CHD5 may be a promising novel bio-marker for the diagnosis of lung cancer patients

Jun Yang
The First Affiliated Hospital of Xinxiang Medical University
Hua Zhong
The First Affiliated Hospital of Xinxiang Medical University
Qinghui Yang
The First Affiliated Hospital of Xinxiang Medical University
Jian Yu
The First Affiliated Hospital of Xinxiang Medical University
Cailing Jin
The First Affiliated Hospital of Xinxiang Medical University
Xiaorui Li (hcyxkg@yeah.net)
The First Affiliated Hospital of Xinxiang Medical University https://orcid.org/0000-0001-5254-2862

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Abstract

Background

Chromodomain helicase DNA binding protein 5 (*CHD5*) is a new tumor suppressor gene in various types of cancer. And it is still not clear about the role of *CHD5* in lung cancer. In this study, we aim to assess diagnostic value of *CHD5* in patients with lung cancer.

Methods

CHD5 expression in 108 lung cancer serum samples and 65 healthy controls were determined by quantitative Real-Time PCR (qRT-PCR). A receiver operating characteristic (ROC) curve was established to analyze the effect of *CHD5* in the diagnosis of lung cancer.

Results

The expression of *CHD5* was significantly decreased in lung cancer samples compared with the healthy controls ($P < 0.0001$). Advanced TNM stage ($P = 0.020$), gender ($P = 0.001$) and smoking history ($P = 0.000$) were associated with the decreased *CHD5* expression. Besides, the results of ROC analysis showed that the area under ROC curve (AUC) was 0.855 with a sensitivity of 87.1% and a specificity of 81.3%.

Conclusions

In conclusion, this study suggested that *CHD5* expression is down-regulated in lung cancer. Furthermore, *CHD5* expression could be a potential diagnosis bio-marker in lung cancer patients.

Background

Lung cancer, a serious health-care problem, is one of the deadliest types of cancers in the world [1]. Although the molecular network of lung carcinogenesis at the levels of gene and protein has been studied in recent decades, the high mortality of lung cancer is not markedly changed. One of significant factors is diagnosis at late stage, since no obvious symptom was observed at early stage. Besides, due to the metastasis character the tumor often rapidly spreads to distant organs [2]. Although the recent advancements in imaging modalities have improved the detection and evaluation of lung cancer, these non-invasive methods do not provide definitive confirmation of the disease. There is several limitations by using CT, positron emission tomography and X-ray for early diagnosis [3-5]. Therefore, it is urgent and necessary to find novel non-invasive bio-markers for early diagnosis of lung cancer.

The chromodomain helicase DNA binding domain 5 (*CHD5*) gene, a member of Chromodomain (*CHD*) gene family, is known as a part of the lp36 chromosome [6]. The normal mechanism of cancer prevention will be cut off if the *CHD5* not work. Like a circuit breaker, *CHD5* regulates the tumor suppressor ability of our cells in our body, When it is disconnected, the tumor happens. As a nuclear protein, *CHD5* may be difficult to target. However, *CHD5* is usually silenced by promoter methylation, which is easy to be modified. The suppression of clonigenicity and tumorigenicity, as well as correlation with risk factors and outcome, suggested that *CHD5* is a tumor suppressor gene [7-10]. *CHD5* has been found in the breast, colon, ovarian and glioma tumors, revealed that *CHD5* acted important roles in tumorigenesis and progression of several cancers [11-13]. Moreover, in a recent study, *CHD5* is identified as a novel tumor suppressor gene in lung cancer [14], but the diagnostic role of *CHD5* in lung cancer patients is still unclear.

In the current study, we sought to examine the expression level of *CHD5* in lung cancer serum samples compared with normal controls. Besides the association between *CHD5* expression and clinical features was analyzed. Furthermore, receiver operating characteristics (ROC) curves was generated for the diagnostic value of *CHD5* in lung cancer patients.

Methods

Patients and specimens

In the current study, a total of 173 participants were recruited from The First Affiliated Hospital of Xinxiang Medical University, which included 108 lung cancer patients and 65 healthy controls. The inclusion criteria of lung cancer patients was no previous history of cancer-related diseases and did not receive radiotherapy or chemotherapy prior to research. The healthy control individuals were without tumor-associated lesions before the study. Total of 5ml peripheral blood samples from lung cancer patients and healthy controls were obtained and centrifuged and then stored at -80℃ for further experiments. This study was approved by the Ethical Committee of the hospital and all enrolled patients provided written informed consents prior to sample collection. The clinicopatholgical features for these patients were listed in Table 1.

Table 1. Relationship between *CHD5* expression and clinical features of lung cancer patients

Features	All cases	*CHD5* expression		*P* Value
		low	high	
Total	108	52	56	
Age (Years)				0.332
≤ 60	53	23	30	
> 60	55	29	26	
Gender				0.001
Female	48	32	16	
Male	60	20	40	
Tumor size (cm)				0.316
≤5	49	21	28	
>5	59	31	28	
Smoking history				0.000
≤10	51	35	16	
>10	57	17	40	
TNM stage				0.020
I - II	56	33	23	
III - IV	52	19	33	

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from the serum samples using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions and the purity of RNAs was measured using a NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE, USA). After purification cDNA was synthesized using the First-Strand cDNA Synthesis Kit (ReverTra Ace-a, FSK-100, Toyobo, Osaka, Japan) according to the manufacture's protocol. The real-time PCR was conducted using the 7300 Real-Time PCR System (Applied Biosystems, USA). *GAPDH* was used as the internal control and each sample was examined in triplicate.

Statistical analysis

In the study, SPSS 21.0 (SPSS Inc., Chicago, IL, USA) software was used to perform all the statistical analyses and GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA) software was used to generate all the graphs. The Student's t test was used to assess the difference of *CHD5* expression level

in lung cancer samples and healthy control samples. The relationship between *CHD5* expression and clinicopathological characteristics of lung cancer patients was assessed by Chi-square test. The ROC curve was established to explore the diagnostic value of *CHD5* in lung cancer patients. *P* value less than 0.05 was considered statistically significant.

Results

The expression of *CHD5* was reduced in lung cancer patients

The qRT-PCR was performed to evaluate the expression of *CHD5* in lung cancer samples and healthy controls. The results demonstrated that the expression of *CHD5* in lung cancer patients significantly reduced compared with that of the healthy controls ($P < 0.05$, **Figure 1**).

Association of *CHD5* expression with clinicopathological features of lung cancer patients

To verify the relationship between *CHD5* expression and development of lung cancer, we estimated the association of *CHD5* expression with clinicopathological data of lung cancer patients using Chi-square test. The results in **Table 1** revealed that the expression level of *CHD5* was associated with advanced TNM stage (P=0.020), gender (P=0.001) and smoking history (P=0.000), but there was no significant differences with the age (P=0.332) and tumor size (P=0.316) (**Table 1**).

Diagnostic accuracy of *CHD5* in patients with lung cancer

In order to examine the diagnostic value of *CHD5* in lung cancer patients ROC analysis was applied. The ROC curve analysis revealed that the area under the curve (AUC) was 0.855, with the sensitivity of 87.1% and specificity of 81.3%. The optimal cut-off point was 3.880. All these results above suggested that the *CHD5* expression was closely correlated with the diagnosis of lung cancer (**Figure 2**).

Discussion

As a major global health problem, lung cancer is a leading cause of cancer-related death worldwide, accounting for more than 1.4 million deaths per year [15]. Non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC) are two main histological subtypes of lung cancer [16]. One of the primary risk factors for lung cancer is smoking tobacco [17]. In spite of significant improvements in different kinds of therapeutic methods, the 5-year overall survival rate was low [18]. The 30% of patients were usually recommended adjuvant chemotherapy, who experienced relapse after surgery with stage I lung cancer [19]. Recent advances in the field of lung cancer had showed that several biomarkers acted as non-invasive and cost-effective tools for early-stage prognosis of lung cancer [20]. Although the diagnosis of lung cancer is gradually improving, the detection of tumors at an early stage is still very poor.

Chromodomain (CHD) protein family play important functions in chromatin organization, regulation of transcription and cancer prevention [21]. As one of nine members of the CHD family, *CHD5* has been

confirmed as the tumor suppressor in various malignant tumors. For example, Ma et al. reported that the expression of *CHD5* was significantly suppressed in breast cancer tissues and downregulation of *CHD5* may contribute to the development and progression of breast cancer [22]. Liu et al. showed that *CHD5* mRNA was positively correlated with protein expression and *CHD5* gene methylation is associated with clinical and pathological features of colorectal cancer patients [23]. Moreover, in the study of Du et al., they found that *CHD5* functioned as a novel tumor suppressor gene for renal cell carcinoma [24]. The tumor suppressor role of *CHD5* is also found in other cancers including gastric cancer and neuroblastomas [25, 26]. However, only few literatures have investigated the *CHD5* gene in lung cancer. Zhao et al. have reported that *CHD5* is down-regulated in lung cancer cell lines and primary tumor tissues, and it is a potential tumor suppressor gene in lung cancer [14]. The study of Baykara O et al. showed *CHD5* is a potential tumor suppressor in non small cell lung cancer [27]. However, little is known about the diagnostic value of *CHD5* in lung cancer patients.

In the current study, the expression of *CHD5* in lung cance was decreased compared with the healthy controls. Besides, the Chi-square test was performed to examine the relationship between *CHD5* expression level and clinicopathological features of lung cancer patients. The expression of *CHD5* in lung cancer is significantly associated with TNM stage, gender and smoking history. The results of ROC analysis showed that the value of AUC was high as well as with high sensitivity and specificity. These results suggested *CHD5* was a useful diagnostic biomarker for lung cancer patients.

Promoter hypermethylation is one mechanism for the transcriptional inactivation of many tumor suppressor genes. Methylation of the *CHD5* gene promoter had been identified in several types of cancers [23, 24, 28]. Previous studies also found that down-regulation of *CHD5* may be due to different epigenetic silencing mechanism possibly by miRNAs. Recently, Naraparaju et al. identified three different miRNA target regions in the 3’-UTR region of the *CHD5* gene using two different prediction algorithms and they also confirmed seven miRNAs that significantly downregulated *CHD5* expression in Neuroblastoma [29]. The above outcome provides insights for our future studies on the molecular mechanism of *CHD5* in lung cancer.

Conclusions

In summary, the expression levels of *CHD5* are down-regulated in lung cancer compare with the healthy controls. And *CHD5* might serve as a novel biomarkers for early detection of lung cancer. Although all the data suggesting *CHD5* may play a broader role in lung cancer, further studies are required to improve the diagnostic ability in lung cancer patients.

Abbreviations

Chromodomain helicase DNA binding protein 5 (CHD5)

quantitative Real-Time PCR (qRT-PCR)

receiver operating characteristic (ROC)

area under curve (AUC)

Non-small-cell lung cancer (NSCLC)

small-cell lung cancer (SCLC)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of The First Affiliated Hospital of Xinxiang Medical University and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

J.Y. design of the work; H.Z. the acquisition, analysis, Q.Y interpretation of data; J.Y. the creation of new software used in the work; C.J., X.L. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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References

1. Kaneko M, Eguchi K, Ohmatsu H, Kakinuma R, Naruke T, Suemasu K, Moriyama N: Peripheral lung cancer: screening and detection with low-dose spiral CT versus radiography. *Radiology* 1996, 201(3):798-802.
2. Wu X, Piper-Hunter MG, Crawford M, Nuovo GJ, Marsh CB, Otterson GA, Nana-Sinkam SP: MicroRNAs in the pathogenesis of Lung Cancer. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer* 2009, 4(8):1028-1034.
3. Kirienko M, Gallivanone F, Sollini M, Veronesi G, Voulaz E, Antunovic L, Leonardi L, Testanera G, Castiglioni I, Chiti A: FDG PET/CT as theranostic imaging in diagnosis of non-small cell lung cancer. *Front Biosci (Landmark Ed)* 2017, 22:1713-1723.
4. Ehlerding EB, England CG, Majewski RL, Valdovinos HF, Jiang D, Liu G, McNeel DG, Nickles RJ, Cai W: ImmunoPET Imaging of CTLA-4 Expression in Mouse Models of Non-small Cell Lung Cancer. *Molecular pharmaceutics* 2017, 14(5):1782-1789.
5. Scherer K, Yaroshenko A, Bolukbas DA, Gromann LB, Hellbach K, Meinel FG, Braunagel M, Berg JV, Eickelberg O, Reiser MF *et al*: X-ray Dark-field Radiography - In-Vivo Diagnosis of Lung Cancer in Mice. *Scientific reports* 2017, 7(1):402.
6. Kolla V, Zhuang T, Higashi M, Naraparaju K, Brodeur GM: Role of CHD5 in human cancers: 10 years later. *Cancer research* 2014, 74(3):652-658.
7. Garcia I, Mayol G, Rodriguez E, Sunol M, Gershon TR, Rios J, Cheung NK, Kieran MW, George RE, Perez-Atayde AR *et al*: Expression of the neuron-specific protein CHD5 is an independent marker of outcome in neuroblastoma. *Molecular cancer* 2010, 9:277.
8. Fujita T, Igarashi J, Okawa ER, Gotoh T, Manne J, Kolla V, Kim J, Zhao H, Pawel BR, London WB *et al*: CHD5, a tumor suppressor gene deleted from 1p36.31 in neuroblastomas. *Journal of the National Cancer Institute* 2008, 100(13):940-949.
9. Garcia I, Mayol G, Rios J, Domenech G, Cheung NK, Oberthuer A, Fischer M, Maris JM, Brodeur GM, Hero B *et al*: A three-gene expression signature model for risk stratification of patients with neuroblastoma. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2012, 18(7):2012-2023.
10. Koyama H, Zhuang T, Light JE, Kolla V, Higashi M, McGrady PW, London WB, Brodeur GM: Mechanisms of CHD5 Inactivation in neuroblastomas. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2012, 18(6):1588-1597.
11. Vogiatzi P, Vindigni C, Roviello F, Renieri A, Giordano A: Deciphering the underlying genetic and epigenetic events leading to gastric carcinogenesis. *Journal of cellular physiology* 2007, 211(2):287-295.
12. Cheng YY, Jin H, Liu X, Siu JM, Wong YP, Ng EK, Yu J, Leung WK, Sung JJ, Chan FK: Fibulin 1 is downregulated through promoter hypermethylation in gastric cancer. *British journal of cancer* 2008, 99(12):2083-2087.
13. Yu J, Tao Q, Cheung KF, Jin H, Poon FF, Wang X, Li H, Cheng YY, Rocken C, Ebert MP *et al*: Epigenetic identification of ubiquitin carboxyl-terminal hydrolase L1 as a functional tumor suppressor and

biomarker for hepatocellular carcinoma and other digestive tumors. *Hepatology* 2008, 48(2):508-518.

14. Zhao R, Yan Q, Lv J, Huang H, Zheng W, Zhang B, Ma W: CHD5, a tumor suppressor that is epigenetically silenced in lung cancer. *Lung Cancer* 2012, 76(3):324-331.
15. Siegel R, Naishadham D, Jemal A: Cancer statistics, 2013. *CA: a cancer journal for clinicians* 2013, 63(1):11-30.
16. Herbst RS, Heymach JV, Lippman SM: Lung cancer. *The New England journal of medicine* 2008, 359(13):1367-1380.
17. Spira A, Ettinger DS: Multidisciplinary management of lung cancer. *The New England journal of medicine* 2004, 350(4):379-392.
18. Secretan B, Straif K, Baan R, Grosse Y, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Guha N, Freeman C, Galichet L *et al*: A review of human carcinogens--Part E: tobacco, areca nut, alcohol, coal smoke, and salted fish. *The Lancet Oncology* 2009, 10(11):1033-1034.
19. Chansky K, Sculier JP, Crowley JJ, Giroux D, Van Meerbeeck J, Goldstraw P: The International Association for the Study of Lung Cancer Staging Project: prognostic factors and pathologic TNM stage in surgically managed non-small cell lung cancer. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer* 2009, 4(7):792-801.
20. Boldrini L, Giordano M, Servadio A, Niccoli C, Bertoglio P, Lucchi M, Melfi F, Mussi A, Fontanini G: Prognostic role of TPL2 in earlystage nonsmall cell lung cancer. *Molecular medicine reports* 2017.
21. Potts RC, Zhang P, Wurster AL, Precht P, Mughal MR, Wood WH, 3rd, Zhang Y, Becker KG, Mattson MP, Pazin MJ: CHD5, a brain-specific paralog of Mi2 chromatin remodeling enzymes, regulates expression of neuronal genes. *PloS one* 2011, 6(9):e24515.
22. Ma Z, Song J, Liu S, Han L, Chen Y, Wang Y, Yu C, Hou L: Decreased expression of the CHD5 gene and its clinicopathological significance in breast cancer: Correlation with aberrant DNA methylation. *Oncology letters* 2016, 12(5):4021-4026.
23. Liu JB, Zhou QB, Xu JZ, Wang GX, Yuan WT: Influence of colorectal cancer tumor suppressor gene CHD5 methylation on its clinical and pathological characteristics. *Journal of biological regulators and homeostatic agents* 2015, 29(4):889-893.
24. Du Z, Li L, Huang X, Jin J, Huang S, Zhang Q, Tao Q: The epigenetic modifier CHD5 functions as a novel tumor suppressor for renal cell carcinoma and is predominantly inactivated by promoter CpG methylation. *Oncotarget* 2016, 7(16):21618-21630.
25. Wang X, Lau KK, So LK, Lam YW: CHD5 is down-regulated through promoter hypermethylation in gastric cancer. *Journal of biomedical science* 2009, 16:95.
26. Okawa ER, Gotoh T, Manne J, Igarashi J, Fujita T, Silverman KA, Xhao H, Mosse YP, White PS, Brodeur GM: Expression and sequence analysis of candidates for the 1p36.31 tumor suppressor gene deleted in neuroblastomas. *Oncogene* 2008, 27(6):803-810.
27. Baykara O, Tansarikaya M, Bulut P, Demirkaya A, Buyru N: CHD5 is a potential tumor suppressor in non small cell lung cancer (NSCLC). *Gene* 2017.

28. Zhao R, Wang N, Huang H, Ma W, Yan Q: CHD5 a tumour suppressor is epigenetically silenced in hepatocellular carcinoma. *Liver international : official journal of the International Association for the Study of the Liver* 2014, 34(6):e151-160.
29. Naraparaju K, Kolla V, Zhuang T, Higashi M, Iyer R, Kolla S, Okawa ER, Blobel GA, Brodeur GM: Role of microRNAs in epigenetic silencing of the CHD5 tumor suppressor gene in neuroblastomas. *Oncotarget* 2016, 7(13):15977-15985.

Figures

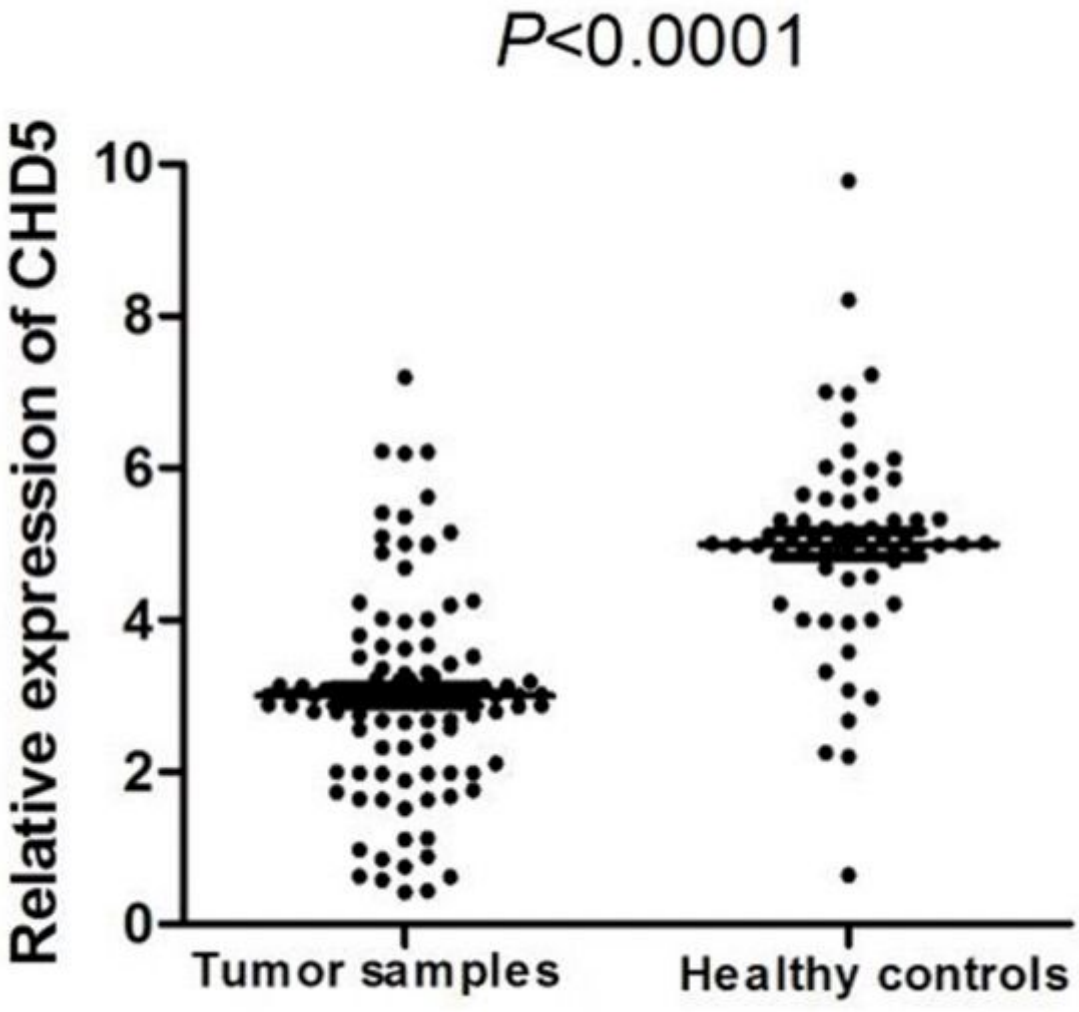

Figure 1

The relative expression of CHD5 in lung cancer samples and healthy controls. The expression of CHD5 with lung cancer was significantly lower than that in healthy controls (P<0.0001).

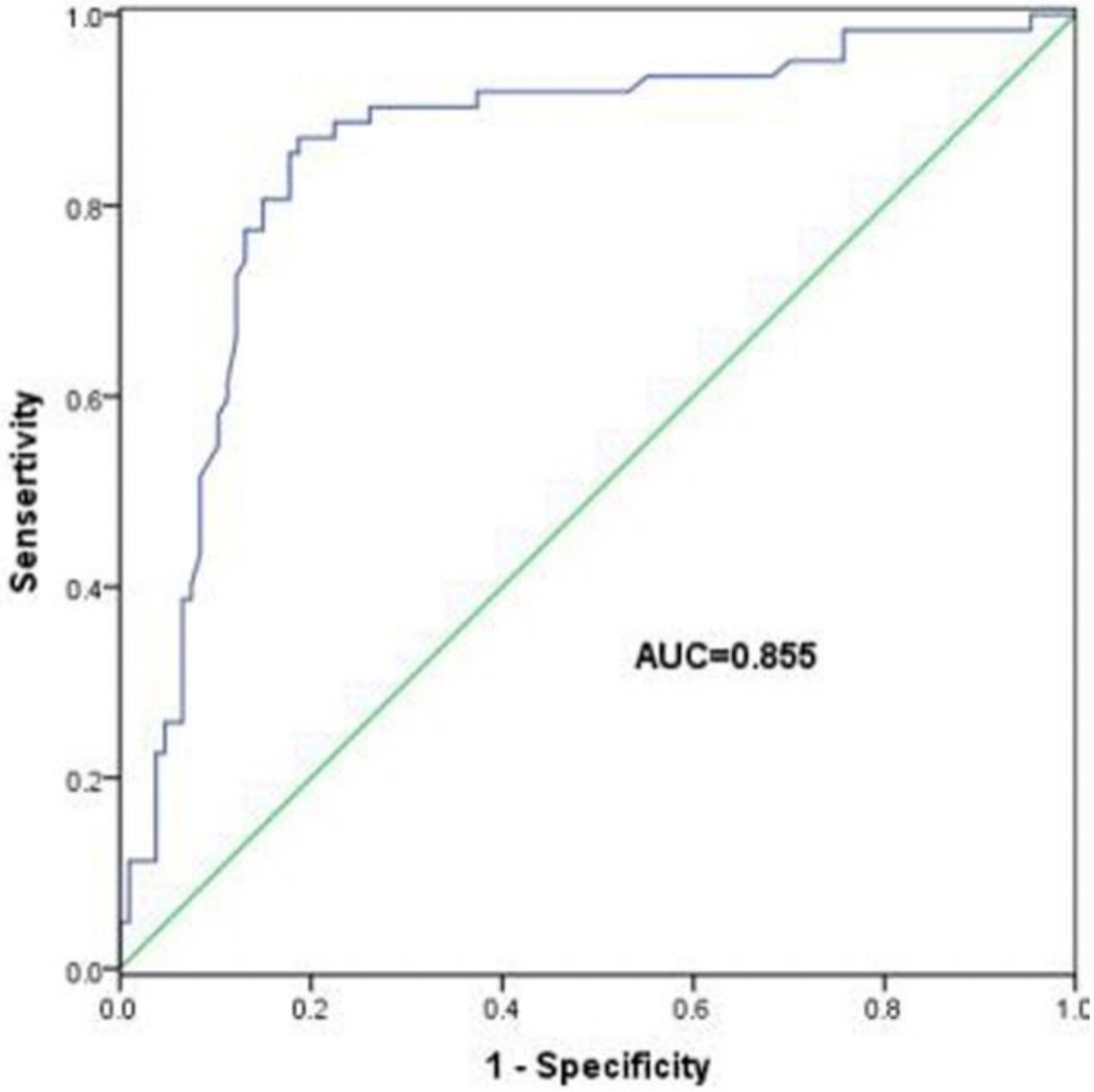

Figure 2

The ROC curve based on the expression of CHD5 in lung cancer. The result showed the AUC was 0.855 with a sensitivity of 87.1% and specificity of 81.3%.