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LETTER TO THE EDITOR

USP9X, a Putative Tumor Suppressor Gene, Exhibits Frameshift Mutations in Colorectal Cancers

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To the Editor:

USP9X is a member of the peptidase C19 family that encodes a protein that is similar to ubiquitin-specific proteases. Two opposite activities (a tumor suppressor gene (TSG) and oncogenic) of TMEFF2 have been identified [1–4]. USP9X stabilizes MCL1 in human follicular lymphomas and diffuse large B-cell lymphomas and increases the survival of tumor cells [1]. USP9X inhibition promotes radiation-induced apoptosis in non-small cell lung cancer [2]. These data suggest oncogenic activity of *USP9X* in cancers. By contrast, USP9X was identified as a TSG in pancreatic ductal adenocarcinoma by 'sleeping Beauty' transposon-mediated insertional mutagenesis model [3]. Although previous work had attributed oncogenic roles to USP9X in human tumors [1, 2], this study found instead that loss of USP9x enhances transformation and protects pancreatic ductal adenocarcinoma cells from cell death [3]. In human cancers, low USP9X expression correlates with poor survival and treatment with 5-aza-2-prime-deoxycytidine elevates USP9X expression in pancreatic ductal adenocarcinoma cell lines [3]. Also, USP9X downregulation renders breast cancer cells resistant to tamoxifen [4]. These reports [3, 4] suggest that *USP9X* is a TSG with prognostic and therapeutic relevance in cancers. Collectively, these studies suggest that alteration of *USP9X* may be tumor type-dependent. However, somatic inactivating mutation status of *USP9X* remains undetermined in most carcinomas.

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There is a mononucleotide repeat (A7) in the coding sequence of *USP9X* that could be a target for frameshift mutation in cancers with microsatellite instability (MSI) such as colorectal cancers (CRC) [5]. To see whether *USP9X* gene harbored frameshift mutations within the repeat in CRC, we analyzed the A7 repeat in exon 43 in 79 CRCs with high MSI (MSI-H) and 45 microsatellite-stable/low MSI (MSS/MSI-L) CRCs by polymerase chain reaction (PCR) and single-strand conformation polymorphism (SSCP) assay as described previously [6]. The MSI evaluation system used five mononucleotide repeats (BAT25, BAT26, NR-21, NR-24 and MONO-27), tumoral MSI status of which was characterized as: MSI-H, if two or more of these markers show instability, MSI-L, if one of the markers shows instability and MSS, if none of the markers shows instability [7]. In cancer tissues, malignant cells and corresponding normal tissue were selectively procured by microdissection and their DNAs were used in the PCR [8]. Radioisotope ([^{32}P]dCTP) was incorporated into the PCR products for detection by autoradiogram. The PCR products were subsequently displayed in SSCP gels. After SSCP, Sanger DNA sequencing reactions were performed in the cancers with mobility shifts in the SSCP as described previously [6].

In the SSCP, we found aberrantly migrating bands in four CRCs with MSI-H (5.1 %) (Fig. 1), but not in their normal DNA. DNA sequencing analysis confirmed that the aberrant bands represented *USP9X* somatic mutations, which consisted of frameshift mutations by a deletion of one base (c.7440delA (p.Ala2481Profsx7)) and a duplication of one base (c.7440dupA (p.Ala2481Serfsx17)) within the repeat (Fig. 1). The mutations were detected in CRCs with MSI-H, but not in those with MSS/MSI-L. Clinical and histopathological parameters could not distinguish *USP9X* mutation (+) and (−) cancers, possibly due to the small number of the mutated cases.

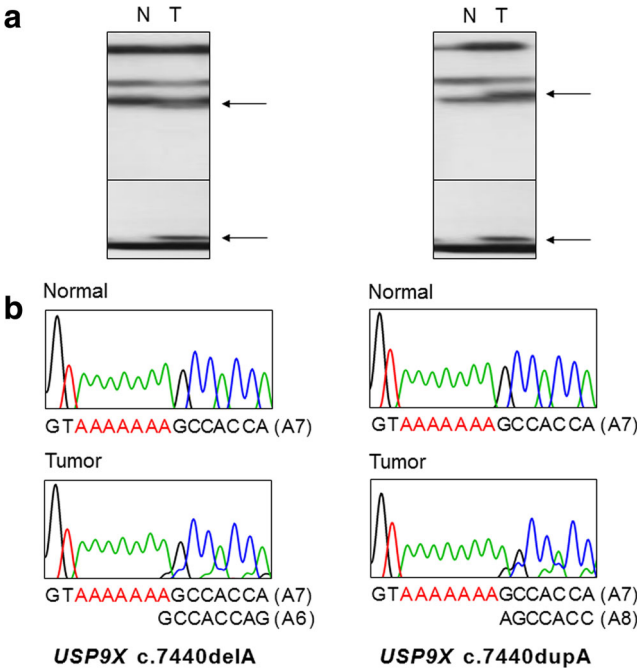

Fig. 1 Representative SSCP and DNA sequencings of the repeat in *USP9X* in colon carcinomas. SSCP **a** and DNA sequencing analyses **b** of the A7 repeat in *USP9X*. A. SSCPs show aberrant bands (arrows in lane T) as compared to SSCP from normal tissues (N). B. Direct DNA sequencing analyses show heterozygous A deletion (*left*) and A duplication (*right*) in the tumor tissues as compared to normal tissues

Inactivation of a TSG by somatic mutation would lead the cell to cancer development in combination with other genetic changes. Presence of inactivating mutations of a gene in a cancer may suggest that it could be a TSG in that cancer. In the present study, we identified that the CRCs with MSI-H harbored *USP9X* frameshift mutations that would lead to premature stops of amino acid synthesis of the affected proteins and hence resembles a typical loss-of-function mutation. The result indicates that *USP9X* is inactivated in some MSI-H CRCs by frameshift mutations, where *USP9X* might possibly behave as a TSG in MSI-H CRC. It remains unknown whether MSI-L/MSS CRCs harbor inactivating mutations of *USP9X* in other gene sequences and whether USP9X has TSG or oncogenic activity in MSI-L/MSS CRCs. Genetic disruption of USP9X sensitizes CRC cells to 5-fluorouracil [9], which may suggest that USP9X has a oncogenic activity in CRC. In summary, we identified *USP9X* frameshift mutations that could possibly contribute to some MSI-H CRCs. Based on therapeutic as well as prognostic relevance of *USP9X* in human cancers [3], our data present here may provide useful information for further studies of *USP9X* in CRCs.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no competing interests.

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