Supplementary Material 1. Molecules involved in clear cell sarcoma of the kidney (CCSK).

BCL6 co-repressor (BCOR). BCOR gene is located on chromosome Xp11.4 and is primarily expressed in the cell nucleus. It is a corepressor that interacts with BCL6. The protein encoded by this gene can selectively interact with the POZ domain of BCL6 (1,2). BCOR is associated with growth and development of children. BCOR acts as a negative regulator of osteogenic ability in adult stem cells by inhibiting transcription factor AP-2α (TFAP2A) (3) and promoting osteoblast division. In CCSK, the abnormal expression of BCOR (such as internal tandem repeat amplification) leads to an enhanced inhibitory effect on TFAP2A. This may disrupt the cellular balance in the bone microenvironment, making it easier for tumor cells to colonize and grow in the bone. In addition, the inhibition of TFAP2A may also affect the normal function of bone cells, such as the activity of osteoblasts and osteoclasts, thereby promoting the occurrence of bone metastasis (3). This also explains why CCSK is prone to bone metastasis. In 2015, Ueno-Yokohata *et al* (4) first discovered that internal tandem duplication (ITD)-BCOR is the cause of CCSK. Specifically, different ITDs involve the region of 1,700-1,755 amino acids, with the number of repeated amino acids ranging from 22 to 38. This indicates that high-frequency mutations occur in a relatively small but functionally specific region (1-3). Zhang *et al* (5) found that in cases of BCOR-ITD in CCSK, there are also deletions or mutations of TP53. TP53 aberration has been confirmed to be associated with the development of various malignant tumors, such as lung, breast, colorectal cancer, ovarian cancer, bladder cancer (5,6). Children with BCOR-ITD and TP53 aberrations typically have poor prognosis (5), indicating TP53 mutations serve an important role in the prognosis of CCSK. However, BCOR-ITD does not occur in all CCSK cases. A retrospective study by Astolfi *et al* (1) found that ≤75% of CCSK cases have BCOR-ITD and few cases exhibit tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein ε-NUT family member 2 (YWHAE-NUTM2) fusion (6).

YWHAE. In humans, YWHAE is a highly conserved gene located on chromosome 17p13.3 that serves as a phosphoserine/phosphothreonine-binding protein (7). It is involved in numerous important cell regulatory processes such as metabolism, protein transport, signal transduction, apoptosis and cell cycle regulation (7). It is also highly expressed in various organs, such as brain, thyroid, kidney and testis. However, the chromosomal region where this gene is located is prone to allelic deletion and it is abnormally expressed in renal tumors (8), ovarian (9), breast (10) and prostate cancer (11), CCSK and uterine leiomyosarcoma. YWHAE promotes the proliferation of prostate cancer cells by phosphorylating and activating the PI3K/AKT/Bcl-2 signaling pathway (11). Yan *et al* (12) discovered that YWHAE promotes tumor growth by increasing the levels of phosphorylated phosphatidylethanolamine-binding Protein 1) and ERK, thereby activating the ERK/MAPK pathway. These findings indicate that YWHAE phosphorylates multiple members of key oncogenic signaling pathways, ultimately leading to tumor development. Research has shown that YWHAE is prone to fusion with NUTM2 in soft tissue sarcoma. This fusion gene was initially used to diagnose uterine leiomyosarcoma but is also detected in CCSK sequencing, suggesting an association between CCSK and maternal inheritance, highlighting the necessity of detecting YWHAE-NUTM2 fusion during early pregnancy screening in clinical practice (13). In a study by Li *et al* (9) on CCSK, it was found that cells expressing the YWHAE-NUTM2 fusion show more significant cell migration and MAPK/PI3K/AKT signaling pathway dysregulation. Importantly, the same pathway activation was observed in CCSK tumor samples, confirming that YWHAE promotes tumor cell proliferation in CCSK by phosphorylating the MAPK/PI3K/AKT signaling pathway (14). In addition to promoting tumor cell proliferation, YWHAE also facilitates the osteogenic differentiation of mesenchymal stem cells, which is consistent with the role of BCOR (6,9-12). Therefore, it was hypothesized that YWHAE and BCOR act on the same pathway to inhibit TFAP2A and promote osteoblast differentiation. YWHAE can regulate TFAP2A activity by binding phosphorylated proteins (such as transcription factors or signaling molecules). If YWHAE directly binds to TFAP2A and inhibits its transcriptional activity, or recruits histone-modifying enzymes (such as histone deacetylases) to change the chromatin state of TFAP2A target genes, thereby inhibiting its expression, it may indirectly promote osteogenic differentiation. BCOR may be recruited to the TFAP2A promoter region by sequence-specific DNA-binding proteins (such as BCL6 or MLLT3), and directly inhibit TFAP2A expression through epigenetic modification, thereby relieving its inhibition of osteogenic differentiation.

TLE family member 1, transcriptional corepressor (TLE1). TLE1 is one of four transducin-like enhancer genes involved in the regulation of hematopoiesis, neuronal differentiation and terminal epithelial differentiation and has high sensitivity and specificity for the diagnosis of synovial sarcoma (15). TLE1 is a transcriptional co-repressor of the Wnt signaling pathway and affects the occurrence and development of tumors through the classical Wnt pathway. Previous studies (16,17) have found that inhibiting the expression of TLE1 reverses the transduction of the Wnt pathway and improves the extensive crosstalk and mutual antagonism between the two signaling pathways in B cell precursor acute lymphoblastic leukemia. Moreover, TLE1 regulates the Wnt pathway and serves an important role in gastrointestinal stromal tumors (17). IHC of TLE1 positivity is not limited to synovial sarcoma. Ali *et al* (15) found that TLE1 expression is also positive in many soft tissue tumors, including CCSK. However, its specificity is relatively poor (15).

CCND1. CCND1 shows notable periodicity in protein abundance throughout the cell cycle. As regulatory factors of cyclin dependent kinase kinases, cyclins exhibit different expression and degradation patterns that contribute to the temporal coordination of each mitotic event (18). CCND1 is regarded as a pan-cancer factor; its mutation and amplification alter the cell cycle process and promote tumorigenesis (18-20). CCND1 shows extensive and strong expression characteristics in CCSK cells and cases, as well as in cases with YWHAE-FAM22 translocation, YWHAE-NUTM2 fusion, and BCOR-ITD status (20,21). This indicates that the positive expression of CCND1 is useful in differentiating CCSK from Wilms' tumor,

as CCND1 highlights the epithelial component. Nevertheless, CCND1 cannot distinguish CCSK from neuroblastoma and mesoblastic nephroma. Therefore, examination of histological features is key for the accurate diagnosis of CCSK.

References

1. Astolfi A, Fiore M, Melchionda F, Indio V, Bertuccio SN and Pession A: BCOR involvement in cancer. Epigenomics 11: 835-855, 2019.
2. Huynh KD, Fischle W, Verdin E and Bardwell VJ: BCoR, a novel corepressor involved in BCL-6 repression. Genes Dev 14: 1810-1823, 2000.
3. Taha Najim R, Topa A, Jugård Y, Casslén B, Odersjö M and Andersson Grönlund M: Children and young adults with anophthalmia and microphthalmia: Diagnosis and management. Acta Ophthalmol 98: 848-858, 2020.
4. Ueno-Yokohata H, Okita H, Nakasato K, Akimoto S, Hata J, Koshinaga T, Fukuzawa M and Kiyokawa N: Consistent in-frame internal tandem duplications of BCOR characterize clear cell sarcoma of the kidney. Nat Genet 47: 861-863, 2015.
5. Zhang M, Yao X, Guan X, Jia C, Zhang R, Wang H, Guo Y, Ni X, Yu Y and He L: Clinical relevance of BCOR internal tandem duplication and TP53 aberration in clear cell sarcoma of the kidney. Hum Pathol 134: 45-55, 2023.
6. Kenny C, Bausenwein S, Lazaro A, Furtwängler R, Gooskens SL, van den Heuvel Eibrink M, Vokuhl C, Leuschner I, Graf N, Gessler M and O'Sullivan MJ: Mutually exclusive BCOR internal tandem duplications and YWHAE-NUTM2 fusions in clear cell sarcoma of kidney: not the full story. J Pathol 238: 617-620, 2016.
7. Zhang Y, Yan M, Yu Y, Wang J, Jiao Y, Zheng M and Zhang S: 14-3-3ε: A protein with complex physiology function but promising therapeutic potential in cancer. Cell Commun Signal 22: 72, 2024.
8. Liang S, Xu Y, Shen G, Liu Q, Zhao X, Xu Z, Xie X, Gong F, Li R and Wei Y: Quantitative protein expression profiling of 14-3-3 isoforms in human renal carcinoma shows 14-3-3 epsilon is involved in limitedly increasing renal cell proliferation. Electrophoresis 30: 4152-4162, 2009.
9. Li X, Wang C, Wang S, Hu Y, Jin S, Liu O, Gou R, Nie X, Liu J and Lin B: YWHAE as an HE4 interacting protein can influence the malignant behaviour of ovarian cancer by regulating the PI3K/AKT and MAPK pathways. Cancer Cell Int 21: 302, 2021.
10. Yang YF, Lee YC, Wang YY, Wang CH, Hou MF and Yuan SF: YWHAE promotes proliferation, metastasis, and chemoresistance in breast cancer cells. Kaohsiung J Med Sci 35: 408-416, 2019.
11. Zhao J, Xu H, Duan Z, Chen X, Ao Z, Chen Y, Ruan Y and Ni M: miR-31-5p regulates 14-3-3 ε to inhibit prostate cancer 22RV1 cell survival and proliferation via PI3K/AKT/Bcl-2 signaling pathway. Cancer Manag Res 12: 6679-6694, 2020.
12. Yan L, Gu H, Li J, Xu M, Liu T, Shen Y, Chen B and Zhang G: RKIP and 14-3-3ε exert an opposite effect on human gastric cancer cells SGC7901 by regulating the ERK/MAPK pathway differently. Dig Dis Sci 58: 389-396, 2013.
13. Gooskens SL, Kenny C, Lazaro A, O'Meara E, van Tinteren H, Spreafico F, Vujanic G, Leuschner I, Coulomb-L'Herminé A, Perotti D, *et al*: The clinical phenotype of YWHAE-NUTM2B/E positive pediatric clear cell sarcoma of the kidney. Genes Chromosomes Cancer 55: 143-147, 2016.
14. Kenny C, McDonagh N, Lazaro A, O'Meara E, Klinger R, O'Connor D, Roche F, Hokamp K and O'Sullivan MJ: Dysregulated mitogen-activated protein kinase signalling as an oncogenic basis for clear cell sarcoma of the kidney. J Pathol 244: 334-345, 2018.
15. Ali Z, Haroon Khan A, Rehman U, Faisal M, Ahmad IN, Mamoon N, Nasir H and Hameed Z: Is TLE1 expression limited to synovial sarcoma? Our experience at Shifa International Hospital, Pakistan. Cureus 11: e6259, 2019.
16. Bergeron BP, Barnett KR, Bhattarai KR, Mobley RJ, Hansen BS, Brown A, Kodali K, High AA, Jeha S, Pui CH, *et al*: Mutual antagonism between glucocorticoid and canonical Wnt signaling pathways in B-cell acute lymphoblastic leukemia. Blood Adv 7: 4107-4111, 2023.
17. Hasbay B and kayaselCuk F: Expression of TLE-1 in gastrointestinal stromal tumour and its relationship to clinicopathological parameters. J Coll Physicians Surg Pak 33: 286-291, 2023.
18. Milman T, Eiger-Moscovich M, Henry RK, Ida CM, Ruben M, Shields CL, Lally SE, Penne RB, Stefanyszyn MA, Bilyk JR, *et al*: Cyclin D1 expression and molecular genetic findings in periocular histiocytoses and neoplasms of macrophage-dendritic cell lineage. Am J Ophthalmol 242: 36-51, 2022.
19. Mirkovic J, Calicchio M, Fletcher CD and Perez-Atayde AR: Diffuse and strong cyclin D1 immunoreactivity in clear cell sarcoma of the kidney. Histopathology 67: 306-312, 2015.
20. Uddin N, Minhas K, Abdul-Ghafar J, Ahmed A and Ahmad Z: Expression of cyclin D1 in clear cell sarcoma of kidney. Is it useful in differentiating it from its histological mimics? Diagn Pathol 14: 13, 2019.
21. Singh V, Gupta K, Saraswati A, Peters NJ and Trehan A: Role of cyclin D1 and BCOR immunohistochemistry in differentiating clear cell sarcoma of kidney from its mimics. J Pediatr Hematol Oncol 43: 294-300, 2021.

Table SI. Primary antibodies used for immunohistochemistry.

Antibody	Dilution	Antibody type	Clone no.	Cat. no.	Supplier
Ki67	1:1,000	Rabbit monoclonal	LBP2-Ki67	IR098	Guangzhou Anbiping Pharmaceutical Technology Co., Ltd.
Soluble protein-100	1:400	Mouse monoclonal	LBP1-S100P	IM193	Guangzhou Anbiping Pharmaceutical Technology Co., Ltd.
Paired box gene 2	1:1,000	Rabbit monoclonal	LBP2-Pax2	IR225	Guangzhou Anbiping Pharmaceutical Technology Co., Ltd.
Cyclin D1	1:500	Rabbit monoclonal	EP180	IR392	Guangzhou Anbiping Pharmaceutical Technology Co., Ltd.
BCL6 co-repressor	1:100	Rabbit polyclonal	CSB-PA000988	Q6W2J9	Cusabio Technology, LLC
CD99	1:50	Rabbit monoclonal	LBP2-CD99	IR045	Guangzhou Anbiping Pharmaceutical Technology Co., Ltd.
BCL-2	1:1,000	Mouse monoclonal	LBP1-BCL2	IM009	Guangzhou Anbiping Pharmaceutical Technology Co., Ltd.
Transducin-like enhancer protein 1	1:50	Mouse monoclonal	LBP1-TLE1	IM401	Guangzhou Anbiping Pharmaceutical Technology Co., Ltd.
Special AT-rich sequence-binding protein 2	1:600	Rabbit monoclonal	E8R8H	CST39229	Cell Signaling Technology, Inc.
Integrase interactor-1	1:2,000	Rabbit monoclonal	D8M1X	CST91735	Cell Signaling Technology, Inc.
Wilms' tumor 1	1:100	Rabbit monoclonal	LBP2-WT1	IR346	Guangzhou Anbiping Pharmaceutical Technology Co., Ltd.
CD56	1:400	Rabbit monoclonal	MRQ-42	IR040	Guangzhou Anbiping Pharmaceutical Technology Co., Ltd.
Vimentin	1:400	Rabbit monoclonal	LBP2-Vim	IR142	Guangzhou Anbiping Pharmaceutical Technology Co., Ltd.
Brahma-related gene 1	1:500	Rabbit polyclonal	CSB-PA851527	LA11HU	Cusabio Technology, LLC