ARRAY COMPARATIVE GENOMIC HYBRIDIZATION IN SARCOMAS

SIPPY KAUR

Department of Pathology
Haartman Institute and HUSLAB
University of Helsinki and Helsinki University Central Hospital
Finland

Academic Dissertation

To be publicly discussed with the permission of the Faculty of Medicine, University of Helsinki, in the lecture hall of the Department of Oncology, Helsinki University Central Hospital, Haartmaninkatu 4, on June 15th 2007, at 12 noon.

Helsinki 2007

Supervisors:

Sakari Knuutila, Ph.D.
Professor h.c.
Department of Pathology
Haartman Institute and HUSLAB
University of Helsinki and
Helsinki University Central Hospital
Helsinki, Finland

Marcelo L. Larramendy, Ph.D.
Professor
Principal researcher
National Council of Scientific and
Technological Research (CONILET)
Faculty of Natural Sciences and Museum
National University of La Plata
La Plata, Argentina

Reviewers:

Merja Perälä, Ph.D.
Docent
VTT Technical Research Centre of Finland
Medical Biotechnology
Turku, Finland

Arto Mannermaa, Ph.D.
Docent
Institute of Clinical Medicine
Pathology and Forensic Medicine
University of Kuopio
Kuopio, Finland

Official Opponent:

Ylermi Soini, M.D., Ph.D.
Professor
Department of Pathology and Forensic Medicine
University of Kuopio
Kuopio, Finland

ISBN 978-952-92-2200-1 (paperback)
ISBN 978-952-10-3990-4 (PDF)
http://ethesis.helsinki.fi

Yliopistopaino
Helsinki 2007

CONTENTS

ABBREVIATIONS

aCGH	array comparative genomic hybridization
AIDS	acquired immunodeficiency syndrome
BAC	bacterial artificial chromosome
bp	base pairs
BFB	breakage fusion bridge
cCGH	chromosomal comparative genomic hybridization
cDNA	complementary deoxyribonucleic acid
CGH	comparative genomic hybridization
CNV	copy number variation
DAPI	4',6-diamino-2-phenylindole
DFSP	dermatofibrosarcoma protuberans
DNA	deoxyribonucleic acid
FISH	fluorescence *in situ* hybridization
FITC	fluorescein isothiocyanate
GO	gene ontology
kb	kilobase
LMS	leiomyosarcoma
Mb	megabase
MFH	malignant fibrous histiocytoma
MLPA	multiplex ligation-dependent probe amplification
OS	osteosarcoma
qRT-PCR	quantitative real-time polymerase chain reaction
RNA	ribonucleic acid
SNP	single nucleotide polymorphisim
SV40	simian virus 40
ULMS	uterine leiomyosarcoma

All gene symbols can be found at (http://www.ncbi.nlm.nih.gov/entrez)

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals.

I Larramendy ML*, Kaur S*, Svarvar C, Böhling T, Knuutila S. 2006. Gene copy number profiling of soft tissue leiomyosarcoma by array-CGH. Cancer Genet Cytogenet 169:94-101.

II Kaur S, Vauhkonen H, Böhling T, Mertens F, Mandahl N, Knuutila S. 2006. Gene copy number changes in dermatofibrosarcoma protuberans – a fine-resolution study using array comparative genomic hybridization. Cytogenet Genome Res 115:283-288.

III Atiye J, Wolf M, Kaur S, Monni O, Böhling T, Kivioja A, Tas E, Serra M, Tarkkanen M, Knuutila S. 2005. Gene amplifications in osteosarcoma-CGH microarray analysis. Genes Chromosomes Cancer 42:158-163.

IV Kaur S, Larramendy ML, Vauhkonen H, Böhling T, Knuutila S. 2007. Loss of *TP53* in sarcomas with 17p11~p12 gain. A fine-resolution oligonucleotide array comparative genomic hybridization study. Cytogenet Genome Res 116:153-157.

V Kaur S, Larramendy ML, Gentile M, Svarvar C, Koivisto-Korander R, Vauhkonen H, Scheinin I, Leminen A, Bützow R, Böhling T, Knuutila S. 2006. New Insights into the Cellular Pathways Affected in Primary Uterine Leiomyosarcoma**.** Cancer Genomics Proteomics 3:347-354.

*These authors contributed equally to the study.

ABSTRACT

Over the past years, much research on sarcomas based on low-resolution cytogenetic and molecular cytogenetic methods has been published, leading to the identification of genetic abnormalities partially underlying the tumourigenesis. Continued progress in the identification of genetic events such as copy number aberrations relies upon adapting the rapidly evolving high-resolution microarray technology, which will eventually provide novel insights into sarcoma biology, and targets for both diagnostics and drug development.

The aim of this Thesis was to characterize DNA copy number changes that are involved in the pathogenesis of soft tissue leiomyosarcoma (LMS), dermatofibrosarcoma protuberans (DFSP), osteosarcoma (OS), malignant fibrous histiocytoma (MFH), and uterine leiomyosarcoma (ULMS) by applying fine resolution array comparative genomic hybridization (aCGH) technology.

Both low- and high-grade LMS tumours showed distinct copy number patterns, in addition to sharing two minimal common regions of gains (15q26-qter and 17p13.1-q11) and losses (6p12-p21.3 and 13q14.3-qter). Small aberrations were detected by aCGH, which were beyond the resolution of chromosomal comparative genomic hybridization (cCGH). DFSP tumours analysed by aCGH showed gains in 17q (100%), 22q (43%), and 21 additional gained regions, but only one region (22q) with copy number loss. Recurrent amplicons identified in OS by aCGH were 12q11-q15, 8q, 6p12-p21, and 17p. Amplicons 12q and 17p were further characterized in detail. Possible target genes identified within amplicon 12q were *OS9, CYP27B1, DKFZP586D0919*, and *TSFM*. Genes such as *COX10, PMP22, ADORA2B, ZNF287, MRIP, COPS3, PEMT, SREBF1, TOM1L2, TOP3A, GRAP,* and *MAPK7* were considered as possible targets harboured within the 17p amplicon.

The amplicon at 17p was characterized by aCGH in low- and high-grade LMS, OS, and MFH. In all but one case this amplicon, with minimal common regions of gains at 17p11-p12, started with the distal loss of 17p13-pter. OS and high-grade LMS were grouped together as they showed a complex pattern of copy number gains and amplifications at 17p, whereas MFH and low-grade LMS showed a continuous pattern of copy number gains and amplification at 17p. In addition to the commonly gained (1p, 1q, 2p, 3p, 6p, 8q, 10q, 18q) and lost regions (2q, 4q, 6p, 6q, 7p, 7q, 13q, 14p, 16q, 19p, Xp, Xq) identified in ULMS by aCGH, various biological processes affected by these copy number changes were also indicated by pathway analysis. The most interesting and statistically significant pathways which might contribute to tumourigenesis in ULMS were the G1/S transition of the mitotic cycle, co-translational protein targeting to membranes, actin filament polymerization, positive regulation of cytokine biosynthesis, DNA replication, chromatin modification, telomere maintenance, meiosis, mitosis, and angiogenesis.

The three novel findings obtained in this work were: characterization of amplicon 17p in low- and high-grade LMS and MFH, profiles of DNA copy number changes in LMS, and detection of various pathways affected by copy number changes in ULMS. These studies have not been undertaken previously by aCGH technology, thus this Thesis adds new information regarding DNA copy number changes in sarcomas.

In conclusion, the aCGH technique used in this Thesis has provided new insights into the genetics of sarcomas by detecting the precise regions affected by copy number changes and some potential candidate target genes within those regions, which had not been uncovered by previously applied low resolution techniques. The candidate genes identified here can be thus be used for further studies for understanding the genetic causes underlying sarcomas.

INTRODUCTION

Numerous genetic alterations such as gene copy number changes, point mutations, and both numerical and structural chromosomal alterations underlie the development of cancer. In the majority of cases the development of cancer is mainly due to somatically acquired alterations, whereas a minority develop due to an inherited predisposing genotype.

Sarcomas are a heterogeneous group of rare malignancies of mesenchymal origin accounting for approximately 1% of all malignancies. They are divided into two major types: bone and soft tissue sarcomas. These tumours occur mostly in the long bones, extremities, trunk, retroperitoneum and abdomen. Sarcomas are uncommon even though soft tissue and bone comprise almost two thirds of the mass of the human body. In Finland, with a population of 5.2 million inhabitants, bone and soft tissue sarcomas comprise less than 1.5% of all new cancers diagnosed during 1953-2000 (data obtained from the Finnish Cancer Registry Database). Based on previous comparative genomic hybridization (CGH) and cytogenetic studies, sarcomas can be divided into two major groups: one group possessing simple karyotypes including translocations, and the other characterised by complex karyotypes (Borden *et al.* 2003).

Cytogenetic and molecular genetic techniques such as G-banding, FISH, quantitative real time PCR, and cCGH have lead to enhanced diagnostic accuracy, especially when tumours display overlapping clinicohistopathological features or unusual phenotypes. For example, supernumerary ring chromosomes are frequently present as the sole karyotypic abnormality in well-differentiated liposarcoma, myxoid malignant fibrous histiocytoma and parosteal osteosarcoma. This abnormality may be used as a marker to differentiate the tumours from other types of liposarcoma, malignant fibrous histiocytoma and osteosarcoma. Over the past years many tumour-specific alterations have been detected by cytogenetic and molecular methods, thus providing accurate diagnosis in cases where the histological appearance is uncertain or confusing (Busam and Fletcher 1997; Lazar *et al.* 2006).

Chromosomal imbalances are frequently found in sarcomas. These alterations have been detected previously by standard cytogenetic analysis and chromosomal comparative genomic hybridization (cCGH), where the resolution of each method is at the level of chromosomal bands. It is suggested that tumour suppressor genes are located within regions showing DNA copy number losses whereas oncogenes are found in regions showing DNA copy number gains/amplifications (Snijders *et al.* 2003). Thus, genome-wide gene copy number analysis is crucial for cancer genetics for the identification of target oncogenes and tumour suppressor genes that are involved in the multi-step process of cancer development. In this Thesis, array comparative genomic hybridization (aCGH) was applied to analyse sarcomas due to the ability of this technique to determine the copy number changes at the gene level. aCGH, a high-throughput and high-resolution technique, has the potential in the near future to be used routinely for research and diagnostic purposes in detecting gene copy number imbalances.

Previous studies of sarcoma have identified recurrent chromosomal regions of amplifications and losses (see review of Knuutila *et al.* 1998, 1999, and references therein). But the knowledge of the target oncogenes and tumour suppressor genes involved in the copy number changes is still limited, although it's essential for better

understanding of the biology behind sarcomas. The aim of this Thesis was to apply the high resolution aCGH technology in order to identify and characterize the copy number changes, and the genes within those regions that remained undetected by low resolution methods, that are involved in the pathogenesis of sarcomas.

REVIEW OF THE LITERATURE

1. SARCOMAS

1.1. Soft tissue sarcomas

Soft tissue sarcomas are defined clinically and histologically as a heterogeneous group of malignant neoplasms of mesenchymal origin. They develop in connective tissues other than bone, such as skeletal muscle, fat, tendons, fibrous tissue, smooth muscle and the neurovascular elements that support these components (Enzinger and Weiss 2001b). Soft tissue sarcomas occur in a wide variety of anatomical sites. The majority arise in lower extremities (59%), trunk (19%), retroperitoneum (15%), and head and neck (9%), with a slightly higher incidence in males. Sarcomas metastasize most frequently to the lungs (Cormier and Pollock 2004). Metastatic spread to lymph nodes is rare except in synovial sarcoma and rhabdomyosarcoma where there is a significant risk for lymph node metastasis (Conrad and Enneking 1990). Soft tissue sarcomas are generally classified according to the resemblance of the tumour to the normal cell type. The most accepted classification system for soft tissue sarcoma is that of the World Health Organization (Fletcher *et al.* 2002).

The grade of the tumour is an estimator for the degree of sarcoma malignancy. Histological parameters used for the grading of the soft tissue sarcomas provide information regarding prognostic prediction, metastatic risk and treatment. Currently, the most widely used grading systems are the French 3-step grading (French Federation of Cancer Centers) and National Cancer Institute grading (Coindre 2006; Guillou *et al.* 1997; Gustafson *et al.* 2003; Oliveira and Nascimento 2001; van Unnik *et al.* 1993). The former is based on tumour differentiation, mitotic index and tumour necrosis whereas the latter takes into account the histologic type and subtype, location and the amount of tumour necrosis, and for some tumour types cellularity, nuclear pleomorphisim, and mitotic index. Reproducibility, validity, risk management and prognostic capabilities could be improved in a future grading system by incorporation of the advances in imaging techniques, molecular genetics and determination of genome wide expression of oncogenes and tumour suppressor proteins (Oliveira and Nascimento 2001).

The incidence rate of the different types of soft tissue sarcomas varies among different reports (Table 1). However, the incidences of the most common soft tissue sarcomas according to Hashimoto *et al* (1992) were malignant fibrous histiocytoma (MFH) (25.1%) and liposarcoma (11.6%), followed by rhabdosarcoma (9.7%), leiomyosarcoma (LMS) (9.1%), synovial sarcoma (6.5%), malignant schwannoma (5.9%), and fibrosarcoma (5.2%). The etiology of soft tissue sarcomas is relatively poorly understood. The implicated etiologic agents are genetic, environmental and iatrogenic factors (therapy associated), but there is no conclusive evidence to support such claims (Conrad and

Enneking 1990). Radiation-induced sarcomas in adults are rare, mainly observed after radiotherapy treatment for breast, prostate, and cervical cancer (Mark *et al.* 1994; Mertens *et al.* 2000; Travis 2002). However, radiation-induced sarcomas in childhood are the most frequent secondary cancers (Menu-Branthomme *et al.* 2004). The most common radiation-induced soft tissue sarcomas are MFH, osteosarcoma (OS), fibrosarcoma, and rarely malignant peripheral nerve sheath tumour and angiosarcoma (Mark *et al.* 1994; Wiklund *et al.* 1991).

The role of oncogenic viruses in the development of soft tissue sarcomas is not clearly known. The involvement of simian virus 40 (SV40) in the development of OS is controversial (Shah *et al.* 2004). DNA viruses such as human herpesvirus 8 is a well known aetiological agent of Kaposi sarcoma in AIDS patients (Rezaee *et al.* 2006) and Epstein-Barr virus has been shown to infect smooth-muscle cells in immunodeficient or immunosuppressed patients, and may thus contribute to the pathogenesis of LMS in children with HIV infection (Jenson *et al.* 1999).

Traditionally, soft tissue sarcomas are diagnosed with the aid of morphological findings e.g. by microscopy and immunophenotyping. However, the morphological heterogeneity, genetic complexity and rare occurrence lead to low validity and non-optimal reproducibility of the diagnosis and grading (Suehara *et al.* 2006). At present, microarray technologies such as gene expression profiling and protein expression studies are being used for research purposes for the identification of important genes/proteins for the molecular classification of sarcomas, which might lead to the development of novel therapeutic strategies (Baird *et al.* 2005; Suehara *et al.* 2006).

1.2. Bone sarcomas

The occurrence of bone sarcomas is rare as compared with soft tissue sarcomas. Bone sarcomas are bimodal regarding their incidence rates and age specific frequencies. The first incidence peak occurs during the second decade of life, while the second occurs after the age of 60 (Fletcher *et al.* 2002). Incidence rates of the most common bone sarcomas such as OS, chondrosarcoma and Ewing sarcoma are shown in Table 1.

The major malignant bone tumours are OS (31%), chondrosarcoma (31%) and Ewing's sarcoma (14%) (Bauer *et al.* 1999). OS typically occurs in the second decade of life, and arises in the long bones of extremities. Overall survival rates for OS patients have improved remarkably after the introduction of preoperative chemotherapy, but have remained constant over the past 10-15 years, with no substantial improvements (Weber 2005). The incidence rate is age-specific in chondrosarcoma, showing a gradual increase up to age 75. This is a malignant cartilage-forming tumour of bone, which is frequently located in the pelvis and femur (Papachristou and Papavassiliou 2007). Since chemotherapy and radiotherapy have not substantially improved the survival of chondrosarcoma patients, surgical treatment is so far the only effective form of treatment (Weber 2005). Tumour grading based on light microscopy analysis is currently the best prognostic indicator for chondrosarcomas (Bovee *et al.* 2005; Rozeman *et al.* 2002). The most common primary bone tumour seen in children is Ewing sarcoma. Surgery is a major tool for its treatment, but for patients with localized tumours chemotherapy increases survival rates from 5% up to 70% and for the metastatic tumours from 25% up to 30%. Radiotherapy alone or in addition to surgery is also beneficial (Marec-Berard

and Philip 2004). The less common malignant bone tumours are fibrosarcoma, LMS and MFH. The most important criteria for the histological grading in bone tumours are cellularity and the nuclear features of the tumour cells. Mitotic patterns and necrosis are other additional parameters for grading (Heck *et al.* 2006).

Table 1: Incidence rates of specific histologic types of soft tissue and bone sarcomas based on the M.D Anderson Cancer Center ([a]MDACC in adolescent and young adult and [b]MDACC in the total population) tumour registry from 1990-2003 in the U.S.A., and the Scandinavian Sarcoma Group Register (SSGR) from 1986-1997 (Modified from Herzog 2005).

Soft tissue sarcoma	**[a]MDACC**	**[b]MDACC**	**SSGR**
Unclassified and other	22%	22%	11%
Leiomyosarcoma	7%	20%	8%
Malignant fibrous histiocytoma	5%	18%	45%
Liposarcoma	5%	11%	14%
Rhabdomyosarcoma	15%	6%	1%
Synovial sarcoma	16%	6%	7%
Neurofibrosarcoma	5%	2%	5%
Extraosseous Ewing	6%	2%	1%
Epitheliod sarcoma	4%	2%	1%
Alveolar soft-parts sarcoma	4%	1%	1%
Dermatofibrosarcoma protuberans	3%	3%	-
Fibrosarcoma	3%	2%	4%
Hemangiopericyoma	<1%	<1%	1%
Angiosarcoma	4%	5%	-
Bone sarcoma	**[a]MDACC**	**[b]MDACC**	**SSGR**
Osteosarcoma	57%	45%	31%
Chondrosarcoma	10%	28%	31%
Ewing sarcoma	25%	16%	14%
Malignant fibrous histiocytoma	2%	4%	6%
Other	6%	7%	18%

2. GENETIC CHARACTERISTICS OF SARCOMAS

Alterations seen at the cytogenetic level in sarcomas are generally complex, exhibiting both numerical and structural aberrations (Skapek and Chui 2000). General features of the sarcomas studied in this work are shown in Table 2.

On the basis of previous cytogenetic and molecular studies, sarcomas can be genetically segregated into two different groups. One group has simple karyotypes showing specific translocations leading to the formation of fusion genes, as shown in Table 3. The other group has complex karyotypes with numerous structural and numerical changes (Borden *et al.* 2003). Sarcomas lacking specific translocations, such as OS, MFH, LMS, and liposarcoma, possess numerous gains and losses. The biological features that differentiate sarcomas with and without precise genetic alterations are listed in Table 4. The major

biological differentiating factor between the two sarcoma types is inactivation of the *TP53* pathway. Alterations in this pathway are rare in sarcomas that display characteristic translocations, but when present they are associated with decreased survival rates e.g., in synovial sarcoma, myxoid liposarcoma, and Ewing sarcoma (Borden *et al.* 2003). The *TP53* pathway is always altered in those sarcomas with non-specific alterations, but it has no or very little value in the prognosis.

Unlike most solid tumours (Aman 1999), translocations are detected in one-third of all sarcomas (Mitelman 2000), and in some haematological malignancies (Aplan 2006). As translocation-associated fusion genes are specific for particular sarcoma types they are used as specific and powerful diagnostic markers. Fusion genes produced by translocation produce chimeric proteins, most of which encode abnormal transcription factor/s. Based on cell line studies conducted on various sarcoma types, e.g. alveolar rhabdomyosarcoma, Ewing sarcoma, and synovial sarcoma (Arvand *et al.* 2001; Nagai *et al.* 2001; Scheidler *et al.* 1996), it seems evident that the chimeric proteins are oncogenic, i.e., are capable of promoting uncontrolled cell growth, inhibiting apoptosis, and promoting tumour formation. Studies of mouse models with alveolar rhabdomyosarcoma possessing a recurrent translocation t(2;13)(1;13) demonstrated that possessing only the chimeric transcript is insufficient for tumourigenesis, and that additional genetic changes are needed as synergetic events for the formation of the tumour (Lagutina *et al.* 2002).

Table 2: General features of DFSP, LMS, ULMS, MFH and OS.

Sarcoma	**DFSP**	**LMS**	**ULMS**	**MFH**	**OS**
Type	Soft tissue	Soft tissue	Soft tissue	Soft tissue	Bone
Origin	Mesenchymal	Mesenchymal	Mesenchymal	Mesenchymal	Bone forming mesenchymal cells
Age	Not specific, usually at 30-50 years	Not specific, usually at 50-60 years and also in young adults and children	Postmenopausal women	Late adult life	Children
Characteristic cytogenetic feature	t(17;22)(q22;13q)	Complex karyotypes Losses: 10q, 13q Gains: Xp, 1q, 17p	Complex karyotypes Losses: 14q, 22q, 13q, 16q Gains: 1, 8, 17p, Xp	Complex karyotypes, 19p+ marker, unidentified ring chromosomes Losses: 9p, 10q, 13q Gains: 1q, 17, 20q	Complex karyotypes Losses: 2q, 3p, 6q, 8p, 10p Gains: 8q, 6p, 12q, 17p, 19q
Recurrence	Yes	Yes	Yes	Yes	Yes
Metastasis	Rarely	Yes	Yes	Yes	Yes
Cure	Surgery	Surgery	Surgery	Surgery	Surgery and chemotherapy

Table 3: Chromosomal translocations and their associated fusion genes in sarcomas (Modified from Xia and Barr 2005).

Tumour type	Translocation/ Fusion gene	References
Alveolar rhabdomyosarcoma	t(2;13)(q35;q14) *PAX3-FKHR*	Galili *et al.* 1993
	t(1;13)(p36;q14) *PAX7-FKHR*	Davis *et al.* 1994
Alveolar soft part sarcoma	t(X;17)(p11;q25) *TFE3-ASPL*	Ladanyi *et al.* 2001
Angiomatoid fibrous histiocytoma	t(12;16)(q13;p11) *FUS-ATF1*	Waters *et al.* 2000
Clear-cell sarcoma	t(12;22)(q13;q12) *EWS-ATF1*	Zucman *et al.* 1993a
Dermatofibrosarcoma protuberans	t(17;22)(q22;q13) *COL1A1-PDGFB*	Simon *et al.* 1997
Desmoplastic small-round cell tumours	t(11;22)(p13;q12) *EWS-WT1*	Ladanyi and Gerald 1994
Endometrial stromal sarcoma	t(7;17)(p15;q21) *JAZF1-JJAZ1*	Koontz *et al.* 2001
Ewing family tumours	t(11;22)(q24;q12) *EWS-FLI1*	Delattre *et al.* 1992
	t(21;22)(q22;q12) *EWS-ERG*	Zucman *et al.* 1993b
	t(7;22)(p22;q12) *EWS-ETV1*	Jeon *et al.* 1995
	t(2;22)(q33;q12) *EWS-E1AF*	Kaneko *et al.* 1996
	t(17;22)(q12;q12) *EWS-FEV*	Peter *et al.* 1997
	t(16;21)(p11;q22) *FUS-ERG*	Shing *et al.* 2003
Infantile fibrosarcoma	t(12;15)(p13;q25) *ETV6-NTRK3*	Knezevich *et al.* 1998
Inflammatory myofibroblastic tumour	t(1;2)(q22;p23) *TPM3-ALK*	Lawrence *et al.* 2000
	t(2;19)(p23;p13) *TPM4-ALK*	Lawrence *et al.* 2000
	t(2;17)(p23;q23) *CLTC-ALK*	Bridge *et al.* 2001
Low grade fibromyxoid sarcoma	t(7;16)(q33;p11) *FUS-CREB312*	Panagopoulos *et al.* 2004
Myxoid chondrosarcoma	t(9;22)(q22;q12) *EWS-CHN*	Labelle *et al.* 1995
	t(9;17)(q22;q11) *TAF2N-CHN*	Sjogren *et al.* 1999
	t(9;15)(q22;q21) *TCF12-CHN*	Sjogren *et al.* 2000
Myxoid liposarcoma	t(12;16)(q13;p11) *FUS-CHOP*	Crozat *et al.* 1993
	t(12;22)(q13;p12) *EWS-CHOP*	Panagopoulos *et al.* 1996
Synovial sarcoma	t(X;18)(p11;q11) *SYT-SSX1*	Crew *et al.* 1995
	SYT-SSX2	Crew *et al.* 1995
	SYT-SSX4	Skytting *et al.* 1999

Table 4: Biological features of sarcomas with or without precise alterations (Modified from Borden et al. 2003).

Feature	**Sarcomas with precise changes**	**Sarcoma without precise changes**
Karyotypes	Often simple	Usually complex
Translocations	Reciprocal and specific	Usually not, or nonreciprocal and nonspecific
Average age at diagnosis	27	57
Prevalence of *TP53* pathway alterations	Relatively low	High
Prognostic impact of *TP53* pathway alterations	Strong	Weak to moderate
Incidence in bilateral retinoblastoma and Li-Fraumeni syndrome	Rare	Common
Incidence among radiation-induced sarcomas	Rare	Common

Table 5: Mutations causing sarcomas and their diagnostic utility (Slominski et al. 1999).

Gene name	**Location**	**Sarcoma type**	**Diagnostic utility**
TP53	17p13	Various	Possible
Rb1	13q14	Various	Possible
CDKN2A	9p12	OS and LMS	Possible
RAS	Various	Various	No

In addition to chromosomal-scale abnormalities, the majority of sarcomas show sporadic point mutations. Table 5 shows the most commonly mutated genes with possible diagnostic applications. However there is also a subset of sarcomas arising due to inherited predisposition. For example, Li-Fraumeni syndrome, which is caused by germline *TP53* mutations, predisposes individuals to different types of cancers and sarcomas of bone and soft tissue in early childhood (Malkin *et al*. 1990). Patients with hereditary retinoblastoma, harbouring germline mutation in *Rb*, are susceptible to various secondary malignancies and are also at increased risk of developing osteogenic sarcomas (Abramson *et al.* 1984). Neurofibromatosis type I syndrome, caused by germline mutation in *NF1*, leads to the development of neurofibromas and malignant peripheral-nerve-sheath tumour in a minority of patients (King *et al*. 2000).

3. GENERAL FEATURES OF SARCOMAS STUDIED IN THIS THESIS

3.1. Dermatofibrosarcoma protuberans (DFSP)

DFSP is a relatively rare dermal and subcutaneous spindle-shaped neoplasm of intermediate malignancy. Clinically, DFSP is characterized by aggressive growth accompanied by a high rate of local recurrence near the same site due to the extensive infiltrative growth, but it rarely metastasizes. Adverse clinical outcomes are associated with the fibrosarcomatous transformation of DFSP, which is a form of high-grade tumour progression (Sandberg and Bridge 2003a; Sirvent *et al.* 2003). DFSP can occur in any part of the body but it usually arises as a firm nodule or plaque on the trunk and proximal extremities of young to middle age adults, more frequently in males (Enzinger and Weiss 2001a). However, in a recent study the DFSP incidence rates were stated to be higher in women than in men (Criscione and Weinstock 2006). As shown in Figure 1, the cells of DFSP are composed of spindle-shaped nuclei arranged in a distinct storiform or cartwheel growth pattern. The tumour displays low to moderate mitotic activity. Necrosis, which is a common feature of MFH, is rarely seen in this tumour type (Enzinger and Weiss 2001a). DFSP is distinguished from other spindle cell sarcomas by its strong CD34 immunopositivity (Sonobe *et al.* 1999). The current treatment is a wide local excision (Mendenhall *et al.* 2004).

Cytogenetically DFSP is characterized by a reciprocal chromosomal translocation t(17;22)(q22;q13) resulting in the fusion of two genes, *COL1A1* and *PDGFB,* which are located on chromosomes 17q22 and 22q13, respectively (Pedeutour *et al.* 1996). This fusion of the *COL1A1* promotor to coding sequence of *PDGFB* causes the stimulation of PDGFBR in an autocrine manner, suggested to contribute to the disease development (Greco *et al.* 1998; Shimizu *et al.* 1999; Sjoblom *et al.* 2001). In one-third of DFSP cases a supernumerary ring chromosome derived from segments of chromosome 17 and 22 is also observed (Naeem *et al.* 1995; Pedeutour *et al.* 1994; Pedeutour *et al.* 1995). Other frequent numerical chromosomal abnormalities seen in DFSP are trisomies of chromosomes 5 and 8, whereas trisomies of chromosomes 4, 7, 11, 12, 13, 14, 15, 16, and 18 are seen less frequently (Sirvent *et al.* 2003). Figure 2 illustrates a DFSP karyotype.

3.2. Soft tissue leiomyosarcoma (LMS)

LMSs are malignant mesenchymal tumours comprised of cells resembling well-differentiated smooth-muscle cells. LMS, which accounts for 10% of soft tissue sarcomas (Enzinger and Weiss 2001c; Hu *et al.* 2005; Sandberg 2005), occurs in a wide variety of anatomical locations, and is mainly found in elderly individuals although it may also develop in young adults and children (Miettinen and Fetsch 2006). The high incidence of recurrence and metastasis in these aggressive tumours are related to the tumour size and grade (Wang *et al.* 2001). LMSs arise mainly in the retroperitoneal space and abdominal cavity, and around half arise in retroperitoneum of which about two-thirds of cases occur in women (Enzinger and Weiss 2001c).

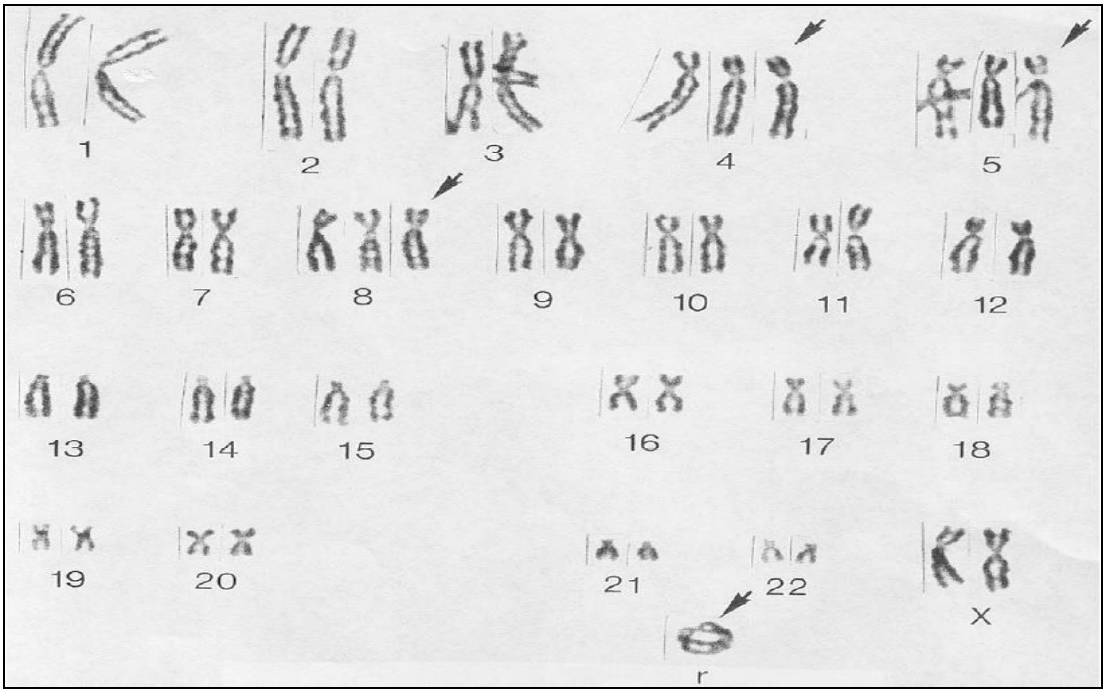


Figure 2: Karyotype of a DFSP tumour with trisomies of chromosomes 4, 5, and 8, and a ring chromosome (r) indicated by an arrow. "Reprinted from Pedeutour et al (1994), with permission from Elsevier."

In contrast to other sarcoma entities, LMSs are very rarely induced by radiotherapy (Brady *et al.* 1992; Patel *et al.* 1999). Histologically the spindle shaped LMS cells contain elongated cigar-shaped nuclei (Figure 1). The diagnosis of this sarcoma type is based on immunohistochemical staining with desmin, actin and H-caldesmon (Miettinen and Fetsch 2006). So far the karyotypes reported in LMSs are complex, diverse, and incomplete with no common aberrations seen in most or all of the cases (Sandberg 2005) (Figure 3).

3.3. Uterine leiomyosarcoma (ULMS)

ULMS is a rare aggressive tumour that is more frequent than all other LMSs, and occurs predominantly in postmenopausal women. It accounts for 1% of all gynecologic malignancies and is associated with an extremely poor prognosis (Echt *et al.* 1990; Matsumura *et al.* 2006). The 5-year survival is between 20% and 75% and the rate of recurrence ranges from 45% up to 73% (Giuntoli *et al.* 2003). Resistance to chemotherapy and radiotherapy makes surgery the primary treatment method for this sarcoma (Benoit *et al.* 2005). Therefore, identification of the target genes that are involved in the pathogenesis is needed in order to develop new therapeutic treatment. Previously, it has been reported that ULMS may either arise *de novo* from smooth-muscle cells of the uterus or from pre-existing uterine leiomyoma (Jatoi 2003; Lee *et al.* 1994). The karyotypes of ULMS are very complex, showing both numerical and structural aberrations.

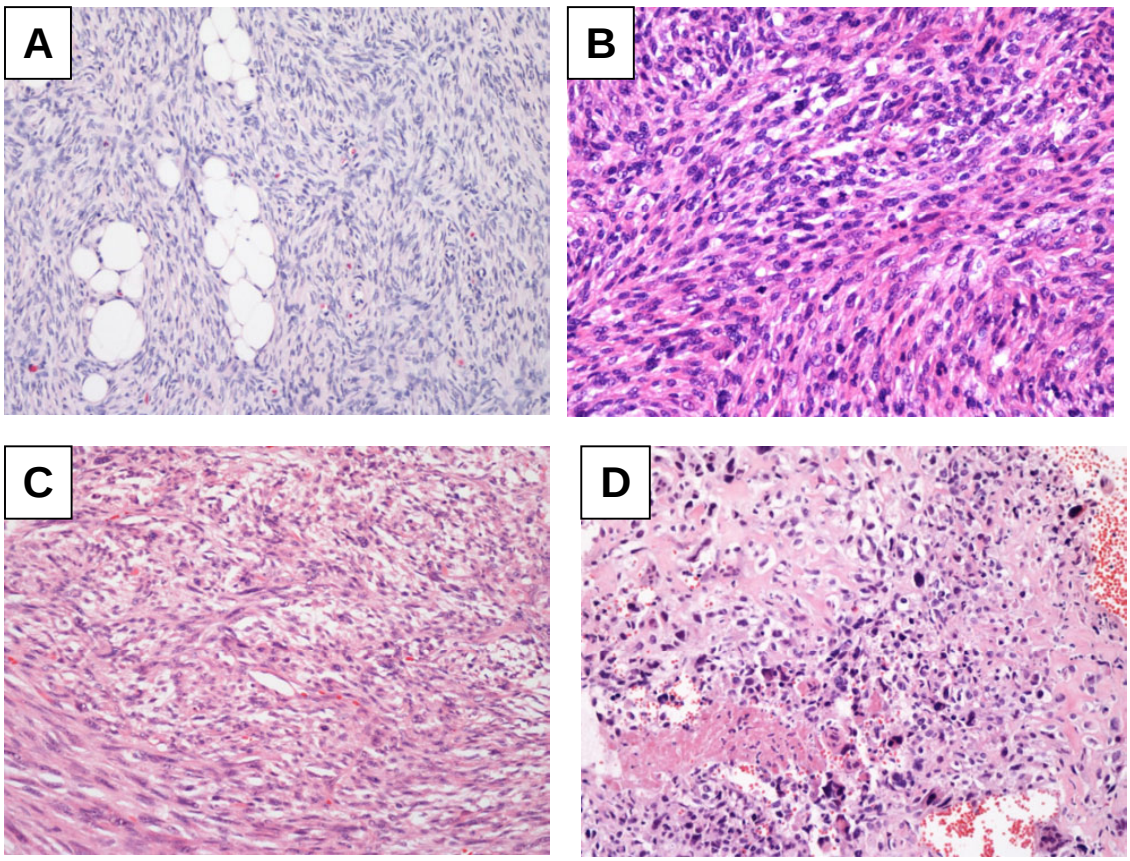


Figure 1: ***Morphological features typical for DFSP, LMS, MFH and OS****.* ***A)*** *Spindle cells, with regular small nuclei infiltrating into subcutaneous fat, representing a typical DFSP.* ***B)*** *A tumour composed of spindle cells with atypical hyper chromatic nuclei and eosinophilic cytoplasm representing a high-grade LMS.* ***C)*** *A spindle cell tumour forming storiform structures representing a MFH.* ***D)*** *A tumour composed of large atypical cells, surrounded by immature osteoid, representing a high-grade osteoblastic OS. All figures are stained by Haematoxylin-Eosin, original magnification X 200.*

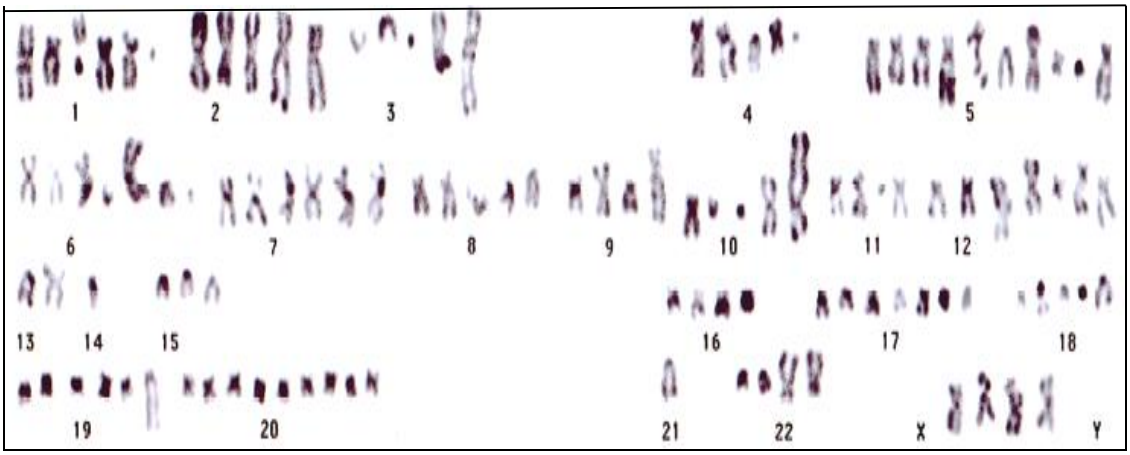


Figure 3: Inverted DAPI-banded image of a grade III LMS tumour showing complex rearrangements and numerical aberrations in all chromosomes. "Reprinted from Wang et al (2001), with permission of Wiley-Liss, Inc. a subsidiary of John Wiley & Sons, Inc."

3.4. Malignant fibrous histiocytoma (MFH)

MFH is the most common type of soft tissue sarcoma. In the current WHO classification MFH is now regarded as synonymous with undifferentiated pleomorphic sarcoma (Fletcher *et al.* 2002). It is diagnosed in late adult life and occurs as the most common type of post irradiation sarcoma (Enzinger and Weiss 2001d). This heterogeneous group of tumours displays a broad range of histological appearances and can be divided into four subtypes, such as storiform-pleomorphic, myxoid, giant cell, and inflammatory types. The two first tumour types are the most common and the latter two are rare (Enzinger and Weiss 2001d). These tumours are usually located in the extremities and retroperitoneum, and occur in both sexes with a slight predominance in males (Walter *et al.* 1997). Although MFH is classified as a soft tissue sarcoma, it may also occur in bone (Senel *et al.* 2006; Tarkkanen *et al.* 2006).
Morphological features of MFH are shown in Figure 1. To date, there is little cytogenetic data on MFH and the reported karyotypes are complex with numerous numerical and structural abnormalities (Simons *et al.* 2000). Structural rearrangements seen in MFH are ring chromosomes, double minutes, homogenously staining regions, telomeric associations, dicentric chromosomes, and ploidy changes (Figure 4). Despite a number of cytogenetic analyses, no consistent chromosomal aberrations have been reported (Szymanska *et al.* 1995; Walter *et al.* 1997). Certain chromosomal abnormalies, such as a 19p+ marker chromosome, may indicate high risk of local recurrence and metastasis (Simons *et al.* 2000).

3.5. Osteosarcoma (OS)

OS is the most common nonhaematopoetic malignant bone tumour of children and adults, deriving from the primitive bone forming mesenchymal cells. The tumour typically arises in the long bones of the appendicular skeleton such as distal femur, proximal tibia, and proximal humerus (Sandberg and Bridge 2003b). The incidence of OS is higher in males

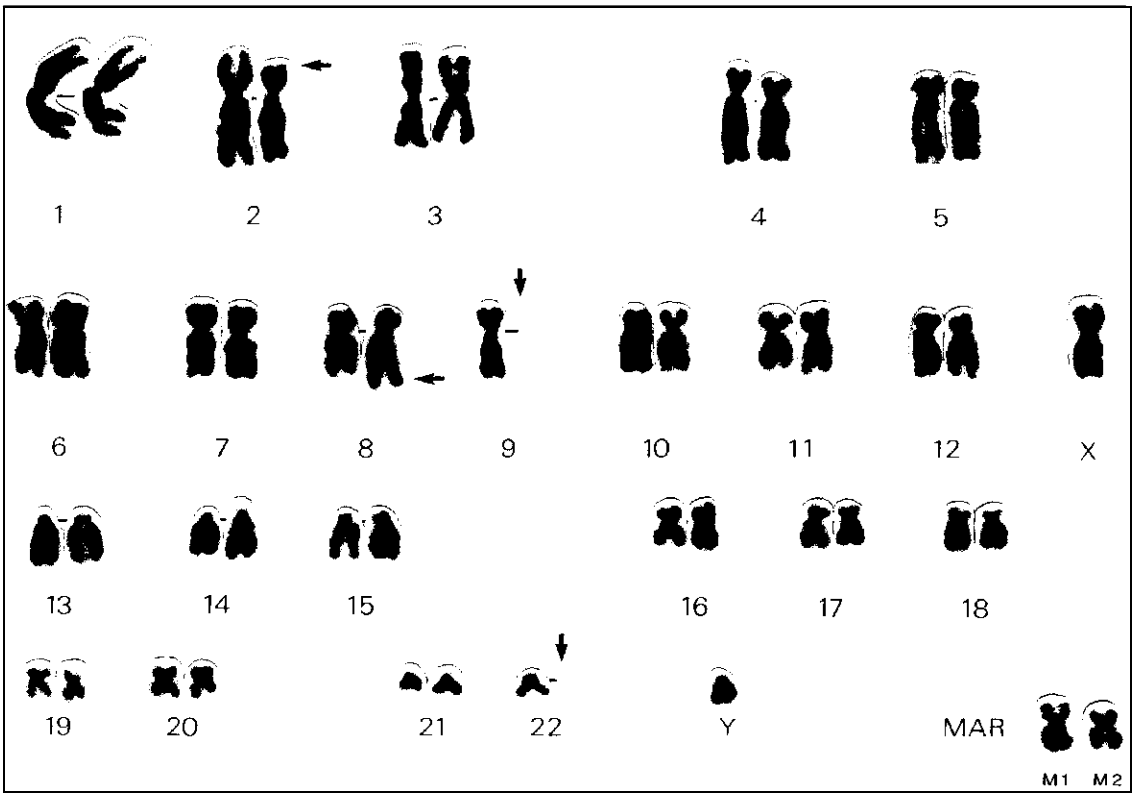


Figure 4: G-band karyotype of a grade IV MFH indicating 46,XY,del(2)(p16), add(8)(q?),-9,-22,+mar1,+mar2. "Reprinted from Szymanska et al (1995), with permission from Elsevier."

with an overall 5-year survival rate ranging between 20% and 70% (Stock *et al.* 2000). Histologically OS is a complex mixture of various cell types ranging from spindle cells to epitheliod or mono- or multinucleated giant cells (Ragland *et al.* 2002) (Figure 1). OS has no characteristic recurrent translocation but can be characterized by the occurrence of complex chromosomal rearrangements exhibiting multiple marker chromosomes, double minutes, homogenously stained regions, and polyploidy involving various tumour suppressors and oncogenes (Bridge *et al.* 1997; Fletcher *et al.* 1994; Hoogerwerf *et al.* 1994). A karyotype of OS is shown in Figure 5. The regions most frequently involved in chromosomal rearrangements in OS identified by spectral karyotyping are located in chromosomes 6, 8, 17 and 20 (Bayani *et al.* 2003; Ozaki *et al.* 2003).
Due to the heterogeneity or cell-to-cell variation, cytogenetic studies in OS have yielded conflicting results insufficient for proper diagnosis and for determining the prognosis of the disease.

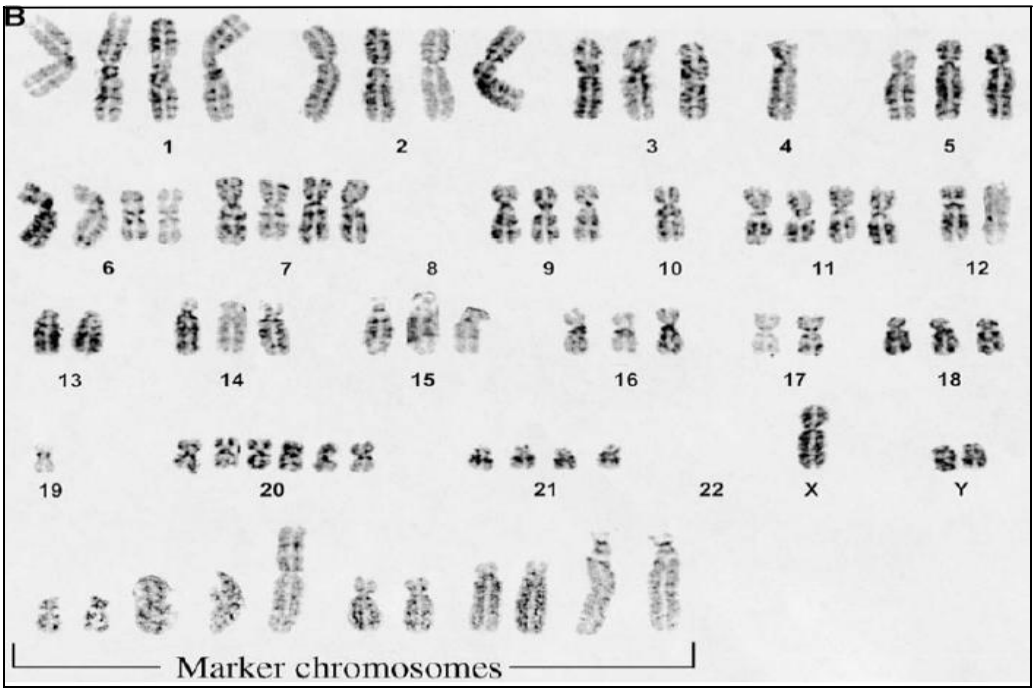


Figure 5: G-banded karyotype from a conventional OS indicating numerous marker chromosomes and multiple numerical and structural abnormalities. "Reprinted from Sandberg and Bridge (2003b), with permission from Elsevier."

4. MOLECULAR GENETIC ANALYSIS OF SARCOMA

4.1. Copy number changes by comparative genomic hybridization

In contrast with leukaemia and lymphoma where cytogenetic methods have been successfully applied to investigate chromosomal changes, analysing solid tumours by conventional cytogenetics is challenging. Tumours cells are difficult to culture and harvest and the few metaphase spreads obtained are either inadequate or too complex to determine banding patterns. Chromosomal comparative genomic hybridization (cCGH), a molecular cytogenetic technique introduced by Kallioniemi and co-workers in 1992, enabled the comprehensive scanning of DNA copy number changes across the whole genome at the chromosomal level. CGH analyses on large numbers of tumour samples enabled the identification of recurrent regions of copy number changes specific for particular tumour types, leading to the identification of regions containing genes involved in cancer development (Knuutila *et al.* 1998; 1999). As illustrated in Figure 6, in cCGH tumour genomic DNA and reference DNA samples are differentially labeled by nick translation with different dyes in order to distinguish between the two genomes hybridized on the metaphase chromosome.
Several disadvantages of cCGH have to be taken into the account while analysing the results. It cannot detect balanced chromosomal translocations, inversions, polyploidy or mosaicism. In addition, copy number changes can only be detected when tumour content of the sample is at least 50%. Aberrations are detected with an approximate resolution of

5Mb (Kallioniemi *et al.* 1994). Smaller areas (1Mb) are detectable only if they are highly amplified (Forozan *et al.* 1997).

Development of array comparative genomic hybridization (aCGH) has overcome the limited resolution of cCGH, enabling mapping of changes directly into genomic sequences and thus detecting the changes at the gene level. As illustrated in Figure 6, aCGH is based on the same principles as cCGH but the DNAs are labeled with fluorescent dyes by random priming and metaphase chromosomes are replaced by known genomic sequences (BAC, cDNA clones, or oligonucleotides) with known chromosomal locations directly spotted onto microscope slides. This approach (aCGH) has paved the way for higher resolution and specificity of array analysis. In the early applications of aCGH, large size BAC clones were used which provided sufficiently intense signal for accurate measurement specifically for detecting single copy number changes and homozygous deletions (Albertson and Pinkel 2003; Pinkel *et al.* 1998). Another approach for performing aCGH comprised of mapped cDNA clones. The use of cDNA arrays for measuring copy number changes was pioneered by Pollack in 1999.

In cDNA arrays short exonic sequence is spotted onto slides, excluding promoter, intronic and intergenic sequences. Use of cDNA arrays for aCGH analysis is appealing as both DNA copy number and expression studies can be done in parallel on the cDNA platform, thus combining genomic data with expression data to facilitate the identification of target genes (Hyman *et al.* 2002; Kauraniemi *et al.* 2001; Monni *et al.* 2001; Pollack *et al.* 2002). Oligonucleotide arrays are the latest approach for performing aCGH, and allow also the detection of introns and intragenic regions (Barrett *et al.* 2004; Brennan *et al.* 2004; Carvalho *et al.* 2004). Oligonucleotide array analyses are performed on very small amounts of DNA (1.5 μg), which is a limiting factor when using cDNA arrays where 20 μg of DNA is required as starting material. Clone sizes and the corresponding resolution for BAC, cDNA and oligonucleotide arrays are shown in Table 6.

Table 6: Details regarding the size of the spotted clones and the resolution of different microarray platforms.

Clone type	**Year of first publication**	**Clone size**	**Resolution**	**References**
BAC	1998	~100 kb	1.3 Mb	Pinkel *et al.* 1998
cDNA	1999	~300 bp	0.1 Mb	Pollack *et al.* 1999
Oligonucleotide	2004	60 bp	~35 kb	Barrett *et al.* 2004

Oligonucleotide aCGH with a dense representation of sequences allows the fine resolution identification of copy number aberrations at the gene level, and is specifically used for the detection of microdeletions and very small novel alterations that may be important for the disease but were not detected by low resolution methods. However, aCGH still lacks the ability to track balanced translocations that do not lead to copy number change.

The most recently developed array platform, single nucleotide polymorphism (SNP) arrays, offers the ability to characterize simultaneously the copy number changes and loss of heterozygosity events in a tumour sample (Bignell *et al.* 2004; Zhao *et al*. 2004; Pfeifer *et al.* 2007). These arrays in the future are likely to be invaluable tools for the comprehensive characterization of tumours (Dutt and Beroukhim 2007).
Copy number variation (CNV) is ubiquitous, submicroscopic copy number variation of DNA segments. CNVs include deletions, insertions, and duplications larger than 1kb up to several Mb. Several thousands of CNVs exist within the human genome and still more remain to be identified (Freeman *et al*. 2006; Rendon *et al*. 2006; Wong *et al*. 2007). Due to the ubiquity of CNV in the genome and their presence in genomes of healthy individuals, discrimination between normal and pathological CNV is very important. At present all identified CNVs are registered in the Database of Genomic Variants (http://projects.tcag.ca/variation) (Rendon *et al.* 2006). While making clinical diagnostic interpretations CNV should be taken into consideration. aCGH detects only cumulative but not allele specific CNV. In order to detect CNV inheritance patterns, development of locus and allele specific quantitative assays for DNA copy number evaluation is needed (Freeman *et al*. 2006). In order to precisely interpret the genomic data concerning cancer and other malignancies precise detection of all CNVs is needed which could be achieved by high throughput genome mapping and association analysis.

4.2. Application of comparative genomic hybridization in sarcoma genetics

Since the development of cCGH it has been used extensively as a research tool in cancer genetics. It has been a valuable diagnostic tool for patients with multiple tumours (Weiss *et al.* 2003a) and in the differential diagnosis of benign and malignant bone tumours, where immunohistochemistry was inadequate due to the unavailability of reliable markers (Gebert *et al.* 2006). cCGH enabled the detection of tumour specific recurrent changes and the implication of specific genes in the development of cancer, thus providing the starting point for the further characterization of the aberrant regions (Knuutila *et al.* 1998; 1999). cCGH analysis on MFH of bone, a relatively rare malignant tumour, supported the concept that bone MFH is an individual bone tumour entity with characteristic aberration patterns distinct from OS, bone fibrosarcoma, and soft tissue MFH (Tarkkanen *et al.* 2006). Some aberrant chromosomal regions are also clinically relevant, for example gain of 13q in liposarcoma correlates with poor prognosis (Schmidt *et al.* 2005). Gain of 12q correlates with poor overall survival rate in adult fibrosarcoma (Schmidt *et al.* 2002). Gains in 6p are associated with poor prognosis in Ewing sarcomas and related tumours (Tarkkanen *et al.* 1999b). Gains at 8q are assumed as a potential marker for poor prognosis in high-grade OS (Tarkkanen *et al.* 1999a) and loss of 10q and gain of 5p are associated with aggressive outcome in LMS (Hu *et al.* 2005). Classification of various cancers based on copy number profiles obtained by aCGH technology is more informative, for example, in sarcomas, histologically similar tumours such as GIST and LMS are differentiated based on their copy number profiling by aCGH (Meza-Zepeda *et al.* 2006).

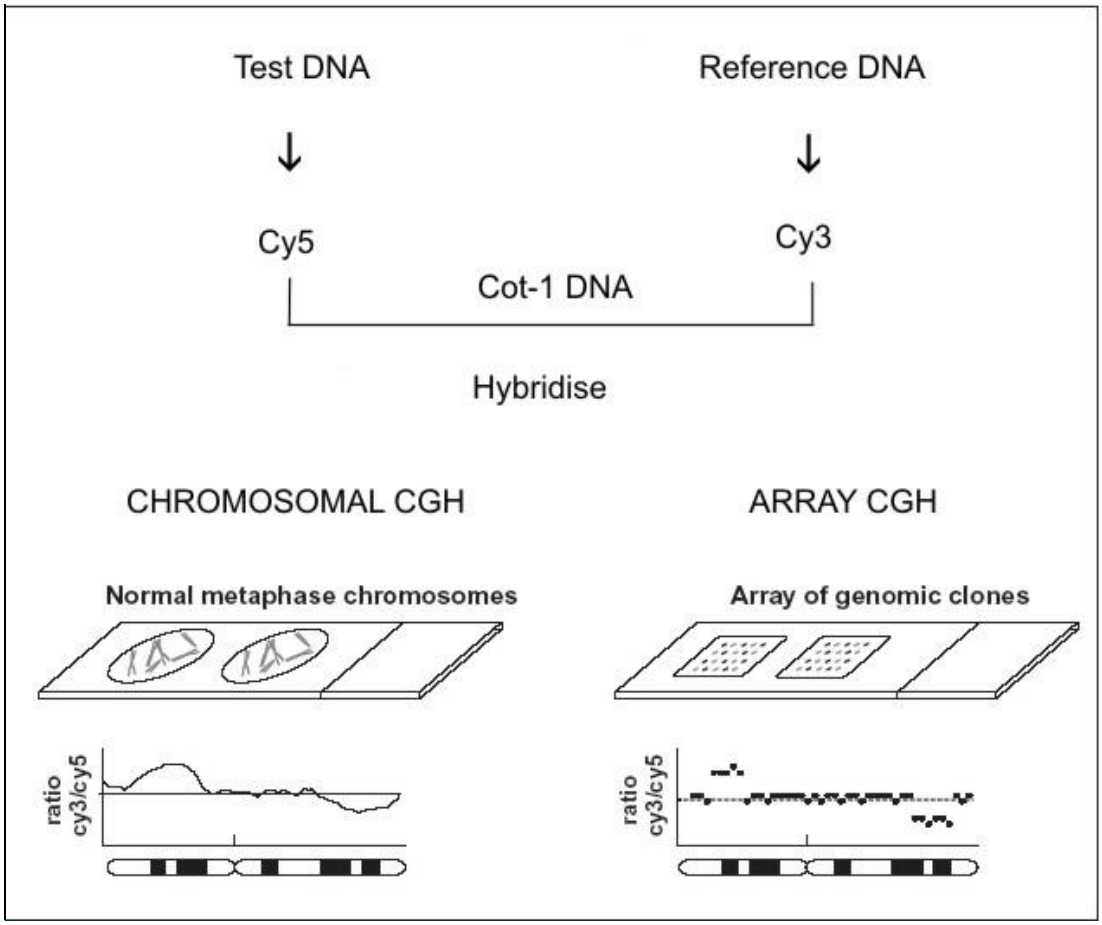


Figure 6: Schematic representation of the comparative genomic hybridization (cCGH and aCGH) technique (Modified from Snijders et al. 2003). Equal amounts of tumour and reference DNAs are labeled with different dyes and hybridized onto slides with Cot-1 DNA for blocking the repetitive sequences. cCGH uses metaphase chromosomes as hybridization targets, whereas aCGH uses genomic sequences on slides. Hybridized cCGH slides are then analysed by automated digital image analysis software in order to determine signals indicating chromosomal gains, high-level amplifications and losses. In aCGH intensity differences in the hybridization patterns indicating gains, high-level amplifications, losses and homozygous deletions are measured by using appropriate software for example, CGH analytic software (Agilent Technologies).

In various cancers genomic profiling by aCGH has proven to be a very feasible approach for accurate diagnosis of the cancer, predicting prognosis, and differentiating between subsets of tumours, which in the end lead to the proper therapeutic treatments (Callagy *et al.* 2005; Weiss *et al.* 2003b; Wilhelm *et al.* 2002). In liposarcomas, in contrast to RNA expression analysis, copy number profiles obtained from aCGH analyses appeared to be more powerful for discriminating between dedifferentiated and pleomorphic subtypes (Fritz *et al.* 2002).

Genome-wide aCGH has introduced new dimensions in terms of resolution and precision for detecting DNA copy number changes, thus making it a reliable and powerful method for detecting disease-associated genes. aCGH analysis is able to detect known unbalanced translocations with better resolution as compared with cytogenetic methods, which fail to detect the precise breakpoints. Buckley and coworkers (2002) used aCGH for the detection of unbalanced translocations in two cases of DFSP. Only by the use of aCGH has it been shown that cytogenetically balanced translocations are indeed frequently associated with small gains and losses (Watson *et al.* 2007), thus highlighting the usefulness of aCGH for diagnostic, therapeutic and research purposes for defining translocation breakpoints with precision.

In conclusion, aCGH is a powerful tool for precisely mapping the boundaries of the amplicon and translocation breakpoints. The candidate gene within the amplicon could then be further confirmed based on RNA/protein expression studies which would allow discrimination between driver genes (which are the target genes) from the passenger genes (copy numbers of which are changed due to the proximity of driver genes).

The most recurrent DNA copy number changes and putative target genes detected by both cCGH and aCGH in DFSP, LMS, ULMS, MFH, and OS are shown in Table 7.

Table 7. Most recurrent copy number changes in sarcomas detected by cCGH and aCGH.

Sarcoma type	Translocation	cCGH	References	aCGH	References	Target genes
DFSP	17;22 (q22;q13)	**Gains**: 17q, 22q, 8q, 5 **Losses**: ND	Nishio *et al.* 2002; Sirvent *et al.* 2003	**Gains**: 8, 5, 22q, 17q, 7p, 18, 21 **Losses**: 19, 22q	Buckley *et al.* 2002; Linn *et al.* 2003	*COL1A1, PDGFB*
LMS	No	**Gains**: 1q21, 4p16, 5p, 12q13-q14, 17p, 20q13.1, 22q, Xp **Losses**: 4q, 11q, 13q, 10q, 2q, 16q, Xq	El-Rifai *et al.* 1998; Hu *et al.* 2005; Otano-Joos *et al.* 2000; Sandberg 2005; Svarvar *et al.* 2006; Wang *et al.* 2001; Wang *et al.* 2003	ND	ND	ND
ULMS	No	**Gains**: 17p, Xp, 1q, 8 **Losses**: 13q, 10q, 16q, 12p, 2p, 14q, 22q	Hu *et al.* 2001; Levy *et al.* 2000; Packenham *et al.* 1997, Svarvar *et al.* 2006	**High-level gains**: 7q36.3, 7q33-q35, 12q13-q15, 12q23.3 **Losses:** 1p21.1-p21.2, 1p22.3-p31.1, 9p21.2-p22.2, 10q25-q25.2, 11q24.2-q25, 13q12-q12.13, 14q31.1-q31.3, 14q32.32-q32.33, 15q11-q12, 15q13-q14, 18q12.1-q12.2, 18q22.1-q22.3, 20p12.1 and 21q22.12-q22.13	Cho *et al.* 2005	*HMGI-C, MDM2, SAS, TIMI, DCC*
MFH	t(5;7)(q31;q22), t(13;14)(q10;q10), t(1;10) In subset of case	**Gains:** 1p31, 9q31, 5p14-pter, 7q32, 7p15-pter, 1q21-q22, 17q23-qter, 20q, 8p21-pter, 12q13-q21, 6q21-q23, 8p21-pter, 8q24-qter, 12q13-q21 **Losses**: 13q21, 13q22, 9p21-pter, 10q, 11q23-qter, 13q10-q31	Larramendy *et al.* 1997; Simons *et al.* 2000; Weng *et al.* 2004	**Gains:** 6q21-q23, 8p21-pter, 8q24-qter, 12q13-q21 **Losses:** ND	Weng *et al.* 2004	*CDKN2A, RBI, TP53, MDM2, MASL1, CDK4*
OS	No	**Gains:** 1q21, 3q26, 4q, 7q31-32, 5p13-14, 6p, 8q, 12q12-13, 14q24-qter, 17p11-12, Xp11.2-21, Xq12, 20q **Losses**: 2q, 3p, 9, 12q, 13q, 14q, 15q, 16, 17p, 18q, 6q, 8p, 10p	Forus *et al.* 1995; Hulsebos *et al.* 1997; Lau *et al.* 2004; Stock *et al.* 2000; Tarkkanen *et al.* 1995; Tarkkanen *et al.* 1999a	**Gains**: 6p, 8q12-q21.3, 8q22-q24, 17p, 1p36.32, 6p21.1, 8q24, 12q14.3, 16p13, X, 1p, 12q **Losses**: 17p13, 17p12, 17q12, 6q27, 13q12.2, 13q, 10q,	Lim *et al.* 2004; Man *et al.* 2004; Squire *et al.* 2003; Zielenska *et al.* 2004	*RBI, CDK4, TP53, MDM2, SAS, MYC, PMP22, TOP3A, MAPK7, COPS3*

4.3. Quantitative analysis of genetic alterations

Despite the utility of the array methods, definite quantitative results of the genomic regions are not obtained. Molecular methods for detecting the quantitative genetic alterations in cancer cells are quantitative real-time polymerase chain reaction (qRT-PCR), locus specific FISH and multiplex ligation-dependent probe amplification (MLPA).

qRT-PCR is the most common method used for quantifying nucleic acids (Bustin *et al.* 2005). It is widely used in diagnostic and research applications for the diagnosis of tumours, such as in monitoring minimal residual diseases (Marcucci *et al.* 1998; Krauter *et al*. 2001), in identification of micrometastases in neuroblastoma, colorectal cancer, and prostate cancer (Cheung *et al.* 2001; Bustin *et al.* 1999; Gelmini *et al.* 2001). This method is also used for the quantification of amplifications of oncogenes (Bieche *et al.* 1999; Konigshoff *et al.* 2003). Locus specific FISH is also an effective technique for determining the number of copies of genes altered. This technique is commonly used to identify oncogene amplification (Sartelet *et al*. 2002; Tajiri *et al*. 1999). The recently developed MLPA method (Schouton *et al*. 2002) is used for copy number detection, methylation quantification, detection of unknown mutations, SNPs, and for mRNA profiling. This method is applied to a broad range of cancers for research purposes and in routine diagnostics (Monfort *et al*. 2006; Rooms *et al.* 2006). The use of the above methods not only enhances accuracy of diagnoses of various tumour types but can also be used for the validation of DNA microarray results.

5. IMPACT OF DNA COPY NUMBER CHANGES

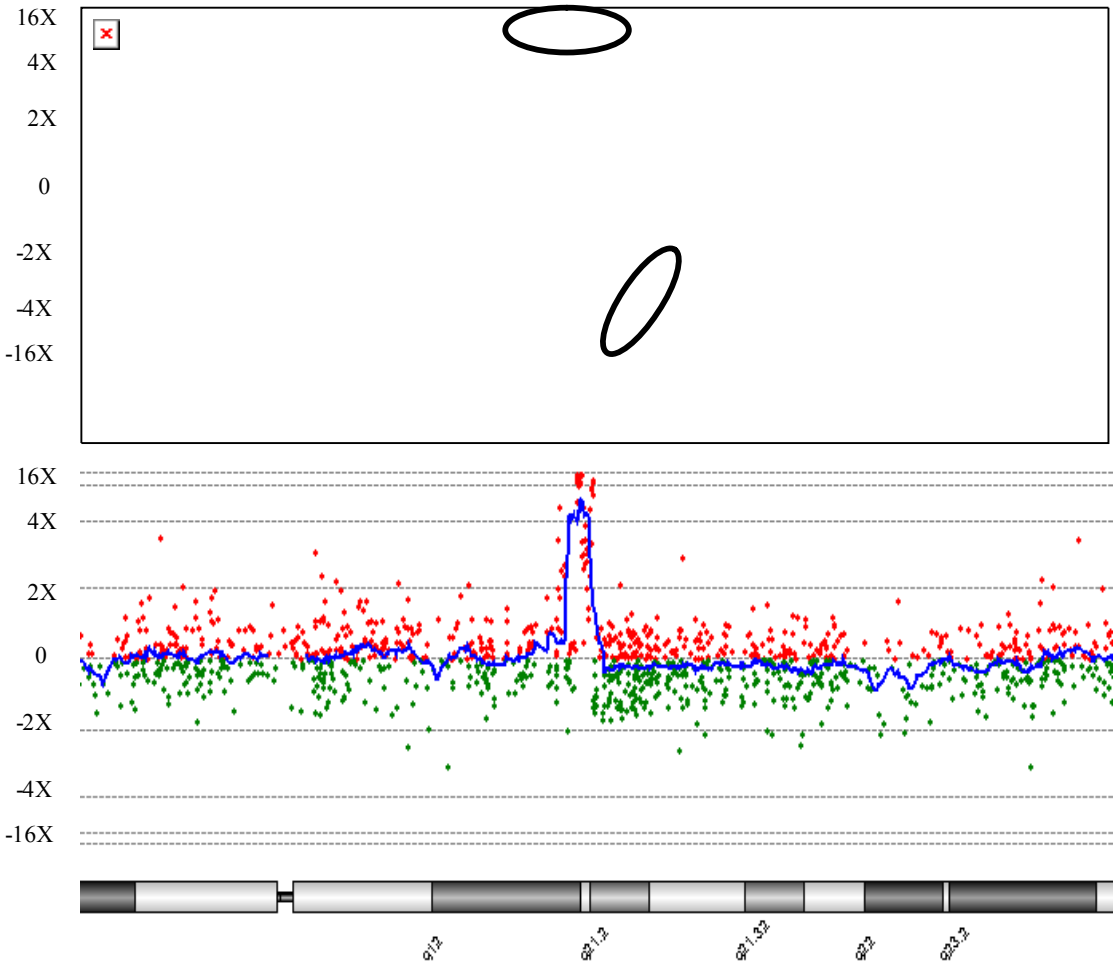
5.1. Oncogene activation by DNA copy number amplifications

Genetic alterations such as DNA copy number amplification and losses are known to be involved in the activation of proto-oncogenes and in the inactivation of tumour suppressor genes, respectively. It is well known that accumulation of these genetic changes disturbs the normal cellular growth controlling mechanism leading to the development of tumours. Proto-oncogenes are the genes involved in normal cellular growth and differentiation. Alterations such as chromosomal translocations, point mutations or gene amplifications can change the proto-oncogenes into active oncogenes, leading to uncontrolled cell growth and proliferation, and hence to the development of cancer. Alteration of a single allele is sufficient for the oncogenic effects, as oncogenes act in a dominant manner. The most frequent location for proto-oncogenes is at or near chromosomal translocation breakpoints, as shown in Table 3. Proto-oncogene activation by gene amplification is commonly seen in sarcomas and other cancers. The extent of gene amplification has proven to be useful for predicting prognosis and targeted therapies, for example, MYCN amplification in neuroblastoma (Savelyeva and Schwab 2001) and *ERBB2* amplification in breast cancer (Al-Kuraya *et al.* 2004). The percentage of genes over-expressed due to gene amplification varies between different types of cancer (Hyman *et al.* 2002; Pollack *et al.* 2002; Wolf *et al.* 2004).

High level gene amplification can be detected in cytogenetic analysis in the form of double minutes, homogenously staining regions or ring chromosomes (Cowell 1982). Amplicons are small chromosomal regions containing multiple copies of amplified DNA (0.5-10 Mb), whereas larger chromosomal areas showing copy number increases are considered as gains (Lengauer *et al.* 1998). Various models for gene amplifications have been proposed but the exact underlying mechanism is not fully understood. According to the onion skin (overreplication) model, a replication bubble or overreplicated strands are formed during the S-phase of mitosis due to multiple points of initiation of DNA replication in a single cell cycle. The overreplicated strands can be then resolved by recombination into extrachromosomal elements or linear sequence. However, not all amplicons display amplification patterns generated by this model (Botchan *et al.* 1979; Sambrook *et al.* 1975). The breakage-fusion-bridge (BFB) model of gene amplification has explained the genomic instabilities in cancer, and the generation of a variety of gene amplifications (Albertson *et al.* 2003; Fenech 2002; Masuda and Takahashi 2002; Von Hoff 1991). Furthermore, it has also been reported to be responsible for intratumour genetic heterogeneity (Gisselsson *et al.* 2000). This model requires a double-strand break or telomere erosion for its initiation followed by replication and fusion of two uncapped sister chromatids, resulting in the formation of unstable dicentric chromosomes, thus leading to another breakage. Many cycles of BFB result in the multiplication of genes near the break point (Murnane 2006; Myllykangas and Knuutila 2006; Shimizu *et al.* 2005).

Gene amplification by aCGH can be detected at the gene level where the growth promoting gene(s) can be seen. As compared to the locus specific FISH, where the exact number of gene copies can be counted, aCGH does not provide any good estimate or signal intensity for gains and amplifications and thus the criteria for differentiating gains from amplifications vary in different reports. The tumour cell content (i.e., the level of contaminative normal tissue present in the sample) and the tumour tissue heterogeneity should be considered while analyzing the results of aCGH, as they might cause variation in the estimates of copy numbers. Figure 7 demonstrates an example of oncogene amplification detected by aCGH.

GENE VIEW



CHROMOSOME VIEW

Figure 7: aCGH analysis by CGH analytics software of a gastric cancer sample, indicating a high level amplification of the proto-oncogene ERBB2 and 10 other genes located on region 17q21.1 (Vauhkonen et al, unpublished data). Base pair positions from pter to qter are indicated on the X-axis and the intensity ratios indicating copy number fold changes are shown on the Y-axis (for example +2X represents a 2-fold increase as compared to normal level).

5.2. Tumour suppressor gene inactivation by DNA copy number loss

Tumour suppressor genes constrain cell growth. Therefore, the loss of their function releases the growth constraints, leading to uncontrolled cell growth. Based on epidemiologic studies, Alfred Knudson (1971) proposed the 'two-hit' hypothesis in the development of retinoblastoma, an aggressive childhood eye tumour. According to his theory 'two hits' or mutagenic events are required to inactivate the tumour suppressor alleles. In familial cancers one hit is inherited through the germline and a second somatic

mutation is needed, whereas in sporadic cancers both mutations are somatic. The functional suppression of both alleles is required for the tumour suppressor gene to lose its ability to inhibit the malignant transformation of the cell. In addition to copy number loss, tumour suppressor genes can be inactivated by mutations (Lengauer *et al*. 1998; Futreal *et al*. 2004), loss of heterozygosity (Cho and Vogelstein 1992; Koorey and McCaughan 1993), and by methylation of the promoter region (Jones and Baylin 2007; Nephew and Huang 2003). Deregulated cell growth is the final result of multiple sequential alterations in both oncogenes and tumour suppressor genes (Lengauer *et al.*1998). An example of a loss and microdeletion is shown in Figure 8, which depicts the powerful resolution of aCGH for detecting both losses and microdeletions, which are beyond the resolution of cCGH. Well-known proto-oncogenes and tumour suppressor genes involved in tumourigenesis that are activated and inactivated by copy number amplifications and losses are listed in Table 8.

Table 8: Well-known oncogenes and tumour suppressors amplified and lost in various cancers.

Oncogenes	**Cancer type**	**References**
ERBB2	Breast, ovarian	Slamon *et al.* 1987
MDM2	Sarcoma	Leach *et al*. 1993
MYCN	Neuroblastoma	Schwab 1993; Seeger *et al.* 1985
AKT2	Ovarian cancer	Cheng *et al*. 1992
Tumour suppressors		
TP53	Various cancers	Donehower *et al.* 1992; Livingstone *et al.* 1992
BRCA1	Breast and ovarian cancer	Katsama *et al*. 2000; Rio *et al*. 1998
APC	Colorectal cancer	Flintoff *et al.* 2001; Su *et al*. 2000
DCC	Colorectal cancer	Cawkwell *et al.* 1994; Gerdes *et al.* 1995

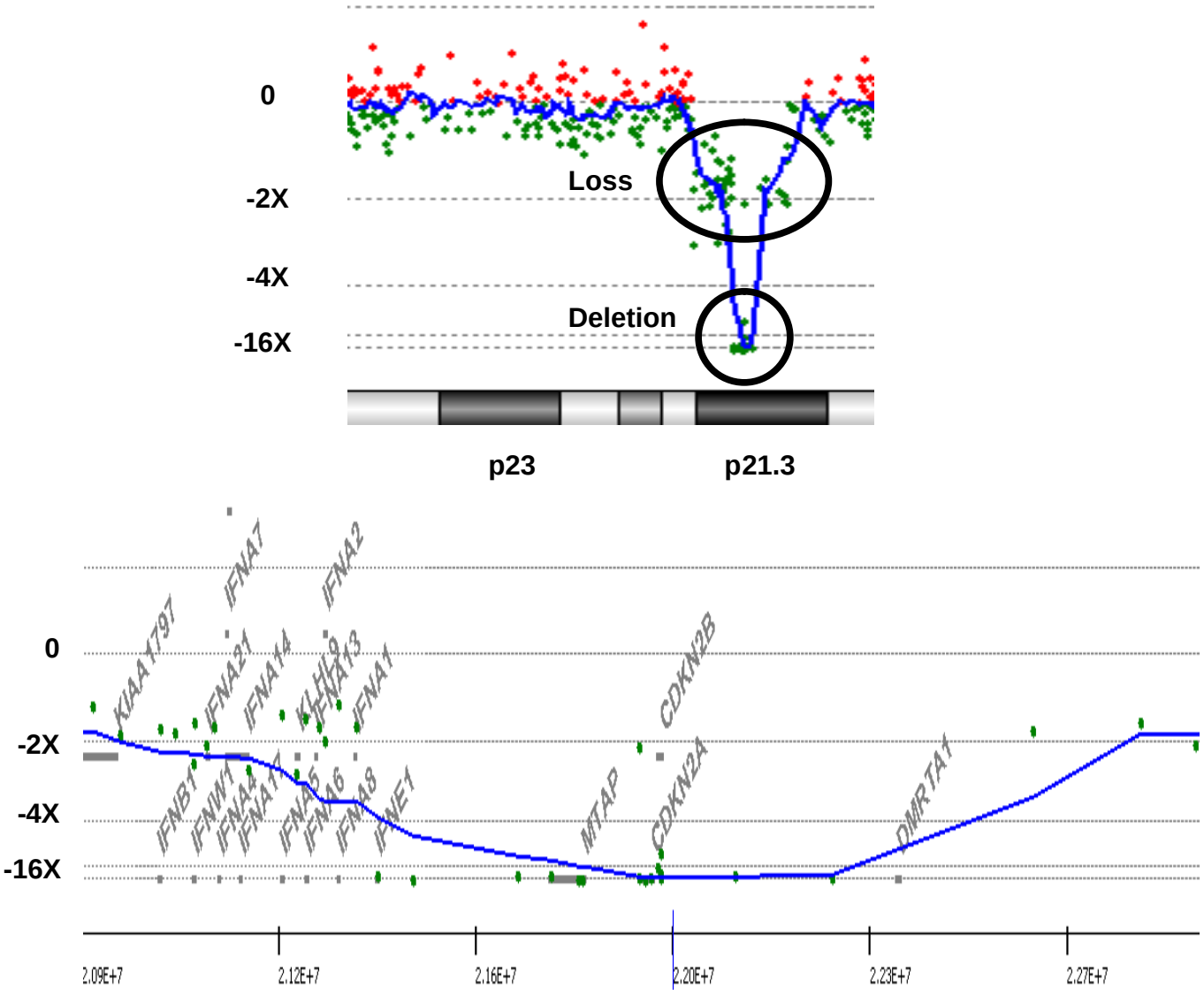


Figure 8: DNA copy number loss of various genes and microdeletions at 9p21.3 harbouring CDKN2A and MTAP detected by aCGH in an acute lymphoblastic leukaemia patient (Usvasalo, unpublished data). Base pair positions from pter to qter are indicated on the X-axis and the intensity ratios indicating copy number fold changes are shown on the Y-axis (for example +2X represents a 2-fold increase as compared to normal level).

5.3. Pathway analysis based on aCGH data

Cancer is a complex genetic disease and genes operate through the interaction of various pathways. Although individual genes with copy number changes may allow the progression or initiation of tumours, these copy number changes ultimately affect their protein products and their functions. These proteins do not operate on their own, but rather are involved in large interaction networks, or pathways, to achieve a particular role

at the cellular level. It is hence highly useful to study copy number alterations in terms of biologically meaningful pathways, rather than the traditional approach at the single gene level. Pathway level analysis thus provides a more comprehensive view of the biological mechanisms underlying the disease.

High-throughput technologies such as aCGH generate large-scale, genome-wide datasets regarding gene copy number changes. Sets of affected genes can be assessed for statistical significance using a variety of methods, the majority of which evaluate the degree of enrichment relative to what is expected by chance. There are many tools available on-line for performing this type of analysis (http://www.geneontology.org/GO.tools.shtml), one of the feature-rich tools used in Study V being WEBGESTALT (Zhang *et al.* 2005). Figure 9 shows an example of an analysis result, displayed as an acyclic graph, generated by WEBGESTALT for the genes gained in ULMS using pathway definitions provided by the Gene Ontology (GO) consortium (http://www.geneontology.org). A number of pathways belonging to the biological processes category were identified to be significantly affected, Figure 9 showing a few of the pathways affected by copy number gains.

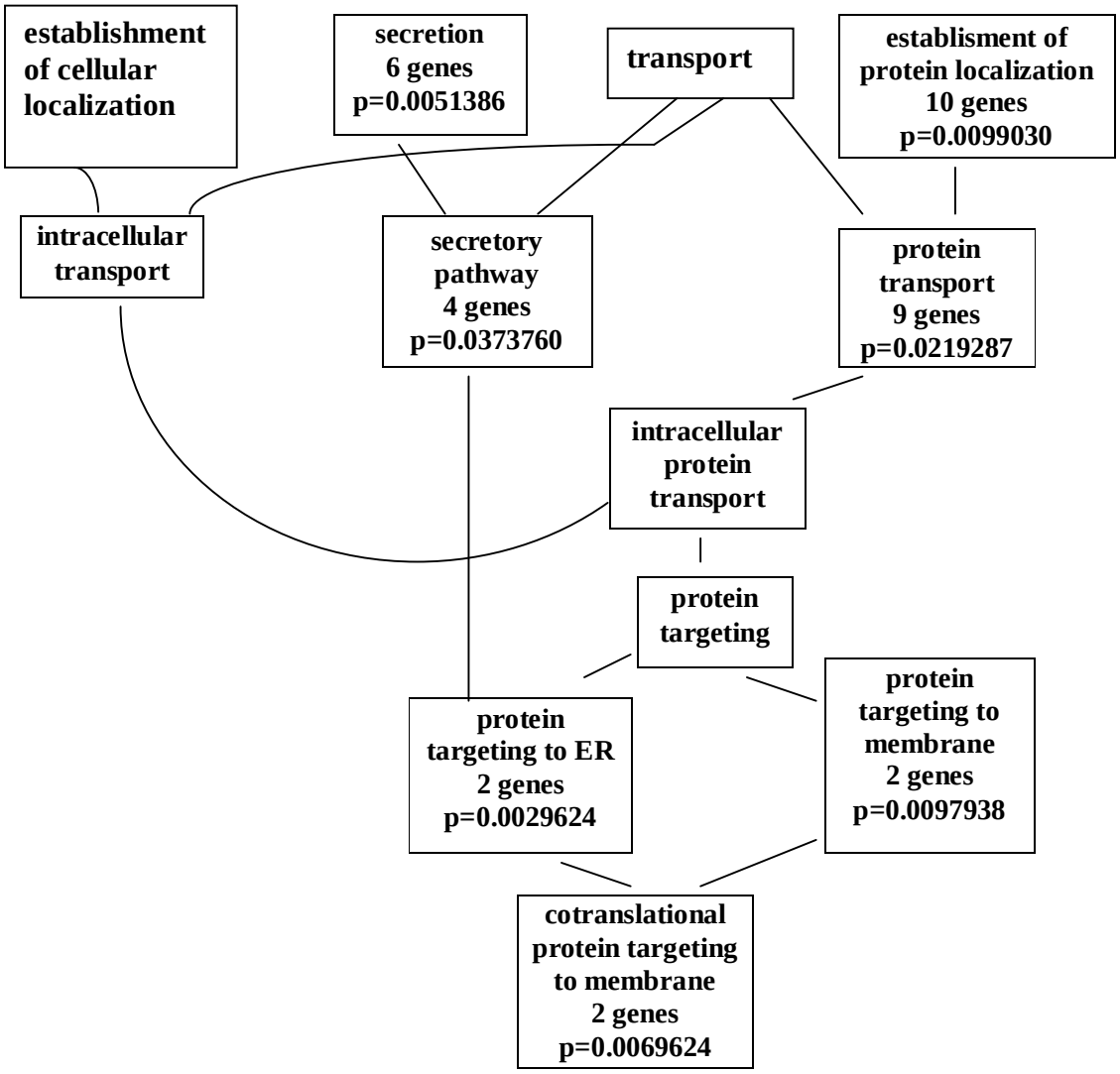


Figure 9: Pathway analysis on the genes affected by copy number gains performed by WEBGESTALT tool. The figure depicts the partial acyclic graph for pathways under the biological processes category. Listed in the boxes are the pathway names provided by GO, the number of genes affected and the P-value from the statistical test for enrichment.

.

AIMS OF THE STUDY

The aim of the present Study was to analyse the DNA copy number changes in selected types of rare soft tissue sarcomas and osteosarcoma using high-throughput microarray comparative genomic hybridization (aCGH) technology.

The specific aims were:

a) To screen novel gene copy number changes and detect precise boundaries of gene copy number aberrations and characterize their target genes in soft tissue leiomyosarcoma, dermatofibrosarcoma protuberans and osteosarcoma (I, II, III).

b) To uncover the biological networks involved in the malignant progression of uterine leiomyosarcoma (V).

c) To characterize the 17p amplicon shared by leiomyosarcoma, malignant fibrous histiocytoma and osterosarcoma (IV).

MATERIALS AND METHODS

1. Sarcoma specimens and DNA extraction (I-V)

In Study I: Fourteen formalin-fixed paraffin-embedded tissue sections from primary LMS from 14 patients were collected from the Helsinki University Central Hospital, Helsinki, Finland. Appropriate immunohistochemical analyses and histopathologic reviews were performed to confirm the diagnoses. The Scandinavian Sarcoma group grading system was applied where tumours were classified as low grade (I-II) or high grade (III-IV). Among these 14 samples, 3 were low-grade with changes in chromosome 17p, 4 were low-grade without changes in chromosome 17p, 3 were high-grade with changes in chromosome 17p, and 4 were high-grade without changes in chromosome 17p. For aCGH analysis samples were pooled (Table 9) into four categories according to the grade and changes in chromosome 17p.

Table 9: Details of the 4 pools used in Study I.

Pool A (case 1-3)	Low-grade LMS with changes in chromosome 17p
Pool B (case 4-7)	Low-grade LMS without changes in chromosome 17p
Pool C (case 8-10)	High-grade LMS with changes in chromosome 17p
Pool D (case 11-14)	High-grade LMS without changes in chromosome 17p

In Study II: Six formalin-fixed paraffin-embedded tissues from DFSP specimens were collected from the Orthopedic Centers at the Helsinki University Central Hospital, Helsinki, Finland and 4 frozen samples were obtained from the Lund University Hospital, Sweden. Re-evaluation of the diagnosis was done by histological examination and immunohistochemistry using CD34 antibody.

In Study III: Twenty-one fresh frozen OS samples (14 conventional, 2 soft-tissue, 3 periosteal, 1 fibrohistiocytic and 1 small cell OS) were collected from Helsinki University Central Hospital, Helsinki, Finland, and one cell line was obtained from the Laboratory of Oncologic Research, Rizzoli Orthopaedic Institute, Bologna, Italy.

In Study IV: Formalin-fixed paraffin-embedded tissue sections from 2 low- grade and 2 high- grade soft tissue LMS, 3 high-grade OS, and 2 high-grade MFH were obtained from Helsinki University Central Hospital, Helsinki, Finland. The samples selected for the study showed gains in chromosome 17p as revealed by cCGH analysis.

In Study V: Eighteen formalin-fixed paraffin-embedded tissue sections from 18 primary ULMS samples from 18 Finnish patients were obtained from Helsinki University Central Hospital, Helsinki, Finland. Of these 18 samples, 8 were low-grade and 10 were high-grade LMS. Grading was done as described in Study I. Diagnosis and grading was confirmed by histopathological analyses.

All the above-mentioned primary sarcomas selected for these studies were collected from untreated patients. Clinical data of the sarcoma entities is shown in Table 10.
DNA was extracted using standard protocols (Miller *et al*. 1988; Sambrook *et al*. 1989). Reference DNA used in both chromosomal and array comparative genomic hybridization was extracted from pooled peripheral blood samples (male and female pool) of healthy individuals.

2. Chromosomal comparative genomic hybridization (I-V)

In order to determine the genome-wide DNA copy number changes at the chromosome level, cCGH was performed on all the sarcoma samples from Studies I to V. The protocol for cCGH was according to the method described by Kallioniemi and co-workers (1992, 1994) with slight modifications (El-Rifai *et al.* 1997). Briefly, tumour DNA and reference DNA were labeled by nick translation. Equal amounts of tumour DNA labeled with fluorescein isothiocyanate FITC-dCTP and FITC-dUTP (1:1; DuPont, Boston, MA, USA) and reference DNA labelled with Texas-Red-dCTP and Texas Red-dUTP (1:1; DuPont), along with unlabeled Cot-1 DNA to block the binding of repetitive DNA sequences, were hybridized onto normal metaphase spreads. After 48-hour hybridization at 37°C, the slides were washed and counterstained with DAPI in an antifading solution.
An Olympus fluorescence microscope and the ISIS digital image analysis system (Metasystems GmbH, Altlussheim, Germany) were used to analyse the hybridized slides. For each sample, three-colour images of 10-12 good quality metaphases were taken. Changes in the chromosomal regions were interpreted by calculating the signal intensity ratios of red to green colours along each chromosome. The cut-off value for gains was defined as when the ratio exceeded 1.17, losses when the ratio fell below 0.85, and high-level amplification when the ratio exceeded 1.5.

Table 10: Clinical data of the sarcomas cases analysed in Studies I-V.

Case/Study/ tumour type	Sex/ age	Grade	Tumour site/size (cm)	Case/Study/ tumour type	Sex/ age	Grade	Tumour site/size (cm)
1/I/LMS	F/44	LG	Subcutaneous/6.0	1/IV/LMS	M/36	LG	Cutaneous/1.2
2/I/LMS	M/36	LG	Cutaneous/1.2	2/IV/LMS	F/64	LG	Subcutaneous/3
3/I/LMS	F/64	LG	Subcutaneous/3.0	3/IV/LMS	F/80	HG	Subcutaneous/2
4/I/LMS	M/57	LG	Cutaneous/2.0	4/IV/LMS	M/46	HG	Cutaneous/2.3
5/I/LMS	M/78	LG	Cutaneous/0.7	5/IV/OS	M/31	HG	Femur/NA
6/I/LMS	F/25	LG	Cutaneous/1.0	6/IV/OS	F/22	HG	Femur/NA
7/I/LMS	M/65	LG	Cutaneous/0.9	7/IV/OS	M/16	HG	Femur/NA
8/I/LMS	F/80	HG	Subcutaneous/2.0	8/IV/MFH	F/63	HG	Soft tissue/NA
9/I/LMS	M/61	HG	Deep/8.0	9/IV/MFH	M/64	HG	Soft tissue/NA
10/I/LMS	M/46	HG	Cutaneous/2.3	1/V/ULMS	F/64	LG	Uterus/10
11/I/LMS	F/91	HG	Subcutaneous/13	2/V/ULMS	F/75	LG	Uterus/7
12/I/LMS	F/55	HG	Subcutaneous/2.0	3/V/ULMS	F/42	LG	Uterus/5
13/I/LMS	F/77	HG	Deep/6.0	4/V/ULMS	F/40	LG	Uterus/5
14/I/LMS	M/35	HG	Deep/10	5/V/ULMS	F/48	LG	Uterus/NA
1/II/DFSP	M/27	NA	Forearm/2.8	6/V/ULMS	F/49	LG	Uterus/6
2/II/DFSP	M/46	NA	Groin/7	7/V/ULMS	F/34	LG	Uterus/4
3/II/DFSP	M/28	NA	Chest/2	8/V/ULMS	F/69	LG	Uterus/NA
4/II/DFSP	M/40	NA	Back/6	9/V/ULMS	F/58	HG	Uterus/4
5/II/DFSP	M/52	NA	Scalp/NA	10/V/ULMS	F/71	HG	Uterus/>20
6/II/DFSP	F/36	NA	Back/8	11/V/ULMS	F/70	HG	Uterus/NA
7/II/DFSP	M/42	NA	Arm/7	12/V/ULMS	F/64	HG	Uterus/5
8/II/DFSP	M/56	NA	Groin/4	13/V/ULMS	F/38	HG	Uterus/4
9/II/DFSP	M/60	NA	Abdominal/4	14/V/ULMS	F/64	HG	Uterus/8
10/II/DFSP	M/64	NA	Shoulder/11	15/V/ULMS	F/69	HG	Uterus/NA
1/III/OS	M	HG	Cell line/NA	16/V/ULMS	F/38	HG	Uterus/>20
2/III/OS	M/27	HG	Femur/NA	17/V/ULMS	F/53	HG	Uterus/10
3/III/OS	M/31	HG	Femur/NA	18/V/ULMS	F/81	HG	Uterus/14
4/III/OS	M/18	HG	Femur/NA				
5/III/OS	F/19	HG	Femur/NA				
6/III/OS	M/37	HG	Femur/NA				
7/III/OS	M/45	HG	Tibia/NA				
8/III/OS	M/16	HG	Femur/NA				
9/III/OS	F/48	HG	Femur/NA				
10/III/OS	F/16	HG	Tibia/NA				
11/III/OS	M/23	HG	Femur/NA				
12/III/OS	F/22	HG	Femur/NA				
13/III/OS	M/22	HG	Femur/NA				
14/III/OS	F/19	HG	Femur/NA				
15/III/OS	F/32	HG	Femur/NA				
16/III/OS	M/63	HG	Femur/NA				
17/III/OS	F/77	HG	Femur/NA				
18/III/OS	F/25	HG	Femur/NA				
19/III/OS	M/44	HG	Femur/NA				
20/III/OS	F/91	HG	Femur/NA				
21/III/OS	F/21	LG	Femur/NA				
22/III/OS	M/19	HG	Femur/NA				

LG=low-grade; HG=high-grade; NA= Not available

3. Array comparative genomic hybridization (I-V)

DNA copy number changes at the gene level were detected by performing high-resolution genome-wide aCGH in all Studies (I-V). Due to the rapid development of the aCGH technology, three different array platforms were used. cDNA arrays containing 13,000 clones (Agilent Technologies, Palo Alto, CA, USA) were used in Studies I, II and III. Customized cDNA arrays containing 16,000 clones (http://microarrays.btk. utu.fi) in Study V and oligonucleotide arrays with 44,000 targets (Agilent Technologies) in Study II and IV were used to perform the array experiments. In order to avoid the detection of copy number polymorphisms in the reference sample, pooled sex-matched reference (female reference for female sample) DNA samples were used. Performing four sex-mismatched and sex-matched normal versus normal hybridizations validated the quality of arrays. The quality of genomic DNA has a substantial effect on the data, thus quality and purity of the DNA was measured by gel electrophoresis and optical density, respectively. For the oligonucleotide arrays, quantification of the labelled genomic DNA was performed using an ND-1000 Spectrophotometer (NanoDrop Technologies, Rockland, DE). aCGH on cDNA arrays was conducted according to the protocol described by Pollack *et al.* (1999) and Monni *et al.* (2001), with slight modifications. For cDNA arrays, 6.0 μg of *AluI* and *RsaI* digested genomic DNA, and for oligonucleotide arrays 1.5 μg of digested DNA, were used for the hybridization. The DNA was labeled by random priming reactions. Tumour and reference DNA were labeled with Cy5-dUTP and Cy3-dUTP, respectively (Amersham Biosciences, Piscataway, NJ). Hybridizations and washing of 13K and 16K cDNA slides were performed as described elsewhere (Monni *et al.* 2001), with minor changes in the duration of washes. Microarray slides were then dried and scanned using a laser confocal scanner (Agilent Technologies) to measure the signal intensities. Agilent's Feature Extraction software was used to extract the data from the microarray images. Agilent oligonucleotide arrays were performed following the manufacturer's instruction (Aligent Technologies).

4. Data analysis (I-V)

For 13K and 16K cDNA arrays (Studies I and II) DNA copy number aberrations and their associated breakpoints were identified, in addition to visual analysis, with aCGH-smooth software (Jong *et al.* 2004). For oligonucleotide arrays (Studies II and IV) CGH analytics software (Agilent Technologies) was used. For Study III the thresholds for gains (1.3) and losses (0.7) were defined by self versus self hybridizations. GeneSpring 7.2 software (Redwood City, CA) for filtering the data and CGH-explorer 2.5 for the identification of gains and losses at a false discovery rate of 1% were used in Study V. Genomic base-pair localizations for each cDNA clone for both the 13K and 16K cDNA arrays were retrieved from the University of California Santa Cruz Genome Browser database (http://www.genome.ucsc.edu/) as described previously by Hyman and co-workers (2002). In Study V, biologically relevant associations for the statistically significant gained or lost genes were assessed using the WEBGESTALT tool (http://bioinfo.vanderbilt.edu/webgestalt/).

5. Statistical analysis (V)

Multivariate statistics were used in Study V for assessing patterns of DNA copy number changes for association with clinical parameters i.e., tumour grade, tumour size, and the patients' last follow-up.

6. Ethical permissions (I-V)

For Study I and Study V ethical permissions were granted by the Ethics Review Board, Department of Surgery, Hospital District of Helsinki and Uusimaa (610/E6/2001) and the National Authority for Medicolegal Affairs (1745/32/300/03). The HUS coordinating Ethics Review Board HUS 328/E0/05 and HUS 329/E0/05 (both on 27.9.2005) granted permission for Studies II, III and IV.

RESULTS

1. DNA copy number profiling of sarcomas (I-III)

In Studies I-III, 14 low- and high-grade LMS, seven DFSP and 22 OS cases were analysed by aCGH. Due to the rapid development in microarray technology, three different platforms were used. In Studies I and III 13K arrays were used, and in Study II 13K, 16K and 44K arrays were used. The aCGH results of Studies I-III are summarized in Table 11 and Figure 10. In Study III losses and gains were excluded from the analysis, as the main focus was to identify recurrent amplicons and the target genes therein. The gains/amplifications seen in at least 20% of the sarcomas were at chromosomes 6p12-p21, 8q, 12q11-q15, 17 and 22q. Of these recurrent changes, amplifications of 6p12-p21, 8q, and 12q11-q15 were restricted to OS, while gain/amplification of 17 were seen in DFSP, OS, and LMS. Gain/amplification of 22q occurred in DFSP and OS.

Table 11: DNA copy number changes detected by aCGH in LMS, DFSP, and OS. DNA copy number gains and losses are marked with (+) and (-), amplification is indicated in boldface. In Study III only amplifications were scored.

Study/case	DNA copy number changes
LMS	
I/pool A	-2p25, -6p12-pter, +6p12-qter, -10q26.3-qter, -12p13.3, -13q14-q34, +15q26, +17p13.1-q11, -19q13.33-q13.43, -22q11.21
I/pool B	-13q11-qter
I/pool C	+10pter-q11.22, -10q11.22-q24.3, -13q14.3-q34, +15q11-q26.3, +17p13.1-q11, -17q25.3 +19p13.2-p13.3, +20p11, -Xq13.2-q28
I/pool D	-6p21-q26, -10p12.32-pter, +20pter-q11.2, + 20q13-qter
DFSP	
II/2	+5q35.3, +17q23.2-qter, +18q21.1-q21.3, +19q12-pter, + 22q22.3
II/3	+3p21.31-p21.1, +8q24.3, +17q21.33-qter, +20q13.33, +22cen-q13.1
II/6	+1p35.3-pter, +1q21.2-qter, +17q21.31-q25.3/**17q24.1-q25.3**, +18q21.33-pter, + Xq28
II/7	+11p15.5, + 17q23.2-qter, -22q13.2-qter
II/8	+17q24.2-qter
II/9	+6p21.2-p21.3, +17q21.2-qter, +19q13.1-q13.2, +19q13.42-q13.43, 22cen-q13.1
II/10	+8q24.2-q24.3, +13q34, +17q21.32-qter, + 22cen-q13.1
OS	
III/1	**+6p12-p21, +7q31-q34, +9p13, +12p13, +17p**
III/2	**+8p11, +17q25**
III/3	**+3p25, +5p, +6p12-p21, +7q31-q34, +8q, +15q23-q26, +16q12-q24, +17p, +18q, +17q25, + 21q22**
III/5	**+1p36, +12q11-q15, + 19q13, +20q**
III/6	**+12q11-q15**
III/8	**+17p, +19q13**
III/10	**+8q**
III/11	**+1p13, +1q21, + 6p12-p21, +7q22, +8q, +9q34, +11q13, +12q11-q15, +14q11, +16p13, +20q, +17q25, +19q13, +21q22, +22q**
III/12	**+12q11-q15, +17p**
III/13	**+1p22, +1q21, +1q23, +8q, +6p12-p21**
III/14	**+2q11-q12**
III/15	**+12p13, +12q11q15, +14q11**
III/16	**+2p11, +4p12-q12, +8q, +14q11, +15q23-q26, +20p11-p12**
III/17	**+1p36**
III/18	**+5p12-p15, +8q, +19p13**
III/19	**+1p34-p36, +1q11-q24, +12q11-q15, +16p12-p13, +19p12, +19q13, +20p12, +20q12, +21q12, +22q12**
III/20	**+12p13, +12q11-q15,+14q11, +22q**
III/21	**+12q11-q15**
III/22	**+1p13, +1q32, +1q21, +6p12-p21, +22q**

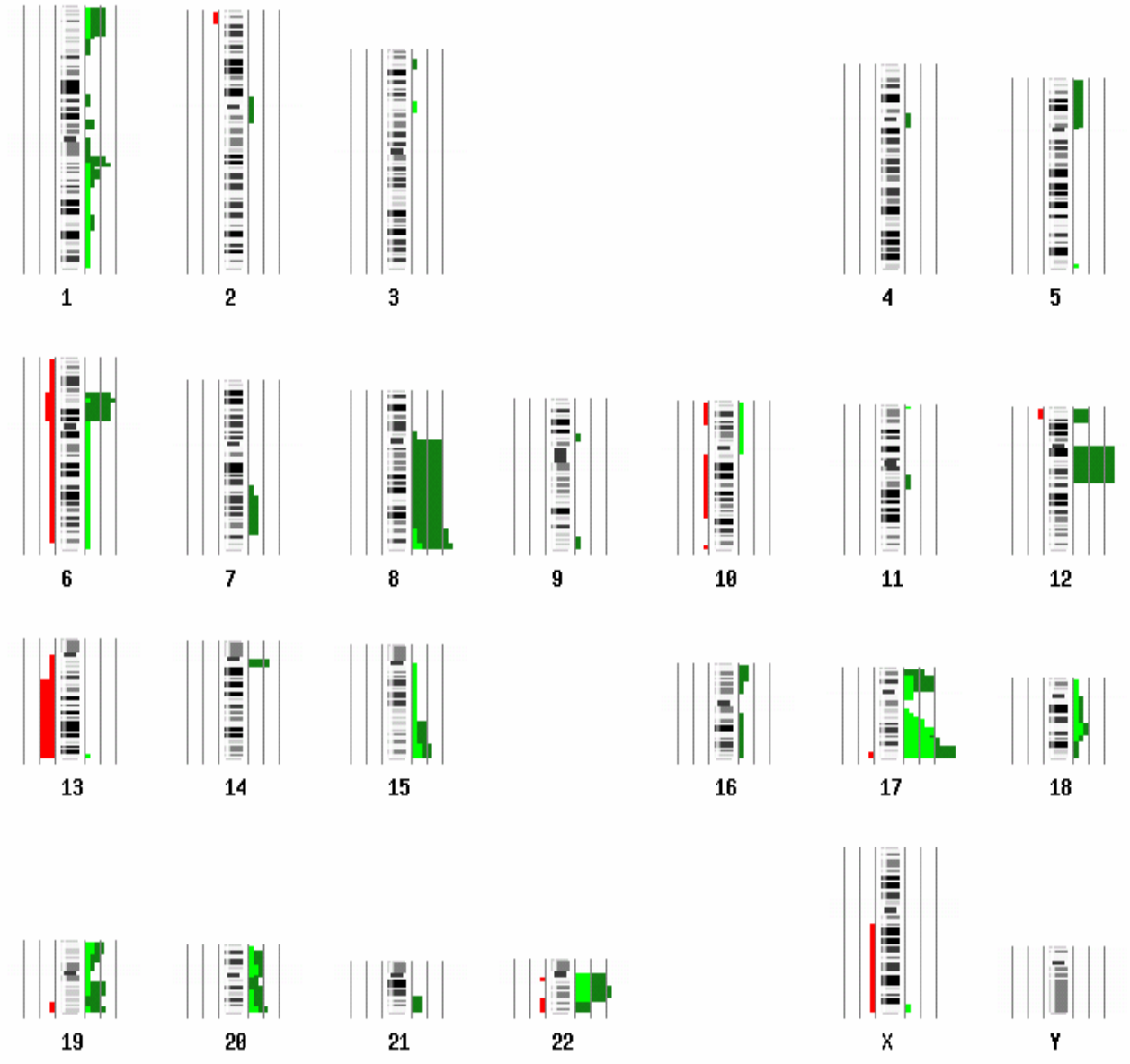


Figure 10: *A summary of DNA copy number changes throughout the genome detected by aCGH in low- and high-grade LMS (14 cases), DFSP (7 cases), and OS (19 cases). Losses are marked as areas drawn on the left side of the chromosome idiogram, and gains on right side. Amplifications are shown as darker areas on the right side. Chromosomal idiogram was generated with the profilebase software (www.progenetix.net). The three vertical thin lines at the left and right of the chromosome idiogram indicate the frequency of losses and gains at cut-off of 5%, 10%, and 20%, respectively.*

2. Association of DNA copy number changes in ULMS to biological processes and the clinical data (V)

aCGH analysis on 18 ULMS cases using customized 16K microarrays revealed a complex pattern of copy number changes in all chromosomes with a slightly higher proportion of losses than of gains. After rigorous filtering of the data, bringing the number of genes included for statistical analysis down to 10,590 genes, 4,387 and 4,518 genes were detected gained and lost, respectively, in at least one case. As the aim of the Study was to focus on the more representative aberrations for ULMS, the loci affected in at least 20% of cases were considered, resulting in 231 and 265 genes being identified as gained or lost. In order to obtain a more expansive view of the biological processes affected by the underlying complex aberration pattern, pathway analysis was additionally performed. A high proportion of genes with DNA copy number gains and losses were observed for a number of pathways in the biological processes defined by GO. The most intriguing pathways affected by copy number changes, with obvious relevance to cancer development and/or progression, are listed in Table 12.
Multidimensional scaling analysis was performed in order to detect any correlation between DNA copy number change and clinical parameters such as tumour grade, tumour size, and status at last follow-up. No evidence of correlation between aberration patterns and clinical parameters were detected.

Table 12: Some of the pathways affected by copy number gains and losses.

Gains	**Pathways affected**	**Losses**	**Pathways affected**
1p22, 1q12-q32, 2p23.3, 3p24-p26, 6p21.3, 8q22-q24, 10q26, 18p11.32	G1/S transition of mitotic cycle (p=0.016), co-translational protein targeting to membrane (p= 0.006), actin filament polymerization (p=0.016), positive regulation of cytokine biosynthesis (p=0.018)	2q33.1, 4q13-q35, 6p21, 6q14.1, 7p15.2, 7q22.1, 13q11-q31, 14q32.3, 16q22-q24, 19p13, Xp22.3, Xq26-q28	DNA replication (p= 0.021), chromatin modification (p=0.017), telomere maintaining (p= 0.006), meiosis (p=0.020), mitosis (p=0.036), angiogenesis (p=0.040)

3. Characterization of the copy number changes in the p-arm of chromosome 17 by aCGH (IV)

The detailed DNA copy number aberrations in chromosome 17p were analysed by oligonucleotide-based aCGH in nine cases belonging to three sarcoma entities (OS, LMS, MFH). The inclusion of samples was based on gains at 17p detected by previous cCGH analysis. As shown in Figure 11, in all cases except for one case of MFH (case 8), the p arm of chromosome 17 started with a p-terminal loss, *TP53-C17orf25* being the minimal common lost distal region and *TP53-GAS7* the minimal common proximal lost region. The aforementioned p-terminal losses were followed by gains and amplifications in all

cases. Copy number losses detected by aCGH were not detected by cCGH due to the restricted resolution of this technique. Gains and amplifications in 17p11.2-p12 were detected in all samples, containing 63 genes spanning from *FLJ45455* to *ULK2*. Low-grade LMS and MFH showed a continuous copy number pattern at 17p11.2-p12, whereas high-grade LMS and OS showed a discontinuous pattern.

.

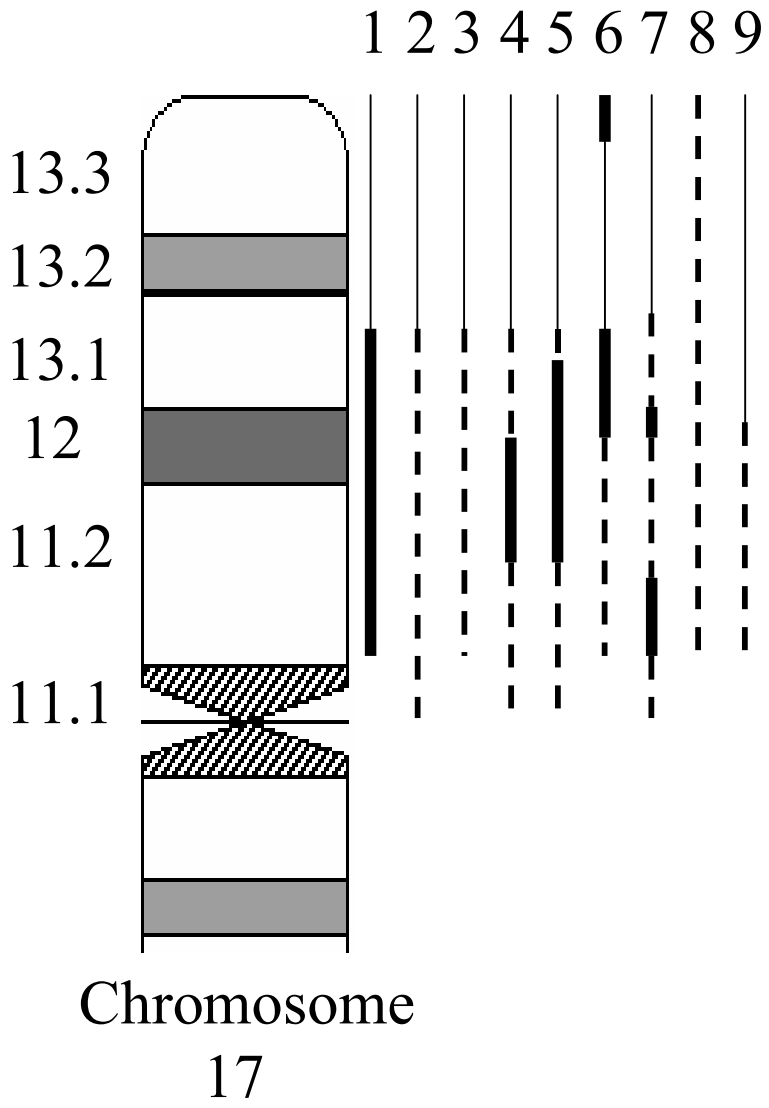


Figure 11: Fine structure of 17p DNA copy number changes found by aCGH in low- and high-grade LMS (cases 1-4), OS (cases 5-7) and MFH (cases 8-9). Thin lines indicate losses, dotted lines indicate gains and bold lines indicate amplifications.

DISCUSSION

1. Impact of aCGH technology in understanding sarcomas (I-V)

aCGH has been used in various cancers as a tool for measuring copy number status of tumour DNA. The aim of the present work was to apply aCGH as a tool to screen novel DNA copy number aberrations and the genes involved in these aberrations in sarcomas not detectable by low-resolution cytogenetic methods, thereby gaining new insights into the molecular genetic changes that occur in sarcomas. Further aims were to to identify the tumour-specific copy number changes and the genes therein with accurate breakpoint boundaries, which are not precisely detected by cCGH. This Thesis provided new information regarding the precise DNA copy number changes, thus adding new information to results previously described by others. The novelty of this Thesis lies in providing information regarding the biological pathways involved in pathogenesis for some sarcoma entities. Thus, application of the aCGH technique in the rare sarcomas studied in this Thesis proved to be highly relevant in identification of potential target genes for further studies and also in understanding the biology of sarcomas.

In Studies I-V aCGH was applied to the DNA obtained from fresh frozen and paraffin-embedded tissue. Regardless of the DNA source, successful aCGH results were obtained from all samples. Thus, Studies I-V demonstrated the applicability of the enhanced resolution achieved by aCGH, which enabled the identification of precise boundaries of copy number changes and their breakpoints, candidate cancer related genes, and detection of recurrent aberrations. As an example, Figure 12 demonstrates the aCGH results of low-grade LMS. Fold level changes in signal intensity, reflecting copy number losses and gains, and the genes that occur within these regions are indicated. Accordingly, the results demonstrated the power of aCGH to provide the in-depth information of aberrations regarding the genes and the breakpoints of chromosomal gains, losses, and amplifications.

In Study I, gene copy number profiling of low- and high-grade LMS by aCGH was conducted for the first time, highlighting the differences and similarities between the two tumour grades.

The recently developed 60-bp oligonucleotide microarrays need very little DNA (1.5 μg) allowing the analysis of individual tumours, which is sometimes not possible with cDNA arrays where 20 μg of DNA is required as the starting material for the analysis. Oligonucleotide arrays were used in Study IV and their high resolution provided in-depth information regarding the 17p amplicon structure in sarcomas. One of the most striking findings by aCGH in this Study was the copy number loss before the 17p amplicon that has not been observed by cCGH due to its limited resolution. Copy number profiling of 17p done in this Study for MFH and LMS, to our knowledge, has never been previously reported. Study V provided in-depth information regarding the various, not previously reported, biological pathways that were altered by copy number changes.

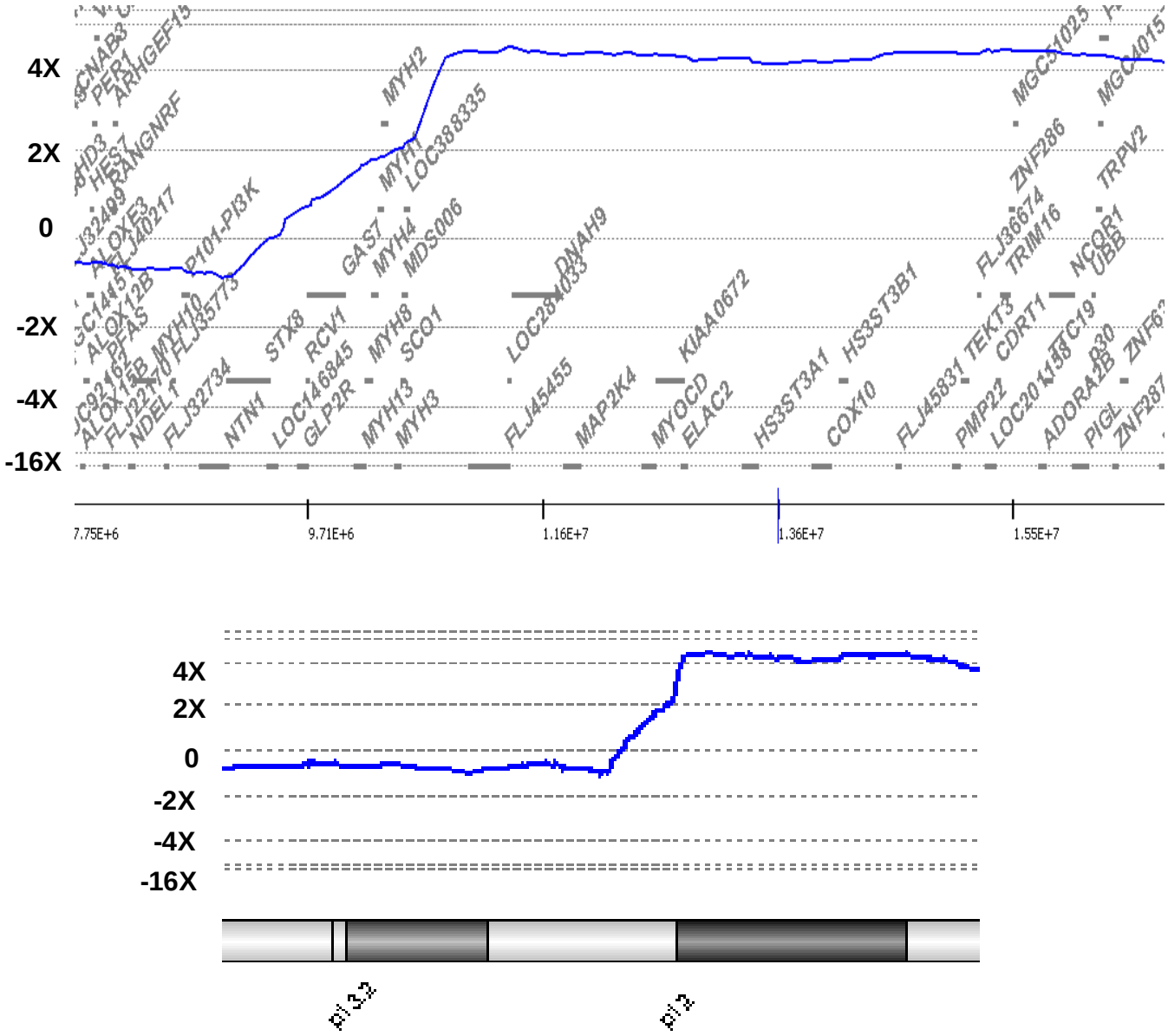


Figure 12: DNA copy number losses and gains detected in chromosome 17p in low grade LMS. aCGH detects both genes and the breakpoint region involved in the aberrations. On the Y-axis zero indicates the baseline and intensity ratios indicating copy number fold changes are shown (for example +2X represents a 2-fold increase as compared to normal level). Base pair positions are indicated on the X-axis.

In this Thesis aCGH analyses were performed on only a small number of cases from each sarcoma entity due to the rarity of these sarcomas. Nevertheless, aCGH analysis provided

the leads to the identification of target genes that in the future need to be further investigated. Details of these results are discussed below.

2. High- and low-grade LMS show distinctive aberration patterns (I)

aCGH was performed on 13K arrays, which require 20 μg of DNA as starting material. As there was not enough tissue to meet the requirements for making individual arrays, the DNA of individual samples was pooled. Use of sample pools in microarray experiments in order to detect relevant changes common to all samples has been successfully applied in many microarray studies (Craig *et al.* 2005; Kendziorski *et al.* 2005; Zhang and Gant 2005a). However, the small changes that vary from case to case remain undetected in sample pools. aCGH detected two common regions of gains (15q26-qter and 17p13.1-q11) and two of losses (6p12-p21.3 and 13q14.3-qter) which were shared between low- and high-grade LMS, and nine and 12 different regions altered only in the low- and high-grade sample pools, respectively. All of these regions were undetected by cCGH, thus aCGH proved to be an effective tool in differentiating between low- and high-grade LMS based on their aberration patterns. More gains (three-fold) were detected in high-grade as compared with low-grade LMS, and the numbers of losses were about two-fold higher in low-grade as compared to high-grade LMS. This might indicate that losses are the initiation factor for tumourigenesis and that gain is required for tumour progression. 17p13.1-q11 was gained in both low- and high-grade LMS, which supports previously published data which indicates that gain of this region is a frequent phenomenon in LMS. Pools of low- and high-grade LMS with changes in chromosome 17p showed a large number of aberrations as compared to low- and high-grade pools without changes in chromosome 17p. This might suggest that tumours with changes in chromosome 17p are more complex and that the region might contain some important genes which cause the complexity in the karyotype.

3. aCGH detected the exact translocation breakpoints at the exon level in DFSP (II)

The characteristic feature of DFSP is a translocation, t(17;22)(q22;q13.1), that is most often unbalanced, resulting in the fusion of *COL1A1* and *PDGFB*. The major advantage of aCGH performed on high-resolution (60 bp) 44K chips lies in the knowledge of the exact base pair location for every probe. Previous analysis has shown the chromosome band 17q22 as the location for *COL1A1* and 22q13 as the location for *PDGFB*. By aCGH analysis 17q21.33 was defined as the breakpoint region for *COL1A1* and 22q13.1 for *PDGFB*. It was earlier reported that the location of the breakpoint within *COL1A1* varies greatly whereas *PDGFB* has a constant breakpoint (O'Brien *et al.* 1998). By the aCGH approach the detection of the exact breakpoint within the COL1A1 gene became possible. Recurrent gains of 17q21.33-qter and 22q-q13.1 could be seen by aCGH, consistent with previous cCGH studies (Kiuru-Kuhlefelt *et al.* 2001; Nishio *et al.* 2002; Sirvent *et al.* 2003). aCGH analysis thus provided novel information about the genes involved in the gained regions, which need to be further investigated. DNA copy losses in DFSP were not as frequent as in other sarcomas. In one case aCGH detected loss in 22q13.2-qter that might indicate an unbalanced translocation. This terminal loss had previously been identified by Buckley and co-workers (2002) in two DFSP tumours using chromosome 22 BAC arrays. Exact breakpoints and the genes involved were determined for the

additional changes reported by aCGH in Study II that might be involved in the pathogenesis of DFSP.

4. aCGH defined 12q11-q15, 8q, 6p12-p21 and 17p as recurrent amplicons in OS (III)

In contrast to some other sarcoma entities that are associated with some specific, well-defined chromosomal translocations, OS presents a unique challenge as it lacks any recurrent translocation or chromosomal changes, and possesses instead multiple chromosomal abnormalities. This Study aimed to analyse only the recurrent amplicon and their target genes, which might lead to the identification of the target genes driving the amplifications. aCGH detected recurrent amplicons with high levels of amplification at 12q11-q15, 8q, 6p12-p21, and 17p, and less frequent small amplicons showing lower levels of amplification at 1p34-p36, 1q21, 19q13, and 21q22. These amplicons had discontinuous structures accompanied by DNA copy number losses, gains and no copy number changes. These amplicons have been also detected in previous studies by cCGH (Sandberg and Bridge 2003b).
The amplicons at 12q and 17p were further analysed in detail as these amplicons contained genes with very high copy numbers ratios and were recurrent in all the samples. The possible target genes of amplicon 12q, based on the very high amplification ratios, were *OS9, CYP27B1, DKFZP586D0919*, and *TSFM*. Genes such as *COX10, PMP22, ADORA2B, ZNF287, MRIP, COPS3, PEMT, SREBF1, TOM1L2, TOP3A, GRAP* and *MAPK7* were considered as possible targets within the 17p amplicon, as these were the most recurrent genes gained in all samples. Some of the target genes amplified in our Study has also been detected in other studies (van Dartel *et al.* 2002). Based on expression profiling of an OS cell line (Monni *et al* personal communication), *TOM1L2* could be considered as a candidate target gene since this gene was both amplified and expressed. TOM1L2 is involved in intracellular protein transport (http://www.geneontology.org). Further studies are needed to investigate the role of the other possible target genes mentioned in order to understand the contribution of those genes in OS.

5. aCGH highlights the involvement of various cellular pathways in ULMS (V)

To understand the various biological processes underlying cancer, pathway analysis based on gene expression data has been extensively used in various cancers (Furge *et al.* 2007; Hong *et al.* 2007; Oishi *et al.* 2007). To date, studies adopting similar analysis techniques for aCGH data are scarce, especially regarding ULMS. To our knowledge there is, in fact, only one such study, limited to only seven ULMS tumours and using a lower resolution BAC-based array (Cho *et al.* 2005). Accordingly, in the present Study the GO classification system (http://www.geneontology.org) was used to describe the pathways that were ascribed to the gained or lost genes. GO provides a formal, controlled and precisely defined vocabulary in order to describe the roles of genes or gene products in organisms. The GO definitions are organized into three categories: molecular function, biological processes and cellular component. By analyzing the complex patterns of copy number gains and losses by adopting a pathway approach, we aimed to gain understanding about the cellular mechanisms that underlie ULMS. The idea was that the

analysis results would not only help to pinpoint the processes implicated in the development and/or progression of the cancer, but also shed some light on the resistance of ULMS to both radiotherapy and chemotherapy. Our approach provided results that are biologically interpretable and statistically robust. Various biological processes were identified as being statistically significantly affected by gene copy number gains and losses, of which perhaps the most striking were chromatin modification, mitosis, DNA replication, meiosis, angiogenesis, and G1/S transition of mitotic cycle**.** These pathways featured two well-established tumour suppressor genes (*BRCA2, EREG*) and one proto-oncogene (*GFI1).* These findings provided a clear view of the various deregulated biological pathways, such as DNA replication, chromatin modification, telomere maintenance, meiosis and mitosis, among others, which might contribute to the tumourigenesis in ULMS.
The aberration patterns showed no correlation with clinical parameters, as observed also in previous studies including various types of sarcomas (Mandahl *et al.* 2000).

6. DNA copy number changes of 17p in sarcomas (IV)

Amplification or gains in the p arm of chromosome 17 is a characteristic feature shared by sarcoma entities such as OS, LMS, and MFH. This might indicate the residence of the putative oncogene (s) in this zone. A few target genes for OS have been suggested (van Dartel *et al.* 2002) but no specific targets have been defined for LMS or MFH. We applied aCGH to explore the copy number status of chromosome 17 in detail in the aforementioned sarcoma entities, work previously not done using fine resolution arrays.
Precise boundaries of aberrations were defined. With the exception of one MFH case, all cases showed losses distal to gains or amplifications. In all cases, a minimal common region of *TP53-C17orf25* was defined as a distal region of loss and *TP53-GAS7* as a proximal region of loss before the gain or amplification. These losses have not been detected by cCGH method previously, thus aCGH proved to be effective tool for screening losses and gains upto the kb level, which helps in the characterization of amplicons. Identification of DNA copy number loss before the 17p amplicon is still an open question in understanding whether DNA copy number loss is an early initiating event before the amplification or is a result of the amplification. *TP53* functions as a guardian of the genome, and is involved in pathways including detection of DNA damage, DNA repair, cell cycle arrest, and apoptosis. It responds to DNA damage signals by inducing apoptosis and thus maintaining genome integrity (Attardi 2005; Hussain and Harris 2006; Janus *et al.* 1999). Deletions at *TP53* reported in the mutation database in MFH, LMS, and OS were at frequencies of 8.43%, 2.73%, and 13.4%, respectively, whereas point mutations in *TP53* were reported with frequencies of 19.2%, 20.5%, and 26.9%, respectively (Olivier *et al.* 2002).
Based on the copy number profiling at chromosome 17p by aCGH, low-grade LMS and MFH were grouped together due to the similar continuous pattern of gains, whereas high-grade LMS and OS were grouped together due to their non-continuous amplification patterns (Figure 13). Based on aCGH results it can thus be speculated that variation in the copy number pattern such as continuous and non-continuous gain/amplification patterns lead to different mechanisms of the establishment of amplicons. In Study III all OS showed the same starting and ending point for 17p amplicons by 13K arrays. Study IV

resolved the DNA copy number trend in OS using oligonucleotide arrays and thus demonstrated a complex amplification pattern interrupted by gains and losses, which were not detected by the lower resolution 13K arrays. Beside gains/amplification at 17p in OS, LMS, MFH, complex aberration patterns were observed in these sarcomas. This is attributed to the breakage-fusion-bridge cycle that is responsible for the chromosomal instability, by causing excess structural and numerical alterations, gene amplification, complex rearrangements, ploidy changes and cell-to-cell cytogenetic variation (Gisselsson *et al.* 2000).

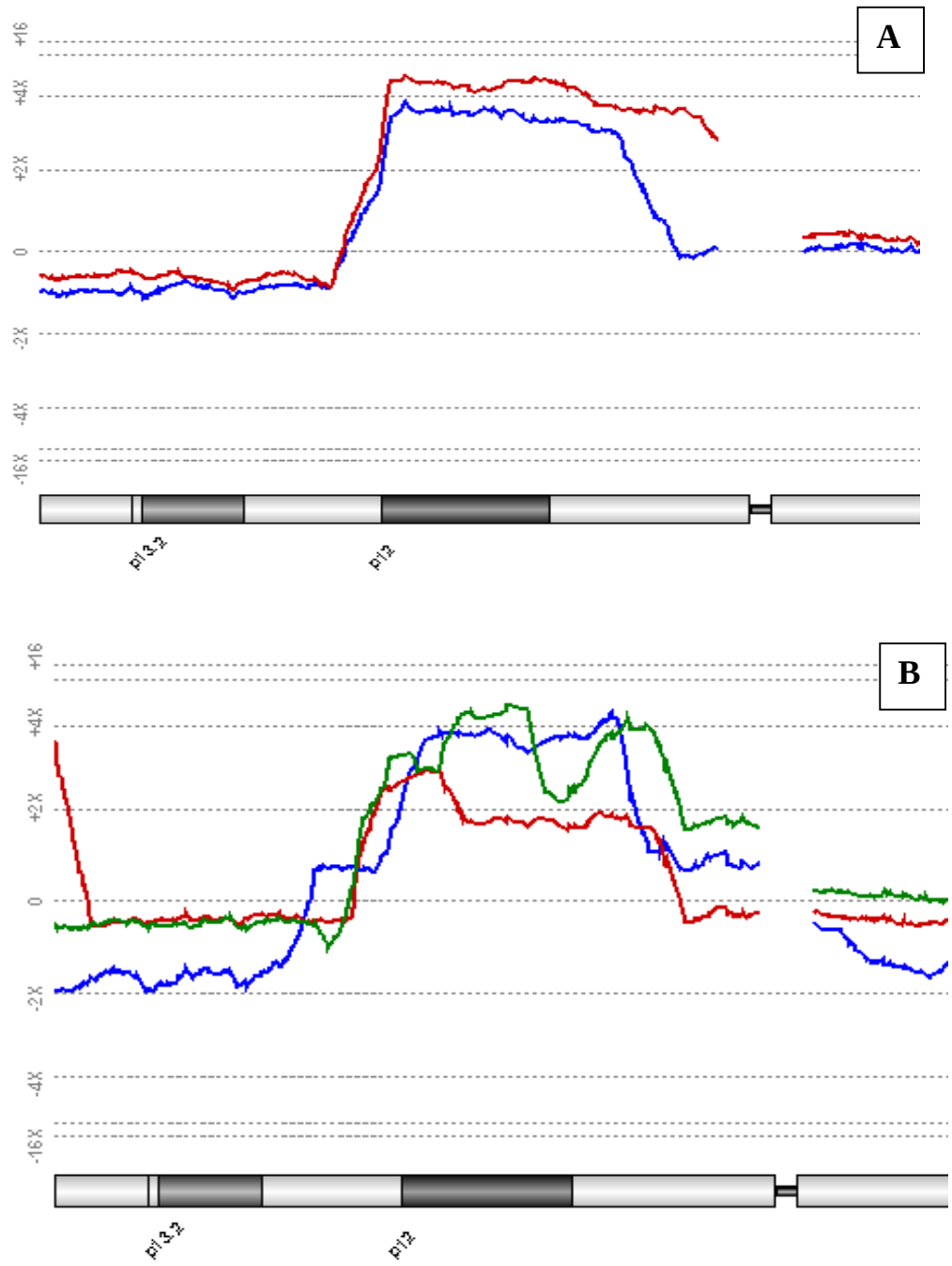


Figure 13: Continuous (A) and discontinuous (B) patterns of copy number changes at 17p in low-grade LMS and OS, respectively, detected by aCGH. On the X-axis the cytogenetic position of chromosome 17p is shown and on the Y-axis the intensity ratios indicating copy number fold changes are shown (for example +2X represents a 2-fold increase as compared to normal levels).

CONCLUSIONS

The aim of this Thesis was to detect the DNA copy number changes in soft tissue (LMS, DFSP, ULMS, and MFH) and bone sarcomas (OS) by high-resolution aCGH. aCGH was proven to be an effective tool for screening the copy number changes regardless of the source of the DNA. aCGH discriminated between low- and high-grade LMS based on their copy number patterns. In DFSP, 21 changes were detected in addition to the well-known gains in 17q and 22q. In OS recurrent amplicons were detected at 12q11-q15, 8q, 6p12-p21 and 17p. The target genes identified in the amplicons at 12q and 17q were *OS9, CYP27B1, DKFZP586D0919*, and *TSFM*, *COX10*, *PMP22, ADORA2B, ZNF287, MRIP, COPS3, PEMT, SREBF1, TOM1L2, TOP3A, GRAP,* and *MAPK7*. The amplicon at 17p characterized by aCGH in low- and high-grade LMS, OS, and MFH revealed 17p11-p12 as the minimal commonly gained region in all cases. A distal loss at 17p13-pter followed by gains or amplification was detected in all but one case. A complex pattern of gains and amplification at 17p separated OS and high-grade LMS from MFH and low-grade LMS, which showed more continuous regions of copy number gains and amplification at 17p. In ULMS commonly gained (1p, 1q, 2p, 3p, 6p, 8q, 10q, 18q) and lost (2q, 4q, 6p, 6q, 7p, 7q, 13q, 14p, 16q, 19p, Xp, Xq) regions were identified by aCGH. To understand the pathogenesis behind these copy number changes, pathway analysis on the genes with altered copy number changes provided clues to the various biological processes underlying the tumourigenesis.

For copy number changes analysis beside aCGH, cCGH was also applied in all studies (I-V) and in Study II in some cases G-banding was used. Results from all these Studies clearly showed that aCGH detected additional changes undetected by other low-resolution methods used. aCGH, due to ultra fine resolution, has a promising future in clinical diagnostics as a powerful tool in scanning the entire genome for DNA copy number changes and detecting the genes therein, which could be used as specific markers.

Application of aCGH technology with improved resolution was shown to be an appropriate method for detecting the exact boundaries of the DNA copy number changes and the corresponding target genes. Furthermore, aCGH was also found to be suitable in providing information regarding the biological processes that were affected by DNA copy number changes that might be involved in the pathogenesis of sarcomas.

Not all cytogenetic techniques can be used to analyse mesenchymal tumours due to difficulties in obtaining enough mitoic cells from cell cultures. FISH and RT-PCR are used to detect specific known genes, but it's extremely difficult to analyse entire genomes in a reasonable time. Thus, cCGH and aCGH are only two methods available for genome wide analysis. aCGH, due to its ultra fine resolution, provides the gene level information regarding the precise copy number changes and can detect the tumour-specific markers/genes. Thus, in the future this technique has potential in clinical diagnostics. In the future FISH, RT-PCR, and tissue microarray analyses are needed to confirm the present findings on large sample collections and also to determine the clinical significance of the identified genes. Transcriptomics and proteomics analysis are needed to understand the biology behind sarcomas and also in identification of target gene underlying the development/progression of sarcomas, which could lead to the identification of molecular targets for effective cancer treatment.

ACKNOWLEDGMENTS

This Study was carried out at the Department of Pathology in Haartman Institute, University of Helsinki during years January 2004-June 2007. It was financially supported by the special state appropriations of the Helsinki and Uusimaa Hospital District, the Cancer Society and the Sigrid Jusélius Foundation.

I wish to express my gratitude to all those who made this Study possible and wish to express my sincere gratitude especially to:

My supervisor Professor Sakari Knuutila for providing excellent facilities and giving me the opportunity to carry out this work. I am grateful not only for the terrific guidance and inspiration he gave me during all these years but also for showing that there is always another side of the coin when things don't work out. Professor Marcelo Larramendy for being a catalyst.

Docent Merja Perälä and Docent Arto Mannermaa, the official pre-examiners of this thesis, for their constructive comments. Jodie Painter for her friendship and for the excellent language revision.

Dr. Hanna Vauhkonen (unofficial supervisor) and Docent Tom Böhling for being always there for me and having enough patience to answer my questions of all sorts. Without their endless encouragement, support and scientific discussions I would have never completed this work. Massimiliano Gentile for his huge support in Study V.

Pirjo Pennanen, an extraordinary personality. She has been looking over my shoulder all these years and could never be thanked enough for her support in language revision of all my papers and for all other practical matters.

Salla Ruosaari, for scientific and non-scientific discussions during the long evenings in the lab. Anu Usvasalo (Stony) for her joyful character. Tuija Lundán for spicy discussions during the long evening walks. Pamela Lindholm for always ready to offer help and assistance and for our unforgettable conference trip. Other members of the CMG group are also acknowledged for creating a friendly working atmosphere. My friends outside the CMG group, whose names are not mentioned here due to limited space, are thanked for the wonderful times I have spent with them.

To my Mom, Dad, brother, sister, and my husband. I am so lucky to have you. I wish I could spend more time with you all. Support from my family was nothing less than extraordinary.

I dedicate this work to my parents, the treasure of my life.

Helsinki, June 2007
Sippy Kaur

REFERENCES

Abramson DH, Ellsworth RM, Kitchin FD, Tung G. 1984. Second nonocular tumors in retinoblastoma survivors. Are they radiation-induced?. Ophthalmology 91:1351-1355.

Albertson DG, Collins C, McCormick F, Gray JW. 2003. Chromosome aberrations in solid tumors. Nat Genet 34:369-376.

Albertson DG and Pinkel D. 2003. Genomic microarrays in human genetic disease and cancer. Hum Mol Genet 12:145-152.

Al-Kuraya K, Schraml P, Torhorst J, Tapia C, Zaharieva B, Novotny H, Spichtin H, Maurer R, Mirlacher M, Köchli O, Zuber M, Dieterich H, Mross F, Wilber K, Simon R, Sauter G. 2004. Prognostic relevance of gene amplifications and coamplifications in breast cancer. Cancer Res 64:8534-8540.

Aman P. 1999. Fusion genes in solid tumors. Semin Cancer Biol 9:303-318.

Aplan PD. 2006. Causes of oncogenic chromosomal translocation. Trends Genet 22:46-55.

Arvand A, Welford SM, Teitell MA, Denny CT. 2001. The COOH-terminal domain of FLI-1 is necessary for full tumorigenesis and transcriptional modulation by EWS/FLI-1. Cancer Res 61:5311-5317.

Attardi LD. 2005. The role of p53-mediated apoptosis as a crucial anti-tumor response to genomic instability: lessons from mouse models. Mutat Res 569:145-157.

Baird K, Davis S, Antonescu CR, Harper UL, Walker RL, Chen Y, Glatfelter AA, Duray PH, Meltzer PS. 2005. Gene expression profiling of human sarcomas: insights into sarcoma biology. Cancer Res 65:9226-9235.

Barrett MT, Scheffer A, Ben-Dor A, Sampas N, Lipson D, Kincaid R, Tsang P, Curry B, Baird K, Meltzer PS, Yakhini Z, Bruhn L, Laderman S. 2004. Comparative genomic hybridization using oligonucleotide microarrays and total genomic DNA. Proc Natl Acad Sci USA 101:17765-17770.

Bauer HC, Alvegard TA, Berlin O, Erlanson M, Gustafson P, Kivioja A, Klepp R, Lehtinen T, Lindholm P, Moller TR, Rydholm A, Saeter G, Trovik CS, Wahlstrom O, Wiklund T. 1999. The Scandinavian Sarcoma Group Register. Acta Orthop Scand Suppl 285:41-44.

Bayani J, Zielenska M, Pandita A, Al-Romaih K, Karaskova J, Harrison K, Bridge JA, Sorensen P, Thorner P, Squire JA. 2003. Spectral karyotyping identifies recurrent complex rearrangements of chromosomes 8, 17, and 20 in osteosarcomas. Genes Chromosomes Cancer 36:7-16.

Benoit L, Arnould L, Cheynel N, Goui S, Collin F, Fraisse J, Cuisenier J. 2005. The role of surgery and treatment trends in uterine sarcoma. Eur J Surg Oncol 31:434-442.

Bieche I, Laurendeau I, Tozlu S, Olivi M, Vidaud D, Lidereau R, Vidaud M. 1999. Quantitation of *MYC* Gene Expression in Sporadic Breast Tumors with a Real-Time Reverse Transcription-PCR Assay. Cancer Res 59:2759-2765.

Bignell GR, Huang J, Greshock J, Watt S, Butler A, West S, Grigorova M, Jones KW, Wei W, Stratton MR, Futreal PA, Weber B, Shapero MH, Wooster R. 2004. High-Resolution Analysis of DNA Copy Number Using Oligonucleotide Microarrays. Genome Res 14:287-295.

Borden EC, Baker LH, Bell RS, Bramwell V, Demetri GD, Eisenberg BL, Fletcher CD, Fletcher JA, Ladanyi M, Meltzer P, O'Sullivan B, Parkinson DR, Pisters PW, Saxman S, Singer S, Sundaram M, van Oosterom AT, Verweij J, Waalen J, Weiss SW, Brennan MF. 2003. Soft tissue sarcomas of adults: state of the translational science. Clin Cancer Res 9:1941-1956.

Botchan M, Topp W, Sambrook J. 1979. Studies on simian virus 40 excision from cellular chromosomes. Cold Spring Harb Symp Quant Biol 43 Pt 2:709-719.

Bovee JV, Cleton-Jansen AM, Taminiau AH, Hogendoorn PC. 2005. Emerging pathways in the development of chondrosarcoma of bone and implications for targeted treatment. Lancet Oncol 6:599-607.

Brady MS, Gaynor JJ, Brennan MF. 1992. Radiation-associated sarcoma of bone and soft tissue. Arch Surg 127:1379-1385.

Brennan C, Zhang Y, Leo C, Feng B, Cauwels C, Aguirre AJ, Kim M, Protopopov A, Chin L. 2004. High-resolution global profiling of genomic alterations with long oligonucleotide microarray. Cancer Res 64:4744-4748.

Bridge JA, Kanamori M, Ma Z, Pickering D, Hill DA, Lydiatt W, Lui MY, Colleoni GW, Antonescu CR, Ladanyi M, Morris SW. 2001. Fusion of the ALK gene to the clathrin heavy chain gene, CLTC, in inflammatory myofibroblastic tumor. Am J Pathol 159:411-415.

Bridge JA, Nelson M, McComb E, McGuire MH, Rosenthal H, Vergara G, Maale GE, Spanier S, Neff JR. 1997. Cytogenetic findings in 73 osteosarcoma specimens and a review of the literature. Cancer Genet Cytogenet 95:74-87.

Buckley PG, Mantripragada KK, Benetkiewicz M, Tapia-Paez I, Diaz De Stahl T, Rosenquist M, Ali H, Jarbo C, De Bustos C, Hirvela C, Sinder Wilen B, Fransson I, Thyr C, Johnsson BI, Bruder CE, Menzel U, Hergersberg M, Mandahl N, Blennow E, Wedell A, Beare DM, Collins JE, Dunham I, Albertson D, Pinkel D, Bastian BC, Faruqi AF, Lasken RS, Ichimura K, Collins VP, Dumanski JP. 2002. A full-coverage, high-resolution human chromosome 22 genomic microarray for clinical and research applications. Hum Mol Genet 11:3221-3229.

Busam KJ and Fletcher CDM. 1997. The clinical role of molecular genetics in soft tissue tumor pathology. Cancer Metastatis Rev 16:207-227.

Bustin SA, Gyselman VG, Williams NS, Dorudi S. 1999. Detection of cytokeratins 19/20 and guanylyl cyclase C in peripheral blood of colorectal cancer patients Br J Cancer 79: 1813-1820.

Bustin SA and Mueller R. 2005. Real-time reverse transcription PCR (qRT-PCR) and its potential use in clinical diagnosis. Clin Sci 109:365-379.

Callagy G, Pharoah P, Chin SF, Sangan T, Daigo Y, Jackson L, Caldas C. 2005. Identification and validation of prognostic markers in breast cancer with the complementary use of array-CGH and tissue microarrays. J Pathol 205:388-396.

Carvalho B, Ouwerkerk E, Meijer GA, Ylstra B. 2004. High resolution microarray comparative genomic hybridisation analysis using spotted oligonucleotides. J Clin Pathol 57:644-646.

Cawkwell L, Lewis FA, Quirke P. 1994. Frequency of allele loss of DCC, p53, RBI, WT1, NF1, NM23 and APC/MCC in colorectal cancer assayed by fluorescent multiplex polymerase chain reaction. Br J Cancer 70:813-818.

Cheng JQ, Godwin AK, Bellacosa A, Taguchi T, Franke TF, Hamilton TC, Tsichlis PN, Testa JR. 1992. AKT2, a putative oncogene encoding a member of a subfamily of protein-serine/threonine kinases, is amplified in human ovarian carcinomas. Proc Natl Acad Sci USA 89:9267-9271.

Cheung IY and Cheung NKV. 2001. Quantitation of Marrow Disease in Neuroblastoma by Real-Time Reverse Transcription-PCR. Clin Cancer Res 7:1698-1705.

Cho KR and Vogelstein B. 1992. Genetic alterations in the adenoma--carcinoma sequence. Cancer 15:1727-1731.

Cho YL, Bae S, Koo MS, Kim KM, Chun HJ, Kim CK, Ro DY, Kim JH, Lee CH, Kim YW, Ahn WS. 2005. Array comparative genomic hybridization analysis of uterine leiomyosarcoma. Gynecol Oncol 99:545-551.

Coindre JM. 2006. Grading of soft tissue sarcomas: review and update. Arch Pathol Lab Med 130:1448-1453.

Conrad EU,3rd and Enneking WF. 1990. Common soft tissue tumors. Clin Symp 42:2-32.

Cormier JN and Pollock RE. 2004. Soft tissue sarcomas. CA Cancer J Clin 54:94-109.

Cowell JK. 1982. Double minutes and homogeneously staining regions: gene amplification in mammalian cells. Ann Rev Genet 16:21-59.

Craig DW, Huentelman MJ, Hu-Lince D, Zismann VL, Kruer MC, Lee AM, Puffenberger EG, Pearson JM, Stephan DA. 2005. Identification of disease causing loci using an array-based genotyping approach on pooled DNA. BMC Genomics 6:138.

Crew AJ, Clark J, Fisher C, Gill S, Grimer R, Chand A, Shipley J, Gusterson BA, Cooper CS. 1995. Fusion of SYT to two genes, SSX1 and SSX2, encoding proteins with homology to the Kruppel-associated box in human synovial sarcoma. EMBO J 14:2333-2340.

Criscione VD and Weinstock MA. 2007. Descriptive epidemiology of dermatofibrosarcoma protuberans in the United States, 1973 to 2002. J Am Acad Dermatol 56:968-973.
Crozat A, Aman P, Mandahl N, Ron D. 1993. Fusion of CHOP to a novel RNA-binding protein in human myxoid liposarcoma. Nature 363:640-644.
Davis RJ, D'Cruz CM, Lovell MA, Biegel JA, Barr FG. 1994. Fusion of PAX7 to FKHR by the variant t(1;13)(p36;q14) translocation in alveolar rhabdomyosarcoma. Cancer Res 54:2869-2872.
Delattre O, Zucman J, Plougastel B, Desmaze C, Melot T, Peter M, Kovar H, Joubert I, de Jong P, Rouleau G. 1992. Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumours. Nature 359:162-165.
Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA Jr, Butel JS, Bradley A. 1992. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature 356:215-221.
Dutt A and Beroukhim R. 2007. Single nucleotide polymorphism array analysis of cancer. Curr Opin Oncol 19:43–49.
Echt G, Jepson J, Steel J, Langholz B, Luxton G, Hernandez W, Astrahan M, Petrovich Z. 1990. Treatment of uterine sarcomas. Cancer 66:35-39.
El-Rifai W, Larramendy ML, Bjorkqvist AM, Hemmer S, Knuutila S. 1997. Optimization of comparative genomic hybridization using fluorochrome conjugated to dCTP and dUTP nucleotides. Lab Invest 77:699-700.
El-Rifai W, Sarlomo-Rikala M, Knuutila S, Miettinen M. 1998. DNA copy number changes in development and progression in leiomyosarcomas of soft tissues. Am J Pathol 153:985-990.
Enzinger FM and Weiss SW. 2001a. Fibrohistiocytic tumors of intermediate malignancy. In: Weiss SW and Goldblum JR, editors. Soft tissue tumors. 4th ed. St Louis : Mosby. pp 491-534.
Enzinger FM and Weiss SW. 2001b. General considerations. In: Weiss SW and Goldblum JR, editors. Soft tissue tumors. 4th ed. St Louis : Mosby. pp 1-19.
Enzinger FM and Weiss SW. 2001c. Leiomyosarcoma. In: Weiss SW and Goldblum JR, editors. Soft tissue tumors. 4th ed. St Louis : Mosby. pp 727-748.
Enzinger FM and Weiss SW. 2001d. Malignant Fibrohistiocytic tumors. In: Weiss SW and Goldblum JR, editors. Soft tissue tumors. 4th ed. St Louis : Mosby. pp 535-569.
Fenech M. 2002. Chromosomal biomarkers of genomic instability relevant to cancer. Drug Discov Today 7:1128-1137.
Fletcher CDM, Unni KK, Mertens F. 2002. World Health Organization Classification of Tumors. Pathology and Genetics of Tumors of Soft Tissue and Bone. Lyon: IARC Press.
Fletcher JA, Gebhardt MC, Kozakewich HP. 1994. Cytogenetic aberrations in osteosarcomas. Nonrandom deletions, rings, and double-minute chromosomes. Cancer Genet Cytogenet 77:81-88.
Flintoff KJ, Sheridan E, Turner G, Chu CE, Taylor GR. 2001. Submicroscopic deletions of the APC gene: a frequent cause of familial adenomatous polyposis that may be overlooked by conventional mutation scanning. J Med Genet 38:129-132.
Forozan F, Karhu R, Kononen J, Kallioniemi A, Kallioniemi OP. 1997. Genome screening by comparative genomic hybridization. Trends Genet 13:405-409.
Forus A, Weghuis DO, Smeets D, Fodstad O, Myklebost O, Geurts van Kessel A. 1995. Comparative genomic hybridization analysis of human sarcomas: II. Identification of novel amplicons at 6p and 17p in osteosarcomas. Genes Chromosomes Cancer 14:15-21.
Freeman JL, Perry GH, Feuk L, Redon R, McCarroll SA, Altshuler DM, Aburatani H, Jones KW, Tyler-Smith C, Hurles ME, Carter NP, Scherer SW, Lee C. 2006. **C**opy number variation: New insights in genome diversity. Genome Res 16:949-961.
Fritz B, Schubert F, Wrobel G, Schwaenen C, Wessendorf S, Nessling M, Korz C, Rieker RJ, Montgomery K, Kucherlapati R, Mechtersheimer G, Eils R, Joos S, Lichter P. 2002. Microarray-based copy number and expression profiling in dedifferentiated and pleomorphic liposarcoma. Cancer Res 62:2993-2998.

Furge KA, Tan MH, Dykema K, Kort E, Stadler W, Yao X, Zhou M, Teh BT. 2007. Identification of deregulated oncogenic pathways in renal cell carcinoma: an integrated oncogenomic approach based on gene expression profiling. Oncogene 26:1346-1350.

Futreal PA, Coin L, Marshall M, Down T, Hubbard T, Wooster R, Rahman N, Stratton MR. 2004. A census of human cancer genes. Nat Rev Cancer 4:177-183.

Galili N, Davis RJ, Fredericks WJ, Mukhopadhyay S, Rauscher FJ,3rd, Emanuel BS, Rovera G, Barr FG. 1993. Fusion of a fork head domain gene to PAX3 in the solid tumour alveolar rhabdomyosarcoma. Nat Genet 5:230-235.

Gebert C, Brinkschmidt C, Bielack S, Bernhardt T, Jurgens H, Bocker W, Winkelmann W, Burger H, Gosheger G. 2006. The potential of comparative genomic hybridization as a tool in the differential diagnosis of matrix-producing bone lesions. Int J Surg Pathol 14:187-192.

Gelmini S, Tricarico C, Vona G, Livi L, Melina AD, Serni S, Cellai E, Magrini S, Villari D, Carini M, Serio M, Forti G, Pazzagli M, Orlando C. 2001. Real-Time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) for the measurement of prostate-specific antigen mRNA in the peripheral blood of patients with prostate carcinoma using the taqman detection system. Clin Chem Lab Med 39:385-391.

Gerdes H, Chen Q, Elahi AH, Sircar A, Goldberg E, Winawer D, Urmacher C, Winawer SJ, Jhanwar SC. 1995. Recurrent deletions involving chromosomes 1, 5, 17, and 18 in colorectal carcinoma: possible role in biological and clinical behavior of tumors. Anticancer Res 15:13-24.

Gisselsson D, Pettersson L, Hoglund M, Heidenblad M, Gorunova L, Wiegant J, Mertens F, Dal Cin P, Mitelman F, Mandahl N. 2000. Chromosomal breakage-fusion-bridge events cause genetic intratumor heterogeneity. Proc Natl Acad Sci USA 97:5357-5362.

Giuntoli RL,2nd, Metzinger DS, DiMarco CS, Cha SS, Sloan JA, Keeney GL, Gostout BS. 2003. Retrospective review of 208 patients with leiomyosarcoma of the uterus: prognostic indicators, surgical management, and adjuvant therapy. Gynecol Oncol 89:460-469.

Greco A, Fusetti L, Villa R, Sozzi G, Minoletti F, Mauri P, Pierotti MA. 1998. Transforming activity of the chimeric sequence formed by the fusion of collagen gene COL1A1 and the platelet derived growth factor b-chain gene in dermatofibrosarcoma protuberans. Oncogene 17:1313-1319.

Guillou L, Coindre JM, Bonichon F, Nguyen BB, Terrier P, Collin F, Vilain MO, Mandard AM, Le Doussal V, Leroux A, Jacquemier J, Duplay H, Sastre-Garau X, Costa J. 1997. Comparative study of the National Cancer Institute and French Federation of Cancer Centers Sarcoma Group grading systems in a population of 410 adult patients with soft tissue sarcoma. J Clin Oncol 15:350-362.

Gustafson P, Akerman M, Alvegard TA, Coindre JM, Fletcher CD, Rydholm A, Willen H. 2003. Prognostic information in soft tissue sarcoma using tumour size, vascular invasion and microscopic tumour necrosis-the SIN-system. Eur J Cancer 39:1568-1576.

Hashimoto H, Daimaru Y, Takeshita S, Tsuneyoshi M, Enjoji M. 1992. Prognostic significance of histologic parameters of soft tissue sarcomas. Cancer 70:2816-2822.

Heck RK, Jr, Peabody TD, Simon MA. 2006. Staging of primary malignancies of bone. CA Cancer J Clin 56:366-375.

Herzog CE. 2005. Overview of sarcomas in the adolescent and young adult population. J Pediatr Hematol Oncol 27:215-218.

Hong Y, Ho KS, Eu KW, Cheah PY. 2007. A susceptibility gene set for early onset colorectal cancer that integrates diverse signaling pathways: implication for tumorigenesis. Clin Cancer Res 13:1107-1114.

Hoogerwerf WA, Hawkins AL, Perlman EJ, Griffin CA. 1994. Chromosome analysis of nine osteosarcomas. Genes Chromosomes Cancer 9:88-92.

Hu J, Khanna V, Jones M, Surti U. 2001. Genomic alterations in uterine leiomyosarcomas: potential markers for clinical diagnosis and prognosis. Genes Chromosomes Cancer 31:117-124.

Hu J, Rao UN, Jasani S, Khanna V, Yaw K, Surti U. 2005. Loss of DNA copy number of 10q is associated with aggressive behavior of leiomyosarcomas: a comparative genomic hybridization study. Cancer Genet Cytogenet 161:20-27.

Hulsebos TJ, Bijleveld EH, Oskam NT, Westerveld A, Leenstra S, Hogendoorn PC, Bras J. 1997. Malignant astrocytoma-derived region of common amplification in chromosomal band 17p12 is frequently amplified in high-grade osteosarcomas. Genes Chromosomes Cancer 18:279-285.

Hussain SP and Harris CC. 2006. p53 biological network: at the crossroads of the cellular-stress response pathway and molecular carcinogenesis. J Nippon Med Sch 73:54-64.

Hyman E, Kauraniemi P, Hautaniemi S, Wolf M, Mousses S, Rozenblum E, Ringner M, Sauter G, Monni O, Elkahloun A, Kallioniemi OP, Kallioniemi A. 2002. Impact of DNA amplification on gene expression patterns in breast cancer. Cancer Res 62:6240-6245.

Janus F, Albrechtsen N, Dornreiter I, Wiesmuller L, Grosse F, Deppert W. 1999. The dual role model for p53 in maintaining genomic integrity. Cell Mol Life Sci 55:12-27.

Jatoi N. 2003. Leiomyosarcoma: a rare malignant change in a leiomyoma. J Coll Physicians Surg Pak 13:106-107.

Jenson HB, Montalvo EA, McClain KL, Ench Y, Heard P, Christy BA, Dewalt-Hagan PJ, Moyer MP. 1999. Characterization of natural Epstein-Barr virus infection and replication in smooth muscle cells from a leiomyosarcoma. J Med Virol 57:36-46.

Jeon IS, Davis JN, Braun BS, Sublett JE, Roussel MF, Denny CT, Shapiro DN. 1995. A variant Ewing's sarcoma translocation (7;22) fuses the EWS gene to the ETS gene ETV1. Oncogene 10:1229-1234.

Jones PA and Baylin SB. 2007. The epigenomics of cancer. Cell 128:683-692.

Jong K, Marchiori E, Meijer G, Vaart AV, Ylstra B. 2004. Breakpoint identification and smoothing of array comparative genomic hybridization data. Bioinformatics 20:3636-3637.

Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, Waldman F, Pinkel D. 1992. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 258:818-821.

Kallioniemi OP, Kallioniemi A, Piper J, Isola J, Waldman FM, Gray JW, Pinkel D. 1994. Optimizing comparative genomic hybridization for analysis of DNA sequence copy number changes in solid tumors. Genes Chromosomes Cancer 10:231-243.

Kaneko Y, Yoshida K, Handa M, Toyoda Y, Nishihira H, Tanaka Y, Sasaki Y, Ishida S, Higashino F, Fujinaga K. 1996. Fusion of an ETS-family gene, EIAF, to EWS by t(17;22)(q12;q12) chromosome translocation in an undifferentiated sarcoma of infancy. Genes Chromosomes Cancer 15:115-121.

Katsama A, Sourvinos G, Zachos G, Spandidos DA. 2000. Allelic loss at the BRCA1, BRCA2 and TP53 loci in human sporadic breast carcinoma. Cancer Lett 150:165-170.

Kauraniemi P, Barlund M, Monni O, Kallioniemi A. 2001. New amplified and highly expressed genes discovered in the ERBB2 amplicon in breast cancer by cDNA microarrays. Cancer Res 61:8235-8240.

Kendziorski C, Irizarry RA, Chen KS, Haag JD, Gould MN. 2005. On the utility of pooling biological samples in microarray experiments. Proc Natl Acad Sci USA 102:4252-4257.

King AA, Debaun MR, Riccardi VM, Gutmann DH. 2000. Malignant peripheral nerve sheath tumors in neurofibromatosis 1. Am J Med Genet 93:388-392.

Kiuru-Kuhlefelt S, El-Rifai W, Fanburg-Smith J, Kere J, Miettinen M, Knuutila S. 2001. Concomitant DNA copy number amplification at 17q and 22q in dermatofibrosarcoma protuberans. Cytogenet Cell Genet 92:192-195.

Knezevich SR, McFadden DE, Tao W, Lim JF, Sorensen PH. 1998. A novel ETV6-NTRK3 gene fusion in congenital fibrosarcoma. Nat Genet 18:184-187.

Knudson AG, Jr. 1971. Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci USA 68:820-823.

Knuutila S, Aalto Y, Autio K, Bjorkqvist AM, El-Rifai W, Hemmer S, Huhta T, Kettunen E, Kiuru-Kuhlefelt S, Larramendy ML, Lushnikova T, Monni O, Pere H, Tapper J, Tarkkanen M, Varis A, Wasenius VM, Wolf M, Zhu Y. 1999. DNA copy number losses in human neoplasms. Am J Pathol 155:683-694.

Knuutila S, Bjorkqvist AM, Autio K, Tarkkanen M, Wolf M, Monni O, Szymanska J, Larramendy ML, Tapper J, Pere H, El-Rifai W, Hemmer S, Wasenius VM, Vidgren V, Zhu Y. 1998. DNA copy number amplifications in human neoplasms: review of comparative genomic hybridization studies. Am J Pathol 152:1107-1123.

Konigshoff M, Wilhelm J, Bohle RM, Pingoud A, Hahn M. 2003**.** *HER-2/neu* Gene Copy Number Quantified by Real-Time PCR: Comparison of Gene Amplification, Heterozygosity, and Immunohistochemical Status in Breast Cancer Tissue. Clin Chem 49:219-29.

Koontz JI, Soreng AL, Nucci M, Kuo FC, Pauwels P, van Den Berghe H, Cin PD, Fletcher JA, Sklar J. 2001. Frequent fusion of the JAZF1 and JJAZ1 genes in endometrial stromal tumors. Proc Natl Acad Sci USA 98:6348-6353.

Koorey DJ and McCaughan GW. 1993. Tumour suppressor genes and colorectal neoplasia. J Gastroenterol Hepatol 8:174-184.

Krauter J, Heli G, Ganser A. 2001. The AML1/MTG8 Fusion Transcript in t(8;21) Positive AML and its Implication for the Detection of Minimal Residual Disease; Malignancy. Hematol 5: 369-381.

Labelle Y, Zucman J, Stenman G, Kindblom LG, Knight J, Turc-Carel C, Dockhorn-Dworniczak B, Mandahl N, Desmaze C, Peter M. 1995. Oncogenic conversion of a novel orphan nuclear receptor by chromosome translocation. Hum Mol Genet 4:2219-2226.

Ladanyi M and Gerald W. 1994. Fusion of the EWS and WT1 genes in the desmoplastic small round cell tumor. Cancer Res 54:2837-2840.

Ladanyi M, Lui MY, Antonescu CR, Krause-Boehm A, Meindl A, Argani P, Healey JH, Ueda T, Yoshikawa H, Meloni-Ehrig A, Sorensen PH, Mertens F, Mandahl N, van den Berghe H, Sciot R, Cin PD, Bridge J. 2001. The der(17)t(X;17)(p11;q25) of human alveolar soft part sarcoma fuses the TFE3 transcription factor gene to ASPL, a novel gene at 17q25. Oncogene 20:48-57.

Lagutina I, Conway SJ, Sublett J, Grosveld GC. 2002. Pax3-FKHR knock-in mice show developmental aberrations but do not develop tumors. Mol Cell Biol 22:7204-7216.

Larramendy ML, Tarkkanen M, Blomqvist C, Virolainen M, Wiklund T, Asko-Seljavaara S, Elomaa I, Knuutila S. 1997. Comparative genomic hybridization of malignant fibrous histiocytoma reveals a novel prognostic marker. Am J Pathol 151:1153-1161.

Lau CC, Harris CP, Lu XY, Perlaky L, Gogineni S, Chintagumpala M, Hicks J, Johnson ME, Davino NA, Huvos AG, Meyers PA, Healy JH, Gorlick R, Rao PH. 2004. Frequent amplification and rearrangement of chromosomal bands 6p12-p21 and 17p11.2 in osteosarcoma. Genes Chromosomes Cancer 39:11-21.

Lawrence B, Perez-Atayde A, Hibbard MK, Rubin BP, Dal Cin P, Pinkus JL, Pinkus GS, Xiao S, Yi ES, Fletcher CD, Fletcher JA. 2000. TPM3-ALK and TPM4-ALK oncogenes in inflammatory myofibroblastic tumors. Am J Pathol 157:377-384.

Lazar A, Abruzzo LV, Pollock RE, Lee S, Czerniak B. 2006. Molecular diagnosis of sarcomas: chromosomal translocations in sarcomas. Arch Pathol Lab Med 130:1199-1207.

Leach FS, Tokino T, Meltzer P, Burrell M, Oliner JD, Smith S, Hill DE, Sidransky D, Kinzler KW, Vogelstein B. 1993. p53 Mutation and MDM2 amplification in human soft tissue sarcomas. Cancer Res 53:2231-2234.

Lee WY, Tzeng CC, Chou CY. 1994. Uterine leiomyosarcomas coexistent with cellular and atypical leiomyomata in a young woman during the treatment with luteinizing hormone-releasing hormone agonist. Gynecol Oncol 52:74-79.

Lengauer C, Kinzler KW, Vogelstein B. 1998. Genetic instabilities in human cancers. Nature 396:643-649.

Levy B, Mukherjee T, Hirschhorn K. 2000. Molecular cytogenetic analysis of uterine leiomyoma and leiomyosarcoma by comparative genomic hybridization. Cancer Genet Cytogenet 121:1-8.

Lim G, Karaskova J, Vukovic B, Bayani J, Beheshti B, Bernardini M, Squire JA, Zielenska M. 2004. Combined spectral karyotyping, multicolor banding, and microarray comparative genomic hybridization analysis provides a detailed characterization of complex structural chromosomal rearrangements associated with gene amplification in the osteosarcoma cell line MG-63. Cancer Genet Cytogenet 153:158-164.

Linn SC, West RB, Pollack JR, Zhu S, Hernandez-Boussard T, Nielsen TO, Rubin BP, Patel R, Goldblum JR, Siegmund D, Botstein D, Brown PO, Gilks CB, van de Rijn M. 2003. Gene expression patterns and gene copy number changes in dermatofibrosarcoma protuberans. Am J Pathol 163:2383-2395.

Livingstone LR, White A, Sprouse J, Livanos E, Jacks T, Tlsty TD. 1992. Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. Cell 70:923-935.

Malkin D, Li FP, Strong LC, Fraumeni JF, Nelson CE, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky MA, Friend SH. 1990. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 250:1233-1238.

Man TK, Lu XY, Jaeweon K, Perlaky L, Harris CP, Shah S, Ladanyi M, Gorlick R, Lau CC, Rao PH. 2004. Genome-wide array comparative genomic hybridization analysis reveals distinct amplifications in osteosarcoma. BMC Cancer 4:45.

Mandahl N, Fletcher CD, Dal Cin P, De Wever I, Mertens F, Mitelman F, Rosai J, Rydholm A, Sciot R, Tallini G, Van Den Berghe H, Vanni R, Willen H. 2000. Comparative cytogenetic study of spindle cell and pleomorphic leiomyosarcomas of soft tissues: a report from the CHAMP Study Group. Cancer Genet Cytogenet 116:66-73.

Marcucci G, Strout MP, Bloomfield CD, Caligiuri MA. 1998. Detection of Unique ALL1(MLL) Fusion Transcripts in Normal Human Bone Marrow and Blood: Distinct Origin of Normal versus Leukemic ALL1 Fusion Transcripts1. Cancer Res 58:790-793.

Marec-Berard P and Philip T. 2004. Ewing sarcoma: the pediatrician's point of view. Pediatr Blood Cancer 42:477-480.

Mark RJ, Poen J, Tran LM, Fu YS, Selch MT, Parker RG. 1994. Postirradiation sarcomas. A single-institution study and review of the literature. Cancer 73:2653-2662.

Masuda A and Takahashi T. 2002. Chromosome instability in human lung cancers: possible underlying mechanisms and potential consequences in the pathogenesis. Oncogene 21:6884-6897.

Matsumura N, Mandai M, Miyanishi M, Fukuhara K, Baba T, Higuchi T, Kariya M, Takakura K, Fujii S. 2006. Oncogenic property of acrogranin in human uterine leiomyosarcoma: direct evidence of genetic contribution in in vivo tumorigenesis. Clin Cancer Res 12:1402-1411.

Mendenhall WM, Zlotecki RA, Scarborough MT. 2004. Dermatofibrosarcoma protuberans. Cancer 101:2503-2508.

Menu-Branthomme A, Rubino C, Shamsaldin A, Hawkins MM, Grimaud E, Dondon MG, Hardiman C, Vassal G, Campbell S, Panis X, Daly-Schveitzer N, Lagrange JL, Zucker JM, Chavaudra J, Hartman O, de Vathaire F. 2004. Radiation dose, chemotherapy and risk of soft tissue sarcoma after solid tumours during childhood. Int J Cancer 110:87-93.

Mertens F, Larramendy M, Gustavsson A, Gisselsson D, Rydholm A, Brosjo O, Mitelman F, Knuutila S, Mandahl N. 2000. Radiation-associated sarcomas are characterized by complex karyotypes with frequent rearrangements of chromosome arm 3p. Cancer Genet Cytogenet 116:89-96.

Meza-Zepeda LA, Kresse SH, Barragan-Polania AH, Bjerkehagen B, Ohnstad HO, Namlos HM, Wang J, Kristiansen BE, Myklebost O. 2006. Array comparative genomic hybridization reveals distinct DNA copy number differences between gastrointestinal stromal tumors and leiomyosarcomas. Cancer Res 66:8984-8993.

Miettinen M and Fetsch JF. 2006. Evaluation of biological potential of smooth muscle tumours. Histopathol 48:97-105.

Miller SA, Dykes DD, Polesky HF. 1988. A simple salting out procedure for extracting DNAs from human nucleated cells. Nucleic Acid Res 16:1215.

Mitelman F. 2000. Recurrent chromosome aberrations in cancer. Mutat Res 462:247-253.

Monfort S, Orellana C, Oltra S, Rosell M, Guitart M, Martínez F. 2006. Evaluation of MLPA for the detection of cryptic subtelomeric rearrangements. J Lab Clin Med 147:295-300.

Monni O, Barlund M, Mousses S, Kononen J, Sauter G, Heiskanen M, Paavola P, Avela K, Chen Y, Bittner ML, Kallioniemi A. 2001. Comprehensive copy number and gene expression profiling of the 17q23 amplicon in human breast cancer. Proc Natl Acad Sci USA 98:5711-5716.

Murnane JP. 2006. Telomeres and chromosome instability. DNA Repair 5:1082-1092.

Myllykangas S and Knuutila S. 2006. Manifestation, mechanisms and mysteries of gene amplifications. Cancer Lett 232:79-89.

Naeem R, Lux ML, Huang SF, Naber SP, Corson JM, Fletcher JA. 1995. Ring chromosomes in dermatofibrosarcoma protuberans are composed of interspersed sequences from chromosomes 17 and 22. Am J Pathol 147:1553-1558.

Nagai M, Tanaka S, Tsuda M, Endo S, Kato H, Sonobe H, Minami A, Hiraga H, Nishihara H, Sawa H, Nagashima K. 2001. Analysis of transforming activity of human synovial sarcoma-associated chimeric protein SYT-SSX1 bound to chromatin remodeling factor hBRM/hSNF2 alpha. Proc Natl Acad Sci USA 98:3843-3848.

Nephew KP and Huang TH. 2003. Epigenetic gene silencing in cancer initiation and progression. Cancer Lett 190:125-133.

Nishio J, Iwasaki H, Ohjimi Y, Ishiguro M, Isayama T, Naito M, Iwashita A, Kikuchi M. 2002. Overrepresentation of 17q22-qter and 22q13 in dermatofibrosarcoma protuberans but not in dermatofibroma: a comparative genomic hybridization study. Cancer Genet Cytogenet 132:102-108.

O'Brien KP, Seroussi E, Dal Cin P, Sciot R, Mandahl N, Fletcher JA, Turc-Carel C, Dumanski JP. 1998. Various regions within the alpha-helical domain of the COL1A1 gene are fused to the second exon of the PDGFB gene in dermatofibrosarcomas and giant-cell fibroblastomas. Genes Chromosomes Cancer 23:187-193.

Oishi Y, Nagasaki K, Miyata S, Matsuura M, Nishimura S, Akiyama F, Iwai T, Miki Y. 2007. Functional pathway characterized by gene expression analysis of supraclavicular lymph node metastasis-positive breast cancer. J Hum Genet 52:271-279.

Oliveira AM and Nascimento AG. 2001. Grading in soft tissue tumors: principles and problems. Skeletal Radiol 30:543-559.

Olivier M, Eeles R, Hollstein M, Khan MA, Harris CC, Hainaut P. 2002. The IARC TP53 database: new online mutation analysis and recommendations to users. Hum Mutat 19:607-614.

Otano-Joos M, Mechtersheimer G, Ohl S, Wilgenbus KK, Scheurlen W, Lehnert T, Willeke F, Otto HF, Lichter P, Joos S. 2000. Detection of chromosomal imbalances in leiomyosarcoma by comparative genomic hybridization and interphase cytogenetics. Cytogenet Cell Genet 90:86-92.

Ozaki T, Neumann T, Wai D, Schafer KL, van Valen F, Lindner N, Scheel C, Bocker W, Winkelmann W, Dockhorn-Dworniczak B, Horst J, Poremba C. 2003. Chromosomal alterations in osteosarcoma cell lines revealed by comparative genomic hybridization and multicolor karyotyping. Cancer Genet Cytogenet 140:145-152.

Packenham JP, du Manoir S, Schrock E, Risinger JI, Dixon D, Denz DN, Evans JA, Berchuck A, Barrett JC, Devereux TR, Ried T. 1997. Analysis of genetic alterations in uterine leiomyomas and leiomyosarcomas by comparative genomic hybridization. Mol Carcinog 19:273-279.

Panagopoulos I, Hoglund M, Mertens F, Mandahl N, Mitelman F, Aman P. 1996. Fusion of the EWS and CHOP genes in myxoid liposarcoma. Oncogene 12:489-494.

Panagopoulos I, Storlazzi CT, Fletcher CD, Fletcher JA, Nascimento A, Domanski HA, Wejde J, Brosjo O, Rydholm A, Isaksson M, Mandahl N, Mertens F. 2004. The chimeric FUS/CREB3l2 gene is specific for low-grade fibromyxoid sarcoma. Genes Chromosomes Cancer 40:218-228.

Papachristou DJ and Papavassiliou AG. 2007. Osteosarcoma and chondrosarcoma: New signaling pathways as targets for novel therapeutic interventions. Int J Biochem Cell Biol 39:857-862.

Patel SG, See AC, Williamson PA, Archer DJ, Evans PH. 1999. Radiation induced sarcoma of the head and neck. Head Neck 21:346-354.

Pedeutour F, Coindre JM, Sozzi G, Nicolo G, Leroux A, Toma S, Miozzo M, Bouchot C, Hecht F, Ayraud N. 1994. Supernumerary ring chromosomes containing chromosome 17 sequences. A specific feature of dermatofibrosarcoma protuberans? Cancer Genet Cytogenet 76:1-9.

Pedeutour F, Simon MP, Minoletti F, Barcelo G, Terrier-Lacombe MJ, Combemale P, Sozzi G, Ayraud N, Turc-Carel C. 1996. Translocation, t(17;22)(q22;q13), in dermatofibrosarcoma protuberans: a new tumor-associated chromosome rearrangement. Cytogenet Cell Genet 72:171-174.

Pedeutour F, Simon MP, Minoletti F, Sozzi G, Pierotti MA, Hecht F, Turc-Carel C. 1995. Ring 22 chromosomes in dermatofibrosarcoma protuberans are low-level amplifiers of chromosome 17 and 22 sequences. Cancer Res 55:2400-2403.

Peter M, Couturier J, Pacquement H, Michon J, Thomas G, Magdelenat H, Delattre O. 1997. A new member of the ETS family fused to EWS in Ewing tumors. Oncogene 14:1159-1164.

Pfeifer D, Pantic M, Skatulla I, Rawluk J, Kreutz C, Martens UM, Fisch P, Timmer J, Veelken H. 2007. Genome-wide analysis of DNA copy number changes and LOH in CLL using high-density SNP arrays. Blood 109:1202-1210.

Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo WL, Chen C, Zhai Y, Dairkee SH, Ljung BM, Gray JW, Albertson DG. 1998. High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. Nat Genet 20:207-211.

Pollack JR, Perou CM, Alizadeh AA, Eisen MB, Pergamenschikov A, Williams CF, Jeffrey SS, Botstein D, Brown PO. 1999. Genome-wide analysis of DNA copy-number changes using cDNA microarrays. Nat Genet 23:41-46.

Pollack JR, Sorlie T, Perou CM, Rees CA, Jeffrey SS, Lonning PE, Tibshirani R, Botstein D, Borresen-Dale AL, Brown PO. 2002. Microarray analysis reveals a major direct role of DNA copy number alteration in the transcriptional program of human breast tumors. Proc Natl Acad Sci USA 99:12963-12968.

Ragland BD, Bell WC, Lopez RR, Siegal GP. 2002. Cytogenetics and molecular biology of osteosarcoma. Lab Invest 82:365-373.

Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, Fiegler H, Shapero MH, Carson AR, Chen W, Cho EK, Dallaire S, Freeman JL, Gonzalez JR, Gratacos M, Huang J, Kalaitzopoulos D, Komura D, MacDonald JR, Marshall CR, Mei R, Montgomery L, Nishimura K, Okamura K, Shen F, Somerville MJ, Tchinda J, Valsesia A, Woodwark C, Yang F, Zhang J, Zerjal T, Zhang J, Armengol L, Conrad DF, Estivill X, Smith CT, Carter NP, Aburatani H, Lee C, Jones KW, Scherer SW, Hurles ME. 2006.Global variation in copy number in the human genome. Nature 444:444-454.

Rezaee SA, Cunningham C, Davison AJ, Blackbourn DJ. 2006. Kaposi's sarcoma-associated herpesvirus immune modulation: an overview. J Gen Virol 87:1781-1804.

Rio PG, Pernin D, Bay JO, Albuisson E, Kwiatkowski F, De Latour M, Bernard-Gallon DJ, Bignon YJ. 1998. Loss of heterozygosity of BRCA1, BRCA2 and ATM genes in sporadic invasive ductal breast carcinoma. Int J Oncol 13:849-853.

Rooms L, Reyniers E, Wuyts W, Storm K, van Luijk R, Scheers S, Wauters J, van den Ende J, Biervliet M, Eyskens F, van Goethem G, Laridon A, Ceulemans B, Courtens W, Kooy RF. 2006. Multiplex ligation-dependent probe amplification to detect subtelomeric rearrangements in routine diagnostics. Clin Genet 69:58-64.

Rozeman LB, Hogendoorn PC, Bovee JV. 2002. Diagnosis and prognosis of chondrosarcoma of bone. Expert Rev Mol Diagn 2:461-472.

Sambrook J, Botchan M, Gallimore P, Ozanne B, Pettersson U, Williams J, Sharp PA. 1975. Viral DNA sequences in cells transformed by simian virus 40, adenovirus type 2 and adenovirus type 5. Cold Spring Harb Symp Quant Biol 39 Pt 1:615-632.

Sambrook J, Fritsch EF, Maniatis T. 1989. Molecular cloning. A laboratory manual. Cold Spring Harbour Laboratory Press:New York.

Sandberg AA. 2005. Updates on the cytogenetics and molecular genetics of bone and soft tissue tumors: leiomyosarcoma. Cancer Genet Cytogenet 161:1-19.

Sandberg AA and Bridge JA. 2003a. Updates on the cytogenetics and molecular genetics of bone and soft tissue tumors. Dermatofibrosarcoma protuberans and giant cell fibroblastoma. Cancer Genet Cytogenet 140:1-12.

Sandberg AA and Bridge JA. 2003b. Updates on the cytogenetics and molecular genetics of bone and soft tissue tumors: osteosarcoma and related tumors. Cancer Genet Cytogenet 145:1-30.

Sartelet H, Grossi L, Pasquier D, Combaret V, Bouvier R, Ranchere D, Plantaz D, Munzer M, Philip T, Birembaut P, Zahm JM, Bergeron C, Gaillard D, Pasquier B. 2002. Detection of N-*myc* amplification by FISH in immature areas of fixed neuroblastomas: more efficient than Southern blot/PCR. J Pathol 198:83-91.

Savelyeva L and Schwab M. 2001. Amplification of oncogenes revisited: from expression profiling to clinical application. Cancer Lett 167:115-23.

Scheidler S, Fredericks WJ, Rauscher FJ,3rd, Barr FG, Vogt PK. 1996. The hybrid PAX3-FKHR fusion protein of alveolar rhabdomyosarcoma transforms fibroblasts in culture. Proc Natl Acad Sci USA 93:9805-9809.

Schmidt H, Bartel F, Kappler M, Wurl P, Lange H, Bache M, Holzhausen HJ, Taubert H. 2005. Gains of 13q are correlated with a poor prognosis in liposarcoma. Mod Pathol 18:638-644.

Schmidt H, Taubert H, Wurl P, Kappler M, Lange H, Bartel F, Bache M, Holzhausen HJ, Hinze R. 2002. Gains of 12q are the most frequent genomic imbalances in adult fibrosarcoma and are correlated with a poor outcome. Genes Chromosomes Cancer 34:69-77.

Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. 2002. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res 30:e57.

Schwab M. 1993. Amplification of N-myc as a prognostic marker for patients with neuroblastoma. Semin Cancer Biol 4:13-18.

Seeger RC, Brodeur GM, Sather H, Dalton A, Siegel SE, Wong KY, Hammond D. 1985. Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastomas. N Engl J Med 313:1111-1116.

Senel FC, Bektas D, Caylan R, Onder E, Gunhan O. 2006. Malignant fibrous histiocytoma of the mandible. Dentomaxillofac Radiol 35:125-128.

Shah KV, Galloway DA, Knowles WA, Viscidi RP. 2004. Simian virus 40 (SV40) and human cancer: a review of the serological data. Rev Med Virol 14:231-239.

Shimizu A, O'Brien KP, Sjoblom T, Pietras K, Buchdunger E, Collins VP, Heldin CH, Dumanski JP, Ostman A. 1999. The dermatofibrosarcoma protuberans-associated collagen type Ialpha1/platelet-derived growth factor (PDGF) B-chain fusion gene generates a transforming protein that is processed to functional PDGF-BB. Cancer Res 59:3719-3723.

Shimizu N, Shingaki K, Kaneko-Sasaguri Y, Hashizume T, Kanda T. 2005. When, where and how the bridge breaks: anaphase bridge breakage plays a crucial role in gene amplification and HSR generation. Exp Cell Res 302:233-243.

Shing DC, McMullan DJ, Roberts P, Smith K, Chin SF, Nicholson J, Tillman RM, Ramani P, Cullinane C, Coleman N. 2003. FUS/ERG gene fusions in Ewing's tumors. Cancer Res 63:4568-4576.

Slominski A, Wortsman J, Carlson A, Mihm M, Nickoloff B, McClatchey K. 1999. Molecular Pathology of Soft Tissue and Bone Tumors. Arch Pathol Lab Med 123:1246-1259.

Simon MP, Pedeutour F, Sirvent N, Grosgeorge J, Minoletti F, Coindre JM, Terrier-Lacombe MJ, Mandahl N, Craver RD, Blin N, Sozzi G, Turc-Carel C, O'Brien KP, Kedra D, Fransson I, Guilbaud C, Dumanski JP. 1997. Deregulation of the platelet-derived growth factor B-chain gene via fusion with collagen gene COL1A1 in dermatofibrosarcoma protuberans and giant-cell fibroblastoma. Nat Genet 15:95-98.

Simons A, Schepens M, Jeuken J, Sprenger S, van de Zande G, Bjerkehagen B, Forus A, Weibolt V, Molenaar I, van den Berg E, Myklebost O, Bridge J, van Kessel AG, Suijkerbuijk R. 2000.

Frequent loss of 9p21 (p16(INK4A)) and other genomic imbalances in human malignant fibrous histiocytoma. Cancer Genet Cytogenet 118:89-98.

Sirvent N, Maire G, Pedeutour F. 2003. Genetics of dermatofibrosarcoma protuberans family of tumors: from ring chromosomes to tyrosine kinase inhibitor treatment. Genes Chromosomes Cancer 37:1-19.

Sjoblom T, Shimizu A, O'Brien KP, Pietras K, Dal Cin P, Buchdunger E, Dumanski JP, Ostman A, Heldin CH. 2001. Growth inhibition of dermatofibrosarcoma protuberans tumors by the platelet-derived growth factor receptor antagonist STI571 through induction of apoptosis. Cancer Res 61:5778-5783.

Sjogren H, Meis-Kindblom J, Kindblom LG, Aman P, Stenman G. 1999. Fusion of the EWS-related gene TAF2N to TEC in extraskeletal myxoid chondrosarcoma. Cancer Res 59:5064-5067.

Sjogren H, Wedell B, Meis-Kindblom JM, Kindblom LG, Stenman G. 2000. Fusion of the NH2-terminal domain of the basic helix-loop-helix protein TCF12 to TEC in extraskeletal myxoid chondrosarcoma with translocation t(9;15)(q22;q21). Cancer Res 60:6832-6835.

Skapek SX and Chui CH. 2000. Cytogenetics and the biologic basis of sarcomas. Curr Opin Oncol 12:315-322.

Skytting B, Nilsson G, Brodin B, Xie Y, Lundeberg J, Uhlen M, Larsson O. 1999. A novel fusion gene, SYT-SSX4, in synovial sarcoma. J Natl Cancer Inst 91:974-975.

Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. 1987. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235:177-182.

Snijders AM, Pinkel D, Albertson DG. 2003. Current status and future prospects of array-based comparative genomic hybridisation. Brief Funct Genomic Proteomic 2:37-45.

Sonobe H, Furihata M, Iwata J, Ohtsuki Y, Chikazawa M, Taguchi T, Shimizu K. 1999. Dermatofibrosarcoma protuberans harboring t(9;22)(q32;q12.2). Cancer Genet Cytogenet 110:14-18.

Squire JA, Pei J, Marrano P, Beheshti B, Bayani J, Lim G, Moldovan L, Zielenska M. 2003. High-resolution mapping of amplifications and deletions in pediatric osteosarcoma by use of CGH analysis of cDNA microarrays. Genes Chromosomes Cancer 38:215-225.

Stock C, Kager L, Fink FM, Gadner H, Ambros PF. 2000. Chromosomal regions involved in the pathogenesis of osteosarcomas. Genes Chromosomes Cancer 28:329-336.

Su LK, Steinbach G, Sawyer JC, Hindi M, Ward PA, Lynch PM. 2000. Genomic rearrangements of the APC tumor-suppressor gene in familial adenomatous polyposis. Hum Genet 106:101-107.

Suehara Y, Kondo T, Fujii K, Hasegawa T, Kawai A, Seki K, Beppu Y, Nishimura T, Kurosawa H, Hirohashi S. 2006. Proteomic signatures corresponding to histological classification and grading of soft-tissue sarcomas. Proteomics 6:4402-4409.

Svarvar C, Larramendy ML, Blomqvist C, Gentile M, Koivisto-Korander R, Leminen A, Butzow R, Bohling T, Knuutila S. 2006. Do DNA copy number changes differentiate uterine from non-uterine leiomyosarcomas and predict metastasis? Mod Pathol 19:1068-1082.

Szymanska J, Tarkkanen M, Wiklund T, Virolainen M, Blomqvist C, Asko-Seljavaara S, Tukiainen E, Elomaa I, Knuutila S. 1995. A cytogenetic study of malignant fibrous histiocytoma. Cancer Genet Cytogenet 85:91-96.

Tarkkanen M, Elomaa I, Blomqvist C, Kivioja AH, Kellokumpu-Lehtinen P, Bohling T, Valle J, Knuutila S. 1999a. DNA sequence copy number increase at 8q: a potential new prognostic marker in high-grade osteosarcoma. Int J Cancer 84:114-121.

Tarkkanen M, Karhu R, Kallioniemi A, Elomaa I, Kivioja AH, Nevalainen J, Bohling T, Karaharju E, Hyytinen E, Knuutila S. 1995. Gains and losses of DNA sequences in osteosarcomas by comparative genomic hybridization. Cancer Res 55:1334-1338.

Tarkkanen M, Kiuru-Kuhlefelt S, Blomqvist C, Armengol G, Bohling T, Ekfors T, Virolainen M, Lindholm P, Monge O, Picci P, Knuutila S, Elomaa I. 1999b. Clinical correlations of genetic

changes by comparative genomic hybridization in Ewing sarcoma and related tumors. Cancer Genet Cytogenet 114:35-41.
Tarkkanen M, Larramendy ML, Bohling T, Serra M, Hattinger CM, Kivioja A, Elomaa I, Picci P, Knuutila S. 2006. Malignant fibrous histiocytoma of bone: analysis of genomic imbalances by comparative genomic hybridisation and C-MYC expression by immunohistochemistry. Eur J Cancer 42:1172-1180.
Tajiri T, Shono K, Fujii Y, Noguchi S, Kinoshita Y, Tsuneyoshi M, Suita S. 1999. Highly sensitive analysis for *N-myc* amplification in neuroblastoma based on fluorescence in situ hybridization. J Pediatr Surg 34:1615-1619.
Travis LB. 2002. Therapy-associated solid tumors. Acta Oncol 41:323-333.
van Dartel M, Cornelissen PW, Redeker S, Tarkkanen M, Knuutila S, Hogendoorn PC, Westerveld A, Gomes I, Bras J, Hulsebos TJ. 2002. Amplification of 17p11.2 approximately p12, including PMP22, TOP3A, and MAPK7, in high-grade osteosarcoma. Cancer Genet Cytogenet 139:91-96.
van Unnik JA, Coindre JM, Contesso C, Albus-Lutter CE, Schiodt T, Sylvester R, Thomas D, Bramwell V, Mouridsen HT. 1993. Grading of soft tissue sarcomas: experience of the EORTC Soft Tissue and Bone Sarcoma Group. Eur J Cancer 29:2089-2093.
Von Hoff DD. 1991. New mechanisms of gene amplification in drug resistance (the episome model). Cancer Treat Res 57:1-11.
Walter TA, Weh HJ, Schlag PM, Zornig C, Hossfeld DK. 1997. Cytogenetic studies in malignant fibrous histiocytoma. Cancer Genet Cytogenet 94:131-134.
Wang R, Lu YJ, Fisher C, Bridge JA, Shipley J. 2001. Characterization of chromosome aberrations associated with soft-tissue leiomyosarcomas by twenty-four-color karyotyping and comparative genomic hybridization analysis. Genes Chromosomes Cancer 31:54-64.
Wang R, Titley JC, Lu YJ, Summersgill BM, Bridge JA, Fisher C, Shipley J. 2003. Loss of 13q14-q21 and gain of 5p14-pter in the progression of leiomyosarcoma. Mod Pathol 16:778-785.
Waters BL, Panagopoulos I, Allen EF. 2000. Genetic characterization of angiomatoid fibrous histiocytoma identifies fusion of the FUS and ATF-1 genes induced by a chromosomal translocation involving bands 12q13 and 16p11. Cancer Genet Cytogenet 121:109-116.
Watson SK, Deleeuw RJ, Horsman DE, Squire JA, Lam WL. 2007. Cytogenetically balanced translocations are associated with focal copy number alterations. Hum Genet 120:795-805.
Weber KL. 2005. What's new in musculoskeletal oncology. J Bone Joint Surg Am 87:1400-1410.
Weiss MM, Kuipers EJ, Meuwissen SG, van Diest PJ, Meijer GA. 2003a. Comparative genomic hybridisation as a supportive tool in diagnostic pathology. J Clin Pathol 56:522-527.
Weiss MM, Kuipers EJ, Postma C, Snijders AM, Siccama I, Pinkel D, Westerga J, Meuwissen SG, Albertson DG, Meijer GA. 2003b. Genomic profiling of gastric cancer predicts lymph node status and survival. Oncogene 22:1872-1879.
Weng WH, Wejde J, Ahlen J, Pang ST, Lui WO, Larsson C. 2004. Characterization of large chromosome markers in a malignant fibrous histiocytoma by spectral karyotyping, comparative genomic hybridization (CGH), and array CGH. Cancer Genet Cytogenet 150:27-32.
Wiklund TA, Blomqvist CP, Raty J, Elomaa I, Rissanen P, Miettinen M. 1991. Postirradiation sarcoma. Analysis of a nationwide cancer registry material. Cancer 68:524-531.
Wilhelm M, Veltman JA, Olshen AB, Jain AN, Moore DH, Presti JC,Jr, Kovacs G, Waldman FM. 2002. Array-based comparative genomic hybridization for the differential diagnosis of renal cell cancer. Cancer Res 62:957-960.
Wolf M, Mousses S, Hautaniemi S, Karhu R, Huusko P, Allinen M, Elkahloun A, Monni O, Chen Y, Kallioniemi A, Kallioniemi OP. 2004. High-resolution analysis of gene copy number alterations in human prostate cancer using CGH on cDNA microarrays: impact of copy number on gene expression. Neoplasia 6:240-247.
Wong KK, deLeeuw RJ, Dosanjh NS, Kimm LR, Cheng Z, Horsman DE, MacAulay C, Ng RT, Brown CJ, Eichler EE, Lam WL. 2007. A Comprehensive Analysis of Common Copy-Number Variations in the Human Genome. Am J Hum Genet: 80:91-104.

Xia SJ and Barr FG. 2005. Chromosome translocations in sarcomas and the emergence of oncogenic transcription factors. Eur J Cancer 41:2513-2527.

Zhang B, Kirov S, Snoddy J. 2005. WebGestalt: an integrated system for exploring gene sets in various biological contexts. Nucleic Acids Res 33:741-748.

Zhang SD and Gant TW. 2005a. Effect of pooling samples on the efficiency of comparative studies using microarrays. Bioinformatics 21:4378-4383.

Zhao X, Li C, Paez JG, Chin K, Janne PA, Chen TH, Girard L, Minna J, Christiani D, Leo C, Gray JW, Sellers WR, Meyerson M. 2004. An Integrated View of Copy Number and Allelic Alterations in the Cancer Genome Using Single Nucleotide Polymorphism Arrays. Cancer Res 64:3060-3071.

Zielenska M, Marrano P, Thorner P, Pei J, Beheshti B, Ho M, Bayani J, Liu Y, Sun BC, Squire JA, Hao XS. 2004. High-resolution cDNA microarray CGH mapping of genomic imbalances in osteosarcoma using formalin-fixed paraffin-embedded tissue. Cytogenet Genome Res 107:77-82.

Zucman J, Delattre O, Desmaze C, Epstein AL, Stenman G, Speleman F, Fletchers CD, Aurias A, Thomas G. 1993a. EWS and ATF-1 gene fusion induced by t(12;22) translocation in malignant melanoma of soft parts. Nat Genet 4:341-345.

Zucman J, Melot T, Desmaze C, Ghysdael J, Plougastel B, Peter M, Zucker JM, Triche TJ, Sheer D, Turc-Carel C. 1993b. Combinatorial generation of variable fusion proteins in the Ewing family of tumours. EMBO J 12:4481-4487.