USP9X Increased Tumor Angiogenesis in Mantle Cell Lymphoma by Upregulation of CCND1- mediated SOX11

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Abstract. Mantle cell lymphoma (MCL) is an aggressive lymphoid malignancy with a poor prognosis. Ubiquitin-specific peptidase 9, X-linked (USP9X), has been associated with multiple physiological pathways and regulates various cellular activities. In this study, we explored the role of USP9X in MCL in vitro and in vivo. USP9X was verified to be increased in peripheral blood mononuclear cells (PBMCs) of MCL patients and MCL cells. Moreover, CCND1 and SOX11 were also upregulated in PBMCs of MCL patients. The positive correlation between USP9X and CCND1, USP9X and SOX11, and CCND1 and SOX11 were identified. Further, USP9X overexpression and knockdown were performed in MCL cells. We proved that USP9X overexpression promoted proliferation and cell cycle and suppressed cell apoptosis in MCL cells. Upregulation of angiogenesis and cell migration were induced by USP9X overexpression in MCL cells. However, the USP9X knockdown showed opposite effects. In addition, USP9X was discovered to decrease Cyclin D1 (CCND1)-mediated SOX11 expression in MCL cells. We demonstrated that SOX11 overexpression reversed USP9X knockdown-mediated angiogenesis in MCL cells. Besides, tumor formation was inhibited by USP9X knockdown in mice in vivo. In conclusion, these results revealed that USP9X promoted tumor angiogenesis in MCL via increasing CCND1-mediated SOX11.

Keywords: Mantle cell lymphoma, USP9X, Angiogenesis, CCND1, SOX11.

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Introduction. Mantle cell lymphoma (MCL) is a relatively rare subtype of highly aggressive B-cell lymphoma with approximately 7% of the non-Hodgkin lymphoma incidence rate.¹ Pathologically, it is featured by the chromosomal translocation t (11;14) (q13; q32).² MCL has a poor prognosis compared with other types of non-Hodgkin lymphoma, with a median survival time of only 3-5 years.³ When most patients are diagnosed with stage III-IV, tumor infiltration usually occurs in lymph nodes, bone marrow, spleen, peripheral blood, and digestive tract.⁴,⁵ In MCL, tumor cells can shape their microenvironment and activate a complex network of chemokines to promote tumor progression. For example, tumor stroma can increase endothelial cell proliferation and microvascular formation and enhance the aggressiveness of MCL.⁶ Although a series of novel treatment approaches are applied to the improvement of survival rates of MCL patients,⁷ about half of patients...
still suffer from recurrent MCL within 10 years. Thus, treatment targets of MCL are necessary to be further explored.

Ubiquitin-specific peptidase 9, X-linked (USP9X), acts as a member of the USP family to be involved in multiple physiological pathways and regulate a variety of cellular activities. For example, USP9X was highly expressed in pancreatic cancer, promoting epithelial mesenchymal transformation and inhibiting cell apoptosis. Moreover, USP9X was associated with regulating tumor microenvironment and angiogenesis in breast cancer. Additionally, it has been reported that USP9X expression was high in human invasive B-cell lymphoma samples and enhanced the chemoresistance of invasive B-cell lymphoma. However, the underlying mechanism function of USP9X in MCL is still unclear.

CCND1, as a cell cycle regulatory protein, is located in chromosome 11q13 and directly regulated by p53. Its upregulation was closely related to more advanced tumors and tumor metastasis. Furthermore, CCND1 participated in regulating the malignant progression of MCL. USP9X was proved to upregulate the expression of cell cycle-related protein CCND1, suggesting that USP9X might function in MCL through CCND1.

Here, this study aims to investigate the effects of USP9X on the development of MCL. In addition, we confirmed the specific mechanism of USP9X on MCL through CCND1 in MCL, which provides a potential therapeutic target for MCL.

Materials and Methods

Clinical sample. All specimens were obtained from 50 treatment-naïve MCL patients and 20 healthy volunteers. Informed consent was gained from all volunteers. This study was approved by the World Medical Association Declaration of Helsinki and Yuebei people’s Hospital. Ficoll gradient centrifugation acquired peripheral blood mononuclear cells (PBMCs) of all volunteers, then PBMCs were purified through anti-CD19 magnetic beads. Finally, the samples exhibited at least 90% CD19+ cells identified by flow cytometry (BD Biosciences).

Cell culture and transfection. MCL cells (Z-138, Mino, REC-1, Jeko-1, and JVM2) were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) under 5% CO2 at 37°C. Z-138 and Jeko-1 cells were transiently transfected with USP9X overexpression or knockdown via Lipofectamine 2000 (Life Technologies Corp., USA) for 24 h. Moreover, Z-138 and Jeko-1 cells were transiently transfected with USP9X overexpression and CCND1 knockdown, or USP9X knockdown and CCND1 overexpression. Additionally, Z-138 and Jeko-1 cells were transiently transfected with USP9X knockdown and SOX11 overexpression.

Plasmids. For USP9X, CCND1, or SOX11 overexpression, human USP9X, CCND1, or SOX11 cDNA was cloned into the vector pcDNA3.0, respectively. USP9X or CCND1 knockdowns were purchased from JTSBIO (Wuhan, China). The USP9X or CCND1 shRNA target sequences and scrambled sequences were inserted into the expression siRNA vector-pRNAH1.1 vector.

Quantitative real-time PCR (qRT-PCR). Total RNA from peripheral blood mononuclear cells of MCL patients or MCL cells was extracted using TRIZol (Takara, Dalian, China). Then, cDNA was synthesized through universal cDNA synthesis kit II (Applied Biosystems, Foster City, CA, USA). Relative expression of USP9X was detected and calculated using the 2−ΔΔCT method. Primers were used for this study as follows: USP9X-F, 5′-CAATGGATAGATCGCTTTATA-3′; USP9X-R, 5′-CTTTCTTGCCATGGCCTTAAAT-3′.

Cell viability assay. The cell viability was measured through a Cell Counting Kit-8 (CCK-8, Beyotime, Shanghai, China). Cells were seeded into 96 plates, and cell viability was examined at different periods (0, 24, 48, and 72 h). The OD value was detected using an MRX II microplate reader at 450 nm.

BrdU assay. Cells were fixed with 4% paraformaldehyde and then cut into 5 μm sections. Sections were deparaffinized and rehydrated and stained with BrdU. The images were captured under a light microscope.

Cell cycle analysis. Cells were collected and resuspended through pre-cooled 75% ethanol. Then,
500ul PBS containing 20ul RNaseA was added to cells for 30 min. Finally, cells were stained with PI (50 μg/mL) for 25 minutes. Flow cytometry was used for cell cycle analysis.

**Cell apoptosis assay.** Cells were collected and resuspended with 100 μL of binding buffer. Then, cells were stained with Annexin V-FITC and PI for 15 min. Finally, cell apoptosis was analyzed through a flow cytometer.

**Transwell assay.** For cell invasion analysis, Transwell chambers were added with Matrigel. For cell migration analysis, Transwell chambers were not added with Matrigel. Briefly, 4×10^5 cells were collected and resuspended with an FBS-free medium. Then, 200 μL of suspension was added to the top chamber and 500 μL of medium supplemented with 10% FBS was added to that lower. The cells were incubated for 48 h at 37°C, and cells were fixed and stained with 0.1% crystal violet. The images were observed under a microscope.

**Animals.** BALB/c nude mice (~4–5 weeks old) were divided into two groups (n=6/group): shNC; shUSP9X-2#. Then, 1 × 10^6 Z-138 cells with USP9X knockdown (shUSP9X-2#) and control cells (shNC) were subcutaneously injected into mice. Tumor growth was monitored for 30 days. Last, tumor tissues were collected and photographed when mice were sacrificed. According to Guide for the Care and Use of Laboratory Animals and (Yuebei people’s Hospital), all experiments were conducted in.

**Tube formation assay.** Matrigel (50 microliters) was plated in 96-well plates. Human umbilical vein endothelial cells (HUVECs) were grown on Matrigel with different treatments and then incubated at 37°C for four h. Finally, tube formation was observed using microscopy and the branch points were calculated.

**Immunohistochemistry and microvessel density (MVD) analysis.** Tissues were fixed with 4% paraformaldehyde, and then paraffin embedding was conducted. Paraffin-embedded tissues were cut into 5-μm-thick slides. After blocking with 1% BSA, the samples were incubated with primary antibodies, including TUNEL (1:1000), Ki67 (1:1500), USP9X (1:1000), CCND1 (1:1000), SOX11 (1:1000), and CD31 (1:1000) (all from Abcam, Cambridge, MA, USA) for 60 min at 37°C. Then, tissues were fixed with 4% paraformaldehyde for 30 min. Finally, tissues were incubated with goat anti-mouse secondary antibody for 50 min. Cell nuclei were stained with DAPI. Images were acquired under microscopy. Additionally, CD31-positive MVD areas were calculated as the sum of areas of MVD (μm^2) evaluated divided by the total size of the core analyzed (μm^2) as previously described. 23

**Statistical analysis.** Experimental statistical analyses were performed using

Measurement data were presented as mean ± SD. Two groups were compared with unpaired t-tests, and comparisons among multiple groups were conducted using ANOVA by Turkey. P less than 0.5 was considered a statistical significance.

**Results.**

**USP9X was increased in PBMCs of MCL patients.** The expression of USP9X, CCND1 and SOX11 in PBMCs of MCL patients was measured by qRT-PCR and western blotting. The results showed that USP9X, CCND1, and SOX11 mRNA levels and protein levels were high in peripheral blood mononuclear cells of MCL patients compared to the normal sample (Figure 1A, B). Moreover, a positive correlation between USP9X and CCND1, USP9X and SOX11, and CCND1 and SOX11 was proved by qRT-PCR, respectively (Figure 1C). Additionally, qRT-PCR and western blotting demonstrated that USP9X expression was enhanced in MCL cell lines (Z-138, Mino, REC-1, Jeko-1, or JVM2) (Figure 1E). These findings suggested that USP9X was increased in PBMCs of MCL patients and MCL cells.

**USP9X promoted proliferation and cell cycle in MCL cells.** The effect of USP9X on proliferation and cell cycle was detected in MCL cells by inducing either the USP9X overexpression or knockdown. Western blotting showed that USP9X overexpression increased USP9X protein level, and USP9X knockdown decreased USP9X protein level in Z-138 and Jeko-1 (Figure 2A). CCK-8 assay revealed that cell proliferation was promoted by USP9X overexpression, whereas it was inhibited by USP9X knockdown in Z-138 and Jeko-1 (Figure 2B). Accordingly, USP9X overexpression elevated BrdU positive cells and USP9X knockdown reduced BrdU positive cells (Figure 2C). Further, flow cytometry demonstrated that USP9X overexpression inhibited the G1 stage and promoted the S stage, whereas USP9X knockdown exerted opposite effects (Figure 2D). Furthermore, western blotting verified that USP9X overexpression increased CDK4, CDK6, and PCNA protein levels and decreased P21 protein level. However, USP9X knockdown reversed these protein levels (Figure 2E). These results indicated that USP9X promoted proliferation and cell cycle in MCL cells.

**USP9X inhibited cell apoptosis in MCL cells.** The effect of USP9X on cell apoptosis was explored in MCL cells. The results from flow cytometry was proved that USP9X overexpression suppressed cell apoptosis, and USP9X knockdown enhanced cell apoptosis in MCL cells (Z-138 and Jeko-1) (Figure 3A). Western blotting demonstrated that USP9X overexpression upregulated Bcl-2 protein levels and downregulated Bax and Cleaved caspase 3
USP9X contributed to angiogenesis and promoted cell migration in MCL cells. The role of USP9X in angiogenesis and cell migration was investigated. We found that tube formation was increased in HUVECs treated by USP9X overexpression-positive cell medium (CM), and it was decreased in HUVECs exposed to USP9X knockdown-positive CM (Figure 4A). Western blotting verified that USP9X overexpression elevated ANGPT2, FGF1, PDGFA, and VEGF protein levels, whereas USP9X knockdown repressed these protein levels in MCL cells (Z-138 and Jeko-1) (Figure 4B). In addition, cell migration and invasion were promoted by USP9X overexpression. However, USP9X knockdown inhibited cell migration and invasion in Z-138 and Jeko-1 (Figure 4C, D). BrdU revealed that USP9X knockdown decreased cell proliferation. CCND1 overexpression increased USP9X knockdown-caused cell proliferation and CCND1 T286A further promoted cell proliferation (Supplement Figure 1A). Flow cytometry proved that the cell cycle inhibited by USP9X knockdown was enhanced by CCND1 overexpression. In addition, CCND1 T286A further promoted the cell cycle (Supplement Figure 1B). These results indicated that USP9X contributed to angiogenesis and enabled cells to migrate in MCL tissue.

USP9X downregulated CCND1-mediated SOX11 expression in MCL cells. We observed how USP9X could affect CCND1-mediated SOX11 expression in MCL cells. Western blotting demonstrated that USP9X overexpression elevated USP9X, CCND1, and SOX11 protein levels, whereas USP9X knockdown reduced these protein levels in Z-138 and Jeko-1 (Figure 5A). Furthermore, USP9X overexpression increased USP9X, CCND1, and SOX11 protein levels, and WP1130, a
Figure 2. USP9X promoted proliferation and cell cycle in MCL cells. USP9X overexpression or knockdown were conducted, then (A) USP9X and β-actin protein levels were detected using western blotting in Z-138 and Jeko-1. (B) Cell proliferation was examined through CCK-8 in Z-138 and Jeko-1. (C) BrdU assay was used to measure cell proliferation in Z-138 and Jeko-1. (D) Flow cytometry was carried out to measure cell cycle in Z-138 and Jeko-1. (E) Western blotting was conducted to detect CDK4, CDK6, P21, PCNA and β-actin protein levels in Z-138 and Jeko-1. n=3. *, p<0.05. **, p<0.01. ***, p<0.001. & vs Control. && vs shNC. & vs shNC. Partially selective deubiquitinases (DUB) inhibitor, decreased USP9X, CCND1, and SOX11 protein levels. However, USP9X overexpression could promote WP1130-mediated these protein levels (Figure 5B). Further, western blotting also showed that CCND1 knockdown inhibited USP9X overexpression-mediated CCND1 and SOX11 protein levels, and CCND1 overexpression promoted USP9X knockdown-mediated CCND1 and SOX11 protein levels (Figure 5C). These findings suggested that USP9X downregulated CCND1-
Figure 3. USP9X inhibited cell apoptosis in MCL cells. USP9X overexpression or knockdown were performed. Subsequently, (A) Cell apoptosis was detected using flow cytometry in Z-138 and Jeko-1. (B) Bcl-2, Bax, Cleaved caspase 3 and β-actin protein levels were analyzed through western blotting in Z-138 and Jeko-1. n=3. *, p<0.05. **, p<0.01. & p<0.05. &&, p<0.01. &&&, p<0.001. * vs Control. & vs shNC.

mediated SOX11 expression in MCL cells.

SOX11 overexpression reversed USP9X knockdown-mediated angiogenesis in MCL cells. SOX11 overexpression was induced in MCL cells with USP9X knockdown to explore whether SOX11 can regulate...
Figure 4. **USP9X contributed to angiogenesis and promoted cell migrate in MCL cells.** HUVECs were treated with USP9X overexpression or knockdown-positive cell medium (CM), then (A) Tube branch points were observed and analyzed using tube formation assay. USP9X overexpression or knockdown were performed in Z-138 and Jeko-1, then (B) ANGPT2, FGF1, PDGFA, VEGF and β-actin protein levels were detected via western blotting. (C, D) Cell migration and invasion were measured Transwell migration assay. n=3. *, p<0.05. **, p<0.01. ***, p<0.001. & vs Control. &&, p<0.05. &&, p<0.01. &&&, p<0.001. * vs Control. & vs shNC.

USP9X knockdown-mediated angiogenesis in MCL cells. Western blotting verified that SOX11 protein level was inhibited in USP9X knockdown MCL cells and enhanced in SOX11 overexpression MCL cells. USP9X protein level was repressed in USP9X knockdown MCL cells, whereas SOX11 overexpression did not affect USP9X protein level in MCL cells. However, SOX11 overexpression in USP9X knockdown MCL cells suppressed SOX11 overexpression-mediated USP9X and SOX11 protein levels (**Figure 6A**). Compared to the USP9X knockdown group, tube formation was enhanced in HUVECs treated with SOX11 overexpression and USP9X knockdown positive CM (**Figure 6B**). Western blotting demonstrated that USP9X knockdown decreased USP9X protein level, and SOX11 overexpression showed no effect on USP9X protein level in MCL cells. However, SOX11 overexpression repressed SOX11 overexpression-mediated USP9X protein levels. Additionally, SOX11 overexpression elevated ANGPT2, FGF1, PDGFA, and VEGF protein levels in USP9X knockdown MCL cells (**Figure 6C**). Further, tube formation was inhibited in HUVECs treated with USP9X knockdown, whereas CCND1 overexpression enhanced this tube formation (**Supplement Figure 2A**). USP9X knockdown repressed ANGPT2, FGF1, PDGFA and VEGF protein levels. However, CCND1 overexpression showed opposite effects on these protein levels (**Supplement Figure 2B**). These results indicated that SOX11 overexpression or CCND1 reversed USP9X knockdown-mediated angiogenesis in MCL cells.
Figure 5. USP9X downregulated CCND1-mediated SOX11 expression in MCL cells. USP9X overexpression or knockdown were carried out in Z-138 and Jeko-1, then (A) USP9X, CCND1, SOX11 and β-actin protein levels were detected through western blotting. USP9X overexpression was conducted in Z-138 and Jeko-1 treated with WP1130 (a partially selective deubiquitinases (DUB) inhibitor); then (B) Western blotting was performed to measure USP9X, CCND1, SOX11 and β-actin protein levels. USP9X overexpression and CCND1 knockdown, or USP9X knockdown and CCND1 overexpression were performed in Z-138 and Jeko-1, subsequently, (C) Western blotting was used to examine USP9X, CCND1, SOX11 and β-actin protein levels. n=3. **, p<0.01. &&, p<0.01. &&&, p<0.001. $, p<0.05. $$$, p<0.001. * vs Control or NC+shNC. & vs shNC or USP9X or USP9X+shNC. $ vs WP1130 or shUSP9X-2#.&&NC.

USP9X knockdown inhibited tumor formation in mice. The effect of USP9X knockdown on tumor formation was explored in mice. Figure 7A and B shows that USP9X knockdown suppressed tumor volume and weight. IHC showed that USP9X knockdown increased TUNEL expression and decreased Ki67, USP9X, CCND1, and SOX11 expression in tumor tissues (Figure 7C). Further, MVD revealed that USP9X knockdown repressed CD31 protein level using IHC (Figure 7D). The data suggested that USP9X knockdown inhibited tumor formation in mice.

Discussion. Here, USP9X, CCND1, and SOX11 were upregulated in PBMCs of MCL patients. USP9X level was increased in MCL cells. The positive correlation between USP9X and CCND1, USP9X and SOX11, and CCND1 and SOX11 were demonstrated. USP9X was identified to promote proliferation and cell cycle and inhibited cell apoptosis in MCL cells. Moreover, we found that USP9X contributed to angiogenesis and increased cell migration in MCL cells. Further, USP9X downregulated CCND1-mediated SOX11 expression in MCL cells. SOX11 overexpression was verified to reverse USP9X knockdown-mediated angiogenesis in MCL cells. In vivo, the results demonstrated that USP9X knockdown suppressed tumor formation in mice.

Previous studies have revealed that USP9X could target several cytosolic proteins and then be involved in regulating multiple cellular activities, such as cell apoptosis, cell growth, and migration.24-26 The physical association of USP9X with centrosomes was dependent on the cell cycle and mostly measured in S and G2 phases.27 Accordingly, our study demonstrated that USP9X increased proliferation and cell cycle in MCL cells. Furthermore, cell apoptosis was repressed by USP9X in MCL cells, evidenced by the upregulation of Bcl-2 protein level and downregulation of Bax and Cleaved caspase 3 protein levels. In addition, USP9X was also verified to contribute to angiogenesis and enhanced cell migration in MCL cells. These findings suggest that USP9X exerts a key role in MCL cells.

A recent study showed that the gain-of-function of
Figure 6. SOX11 overexpression reversed USP9X knockdown-mediated angiogenesis in MCL cells. USP9X knockdown and SOX11 overexpression were performed in Z-138 and Jeko-1. (A) Western blotting was performed to measure USP9X, SOX11 and β-actin protein levels. HUVECs were treated with USP9X knockdown and SOX11 overexpression-positive cell medium (CM), then (B) Tube formation assay was used to observe tube branch points. USP9X knockdown and SOX11 overexpression were conducted in Z-138 and Jeko-1, then (C) USP9X, ANGPT2, FGF1, PDGFA, VEGF and β-actin protein levels were examined using western blotting. n=3. *, p<0.05. **, p<0.01. ***, p<0.001. &&&. p<0.001. $, p<0.05. $$, p<0.01. * vs NC+shNC or NC+shNC CM. & vs shUSP9X-2#+NC or shUSP9X-2#+NC CM. $ vs SOX11+shNC or SOX11+shNC CM.

USP9X took part in increasing CCND1 and decreasing CDKN1A in breast cancer tissue and cells. CCND1 overexpression was an early and unifying oncogenic event in MCL. Davis et al. discovered that CCND1 functioned in the cell cycle in colorectal cancer and MCL. Chinen et al. revealed that transcript variants of CCND1 were companied with chromosome 11q13 abnormalities in MCL. Our study proved that USP9X overexpression promoted CCND1 expression in MCL cells. USP9X knockdown decreased cell proliferation and cycle. CCND1 overexpression increased USP9X knockdown-caused cell proliferation and cycle, suggesting that USP9X affects cell proliferation and cycle through CCND1. However, compared with CCND1 WT, CCND1 T286A further promoted the USP9X knockdown-mediated cell proliferation and cycle, indicating that USP9X regulates cell proliferation and cycle by preventing degradation of CCND1 via the removal of conjugated ubiquitin. Besides, we found that USP9X overexpression also increased SOX11 protein levels. Accumulating evidence has shown that SOX11 expression was regulated by CCND1 and STAT3 in MCL. SOX11 has been identified to enhance tumor angiogenesis through PDGFA in MCL. The relationship between angiogenesis and SOX11 expression has been revealed, and SOX11 was demonstrated to elevate an angiogenic phenotype in primary MCL. Interestingly, our research verified that CCND1 knockdown repressed USP9X overexpression-mediated SOX11 protein level, suggesting that USP9X
downregulated CCND1-mediated SOX11 expression in MCL cells. USP9X overexpression could promote WP1130-mediated USP9X, CCND1, and SOX11 protein levels, indicating that USP9X exerts its function by deubiquitinating Cyclin D1 and/or SOX11. Additionally, SOX11 overexpression was also found to reverse USP9X knockdown-mediated angiogenesis in MCL cells. Further analysis showed that USP9X knockdown could play a suppressive role in tumor formation in mice. USP9X knockdown reduced tumor volume and weight, inhibited TUNEL expression, and repressed Ki67, USP9X, CCND1, and SOX11 expression in tumor tissues. The data indicated that USP9X affected tumor angiogenesis in MCL through regulation of CCND1-mediated SOX11.

However, the mechanism of USP9X functions in MCL needs to be further explored. Additionally, the USP9X application in the clinical sample will be evaluated. Thus, more experiments will be conducted in the near future. In conclusion, we demonstrated that USP9X contributed to tumor angiogenesis. Furthermore, USP9X functioned in MCL through upregulating CCND1-mediated SOX11, which provides a new target for MCL patients.

Ethics approval. All procedures performed in studies involving human participants were in accordance with the standards upheld by the Ethics Committee of Yuebei people’s Hospital and with those of the 1964 Helsinki Declaration and its later amendments for ethical research involving human subjects.

The Ethics Committee approved all animal experiments of Yuebei people’s Hospital for the use of animals and conducted in accordance with the National Institutes of Health Laboratory Animal Care and Use Guidelines.

Statement of Informed Consent. Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

Authors’ contributions. Gang Huang and Jianjun Liao designed the study, supervised the data collection, Mingli Wang analyzed the data, interpreted the data, and Yali Huang, Mingjie Tang, and Yanyan Hao prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

References:
1. Fakhri B, Kahil B. Current and emerging treatment options for mantle cell lymphoma. Therapeutic Advances in Hematology. 2017; 8(8): 223-34. https://doi.org/10.1177/204626717719616
PMID:28811872 PMCid:PMC5544150
2. Campo E, Rule S. Mante cell lymphoma: evolving management strategies. Blood. 2015; 125(2): 48-55. https://doi.org/10.1182/blood-2014-05-521898
PMID:25499451
3. Eskeland CW, Kolstad A, Jerkeman M, Raty R, Laurell A, Eloranta S, Smedby KE, Hussy S, Pedersen LB, Andersen NS, Eriksson M, Kimby E, Bentzen H, Kuitinen O, Lauritsen GF, Nilsson-Ehle H, Ralfkiaer E, Ehinger M, Sundstrom C, Delabie J, Karjalainen-Lindsberg ML, Workman CT, Garde C, Elonen E, Brown P, Gronbaek K, Geisler CH. 15-year follow-up of the Second Nordic Mantle Cell Lymphoma trial (MCL2): prolonged remissions without survival plateau. British Journal of Haematology. 2016; 175(3): 410-419. https://doi.org/10.1111/bjh.14241
PMID:27378674
4. Cortelazzo S, Ponzones M, Ferreri AJM, Dreyling M. Mante cell lymphoma. Critical Reviews in Oncology/Hematology. 2020; 153: 103038. https://doi.org/10.1016/j.critrevonc.2020.103038
PMID:32738930
5. Hanel W, Epperla N. Emerging therapies in mantle cell lymphoma. Journal of Hematology & Oncology. 2020; 13(1): 79. https://doi.org/10.1186/s12885-020-00914-1
PMID:32552760 PMCid:PMC7025587
6. Amnese T, Ingravallo G, Tamma R, De Giorgis M, Maiorano E, Perrone R, Albano F, Specchia G, Ribatti D. Inflammatory Infiltrate and Angiogenesis in Mantle Cell Lymphoma. Translational Oncology. 2020; 13(3): 1007-1044. https://doi.org/10.1016/j.tranon.2020.100744
PMID:32110334 PMCid:PMC7052512
7. Dreyling M, Amador V, Callanan M, Jerkeman M, Le Gouill S, Pott C, Rule S, Zaja F. Update on the molecular pathogenesis and targeted strategies of mantle cell lymphoma: summary of the 12th annual conference of the European Mantle Cell Lymphoma Network. Leukemia. 2015; 56(4): 866-76. https://doi.org/10.1038/leu.2014.90584
PMID:25015778
8. Wu C, de Miranda NF, Chen L, Wasik AM, Mansouri L, Jurczak W, Hsu H, Sun T, Ren Z. Deubiquitinase USP9X promotes cell migration, invasion and inhibits apoptosis of human hematopoietic cancer. Oncology Reports. 2017; 38(6): 3531-3537. https://doi.org/10.3892/or.2017.6500
PMID:29130109
9. Pal A, Dzubinski M, Di Magliano MP, Simeone DM, Owens S, Thomas D, Peterson L, Potu H, Talpaz M, Donato NJ. USp9X Promotes Survival in Human Pancreatic Cancer and Its Inhibition Suppresses Pancreatic Ductal Adenocarcinoma In Vivo Tumor Growth. Neoplasia. 2018; 20(2): 152-64. https://doi.org/10.1016/j.neo.2017.11.007
PMID:29248719 PMCid:PMC5735260
10. Shen Q, Reedijk M. Notch Signaling and the Breast Cancer Microenvironment. Advances in Experimental Medicine and Biology. 2021; 1287: 183-200. https://doi.org/10.1007/978-3-030-55034-8_12
PMID:33034033
11. Engel K, Rudelius M, Slavova J, Jacobs L, Ahanargian Abhari B, Altmann B, Kurutz J, Rathakrishana A, Fernandez-Saiz V, Brunner A, Targosz BS, Loewecke F, Gloczew CL, Ueffing M, Fulda S. Pteunendschuh M, Trumper L, Klapper W, Keller U, Jost PJ, Rosenwald A, Feschel C, Basmannen F. USP9X stabilizes XIAP to regulate mitotic cell death and chemoresistance in aggressive B-cell lymphoma. EMBO Molecular Medicine. 2016; 8(8): 851-62. https://doi.org/10.15252/emmm.201506047
PMID:27317434 PMCid:PMC4967940
12. Hermeking H. The mir-334 family in cancer and apoptosis. Cell Death and Differentiation. 2010; 17(2): 193-9. https://doi.org/10.1038/cdd.2009.56
PMID:19461653
13. Sun F, Fu H, Liu Q, Tie Y, Zhu J, Xing R, Sun Z, Zheng X. Downregulation of CCND1 and CDK6 by miR-34a induces cell cycle arrest. Plos Biology. 2008; 6(10): 1564-8. https://doi.org/10.1371/journal.pbio.0060157
PMID:18406535
14. Jin Y, Jin C, Wannerberg J, Hoglund M, Mertens F. Cyclin D1 amplification in chromosomal band 11q13 is associated with overrepresentation of 3q21-q23 in head and neck carcinomas. International Journal of Cancer. 2002; 98(3): 475-9. https://doi.org/10.1002/ijc.10225
PMID:11920603
15. Ahlin C, Lundgren C, Embrtens-Varro E, Jirstrom K, Blomqvist C, Fjallskog M. High expression of cyclin D1 is associated to high proliferation rate and increased risk of mortality in women with ER positive but not in ER-negative breast cancers. Breast Cancer Research and Treatment. 2017; 164(3): 667-78. https://doi.org/10.1007/s10549-017-4294-5
PMID:28352430 PMCid:PMC495873
16. Lee E, Jin D, Lee BB, Kim Y, Han J, Shin YM, Kim DH. Negative effect of cyclin D1 overexpression on recurrence-free survival in stage II-IIIA lung adenocarcinoma and its expression modulation by vorinostat in vitro. BMC Cancer. 2015; 15: 982. https://doi.org/10.1186/s12885-015-0901-7
PMID:26681159 PMCid:PMC4683846
17. Davis MI, Pragani R, Fox JT, Shen M, Parmar K, Gaudiano EF, Liu L, Tanega C, McGee L, Hall MD, McKnight C, Shinn P, Nelson H, Chattopadhyay D, A’andrea AD, Auld DS, DeLucas LJ, Li Z, Boxer MB, Simeonov A. Small Molecule Inhibition of the Ubiquitin-specific Protease USP2 Accelerates cyclin D1 Degradation and Leads to Cell Cycle Arrest in Colorectal Cancer and Mantle Cell Lymphoma Models. The Journal of Biological Chemistry. 2016; 291(47): 24628-40. https://doi.org/10.1074/jbc.M116.738567
PMID:27681596 PMCid:PMC5114414
18. Li H, Zheng B. Overexpression of the Ubiquitin-Specific Peptidase 9 X-Linked (USP9X) Gene is Associated with Upregulation of Cyclin D1 (CCND1) and Downregulation of Cyclin-Dependent Kinase 1A (CDK1A1) in Breast Cancer Tissue and Cell Lines. Medical science monitor: International Medical Journal of Experimental and Clinical Research. 2019; 25: 4207-16. https://doi.org/10.15338/msm.8_12
PMID:31160265 PMCid:PMC6568031
19. Cardesa-Salzmann TM, Colombo L, Gutierrez G, Chan WC, Weisenburger D, Climent F, Gonzalez-Barca E, Mercadal S, Arellanas L, Serrano S, Tubbs R, Delabie J, Gasconye RD, Connors JM, Mate JL, Rimsza L, Duval R, Rosenwald A, Lenz G, Wright G, Iaff ES, Staudt L, Jares P, Lopez-Guillermo A, Campo E. High microsvessle density determines a poor outcome in patients with diffuse large B-cell lymphoma treated with rituximab plus chemotherapy. Haematologica. 2011; 96(7): 996-1001. https://doi.org/10.3324/haematol.2010.037408
PMID:21564560 PMCid:PMC3128218
20. Nagai H, Noguchi T, Homma K, Katagiri K, Takeda K, Matsuzaka A, Ichijo H. Ubiquitin-like sequence in ASK1 plays critical roles in the recognition and stabilization by USP9X and oxidative stress-induced cell death. Molecular Cell. 2009; 36(5): 805-18. https://doi.org/10.1016/j.molcel.2009.01.016
PMID:20005844
21. Homan CC, Kumar R, Nguyen LS, Haan E, Raymond FL, Abidi F, Raynaud M, Schwartz CE, Wood SA, Gezz J, Lolly LA. Mutations in USP9X are associated with X-linked intellectual disability and disrupt
neuronal cell migration and growth. American Journal of Human Genetics. 2014; 94(3): 470-8 https://doi.org/10.1016/j.ajhg.2014.02.004 PMid:24607389 PMCid:PMC3951929

26. Dupont S, Mamidi A, Cordenonsi M, Montagner M, Zacchigna L, Adorno M, Martello G, Stinchfield MJ, Soligo S, Morsut L, Inui M, Moro S, Modena N, Argenton F, Newfeld SJ, Piccolo S, FAM/USP9x, a deubiquitinating enzyme essential for TGFbeta signaling, controls Smad4 monoubiquitination. Cell. 2009; 136(1): 123-35 https://doi.org/10.1016/j.cell.2008.10.051 PMid:19135894

27. Li X, Song N, Liu L, Liu X, Ding X, Song X, Yang S, Shan L, Zhou X, Su D, Wang Y, Zhang Q, Cao C, Ma S, Yu N, Yang F, Yao Z, Shang Y, Shi L, USP9X regulates centrosome duplication and promotes breast carcinogenesis. Nature Communications. 2017; 8: 14866 https://doi.org/10.1038/s41467-016-014866 PMid:28361952 PMCid:PMC5380967

28. Chinen Y, Tsukamoto T, Maegawa-Matsui S, Matsumura-Kimoto Y, Takimoto-Shimomura T, Tanba K, Mizuno Y, Fujibayashi Y, Kuwahara-Ota S, Shimura Y, Kobayashi T, Horinke S, Taniwaki M, Kuroda J, Tumor-specific transcript variants of cyclin D1 in mantle cell lymphoma and multiple myeloma with chromosome 11q13 abnormalities. Experimental Hematology. 2020; 84: 45-53 e1 https://doi.org/10.1016/j.exphem.2020.02.004 PMid:32145384

29. Mohanty A, Sandoval N, Phan A, Nguyen TV, Chen RW, Budde E, Mei M, Poppiewell L, Pham LV, Kwak LW, Weisenburger DD, Rosen ST, Chan WC, Muschen M, Ngo VN. Regulation of SOX11 expression through CCND1 and STAT3 in mantle cell lymphoma. Blood. 2019; 133(4): 306-18 https://doi.org/10.1182/blood-2018-05-851667 PMid:30530749 PMCid:PMC6347093

30. Palomero J, Vegliante MC, Rodriguez ML, Eguileor A, Castellano G, Planas-Rigol E, Jares P, Ribera-Cortada I, Cid MC, Campo E, Amador V, SOX11 promotes tumor angiogenesis through transcriptional regulation of PDGFA in mantle cell lymphoma. Blood. 2014; 124(14): 2235-47 https://doi.org/10.1182/blood-2014-04-569566 PMid:25092176

31. Petakis G, Veloza L, Clot G, Gine E, Gonzalez-Farre B, Navarro A, Bea S, Martinez A, Lopez-Guillermo A, Amador V, Ribera-Cortada I, Campo E. Increased tumour angiogenesis in SOX11-positive mantle cell lymphoma. Histopathology. 2019; 75(5): 704-14 https://doi.org/10.1111/his.13935 PMid:31173643
Supplementary files:

Supplement Figure 1. Cell proliferation and cycle were analyzed in MCL cells. USP9X knockdown was conducted in Z-138 treated with CCND1 WT or CCND1 T286A. (A) BrdU assay was performed to detect cell proliferation. (B) Cell cycle was measured using flow cytometry. n=3. ***, p<0.001. &, p<0.05. &&, p<0.01. &&&, p<0.001. * vs Control. & vs shUSP9X-2#. 
Supplement Figure 2. Angiogenesis was measured in MCL cells. USP9X knockdown and CCND1 overexpression were conducted in Z-138. (A) Tube branch points were analyzed using tube formation assay. (B) Western blotting was performed to detect ANGPT2, FGF1, PDGFA, VEGF and β-actin protein levels. n=3. ***, p<0.001. **, p<0.01. &&& p<0.001. * vs NC+shNC. & vs shUSP9X-2#+NC.