No Expression of Human Leukocyte Antigen G (HLA-G) in Colorectal Cancer Cells

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Although there is a specific antitumor immune response in the body, colorectal cancer cells progressively develop. This fact indicated that the cancer cells could have a variety of mechanisms to evade or escape the immune system. HLA-G is identified to inhibit the recognition of NK-cell in various kinds of cancers. This study investigated the expression of HLA-G in colorectal cancer. Eighty five specimens of colorectal cancer, carcinoma in situ and adenomatous polyp were examined by immunohistochemistry and RT-PCR for the detection of human leukocyte antigen (HLA)-G. The expression of HLA-G was not found in all colorectal specimens (85/85) both protein level and transcription level, suggesting that the expression of HLA-G is not a possible immune evasion mechanism of colorectal cancer cell.

Keywords : *Immune evasion, Colorectal cancer, Adenomatous polyp, HLA-G*

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The expression of HLA-G in cancer cells has attracted increasing attention in recent year. These non-classical class I histocompatibility HLA-G molecules share some properties with the classical HLA class Ia antigens such as association with β_2-microglobulin (β_2 m), and conservation of the CD8 binding loop[1]. The HLA-G molecule interferes with the function of immunocompetent Natural killer-(NK) and T-cell by acting as a major inhibitory ligand. This molecule selectively was expressed on some tissues including trophoblastic cells and was believed to be involved in the tolerance of maternal immune response[2]. Recently, there has been evidence that several kinds of cancers including lung cancer[3], malignant melanoma[4] and lymphoma[5] express HLA-G as a role in their escape from host immune surveillance. However, until now, there are few studies in the area of the expression of HLA-G in colorectal cancer. This paper investigated the presence of HLA-G in colorectal cancer specimens using immunohistochemical staining to identify the levels of protein expression and RT-PCR to determine the mRNA transcription.

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Material and Method

Specimens

Surgically resected colorectal cancer specimens as well as colorectal adenomatous polyps were collected from 85 patients (60 patients with primary colorectal cancer and 25 patients with colorectal adenomatous polyps) treated at the department of surgery, Rajavithi Hospital (Thailand). The pathological stages of the tumor were classified according to the TNM classification.

All specimens were separated into two parts; the first part was submerged in RNAlater solution (Ambion) and kept at -2O C for RNA extraction. Another was fixed in 10% formalin and embedded in paraffin wax for immunohistochemical examination. Trophoblastic specimens were used as positive control.

Immunohistochemistry

The 4 mm sections of specimens were deparaffinized in xylene and rehydrated through a graded series of ethanol. Endogenous peroxidase activity was blocked with 0.5% hydrogen peroxide. To retrieve antigen, the slides were boiled in 10mM citrate buffer (pH 6.0), for 4 minutes. The specimens were then saturated in 3% normal horse serum for 20 minutes and incubated at room temperature for 30 minutes with the anti-HLA-G (Abcam, USA). After washing in PBS, a biotinylated goat antibody to mouse immunoglobulin (DAKO) was applied and incubated at room temperature for 30 minutes. The slides were then incubated with an avidin-biotin-peroxidase conjugate (ABC Elite) for 30 minutes. The immunohistochemical reactions were developed with freshly prepared 3,3'-diaminobenzidine tetrahydrochloride solution (Histofine SAB-PO kit). The slides were counterstained with hematoxylin, dehydrated through alcohol and cleared in xylene before mounting.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

1 g of total RNA was subjected to RT-PCR using the OneStep RT-PCR kit (QIAGEN), according to the manufacturer's protocol. The sequences of forward and reverse primer were 5'- GGAAGAGGAGA CACGGAACA-3' and 5'-TGAGACAGAGACGGAG AC AT-3', respectively. 30 PCR cycles were 1 minute at 94 C, 1 minute at 60 C and 1 minute at 72 C. The resulting RT-PCR products were analyzed on a 1% agarose gel.

Results

Immunohistochemical localization of HLA-G

All specimens including colorectal cancer, carcinoma in situ and adenomatous polyps were negative for HLA-G immunostaining while placenta specimens, the positive control, were demonstrated positive staining. Fig. 1A represents a typical photomicrograph of negative immunohistochemical staining for HLA-G in colon cancer specimen and Fig. 1B represents a typical positive staining for HLA-G in trophoblastic specimens.

Expression of HLA-G mRNA

To confirm the results obtained from the immunohistochemical study, the expression of HLA-G mRNA was evaluated by RT-PCR. Although, HLA-G cDNA products from RT-PCR were clearly identified in placenta specimens, none of the colorectal specimens demonstrated the expression of HLA-G (Fig.2).

Discussion

Published data indicated the expression of HLA-G in certain sites of immune privilege including placenta and thymus. These molecules protect cells lacking HLA class Ia expression from NK-cell mediated cytolysis through interaction with specific killer inhibitor receptors (KIRs) expressed on NK-cells[1,2,6]. In addition, a previous study demonstrated that HLA-G expression is tightly regulated and the processes of reversal of DNA methylation-mediated repression directly induce HLA-G expression[6].

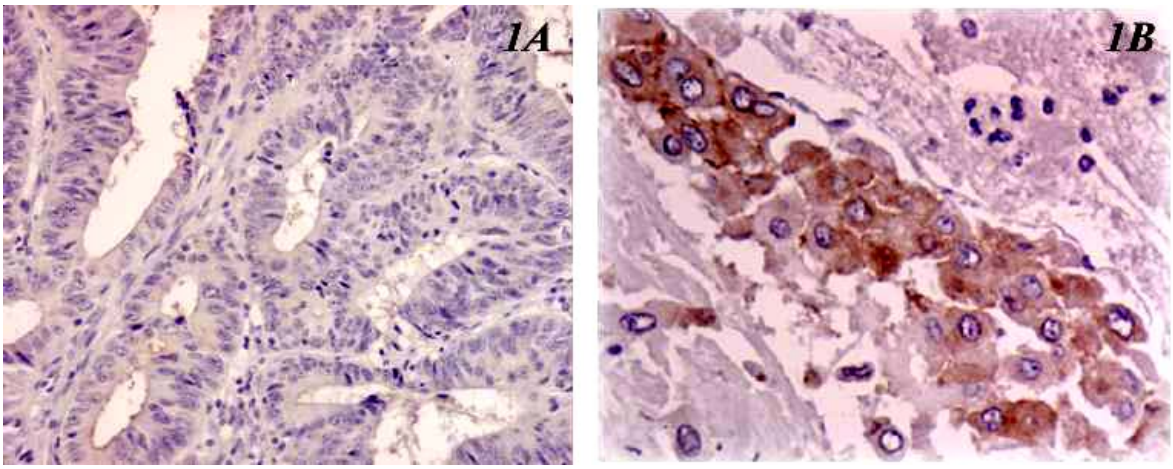

Fig. 1 Immunohistochemical staining for HLA-G
A: the negative staining in colorectal cancer specimens, B: the positive staining in trophoblastic cells

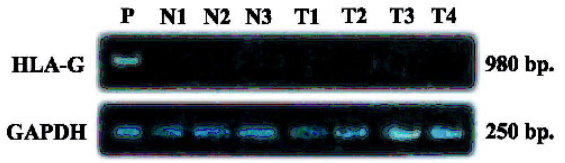

Fig. 2 A typical result of RT-PCR of HLA-G. There was no HLA-G mRNA expression in the colorectal specimens. P indicated the sample taken from placenta and N1, N2, N3 and N4 indicated the samples taken from normal epithelium. T1, T2, T3, and T4 indicated the samples taken from colorectal cancer stages I, II, III, and IV, respectively. GAPDH mRNA was amplified for internal control

A recent paper identified the expression of HLA-G in 9/34 cases of lung cancer, 11/18 cases of renal cell carcinoma, 5/40 cases of melanoma, and 23/45 cases of cutaneous lymphoma[7]. However, the present study did not find the expression of HLA-G (both protein expression and mRNA expression) in all cases of colorectal cancer including colorectal adenomatous polyp and carcinoma in situ lesion. This result suggested that HLA-G does not involve the immune escape mechanisms for colorectal cancer.

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เซลล์มะเร็งลำไส้ใหญ่ไม่ได้สร้าง เอชแอลเอ-จี (HLA-G) โมเลกุล ในการหลบหลีกภูมิคุ้มกัน

กวิญ ลีละวัฒน์, สุรางค์ อิงประเสริฐ, อุไร พงศ์ชัยฤกษ์, สุภาทิพย์ ตู้จินดา, ชีพสุมน สุทธิพินทะวงศ์, วิจิตรา เลิศกมลกาญจน์

เอชแอลเอ-จี (HLA-G) โมเลกุล มีบทบาทในการยับยั้ง เอนเค-เซลล์ (NK-Cell) เข้ามาทำลายเซลล์ที่สร้างโมเลกุลชนิดนี้ พบว่า เซลล์ของรกในมารดา สามารถสร้าง เอชแอลเอ-จี โมเลกุล เพื่อป้องกันไม่ให้ เอนเค-เซลล์เข้ามาทำลายทารกในครรภ์ ต่อมานักวิทยาศาสตร์ พบว่าเซลล์มะเร็งหลายชนิด เช่นมะเร็งปอด, มะเร็งเม็ดเลือดขาว สามารถสร้าง เอชแอลเอ-จี โมเลกุลได้ ซึ่งน่าจะมีส่วนช่วยให้เซลล์มะเร็งหลบเลี่ยงภูมิคุ้มกันได้

ได้ทำการศึกษาการแสดงออกของ เอชแอลเอ-จี (HLA-G) โมเลกุล ในเซลล์มะเร็งลำไส้ใหญ่ที่ได้จากการผ่าตัดผู้ป่วย ที่มารับการรักษา ที่ภาควิชาศัลยกรรม โรงพยาบาลราชวิถี จำนวน 85 ราย โดยใช้วิธีย้อมพิเศษเพื่อหาโปรตีน (Immuohistochemical staining) และการตรวจหาอาร์เอนเอ (RNA) โดยใช้วิธี อาร์ที-พีซีอาร์ (RT-PCR) ผลการศึกษาไม่พบโมเลกุล HLA-G และ HLA-G mRNA ในเซลล์มะเร็งลำไส้ใหญ่ ตลอดระยะการดำเนินโรค ตั้งแต่ระยะที่ 1 ถึงระยะที่ 4 เลย ดังนั้น เซลล์มะเร็งลำไส้ใหญ่น่าจะใช้กลไกอื่นในการหลบหลีกภูมิคุ้มกันในร่างกาย ซึ่งจะต้องมีการศึกษาต่อไปในอนาคต