CHAPTER 5

MOLECULAR KARYOTYPING IN SARCOMA DIAGNOSTICS AND RESEARCH

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Abstract: Conventional cytogenetic and molecular genetic studies have both clinical and biological significance in sarcomas. However, the resolution of these methods does not always suffice to screening of novel, specific genetic changes, such as small deletions, amplifications, and fusion genes. Tumor-specific chromosomal translocations revealed by cytogenetic and molecular methods play a decisive role in the differential diagnosis of sarcomas. The novel molecular karyotyping techniques have proven to be powerful in the screening of clinically and biologically relevant molecular changes in human neoplasias. A variety of platforms for molecular karyotyping is available, e.g., arrayed cDNA clones or oligonucleotides that can be used in microarray-based comparative genomic hybridization (CGH) and gene expression analysis. We review here the clinically most relevant cytogenetic and molecular changes in sarcomas and describe latest microarray techniques for screening of clinically relevant gene copy number and expression changes.

1. SARCOMAS AND GENETIC ALTERATIONS

Bone and soft tissue sarcomas are a heterogeneous group of rare mesenchymal tumors comprising only 1% of all human neoplasms. In most sarcoma types, the cell of origin remains undefined, but there is some evidence that tumor cells in Ewing's sarcoma are derived from neural crest cells [1]. Osteosarcoma, chondrosarcoma, and Ewing's sarcoma as the three most common forms of bone sarcoma, and the most prevalent soft tissue sarcomas are malignant fibrous histiocytoma, liposarcoma, leiomyosarcoma, and synovial sarcoma [2,3]. The

patients' age distribution varies according to the histological type of the tumor, but bone tumors frequently affect teenagers and young adults, whereas soft tissue sarcomas usually show a gradual increase of incidence with advancing age [3]. Although the etiology and pathogenesis of specific sarcomas are still largely unknown, in recent years considerable progress has been made in the genetic typing of solid tumors, including sarcomas. Based on cytogenetics, the sarcomas can be divided into two groups: (1)_ cancers with translocations (e.g., Ewing's sarcoma, desmoplastic round cell tumor, alveolar rhabdomyosarcoma, and synovial sarcoma) and (2) cancers with complex karyotypes without any uniform translocations (e.g., osteosarcoma, malignant fibrous histiocytoma, leiomyosarcoma, and chondrosarcoma). Small round cell tumors include histologically indistinguishable sarcomas, neuroblastomas, and lymphomas, which makes differential diagnosis difficult (for review, see Tarkkanen and Knuutila [4]). To ensure proper prognostic and therapeutic assessment, early and accurate diagnosis can be aided using cytogenetic and molecular methods.

Specific chromosomal translocations have proven to be useful in diagnostics and monitoring residual disease [4]. By coupling a transcriptional activation domain with a DNA-binding domain, the translocation results often in creation of novel fusion protein with oncogenic properties. Table 5.1 shows a summary of selected sarcoma types and their characteristic translocations. A classical example in the Ewing family of tumors is the t(11;22)(q24;q12) translocation, which leads to production of the EWS-FLI1 fusion protein [5]. This translocation is seen in about 85% of Ewing's sarcoma patients while the remaining 15% display other nonrandom gene rearrangements with *EWS* (for review see Burchill [6]), as shown in Table 5.1. Gains of chromosomes 8, 12, and 1q are detected secondary aberrations in Ewing tumors and a deletion at 9p21 (encompassing the *p14* and *p16* genes) has been suggested to be a marker for poor prognosis [7,8]. Although the role of secondary aberrations is not fully understood, they may have a role in tumor progression.

The sarcomas with complex karyotypes show numerous chromosomal aberrations, e.g., translocations, inversions, gains, losses, and amplifications of genetic material [9–11]. This limits the potential of conventional cytogenetic analysis, especially when mitotic cells are scarce and yield poor chromosome banding and morphology. Many specific aberrations can be detected by molecular methods, i.e., fluorescence and chromogenic in situ hybridization (FISH and CISH), PCR, CGH, and newly developed microarray-based techniques [4,12].

Clinical and diagnostic features often fail to classify the tumor in time. Rapid diagnosis is particularly crucial for sarcoma patients whose treatment strategy to eradicate the disease should be determined as the optimal combination of chemo-and/or radiotherapy together with extensive surgery. Detection of residual disease and distinct prognostic markers for recurrence or metastasis are also of clinical importance. Today novel molecular karyotyping methods can be used for high-resolution genome-wide screening for molecular signatures that assist us in tumor classification and offer new reliable prognostic parameters in

Table 5.1. Cytogenetic and molecular genetic features of selected sarcomas

Bone and soft tissue tumors	Translocation	Gene fusion	Other cytogenetic characteristics
Alveolar soft part sarcoma	t(X;17)(p11;q25)	*ASPL/TFE3*	–
Clear cell sarcoma	t(12;22)(q13;q12)	*EWS/ATF1*	+7, +8
Congenital fibrosarcoma/ Infantile fibrosarcoma	t(12;15)(p13;q25)	*ETV6/NTRK3*	+8, +11, +17, +20
Desmoplastic small round cell tumor	t(11;22)(p13;q12)	*EWS/WT1*	–
Dermatofibrosarcoma protuberans	t(17;22)(q22;q13)	*CO1LA1/PDGFB*	Amp (17q,22q)
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12)	*NR4A3/EWS*	–
	t(9;17)(q22;q11)	*NR4A3/RBP56*	–
Ewing sarcoma/PNET	t(11;22)(q24;q12) main	*FLI1/EWSR1*	+1q, +8, +12
	t(21;22)(q22;q12)	*ERG/EWSR1*	–
	t(7;22)(p22;q12)	*ETV1/EWSR1*	–
	t(17;22)(q21;q12)	*ETV4/EWSR1*	–
	t(2;22)(q33;q12)	*FEV/EWSR1*	–
Lipoma	t(3;12)(q28;q14)	*HMGIC/LPP*	Changes in 12q13–15, 6p and 13q
Liposarcoma, myxoid	t(12;16)(q13;p11)	*FUS/DDIT3*	–
	t(12;22)(q13;12)	*EWS/DDIT3*	–
Rhabdomyosarcoma, alveolar	t(2;13)(q35;q14)	*PAX3/FKHR*	12q13–15 amplification, MYCN amplification
	t(1;13)(p36;q14)	*PAX7/FKHR*	
Synovial sarcoma	t(X;18)(p11;q11)	*SS18/SSX1*	–3, +7, +8, +12
	–	*SS18/SSX2*	–
	–	*SS18/SSX4*	–

diagnosis. In the following sections we will briefly describe the microarray-based molecular karyotyping methods and discuss its clinical potentials.

2. METHODOLOGICAL CONSIDERATIONS

2.1. Cytogenetics and molecular genetics

In addition to chromosomal translocations, alterations in DNA copy numbers are important in the progression of carcinogenesis, as reviewed by Knuutila [12]. The activation of cellular proto-oncogenes may occur by increase in the copy number of the corresponding genomic region, and the inactivation of tumor suppressor genes may occur by copy number decrease. CGH is a powerful method in genome-wide detection of copy number changes, e.g., gains, losses, and amplifications [13]. Differentially labeled tumor and normal tissue DNAs are hybridized competitively on a metaphase spread, and based on the tumor tissue copy numbers in relation to normal tissue, specific chromosomal regions appear as gained or lost. The tissue used for the analysis may be fresh or fresh-frozen tumor, or even paraffin-embedded fixed tumor material [14]. An illustration of the CGH methods is

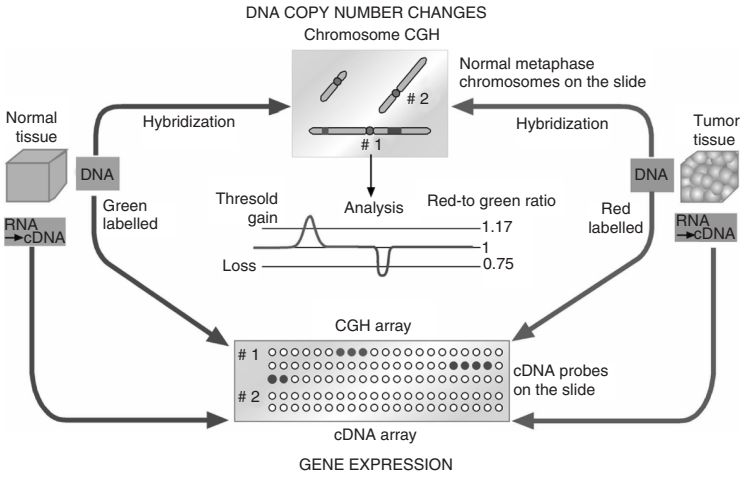

Figure 5.1. Comparative genomic hybridization (CGH) and gene expression analysis. DNA or RNA is extracted from tumor tissue and labeled with a fluorescent dye. Before labeling RNA can be converted to cDNA. A reference DNA or RNA with normal DNA copy number or expression level is labeled with another dye. The samples are combined and hybridized on a metaphase spread (e.g., chromosomal CGH) or on arrayed cDNA clones (e.g., array CGH, expression array). The resulting images are analyzed by compatible software, which shows over- and underrepresentation of tumor DNA or RNA as compared with the control sample. Thus, the results can be interpreted as gains or losses of genomic regions (DNA analysis) or as over- or underexpression of particular genes (RNA analysis).

shown in Figure 5.1. A comprehensive data compilation of copy number changes is publicly accessible at www.helsinki.fi/cmg/cgh_data.html [15]. Figure 5.2 shows recurrent amplicons seen in all sarcomas.

Given that the resolution of conventional chromosomal CGH is at the level of chromosomal bands (10–20 Mb), the method is not sufficient to detect specific amplification or deletion targets at gene level. Therefore microarray methods have been developed using BAC or cDNA-based targets [16,17]. In cDNA-based platforms, cDNA clones arrayed on a glass slide yield genome-wide gene-level information, as exemplified by our osteosarcoma array results [18]. Figure 5.3 shows a genome-wide copy number profile of an osteosarcoma sample, analyzed using cDNA array CGH. The latest technological advance arrays 30–80-mer oligonucleotides representing different exons of a specific gene or intergenic regions on a single platform [19,20]. The oligonucleotide-based CGH arrays give an average resolution of 35 kb (Human Genome CGH microarray Kit 44B, Agilent, Palo Alto, CA), which reveals aberrations not visible by other techniques, e.g., small deletions or amplifications. As shown in Figure 5.4, a sample with *MLL-ARG* fusion indicates that also apparently balanced translocations may harbor cryptic deletions [21].

The CGH techniques have certain limits, e.g., balanced translocations cannot be detected since the copy number levels are not altered. It has been argued

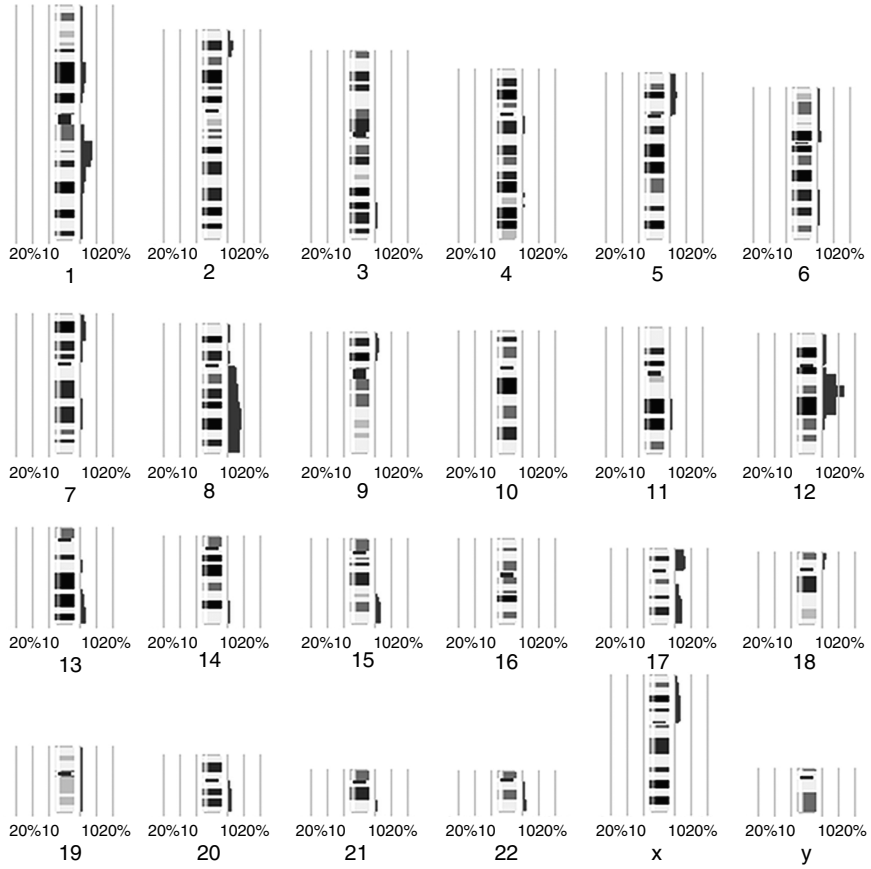

Figure 5.2. Recurrent amplicons seen in all sarcomas (www.helsinki.fi/cmg/cgh_data.html) [15].

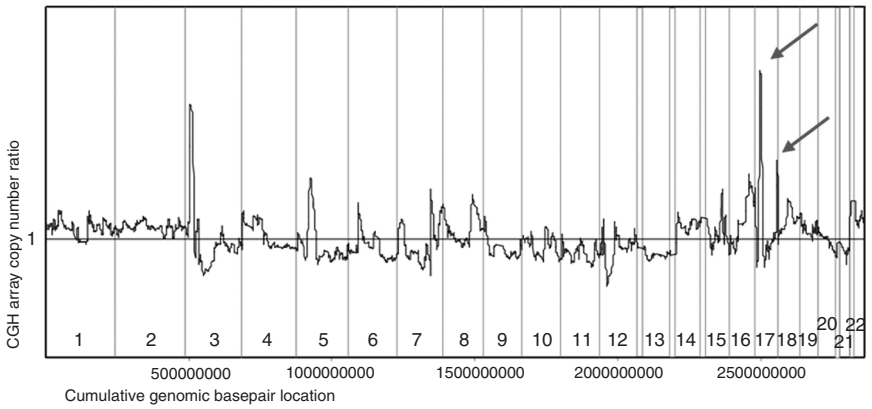

Figure 5.3. Example of a cDNA array CGH result of an osteosarcoma sample [18]. The cumulative base pair locations of the genome (1pter-Xqter) are indicated on the *X*-axis and the relative fluorescence ratios (tumor vs normal tissue) on the *Y*-axis. In addition to the well-known amplification at 17p11–13 (*arrow*), a novel region of amplification was found at 17q25 (*arrow*).

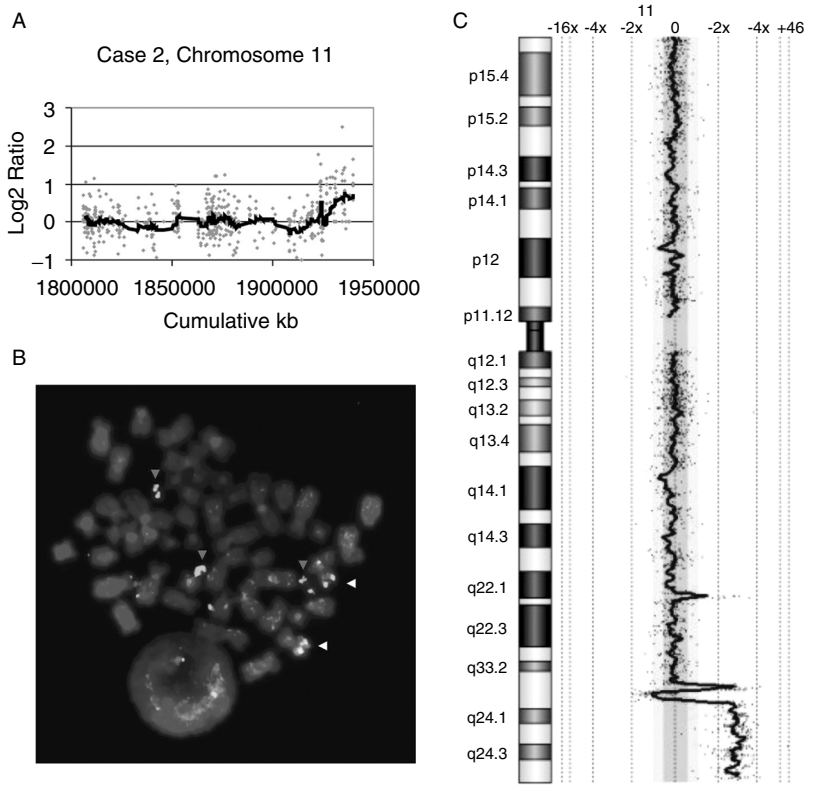

Figure 5.4. A deletion observed within a fusion gene in leukemia [21]. Panel A shows the results obtained using cDNA arrays and panel B shows the presence of the fusion gene with FISH (white arrowheads). The results shown in Panel C are produced using CGH on oligoarray platforms. The 1.8-Mb deletion containing approximately 30 genes (*arrow*) was not detected in conventional CGH or cDNA aCGH. The result is a fusion gene in chromosome 11q (*MLL-LARG*), which is thereafter amplified. The oligoarray results in Panel C are shown as Log2 ratios of fluorescence intensities with the corresponding genomic location in the ideogram. The losses are shown on the left side (negative Log2 values) and gains on the right side of the *X*-axis (positive Log2 values).

that CGH methods are less useful in detecting primary events, for instance translocations in Ewing's sarcoma. Instead, CGH is capable of revealing gains and losses, which are thought to be secondary events [7]. Whether apparently balanced translocations in sarcomas contain small regions of deletion or small amplifications has yet to be shown. But, the secondary changes are valuable in the prognostification of the disease. Applied to sarcoma research, the oligonucleotide-based platform will provide novel information of small copy number gains and losses not detected by other methods and it will possibly help to identify novel fusion genes that can be generated by minor aberrations, e.g., small deletions.

2.2. Gene expression analysis

The gene expression of a tissue sample is assessed by analyzing its mRNA content on arrays [22,23] (Figure 5.1), using either cDNA or oligonucleotide platforms. The observation of up- or downregulated genes in tumors supports diagnosis and prognosis and helps to specify targets for drug development. Furthermore, molecular signatures of sarcomas, which have been obtained using gene expression profiling, can be applied in diagnosis, prognosis, and treatment of these malignancies. Systems biology approach, integration of genome-wide gene expression data (coregulated transcripts) with gene function annotations (e.g., GO, KEGG, and Biocarta databases) and genomic sequence (regulatory DNA elements), provides knowledge of functional networks and activated signal transduction pathways in sarcomas. Holistic inferences of gene functionality and gene expression regulating machinery are essential in understanding carcinogenesis of sarcomas at molecular level. A variety of sarcomas have been subjected to expression analysis, e.g., osteosarcoma, synovial sarcoma, clear cell sarcoma, chondrosarcoma, synovial sarcoma, and liposarcoma [24–29]. The main aim of these works has been to obtain gene expression signatures and novel marker genes for specific tumor types, aiding their separation from other solid tumors with similar appearance [30,31]. As an example of studies with clinical aims, expression profile-based clustering of Ewing's sarcomas [32] and leiomyosarcomas [33] has distinguished low-risk patients from patients with high risk of relapse or metastasis.

2.3. Laboratory practice

Preparation of samples requires special care because mRNA degrades at a rapid rate. Accordingly, expression analysis is generally not applicable on paraffin-embedded material. It should be further emphasized that expression analysis covers only a cross-section of the mRNA content at the time point of the tumor excision. In addition, rapidly degrading mRNA molecules, which can also be up- or downregulated, may not necessarily show the actual state of the transcriptome in the tumor. Besides, the heterogeneity of the tumor sample including, differential cancer cell clones, contaminating normal tissue or inflammatory cells may interfere with interpretation [34]. Still, the gene expression profiles of different macroscopic parts of a tumor have been shown to cluster together and to give a distinct profile to individual tumors [35].

2.4. Tissue microarrays

Tissue microarrays can be used to validate the clinical significance of targets found in aCGH and expression array studies [36]. A tissue microarray consists of a large number of tissue samples with known histology and patient history. The samples are embedded in a single paraffin block, of which sections are sliced to

represent all of the samples. Then FISH, CISH, or immunohistochemistry is performed on the tissue sections to facilitate the association of molecular karyotyping results with the clinical parameters and the validation of specific targets [30]. The presence of translocations can be tested using breakapart probe-FISH [37,38], thus avoiding the need for immunohistochemistry or PCR. Engellau et al. [39] used 140 arrayed soft tissue sarcoma samples with different clinicopathological characteristics and found that immunopositivity of Ki-67, β-catenin, CD44, and Pgp has prognostic value for tumor metastasis. In another example, the prognostic significance of a novel amplicon in breast cancer was validated using 262 arrayed tissue samples [38]. The amplicon was found to reside close to a rearranged gene and to co-occur in the same samples with the rearrangement. Furthermore, the novel amplicon was confirmed to be associated with poor prognosis using FISH and breakapart probe-FISH on the tissue microarray [38]. Similarly, the results obtained from DNA copy number and expression studies of sarcoma samples could be tested on a tissue microarray with large sample populations to evaluate the clinical significance of the findings.

3. CLINICAL APPLICATIONS

Sarcomas are divided into prognostic subtypes according to histopathological features (e.g., tumor size, location, and number of mitotic cells). Specific translocations are determined by cytogenetic analysis, fusion gene transcripts by RT-PCR, and chromosomal gains or losses by FISH or conventional CGH. Table 5.1 gives an overview of sarcoma-specific translocations. However, these methods are not accurate enough to give sufficient gene-level information for diagnostic purposes, classification of the tumors, or prognostification. The molecular profiles generated by array-based methods have provided predictive markers for treatment response in breast cancer [40–42], molecular classification of tumors in lung adenocarcinomas [43] and diffuse large B-cell lymphomas [44], and markers for resistance of treatment in pediatric osteosarcomas [29]. The methodology enables also the detection of novel fusion genes [21], which may be useful in cancer diagnostics, and the detection of residual disease after primary tumor eradication [45]. However, further clinical trials should be conducted to validate these findings.

4. CONCLUSIONS AND FUTURE PROSPECTS

Molecular karyotyping methods include genome-wide techniques to assess the DNA copy-number and gene expression changes in tumor samples. The results provide distinct molecular signatures or single targets, which can be used for tumor classification, prognosis, and prediction of treatment response and occurrence of residual disease. Gene expression microarrays and systems biology tools can be used to identify novel drug targets, genes, which are actively

transcribed in sarcomas, and signaling proteins and transcription factors, which promote sarcomagenesis.

REFERENCES

1. MS. Staege, C. Hutter, I. Neumann, S. Foja, UE. Hattenhorst, G. Hansen, D. Afar and SE. Burdach. DNA microarrays reveal relationship of Ewing family tumors to both endothelial and fetal neural crest-derived cells and define novel targets. *Cancer Res* 64: 8213–8321 (2004).
2. HC. Bauer, TA. Alvegard, O. Berlin, M. Erlanson, P. Gustafson, A. Kivioja, R. Klepp, T. Lehtinen, P. Lindholm, TR. Moller, A. Rydholm, G. Saeter, CS. Trovik, O. Wahlstrom and T. Wiklund. The Scandinavian Sarcoma Group Register. *Acta Orthop Scand Supp.* 285: 41–44 (1999).
3. CE. Herzog. Overview of sarcomas in the adolescent and young adult population. *J Pediatr Hematol Oncol* 27: 215–218 (2005).
4. M. Tarkkanen and S. Knuutila. The diagnostic use of cytogenetic and molecular genetic techniques in the assessment of small round cell tumours. *Current Diagnostic Pathology* 8: 338–348 (2002).
5. O. Delattre, J. Zucman, B. Plougastel, C. Desmaze, T. Melot, M. Peter, H. Kovar, I. Joubert, P. de Jong, G. Rouleau, et al. Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumours. *Nature* 359: 162–165 (1992).
6. SA. Burchill. Ewing's sarcoma: diagnostic, prognostic, and therapeutic implications of molecular abnormalities. *J Clin Pathol* 56: 96–102 (2003).
7. CM. Hattinger, U. Potschger, M. Tarkkanen, J. Squire, M. Zielenska, S. Kiuru-Kuhlefelt, L. Kager, P. Thorner, S. Knuutila, FK. Niggli, PF. Ambros, H. Gadner and DR. Betts. Prognostic impact of chromosomal aberrations in Ewing tumours. *Br J Cancer* 86: 1763–1769 (2002).
8. G. Wei, CR. Antonescu, E. de Alava, D. Leung, AG. Huvos, PA. Meyers, JH. Healey and M. Ladanyi. Prognostic impact of INK4A deletion in Ewing sarcoma. *Cancer* 89: 793–799 (2000).
9. M. Tarkkanen, A. Kaipainen, E. Karaharju, T. Bohling, J. Szymanska, H. Helio, A. Kivioja, I. Elomaa and S. Knuutila. Cytogenetic study of 249 consecutive patients examined for a bone tumor. *Cancer Genet Cytogenet* 68: 1–21 (1993).
10. J. Szymanska, M. Tarkkanen, T. Wiklund, M. Virolainen, C. Blomqvist, S. Asko-Seljavaara, E. Tukiainen, I. Elomaa and S. Knuutila. Gains and losses of DNA sequences in liposarcomas evaluated by comparative genomic hybridization. *Genes Chromosomes Cancer* 15: 89–94 (1996).
11. S. Kiuru-Kuhlefelt, W. El-Rifai, M. Sarlomo-Rikala, S. Knuutila and M. Miettinen. DNA copy number changes in alveolar soft part sarcoma: a comparative genomic hybridization study. *Mod Pathol* 11: 227–231 (1998).
12. S. Knuutila. Cytogenetics and molecular pathology in cancer diagnostics. *Ann Med.* 36: 162–171 (2004).
13. A. Kallioniemi, OP. Kallioniemi, D. Sudar, D. Rutovitz, JW. Gray, F. Waldman and D. Pinkel. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science* 258: 818–821 (1992).
14. M. Zielenska, P. Marrano, P. Thorner, J. Pei, B. Beheshti, M. Ho, J. Bayani, Y. Liu, BC. Sun, JA. Squire and XS. Hao. High-resolution cDNA microarray CGH mapping of genomic imbalances in osteosarcoma using formalin-fixed paraffin-embedded tissue. *Cytogenet Genome Res* 107: 77–82 (2004).
15. S. Knuutila, K. Autio and Y. Aalto. Online access to CGH data of DNA sequence copy number changes. *Am J Pathol* 157: 689 (2000).
16. JR. Pollack, CM. Perou, AA. Alizadeh, MB. Eisen, A. Pergamenschikov, CF. Williams, SS. Jeffrey, D. Botstein and PO. Brown. Genome-wide analysis of DNA copy-number changes using cDNA microarrays. *Nat Genet* 23: 41–46 (1999).
17. D. Pinkel, R. Segraves, D. Sudar, S. Clark, I. Poole, D. Kowbel, C. Collins, WL. Kuo, C. Chen, Y. Zhai, SH. Dairkee, BM. Ljung, JW. Gray and DG. Albertson. High resolution analysis of

DNA copy number variation using comparative genomic hybridization to microarrays. *Nat Genet* 20: 207–211 (1998).

18. J. Atiye, M. Wolf, S. Kaur, O. Monni, T. Böhling, A. Kivioja, É. Tas, M. Serra, M. Tarkkanen and S. Knuutila. Gene amplifications in osteosarcoma — CGH microarray analysis. *Genes Chromosomes Cancer* 42: 158–163 (2005).
19. C. Brennan, Y. Zhang, C. Leo, B. Feng, C. Cauwels, AJ. Aguirre, M. Kim, A. Protopopov and L. Chin. High-resolution global profiling of genomic alterations with long oligonucleotide microarray. *Cancer Res* 64: 4744–4748 (2004).
20. AJ. Aguirre, C. Brennan, G. Bailey, R. Sinha, B. Feng, C. Leo, Y. Zhang, J. Zhang, JD. Gans, N. Bardeesy, C. Cauwels, C. Cordon-Cardo, MS. Redston, RA. DePinho and L. Chin. High-resolution characterization of the pancreatic adenocarcinoma genome. *Proc Natl Acad Sci USA* 101: 9067–9072 (2004).
21. A. Tyybäkinoja, U. Saarinen-Pihkala, E. Elonen and S. Knuutila. Amplified, lost, and fused genes in 11q23-25 amplicon in acute myeloid leukemia -array-CGH study. *Genes Chromosomes Cancer*, in press (2005).

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22. M. Schena, D. Shalon, R. Heller, A. Chai, PO. Brown and RW. Davis. Parallel human genome analysis: microarray-based expression monitoring of 1000 genes. *Proc Natl Acad Sci USA* 93: 10614–10619 (1996).
23. M. Schena, D. Shalon, RW. Davis and PO. Brown. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 270: 467–470 (1995).
24. S. Nagayama, T. Katagiri, T. Tsunoda, T. Hosaka, Y. Nakashima, N. Araki, K. Kusuzaki, T. Nakayama, T. Tsuboyama, T. Nakamura, M. Imamura, Y. Nakamura and J. Toguchida. Genome-wide analysis of gene expression in synovial sarcomas using a cDNA microarray. *Cancer Res* 62: 5859–5866 (2002).
25. KL. Schaefer, K. Brachwitz, DH. Wai, Y. Braun, R. Diallo, E. Korsching, M. Eisenacher, R. Voss, F. Van Valen, C. Baer, B. Selle, L. Spahn, SK. Liao, KA. Lee, PC. Hogendoorn, G. Reifenberger, HE. Gabbert and C. Poremba. Expression profiling of t(12;22) positive clear cell sarcoma of soft tissue cell lines reveals characteristic up-regulation of potential new marker genes including ERBB3. *Cancer Res* 64: 3395–3405 (2004).
26. KM. Skubitz, EY. Cheng, DR. Clohisy, RC. Thompson and AP. Skubitz. Differential gene expression in liposarcoma, lipoma, and adipose tissue. *Cancer Invest* 23: 105–118 (2005).
27. S. Subramanian, RB. West, RJ. Marinelli, TO. Nielsen, BP. Rubin, JR. Goldblum, RM. Patel, S. Zhu, K. Montgomery, TL. Ng, CL. Corless, MC. Heinrich and M. van de Rijn. The gene expression profile of extraskeletal myxoid chondrosarcoma. *J Pathol* 206: 433–444 (2005).
28. CM. Schorle, F. Finger, A. Zien, JA. Block, PM. Gebhard and T. Aigner. Phenotypic characterization of chondrosarcoma-derived cell lines. *Cancer Lett* 226: 143–54 (2005).
29. MB. Mintz, R. Sowers, KM. Brown, SC. Hilmer, B. Mazza, AG. Huvos, PA. Meyers, B. Lafleur, WS. McDonough, MM. Henry, KE. Ramsey, CR. Antonescu, W. Chen, JH. Healey, A. Daluski, ME. Berens, TJ. Macdonald, R. Gorlick and DA. Stephan. An expression signature classifies chemotherapy-resistant pediatric osteosarcoma. *Cancer Res* 65: 1748–1754 (2005).
30. TO. Nielsen, RB. West, SC. Linn, O. Alter, MA. Knowling, JX. O'Connell, S. Zhu, M. Fero, G. Sherlock, JR. Pollack, PO. Brown, D. Botstein and M. van de Rijn. Molecular characterisation of soft tissue tumours: a gene expression study. *Lancet* 359: 1301–1307 (2002).
31. M. van de Rijn and BP. Rubin. Gene expression studies on soft tissue tumors. *Am J Pathol* 161: 1531–1534 (2002).
32. A. Ohali, S. Avigad, R. Zaizov, R. Ophir, S. Horn-Saban, IJ. Cohen, I. Meller, Y. Kollender, J. Issakov and I. Yaniv. Prediction of high risk Ewing's sarcoma by gene expression profiling. *Oncogene* 23: 8997–9006 (2004).
33. YF. Lee, M. John, A. Falconer, S. Edwards, J. Clark, P. Flohr, T. Roe, R. Wang, J. Shipley, RJ. Grimer, DC. Mangham, JM. Thomas, C. Fisher, I. Judson and CS. Cooper. A gene expression signature associated with metastatic outcome in human leiomyosarcomas. *Cancer Res* 64: 7201–7204 (2004).

34. M. O'Sullivan, V. Budhraja, Y. Sadovsky and JD. Pfeifer. Tumor heterogeneity affects the precision of microarray analysis. *Diagn Mol Pathol* 14: 65–71 (2005).
35. P. Francis, J. Fernebro, P. Eden, A. Laurell, A. Rydholm, HA. Domanski, T. Breslin, C. Hegardt, A. Borg and M. Nilbert. Intratumor versus intertumor heterogeneity in gene expression profiles of soft-tissue sarcomas. *Genes Chromosomes Cancer* 43: 302–308 (2005).
36. J. Kononen, L. Bubendorf, A. Kallioniemi, M. Barlund, P. Schraml, S. Leighton, J. Torhorst, MJ. Mihatsch, G. Sauter and OP. Kallioniemi. Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat Med* 4: 844–847 (1998).
37. J. Terry, TS. Barry, DE. Horsman, FD. Hsu, AM. Gown, DG. Huntsman and TO. Nielsen. Fluorescence in situ hybridization for the detection of t(X;18)(p11.2;q11.2) in a synovial sarcoma tissue microarray using a breakapart-style probe. *Diagn Mol Pathol* 14: 77–82 (2005).
38. LM. Prentice, A. Shadeo, VS. Lestou, MA. Miller, RJ. Deleeuw, N. Makretsov, D. Turbin, LA. Brown, N. Macpherson, E. Yorida, MC. Cheang, J. Bentley, S. Chia, TO. Nielsen, CB. Gilks, W. Lam and DG. Huntsman. NRG1 gene rearrangements in clinical breast cancer: identification of an adjacent novel amplicon associated with poor prognosis. *Oncogene* [Epub ahead of print] (2005). [Au 2]
39. J. Engellau, PO. Bendahl, A. Persson, HA. Domanski, M. Akerman, P. Gustafson, TA. Alvegard, M. Nilbert and A. Rydholm. Improved prognostication in soft tissue sarcoma: independent information from vascular invasion, necrosis, growth pattern, and immunostaining using whole-tumor sections and tissue microarrays. *Hum Pathol* 36: 994–1002 (2005).
40. LJ. van't Veer, H. Dai, MJ. van de Vijver, YD. He, AA. Hart, M. Mao, HL. Peterse, K. van der Kooy, MJ. Marton, AT. Witteveen, GJ. Schreiber, RM. Kerkhoven, C. Roberts, PS. Linsley, R. Bernards and SH. Friend. Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 415: 530–536 (2002).
41. T. Sorlie, CM. Perou, R. Tibshirani, T. Aas, S. Geisler, H. Johnsen, T. Hastie, MB. Eisen, M. van de Rijn, SS. Jeffrey, T. Thorsen, H. Quist, JC. Matese, PO. Brown, D. Botstein, P. Eystein Lonning and AL. Borresen-Dale. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA* 98: 10869–10874 (2001).
42. CM. Perou, T. Sorlie, MB. Eisen, M. van de Rijn, SS. Jeffrey, CA. Rees, JR. Pollack, DT. Ross, H. Johnsen, LA. Akslen, O. Fluge, A. Pergamenschikov, C. Williams, SX. Zhu, PE. Lonning, AL. Borresen-Dale, PO. Brown and D Botstein. Molecular portraits of human breast tumours. *Nature* 406: 747–752 (2000).
43. T. Shibata, S. Uryu, A. Kokubu, F. Hosoda, M. Ohki, T. Sakiyama, Y. Matsuno, R. Tsuchiya, Y. Kanai, T. Kondo, I. Imoto, J. Inazawa and S. Hirohashi. Genetic classification of lung adenocarcinoma based on array-based comparative genomic hybridization analysis: its association with clinicopathologic features. *Clin Cancer Res* 11: 6177–6185 (2005).
44. H. Tagawa, M. Suguro, S. Tsuzuki, K. Matsuo, S. Karnan, K. Ohshima, M. Okamoto, Y. Morishima, S. Nakamura and M. Seto. Comparison of genome profiles for identification of distinct subgroups of diffuse large B-cell lymphoma. *Blood* 106: 1770–1777 (2005).
45. S. Avigad, IJ. Cohen, J. Zilberstein, E. Liberzon, Y. Goshen, S. Ash, I. Meller, Y. Kollender, J. Issakov, R. Zaizov and I. Yaniv. The predictive potential of molecular detection in the non-metastatic Ewing family of tumors. *Cancer* 100: 1053–1058 (2004).