

Histopathology and Molecular Pathology of Bone and Extraskkeletal Osteosarcomas

Helen Trihia and Christos Valavanis

*"Metaxa" Cancer Hospital, Department of Pathology and Molecular Pathology Unit
Greece*

1. Introduction

Osteosarcoma has been recognized for almost two centuries and is the most common primary, non-haemopoietic malignant tumour of the skeletal system. It is thought to arise from primitive mesenchymal bone-forming cells and its histologic hallmark is the production of osteoid. Other cell populations may also be present, as these types of cells may also arise from pluripotential mesenchymal cells, but any area of bone or osteoid synthesized by malignant cells in the lesion establishes the diagnosis of osteosarcoma (Acchiappati et al, 1965).

It accounts for approximately 20% of all primary malignant bone tumours. In the United States, the incidence of osteosarcoma is 400 cases per year (4.8 per million population <20 y). The incidence is slightly higher in blacks than in whites (Huvos et al, 1983), in males than in females. It has a bimodal age distribution and a propensity to develop in adolescents and young adults (Dix et al, 1983; Soloviev, 1969; Wilimas, et al, 1977, Dorfman & Czerniak, 1995), with 60% of tumours occurring in patients younger than 25 years of age and only 13% to 30% in patients older than 40 years (de Santos et al, 1982; Huvos, 1986). Osteosarcoma is very rare in young children (0.5 cases per million per year in children <5y).

Osteosarcoma can occur in any bone, but most tumours originate in the long bones of the appendicular skeleton, near metaphyseal growth plates, especially the distal femur (42%, 75% of which in the distal femur), followed by the proximal tibia (19%, 80% of which in the proximal tibia) and proximal humerus (10%, 90% of which in the proximal humerus) (Goes M, 1952). In the long bones the tumour is most frequently centered in the metaphysis (90%), infrequently in the diaphysis (9%) and rarely in the epiphysis (Tsuneyoshi & Dorfman, 1987). Other significant locations are the skull and jaw (8%) and pelvis (8%) (Benson et al, 1984).

The clinicopathologic features of osteosarcoma form the basis of its classification, most importantly including the histologic features, the biologic potential (grade), relation to bone (intramedullary or surface), multiplicity (solitary and multifocal) and the pre-existing state of underlying bone (primary or secondary).

Osteosarcomas have a wide range of radiographic and histologic appearances. For example, some may be radiolucent or radiodense. Some are confined to the medullary cavity, or originate and grow on the bone surface. Some arise on normal bone (*de novo* osteosarcoma), others arise in the setting of Paget disease or radiation (secondary osteosarcoma). Most arise

in genetically normal individuals but rare cases have been seen in patients' various genetic syndromes (such as Rothmund-Thompson, Li Fraumeni and Retinoblastoma gene mutation) (Dick et al, 1982; Hansen et al, 1985; Kozlowski et al, 1980). The vast majority are solitary lesions, although rare cases of multifocal osteosarcomas have been reported (Ackermann, 1948, Amstutz, 1969, Laurent et al, 1973).

According to the mode of growth, osteosarcomas are subdivided into intramedullary and surface osteosarcomas. Intramedullary osteosarcomas are further subdivided into typical intramedullary, telangiectatic and highly differentiated. Surface osteosarcomas are subdivided into periosteal and parosteal osteosarcomas and high-grade surface osteosarcomas. According to the prevalent cell in the neoplastic tissue are subdivided into osteoblastic, chondroblastic, fibroblastic, giant cell, small cell and mixed (McDonald & Budd, 1943).

Most osteosarcomas can be categorized into four major groups: 1) conventional, high grade osteosarcoma and its histologic subtypes (75%-85%), 2) high grade osteosarcoma that arises in a diseased bone (10%), 3) intramedullary, well-differentiated (1%) and 4) surface osteosarcoma (5%-10%).

Bone tumour grading has traditionally been based on a combination of histologic diagnosis and the Broders grading system which assesses cellularity and degree of anaplasia (Broders, 1920; Inwards & Unni, 1995). The 7th edition of the AJCC Cancer Staging Manual recommends a 4-grade system (Edge et al, 2009), with grades 1 and 2, considered as 'low grade' and grades 3 and 4 'high grade'. The 2009 CAP Bone Tumour Protocol recommends a pragmatic approach, based principally on histologic classification. Under this system, central low grade osteosarcoma and parosteal osteosarcoma are considered Grade 1 sarcomas, with periosteal osteosarcoma considered Grade 2, and all other osteosarcomas considered Grade 3 (Dorfman et al, 1998, 2002).

The exact cause of osteosarcoma is unknown. The best known causative association - environmental risk factor is exposure to radiation (Huvos, 1986). Its causal relation was first documented in radium dial painters. Osteosarcoma after therapeutic irradiation is an uncommon complication, and usually develops after approximately 15 (range 3 to 55) years.

Osteosarcoma is known to affect approximately 1% of patients with Paget disease of bone, which reflects a several thousand-fold increase in risk in comparison with that of the general population (Wick et al, 1981).

Osteosarcoma may also arise in sites of previous bone infarction (Mirra et al, 1974), chronic osteomyelitis (Bartkowski & Klenczynski, 1974; Spyra, 1976), pre-existing primary benign bone tumours (osteochondroma, enchondroma, fibrous dysplasia, giant cell tumour, osteoblastoma, aneurysmal bone cyst and unicameral bone cyst) and adjacent to metallic implants (Johnson et al, 1962; Koppers et al, 1977, Ruggieri 1995; Schweitzer & Pirie, 1971; Smith et al, 1986). These secondary osteosarcomas account for only a small percentage of osteosarcomas and their pathogenesis is likely related to chronic cell turnover that is associated to the underlying bone disease.

The diagnosis of osteosarcoma, like all bone-tumours, emphasizes the necessity of close cooperation of all involved disciplines for diagnosis and therapy, including clinicians, radiologists and pathologists, as the correct diagnosis relies on their clinico-pathological appearance.

2. Pathologic features

2.1 Conventional osteosarcoma

Conventional osteosarcoma, is solitary, arises in the medullary cavity of an otherwise normal bone, is of high grade and produces neoplastic bone with or without cartilaginous or fibroblastic components. The gross findings are variable depending on the amount of bone and other components present. It manifests as a large, metaphyseal, intramedullary and tan-gray-white, gritty mass. Tumours that are producing abundant mineralized bone are tan-gray and hard, whereas non-mineralized, cartilaginous components are glistening, gray, and may be mucinous if the matrix is myxoid, or more rubbery if hyaline in nature. It can be necrotic, hemorrhagic and cystic. Intramedullary involvement is often considerable and the tumour usually destroys the overlying cortex and forms an eccentric or circumferential soft tissue component that displaces the periosteum peripherally. In the proximal and distal portions of the tumour the raised periosteum deposits a reactive bone, known as Codman's triangle. In some cases, the tumour grows into the joint space, resulting in coating of the peripheral portions of the articular cartilage by the sarcoma. Solitary or multiple skip metastases appear as intramedullary nodules in the vicinity of or far from the main mass. Furthermore, not all osteosarcomas arise in a solitary fashion, as multiple sites may become apparent within a period of about 6 months (synchronous osteosarcoma), or multiple sites may be noted over a period longer than 6 months (metachronous osteosarcoma). Such multifocal osteosarcoma is decidedly rare, but when it occurs, it tends to be in patients, younger than 10 years.

The diagnosis of osteosarcoma is based on the accurate identification of osteoid. Osteoid is unmineralized bone matrix that histologically appears as eosinophilic, dense, homogeneous, amorphous and curvilinear intercellular material, somewhat refractile. It must be distinguished from other eosinophilic extra-cellular materials such as fibrin and amyloid. Unequivocal discrimination between osteoid and non-osseous collagen may be difficult, or sometimes arbitrary (Fornasier, 1977). Non-osseous collagen tends to be linear, fibrillar and compresses between neoplastic cells. In contrast, osteoid is curvilinear with small nubs, arborisation and what appears to be abortive, lacunae formation. The thickness of the osteoid is highly variable with the 'thinnest' variant referred to as 'filigree', whereas osteoid seams are flat and thick. Osseous matrix has also the predisposition for appositional deposition upon previously existing normal bone trabeculae ('scaffolding').

Conventional osteosarcoma can produce varying amounts of cartilage and/or fibrous tissue. The algorithm is: identify the presence or absence of matrix, and if significant matrix is present, determine the matrix form and therefore subclassify into osteoblastic, chondroblastic, fibroblastic and mixed types, by virtue of the predominance of the neoplastic component.

Histologic subtyping may not always be possible, even with generous sampling. Such lesions are better categorized under the term 'osteogenic sarcoma with no predominant growth pattern'. Furthermore, sub-classification on local recurrence or following chemotherapy or irradiation can provide false results, because treatment can alter tumour appearance.

In addition, osteosarcoma may be of any histologic grade. Some contain highly pleomorphic cells and abundant mitotic figures, whereas others may be difficult to differentiate from benign neoplasms.

2.1.1 Osteoblastic osteosarcoma

Conventional osteosarcoma is usually of the osteoblastic type. In osteoblastic osteosarcoma the predominant matrix is bone and/or osteoid. It contains pleomorphic malignant cells and coarse neoplastic woven bone. The tumour cells are intimately related to the surface of the neoplastic bone, which is woven in architecture, varies in quantity and is deposited as primitive, disorganized trabeculae in a coarse, lace-like pattern, thin, arborising lines of osteoid (filigree) interweaving between neoplastic cells, or broad, large sheets of coalescing, trabeculae, as seen in the sclerosing variant. Depending on its state of mineralization, the bone can be eosinophilic, or basophilic and may have a pagetoid appearance caused by haphazardly deposited cement lines.

2.1.2 Chondroblastic osteosarcoma

Chondroid matrix is predominant in chondroblastic osteosarcoma, intimately associated with non-chondroid elements. The neoplastic chondrocytes are mostly characterized by severe cytologic atypia and reside in lacunar spaces, hyaline matrix or float singly or in cords in myxoid matrix. Myxoid and other forms of cartilage are uncommon, except in the jaws and in the pelvis.

2.1.3 Fibroblastic osteosarcoma

Typically, is composed of fusiform highly pleomorphic malignant cells, arranged in a herringbone or storiform pattern, similar to fibrosarcoma or malignant fibrous histiocytoma, with minimal osseous matrix. Although, the degree of atypia is variable, is frequently severe, with numerous mitoses, including atypical forms. In general, the lack of significant amounts of osteoid, bone or cartilage, relegates them to subtypes of fibroblastic osteosarcoma.

Although, there is a tendency for metastases of osteosarcomas to mimic the primary, exceptions are frequent and is a higher than expected incidence of fibroblastic differentiation in metastases.

2.2 Small cell osteosarcoma

It comprises 1.5% of osteosarcomas, with slight predilection for females and is composed of small cells with variable degree of osteoid production (Sim et al, 1979). According to the predominant cell pattern, tumours are classified to round cell type or short spindle cell type. Nuclei can be very small to medium sized, with scanty amount of cytoplasm, comparable to those of Ewing sarcoma and to large cell lymphoma respectively. The chromatin distribution can be fine or coarse and the mitoses range from 3 to 5/per high power field-HPF. Lace-like osteoid production is always present, although particular care must be taken to differentiate osteoid from fibrin deposits that may be seen among Ewing sarcoma cells.

2.3 Telangiectatic osteosarcoma

When first recognized, telangiectatic osteosarcoma was considered a distinct clinical and pathologic entity (Gaylord, 1903). On the basis of subsequent findings, telangiectatic osteosarcoma was considered a variant of osteosarcoma (Ewing, 1922, 1939).

It is rare, less than 4% of all cases of osteosarcomas and more frequent in the second decade of life. Although, most frequently affects long tubular bones, has also been noted to arise in extraosseous soft tissues in the forearm, thigh and popliteal fossa (Mirra et al, 1993).

Radiographically, is characterized by typically purely lytic destructive process, without matrix mineralization. Clinically, a pathological fracture is present in one fourth of the cases. On gross examination, there is a dominant cystic architecture in the bone medulla. The cystic space is filled incompletely with blood clot, described as 'a bag of blood'. Histologically, the tumour is characterized by dilated, blood-filled or empty spaces, separated by thin septae, simulating aneurysmal bone cyst. The cystic spaces are lined by benign osteoclast-like giant cells, without endothelial lining. The septae are cellular, containing highly malignant, atypical mononuclear tumour cells with high mitotic activity, including atypical mitoses. The amount of osteoid varies, but usually is fine, lace-like and of minimal amount, although it tends to be more prominent in metastatic foci. Therefore, it is imperative that the entire specimen is meticulously examined histologically.

Telangiectatic osteosarcomatous differentiation has been reported in parosteal osteosarcoma (Wines et al,2000), in dedifferentiated chondrosarcoma arising in the background of osteochondroma (Radhi & Loewy, 1999), in association with aneurysmal bone cysts (Adler, 1980; Kyriakos & Hardy, 1991), in osteitis deformans, as well as in cases of malignant phylloides tumour of the breast (Gradt et al, 1998) and in ovarian sarcomas (Hirakawa et al, 1998).

2.4 Low grade central (well differentiated intraosseous/intramedullary) osteosarcoma

It accounts for 1-2% of all osteosarcomas and is the medullary equivalent to parosteal osteosarcoma (Kurt et al, 1990; Unni et al, 1977). There is a tendency for a slightly older age and slightly longer symptomatology in comparison to the conventional osteosarcoma. It is important to recognize this subtype, since the patient tends to do much better, than those with conventional osteosarcoma. It is a low grade fibroblastic osteosarcoma and is histologically identical to parosteal osteosarcoma.

Histologically, contains long, parallel trabeculae or round islands of woven bone intimately associated with a mildly to moderately cellular population of cytologically bland neoplastic spindle cells with variable amounts of osteoid production. Nuclear enlargement and hyperchromasia are generally evident with occasional mitotic figures (1-2 mitoses per 10 high power fields-HPFs). When involving the medullary canal it may be confused with fibrous dysplasia (FD). Radiologically, FD is homogeneous ('ground glass' appearance), whereas the well differentiated osteosarcoma is less so, and may show trabeculations. Furthermore, a permeative growth pattern into the pre-existing lamellar bone or fatty marrow is diagnostic. Microscopically, the trabeculae of FD tend to be rather short and curled, whereas those of well differentiated osteosarcoma are longer and may be arranged in parallel arrays. Again, cytologic atypia should be sought and is diagnostic if found.

Conventional osteosarcomas often have better differentiated, innocuous 'normalized' areas. These should not be underdiagnosed as low grade osteosarcoma, since biologically they behave accordingly to their more aggressive foci.

Desmoplastic fibromas may infiltrate the bone and can be diagnostically challenging. In these cases, osteoid production should be sought. Radiographic evidence of matrix production is helpful in establishing the correct diagnosis.

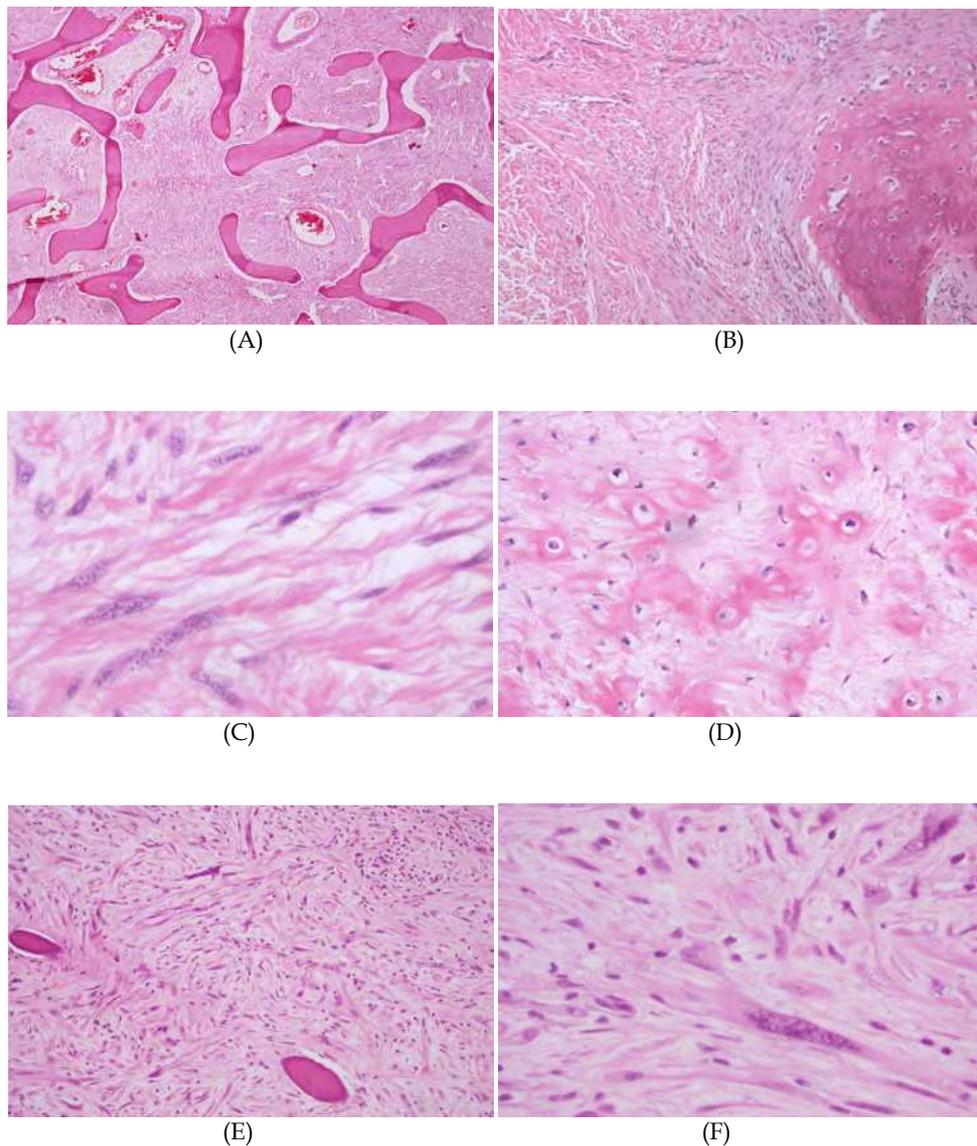


Fig. 1. **(A-D) Intramedullary (central) low grade osteosarcoma** (H&E stain). A. Long parallel neoplastic bony trabeculae associated with spindle cell stroma. B. Hypocellular spindle cell collagenized stroma with osteoid production C. Scattered fusiform cells with nuclear enlargement (high magnification). D. Area of chondroblastic differentiation. **(E, F) Osteosarcoma fibroblastic type** (H&E stain). E. Neoplastic bony trabeculae surrounded by spindle cell stroma exhibiting scattered cytologic atypia prominent at low magnification. F. Prominent cytologic atypia (high magnification)

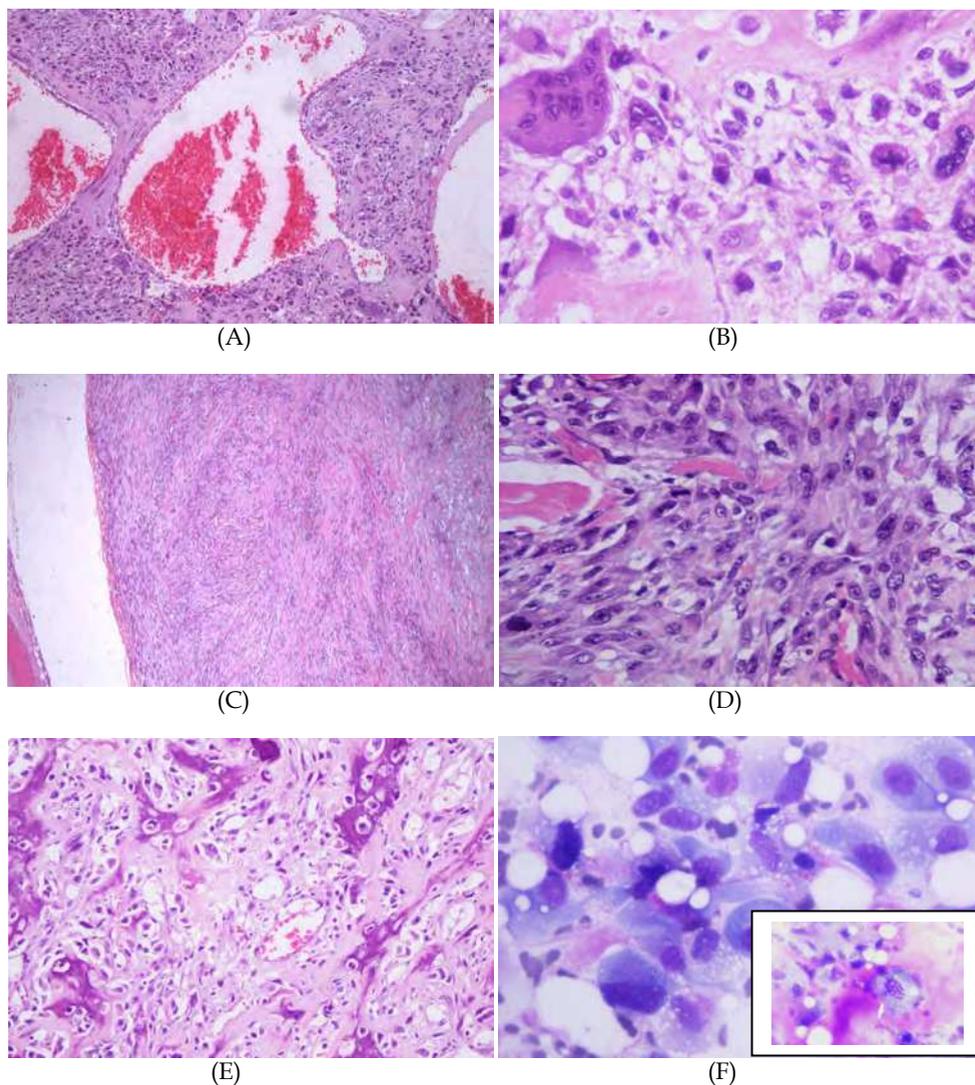


Fig. 2. **(A, B) Telangiectatic osteosarcoma** (H&E stain). (A) Blood-filled cystic spaces, separated by septae with abundant osteoclastic-type giant cells. (B) Giant cell-rich area with high cellular pleomorphism and patchy osteoid formation. **(C-E) Extraskkeletal osteosarcoma of the breast**. (C) Well circumscribed margins, peripheral spindle cell component and chondroblastic area (on the right). (D) Highly pleomorphic tumor cells including mitoses. (E) Osteoblastic osteosarcoma area with abundant mineralized osteoid. **(F) Fine needle aspirate from extraskkeletal osteosarcoma of the breast** (MGG stain). Cellular smear displaying epithelioid cells with plasmacytoid appearance. (Inset) Enlarged pleomorphic cell with fine cytoplasmic vacuolization, reminiscent of neoplastic chondroblast in association with red-purple amorphous material (reproduced with permission from S. Karger A.G., Medical and Scientific Publishers, first published by Trihia et al, *Acta Cytologica*, 2007;51:443-450)

2.5 Secondary osteosarcomas

Secondary osteosarcomas are bone forming sarcomas occurring in bones that are affected by preexisting abnormalities, the most common being Paget disease and radiation change.

2.5.1 Paget osteosarcoma

Incidence of sarcomatous change in Paget disease is estimated to 0.7-0.95%, with osteosarcomas representing 50-60% of Paget sarcomas (Haibach et al, 1985; Huvos et al, 1983; Schajowicz et al, 1983). It is more common in men, with median age of 64 years and is usually observed in patients with widespread Paget disease (70%). Most tumours arise in the medulla. Histologically are high grade sarcomas, mostly osteoblastic or fibroblastic. A great number of osteoclast-like giant cells may be found. Telangiectatic and small cell osteosarcomas have been reported.

2.5.2 Post-radiation osteosarcoma

They constitute 3.4-5.5% of all osteosarcomas and 50-60% of radiation-induced sarcomas. It is estimated that the risk of developing osteosarcoma in irradiated bone is 0.03-0.8% (Huvos et al, 1985; Mark et al, 1994). Children treated with high-dose radiotherapy and chemotherapy are at the greater risk, and the prevalence of post-radiation osteosarcomas is increasing as children survive treatment for their malignant tumour. It can develop in any irradiated bone, with most common locations the pelvis and the shoulder. The modified criteria for postradiation cancer initially promulgated in 1948 by Cahan and associates are as follows : (1) the patient received irradiation, (2) the neoplasm occurred in the radiation field, (3) a latent period of years had elapsed, (4) histologic or radiographic evidence for the pre-existent osseous lesion, a benign tumour or non-bone forming malignancy (Cahan et al, 1948).

Histologically, high grade osteosarcomas predominate.

Many of the reported cases of osteosarcoma arising in fibrous dysplasia have also been complicated by radiation therapy.

2.5.3 Osteosarcomas in other benign precursors

Other rare instances of secondary osteosarcomas have included cases arising in association with bone infarcts, endoprostheses and already mentioned fibrous dysplasia. Infarct associated sarcomas as well as malignant tumours at the site of prosthetic replacements (Brien et al, 1990), or at the site of prior fixation, most commonly show the histological pattern of malignant fibrous histiocytoma (MFH), with a minority being osteosarcomas.

2.6 Parosteal osteosarcoma

It is the most common type of osteosarcoma of the bone surface, although it accounts for about 5% of all osteosarcomas, with a slight female predominance (Okada et al, 1995). It is a low grade, slow growing neoplasm, with a predilection for the posterior aspect of the distal femur, where presents as a hard lobulated mass attached to the underlying cortex with a broad base. Histologically, it is a well differentiated fibro-osseous neoplasm, consisting of well formed bony trabeculae embedded in a hypocellular fibrous stroma. The bony

trabeculae can be arranged in parallel strands, simulating normal bone and may or may not show osteoblastic rimming. The intertrabecular stroma is hypocellular with minimal atypia, whereas it can be more cellular with moderate cytologic atypia in 20% of cases. About 50% of the tumours will show cartilaginous differentiation, in the form of hypercellular nodules of cartilage within the tumour substance or as a cup on the surface. When present, the cartilage cup is mildly cellular with mild cytologic atypia and lacks the 'columnar' appearance seen in osteochondromas.

About 15% of the tumours will show high grade spindle cell sarcoma (dedifferentiation), more often at the time of recurrence, in the form of osteosarcoma, fibrosarcoma or MFH (Wold et al, 1984).

Its diagnosis can be very difficult and the differential diagnosis has included diverse entities, such as: myositis ossificans, fracture callus, ossifying haematoma, osteochondroma, extraosseous osteosarcoma, parosteal chondroma, desmoplastic fibroma and osteoma. Therefore, parosteal osteosarcoma, like no other tumour, implies the necessity of close cooperation of all involved disciplines for the correct diagnosis.

2.7 Periosteal osteosarcoma

It accounts for less than 2% of all osteosarcomas, more common than high grade surface osteosarcoma, but about one third as common as parosteal osteosarcoma (Unni et al, 1976). Unlike parosteal osteosarcoma, which extends from the cortex like a bony knob, periosteal osteosarcoma tightly encases the bone, like a glove. Unlike other osteosarcomas, it tends to involve the diaphysis. Radiologically, it is a circumferential surface mass, less radiodense than parosteal osteosarcoma. Mineralization occurs as ring-shaped radiodensities or as streaks of reactive bone radiating from the surface. Lesions can be best visualized by MRI scan. A sub-periosteal lesion with a bright white signal is present, indicating its high cartilage content. Histologically, it has the appearance of a moderately differentiated, grade 2/3 chondroblastic osteosarcoma. It consists almost entirely of lobules of cellular, atypical cartilage, with bone formation in the centre of the lobules, separated by thin strands of fibrous tissue. Careful scrutiny of the fibrous component, reveals seams of neoplastic osteoid, usually at the outer surface of the neoplasm, which distinguishes it from surface chondrosarcoma.

2.8 High grade surface osteosarcoma

A high grade, bone-forming malignant tumour, arising from the bone surface, which comprises less than 1% of all osteosarcomas. Histologically, is similar to conventional osteosarcoma. Regions of predominantly osteoblastic, chondroblastic, or fibroblastic differentiation may predominate. However all tumours will show high cytologic atypia and lace-like osteoid. The pattern of osteoid production and the high grade cytologic atypia help to separate it from parosteal osteosarcoma. High grade surface osteosarcoma with chondroblastic differentiation may be confused with periosteal osteosarcoma. The degree of cytologic atypia is greater in high grade surface osteosarcoma and the tumours generally show larger spindle cell areas. Finally, unlike dedifferentiated parosteal osteosarcomas, low grade regions are not found in high grade surface osteosarcomas.

Cortical destruction and invasion into the medullary canal in high-grade juxtacortical (periosteal and high-grade surface) osteosarcomas, is often absent, but when present, is only focal. If extensive, it becomes difficult, if not impossible to distinguish an intramedullary tumour with an eccentric soft tissue component from a surface neoplasm with extensive invasion of the medullary canal.

2.9 Extraskkeletal osteosarcomas

Extraskkeletal osteosarcomas (EOs) or soft tissue osteosarcomas (STOs) are rare sarcomas arising in extraskkeletal somatic soft tissue, in which the neoplastic cells produce osteoid and/or bone matrix, therefore recapitulating the phenotype of osteoblasts. By definition, it is a high grade mesenchymal neoplasm that produces osteoid, bone and chondroid material, shows no evidence of epithelial component and is located in soft tissues, without attachment to bone or periosteum, as determined by X-ray findings or inspection during the operative procedure. It is significantly less frequent than its osseous counterpart. They account for 1.2% of soft tissue sarcomas and 4% of osteosarcomas (Bane et al, 1990; Sordillo et al, 1983). They typically arise in the deep soft tissues of the proximal extremities, with most common locations the deep soft tissues of the thigh and buttocks, followed in descending order by the upper limb, retroperitoneum, trunk, head and neck (Chung & Enzinger, 1987; Lee et al, 1995; Lidang et al, 1998; Sordillo et al, 1983). Fewer than 10% are superficial, originating in the dermis or subcutis. Cases of EOs arising in unusual sites have been reported, such as the breast, larynx, thyroid gland, parotid, abdominal viscera, including esophagus, small intestine, omentum majum, liver, heart, the urogenital system, including urinary bladder, ureter the prostate and the penis, pleura, mediastinum, ectopic thymus, pulmonary artery, pilonidal area and aorta (Baydar, et al, 2009; Burke & Virmani, 1991; Kemmer et al, 2008; Loose et al, 1990; Micolonghi et al, 1984; Pisciole et al, 1985; Shui et al, 2011; Silver & Tavassoli, 1998; Trowell & Arkell, 1976; Young & Rosenberg, 1987; Wegner et al, 2010; Greenwood & Meschter, 1989). Fewer than 300 cases have been reported to date and their aetiology is essentially unknown. Unlike osseous conventional osteosarcoma, it typically occurs in older adults, with a peak incidence in the fifth and sixth decades of life, in contrast with skeletal osteosarcomas that most commonly affect young adults (Sordillo et al, 1983). Males are affected more frequently than females at a ratio of 1.9:1. Some cases have been associated with a history of prior therapeutic irradiation at the site of the tumour (Logue & Cairnduff, 1991) and trauma (Allan & Soule, 1971). In one case report of primary osteosarcoma of the urinary bladder, prolonged treatment with immunosuppressive medications, including cyclophosphamide for active systemic lupus erythematosus (SLE) has been reported (Baydar et al, 2009). The diagnosis is generally delayed and prognosis is poor, with most patients died within months of diagnosis and with a cause-specific survival rate at 5 years less than 25%. Clinically and mammographically can mimic a benign tumour. Like most of soft tissue sarcomas, tumours may appear grossly circumscribed, however they are microscopically infiltrative. Imaging studies usually reveal compact calcifications/variable mineralization within the mass. Primary extraosseous osteosarcoma should always be included in the differential diagnosis, in the view of a well demarcated calcified mass on image analysis. In the case of extraskkeletal osteosarcoma of the breast, an underdiagnosis as a calcified fibroadenoma, should be avoided.

STOs are usually large, ranging in size between 5 and 10 cm, well-circumscribed, grossly heterogeneous tumours that exhibit areas of haemorrhage and/or necrosis. Microscopically,

STOs constitute highly cellular, cytologically pleomorphic, mitotically active sarcomas, spindle cell to epithelioid in appearance. The defining feature is the presence of neoplastic osteoid and/or bone. The latter usually presents in a 'lace like' manner, although solid sheets of amorphous osteoid can also be found. When the bone/osteoid matrix is found only focally in the tumour, largely demonstrates a nonspecific, undifferentiated, spindle cell sarcomatous appearance. Lobules of highly cellular, atypical hyaline/fibrocartilage may also present. Osteoclast-like giant cells have also been described. Various histologic subtypes of bone osteosarcoma can be seen in extraskkeletal osteosarcoma. Osteoblastic variant is the most common type, followed by fibroblastic, chondroid, telangiectatic, small cell and well-differentiated variants. Common to all variants is the production of osteoid, intimately associated with tumour cells, which may be deposited in a lacy, trabecular or sheet-like pattern. Neoplastic bone formation is more prominent in the centre of the tumour with the peripheral areas being more cellular, a reverse pattern of myositis ossificans. In essence, any of the microscopic patterns of high-grade intraosseous osteosarcoma may be seen in EO.

The differential diagnosis should always include spindle cell (sarcomatoid) carcinomas and the exclusion of metastasis from a primary osteosarcoma, as well as carcinosarcomas, malignant tumours with osseous metaplasia and sarcomas. Metaplastic ossification, ranging from osteoid to woven bone formation can be seen in synovial sarcoma, epithelioid sarcoma, liposarcoma and carcinosarcoma. Osteogenic differentiation can also be seen as a phenomenon of dedifferentiation in various soft tissue sarcomas. In the above tumours, other histologic lineages or histologic characteristics of original tumours are usually present. The key to diagnosis, is the identification of the matrix surrounding the tumour cells and lack of epithelial differentiation. Furthermore, except osteoblastic, chondroblastic and fibroblastic differentiation, no other lineages of histologic differentiation should be detectable in EOs. Diagnostic confirmation using immunohistochemical markers is necessary to ensure the absence of an epithelial component and exclude the neoplasms of biphasic origin.

Immunohistochemical studies indicate that the EOs' immunophenotype is similar to bone osteosarcoma. EOs are uniformly positive for vimentin, they express smooth muscle actin (68%), desmin (25%), S-100 protein (20%), including non-cartilaginous areas, EMA (52%), Keratin (8%) and are negative for PLAP.

Differential diagnosis from recurrent or metastatic osteosarcoma is based on clinical history.

3. The use of cytology in the preoperative investigation of bone tumours with emphasis in osteosarcoma

Fine needle aspiration (FNA) of bone lesions has been performed ever since the technique was introduced (Coley et al, 1931). It has certain advantages over open biopsy, as it is less disruptive to bone and permits multiple sampling without complications. Its main use is to confirm malignancy. Although, cytomorphology of primary bone tumours has been extensively described in correlation with histology (Hajdu, 1975; Stormby & Akerman, 1973; Walaas & Kindblom, 1990; White et al, 1988), FNA is not as good for diagnosing primary bone tumours.

There are two main indications of FNA of soft tissue and bone lesions: preoperative diagnosis before definitive treatment and the investigation of lesions suspicious of tumour recurrence or metastasis. Eventhough, the first use has limitations, however, at present, the

use of FNA as the diagnostic, pre-treatment tool for musculoskeletal tumours is accepted in many orthopaedic centres, provided that certain requirements are fulfilled and that the final cytologic diagnosis is based on the combined evaluation of clinical data, radiographic findings and cytomorphology (Domansky et al, 2010). One important reason, why FNA is preferred upon open biopsy, is when the preoperative evaluation of a lesion as malignant is of most importance, rather the subtype of the lesion. On the other hand, in the use of neo-adjuvant treatment (radio- or chemotherapy), before surgery, the FNA diagnosis is of major importance, analogous to histopathologic examination, in regard to subtype and tumour grade. This is mostly the case with small round cell sarcomas. Regarding bone lesions, FNA may also replace open biopsy in the primary diagnosis. It is the task of the pathologist to distinguish benign and malignant primary bone tumours from metastatic deposits and from the wide range of benign and inflammatory reactive lesions of the bone. Furthermore, the pathologist should give a confident diagnosis of the various benign bone tumours and sarcomas, if open biopsy is to be avoided. Skeletal aspiration is not different from other aspirations, but it is important to remember that intact cortical bone cannot be penetrated by regularly used (22 gauge) needles, something that can be easily done with partly destroyed or eroded bone. A 18-gauge needle can be used in intact bone, under anaesthesia and multiple passes can be performed. Many malignant bone tumours have palpable soft tissue involvement, which can be much easier penetrated by the needle. In any case, it is strongly recommended that the pathologist is familiar with the radiological findings, discuss the best approach to the tumour with the radiologist and that non-palpable lesions are aspirated under image-guided techniques. Essentially, when the aspirated material is technically satisfactory, it is suitable for use of specialized techniques, likewise biopsy material, to assist in the diagnosis.

The FNA cytological findings of osteosarcoma have thoroughly been described (Mertens et al, 1982; Walaas & Kindblom, 1990; White et al, 1988). They include mixture of cell clusters and dispersed cells, pleomorphic, spindle and rounded cells with frequent mitoses, including atypical forms, intercellular tumour matrix of osteoid, benign osteoclast-like giant cells, epithelioid tumour cells, which may be of osteoblastic type, or resemble chondroblasts, in osteoblastic and chondroblastic variants respectively, atypical spindle-shaped cells in fibroblastic subtypes. The presence of osteoid-like material can be 'prominent, or scarce, dissociated, or in association with cell clusters, either as moderate-sized fragments, or not easily discernible globule-like particles'. This material could be associated either with the predominant histologic pattern or with the sampled area represented in the smears. Osteoid-like matrix material has been originally described as 'homogeneous or vacuolated eosinophilic plaques' or pink-purple acellular material in May Grunwald Giemsa (MGG) smears and green-blue in Papanicolaou-stained (PAP) smears. Another cause of difficulty in accurately assessing the presence of osteoid in the smears is that osteoid, cartilage and dense collagen fibers appear very similar in both Diff-Quick and PAP stains. Furthermore, osteoid resists the suction, during aspiration, and when aspirated, the small, disrupted fragments lack the characteristic lattice-like pattern observed in histological sections (Mertens & Langnickel, 1982; Nikol et al, 1998).

FNA has proven to be of value in the preoperative diagnosis of breast EOs (Trihia et al, 2007). Its diagnosis is often delayed because of a deceptively benign clinical and radiologic appearance (Watt et al, 1984). Although differential diagnosis from metaplastic carcinoma may not be possible on cytological grounds alone, FNA has proven to be of value in preoperative

diagnosis of malignancy and prompt surgical intervention, as clinically and mammographically these lesions can be underdiagnosed as a benign tumour (Trihia et al, 2007).

4. The role of immunohistochemistry in the diagnosis of osteosarcoma

The diagnostic algorithm of primary bone tumors is, and always has been, a collaborative effort in which clinical, radiologic, and pathologic findings have to be considered. In the majority of cases, the pathologist can rely exclusively on histopathologic examination to provide an accurate diagnosis. In some cases, however, ancillary studies have to be employed to distinguish entities that share morphologic characteristics.

When evaluating a bone tumor, the pathologist is confronted with several difficulties. Bone tumors are rare entities, and not all pathologists are exposed to bone pathology with the frequency needed to gain the necessary level of diagnostic expertise. Also, certain osseous tumors share histopathologic features and, in many cases, important diagnostic features may not be readily evident in small specimens. Finally, intramedullary lesions often must be decalcified, a process that may be associated with loss in cellular morphologic detail. All of these factors complicate the diagnostic process. Diagnosis for many entities can be reached by the evaluation of histopathologic features alone, or can be interpreted in the context of clinico-radiologic findings, but for others, only a differential diagnosis can be reached without ancillary studies.

Several studies have shown that gentle decalcification methods preserve antigenicity relatively well for the most commonly used markers.

One of the greatest challenges in bone and soft tissue pathology is the reliable recognition of osseous matrix production in malignant lesions. Although several antigens have been explored, unfortunately, little is known about the antigenic specificity of normal bone tissue and bone neoplasms and currently there is no specific marker to distinguish the bone matrix from its collagenous mimics.

Since late 1990's, due to their central function in the process of mineralization, a group of proteins have been proposed for tumor diagnosis: alkaline phosphatase, osteonectin, and osteocalcin. Osteocalcin (OCN) and osteonectin (ONN) have been applied to paraffin sections and been used to highlight osteoid. OCN is one of the most prevalent intraosseous proteins and is produced exclusively by bone-forming cells-osteoblasts and therefore has received special attention as a specific marker (Fanburg et al, 1997, 1999; Takada et al, 1992). In the detection of bone-forming tumors, osteocalcin has been associated with 70% sensitivity and 100% specificity, compared with the 90% sensitivity and 54% specificity reported for ONN. Nevertheless, OCN is rarely used in clinical practice. ONN is a protein that is implicated in regulating the adhesion of osteoblasts and platelets to their extracellular matrix, as well as early mineralization and should only be used as part of a panel of reagents, directed at several lineage-related proteins (Serra et al, 1992; Wuisman et al, 1992).

Strong labeling of the osseous isozyme of alkaline phosphatase has been used to distinguish EO from other pleomorphic sarcomas. The major drawback of this marker is that it can only be used on cryostat sections and imprint smears.

Ancillary techniques have a limited role in diagnosing osteosarcoma, as the tumour is largely recognized by its morphologic features. Because of the many varieties of osteosarcoma, diverse tumours are considered in its differential diagnosis.

Currently, immunohistochemistry has limited application in the differential diagnosis of primary bone tumors. In general, osteosarcoma has a broad immunoprofile that lacks diagnostic specificity. Vimentin, OCN, ONN, S-100 protein, muscle protein smooth muscle actin (SMA), neuron specific enolase (NSE) and CD99 are some of the antigens that are commonly expressed. Importantly, some tumours also stain with antibodies to keratin and epithelial membrane antigen (EMA). Also, it's worth noticing, that osteosarcoma usually does not stain with antibodies to factor VIII and CD31.

Osteosarcoma is distinguished from benign tumours and fibro-osseous lesions, by virtue of its infiltrative growth pattern, with the tumour replacing the marrow space and surrounding bony trabeculae, which may also serve as scaffolding for the deposition of neoplastic bone.

Telangiectatic osteosarcoma differs from aneurysmal bone cyst because it contains cytologically malignant cells within the cyst walls, whereas the cells in aneurysmal bone cyst are banal in appearance.

Biopsies of osteosarcoma that lack neoplastic bone can be problematic because its immunophenotype can generate a broad list of differential diagnoses that include Ewing's sarcoma/Primitive Neuroectodermal Tumour-PNET, metastatic carcinoma and melanoma, leiomyosarcoma and malignant peripheral nerve sheath tumour.

The subtype of osteosarcoma that most likely will benefit from the application of an immunohistochemistry panel is the "small-cell" type. The diagnosis of this entity is difficult due to the paucity of osteoid and the similarity to other small round-cell tumors. Although the antigenic profile of small-cell osteosarcoma is unknown, expression of markers specific for other small-cell tumors, help in ruling out this diagnosis.

In these circumstances, immunohistochemical analysis, electron microscopic evaluation and molecular studies may be helpful. Small cell osteosarcoma is distinguished from Ewing's sarcoma by the presence of neoplastic bone and dilated rough endoplasmic reticulum by electron microscopy, as osteosarcoma cells have the features of mesenchymal cells with abundant endoplasmic reticulum and the matrix contains collagen fibres, which may show calcium hydroapatite crystal deposition and the absence of t(11;22) translocation, or its variants, which is diagnostic of Ewing's sarcoma.

MIC-2 gene product (CD99), which is located in the short arm of the sex chromosome, encodes a surface protein, first described in T-cell and null-cell acute lymphoblastic leukemia. Osteosarcoma usually has a diffuse moderate to strong intracytoplasmic staining for CD99. Tumour cells of the small cell variant of osteosarcoma may be positive for CD99, vimentin, osteocalcin, osteonectin, smooth muscle actin, Leu-7 and KP1.

The differential diagnosis of osteosarcoma from other sarcomas (e.g., malignant fibrous histiocytoma, fibrosarcoma) is important because of the specific therapy available for osteosarcoma patients. Most osteosarcomas express vimentin and, according to some authors, some tumors focally express cytokeratin and desmin, although these findings have not been widely confirmed. Bone matrix proteins, such as OCN, alkaline phosphatase, and ONN, are expressed in osteosarcomas. However, their presence has also been detected in chondrosarcomas, Ewing's sarcoma, fibrosarcomas, and malignant fibrous histiocytomas. Caution should also be used in the interpretation of focal expression of a variety of markers

(e.g., S-100, actin, epithelial membrane antigen) found occasionally in otherwise typical osteosarcomas. Extraskkeletal osteosarcomas of the fibroblastic subtype often have sparse amounts of osteoid and can be differentiated from malignant fibrous histiocytoma on the basis of strong expression of alkaline phosphatase. Chondroblastic osteosarcoma and chondrosarcoma, however, cannot be distinguished immunohistochemically.

The different types of collagen present in the bone matrix are also produced by other tumors and therefore have no application in differential diagnosis. The basic calponin gene, a smooth muscle differentiation-specific gene that encodes an actin-binding protein involved in the regulation of smooth muscle contractility, is expressed in osteosarcomas (Yamamura, et al, 1998).

The list of entities included in the differential diagnosis of MFH is extensive. Immunohistochemistry helps in the distinction of MFH (CD68+, OCN-, alkaline phosphatase-) from leiomyosarcoma (CD68-), malignant neurilemmoma (S-100+) and from fibroblastic osteosarcoma (occasionally positive for both OCN, alkaline phosphatase). The distinction of cytokeratin-positive MFH from sarcomatoid carcinoma may be impossible by immunohistochemistry and is best accomplished by electron microscopy.

Finally, stress fracture and accompanying callus can sometimes be confused with osteosarcoma because the reactive bone and cartilage are deposited around pre-existing bony trabeculae, mimicking an infiltrative pattern of growth. However, the cells in reactive tissues are banal and osteoblastic rimming is usually present.

In conclusion, although immunohistochemistry does not currently play an important diagnostic role in primary bone tumors as it does in soft-tissue counterparts, research efforts to characterize the histogenesis of many of these neoplasias may offer new alternatives for diagnosis in the near future. For the distinction of primary tumors versus metastases of non-osseous origin and for the characterization of a small subset of neoplasias, such as those with small round-cell morphology, immunohistochemistry remains the technique of choice.

5. Molecular pathology of osteosarcoma

Traditionally, our understanding of osteosarcoma has been largely based on anatomic and histologic features. However, recent studies in the molecular pathology of osteosarcoma have provided new insight into its pathogenesis. Through the identification of molecular pathways of osteosarcoma development and progression, the roles played by mutated tumor suppressor genes, oncogenes, and cell cycle regulatory molecules in bone oncogenesis, differentiation, cell death and cell migration have been explored. Furthermore, numerous cytogenetic abnormalities have been associated with osteosarcoma, including chromosomal amplifications, deletions, rearrangements, and translocations.

Thus, the diagnostic and prognostic significance of molecular aberrations are beginning to be evaluated and novel approaches for therapeutic interventions of osteosarcoma are being developed.

5.1 Bone growth, differentiation and osteosarcoma tumorigenesis

It is well known that osteosarcoma has a propensity for developing in bone growth plates characterized by rapid bone turnover during childhood and adolescence (Broadhead et al., 2011; Gelberg et al., 1997)

Additionally, patients affected by Paget's disease, a disorder characterized by both excessive bone formation and breakdown, also have a higher incidence of osteosarcoma (Vigorita, 2008). These observations, along with the knowledge that normal bone differentiation process occurs in the epiphyseal growth plates, strongly suggest that osteosarcoma is caused by genetic and epigenetic disturbances in the osteoblast proliferation and differentiation pathways (Tang et al., 2008; Thomas et al., 2006).

Osteogenesis results from a regulated sequence of events involving epithelial mesenchymal interactions, condensation, and terminal differentiation. Several major signal transduction pathways, such as Wnt, BMP, FGF, and hedgehog signaling, play an important role in regulating osteogenic differentiation (Glass & Karsenty, 2007; Luu et al., 2007; Reya & Clevers, 2005). Bone lineage commitment and terminal differentiation are regulated by several osteogenic transcriptional factors such as, Runx2, Osterix, ATF4, and TAZ (Chien & Karsenty, 2005; Deng et al., 2008; Kansara & Thomas, 2007; Karsenty, 2000; Karsenty, 2002; Karsenty, 2003; Hong et al., 2005; Yang & Karsenty, 2004) in coordination with their activators, like Rb which transactivates Runx2 (Ogasawara et al., 2004; Thomas et al., 2001) and their repressors, like WWOX which suppresses RUNX2 transcriptional activity (Del Mare et al., 2011).

Among these factors, Runx2 is the most important regulator of bone development and serves as a hub to direct progenitors to osteogenic lineage through BMP-induced osteogenesis, synergistically inducing many terminal differentiation markers (Lian et al., 2006; Nakashima et al., 2002; Thomas et al., 2004; Yamaguchi et al., 2000). Additionally, Runx2 associates with p27KIP1 protein and through interaction with the hypophosphorylated form of Rb and transactivation of Osterix transcription factor, promotes terminal cell cycle exit and the formation of a differentiated osteoblastic phenotype (Nakashima et al., 2002; Nishio et al., 2006; Thomas et al., 2004).

Furthermore, the canonical Wnt pathway has been identified to play a crucial role in osteoblast differentiation, as evidenced by the fact that Wnt3a expression leads to cell proliferation and suppression of osteogenic differentiation in adult mesenchymal stem cells (Boland et al., 2004), and that multiple aberrations in the Wnt signaling pathway have been associated with osteosarcoma tumorigenesis (Haydon et al., 2002, Reya & Clevers, 2005, Clevers, 2006).

Disruption of the well-coordinated balance between osteogenic progenitors proliferation and differentiation may lead to osteosarcoma development. The defects caused by genetic (eg, activation of oncogenes or inactivation of p53 and RB tumor suppressor genes) and epigenetic alterations may occur at different stages of osteogenic differentiation leading to more or less aggressive tumorigenic phenotypes (Kansara and Thomas, 2007).

Moreover, osteosarcoma cells utilize an alternative lengthening of telomere (ALT) pathway that prevents telomere shortening, allowing the tumor cells to evade senescence and resemble their stem cell progenitors (Wang, 2005).

5.2 Osteosarcoma invasion and metastasis

Osteosarcoma is an aggressive tumor with high metastatic potential. Osteosarcoma invasion of bone and metastasis to other organs relies on complex cell-cell and cell-matrix interactions. The

invasion and metastatic sequence involves the detachment of osteosarcoma cells from the primary site, lysis of bone matrix, local migration, invasion through stromal tissue, intravasation, and extravasation. In this process, interactions between osteosarcoma cells, osteoblasts and osteoclasts are the main events leading to the substantial osteolysis exhibited by some osteosarcomas as a result of increased osteoclastic activity.

During the initial stages of osteosarcoma invasion, TGF- β is released from the degraded bone matrix and acts on osteosarcoma cells, stimulating the release of PTHrP, interleukin-6 (IL-6) and interleukin-11 (IL-11) (Quinn et al., 2001). These cytokines then stimulate osteoclasts, facilitating further invasion and release of proresorptive cytokines. Osteoblasts function as mediators in this process of bone resorption. Osteosarcoma cells release endothelin-1 (ET-1), VEGF, and PDGF in response to the hypoxic and acidotic conditions leading to angiogenesis and stimulation of osteoblastic function (Kingsley et al., 2007; Chirgwin et al., 2007). PTHrP and IL-11 also act on osteoblasts, stimulating increased expression of receptor activator of nuclear factor κ B ligand (RANKL). RANKL is a main mediator of osteoclast differentiation and activity, and osteosarcoma cells produce RANKL independently (Kinpara, 2000). RANKL activates osteoclasts through binding to RANK on the osteoclast surface. RANK expression is regulated by cytokines IL-1, IL-6, IL-8, tumour necrosis factor- α (TNF- α), PTHrP, and TGF- α (Hofbauer & Heufelder, 1998).

Receptor-ligand binding leads to activation of both NF κ B and MAPK pathways, with a resulting increase in nuclear factor of activated T cells (NFATc1) activity. RANK/RANKL also activates the c-Fos component of AP-1, resulting in additional NFATc1 upregulation. NFATc1 then activates the transcription of genes involved in osteoclast activity and maturation (Takayanagi, 2007).

Activated osteoclasts release proteases such as cathepsin K (Cat K) necessary for breakdown of collagen I, osteopontin, and osteonectin (Stoch & Wagner, 2008) aiding the invasion process (LeGall et al., 2007). This protease is essential for osteoclast function in normal bone remodelling and also in pathological states of osteolysis. For patients with high-grade metastatic osteosarcoma, low Cat K levels at the time of diagnosis confers a better prognosis (Husmann et al., 2008).

Invasion of the surrounding tissues by osteosarcoma also involves degradation of the extracellular matrix. Matrix metalloproteinases (MMPs) are principally involved in the breakdown of the extracellular matrix and are regulated by natural inhibitors such as tissue inhibitors of MMPs (TIMPs), RECK, and α 2 macroglobulin (Birkedal-Hansen et al., 1993; Chakraborti et al., 2003). In the setting of osteosarcoma, MMPs break down extracellular collagens, facilitating both tumour and endothelial cell invasion. MMPs play also a role in angiogenesis aiding further the metastatic process.

The urokinase plasminogen activator (uPA) and its receptor (uPAR) system is another key regulator of osteosarcoma invasion, which acts as an activator of pro-MMPs. An inverse relationship between uPA levels and overall survival has been demonstrated in osteosarcoma cases (Choong et al., 1996).

5.3 Syndromes associated with osteosarcoma

A variety of genetically based diseases and syndromes show a susceptibility to the development of osteosarcoma, such as Paget's disease, Rothmund-Thomson, Bloom, Werner and Li-Fraumeni syndromes, as well as Hereditary Retinoblastoma.

In patients affected by Paget's, a hereditary disorder characterized by rapid bone remodelling leading to dysregulated bone turnover, about 1 % of them will develop osteosarcoma. This percentage accounts for a substantial fraction of the osteosarcoma cases diagnosed over 60 years of age (McNairn et al., 2001). Although the complete pattern of genetic aberrations leading to Paget's disease remains unclear, LOH18CRI located at 18q21-q22 locus has been identified as the major underlying genetic abnormality linked to Paget osteosarcoma (Cody et al., 1997; Good et al., 2002; Hansen et al., 1999; Nelissery et al., 1998). Furthermore, the genes SQSTM1 at 5q31 and MAPK8 at 5q35qter, which are implicated in IL-1/TNF and RANK signalling pathways, respectively, seem to be involved in Paget osteosarcoma pathogenesis (Kansara & Thomas, 2007).

Rothmund-Thomson, Bloom and Werner syndromes are characterized by germline mutations in RecQ helicases genes whose products are responsible for separation of double-stranded DNA prior to replication (German, 1993; Goto et al., 1996; Hickson, 2003). Among them Rothmund-Thomson syndrome, an autosomal recessive disorder, is related to the highest osteosarcoma incidence (32 %) with a tendency to occur at a younger age (Tang et al., 2008; Wang, 2001; Wang et al., 2003; Wang et al., 2005).

Li-Fraumeni syndrome patients are characterized by germline mutations in p53 gene and have an increased risk in developing osteosarcomas among other malignancies (Malkin et al., 1990).

Hereditary Retinoblastoma patients have germline mutations in Rb1 tumor suppressor gene and are predisposed to develop osteosarcoma (Araki et al., 1991). Such bone sarcomas are likely to show LOH at 13q and molecular alterations of the Rb1 gene (Andreassen et al., 1993; Wadayama et al., 1994; Wunder et al., 1991).

5.4 Cytogenetic aberrations

5.4.1 Conventional osteosarcoma

5.4.1.1 Cytogenetics

Although the germline mutations explain part of the osteosarcoma cases, most osteosarcomas are sporadic. Sporadic osteosarcomas have a wide range of genetic abnormalities and display extensive genetic heterogeneity. However involvement of certain chromosomal loci is recurrent and chromosomal regions 1p11-13, 1q11-12, 1q21-22, 11p14-15, 14p11-13, 15p11-13, 17p and 19q13 are most frequently affected (Mertens et al., 1993; Tarkkanen et al., 1993). Numerical chromosomal abnormalities associated with conventional osteosarcoma include loss of chromosomes 6q, 9, 10, 13, and 17 as well as gain of chromosome 1 (Boehm et al., 2000; Bridge et al., 1997; Stock et al., 2000).

Recent studies have identified amplifications of chromosomes 6p21, 8q24, and 12q14, as well as loss of heterozygosity of 10q21.1, as being among the most common genomic alterations in osteosarcoma. Furthermore, patients carrying these aberrations had a poorer prognosis (Smida et al., 2010).

Cytogenetic features of gene amplification, such as ring chromosomes, double minutes, and homogeneously staining regions are frequently identified in conventional osteosarcomas (Menghi-Sartorio et al., 2001).

Despite intensive research, no consensus specific chromosomal aberrations that could be used diagnostically in osteosarcoma tumors have been identified.

5.4.1.2 DNA copy numbers

Osteosarcoma tumors contain multiple random chromosomal aberrations and only a few deletions and amplifications appear common to comparative genomic hybridization (CGH) studies (Atiye et al., 2005; Selvarajah et al., 2008).

Comparative genomic hybridization analysis reveals that chromosomal regions 3q26, 4q12-13, 5p13-14, 7q31-32, 8q21-23, 12q12-13, 12q14-15, and 17p11-12 are most frequently gained (Menghi-sartorio et al., 2001; Stock et al., 2000; Tarkannen et al., 1995). Gain of 8q23 is detected in 50% of tumors (Stock et al., 2000) and is correlated with poor prognosis (Tarkannen et al., 1989). Increased copy number of the MYC gene localized to 8q24 was detected by in situ hybridization in 44% of cases (Stock et al., 2000). The most frequent losses are found at 2q, 6q, 8p, and 10p (Knutila et al., 2000; Tarkannen et al., 1995).

In a recent study with 10 osteosarcomas, CGH whole-genome analysis showed changes including: hypomethylation, gain, and overexpression of histone cluster 2 genes at chromosome 1q21.1-q21.3; loss of chromosome 8p21.2-p21.3 and underexpression of DOCK5 and TNFRSF10A/D genes; and amplification-related overexpression of RUNX2 at chromosome 6p12.3-p21.1. Amplification and overexpression of RUNX2 could disrupt G2/M cell cycle checkpoints and bone differentiation leading to genomic instability. Disruption of DOCK5-signaling, together with p53 and TNFRSF10A/D related cell cycle and death pathways, may play a critical role in abrogating apoptosis (Sadovicic et al., 2009).

Diploid ploidy pattern by DNA fluocytometry has been reported to be a poor prognostic sign (Kusuzaki et al., 1999).

5.4.1.3 Loss of heterozygosity (LOH)

Loss of heterozygosity are detected more frequently at the long arms of chromosomes 3, 13 and 18 and at the short arm of chromosome 17 (Kruzelock et al., 1997). As the incidence of LOH is high at 3q26.6-26.3, this area has been suggested to harbour a putative suppressor gene (Kruzelock et al., 1997).

5.4.2 Telangiectatic osteosarcoma

A limited number of cases show highly complex chromosomal changes, and only one case is characterized by trisomy 3 (Bridge et al., 1997; Fletcher et al., 1994; Hoogerwerf et al., 1994). Mutations in the TP53 and RAS genes, LOH at the TP53, CDKN2A and RB1 loci, and amplification of the MDM2 and MYC genes are rare in telangiectatic osteosarcomas (Radig et al., 1998).

Telangiectatic osteosarcoma can be distinguished from aneurysmal bone cyst by the balanced translocations between the short arm of chromosome 17 and the long arm of chromosome 16 (Panoutsakopoulos et al., 1999). This rearrangement is characteristic of aneurysmal bone cyst. However, there are many variations on this theme, and at least five different chromosomes can serve as translocation partners with chromosome 17 (Dal Cin et al., 2000; Herens et al., 2001; Wyatt-Ashmead et al., 2001).

5.4.3 Small cell osteosarcoma

No specific cytogenetic abnormalities have been detected in small cell osteosarcoma.

Small cell osteosarcoma can be distinguished from Ewing sarcoma by the presence of neoplastic bone and the absence of the t(11:22) translocation or one of its variants.

5.4.4 Low grade central osteosarcoma

Low grade central (intramedullary) osteosarcoma show minimal chromosomal imbalances compared with the complex cytogenetic aberrations identified in high grade osteosarcoma. Cytogenetic studies revealed ring chromosomes of amplified regions 12q13-15, as well as abnormalities in chromosome 6p, 14 and 15 (Werner et al., 1998).

Comparative genomic hybridization demonstrate recurrent gains in chromosomal regions at 12q13-14, 12p, and 6p21 (Tarkkanen et al., 1998). MDM2, CDK4, and SAS at the 12q13-15 amplicon have been reported to be amplified at frequencies of 35%, 65% and 15%, respectively (Ragazzini et al., 1999).

5.4.5 Postradiation osteosarcoma

Cytogenetic and DNA copy number changes are complex and similar to those in conventional osteosarcomas (Mertens et al.; 2000). Postradiation osteosarcomas frequently exhibit 3p and 1p chromosomal losses compared to sporadic osteosarcoma which show more gains than losses (Mertens et al., 2000; Tarkkanen et al., 2001).

In one study, a high (58%) incidence of TP53 mutations was found (Nakanishi et al., 1998).

5.4.6 Parosteal osteosarcoma

Chromosomal alterations in parosteal osteosarcomas (low grade surface osteosarcomas) are characterized by one or more supernumerary ring chromosomes, often as the sole alteration (Mertens et al., 1993; Örndal et al., 1993; Sinovic et al., 1992).

Comparative genomic hybridization studies indicate 12q13-15 amplified region in the chromosomal rings (Szymanska et al., 1996). The SAS, CDK4, and MDM2 genes are coamplified and overexpressed in the majority of cases (Wunder et al., 1999).

Mutations in RB1 (Wadayama et al., 1994) or microsatellite instability (Tarkkanen et al., 1996) have not been found to be present in parosteal osteosarcoma.

5.4.7 Periosteal osteosarcoma

Most of the periosteal osteosarcomas show complex karyotypic aberrations (Gisselsson et al., 1998; Hoogerwerf et al., 1994; Tarkkanen et al., 1993).

5.4.8 Extraskkeletal osteosarcomas

Clonal chromosomal aberrations with complex patterns have been reported (Mandahl et al., 1989; Mertens et al. 1998). So far, no genetic differences between osteosarcomas of bone and extraskkeletal origin have been identified.

5.5 Molecular genetic alterations

A number of molecular defects in tumor suppressor genes, oncogenes, bone differentiation genes and genes involved in cell migration have been observed in osteosarcomas.

5.5.1 Tumor suppressor genes

The p53 and retinoblastoma (Rb) genes are the major tumor-suppressor genes affected in osteosarcoma (Marina et al., 2004). The p53 gene is mutated in 22% of osteosarcomas (Ta et al., 2009) and its expression seems to be higher in low grade osteosarcomas and correlate with reduced metastatic disease and improved survival (Hu et al., 2010). p53 mutation has also been shown to be more common in high-grade conventional osteosarcomas versus low grade central osteosarcomas (Park et al., 2004). However, other studies showed no correlation between survival and the p53 protein, while coexpression of p53 and P-glycoprotein was associated with a poorer prognosis (Lonardo et al., 1997; Park et al., 2001).

In addition to p53, the Rb tumour suppressor has also been implicated in the tumorigenesis of osteosarcoma. Both germ-line and somatic mutations of Rb confer an increased risk of osteosarcoma (Benassi et al., 1999). Loss of heterozygosity for Rb has been reported to confer both an improved and poorer prognosis for patients (Heinsohn et al., 2007; Wadayama et al., 1994; Feugeas et al., 1996).

5.5.2 Oncogenes

Transcription factors c-Fos and c-Jun are significantly upregulated in high-grade osteosarcomas compared with benign osteogenic lesions and low-grade osteosarcomas (Wu et al., 1990; Franchi et al., 1998) and are associated with the propensity to develop metastases (Gamberi et al., 1998).

Another transcription factor involved in cell proliferation, is c-Myc, whose amplification has been implicated in osteosarcoma pathogenesis and resistance to chemotherapeutic drugs. Overexpression of Myc in bone marrow stromal cells leads to osteosarcoma development and loss of adipogenesis (Shimizu et al., 2010).

Overexpression of MET (Ferracini et al., 1995; Rong et al., 1993) and c-Fos (Wu et al., 1990) has been reported in more than 50% of osteosarcoma cases, whereas c-Myc is overexpressed in less than 15% of cases (Barrios et al., 1993; Ladanyi et al., 1993). c-Myc, c-Fos, and cathepsin L have been shown to be overexpressed in a high proportion of relapsed tumours and metastases (Gamberi et al., 1998; Park et al., 1996).

Transforming growth factor beta (TGF- β) family proteins are also implicated in osteosarcomagenesis through impairment of osteoblast proliferation, differentiation and cell death. High-grade osteosarcomas are found to express TGF- β 1 in significantly higher amounts than low-grade osteosarcomas (Franchi et al., 1998).

IGF (insulin-like growth factor)-I and IGF-II are growth factors that are often overexpressed by osteosarcomas (Rikkof et al., 2009).

Connective tissue growth factor (CTGF) is related to a number of proteins in the CCN family (CTGF/Cyr61/ Cef10/NOVH). This protein family act via integrin signalling pathways (Lau et al., 1999) and, is involved in a diverse range of functions including adhesion,

migration, proliferation, survival, angiogenesis, and differentiation. A related protein, CCN3, was found to be overexpressed in osteosarcoma and associated with a worse prognosis (Perbal et al., 2008).

In one study, a substantial percentage (42.6%) of osteosarcomas displayed high levels of HER2/neu (c-erbB2,ERBB2) expression, relative to adjacent normal tissues (Gorlick et al., 1999).

5.5.3 Genes amplifications

Amplifications at 1q21-23 and at 17p are frequent findings in conventional osteosarcoma (Knuutila et al., 2000). Several genes have been reported to be involved in the 1q21-23 amplicon (Forus et al., 1998; Meza-Zepeda et al., 2002). Similarly, a variety of genes in the 12q13-15 region are co-amplified (Berner et al., 1997; Forus et al., 1994; Khatib et al., 1993; Momand et al., 1992; Oliner et al., 1992; Roberts et al., 1989; Smith et al., 1992; Yotov et al., 1999).

FISH analysis has revealed that genes, including CCND2, ETV6, and KRAS2, at 12p and MDM2 at 12q were differently amplified in low grade osteosarcomas (parosteal osteosarcoma) and high grade osteosarcomas (Gisselson et al., 2002).

MDM2 (Ladanyi et al., 1993; Oliner et al., 1992) and PRIM1 (Yotov et al., 1999) amplifications have been detected in 14-27% and 41% of osteosarcoma cases, respectively. In aggressive osteosarcomas CDK4 is most consistently amplified, alone or together with MDM2 (Berner et al., 1996; Forus et al., 1994; Maelandsmoet et al., 1995).

Recently, it was shown that APEX1 gene was amplified in osteosarcomas and that APEX1 expression was an independent predictor of the osteosarcoma local recurrence and/or metastasis (Yang et al., 2010).

5.5.4 Gene expression

Osteosarcoma cells share many similar features to undifferentiated osteoprogenitors, including a high proliferative capacity, resistance to anoikis, and expression of many osteogenic markers, such as CTGF, Runx2, ALP, Osterix, Osteocalcin and Osteopontin (Tang et al., 2008; Haydon et al., 2007; Luu et al., 2007). Furthermore, the more aggressive osteosarcoma phenotypes often have features of early osteogenic progenitors, while less aggressive tumors seem to be more similar to osteogenic mesenchymal stem cells that have progressed further along the differentiation cascade (Broadhead et al., 2011; He et al., 2010).

Osteogenic differentiation of mesenchymal stem cells can be monitored by using bone morphogenetic proteins (BMPs) and their downstream mediators, such as Id proteins and connective tissue growth factor (CTGF), as early markers, alkaline phosphatase and Osterix as early/middle markers and osteocalcin and osteopontin as late markers of bone formation (Tang et al., 2008).

Analysis of the expression of these osteogenic markers in osteosarcoma cells demonstrates a much lower alkaline phosphatase expression in tumor cells when compared to committed osteoblastic cell lines (Harris et al., 1995; Luo et al., 2008). Similarly, the late osteogenic

markers osteopontin and osteocalcin are highly expressed in mature, differentiated osteoblasts, but are minimally expressed in both primary OS tumors and OS cell lines (Cheng et al., 2003; Luu et al., 2007). CTGF, a multifunctional growth factor that is normally upregulated at the earliest stages of osteogenic differentiation, also shows elevated basal expression in human osteosarcoma cells (Luo et al., 2004).

Recent studies have revealed that *WWOX*, a gene involved in bone differentiation, is deleted in 30% of osteosarcoma cases and *WWOX* protein is absent or reduced in ~60% of osteosarcoma tumors (Del Mare et al., 2011). *WWOX* associates with *Runx2*, a key regulator of bone differentiation. Interestingly, *Runx2* has a very low expression in osteosarcoma cell lines. *Runx2* also associates with *BMPs*, *Rb*, and *p27KIP1* playing a crucial role in cell cycle and bone differentiation regulatory pathways. Thus, it is natural that any alterations would lead to uncontrolled proliferation and loss of differentiation. Accordingly, high-grade osteosarcomas show decreased expression of *p27KIP1*, while lower-grade tumors have detectable *p27KIP1* levels (Thomas et al., 2004).

Bone morphogenetic proteins (*BMPs*) such as bone morphogenetic protein-6 and bone morphogenetic protein receptor 2 are expressed in more than 50% of osteosarcomas and related to poor prognosis (Gobbi et al., 2002; Guo et al., 1999; Yoshikawa et al., 2004).

As above mentioned, Wnt pathway plays a crucial role in osteoblast differentiation and elevated levels of β -Catenin, an important regulator of the Wnt pathway, are correlated with osteoprogenitor proliferation and osteosarcoma metastasis (Iwaya et al., 2003). In addition, osteosarcoma tumors overexpressing *LRP5*, a Wnt co-receptor, are associated with a poorer prognosis and decreased patient survival (Hoang et al., 2004). Furthermore, Wnt mutations causing excessive Wnt (Wingless and int) signalling are associated to decreased survival and increased metastatic capacity (Hoang et al., 2004; Iwaya et al., 2003; Kansara and Thomas, 2007).

Other genes with altered expression associated to osteosarcoma include overexpression of *MAGE* genes (Sudo et al., 1997), *p19INK4D* (a cyclin dependent kinase inhibitor involved in cell cycle regulation, found in 7 % of osteosarcomas) (Miller et al., 1997), transforming growth factor-beta (*TGF β*) isoforms, of which *TGF β 3* is strongly related to disease progression (Kloen et al., 1997) and vascular endothelial growth factor (*VEGF*, expression correlated with survival) (Jung et al., 2005). Antiangiogenic proteins such as thrombospondin 1, *TGF- β* , troponin I, pigment epithelial-derived factor (*PEDF*), and reversion-inducing cysteine rich protein with Kazal motifs (*RECK*) are downregulated in osteosarcoma (Ren et al., 2006; Cai et al., 2006; Clark et al., 2007).

Overexpression of *Ezrin*, a protein involved in cell movement, correlates with poor outcome in paediatric osteosarcoma (Khanna et al., 2004) and according to animal studies, is overexpressed in highly metastatic osteosarcoma compared to tumors with lower metastatic potential (Khanna et al., 2000).

MMP2 and *MMP9* were overexpressed in osteosarcoma cells and associated with the ability of the cells to metastasize (Bjornland et al., 2005). Increased expression of membrane-type *MMP1* has been correlated with poor prognosis (Ushibori et al., 2006) and upregulation of *TIMP1* is associated with poor clinical outcome (Ferrari et al., 2004).

5.6 Epigenetics

Compared to large number of studies describing genetic alterations in osteosarcoma, relatively few studies investigating epigenetic alterations have been reported so far (Benassi et al., 2001; Benassi et al., 1999; Harada et al., 2002; Hou et al., 2006; Thomas and Kansara, 2006; Tsuchiya et al., 2000).

Recent studies have identified a close association between the aberrant methylation of histone cluster 2 genes, p14ARF/CDKN2A, p16 and Ras effector homologue (RASSF1A) genes and osteosarcoma tumorigenesis (Hou et al., 2006; Rao-Bindal & Kleinerman, 2011). It is believed that hypermethylation and subsequent transcriptional silencing of tumor suppressor genes contributes to the neoplastic process by increasing the mutation rate (Jones & Baylin, 2002; Wajed et al., 2001).

5.7 Epilogue

Understanding of the molecular pathogenesis of osteosarcoma has advanced considerably over recent decades. The processes involved in osteosarcoma oncogenesis have been outlined above with emphasis on the disruption of bone differentiation machinery.

However, the study of pathogenic mechanisms is in itself not enough. Translational studies are critical if an effective treatment for osteosarcoma is to arise from this understanding of osteosarcoma molecular pathology. If osteosarcoma results from bone differentiation defects, future research should focus on identifying relevant biomarkers and therapies targeting cellular differentiation thereby avoiding complications associated with conventional chemotherapy and be most effective in treatment of this debilitating tumor.

6. References

- Acciapatil G, Randelli G, & Randelli M (1965). Observations on osteogenous- osteogenic sarcoma. *Arch Orthop*, 78:57-156
- Ackerman A.J (1948). Multiple osteogenic sarcoma. Report of two cases. *Am J Roentgenol Radium Ther Nucl Med*, 60:623-632
- Adler C.P (1980). Case report 111. *Skeletal Radiol*, 5(1):56-60
- Amstutz H.C (1969). Multiple osteogenic osteosarcomata-metastatic or multicentric? Review of two cases and review of the literature. *Cancer*, 24:923-931
- Allan C.J, Soule E.H (1971). Osteogenic sarcoma of the somatic soft tissues. Clinicopathologic study of 26 cases and review of the literature. *Cancer*, 27:1121-1133
- Andreassen A, Oyjord T, Hovig E, Holm R, Florenes VA, Nesland JM, Myklebost O, Hoie J, Bruland OS & Borresen AL. (1993). p53 abnormalities in different subtypes of human sarcomas. *Cancer Res*, 53: 468-471.
- Araki N, Uchida A, Kimura T, Yoshikawa H, Aoki Y, Ueda T, Takai S, Miki T & Ono K (1991). Involvement of the retinoblastoma gene in primary osteosarcomas and other bone and soft-tissue tumors. *Clin Orthop*, 270:271-277.
- Atiye J, Wolf M, Kaur S, Monni O, Bohling 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.

- Bane B.L, Evans H.L, Ro J.Y, et al (1990). Extraskelletal osteosarcoma. A clinicopathologic review of 26 cases. *Cancer*, 65:2762-2770
- Barrios C, Castresana JS, Ruiz J, Kreicbergs A (1993). Amplification of c-myc oncogene and absence of c-Ha-ras point mutation in human bone sarcoma. *J Orthop Res*, 11: 556-563
- Bartkowski S & Kleczynski A (1974). A case of sarcoma developing in the course of chronic non specific osteitis. *Pol Przegl Chir*, 46:783-785
- Baydar D.E, Himmetoglu C.,Yazici S., et al. (2009). Primary osteosarcoma of the urinary bladder following cyclophosphamide therapy for systemic lupus erythematosus: a case report. *Journal of Medical Case Reports*, 3(39):1-5
- Benassi, M. S., Molendini, L., Gamberi, G., Magagnoli, G., Ragazzini, P., Gobbi, G. A., Sangiorgi, L., Pazzaglia, L., Asp, J., Brantsing, C., & Picci, P. (2001). Involvement of INK4A gene products in the pathogenesis and development of human osteosarcoma. *Cancer* 92: 3062-3067
- Benassi, M. S., Molendini, L., Gamberi, G., Ragazzini, P., Sollazzo, M. R., Merli, M., Asp, J., Magagnoli, G., Ballardelli, A., Bertoni, F., & Picci, P. (1999). Alteration of pRb/p16/cdk4 regulation in human osteosarcoma. *Int J Cancer*, 84: 489-493
- Benson J.E, Goske M, Han J.S, et al (1984). Primary osteogenic sarcoma of the calvaria. *Am J Neuroradiol*, 5:810-813
- Berner JM, Forus A, Elkahloun A, Meltzer PS, Fodstad O & Myklebost O (1996). Separate amplified regions encompassing CDK4 and MDM2 in human sarcomas. *Genes Chromosomes Cancer* 17: 254-259
- Berner JM, Meza-Zepeda LA, Kools PF, Forus A, Schoenmakers EF, Van de Ven WJ, Fodstad O & Myklebost O (1997). HMGIC, the gene for an architectural transcription factor, is amplified and rearranged in a subset of human sarcomas. *Oncogene*, 14: 2935-2941
- Bjornland K, Flatmark K, Pettersen S, Aaasen AO, Fodstad O & Maelandsmo GM. (2005). Matrix metalloproteinases participate in osteosarcoma invasion. *J Surg Res.*, 127:151-156
- Birkedal-Hansen H., W. G. I. Moore, M. K. Bodden et al. (1993). Matrix metalloproteinases: a review. *Critical Reviews in Oral Biology and Medicine*, 4(2):197-250
- Boehm AK, Neff JR, Squire JA, Bayani J, Nelson M & Bridge JA (2000). Cytogenetic findings in 36 osteosarcoma specimens and a review of the literature. *Ped Pathol Mol Med*, 19: 359-376
- Boland G. M., G. Perkins, D. J. Hall, and R. S. Tuan (2004). Wnt 3a promotes proliferation and suppresses osteogenic differentiation of adult human mesenchymal stem cells. *Journal of Cellular Biochemistry*, 93(6):1210-1230
- Brien W.W, Salvati E.A, Healey J.H, et al (1990). Osteogenic sarcoma arising in the area of a total hip replacement. A case report. *J Bone Joint Surg Am*, 72:1097-1099
- 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.
- Broadhead M. L., Clark J. C. M., Myers D. E., Dass C. R., & Choong P. F. M. (2011). The Molecular Pathogenesis of Osteosarcoma: A Review. *Sarcoma*, 2011: Article ID 959248, 12 pp
- Brodgers A.C (1920). Squamous cell epithelioma of the lip: A study of 537 cases. *JAMA*, 74:659-664
- Burke A. P, Virmani R. (1991). Osteosarcoma of the heart. *Am J Surg Pathol*, 15:289-295

- Cahan W.G., Woodard H.Q., Higinbotham N.L., & al. (1948). Sarcoma arising in irradiated bone. Report of eleven cases. *Cancer*, 1 :3-29
- Cai J., Parr C., Watkins G., Jiang W. G., & Boulton M.(2006). Decreased pigment epithelium-derived factor expression in human breast cancer progression. *Clinical Cancer Research*, 12(11): 3510-3517
- Coley B.L, Sharp G.S & Ellis E.B (1931). Diagnosis of bone tumours by aspiration. *Am J Surg Pathol*, 13:215-224
- Chakraborti S, Mandal M., Das S., Mandal A, & Chakraborti T. (2003). Regulation of matrix metalloproteinases. An overview. *Molecular and Cellular Biochemistry*, 253(1-2):269-285
- Cheng H., W. Jiang, F. M. Phillips et al. (2003). Osteogenic activity of the fourteen types of human bone morphogenetic proteins (BMPs). *Journal of Bone and Joint Surgery A*, 85(8):1544-1552
- Chien KR & Karsenty G.(2005). Longevity and lineages: toward the integrative biology of degenerative diseases in heart, muscle and bone. *Cell*,120:533-544
- Chirgwin J. M. & T. A. Guise (2007). Skeletal metastases: decreasing tumor burden by targeting the bone microenvironment. *Journal of Cellular Biochemistry*, 102(6):1333-1342
- Choong P. M., Ferno M., Akermans M. et al. (1996). Urokinaseplasminogen-activator levels and prognosis in 69 soft-tissue sarcomas. *International Journal of Cancer*, 69(4):268-272
- Chung E.B, Enzinger F.M (1987). Extraskelatal osteosarcoma. *Cancer*, 60:1132-1142
- Clark J. C. M., D. M. Thomas, P. F. M. Choong, & C.R. Dass (2007). RECK—a newly discovered inhibitor of metastasis with prognostic significance in multiple forms of cancer. *Cancer and Metastasis Reviews*, 26(3-4):675-683
- Clevers H. (2006). Wnt/ β -catenin signaling in development and disease. *Cell*, 127(3):469-480
- Cody JD, Singer FR, Roodman GD, Otterund B, Lewis TB, Leppert M & Leach RJ (1997). Genetic linkage of Paget disease of the bone to chromosome 18q. *Am J Hum Genet*, 61: 1117-1122
- Dal Cin P, Kozakewich HP, Goumnerova L, Mankin HJ, Rosenberg AE, & Fletcher JA (2000). Variant translocations involving 16q22 and 17p13 in solid variant and extraosseous forms of aneurysmal bone cyst. *Genes Chromosomes Cancer*, 28: 233-234.
- Dass C. R., Nadesapillai A. P. W., Robin D. et al. (2005). Downregulation of uPAR confirms link in growth and metastasis of osteosarcoma. *Clinical and Experimental Metastasis*, 22(8):643-652
- Del Mare S., Kurek K.C., Stein G.S, Lian J.B., & Aqeilan R.I. (2011). Role of the WWOX tumor suppressor gene in bone homeostasis and the pathogenesis of osteosarcoma. *Am J Cancer Res.*, 1(5): 585-594
- Deng ZL, Sharff KA, Tang N, Song WX, Luo JXL, Chen J, Bennett E & Reid R. Manning D, Xue A, Montag AG, Luu HH, Haydon RC, He T-C. (2008). Regulation of osteogenic differentiation during skeletal development. *Frontiers in Biosci.*, 13:2001-2021
- De Santos L.A, Rosengren J-E, Wooten W.B, et al (1978). Osteogenic sarcoma after the age of 50: a radiographic evaluation. *Am J Roentgenol*, 131:481-484
- Dick D.C, Morley W.N & Watson J.J (1982). Rothmund-Thompson syndrome and osteogenic sarcoma. *Clin Exp Dermatol*, 7:119-123
- Dix D., McDonald M., & Cohen P. (1983). Adolescent bone cancer: is the growth spurt implicated? (Letter to the Editor) *Eur J Cancer Clin Oncol*, 19:859-860

- Domanski H., Akerman M. & Silverman J. (2010). Soft tissue and musculoskeletal system In: *Diagnostic Cytopathology* by W. Gray and G. Kocjan, Elsevier editions
- Dorfman H.D & Czerniak B. (1005). Bone cancers. *Cancer*, 75:201-210,
- Dorfman H.D & Czerniak B. (1998). Osteosarcoma In: *Bone Tumors*. Mosby, pp.128-252, St.Louis, USA
- Dorfman H.D, Czerniak B. & Kotz R. (2002). WHO classification of tumors of bone: Introduction In: *Pathology and Genetics. Tumors of soft tissue and bone*, IARCPress, 226-232, Lyon, France
- Edge S.B, Byrd D.R, Carducci M.A, et al: eds (2009). *AJCC Cancer Staging Manual 7th ed.*, Springer, New York,USA
- Ewing J (1922). A review and classification of bone sarcomas. *Arch Surg*, 4:483-533
- Ewing J. (1939). A review of the classification of bone tumors. *Bull Am Coll Surg*, 24:290-295
- Fanburg J.C, Rosenberg A.E, Weaner D.L, et al (1997). Osteocalcin and osteonectin immunoreactivity in the diagnosis of osteosarcoma. *Am J Clin Pathol*, 108:464-473
- Fanburg-Smith J.C, Bratthauer G.L & Miettinen M. (1999). Osteocalcin and osteonectin immunoreactivity in extraskkeletal osteosarcoma: A study of 28 cases. *Hum Pathol*, 30:32-38
- Ferracini R, Di Renzo MF, Scotlandi K, Baldini N, Olivero M, Lollini P, Cremona O, Campanacci M & Comoglio PM (1995). The Met/HGF receptor is over-expressed in human osteosarcomas and is activated by either a paracrine or an autocrine circuit. *Oncogene*, 10: 739-749.
- Ferrari C, Benassi S, Ponticelli F, Gamberi G, Ragazzini P, Pazzaglia L, Balladelli A, Bertoni F & Picci P. (2004). Role of MMP-9 and its tissue inhibitor TIMP-1 in human osteosarcoma: findings in 42 patients followed for 1-16 years. *Acta Orthop Scand.*, 75:487-491
- Feugeas O., N. Guriec, A. Babin-Boilletot et al. (1996). Loss of heterozygosity of the RB gene is a poor prognostic factor in patients with osteosarcoma. *Journal of Clinical Oncology*, 14(2):467-472
- Fletcher JA, Gebhardt MC & Kozakewich HP (1994). Cytogenetic aberrations in osteosarcomas. Nonrandom deletions, rings, and double-minute chromosomes. *Cancer Genet Cytogenet*, 77: 81-88.
- Fornasier V.L: Osteoid: An ultrastructural study(1997). *Hum Pathol* 8:243-254
- Forus A, Berner JM, Meza-Zepeda LA, Saeter G, Mischke D, Fodstad O, & Myklebost O (1998). Molecular characterization of a novel amplicon at 1q21-q22 frequently observed in human sarcomas. *Br J Cancer*, 78: 495-503
- Forus A, Florenes VA, Maelandsmo GM, Fodstad O & Myklebost O (1994). The protooncogene CHOP/GADD153, involved in growth arrest and DNA damage response, is amplified in a subset of human sarcomas. *Cancer Genet Cytogenet*, 78:165-171.
- Forus A, Flørenes VA, Maelandsmo GM, Meltzer PS, Fodstad O & Myklebost O. (1993). Mapping of amplification units in the q13-14 region of chromosome 12 in human sarcomas: some amplicons do not include MDM2. *Cell Growth Differ.*, 4(12):1065-70
- Franchi A., L. Arganini, G. Baroni et al. (1998). Expression of transforming growth factor β isoforms in osteosarcoma variants: association of TGF β 1 with high-grade osteosarcomas. *Journal of Pathology*, 185(3):284-289

- Franchi A., A. Calzolari & G. Zampi (1998). Immunohistochemical detection of c-fos and c-jun expression in osseous and cartilaginous tumours of the skeleton. *Virchows Archiv*, 432(6):515-519
- Gamberi G, Benassi MS, Böhling T, Ragazzini P, Molendini L, Sollazzo MR, Pompetti F, Merli M, Magagnoli G, Balladelli A & Picci P (1998). C-myc and c-fos in human osteosarcoma: prognostic value of mRNA and protein expression. *Oncology*, 55: 556-563.
- Gaylord H.R (1903). On the pathology of so-called bone aneurism. *Ann Surg*, 37:834-847
- Gelberg K. H., E. F. Fitzgerald, S. A. Hwang & R. Dubrow (1997). Growth and development and other risk factors for osteosarcoma in children and young adults. *International Journal of Epidemiology*, 26(2):272-278.
- German, J. (1993). Bloom syndrome: a mendelian prototype of somatic mutational disease. *Medicine (Baltimore)*, 72: 393-406.
- Gisselsson D, Höglund M, Mertens F, Mitelman F & Mandahl N (1998). Chromosomal organization of amplified chromosome 12 sequences in mesenchymal tumors detected by fluorescence in situ hybridization. *Genes Chromosomes Cancer*, 23: 203-212
- Gisselsson D, Pålsson E, Höglund M, Domanski H, Mertens F, Pandis N, Sciort R, Dal Cin P, Bridge JA & Mandahl N (2002). Differentially amplified chromosome 12 sequences in low- and high-grade osteosarcoma. *Genes Chromosomes Cancer*, 33: 133-140
- Glass DA 2nd & Karsenty G. (2007). In vivo analysis of Wnt signaling in bone. *Endocrinology*, 148:2630-2634
- Gobbi, G., Sangiorgi, L., Lenzi, L., Casadei, R., Canaider, S., Strippoli, P., Lucarelli, E., Ghedini, I., Donati, D., Fabbri, N., et al. (2002). Seven BMPs and all their receptors are simultaneously expressed in osteosarcoma cells. *Int J Oncol*, 20:143-147
- Goes M., (1952) Knochenwachstum und osteogenes sarcom. *Strahlentherapie*, 89:194-210
- Good DA, Busfield F, Fletcher BH, Duffy DL, Kesting JB, Andersen J & Shaw JT (2002). Linkage of Paget disease of bone to a novel region on human chromosome 18q23. *Am J Hum Genet*, 70: 517-525.
- Gorlick R, Huvos AG, Heller G, Aledo A, Beardsley GP, Healey JH & Meyers PA. (1999). Expression of HER2/c-erbB-2 correlates with survival in osteosarcoma. *J Clin Oncol.*, 17:2781-2788
- Goto, M., Miller, R. W., Ishikawa, Y. & Sugano, H. (1996). Excess of rare cancers in Werner syndrome (adult progeria). *Cancer Epidemiol Biomarkers Prev*, 5:239-246
- Gradt van Roggen J.F, Zonderland H.M, Welvaart K., et al (1998). Local recurrence of a phylloides tumour of the breast presenting with widespread differentiation to a telangiectatic osteosarcoma. *J Clin Pathol*, 51(9):706-708
- Greenwood S.M, & Meschter S.C (1989). Extraskelatal osteogenic sarcoma of the mediastinum. *Arch Pathol Lab Med*, 1113:430-433
- Guo W, Gorlick R, Ladanyi M, Meyers PA, Huvos AG, Bertino JR & Healey JH (1999). Expression of bone morphogenetic proteins and receptors in sarcomas. *Clin Orthop Relat Res.*, 365: 175-183.
- Haibach H., Farrell C. & Dittrich F.J (1985). Neoplasms arising in Paget's disease of bone. A study of 82 cases. *Am J Clin Pathol*, 83:594-600
- Hansen M.F, Koufos A., Gallie B.L, et al (1985). Osteosarcoma and retinoblastoma: a shared chromosomal mechanism revealing recessive predisposition. *Proc Natl Acad Sci USA*, 82:6216-6220

- Hajdu S.I (1975). Aspiration biopsy of primary malignant bone tumours. *Front Radiat Ther Oncol*, 10:73-81
- Hansen MF, Nellissery MJ & Bhatia P (1999). Common mechanisms of osteosarcoma and Paget's disease. *J Bone Miner Res*, 14 (Suppl 2): 39-44.
- Harada, K., Toyooka, S., Maitra, A., Maruyama, R., Toyooka, K. O., Timmons, C. F., Tomlinson, G. E., Mastrangelo, D., Hay, R. J., Minna, J. D. & Gazdar, A. F. (2002). Aberrant promoter methylation and silencing of the RASSF1A gene in pediatric tumors and cell lines. *Oncogene* 21, 4345-4349
- Harris S. A., R. J. Enger, B. L. Riggs & T. C. Spelsberg (1995). Development and characterization of a conditionally immortalized human fetal osteoblastic cell line. *Journal of Bone and Mineral Research*, 10(2):178-186
- Haydon R. C., A. Deyrup, A. Ishikawa et al. (2002). Cytoplasmic and/or nuclear accumulation of the β -catenin protein is a frequent event in human osteosarcoma. *International Journal of Cancer*, 102(4): 338-342
- Haydon R. C., Luu H. H. & He T. C. (2007). Osteosarcoma and osteoblastic differentiation: a new perspective on oncogenesis. *Clinical Orthopaedics and Related Research*, 454:237-246
- He B. C., L. Chen, G. W. Zuo et al. (2010). Synergistic antitumor effect of the activated PPAR γ and retinoid receptors on human osteosarcoma. *Clinical Cancer Research*, 16(8):2235-2245
- Heinsohn S., U. Evermann, U. Zur Stadt, S. Bielack, and H. Kabisch, (2007). Determination of the prognostic value of loss of heterozygosity at the retinoblastoma gene in osteosarcoma. *International Journal of Oncology*, 30(5):1205-1214
- Herens C, Thiry A, Dresse MF, Born J, Flagthier C, Vanstraelen G, Allington N & Bex V (2001). Translocation (16;17)(q22;p13) is a recurrent anomaly of aneurysmal bone cysts. *Cancer Genet Cytogenet*, 127: 83-84.
- Hickson, I. D. (2003). RecQ helicases: caretakers of the genome. *Nat Rev Cancer*, 3:169-178
- Hirakawa T., Tsuneyoshi M, Enjoji M., et al (1998). Ovarian stroma with histologic features of telangiectatic osteosarcoma of the bone. *Am J Surg Pathol*, 12(7):567-572
- Hoang, B. H., Kubo, T., Healey, J. H., Sowers, R., Mazza, B., Yang, R., Huvos, A. G., Meyers, P.A. & Gorlick, R. (2004). Expression of LDL receptor-related protein 5 (LRP5) as a novel marker for disease progression in high-grade osteosarcoma. *Int J Cancer*, 109:106-111.
- Hofbauer L. C. & A. E. Heufelder (1998). Osteoprotegerin and its cognate ligand: a new paradigm of osteoclastogenesis. *European Journal of Endocrinology*, 139(2):152-154
- Hong JH, Hwang ES, McManus MT, Amsterdam A, Tian Y, Kalmukova R, Mueller E, Benjamin T, Spiegelman BM, Sharp PA, Hopkins N & Yaffe MB.(2005). TAZ, a transcriptional modulator of mesenchymal stem cell differentiation. *Science*, 309:1074-1078
- Hoogerwerf WA, Hawkins AL, Perlman EJ & Griffin CA (1994). Chromosome analysis of nine osteosarcomas. *Genes Chromosomes Cancer*, 9: 88-92.
- Hou, P., Ji, M., Yang, B., Chen, Z., Qiu, J., Shi, X. & Lu, Z. (2006). Quantitative analysis of promoter hypermethylation in multiple genes in osteosarcoma. *Cancer* 106:1602-1609
- Hu X., A. X. Yu, B. W. Qi et al. (2010). The expression and significance of IDH1 and p53 in osteosarcom. *Journal of Experimental & Clinical Cancer Research*, 29: 43

- Husmann K., R. Muff, M. E. Bolander, G. Sarkar, W. Born & B. Fuchs (2008). Cathepsins and osteosarcoma: expression analysis identifies cathepsin K as an indicator of metastasis. *Molecular Carcinogenesis*, 47(1):66-73
- Huvos A.G (1986). Osteogenic sarcoma of bones and soft tissues in older persons. A clinicopathologic analysis of 117 patients older than 60 years. *Cancer*, 57:1442-1449
- Huvos A.G, Butler A. & Bretsky S.S (1983). Osteogenic sarcoma in the American black. *Cancer*, 52:1959-1965
- Huvos A.G, Butler A. & Bretsky S.S (1983). Osteogenic sarcoma associated with Paget's disease of bone. A clinicopathologic study of 65 cases. *Cancer*, 52:1489-1495
- Huvos A.G, Woodard H.Q, Cahan W.G, et al (1985). Postirradiation osteogenic sarcoma of bone and soft tissues. A clinicopathologic study of 66 patients. *Cancer*, 55:1244-1255
- Inwards C.Y & Unni K. (1995). Classification and grading of bone sarcomas. *Haematol Oncol Clin North Am*, 9(3):545-569
- Iwaya, K., Ogawa, H., Kuroda, M., Izumi, M., Ishida, T. & Mukai, K. (2003). Cytoplasmic and/or nuclear staining of beta-catenin is associated with lung metastasis. *Clin Exp Metastasis*, 20:525-529.
- Johnson L.C, Vetter H. & Putschar W.G.J (1962). Sarcoma arising in bone cysts. *Virchows Arch (Pathol. Anat.)*, 335:428-451
- Jones, P. A., and Baylin, S. B. (2002). The fundamental role of epigenetic events in cancer. *Nat Rev Genet*, 3:415-428
- Jung, S. T., Moon, E. S., Seo, H. Y., Kim, J. S., Kim, G. J. & Kim, Y. K. (2005). Expression and significance of TGF-beta isoform and VEGF in osteosarcoma. *Orthopedics* 28: 755-760
- Kansara, M. & Thomas, D. M. (2007). Molecular pathogenesis of osteosarcoma. *DNA Cell Biol*, 26:1-18
- Karsenty G. (2000). Bone formation and factors affecting this process. *Matrix Biol.*, 19:85-89
- Karsenty G. (2003). The complexities of skeletal biology. *Nature*, 423: 316-318
- Karsenty G & Wagner EF. (2002). Reaching a genetic and molecular understanding of skeletal development. *Dev Cell*, 2:389-406
- Kemmer H., Grass C., Siemer S., et al (2008). First case of a primary osteosarcoma of the ureter: diagnostic findings, course of disease and treatment. *Q J Med*, 101:663-665
- Khanna, C., Wan, X., Bose, S., Cassaday, R., Olomu, O., Mendoza, A., Yeung, C., Gorlick, R., Hewitt, S. M. & Helman, L. J. (2004). The membrane-cytoskeleton linker ezrin is necessary for osteosarcoma metastasis. *Nat Med* 10: 182-186
- Khanna, C., Prehn, J., Yeung, C., Caylor, J., Tsokos, M. & Helman, L. (2000). An orthotopic model of murine osteosarcoma with clonally related variants differing in pulmonary metastatic potential. *Clin Exp Metastasis*, 18:261-271
- Khatib ZA, Matsushime H, Valentine M, Shapiro DN, Sherr CJ & Look AT (1993). Coamplification of the CDK4 gene with MDM2 and GLI in human sarcomas. *Cancer Res.*, 53: 5535-5541.
- Kingsley L. A., P. G. J. Fournier, J. M. Chirgwin & T.A. Guise (2007). Molecular biology of bone metastasis. *Molecular Cancer Therapeutics*, 6(10):2609-2617
- Kinpara K. (2000). Osteoclast differentiation factor in human osteosarcoma cell line. *Journal of Immunoassay*, 21(4):327-340
- Kloen, P., Gebhardt, M. C., Perez-Atayde, A., Rosenberg, A. E., Springfield, D. S., Gold, L. I. & Mankin, H. J. (1997). Expression of transforming growth factor-beta (TGF-beta)

- isoforms in osteosarcomas: TGF-beta3 is related to disease progression. *Cancer*, 80: 2230-2239.
- Knuutila S, Autio K & Aalto Y (2000). Online access to CGH data of DNA sequence copy number changes. *Am J Pathol*, 157: 689
- Koppers B., Rakow D. & Schmidt L. (1977). Osteogenic sarcoma combined with non-ossifying fibroma in one bone. *Roentgenblaetter*, 30:261-266
- Kozlowski K., Scougall J.S & Oates R.K (1980). Osteosarcoma in a boy with Rothmund-Thomson syndrome. *Pediatr*, 10:42-45
- Kruzelock RP, Murphy EC, Strong LC, Naylor SL & Hansen MF (1997). Localization of a novel tumor suppressor locus on human chromosome 3q important in osteosarcoma tumorigenesis. *Cancer Res*, 57: 106-109.
- Kurt A.M, Unni K.K, McLeod R.A, et al (1990). Low-grade intraosseous osteosarcoma. *Cancer*, 65:1418-1428
- Kusuzaki K, Takeshita H, Murata H, Hirata M, Hashiguchi S, Ashihara T & Hirasawa Y (1999). Prognostic significance of DNA ploidy pattern in osteosarcomas in association with chemotherapy. *Cancer Lett*, 137: 27-33.
- Kyriakos M. & Hardy D. (1991). Malignant transformation of aneurismal bone cyst, with an analysis of the literature. *Cancer*, 68(8):1770-1780
- Ladanyi M, Cha C, Lewis R, Jhanwar SC, Huvos AG & Healey JH (1993). MDM2 gene amplification in metastatic osteosarcoma. *Cancer Res*, 53: 16-18
- Ladanyi M, Park CK, Lewis R, Jhanwar SC, Healey JH & Huvos AG (1993). Sporadic amplification of the MYC gene in human osteosarcomas. *Diagn Mol Pathol*, 2: 163-167
- Lau L. F. & S. C. T. Lam (1999). The CCN family of angiogenic regulators: the integrin connection. *Experimental Cell Research*, 248(1):44-57
- Laurent H-R, Rebonl J., Picot A, et al (1973). Multicentric osteogenic sarcoma. *Bordeaux Med*, 17: 2685-2694
- Lee J.S, Fetsch J.F, Wasdhal D.A, et al (1995). A review of 40 patients with extraskelatal osteosarcoma. *Cancer*, 76: 2253-2259
- Le Gall C., A. Bellahcene, E. Bonnelye et al. (2007). A cathepsin K inhibitor reduces breast cancer-induced osteolysis and skeletal tumor burden. *Cancer Research*, 67(20) : 9894-9902
- Lian JB, Stein GS, Javed A, van Wijnen AJ, Stein JL, Montecino M, Hassan MQ, Gaur T, Lengner CJ & Young DW. (2006). Networks and hubs for the transcriptional control of osteoblastogenesis. *Rev Endocr Metab Disord.*, 7:1-16
- Lidang J.M, Schumacher B., Myhre J.O, et al (1998). Extraskelatal osteosarcomas: a clinicopathologic study of 25 cases. *Am J Surg Pathol*, 22:588-594
- Logue J.P & Cairnduff F. (1991). Radiation induced extraskelatal osteosarcoma. *Br J Radiol*, 64:171-172
- Lonardo F., T. Ueda, A. G. Huvos, J. Healey & M. Ladanyi (1997). p53 and MDM2 alterations in osteosarcomas: correlation with clinicopathologic features and proliferative rate. *Cancer*, 79(8):1541-1547
- Loose J.H, El-Naggar A.K, Ro J.Y, et al (1990). Primary osteosarcoma of the lung: Report of two cases and review of the literature. *J Thorac Cardiovasc Surg*, 100:867-873
- Luo X., J. Chen, W. X. Song et al. (2008). Osteogenic BMPs promote tumor growth of human osteosarcomas that harbor differentiation defects. *Laboratory Investigation*, 88(12):1264-1277

- Luo Q., Q. Kang, W. Si et al. (2004). Connective tissue growth factor (CTGF) is regulated by Wnt and bone morphogenetic proteins signaling in osteoblast differentiation of mesenchymal stem cells. *Journal of Biological Chemistry*, 279(53):55958–55968
- Luu HH, Song WX, Luo X, Manning D, Luo J, Deng ZL, Sharff KA, Montag AG, Haydon RC & He TC. (2007). Distinct roles of bone morphogenetic proteins in osteogenic differentiation of mesenchymal stem cells. *J Orthop Res.*, 25:665–677
- Maeldandsmo GM, Berner JM, Florenes VA, Forus A, Hovig E, Fodstad O & Myklebost O (1995). Homozygous deletion frequency and expression levels of the CDKN2 gene in human sarcomas – relationship to amplification and mRNA levels of CDK4 and CCND1. *Br J Cancer*, 72: 393-398
- Malkin, D., Li, F. P., Strong, L. C., Fraumeni, J. F., Jr., Nelson, C. E., Kim, D. H., Kassel, J., Gryka, M. A., Bischoff, F. Z., Tainsky, M. A., et al. (1990). Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science*, 250: 1233-1238
- Mandahl N, Heim S, Willén H, Rydholm A, Eneroth M, Nilbert M, Kreibergs A & Mitelman F (1989). Characteristic karyotypic anomalies identify subtypes of malignant fibrous histiocytoma. *Genes Chromosomes Cancer*, 1: 9-14
- Marina N., M. Gebhardt, L. Teot & R. Gorlick (2004). Biology and therapeutic advances for pediatric osteosarcoma. *Oncologist*, 9(4):422–441
- Mark R.J, Poen J., Tran L.M, et al (1994). Postirradiation sarcomas. A single-institution study and review of the literature. *Cancer*, 73:2653-2662
- McDonald J. & Budd J.W (1943). Osteogenic sarcoma: I. A modified nomenclature and a review of 118 five-year cures. *Surg Gynecol Obstet*, 76:413-421
- McNairm J. D. K., T. A. Damron, S. K. Landas, J. L. Ambrose & A. E. Shrimpton (2001). Inheritance of osteosarcoma and Paget's disease of bone: a familial loss of heterozygosity study. *Journal of Molecular Diagnostics*, 3(4):171–177
- Menghi-Sartorio S, Mandahl N, Mertens F, Picci P & Knuutila S (2001). DNA copy number amplifications in sarcomas with homogeneously staining regions and double minutes. *Cytometry*, 46: 79-84
- Mertens F, Fletcher CD, Dal Cin P, de Wever I, Mandahl N, Mitelman F, Rosai J, Rydholm A, Sciò R, Tallini G, van den Berghe H, Vanni R & Willén H (1998). Cytogenetic analysis of 46 pleomorphic soft tissue sarcomas and correlation with morphologic and clinical features: a report of the CHAMP study group. *Genes Chromosomes Cancer*, 22: 16-25.
- Mertens H.H, Langnickel D. & Staedtler F. (1982). Primary osteogenic sarcoma of the breast. *Acta Cytol*, 26:512-516
- Mertens F, Larramendy M, Gustavsson A, Gisselsson D, Rydholm A, Brosjö 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.
- Mertens F, Mandahl N, Örndal C, Baldetorp B, Bauer HC, Rydholm A, Wiebe T, Willén H, Åkerman M, Heim S & Mitelman F (1993). Cytogenetic findings in 33 osteosarcomas. *Int J Cancer*, 55: 44-50
- Meza-Zepeda LA, Forus A, Lygren B, Dahlberg AB, Godager LH, South A, Serra M, Nezetic D, Tarkkanen M, Knuutila S & Myklebost O (2002). Positional cloning identifies a novel cyclophilin as a candidate amplified oncogene in 1q21. *Oncogene*, 21: 2261-2269

- Micolonghi T.S, Liang D. & Schwarz S. (1984). Primary osteosarcoma of the kidney. *J Urol*, 131:1164-1166
- Miller, C. W., Yeon, C., Aslo, A., Mendoza, S., Aytac, U. & Koeffler, H. P. (1997). The p19INK4D cyclin dependent kinase inhibitor gene is altered in osteosarcoma. *Oncogene*, 15:231-235
- Mirra J.M, Bullough P.G, Marcove R.C, et al (1974). Malignant fibrous histiocytoma and osteosarcoma in association with bone infarcts. Report of four cases, two in caisson workers. *J Bone Joint Surg (Am)*, 56:932-940
- Mohamed AN, Zalupski MM, Ryan JR, Koppitch F, Balcerzak S, Kempf R & Wolman SR (1997). Cytogenetic aberrations and DNA ploidy in soft tissue sarcoma. A Southwest Oncology Group Study. *Cancer Genet Cytogenet*, 99: 45-53
- Momand J, Zambetti GP, Olson DC, George D & Levine AJ (1992). The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. *Cell*, 69: 1237-1245
- Nakanishi H, Tomita Y, Myoui A, Yoshikawa H, Sakai K, Kato Y, Ochi T & Aozasa K (1998). Mutation of the p53 gene in postradiation sarcoma. *Lab Invest*, 78: 727-733.
- Nakashima K, Zhou X, Kunkel G, Zhang Z, Deng JM, Behringer RR & de Crombrughe B. (2002). The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. *Cell*, 108:17-29
- Nellisery MJ, Padalecki SS, Brkanac Z, Singer FR, Roodman GD, Unni KK, Leach RJ & Hansen MF (1998). Evidence for a novel osteosarcoma tumor-suppressor gene in the chromosome 18 region genetically linked with Paget disease of bone. *Am J Hum Genet*, 63: 817-824
- Nikol K.K, Ward W.G, Savage P.D, et al (1998). Fine-needle aspiration biopsy of skeletal versus extraskelatal osteosarcoma. *Cancer (Cancer Cytopathol)*, 84:176-185
- Nishio, Y., Dong, Y., Paris, M., O'Keefe, R. J., Schwarz, E. M. & Drissi, H. (2006). Runx2-mediated regulation of the zinc finger Osterix/Sp7 gene. *Gene*, 372: 62-70
- Nitzsche E.U, Seeger L.L, Klosa B., et al (1992). Primary osteosarcoma of the thyroid gland. *J Nucl Med*, 33:1399-1401
- Ogasawara, T., Kawaguchi, H., Jinno, S., Hoshi, K., Itaka, K., Takato, T., Nakamura, K. & Okayama, H. (2004). Bone morphogenetic protein 2-induced osteoblast differentiation requires Smad-mediated down-regulation of Cdk6. *Mol Cell Biol*, 24: 6560-6568
- Okada K., Frassica F.J, Sim F.H, et al (1994). Parosteal osteosarcoma. A clinicopathological study. *J Bone Joint Surg Am*, 76:366-378
- Oliner JD, Kinzler KW, Meltzer PS, George DL & Vogelstein B (1992). Amplification of a gene encoding a p53-associated protein in human sarcomas. *Nature*, 358: 80-83
- Örndal C, Mandahl N, Rydholm A, Willén H, Brosjö O & Mitelman F (1993). Chromosome aberrations and cytogenetic intratumor heterogeneity in chondrosarcomas. *J Cancer Res Clin Oncol*, 120: 51-56
- Panoutsakopoulos G, Pandis N, Kyriazoglou I, Gustafson P, Mertens F & Mandahl N (1999). Recurrent t(16;17)(q22;p13) in aneurysmal bone cysts. *Genes Chromosomes Cancer*, 26: 265-266
- Park IC, Lee SY, Jeon DG, Lee JS, Hwang CS, Hwang BG, Lee SH, Hong WS, Hong SI (1996). Enhanced expression of cathepsin L in metastatic bone tumors. *J Korean Med Sci*, 11: 144-148

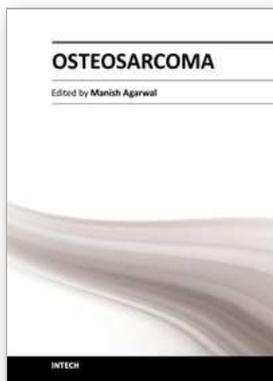
- Park Y. B., H. S. Kim, J. H. Oh & S. H. Lee (2001). The co-expression of p53 protein and P-glycoprotein is correlated to a poor prognosis in osteosarcoma. *International Orthopaedics*, 24(6):307-310
- Park H. R., W. Won Jung, F. Bertoni et al. (2004). Molecular analysis of p53, MDM2 and H-ras genes in low-grade central osteosarcoma. *Pathology Research and Practice*, 200(6):439-445
- Perbal B., M. Zuntini, D. Zambelli et al. (2008). Prognostic value of CCN3 in osteosarcoma. *Clinical Cancer Research*, 14(3):701-709
- Piscioli F., Govoni E., Polla E., et al (1985). Primary osteosarcoma of the uterine corpus: Report of a case and a critical review of the literature. *Int J Gynaecol Obstet*, 23:377-385
- Quinn J. M. W., Itoh K., Udagawa N. et al. (2001). Transforming growth factor β affects osteoclast differentiation via direct and indirect actions. *Journal of Bone and Mineral Research*, 16(10):1787-1794
- Radhi J.M, Loewy J. (1999). Dedifferentiated chondrosarcoma with features of telangiectatic osteosarcoma. *Pathology*, 31(4):428-430
- Radig K, Schneider-Stock R, Mittler U, Neumann HW & Roessner A (1998). Genetic instability in osteoblastic tumors of the skeletal system. *Pathol Res Pract*, 194: 669-677
- Ragazzini P, Gamberi G, Benassi MS, Orlando C, Sestini R, Ferrari C, Molendini L, Sollazzo MR, Merli M, Magagnoli G, Bertoni F, Böhlting T, Pazzagli M & Picci P (1999). Analysis of SAS gene and CDK4 and MDM2 proteins in low-grade osteosarcoma. *Cancer Detect Prev*, 23: 129-136
- Rao-Bindal K. & Kleinerman E.S. (2011). Epigenetic Regulation of Apoptosis and Cell Cycle in Osteosarcoma. *Sarcoma*, 2011, ID article 679457, 5 pages
- Reya T & Clevers H. (2005). Wnt signalling in stem cells and cancer. *Nature*, 434:843-850
- Ren B., K. O. Yee, J. Lawler & R. Khosravi-Far (2006). Regulation of tumor angiogenesis by thrombospondin-1. *Biochimica et Biophysica Acta*, 1765(2):178-188
- Rikhof B., S. De Jong, A. J.H. Suurmeijer, C.Meijer & W. T. A. van der Graaf (2009). The insulin-like growth factor system and sarcomas. *Journal of Pathology*, 217(4):469-482
- Roberts WM, Douglass EC, Peiper SC, Houghton PJ & Look AT (1989). Amplification of the gli gene in childhood sarcomas. *Cancer Res*, 49: 5407-5413
- Rong S, Jeffers M, Resau JH, Tsarfaty I, Oskarsson M & Vande Woude GF (1993). Met expression and sarcoma tumorigenicity. *Cancer Res*, 53: 5355-5360
- Ruggieri P., Sim F.H, Band J.R, et al (1995). Osteosarcoma in a patient with polyostotic fibrous dysplasia and Albright's syndrome. *Orthopedics*, 18:71-75
- Sadikovic B., M. Yoshimoto, S. Chilton-MacNeill, P. Thorner, J. A. Squire & M. Zielenska (2009). Identification of interactive networks of gene expression associated with osteosarcoma oncogenesis by integrated molecular profiling. *Human Molecular Genetics*, 18(11):1962-1975
- Schajowicz F., Santini A.E & Berenstein M. (1983). Sarcoma complicating Paget's disease of bone. A clinicopathological study of 62 cases. *J Bone Joint Surg Br*, 65:299-307
- Schweitzer G. & Gand Pirie D. (1971). Osteosarcoma arising in a solitary osteochondroma. *S Afr Med J*, 45:810-811
- Selvarajah S, Yoshimoto M, Ludkovski O, Park PC, Bayani J, Thorner P, Maire G, Squire JA & Zielenska M. (2008). Genomic signatures of chromosomal instability and osteosarcoma progression detected by high resolution array CGH and interphase FISH. *Cytogenet Genome Res.*, 122:5-15

- Serra M., Morini M.C, Scotlandi K., et al (1992). Evaluation of osteonectin as a diagnostic marker of osteogenic bone tumours. *Hum Pathol*, 23:1326-1331
- Shimizu T., T. Ishikawa, E. Sugihara et al. (2010). c-MYC overexpression with loss of Ink4a/Arf transforms bone marrow stromal cells into osteosarcoma accompanied by loss of adipogenesis. *Oncogene*, 29(42):5687-5699
- Shui-Xiang Tao, Guo-Quin Tian, Meng-Hua Ge, et al (2011). Primary extraskkeletal osteosarcoma of omentum majus. *World J of Surg Oncol*, 9:25-27
- Silver SA & Tavassolli FA (1980). Primary osteogenic sarcoma of the breast: A clinicopathologic analysis of 50 cases. *Am J Surg Pathol*, 22:925-933
- Sinovic JF, Bridge Jab & Neff JR (1992). Ring chromosome in parosteal osteosarcoma. Clinical and diagnostic significance. *Cancer Genet Cytogenet*, 62: 50-52
- Siu F.H, Unni K.K, Beabout J.W, et al (1979). Osteosarcoma with small cells simulating Ewing's tumor. *J Bone Joint Surg (Am)*, 61:201-215
- Smida J., Baumhoer D., Rosemann M. et al. (2010). Genomic alterations and allelic imbalances are strong prognostic predictors in osteosarcoma. *Clinical Cancer Research*, 16(16):4256-4267
- Smith G.D, Chalmer J. & McQueen M.M (1986). Osteosarcoma arising in relation to an echondroma. A report of three cases. *J Bone Joint Surg (Br)*, 68:315-319
- Smith SH, Weiss SW, Jankowski SA, Coccia MA & Meltzer PS (1992). SAS amplification in soft tissue sarcomas. *Cancer Res*, 52: 3746-3749
- Stoch S. A. & Wagner J. A (2008). Cathepsin K inhibitors: a novel target for osteoporosis therapy. *Clinical Pharmacology and Therapeutics*, 83(1):172-176
- 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
- Soloviev, Yu. N (1969). On the relationship between the rate of skeleton growth and occurrence of primary osteogenic sarcoma. *Vopr Onkol*, 15(5):3-7
- Sordillo P.P, Hadju S.I, Magill G.B, et al (1983). Extraosseous osteogenic sarcoma. A review of 48 patients. *Cancer*, 51:727-734
- Spyra S. (1976). Osteogenic sarcoma in chronic osteitis. *Chir Narzadow Ruchu Ortop Pol*, 41:99-100
- Stormby N. & Akerman M (1973). Cytodiagnosis of bone lesions by means of fine needle aspiration biopsy. *Acta Cytol*, 17:166-172
- Sudo T, Kuramoto T, Komiya S, Inoue A & Itoh K (1997). Expression of MAGE genes in osteosarcoma. *J Orthop Res*, 15: 128-132
- Szymanska J, Mandahl N, Mertens F, Tarkkanen M, Karaharju E & Knuutila S (1996). Ring chromosomes in parosteal osteosarcoma contain sequences from 12q13-15: a combined cytogenetic and comparative genomic hybridization study. *Genes Chromosomes Cancer*, 16: 31-34
- Ta H. T., Dass C. R., Choong P. F. M. & Dunstan D. E. (2009). Osteosarcoma treatment: state of the art. *Cancer and Metastasis Reviews*, 28(1-2):247-263
- Takada J., Ishii S., Ohta T., et al (1992). Usefulness of a novel monoclonal antibody against human osteocalcin in immunohistochemical diagnosis. *Virchows Arch A*, 420:507-511
- Takayanagi H. (2007). The role of NFAT in osteoclast formation. *Annals of the New York Academy of Sciences*, 1116: 227-237
- Tang, N., Song, W. X., Luo, J., Haydon, R. C. & He, T. C. (2008). Osteosarcoma development and stem cell differentiation. *Clin Orthop Relat Res*, 466: 2114-2130

- Tarkkanen M, Aaltonen LA, Böhling T, Kivioja A, Karaharju E, Elomaa I & Knuutila S (1996). No evidence of microsatellite instability in bone tumours. *Br J Cancer*, 74: 453-455
- Tarkkanen M, Böhling T, Gamberi G, Ragazzini P, Benassi MS, Kivioja A, Kallio P, Elomaa I, Picci P & Knuutila S (1998). Comparative genomic hybridization of low grade central osteosarcoma. *Mod Pathol*, 11: 421-426
- Tarkkanen M, Elomaa I, Blomqvist C, Kivioja AH, Kellokumpu-Lehtinen P, Böhling T, Valle J & Knuutila S (1999). DNA sequence copy number increase at 8q: a potential new prognostic marker in high grade osteosarcoma. *Int J Cancer*, 84: 114-121
- Tarkkanen M, Kaipainen A, Karaharju E, Böhling T, Szymanska J, Helio H, Kivioja A, Elomaa I & Knuutila S (1993). Cytogenetic study of 249 consecutive patients examined for a bone tumor. *Cancer Genet Cytogenet*, 68:1-21
- Tarkkanen M, Karhu R, Kallioniemi A, Elomaa I, Kivioja AH, Nevalainen J, Böhling T, Karaharju E, Hyytinen E, Knuutila S & Kalioniemi OP (1995). Gains and losses of DNA sequences in osteosarcomas by comparative genomic hybridization. *Cancer Res*, 55: 1334-1338
- Tarkkanen M, Wiklund TA, Virolainen MJ, Larramendy M, Mandahl N, Mertens F, Blomqvist C, Tukiainen E, Miettinen M, Elomaa I & Knuutila S (2001). Comparative genomic hybridization of postirradiation sarcomas. *Cancer*, 92: 1992-1998
- Thomas, D. M., Carty, S. A., Piscopo, D. M., Lee, J. S., Wang, W. F., Forrester, W. C. & Hinds, P. W. (2001). The retinoblastoma protein acts as a transcriptional coactivator required for osteogenic differentiation. *Mol Cell*, 8: 303-316.
- Thomas D. M., Johnson S. A., Sims N. A. et al. (2004). Terminal osteoblast differentiation, mediated by runx2 and p27, is disrupted in osteosarcoma. *Journal of Cell Biology*, 167(5):925-934
- Thomas, D. & Kansara, M. (2006). Epigenetic modifications in osteogenic differentiation and transformation. *J Cell Biochem*, 98: 757-769
- Trihia H., Valavanis C., Markidou S., et al (2007). Primary osteogenic sarcoma of the breast. Cytomorphologic study of three cases and histologic correlation. *Acta Cytologica*, 51(3): 443-450
- Trowell J.E & Arkell D.G (1976). Osteosarcoma of the thyroid gland. Case report. *J Pathol*, 119:123-127
- Tsuchiya, T., Sekine, K., Hinohara, S., Namiki, T., Nobori, T. & Kaneko, Y. (2000). Analysis of the p16INK4, p14ARF, p15, TP53, and MDM2 genes and their prognostic implications in osteosarcoma and Ewing sarcoma. *Cancer Genet Cytogenet*, 120:91-98.
- Tsuneyoshi M. & Dorfman H.D (1987). Epiphyseal osteosarcoma: distinguishing features from clear cell chondrosarcoma, chondroblastoma and epiphyseal echondroma. *Hum Pathol*, 18:664-651
- Uchibori M, Nishida Y, Nagasaka T, Yamada Y, Nakanishi K & Ishiguro N. (2006). Increased expression of membrane-type matrix metalloproteinase-1 is correlated with poor prognosis in patients with osteosarcoma. *Int J Oncol*. 28:33-42.
- Unni K.K, Dahlin D.C & Beabout J.W (1976). Periosteal osteosarcoma. *Cancer*, 37:2476-2485
- Unni K.K, Dahlin D.C, McLeod R.A, et al (1977). Intra-osseous well-differentiated osteosarcoma. *Cancer*, 40:1337-1347
- Vigorita V. J. (2008). *Orthopaedic Pathology*, Lippincott, Williams & Wilkins, Philadelphia, Pa, USA

- Wadayama B, Toguchida J, Shimizu T, Ishizaki K, Sasaki MS, Kotoura Y, & Yamamuro T (1994). Mutation spectrum of the retinoblastoma gene in osteosarcomas. *Cancer Res*, 54: 3042-3048
- Wajed, S. A., Laird, P. W. & DeMeester, T. R. (2001). DNA methylation: an alternative pathway to cancer. *Ann Surg*, 234:10-20
- Walaas L. & Kindblom L-G (1990). Light and electron microscopic examinations of fine needle aspirates in the preoperative diagnosis of osteogenic tumours. *Diagn Cytopath*, 6:27-38
- Wang, L. L. (2005). Biology of osteogenic sarcoma. *Cancer J*, 11:294-305
- Wang, L.L., Gannavarapu, A., Kozinetz, C.A., Levy, M.L., Lewis, R.A., Chintagumpala, M. M., Ruiz-Maldonado, R., Contreras-Ruiz, J., Cunniff, C., Erickson, R. P., et al. (2003). Association between osteosarcoma and deleterious mutations in the RECQL4 gene in Rothmund-Thomson syndrome. *J Natl Cancer Inst*, 95:669-674
- Wang, L. L., Levy M. L., Lewis, R. A., Chintagumpala, M. M., Lev, D., Rogers, M. & Plon, S.E. (2001). Clinical manifestations in a cohort of 41 Rothmund-Thomson syndrome patients. *Am J Med Genet*, 102:11-17
- Watt A.C, Hagggar A.M & Krasicky G.A (1984). Extraosseous osteogenic sarcoma of the breast. Mammographic and pathologic findings. *Radiology*, 150:34
- Wegner R.E, McGrath K.M, Luketoch J.D, et al (2010). Extraosseous osteosarcoma of the esophagus: a case report. *Sarcoma*, vol 2010, article ID 907127, 3 pages
- Werner M, Rieck J & Delling G. (1998). Cytogenetic changes in low grade central osteosarcomas. *Verh Dtsch Ges Pathol.*, 82:189-94
- White V.A, Fanning C.V, Ayala A, et al (1988). Osteosarcoma and the role of fine-needle aspiration: a study of 51 cases. *Cancer*, 62:1238-1246
- Wick M.R, Siegal G.P, Unni K.K, et al (1981). Sarcoma of bone complicating osteitis deformans (Paget' disease): fifty years' experience. *Am J Surg Pathol*, 5:47-59
- Wilimas J., Barrett G. & Pratt C. (1977). Osteosarcoma in two very young children. *Clin Pediatr*, 16:548-551
- Wines A., Bonar F. & Lam P. (2000). Telangiectatic differentiation of a parosteal osteosarcoma. *Skeletal Radiol.*, 29(10):597-600
- Wold L.E, Unni K.K & Beabout J.W, et al (1984). Dedifferentiated parosteal osteosarcoma. *J Bone Joint Surg Am*, 66:53-59
- World Health Organization Classification of Tumours. *Pathology and Genetics of Tumours of Soft Tissue and Bone*. Lyon: IARC Press: 2002
- Wu J. X., Carpenter P. M., Gresens C. et al. (1990). The protooncogene c-fos is over-expressed in the majority of human osteosarcomas. *Oncogene*, 5(7):989-1000
- Wuisman P., Roessner A., Bosse A., et al (1992). Osteonectin in osteosarcomas: A marker of differential diagnosis and/or prognosis? *Ann Oncol*, 3(2):533-535
- Wunder JS, Czitrom AA, Kandel R & Andrulis IL (1991). Analysis of alterations in the retinoblastoma gene and tumor grade in bone and soft-tissue sarcomas. *J Natl Cancer Inst*, 83: 194-200
- Wunder JS, Eppert K, Burrow SR, Gokgoz N, Bell RS, Andrulis IL & Gogkoz N (1999). Co-amplification and overexpression of CDK4, SAS and MDM2 occurs frequently in human parosteal osteosarcomas. *Oncogene*, 18: 783-788

- Wyatt-Ashmead J, Bao L, Eilert RE, Gibbs P, Glancy G & McGavran L (2001). Primary aneurysmal bone cysts: 16q22 and/or 17p13 chromosome abnormalities. *Pediatr Dev Pathol*, 4: 418-419
- Yamaguchi A, Komori T & Suda T. (2000). Regulation of osteoblast differentiation mediated by bone morphogenetic proteins, hedgehogs, and Cbfa1. *Endocr Rev.*, 21:393-411
- Yamamura H., Yashikawa H., Tatsuta M., et al (1998). Expression of the smooth muscle calponin gene in human osteosarcoma and its possible association with prognosis. *Int J Cancer*, 79:245-250
- Yang X & Karsenty G. (2004). ATF4, the osteoblast accumulation of which is determined post-translationally, can induce osteoblast specific gene expression in non-osteoblastic cells. *J Biol Chem.*, 279:47109-47114
- Yang J, Yang D, Cogdell D, Du X, Li H, Pang Y, Sun Y, Hu L, Sun B, Trent J, Chen K & Zhang W. (2010). APEX1 gene amplification and its protein overexpression in osteosarcoma: correlation with recurrence, metastasis, and survival. *Technol Cancer Res Treat.*, 9(2):161-9.
- Yoshikawa, H., Nakase, T., Myoui, A., & Ueda, T. (2004). Bone morphogenetic proteins in bone tumors. *J Orthop Sci* 9:334-340
- Yotov WV, Hamel H, Rivard GE, Champagne MA, Russo PA, Leclerc JM, Bernstein ML & Levy E (1999). Amplifications of DNA primase 1 (PRIM1) in human osteosarcoma. *Genes Chromosomes Cancer*, 26: 62-69
- Young R.H & Rosenberg A.E (1987). Osteosarcoma of the urinary bladder: Report of a case and review of the literature. *Cancer*, 59:174-178



Osteosarcoma

Edited by Dr. Manish Agarwal

ISBN 978-953-51-0506-0

Hard cover, 174 pages

Publisher InTech

Published online 11, April, 2012

Published in print edition April, 2012

This book is aimed at quickly updating the reader on osteosarcoma, a dreaded primary bone cancer. Progress in management of osteosarcoma has been slow after the evolution of chemotherapy and limb salvage surgery. Research is now directed towards identifying molecular targets for systemic therapy. Availability of chemotherapy drugs and low cost implants in developing world have allowed limb salvage surgery to develop. This book looks at current basic knowledge on osteosarcoma and some of the developments in research which have the potential to change the prognosis.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Helen Trihia and Christos Valavanis (2012). Histopathology and Molecular Pathology of Bone and Extraskelatal Osteosarcomas, Osteosarcoma, Dr. Manish Agarwal (Ed.), ISBN: 978-953-51-0506-0, InTech, Available from: <http://www.intechopen.com/books/osteosarcoma/histopathology-and-molecular-pathology-of-bone-and-extraskelatal-osteosarcomas>

INTECH

open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821