

## IQGAP2 inactivation through aberrant promoter methylation and promotion of invasion in gastric cancer cells

Shun-Hua Jin<sup>1</sup>, Yoshimitsu Akiyama<sup>1</sup>, Hiroshi Fukamachi<sup>1</sup>, Kazuyoshi Yanagihara<sup>2</sup>, Takumi Akashi<sup>3</sup> and Yasuhito Yuasa<sup>1\*</sup>

<sup>1</sup>Department of Molecular Oncology, Graduate School of Medicine and Dentistry, Tokyo Medical and Dental University, Tokyo, Japan

<sup>2</sup>Central Animal Laboratory, National Cancer Center Research Institute, Tokyo, Japan

<sup>3</sup>Department of Pathology, Tokyo Medical and Dental University, Tokyo, Japan

Invasion and metastases of cancer cells are the main causes of treatment failure in cancer. IQ motif-containing GTPase activating protein 1 (IQGAP1), plays pivotal roles in intercellular adhesion, migration, invasion and metastases in various cancer cells. However, the role of another family member, IQGAP2, in carcinogenesis remains unknown. Here, we investigated IQGAP2 functions in gastric cancers. We found that IQGAP2 protein expression was lost in 5 of the 9 gastric cancer cell lines. Through analysis by the methylation-specific PCR, aberrant IQGAP2 methylation was detected in 3 gastric cancer cell lines. IQGAP2 mRNA was found to be activated after 5-aza-2'-deoxycytidine treatment of the methylation-positive cells. Moreover, IQGAP2 methylation was detected in 28 of the 59 (47%) primary gastric cancer tissues, but not in 12 normal gastric mucosa samples. Immunohistochemical staining revealed that 7 of the 8 (88%) gastric cancer tissues without methylation signals displayed IQGAP2 expression, whereas among 10 with methylation signals none expressed IQGAP2 ( $p = 0.0002$ ), indicating that IQGAP2 methylation is highly associated with loss of the IQGAP2 expression in the primary gastric cancer tissues as well as gastric cancer cell lines. Furthermore, IQGAP2 methylation was also associated with tumor invasion and a poor prognosis. IQGAP2 knockdown with small interfering RNA increased the invasive capacity of a gastric cancer cell line. These results suggest that silencing of IQGAP2 by promoter methylation may contribute to gastric cancer development.

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**Key words:** IQGAP2; methylation; gastric cancer; invasion; prognosis

According to WHO, gastric cancer is the fourth most common malignancy worldwide, ~870,000 new cases occurring yearly.<sup>1</sup> Uncontrollable tumor invasion and dissemination of cancer cells around the primary organ comprise the neoplastic process responsible for most deaths from cancer because of inadequacy of surgical removal.<sup>2</sup> Invasive and metastatic cancer cells undergo numerous genetic and epigenetic changes, manifested as adhesion molecules, motility factors, growth factors, and expression of proteolytic enzymes that degrade the basement membrane and migrate into the surrounding extracellular matrices of various tissues, including the stomach.<sup>3–6</sup>

Inactivation of gene expression through abnormal methylation of CpG islands can act as a "hit" for tumor generation.<sup>7,8</sup> It has been recognized that aberrant hypermethylation events can occur early in tumorigenesis, predisposing cells to malignant transformation. Given the importance of gene expression and invasion, the determination of their relationship seems to be essential for a better understanding of tumor biology and for the development of new treatment strategies. However, the relationship between the two is poorly characterized.

The Rho-family small GTPases, especially Rac1 and Cdc42, regulate actin cytoskeletal dynamics by interacting with a number of effectors, including the IQGAPs.<sup>9,10</sup> IQGAPs are so named because they possess IQ domains, which are tandem repeats of 4 IQ motifs (tandem isoleucine and glutamine residues), and Ras GTPase-activating protein (GAP)-related domains.<sup>10</sup> The IQGAP gene family consists of 3 members, IQGAP1, IQGAP2, and IQGAP3.<sup>11</sup> Mammalian IQGAP1 is considered to be a scaffolding protein at the crossroads of several signaling pathways involved in

the control of cell adhesion,<sup>12,13</sup> polarization<sup>14,15</sup> and directional migration.<sup>15,16</sup> IQGAP1 promotes invasion in a breast cancer cell line.<sup>17</sup> Among gastric cancers, diffuse type gastric cancers exhibit a higher frequency of invasion with dissemination to the peritoneum and lymph node metastasis compared to intestinal type gastric ones.<sup>18,19</sup> IQGAP1 was upregulated by gene amplification in 2 diffuse type gastric cancer cell lines.<sup>20</sup> Furthermore, there seems to be a correlation between dysfunction of E-cadherin-mediated adhesion and increased membrane localization of IQGAP1 in gastric cancer.<sup>21</sup> Similarly, IQGAP1 expression increases in human colorectal cancers, particularly at the invasion front.<sup>22</sup> This observation raised the possibility that IQGAP1 promotes invasiveness of gastrointestinal tract cancer cells.

A comparison of the structure and functions of IQGAP2 with those of IQGAP1 revealed some similarities and several differences. The human IQGAP2 protein exhibits 59% identity to IQGAP1, and their domain structures are well conserved.<sup>11,23</sup> Although IQGAP2 expression is widely distributed in various tissues and cellular processes,<sup>24–26</sup> the roles of IQGAP2 in gastric carcinogenesis remain unclear. In this study, we found that IQGAP2 was frequently inactivated in gastric cancers and that the major mechanism was aberrant methylation of IQGAP2. Furthermore, we showed that inactivation of IQGAP2 was correlated with invasion of gastric cancer cells and a poor prognosis.

### Material and methods

#### Cell lines and tissue samples

The human gastric cancer cell lines, except for HSC-44, HSC-39 and HSC-59,<sup>27</sup> i.e., MKN28, NUGC-4, MKN7, MKN45, TGBC11TKB and KATOIII, were obtained from the Human Science Research Resources Bank (Osaka, Japan) or Riken Cell Bank (Tsukuba, Japan). Surgically resected specimens from 59 primary gastric cancer patients were randomly obtained from the Affiliated Hospital of School of Medicine, Tokyo Medical and Dental University. Informed consent was obtained from all patients, and the study was approved by the appropriate institutional review committee. Histological classification was performed according to the general rules established by the Japanese Gastric Cancer Association.<sup>28</sup>

#### Cell culture

The gastric cancer cell lines were grown in Dulbecco's modified Eagle's medium or RPMI1640 supplemented with 10% fetal

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\*Correspondence to: Department of Molecular Oncology, Graduate School of Medicine and Dentistry, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan.

Fax: +81-3-5803-0125. E-mail: yuasa.monc@tmd.ac.jp

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