Pathol. Oncol. Res. (2011) 17:639–644
DOI 10.1007/s12253-011-9364-0

RESEARCH

Immunoexpression of SALL4 in Wilms Tumors and Developing Kidney

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Received: 5 November 2010 / Accepted: 6 January 2011 / Published online: 22 January 2011

Abstract SALL4 is a zinc finger transcription factor that plays a role in the maintainence and pluripotency of embryonic stem cell and is important in renal development where *SALL4* mutations give rise to renal malformations. Because Wilms tumor recapitulates renal embryogenesis, we hypothesized that Wilms tumor cells may also express SALL4. We performed immunohistochemistry for SALL4 on tissue microarray sections of Wilms tumors, nephrogenic rests, and fetal renal cortices. Half (26 out of 52) of the Wilms tumors showed SALL4 immunoreactivity, ranging from strong and diffuse to focal and weak. Blastemal, epithelial, and combined blastemal and epithelial patterns of immunoreactivity were identified. No cases showed stromal staining. In the fetal kidney, SALL4 expression was restricted to the blastema and primitive epithelium at 15 weeks' gestation. SALL4 staining was not seen at later gestational ages, in non-neoplastic postnatal kidneys, or in nephrogenic rests. Our study is the first to demonstrate SALL4 immunoreactivity in Wilms tumors and in developing fetal kidney. The absence of SALL4 staining in nephrogenic rests, the presumed precursors of Wilms tumors, is intriguing and suggests that Wilms tumors have a pluripotency quality that may be lacking in nephrogenic rests.

Keywords Immunohistochemistry · Kidney development · Nephrogenic rest · SALL4 · Wilms tumor

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Introduction

Wilms tumor is a primitive multilineage malignant neoplasm of embryonic renal precursor cells that recapitulates renal embryogenesis and is often associated with and presumed to arise from persistent foci of embryonic renal tissue called nephrogenic rests. Since Wilms tumors have the potential for multilineage differentiation, it is likely that the Wilms tumor cells have a stem cell phenotype. Indeed, the presence of cells with stem cell phenotype may be responsible for resistance to chemo- and radiotherapy and post-therapy relapse in Wilms tumors [1]. SALL4 is a zinc finger transcription factor, which, along with Oct4, Pou5f1, and NanoG, plays a role in the maintainence and pluripotency of embryonic stem cells [2, 3]. Pertinently, SALL4 is important in renal development, and *SALL4* mutations produce renal developmental abnormalities as a part of multiple congenital anomaly syndromes such as the DRRS (Duane-radial ray syndrome) and the IVIC (Instituto Venezolano de Investigaciones Científicas) syndrome. We hypothesized that SALL4 might be expressed by Wilms tumor cells. Therefore, we examined immunohistochemical staining for SALL4 in Wilms tumors, nephrogenic rests, and fetal kidneys.

Materials and Methods

Formalin-fixed and Paraffin-embedded Tissue

This study was conducted with the approval of UT Southwestern Institutional Review Board. Immunohistochemistry for SALL4 was performed on tissue microarray sections of 52 Wilms tumor, 6 nephrogenic rests, and 13 fetal renal cortices spanning 15 to 39 weeks' gestation. The Wilms tumor and fetal renal cortex tissue microarrays

contain 2 mm cores of each case. The nephrogenic rest tissue microarray contains 3 mm cores of each case. All tissue microarrays contain cores of non-neoplastic postnatal renal cortices as controls.

Immunohistochemistry

Anti-SALL4 antibody (Abnova Corporation, Taipei City, Taiwan) was used at a dilution of 1:200. Immunohistochemistry was performed on a Ventana BenchMark XT automated immunostainer (Ventana Medical Systems, Tucson, AZ) using standard immunoperoxidase techniques and hematoxylin counterstaining. Appropriate positive and negative controls were utilized for each run of immunostains. Only nuclear reactivity was considered positive. The staining intensity was graded on a semiquantitative scale as weak, moderate, or strong. For Wilms tumors and nephrogenic rests, the percentage of positively staining cells was graded as 0 (no staining), 1+ (>0 and ≤25% of cells positive), 2+ (>25 and ≤50% of cells positive), 3+ (>50 and ≤90% of cells positive), or 4+ (>90% of cells positive). In addition, a note was made of the histologic cell types (blastemal, epithelial, stromal) that showed or did not show SALL4 staining. For non-neoplastic fetal and postnatal renal cortices, the staining intensity and the types of cells/structures that stained for SALL4 were noted.

Statistical Analyses

The Fisher exact test was used to compare the SALL4 staining results to the clinicopathologic findings of histologic grade, lymph node involvement, capsular invasion, renal sinus invasion, and surgical margin involvement. Analysis of variance was used to compare the SALL4 staining results and the local stage at diagnosis. For both sets of analyses, P value of less than 0.05 was considered statistically significant.

Results

Wilms Tumor Characteristics

The 52 Wilms tumors were from pediatric patients seen at our institution over a 10-year period between 2000 and 2009. The patients ranged in age from 4 months to 16 years/3 months (mean age of 3 years/7 months; median age of 3 years/4 months). There were 25 males and 27 females. Twenty five tumors occurred in the right kidney and 27 in the left. There were no bilateral tumors. Forty-seven (90%) tumors were unifocal and 5 (10%) were multifocal. The tumor size varied from 2.5 cm to 20 cm (mean size of 10.9 cm; median size of 11 cm). Forty-seven (90%) tumors were classified as having favorable histology, while 5 (10%) showed diffuse anaplasia. In 18 (35%) cases, nephrogenic rests were identified. By pathologic examination, 12 (23%) tumors were stage I, 21 (40%) were stage II, and 19 (37%) were stage III.

Immunohistochemical Staining for SALL4 in Wilms Tumors

Of the 52 Wilms tumors, 26 (50%) showed unequivocal nuclear staining for SALL4. Of these, 15 (29%) tumors showed SALL4 labeling of the epithelial and blastemal elements. Blastemal staining alone was seen in 8 (15%) cases, while the staining was restricted to the epithelial elements in 3 (6%) cases. No cases showed any staining of the stromal elements. Sixteen (31%) cases showed 1+ staining, 5 cases (9%) showed 2+ staining, 4 (8%) cases showed 3+ staining, and 1 case (2%) showed 4+ staining (Fig. 1).

When compared to clinicopathologic criteria used for the grading and staging of Wilms tumors, there were no significant differences between cases that expressed SALL4 and those that did not.

Immunohistochemical Staining for SALL4 in Nephrogenic Rests

None of the 6 nephrogenic rests (4 perilobar and 2 intralobar nephrogenic rests) showed SALL4 immunoreactivity (Fig. 2).

Immunohistochemical Staining for SALL4 in Fetal and Mature Kidney

Nuclear staining for SALL4 was restricted to focal strong staining of the primitive epithelial structures and weak staining of the adjacent blastemal mesenchyme in the fetal renal cortex at 15 weeks' gestation (Fig. 3). SALL4 staining was not observed in the fetal kidneys at later gestational ages or in postnatal mature kidneys.

Discussion

The Drosophilia spalt is a multiple double zinc finger motif transcription factor important for promoting terminal differentiation of the anterior and posterior compartments of Drosophilia [4]. Currently, four human spalt gene homologues have been identified (*SALL1-4*) that play roles in fetal development. Of these *SALL1* and *SALL4* are clearly implicated in renal development. *SALL1* gene mutations cause Townes-Brocks syndrome (OMIM #107480), an autosomal dominant disease characterized

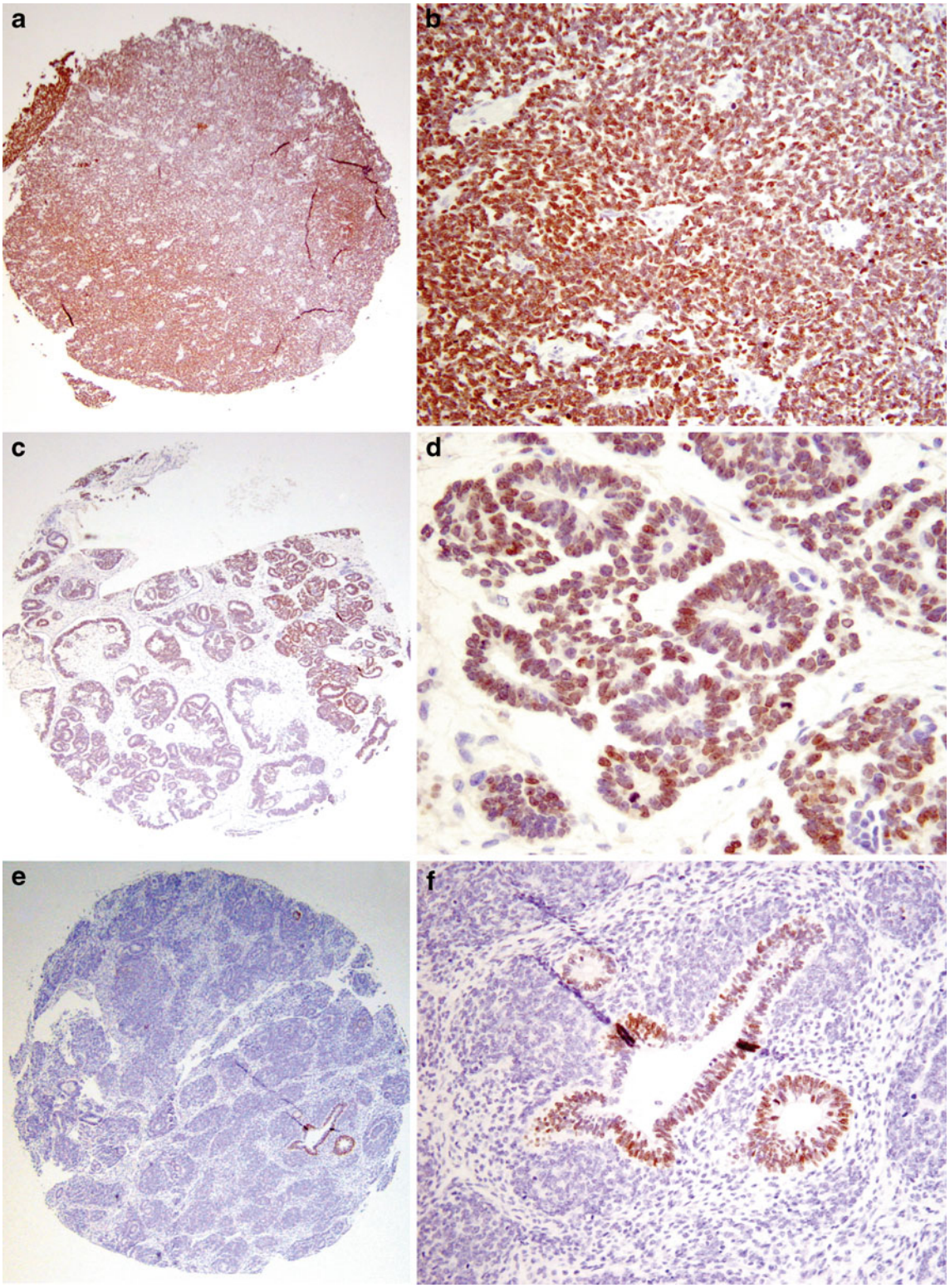

Fig. 1 SALL4 immunostaining in Wilms tumors. **a**, **b**. Diffuse and strong blastemal and epithelial staining. **c**, **d**. Moderate epithelial staining with no staining of stromal cells. **e**, **f**. Focal epithelial component. (original magnification ×40 for **a**, **c**, **d**, ×100 for **b**, **f**, and ×200 for **d**)

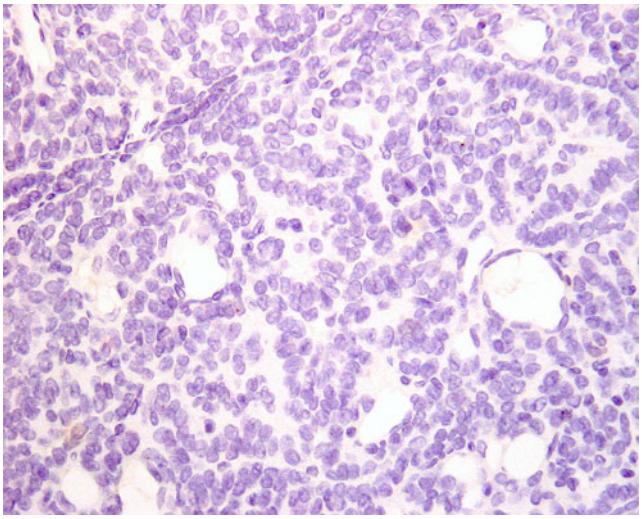

Fig. 2 SALL4 immuostaining in a nephrogenic rest. No staining was seen in six of six nephrogenic rests studied. (original magnification ×200)

by urogenital, anal, limb, ear, and cardiac abnormalities. The renal malformations include hypoplastic or dysplastic kidneys, renal agenesis, and multicystic kidneys [5, 6]. *SALL1* may be a renal stem cell marker and is expressed in Wilms tumors and in ureteric buds, immature tubules, and adjacent metanephric mesenchyme in human fetal kidney at 12 weeks' gestation [7–9]. It is a putative downstream target of *WT1*, which suggests that *SALL1* dysregulation may play a role in Wilms tumorigenesis [10].

SALL4, the most recently described human spalt homologue, is located on chromosome region 20q13.3. It is a stem cell transcription factor that plays a role in maintainence of pluripotency by forming a regulatory network with Oct4, Pou5f1, and NanoG [3, 11]. *SALL4* gene mutations cause DRRS and IVIC syndrome. The autosomal dominant DRRS, also known as Okihiro or acro-renal-ocular syndrome (OMIM #607323), is characterized by the Duane anomaly (restricted lateral eye movement because of abducens nerve palsy) in combination with upper limb and renal abnormalities and occasionally anogenital abnormalities and colonic aganglionosis [12–20]. The IVIC syndrome (OMIM #147750) is similar to DRRS, with the additional findings of cardiac malformations, carpal osseous fusion, thrombocytopenia, and leukocytosis [21].

Constituitive expression of *SALL4* represses the activities of the *SALL1* and *PTEN*, both of which are important in renal development [22]. SALL4 also binds to SALL1, forming heterodimers in the brain, heart, and anogenital region. Truncated SALL1 inhibited SALL4 activity, which suggests that the abnormalities seen in association with *SALL1* mutations (Townes-Brocks syndrome) may be due to SALL4 inhibition [23]. Here, we demonstrate, for the first time, immunohistochemical expression of SALL4 in human fetal kidney. SALL4 immunoreactivity was seen in human fetal kidney at 15 weeks' gestation. SALL1 expression has previously been identified in human fetal kidney at 12 weeks' gestation. Since SALL4 and SALL1 interact with each other, it would be expected that they would have similar temporal and spatial expression patterns.

To the best of our knowledge, this is the first report of SALL4 expression in Wilms tumors. Because SALL1 and SALL4 play a role in renal development, and the *SALL* gene complex is putative downstream target of *WT1*, it is no surprise that *SALL* genes are dysregulated in Wilms tumors. Of note, SALL1 expression has been demonstrated in blastemal and epithelial components, but not in the stromal component, in three Wilms tumors [8]. This staining pattern is similar to the staining pattern in SALL4-positive Wilms tumors.

SALL4 is consistently expressed by neoplastic cells in most germ cell tumors, namely choriocarcinomas, germinomas, embryonal carcinomas, yolk sac tumors, gonadoblastomas, and intratubular germ cell neoplasia (ITGCN). Focal weak epithelial reactivity has been described in immature and mature teratoms [24–29]. In addition to germ cell tumors, SALL4 may occasionally be expressed by carcinomas, sarcomas, peripheral neuroectodermal tumors (PNET), and ovarian clear cell carcinomas [24–26]. In a recent study, SALL4 stained all cases of alpha-fetoprotein (AFP) producing gastric carcinoma. [30] SALL4 is also overexpressed in leukemias and myelodysplastic syndrome [11, 31–33]. Our results add Wilms tumors to the growing list of tumors that express SALL4.

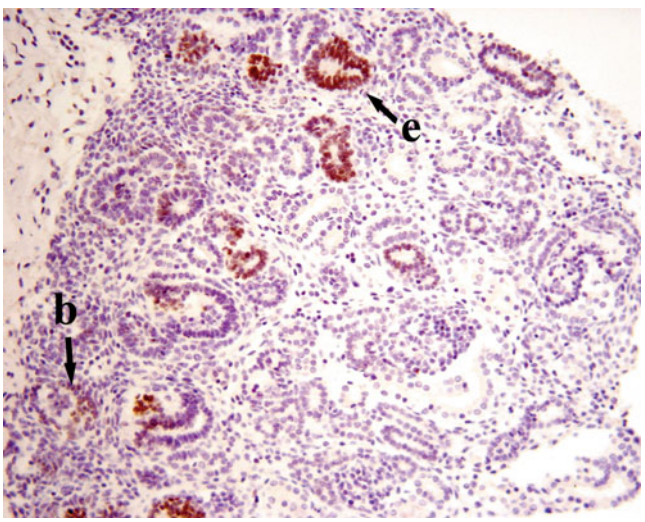

Fig. 3 SALL4 immunostaining in fetal renal cortex at 15 weeks' gestation. There is focal strong staining of the primitive epithelial structures (**e**) and focal weak staining of the blastemal mesenchyme (**b**). (original magnification ×200)

Interestingly, none of 6 nephrogenic rests studied here showed any immunoexpression of SALL4. All the nephrogenic rests stained in this study were from kidneys with Wilms tumors and therefore represented nephroblastomatosis. Wilms tumors are often associated with and thought to arise from nephrogenic rests. Our data, limited by the small number of nephrogenic rests studied, suggests that one of the differences between nephrogenic rests and Wilms tumors may be the lack of stem-cell like characteristics in nephrogenic rests and the presence of stem-cell like characteristics in Wilms tumors. Further investigations in this direction may yield a greater understanding of the molecular events that orchestrate the progression of nephrogenic rests to Wilms tumors. SALL4 immunostaining may be of value in distinguishing nephrogenic rests from Wilms tumors in small biopsies.

Acknowledgements The data in this manuscript was presented at the 2010 Fall Meeting of the Society for Pediatric Pathology held in Banff, Alberta, Canada. The abstract titled "Immunohistochemical expression of SALL4 in Wilms tumors, nephrogenic rests, and fetal and postnatal renal cortices" earned Dr Jeremy Deisch the Gordon L. Vawter Award for the best presentation by a trainee.

The authors thank Ping Shang HT(ASCP)QIHC at the University of Texas Southwestern Immunohistochemistry Laboratory for the excellent immunohistochemical staining.

References

1. Pode-Shakked N, Metsuyanim S, Rom-Gross E et al (2009) Developmental tumourigenesis: NCAM as a putative marker for the malignant renal stem/progenitor cell population. J Cell Mol Med 13:1792–1808
2. Yang J, Chai L, Fowles TC et al (2008) Genome-wide analysis reveals Sall4 to be a major regulator of pluripotency in murine-embryonic stem cells. Proc Natl Acad Sci USA 105:19756–19761
3. Zhang J, Tam WL, Tong GQ et al (2006) Sall4 modulates embryonic stem cell pluripotency and early embryonic development by the transcriptional regulation of Pou5f1. Nat Cell Biol 8:1114–1123
4. Kuhnlein RP, Frommer G, Friedrich M et al (1994) Spalt encodes an evolutionarily conserved zinc finger protein of novel structure which provides homeotic gene function in the head and tail region of the Drosophila embryo. EMBO J 13:168–179
5. Kohlhase J, Taschner PE, Burfeind P et al (1999) Molecular analysis of SALL1 mutations in Townes-Brocks syndrome. Am J Hum Genet 64:435–445
6. Kohlhase J, Wischermann A, Reichenbach H, Froster U, Engel W (1998) Mutations in the SALL1 putative transcription factor gene cause Townes-Brocks syndrome. Nat Genet 18:81–83
7. Metsuyanim S, Harari-Steinberg O, Buzhor E et al (2009) Expression of stem cell markers in the human fetal kidney. PLoS ONE 4:e6709
8. Ma Y, Singer DB, Gozman A et al (2001) Hsal 1 is related to kidney and gonad development and is expressed in Wilms tumor. Pediatr Nephrol 16:701–709
9. Metsuyanim S, Pode-Shakked N, Schmidt-Ott KM et al (2008) Accumulation of malignant renal stem cells is associated with epigenetic changes in normal renal progenitor genes. Stem Cells 26:1808–1817
10. Chai L, Yang J, Di C et al (2006) Transcriptional activation of the SALL1 by the human SIX1 homeodomain during kidney development. J Biol Chem 281:18918–18926
11. Yang J, Chai L, Gao C et al (2008) SALL4 is a key regulator of survival and apoptosis in human leukemic cells. Blood 112:805–813
12. Al-Baradie R, Yamada K, St Hilaire C et al (2002) Duane radial ray syndrome (Okihiro syndrome) maps to 20q13 and results from mutations in SALL4, a new member of the SAL family. Am J Hum Genet 71:1195–1199
13. Borozdin W, Boehm D, Leipoldt M et al (2004) SALL4 deletions are a common cause of Okihiro and acro-renal-ocular syndromes and confirm haploinsufficiency as the pathogenic mechanism. J Med Genet 41:e113
14. Borozdin W, Wright MJ, Hennekam RC et al (2004) Novel mutations in the gene SALL4 provide further evidence for acro-renal-ocular and Okihiro syndromes being allelic entities, and extend the phenotypic spectrum. J Med Genet 41:e102
15. Kohlhase J, Chitayat D, Kotzot D et al (2005) SALL4 mutations in Okihiro syndrome (Duane-radial ray syndrome), acro-renal-ocular syndrome, and related disorders. Hum Mutat 26:176–183
16. Kohlhase J, Heinrich M, Schubert L et al (2002) Okihiro syndrome is caused by SALL4 mutations. Hum Mol Genet 11:2979–2987
17. Kohlhase J, Schubert L, Liebers M et al (2003) Mutations at the SALL4 locus on chromosome 20 result in a range of clinically overlapping phenotypes, including Okihiro syndrome, Holt-Oram syndrome, acro-renal-ocular syndrome, and patients previously reported to represent thalidomide embryopathy. J Med Genet 40:473–478
18. Miertus J, Borozdin W, Frecer V et al (2006) A SALL4 zinc finger missense mutation predicted to result in increased DNA binding affinity is associated with cranial midline defects and mild features of Okihiro syndrome. Hum Genet 119:154–161
19. Terhal P, Rosler B, Kohlhase J (2006) A family with features overlapping Okihiro syndrome, hemifacial microsomia and isolated Duane anomaly caused by a novel SALL4 mutation. Am J Med Genet A 140:222–226
20. Okihiro MM, Tasaki T, Nakano KK, Bennett BK (1977) Duane syndrome and congenital upper-limb anomalies. A familial occurrence. Arch Neurol 34:174–179
21. Paradisi I, Arias S (2007) IVIC syndrome is caused by a c.2607delA mutation in the SALL4 locus. Am J Med Genet A 143:326–332
22. Lu J, Jeong HW, Kong N et al (2009) Stem cell factor SALL4 represses the transcriptions of PTEN and SALL1 through an epigenetic repressor complex. PLoS ONE 4:e5577
23. Sakaki-Yumoto M, Kobayashi C, Sato A et al (2006) The murine homolog of SALL4, a causative gene in Okihiro syndrome, is essential for embryonic stem cell proliferation, and cooperates with Sall1 in anorectal, heart, brain and kidney development. Development 133:3005–3013
24. Cao D, Guo S, Allan RW, Molberg KH, Peng Y (2009) SALL4 is a novel sensitive and specific marker of ovarian primitive germ cell tumors and is particularly useful in distinguishing yolk sac tumor from clear cell carcinoma. Am J Surg Pathol 33:894–904
25. Cao D, Humphrey PA, Allan RW (2009) SALL4 is a novel sensitive and specific marker for metastatic germ cell tumors, with particular utility in detection of metastatic yolk sac tumors. Cancer 115:2640–2651
26. Cao D, Li J, Guo CC, Allan RW, Humphrey PA (2009) SALL4 is a novel diagnostic marker for testicular germ cell tumors. Am J Surg Pathol 33:1065–1077
27. Liu A, Cheng L, Du J et al (2010) Diagnostic utility of novel stem cell markers SALL4, OCT4, NANOG, SOX2, UTF1, and TCL1

in primary mediastinal germ cell tumors. Am J Surg Pathol 34:697–706
28. Mei K, Liu A, Allan RW et al (2009) Diagnostic utility of SALL4 in primary germ cell tumors of the central nervous system: a study of 77 cases. Mod Pathol 22:1628–1636
29. Wang F, Liu A, Peng Y et al (2009) Diagnostic utility of SALL4 in extragonadal yolk sac tumors: an immunohistochemical study of 59 cases with comparison to placental-like alkaline phosphatase, alpha-fetoprotein, and glypican-3. Am J Surg Pathol 33:1529–1539
30. Ushiku T, Shinozaki A, Shibahara J et al (2010) SALL4 represents fetal gut differentiation of gastric cancer, and is diagnostically useful in distinguishing hepatoid gastric carcinoma from hepatocellular carcinoma. Am J Surg Pathol 34:533–540
31. Shuai X, Zhou D, Shen T et al (2009) Overexpression of the novel oncogene SALL4 and activation of the Wnt/beta-catenin pathway in myelodysplastic syndromes. Cancer Genet Cytogenet 194:119–124
32. Cui W, Kong NR, Ma Y, Amin HM, Lai R, Chai L (2006) Differential expression of the novel oncogene, SALL4, in lymphoma, plasma cell myeloma, and acute lymphoblastic leukemia. Mod Pathol 19:1585–1592
33. Ma Y, Cui W, Yang J et al (2006) SALL4, a novel oncogene, is constitutively expressed in human acute myeloid leukemia (AML) and induces AML in transgenic mice. Blood 108:2726–2735