Published in final edited form as:

Cell Mol Life Sci. 2016 October ; 73(19): 3711–3718. doi:10.1007/s00018-016-2266-2.

Liposarcoma: molecular targets and therapeutic implications

Kate Lynn J. Bill^{#1,2}, Lucia Casadei^{#1,2}, Bethany C. Prudner^{1,2}, Hans Iwenofu^{1,3}, Anne M. Strohecker^{1,2,4}, Raphael E. Pollock^{1,2}

¹The James Comprehensive Cancer Center, The Ohio State University, Columbus, OH, USA

²Division of Surgical Oncology, Department of Surgery, Wexner Medical Center, The Ohio State University, 410W 10th Ave., Columbus, OH 43210, USA

³Department of Pathology, The Ohio State University, Columbus, OH, USA

⁴Department of Molecular Virology, Immunology, and Medical Genetics, The Ohio State University, Columbus, OH, USA

These authors contributed equally to this work.

Abstract

Liposarcoma (LPS) is the most common soft tissue sarcoma and accounts for approximately 20 % of all adult sarcomas. Current treatment modalities (surgery, chemotherapy, and radiotherapy) all have limitations; therefore, molecularly driven studies are needed to improve the identification and increased understanding of genetic and epigenetic deregulations in LPS if we are to successfully target specific tumorigenic drivers. It can be anticipated that such biology-driven therapeutics will improve treatments by selectively deleting cancer cells while sparing normal tissues. This review will focus on several therapeutically actionable molecular markers identified in well-differentiated LPS and dedifferentiated LPS, highlighting their potential clinical applicability.

Keywords

Dedifferentiated liposarcoma; Biomarker; miRNAs; MDM2; Exosome; 12q13–15 amplicons; Molecular-cytogenetic analysis

Introduction

Soft tissue sarcomas (STSs) are a rare (<1 % tumors in adults) heterogeneous group of diseases arising from mesenchymal cells. In the United States, there are an estimated 11,930 new cases of STS and 4870 deaths annually [1]. Liposarcoma (LPS) is the most common STS, accounting for 24 % of extremity and 45 % of retroperitoneal occurrences [2]. LPS is a heterogeneous group of malignant adipocytic tumors that are morphologically classified into four subtypes: (1) well differentiated, (2) dedifferentiated, (3) myxoid, and (4) pleomorphic [2, 3]. Depending on the specific subtype and location, liposarcomas can recur in over 80 % of cases, and mortality rates that range from 1 to 90 % highlight the importance of accurate classification [4, 5].

WD and DDLPS embody the most common liposarcoma histologies observed clinically and are the focus of this review. These two subtypes have both common and unique features that contribute to their clinical behavior. Identifying the molecular underpinnings of LPS is a critical step in understanding the biology of LPS. Recent developments in genomic approaches have provided critical information regarding genetic aberrations driving tumorigenesis, histological characterization, and identification of new biomarkers potentially useful for targeted drug therapies in patients.

Clinical presentation

WDLPS accounts for approximately 40–45 % of all LPS and is characterized as a low-grade tumor with a high local recurrence rate of 60 % at 5 year follow-up [6, 7]. The majority of WDLPS arises in the retroperitoneum; however, it can be found in the deep soft tissue of the thigh, paratesticular area, and the mediastinum [8, 9]. The location of this tumor is an important prognostic factor as tumors found in the retroperitoneum are more capable of recurrence compared with the extremities (~40 % compared with ~2 %; respectively) [2, 8, 10]. Importantly, in as many as 40 % of recurrent lesions within the retroperitoneum, WDLPS manifests a dedifferentiated component [8–11].

In 1979, Evans first described the histopathological features of dedifferentiated regions that were observed in recurrent, initially well-differentiated liposarcomas [12]. DDLPS is categorized as an intermediate—to high-grade lesion and may arise as a primary, or “de novo” lesion (90 % of cases) or as recurrence of a prior WDLPS (10 % of cases, i.e., secondary DDLPS) [7, 13–15]. Similar to WD, DDLPS commonly originates in the retroperitoneum and exhibit high local recurrence [13, 16]. Clinically, DDLPS is much more aggressive than WDLPS with a metastatic rate of approximately 15–20 % and a dismal 30 % 5-year overall survival (OS) compared with 90 % OS for WD [2, 7, 9, 11, 13, 17].

The WD/DDLPS dichotomy

Morphologically, these tumors are remarkably different in other regards as well. WDLPS is composed of malignant adipocytes and spindle cells showing fibroblastic/myofibroblastic differentiation with aberrantly enlarged and hyperchromatic nuclei (Fig. 1a, b). On gross examination, these tumors show solid, highly cellular, non-lipomatous dedifferentiation areas abruptly demarcated from the often multi-nodular and yellow mass that constitutes the well-differentiated portion of the tumor (Fig. 1c) [2, 13, 17–20]. In most cases, DDLPS is no longer recognizably adipocytic; however, lipogenic features similar to those seen in pleomorphic liposarcoma have been observed [21, 22].

These morphological and clinical observations give rise to an unexplained conundrum; WD/DDLPS has a common origin and entail a process of progressive evolution from WD to DDLPS or do they ascend from two different cellular populations, representing two distinct malignancies cohabitating in the same anatomic locus? To address these possibilities, profiling studies seeking to identify the unique drivers of each subtype have been performed.

In support to the first theory, genomic profiling has identified common molecular aberrations in WD/DDLPS. Supernumerary ring and/or giant rod chromosomes containing amplified

segments from the 12q13–15 region are observed in both WD and DDLPS [2, 23]. This alteration can be utilized for pathological diagnosis using comparative genomic hybridization to detect DNA sequence copy number changes, thereby distinguishing WD and DDLPS from benign lipomas [24] (Fig. 1d). Additional molecular studies have identified common molecular underpinnings in LPS [25]. In both WD and DDLPS, murine double minute type 2 (*MDM2*) is the most frequently amplified gene (100 % of DDLPS cases), followed closely by cyclin-dependent kinase 4 (*CDK4*; amplified in [90 % of cases] [2, 26]. It has been proposed that co-amplification of *MDM2* and *CDK4*, which both reside within the 12q13–15 amplicon, are potentially the initiating “driving” factor in these tumors [26–29]. As a result, extensive studies targeting the *MDM2* and *CDK4* oncogenes as an anti-LPS therapy have been conducted.

To further explain the potential WD/DDLPS disease progression, Shimoji et al. investigated the heterogeneity of LPS tumors, seeking to identify more aggressive cellular populations. Using cDNA microarray analysis, they observed that the WDLPS component of a DDLPS lesion clustered independently from that of a pure WDLPS tumor, suggesting that perhaps, only a subset of WDLPS is capable of progressing to DDLPS [30].

Although similar to WD, DDLPS has an overall more complex genetic profile, which may contribute to its more aggressive phenotype. In support of this second theory, studies considering the differences between WD and DDLPS as separate entities have been performed. Matushansky et al. demonstrated the genomic resemblance of liposarcoma histologies to the various differentiation stages of mesenchymal stem cells to mature adipose tissue, reporting that DDLPS and WDLPS potentially arise from different stages of development [31].

Molecular targets and therapeutic implications

Despite the two conflicting theories of disease origin, the question is still unresolved about which additional molecular alterations may account for the very different behavior and prognosis of WD vs DD LPS. Several studies have focused on the underlying mechanisms driving these two distinct LPS subtypes and their therapeutic implications. To date, surgery remains the cornerstone of potentially curative treatment for both WD and DDLPS [32]. However, in either disease, the majority of patients will manifest local recurrence and require additional therapeutic management [7]. The poor response to systemic chemotherapy emphasizes the necessity to therapeutically target molecular aberrations as possible new strategies for WD/DDLPS patients and, hence, the effort at identification of actionable targets and biology-driven therapies in this disease.

MDM2 amplification leads to the ubiquitination and degradation of tumor suppressor p53 (*TP53*); as a result, p53-reactivation via MDM2 inhibition may comprise an ideal target for DDLPS therapy. Recent work performed in our laboratory exploited this possibility by utilizing a small-molecule MDM2 inhibitor, SAR405838 (Sanofi-Aventis) to disrupt this specific protein–protein interaction [33]. This approach restored the p53 pathway, inducing cell cycle arrest and ultimately apoptosis. SAR405838 was compared with Nutlin-3a and MI-219, which are other small molecules that target MDM2. These preclinical results

highlight the contribution of the MDM2-p53 axis in DDLPS tumorigenesis and the potential utilization of SAR405838 as a therapeutic option for patients suffering from this disease.

CDK4, the second most observed molecular irregularity in WD and DDLPS, has also been implicated as a therapeutic target. Inhibition of CDK4 with NVP-LEE011 (LEE011; Novartis) decreases cell proliferation via G₁/G₀ arrest, due to the reduction of phosphorylation on retinoblastoma (pRb) [34]. Although continuous exposure to LEE011 eventually led to reentry into the cell cycle, a repeat exposure to this drug reinstated the cell cycle arrest and halted in proliferation. This points to the ability of cancer cells to mount a dynamic mechanism that can overcome drug sensitivity, and also suggests the importance of dosage scheduling and the applicability of dual-target strategy to elicit a cytotoxic synergy as an additional relevant option.

The contribution to tumorigenesis and disease progression from additional oncogenes residing in the 12q amplicon (*HMGA2*, *FRS2*, *STAT6*, and so on) has been an on-going investigation of many researchers [28, 35, 36]. This delineated chromosomal amplification exemplifies the genomic complexity and heterogeneity in LPS. In addition, genomic amplifications in 1p32, 1q21–24, and/or 6q23 (which reside the oncogenes *JUN*, *ASK1*, and *MAP3K5*, respectively) are observed frequently in DDLPS and may contribute to the more aggressive and stem-like phenotype that could contribute to worse prognosis [37–41]. Added targets that reside outside the 12q13–15 amplicon, such as c-JUN and ASK1, have also been investigated [40]. Both proteins act downstream on peroxisome proliferator-activated receptor gamma (PPAR- γ), suggesting a key role in adipocyte differentiation into DD or WD [38, 39, 42]. Together, these results demonstrate that high expression of c-JUN may play a role in the loss of early stage adipogenesis, and consequently, its inhibition may offer therapeutic implication in DDLPS.

Tyrosine kinase receptors have been utilized as key targets for molecular based therapies due to their direct impact on signaling pathways. Aberrant activation of the Met pathway has been implicated in multiple tumor types, including sarcoma [43–47], and combination treatment with standard chemotherapy has been demonstrated to be effective against proliferation in multiple myeloma [48]. In LPS, increased Met pathway activation has been observed [49]. Ectopic expression of the only known activating Met ligand, hepatocyte growth factor (HGF) results in increased proliferation and motility in DDLPS cells, while Met inhibition impairs the growth of DDLPS cell lines in vivo [50]. Thus, the Met pathway may be a potential therapeutic target for DDLPS treatment.

Taken together, identification of molecular aberrations potentially driving LPS tumor establishment and progression has demonstrated clinical application; however, there is still much to be learned. For example, the degree of MDM2 amplification is variable, and identifying a specific threshold may identify a subset of patients who benefit better from specific therapeutic combinations [51]. This study stratified DDLPS cell lines by their relative degree of MDM2 amplification and found that doxorubicin was more effective in the lower expressing MDM2 subset, while small molecule MDM2 inhibitors were more potent when MDM2 was expressed at higher levels. This study exemplifies the need to carefully evaluate the contribution of a molecular event and also illustrates the limitations of such

molecular studies, i.e., even the most frequently studied LPS molecular aberration (MDM2) requires better characterization if it is to serve as a meaningful predictive and prognostic marker in LPS.

miRNAs contribution to liposarcoma progression

Recently, not only genes and proteins, but also miRNAs have been shown to be useful in understanding the WDLPS/DDLPS dilemma and to gain insight into the basis of liposarcoma progression. miRNAs are short (circa 22 nucleotides in length) non-coding RNAs (ncRNAs) that regulate gene expression by binding to specific mRNA targets and promoting their degradation and/or translational inhibition. A central role in LPS has been shown for miR-143; miR-143, which is abundant in normal adipose tissue, was found to be underexpressed in WDLPS, and its expression decreased further as the tumor progressed to DDLPS. Restoring miR-143 expression in DDLPS cells inhibited proliferation and induced apoptosis by targeting the gene network that includes: B-cell lymphoma 2 (*BCL2*), DNA Topoisomerase II (*TOP2A*), protein regulator of cytokinesis 1 (*PRCI*), and polo-like kinase 1 (*PLK1*) [52].

miR-145 and miR-451 have been also proposed to have important role in LPS as they can act as tumor suppressors in adipose tissue; overexpression of miR-145 and miR-451 in LPS cell lines decreases cellular proliferation rate, impairs cell cycle progression, and induces apoptosis [53]. In 2013, an interesting work by Lee et al. revealed the contribution of miR-26a-2 to LPS tumorigenesis, identifying a new target gene of miR-26a-2, regulator of chromosome condensation and BTB domain-containing protein 1 (*RCBTB1*). *RCBTB1* is located at 13q12.3–q14.3, a region responsible for loss of heterozygosity in LPS. As a regulator of the DNA damage/repair pathway and apoptosis, inhibition of *RCBTB1* by overexpression of miR-26a-2 rendered LPS cells more resistant to apoptotic stress [54].

The important function of miR-155 and β -catenin signaling in progression of WDLPS/DDLPS was established by Zhang et al. They found that miR-155 is highly expressed in human WD/DDLPS and contributes to the tumorigenic phenotype of these malignancies through the regulation of casein kinase 1 α (CK1 α), which results in the activation of the β -catenin pathway. β -catenin and its downstream effector cyclin D1 were found to be overexpressed in all human DDLPS cell lines compared with preadipocytes and adipocytes and were also shown to induce DDLPS cell proliferation and cell cycle progression [55].

To add further complexity to this picture, it has been shown that extracellular vesicles, such as exosomes, macrovesicles, and apoptotic bodies, contain miRNAs with LPS-relevant functions [56]. It is now well established that transfer of genetic information in the form of RNA exists and that this form of transfer between cells is functional relevant by causing gene silencing in recipient cells. This newly discovered manner of genetic exchange between cells opens a new possibility of how adjacent cells within an organ may communicate and how miRNAs can affect a cell type or a tissue remote from where it is produced [57]. Increasing evidence suggests that this form of communication occurs in various processes and in pathological situations, including tumor development. In particular, extracellular miRNAs could be important regulators of tumor microenvironment [58]. This field of

research is still growing, and our group is focusing on how exosomal miRNAs associated with LPS could perhaps enhance liposarcomagenesis and dedifferentiation by delivering tumor-promoting miRNAs to adjacent and distant recipient cells, hopefully lending new insight into our understanding of the growth, and malignant behavior of liposarcoma.

Biomarker potential in WD/DDLPS

Early biomarker cancer detection remains a major challenge in malignant disease but holds the promise of resulting in more favorable disease outcomes. In WD/DDLPS, in particular, new biomarkers could help to diagnose recurrence (and dedifferentiation) prior to the currently available radiologic detectability time points. Unfortunately, early predictive/prospective biomarker signatures are not yet developed for those patients, many of whom will ultimately dedifferentiate, and are, therefore, at especially high mortality risk.

To understand the role of biomarkers in clinical research, it is important to recall that the term “biomarker” was defined by the National Institutes of Health as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” or “any substance, structure, or process, that can be measured in the body or its products and influence or predict the incidence and outcome of disease” [59].

Prognostic markers have been associated with overall survival in soft tissue sarcoma, e.g., tumor size and depth, site, grade, and resection margins. Recently, hemoglobin, alkali phosphatase, and C-reactive protein were found to predict the patient outcome, as well as serum creatinine and albumin in liposarcoma [60, 61].

Understanding the relationship between measurable biological processes and clinical outcomes is vital to expand treatment for disease; therefore, several important questions to be considered include whether or not a biomarker expression correlates with tumor diagnosis and prognosis independent of the treatment received, and also whether or not a biomarker can predict a treatment effect if such effects are different for biomarker-positive vs biomarker-negative patients [62].

The assessment of multiple miRNA expression levels (also referred to as miRNA signatures) can accurately predict prognosis in a variety of cancers, and miRNAs expression patterns may be uniquely differing between cancer and normal tissues [63]. For example, miRNAs profiling in liposarcoma has been shown to discriminate WD and DDLPS from normal adipose tissue [64–67] and also to distinguish WDLPS from DDLPS [52, 67, 68].

A comprehensive miRNA expression database for a variety of human sarcoma types was generated by Sarver–Subramanian: the sarcoma microRNA expression database (S-MED) (<http://www.oncomir.umn.edu/>). These studies do not always report common and consistent results; discrepancies and differences reported in miRNAs signature can be due not only to the intratumoral heterogeneity that is the major cause for misinterpretation in most molecular test [69], but also because of the different subtypes of liposarcoma that may be considered together or separately and in comparison with normal fat, lipoma, or other liposarcoma subtypes.

miRNAs have become more attractive as biomarker given their easily retrievable presence in plasma and other body fluids, such as urine, saliva, and so on. From their first discovery in body fluids, multiple studies in a wide range of malignancies have shown unique miRNA biomarkers in the plasma or serum of cancer patients, potentially making them useful for routine clinical diagnosis.

MicroRNAs are considered to enter body fluids via three major mechanisms: (1) passive release from damaged tissue, (2) active secretion in cell-borne membrane vesicles (exosomes, microvesicles, and apoptotic bodies), (3) active secretion in macromolecular complexes associated with Argonaute (Ago) proteins (among other still unidentified proteins) and high-density lipoprotein (HDL) particles [70]. The biological relevance of blood-borne microRNAs is largely unknown, but such entities might be regarded as analogous to circulating hormones implicated in cell-to-cell communication within the tumor, and affecting the immune responses, facilitating angiogenesis, tumor invasion, and even metastatic propagation [71, 72]. However, the possibility that patient-derived serum or plasma miRNAs might serve as novel biomarkers for monitoring stage and diagnosis of sarcomas has not yet been extensively considered especially in WD/DDLPS [73–75].

In light of this reality, our group is putting much effort in identifying miRNAs as potential biomarkers in the peripheral blood of WD/DDLPS patients. Our preliminary observations demonstrate a typical circulating miRNA signature in WD/DDLPS patients that may be able to discriminate between healthy individual and patients with WD/DDLPS and also serve as biomarkers of drug resistance, which could help optimize individual treatment options (manuscript in preparation).

Conclusion

WD and DDLPS embody the most common liposarcoma histology. These two subtypes have both common and unique features, and the clinical outcome are very different: long-term survival is common in WDLPS, whereas DDLPS is markedly more aggressive and has metastatic possibilities. This WD/DDLPS dichotomy is not yet completely understood.

Unique in solid tumor oncology, the WD/DDLPS dichotomy in morphology, clinical behavior, molecular biology, and prognosis unequivocally identifies the need for biomarkers that enable earlier detection of clinical differences relevant to therapeutic intervention. Such efforts, already underway, hold the promise of new therapies and earlier detection of WD/DDLPS recurrences or progression, a critical effort if we are to improve outcomes for patients burdened by the specter of this very unfavorable malignant condition.

Acknowledgments

The authors would like to acknowledge Dr. Julie Bridge from the Department of Pathology, University of Nebraska who graciously provided the Fluorescence In Situ Hybridization (FISH) image.

Funding This review was supported by the NCI U54CA168512 to REP.

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Fig. 1. Well-differentiated and dedifferentiated liposarcoma. **a** Hematoxylin and eosin stain of WDLPS (magnification 9200). Image shows a lipomatous tumor with broad and cellular septa containing hyperchromatic cells. **b** Hyperchromasia of the atypical cells, consistent with atypical lipomatous tumor/well-differentiated liposarcoma (magnification 9400). **c** Hematoxylin and eosin stain of DDLPS (magnification 9200). Image demonstrates the biphasic malignant neoplasm composed of a high-grade spindle cell sarcoma separated from a well-differentiated fatty tumor by a fibrous capsule. The *inset on the lower left* shows scattered single hyperchromatic cells within the fatty tumor consistent with the WDLPS component of DDLPS. **d** Positive *MDM2* gene amplification by fluorescence in situ hybridization observed in both WD and DDLPS