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**Scientific Research Process**

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**Title:** Detection of metastatic cancer cells in mesentery of colorectal cancer patients

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## 1 What did this study explore?

This study demonstrates the existence of isolated cancer cells in the mesentery of colorectal (Metastasis V), and investigate its clinical significance for colorectal cancer patients.

## 2 How did the authors perform all experiments?

Sixty-three colorectal cancer patients underwent laparoscopy-assisted radical colectomy (CME) or proctectomy (TME) with R0 excision were included. Surgical samples were removed for hematoxylin-eosin staining and immunohistochemistry (cytokeratin 19) by histological slides. The preoperative serum CEA level was also recorded.

## 3 How did the authors process all experimental data?

### 3.1 Immunohistochemistry image analysis

Image Pro Plus Software 6.0 (Media Cybernetics, CA, USA) was used to semi-quantitatively measure the concentration of the cytokeratin 19 immunohistochemistry.

### 3.2 Statistical analysis

The Fisher's exact test,  $X^2$ test, and Mann-Whitney U test were used to inspect the significance of the differences between the variances. We considered a P-value of less

than 0.05 statistically significant. Standard statistical analyses were executed by SPSS version 20.0.

#### 4 How did the authors deal with the pre-study hypothesis?

Our previous study has proposed a novel type of tumor metastasis designated as Metastasis V in gastric cancer [8]. Metastasis V is defined as the appearance of cancer cells in the mesogastrium with perigastric adipose tissue, and is a risk factor for patient survival after radical gastrectomy. Here, in this study, we expand our hypothesis and further explore whether metastasis V can be detected in the mesocolorectum, and examined its clinic significance in colorectal patients.

#### 5 What are the novel findings of this study?

In this study, we firstly reported that Metastasis V was detected in 14 out of the total 63 (22.2%) colorectal cancer patients by immunohistochemistry (IHC) staining. Metastasis V was more likely to occur in poorly differentiated tumor (5/11; 45.5%) rather than moderately (8/46; 17.4%) and well differentiated tumors (1/6; 16.7%). The incidence of Metastasis V in N2 stage (9/14; 64.3%) was more frequent than in the N0 stage (3/35; 8.6%) or N1 stages (2/14; 14.3%). Preoperative serum CEA level in Metastasis V-positive patients was significantly higher than Metastasis V-negative patients (4.27 vs. 3.00 ng/ml).

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