

## Immunohistochemistry Division

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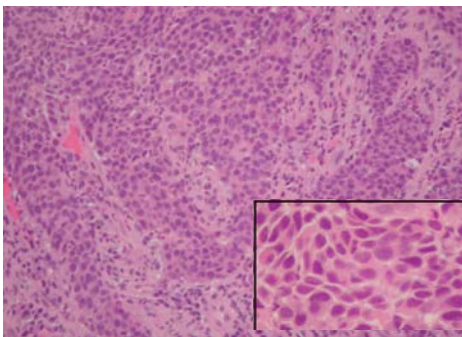
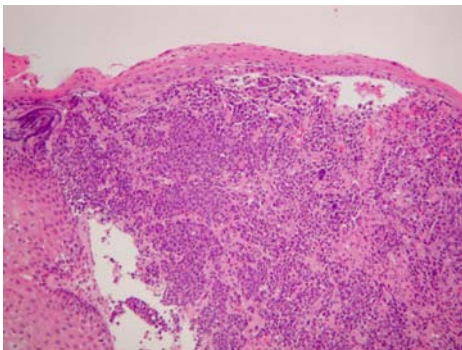
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## Focus on Antibodies – October 2000

### Squamous Carcinoma vs. Poorly Differentiated GI Adenocarcinoma vs. Small Cell Carcinoma

When surgical pathologists receive small endoscopic biopsy specimens that may be crushed or otherwise distorted, it can be difficult in some cases to distinguish poorly differentiated "small cell" squamous carcinoma from poorly differentiated GI adenocarcinoma and small cell (neuroendocrine) carcinoma. Mucin stains may be helpful, but when negative (as they often are in poorly differentiated adenocarcinoma), a battery of immunostains can allow a definitive classification of tumor to be rendered with a high degree of certainty. An example of such a case is illustrated below.

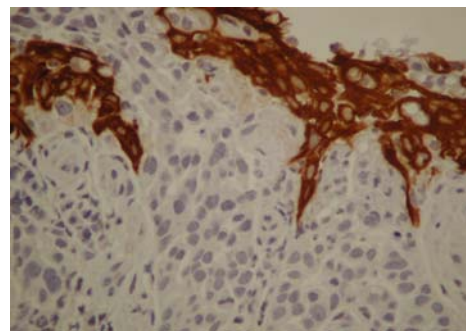


H&E sections of esophageal mass biopsy on 72 year old male. The differential diagnosis was squamous ca. vs. adenoca. vs. small cell ca.

Of the three pathologists who initially viewed the H&E's two favored the diagnosis of a poorly differentiated squamous carcinoma composed of small cells, and the other favored small cell

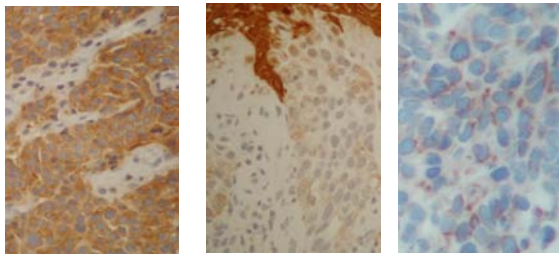
carcinoma. The battery of immunostains selected included the following: Cytokeratin 5/6, Cytokeratin LMW (8,18), Cytokeratin HMW (34bE12), Chromogranin, Synaptophysin, and Villin. The rationale is discussed below:

**Cytokeratin 5/6:** Characteristically, squamous carcinomas express cytokeratin 5/6 (CK 5/6) in a strong and diffuse fashion. In fact, we are hesitant to make the diagnosis of squamous carcinoma in the absence of this finding (unless there is unequivocal keratinization or intercellular bridge formation). Scattered individual CK 5/6 positive cells are common in a wide variety of tumors (including adenocarcinomas), but if a tumor is truly a squamous carcinoma, it is expected to express strong and diffuse CK 5/6. (There are a few other tumors that may show this pattern of expression with this marker, including epithelial mesothelioma, basal cell carcinoma of skin, and also some cutaneous adnexal tumors.) Therefore, in the situation under consideration, the lack of expression of strong CK 5/6 in this case argues strongly against the interpretation of poorly differentiated squamous carcinoma.



CK 5/6 immunostain highlights benign surface squamous epithelium, but the underlying invasive tumor is negative, making a squamous carcinoma highly unlikely.

**Cytokeratin LMW (8,18) and Cytokeratin HMW (34bE12):** These two antibodies are very useful in cases in which small cell carcinoma is in the differential diagnosis. In nearly all cases of small cell carcinoma, the tumor cells express low molecular weight cytokeratin (CK-lmw), and they express this marker in a very characteristic distribution. Small perinuclear dots of CK-lmw immunoreactivity are typical of small cell carcinoma, and we are reluctant to make a diagnosis of small cell carcinoma unless we can identify this finding, at least focally. If a tumor expresses strong and diffuse CK-lmw, it is probably not a small cell carcinoma. In contrast, expression of high molecular weight cytokeratin (CK-hmw) in small cell carcinoma is virtually always substantially weaker than CK-lmw, and frequently high molecular weight cytokeratin is completely negative. Occasionally one may observe a small minority of cells with small perinuclear dots on the CK-hmw immunostain. Adenocarcinomas may or may not express substantial CK-hmw, and squamous carcinomas always express strong CK-hmw.

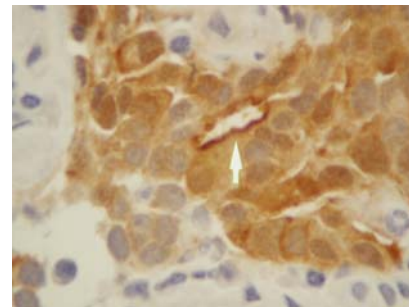


Immunostains for CK-lmw (left) and CK-hmw (center) on the esophageal biopsy. The esophageal tumor expresses uniform CK-lmw (left), unlike small cell carcinoma. Note that the overlying benign squamous epithelium is strongly positive for CK-hmw (middle), but the invasive tumor is only weakly positive for CK-lmw. This renders a squamous tumor highly unlikely. For comparison, a CK-lmw immunostain on a pulmonary small cell carcinoma is illustrated on the right, showing the characteristic perinuclear dot-like pattern with CK-lmw, characteristic of small cell carcinoma.

**Neuroendocrine markers: Chromogranin and synaptophysin:** Chromogranin and synaptophysin are reliable neuroendocrine markers, and with good technique, expression of one or both of these markers can be demonstrated in 85-90% of cases of small cell carcinoma, supporting the interpretation of a neuroendocrine tumor. *(This juncture provides a convenient point for editorializing about another putative neuroendocrine marker, so-called neuron-specific enolase (NSE) Some pathologists use NSE as a marker for neuroendocrine differentiation, but in our opinion, this is a seriously flawed practice. At ProPath we routinely thoroughly evaluate and validate all antibodies before putting them into diagnostic use, and we have repeatedly found*

*that the specificity of NSE for true neuroendocrine differentiation is very poor. We've discovered that we have a similar chance of "getting it right" by flipping a coin.)* In the esophageal tumor in question, both chromogranin and synaptophysin were negative, so there is no evidence of neuroendocrine differentiation in this tumor.

**Villin:** Villin is a GI-related cytoskeletal protein associated with brush border microfilaments, and it can be useful for many aspects of diagnostic immunohistochemistry. The vast majority of GI adenocarcinomas express villin, so its absence argues against a GI adenocarcinoma. An additional feature of this marker is that it can assist greatly in highlighting small glandular lumina that may not be apparent on standard H&E examination or with standard mucin stains. Often, there is luminal accentuation of immunoreactivity, which correlates with the presence of a "brush border" in glandular lumina noted on electron microscopy.



This photomicrograph illustrates the expression of villin by the esophageal tumor cells, and also highlights a glandular lumen (arrow) that displays a brush border pattern of reactivity, indicative of glandular differentiation.

**CONCLUSION:** The immunophenotypic findings in the esophageal tumor biopsy conclusively establish the diagnosis of poorly differentiated adenocarcinoma, and small cell carcinoma and squamous cell carcinoma are excluded.

**SUMMARY:** In situations where the differential diagnosis of small cell carcinoma vs. squamous carcinoma vs. GI adenocarcinoma is difficult or impossible on H&E, a battery of immunostains including cytokeratin 5/6, CK-lmw, CK-hmw, synaptophysin, chromogranin, and villin frequently allow a definitive interpretation to be rendered with a high degree of certainty.

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