on the GM2AP using AAL lectin staining. The GM2AP spot was transferred to PVDF membrane prior lectin blotting procedure. (A) Urine sample of healthy control. (B) Urine sample of lung cancer patient.

3.4 Determination the glycan structure of GM2AP in lung cancer

In order to determine the glycan structure of GM2AP, the GM2AP spots from 2-DE were in-gel digested with trypsin and chymotrypsin. The resulted peptides were further analyzed by nanoLC-MS/MS. The mass spectra showed the oxonium ion at m/z 204.087 and Y_1 ion, peptide with a HexNac was present in HCD. The parent ion at m/z 1202.1025 and peptide molecular mass of 1363.813 Da (PIIVPGNVTLSVVG) of

GM2AP was calculated; in the meantime, glycan composite $(Hex)_3(HexNAc)_2(Fuc)_1$ was also determined as shown in **Figure 3.18**.

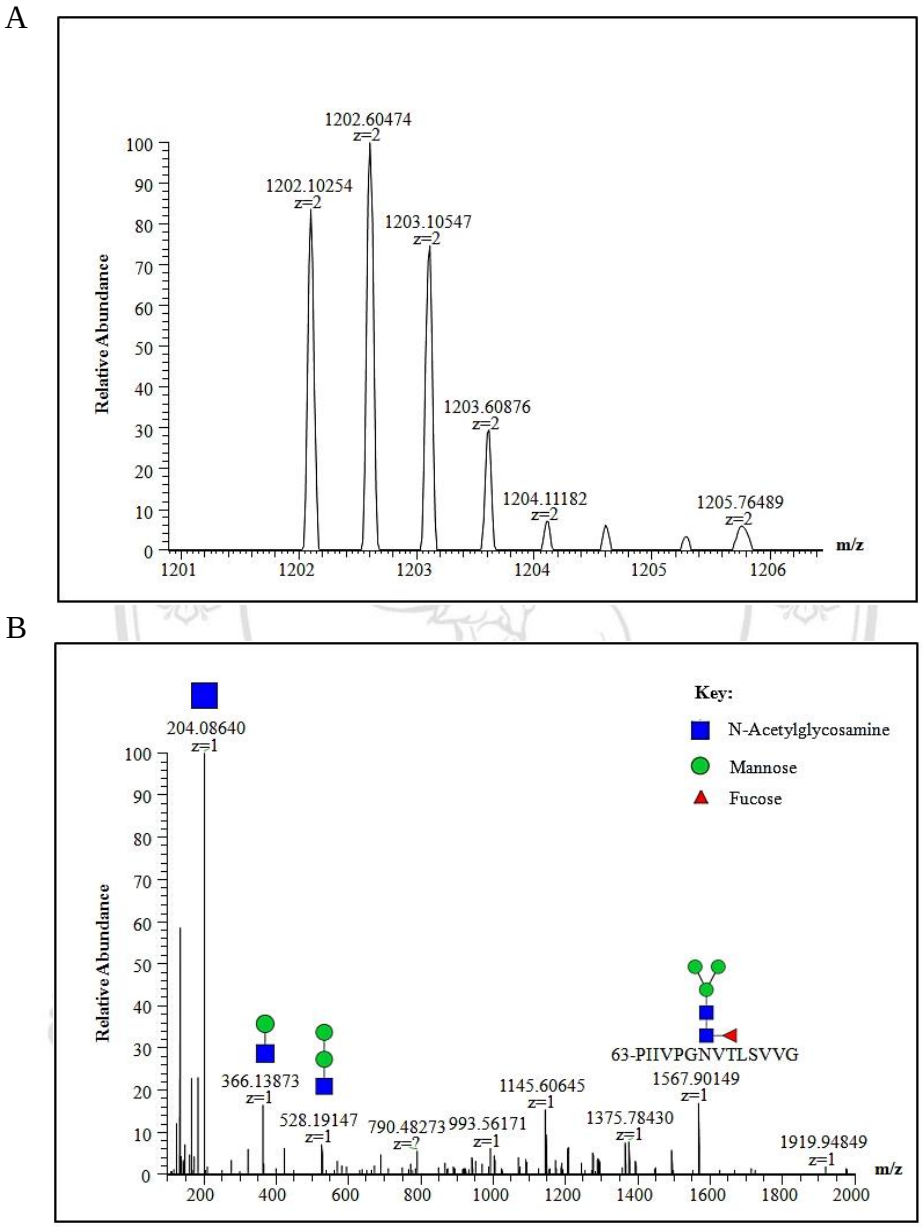


Figure 3.18 Mass spectra of the glycopeptides of urinary GM2AP in lung cancer patients. (A) The parent ion at m/z 1202.1025. (B) Fragment ion chromatography of *N*-linked glycan structure of (2HexNAc3Hex1dHex) linked with peptide (PIIVPGNVTLSVVG) of GM2AP in lung cancer urine samples.