Chapter 1

General Introduction
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1.1 Myxoid tumours of soft tissue

1.1.1. Classification and grading of soft tissue tumours
Uniform annotation of bone-, and soft tissue tumours is performed on the basis of the international consensus guidelines of the World Health Organization (12). Hereby, soft tissue tumours are classified according to their cell type of differentiation, e.g. adipocytic, fibroblastic, pericytic or vascular. This concept is only partial true for bone tumours which main entities are basically classified according to the dominant pattern of extracellular matrix (ECM) formation on microscopy, i.e. cartilage or osteoid. In this respect myxoid tumours of soft tissue are not a well-defined group as such in the WHO classification and rather a historically based "hybrid" concept of soft tissue tumours named after the predominant microscopy of their ECM. On the basis of their biological potential, soft tissue tumours are divided into the following four categories: (1) benign, (2) intermediate (locally aggressive), (3) intermediate (rarely metastasizing) or (4) malignant. In case of malignancy, a histological grade should be provided aiming at predicting the level of aggressiveness of the tumour (23). The world wide standard grading occurs according to the (modified) criteria of the Fédération Nationale des Centres de Lutte Contre le Cancer (25).

1.1.2. Definition of myxoid tumours of soft tissue
Myxoid tumours of soft tissue are mesenchymal tumours characterized by the presence of abundant so-called "myxoid" extracellular matrix (ECM) at microscopy (24). The term "myxoid" was first used by Rudolf Virchow to describe tumours that histologically resembled the structure of the umbilical cord, referring to the substance of Wharton's jelly (figure 1) (69). Myxoid/mucoid appearance of the ECM on light microscopy can be a feature of a variety of both epithelial and mesenchymal tumours (75). In myxoid tumours of soft tissue this myxoid ECM is intrinsic to the entity though often not directional for diagnosis: this group comprises a broad spectrum of tumours with overlapping histology but different clinical behavior ranging from truly benign to frankly malignant warranting adequate recognition of the entities (24, 75).

1.1.3. Challenges in differential diagnosis
The differential diagnosis of myxoid tumours of soft tissue can be very challenging especially in biopsies because of significant histological overlap between the different entities at the light microscopical level (75). As for all (soft tissue) tumours, integration of microscopy with microscopy including immunohistochemistry as well as extensive additional clinical and radiological data is essential to render the correct diagnosis. Moreover, during the recent years, many sarcomas have been shown to harbour tumour-specific genetic alterations which do not only give insight into their biology; they also provide helpful tools in differential diagnosis and treatment (table 1) (13, 23). Myxofibrosarcoma and intramuscular myxoma are relatively common soft tissue
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Figure 1: Characteristic macroscopy and histomorphology of the myxoid ECM.

Rudolph Virchow introduced the term myxoma for those tumours morphologically resembling Wharton's jelly of the umbilical cord (a), which contains large amounts of GAGs as detected by Alcian Blue (b). High-power image of Wharton's jelly showing abundant myxoid ECM containing fibrillary collagens, interspersed between myofibroblast-like stroma cells (c). Intramuscular myxoma characteristically has a gelatinous appearance on cut surface (d) and is well circumscribed towards its peripheral tissue (e). On higher magnification, it shows the same abundant myxoid ECM as the umbilical cord (c) and no significant atypia of the sparse tumour cells (f). Histological criteria are still a hallmark of diagnosis, showing characteristic lobulated, hypocellular morphology of grade I myxofibrosarcoma at low magnification (g). Curvilinear blood vessels are quite specific for grade I myxofibrosarcoma (but are not diagnostic), whereas tumour cells show vesicular, slightly atypical nuclei compared to intramuscular myxoma (h). Another hallmark of myxofibrosarcoma is areas with abrupt transition of grade (i) which was already mentioned by Mentzel et al. (41).

tumours, usually occurring in the extremities of adult or older patients. Accurate diagnosis and grading is crucial for the decision on adjuvant therapy. According to the 2010 ESMO guidelines adjuvant radiation therapy is a standard for soft tissue tumours of high-grade deep seated tumours regardless of diameter (< or > 5 cm). Radiation therapy is added in selected cases of low-grade, superficial, >5 cm, and low-grade, deep, <5 cm soft tissue tumours. In the case of low-grade, deep, >5 cm soft tissue sarcoma, radiation therapy is recommended to be discussed in a multidisciplinary fashion. Radiation therapy is also recommendatory following marginal or R1-R2
excisions, if these cannot be rescued through re-excision (11). Compartmental resection of an intracompartmental tumour, does not require adjuvant radiation therapy. Adjunctive chemotherapy is not standard treatment in adult-type soft tissue sarcomas, although it is proposed by some as an option in high-risk patients with tumours of intermediate- or high grade, deep seated and >5 cm) (51). Some histological types are more chemosensitive and the histotype may therefore be considered in the decision-making (11). Accurate diagnosis is thus essential warranting the need of additional diagnostic tools with high specificity, both for assessment of prognosis as well as for tailoring therapy and metastatic disease.

Table 1: Clinicopathological and (cyto) genetic characteristics of myxoid tumours of soft tissue
1.2 Molecular genetics and cytogenetics of myxoid tumours of soft tissue

Based on molecular genetics and cytogenetics, sarcomas can be divided into two major groups: (a) sarcomas with relatively "simple" karyotypes showing specific genetic alterations (such as balanced translocations) with the formation of tumour specific fusion genes (table 2), or specific genetic mutations (often in proto-oncogenes), and (b) sarcomas with non-specific gene alterations and very complex karyotypes with structural and numerical aberrations (49). Overlap exists between groups (a) and (b) with sometimes additional/secondary complex karyotypic aberrations superimposed upon initial specific driver mutations (e.g. gastrointestinal stromal tumours (GIST)) (58).

Table 2: Molecular genetics and cytogenetics of myxoid tumours of soft tissue

<table>
<thead>
<tr>
<th>Sarcoma</th>
<th>Abnormality</th>
<th>Molecular genetic consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myxoid liposarcoma</td>
<td>Ring chromosome/giant marker</td>
<td>Amplification of, e.g., MYC, CCND1, HMGA2, GLI and SAS</td>
</tr>
<tr>
<td>Myxoid liposarcoma</td>
<td>t(12;16)</td>
<td>FUS-DDIT3</td>
</tr>
<tr>
<td>Solitary fibrous tumour of meningotheal origin</td>
<td>t(12;22)</td>
<td>EWS-FLI1</td>
</tr>
<tr>
<td>Inflammatory myofibroblastic tumor</td>
<td>t(2;13)</td>
<td>TPM3-ALK</td>
</tr>
<tr>
<td>Soft tissue sarcoma</td>
<td>t(11;19)</td>
<td>Unknown</td>
</tr>
<tr>
<td>Intermediate fibrosarcoma</td>
<td>t(12;15)</td>
<td>ETVB6-NF9K3</td>
</tr>
<tr>
<td>Myxoid inflammatory fibroblastic sarcoma</td>
<td>t(13;24)</td>
<td>Deactivation of FGFR1 and NF123</td>
</tr>
<tr>
<td>Low-grade fibromyxoid sarcoma</td>
<td>t(7;16)</td>
<td>FUS-CREB12</td>
</tr>
<tr>
<td>Dermatofibrosarcoma protuberans</td>
<td>t(11;14)</td>
<td>FUS-CREB1</td>
</tr>
<tr>
<td>Pleomorphic liposarcoma</td>
<td>t(11;13)</td>
<td>FUS-CREB1</td>
</tr>
<tr>
<td>Angiomyxoid fibrous histiocytoma</td>
<td>t(2;12)</td>
<td>EWS1-ATF1</td>
</tr>
<tr>
<td>Synovial sarcoma</td>
<td>t(2;8)</td>
<td>FUS-ATF1</td>
</tr>
<tr>
<td>Clear cell sarcoma</td>
<td>t(2;13)</td>
<td>FUS-ATF1</td>
</tr>
<tr>
<td>Liposarcoma</td>
<td>t(12;16)</td>
<td>FUS-ATF1</td>
</tr>
<tr>
<td>Extraskeletal myxoid chondrosarcoma</td>
<td>t(2;3)</td>
<td>FUS-ATF1</td>
</tr>
<tr>
<td>Axillary soft part sarcoma</td>
<td>t(4;17)</td>
<td>FUS-ATF1</td>
</tr>
</tbody>
</table>
**Figure 2:** Activating mutations in GNAS1 lead to constitutive activation of protein kinase A

G-proteins transmiss signals from activated seven transmembrane spanning receptors to intracellular effectors, e.g. adenylate cyclase. Activation of adenylate cyclase converts ATP to cAMP which subsequently activates protein kinase A and cAMP responsive genes. In the inactive state, the G-protein is a αβγ heterotrimer with the α subunit bound to guanosine triphosphate (GTP). Binding of GTP leads to a conformational change of the α-subunit which then dissociates from the complexed βγ dimer and increased affinity for the receptor and the intracellular effector. Hydrolysis of GTP to guanosine diphosphate by the intrinsic GTPase of the G-protein leads to the re-formation of the heterotrimeric complex and subsequent ending of the activation signal. Activating mutations in codon 201 or codon 227 reduce the α subunit’s GTPase activity. Hereby it prevents hydrolysis of the GTP bound to the α subunit and causes consecutive activation of adenylate cyclase and downstream cell signaling. Adapted from: Lania AG et al (36).

protein leads to the temporarily activation of cell signaling, which stops at dephosphorylation. The responsible phosphorylase which can be an intrinsic part of the protein, thereby acts as an “on-off” switch in cell signaling and transduces extracellular signals via ligand-receptor binding to a downstream target (31, 54). Activating mutations occur mostly in the binding pocket of the phosphorylase and thereby block dephosphorylation of the protein (figure 2). This results in constitutive cell signaling and continuous stimulation of pathways involved in cell growth. Activating mutations in sarcomas are exemplary (e.g. KIT and PDGFR in GIST) and are also found in mesenchymal tumours-related syndromes (9, 18, 68). Somatic and germline mutations in GNAS1 gene have been described in fibrous dysplasia, both in isolated lesions as well patients suffering Mazabraud syndrome (mono/polyostotic fibrous dysplasia and...
intramuscular myxoma) and McCune-Albright syndrome (cafe-au-lait spots, precocious puberty and fibrous dysplasia) (71). This leads to downstream activation of cFos which acts as a transcription factor (10). Activating mutations in codon 12/13 of KRAS also lead to downstream activation of c-Fos. KRAS-activating mutations have been described in both mouse and human sarcomas. Kirsch et al. showed that KRAS and TP53 mutations were sufficient to initiate high-grade sarcomas with myofibroblastic features in mice (33). P53 is a major cellular gatekeeper for cell growth and division (37). Inactivating TP53 mutations are relatively common in sarcomas with nonspecific genetic aberrations compared with sarcomas with reciprocal specific translocations (8). This was sustained by previously published data that p53 immunohistochemical staining was predominantly found in myxofibrosarcoma of grade II and III harboring non-specific cytogenetic aberrations compared to grade I tumours which have less aberrant, sometimes normal karyotypes (47, 74). Another important gene involved in cell cycle regulation is P16. Inactivation of P16 (either by promoter hypermethylation, inactivating mutations or deletions) has been extensively described in many sarcomas (43, 45, 61). Significant reduction in p16 expression has been found in the (more aggressive and therefore grade determining) round cell component of myxoid liposarcoma and is partly due to promoter hypermethylation and mutation (48). Also in myxofibrosarcoma, reduced p16 expression correlates with worse prognosis (47), suggesting that p16 might play an important role in tumour progression in these tumours.

1.2.2. Balanced translocations
Balanced translocations have been described in both benign and malignant tumours, especially in hematological malignancies and sarcomas, and are increasingly recognized in epithelial tumours (5, 42, 44, 52, 67). Though the involved genes are often (but not always!) tumour specific, their fusion partners are mostly restricted to a certain group of genes. For example, EWSR1 (the Ewing sarcoma breakpoint region 1, a.k.a. EWS) is not only translocated in Ewing sarcoma (17, 59), but also in desmoplastic small round cell tumour (35), clear cell sarcoma (20) angiomatoid fibrous histiocytoma (3, 60), extraskeletal myxoid chondrosarcoma (66), and a small subset of myxoid liposarcoma (50). Interestingly, one and the same gene can be translocated in both epithelial and mesenchymal tumours, such as the Xp11.2 gene, coding for tfe3 which is translocated in both paediatric renal cell carcinoma and alveolar soft part sarcoma (30, 72). Balanced translocations can drive tumorigenesis by different mechanisms. First, the transcribed fusion protein can act as a kinase or transcription factor and thereby activate transcription of genes and proteins involved in cell cycle, growth, angiogenesis etc (42). Hereby, they do not only play a role in tumour proliferation but sporadically also in driving tumour morphology such as FUS/DDIT3 in myxoid liposarcoma (56, 57). Secondly, balanced translocations can cause a promoter swap in which one gene involved in the translocation is placed under the transcriptional control of the promoter of an other (highly transcribed) gene. For example, in (myxoid)
dermatofibrosarcoma protuberans, the COL1A1-PDGFB fusion leads to PDGF overexpression, increased autocrine stimulation and subsequent cell proliferation (46, 64).

1.2.3. Gene specific amplification.
Next to non-specific randomly occurring gene amplifications, some sarcomas are characterized by gene specific amplifications, such as of CDK4 and MDM2. These amplifications are (not exclusively) present in the majority of well- and dedifferentiated liposarcoma and believed to play a role in their genesis (15, 29). Detection of these amplifications by FISH or their transcribed proteins by immunohistochemistry can be used in their differential diagnosis (29).

1.2.4. Non-specific karyotypic aberrations
The more frequent occurring sarcomas show non-specific numerical and structural cytogenetic changes which are the reflection of genetic instability (13). These complex karyotypic aberrations increase upon tumour progression suggesting a multistep tumour progression model and are often associated with functional and/or structural loss of genes involved in guarding the genome, such as TP53 or RB (40, 62). Superimposed, often non-specific karyotypic aberrations such as observed in myxofibrosarcoma and osteosarcoma can also be seen in translocation-driven sarcomas (16, 43, 47).

1.2.5. Hereditary syndromes involving the occurrence of mesenchymal tumours
During the recent years, an increased number of (Mendelian) inherited sarcoma-related syndromes has been reported. Subsequent molecular-genetic knowledge of genes predisposing to these syndromes, provide not only insight into their genetic pathways; they also serve as a solid basis for genetic counseling. Some of these relatively frequent syndromes are associated with mesenchymal and epithelial neoplasms, both benign and malignant. A not exhaustive list is summarized in table 3 and includes more general cancer syndromes such as Li Fraumeni-, and Retinoblastoma syndrome, caused by mutations in tumour suppressor genes involved in cell cycle check point regulation (such as TP53 and RB). Interestingly, hereditary syndromes including (intramuscular) myxomas involve activation of the G-protein-prkar alpha1 axis. This activation is either caused by (1) activating mutations in GNAS1 gene coding for (the alpha subunit of) the G-protein, such as in Mazabraud syndrome and McCune-Albright syndrome, or (2) activating mutations in the PRKAR gene, coding for the downstream protein kinase A receptor, such as in Carney complex. Interestingly, mutations in the GNAS1 gene are also involved in mesenchymal tumour-related syndromes without myxomas such as in Albright hereditary osteodystrophy. Interestingly, this latter syndrome is caused by inhibiting (and not activating) mutations in the GNAS1 gene (55).
1.3 Analytical tools for extracellular matrix analysis

The classification of soft tissue tumours by microscopical features (i.e. on the basis of their normal cellular counterpart) corresponded wonderfully well with the increasing differential biological/molecular genetic data of these different tumour types. In this respect, the more historically originated concept of naming tumours after their (myxoid) ECM, turned out to be not so adequate. This might partially be explained by the restrictive discriminative power of examination of the ECM by microscopy alone, as the constituents which can be identified by (immuno) histochemistry are often not tumour specific (such as collagens, GAGs). Though Rudolf Virchow already mentioned that the ECM might influence the biology of (cancer) cells, study of ECM molecules was rather restricted. Indeed 150 years after the introduction of the term "myxoid" as an ubiquitous microscopical feature, knowledge of the exact constituents and possible function of this so-called myxoid ECM are still very limited. However, during the last decade, recognition of the importance of the ECM and its interactions with-tumour cells in their development and maintenance, has led to a more profound study of the ECM and its (low-abundant) molecules.

1.3.1. Alcian Blue staining

Discovered in 1950, Alcian Blue (AB) is a phthalocyanine cationic dye containing copper ions and non-covalently binding negatively charged macromolecules. It was John Scott who used this staining to distinguish different glycosaminoglycans in tissue sections by varying the electrolyte concentration. By adding gradual increasing concentrations of Mg2+ which competes with AB for binding to mucopolysaccharides and glycosaminoglycans, AB selectively identifies neutral, sulphated and phosphated mucopolysaccharides (63). Kindblom et al showed that the myxoid ECM of various

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Inheritance</th>
<th>Gene locus</th>
<th>Gene</th>
<th>Sarcoma's</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albright hereditary osteodystrophy</td>
<td>AD</td>
<td>20q13</td>
<td>GNAS1</td>
<td>Soft tissue calcifications and osteomas</td>
</tr>
<tr>
<td>Beckwith-Wiedemann syndrome</td>
<td>Sporadic/AD</td>
<td>11p15</td>
<td>Multigenic, incl. CDKN1C and IKF2</td>
<td>Embryonal rhabdomyosarcoma, myxomas, fibromas, hamartomas</td>
</tr>
<tr>
<td>Camey complex</td>
<td>AD</td>
<td>17q23-24</td>
<td>PRKAR1A/PRKAR1K</td>
<td>Cardiac and other myxomas, melanotic schwannomas</td>
</tr>
<tr>
<td>Li-Fraumeni syndrome</td>
<td>AD</td>
<td>17p13</td>
<td>TPS3</td>
<td>Osteosarcoma, rhabdomyosarcoma and other sarcomas</td>
</tr>
<tr>
<td>Mazabraud syndrome</td>
<td>Sporadic</td>
<td>20q13</td>
<td>GNAS1</td>
<td>Polysarotic fibrous dysplasia, osteosarcomas, intramuscular myxomas</td>
</tr>
<tr>
<td>McCune Albright syndrome</td>
<td>Sporadic</td>
<td>20p13</td>
<td>GNAS1</td>
<td>Polysarotic fibrous dysplasia, osteosarcomas</td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>AD</td>
<td>13q14</td>
<td>RB1</td>
<td>Osteosarcomas and other sarcomas</td>
</tr>
</tbody>
</table>

Table 3: Molecular genetics of syndromes involving myxoid tumours of soft tissue
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(non) neoplastic lesions contained various amounts of the different GAGs (36). It has become clear during the years that myxoid changes of the extracellular matrix can be found in reactive and neoplastic (benign and malignant) lesions of both epithelial and mesenchymal origin (75). Thereby myxoid ECM is not specific for any tumour type at all (neither from mesenchymal nor epithelial origin), and nowadays AB staining of the ECM is not of much use in discriminating sarcomas anymore.

1.3.2. Immunohistochemistry

Immunohistochemistry is a crucial adjunct technique in routine diagnostics as well as in research. Though more expensive, the epitopes recognized by immunohistochemistry are usually much more specific than histochemical stainings (although over time this specificity always tends to be less than initially claimed, or hoped for). Immunohistochemical stainings bind to a still increasing number of epitopes identified (e.g. the cluster of differentiation) so its potential is still emerging and includes a large and broad series of validated diagnostic, predictive and prognostic markers. Proteins (i.e. their epitopes) recognized by immunohistochemical stainings can be categorized in different types: structural ECM molecules (e.g. collagens, decorin, vimentin), cell cycle related proteins (e.g. p53, cyclin D, Ki67), proteins involved in cell maturation/differentiation (e.g. CD2, CD3, CD4, CD5, CD7, CD8), receptors (e.g. ER, PR, Her2Neu) and secretory proteins (e.g. gastrin, thyreoglobulin, ACTH, insulin). Hereby, immunohistochemistry, much more than histochemistry, links protein expression to tumour biology and bridges a gap between morphology and molecular genetics. Because of their mesenchymal origin, myxoid tumours of soft tissue nearly always express vimentin, whereas other markers are helpful for more specific classification and diagnosis, depending on the immunohistochemical expression of epitopes often reflecting the cell type of differentiation (e.g. desmin and MS actin in smooth muscle cell tumours; CD31 and CD34 in vascular tumours). In the future, protein screens of (myxoid) tumours (of soft tissue) without a priori knowledge might lead to the discovery of new biomarkers useful in their differential diagnosis.

1.3.3. Liquid-based Chromatography Mass Spectrometry

Besides more conventional ways of studying the proteome, there is a tendency to incorporate more high-tech procedures in the analysis of (soft tissue) tumours during the last years. With its origins in chemistry, mass spectrometry (MS) has recently entered the field of tumour biology. Recognition of the identification of many molecules (including peptides, proteins or lipids) in one single experiment makes MS a promising technique in cancer research. In contrast to (immuno) histochemistry, it uses masses (m/z values) and not charge or structure (e.g. epitope) to discriminate between molecules. Hereby, it allows the identification of many molecules (up to hundreds to thousands) without a priori knowledge of the targeted molecule. A standard mass spectrometry experiment consists mainly out of three elements: (a) creation of the ions by an ion
Figure 3: Simplified methodological overview of the ionisation process by LC-MS/MS

The ion sources routinely used in mass spectrometry research are matrix assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI). In MALDI the sample of interest is co-crystallized with a matrix, overall organic acid. The identity depends on the molecules and mass range of interest (e.g. peptide/proteins, lipids). By irradiating the matrix crystals with a pulsed laser beam, the analytes are desorbed from the matrix and ionized after which they enter the mass analyzer. In ESI the liquid sample containing the analytes of interest are passed through a needle hold at high potential. The electric field between the needle and an other electrode leads to the formation of a Taylor cone at the needle orifice, from which emerges a jet of charged droplets. Sequential cycles of solvent evaporation/Rayleigh instability lead to the generation of very small, highly charged droplets which enter the mass spectrometer. Evaporation of the remaining solvent leads to gas-phase molecular ions. ESI circumvents the need for matrix application, can be perfectly preceded by a first separation step by liquid chromatography and allows a continuous introduction of the ions into the mass spectrometer. Adapted from: Ruedi Aebersold and Matthias Mann (1).

source, (b) the mass analysis and (c) the detection of the masses. A simplified methodological overview of the ionisation process by LC-MS/MS is depicted in figure 3. Basically, two types of ion sources are used: matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI) (figure 3). Different mass spectrometers are currently available, such as Time of flight (ToF), quadrupoles (such as ion trap) and Fourier Transformed (FT) techniques, such as Fourier Transformed Ion Cyclotron Resonance or Orbitrap). Each of these mass spectrometers has its own advantages and relative shortcomings so that the specific mass spectrometer of choice largely depends on the research question(s) imposed to address. A schematic overview of a routine LC-MS/MS experiment is depicted in figure 4. After data acquisition, the mass spectra are analyzed by matching the multiple peptide/protein fragments to a sequence database. Based on the amount of structural overlap of these fragments, a
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1.3.4. Imaging Mass Spectrometry

Recently, imaging mass spectrometry was developed, a technique which combines conventional mass spectrometry (e.g. MALDI-ToF) with spatial resolution and relates the obtained spectra to their exact location in the tissue (figure 5). Imaging mass spectrometry has also entered the field of cancer research. Its particular strengths, such as the analysis of multiple molecules at the same time, in the same tissue and related this information to the spatial resolution of these molecules, without a priori

Figure 4: Schematic overview of a routine LC-MS/MS experiment

For the study of tumour tissues or cell lysates, the (often very complex) samples are analyzed with LC-MS/MS following a 1D or 2D gel electrophoresis. After isolating the spots or bands from the gel (“spot picking”), these (still complex) samples are digested using a protease (commonly trypsin). Trypsin is a serine protease, specifically cleaving the carboxyl end of lysine and arginine. The resulting tryptic peptide samples are typically separated by liquid chromatography (LC) and then ionized using electrospray ionization (ESI). After mass analysis peptides are automatically selected for tandem mass spectrometry (MS/MS). This involves the isolation of the selected peptide ion followed by its fragmentation. Peptide fragmentation follows known rules and so the peptide can be identified by comparing the experimental MS/MS spectra with a peptide (MS/MS) database. Adapted from koler et al. (34).

probability score (so-called “MASCOT” score) is calculated of the reconstructed peptide/protein.

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Figure 5: Schematic overview of a imaging MS experiment

Workflow of a MALDI imaging MS experiment. A) The tissue is prepared for MALDI MS analysis by depositing a matrix solution onto the tissue. Peptides and proteins dissolved by the matrix solution become incorporated into the matrix crystals as the solvent evaporates. B) Irradiation of the matrix crystals with a UV laser leads to efficient production of gas-phase peptide and protein ions, which can then be mass analyzed in a mass spectrometer. MALDI MS analysis of an array of positions covering the tissue provides the spatial distribution of many peptides and proteins. C) The data can be analyzed to reveal the peptide and protein content of defined regions of the tissue or to reveal the distributions of specific proteins. Adapted from McDoneel LA et al (with permission)(34).

knowing them- makes it a very promising tool, at least in theory (34). A crucial step for each imaging mass spectrometry experiment, as for all mass spectrometry experiments, is the quality of the samples (“garbage in = garbage out”), the applied matrix and the matrix application itself. Depending on the range of spectra one is interested in (e.g. <1,000 Da, 3,000-20,000 Da, >20 kDa) as well as the nature of the molecules (e.g. lipids, peptides or proteins), different matrices can be applied. Matrix application can be done manually but for optimal control and reproducibility is best done in an automated fashion by computer assistance. The volume of the droplets is a delicate balance between resolution and quality of the obtained spectra: a larger droplet volume gives better extraction and thus a richer spectrum, but it decreases the resolution (and visa versa).
1.4 Defining therapeutic targets

The revelation of cell signaling pathways in cancer (cells) does not only provide fundamental insight into the mechanisms and biology of cancer. It has also shown to provide excellent clues for more rational-based molecular targeting of specific signaling pathways. Many cancers arise from aberrant cell signaling, which is predominantly regulated by post-translational modifications, such as phosphorylation by kinases. Kinases activate proteins by phosphorylation of the amino acid residues: tyrosine, serine, or threonine. The possibilities of interfering this aberrant cell signaling by inhibitors of these kinases, opens a new era of targeted and more cancer cell specific therapy. The search for pathway discovery, including the activated kinases and their subsequent inhibitors, is especially relevant in sarcoma patients and has been underscored in the treatment of GIST. The tyrosine kinase inhibitor imatinib has quadrupled the median survival of patients with metastatic GIST. However, most patients inevitably develop resistance, which is mostly conferred by secondary mutations within the split kinase domain (exon 13 and 17) of KIT (26, 27, 70). Although mutations within the ATP-binding pocket (exon 13, exon 14) are generally sensitive to secondary generation direct KIT inhibitors such as sunitinib and nilotinib, mutations within the activation loop (exon 17) are often cross resistant to these newer generation KIT inhibitors (7, 53). In sarcoma patients, surgery and irradiation are the mainstay of curative therapy for local disease. Treatment options for patients with advanced (metastatic), or inoperable disease is rather poor (28). Conventional chemotherapy is limited and can have serious side effects, whereas kinase-inhibitors act on more specific targets and subsequently have less severe side effects (73). Downstream signalling targets, including activated kinases have been recently elucidated for well and dedifferentiated liposarcoma (29, 65), low-grade fibromyxoid sarcoma (39), extraskeletal myxoid chondrosarcoma (19), malignant peripheral nerve sheath tumours and alveolar soft part sarcoma (2).

1.5 Aims of the thesis

Originating from mesenchymal precursor cells, myxoid tumours of soft tissue are characterized by their loose myxoid texture of extracellular matrix. In this group, intramuscular myxoma including its cellular variant (a.k.a. cellular myxoma), myxofibrosarcoma and myxoid liposarcoma are the most common ones. Though a hallmark at microscopy, the exact composition of the myxoid ECM is not known. Interactions between tumour cells and their surrounding ECM play an important role in tumour biology. The clinical behavior of myxoid tumours of soft tissue ranges form truly benign to frankly malignant with metastatic potential. On one hand, this might suggest that the ECM of these tumours is not homogeneous and that ECM constituents...
might play a role in this different tumour biology (14). On the other hand, this warrants the need of further (molecular genetic) research to define tumour-specific genetic aberrations which not only give insight in their biology, but also provide diagnostic clues for differential diagnosis and more targeted therapy. The research questions addressed in this thesis are:

(1) what is the exact constitution of the so-called myxoid extracellular matrix and does it play a potential role in the biology of these tumours, outlined in chapters 2, 4, 5 and 6;

(2) which molecular and cytogenetic events characterize these different myxoid tumours of soft tissue, addressed in chapters 2, 3 and 5;

(3) what is the role of these molecular aberrations in their tumourigenesis and do they offer clues to tumour specific targeting, studied in chapters 3, 5 and 7.

References

Introduction

Chapter 1

63. Scott JE, Dorling J: Differential staining of acid glycosaminoglycans (mucopolysaccharides) by alcian blue in salt solutions. Histochemie 5:221-233, 1965