DNA methylation analysis of undifferentiated pleomorphic sarcoma of bone

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Significance

Undifferentiated pleomorphic sarcoma of bone (UPS-B) is a diagnostic and treatment challenge: although it arises in bones, it microscopically resembles the more common undifferentiated pleomorphic sarcoma of soft tissues (UPS-ST). Like UPS-ST, UPS-B lacks reliable immunohistochemical markers for diagnosis. Optimal treatment strategies are similarly unclear, and it carries a poor prognosis.

Innovation

DNA methylation studies have been shown to be a promising diagnostic adjunct in bone and soft tissue sarcomas; however, this technology has not been utilized to study or classify UPS-B. Improved characterization of these tumors may lead to improved prognostic capabilities and clinical decision-making for both surgical and medical oncologists regarding treatment of this devastating disease with an unmet medical need.

Approach

Eleven samples of untreated UPS-B, 10 of UPS-ST, and 11 of adult-onset (>40 years) osteosarcoma, all histologically confirmed by two bone/soft tissue pathologists, underwent genome-wide methylation sequencing using the Illumina Infinium Human Methylation EPICv2 array interrogating 935,000 CpG sites. Methylation data were inputted into a publicly available sarcoma methylation classifier (Heidelberg Epignostix Sarcoma Classifier, v13.1). This utilizes a random forest algorithm based on a training set of >1,000 sarcoma samples. Imputation of a tumor's methylation profile returns a most-likely diagnostic class and a confidence score (CS) for the diagnosis between 0 and 1.

Results

All cases passed genetic and quantitative quality control metrics. A mapping of histologic to methylation-based diagnoses are seen in Figure 1.

Of UPS-B, 64% (7/11) were predicted to be of the UPS/myxofibrosarcoma spectrum, though only two of these had high (>0.9) CSs (Table 1). Of six secondary UPS-B, four were radiation-associated, two (including one of the radiation-associated) were associated with bone infarct, and one with fibrous dysplasia. These were predicted to be different classes (UPS/MFS spectrum [3], malignant peripheral nerve sheath tumor (MPNST) [2], and histiocytic sarcoma [1]); only one had a high confidence level (0.983) which was mapped to "MPNST." UPS-ST were consistently (90%; 9/10) predicted by the classifier as the UPS/myxofibrosarcoma spectrum; all nine had confidence levels >0.9.

Only 27% (3/11) of osteosarcomas had confidence levels above 0.9. Five tumors in the cohort were predicted to be osteosarcoma by the methylation classifier. Upon later review, one of tumor mapped to osteoblastoma was histologically re-classified as "osteoblastoma-like osteosarcoma."

Discussion

This is the only study to date of UPS-B methylation profiles. The Epignostix methylation classifier did not include UPS-B in their initial training cohort, which could account for the fact that only a third had high CSs in

their predictions. These results may imply that many UPS-B tumors occupy separate classes in a methylation-based ontology, or of significant epigenomic heterogeneity within the histological umbrella of "UPS-B," particular for secondary tumors. However, it did prove useful in re-classifying two tumors as MPNST of bone. We strongly conclude that UPS-B samples should be included in future classifier training sets.

Next steps include copy number variation analysis, pathway analysis of highly differentially methylated regions, and unbiased clustering analysis of samples. These results can help drive future studies on a larger cohort via multi-institutional cooperation.

Figure 1: Tumor samples histological (left) and methylation-predicted diagnostic classes (right). Solid lines have predicted confidence >0.9.

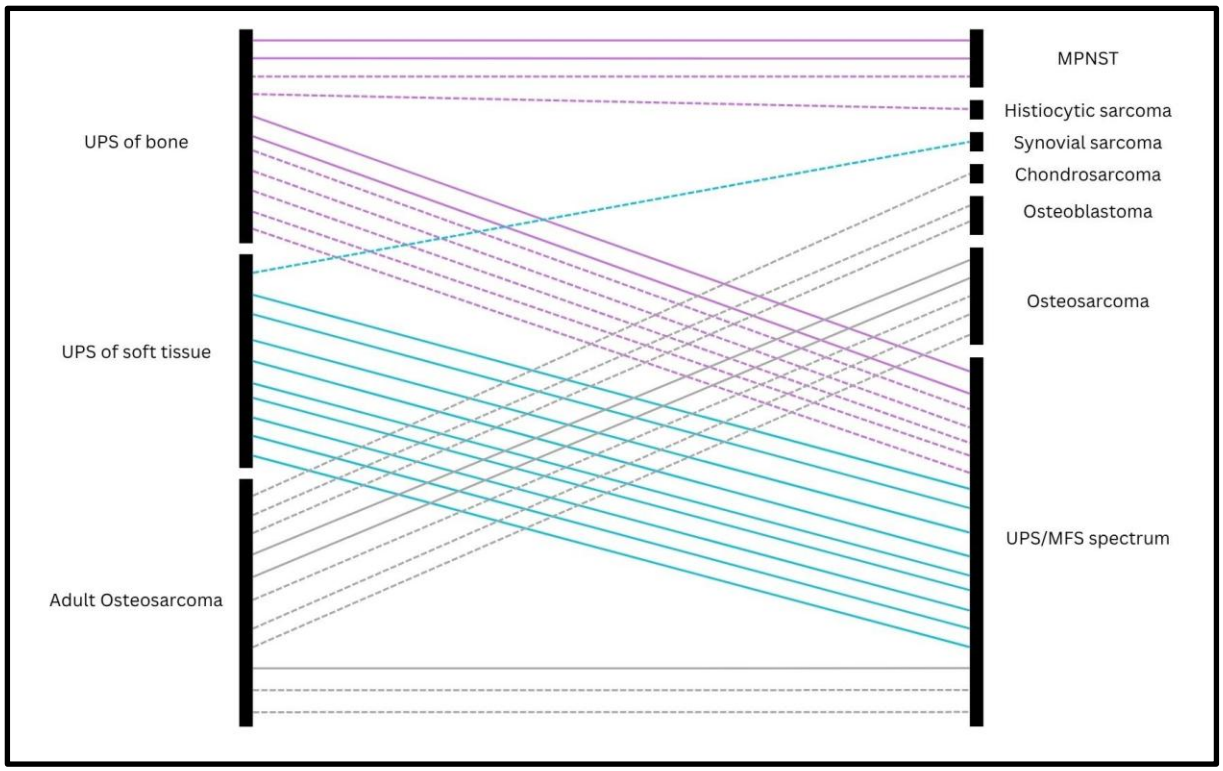

Table 1: Tumor samples identified by histology and methylation classifier predicted diagnostic classification

Case	Histologic diagnosis	Anatomical location	Class prediction	Confidence score (0-1)	Concordance
1	UPS-B $^{\alpha}$	Tibia	UPS/MFS spectrum	0.2256	Indeterminate
2	UPS-B $^{\alpha\,\dagger}$	Femur	MPNST	0.8132	Indeterminate
3	UPS-B	Femur	UPS/MFS spectrum	0.4902	Indeterminate
4	UPS-B $^{\alpha\,\dagger}$	Femur	UPS/MFS spectrum	**0.9093**	Concordant
5	UPS-B	Femur	UPS/MFS spectrum	**0.9991**	Concordant
6	UPS-B	Pelvis	MPNST	**0.9939**	Discrepant - reclassification

8	UPS-B $^{\alpha\,\dagger}$	Tibia	MPNST	**0.9832**	Discrepant - reclassification
9	UPS-B $^{\alpha\,\dagger}$	Pelvis	Histiocytic sarcoma	0.1913	Indeterminate
10	UPS-B	Tibia	UPS/MFS spectrum	0.1914	Indeterminate
11	UPS-B $^{\alpha}$	Tibia	UPS/MFS spectrum	0.6367	Indeterminate
12	UPS-B	Tibia	UPS/MFS spectrum	0.8931	Indeterminate
13	UPS-ST	Gluteal	UPS/MFS spectrum	**0.9858**	Concordant
14	UPS-ST	Thigh	UPS/MFS spectrum	**0.9977**	Concordant
15	UPS-ST	Calf	UPS/MFS spectrum	**0.9995**	Concordant
16	UPS-ST	Calf	UPS/MFS spectrum	**0.9439**	Concordant
17	UPS-ST	Thigh	UPS/MFS spectrum	**0.9996**	Concordant
18	UPS-ST	Thigh	UPS/MFS spectrum	**0.9996**	Concordant
19	UPS-ST	Calf	UPS/MFS spectrum	**0.9761**	Concordant
20	UPS-ST	Shoulder	Synovial Sarcoma	0.2089	Indeterminate
21	UPS-ST	Flank	UPS/MFS spectrum	**0.9564**	Concordant
22	UPS-ST	Gluteal	UPS/MFS spectrum	**0.9998**	Concordant
23	OS	Femur	OS	0.3375	Indeterminate
24	OS	Femur	OS	**0.9999**	Concordant
25	OS	Femur	Osteoblastoma	0.6791	Indeterminate
26	OS	Femur	Chondrosarcoma	0.6200	Indeterminate
27	OS	Femur	UPS/MFS spectrum	0.6027	Indeterminate
28	OS	Femur	UPS/MFS spectrum	0.8284	Indeterminate
29	OS	Femur	UPS/MFS spectrum	**0.9264**	Discrepant - misleading
30	OS	Tibia	OS	**0.9770**	Concordant
31	OS	Femur	Osteoblastoma	0.7534	Indeterminate
32	OS	Femur	OS	0.5418	Indeterminate
33	OS	Humerus	OS	0.1646	Indeterminate

α: secondary (vs de novo) tumors; †: radiation-associated sarcoma; UPS-B: undifferentiated pleomorphic of bone; UPS-ST: undifferentiated pleomorphic sarcoma of soft tissues; OFMT: ossifying fibromyxoid tumor; OS: osteosarcoma; MPNST: malignant peripheral nerve sheath tumor; “UPS /MFS spectrum” encompasses

undifferentiated pleomorphic sarcoma, myxofibrosarcoma, pleomorphic liposarcoma, and pleomorphic rhabdomyosarcoma.

Concordant=CS >0.9 and histology and classifier prediction agree; Indeterminate= CS <0.9; Discrepant/reclassification= re-review of histology resulted in changing the original diagnosis; Discrepant/misleading= CS>0.9 and re-review of the histology strongly supported the original diagnosis rather than the conflicting classifier prediction.