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# ORIGINAL ARTICLE

# E-cadherin downregulation at the infiltrating tumour front is associated with histological grade and stage in colorectal carcinoma of Malaysians

Serena Diane DASS *MD*, Phaik-Leng CHEAH *MBBS, FRCPath*, Diana Bee-Lan ONG *MBChB, MPath*, Kean-Hooi TEOH *MBChB, MPath* and Lai-Meng Looi *FRCPA, FRCPath*

*Department of Pathology, Faculty of Medicine, University of Malaya.*

*Abstract*

Loss of E-cadherin, a 120 kDA transmembrane glycoprotein responsible for cell-cell adhesion, is one of the hallmarks of epithelial-mesenchymal-transition (EMT). E-cadherin expression was immunohistochemically studied in 94 histopathologically re-confirmed colorectal carcinomas (CRC) using a monoclonal antibody to E-cadherin (Dako: Clone NCH-38) on a Ventana Benchmark XT automated system. Each case was assessed for E-cadherin immunopositivity at two separate locations viz the tumour centre (TC) as well as the infiltrating front (IF). Expression was semiquantitated for proportion of immunopositive malignant cells as 0 (negative), 1 (1-25% staining), 2 (26-50% staining), 3 (51-75% staining) and 4 (>75% staining) and staining intensity: 0 (negative), 1 (weak), 2 (moderate) and 3 (strong). The final histoscore of E-cadherin immunopositivity was arbitrarily computed as proportion of immunopositivity multiplied by staining intensity of the malignant cells. E-cadherin histoscores were significantly lower at the IF (4.5 ± 2.5) compared with TC (10.7 ± 2.4). Furthermore, the histoscores were significantly reduced at the IF of 49 TNM III+IV tumours (3.6 ± 2.5) compared with 45 II+III CRC (5.4 ± 2.2). Reduction of E-cadherin expression was also noted in the 23 high grade (TC=8.6 ± 3.2; IF=2.6 ± 2.3) compared with 71 low grade tumours (TC = 11.4 ± 1.5; IF = 5.1 ± 2.3). E-cadherin is downregulated at the infiltrating front of CRC, possibly marking for EMT at this location. The downregulation is further enhanced amongst late stage and high grade tumours compared with earlier stage and low grade tumours; findings which are similar to that noted in CRC of other populations.

*Keywords*: e-cadherin, colorectal carcinoma, tumour stage, tumour grade, Malaysian

## INTRODUCTION

Colorectal carcinoma (CRC) is the third most common malignancy worldwide with an estimated 103,000 new cases per year.[1] Although much has been learnt about the pathogenesis of CRC and survival rate has improved over the years, to date almost 30% of patients still develop recurrent and metastatic disease.[2,3] In recent years, epithelial-mesenchymal transition (EMT) has been recognised as a process which may influence the invasive properties of neoplastic cells. EMT is basically a process whereby epithelial cells acquire mesenchymal characteristics, with loss of cellular polarity while increasing their motility and invasive characteristics.[4] Epithelial-cadherin (E-cadherin) first named by Takeichi *et al* in the 1980s,[5,6] is a member of the classical cadherin family and its loss is one of the hallmarks of EMT.[7,8] E-cadherin has also been described as liver cell adhesion molecules in chickens[9] and uvomorulin in mice.[10] Many studies have since been carried out which show an association of E-cadherin expression with prognosis in CRC.[11-13] It is undoubtedly interesting that He *et al* in a recent meta-analysis noted that loss of E-cadherin is associated with worse prognosis amongst Asian patients with CRC compared with Europeans.[14] This observation prompted us to study E-cadherin expression in Malaysian CRC, acknowledging the paucity of information in this area amongst Malaysians. As part of an ongoing project on EMT, we initiated a study to establish

*Address for correspondence:* Professor Phaik-Leng Cheah, Department of Pathology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia. E-mail: cheahpl@ummc.edu.my

the immunohistochemical (IHC) expression pattern of E-cadherin, focussing on whether known predictive histopathological factors[15] i.e. tumour stage and grade are associated with E-cadherin expression in a set of Malaysian CRC and whether the associations, if they exist, differ from other populations.

## MATERIALS AND METHODS

Fifty consecutive cases of non-metastatic colorectal carcinoma (TNM stage I and II) and 50 metastatic colorectal carcinoma (TNM stage III and IV) diagnosed for the first time at the Department of Pathology, University of Malaya Medical Centre, Kuala Lumpur and who had undergone surgical resection, without any prior adjuvant therapy, were retrieved from the departmental archives. The demographic profiles of the patients were obtained from the histopathological examination request forms.

All cases were reviewed for the histopathological diagnosis and staging according to the TNM staging system.[16,17] Grading was based on a two-tiered system dividing the CRC into low and high grade carcinoma.[18] Only reconfirmed cases were considered for entry into the study. For each case a paraffin block of the formalin-fixed CRC which included some adjacent normal colonic tissue was selected during the review for IHC staining. Only cases where it could be ensured that sufficient tissue remained in the paraffin block for future review, following sectioning for this study, were finally admitted. One 4-µm section was cut from the selected paraffin block for each case on to a platinum coated slide (Matsunami Glass Industries, Japan) for IHC staining using a mouse monoclonal antibody to E-cadherin (1:50; Dako: Clone NCH-38) on a Ventana Benchmark XT automated system. The normal colonic tissue served as an internal positive control.

*E-cadherin histoscore*

Each case was assessed for E-cadherin immunopositivity (Figure 1) at two separate locations viz the tumour centre (TC) as well as the infiltrating front (IF). For purpose of this study, IF included tumour buds (clusters of 1-4 cells)[19, 20] as well as non-buds (≥5 cells). E-cadherin staining was considered positive when the cytoplasmic membrane was completely stained. Expression was semiquantitated for

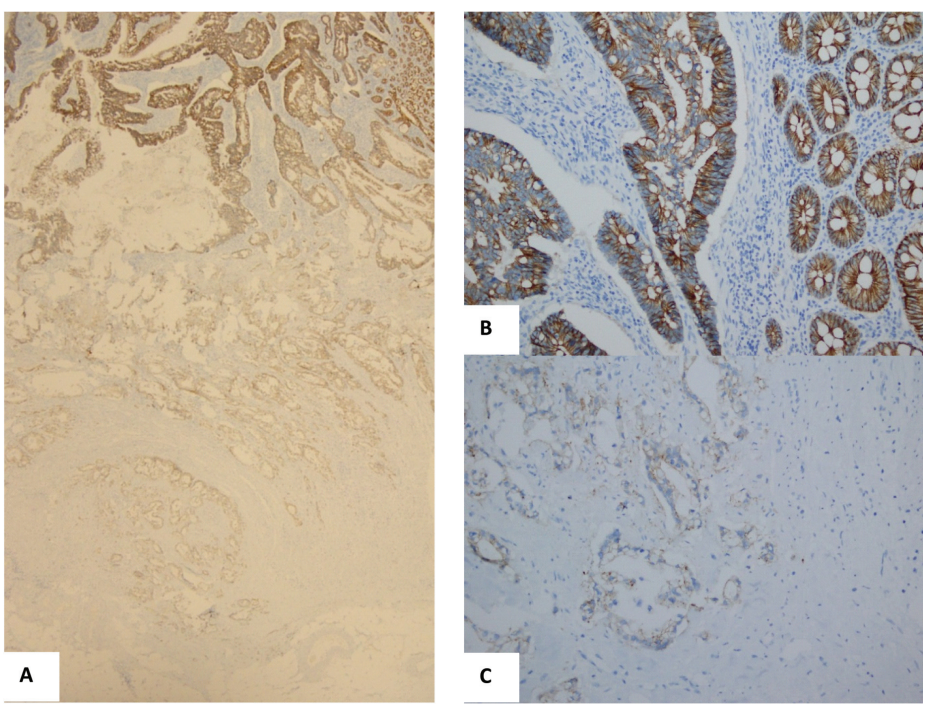


FIG. 1. (A): Low power view of colorectal carcinoma showing differential expression of E-cadherin between tumour centre (top) and infiltrating front (bottom). Note normal mucosa at top right. IHC for ecad x20. (B): Higher power view of tumour centre. Note normal mucosa at right. IHC for ecad X200. (C): Higher power view of tumour infiltrating front. IHC for ecad X200

(a) proportion of malignant cells which were immunopositive for E-cadherin as 0 (negative), 1 (1-25% staining), 2 (26-50% staining), 3 (51-75% staining) and 4 (>75% staining) and (b) intensity of staining: 0 (negative), 1 (weak), 2 (moderate) and 3 (strong). The final histoscore of E-cadherin immunopositivity was arbitrarily taken as proportion of immunopositivity multiplied by intensity of staining of the malignant cells. The histoscore was evaluated for TC and IF for each case.

Statistical analysis was performed using the t-test (SPSS, version 20.0) with statistical significance levels set as $p<0.05$.

## RESULTS

A total of 94 cases of colorectal adenocarcinoma were finally admitted into the study. 49 cases were in TNM stage I or II and considered non-metastatic, while 45 were in stage III or IV and deemed CRC with metastatic disease. 71 were low grade and 23 high grade tumours.

The histoscores of E-cadherin expression at the TC and IF of the CRC in this study as stratified by stage and tumour differentiation are illustrated in Table 1. E-cadherin was expressed in the TC of all cases of CRC. The histoscores showed a range of 1-12 with a mean ± SD of 10.7 ± 2.4. In comparison, the histoscores of E-cadherin expression at the IF ranged from 0-9 with a mean ± SD of 4.5 ± 2.5, being significantly lower than those observed at the TC.

For metastatic CRC (TNM stage III and IV), the histoscores of E-cadherin expression at the TC ranged between 1-12 with a mean ± SD of 10.2 ± 2.7, was not significantly lower than that at the TC of non-metastatic CRC (TNM stage I and II) which ranged between 3-12 with a mean ± SD of 11.1±2.0. In contrast, the histoscores of E-cadherin expression at the IF of metastatic CRC (range = 0-9; mean ± SD = 3.6 ± 2.5) was significantly lower than that of non-metastatic CRC (range = 1-9; mean ± SD = 5.4 ± 2.2).

Histoscores at the TC of high grade CRC (range = 1-12; mean ± SD = 8.6 ± 3.2) was significantly lower than that at the TC of low grade tumours (range = 6-12; mean ± SD = 11.4 ± 1.5). This significant difference was also noted at the IF where histoscores of high grade CRC (range = 0-6; mean ± SD = 2.6 ± 2.3) were lower compared to those of the low grade varieties (range = 0-9; mean ± SD = 5.1 ± 2.3).

## DISCUSSION

The 120 kDA E-cadherin glycoprotein encoded by the *CDH1* gene on chromosome 16q22.1, is made up of 3 main domains viz an extracellular, a transmembrane and a cytoplasmic domain. The extracellular domain has 5 tandem repeats, EC1-EC5 which bind to $Ca^{2+}$, and together are responsible for cell-cell adhesion between a cell and the adjacent ones. The cytoplasmic domain interacts with catenins (α-, β-, γ- and p120 catenin) which are anchored to the actin

**TABLE 1: Histoscore of E-cadherin at the tumour centre (TC) versus infiltrating front (IF) of colorectal adenocarcinoma and at the TC and IF according to stage and tumour grade**

| | Histoscore | | | | |
|---|---|---|---|---|---|
| | N | Range | Mean | SD | p-value |
| TC | 94 | 1-12 | 10.7 | 2.4 | <0.0000001 |
| IF | 94 | 0-9 | 4.5 | 2.5 | |
| TC (TNM stage I+II) | 49 | 3-12 | 11.1 | 2.0 | 0.071 |
| TC (TNM stage III+IV) | 45 | 1-12 | 10.2 | 2.7 | |
| IF (TNM stage I+II) | 49 | 1-9 | 5.4 | 2.2 | 0.00037 |
| IF (TNM stage III+IV) | 45 | 0-9 | 3.6 | 2.5 | |
| TC (low grade) | 71 | 6-12 | 11.4 | 1.5 | 0.00045 |
| TC (high grade) | 23 | 1-12 | 8.6 | 3.2 | |
| IF (low grade) | 71 | 0-9 | 5.1 | 2.3 | 0.000065 |
| IF (high grade) | 23 | 0-6 | 2.6 | 2.3 | |

cystoskeleton of the cell.[8, 21] Loss of E-cadherin is usually associated with loss of cell-cell adhesion, epithelial-to-mesenchymal transformation of the cell, and invasion. Hence E-cadherin is often considered a "tumour suppressor" protein.[22,23] In this study, E-cadherin expression was significantly reduced at the IF of the colorectal carcinomas (histoscore = 4.5 ± 2.5) when compared with the TC (histoscore = 10.7 ± 2.4). This observation is in line with that noted in other studies,[23-25] and to be expected as tumour cells at the infiltrating front should presumably possess features which endow them with properties that propagate infiltration compared with those at the centre of the tumour.[25-28] The loss of E-cadherin at the IF would equate enhancement of EMT which facilitates invasion.

In our study, metastases to lymph nodes and distant organs appear to be associated with further reduction in E-cadherin expression, especially at the IF. Although the E-cadherin decrease at the TC did not reach statistical significance between TNM stage III +IV (histoscore = 10.2 ± 2.7) and stage I + II tumours (histoscore = 11.1 ± 2.0), the reduction was statistically significant at the IF i.e (3.6 ±2.5 versus 5.4 ± 2.2). This observation that late stage tumours have further lowered E-cadherin has also been borne out by other studies.[29, 30] That this phenomenon is more pronounced at the IF is also interesting and would support the importance of EMT in the progression of CRC. Nonetheless, it is noteworthy to consider emerging evidence of over-expression of E-cadherin, rather than reduction, being associated with progression of some cancers, including CRC.[31-34] Various theories have been put forth to explain this new conflicting finding. Chao *et al* working on breast cancers suggested that E-cadherin expression could be dynamically regulated with suppression during epithelial-mesenchymal transition and re-expression during mesenchymal-epithelial transition (MET) in the process of infiltration and metastases, hence levels of expression may vary and be the final resultant of a balance of the EMT or MET.[35] Other suggestions include the possibility of other E-cadherin roles which are non-silencing being invoked during tumour progression.[36]

Besides tumour stage, tumour grade and differentiation also appears to affect E-cadherin expression in our cases of CRC. High grade tumours were noted to show significantly reduced E-cadherin expression both at the TC (histoscore = 8.6 ± 3.2 ) and IF (histoscore = 2.6 ± 2.3) compared with low grade tumours (TC = 11.4 ± 1.5; IF = 5.1 ± 2.3). While this finding concurs with that of reported by some,[37-39] others have not been able to demonstrate this association.[40]

In summary, E-cadherin expression was significantly decreased at the IF of CRC cases in this study. Reduction of E-cadherin was more pronounced in metastatic than non-metastatic CRC, in particular at the IF. High grade tumours also had lower E-cadherin expression compared with low grade CRC. These findings are fairly similar to those from most other studies in this area. As the aforementioned studies encompassed both Asian and Western populations, it appears that E-cadherin immunohistochemical morphological expression pattern in Malaysian CRC patients does not differ significantly from either their Asian or Western counterparts. It is consequential therefore to seek further clarification regarding He *et al*'s,[14] observation of downregulated E-cadherin expression carrying a worse prognosis for Asian CRC patients, in a background from this study where the morphological E-cadherin immunohistochemical profile seems similar between Asian and Western patients.

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## REFERENCES

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin. 2012; 62(1): 10-29.
2. Andre N, Schmiegel W. Chemoradiotherapy for colorectal cancer. Gut. 2005; 54(8): 1194-202.
3. Lurje G, Zhang W, Lenz HJ. Molecular prognostic markers in locally advanced colon cancer. Clin Colorectal Cancer. 2007; 6(10): 683-90.
4. Thiery JP, Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol. 2006; 7(2): 131-42.
5. Ogou SI, Yoshida-Noro C, Takeichi M. Calcium-dependent cell-cell adhesion molecules common to hepatocytes and teratocarcinoma stem cells. J Cell Biol. 1983; 97(3): 944-8.
6. Yoshida-Noro C, Suzuki N, Takeichi M. Molecular nature of the calcium-dependent cell-cell adhesion system in mouse teratocarcinoma and embryonic cells studied with a monoclonal antibody. Dev Biol. 1984; 101(1): 19-27.
7. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest. 2009; 119(6): 1420-8.

8. Gall TM, Frampton AE. Gene of the month: E-cadherin (CDH1). J Clin Pathol. 2013; 66(11): 928-32.
9. Gallin WJ, Edelman GM, Cunningham BA. Characterization of L-CAM, a major cell adhesion molecule from embryonic liver cells. Proc Natl Acad Sci U S A. 1983; 80(4): 1038-42.
10. Schuh R, Vestweber D, Riede I, *et al*. Molecular cloning of the mouse cell adhesion molecule uvomorulin: cDNA contains a B1-related sequence. Proc Natl Acad Sci U S A. 1986; 83(5): 1364-8.
11. Repetto O, De Paoli P, De Re V, Canzonieri V, Cannizzaro R. Levels of soluble E-cadherin in breast, gastric, and colorectal cancers. BioMed Res Int. 2014; 2014: 408047.
12. Yun JA, Kim SH, Hong HK, *et al*. Loss of E-Cadherin expression is associated with a poor prognosis in stage III colorectal cancer. Oncology. 2014; 86(5-6): 318-28.
13. Bruun J, Kolberg M, Nesland JM, Svindland A, Nesbakken A, Lothe RA. Prognostic Significance of beta-Catenin, E-Cadherin, and SOX9 in Colorectal Cancer: Results from a Large Population-Representative Series. Front Oncol. 2014; 4: 118.
14. He X, Chen Z, Jia M, Zhao X. Downregulated E-cadherin expression indicates worse prognosis in Asian patients with colorectal cancer: evidence from meta-analysis. PLoS One. 2013; 8(7): e70858.
15. Freedman LS, Macaskill P, Smith AN. Multivariate analysis of prognostic factors for operable rectal cancer. Lancet. 1984; 2(8405): 733-6.
16. Edge S, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A, editors. AJCC Cancer Staging Manual. 7th ed. Chicago, IL: Springer; 2010.
17. Dukes CE, Bussey HJ. The spread of rectal cancer and its effect on prognosis. Br J Cancer. 1958; 12(3): 309-20.
18. Compton CC, Fielding LP, Burgart LJ, *et al*. Prognostic factors in colorectal cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med. 2000; 124(7): 979-94.
19. Prall F. Tumour budding in colorectal carcinoma. Histopathology. 2007; 50(1): 151-62.
20. Karamitopoulou E, Lugli A, Panayiotides I, *et al.* Systematic assessment of protein phenotypes characterizing high-grade tumour budding in mismatch repair-proficient colorectal cancer. Histopathology. 2010; 57(2): 233-43.
21. Liu X, Chu KM. E-cadherin and gastric cancer: cause, consequence, and applications. Biomed Res Int. 2014; 2014: 637308.
22. Kanczuga-Koda L, Wincewicz A, Fudala A, *et al*. E-cadherin and beta-catenin adhesion proteins correlate positively with connexins in colorectal cancer. Oncol Lett. 2014; 7(6): 1863-70.
23. Lee SJ, Choi SY, Kim WJ, *et al*. Combined aberrant expression of E-cadherin and S100A4, but not beta-catenin is associated with disease-free survival and overall survival in colorectal cancer patients. Diagn Pathol. 2013; 8: 99.
24. Garcia-Solano J, Conesa-Zamora P, Trujillo-Santos J, Torres-Moreno D, Makinen MJ, Perez-Guillermo M. Immunohistochemical expression profile of beta-catenin, E-cadherin, P-cadherin, laminin-5gamma2 chain, and SMAD4 in colorectal serrated adenocarcinoma. Hum Pathol. 2012; 43(7): 1094-102.
25. Karamitopoulou E, Zlobec I, Panayiotides I, *et al*. Systematic analysis of proteins from different signaling pathways in the tumor center and the invasive front of colorectal cancer. Hum Pathol. 2011; 42(12): 1888-96.
26. Cianchi F, Cuzzocrea S, Vinci MC, *et al*. Heterogeneous expression of cyclooxygenase-2 and inducible nitric oxide synthase within colorectal tumors: correlation with tumor angiogenesis. Dig Liver Dis. 2010; 42(1): 20-7.
27. Brabletz T, Jung A, Hermann K, Gunther K, Hohenberger W, Kirchner T. Nuclear overexpression of the oncoprotein beta-catenin in colorectal cancer is localized predominantly at the invasion front. Pathol Res Pract. 1998; 194(10): 701-4.
28. Baldus SE, Monig SP, Huxel S, *et al*. MUC1 and nuclear beta-catenin are coexpressed at the invasion front of colorectal carcinomas and are both correlated with tumor prognosis. Clin Cancer Res. 2004; 10(8): 2790-6.
29. Lampropoulos P, Zizi-Sermpetzoglou A, Rizos S, Kostakis A, Nikiteas N, Papavassiliou AG. Prognostic significance of transforming growth factor beta (TGF-beta) signaling axis molecules and E-cadherin in colorectal cancer. Tumour Biol. 2012; 33(4): 1005-14.
30. Ozguven BY, Karacetin D, Kabukcuoglu F, Taskin T, Yener S. Immunohistochemical study of E-cadherin and beta-catenin expression in colorectal carcinomas. Pol J Pathol. 2011; 62(1): 19-24.
31. Lewis-Tuffin LJ, Rodriguez F, Giannini C, *et al*. Misregulated E-cadherin expression associated with an aggressive brain tumor phenotype. PLoS One. 2010; 5(10): e13665.
32. Qureshi HS, Linden MD, Divine G, Raju UB. E-cadherin status in breast cancer correlates with histologic type but does not correlate with established prognostic parameters. Am J Clin Pathol. 2006; 125(3): 377-85.
33. Truant SC, Gouyer VP, Leteurtre EA, Zerimech F, Huet GM, Pruvot FR. E-cadherin and beta-catenin mRNA levels throughout colon cancer progression. J Surg Res. 2008; 150(2): 212-8.
34. Chung YC, Wei WC, Huang SH, *et al*. Rab11 regulates E-cadherin expression and induces cell transformation in colorectal carcinoma. BMC Cancer. 2014; 14: 587.
35. Chao YL, Shepard CR, Wells A. Breast carcinoma cells re-express E-cadherin during mesenchymal to epithelial reverting transition. Mol Cancer. 2010; 9: 179.
36. Rodriguez FJ, Lewis-Tuffin LJ, Anastasiadis PZ. E-cadherin's dark side: possible role in tumor progression. Biochim Biophys Acta. 2012; 1826(1): 23-31.
37. Bendardaf R, Elzagheid A, Lamlum H, Ristamaki R, Collan Y, Pyrhonen S. E-cadherin, CD44s and CD44v6 correlate with tumour differentiation in colorectal cancer. Oncol Rep. 2005; 13(5): 831-5.

38. Matsuoka T, Mitomi H, Fukui N, *et al*. Cluster analysis of claudin-1 and -4, E-cadherin, and beta-catenin expression in colorectal cancers. J Surg Oncol. 2011; 103(7): 674-86.
39. Karatzas G, Karayiannakis AJ, Syrigos KN, *et al*. E-cadherin expression correlates with tumor differentiation in colorectal cancer. Hepatogastroenterology. 1999; 46(25): 232-5.
40. Elzagheid A, Buhmeida A, Laato M, *et al*. Loss of E-cadherin expression predicts disease recurrence and shorter survival in colorectal carcinoma. APMIS. 2012; 120(7): 539-48.