Published in final edited form as:
Hum Pathol. 2012 November ; 43(11): 1799–1807. doi:10.1016/j.humpath.2012.03.019.

Immunohistochemical Panel for Distinguishing Esophageal Adenocarcinoma from Squamous Cell Carcinoma: A Combination of p63, Cytokeratin 5/6, MUC5AC, and AGR2 Allows Optimal Subtyping

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Abstract

Distinguishing adenocarcinoma and squamous cell carcinoma of the esophagus is often based on morphologic criteria and can be difficult in small biopsies. We analyzed commonly used immunohistochemical markers (p63, cytokeratin 5/6, cytokeratin 7, CDX2, MUC2, and MUC5AC) and two new markers, AGR2 and SOX2, in esophageal carcinomas to establish the best panel to distinguish these tumors. Tissue microarrays with 69 esophageal adenocarcinomas and 41 whole sections of esophageal squamous cell carcinomas were stained for these markers and semiquantitatively scored. Sensitivities and specificities were calculated for individual markers and select combinations using the morphologic diagnosis as a gold standard. All squamous cell carcinomas expressed p63 with 38/41 demonstrating reactivity in >75% of tumor cells. Cytokeratin 5/6 expression was seen in 40/41 squamous cell carcinomas with 39/41 demonstrating reactivity >75% of tumor cells. SOX2 expression was present in 35/41 of squamous cell carcinomas but also in 24/69 of adenocarcinomas, frequently demonstrating extensive reactivity in adenocarcinomas. AGR2 was highly sensitive for adenocarcinoma and present in 68/69 of cases, but AGR2 reactivity was also identified in 15/41 of squamous cell carcinomas, typically demonstrating focal reactivity in squamous cell carcinoma. MUC5AC expression was seen almost exclusively in adenocarcinomas with only a single squamous cell carcinoma demonstrating focal MUC5AC staining. Overall, the dual expression of both p63 and cytokeratin 5/6 was 99% specific and 98% sensitive for squamous cell carcinoma. In addition, AGR2 and MUC5AC are useful positive markers of adenocarcinoma in the setting of absent or diminished p63 and cytokeratin 5/6 staining.

Keywords

Esophagus; carcinoma; immunohistochemistry; subtyping

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INTRODUCTION

Esophageal carcinoma is one of the most commonly diagnosed cancers worldwide with an estimated 480,000 new cases and 400,000 deaths in 2008. The major subtypes are squamous cell carcinoma, which commonly occurs in the upper two-thirds of the esophagus, and adenocarcinoma, which is most frequent in the lower third and at the gastroesophageal junction. The distinction of these two subtypes impacts clinical management. Recent data suggests optimal management of esophageal adenocarcinoma with either neoadjuvant chemotherapy or neoadjuvant chemoradiation. Peri-operative chemotherapy is also utilized in patients with operable adenocarcinoma of the lower third or gastroesophageal junction. In contrast, squamous cell carcinoma of the esophagus may be treated by definitive chemoradiation alone, with surgery reserved for locally persistent disease or relapse.

Traditional immunohistochemical markers of squamous differentiation include p63 and cytokeratin 5/6, which have demonstrated diagnostic utility in squamous cell carcinoma of multiple anatomic sites. Glickman et al. analyzed a series of esophageal adenocarcinomas and squamous cell carcinoma and demonstrated p63 expression in 100% of esophageal squamous cell carcinomas with no expression identified in esophageal adenocarcinomas. A reliable and specific positive marker for esophageal adenocarcinomas has not been identified but putative markers include CDX2 and cytokeratin 7. In one series, 67% of esophageal adenocarcinomas expressed CDX2. Cytokeratin 7 expression is also commonly observed in esophageal adenocarcinomas, with reports ranging from 82% to 89%. Less commonly used in clinical practice are antibodies to mucin peptide core antigens, MUC2 and MUC5AC. In one analysis, MUC2 was expressed in 17% and MUC5AC in 83% of distal esophageal adenocarcinomas. These two markers have also been used to elucidate the cellular origin of adenocarcinomas arising in Barrett's esophagus with MUC5AC expression more specific to gastric type adenocarcinoma and MUC2 reflective of intestinal type adenocarcinoma. No expression of either marker is seen in normal squamous mucosa of the esophagus.

More recent literature reports have characterized new immunohistochemical markers with possible diagnostic utility in differentiating squamous cell carcinomas and adenocarcinomas. SOX2 is a transcription factor involved in maintaining pleuripotency of embyronic stem cells as well as differentiation of neural stem cells. It is expressed in endoderm of the developing foregut with highest levels in the esophagus and stomach. In a recent analysis, Long and Hornick demonstrated that 80% of esophageal and 90% of anal squamous cell carcinomas express SOX2, with less than 20% of adenocarcinomas at these sites demonstrating expression. *Anterior Gradient Homolog 2* (*AGR2*) encodes a 17 kDa protein expression of which has been identified in adenocarcinomas of varying sites, including lung, breast, and prostate. We recently identified increased levels of expression of AGR2 in esophageal adenocarcinoma.

In small endoscopic biopsies, distinguishing esophageal carcinomas may be difficult as a result of scant cellularity and biopsy artifact. Classification is especially difficult in poorly differentiated tumors. As histologic subtype will often impact clinical management, correct classification is important. Herein, we examined the expression of eight immunohistochemical markers, including the novel markers SOX2 and AGR2, in a large series of esophageal adenocarcinomas and squamous cell carcinomas in an attempt to determine the optimal panel of immunohistochemical markers for distinguishing these tumors.

METHODS

Study Population and Pathologic Evaluation

Patients with radical resection of primary esophageal and gastroesophageal junction adenocarcinoma accessioned at the Department of Pathology, Stanford University Hospital from January 2000 to September 2009 were reviewed. Patients with either endoscopic biopsies or radical resections of primary esophageal squamous cell carcinoma or neuroendocrine carcinoma accessioned at the Department of Pathology, Stanford University Hospital from January 2004 to September 2011 were reviewed. A total of 69 esophageal and gastroesophageal junction adenocarcinomas, 41 esophageal squamous cell carcinomas (27 biopsies, 14 excisions), and 6 high-grade (large cell) neuroendocrine carcinomas (2 biopsies, 4 resections) were identified and comprise our study group. Hematoxylin and eosin stained sections were examined by two pathologists (MAD, RKP) and histologic subtyping of the esophageal tumors was confirmed using previously published criteria. The grade of the tumors was assigned using previously published criteria. This study was approved by the Institutional Review Board of Stanford University Medical Center.

Tissue Microarray Construction

Two tissue microarrays containing a total of 323 tissue cores, each measuring 1.0 mm, were created using a tissue arrayer (Beecher Instruments, Silver Spring, MD) according to a previously described method. Representative areas from each invasive esophageal adenocarcinoma were selected for the microarray from paraffin blocks based on hematoxylin and eosin (H&E) stained sections. For each adenocarcinoma, two distinctly different areas of invasive carcinoma at least 1.0 cm apart were selected for inclusion in the tissue microarray. In cases with mixed tumor morphology, tissue cores were selected from each morphologically distinct area.

Immunohistochemical and Histochemical Analysis

Immunohistochemistry was performed on the tissue microarrays of esophageal adenocarcinoma and whole sections of esophageal squamous cell carcinoma and neuroendocrine carcinoma by standard protocol on Ventana and Dako automated stainers. Primary monoclonal antibodies against p63 (clone 4A4, Cell Marque, 1:200 dilution, Leica Bond ER2 (EDTA) retrieval), cytokeratin 5/6 (clone D5/16B4, Cell Marque, 1:100 dilution, Leica Bond ER2 (EDTA) retrieval), cytokeratin 7 (clone OV-TL12/30, Dako, 1:800 dilution, Leica Bond Enzyme 1), CDX2 (clone CDX2-88, BioGenex, 1:10 dilution, Leica Bond ER2 (EDTA) retrieval), MUC2 (clone CCP58, Fitzgerald Industries, 1:100 dilution, Dako-Citrate retrieval), MUC5AC (clone 45M1, Biocare Medical, 1:40 dilution, Leica Bond Enzyme 1) were applied to 4 micron thick formalin-fixed, paraffin embedded sections. The sections were deparaffinized in xylene, and rehydrated through graded alcohols to distilled water before undergoing antigen retrieval. Immunohistochemical staining for AGR2 was performed as previously described. Briefly, antigen retrieval was enhanced by heating in citrate buffer (pH 6.0) followed by blocking with 5% normal goat serum diluted in phosphate buffered saline. Primary antibody against AGR2 (1:200 dilution) was applied for 30 minutes and visualization was achieved using using the horseradish peroxidase substrate (DAKO, Carpinteria, CA). Immunohistochemical staining for SOX2 was performed as previously described. Briefly, antigen retrieval was enhanced by heating in citrate buffer (pH 6.0) following by blocking with 5% normal goat serum diluted in PBS. Primary antibody against SOX2 (1:1000 dilution, Chemicon/Millipore, rabbit polyclonal) was applied for 22 hours and visualization was achieved using using the horseradish peroxidase substrate (DAKO, Carpinteria, CA). The tissue microarrays of esophageal adenocarcinoma and whole sections of esophageal squamous cell carcinoma were also evaluated for mucin expression using mucicarmine and periodic acid-Schiff (PAS) with diastase (PASd)

histochemical stains. Esophageal neuroendocrine carcinomas were tested for the following limited panel of stains: AGR2, SOX2, MUC5AC, cytokeratin 5/6, PASd, and mucicarmine.

Reactivity was semiquantitatively and independently scored by two pathologists. Percentage of reactive tumor cells was quantified as follows: 0 (no reactivity), 1+ (1–25% staining), 2+ (26–50%), 3+ (51–75%), or 4+ (76–100%). Staining was also classified as strong (clearly seen with 4x or 10x objective lens) or weak (visible at 20x objective or higher). For p63, CDX2 and SOX2, only nuclear labeling was considered positive. For all other immunohistochemical markers, cytoplasmic staining was considered positive. For mucicarmine and PASd staining, only cytoplasmic reactivity was considered positive.

Statistical Analysis

For each marker and select combinations of markers, sensitivity and specificity were calculated against the gold standard represented by the morphologic diagnosis.

RESULTS

Reactivities for the various markers in carcinomas of the esophagus are shown in Table 1. Squamous cell carcinomas consistently expressed cytokeratin 5/6 (40/41, 98%) and p63 (41/41, 100%). Cytokeratin 5/6 (39/41, 95%) and p63 (38/41, 93%) typically demonstrated 4+ immunoreactivity in squamous cell carcinomas. Interestingly, in those squamous cell carcinomas lacking either p63 or cytokeratin 5/6 reactivity, cytokeratin 5/6 and p63 were typically expressed independently of each other. The one case of squamous cell carcinoma lacking cytokeratin 5/6 expression demonstrated 3+, strong p63 expression, and the two squamous cell carcinomas demonstrating 1+ p63 staining had 4+, strong cytokeratin 5/6 expression. SOX2 expression was observed in 35 of 41 cases (85%), similar to what has been previously reported (Figure 1). MUC5AC expression was absent in cases of squamous cell carcinoma, with the exception of a single case (1/41, 2%) with 1+ staining (Figure 2). AGR2 was expressed in 15 of 41 cases (37%) of squamous cell carcinoma, and AGR2 most frequently demonstrated only 1+ immunoreactivity in squamous cell carcinoma. Importantly, none of the squamous cell carcinomas with no or focal (1+ or 2+) p63 and/or cytokeratin 5/6 immunoreactivity demonstrated AGR2 or MUC5AC positivity. Cytokeratin 7, CDX2, and MUC2 expression were found in 34%, 27%, and 20% of squamous cell carcinoma cases, respectively, and most demonstrated 1+ reactivity. PASd and mucicarmine staining was infrequently observed in squamous cell carcinoma (7/41, 17% and 2/41, 5%, respectively). All squamous cell carcinomas with PASd and mucicarmine staining had areas of unequivocal in-situ squamous cell carcinoma which also demonstrated scattered cytoplasmic reactivity.

The most consistently expressed antigen in esophageal adenocarcinomas was AGR2, with immunoreactivity in 68 of 69 cases (99%). The vast majority (63/69, 91%) of these cases demonstrated 3+ or 4+, strong expression. Cytokeratin 7 expression was also found in 62 of 68 adenocarcinomas (91%), with a majority (48/68, 71%) of cases demonstrating 4+ expression. Expression of the mucin peptide core antigens MUC2 and MUC5AC in esophageal adenocarcinomas was variable with MUC5AC expression in 64% and MUC2 expression in 41% of cases. These antigens were more commonly expressed in a variable pattern often with less than 50% of the tumor demonstrating immunoreactivity. CDX2 was present 59% of esophageal adenocarcinomas. Sixty-six of 69 (96%) adenocarcinomas demonstrated cytoplasmic PASd staining; however, the staining was often seen in only a minor subset of tumor cells (1+ staining, 25/69). Mucicarmine revealed cytoplasmic mucin in only 46/69 (67%) of adenocarcinomas with 1+ reactivity being most common (27/69, 39%). SOX2, p63, and cytokeratin 5/6 expression were found in 35%, 10%, and 13% of adenocarcinomas, with 1+ immunoreactivity being the most common pattern of expression.

Importantly, p63 and cytokeratin 5/6 expression in adenocarcinomas were independent of each other. Of the nine cytokeratin 5/6-positive esophageal adenocarcinomas, only one case demonstrated concurrent 1+ p63 immunoreactivity. Conversely, of the seven p63-positive esophageal adenocarcinomas, only one case demonstrated concurrent 1+ cytokeratin 5/6 staining.

All of the esophageal neuroendocrine carcinomas in our study were large cell neuroendocrine carcinomas with neuroendocrine differentiation confirmed by positive staining with synaptophysin and/or chromogranin immunohistochemistry. Esophageal large cell neuroendocrine carcinomas frequently expressed SOX2 (6/6, 100%) and the staining was typically diffuse (3+ or 4+) with strong staining intensity (6/6, 100%). Immunoreactivity for p63 was also observed in a subset of esophageal large cell neuroendocrine carcinomas (4/6, 66%), although all tumors demonstrated either 1+ or 2+ extent of staining. Importantly, only one esophageal large cell neuroendocrine carcinoma demonstrated cytokeratin 5/6 staining in a small proportion of tumor cells (1+). MUC5AC, PASd, and mucicarmine staining was not observed in esophageal large cell neuroendocrine carcinomas.

Subset analysis of the poorly differentiated carcinomas (n=55) demonstrated largely similar results (Table 2). Among 15 poorly differentiated squamous cell carcinomas, 100% expressed p63, 93% expressed cytokeratin 5/6, and 87% expressed SOX2. A higher proportion of poorly differentiated squamous cell carcinomas expressed AGR2 (47%), compared to all squamous cell carcinomas. Cytokeratin 7 expression (40%) and CDX2 expression (20%) in poorly differentiated squamous cell carcinomas was similar to all squamous cell carcinomas. Rare poorly differentiated squamous cell carcinomas demonstrated 1+ staining with PASd (3/15, 20%) and mucicarmine (1/15, 7%). Poorly differentiated adenocarcinomas also had similar immunohistiochemical profiles in comparison to all esophageal adenocarcinoma with AGR2 and cytokeratin 7 expression in 98% and 90% of cases, respectively. Expression of MUC5AC (55%), CDX2 (63%), SOX2 (33%), p63 (10%) and cytokeratin 5/6 (8%) in poorly differentiated adenocarcinoma were generally similar to all adenocarcinomas. PASd and mucicarmine staining was seen in 95% and 57% of poorly differentiated adenocarcinomas, respectively.

Sensitivities and specificities of the individual markers as well as select marker combinations were calculated for both esophageal adenocarcinoma and squamous cell carcinoma (Table 3). Individual markers most sensitive for the diagnosis of squamous cell carcinoma were the presence of any p63 or cytokeratin 5/6 staining, with sensitivities of 100% and 98%, respectively. The presence of any AGR2 staining was 99% sensitive but 63% specific for the diagnosis of adenocarcinoma. In contrast, any MUC5AC staining was 64% sensitive and 98% specific for the diagnosis of adenocarcinoma. Examination of select combinations of markers showed the combination of any p63 and any cytokeratin 5/6 staining was 98% sensitive and 99% specific for the diagnosis of squamous cell carcinoma. Adjusting the cutoff to greater than 50% staining p63 and cytokeratin 5/6 resulted in 93% sensitivity but 100% specificity. The presence of either p63 or cytokeratin 5/6 staining in greater than 50% of tumor cells was also highly specific (100%) and sensitive (95%) for squamous cell carcinoma. As p63 and cytokeratin 5/6 appeared to perform better in the diagnosis of squamous cell carcinoma, we determined if the addition of AGR2 and MUC5AC to the panel would increase diagnostic accuracy. For adenocarcinomas, any AGR2 staining in the presence of any MUC5AC staining was 62% sensitive but 98% specific for adenocarcinoma. In the presence of diminished (<50%) immunoreactivity with p63 and cytokeratin 5/6, positive staining with either AGR2 or MUC5AC is highly sensitive (100%) and specific (95%) for a diagnosis of adenocarcinoma. Similarly, in the presence of diminished (<50%) immunoreactivity with p63 and cytokeratin 5/6, any PASd reactivity

was 96% sensitive and 100% specific for a diagnosis of adenocarcinoma. Subset analysis of marker combinations in poorly differentiated carcinomas showed similar findings, in part because poorly differentiated carcinomas comprised 55 of the 110 carcinomas in this study (50%).

DISCUSSION

The classification of esophageal carcinomas in scant biopsies can be problematic, especially in the setting of poorly differentiated tumors. To our knowledge, an optimal panel of immunohistochemical markers for differentiating esophageal adenocarcinomas and squamous cell carcinomas has not been reported. In this study, we examined a panel of traditional markers and characterized two newly described immunohistochemical markers, AGR2 and SOX2, to determine their utility in differentiating these tumors. Our results demonstrate the combination of p63 and cytokeratin 5/6 is highly sensitive and specific for distinguishing squamous cell carcinoma from adenocarcinoma. No other markers in this panel had comparable sensitivity and specificity for squamous cell carcinoma. SOX2 expression has been previously described in esophageal and anorectal squamous cell carcinomas in one study, which also demonstrated staining in approximately 15% of adenocarcinomas of these locations. Our analysis examined a larger number of esophageal carcinomas and found expression of SOX2 in 35% of esophageal adenocarcinomas. While SOX2 may be sensitive for the diagnosis of esophageal squamous cell carcinoma, it is not as sensitive as either p63 or cytokeratin 5/6, and may be observed in a large number of esophageal adenocarcinomas and large cell neuroendocrine carcinomas, limiting its diagnostic utility. The traditional markers of adenocarcinoma, including CDX2 and cytokeratin 7, are immunoreactive with a large fraction of squamous cell carcinomas and may demonstrate extensive staining in some cases. In our analysis, MUC5AC appeared to be the most specific marker of adenocarcinoma but suffers from relatively low sensitivity for adenocarcinoma. In contrast, AGR2 is a highly sensitive marker of adenocarcinoma but is immunoreactive in a subset of squamous cell carcinomas. The histochemical stain PASd was highly sensitive (96%) for a diagnosis of adenocarcinoma. However, interpretation of PASd staining is often difficult given the frequent occurrence of intense staining of extracellular proteinaceous material. In addition, PASd staining is often very focal with only a limited number of tumor cells demonstrating cytoplasmic staining making discrimination between poorly differentiated adenocarcinoma and squamous cell carcinoma difficult on limited biopsy samples. Mucicarmine displayed diminished sensitivity for a diagnosis of adenocarcinoma, limiting its utility as an ancillary diagnostic study.

Given the utility of p63 and cytokeratin 5/6 in detecting squamous cell carcinomas, exclusion of squamous carcinoma by the absence of these markers supports the diagnosis of adenocarcinoma. Importantly, although p63 or cytokeratin 5/6 staining can be observed in adenocarcinoma, these are typically expressed independently of each other. Of 69 adenocarcinomas, only a single case of adenocarcinoma, which was poorly differentiated, demonstrated dual staining with p63 and cytokeratin 5/6, both of which involved less than 25% of the tumor. In addition, p63 and cytokeratin 5/6 were expressed independently of each other in the limited number of neuroendocrine carcinomas included in this study. p63 immunohistochemical staining was frequently identified in neuroendocrine carcinomas; however, only a single case of neuroendocrine carcinoma demonstrated cytokeratin 5/6 staining in fewer than 5% of tumor cells. Due to the independence of these markers in adenocarcinoma and neuroendocrine carcinomas, both p63 and cytokeratin 5/6 immunohistochemistry should be performed when attempting to establish differentiation in a poorly differentiated esophageal carcinoma of uncertain differentiation. By examination of the subset of poorly differentiated carcinomas, we observed little difference in the

sensitivities and specificities of the individual markers and marker combinations. This is largely due to the high number of poorly differentiated tumors in this series.

Based on this analysis, we propose an algorithmic approach for differentiating esophageal adenocarcinoma from squamous cell carcinoma, as depicted in Figure 3. Immunoreactivity for p63 or cytokeratin 5/6 in greater than 50% of tumor cells is 100% specific for squamous cell carcinoma. Typically, both p63 and cytokeratin 5/6 will exhibit diffuse, strong reactivity in squamous cell carcinoma but occasionally only one of these markers will be positive. Importantly, those squamous cell carcinomas lacking cytokeratin 5/6 reactivity typically retain diffuse p63 immunoreactivity and vice versa. Thus, both p63 and cytokeratin 5/6 should be performed together as the first-line panel of immunohistochemical stains to establish differentiation in esophageal carcinoma. While neuroendocrine carcinomas can demonstrate p63 immunoreactivity, the staining is often focal, involving <50% of tumor cells. However, if the histologic features raise concern for the possibility of neuroendocrine carcinoma, synaptophysin and chromogranin immunohistochemistry should also be performed. If p63 and cytokeratin 5/6 demonstrate immunoreactivity in a minority of the tumor cells or if both are negative, our results suggest that additional studies with AGR2, MUC5AC, and/or PASd may help to confirm the diagnosis of esophageal adenocarcinoma. The presence of diffuse AGR2 expression is highly sensitive for adenocarcinoma, particularly in the setting of negative or diminished p63 and cytokeratin 5/6 positivity. However, neuroendocrine carcinomas can demonstrate focal (<50%) AGR2 staining is a subset of cases. MUC5AC is highly specific for esophageal adenocarcinoma, and the presence of MUC5AC positivity in a tumor lacking diffuse p63 and cytokeratin 5/6 can provide evidence of adenocarcinoma. We realize that many pathology laboratories do not have AGR2 and MUC5AC available in their panel of immunohistochemical antibodies. Our results show that PASd histochemical staining is a useful alternative to confirm the diagnosis of adenocarcinoma and exclude the possibility of neuroendocrine carcinoma in the setting of negative or diminished p63 and cytokeratin 5/6 reactivity. However, interpretation of PASd staining is often difficult given the limited and focal nature of reactivity within poorly differentiated carcinomas. If all markers in this proposed panel (p63, cytokeratin 5/6, AGR2, MUC5AC, and PASd) are negative, consideration for other esophageal carcinoma subtypes, including neuroendocrine carcinoma, should be considered, and if excluded, the carcinoma may be typed as undifferentiated.

In summary, we retrospectively examined a large number of esophageal carcinomas to determine the optimal panel of immunohistochemical markers for distinguishing adenocarcinomas from squamous cell carcinomas. We demonstrated the two marker panel of p63 and cytokeratin 5/6 is highly sensitive and specific for distinguishing squamous cell carcinoma from adenocarcinoma. The presence of MUC5AC and diffuse AGR2 immunoreactivity, or alternatively positive staining with PASd, may help establish the diagnosis of adenocarcinoma in the setting of negative or diminished staining with p63 and cytokeratin 5/6.

Acknowledgments

This work was supported in part by the National Institutes of Health Award DK063624 (to A. W. L.)

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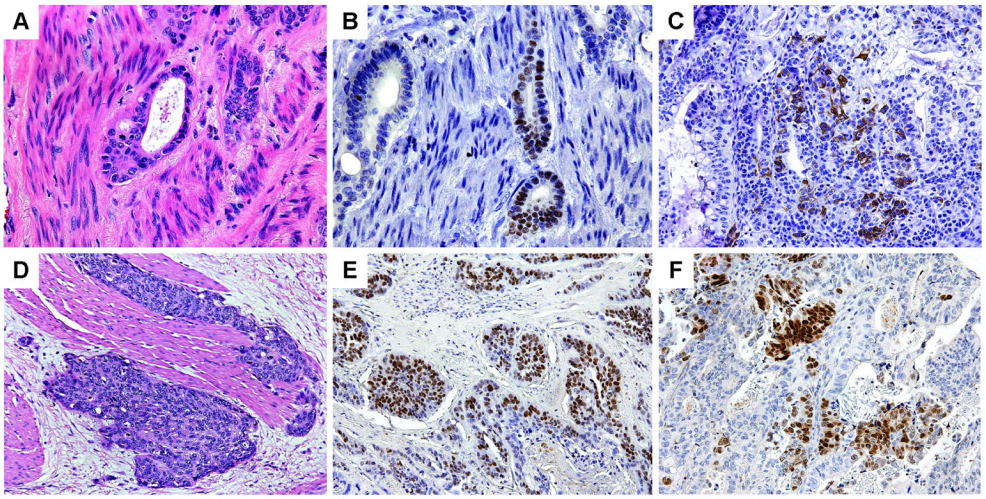

Figure 1.
Well differentiated esophageal adenocarcinoma (A) invasive to the muscularis propria which demonstrates focal expression of p63 in neoplastic nuclei (B). Focal expression of cytokeratin 5/6 expression was observed in some cases of adenocarcinoma (C). Invasive squamous cell carcinoma of the esophagus (D) demonstrates strong nuclear expression of SOX2 (E). Some cases of esophageal adenocarcinoma also expressed nuclear SOX2 (F).

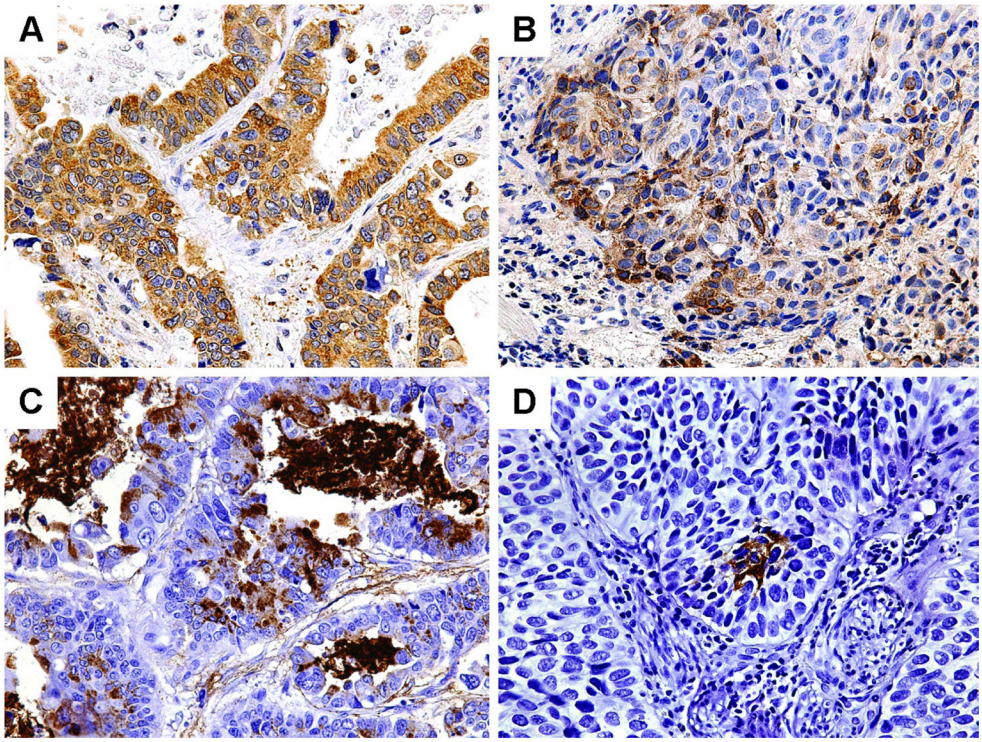

Figure 2.

Strong expression of AGR2 in esophageal adenocarcinoma (A) and in esophageal squamous cell carcinoma (B). MUC5AC expression in adenocarcinoma is accentuated in apical cytoplasm of neoplastic epithelial cells (C). Focal MUC5AC expression was observed in a single case of squamous cell carcinoma (D).

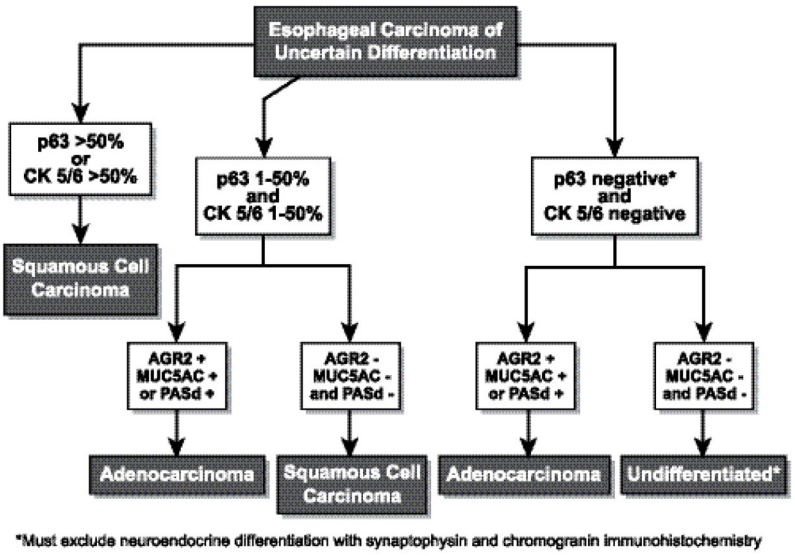

Figure 3.
Diagnostic algorithm for esophageal carcinoma of uncertain differentiation.

Hum Pathol. Author manuscript; available in PMC 2013 November 01.

Table 1

Immunoreactivity of Markers in Esophageal Carcinomas (Includes all tumor grades)

Marker	Diagnosis (No. Tested)	0 (0%) (%)	1+ (1–25%) (%)	2+ (26–50%) (%)	3+ (51–75%) (%)	4+ (>75%) (%)
p63	ADC (69)	62 (90)	7 (10)	0	0	0
	SCC (41)	0	2 (5)	1 (2)	0	38 (93)
	NEC (6)	2 (33)	2 (33)	2 (33)	0	0
CK5/6	ADC (69)	60 (87)	7 (10)	2 (3)	0	0
	SCC (41)	1 (2)	1 (2)	0	0	39 (95)
	NEC (6)	5 (83)	1 (17)	0	0	0
SOX2	ADC (69)	45 (65)	12 (17)	1 (1)	4 (6)	7 (10)
	SCC (41)	6 (15)	3 (7)	4 (10)	6 (15)	22 (54)
	NEC (6)	0	1 (17)	0	2 (33)	3 (50)
CK7	ADC (68)	6 (9)	6 (9)	3 (4)	5 (7)	48 (71)
	SCC (41)	27 (66)	6 (15)	3 (7)	2 (5)	3 (7)
MUC2	ADC (69)	41 (59)	17 (25)	8 (12)	0	3 (4)
	SCC (41)	33 (80)	7 (17)	1 (2)	0	0
MUC5AC	ADC (69)	25 (36)	21 (30)	8 (12)	5 (7)	10 (14)
	SCC (41)	40 (98)	1 (2)	0	0	0
	NEC (6)	6 (100)	0	0	0	0
CDX2	ADC (69)	28 (41)	21 (30)	6 (9)	4 (6)	10 (14)
	SCC (41)	30 (73)	4 (10)	1 (2)	4 (10)	2 (5)
AGR2	ADC (69)	1 (1)	1 (1)	4 (6)	1 (1)	62 (90)
	SCC (41)	26 (63)	9 (22)	2 (5)	3 (7)	1 (2)
	NEC (6)	3 (50)	1 (17)	2 (33)	0	0
PAS-d	ADC (69)	3 (4)	25 (36)	14 (20)	20 (29)	7 (10)
	SCC (41)	34 (83)	7 (17)	0	0	0
	NEC (6)	6 (100)	0	0	0	0
Mucicarmine	ADC (69)	23 (33)	27 (39)	8 (12)	8 (12)	3 (4)
	SCC (41)	39 (95)	2 (5)	0	0	0
	NEC (6)	6 (100)	0	0	0	0

SCC, squamous cell carcinoma; ADC, adenocarcinoma; NEC, neuroendocrine carcinoma; CK, cytokeratin

Table 2

Immunoreactivity of Markers in Poorly-Differentiated Esophageal Adenocarcinoma and Squamous Cell Carcinoma

Marker	Diagnosis (No. Tested)	0 (0%) (%)	1+ (1–25%) (%)	2+ (26–50%) (%)	3+ (51–75%) (%)	4+ (>75%) (%)
p63	ADC (40)	36 (90)	4 (10)	0	0	0
	SCC (15)	0	2 (13)	1 (7)	0	12 (80)
CK5/6	ADC (40)	37 (93)	2 (5)	1 (3)	0	0
	SCC (15)	1 (7)	1 (7)	0	0	13 (87)
SOX2	ADC (40)	27 (68)	5 (13)	0	3 (8)	5 (13)
	SCC (15)	2 (13)	2 (13)	2 (13)	0	9 (60)
CK7	ADC (39)	4 (10)	4 (10)	2 (5)	4 (10)	25 (64)
	SCC (15)	9 (60)	1 (7)	2 (13)	1 (7)	2 (13)
MUC2	ADC (40)	25 (63)	9 (23)	3 (8)	0	3 (8)
	SCC (15)	9 (60)	5 (33)	1 (7)	0	0
MUC5AC	ADC (40)	18 (45)	9 (23)	6 (15)	4 (10)	3 (8)
	SCC (15)	14 (93)	1 (7)	0	0	0
CDX2	ADC (40)	15 (38)	14 (35)	4 (10)	1 (3)	6 (15)
	SCC (15)	12 (80)	2 (13)	0	1 (7)	0
AGR2	ADC (40)	1 (3)	1 (3)	2 (5)	0	36 (90)
	SCC (15)	8 (53)	3 (20)	1 (7)	3 (20)	0
PAS-d	ADC (40)	2 (5)	19 (48)	5 (13)	8 (20)	6 (15)
	SCC (15)	12 (80)	3 (20)	0	0	0
Mucicarmine	ADC (40)	17 (43)	15 (38)	3 (8)	3 (8)	2 (5)
	SCC (15)	14 (93)	1 (7)	0	0	0

SCC, squamous cell carcinoma; ADC, adenocarcinoma; CK, cytokeratin

Table 3

Sensitivity and Specificity of Various Marker Combinations

Marker Reactivity		Predicted Tumor Type	Sensitivity % (95% CI)*	Specificity % (95% CI)*
Individual Markers	p63 (any)	SCC	100 (89, 100)	90 (80, 96)
	CK5/6 (any)	SCC	98 (86, 100)	87 (76, 94)
	SOX2 (>50%)	SCC	68 (52, 81)	84 (73, 91)
	AGR2 (any)	ADC	99 (91, 100)	63 (47, 77)
	MUC5AC (any)	ADC	64 (51, 75)	98 (86, 100)
	MUC2 (>50%)	ADC	4 (1, 13)	100 (89, 100)
	CDX2 (>50%)	ADC	20 (12, 32)	85 (70, 94)
	CK7 (>50%)	ADC	78 (66, 87)	88 (73, 95)
	PAS-d (any)	ADC	96 (87, 99)	83 (67, 92)
	Mucicarmine (any)	ADC	67 (54, 77)	95 (82, 99)
Marker Combinations	p63 (any) and CK5/6 (any)	SCC	98 (86, 100)	99 (91, 100)
	p63 (>50%) and CK5/6 (>50%)	SCC	93 (79, 98)	100 (93, 100)
	p63 (>50%) or CK5/6 (>50%)	SCC	95 (82, 99)	100 (93, 100)
	AGR2 (any) and MUC5AC (any)	ADC	62 (50, 74)	98 (86, 100)
	p63 (−) and CK5/6 (−) AND AGR2 (any) or MUC5AC (any)	ADC	78 (66, 87)	100 (89, 100)
	p63 (<50%) and CK5/6 (<50%) AND AGR2 (any) or MUC5AC (any)	ADC	100 (93, 100)	95 (82, 99)
	PAS-d (any) or mucicarmine (any)	ADC	96 (87, 99)	78 (62, 89)
	p63 (−) and CK5/6 (−) and PAS-d (any)	ADC	74 (62, 83)	100 (89, 100)
	p63 (<50%) and CK5/6 (<50%) and PASd (any)	ADC	96 (87, 99)	100 (89, 100)

SCC, squamous cell carcinoma; ADC, adenocarcinoma; CK, cytokeratin; CI, confidence interval

*Sensitivity and specificity were calculated only for squamous cell carcinoma and adenocarcinoma and includes all tumor grades. Neuroendocrine carcinomas were not included in the sensitivity and specificity analysis.