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TITLE: Classification and Genetics of Sarcomas

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Slide 1:
Hello, my name is Olena Morozova. I am a Clinical Molecular Genetics Fellow at University of California, San Francisco. Welcome to this Pearl of Laboratory Medicine on “Classification and Genetics of Sarcomas”.

Slide 2:
Sarcomas are a heterogeneous group of tumors of mesenchymal origin. Sarcomas can be grouped into two main classes: bone sarcomas and soft-tissue sarcomas. There are more than 100 different histopathological subtypes of sarcomas defined, and about 50% of them are associated with characteristic somatic molecular abnormalities, often gene fusions. In addition, sarcomas are often associated with a germline predisposition syndrome.

Slide 3:
Bone sarcomas are very rare and represent less than 0.2% of human cancers. There are about 3000 diagnoses and 1500 deaths a year. Bone sarcomas most often occur in older children, as well as adolescents and young adults. Most common subtypes of bone sarcomas are osteosarcoma (about 35% of cases), chondrosarcoma (about 30% of cases), and Ewing sarcoma (about 16% of cases). Ewing sarcomas are associated with a gene fusion involving EWS and FLI1 genes.

Slide 4:
Soft tissue sarcomas are a bit more common than bone sarcomas and represent less than 1% of human cancers. There are about 12,000 diagnoses and 4,500 deaths a year. Soft-tissue sarcomas are diverse with over 100 different subtypes that can occur in different age groups. Roughly half of the soft tissue sarcoma subtypes are associated with a recurrent cytogenetic abnormality. Some subtypes with the associated genetic abnormality are listed on this slide.

Slide 5:
The next few slides show examples of different types of sarcomas. Here is an H&E image demonstrating a monotonous population of small blue round cells in a soft tissue tumor. This is an example of a Ewing sarcoma.

Slide 6:
The next H&E depicts a well-differentiated liposarcoma with variable-sized adipocytes with nuclear atypia.

Slide 7:
This slide shows an H&E demonstrating a proliferation of atypical spindle cells with laying down of malignant osteoid. This is an osteosarcoma.

Slide 8:
And finally, this H&E shows an atypical fascicular proliferation of spindle cells with increased mitotic index. This image is characteristic of an undifferentiated pleomorphic sarcoma or UPS.

Slide 9:
Sarcoma genomes fall into one of three classes: those with a simple karyotype with a defining translocation/gene fusion; those with a simple or complex karyotype with a specific mutation; and those with a complex karyotype with multiple chromosomal rearrangements, duplications and deletions.

Slide 10:
Gene fusions are common molecular events in sarcomas. As a result of some sort of genomic rearrangement, two genes are juxtaposed together. They are then transcribed as one chimeric product creating a fusion transcript. Gene fusions can result in a loss of function of one or both partners, a gain of function of one partner or a completely novel function.

Slide 11:
Gene fusions can be detected using a variety of molecular assays. Fluorescence in-situ hybridization (FISH), specifically break-apart FISH, is a molecular test, used clinically for detecting gene fusions in sarcomas. This test employs two fluorescent probes of different colors that hybridize to two different regions on a chromosome. The two probes are designed such that they map proximal and distal to the fusion breakpoints in the gene of interest. When the probes are fused, it indicates a normal gene; if the probes are apart, it indicates a break in the gene, irrespective of the fusion partner. Specific gene fusions can also be detected using PCR, performed on either the DNA or RNA material. Next-generation DNA sequencing can be used to detect a variety of different fusions in a sample. Gene panel sequencing approaches can detect fusions in genes of interest. An important gene panel design consideration is to include intronic sequence,

as fusion breakpoints often occur in introns. Whole genome sequencing is an attractive comprehensive approach, which is currently used in a research setting. Finally, RNA sequencing can be also used in a panel format or as a whole transcriptome to detect both known and novel fusions. RNA sequencing may have an added advantage of being able to quantify the amount of the fusion transcript.

Slide 12:
This image demonstrates a sarcoma positive for an *EWSR1* rearrangement, assayed by break-apart FISH. Green and red fluorescently labeled probes flank the *EWSR1* locus. Cells negative for an *EWSR1* gene fusion have two yellow signals, produced from the overlap of the green and red signals. Cells with split red and green signals are positive for an *EWSR1* gene fusion.

Slide 13:
This slide illustrates the principle of detecting gene fusions using next-generation DNA or RNA sequencing. In both of these approaches, sequencing reads are mapped to a reference genome and then the alignments are examined to assign the reads into three different categories. An important note here is that paired-end sequencing, where nucleic acid fragments are sequenced from both ends, is typically used for detecting gene fusions. Concordant reads are those that have mate pairs (reads from the two ends of the same fragment) mapping to same gene. Split reads are those that map across a fusion breakpoint (these reads may be lost when mapping to a reference), while spanning reads are those that have mate pairs mapping to two different genes (fusion partners). The detection of both spanning and split reads would indicate the presence of a gene fusion.

Slide 14:
Molecular testing has several applications for the management of patients with sarcomas: arriving at diagnosis for cases with ambiguous histology (for example. diagnosis of small round cell tumors that have similar appearance under the microscope), refining molecular subtype of the disease (for example, alveolar subtype of rhabdomyosarcoma is associated with PAX-FOXO fusions, while embryonal rhabdomyosarcoma is not); Identifying treatment options (for examples, KIT and PDGFRA mutations in Gastro-Intestinal Stromal Tumors (GIST) are associated with response to Imatinib); confirmation of lesions with an unusual presentation (for example, confirmation of a synovial sarcoma in a 76-year-old individual), and finally aiding in diagnosis for cases with de-differentiated histology.

Slide 15:
I would like to highlight further one particular application of molecular testing for the management of sarcomas. SRCTs have homogeneous light microscopic appearance of small round-cell neoplasms like we saw on one of the previous slides. Several entities may present in this way: Ewing sarcoma, rhabdomyosarcoma, mesenchymal chondrosarcoma, desmoplastic small round cell tumor, round cell liposarcoma, poorly

differentiated synovial sarcoma, and neuroblastoma. Molecular testing can be used to identify a characteristic gene fusion can help make the diagnosis (for example, the identification of an EWS-FLI1 fusion would be diagnostic of a Ewing sarcoma)

Slide 16:
Several cancer predisposition syndromes are associated with an increased risk of sarcomas. Li-Fraumeni syndrome, caused by germline heterozygous inactivating mutations in TP53 is associated with childhood-onset sarcomas. Retinoblastoma syndrome, caused by germline heterozygous inactivating mutations in RB1 is associated with osteosarcomas. Familial adenomatous polyposis (FAP), caused by germline heterozygous inactivating mutations in APC
Is associated with desmoid tumors.

Slide 17:
There are also several genetic syndromes, which have many different phenotypic manifestations, including a predisposition to sarcomas. Some examples of these conditions are listed on the slide.

Slide 18:
Here are some take home messages from my talk. Sarcomas are a heterogeneous group of diseases with over 100 subtypes defined by histopathology. Genetic testing can help with the diagnosis, molecular subtyping, and treatment selection
A significant fraction of sarcomas are associated with either a germline cancer predisposition syndrome or a genetic syndrome with other phenotypic features

Slide 19: References

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Slide 20: Disclosures
I have no disclosures to make or conflicts of interest.

Slide 21: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on “Classification and Genetics of Sarcomas.”