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ORIGINAL ARTICLE

CD133 marks for colorectal adenocarcinoma

Man-Fong CHEW *MBBS*, Kean-Hooi TEOH *MBChB, MPath,* Phaik-Leng CHEAH *FRCPath, MD*

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

Abstract

CD133, a marker which has been advocated to mark colorectal carcinoma "stem or tumour initiating cells" is amongst the frequently studied markers in colorectal cancer. A study was conducted at the Department of Pathology, University of Malaya Medical Centre to determine the expression of CD133 in 56 archived, formalin-fixed, paraffin-embedded colorectal adenocarcinoma in comparison with adjacent benign colorectal epithelium by immunohistochemical staining for CD133 expression. CD133 immunopositivity was determined as staining at the glandular luminal surface or in the intraluminal debris. Expression was semiquantitated for (1) proportion of CD133 immunopositivity in the malignant or adjacent benign colorectal epithelium and (2) intensity of staining. The final score of CD133 immunopositivity was arbitrarily taken as proportion of CD133 immunopositivity multiplied by intensity of staining in both the malignant and adjacent benign colorectal epithelium. CD133 expression was observed in significantly increased frequency in 49 (87.5%) colorectal adenocarcinoma compared with 15 (26.8%) of the adjacent benign colorectal epithelium ($p<0.05$). In terms of immunopositivity score (proportion of CD133 immunopositivity multiplied by intensity of staining), colorectal adenocarcinoma had a mean arbitrary score of 8.5 which was significantly higher than the mean immunopositivity score of 0.5 of the adjacent benign colorectal epithelium ($p<0.05$). In addition, the maximum immunopositivity score for the adjacent benign colorectal epithelium was 4, while 38 (67.9%) of colorectal adenocarcinoma had scores >4. This study shows that CD133 is able to mark colorectal adenocarcinoma but it is still unclear at this juncture whether CD133 is indeed a marker for colorectal adenocarcinoma "stem cells".

Key words: Colorectal adenocarcinoma, CD133, immunohistochemistry

INTRODUCTION

Colorectal cancer is the third most common cancer on a global scale.[1] In Malaysia, it is the most common cancer in males, and second to breast cancer in females.[2] The most important established risk factor for colorectal cancer is age, being rare before the age of 40 years.[3] As Malaysia continues to progress towards a developed nation, life expectancy is expected to further improve with resultant increase in incidence of colorectal cancer and its importance. The existence of a subset of cells which are self-sustaining and refractory to current cytotoxic treatment has been put forth in tumour formation and progression. These cells variously known as "cancer stem cells" or "tumour initiating cells" are defined as "cells within a tumor that possess the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumour."[4] Although known as "cancer stem cells" they need not necessarily be prototype progenitor stem cells but can result from dedifferentiation of differentiated cancer cells.[5]

First characterized in 1997,[6,7] CD133 is a cell surface marker for hematopoietic and neural stem cells and considered a marker of cancer stem cell in brain tumours.[8-10] CD133 marks for AC133 antigen which is the human homologue of murine Prominin-1. Although there is no consensus about the 'best marker' which identifies cancer stem cells in any particular cancer,[11] CD133 is one of the most studied in colorectal carcinoma.[12–14] As such we were interested to ascertain the immunohistochemical pattern of CD133 expression in colorectal adenocarcinoma, the most common histological type of colorectal cancer, compared with adjacent benign colorectal

Address for correspondence and reprint requests: Dr Man-Fong Chew, Division of Anatomical Pathology, Department of Pathology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.

epithelium for further understanding of the role of CD133 in colorectal carcinogenesis.

MATERIALS AND METHODS

All cases of colorectal carcinoma diagnosed between 1st January 2005 and 31st December 2007 were retrieved from the archives of the Department of Pathology, University of Malaya Medical Centre, Kuala Lumpur. Patients' demographic information was obtained from the histopathology request forms. Only cases histologically-diagnosed as colorectal adenocarcinoma were considered. All other histological types of colorectal cancers were not included. Cases diagnosed on biopsy but not subsequently resected were also not included. The study was approved by the institutional Medical Ethics Committee. All histological slides of the cases were retrieved and reviewed and histologically re-confirmed cases were entered into the study. In addition, each case had to have at least one formalin-fixed, paraffin-embedded tissue block containing colorectal adenocarcinoma and adjacent benign colorectal epithelium.

Immunohistochemistry

The formalin-fixed, paraffin-embedded tissue block would be selected during the review for immunohistochemical staining. Four- μm sections were cut from the selected paraffin block for staining using a rabbit polyclonal antibody to CD133 (1:200; Abcam, ab19898) on a Ventana Benchmark XT automated system. A case of glioblastoma, earlier shown to be immunopositive for CD133 was used as a positive control and run with each batch. CD133 immunopositivity was considered when there was staining at the glandular luminal surface or in the intraluminal debris (Figure 1).[15]

Expression was semiquantitated for (1) proportion of CD133 immunopositivity in the malignant or adjacent benign colorectal epithelium as 0 (negative), 1 (< 5% of malignant or benign colorectal epithelial staining), 2 (6-25% staining), 3 (26-50% staining), 4 (51-75% staining) and 5 (> 75% staining) and (2) intensity of staining 0 (negative), 1 (weak), 2 (moderate) and 3 (strong). The final score of CD133 immunopositivity was arbitrarily taken as proportion of CD133 immunopositivity multiplied by intensity of staining in both the malignant and adjacent benign colorectal epithelium.

Statistical analysis was performed using the chi-square and t-tests (SPSS, version 19.0) with statistical significance set as $p < 0.05$.

RESULTS

A total of 56 histologically re-confirmed cases of colorectal adenocarcinoma were finally included in this study. The ages of the patients ranged from 22 years to 85 years (mean=62 years; median=64 years). Of the patients, 33 were male (59%), and 23 were female (41%) with a M:F ratio of 1.4:1. Ethnically, there were 25 Chinese, 18 Malay, 12 Indian and one of other ethnicity.

CD133 immunohistochemistry

CD133 immunopositivity was noted in 49 (87.5%) colorectal adenocarcinoma and 15 (26.8%) of the adjacent benign colorectal epithelium.

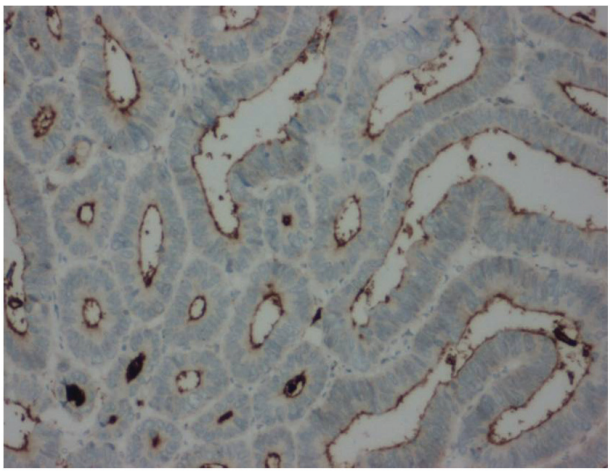

FIG 1: A case of colorectal adenocarcinoma showing CD133 immunopositivity on the glandular luminal surface and in the intraluminal debris

TABLE 1: CD133 immunopositivity scores (proportion of CD133 immunopositivity multiplied by intensity of staining) of colorectal adenocarcinoma compared with adjacent benign colorectal epithelium (n=56)

	CD133 immunopositivity score		
	Colorectal adenocarcinoma	Benign colorectal epithelium	
Range (mean)	0-15 (8.5)	0-4 (0.5)	$p<0.05$

Immunopositivity was significantly increased in colorectal adenocarcinoma compared with adjacent benign colorectal epithelium ($p<0.05$). In terms of immunopositivity score (proportion of CD133 immunopositivity multiplied by intensity of staining), colorectal adenocarcinoma had a mean arbitrary score of 8.5 (standard deviation=5.45). In comparison, adjacent benign colorectal epithelium showed a significantly decreased ($p<0.05$) mean immunopositivity score of 0.5 (standard deviation=0.95). Table 1 illustrates the immunopositivity scores of colorectal adenocarcinoma compared with the adjacent benign colorectal epithelium. Furthermore, the maximum immunopositivity score for the adjacent benign colorectal epithelium was 4, while 38 (67.9%) of colorectal adenocarcinoma had scores >4.

DISCUSSION

The age, sex and ethnic distribution of the cases of colorectal carcinoma in this study do not differ from that reported in other Malaysian patients.[2,16] From this study, CD133 shows a significantly increased immunohistochemical expression in colorectal adenocarcinoma compared with the adjacent benign colorectal epithelium, as reported in other studies.[13,14] CD133 was found to be expressed by about 90% of the colorectal adenocarcinoma in this study similar to Yang *et al*'s study.[17] As observed by Wang *et al*,[13] CD133 was only noted in the glandular luminal surface of the crypts, if at all, in the adjacent benign colorectal epithelium. Intensity of staining was generally weak with only 3/15 (20.0%) cases demonstrating moderate CD133 staining in the benign colorectal epithelium. None of the benign colorectal epithelium showed strong immunopositivity. In comparison, 29/49 (59.2%) colorectal adenocarcinoma demonstrated strong immunostaining.

Although CD133 has been advocated as a "cancer stem or initiating cell" marker, the findings of this study can only attest to colorectal adenocarcinoma showing an increased expression of CD133 compared with the adjacent benign colorectal epithelium. In this context, it appears that CD133 is a marker for colorectal adenocarcinoma in comparison to normal benign colorectal epithelium. It is unfortunate that the current design of the study precludes confirmation of CD133 as a colorectal adenocarcinoma stem cell marker, an issue which still remains controversial and not yet proven in various studies in this area. Until it can be shown that colorectal adenocarcinoma with CD133 expression indeed have the capacity for self-renewal of "clinically cured" tumours, it may be difficult to conclude that CD133 is a stem cell marker of colorectal adenocarcinoma.

Another issue to resolve would be the high percentage of colorectal adenocarcinomas with CD133 expression. If taken as immunopositivity at any level, then it would appear that about 90% of the colorectal adenocarcinoma carry stem cells. If taken that presence of cancer stem cells are evidenced when CD133 immunopositivity score is >4 (the maximum score shown by adjacent benign colorectal epithelium), it would appear that 67.9% of colorectal adenocarcinoma have colorectal adenocarcinoma stem cells. If this hypothesis is correct, the chance of "cure" in colorectal adenocarcinoma would be difficult to achieve unless the inherent "cancer stem cells" are eradicated.

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