SY-20 – Soft Tissue and Bone Pathology: Diagnostic molecular pathology of bone and soft tissue tumors: techniques, translation and targets – Genomic insights in sarcomas

31st ECP; Nice, France
Tuesday 10 September 2019; 14:45 – Athena Auditorium

Alexander Lazar MD/PhD
Professor
Departments of Pathology, Genomic Medicine & Dermatology
Sarcoma Research Center
I have the following financial relationships to disclose:


These relationships are **NOT** relevant to the educational content of this lecture.
Road Map of the Talk

• Quick sarcoma introduction

• Sarcoma and Cancer Genome introduction

• The Cancer Genome Atlas (TCGA) in a nutshell:
  – Sarcoma Biomarker
  – Clinical implications of Sarcoma TCGA
  – Related sarcoma studies
Sarcoma Classification

• Can be challenging

• Heterogeneous group of tumors with >50 histological subtypes, each with varying clinical phenotypes and behavior

• Some tumors are unclassifiable

• Many benign entities (100:1), some of which can be confused for sarcomas
Sarcoma Classification:
Histogenesis/Line of Differentiation

- Osteosarcoma
- Chondrosarcoma
- Angiosarcoma
- Liposarcoma
- Synovial Sarcoma
- UPS
- Leiomyosarcoma
- Rhabdomyosarcoma
- MPNST
- Ewing Sarcoma/PNET
- ASPS

Courtesy of Dr. Brian Rubin, CCF
Sarcoma Classification: Line of Differentiation

- Morphologically or by immunohistochemical studies
- Beware of mimics
- Some tumors lack a normal counterpart (i.e. synovial sarcoma)
- Not all tumors with similar differentiation will behave the same
## FNCLCC Grading

- **Mitotic Count.** In the most mitotically active area, ten successive high-power fields (at 400x magnification=0.1734 mm²) using a 40x objective.
  - 1 0-9 mitoses per 10 HPFs
  - 2 10-19 mitoses per 10 HPFs
  - 3 >20 mitoses per 10 HPFs

- **Tumor necrosis.** Evaluated on gross examination and validated with histological sections
  - 0 No tumor necrosis
  - 1 <50% tumor necrosis
  - 2 >50% tumor necrosis

- **Degree of Differentiation.** 1-3

### Grading

- **Grade 1:** 2-3
- **Grade 2:** 4-5
- **Grade 3:** 6-8

- All three numbers are summated to determine degree of differentiation

- Proven to correlated well with survival
Sarcoma Genomic Classification

• **Simple karyotype (recurrent)**
  – Translocation (SS, RMS, DFSP, MLPS, etc)
  – Activating Gene Mutations (GIST, desmoid)

• **Intermediate (recurrent)**
  – WD/DD liposarcoma

• **Complex karyotype**
  – *TP53*, disrupted chromosome management
  – UPS, MFS, pleomorphic liposarcoma
TCGA – Integrative Analysis

Modified from The Cancer Genome Atlas Pan-Cancer analysis project
Pan-Cancer 33 Effort

Multiplatform Analyses

- Copy Number
  - 5' to 3'
  - Whole Exome

- DNA Methylation
  - 5' to 3'
  - Whole Genome

- mRNA
- miRNA
- Protein
- Histology
- Clinical Data

Data Normalization

- PanCancer analysis
- PanGYN
- PanSquamous
- Immune landscape
- Driver mutation discovery
- Tumor heterogeneity
- Pathway analyses

Gynecologic
- USC, UCEC, CESC, OV, BRCA

Genitourinary
- PRAD, TCGT

Other endocrine
- ACC, PCPG

THCA, THYM, MESO, LIHC, CHOL, KICH, KIRC, KIRP, PAAD, DLBCL, SARC, LGG, GBM, UVM, HNSCC, ESCA, SKCM, LUAD, LUSC, STAD, COAD, BLCA, READ, AML
Comprehensive and Integrated Genomic Characterization of Adult Soft Tissue Sarcomas

The Cancer Genome Atlas Research Network1,2,*
1Cancer Genome Atlas Program Office, National Cancer Institute at NIH, 31 Center Drive, Bldg. 31, Suite 3A20, Bethesda, MD 20892, USA
2Lead Contact (Alexander J. Lazar)
*Correspondence: elizabeth.demicco@sinahealthsystem.ca (Elizabeth G. Demicco), lding@wustl.edu (Li Ding), ladanya@mskcc.org (Marc Ladany), alazar@mdanderson.org (Alexander J. Lazar), singers@mskcc.org (Samuel Singer)

https://doi.org/10.1016/j.cell.2017.10.014

Graphical Abstract

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Leiomyosarcoma
- Uterine LMS poor prognosis
- Soft tissue LMS
- \textsuperscript{117}p11.2-p12 methylation
- inflam. score
- methylation
- STLMS C1 poor prognosis
- STLMS C2 good prognosis

Myxofibrosarcoma
- Undifferentiated Pleomorphic Sarcoma
- MFS
- UPS
- Molecular spectrum
- Methylated predicts survival

Dedifferentiated Liposarcoma
- hypo-methylated
- hyper-methylated
TCGA Sarcoma Case Selection

Initial Diagnoses
- LMS (n=94)
- DDLPS (n=55)
- UPS (n=48)
- MFS (n=20)
- MPNST (n=8)
- SS (n=10)
- DESMO (n=2)

Final Diagnoses
- LMS (n=80)
- DDLPS (n=50)
- UPS (n=44)
- MFS (n=17)
- MPNST (n=5)
- SS (n=10)
- DESMO (n=2)
- Not Sarcoma (n=6)
- Uncertain Type (n=17)
- Inappropriate Type (n=5)
- 2nd Malignancy (n=1)

The Cancer Genome Atlas
Tumor Map – Josh Stuart, UCSC
Tumor Map
Unbalanced Copy Number
COSMIC Mutational Signatures in Sarcoma
Mutations in Sarcoma
Specific Mutations in SMGs
Sarcoma SMGs are tumor suppressors
Sarcomas have low and high CNV states
Image Analysis & Mutational Signatures

**B**

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<th>Whole genome doublings</th>
<th>Area Variance</th>
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<tr>
<td>0</td>
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<tr>
<td>2</td>
<td>15000</td>
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<td>3</td>
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*p = 0.0003*

**C**

- **Histology**
  - DDLPS
  - MFS
  - MPNST
  - SS
  - STLMS
  - ULMS
  - UPS

- **Whole genome doublings**
  - 0
  - 1
  - 2

- **Samples**
  - TCGA-3B-A913
  - TCGA-IE-A3OV
  - TCGA-QQ-A5V9

**Graphs**

- **Subclonal Genome Fraction** vs. **Area Variance**
  - Correlation: 0.342
  - Intercept: -0.0471
  - Slope: 8500
  - *p* = 0.0006

- **Aneuploidy Score (Number of Events)** vs. **Area Variance**
  - Correlation: 0.338
  - Intercept: -0.0247
  - Slope: 84.3
  - *p* = 0.0006

*The Cancer Genome Atlas*
Immune Microenvironment

C

CD8

Macrophages

PD-1

PD-L1

D

ULMS - CD8

STLMS - NK cells

MFS - iDC

DDLPS - T_2

E

<table>
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<th>NK cells</th>
<th>ULMS</th>
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Significant Survival Difference:
- Green: Good outcome
- Purple: Poor outcome
- No Significant Survival Difference

p-values:
- P = 0.0172
- P = 0.0364
- P = 0.00418
- P = 0.00244
Pembrolizumab in advanced soft-tissue sarcoma and bone sarcoma (SARC028): a multicentre, two-cohort, single-arm, open-label, phase 2 trial


UPS: pre-tx and at 20 weeks
Leiomyosarcoma

Figure 5

A

Histology: ULMS STLMS
Gender: F M
iCluster LMS: C1 C2
iCluster STLMS: C1 C2
mRNA LMS cluster: C1 C2 C3
Meth LMS cluster: 1 2 3
SCNA LMS cluster: C1 C2 C3
FDR <1e-22 TP53 2e-15 RB1 0.49 PTEN

# Mutations

Mutation type: Nonsense Splice Site Other Non Syn Synonymous
Frameshift Missense In-frame Indel No Mutation
Leiomyosarcoma
Leiomyosarcoma
Leiomyosarcoma
Leiomyosarcoma - RPPA
Examination of Mutations in BRAF, NRAS, and PTEN in Primary Cutaneous Melanoma

Vikas K. Goel¹,²,⁵, Alexander J.F. Lazar²,³,⁵,⁶, Carla L. Warneke⁴, Mark S. Redston²,³ and Frank G. Haluska¹,²

Frequent somatic mutation of v-raf murine sarcoma viral oncogene homolog B (BRAF), a downstream effector of the rat sarcoma oncogene (RAS) signaling pathway, is described in melanoma and other tumors. Our analysis of melanoma cell lines suggests that activating mutations in BRAF can occur simultaneously with inactivation of phosphatase and tensin homolog (PTEN), but neuroblastoma RAS (NRAS) mutations are not coincident. We determined the concurrent prevalence of mutations in BRAF and NRAS, and alteration of PTEN expression in 69 primary cutaneous melanomas. BRAF mutations were seen in 57% of cases, NRAS were mutated in 47% of samples, exclusively in exon 2. Two cases showed concurrent immunohistochemistry, PTEN protein expression was lost or greatly reduced in tumors with reduced PTEN yielded DNA amenable to sequencing, and three had NRAS. In all, 11 (85%) of 13 tumors showing reduced PTEN expression had an association of increasing Breslow thickness and loss or reduction of PTEN (P<0.0001). Mutations in NRAS were not coincident with reduced PTEN mutation of NRAS and BRAF was rare.

**PTEN Loss Correlates with ↑AKT Activation**

- RPPA of 96 OCT-embedded melanoma metastases & 58 cell lines
  - Y-axis, P-AKT expression; Color ~ PTEN expression
- *Compared PI3K-AKT pathway activation to mutation status*

**Tumors (n=96)**

- Loss of PTEN associated with greater activation of PI3K pathway (P-AKT) than NRAS mutations
- ~Results in tumors and cell lines

*Supports that PTEN is the critical regulator pathway in melanoma*

Davies, CCR, 2009
***PTEN IHC on FFPE Melanoma***

Intensity relative to internal (+)ive controls

- **Clonal**
- **Absent**
- **Mildly Reduced**
- **Markedly Reduced**
- **Normal/Increased**
Correlation of PTEN loss in melanoma cells with an immune resistance phenotype.


©2016 by American Association for Cancer Research
Loss of PTEN Is Associated with Resistance to Anti-PD-1 Checkpoint Blockade Therapy in Metastatic Uterine Leiomyosarcoma

Graphical Abstract

Resection of primary uterine leiomyosarcoma → Metastatic disease treated with anti-PD-1 monotherapy → Resection of only resistant metastasis → Sustained tumor control (>2 years)

Immunohistochemical assessment
Whole exome sequencing
Whole transcriptome sequencing
Neotagin prediction

Immunophenotyping
Evaluation of patient T cell reactivity to tumor-specific neoantigens

Discovery of resistance-associated genomic features

PTEN

KGGFPAQQW
RVHPYQQIV

Improved patient selection for immune checkpoint therapy

Authors
Suzanne George, Diana Miao, George D. Demetri, ..., Patrick A. Ott, Kwok-Kin Wong, Eliezer M. Van Allen

Correspondence
eliezerm_vanallen@dfci.harvard.edu

In Brief
George et al. report an exceptional responder to anti-PD-1 monotherapy in uterine leiomyosarcoma and propose mediators of treatment sensitivity and resistance. Neoantigen-directed immunoreactivity was associated with sensitivity to anti-PD-1 monotherapy, whereas resistance was associated with reduction in neoantigen expression consistent with immune evasion, and biallelic PTEN loss was associated with induction of an immunosuppressive microenvironment.
Rearrangement bursts generate canonical gene fusions in bone and soft tissue tumors

A  Break-fusion-bridge

- break
- replication
- fusion
- break
- radiation or chemical damage
- fragile sites
- replication dependent DNA break
- transposon
- eroded telomere

B  Chromothripsis

C  Chromoplexy

Cancer cell nucleus

http://neuropathologyblog.blogspot.com/2015/10/chromothripsis.html
The SS18-SSX Fusion Oncoprotein Hijacks BAF Complex Targeting and Function to Drive Synovial Sarcoma

Graphical Abstract

Authors
Matthew J. McBride, John L. Pulice, Hannah C. Beird, ..., Javed Khan, Alexander J. Lazar, Cigall Kadoch

Correspondence
cigall_kadoch@dfci.harvard.edu

In Brief
Incorporation of the synovial sarcoma SS18-SSX fusion into BAF complexes results in concomitant eviction of BAF47. McBride et al. show that SS18-SSX retargets BAF complexes from enhancers to polycomb domains to oppose PRC2-mediated repression. Repopulation of BAF47 upon suppression of SS18-SSX restores enhancer activation but is not required for proliferative arrest.
A very different picture than in BAF loss-of-function cancer types – this is why these diseases are different! Different BAF complex mechanisms on chromatin!

Nakayama, Pulice, Valencia et al., Nature Genetics 2017
RNAseq reveals distinct SS gene expression signature, consistent with different chromatin changes between SS18-SSX complexes and LOF (i.e. loss of SMARCB1/loss of SMARCA4, etc) BAF complexes.
Conclusions

• Sarcoma genomics is a bit complicated
• Complex karyotype sarcomas are driven by copy number alterations and loss of TSG
• Translocation-associated sarcomas can have complex mechanisms to produce fusions
• Epigenetics is an important component of sarcoma tumor biology
Thanks!