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Chapter 8

Summary and concluding remarks
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This thesis focusses on the molecular pathology of two specific subtypes of soft tissue sarcomas, leiomyosarcoma of soft tissue and myxoid liposarcoma. As described in detail in the introduction, these malignant tumours each have their unique clinical features, histopathology and genetic profile. Soft tissue leiomyosarcomas are often high grade and generally have an aneuploid karyotype with multiple different and non-recurrent translocations. This complex genomic profile makes it hard to identify a common alteration that can be targeted in the search for new treatment strategies. Therefore, to gain insight in the aetiology of tumorigenesis the focus of research should be on the identification of genes and pathways driving and promoting tumour formation. The identified pathway alterations can be candidate targets for new therapeutic options interfering with members of these pathways. Myxoid liposarcomas, on the other hand, harbour a recurrent genetic aberration, most frequently resulting in the fusion protein FUS-DDIT3. This oncogenic fusion product acts as an aberrant transcription factor and influences gene expression resulting in malignant transformation and tumour growth. The presence of a specific genetic alteration makes the translocation product an ideal candidate for targeted treatment, however this is complicated by the fact that targeting a transcription factor is difficult and that probably not all target genes have been identified yet.

Mutations in exon 2 of the mediator complex subunit 12 (MED12), located on chromosome Xq13.1, were first described in around 50%-70% of benign uterine smooth muscle tumours in 2011 (1). In chapter 2 we investigated the presence of MED12 exon 2 mutations in extra-uterine leiomyomas, including piloleiomyomas, abdominal leiomyomas and angioleiomyomas, and both soft tissue and uterine leiomyosarcomas, aiming to obtain more insight in the pathogenesis of these tumours. In line with the previously published study (1), we found a mutation rate of 58% in uterine leiomyomas. In contrast, only one abdominal leiomyoma harboured a MED12 mutation and based on the expression of the oestrogen receptor, this lesion was most likely derived from the uterus. The absence of MED12 mutations in all other extra-uterine leiomyomas highly suggests a different pathogenesis (2). Recently MED12 mutations have been found in fibroadenomas of the breast and phyllodes tumours (3). As these mutations tend to occur in particular in hormone
sensitive tumours, it might be probable that MED12 is only involved in
tumours with activated hormone receptor signalling. We identified one uterine
leiomyosarcoma with a MED12 mutation, this low mutation rate is similar to
what is described in literature (ranging from 2% to 30%) and supports the
hypothesis that the majority of uterine leiomyosarcomas arise de novo while
only rarely a leiomyosarcoma develops in a benign smooth muscle tumour
(4). It has been postulated that in rare cases uterine leiomyomas create an
environment that facilitates wild-type smooth muscle cells to acquire other
genetic alterations and develop into a malignant phenotype, as has been
described for EXT-mutated osteochondroma (5). We found nuclear β-catenin
in half of the MED12-mutated uterine leiomyomas. When translocated to the
nucleus, β-catenin directly interacts with MED12 and regulates transcription of
target genes of the canonical Wnt-signalling pathway. In addition, in the same
series we investigated fumarate hydratase (FH)-deficiency, which is mutually
exclusive with MED12 mutations. One uterine leiomyoma and one cutaneous
leiomyoma from a patient with suspected hereditary leiomyomatosis and
renal cell carcinoma syndrome, and 2 sporadic leiomyosarcomas were positive
for S-(2-succinyl)cysteine (2SC) immunohistochemistry, which is a robust
biomarker for FH mutations (6). Moreover, these FH deficient tumours showed
increased H3K9me3 methylation compared to FH-wild type tumours, which
was expected since alterations in several genes of the tricarboxylic acid cycle
affect histone demethylases. The role of these epigenetic alterations, is not yet
clarified in smooth muscle tumours.

Soft tissue leiomyosarcomas have a complex genetic profile with
numerous non-recurrent aberrations resulting from a high degree of
chromosomal instability. In the search for a better understanding of the
pathogenesis of this tumour and to identify candidate targets for treatment,
we performed COBRA-FISH karyotyping and found a t(6;14) in two cases,
as described in chapter 3. Interestingly we identified in one of the tumours
cryptic rearrangements around HMGA1 and RAD51L1, genes that are also
involved in a subset of leiomyomas. Detailed breakpoint mapping and in depth
characterization with array-CGH profiling of both cases revealed different
translocation breakpoints which, once more, underlines the complex and
heterogeneous genetic profile of leiomyosarcomas. In this study a high grade
leiomyosarcoma was encountered with a relatively simple karyotype, also
in literature this has been described in a subset of tumours. Combination of
modern sequencing techniques might identify different subgroups in these complex heterogeneous tumours, like for example the ‘muscle-enriched’ group characterized by enhanced expression of muscle differentiation related genes. The recent discovery of the expression of LMOD1 in the ‘muscle-enriched’ group and ARL4C in another subgroup, associated with a better and worse disease specific survival, respectively, might be helpful in determination of the prognosis (7). However, additional research is needed to translate these findings into subtype-specific targeted therapy.

Soft tissue leiomyosarcomas are frequently resistant to chemotherapy and therefore the number of treatment options for this aggressive disease is limited. To gain insight in the mechanisms involved in chemoresistance and to search for new therapeutic targets, we investigated the role of the Bcl-2 family proteins in leiomyosarcomas, in chapter 4. The anti-apoptotic protein Bcl-2 was highly expressed in the majority of tumours and moreover a substantial number of tumours showed expression of Bcl-xL and Bcl-w. Single-agent treatment of leiomyosarcoma cell lines with ABT-737, a BH3 mimetic, resulted in only a slight reduction of cell viability. However, combination treatment with the conventional chemotherapeutic agent doxorubicin demonstrated synergism in all tested cell lines by inducing apoptosis. Therefore, inhibition of anti-apoptotic proteins by a BH3 mimetic sensitizes soft tissue leiomyosarcomas to chemotherapy. Before the results of this study can be translated to the practice of the clinical oncologist for the benefit of leiomyosarcoma patients, further validation in in vivo models need to be done. In case of positive and promising results, combination treatment with a Bcl-2 inhibitor and conventional chemotherapeutic agent might be a therapeutic option for patients with Bcl-2 positive tumours. The Bcl-2 inhibitor navitoclax has shown a favourable safety profile in several phase I trials. The antitumour effect of navitoclax monotherapy was disappointing in for example patients with small cell lung cancer and therefore clinical studies tend to focus on the combination with other chemotherapeutic agents (8, 9). A limited treatment effect might be attributed to expression MCL1 or galectin-3, which are both also antiapoptotic proteins, able to mimic the function of Bcl-2 and acts as a bypass mechanism to avoid apoptosis. A specific sphingosine kinase 2 inhibitor (ABC294640) promotes MCL1 degradation and induced apoptosis in combination with a Bcl-2 inhibitor in multiple myeloma (10). Therefore tumours expressing both Bcl-2 proteins as well as MCL1 or galectin-3, needs to be treated with a combination
of a Bcl-2 inhibitor and a compound that leads to a reduced function of MCL1 or galectin-3 to ensure successful suppression of the antiapoptotic function (11).

In chapter 5 we investigated the protein expression of the cancer-testis antigen NY-ESO-1 (CTAG1B) in a large series of myxoid liposarcomas as well as its expression in several other benign and malignant bone and soft tissue neoplasms using immunohistochemical staining. Cancer-testis antigens are currently under the magnifying glass due to their unique combination of properties, which include expression exclusively in the testis in adults, aberrant expression in certain tumours and highly immunogenic features, rendering them an interesting target for immunotherapy. Strong expression of NY-ESO-1 was found in 88% of myxoid liposarcomas, which was the tumour with the highest proportion of positive cases. In the myxoid liposarcomas pre-treated with chemo- or radiotherapy prior to resection, NY-ESO-1 could be detected in 42% of cases. NY-ESO-1 was also positive in a subset of synovial sarcomas, myxofibrosarcomas and conventional chondrosarcomas, while all benign mesenchymal lesions were negative. Clinical trials investigate the effect of NY-ESO-1 targeted vaccine, autologous T-cell or dendritic cell immunotherapies strategies and these studies should be open to patients with NY-ESO-1 expression. A pilot clinical trial demonstrated an antitumour effect of NY-ESO-1 reactive T-cells in synovial sarcoma patients (12). At this moment several clinical trials are recruiting patients, including myxoid liposarcoma patients, to investigate NY-ESO-1 vaccine or T-cell therapy, either alone or in combination with another drug.

Establishing a cell line from primary myxoid liposarcoma tumour samples is a daunting task reflected by the presence of only two cell lines generated by SV40 virus transformation available world-wide, which hampers the research to this malignant tumour. Chapter 6 focusses purely on myxoid liposarcoma and here we report the establishment and characterization of a new human myxoid liposarcoma cell line (DL-221) with the \textit{FUS-DDIT3} translocation. The new cell line is studied in detail for mutations and pathway alterations frequently encountered in this tumour type, like for example the mutation status of \textit{PIK3CA}, \textit{TERT} and \textit{TP53} and the protein expression of NY-ESO-1. Both the cell line as well as the ancillary xenograft model underwent next-generation whole exome sequencing and they are of pivotal importance to study the biology and novel potential-targeted treatment approaches for myxoid liposarcoma.
In chapter 7 we continue the search for new candidate targets for the treatment of myxoid liposarcoma. A high-throughput drug screen was performed using three myxoid liposarcoma cell lines, including the one presented in chapter 6. As expected, a strong response to the chemotherapeutic agents of the classes of the anthracyclines and taxanes was observed. Survivin (BIRC5) was identified as an interesting novel target in the screen, as a strong decrease in cell viability was observed after treatment with the survivin inhibitor YM155. Survivin has a dual function in the cell; in the nucleus it is involved in cell cycle regulation and in the cytoplasm in the inhibition of apoptosis. We demonstrated that survivin is highly expressed in the nuclei of tumour samples supporting its role in cell cycle regulation in myxoid liposarcoma. Other interesting targets from the drug screen were drugs affecting mTOR, HDAC and HSP90. Further in vivo studies are required to investigate whether YM155 can be a promising therapeutic strategy for patients with advanced myxoid liposarcoma.

Concluding remarks and future perspectives

This thesis presents molecular data for soft tissue leiomyosarcoma, a tumour known for its complex genome and, as a consequence, the difficulty to identify targets for treatment. We here show that MED12 mutations are restricted to uterine smooth muscle tumours, and describe the occurrence of non-recurrent translocations in two cases of soft tissue leiomyosarcomas. How these alterations relate to the three molecular subtypes that have recently been proposed (7) remains to be investigated. In addition, we show high expression of the Bcl family members in leiomyosarcomas, and using cell lines we could demonstrate that these proteins play a role in chemoresistance. Also here it would be interesting to see how the expression of other Bcl family members is related to the molecular subtype. Moreover, we show that treatment with a Bcl-2 inhibitor overcomes the chemoresistance of leiomyosarcoma cells. This combination strategy should be further explored in vivo to investigate whether it might be a promising therapeutic approach for leiomyosarcoma patients. Next, the ongoing research of Next Generation Sequencing in combination with functional studies as metabolomics and proteomics might help in the identification of cellular processes in leiomyosarcomas which
might be used in diagnostics or for therapy. The identification of new, and potential immunogenic, proteins might provide new candidate targets for immunotherapy. A recent study demonstrates that concurrent targeting of the PI3K/AKT/mTOR pathway in combination with doxorubicin treatment results in reduced tumour growth in vivo in leiomyosarcoma xenografts (13).

Myxoid liposarcoma, genetically belonging to the group of tumours with a recurrent translocation, is generally sensitive to conventional chemotherapy and radiation. Trabectedin is initially quite effective in a substantial part of the patients, but this effect might cease after some time. We show expression of the cancer-testis antigen NY-ESO-1 in a large proportion of tumours, providing options for immunotherapy. The use of a dendritic cell vaccine with the NY-ESO-1 protein may teach the patient’s immune system to target and kill tumour cells expressing the NY-ESO-1 protein. Another promising option is the adoptive transfer of autologous T-lymphocytes transduced with a NY-ESO-1-reactive T-cell receptor. As the first myxoid liposarcoma patients have entered clinical trials exploiting NY-ESO-1, soon we will learn whether this is a promising strategy for patients with advanced or metastatic myxoid liposarcoma. So far, preclinical studies on myxoid liposarcoma were hampered by the lack of representative preclinical models. In this thesis we present a novel, spontaneously immortalized myxoid liposarcoma cell line, DL-221, which was used together with the other two available cell lines for a large-scale drug screen. We identified survivin as a promising novel target, which is expressed in 100% of myxoid liposarcomas, and future in vivo studies should reveal whether the surviving inhibitor YM155 is an effective therapeutic option for patients with advanced myxoid liposarcoma.
Summary and concluding remarks

References


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