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Research Article

Significance of CDX2 Expression in Differential Diagnosis of Primary and Secondary Lung Cancers

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Abstract

Background: Lung cancer is one of the deadliest tumors worldwide. It increases in relation to rising levels of air pollution and smoking. Diagnosis of lung cancer whether primary or secondary (metastatic) is not always easy due to histopathological similarities with metastatic adenocarcinomas from the colon, the stomach, the pancreatico-biliary system, the breast and the prostate.

CDX2 acts as a transcription factor, increasing the expression of several gene products associated with mature intestinal epithelial cells, however, it shows a limited range of expression in the spectrum of human tissues and neoplasia and thus may have utility in determining the site of origin of tumors in certain clinical situations.

Methodology: In our study, we evaluated the significance of using CDX2 expression as an indicator for differentiating primary from secondary lung cancer of common primary sites.

Results: In our biopsy specimens; CDX2 was not expressed in any benign or primary malignant lung lesion (0%). CDX2 showed positive expression in all cases of metastatic colonic adenocarcinoma (100%), half the cases of metastatic gastric carcinoma (50%) and in 20% of metastatic pancreatico-biliary carcinoma. Metastatic breast and prostatic carcinomas were all negative for CDX2 expression.

Conclusion: We conclude that, in case of histopathological diagnostic difficulties of lung cancer, immunohistochemical evaluation of CDX2 expression could help -among other tissue markers- in the differentiation between primary and metastatic lung malignant lesions, with positive results indicating strongly gastrointestinal primary origin, most likely colonic.

Keywords: *CDX2 Expression; Lung Cancer*

Introduction

Lung cancer is classified by its histologic appearance into small cell lung cancer (SCLC) or non–small cell lung cancer. NSCLC is divided into adenocarcinoma, squamous cell carcinoma, and large cell carcinoma; these are further sub classified. NSCLC is sometimes poorly differentiated and only distinguishable by immunohistochemically stains and molecular testing [1]. Breast, kidney, bladder, bowel, bone, testicular and aggressive skin cancers are some of the cancers that may spread to the lungs [2]. The majority of patients with solid malignancy die from metastatic burden [3]. Tests, such as computerized tomography (CT) Scan, chest X-ray, bronchoscopy for viewing airways, lung needle biopsy, surgical lung biopsy, and cytological analysis of the pleural fluid, are used for the diagnosis of secondary lung cancer [4].

A variety of diagnostic methods are available that yield cytology samples or small biopsies. The choice of procedure depends on the type, location, and size of the tumor; co-morbidities; and accessibility of metastases in general [5]. Conventional bronchoscopy works best for central lesions, whereas CT-guided transthoracic needle aspiration is typically the first-line method for peripheral lesions. Endobronchial ultrasound and electromagnetic navigation are some of the newer procedures that may increase the diagnostic yield of bronchoscopy for select patients with mediastinal or peripheral lesions [6].

CDX2 acts as a transcription factor, increasing the expression of several gene products associated with mature intestinal epithelial cells [7,8]. CDX2 is required for intestine morphogenesis during embryonic development and functions in an earlier stage in development for trophoblast formation and axial patterning [9]. CDX2 protein product can be detected in epithelial cells from the gastroduodenal junction to the rectum by immunohistochemical staining [10,11]. CDX2 expression showed strong extensive staining in 90% of colonic adenocarcinoma cases, but CDX2 was positive in only 20 - 30% of adenocarcinomas of the stomach, esophagus, and ovary (endometrioid and mucinous types) cases. CDX2 shows a limited range of expression in the spectrum of human tissues and neoplasia and thus may have utility in determining the site of origin of tumors in certain clinical situations [12].

CDX2 is a member of the ParaHox cluster of homeobox genes, is responsible for lung specification and seems critical for lung development [10,11]. The lungs and the intestines are developmentally related, as they are both derived from the developing gut tube. One study presented evidence by using autochthonous KP model of lung adenocarcinoma, derivative cell lines, and human patients that together demonstrate that Nkx2-1, Foxa2, and Cdx2 function collectively to suppress metastatic progression of lung adenocarcinoma [13].

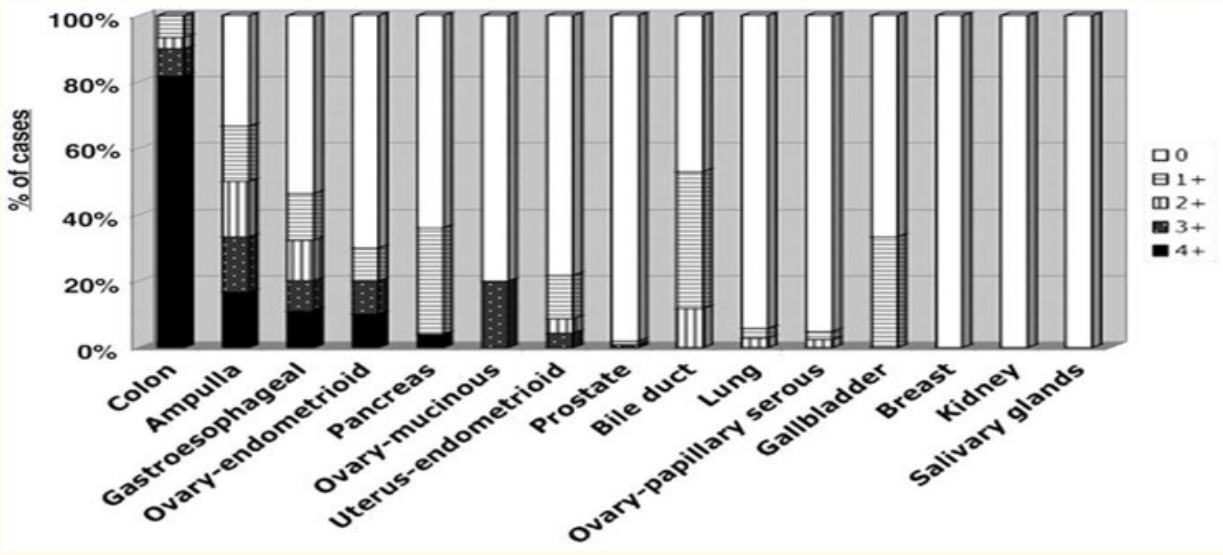

Figure 1: Results of Cdx2 immunohistochemistry in adenocarcinomas. Immunohistochemical staining was graded 0–4+ based on the percent of cells showing nuclear staining for Cdx2. Colorectal adenocarcinomas had the greatest percentage of cases showing strong diffuse Cdx2 staining, followed distantly by ovarian, ampullary, and gastroesophageal adenocarcinomas [10].

Material and Methods

Our study consists of 61 biopsy material from pulmonary lesions suspicious both clinically and radiologically for tumor nature. 36 of our patients were males (59%) and 25 were females (41%). Benign lesions were proved in 9 cases;, while malignant lesions were proved in 52 cases. Bronchoscopic and CT trans-thoracic guided biopsies were taken.

Lung biopsies were delivered to the pathology department of Theodor Bilharz Research Institute from different regional chest hospitals.

Routine histopathological processing and diagnosis was made using hematoxylin and eosin stain. Reporting sheet includes, diagnosis (benign or malignant), with identification of tumor type and grade.

Sections from the archival material was used for immunohistochemical study for CDX2.

Methods

Immunohistochemical Method

Anti- CDX2 antibody ((IR080) DAKO, Denmark)) was used for immunohistochemical (IHC) detection of the expression of CDX2 in tissue. Tissue sections were processed for IHC analysis of as follows. IHC examinations were carried out on 3 μm thick sections. Unmasking was performed with 10 mM sodium citrate buffer, pH 6.0, at 90°C for 30 minutes. Sections were incubated in 0.03% hydrogen peroxide for 10 minutes at room temperature, to remove endogenous peroxidase activity, and then in blocking serum (0.04% bovine serum albumin, A2153, Sigma-Aldrich, Shanghai, China, and 0.5% normal goat serum X0907, Dako Corporation, Carpinteria, CA, USA, in PBS) for 30 minutes at room temperature. Anti-CDX2 antibody (Anti-CDX2 antibody (IR080) was used at a dilution of 1:150. The antibody was incubated overnight at 4°C. Sections were then washed three times for 5 minutes in PBS. Non-specific staining was blocked 5% normal serum for 30 minutes at room temperature. Finally, staining was developed with diaminobenzidine substrate and sections were counterstained with hematoxylin. PBS replaced CDX2 antibody in negative controls.

Quantification of protein expression

The expression of CDX2 was semiquantitatively estimated as the percentage and intensity of nuclear staining. The proportion and intensity of staining was evaluated independently. The proportion of positive cells was calculated in 5 successive HPF and the intensity score represented the staining intensity (score 0: no staining, score 1: weak positive, score 2: moderate positive, score 3: strong positive).

Statistical analysis

SPSS for Windows, version 20 was used for statistical analysis (IBM corporation, Armonk, new York, USA). The comparisons of quantitative variables were performed between two groups using ANOVA and student t-tests. Associations between CDX2 expressions and other studied variables were evaluated by chi square test and fisher test. The P value < 0.05 was considered statistically significant.

Results

Our study consists of 61 biopsy material from pulmonary lesions suspicious both clinically and radiologically for tumor nature. 36 of our patients were males (59%) and 25 were females (41%). Benign lesions were proved in 9 cases; of them 6 were males (66.7%), while malignant lesions were proved in 52 cases; of them 30 were males (57.7%). The mean age of patients with benign fibro-inflammatory lung lesions was (46.33 ± 15.7 years), while the mean age for malignant lung lesions (primary and secondary) was (59.30 ± 13.9 years), with statistically significant difference ($p < 0.05$) (Table 1).

Lesion		Sex		Age (Mean ± SD)
		Females	Males	
Benign (9)	Count	3[a]	6[a]	46.33 ± 15.71
	%	33.3%	66.7%	
Malignant (52)	Count	22[a]	30[a]	59.30* ± 13.92
	%	42.3%	57.7%	
Total	Count	25	36	57.39 ± 14.80
	%	41.0%	59.0%	
Each subscript letter denotes a subset of Sex categories whose column proportions do not differ significantly from each other at the .05 level.				

Table 1: *Sex and age distribution of studied benign and malignant cases.*

** Significant difference with benign cases (p < 0.05).*

Histopathological results of examined lung biopsy specimens revealed nine benign lesions (inflammatory and fibromatous) of them 6 were males and 3 were females, 27 primary lung tumors (squamous cell carcinoma, adenocarcinoma, adenosquamous carcinoma and small cell carcinoma) of them 18 were males and 9 were females and 17 secondary metastatic deposits into the lung (from breast carcinoma, colorectal carcinoma, gastric carcinoma, pancreatico-biliary carcinoma and prostatic carcinoma) of them 12 were males and 5 were females. No statistically significant difference was detected considering the mean age between different studied lesions ($p > 0.05$).

Positive CDX2 expression was detected in 8 out of 52 malignant lung deposits (15.4%), while none of the 9 examined benign lung lesions showed positivity for CDX2 expression, however, the difference between both groups was statistically non-significant ($p > 0.2$) (Table 2).

Positive CDX2 expression was not detected within any case of primary lung cancer (0.0%) or metastatic breast and prostatic cancer. On the other hand, all cases of metastatic colorectal cancer showed positive expression for CDX2 (100%). Secondaries from gastric cancer showed 50% positivity for CDX2 while pancreatico-biliary deposits showed only 20% positivity for CDX2. The differences between groups were statistically significant by Chi square test ($p < 0.05$) (Table 2).

	CDX2 Expression	
	Negative N (%)	Positive N (%)
Malignant (52)	44[a] (84.6%)	8[a] (15.4%)
Lung Adeno Ca (8)	8[a] (100.0%)	0[a] (0.0%)
Lung Adenosquamous Ca (4)	4[a] (100.0%)	0[a] (0.0%)
Lung Squamous cell carcinoma (SCC) (9)	9[a] (100.0%)	0[a] (0.0%)
Lung Neuroendocrine carcinoma (NEC) (6)	6[a] (100.0%)	0[a] (0.0%)
2ry Colorectal cancer (CRC) (4)	0[a] (0.0%)	4[b] (100.0%)
2ry Gastric cancer (GC) (6)	3[a] (50.0%)	3[b](50.0%)
2ry Breast Carcinoma (7)	7[a] (100.0%)	0[a] (0.0%)
2ry Pancreatico-biliary carcinoma (PB) (5)	4[a] (80.0%)	1[a] (20.0%)
2ry Prostatic carcinoma (PCa) (3)	3[a] (100.0%)	0[a] (0.0%)
Fibro-Inflammatory (benign) (9)	9[a] (100.0%)	0[a] (0.0%)
Total (61)	53 (86.9%)	8 (13.1%)

Table 2: *CDX2 expression in studied malignant and benign cases.*

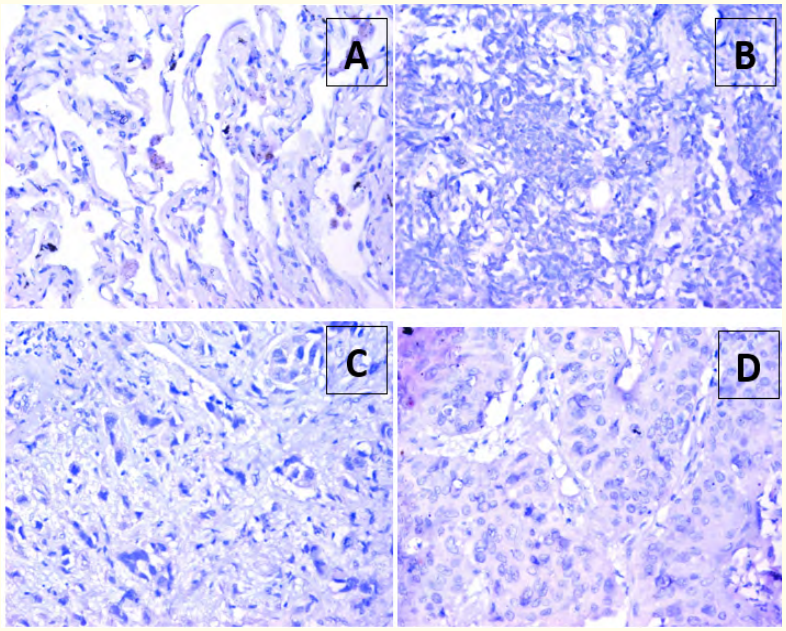

Figure 2: Section from a case of resolving pneumonia showing negative expression for CDX2 in alveolar lining cells (IHC for CDX2, DAB, X200). B: Section in a case of small lung carcinoma, showing negative expression of CDX2 in tumor cells (IHC for CDX2.DAB, X200). C: Lung biopsy in a case of poorly differentiated adenocarcinoma, with negative expression for dcx2 (IHC for DX2, DAB, X200). D: Lung biopsy in a case of poorly differentiated adenosquamous carcinoma, with negative expression for CDX2. (IHC for CDX2, DAB, X200).

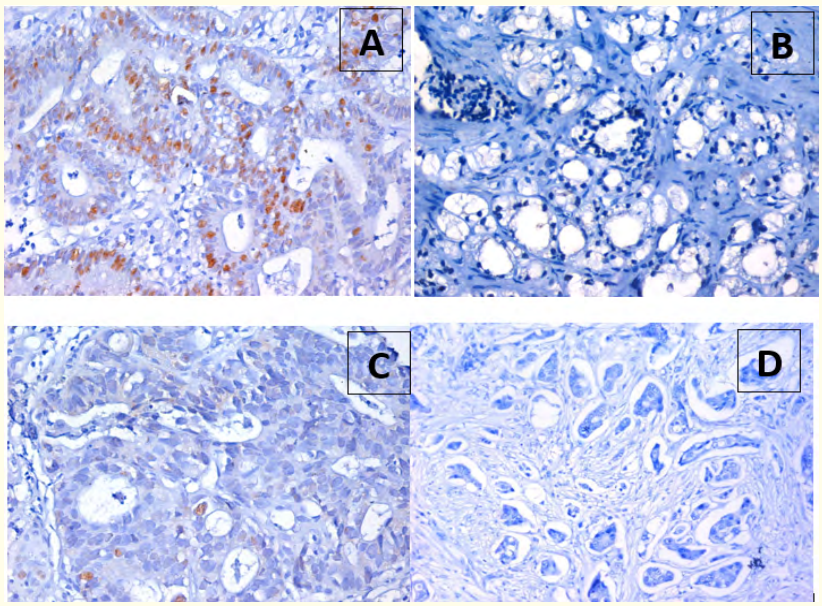

Figure 3: A case of metastatic colonic adenocarcinoma in lung tissue, showing high expression for CDX2 in glandular epithelial cells (IHC for CDX2, DAB, X200). B: A case of metastatic gastric carcinoma in lung tissue, showing mild expression for CDX2 in glandular epithelial cells (IHC for CDX2, DAB, X200). C: A case of metastatic prostatic cancer in lung tissue, showing negative expression for CDX2 in malignant prostatic acini (IHC for CDX2, DAB, X200). D: A case of metastatic breast duct carcinoma in lung tissue, showing negative expression for CDX2 in malignant ductal epithelial cells (IHC for CDX2, DAB, X200).

Discussion

The current study was conducted to test the expression level of CDX2 in lung tissues by immunohistochemical technique. Our study consists of 61 biopsy material from pulmonary lesions suspicious both clinically and radiologically for tumor nature. 36 of our patients were males (59%) and 25 were females (41%). Benign lesions were proved in 9 cases; of them 6 were males (66.7%), while malignant lesions were proved in 52 cases; of them 30 were males (57.7%).

These findings were compared to one of the studies that recorded in out of 20,000 consecutive biopsies, 6534 (32.67%) were neoplastic and 13,466 (67.33%) were non-neoplastic. Of the neoplastic lesions 4726 (72.33%) were malignant, while 1808 (27.67%) were benign [6].

CDX2 expression was not detected within any case of primary lung cancer or any case of metastatic breast and prostatic cancer (0.0%). On the other hand, all cases of metastatic colorectal cancer showed positive expression for CDX2 (100%). Metastasis from gastric cancer showed 50% positivity for CDX2 while pancreatico-biliary deposits showed only 20% positivity for CDX2. The differences between groups were statistically significant by Chi square test ($p < 0.05$).

Our findings were similar to Saad., *et al.*'s study was performed using CDX2 to distinguish adenocarcinoma in situ of the lung from metastatic mucinous colorectal adenocarcinoma. It was recorded that CDX2 expression was found in 19/22 (86%) confirmed metastatic gastrointestinal specimens. All other metastatic adenocarcinomas, from lung, breast, ovaries, pancreas, and prostate sites, were negative for CDX2 [12]. Other study showed that CDX2 was infrequently expressed in pancreatic adenocarcinoma, but was expressed in the majority of cases of rectal adenocarcinoma [14]. De Lott., *et al*. investigated CDX2 expression in tissue microarrays showing positive results in about 72% of colorectal cancers and in only 6% of endometrial carcinomas and 5% of pancreatic adenocarcinoma in cases studied [15].

Our results were against Lie., *et al.* [13] study that showed Cdx2 staining detection in a significant fraction of lung adenocarcinomas (35 of 195; ~20%). Other studies on human lung adenocarcinomas have reported expression of Cdx2 in a subset of patients [16,17].

Conclusion

We concluded that CDX2 could serve as a valuable novel biomarker to be added to the panel used to confirm or to deny primary or secondary (metastatic) lung cancer in cases of histopathological diagnostic difficulties, with positive results for CDX2 staining indicating strongly gastrointestinal origin of the primary tumor, most probably colonic. However, further studies are warranted to clarify the underlying mechanisms of CDX2 expression, thereby contributing to better understanding and further developing its potential use as therapeutic target in some variants of metastatic lung cancer.

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