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Pleomorphic liposarcoma: clinical observations and molecular variables

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Abstract

Background—Pleomorphic liposarcoma (PLS) is a rare high grade sarcoma showing lipoblastic differentiation. We evaluated PLS natural history, patient outcomes, and commonly deregulated protein biomarkers.

Patients and methods—PLS patient medical records (n=155; 1993 to 2010) were reviewed. Univariable and multivariable analyses were conducted to identify independent prognosticators. A PLS tissue microarray (TMA; n=56 human specimens) was constructed for immunohistochemical analysis of molecular markers. *p53* gene sequencing (exon 5–9) was conducted.

Results—Average patient age was 57 years with primary (n=102), recurrent (n=16), and metastatic (n=37) presentations. Lower extremity was the most common site (40%); average tumor size was 11cm. Complete follow-up data were available for 83 patients with 22.6 months median follow-up; the 5Y DSS was 53%. Recurrent disease, unresectability, and microscopic positive margins predicted poor prognosis. Systemic relapse (the strongest poor prognostic determinant) occurred in 35% of localized PLS patients. IHC revealed increased expression of PPARγ (adipogenic marker), BCL2 and survivin (survival factors), VEGF (angiogenic factor),

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MMP2 metalloprotease, and other biomarkers. Frequent loss of Rb expression and high p53 mutation rates (~60%) were identified.

Conclusions—PLS is an aggressive metastasizing sarcoma. Identifying ubiquitous molecular events underlying PLS progression is crucial for progress in patient management and outcomes.

Keywords

Pleomorphic liposarcoma; Clinical outcome; Tissue microarray; Molecular biomarkers; *p53* mutations

Introduction

Pleomorphic liposarcoma (PLS) is a rare high grade pleomorphic sarcoma; the presence of lipoblasts is required for diagnosis (1,2). Due to their rarity (less than 5% of all liposarcomas) knowledge of PLS natural history stems from anecdotal reports and small cohort analyses typically contained within larger liposarcoma studies; only three PLS-specific reports include more than 50 patients (3–5). Such caveats notwithstanding, these studies demonstrate that, as compared to other liposarcoma histological subtypes (well differentiated/dedifferentiated liposarcoma [WDLPS/DDLPS] and myxoid/round cell liposarcoma [MRC]), PLS is the most aggressive -- exhibiting avidity for systemic spread and a poor overall outcome (3–5). Surgical resection is currently the only potentially curative approach to these remarkably chemoresistant tumors; locally advanced and metastatic disease is generally non-curable. This dismal outcome of patients with PLS mandates development of improved (perhaps molecularly-based) therapeutic strategies.

PLS characteristically harbor diverse chromosomal rearrangements and genomic profiles without unifying molecular alterations, a circumstance typical of soft tissue sarcomas (STSs) with complex karyotypes; e.g., leiomyosarcoma, angiosarcoma, and myxofibrosarcoma (6–9). In contrast, WDLPS/DDLPS and MRC commonly exhibit distinctive genetic aberrations; i.e., 12q13-15 chromosomal amplification in WDLPS/DDLPS and a (12;16) translocation resulting in a *FUS-DDIT3* fusion gene in MRC (10–12). This genetic complexity suggests that singular dominant molecular aberrations are unlikely to underlie PLS tumorigenesis and progression. Consequently, it may be more therapeutically relevant to elucidate the currently unknown spectrum of PLS deregulated pathways and/or processes rather than search for a possibly dominant yet non-existent locus of PLS ‘oncogenic addiction’. In light of these knowledge gaps, we chose to investigate the natural history and clinical outcome of a large PLS cohort treated at a single institution, seeking to identify disease-specific survival (DSS) prognosticators and to identify commonly deregulated molecular processes/biomarkers using human PLS specimens assembled in a tissue microarray (TMA).

Patients and methods

Clinical database

This study was conducted with institutional review board (IRB) approval from the University of Texas MD Anderson Cancer Center (UTMDACC). PLS patients seen at UTMDACC from 1/1993 through 1/2010 were identified by search of the UTMDACC prospective sarcoma database, institutional tumor registry, and pathology archives. Only patients with unequivocal PLS histology confirmed by a UTMDACC sarcoma pathologist (AJL) were included in the study (n=155); the presence of lipoblasts was mandatory for diagnosis; cases of “pleomorphic liposarcoma” in the background of well- or dedifferentiated liposarcoma or inconclusive diagnoses were excluded. An initial database was constructed including demographic and tumor associated variables. For patients with

sufficient follow-up information (n=83) treatment and outcome information were included. Only patients with localized PLS were included in the univariable and multivariable analyses.

Tissue microarray (TMA) construction

After evaluation of all potential PLS FFPE samples available, 56 blocks representing tumors derived from 37 patients were selected for inclusion in the TMA. Five FFPE blocks of each of the following histologies: dedifferentiated liposarcoma (DDLPS), myxoid liposarcoma (MLPS), and unclassified pleomorphic sarcoma/malignant fibrous histiocytoma (UPS/MFH) were included as controls. TMA was constructed as previously described (13). In brief H&E-stained sections were reviewed from each tumor block by an institutional sarcoma pathologist (AJL) to define areas of homogeneous, viable tumor. Using an automated TMA apparatus (ATA-27, Beecher Instruments), 0.6 mm punch samples (2 per case) were obtained from each donor block and formatted into a recipient block. Sections (4μm) were cut and verified by H&E.

Immunohistochemistry

Commercially available antibodies against PPARγ (Cell Signaling, Danvers, MA), Adipophilin (Fitzgerald, Acton, MA), BCL2 (Biogenex, Fremont, CA), Survivin (Abcam, Cambridge, MA), p16 (CINtec, Heidelberg, Germany), Rb (BD Pharmingen, San Diego, CA), Cyclin D1 (Labvision, Freemont, CA), CDK4 (Invitrogen, Carlsbad, CA), VEGF (Santa-Cruz, Santa Cruz, CA), MMP2 (Millipore, Billerica, MA), MMP9 (Millipore), β-catenin (BD Biosciences, San Jose, CA), EGFR (Zymed, Carlsbad, CA), MDM2 (EMD, Gibbstown, NJ) and p53 (Dako, Carpenteria, CA) were used for immunohistochemical staining. Spots representing 40 different samples from 37 different patients were applicable for scoring analysis, in three cases both a localized and a metastatic lesion from the same patients was evaluated; labeling intensity was scored by two observers (MPG and AJL) as none, low, or medium-to-high; when pertinent, cytoplasmic and/or nuclear staining were scored separately.

p53 sequencing

Genomic DNA was extracted from paraffin-embedded tissue using a QIAamp DNA Mini Kit (Qiagen Sciences, Valencia, CA) per manufacturer's instructions. Integrity and concentration of the extracted DNA was assessed with the NanoDrop-1000 Spectrophotometer (NanoDrop-Products, Wilmington, DE). DNA sequencing was conducted as previously described (14). In brief: primers were designed for intron sequences flanking exons 5 through 9 of the p53 gene; primers were purchased from Sigma-Genosys Technologies inc. (Woodlands, TX). 100ng of genomic DNA was used as a template for PCR amplification of exonic sequences. PCR reaction was carried out on a Eppendorf Mastercycler® pro thermal cycler (Eppendorf AG, Hamburg, Germany). PCR product sequencing was conducted on an Applied Biosystems (Foster City, CA) 373 automated DNA Sequencer. Sequence analysis was performed using "Sequence Scanner v1.0" (Applied Biosystems).

Statistical analysis

Statistical analysis was conducted as previously described (15). Patient demographics, clinical characteristics, and molecular marker expression levels were summarized using means, medians or proportions as applicable. Median overall- (OS) and disease specific-survival (DSS) were determined using the Kaplan-Meier method. 1-, and 5-year DSS rates (95% CI) were calculated for all evaluable patients. For OS analysis, death was counted as an event. For the DSS analysis, only death due to disease was counted as an event; patients

who survived or died due to other causes were censored at their last follow-up (LFU) date or the date they died from other causes. Cox's proportional hazards regression model was used to test the statistical significance of candidate prognostic factors for DSS in a univariate manner. From this model the hazard ratios for potential prognostic factors were estimated with a 95% confidence interval. All potential prognostic factors with a p-value < 0.10 were then included in a saturated model, and backward elimination was used to remove factors from the model based on the likelihood ratio test in the multiple regression analysis. Fisher's exact and Chi-square tests were used to assess associations between molecular markers. All computations were carried out in SAS.

Results

PLS patient, tumor, and treatment variables

A total of 155 PLS patients were evaluated at UTMDACC during the investigated interval (1993–2010). Only patients with follow up (n=83) are described here. Clinicopathologic variables are summarized in Table 1A. The median presenting age was 53 (range, 14–84) years with slight male predominance. Most patients (80%) presented with localized PLS (primary or recurrent); 20% had metastatic disease, most commonly to the lungs (82%), liver (18%), and skeleton (18%). The most common sites of primary PLS were thigh (34%) and pelvis (15%). The average size of localized primary tumors was ~11cm. Average size of truncal tumors was 8.5cm±4 (1.5–16.5cm) and of extremity tumors was 11.2cm±7.2 (0.7–30cm).

All patients with metastatic PLS (n=17) received chemotherapy (doxorubicin [n=12], ifosfamide [n=12], gemcitabine [n=7], and docetaxel [n=7]). In the majority of cases (n=14) extensive and diffuse metastatic load was present precluding surgical metastasectomy; such was conducted in three cases. Palliative surgery and or/radiotherapy were utilized in six patients patients. Surgery was considered for all patients with localized (primary or recurrent) disease 9 (n=66). Only two tumors (3%) were deemed unresectable after radiation and chemotherapy. Complete macroscopic resection was achieved in 62 of 64 patients with localized disease (97%); negative microscopic margins (R0 resection) were attained in 56 (90%) complete resections. Average tumor size of patients undergoing R0 resection was 9.7cm±6.3 (1.5–29) vs. 11.7cm±6.8 (4.5–25) in patients undergoing R1/R2 resection. R0 resection was achieved in 79% (n=19) of patients with a truncal tumors as compared to 93% (n=37) of those with extremity PLS. Chemotherapy (neoadjuvant and/or adjuvant) was administered to 28 (45%) completely resected patients: agents included doxorubicin (n=24; 89%), ifosfamide (n=19; 70%), docetaxel (n=5; 19%), gemcitabine (n=5; 19%), cyclophosphamide (n=2; 7%) and dacarbazine (n=2; 7%). Radiation was delivered to 43 (69%) completely resected patients: neoadjuvant therapy was administered to 25 patients, 17 patients received post operative adjuvant radiation, and one patient received both pre- and post-operative radiotherapy.

PLS-specific survival and potential outcome-related prognosticators

Median follow-up of patients presenting with metastasis was 8.5 months (2.5–47.2; Table 1B). The median OS for patients with metastasis was 9.1 months; 1-year DSS was 45% and none reached five years. Outcomes of patients with metastatic PLS were markedly worse than for patients with localized tumors ($p<0.0001$; Fig 1A). Median follow-up of patients with localized PLS was 31.5 months (1.5–182). For patients with localized PLS surviving at the end of the study (n=36), median follow up time was 47 months (2–182). Sixteen of the patients with completely resected tumors had local recurrence (25%) and 23 (35%) developed metastatic disease, mainly pulmonary spread. Within the constraints of a relatively short follow up period, the 1- and 5-year DSS of patients with localized PLS were

93% and 65%, respectively. KM analysis further demonstrated that a positive microscopic margin significantly correlated with decreased DSS (p=0.001; Fig 1B). Tumor size (<5cm) and disease status (primary vs. recurrent) did not achieve statistical significance (p=0.07 and 0.06, respectively; Fig 1C&D), possibly due to the small evaluable patient cohort.

A univariable DSS analysis was performed to identify factors predicting poor prognosis in localized PLS patients (Table 2A). No surgical resection, microscopically positive surgical margins, and chemotherapy treatment were significantly associated with decreased DSS. Large tumor size and recurrent disease at presentation to UTMDACC did not achieve statistical significance, possibly due to the small evaluable patient cohort. These variables (except chemotherapy) were included in a multivariate analysis: recurrent disease, no surgical resection, and positive resection margins were identified as independent poor prognostic indicators. It is of note that our analyses are limited but the relatively small patient number and combining datasets from different institutions can enhance the statistical significance of clinical information.

PLS related molecular biomarkers

Little is known about PLS-associated molecular deregulations; such knowledge could enhance disease diagnosis, prognostication, and treatment. Hence, we evaluated protein expression of multiple cancer-related biomarkers using IHC of human PLS specimens assembled in a clinically annotated TMA which included all three PLS subtypes: classical (n=28), myxoid (n=6), and epithelioid (n=6; Figure 2). Marker selection was based on previous reports suggesting their potential relevance to PLS or malignant neoplasms *per se*, including markers of adipogenic differentiation (PPARγ, adipophilin), cell survival (BCL2, survivin), cell cycle regulation (p16, Rb, cyclin D1, CDK4), angiogenesis (VEGF), migration and invasion (MMP2, MMP9) and also β-catenin, EGFR, and p53. The majority of stainings were conducted at the clinical laboratory in using highly validated antibodies and in all cases positive and negative controls were utilized confirming antibody specificity. Table 3 and Figure 2 depict protein expression levels for the entire PLS cohort as well as representative specimen staining photomicrographs; as reflected in the heatmap for every antibody evaluated a spectrum from no staining up to high staining intensity could be identified across the TMA specimens.

Interestingly, PPARγ was expressed in all PLS samples as well as in DDLPS, MLS, and UPS/MFH control specimens; 77% of PLS exhibited moderate-to-high PPARγ expression. Adipophilin expression was found in 80% of PLS, albeit mainly at low expression levels. Of controls, only MLS exhibited adipophilin positivity. Both BCL2 and survivin were commonly expressed in PLS (93% and 100% of specimens, respectively). Moderate-to-high survivin expression was identified in 54% of PLS samples; both cytoplasmic and nuclear staining were noted. p16 was expressed in all PLS samples; 70% exhibited moderate-to-high staining intensity. In contrast, 77% of PLS did not express Rb. Cyclin D1 was expressed in 33% of PLS; in contrast, all DDLPS expressed this protein. Similarly, CDK4 expression was found in 33% of PLS; all DDLPS samples variably expressed CDK4 protein. VEGF expression was found in 68% of PLS. MMP2 and MMP9 were expressed in 93% and 100% of PLS, respectively; moreover, moderate-to-high expression was noted in more than 50% of PLS. 68% of PLS variably expressed β-catenin, although expression was mainly cytoplasmic. Enhanced EGFR expression was noted at either at moderate-to-high (35%) or low (35%) expression levels.

Recently, a high rate of *p53* mutations was reported to occur in PLS (16). To further evaluate p53 mutational status we sequenced the p53 DNA core binding domains (exons 5–9) in DNA extracted from 31 FFPE PLS specimens. p53 mutations were identified in 19 (60%) of the samples and included: exon 5 – R158P, A159V, and R175H; exon 6 - H193R

and R209T; exon 7 - S241Y, G245A, and R248Q; exon 8 – L265P and T304I; exon 9 – S313T. Of these S313T, L265P, and R248Q were the most frequent (Fig 3). We considered that this high mutation rate might be a false positive artifact due to sequencing formalin fixed tissues, so we blindly sequenced two samples whose corresponding frozen samples were available; identical p53 sequence alterations were identified. No statistical correlation between *p53* mutation status and p53 protein expression levels could be identified (Fig 3).

We next evaluated whether biomarker expression levels correlated with PLS patient outcomes. This analysis only included samples from patients with localized disease who underwent complete surgical resection and for whom follow-up information was available (n=22). Univariable analysis failed to identify prognostic value for any markers evaluated, with the caveats inherent to small sample cohorts rendering definitive conclusions problematic. Future attempts would be made to increase the number of samples retrieved from PLS patients undergoing complete resection to enable meaningful evaluation of the prognostic value of a given biomarker.

Discussion

The current study and other major series (3–5,17–20) demonstrate that PLSs exhibit unfavorable outcomes even when managed with multidisciplinary expertise at tertiary cancer centers, reflecting the aggressive biology of this malignancy. PLSs exhibit high rates of local recurrence; as per previous reports (3–5,19,20), 25% of patients undergoing complete surgical resection experienced local failure in our series. Systemic (especially pulmonary) metastases are common; 25% of our patients presented with metastatic spread and more than one-third undergoing complete resection developed distant metastasis during follow-up. This aggressive `behavior is further exemplified by five-year survival rates of ~60% for patients presenting with localized disease (Table 4). Multivariate analysis revealed only recurrent disease and positive microscopic margins as independent outcome prognosticators in this series. Other clinicopathological factors previously shown as potentially predictive include: older age, central tumor location, size larger than 10cm, tumor necrosis, high mitotic rate, and epithelioid morphology (Table 4; 3–5,19,20). A gene expression signature predicting outcome for complex karyotype STS patients was recently devised (21); the validity of this profile should be tested in the PLS patient cohort. If validated, PLS genetic profiling might possibly augment traditional staging, thereby optimizing patient treatment decisions.

Unraveling key deregulations driving PLS inception and progression is essential to establish molecular-based staging systems as suggested above; even more critical is the potential impact such knowledge might have on development of novel and effective anti-PLS targeted therapeutics. Elucidating specific PLS nodes of vulnerability awaits determination of molecular underpinnings of this malignancy; common ‘drugable’ targets are yet to be discovered. The cytogenetic complexity of PLS suggests the probability of multiple molecular aberrations rather than single “oncogenic addictions”. Strategies to disable multiple parallel and/or complementary pathways, rather than single locus “magic bullets”, are likely necessary for effective management of PLS, hence our efforts to identify biomarkers broadly reflecting different cancer-associated therapeutically-relevant processes.

Tumor suppressor pathway deregulations commonly occur in PLS, including Rb (22) and p53 (23). Our study confirms these initial observations, demonstrating loss of Rb expression in a large proportion of PLS samples. In contrast, although well characterized as a tumor suppressor inhibiting progression through the cell cycle by binding to CDK4/6 (24,25), we found increased PLS p16 expression. p16 overexpression has been previously demonstrated in various malignancies, including STS and LPS specifically (26). MDM2 and CDK4 are

amplified in WDLPS/DDLPS as part of the 12q13-15 amplicon identified in these tumors (27); DDLPS samples included as controls on our TMA highly expressed these proteins. In contrast, only a 1/3 of PLS expressed CDK4 and none exhibited MDM2. Clinical trials evaluating the effects of MDM2 and CDK inhibitors (e.g., RO5045337 and PD0332991, respectively) on LPS are currently being initiated. Our findings do not support inclusion of PLS patients in the former study and call for careful PLS patient selection for inclusion in the latter. A recent study has identified *p53* as more frequently mutated in PLS compared to most other STS subtypes (16). Our data recapitulate this observation, demonstrating high rates of *p53* core binding domain mutations in PLS as well as varying levels of p53 protein expression levels. No correlation between *p53* mutation status and p53 protein levels was identifiable, as previously shown for other malignancies (28). Therefore, p53 immunohistochemistry cannot be used as a surrogate methodology to identify *p53* mutations in PLS. p53 mutations contribute to chemoresistance (29), possibly explaining PLS therapeutic resistance and suggesting that reconstituting p53 function in these tumors might be fruitful (30).

PPARγ is a nuclear hormone receptor with a critical role in adipocyte differentiation (31). While counterintuitive given its role in differentiation, data supports a PPARγ pro-tumorigenic function (32). A recent study identified enhanced PPARγ expression in LPS subtypes especially MLS, DDLPS, and PLS (33); our findings support this observation. PPARγ agonists have been shown to induce differentiation and growth inhibition in cancer cells expressing this protein including LPS (34–37). Initial clinical experience demonstrated significant adipocytic differentiation in two PLS patients treated with the PPARγ ligand troglitazone (38). A phase II clinical trial for LPS patients failed to achieve any objective clinical responses, suggesting that PPARγ activation as a solitary approach is insufficient (39); no PLS patients were included in this trial. Further investigation of PPARγ as a therapeutic target for PLS appears warranted, especially in combination with blockade of additional pathways. Molecules contributing to cancer cell survival such as BCL2 and survivin have also been of interest as novel therapeutic targets (40–42); small molecule inhibitors are being evaluated in human clinical trials. Our results demonstrate increased expression of these potential targets in PLS, especially survivin; further preclinical investigation using human cell lines and xenograft models is currently ongoing. Similarly, we identified increased PLS expression of VEGF, the metalloproteases MMP2 and MMP9, as well as the tyrosine kinase receptor EGFR; novel therapies targeting these molecules are currently available (43–45). Our study identifies several potential therapeutic targets as overexpressed in PLS. Further preclinical investigations utilizing relevant PLS experimental models are needed. As expected, we found that a single PLS may exhibit multiple molecular aberrations (see heatmap; Figure 2). This reflects the molecular complexity of PLS where a multitude of genetic and epigenetic deregulations are at play. Consequently, utilizing novel therapeutic combinations rather than single target therapies to block multiple pathways and processes might constitute the best anti-PLS approach.

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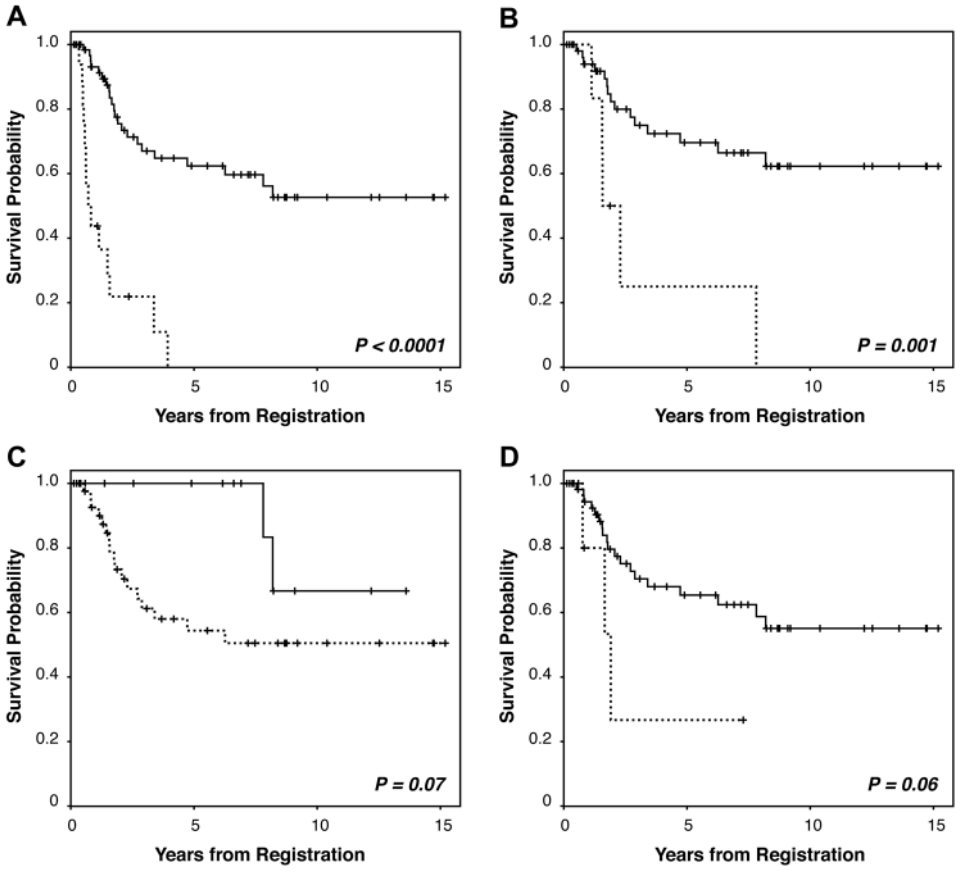

Figure 1. Kaplan-Meier curves for PLS-specific survival

A. stratified for patients treated for localized (solid line) versus metastatic disease (dotted line); *B.* stratified for R0 (solid line) versus R1 (dotted line) resection; *C.* stratified for tumors <5cm (solid line) versus ≥5cm; and, *D.* stratified for primary (solid line) versus recurrent (dotted line) disease at presentation.

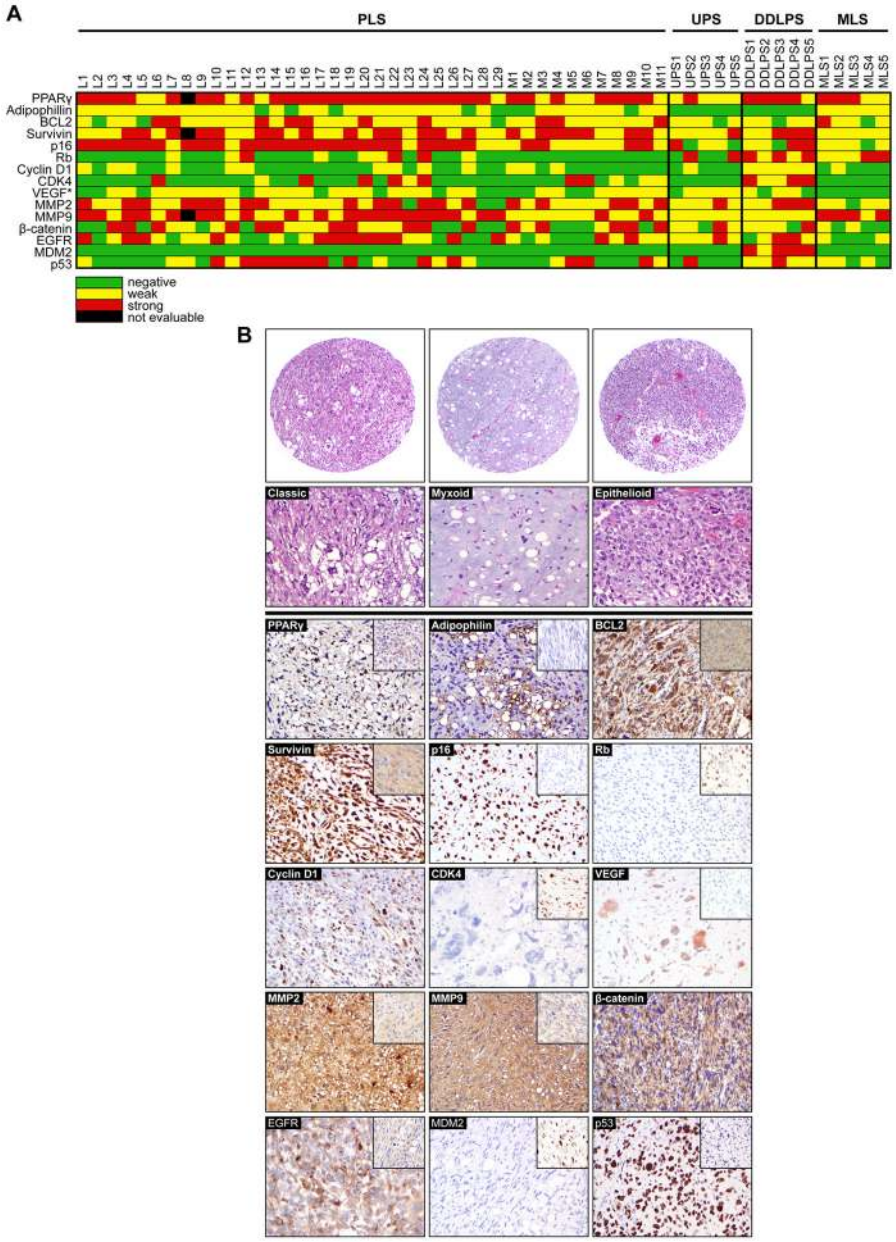

Figure 2. Protein biomarker expression in human PLS

A. "Heatmap" summarizing expression levels of each of the proteins evaluated in all individual scorable samples (* VEGF was only scored as positive or negative); *B.* Immunohistochemical images demonstrating representative levels of evaluated markers in human PLS specimens (controls included as insets: PPARγ = low expressing PLS, adipophilin = DDLPS, BCL2 = low expressing PLS, survivin = low expressing UPS/MFH, p16 = low expressing PLS, Rb = high expressing PLS, CDK4 = DDLPS, VEGF = negative expression PLS, MMP2&9 = negative expressing PLSs, EGFR = low expressing PLS, MDM2 = DDLPS, p53 = low expressing PLS). All original images were captured at ×200 magnification.

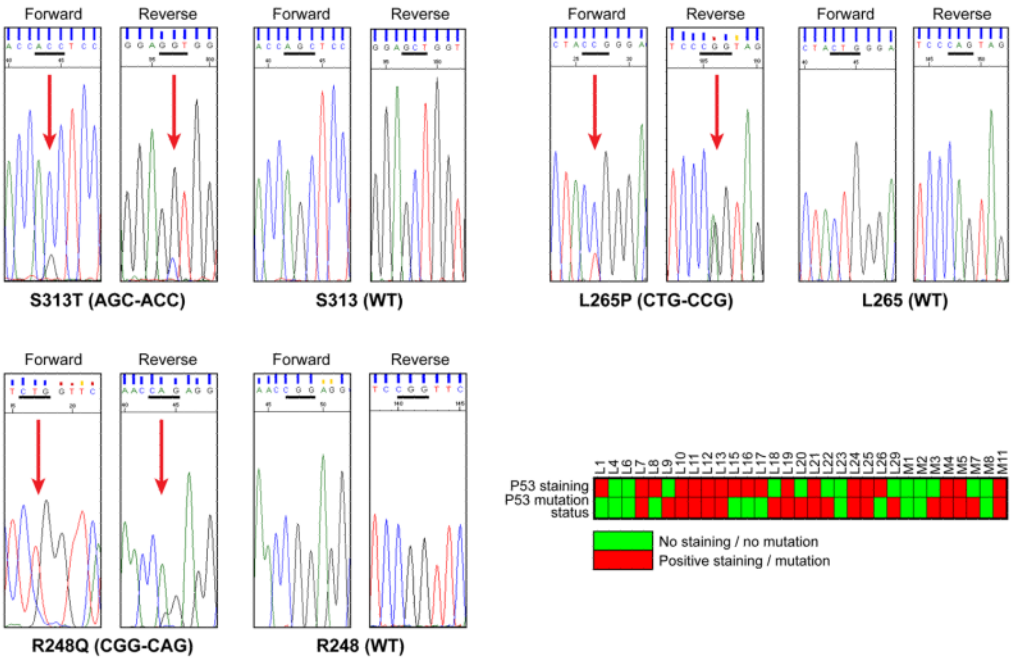

Figure 3. p53 mutations in PLS

Representative sequence chromatograms of the three most commonly occurring p53 mutations in PLS samples and tabulation of p53 mutational status and protein expression levels in individual PLS samples.

Table 1

PLS patient, tumor, treatment and outcome variables

(1A) Patient and tumor variables		
Variable	**Patients with follow-up (n=83) (%)**	
Median age (range)	53.4 ±16.8 (14–84)	
F/M	32/51 (1:1.6)	
Status at presentation		
Primary	60 (73%)	
Recurrent	6 (7%)	
Metastatic[1]	17 (20%)	
Average tumor size (range)[2]	11.1 ±7.25 (0.7–30)	
Tumor site[3,4]		
Extremity	49 (59%)	
Central	34 (41%)	
(1B) Treatment and outcome variables		
Variable	Patients with localized disease (n=66) (%)	Patients with metastatic disease (n=17) (%)
Treatment		
Surgery (Yes/No)	64 / 2 (97%)	6 / 11 (55%)[5]
Type of resection		
Complete surgery	62 (97%)	3/17 (18%)
R0	56 (90%)	
R1	6 (10%)	
Incomplete (R2)	2 (3%)	
Chemotherapy[6]	28 (45%)	17 (100%)
Neoadjuvant[7]	18 (67%)	
Adjuvant[7]	11 (41%)	
Radiotherapy[6]	43 (69%)	3 (18%)[5]
Neoadjuvant[7]	25 (60%)	
Adjuvant[7]	18 (43%)	
Outcome		
Median follow-up in months (range)[8]	31.5 (1.5–182.3)	8.5 (2.5–47.2)
Local recurrence[9]	16 (26%)	NA
Metastasis[10]	23 (35%)	
Site of metastasis[11]		
Lungs/Thorax	16 (70%)	NA
Liver	2 (9%)	
Abdomen	5 (22%)	NA
Skeleton	5 (22%)	
5 year disease specific survival[12]	0.65 (0.52, 0.78)	NA[13]

(1A) Patient and tumor variables		
Variable	**Patients with follow-up (n=83) (%)**	
Median overall survival	7.28 (2.9, 14.7)	0.76 (0.53, 1.58)

[1] Metastatic sites included: lungs 14, liver 3, skeleton 3 and others 1; 4 patients had multiple metastatic sites

[2] Tumor size was available for 53 pts, only primary lesions were considered

[3] Tumor location refers to the location of the primary tumor only; extremities include: upper (5%) and lower arms (3%), shoulder girdle (7%), lower (6%) and upper leg and buttock (34%), central includes: head & neck (4%), thorax (14%), abdomen (12%), pelvis (15%)

[4] Tumor location was not available for 2 patients

[5] Palliative surgery or radiotherapy in patients with metastatic disease

[6] Refers to patients who underwent complete resection (n=62)

[7] %Refer to those that underwent complete resection and received chemotherapy (n=27) or radiotherapy (n=42)

[8] Median follow up for the entire cohort of 83 pts. was 24.7 months

[9] Refers only to patients who underwent complete resection (n=62)

[10] Refers to all pts. with localized disease at presentation (n=66)

[11] Several patients had multiple site metastases; percentages refer to the group with metastatic disease only (23 pts.)

[12] 5YDSS for the entire group was 53%

[13] 1 year DSS for pts. with metastasis was 45%

Table 2

Univariable and multivariable Cox proportional hazard models for PLS disease specific survival

(2A) Univariable analysis			
Variable	**Levels**	**HR (95% CI)**	***P* value**
Tumor size	≥5cm versus <5cm	3.6 (0.83–15.64)	0.09
Status of disease	recurrent versus primary	3.04 (0.88–10.49)	0.08
Surgery	yes versus no	0.07 (0.01–0.38)	0.002
Surgical margins	R1 versus R0	3.96 (1.42–11.02)	0.008
Chemotherapy	yes versus no	2.94 (1.2–7.24)	0.02
(2B) Multivariable analysis			
Variable	Levels	HR (95% CI)	*P* value
Tumor status	recurrent vs. primary	5.05 (1.38–18.51)	0.01
Surgery	yes vs. no	0.04 (0.001–0.23)	0.0003
Margins	R1 vs. R0	5.87 (1.99–17.29)	0.001

Table 3

PLS biomarker expression analysis

Marker	staining positive, n (%)[1]	Biomarker expression level (%)	
		Low	Moderate / High
PPAR γ nuclear	39 (100%)	23	77
Adipophillin	32 (80%)	100	0
BCL2	37 (93%)	73	27
Survivin (cytoplasmic)	39 (100%)	46	54
Survivin (nuclear)	39 (100%)	97	3
p16 nuclear	40 (100%)	30	70
Rb	9 (23%)	67	33
Cyclin D1	13 (33%)	100	0
CDK4	12 (30%)	42	58
VEGF[2]	27 (68%)	--	--
MMP2	37 (93%)	49	51
MMP9	39 (100%)	44	56
Beta catenin	27 (68%)	41	59
EGFR	28 (70%)	50	50
MDM2	0 (0%)	0	0
p53	22 (55%)	41	59

[1] Total number of scorable specimens for each marker varied due to different rates of sample attrition

[2] VEGF staining was scored as positive vs. negative staining only

Table 4

Summary of published PLS clinical series

Ref.#	publication	Patients with FU	5y OS	5y DSS	Disease prognosticators
19	Downes et al, 2001	18	45%	50%	NA
3	Gebhard et al, 2002	48	57%	NA	Age, location, size, margins
4	Hornick et al, 2004	50	63%	NA	Location, size, necrosis, mitotic rate, epithelioid subtype
20	Fiore et al, 2007	60	NA	81%[1] 64%[2]	Size, status[3]
5	Dalal et al. 2006	64	NA	59%	Age, gender, status[3], Location, margins,
Current	Ghadimi et al. 2010	83[4]	49%	53%[5] 65%[6]	Margins, status[1]

[1] DSS of patients with primary disease

[2] DSS of patients with recurrent disease

[3] Status refers to tumor status at presentation (primary versus recurrent localized disease)

[4] Including 66 patients with localized disease and 17 with metastatic disease at presentation

[5] DSS of all evaluable patients

[6] DSS of patients with localized disease