Pathology (January 2019) **51(1)**, pp. 62–66

ANATOMICAL PATHOLOGY

Analysis of chromosome 12p over-representation and clinicopathological features in mediastinal teratomas

Taebum Lee, Youjeong Seo, Joungho Han, Ghee Young Kwon

Department of Pathology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

Summary

Teratomas show diverse biologic behaviour and prognosis as well as variable histological features. Importantly, post-pubertal testicular teratomas composed of mature components have a potential for malignant behaviour, in contrast to ovarian dermoid cysts and pre-pubertal testicular teratomas which are considered almost always benign. On the other hand, the biological behaviour and histogenesis of extragonadal teratomas are still not fully elucidated. In this study of mediastinal mature teratoma (MT), we investigated clinicopathological features and chromosome 12 short arm (12p) status which constitutes a major genetic aberration in the germ cell tumours (GCT) and is indicative of malignant potential. A total of 123 cases of primary mediastinal MT were included, and clinical data were retrieved regarding demographic information, adjuvant treatment, post-operative clinical course, and level of serum tumour markers. Histopathological features were evaluated in 123 cases and 12p status was studied by FISH in 25 cases. Female predilection was identified in the post-pubertal group (38 males vs 77 females), and paediatric teratoma cases had longer follow-up (mean 62.2 months vs 26.5 months). All patients had excellent prognosis with no tumour-associated death, regardless of age and sex. None of the MT cases had cytological atypia and all 21 cases finally evaluated by fluorescence *in situ* hybridisation were negative for 12p over-representation. Our results support the benign nature of mediastinal MT and suggest the possibility that it may share a common histogenesis with pre-pubertal type GCTs.

Key words: Germ cell tumour; teratoma; extragonadal teratoma; mediastinal teratoma; isochromosome 12p.

Received 30 April, revised 16 August, accepted 4 October 2018
Available online 20 November 2018

INTRODUCTION

Germ cell tumour (GCT) is one of the most common cancers in adolescence and comprises a heterogeneous group of tumours.[1,2] While the overwhelming majority arises from the gonads, a small proportion of GCT can be found in extragonadal sites, such as the pineal/intracranial space, mediastinum and sacrococcygeal region.[3,4] The biological behaviour of GCTs depends on various independent factors and extragonadal GCTs have different clinical characteristics from gonadal GCTs even when they contain histologically similar components.[5]

Teratomas are classified as a member of GCTs and, by definition, have tissues or organ components derived from more than one germ layer.[6] Their biological behaviours vary depending on age, sex, site or histological features. The crucial determinant of aggressive behaviour of ovarian teratoma is the presence of immature components or malignant transformation of their elements. However, the appraisal of malignant potential is more complicated in post-pubertal testicular teratoma, which has a potential for malignant behaviour even when the tumours consist entirely of mature components.[7,8] This malignant potential for post-pubertal testicular teratoma is explained by the development of type II non-seminomatous GCT. During the process, seminomatous cells are reprogrammed to totipotent embryonal carcinoma (EC) cells, which may give rise to somatic lineage in varying degrees of maturation.[9] This pathway is different from that of type I or IV GCTs, which originate from direct reprogramming of methylated primitive germ cells (PGCs)/gonocytes or oogonia/oocytes, without prior malignant transformation.[10]

The histogenesis of extragonadal GCT has not been clearly elucidated and there is a limited number of studies regarding extragonadal teratomas.[6,11,12] Two recent series suggest the benign nature of sacrococcygeal teratomas regardless of age, sex or the presence of immature components.[6,11] Intermediate biological behaviour has been reported in mediastinal teratomas but has not been sufficiently validated.[12]

Over-representation of 12p is a landmark finding in the majority of malignant testicular GCTs,[13] found as isochromosome 12p (i12p) in about 80% of invasive testicular GCTs and as 12p amplification due to other chromosomal changes in the remaining 20%.[14,15] Chromosome material on extra copies of 12p is thought to suppress induction of apoptosis of invasive tumour cells.[16] This cytogenetic characteristic has been employed as a molecular indicator of malignancy in some unusual GCTs with uncertain histogenesis. For example, there have been reports on benign teratomas in post-pubertal testis, which suggest the existence of post-pubertal type I GCT in testis.[8] The benign nature of such tumours was supported by the absence of germ cell neoplasia *in situ* and the lack of 12p over-representation as was also the case in sacrococcygeal teratomas.[6,11] To date, there have been only a few reports on 12p status in mediastinal GCT.

Herein, we sought to evaluate the clinicopathological features of mature mediastinal teratomas and analyse 12p status to understand the biological nature and predict

Print ISSN 0031-3025/Online ISSN 1465-3931
DOI: https://doi.org/10.1016/j.pathol.2018.10.002

clinical behaviour, especially in post-pubertal male patients.

MATERIALS AND METHODS

Clinicopathological evaluation

The surgical pathology database at the Samsung Medical Center, Seoul, Korea, was searched for primary mediastinal mature teratoma (MT) cases between 1996 and 2015. Two pathologists (TL and GK) reviewed all haematoxylin and eosin stained slides along with available gross photographs. Cases of benign dermoid cyst were not included, defined as unilocular cyst composed only of stratified squamous epithelium and associated adnexa.[9] Among all 133 primary mediastinal MT cases, 10 cases were excluded because slides and paraffin blocks were not available. Clinical and laboratory data, including age, sex, tumour size, alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), beta-human chorionic gonadotropin (β-hCG), surgical resection, adjuvant chemotherapy, survival, recurrence and follow-up were retrieved from electronic medical records. This study was approved by the institutional review board of Samsung Medical Center.

Construction of tissue microarrays

Twenty-five MT cases focusing on the post-pubertal male group were selected to test for 12p status by fluorescence *in situ* hybridisation (FISH), considering fixation status and representativeness of tumour components. Two, 18 and 5 cases were included from pre-pubertal, post-pubertal male and post-pubertal female groups, respectively. Upon slide review, two histologically representative and diverse areas in each tumour were preferentially selected with a 2 mm diameter. Tissue microarray (TMA) blocks were produced according to the standard procedure,[17] using a manual tissue microarrayer (Quick-Ray UT06; Unitima, Republic of Korea).

For validation of the FISH probe, an additional TMA of gonadal GCT cases was constructed containing one pre-pubertal teratoma, one post-pubertal teratoma, one mixed GCT and two seminoma cases. Nine histologically different areas were selected in a TMA block, containing components of MT, EC, immature teratoma (IT) or seminoma.

Fluorescence *in situ* hybridisation

FISH tests were performed using a CEP 12 control probe (D12Z3; Abbott Molecular-Vysis, USA) and a LSI KRAS probe for 12p12.1 region on chromosome 12 (Abbott). The sections were cut from the TMA blocks 4 μm thick, attached to adhesive-coated slides and dried in an incubator for 16 h at 56°C. After deparaffinisation in xylene and graded ethanol, the slides were co-denaturated with mixed probe at 73°C for 5 min and hybridised overnight at 37°C in humidified atmosphere, using Thermobrite (Abbott). Slides were washed in 0.4x SSC/0.3% NP40 for 2 min, 2x SSC/0.1% NP40 for 1 min at 72°C and for 10 min at room temperature, and counterstained with DAPI. Signals were assessed under BX51TRF microscope (Olympus, Japan). For each tumour, 40–160 individual nuclei were evaluated and a ratio of the averaged 12p to centromere signals above 1.3 was regarded as indicative of 12p over-representation.[18] Among 25 cases, four did not satisfy the minimum nuclear number of evaluation criteria and 21 MT cases were finally evaluated.

RESULTS

Clinicopathological characteristics of 123 mediastinal MT cases are summarised in Table 1. In eight pre-pubertal MT patients, sex predilection was not found (4 boys vs 4 girls); but female predominance was noticed in post-pubertal MT (38 males vs 77 females). The paediatric patients ranged from 1 to 9 years with a median age of 3.5 years, and the post-pubertal patients ranged from 14 to 73 years with a median age of 33 years. The follow-up length was variable, but generally longer in paediatric cases than in post-pubertal cases (mean 62.2 months vs 26.5 months). At the time of diagnosis, age-adjusted serum AFP, CEA and β-hCG levels were within normal limits in all teratoma patients, except one. A 37-year-old woman showed mild elevation in serum AFP level (57 ng/mL) before surgical resection, but she was free of recurrence during the follow-up period (195 months). All patients underwent surgical resection. One 39-year-old female patient received chemotherapy for lung cancer, which was detected 3 years after the diagnosis of MT. Recurrence or metastasis was not identified, and all patients survived during the follow-up period.

The mean tumour size showed no difference regardless of sex and age. Most tumours were well-demarcated cystic and/or solid masses. Forty-four cases (36%) showed unilocular cystic tumour (1 pre-pubertal vs 12 post-pubertal males vs 31 post-pubertal females) which usually contained greasy materials and hair, 56 cases (46%) were multilocular or mixed cystic and solid (4 pre-pubertal vs 19 post-pubertal males vs 33 post-pubertal females), and 14 cases (11%) were solid (3 pre-pubertal vs 3 post-pubertal males vs 8 post-pubertal females). Nine cases (4 post-pubertal males and 5 post-pubertal females) had been submitted in fragmented lumps and gross morphology had not been adequately described. On microscopic examination, the most common component in all 123 MT cases was keratinising or non-keratinising squamous epithelium found in 116 cases (94%), followed by adnexa (78 cases, 63%), respiratory-type epithelium (75 cases, 61%), and fat (68 cases, 55%). In eight pre-pubertal MT cases, the most common component was cartilage, found in seven cases (88%), followed by

Table 1 Summary of clinicopathological features and chromosome 12p data for 123 mature mediastinal teratoma cases

	All	Pre-pubertal MT	Post-pubertal MT male	Post-pubertal MT female
N	123 (100%)	8 (6%)	38 (31%)	77 (63%)
Age range, years (median)	1–73 (33)	1–9 (3.5)	14–70 (39)	14–73 (32)
Follow-up, months	1–195	1–141	1–109	1–195
Survival	100% (123/123)	100% (8/8)	100% (38/38)	100% (77/77)
Recurrence	0% (0/123)	0% (0/8)	0% (0/38)	0% (0/77)
Mean size, cm ± 2SD	7.2 ± 6.4	7.1 ± 5.1	7.3 ± 6	7.3 ± 6.8
AFP	2% (1/47)	0% (0/8)	0% (0/17)	4% (1/22)
CEA	0% (0/38)	0% (0/1)	0% (0/16)	0% (0/21)
β-hCG	0% (0/30)	0% (0/4)	0% (0/8)	0% (0/18)
Chemotherapy	1% (1/123)	0% (0/8)	0% (0/38)	1%[a] (1/77)
Surgical resection	100% (123/123)	100% (8/8)	100% (38/38)	100% (77/77)
Chromosome 12p	0% (0/21)	0% (0/1)	0% (0/12)	0% (0/8)

AFP, alpha-fetoprotein; β-hCG, beta-human chorionic gonadotropin; CEA, carcinoembryogenic antigen; IT, immature teratoma; MT, mature teratoma; NA, not available; ND, not done.
[a] One patient received chemotherapy for incidentally found lung cancer.

squamous epithelium (6 cases, 75%) and fat (6 cases, 75%). Thirty-eight male post-pubertal MT cases showed squamous epithelium in 37 cases (97%), followed by adnexa (24 cases, 63%), respiratory-type epithelium (22 cases, 58%) and fat (21 cases, 55%). Similarly, 77 post-pubertal female MT cases had squamous epithelium in 73 cases (95%), followed by adnexa (51 cases, 66%), respiratory-type epithelium (48 cases, 62%), and fat (41 cases, 53%) (Table 2). There was no significant difference in the predominant component between the male and female groups. None of the cases showed cytological atypia or coexistence of yolk sac tumour (YST) component (Fig. 1).

Over-representation of 12p was studied by FISH and the probes were first validated by the control TMA block containing various GCT from testis. Over-representations of 12p signal were identified with seminoma cases but not in the benign MT cases (Fig. 2). Average 12p to centromere signal ratio of seminoma was 1.31.

None of the 21 cases evaluated by FISH, composed of one pre-pubertal, 15 post-pubertal male and 5 post-pubertal female cases, showed presence of i12p or 12p amplification (Table 1, Fig. 1). Only a few coincident increases in CEP 12 and 12p signals were identified, but 12p/centromere ratio was between 0.77 and 0.93 (Supplementary Table 1, Appendix A).

DISCUSSION

Biological behaviour of teratoma in post-pubertal patients is an interesting issue. Testicular teratomas in post-pubertal patients almost always carry possibilities of invasive growth, recurrence and metastasis, and thus are classified as a malignancy,[7] while dermoid cyst in ovary as well as teratoma in pre-pubertal testis are deemed entirely benign. These divergent behaviours can be comprehended in the context of histogenesis models of germ cell neoplasms, according to their development potential.[19] Pre-pubertal testicular teratoma is classified as type I GCT which probably arises from one pluripotent methylated PGC/gonocyte, and type IV GCT or dermoid cyst of ovary arises from oogonia/oocyte. Except teratoma harbouring a YST component or high degree IT in ovary,[20] all of these tumours, henceforth referred to as 'pre-pubertal type', follow a favourable clinical course and show a normal diploid karyotype lacking chromosomal aberrations.[10] In contrast, post-pubertal testicular teratoma is classified as type II GCT which arises from hypomethylated PGC/gonocyte. Totipotent EC cells, which are reprogrammed from seminomatous cells, lead to all lineages of embryogenesis including somatic lineage.[21,22] The overwhelming majority of testicular teratomas in post-pubertal patients, henceforth referred to as 'post-pubertal type', usually show aneuploidy, chromosomal rearrangements, and cytological atypia.[10]

Unlike testicular teratomas of adolescents and adults, post-pubertal extragonadal MTs are generally regarded as benign.[23] However, since post-pubertal extragonadal teratomas are rare, the clinical evidence is not sufficient and the clinical decisions on treatment are based on a few anecdotal experiences and inferences. Clinicopathological characteristics and genetic aberrations of sacrococcygeal teratomas in two recent studies showed that the tumours behave in a benign manner with favourable outcome,[6,11] whereas in the anterior mediastinum teratomas are not always benign.[12] Therefore, it is clinically important to elucidate the malignant potential of mediastinal MT, which will also help to gain insight into the histogenesis of GCT.

Diverse genetic abnormalities in GCT have been studied.[14,15] Over-representation of 12p is the major genetic aberration in testicular as well as other types of GCT, and is often used as an indicator of malignant potential.[14] Approximately 80% of testicular GCT cases have i12p and the remainder have 12p amplification.[15] A number of post-pubertal testicular teratomas harbour this cytogenetic abnormality independent of histological component, but only a few studies have been conducted regarding 12p status in extragonadal teratomas. In the sacrococcygeal region, no cytogenetic aberrations or over-representation of 12p was identified, in either pre- or post-pubertal teratomas, unless an additional malignant GCT component was present.[6,11,24] Cytogenetic profiles in mediastinal GCTs have been reported in a few articles with conventional cytogenetic analysis[25] or array comparative genomic hybridisation (CGH)[26] performed in a small number of cases, but 12p over-representation status in mediastinal teratomas has not been studied with FISH to the best of our knowledge.

In our study, all mediastinal MT cases had an uneventful clinical course, regardless of age or sex, and therefore surgical resection was sufficient for all patients except one who had lung cancer. None of the components in any cases showed cytological atypia, which is frequently identified in post-pubertal testicular teratoma.[7] In 21 cases we evaluated for FISH, i12p or 12p amplification was totally absent, supporting the benign nature of mediastinal MTs similar to pre-pubertal type GCT.

Table 2 Summary of histological components for 123 mediastinal mature teratomas

	All (123)	Pre-pubertal MT (8)	Post-pubertal MT male (38)	Post-pubertal MT female (77)
Squamous epithelium	116 (94%)	6 (75%)	37 (97%)	73 (95%)
Adnexa	78 (63%)	3 (38%)	24 (63%)	51 (66%)
Respiratory-type epithelium	75 (61%)	5 (63%)	22 (58%)	48 (62%)
Glandular-type epithelium	38 (31%)	4 (50%)	13 (34%)	21 (27%)
Glands, NOS	28 (23%)	2 (25%)	9 (24%)	17 (22%)
Acini-type epithelium	38 (31%)	4 (50%)	12 (32%)	22 (29%)
Fat	68 (55%)	6 (75%)	21 (55%)	41 (53%)
Smooth muscle	30 (24%)	5 (63%)	11 (29%)	14 (18%)
Cartilage	61 (50%)	7 (88%)	19 (50%)	35 (45%)
Bone	14 (11%)	3 (38%)	4 (11%)	7 (9%)

MT, mature teratoma; NOS, not otherwise specified.

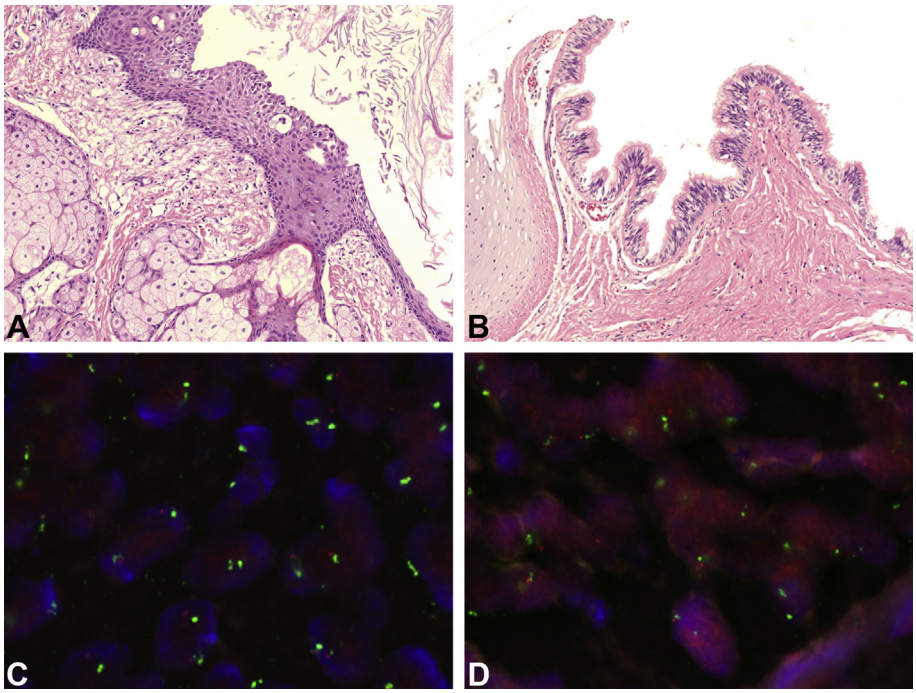

Fig. 1 Microscopic examination and 12p FISH analysis of mediastinal teratomas. (A) Squamous epithelium and adnexa are the most common components, (B) followed by respiratory-type epithelium (H&E stain). None of the cases showed cytological atypia. (C,D) Increased 12p/CEP12 ratio which indicates i12p or 12p amplification was not identified in all selected 21 cases, regardless of age, sex, and type of components.

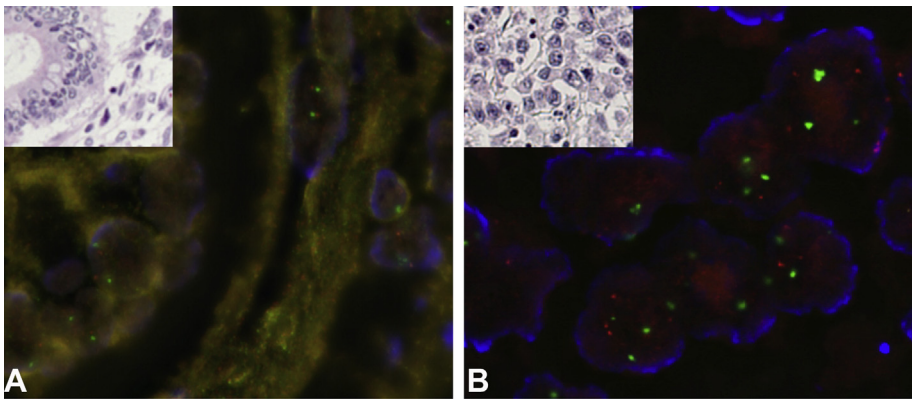

Fig. 2 Validation for 12p FISH test. (Green, CEP 12; Orange, LSI KRAS for 12p). (A) Chromosome 12p to centromere ratio is not increased in FISH, in stratified ciliated epithelium of mature teratoma (inset, H&E stain). (B) Over-representation of 12p is identified in testicular seminoma (inset, H&E).

Pre-pubertal type testicular teratomas have been recently reported in post-pubertal patients.[8,27] Typically these tumours are well differentiated without cytological atypia, germ cell neoplasia *in situ*, or cytogenetic alteration. They share some typical features of pre-pubertal type GCT, showing hair-containing cysts and rare progression to YST.[8] Mediastinal MTs in post-pubertal patients also have similar macroscopic and microscopic appearances,[28] with these features suggesting the possibility of common origin and histogenesis for pre-pubertal type GCT and mediastinal MT.

Considering the existence of a teratoma component in mediastinal mixed GCT, it is reasonable to assume that at least some of these tumours may evolve into mediastinal teratoma of type II GCT. The observation that 12p over-representation was absent from our cases of post-pubertal mediastinal MTs in males implies that all the analysed cases of our series are benign and that MT of type II GCT is vanishingly rare in the mediastinum. However, it cannot be completely excluded based on our observation that histogenesis of mediastinal MT may be different from testicular GCT and that even MT which has evolved from malignant GCT may not have 12p over-representation, as is the case in malignant ovarian GCT. Although cases of malignant mediastinal GCT such as germinoma were not included in our study, a few studies have documented the presence of 12p over-representation in mediastinal GCT. Therefore, taken together, our results suggest that the overwhelming majority of post-pubertal mediastinal MTs in male patients are pre-pubertal type GCTs, unlike testicular teratoma in the post-pubertal age group. A recent study in sacrococcygeal teratomas has suggested the presence of pre-pubertal type in that region[6] and additional studies are required to extrapolate this finding to other extragonadal sites.

Failure to include diverse forms of GCT with teratoma component is a major limitation of this study. It would have provided more information if the cases encompassed

mediastinal IT or malignant mediastinal GCT in pure form or mixed with teratoma. A recent study in the sacrococcygeal region showed absence of i12p and excellent outcome in all nine paediatric IT cases which did not have malignant germ cell component.[11] Malignant potential of mediastinal ITs has not been clearly defined[23] and evaluation of 12p over-representation status in mediastinal IT cases is necessary in further studies.

The small number of cases studied for cytogenetics is another limitation. In the post-pubertal male group we focused on, about 50% (18/38) of cases were tested, with 40% (15/38) finally evaluated by FISH. One of the reasons for this was that unilocular cystic tumours without a sufficient solid area were excluded because the thin wall portion is less likely to be informative in TMA. Alternative techniques such as chromosome banding analysis and array CGH were considered for evaluation of chromosomal alteration; however, they could not be performed due to technical issues and financial constraints.

In summary, this is the first study to our knowledge that has explored clinicopathological data and 12p over-representation status in mediastinal MTs. Our attention was focused on post-pubertal male patients, and mediastinal MTs were deemed benign in that clinical setting unlike post-pubertal testicular teratomas, supported by excellent prognosis, bland histological features, and the absence of 12p over-representation. It is suggested that post-pubertal mediastinal MT in males may share a common origin and histogenesis with pre-pubertal type GCT. Further study is required to make generalised conclusions regarding extragonadal teratomas from other sites.

Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pathol.2018.10.002.

Address for correspondence: Ghee Young Kwon, Department of Pathology, Samsung Medical Center, Sungkyunkwan University School of Medicine, 50 lrwon-dong, Gangnam-gu, Seoul, 135-710, Republic of Korea. E-mail: geeo.kwon@samsung.com

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin* 2016; 66: 7–30.
2. Suijkerbuijk RF, Looijenga L, de Jong B, *et al*. Verification of isochromosome 12p and identification of other chromosome 12 aberrations in gonadal and extragonadal human germ cell tumors by bicolor double fluorescence in situ hybridization. *Cancer Genet Cytogenet* 1992; 63: 8–16.
3. Harms D, Zahn S, Gobel U, *et al*. Pathology and molecular biology of teratomas in childhood and adolescence. *Klin Padiatr* 2006; 218: 296–302.
4. Hainsworth JD, Greco FA. Extragonadal germ cell tumors and unrecognized germ cell tumors. *Semin Oncol* 1992; 19: 119–27.
5. Nichols CR. Mediastinal germ cell tumors. Clinical features and biologic correlates. *Chest* 1991; 99: 472–9.
6. Gurda GT, VandenBussche CJ, Yonescu R, *et al*. Sacrococcygeal teratomas: clinico-pathological characteristics and isochromosome 12p status. *Mod Pathol* 2014; 27: 562–8.
7. Ulbright TM. Gonadal teratomas: a review and speculation. *Adv Anat Pathol* 2004; 11: 10–23.
8. Zhang C, Berney DM, Hirsch MS, *et al*. Evidence supporting the existence of benign teratomas of the postpubertal testis: a clinical, histopathologic, and molecular genetic analysis of 25 cases. *Am J Surg Pathol* 2013; 37: 827–35.
9. Ulbright TM, Amin MB, Balzer B, *et al*. *WHO Classification of Tumours of the Urinary System and Male Genital Organs*. 4th ed. Lyon: IARC Press, 2016; 189–226.
10. Oosterhuis JW, Looijenga LH. *Pathology and biology of human germ cell tumors*. 1st ed. New York: Springer, 2017. Chapter 3, Germ cell tumors from a developmental perspective: cells of origin, pathogenesis, and molecular biology (emerging patterns).
11. Emerson RE, Kao CS, Eble JN, *et al*. Evidence of a dual histogenetic pathway of sacrococcygeal teratomas. *Histopathology* 2017; 70: 290–300.
12. Yalcin B, Demir HA, Tanyel FC, *et al*. Mediastinal germ cell tumors in childhood. *Pediatr Hematol Oncol* 2012; 29: 633–42.
13. Mostert MC, Verkerk AJ, van de Pol M, *et al*. Identification of the critical region of 12p over-representation in testicular germ cell tumors of adolescents and adults. *Oncogene* 1998; 16: 2617–27.
14. Reuter VE. Origins and molecular biology of testicular germ cell tumors. *Mod Pathol* 2005; 18 (Suppl 2): S51–60.
15. Rodriguez E, Houldsworth J, Reuter VE, *et al*. Molecular cytogenetic analysis of i (12p)-negative human male germ cell tumors. *Genes Chromosomes Cancer* 1993; 8: 230–6.
16. Rosenberg C, Van Gurp R, Geelen E, *et al*. Overrepresentation of the short arm of chromosome 12 is related to invasive growth of human testicular seminomas and nonseminomas. *Oncogene* 2000; 19: 5858–62.
17. Kampf C, Olsson I, Ryberg U, *et al*. Production of tissue microarrays, immunohistochemistry staining and digitalization within the human protein atlas. *J Vis Exp* 2012; 63: 3620.
18. Wehle D, Yonescu R, Long PP, *et al*. Fluorescence in situ hybridization of 12p in germ cell tumors using a bacterial artificial chromosome clone 12p probe on paraffin-embedded tissue: clinical test validation. *Cancer Genet Cytogenet* 2008; 183: 99–104.
19. Oosterhuis JW, Looijenga LH. Testicular germ-cell tumours in a broader perspective. *Nat Rev Cancer* 2005; 5: 210–22.
20. De Backer A, Madern GC, Pieters R, *et al*. Influence of tumor site and histology on long-term survival in 193 children with extracranial germ cell tumors. *Eur J Pediatr Surg* 2008; 18: 1–6.
21. Kleinsmith LJ, Pierce Jr GB. Multipotentiality of single embryonal carcinoma cells. *Cancer Res* 1964; 24: 1544–51.
22. Oosterhuis JW, Looijenga LH. The biology of human germ cell tumours: retrospective speculations and new prospectives. *Eur Urol* 1993; 23: 245–50.
23. McKenney JK, Heerema-McKenney A, Rouse RV. Extragonadal germ cell tumors: a review with emphasis on pathologic features, clinical prognostic variables, and differential diagnostic considerations. *Adv Anat Pathol* 2007; 14: 69–92.
24. Veltman I, Veltman J, Janssen I, *et al*. Identification of recurrent chromosomal aberrations in germ cell tumors of neonates and infants using genomewide array-based comparative genomic hybridization. *Genes Chromosomes Cancer* 2005; 43: 367–76.
25. Oosterhuis JW, Rammeloo RH, Cornelisse CJ, *et al*. Ploidy of malignant mediastinal germ-cell tumors. *Hum Pathol* 1990; 21: 729–32.
26. Schneider DT, Schuster AE, Fritsch MK, *et al*. Genetic analysis of mediastinal nonseminomatous germ cell tumors in children and adolescents. *Genes Chromosomes Cancer* 2002; 34: 115–25.
27. Semjen D, Kalman E, Tornoczky T, *et al*. Further evidence of the existence of benign teratomas of the postpubertal testis. *Am J Surg Pathol* 2014; 38: 580–1.
28. Lewis BD, Hurt RD, Payne WS, *et al*. Benign teratomas of the mediastinum. *J Thorac Cardiovasc Surg* 1983; 86: 727–31.