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A.C.Camargo Cancer Center

DNA methylation and mutational signatures suggest dysregulation of developmental pathways in osteosarcomas

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Background

- Osteosarcomas (OS) are the most prevalent malignant bone tumors in children and adolescents since arise more commonly during puberty, a period of rapid bone growth and remodeling, it is plausible to infer the relationship between their tumorigenesis and cell cycle disruption by genetic and epigenetic factors;

- Characterization of OS genomes is incomplete due to rarity and complexity of genomic alterations;

- DNA methylation is involved with tumor progression and aggressiveness, and thus, can provide new insights into OS biology.

Cohort and methods

Cohort

28 osteosarcomas; 9 normal bone tissues;

Fresh-frozen samples (Biobank from the Barretos Cancer Hospital).

Methods

DNA methylation data from tumors and controls was obtained using the Infinium® HumanMethylation450 BeadChip (Illumina), which covers ~450k methylation sites throughout the genome;

Tumor mutational burden was also analyzed, using data from the TruSight clinical exome sequencing panel (Illumina), which covers ~4,800 clinically relevant genes;

Functional enrichment analyses were applied to genes mapped within the DMRs using the Gene Set Enrichment Analysis (GSEA) platform.

Results

- OS samples presented high methylation heterogeneity, although a **global hypomethylation** pattern was detected (*Figure 1*)**;**

- Aberrant DNA methylation and somatic mutations were spread throughout tumor genomes in a pattern suggesting **genomic instability**;

- CpG islands were enriched for **hypermethylation**, a mechanism likely related to the silencing of tumor suppressor genes (*Figure 2*);

- 17 tumor suppressor genes and 10 oncogenes were mapped within the hypermethylated and hypomethylated DMRs, respectively (*Table 1*);

- Regions with differential methylation were located next to genes enriched for **skeletal system development**, **cell proliferation** and **differentiation**, **metabolism** and **RNA-related processes**;

- Several copy number alterations were identified in the families of DNA methylases and demethylases, mainly **gains in *DNMT3B*** (*Figure 3*);

- An enrichment of hypomethylated DMRs was observed at **6p22**, where several histone coding genes are located.

Results

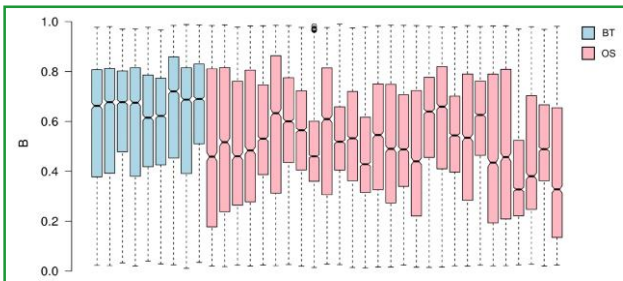

Figure 1. Boxplot of methylation levels detected for normal bone samples (BT) and osteosarcomas (OS), ranging from 0 (unmethylated) to 1 (fully methylated).

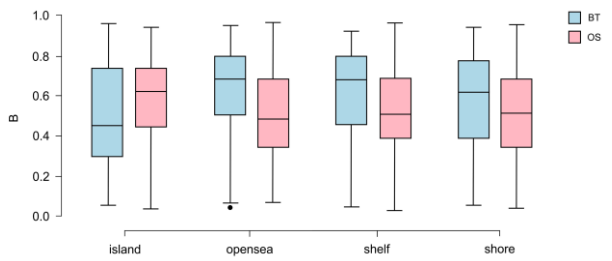

Figure 2. Boxplot of average DMP methylation levels distributed according to the CpG site genomic location.

Table 1. Tumor suppressor genes and oncogenes contained where hypermethylated and hypomethylated DMRs are located, respectively.

Classification*	Genes
Tumor suppressors (Hypermethylated)	*AIF1, DIDO1, DLEC1, DUSP6, FOXC1, GJB2, HIC1, LXN, MIR142, MIR149, PAX6, PROX1, SPI1, STAT5A, TNFAIP8L2, WNT5A, ZIC1*
Oncogenes (Hypomethylated)	*AGAP2, ASPSCR1, MCF2L, MIR380, NANOG, NOTCH4, PAX4, PRDM16, RUNX3, TCL1B*

*: Reference lists of human tumor suppressors and oncogenes were obtained from the Tumor Suppressor Genes Database (https://bioinfo.uth.edu/TSGene/) and the Oncogene Database (http://ongene.bioinfo-minzhao.org/).

Figure 3. Distribution of point mutations and copy number alterations across *DNMT* and *TET* gene families.

Further steps

Evaluating the expression patterns of relevant genes to investigate the functional impact of the detected alterations in osteosarcoma cell lines.

Conclusions

- Aberrant DNA methylation is spread throughout the OS genomes, presented with high variance, like other molecular alterations;

- While the open-sea hypomethylation contributes to genomic instability, enriched CpG island hypermethylation suggests the involvement of mechanisms likely related to silencing tumor suppressor genes in the osteosarcoma tumorigenesis;

- Regions with aberrant methylation are located next to genes enriched for skeletal development and cell differentiation;

- Our data highlight the relevance of *DNMT3B*, probably impacting biological mechanisms associated with early bone development.

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