*Original Article*

# HLA Class I Expressions on Peripheral Blood Mononuclear Cells in Colorectal Cancer Patients

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**ABSTRACT**

**Objective:** To investigate the expression change of human leukocyte antigen (HLA) class I on human peripheral blood mononuclear cells (PBMCs) at both mRNA and protein levels, and to evaluate its roles in the development of colorectal cancer (CRC).

**Methods:** In the present study, 50 patients with CRC, 35 patients with benign colorectal lesion and 42 healthy volunteers were enrolled. Expression levels of HLA class I mRNA and protein were determined using real-time quantitative reverse transcription PCR (RT-PCR) and flow cytometry analysis, respectively.

**Results:** The expression levels of HLA class I mRNA and proteins were not influenced by age and gender. The relative ratios of HLA class I mRNA were 0.99±0.27 in healthy controls, 0.76±0.19 in benign patients, and 0.48±0.21 in CRC patients. Mean fluorescence intensities of HLA class I were 145.58±38.14 in healthy controls, 102.05±35.98 in benign patients and 87.44±34.01 in CRC patients. HLA class I on PBMCs was significantly down-regulated at both mRNA and protein levels in patients with stage III and IV CRC. CRC patients with lymph node metastasis also showed a decreased HLA class I expression at protein level.

**Conclusion:** HLA class I expressions on PBMCs are associated with staging of CRC and lymph node metastasis. Monitoring the expression of HLA class I on PBMCs may provide useful information for diagnosis and metastasis judgement of CRC.

**Key words:** HLA class I; Peripheral blood mononuclear cells; RT-PCR; Flow cytometry; Colorectal cancer

## INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignant tumors. In the USA, CRC is the third most frequently diagnosed cancer in men and the second in women. Deaths from CRC rank third after lung and prostate cancers for men and third after lung and breast cancers for women. The incidence of CRC in China is lower than that in west countries, but has increased in recent years and become a substantial cancer burden. Therefore, it is important to prevent, detect, and treat CRC early enough.

There is a significant difference in survival rates between patients with early-stage CRC and those with advanced CRC[1, 2]. Thus, early diagnosis of CRC is imperative for obtaining a better therapeutic outcome, but still remains a challenge though promising advances in imaging technology and other diagnostic methods have been achieved in recent years.

Human leukocyte antigens (HLA) are cell surface glycoproteins that play critical roles in the regulation of immune responses. These molecules are expressed on the surface of all nucleated cells, necessary for the presentation of peptide antigens to cytotoxic T lymphocytes (CTLs)[3] and for the immune regulatory activity exerted by NK cells[4]. It is widely accepted that total or partial loss of HLA class I molecules on tumor cells was one of the main mechanisms of tumor escape. Studies have demonstrated that HLA class I molecules had the ability to control the metastatic activities of tumor cells[5-7], and had a close

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relationship with patients' prognosis[8, 9], and their polymorphisms influenced tumor susceptibility[10, 11].

Past researches paid more attention to HLA class I on the surface of tumor cells. There are plentiful HLA class I molecules on the surface of peripheral blood mononuclear cells (PBMCs), however, what happens to these molecules during the development of CRC remains unclear. In the present study, we enrolled CRC patients, benign colorectal diseases patients and healthy individuals from Qilu Hospital, collected their peripheral blood and measured HLA class I levels at both mRNA and protein levels, in an attempt to investigate the expression changes of HLA class I during the development of CRC.

## MATERIALS AND METHODS

### Patients

Fifty CRC patients (21 female and 29 male, age range 56–77 years, median 62 years) were enrolled in this study (Table 1). Enrollment took place between Feb 2008 and Oct 2010 at the Department of General Surgery, Qilu Hospital, Jinan, China. Clinical stages and pathological features of primary tumors were defined according to the criteria of the American Joint Commission on Cancer (AJCC). None of these patients had been treated with radiotherapy or chemotherapy prior to enrollment. Benign group of colorectal diseases consisted of 35 patients (12 female and 23 male, age range 52–78 years, median 60 years), including 12 proctitis, 7 ulcerative colitis and 16 colonic polyps. Control volunteers were from the Department of Health Examination Center, and consisted of 42 healthy adults (20 female and 22 male, age range 56–79 years, median 63 years). All subjects complicated with hepatitis B virus (HBV) infection, hepatic cirrhosis, hepatic cancer[12], common cold or influenza were excluded for their potential interference to the expression of HLA class I. Informed consent was obtained from each participating patient. Ethical approval for this study was obtained from the Medical Ethical committee of Qilu Hospital, Shandong University.

### RNA Extraction and Reverse Transcription (RT)

Blood (2 ml) was drawn into sterile heparinized tubes from each patient and control. The blood was centrifuged and heparinized plasma was stored at –80°C until determination of CEA and CA 19-9. Mononuclear cells were isolated from heparinized blood by gradient centrifugation on Ficoll-Hipaque (Haoyang, Tianjin, China). Total RNA was extracted from PBMCs using Trizol reagent (Invitrogen, Carlsbad, CA, USA). cDNA was obtained using a PrimeScript™ reverse transcriptase reagent kit (Takara, Dalian, China) according to the manufacturer's instructions.

**Table 1.** Demographic and clinicopathological characteristics of CRC patients

| Indices | *n* |
|---|---|
| Age (year) | |
| <60 | 15 |
| ≥60 | 35 |
| Gender | |
| Male | 29 |
| Female | 21 |
| Tumor size* | |
| <2 cm | 22 |
| ≥2 cm | 28 |
| Lymph node metastasis | |
| Absent | 17 |
| Present | 33 |
| TNM stage | |
| I | 6 |
| II | 13 |
| III | 24 |
| IV | 7 |

*Tumor size was measured for invasive area by histological examination.

### HLA Class I mRNA Expression Analysis

The expression of HLA class I mRNA was measured by relative quantitative real-time polymerase chain reaction (PCR) using a SYBR Premix Ex Taq™ II kit (Takara) and the ABI 7500 Real-time PCR system (Applied Biosystems, Foster City, CA, USA). Fold expression changes were determined by the $2^{-\Delta\Delta CT}$ method[13]. The primer sequences for HLA class I mRNA were: forward primer 5′-CCTACG-ACGGCAAGGATTAC-3′, reverse primer 5′-TGCC-AGGTCAGTGTGATCTC-3′. The primer sequences for endogenous control (beta-actin) were: forward primer 5′-TTGCCGACAGGATGCAGAA-3′, reverse primer 5′-GCCGATCCACACGGAGTACT-3′. The PCR cycling conditions were: initial reaction at 95°C for 30 s, followed by 40 cycles of 95°C for 5 s, and finally 60°C for 34 s. All reactions were performed in triplicate.

### Flow Cytometry

Heparinized peripheral blood samples (2 ml of each) were taken from all subjects. An amount of 100 μl blood samples was added to polystyrene test tubes (Becton Dickinson, NJ, USA), and then monoclonal antibodies (mAbs) against HLA class I (HLA-I-PE-Cy5, Becton Dickinson) were added. All stainings were conducted under saturating concentrations of mAbs. After an incubation time of 30 min in darkness, red