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January 16, 2020

Dear Editorial Board of *Frontiers in Genetics – Cancer Genetics*

We, along with our co-authors, are submitting the following manuscript entitled, “*MICA \*012:01* Allele Facilitates the Metastasis of Colorectal Cancer Carrying KRAS Mutation” for publication in *Frontiers in Genetics – Cancer Genetics* as a research paper.

The human major histocompatibility complex class I chain-related protein A (MICA) regulates immune surveillance of cancers by binding to its receptor, NKG2D (natural killer group 2D). As a highly polymorphic gene, the genetic association and allele functions of MICA across all exons with colorectal cancer (CRC) have not been explored. In this study, we characterized the alleles present in exons 2-5 of *MICA* in tissue samples from 104 patients with CRC and 536 healthy controls via PCR sequencing, explored the function of MICA alleles in CRC cell lines, and performed a preliminary analysis of the disease-free survival time in patients with CRC. In our study, association analyses revealed that *MICA \*009:01* and \**049* alleles were significantly decreased in patients with CRC (p=0.0049, OR=0.35). In subset analysis of patients, *MICA \*012:01* and *\*045* alleles were associated with CRC that carry KRAS codon 12 mutation (p=0.027, OR=3.33) as well as the protruded type of CRC (p=0.0028, OR=0.07), respectively. In addition, *MICA \*027* was associated with later stage (stage IIIb/IV) CRC as determined by UICC (Union for International Cancer Control; p=0.044, OR=0.14) staging.

Functional experiments with transfected CRC cell lines demonstrated that overexpression of *MICA \*012:01* significantly enhanced proliferation, invasion, and metastasic phenotypes of CRC. Preliminary disease-free survival curve analysis suggest that *MICA \*012:01* allele may be predictive for poor prognosis of patients with KRAS codon 12 mutated CRC. Moreover, no somatic mutation of *MICA* was detected in CRC tumor tissues compared to paracancerous tissues. These results indicate that multiple *MICA* alleles are associated with CRC development and progression in Chinese patients, and suggest that *MICA \*012:01* allele confers susceptibility to patients with CRC carrying KRAS mutation. *MICA* expression may impact disease progression in these patients by alteration of immune surveillance.

We hereby certify that this manuscript consists of original, unpublished work. Thank you for your consideration, and we look forward to hearing from you.

Sincerely,

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