The clinical utility of conventional karyotyping in the detection of cytogenetic abnormalities in soft tissue tumours: an Asian institutional experience

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ABSTRACT

Objectives: To assess the clinical utility of conventional karyotyping as a diagnostic tool in soft tissue tumours amidst the increasing use of molecular cytogenetics.

Design: Case series.

Setting: Singapore General Hospital, an Asian institution.

Participants: A total of 35 participants (18 male and 17 female) aged 15 to 81 years were included in this study. Conventional karyotyping of 35 consecutive fresh soft tissue tumour specimens was performed over 4 years and the results were analysed.

Results: Of the 35 cases of soft tissue tumours reviewed, chromosome abnormalities were detected in 22 (63%) cases, 11 (31%) showed a normal karyotype, and 2 (6%) had culture failure. Of the 22 cases with abnormal karyotype, nine (41%) cases showed recurring aberrations: Ewing's sarcomas (n=2), desmoplastic small round cell tumour (n=1), synovial sarcomas (n=3), myxoid liposarcomas (n=2), and lipoma (n=1). One lipoma case had a t(2;12)(q23;q15) in which 2q23 breakpoint was not reported before. Chromosomal aberration involving 12q15 breakpoint has been shown in a previous study to be indicative of a lipoma-like liposarcoma. Another lipoma case had addition of 5q15 and 9p13 together with a balanced aberration of t(12;13)(q13;q12) which were novel aberrations. One synovial sarcoma case showed t(3;7)(q21;p13) which was an uncharacteristic aberration.

Conclusion: Conventional karyotyping demonstrated utility as a genome-wide screening tool for soft tissue tumours and an adjunct diagnostic tool in the event histopathology results were doubtful. With the more widespread use of karyotyping, novel recurring chromosomal aberrations may be discovered.

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New knowledge added by this study

- To the authors' knowledge, this is the first study on an Asian population documenting the clinical utility of karyotyping in the detection of cytogenetic abnormalities in soft tissue tumours. As compared with a previously published similar American cohort study which had a karyotype detection rate of 48% (n=48), this study had a higher detection rate of 63% (n=35) for chromosomal aberrations in soft tissue tumours.
- This study discovered three novel chromosomal aberration findings not previously documented before in the Mitelman Database of Chromosome Aberrations in Cancer. These comprised one lipoma, one lipoma-like liposarcoma, and one synovial sarcoma.
- This study also demonstrated the importance of karyotyping in the differential diagnosis of soft tissue tumours in cases of borderline histological results and certain cases in which the histological diagnosis did not fit the overall clinical picture.

Implications for clinical practice or policy

- This study advocates the continued clinical use of conventional karyotyping as an adjunct diagnostic tool in addition to molecular cytogenetics and histology in the detection of chromosomal aberrations in soft tissue tumours. In the process, it is hoped that more novel chromosomal findings may be discovered.

Introduction

Soft tissue tumours represent a diverse group of mesenchymal lesions which often present diagnostic challenges to clinicians and pathologists. Histological classification of these tumours is based on their degree of differentiation and metastatic potential: benign, intermediate (locally aggressive), intermediate (rarely metastasising), and malignant.[1]

常規核型分析在檢測軟組織腫瘤中細胞遺傳學異常的臨床應用：一所亞洲機構的經驗

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目的：分子細胞遺傳學的使用越見普及，本研究評估常規核型分析作為軟組織腫瘤診斷工具的臨床應用。

設計：病例系列。

安排：位於亞洲的新加坡中央醫院。

參與者：35名年齡介乎15至81歲人士（18男、17女）參與研究。為連續35個鮮軟組織腫瘤標本進行超過4年的常規核型分析，並分析結果。

結果：共35例軟組織腫瘤的檢測結果中，22例（63%）為染色體異常，11例（31%）正常核型，另2例（6%）的細胞培養失敗。22例異常核型中，9例（41%）表現為經常性畸變，包括尤文氏肉瘤（2例）、促纖維增生性小圓細胞腫瘤（1例）、滑膜肉瘤（3例）、粘液樣脂肪肉瘤（2例）和脂肪瘤（1例）。其中一個脂肪瘤的染色體為t(2;12)(q23;q15)，當中的2q23斷點以往尚未有文獻記載，而涉及12q15斷點的染色體畸變曾於以前的研究報告中證實為代表脂肪瘤樣脂肪肉瘤的存在。另一個脂肪瘤的病例除了有平衡的t(12;13)(q13;q12)，另有5q15和9p13，均證實為新發現的染色體畸變。此外，一個滑膜肉瘤的病例顯示有t(3;7)(q21;p13)的不尋常染色體畸變。

結論：常規染色體核型分析技術適合運用在軟組織腫瘤全基因組篩查分析，而且當對組織病理學結果有懷疑時，這種分析技術可作為輔助的診斷工具。隨着核型分析的廣泛使用，可能會發現新而恆常的染色體畸變。

Recent advances in molecular cytogenetics (fluorescence in-situ hybridisation [FISH]) and molecular assays (reverse transcription–polymerase chain reaction [RT-PCR]) have contributed to the ever-evolving nature of classification and diagnosis of soft tissue sarcomas. Over the past two decades, conventional karyotyping has demonstrated diagnostic utility in detecting a wide range of recurring numerical and structural chromosomal aberrations in soft tissue tumours.

Unlike the newer molecular techniques such as FISH, knowledge of the expected genetic change is not required and this enables karyotyping to function as a genome-wide screening tool. Furthermore, karyotyping can detect any further clonal progression in the event of a tumour relapse. The drawbacks of karyotyping include the dependency on sterile tumour specimens, success of growth culture, and being time-consuming.[2]

Histology, immunohistochemistry, and electron microscopy may sometimes show borderline or non-specific features. An example is that of malignant peripheral nerve sheath tumours which have been historically difficult to distinguish from other spindle cell sarcomas such as synovial sarcomas.[3] Karyotyping has shown the main difference to be the presence of the (X;18) translocation.[3] Many previous studies[4-6] have also demonstrated the role of conventional karyotyping in the detection of clonal aberrations in 68% of malignant fibrous histiocytomas, and 38 to 48% of heterogeneous soft tissue sarcomas. Our study aimed to highlight the use of conventional karyotyping as a genome-wide screening tool, and also as an adjuvant diagnostic tool in the validation of histological diagnosis for soft tissue tumours.

Methods

Cytogenetic analysis involves a coordinated effort between surgical pathologists and cytogenetic laboratory technicians.[6] In our study, fresh tumour samples were collected in sterile bottles from the surgical theatre and transported immediately to the cytogenetics laboratory. Next, the tumour specimens were washed 3 times with media containing Hank's balanced salt solution, and 2% penicillin and streptomycin. After washing, the tissue was minced finely with scalpels and digested in collagenase II (GIBCO, Gaithersburg [MD], US) at a concentration of 1400 units/mL for 1 hour. The disaggregated tissue was then transferred into a centrifuge tube and washed twice with 1X Hank's balanced salt solution and then with Roswell Park Memorial Institute complete medium (culture medium). The cells were centrifuged and transferred to a culture medium containing RPMI 1640, 20% fetal bovine serum, 2% 200 mmol/L L-glutamine, and 2% 5000 U penicillin and 5000 μg streptomycin.

Cells were cultured and harvested according to standard cytogenetic preparations and procedures. The cultures were set up in a 37°C incubator with 5% CO_2. The time of harvesting the cells depended on the degree of cell proliferation in culture. At harvest, 50 μL colcemid (10 μg/mL) was added to the cultures for 3 hours to arrest the cells at metaphase. Cultured cells were detached by treatment with 1X trypsin EDTA and then treated with 0.075 mol/L KCl-0.6% trisodium citrate solution (1:2) for 20 minutes at 37°C. After fixation in two changes of methanol-acetic acid (3:1), chromosome spreads were made by the air-drying method. Chromosomes were stained using the GTG banding method. A total of 20 cells were analysed in each case and karyotype results were designated according to International System of Human Cytogenetic Nomenclature (ISCN 2005, 2009).[7,8]

Conventional karyotyping of 35 consecutive fresh soft tissue tumour specimens was performed in a cytogenetic laboratory at our institution over a period of 4 years from 2005 to 2009. Medical records and histopathology reports for each patient case were reviewed and diagnoses were formulated based on the World Health Organization classification of soft tissue tumours.[1] Recurrent chromosomal

abnormalities were identified using the Mitelman Database of Chromosome Aberrations in Cancer,[9] and with relevant literature search. Any novel chromosomal aberrations were also noted. This research protocol was approved by the ethics committee of our institution, and informed consent from the patients was obtained by the surgeon.

Results

From January 2005 to March 2009, 35 consecutive fresh tissue specimens were harvested from soft tissue tumour surgical specimens. Histopathology results revealed 20 distinct morphologies. There were 29 malignant tumours, five benign tumours, and one of uncertain malignant potential. In our study, there was an almost equal gender representation with 18 males and 17 females, and age ranging from 15 to 81 years. Table 1 shows an overview of the patient's age at diagnosis, tumour site, and tissue type for all 35 cases. The majority of our patients (37%) were in the age-group of 41 to 60 years. The most common tumour location was in the extremities (60%), and adipose tissue (34%) was the most common type. As shown in Table 2, conventional cytogenetic analysis revealed an abnormal karyotype detection rate of 63% (22 of 35 cases). Diagnostic abnormal karyotype was seen in nine (26%) cases—Ewing's sarcomas (n=2), desmoplastic small round cell tumour (DSRCT) [n=1], synovial sarcomas (n=3), myxoid liposarcomas (MLPSs) [n=2], and lipoma (n=1). A normal karyotype (ie 46, XX or 46, XY) was seen in 11 (31%) cases. There were also two (6%) cases of culture failure. Table 3 shows the diagnosis, full karyotype results, and diagnostic utility for all 22 cases with abnormal karyotype.

TABLE 1. Overview of patient age at diagnosis, tumour site, and tissue type in all 35 soft tissue tumour cases

Characteristic	No. (%) of cases
Age (years)	
0-20	4 (11)
21-40	9 (26)
41-60	13 (37)
61-80	7 (20)
>80	2 (6)
Tumour site	
Extremities	21 (60)
Pelvis	6 (17)
Retroperitoneum	2 (6)
Paravertebral	2 (6)
Thorax	2 (6)
Chest wall	1 (3)
Maxilla	1 (3)
Tissue type	
Adipose	12 (34)
Fibrous	9 (26)
Neural	4 (11)
Synovial	4 (11)
Skeletal muscle	3 (9)
Smooth muscle	1 (3)
Mesothelial	1 (3)
Vascular	1 (3)

TABLE 2. Detection rate of abnormal karyotype, diagnostic abnormal karyotype, normal karyotype, and culture failure in all 35 soft tissue tumour cases

Characteristic	Detection rate
Abnormal karyotype	22/35 (63%)
Diagnostic abnormal karyotype	9/35 (26%)
Normal karyotype	11/35 (31%)
Culture failure	2/35 (6%)

Discussion

A wide range of structural and numerical chromosomal abnormalities exists. These aberrations may be characterised by chromosomal gains or losses, balanced or unbalanced translocations, deletions or insertions, ring or marker chromosomes, or multiple complex karyotypes.[6] Sarcomas may be categorised into two major cytogenetic groups: (i) sarcomas with tumour-specific chromosomal alterations and simple karyotypes[2,10,11] or (ii) sarcomas with non-specific chromosomal alterations and complex unbalanced karyotypes.[2] For group (i), karyotypes are considered to be tumour-specific or recurrent if the abnormality is found in two or more cases. For group (ii), a complex karyotype abnormality will not be specific for the diagnosis but is supportive of the diagnosis of malignancy. A ring chromosome may also indicate some form of malignancy. While a marker chromosome is diagnostically non-specific, it is an indicator of clonal progression and further testing by whole chromosome painting (CP) FISH may aid the diagnosis. Chromosome painting refers to the hybridisation of fluorescently labelled chromosome-specific probe pools for the detection of chromosomal aberrations.[12] The simultaneous hybridisation of multiple CP probes, each tagged with a specific fluorochrome, enables the coloured display of all 24 human chromosomes also known as multicolour FISH.[12] The advantages of CP include its ability to detect subtle telomeric translocations and small chromosomal markers, barely the size of a chromosomal band.[12] Despite showing some utility as a genetic screening tool, CP is more straightforward

TABLE 3. Summary of diagnosis and karyotype results for all 22 cases with abnormal karyotype

Case No.	Diagnosis	Abnormal karyotype result	Diagnostic? (Yes / No)
1	Myxoid liposarcoma	46,XX,t(12;16)(q13;p11.2)[18]/46,XX[2]	Y
2	Myxoid liposarcoma	46,XY,t(12;16)(q13p11.2)[20]	Y
3	Ewing's sarcoma/pPNET	46, XX,t(11;22)(q24;q12)[14]/46,XX[1]	Y
4	Ewing's sarcoma/pPNET	38~46,XY,t(2;11;22)(q35;q24;q12),add(17)(p11.2)[cp14]/46,XY[8]	Y
5	Ewing's sarcoma/pPNET	47,XY,+12[cp5]/46,XY[32]	N
6	Atypical lipomatous tumour	47,XX,+r[cp8]/48,idem,+mar[cp3]/46,XX[8]	N
7	Atypical lipomatous tumour	33~50, XX, -4,-9, der(11;22)(q10;q10),-16,-19,+der(?)t(?;15),+mar,+1~3r,+1~5mar[cp11]/78~89,XXXX,-1, -9, -9,-10,-11,-12,+14,-15,-15,-16,-20,-22,-22, +der(?)t(?;15)(?;q15),+der(?)t(?;15)(?;q15),+2~6r,+5mar,inc[cp5]	N
8	Pleomorphic liposarcoma	45,X,-Y[4]/93,XY,der(X)(q26),add(7)(p21),+der(?)t(?,11)(?;q12), +mar1,+mar2x2, +mar3[cp3]/46,XY[17]	N
9	Lipoma	46,XX,add(1)(p36),add(5)(q15),?del(8)(q23),add(9)(p13),dup(10)(p11.2q21),add(12)(q14),der(13)t(12;13)(q13;q12), +der(?)t(7;?)(p11.1;?)t(?;5)(?;q21)[3],inc[cp19]	Y
10	Lipoma	46,XY,t(2;12)(q23;q15)[18]/47,idem,+mar[2]	N
11	Well-differentiated liposarcoma	45~47,XX,dic(13;14)(p11.2;p12),t(8;?)(?q21;?)t(?;11)(?;p13),+1~2r[cp10]/38~48,XX,-X,del(7)(q32),-12,-13, der(14)t(14;?18)(p13;?q11.2),-14,-17,+19,-18,+2~3r, +mar,inc[cp2]/ 46,XX[8]	N
12	Well-differentiated liposarcoma	46,XY[19]	N
13	Sarcoma not otherwise specified	53~55,XY,+i(1)(q10),+5,+8[19],+16,+17,+21,+2~3r[cp20]	N
14	Sarcoma not otherwise specified	85~90,XXYY,add(3)(q27),add(4)(q25),add(22)(q11,2),+1~4mar[cp2]/46,XY[20]	N
15	Collagenous fibroma	44~45,XY,add(3)(q21),add(7)(q32),+8 inc[cp2]/46,XY[15]	N
16	Myxofibrosarcoma	46,XY,add(14)(q25),der(16)t(14;16)(q12;p13.3),+mar[2]/46,XY[27]	N
17	Desmoplastic small round cell tumour	46,XY,t(11;22)(p13;q12)[cp3]/46,XY[16]	Y
18	Synovial sarcoma	45,X,-X,ins(2;?)(p11,2;?),ins(18;?)(p11.2;?)[cp20]	Y
19	Synovial sarcoma	44,XY,+X,t(X;18)(p11;q12),-8,add(19)(p13),-22,-22[1]/53,XY,+X, t(X;18)(p11;q12),+2,-3,+add(4)(p16),+mar[cp5]/46,XY[7]	Y
20	Synovial sarcoma	47,t(X;18)(p11.1;q11.1),Y,t(5,12,13)(q32;q15;p13),+8[19]	Y
21	Synovial sarcoma	54~58,XY,+2,+3,t(3;7)(q21;p13),+7,+10,+10,+12,+13,+13,+14,+15,+15,-16,del(17)(p12),+19,+20,add(22)(q13),+mar1 +1~4mar[cp17]/46,XY [1]	N
22	Stromal tumour of myofibroblastic origin	47,XY,+r[19]/46,XY[20]	N

Abbreviation: pPNET = peripheral primitive neuroectodermal tumour

only when used in conjunction with conventional cytogenetics which provide information on the specific chromosomes involved. This is because CP alone requires the iterative hybridisation of multiple CP probes, which is not always practical due to time constraints and limited specimens.[12]

Of the 22 cases with abnormal karyotype results, nine (41%) cases showed tumour-specific chromosome abnormalities. These nine cases had abnormal karyotypes which were consistent with the Mitelman Database of Chromosome Aberrations in Cancer[9] and previously published literature.[2,11] A normal karyotype was seen in 11 (31%) cases where three tumour tissues were of fibrous origin. One study in our literature search demonstrated a normal karyotype in 42% of cases; the majority of these were soft tissue tumours with a fibrous component or grossly dense matrix.[6] The study rationalised that tumour cells embedded in a dense matrix were more difficult to culture.[6] The two culture failure cases could have been due to specimen contamination or insufficient sample. A study conducted in a single institution in the United States (n=48) had documented an abnormal karyotype detection rate of 48% and a 10% culture failure rate in patients with soft tissue tumours.[6] In contrast, our Asian cohort study had a higher detection rate of 63% (n=35) and a lower culture failure rate of 6%. The small sample size of this study was limited by the disease prevalence (rarity of sarcomas) as well as the logistics of obtaining fresh specimens from the surgical operating room. We intend to conduct future studies with a bigger sample size and explore other cytogenetic aberrations in soft tissue sarcomas

using FISH in conjunction with conventional karyotyping.

Ewing's sarcoma/peripheral primitive neuroectodermal tumour

Of the two cases of Ewing's sarcoma in this study, one was a 42-year-old female (case 3; Table 3) and one a 26-year-old male (case 4; Table 3). This is an unusual clinical age-group for this sarcoma and the histological diagnosis was confirmed by karyotyping. In the male patient, the variant t(2;11;22)(q35;q24;q12) was demonstrated. For case 5 (Table 3), trisomy 12, a non-random secondary aberration, was demonstrated. One study found that the majority of chromosomal aberrations in Ewing's sarcoma appear to be trisomy 8 and trisomy 12, occurring in 44% and 16% of Ewing's sarcoma cases, respectively.[13-15]

Synovial sarcoma

Our study showed two diagnostic cases of synovial sarcoma (cases 19 and 20) with the hallmark translocation t(X;18) seen[16] together with complex cytogenetic aberrations (Table 3). Another two cases of synovial sarcoma (cases 18 and 21) showed structure rearrangement on 2p/18p and translocation t(3;7)(q21;p13), respectively. These abnormalities were uncharacteristic. Histological biopsy of the left distal tibia showed a soft tissue tumour measuring 4 x 3 x 1 cm, composed of large sheets of malignant cells displaying high nuclear cytoplasmic ratio, round or irregular nuclei, nucleoli, scanty cytoplasm, and frequent mitoses. Tumour cells were positive for CD99 (MIC2 gene product), cytokeratin AE1+3 (especially epithelial-like areas), and vimentin. Further immunohistochemical staining with epithelial membrane antigen showed focal positivity. The soft tissue tumour had also invaded the distal tibia on the anteromedial and posteromedial aspects of the left leg with metastasis to the left groin lymph node. Case 21 was reviewed by various histopathologists and the general consensus was that of a high-grade undifferentiated synovial sarcoma. The representative karyogram for case 21 is shown in Figure 1.

In the study by Saboorian et al,[17] there was one case of ambiguous histological results; the stained tissue smears showed densely cellular and tightly cohesive malignant spindle cells without discernible epithelial differentiation. A few differential diagnoses were formulated which included synovial sarcoma and karyotyping confirmed the diagnosis of synovial sarcoma by revealing the presence of t(X;18)(p11.2;q11.2).[17] Another study by Akerman et al,[18] which involved the cytogenetic evaluation of 15 surgical specimens, confirmed the (X;18) translocation as both a specific and sensitive marker for synovial sarcoma. Our study and the above studies serve to highlight the essential supportive role of conventional karyotyping in the confirmation of the diagnosis of synovial sarcoma.

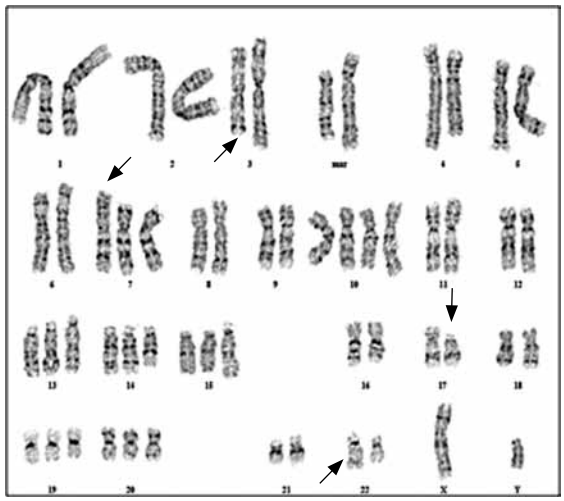

FIG 1. Synovial sarcoma showing hyperdiploid cell with numerical changes and structural rearrangement on 17p and 22q, as well as translocation between chromosomes 3 and 7 in case 21 (arrows)

Liposarcoma

Liposarcoma is the most common soft tissue sarcoma, accounting for 20% of mesenchymal neoplasms.[19] It can be categorised into three subtypes: myxoid and round cell, well-differentiated, and pleomorphic.[19] All three subtypes that were included in our study are discussed below.

Myxoid liposarcoma

Myxoid liposarcoma is the second most common liposarcoma subtype in which two thirds of the cases arise from the thigh musculature.[19] The characteristic translocation t(12;16)(q13;p11) has been well documented in more than 90% of MLPS cases.[19-22] This translocation leads to formation of a TLS-CHOP fusion gene (located at 12q13 and 16p11 respectively) which is highly sensitive and specific for MLPS.[19] A possible trisomy 8 as an additional secondary change has also been reported.[22] Our study demonstrated two cases of MLPS showing the t(12;16)(q13;p11) translocation. As shown in Table 3, this translocation was diagnostic of MLPS in cases 1 and 2. A study by the CHAMP group in which cytogenetic analysis was carried out in 28 MLPS specimens reported the t(12;16)(q13;p11) translocation in 26 cases; this further confirmed its consistency as a genetic marker for MLPS.[20] Conventional karyotyping for t(12;16)(q13;p11) in MLPS was also shown to be useful as an adjunct diagnostic tool in poorly differentiated myxoid neoplasms in another study.[21]

Pleomorphic liposarcoma

Pleomorphic liposarcoma (PLPS) is the rarest (5% of liposarcoma) and most aggressive (highly metastatic) form of liposarcoma.[19] It commonly affects the extremities in the elderly (>50 years) with an equal distribution in both genders.[19] The complex structural abnormalities (unidentified marker chromosomes) and high chromosome counts (polyploidy) make it difficult to detect PLPS-specific aberrations.[19] Our study demonstrated the case of a 77-year-old female (case 8; Table 3) with PLPS which showed complex, structural aberrations on karyotyping which, though not diagnostic, was indicative of a malignant clonal process.

Lipoma

Lipomas are the most common soft tissue tumours and are benign.[23] One study by Sandberg and Bridge[24] had documented rearrangements affecting the 12q13~q15 region as the most common aberration (65% of 188 lipomas). Clonal chromosomal aberrations were also reported in 60% of lipomas, and of these, 70% had normal cytogenetic cells.[24] The most frequent t(3;12)(q27~q28;q13~q15) translocation was seen in 25% of lipoma cases.[24]

Case 10 (Table 3) belonging to a 53-year-old male demonstrated the balanced t(2;12)(q23;q15) translocation which was also novel in that the breakpoint 2q23 has not been previously reported. In this patient, histology showed a large lipoma measuring 14 x 9 x 8 cm. In addition, magnetic resonance imaging suggested a malignant liposarcoma. Szymanska et al[25] found that the overrepresentation of 1q and 12q sequences was a recurrent finding in lipoma-like liposarcomas but not in lipomas. This is consistent with the chromosomal aberration involving 12q15 breakpoint in case 10. The representative karyogram for case 10 is shown in Figure 2.

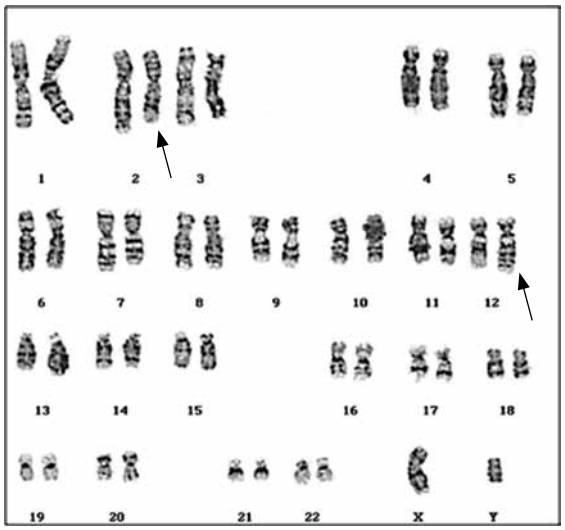

FIG 2. Lipoma showing translocation between chromosomes 2 and 12 in case 10 (arrows)

Atypical lipomatous tumour/well-differentiated liposarcoma

Atypical lipomatous tumour (ALT) is synonymous with well-differentiated liposarcoma (WDLPS) as both exhibit similar cytogenetic findings regardless of location and pathology.[19] Being the most common of all liposarcomas (40%-45%), ALT/WDLPS is an intermediate (locally aggressive) soft tissue sarcoma with mature adipocyte differentiation.[1,19,26] Most ALTs are characterised cytogenetically by the presence of supernumerary ring chromosomes or long marker chromosomes involving chromosome region 12q13-15.[26,27]

Our study demonstrated abnormal karyotypes in two cases of ALT and WDLPS each. Of the two ALTs, case 6 (Table 3) had a ring chromosome as a sole abnormality and case 7 had supernumerary ring chromosomes present in addition to the multiple complex numerical structural aberrations. Case 11 (WDLPS) showed both complex numerical and structural chromosomal rearrangements in which two dicentric chromosomes were present together with ring chromosomes and giant marker chromosomes. Case 12 (WDLPS) belonged to a 65-year-old male; a normal karyotype was seen in 19 cells, one nonclonal abnormal cell was hypodiploid which showed trisomy 12, deletion on 12p, structural rearrangement on 20q as well as a ring chromosome. It is uncertain if this nonclonal abnormal cell is of any clinical significance. Histology had showed a WDLPS measuring 19 x 12 x 4 cm infiltrating the skeletal muscle of the left thigh.

It was reported that virtually all ALT/WDLPS had abnormal cytogenetic results.[26] The CHAMP group conducted a study of 59 ALT/WDLPS and evaluated their relationship and differential diagnoses with other adipose tissue tumours.[28] Clonal chromosomal abnormalities were found in 55 (93%) cases and supernumerary ring or giant marker chromosomes (RGCs) were seen in 37 (63%) cases[28]; RGCs were also shown to have tumour progression potential. Statistical analysis demonstrated a highly significant correlation between ALTs and RGCs ($P<0.0001$).[28] The study reaffirmed the essential role of karyotype analysis in differentiating ALTs from benign lipomas, spindle/PLPS, hibernomas, and MLPS.

Desmoplastic small round cell tumour

Desmoplastic small round cell tumour is a rare and aggressive neoplasm that commonly affects

adolescents and young adults.[29,30] Our study demonstrated the case of a 27-year-old male with DSRCT showing the classic t(11;22)(p13;q12) translocation (case 17; Table 3). In this case, histopathology reports showed no evidence of malignant infiltrates in the tumour specimen but conventional karyotyping confirmed the diagnosis to be DSRCT.

Conclusion

Karyotype analysis detected a majority (63%) of cases with abnormal chromosomes in our Asian cohort study with nine (41%) cases showing 22 abnormal karyotypes. Our study, hence, demonstrated that conventional karyotyping played an essential supportive role in validating histological diagnosis, especially in cases with borderline or complex morphology. Newer molecular techniques such as FISH and RT-PCR techniques may be sensitive but require prior knowledge of the expected genetic change. In view of this, conventional karyotyping is useful as a genome-wide screening tool in detecting single or multiple chromosomal aberrations in each patient. The use of conventional karyotyping is highly encouraged in the pursuit of discovering more novel recurring chromosomal aberrations.

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References

1. Fletcher CD, Unni KK, Mertens F, editors. World Health Organization classification of tumors: pathology and genetics of tumors of soft tissue and bone. Lyon, France: IARC Press; 2002.
2. Antonescu CR. The role of genetic testing in soft-tissue sarcoma. Histopathology 2006;48:13-21.
3. Fletcher JA, Kozakewich HP, Hoffer FA, et al. Diagnostic relevance of clonal cytogenetic aberrations in malignant soft-tissue tumours. N Engl J Med 1991;324:436-43.
4. Mandahl N, Heim S, Wilen H, et al. Characteristic karyotypic anomalies identify subtypes of malignant fibrous histiocytoma. Genes Chromosomes Cancer 1989;1:9-14.
5. Becher R, Wake N, Gibas Z, Ochi H, Sandberg AA. Chromosome changes in soft-tissue sarcomas. J Natl Cancer Inst 1984;72:823-31.
6. Matthews A, Tang M, Cooper K. Cytogenetic aberrations in soft tissue tumours harvested from fresh tissue submitted for surgical pathology: a single institution experience. Int J Surg Pathol 2010;18:260-7.
7. Schaffer LG, Tommerup N, editors. ISCN 2005: an international system for human cytogenetic nomenclature. Basel, Switzerland: S Karger; 2005.
8. Schaffer LG, Slovak ML, Campbell LJ, editors. ISCN 2009: an international system for human cytogenetic nomenclature. Basel, Switzerland: S Karger; 2009.
9. Cancer Genome Anatomy Project. Mitelman database of chromosome aberrations in cancer. Available from: http://cgap.nci.nih.gov/Chromosomes/RecurrentAberrations. Updated August 14, 2009. Accessed 25 Aug 2009.
10. Sreekantaiah C, Ladanyi M, Rodriguez E, Sreekantaiah C. Chromosomal aberrations in soft tissue tumors. Relevance to diagnosis, classification and molecular mechanisms. Am J Pathol 1994;144:1121-34.
11. Dei Tos AP, Dal Cin P. The role of cytogenetics in the classification of soft tissue tumours. Virchows Arch 1997;431:83-94.
12. Reid T, Schrock E, Ning Y, Wienberg J. Chromosome painting: a useful art. Hum Mol Genet 1998;7:1619-26.
13. Roberts P, Burchill SA, Brownhill S, et al. Ploidy and karyotype complexity are powerful prognostic indicators in the Ewing's sarcoma family of tumors: a study by the United Kingdom cancer cytogenetics and the children's cancer and leukaemia group. Genes Chromosomes Cancer 2008;47:207-20.
14. Mugneret F, Lizard S, Aurias A, Turc-Carel C. Chromosomes in Ewing's sarcoma. II. Nonrandom additional changes, trisomy 8 and der(16)t(1;16). Cancer Genet Cytogenet 1988;32:239-45.
15. Maurici D, Perez-Atayde A, Grier HE, Baldini N, Serra M, Fletcher JA. Frequency and implications of chromosome 8 and 12 gains in Ewing's sarcoma. Cancer Genet Cytogenet 1998;100:106-10.
16. Sandberg AA, Bridge JA. Updates on the cytogenetics and molecular genetics of bone and soft tissue tumours. Synovial sarcoma. Cancer Genet Cytogenet 2002;133:1-23.
17. Saboorian MH, Ashfaq R, Vandersteenhoven JJ, Schneider NR. Cytogenetics as an adjunct in establishing a definitive diagnosis of synovial sarcoma by fine-needle aspiration. Cancer 1997;81:187-92.
18. Akerman M, Willén H, Carlén B, Mandahl N, Mertens F. Fine needle aspiration (FNA) of synovial sarcoma: a comparative histological-cytological study of 15 cases, including immunohistochemical, electron microscopic and cytogenetic examination and DNA-ploidy analysis. Cytopathology 1996;7:187-200.
19. Sandberg AA. Updates on the cytogenetics and molecular genetics of bone and soft-tissue tumours: liposarcoma. Cancer Genet Cytogenet 2004;155:1-24.
20. Tallini G, Akerman M, Dal Cin P, et al. Combined morphologic and karyotypic study of 28 myxoid liposarcomas. Implications for a revised morphologic typing (a report from the CHAMP Group). Am J Surg Pathol 1996;20:1047-55.
21. Ohjimi Y, Iwasaki H, Ishiguro M, et al. Myxoid liposarcoma with t(12;16)(q13; p11). Possible usefulness of chromosome analysis in a poorly differentiated sarcoma. Pathol Res Pract 1992;188:736-41.
22. Sreekantaiah C, Karakousis CP, Leong SP, Sandberg AA. Trisomy 8 as a non-random secondary change in myxoid liposarcoma. Cancer Genet Cytogent 1991;51:195-205.
23. Sandberg AA. Updates on the cytogenetics and molecular genetics of bone and soft-tissue tumors: lipoma. Cancer Genet Cytogenet 2004;150:93-115.
24. Sandberg AA, Bridge JA. The cytogenetics of bone and soft

tissue tumours. Austin: RG Landes; 1994.
25. Szymanska J, Virolainen M, Tarkkanen M, et al. Overrepresentation of 1q21-23 and 12q13-21 in lipoma-like liposarcomas but not in benign lipomas: a comparative genomic hybridization study. Cancer Genet Cytogenet 1997;99:14-8.
26. Dei Tos AP, Doglioni C, Piccinin S, et al. Coordinated expression and amplification of the MDM2, CDK4, and HMGI-C genes in atypical lipomatous tumours. J Pathol 2000;190:531-6.
27. Rubin BP, Fletcher CD. The cytogenetics of lipomatous tumours. Histopathology 1997;30:507-11.
28. Rosai J, Akerman M, Dal Cin P, et al. Combined morphologic and karyotypic study of 59 atypical lipomatous tumours. Evaluation of their relationship and differential diagnosis with other adipose tissue tumours (a report of the CHAMP Study Group). Am J Surg Pathol 1996;20:1182-9.
29. Gerald WL, Haber DA. The EWS-WT1 gene fusion in desmoplastic small round cell tumor. Semin Cancer Biol 2005;15:197-205.
30. Lae ME, Roche PC, Jin L, Lloyd RV, Nascimento AG. Desmoplastic small round cell tumour: a clinicopathologic, immunohistochemical and molecular study of 32 tumours. Am J Surg Pathol 2002;26:823-35.

Corrigenda

Immunotherapy for peanut allergy

"Immunotherapy for peanut allergy" (August 2014;20:325–30). On page 329 (left column, lines 11-16), the sentence should have read "It was noted that serum peanut-specific IgE increased in three out of the four children following Xolair and updosing of allergen, when concentrations might have been expected to decrease, as in other forms of allergen-specific desensitisation (Table 2)." rather than "It was noted that serum peanut-specific IgE increased in three out of the four children following Xolair, and there was updosing of allergen when concentrations might have been expected to decrease, as in other forms of allergen-specific desensitisation (Table 2)." as printed. We regret the error. The article is correct at www.hkmj.org.

Halo-pelvic traction: a means of correcting severe spinal deformities

"Halo-pelvic traction: a means of correcting severe spinal deformities" (August 2014;20:358–9). On page 358, the author's affiliation should have read "Guest Author, Education and Research Committee, Hong Kong Museum of Medical Sciences Society" rather than "Member, Education and Research Committee, Hong Kong Museum of Medical Sciences Society" as printed. We regret the error. The article is correct at www.hkmj.org.