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LETTER TO EDITOR

Expressional and Mutational Analysis of *CREBBP* Gene in Gastric and Colorectal Cancers with Microsatellite Instability

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To the Editor:

The *CREBBP* gene encodes a protein CREBBP that binds with cAMP-response element (CRE)-binding protein [1]. The CREBBP has a histone acetyltransferase activity and acts as a scaffold to stabilize additional protein interactions with the transcription complex. This protein shares regions of very high sequence similarity with a protein p300 in its bromodomain, cysteine-histidine-rich regions, and histone acetyltransferase domain [2]. Germline heterozygous mutations in *CREBBP* gene are main cause of Rubinstein-Taybi syndrome that is characterized by mental retardation, postnatal growth deficiency and dysmorphic facial features [3]. To discover cancer-causing genes in transitional cell carcinoma (TCC) of urinary bladder, Gui et al. performed whole-exome sequencing of the genomic DNA from TCC tissues [4]. Among the somatic mutations discovered, the most frequently mutated genes were those implicated in chromatin remodeling, including *ARID1A, EP300, UTX* and *CREBBP*. Somatic mutations of *CREBBP* in the TCC (15/97=15.5 %) consisted of mainly nonsense and missense mutations [4]. The high ratio of inactivating-to-missense mutations (11:4) in *CREBBP* established it as a tumor suppressor gene [4]. Translocations or deletions or somatic mutations of *CREBBP* gene have also been reported in leukemias and non-Hodgkin lymphomas [5–7]. However, it remains known whether *CREBBP* is altered in other cancers.

By analyzing genome sequences in a public database (http://genome.cse.ucsc.edu/), we found a mononucleotide repeat in the coding sequences of human *CREBBP* gene (A7 repeat in exon 16). About 10–30 % of in gastric (GC) and colorectal (CRC) cancers with defective DNA mismatch repair (MMR) show microsatellite instability (MSI) [8]. Frameshift mutation of genes at mononucleotide repeats is a feature of cancers with MSI. One aim of this study was to find frameshift mutations in the repeats of *CREBBP*, which had not been reported in GC and CRC with MSI. Also, we analyzed expression of CREBBP in GC and CRC.

For this, we analyzed somatic mutation in this repeat in *CREBBP* gene in 77 GC and 86 CRC by polymerase chain reaction (PCR) and single-strand conformation polymorphism (SSCP) assay as described previously [9]. The GC consisted of 32 GC with high MSI (MSI-H), 45 GC with stable MSI (MSS), 41 CRC with MSI-H and 45 CRC with MSS. Genomic DNA each from tumor cells and corresponding normal cells were amplified with seven primer pairs for by PCR. Radioisotope ([^{32}P]dCTP) was incorporated into the PCR products for detection by SSCP autoradiogram.

Next, to see whether CREBBP protein is altered in the cancers, we performed immunohistochemistry using tissue microarray (TMA) blocks that contained paraffin-embedded GC (N=100) and CRC (N=100) tissues. Each case has cores representing cancers as well as those representing corresponding normal epithelial tissues. The TMA included CRC with MSI-H (N=20) and GC with MSI-H (N=20). In the immunohistochemistry, we used ImmPRESS System (Vector Laboratories, Burlinggame, CA, USA) with rabbit polyclonal antibody against human CREBBP (Thermo Scientific, Rockford, IL, USA; dilution 1/50). Other procedures for mutation and immunohistochemistry were described in our previous report [10].

SSCP analysis detected aberrantly migrating bands of *CREBBP* in a gastric cancer with MSI-H. The mutation was a frameshift mutation (c.3250delA (p.Ile1084serfsX15)) (Fig. 1) that would lead to a premature stop of amino acid syntheses in CREBBP protein.

In the immunohistochemistry, positive CREBBP immunostaining was observed in 92 % and 93 % of the GC and CRC, respectively. The CREBBP immunostaining, when

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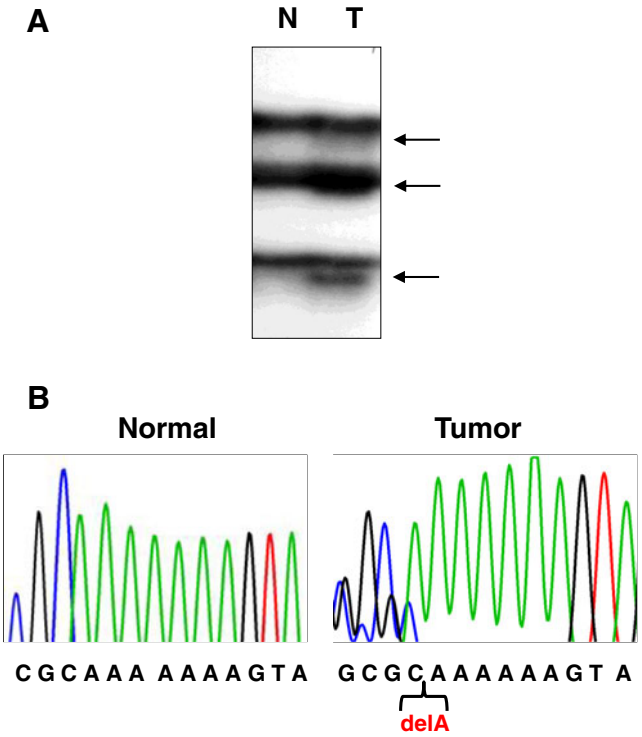

Fig. 1 SSCP and DNA sequencing of *CREBBP*. SSCP (**a**) and DNA sequencing analysis (**b**) of *CREBBP* from a gastric cancer tissue (tumor (T) and normal tissues (N)). **a** In the SSCP, the *arrows* (Lane T) indicate aberrant bands compared to the SSCP from normal tissue (N). **b** Direct DNA sequencing analysis of *CREBBP* show heterozygous deletion of a nucleotide in tumor tissue as compared to normal tissue

present, was found in the nuclei of the cells. Statistically, there was no difference of the immunopositivity between the cancers with MSI-H (37/40) and MSS (148/160) (Fisher's exact test, $p>0.05$). There was no statistical difference of the immunopositivity between GC and CRC (Fisher's exact test, $p>0.05$).

Because CREBBP is involved in the regulation of genome architecture (chromatin remodeling) that is known to underlie general cancer pathogenesis, we hypothesized that *CREBBP* gene might be altered not only in TCC but also in other cancers, including GC and CRC. However, we observed a very low incidence of *CREBBP* mutation in GC and CRC with MSI-H (1.4 %). Also, expressional loss of CREBBP was observed only in 8-9 % of GC and CRC. Although the frameshift mutation and loss of expression of *CREBBP* are novel findings, low incidences of them identified in this study suggest that alteration of *CREBBP* gene by somatic mutation in the nucleotide repeat and expressional loss is not common in GC and CRC, and may not contribute to their cancer development.

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