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LETTER TO THE EDITOR

Mutation and Expression of a Candidate Tumor Suppressor Gene EPB41L3 in Gastric and Colorectal Cancers

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Abstract

Erythrocyte Membrane Protein Band 4.1 Like 3 (*EPB41L3*) is candidate tumor suppressor gene (TSG) in various cancers. EPB41L3 downregulation has been identified in many solid cancers including gastric (GC) and colorectal cancers (CRCs), but somatic inactivating mutation along with protein expression in cancers are largely unexplored. The aim of our study was to find whether *EPB41L3* gene was mutated and expressionally altered in GC and CRC. *EPB41L3* gene has a mononucleotide repeat in the coding sequence that could be mutated in cancers with high microsatellite instability (MSI-H). We analyzed 79 GCs and 124 CRCs, and found that only one CRC with MSI-H (1.3%) harbored the frameshift mutation within the repeat. In immunohistochemistry, loss of EPB41L3 expression was identified in 49% of GCs and 42% of CRCs. Our data may indicate *EPB41L3* that loss of expression but not frameshift mutation may play a role in GC and CRC development by inhibiting TSG functions of *EPB41L3*.

Keywords EPB41L3 · Mutation · Expression · Colon cancer · Gastric cancer

To the editor:

Erythrocyte Membrane Protein Band 4.1 Like 3 (EPB41L3), also known as Protein 4.1B/DAL-1, is a membrane skeletal protein that is involved in many cytoskeleton-associated processes, such as cell motility, adhesion, growth and differentiation [1]. EPB41L3 expression is downregulated in many cancers including gastric (GC) and colorectal (CRC) cancers [1–3]. Both promoter methylation and loss of heterozygosity at its chromosomal locus are known mechanisms responsible for the downregulation [1–3]. Functionally, EPB41L3 inhibits cell growth by inducing apoptosis and cell cycle arrest [1, 4]. These data suggest that *EPB41L3* gene is a candidate tumor suppressor gene (TSG). Although expressional alteration by DNA methylation is important in tumorigenesis [1], other mechanisms underlying *EPB41L3* downregulation in tumorigenesis remain unknown. In this study, we attempted to find whether *EPB41L3* gene might be altered by frameshift mutation along with its expressional alteration in GC and CRC.

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In a public database, there is a mononucleotide repeat in the coding sequences of *EPB41L3* (A7 in exon 4) that might be a mutation target in the cancers with microsatellite instability (MSI) [5]. We analyzed the repeats in 34 GCs with high MSI (MSI-H), 45 GCs with stable MSI (MSS), 79 CRCs with MSI-H and 45 CRCs with MSS by polymerase chain reaction (PCR) and single-strand conformation polymorphism (SSCP) assay as described previously [6]. We found only one frameshift mutation in the CRCs (1/79: 1.3%) with MSI-H. The mutation was a deletion of one base in the A7 repeat (c.519delA) that would cause a premature stop codon, which lead to the termination of translation (p.Lys173AsnfsX14). Frameshift mutation within the A7 was not detected in those with MSS.

To see whether EPB41L3 protein expression was altered, we also analyzed the protein expression in 34 GCs and 79 CRCs with MSI-H, and 45 GCs and 45 CRCs with MSS by immunohistochemistry as described previously [7]. EPB41L3 protein was well expressed in both non-neoplastic gastric and colonic mucosal cells (Fig. 1A and D). Positive expression of EPB41L3 was observed in 17 GCs (50.0%) and 47 CRCs (59.5%) with MSI-H, and 23 GCs (51.1%) and 25 CRCs (55.5%) with MSS (Fig. 1). The EPB41L3 expression was not significantly different with respect to the MSI status (MSI-H Vs. MSS) and not to the cancer type (GC Vs. CRC) (Fisher's exact test, $p > 0.05$). The CRC with the *EPB41L3* frameshift mutation showed negative EPB41L3 immunostaining.

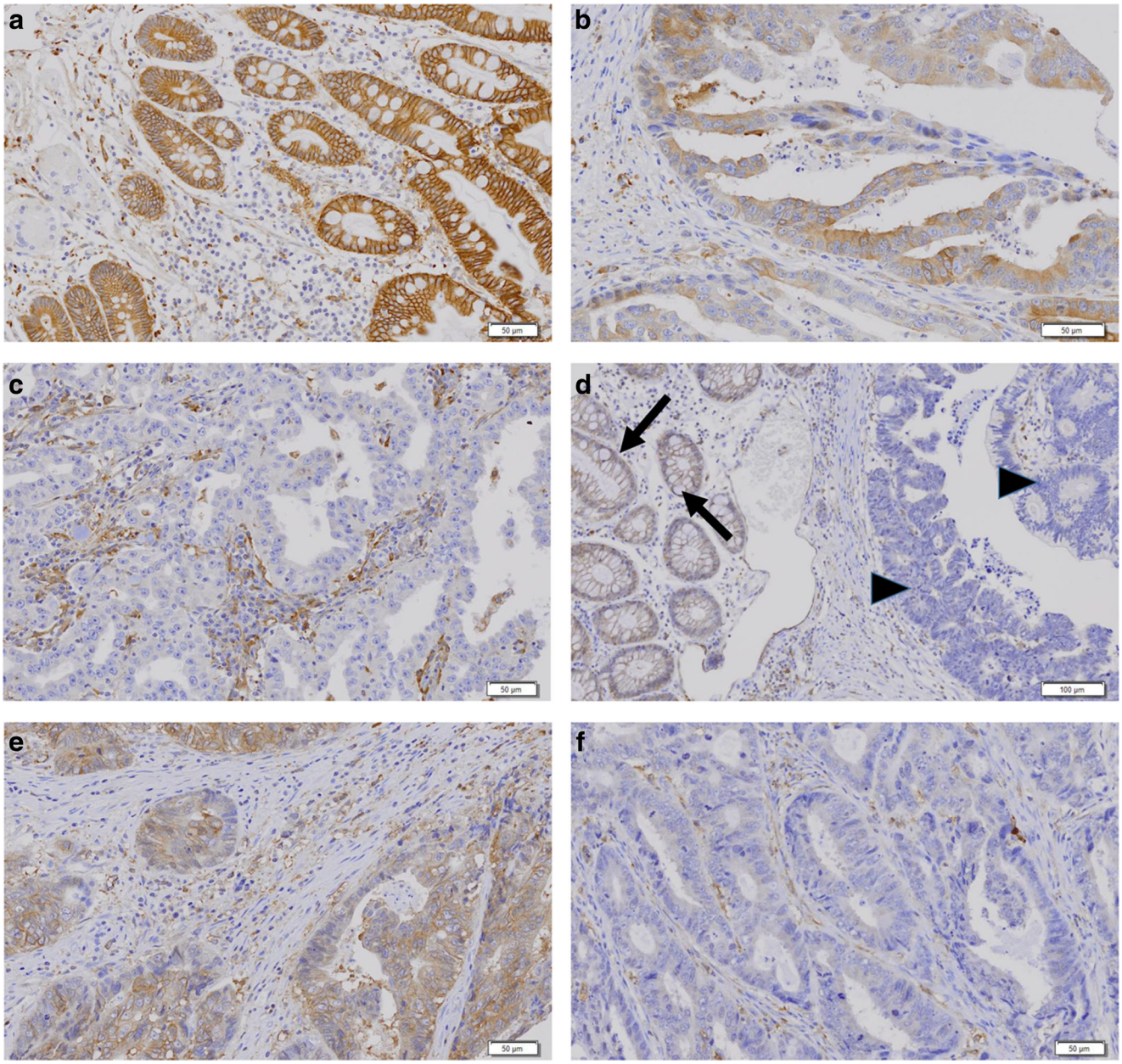

Fig. 1 Expression of EPB41L3 expression in gastric and colorectal cancer tissues by immunohistochemistry. a and d: Normal gastric (**a**) and colonic (**d**) mucosal cells show positive EPB41L3 immunostaining. In d, gastric cancer cells (arrows) exhibit lower EPB41L3 immunostaining intensity than normal colon cells (arrow heads). b and e: Gastric (**b**) and colon (**e**) cancers show positive EPB41L3 immunostaining in the cancer cells. c and f: In a gastric cancer (**c**) and a colon cancer (**f**), the cancer cells show negative EPB41L3 immunostaining

In the present study, we have analyzed and found mutational and expressional alterations of *EPB41L3*, a TSG, in GC and CRC. However, the frameshift mutation was identified in a minor fraction of the cancers, indicating that it may not play a pivotal role in GC and CRC development. The GC and CRC irrespective of the MSI status exhibited expression loss in about half of the cases compared to the abundant expression in normal mucosal cells, which is in agreement with the earlier reports [1–3]. Our data suggest that loss of expression but not frameshift mutation may contribute to GC and CRC pathogenesis by altering it TSG functions. Our findings may provide clues for further researches on *EPB41L3* as well as functional implications of *EPB41L3* alterations in cancers.

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Compliance with Ethical Standards

Conflict of Interest None to declare.

References

1. Wang Z, Zhang J, Ye M, Zhu M, Zhang B, Roy M, Liu J, An X (2014) Tumor suppressor role of protein 4.1B/DAL-1. Cell Mol Life Sci 71:4815–4830
2. Wang H, Xu M, Cui X, Liu Y, Zhang Y, Sui Y, Wang D, Peng L, Wang D, Yu J (2016) Aberrant expression of the candidate tumor suppressor gene DAL-1 due to hypermethylation in gastric cancer. Sci Rep 6:21755
3. Ohno N, Terada N, Murata S, Yamakawa H, Newsham IF, Katoh R, Ohara O, Ohno S (2004) Immunolocalization of protein 4.1B/DAL-1 during neoplastic transformation of mouse and human intestinal epithelium. Histochem Cell Biol 122:579–586
4. Xue F, An C, Chen L, Liu G, Ren F, Guo X, Sun H, Mei L, Sun X, Li J, Tang Y, An X, Zheng P (2019) 4.1B suppresses cancer cell proliferation by binding to EGFR P13 region of intracellular juxtamembrane segment. Cell Commun Signal 17:115
5. Imai K, Yamamoto H (2008) Carcinogenesis and microsatellite instability: the interrelationship between genetics and epigenetics. Carcinogenesis 29:673–680
6. Je EM, Kim MR, Min KO, Yoo NJ, Lee SH (2012) Mutational analysis of MED12 exon 2 in uterine leiomyoma and other common tumors. Int J Cancer 131:E1044–E1047
7. Yoo NJ, Kim HR, Kim YR, An CH, Lee SH (2012) Somatic mutations of the KEAP1 gene in common solid cancers. Histopathology 60:943–952