

CDH17 is a Highly Specific and More Sensitive Marker Than CK20 and CDX2 in Colon Adenocarcinoma and in Stomach Adenocarcinoma

David Tacha and Ding Zhou, Biocare Medical, Concord, CA

Introduction

Metastatic cancers can be difficult to diagnose in a clinical setting with the vast majority being carcinomas.¹ In the past, a panel of CK7, CK20 and CDX2 antibodies has been used to assess gastrointestinal (GI) tumors of unknown primary.^{2, 3} CDX2 is a homeobox gene that encodes an intestine-specific transcription factor and is expressed in the nuclei of epithelial cells throughout the GI tract. Immunohistochemical expression of CDX2 protein in primary and metastatic colorectal carcinomas has been previously documented.^{2, 3} Cadherin 17 (CDH17), also called liver-intestine cadherin, is a novel oncogene which is involved in tumor invasion and metastasis and is expressed in intestinal epithelium.^{4, 5} CDH17 is a highly specific marker in colon cancers and has been shown to be a more sensitive marker than CDX2.^{4, 5} Recently, a mouse monoclonal CDH17 [clone IH3] has been developed as a Class I *In Vitro* Diagnostic (IVD) tool to aid the pathologist in interpreting GI tumors, especially in cases of unknown primary origin. To the best of our knowledge, no immunohistochemical studies have compared CDH17 [clone IH3] with cytokeratin 20 (CK20) and CDX2 antibodies in both colon adenocarcinoma and stomach adenocarcinoma.

Design

CDH17 mouse monoclonal [clone IH3] (Biocare Medical, Concord, CA), CK20 [Ks20.8] and CDX2 [CDX2-88] were evaluated with immunohistochemistry (IHC) for specificity and sensitivity on formalin-fixed, paraffin-embedded tissue microarrays consisting of colon adenocarcinoma (n = 99), stomach adenocarcinoma (n = 69) pancreatic cancer (n = 39), ovarian cancer (n = 70), lung cancer (n = 71), prostate adenocarcinoma (n = 20), breast cancer (n = 13), urothelial carcinoma (n = 20), renal cell carcinoma (n = 12), melanoma (n = 6), liver cancer (n = 57) and in normal tissues (n = 31). Antibody titers were optimized and visualization was accomplished using a polymer-based detection system. A feasibility study using a cocktail of mouse monoclonal CDH17 and CDX2 rabbit monoclonal [EPR2764Y] (Epitomics, Burlingame, CA) was also developed for evaluation. The antibody cocktail was detected with a Double Stain Kit #1 (Biocare Medical), followed by Fast Red and 3,3'-Diaminobenzidine (DAB) chromogens.

Results

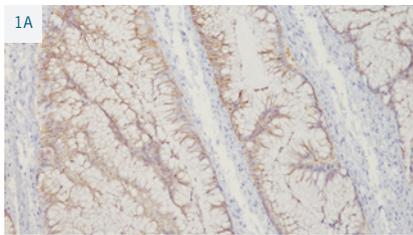
CDH17 stained 100% (99/99) of colon adenocarcinoma, whereas CK20 and CDX2 stained 92% (91/99) and 96% (95/99), respectively (Figure 1A-C and Figure 2A-C). In stomach adenocarcinoma, CDH17 stained 73% (50/69), CK20 28% (19/69) and CDX2 16% (11/69) (Figure 3A-C) (Table 1). CDH17 was positive in 35% (10/29) of pancreatic ductal cell adenocarcinomas (Figure 4A) (data not shown). All cases of pancreatic ductal adenocarcinoma cases that stained positive for CDH17 were low grade (1 and 2), except for one high-grade case that CDH17 and CK20 were both positive and CDX2 was negative (data not shown). One case of high-grade pancreatic adenocarcinoma (1/10) also stained for CDH17 and CK20. In a direct comparison, CK20 was positive in 13% (5/39) of pancreatic cancers and CDX2 was negative in all pancreatic cancers (Table 1). In ovarian cancers, including serous papillary, endometrioid and mucinous adenocarcinoma, CDH17 stained 11% (8/70), and CDX2 and CK20 stained 13% (9/70) and 14% (10/70), respectively. In mucinous adenocarcinoma, CDH17 and CK20 stained 80% (4/5) and CDX2 stained 60% (3/5) (Figure 4B). In lung cancers, CDH17 (M) stained 6% (4/71) and CDX2 and CK20 stained 1% and 3%, respectively. CDH17 stained 12% (4/33) lung adenocarcinomas; however, only low grade (1-2) lung adenocarcinomas stained with CDH17 (Figure 4C), as all other phenotypes of lung cancers were negative including lung squamous cell carcinoma, neuroendocrine carcinoma, large cell carcinoma and small cell carcinoma. CDH17 stained 2% (1/57) in liver cancer; however, HSA and Arginase-1 were negative in the same case. CDH17 was negative in all other cancers types tested (Table 1); and CDH17 was negative in all normal tissues except gastrointestinal epithelial cells (colon and small intestine) and in normal pancreatic ducts.

The CDH17 and CDX2 double stain cocktail stained cytoplasmic/ cell membrane Fast Red (CDH17) and nuclei DAB (CDX2) (Figure 5A-C and Figure 6). Notice in Figure 5D that the base of colon crypts in normal colon lacks CK20 staining (arrows), unlike CDH17 and CDX2.

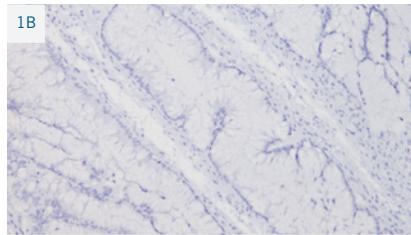
Table 1

Cancer Type	CDH17 (M)	CK20	CDX2
Colon adenocarcinoma	100% (99/99)	92% (91/99)	96% (95/99)
Stomach adenocarcinoma	73% (50/69)	28% (19/69)	16% (11/69)
Pancreatic adenocarcinoma	31% (12/39)	13% (5/39)	0% (0/39)
Ovarian cancers	11% (8/70)	14% (10/70)	13% (9/70)
Lung cancers	6% (4/71)	3% (2/71)	1% (1/71)
Prostate adenocarcinoma	0% (0/20)	0% (0/20)	0% (0/20)
Breast (infiltrating ductal carcinoma)	0% (0/13)	0% (0/13)	0% (0/13)
Urothelial carcinoma	0% (0/20)	0% (0/20)	0% (0/20)
Clear cell renal carcinoma	0% (0/10)	0% (0/10)	0% (0/10)
Melanoma	0% (0/6)	0% (0/6)	0% (0/6)
Liver cancer	2% (1/57)	7% (4/57)	0% (0/57)

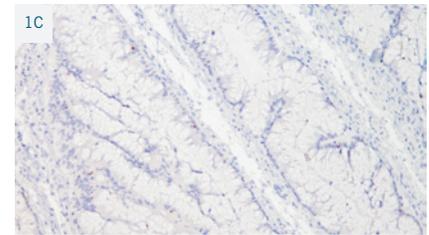
Figure 1: Colon Cancer



CDH17 - Colon Adenocarcinoma, 10x

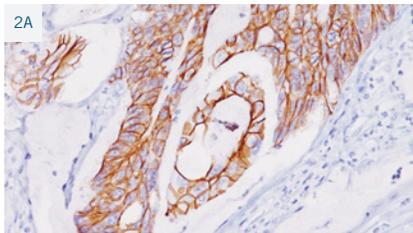


CK20 - Colon Adenocarcinoma, 10x

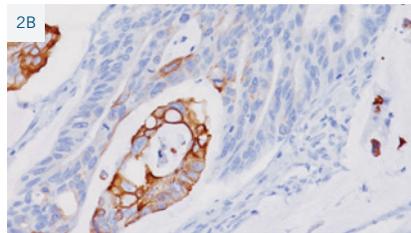


CDX2 - Colon Adenocarcinoma, 10x

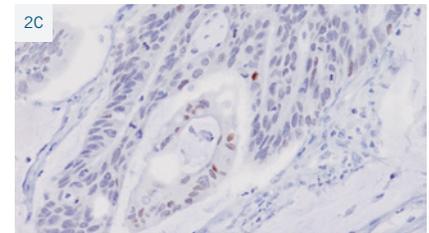
Figure 2: Colon Cancer



CDH17 - Colon Adenocarcinoma, 20x

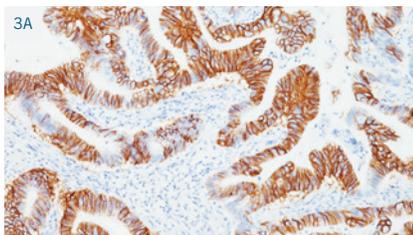


CK20 - Colon Adenocarcinoma, 20x

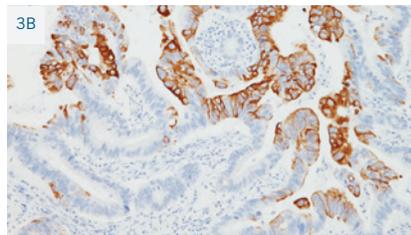


CDX2 - Colon Adenocarcinoma (Focal Expression), 20x

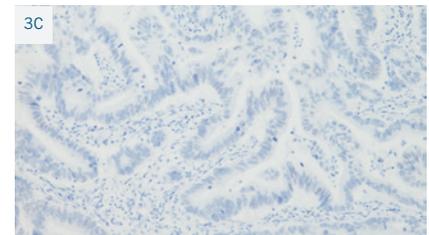
Figure 3: Stomach Adenocarcinoma



CDH17 - Stomach Adenocarcinoma, 10x

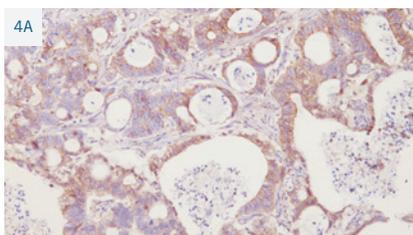


CK20 - Stomach Adenocarcinoma, 10x

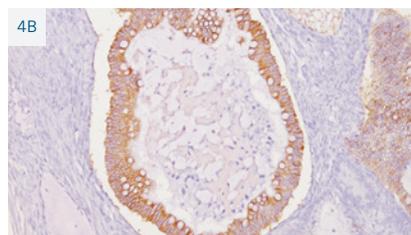


CDX2 - Stomach Adenocarcinoma

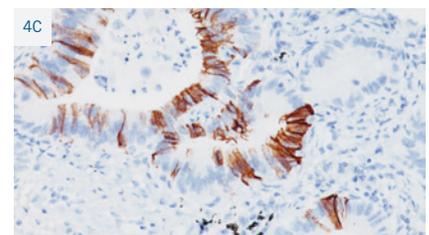
Figure 4: Ovarian Mucinous, Pancreatic Ductal and Lung Adenocarcinomas



CDH17 - Pancreatic Ductal Adenocarcinoma, 10X

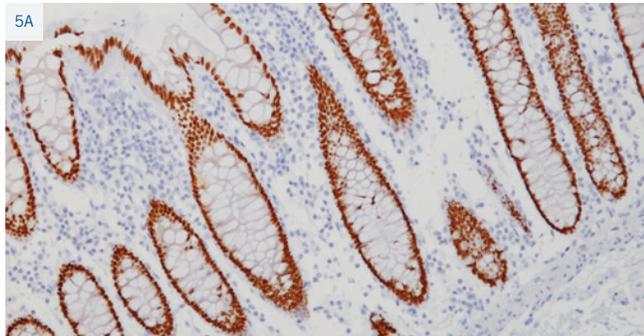


CDH17 - Ovarian Mucinous Adenocarcinoma, 10X

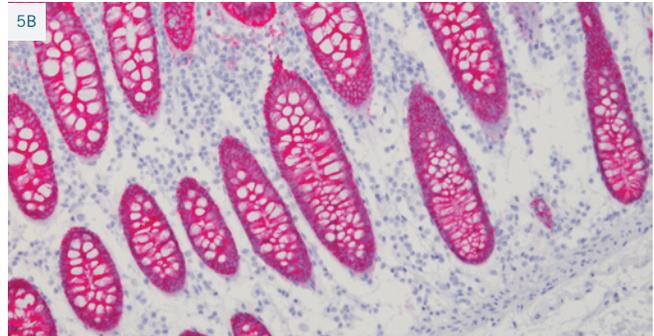


CDH17 - Lung Adenocarcinoma, 20x

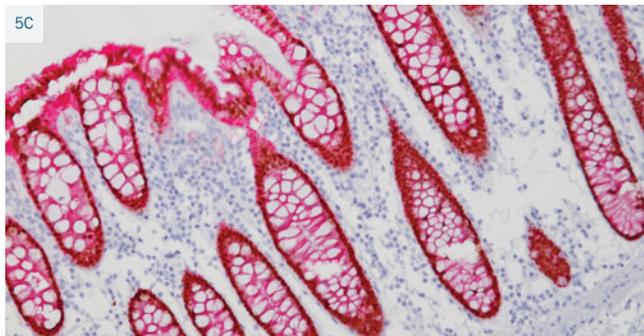
Figure 5: Normal Colon



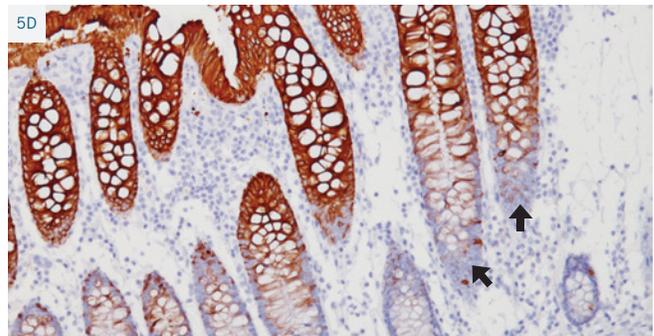
Nuclear Staining Normal Colon with CDX2 (DAB)



Cytoplasmic / Cell Membrane Staining in Normal Colon with CDH17 (Fast Red)



Double Stain with CDX2 (DAB) and CDH17 (Fast Red) in Normal Colon



Cytoplasmic Staining with CK20 (DAB) in Normal Colon (Note: Base of Crypts are Negative for CK20)

Discussion

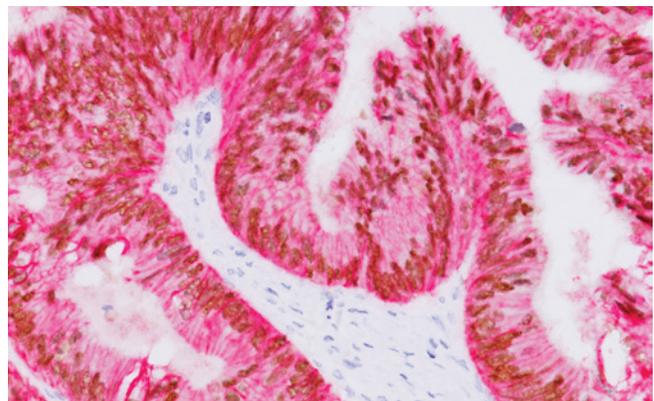
This is the first paper to compare CDH17, CK20 and CDX2 in colon adenocarcinoma and stomach adenocarcinoma. Panarelli NC, *et al.* demonstrated similar data to our study as 100% (161/161) of colon cancers were positive with CDH17 [IH3].⁴

Lin, *et al.* showed CDH17 expression in 98% (123/125) of colon adenocarcinoma and in 13% (26/198) of lung adenocarcinoma, which was almost identical to our findings of 100% and 12%, respectively.⁵ In their study, they also included other cancer types such as angiosarcoma, gastrointestinal stromal tumors, germ-cell tumors, pheochromocytoma, mesothelioma and thyroid cancers (cancer types not included in our study).³ All the above cases were negative for CDH17, thus demonstrating high specificity. In our study, CDH17 stained a high percentage of stomach adenocarcinoma, including 59% (16/27) of poorly differentiated tumors (data not shown). The expression was much higher than CK20 (28%) and CDX2 (16%). In our study, pancreatic cancers also demonstrated similar staining percentages; however, in the Panarelli study, CDX2 was expressed in 27% (8/30) of pancreatic cancers, unlike our study that showed no cases (0/39) of pancreatic cancers positive for CDX2.

Takamura, *et al.* investigated CDH17 [IH3] expression in a large set (n = 102) of patients with pancreatic ductal adenocarcinoma and correlated the findings with patients' survival.⁶ CDH17 expression was seen focally in normal pancreatic ducts. In ductal carcinoma of the pancreas, low grade and/or well-differentiated carcinoma cases strongly expressed CDH17, whereas less differentiated areas and poorly differentiated carcinoma cases expressed less or were negative. This is also correlated with our observations (data not shown). CDH17 mouse monoclonal and CDX2 rabbit monoclonal double stain cocktail was tested on colon adenocarcinoma and normal colon that previously expressed CDH17 and CDX2. Successful double staining was achieved on colon adenocarcinoma (Figures 6), and thus a double stain for IHC on a single tissue biopsy may provide higher sensitivity and the co-expression of both antibodies may provide higher specificity.

On a side note, the prognostic significance for CDH17 expression in colon cancer has been observed. In a study by Kwak JM *et al.*, reduced expression of CDH17 had a significant correlation with tumoral dedifferentiation and short overall survival.⁷ Even though in our studies CDH17 was expressed in 99/99 of colon adenocarcinomas (Table 1), we did observe certain cases with low expression of CDH17 and CDX2 (data not shown).

Figure 6: Double Stain



CDX2 (Nuclear, DAB) + CDH17 (Cytoplasmic/Membrane, FR) in Colon

Conclusion

We conclude that CDH17 compared with CK20 and CDX2 is a highly sensitive and a specific marker for colon and stomach adenocarcinoma in a routine immunohistochemistry, especially in cases with a CK7(-)/CDX2(-)/CK20(-) carcinoma. The combination of CDH17 (+) and CK7 (-) should improve overall specificity of CDH17 and in the future could be the cocktail of choice when stomach adenocarcinoma is suspected in tumors of unknown origin.

References

1. Park SY, *et al.* Expression of cytokeratins 7 and 20 in primary carcinomas of the stomach and colorectum and their value in the differential diagnosis of metastatic carcinomas to the ovary. *Hum Pathol.* 2002 Nov;33(11):1078-85.
2. Werling RW *et al.* CDX2, a highly sensitive and specific marker of adenocarcinomas of intestinal origin: an immunohistochemical survey of 476 primary and metastatic carcinomas. *Am J Surg Pathol.* 2003 Mar;27(3):303-10.
3. Bayrak R, *et al.* The value of CDX2 and cytokeratins 7 and 20 expression in differentiating colorectal adenocarcinomas from extraintestinal gastrointestinal adenocarcinomas: cytokeratin 7-/20+ phenotype is more specific than CDX2 antibody. *Diagn Pathol.* 2012 Jan 23;7:9.
4. Panarelli NC, *et al.* Tissue-specific cadherin CDH17 is a useful marker of gastrointestinal adenocarcinomas with higher sensitivity than CDX2. *Am J Clin Pathol.* 2012 Aug;138(2):211-22.
5. Lin F, *et al.* Cadherin-17 and SATB2 Are Sensitive and Specific Immunomarkers for Medullary Carcinoma of the Large Intestine. *Arch Pathol Lab Med.* 2014 Aug;138(8):1015-26.
6. Takamura M, *et al.* Expression of liver-intestine cadherin and its possible interaction with galectin-3 in ductal adenocarcinoma of the pancreas. *Cancer Sci.* 2003 May;94(5):425-30.
7. Kwak JM, *et al.* The prognostic significance of E-cadherin and liver intestine-cadherin expression in colorectal cancer. *Dis Colon Rectum.* 2007 Nov;50(11):1873-80.