

## The Histone Demethylase RBP2 Is Overexpressed in Gastric Cancer and Its Inhibition Triggers Senescence of Cancer Cells

JIPING ZENG,<sup>\*,†,§</sup> ZHENG GE,<sup>\*</sup> LIXIANG WANG,<sup>||</sup> QIAO LI,<sup>\*,‡</sup> NA WANG,<sup>\*,‡</sup> MAGNUS BJÖRKHOLM,<sup>\*</sup> JIHUI JIA,<sup>‡</sup> and DAWEI XU<sup>\*</sup>

<sup>\*</sup>Department of Medicine, Division of Hematology, Karolinska University Hospital Solna and Karolinska Institutet, Stockholm, Sweden; and <sup>†</sup>Department of Microbiology/Key Laboratory for Experimental Teratology of Chinese Ministry of Education and <sup>§</sup>Department of Biochemistry and <sup>||</sup>Department of Pharmacology, School of Medicine, Shandong University, Jinan, PR China

**BACKGROUND & AIMS:** The aberrant expression of histone-modifying enzymes such as histone deacetylases contributes to oncogenesis. It is unclear whether RBP2, a newly identified histone demethylase, is involved in cancer development/progression. We determined RBP2 expression in gastric cancer and its biologic function in cancer cells. **METHODS:** Cancerous and matched normal gastric specimens from 42 patients with gastric cancer were analyzed for RBP2 expression using quantitative real-time polymerase chain reaction and immunohistochemistry. Gene expression was assessed using quantitative real-time polymerase chain reaction and immunoblotting and depleted with small interference RNA. Clonogenesis and cellular senescence were examined by foci formation and  $\beta$ -Galactosidase staining. Promoter activity was determined by luciferase reporter assay. Chromatin immunoprecipitation was used to detect RBP2 and methylated histone H3-K4 on promoters. **RESULTS:** RBP2 messenger RNA and protein expression were increased in 71.5% (30/42) and 100% (20/20) of gastric cancer specimens, respectively. Significantly diminished foci numbers coupled with massive senescence/growth arrest and elevated expression of cyclin-dependent kinase inhibitors (CDKIs) p21<sup>CIP1</sup>, p27<sup>kip1</sup>, and/or p16<sup>ink4a</sup> occurred in RBP2-depleted gastric and cervical cancer cells. RBP2 depletion-mediated senescence and clonogenic defect were attenuated by inhibiting p21<sup>CIP1</sup> or p27<sup>kip1</sup> expression. The promoter activity of all 3 CDKIs genes was enhanced by RBP2 inhibition. RBP2 occupied these promoters in control cells, and the loss of RBP2 occupancy was accompanied by enhanced H3-K4 trimethylation following RBP2 depletion. **CONCLUSIONS:** RBP2 is overexpressed in gastric cancer, and its inhibition triggers senescence of malignant cells at least partially by derepressing its target genes CDKIs. Histone demethylase inhibition by targeting RBP2 may be an anticancer strategy.

**Keywords:** Cellular Senescence; Histone Demethylation.

Gastric cancer is one of the most common malignancies and second leading cause of cancer-related death worldwide.<sup>1,2</sup> The compelling evidence supports a

role for *Helicobacter pylori* infection in gastric neoplastic transformation. *H pylori* infection triggers an early stage of gastric cancer pathogenesis by causing chronic gastritis; however, a progression into the invasive cancer is clearly a multistep process with numerous alterations involved in cell proliferation and/or survival, whereas the underlying molecular mechanism is largely unclear.<sup>1-6</sup> Additionally, because of poor responses to current therapeutic approaches, the outcomes of gastric cancer remain unfavorable with a low rate of 5-year survival.<sup>2</sup> Therefore, better defining of the pathogenesis of gastric cancer and exploring novel therapeutic targets for treatment are urgently demanding tasks.

The histone-modifying enzymes—responsible for acetylation, phosphorylation, and methylation of histone proteins—play a key role in the regulation of gene transcription by mediating chromatin reconfiguration.<sup>7,8</sup> Among these enzymes, RBP2 is a newly identified member of JARID family proteins with a histone demethylase (HDM) activity, and it specifically targets tri- and dimethylated lysine 4 of histone H3 (H3-K4) for demethylation.<sup>9-11</sup> Recent genome-wide analyses have revealed that RBP2 controls the expression of multiple genes, thereby regulating cell fate determination.<sup>12-15</sup>

The disruption of a balance of histone modifications and aberrant chromatin remodeling has long been implicated in cancer development and progression. One of the best examples is the role of dysregulated histone acetyltransferases and deacetylases (HDACs) in tumorigenesis.<sup>16</sup> Histone acetyltransferases and HDACs catalyze the acetylation and deacetylation of histones H3 and H4, respectively. HDACs have been shown to be overexpressed, aberrantly recruited by oncoproteins, or mutated in malignant cells.<sup>16</sup> Enhanced HDAC activity leads to the transcriptional repression of tumor suppressor genes,

**Abbreviations used in this paper:**  $\beta$ 2-M,  $\beta$ 2-microglobulin; CDKIs, cyclin-dependent kinase inhibitors; GC, gastric cancer; HDACs, histone deacetylases; HDM, histone demethylase; QRT-PCR, quantitative real-time PCR; SA- $\beta$ -Gal, senescence-associated  $\beta$ -Galactosidase; siRNA, small interference RNA.

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#### Reprint requests

Address requests for reprints to: Dawei Xu, MD, Haematology Laboratory, Centre for Molecular Medicine (CMM), L8:00, Karolinska University Hospital Solna, SE-171 76 Stockholm, Sweden. e-mail: [Dawei.Xu@ki.se](mailto:Dawei.Xu@ki.se); fax: (46) 8517 73054 or Jihui Jia, MD, Department of Microbiology, School of Medicine, Shandong University, Jinan, 250012, PR China. e-mail: [jiajihui@sdu.edu.cn](mailto:jiajihui@sdu.edu.cn); fax: (86) 531 8838 2502.

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#### Conflicts of interest

The authors disclose no conflicts.

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