Epigenetic Regulation Of Anoikis-Resistance In Human Osteosarcoma Is Targetable With Vorinostat And 5-Azacytidine

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Background: *Anoikis* is an inducible form of programmed cell death that occurs following detachment from the extracellular matrix. *Anoikis resistance* (*AR*) is a fundamental property of metastatic cells that is governed by tyrosine kinase activation and modulation of FAS/APO-1 death receptor inputs. Epigenetic regulation of *AR* is not well understood in human osteosarcoma. Here, we investigate mechanisms that regulate conversion from anchorage-dependent to anchorage-independent (AI) cell growth using a combination of *in vitro* and *in vivo* models. We identify candidate therapy targets using an integrated genomics approach.

Methods: Established human OS cell lines (n=3) and clinically annotated, patient-derived human OS cell isolates (n=7) were grown in either adherent or AI conditions using ultra-low attachment plates in identical media conditions. We subjected either the adherent or AI-grown cells to doxorubicin or cisplatin treatment and compared the IC_{50} values and cell viability (Wilcoxon signed rank test; $\alpha = 0.05$ with post-hoc adjustment). The relative growth of tumors derived from adherent versus AI-grown cells was compared using an orthotopic in-vivo OS xenograft model (Student's t-test; $\alpha = 0.05$). We next determined fold expression change of key regulatory genes using reverse-transcription PCR, and gene network analysis of the derived expression profiles (Affymetrix U133p2 array). Candidate therapy targets were identified through gene set enrichment analysis and validated using FDA-approved drugs *in vitro*. Mechanisms underlying drug response were verified by Western Blot or, in the case of the DNA Methyl-Transferase inhibitor (DNMTi), 5-azacytidine, alterations in the genome-wide DNA methylation profile (Infinium450 array). The top differentially methylated CpG loci were functionally annotated using a rules-based, gene ontology approach.

Results: We show that AI growth results in a global gene expression profile change that is accompanied by significant chemoresistance (up to 75 fold, $p < 0.05$). AI cells demonstrated consistent alteration of key mediators of mesenchymal differentiation (β-catenin, Runx2), stemness (Sox2), proliferation (c-myc, Akt), and epigenetic regulation (HDAC class 1). AI cells were equally tumorigenic as their adherent counterparts in an orthotopic osteosarcoma xenograft model, however they demonstrated significantly decreased proliferation both *in-vitro* and *in-vivo* ($p < 0.05$). Treatment with the pan-histone deacetylase inhibitor, vorinostat, and 5-azacytidine mitigated AI growth, but only 5-azacytidine sensitized anoikis-resistant cells to doxorubicin ($p < 0.05$). The top differentially methylated CpG loci (adherent vs AI cells and xenografts) were strongly associated with genes that regulate cell adhesion (e.g. ROBO1/2) and axonal guidance and differentiation (e.g. semaphorin gene family).

Conclusions: These data confirm that AR subpopulations maintain a remarkable plasticity accompanied by the rapid development of chemoresistance and altered growth rates that mirror the early stages of metastasis. AR in osteosarcoma is regulated, in part, through epigenetic mechanisms that can be targeted with traditional epigenetic therapies. More work is needed to determine the precise role of genes involved in cell adhesion and differentiation in the process of *AR*.