Lipogenic tumors from a non-lipogenic angle
update on the pathology of fatty tumors

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Lipomatous tumors
lipogenic and non-lipogenic components

- Tumors with obvious lipogenesis:
  lipoma, atypical lipomatous tumor- well differentiated adipocytic liposarcoma (ALT-WDLPS)
- Tumors with “hidden” lipogenesis:
  spindle cell/pleomorphic lipoma, lipoblastoma, dedifferentiated liposarcoma, myxoid/round cell liposarcoma,
  pleomorphic liposarcoma
- The relationship between lipogenic and non-lipogenic cells is poorly understood
- Both components are important not only for diagnosis but also for prognosis and therapy

Lipomatous tumors
the non-lipogenic components

- Spindle cells: spindle cell lipoma, dedifferentiated liposarcoma (DDLPS)
- Pleomorphic stromal cells:
  pleomorphic lipoma, ALT-WDLPS/DDLPS, pleomorphic liposarcoma
- Round cells: myxoid/round cell liposarcoma
- Heterologous components: bone, cartilage, muscle (lipoma, ALT-WDLPS, DDLPS)

Lipogenic tumors
what is the relationship between the lipogenic and the non-lipogenic cells?

- The answer lies in the factors of embryologic fat development and in the specific genetic/molecular alterations of lipogenic tumors
- Adipocytes are not “born” as adipocytes and lipoblasts are not “born” as lipoblasts (they develop from mesenchymal precursor/stem cells)
- Lipogenesis is a complex process and signals differentiation
- Non-lipogenic cellular components represent the proliferative compartment of lipogenic tumors

Lipoma
Translocation: 12q13-15 with no amplification

Lipoma is obviously lipogenic with mature adipocytes but also rare non-lipogenic cells
Prominence of non-lipogenic elements in atrophic adipose tissue
- non-lipogenic spindle cells, branching capillaries

- fibrolipoma
- myxolipoma

- non-lipogenic elements in variants of lipoma
- fibrolipoma
- lipoma

Angiolipoma
- Liposarcoma

Myelolipoma
- Chondroid lipoma

"Heterologous" non-lipogenic differentiation in variants of lipoma

Spindle cell lipoma: prominence of non-lipogenic cells

Spindle cell lipoma: what do the non-lipogenic spindle cells represent?
The spindle cells are not fibroblasts in spindle cell lipoma

fibroblasts for comparison in a desmoplastic fibroblastoma
The spindle cells in reality are CD34 positive (S100 negative) dendritic cells.

Spindle cell lipoma

- 13q14 deletion and loss of RB1 and miR-15a and miR-16-1

- Rb is an important tumor suppressor protein that plays a role in cell cycle progression
- Loss of Rb protein leads to cell proliferation
- Rb also has a role in adipocytic differentiation
- 13q14 deletion in spindle cell and pleomorphic lipomas account not only for tumorigenesis but also focal block of lipogenesis

Spindle cell lipoma and its variants show no expression of MDM2, CDK4, p16 (in contrast to WDLS/DDLS)

- Conventional
- Spindle cell predominant
- Vascular
- Pseudoangiomatous
- Myxoid
- Multiple
- Mixed spindle cell/pleomorphic lipoma

Pseudoangiomatous spindle cell lipoma

Vascular spindle cell lipoma

Myxoid spindle cell lipoma
Myxoid spindle cell lipoma differs from myxolipoma.

Pleomorphic lipoma - myxoid

Loss of nuclear Rb expression

Pleomorphic lipoma - sclerotic

CD34

Floret cells

Lipoblasts

CD34

p16

MDM2

Lipomatous tumors
CD34 positive non-lipogenic cells

- Spindle cell lipoma: all the spindle cells are positive but adipocytes are negative
- Pleomorphic lipoma: both spindle cells and pleomorphic stromal cells are positive
- Well-differentiated liposarcoma: some pleomorphic stromal cells are positive but lipogenic cells are negative
- Key question: what do these CD34 positive non-lipogenic spindle cells represent? Part of the answer can be found in embryonic development of adipose tissue

Embryonic tissue, 8 weeks

Developing adipose tissue, preadipocytic stage

CD34
Adipose tissue stages of prenatal adipose tissue development

- Stage I: spindle cells in myxoid matrix
- Stage II: spindle cells condense around blood vessels
- Stage III: spindle preadipocytes in rich lobular capillary network
- Stage IV: accumulation of multiple lipid droplets in spindle cells (multivacuolated fat cells)
- Stage V: further accumulation of intracytoplasmic lipid (unilocular fat cells)

Embryonic development of adipose tissue

Develops from perivascular non-lipogenic spindle cells:
- Down-regulation of wnt10b, foxa-2 and pref-1
- Up-regulation of C/EBP β, δ, α and PPAR-γ → lipogenesis → development of lipoblasts/adipocytes
- Lipogenesis: associated with deposition of collagen IV and laminin around lipoblasts/adipocytes and expression of S100 protein

Embryonic development of adipose tissue upregulation of adipogenic factors

Mesenchymal progenitor cells → Adipose tissue adipocytes

- PPARγ
- C/EBPβ
- C/EBPα

Developing adipose tissue (18 weeks)

Mostly spindle cells (prelipoblasts) in a richly vascular myxoid matrix - also rare lipoblasts

Developing adipose tissue (21 weeks)

Foxa-2 still upregulated. Spindle cells (prelipoblasts) and multivacuolated lipoblasts

Developing adipose tissue (25 weeks) - Lipogenesis

Mostly multivacuolated lipoblasts. Downregulation of Foxa-2
Malignant lipogenic tumors: conceptual classification based on clinicopathologic and molecular genetic characteristics

- Dedifferentiated liposarcoma - atypical lipomatous tumor/well-differentiated liposarcoma
- Myxoid liposarcoma /round cell liposarcoma
- Pleomorphic liposarcoma

Chromosomal aberrations: malignant lipogenic tumors

- Atypical lipomatous tumor well-differentiated liposarcoma
  - Supernumerary ring chromosomes and long marker chromosomes from amplified segments of 12q14-15
- Dedifferentiated liposarcoma
  - Same as above, but increase in gene dosage effect and ...
- Myxoid/round cell liposarcoma
  - t(12;16)(q13;p11)
  - t(12;22)(q13;q11-12)
- Pleomorphic liposarcoma
  - Complex aberrations

Well-differentiated liposarcoma versus Atypical lipomatous tumor

- Synonyms
- Same morphology, cytogenetics, molecular genetics
- Complete surgical excision is curative
- No metastatic capability
- However, if excision is incomplete, local recurrence is common and 5-15% risk of malignant progression termed dedifferentiation with acquisition of metastatic potential

Preferred diagnostic term according to anatomic sites

Well-differentiated liposarcoma
- Retroperitoneum
- Spermatic cord
- Mediastinum

Atypical lipomatous tumor
- Skin
- Subcutis
- Muscle of extremities

Not to underestimate the significance of such a diagnosis!

Atypical lipomatous tumor well-differentiated liposarcoma
- 40-45% of liposarcomas
- Peak age fifth to seventh decade
- Mostly limbs and retroperitoneum
- Mostly deep-seated
- History of large, long-standing mass
- Recent rapid growth - dedifferentiation
**Atypical lipomatous tumor**
well-differentiated liposarcoma

**Histologic types**
- Adipocytic (lipoma-like)
- Sclerosing
- Inflammatory
- Dedifferentiated

**ALT – WDLPS (adipocyticlipoblast-rich variant**

**Relationship between non-lipogenic and lipogenic cells**

Lipoblasts

atypical non-lipogenic stromal cells

**Cytogenetic changes:** supernumerary ring chromosomes and long marker chromosomes from 12q 14 - 15

**Molecular genetics:** amplifications MDM2 and HMGA2 CDK4

**WD liposarcoma/atypical lipomatous tumor - MDM2**

immunohistochemistry

**WD liposarcoma/atypical lipomatous tumor - CDK4**

over-expression of p16
MDM2 - CDK4 – p16
role of immunohistochemistry in the diagnosis of well differentiated and dedifferentiated liposarcomas

- Most ALT/WDL/DDL express MDM2 (90%) CDK4 (86%) p16 (93%)
- 68% of ALT/WDLs and 72% of DDLS express all three antigens
- 100% of ALT/WDLs and 93% of DDLS express at least two antigens
- Useful in differentiating ALT/WDLs from benign fatty tumors
- Useful in differentiating DDLS from pleomorphic liposarcoma and myxoid liposarcoma
- Useful in differentiating DDLS from other poorly differentiated sarcomas

Thway K et al, Am J Surg Pathol 2012; 36:462-469

WD Liposarcoma - inflammatory

WD Liposarcoma – sclerosing

Scattered non-lipogenic and lipogenic cells

Fine fibrillary collagen

ATL/WD liposarcoma, adipocytic
selected differential diagnoses

- Lipoma variants
- Lipomatous hibernoma
- Lipoma-like angiomyolipoma
- Giant retroperitoneal lipoma
Conventional lipoma-like hibernoma versus hibernoma

WD liposarcoma versus hibernoma

Lipoma-like angiomyolipoma

Conventional
Hibernoma: 11q13

HMB45

Giant retroperitoneal lipoma (32 cm)
no bizarre stromal cells – no lipoblasts in 60 blocks examined

In one block, in the septum: "floret-type" stromal cells in a giant retroperitoneal lipoma

no amplification of MDM2 and CDK4
still a lipoma and NOT a liposarcoma

Dedifferentiated Liposarcoma
 accounts for 10% of liposarcomas
well-differentiated liposarcoma which shows abrupt transition, either in the primary tumor or in a recurrence, to a non-lipogenic sarcoma
90% de novo
10% in recurrences
5-year metastatic risk is 15 - 20%

Dedifferentiated liposarcoma
retroperitoneum, deep-seated tissue, rarely superficial location
numerous morphologic variants:
- high grade pleomorphic non-lipogenic sarcoma
- myxoid non-lipogenic sarcoma resembling low grade myxofibrosarcoma
- low grade spindle cell non-lipogenic sarcoma (fibromatosis-like)
- with meningothelial-like whorls
- richly vascular, nested, paraganglioma-like pattern
- with heterologous differentiation
Dedifferentiated liposarcoma - metastatic rate: 15 - 20%

Dedifferentiated liposarcoma

MDM2

CDK4

p16

Dedifferentiated liposarcoma with meningothelial-like whorls and ossification

SMA and focal Claudin-1 – myofibroblastic and possibly perineurial differentiation

Dedifferentiated liposarcoma – heterologous cartilaginous differentiation

Lipogenic tumors

heterologous elements do not always indicate dedifferentiation

Heterologous differentiation may occur not only in DD liposarcoma but also in ALT/WD liposarcoma and lipoma

- Focal cartilaginous differentiation
- Focal osseous differentiation
- Focal smooth muscle differentiation

In cases of “real” dedifferentiation the heterologous elements are mixed with other dedifferentiated sarcomatous components showing mitotic activity
When to consider a diagnosis of dedifferentiated liposarcoma?

- Not only in the classic histologic setting
- Soft tissue sarcomas which are difficult to classify
- Pleomorphic "undifferentiated" sarcomas
- Sarcomas with heterologous differentiation
- Sarcomas showing meningothelial-like whorls in the absence of other specific lineage of differentiation
- Richly vascular, nested, parangangioma-like neoplasm not expressing neuroendocrine markers

Progression of atypical lipomatous tumor – WD liposarcoma to dedifferentiated liposarcoma

- Atypical lipomatous tumor – WD liposarcoma: composed mainly of lipogenic cells (adipocytes, rare lipoblasts) and scattered non-lipogenic cells (atypical stromal cells)
- Dedifferentiated liposarcoma: composed of mitotically active non-lipogenic cells (spindle cells, pleomorphic cells, or heterologous tissue components)
- Key question: what molecular changes drive tumor progression from a biologically intermediate tumor to an aggressive neoplasm with metastatic capability and absence of lipogenecity?

Progression of ALT-WDLPS to DDLPS traditional concept: further amplification of sequences of 12q14-15 oncogenesis (MDM2, CDK4)

- Nutlin-3 MDM2 inhibitor reactivates the p53 pathway
- Flavopiridol CDK4 inhibitor causes decreased cell proliferation
Progression of WD liposarcoma to dedifferentiated liposarcoma
recent data: better understanding of tumor progression and blocked lipogenecity

- General concept: dedifferentiation of WD liposarcoma likely to occur via multiple alternative genetic alterations
- In addition to the 12q14-15 amplicon, there is co-amplification of JUN at 1p32 or ASK1 at 6q23 in dedifferentiated liposarcoma
- Co-amplification of JUN and ASK1 are mutually exclusive and never seen in pure WDLPS

WD liposarcoma – Dedifferentiated liposarcoma
mechanism of progression

- Amplified c-Jun in dedifferentiated liposarcoma is interspersed with amplified MDM2 in ring and giant marker chromosomes which suggests that c-Jun is amplified at a similar time in the evolution of the tumor
- c-Jun amplification and expression can be found in the well-differentiated component of dedifferentiated liposarcoma, suggesting that c-Jun amplification may occur before dedifferentiation
- c-Jun protein is expressed in the majority of dedifferentiated liposarcomas (91%) and their well-differentiated components (59%), but only in the minority of pure well-differentiated liposarcomas (27%)

Dedifferentiated Liposarcoma
both c-JUN and ASK1 oncogenes can block the adipocyte differentiation program
Dedifferentiated Liposarcoma

Both c-JUN and ASK1 oncogenes can block the adipocyte differentiation program.

Clinical Trials:
Thioredoxin – ASK1 antagonist
Aplidin – JNK activation - apoptosis

Well differentiated liposarcoma

Progression of well differentiated liposarcoma to dedifferentiated liposarcoma

Pathway 1
Amplicon: MDM2, HMGA2
Indolent tumor
Low rate of dedifferentiation
Presents as WD liposarcoma

Pathway 2
Amplicon: MDM2, HMGA2, c-Jun or ASK1
Aggressive tumor
High rate of dedifferentiation
Presents as DD liposarcoma

WD liposarcoma – Dedifferentiated liposarcoma
models of progression
(Fletcher CDM et al, J Pathol 2009)

Model 1: minimum number of oncogene (MDM2 and HMGA2) amplification associated with indolent tumor, low rate of dedifferentiation and WD liposarcoma at presentation

Model 2: not only MDM2 and HMGA2 but also c-Jun or ASK1 amplification providing additional oncogenic stimulus leading to increased proliferation, faster progression and dedifferentiation at the time of diagnosis

Model 2 is in accordance with the observation that all of the c-Jun-amplified tumors published so far presented with dedifferentiation

WD liposarcoma – Dedifferentiated liposarcoma
another suggested mechanism of dedifferentiation
(Helias-Rodzewicz Z et al, Genes Chromosomes & Cancer 2009; 48:943 - 952)

DD liposarcoma: amplified segments of CDK4 gene is often integrated into chromosome arms, which are stable – CDK4 protein promotes cell proliferation, thus precluding adipocytic differentiation

WD liposarcoma: amplified segments of CDK4 gene are located in ring chromosomes - ring chromosomes are unstable and often eliminated in the form of micronuclei

In tissue culture, a dramatic increase of of adipocytic differentiation seen in cells that have eliminated copies of CDK4 gene in micronuclei

Selective elimination of CDK4 sequences in micronuclei correlates with spontaneous adipocytic differentiation in liposarcoma
(Helias-Rodzewicz Z et al, Genes Chromosomes & Cancer 2009; 48:943-952)

Myxoid/round cell liposarcoma

30 - 35% of liposarcomas
Peak age 3rd to 5th decade
Slight male predominance
Predilections for limbs, especially thigh
Mostly deep-seated
Multiple soft tissue metastasis
5-year survival, pure myxoid: 90%
5-year survival, pure round cell: 25%
Myxoid liposarcoma only rare lipoblasts

Variant of myxoid liposarcoma with myxoid pools

Myxoid liposarcoma
differential diagnosis

- Myxofibrosarcoma, low grade
- Well differentiated liposarcoma with myxoid change
- Lipoblastoma
- Myxolipoma
- Intramuscular myxoma
- Spindle cell lipoma, myxoid vascular

Pseudolipoblasts with mucousubstance rather than lipid in their cytoplasm

Myxofibrosarcoma mimicking myxoid liposarcoma

Lipoblastoma – immature myxoid mimicking myxoid liposarcoma
Lipoblastoma – Lipoblastomatosis
chromosomal rearrangements of 8q11-13 with activation of PLAG1

- Lower extremities
- Upper extremities
- Head and neck
- Trunk
- Mediastinum
- Mesentery
- Retroperitoneum

Lipoblastoma - Lipoblastomatosis
- Benign fatty tumor
- Occurs in infancy and early childhood
- 90% of cases present before 3 years of age
- 40% of cases occur before 1 year of age, occasionally at birth
- Sporadic examples above the age of 10 years
- Some lipomas in adults with 8q11-12 aberration could represent fully mature lipoblastomas
- Recurrence rate: 14% (Chung and Enzinger), 13% (Coffin and Williams), 25% (Mentzel et al)

Liposarcomas in children
- Exceedingly rare - most cases reported before 1959 probably represent lipoblastomas
- Nearly 90% of pediatric liposarcomas occur in the second decade of life
- Median age: 13 years (Schmookler and Enzinger, 1983), 18 years (LaQuaglia et al., 1993)
- No credible example below the age of 3 years
- Histology: myxoid liposarcoma (majority), well-differentiated, round cell, pleomorphic (rarely)

Round cell liposarcoma
- a poorly differentiated form of myxoid liposarcoma

Round cell liposarcoma – mostly non-lipogenic cells

S-100

Round cell liposarcoma

Calretinin

PPAR
Myxoid - Round cell liposarcoma – focal lipogenecity

Round cell population Metastasis
- 0 – 5% 23%
- 5 -10% 35%
- >25% 58%

Myxoid/Round cell liposarcoma correlation with clinical outcome

Myxoid/Round cell liposarcoma
- Less than 5% of round cell differentiation: grade I, “myxoid liposarcoma”
- 5 - 25% of round cell differentiation: grade II “mixed myxoid and round cell liposarcoma”
- More than 25% of round cell differentiation: grade III, “round cell liposarcoma”

Myxoid/Round cell liposarcoma genetic aberrations
- t(12;16)(q13;p11) fusion protein FUS(TLS)-DDIT3 (CHOP)
- t(12;22)(q13;q12) fusion protein EWS-DDIT3 (CHOP)

Myxoid liposarcoma “transitional area”
- Histologically between myxoid and round cell liposarcoma
- Hypercellular compared with the low cellularity of typical myxoid liposarcoma
- Tumor cells (mostly non-lipogenic) are separated some myxoid stroma
- Plexiform vascular pattern is discernible

FUS-DDIT3 oncogene of myxoid/round cell liposarcoma blocks the adipocyte differentiation program
Therapy 1: Trabectedin (a natural marine compound) causes detachment of the FUS-DDIT3 chimera from the targeted promoters and induces differentiation in myxoid/round cell liposarcoma.

**Peroxisome Proliferator-Activated Receptor-Gamma (PPAR γ)**
- A member of the nuclear receptor family
- A key transcriptional regulator of cell differentiation and lipid metabolism
- Main role in adipocyte differentiation
- Most round cell, dedifferentiated and pleomorphic liposarcomas express PPAR γ
- Biological receptor for the thiazolidinedione class of antidiabetic drugs (troglitazone, rosiglitazone etc.)

**Pleomorphic liposarcoma**
- Lower extremity (36.5%), upper extremity (16%), thoraco-abdominal wall (9.5%), internal trunk (20.9%)
- "MFH"-like, epithelioid/carcinoma-like, round cell liposarcoma-like
- Median follow-up 38 months: 45% local recurrence rate; 42.5% metastatic rate; 35% mortality

**Diagnosis of liposarcoma**
- Liposarcoma unqualified is not a diagnosis
Diagnosis of liposarcoma

- Reliable diagnosis of liposarcoma can not always be made on histological ground alone, especially on needle core biopsies
- Patient’s age
- Location of tumor
- Tissue plane

Lipoblasts and the diagnosis of liposarcoma

- Identification of lipoblasts is helpful in the diagnosis of liposarcoma
- Lipoblasts do not automatically make the diagnosis of liposarcoma valid
- Liposarcoma can be diagnosed in the absence of lipoblasts
- Lipoblast is only one of the cellular constituents of liposarcoma
- Non-lipogenic tumor cells are equally important during diagnostic considerations

Lipoblast

- Multivacuolated or signet-ring

NUCLEUS
- Normochromatic
- Hyperchromatic
- Single
- Multinucleated
- Must be indented

LIPID VACUOLE
- Perfectly clear
- Sharp margin
- Circular
- Single or multiple

Appropriate histologic background with non-lipogenic tumor cells

Multivacuolated and signet-ring lipoblasts

Pseudolipoblast (myxofibrosarcoma)
Silicon granuloma
Vacuolated GIST

Lipogranuloma (lipid in macrophages)

Imics of lipoblasts
Lipogenic tumors

Summary
- Both lipogenic and non-lipogenic cellular elements are important not only diagnostically and but also biologically
- Non-lipogenic cells represent the proliferative compartment of lipogenic tumors
- Lipogenesis signals differentiation and decreased cell proliferation
- Molecular pathways of lipogenic tumors can be exploited for therapy
- Lipogenic tumors may occur in "disguise" when the lipogenic elements are hidden or only focally present

Lipomatous tumors in "disguise" lipogenicity is hidden or only focally present
- Lipoblastoma/lipoblastomatosis
- WD inflammatory and sclerosing liposarcoma
- Dedifferentiated liposarcoma
- Myxoid/Round cell liposarcoma
- Pleomorphic liposarcoma

Dominance of non-lipogenic elements = increased proliferation rate, metastatic potential