Evaluation of sex-determining region Y box 6 and heart development protein with EGF-like domain 1 in differentiating epithelioid mesothelioma from lung adenocarcinoma

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Background
Malignant mesothelioma can be difficult to be distinguished from lung adenocarcinoma without immunohistochemistry. However, conventional mesothelial markers until now do not have optimal sensitivity and specificity, necessitating the identification of new markers.
Patients and methods
Sex-determining region Y box 6 (SOX6) and heart development protein with EGF-like domain 1 (HEG1) were evaluated by immunohistochemistry in 55 cases of epithelioid mesothelioma and 50 cases of lung adenocarcinoma. All cases were previously immunostained by calretinin and D2-40. Both sensitivity and specificity for distinguishing epithelioid mesothelioma from adenocarcinoma were calculated.
Results
SOX6 expression was present in 54 (98%) of 55 cases of epithelioid mesothelioma, compared with its expression in only four (8%) of 50 cases of lung adenocarcinoma. The sensitivity and specificity of SOX6 expression for differentiating epithelioid mesothelioma from lung adenocarcinoma were 98.2 and 92.0%, respectively. HEG1 expression was present in 52 (94.5%) of 55 cases of epithelioid mesothelioma, compared with its complete negative expression in all studied cases of lung adenocarcinoma. The sensitivity and specificity of HEG1 for differentiation epithelioid mesothelioma from lung adenocarcinoma were 94.5 and 100%, respectively. The sensitivity and specificity of both SOX6 and HEG1 were higher than those of calretinin and D2-40.
Conclusion
SOX6 and HEG1 may be used as immunohistochemical markers for differentiating epithelioid mesothelioma from lung adenocarcinoma.

Keywords:
sex-determining region Y box 6, heart development protein with EGF-like domains, malignant mesothelioma, lung adenocarcinoma

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Introduction

Malignant mesothelioma is ranked one of the highly aggressive tumors, exhibiting poor prognosis. The increasing incidence of mesothelioma worldwide is attributed to environmental asbestos exposure (Langevin *et al.*, 2015).

In the WHO classification, malignant mesothelioma is classified into epithelioid, sarcomatoid, and biphasic forms with multiple various morphologic patterns. Epithelioid mesothelioma displays multiple histomorphological patterns such as acinar, tubulopapillary, solid, trabecular, and micropapillary (Galateau-Salle *et al.*, 2015). Lung adenocarcinomas also show multiple subtypes such as papillary, micropapillary, acinar, solid, and mucinous (Travis *et al.*, 2015).

This histologic variation may lead to difficulty in discriminating lung adenocarcinoma from epithelioid mesothelioma. Management therapeutic protocols and the prognosis for both lung adenocarcinoma and epithelioid mesothelioma differ completely, so it is extremely important to diagnose them accurately (van Zandwijk *et al.*, 2013).

The International Mesothelioma Interest Group recommends Wilms' tumor 1, calretinin, and podoplanin (D2-40) as diagnostic mesothelial markers. The application of these markers had improved the diagnostic accuracy. However, their sensitivity and specificity in distinguishing epithelioid mesothelioma from lung adenocarcinoma are not satisfactory (Husain *et al.*, 2018).

Sex-determining region Y box 6 (SOX6) is a member of the D subfamily of sex-determining region Y-related transcription factors. SOX6 plays important roles in cell fate determination, proliferation, and differentiation. SOX6 has been recognized as a tumor suppressor or an oncogene in different human cancers (Zhou *et al*., 2019).

Some studies have identified SOX6 as a tumor-suppressor gene with downregulation in multiple tumors, including hepatocellular carcinoma, chronic myeloid leukemia, and ovarian cancers (Guo *et al*., 2013; Wang *et al*., 2016; Li *et al*., 2017). In contrast, the oncogenic role of SOX6 was identified in pancreatic carcinoma (Jiang *et al*., 2018) and Ewing sarcoma (Marchetto *et al*., 2020).

Heart development protein with EGF-like domains 1 (HEG1) has been recognized to have vital roles in embryo development, angiogenesis, and cancer progression. The mechanisms of HEG1 in the progression and metastasis of cancer remain unclear (Kreuk *et al*., 2016). HEG1-mediated promotion of HCC invasion, metastasis, and EMT has been approved to be through promotion of β-catenin expression (Zhao *et al*., 2019).

The aim of this study was to evaluate the efficacy of SOX6 and sialylated HEG1 as novel immunohistochemical markers for differentiating epithelioid mesothelioma from lung adenocarcinoma and compare their specificity and sensitivity with the conventional mesothelial markers calretinin and D2-40.

Patients and methods

This retrospective study was performed on 55 selected cases of epithelioid mesothelioma and 50 cases of lung adenocarcinoma in the form of endoscopic specimens. The studied cases included archival formalin-fixed, paraffin-embedded blocks processed from January 2015 to January 2021 from the Pathology Department and Early Cancer Detection Unit, Faculty of Medicine, Benha University, Egypt. As this was a retrospective study, no written informed consent was required. This study was approved by the Research Ethics Committee of Faculty of Medicine, Benha University, Egypt. The approval number is 24-9-2022. All cases were previously immunostained by calretinin and D2-40.

Histopathological study

Hematoxylin and eosin-stained sections of all cases were reviewed for confirmation of the original diagnosis independently by two pathologists. Diagnosis was confirmed through the histologic features and immunohistochemical marker panels and according to the consensus guidelines standard by the 2017 International Mesothelioma Interest Group and the 2015 WHO histologic classification of lung tumors.

Immunohistochemical evaluation

Overall, 4-μm-thick tissue sections were obtained from formalin-fixed, paraffin-embedded tissue blocks on coated slides. The manufacturer's instructions were followed using a standard labeled streptavidin-biotin system (Dako Cytomation A/S, Glostrup, Denmark). Antigen retrieval was performed using 10 mmol/l citrate monohydrate buffer (pH 6.0) and heated for 15 min in the microwave.

The slides of both markers were incubated overnight at 4°C with anti-SOX6 antibody (rabbit monoclonal antibody; Code ab92307, Auburn Ct, Fremont, CA, USA) at dilution of 1 : 50 and monoclonal anti-sialylated HEG1 antibody (SKM9-2; rabbit polyclonal antibody, ab137110; Abcam, Cambridge, UK) at dilution of 1 : 100. Immunoreaction was visualized by adding DAB as a chromogen. Sections of glioma were used as external positive control for SOX6 and colon tissue for HEG1. For negative controls, the primary antibodies were omitted from the staining procedure.

Immunohistochemical assessment

Assessment of sex-determining region Y box 6 expression

Nuclear SOX6 immunostaining was considered positive. The positive immunostaining in malignant cells has been scored as '0' for no expression in tumor cells, '1+' for less than 10% positive tumor cells, '2+' for 10–50% positive tumor cells, and '3+' for more than 50% positive tumor cells. Scores 1, 2, and 3 were considered positive (Kambara *et al*., 2020).

Assessment of heart development protein with EGF-like domain 1 expression

Membranous immunoreactivity was interpreted as positive stain. HEG1 staining intensity was divided into four categories: 0, none; 1, weak; 2, moderate; and 3, strong. HEG1 staining extension was also divided into four categories: 0: 0%; 1: less than 25%; 2: 25–50%; and 3: more than 50%. A staining score was generated by adding the number of both categories of intensity and extension. A negative value was considered when the staining score was less than or equal to 2, and a positive value was considered when the staining score was more than or equal to 3 (Hiroshima *et al*., 2021).

Statistical analysis

Statistical analysis was performed using SPSS, version 16 (SPSS Inc., Chicago, Illinois, USA). Categorical data were presented as number and percentages, whereas quantitative data were expressed as mean±SD. χ^2 test

and Fisher's exact test were used to analyze categorical variables. Differences were considered significant at a calculated *P* value of less than 0.05. Receiver operating characteristic curve analysis was applied to assess optimum sensitivity and specificity.

Results

Sex-determining region Y box 6 expression in epithelioid mesothelioma and lung adenocarcinoma SOX6 expression was predominantly localized in nuclei. Nuclear SOX6 expression was present in 54 (98%) of 55 cases of epithelioid mesothelioma. A total of 44 cases had an immunohistochemical score of 3+, and 10 cases had a score of 2+ (Fig. 1 and Table 1). No epithelioid mesothelioma cases had an immunohistochemical score of 1+.

In contrast, only four (8%) of 50 lung adenocarcinoma cases had weak nuclear expression of SOX6. Of the four positive cases, two had scores of 2+ and two had scores of 1+.

Heart development protein with EGF-like domain 1 expression in epithelioid mesothelioma and lung adenocarcinoma

Positive membranous HEG1 expression was present in 52 (94.5%) of 55 cases of epithelioid mesothelioma. In contrast, all lung adenocarcinoma cases showed negative expression of HEG1 (Fig. 2 and Table 1).

Calretinin expression in epithelioid mesothelioma and lung adenocarcinoma

Nuclear expression of calretinin was present in 53 (96%) of 55 cases of epithelioid mesothelioma. Calretinin was also expressed in 11 (22%) of 50 cases of lung adenocarcinoma (Table 1). Calretinin showed sensitivity of 90.9% and specificity of 78.0% in differentiation between the two diseases (Table 2).

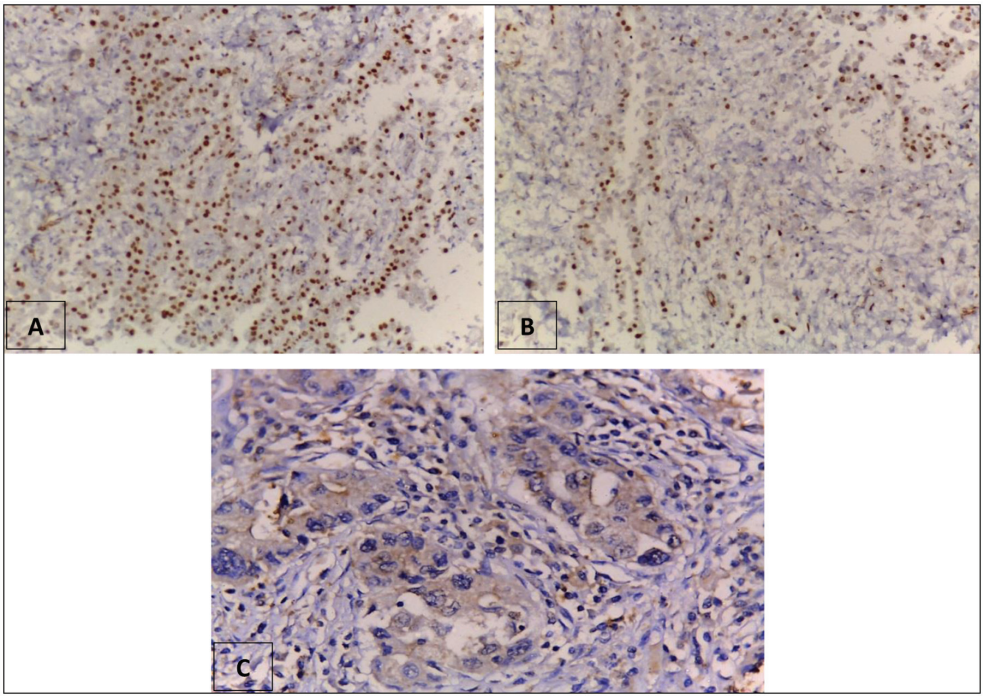
D2-40 expression in epithelioid mesothelioma and lung adenocarcinoma

Membranous expression of D2-40 was present in 53 (98%) of 55 cases of epithelioid mesothelioma. D2-40 was also expressed in five (10%) of 50 cases of lung adenocarcinoma (Table 1). D2-40 showed sensitivity of 96.4% and specificity of 90.0% in differentiation of epithelioid mesothelioma from lung adenocarcinoma (Table 2).

Receiver operating characteristic curve for the validity and predictivity of the studied markers in differentiating malignant mesothelioma and lung adenocarcinoma

SOX6 has sensitivity (98.2%) and specificity (92.0%) in discrimination between mesothelioma and lung adenocarcinoma. HEG1 had sensitivity of 94.5% and specificity of 100% (Table 2, Graph 1).

Figure 1



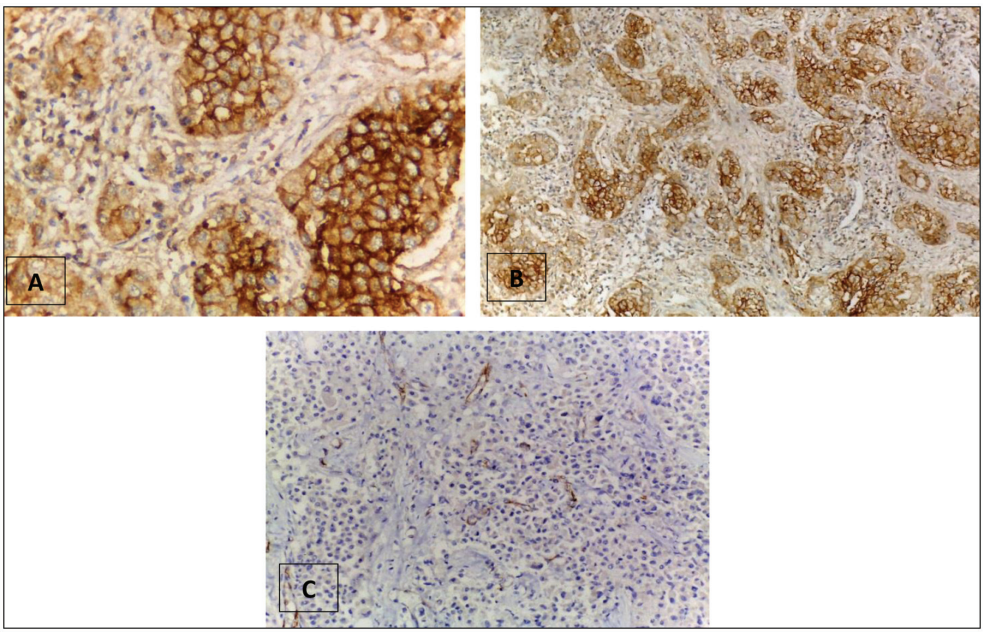
(a) Immunohistochemical expression of SOX6 in epithelioid mesothelioma exhibiting nuclear SOX6 expression score 3 (immunohistochemical ×200). (b) Immunohistochemical expression of SOX6 in epithelioid mesothelioma exhibiting nuclear SOX6 expression score 2 (immunohistochemical ×200). (c) Immunohistochemical expression of SOX6 in lung adenocarcinoma grade 2 exhibiting negative nuclear SOX6 expression score 0 (immunohistochemical ×400). SOX6, sex-determining region Y box 6.

Table 1 Expresion of studied markers in mesothelioma and adenocarcinomaof studied cases

Markers	Epithelioid mesothelioma [*n*/*N* (%)]	Lung adenocarcinoma [*n*/*N* (%)]	*P* value
SOX6			
Positive	54/55 (98)	4/50 (8)	<0.001 (HS)*
Negative	1/55 (2)	46/50 (92)	
HEG1			
Positive	52/55 (94.5)	0/50	<0.001 (HS)*
Negative	3/55 (5.5)	50/50 (100)	
Calretinin			
Positive	53/55 (96.4)	11/50 (22)	<0.001 (HS)*
Negative	2/55 (3.6)	39/50 (78)	
D2-40			
Positive	53/55 (96.4)	5/50 (10)	<0.001 (HS)*
Negative	2/55 (3.6)	45/50 (90)	

HEG1, heart development protein with EGF-like domains; HS, highly significant; SOX6, sex-determining region Y box 6.
*Correlation is significant at the 0.01 level (two tailed).

Figure 2



(a) Immunohistochemical expression of HEG1 in epithelioid mesothelioma showing membranous HEG1 expression score 6 (>50%) (immunohistochemical ×400). (b) Immunohistochemical expression of HEG1 in epithelioid mesothelioma showing membranous HEG1 expression score 5 (>50%) (immunohistochemical ×200). (c) Immunohistochemical expression of HEG1 in lung adenocarcinoma grade 3 showing negative membranous HEG1 expression. (immunohistochemical ×200). HEG1, heart development protein with EGF-like domain 1.

Table 2 Receiver operating characteristic curve results

Markers	AUC	Cutoff point	*P* value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy
SOX6	0.987	≥1	<0.001 (HS)*	98.2	92.0	93.1	97.9	95.2
HEG1	0.979	≥3	<0.001 (HS)*	94.5	100	100	94.3	97.1
Calretinin	0.845	≥1	<0.001 (HS)*	90.9	78.0	82.0	88.6	84.8
D2-40	0.950	≥1	<0.001 (HS)*	96.4	90.0	91.4	95.7	93.3

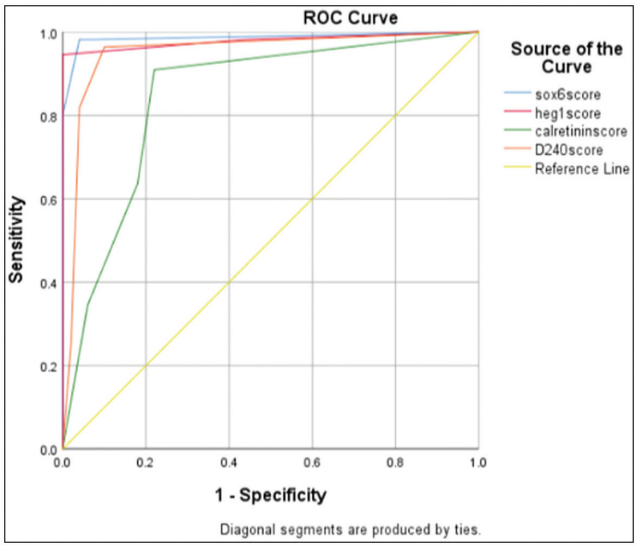
HEG1, heart development protein with EGF-like domains; HS, highly significant; NPV, negative predictive value; PPV, positive predictive value; SOX6, sex-determining region Y box 6.

Sensitivity, specificity, positive predictive value, and negative predictive value of immunohistochemical markers for differentiating epithelioid mesothelioma from lung adenocarcinoma are shown in Table 2.

Discussion

Pleural epithelioid mesothelioma and pulmonary adenocarcinoma have several subtypes that differ in their differential diagnoses. The therapy protocols

Graph 1



SOX6 and HEG1 specificity and sensitivity in ROC curve. ROC, receiver operating characteristic; HEG1, heart development protein with EGF-like domain 1; SOX6, sex-determining region Y box 6.

and prognosis of epithelioid mesothelioma and lung adenocarcinoma are widely variable, so accurate diagnosis of both is imperative.

The International Mesothelioma Interest Group guidelines recommend a panel of positive markers for epithelioid mesothelioma, but the recommended mesothelial markers do not achieve the desired specificity and sensitivity. Previous recent studies reported that SOX6 and HEG1 are highly expressed in different types of malignant mesothelioma (Husain *et al*., 2018).

In this study, we analyzed the immunohistochemical expression of both SOX6 and HEG1 in malignant mesothelioma and lung adenocarcinoma and compare their specificity and sensitivity with the conventional mesothelial markers calretinin and D2-40.

SOX6 belongs to the SOXD family of transcription factors, which contain a highly conserved DNA-binding high-mobility group domain (Jiang *et al*., 2018). SOX6 is a transcription factor involved in tissue differentiation and tumorigenesis in various malignancies (Mehta *et al*., 2019).

The current study showed that SOX6 was expressed in 98% of malignant mesothelioma. The SOX6 staining in epithelioid mesotheliomas was nuclear. SOX6 was expressed in 8% of lung adenocarcinomas. SOX6 expression exhibited high sensitivity (98.2%) and high specificity (92.0%) in demarcation malignant mesothelioma from lung adenocarcinoma. SOX6 sensitivity and specificity values were higher than those of calretinin and D2-40. Therefore, in differentiation of epithelioid mesothelioma from lung adenocarcinoma, SOX6 has potential utility.

In the same context, Kambara *et al*. (2020) declared that SOX6 expression was present in 98% of epithelioid mesothelioma, compared with its expression in 7% of cases of lung adenocarcinoma. The sensitivity and specificity for differentiating epithelioid mesothelioma and lung adenocarcinoma were 98 and 93%, respectively. Naso *et al*. (2021), have published that SOX6 had a sensitivity 85% and specificity 94% for diagnosis of mesothelioma versus adenocarcinoma.

HEG1 plays critical roles in embryonic development of the heart, angiogenesis, and tumor development and progression (Zhao *et al*., 2019).

In the current study, HEG1 was expressed in 94.5% of malignant mesothelioma. The HEG1 staining in most epithelioid mesotheliomas was membranous, strong, and diffuse. HEG1 was not expressed in any of the pulmonary adenocarcinomas. The expression of HEG1 showed high sensitivity (94.5%) and optimal specificity (100%) in demarcation malignant mesothelioma from lung adenocarcinoma. HEG1 sensitivity and specificity values were higher than those of calretinin and D2-40. Therefore, HEG1 has excellent utility for the diagnosis of epithelioid mesothelioma.

In the same context, Tsuji *et al*. (2019) stated that HEG1 was detected in 98% of epithelioid mesothelioma with sensitivity of 92%. Naso *et al*. (2020) declared that HEG1 was seen in 94% of epithelioid mesotheliomas and was completely absent in pulmonary adenocarcinomas. Hiroshima *et al*. (2021) found that HEG1 was detected in 93% of epithelioid mesotheliomas, whereas was negative in lung adenocarcinoma, with sensitivity of 88.8% and specificity of 92.3%. Our results may provide additional confirmation about the important role of SOX6 and HEG1 in the diagnosis of epithelioid mesothelioma.

Conclusion

Immunohistochemical application of SOX6 and HEG1 revealed high sensitivity and specificity for differentiating of epithelioid mesothelioma from lung adenocarcinoma. Further validation of SOX6 and HEG1 is mandatory to verify their potential use as mesothelial markers.

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Nil.

Conflicts of interest
No conflict of interest.

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