IDH Mutations and Epigenetic Regulation of Chondrosarcoma

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Introduction: Cancer metabolism fundamentally differs from normal. It is important to define how often this metabolic reprogramming occurs, and its significance. This study analyzes these changes in chondrosarcoma, from the basic science to the clinical aspects, focusing on genetic changes regulating intermediary metabolism. Somatic mutations in the isocitrate dehydrogenase I (IDH1 and IDH2) genes have been recently described in chondrosarcomas as well as gliomas, acute myeloid leukemias, and other cancers. The IDH family of enzymes catalyze the oxidative decarboxylation of isocitrate to produce alpha ketoglutarate. Mutations in these genes impair IDH1 and IDH2 catalysis of isocitrate to alpha ketoglutarate resulting in the gain of a novel enzymatic activity producing the oncometabolite D2 hydroxyglutarate (2HG). 2HG is normally present at very low levels in cells but has a >100-fold increase in tumors with IDH mutations. The mutations are heterozygous and occur at a single amino acid residue, Arg 132 of IDH1 and the analogous Arg 140 or 172 of IDH2. In addition, a mutation specific antibody targeting IDH1 R132H is now commercially available which can identify protein expression in R132H positive neoplasms. In hematologic tumors and gliomas, IDH mutations are associated with a DNA hypermethylation profile and age expression pattern associated with lineage-specific progenitors (Figueroa et al. 2010; Noushmehr et al. 2010; Turcanetal 2012). The degree of epigenetic changes in chondrosarcoma is unknown. The objective of this project was to retrospectively analyze cartilage neoplasms among over 200 patients treated at MSKCC in the last 20 years (1991-2011) from whom we had prospectively collected snap-frozen tissue samples under an IRB protocol. They were analyzed for mutations of IDH1 and IDH2 and other biologically relevant genetic markers related to IDH1 and IDH2. Specific hypotheses were that: 1) acquisition of IDH1 and IDH2 mutations confer a gain-of-function phenotype to produce 2-HG in chondrosarcoma; 2) these mutations result in epigenetic changes that critically impact cell growth and differentiation.

Methods: Under an IRB protocol, biopsy proven chondrosarcoma specimens were snap frozen and maintained at -70°C. They were genotyped for IDH1 and 2 mutations on the Sequenom Mass-Spectrometry Array Platform. In addition, 271 recurrent point mutations across 27 genes were tested as part of the high throughput Sequenom Mass-Spectrometry Array Platform panel. Various chondrosarcoma cell lines were also obtained and genetically sequenced for IDH 1/2 mutations. Fifty three chondrosarcomas were selected for the study. There were 30 females and 23 males. The age range was 18 to 77 years with a median of 55 years. Histologically, twenty one (21) were classified as grade I/III, 25 as grade II-III/III and 7 as dedifferentiated chondrosarcoma. A separate panel of surgical specimens from 21 patients with chondrosarcomas were compared with samples that were wild type for IDH1/2, IDH1 or IDH2 mutant samples. They were analyzed for epigenetic changes. (Fig.1A). Genome-wide DNA methylation levels were measured using enhanced reduced representation bisulfate sequencing (ERRBS), which was previously demonstrated to provide base pair resolution DNA methylation information and extended genomic coverage beyond CpG island regions compared with traditional RRBS (Akalin et al. 2012a). A minimum cut off of 40% methylation difference, in addition to statistical significance(Q<0.01), was required to identify differentially methylated CpGs between IDH1/2 wild-type and mutant samples.

Results: The IDH mutational status was documented for five chondrosarcoma cell lines. Table 1. In the first training sample, twenty-six of 53 (50%) patients had mutations in IDH1 or IDH2. IDH mutations did not correlate with grade of the tumor. No other mutations were detected in the rest of the gene panel (AKT1, AKT2, AKT3, ALK, BRAF, CDK4, CTNNB1, EGFR, ERBB2, FGFR2, FGFR3, FLT3, GNAQ, HRAS, JAK2, KIT, KRAS, MAP2K1, MET, NOTCH1, NRAS, PDGFRA, PIK3CA, PIK3R1, PTPN11, RET, SMO) by Sequenom Mass Array spectrometry. (Figure 3) In the study subset, 10 of 21 had mutations. Targeted sequencing results revealed a 50% frequency of IDH mutations in chondrosarcomas (seven of the samples had the R132 IDH1 mutation, three had the R172 IDH2 mutation, and 11 were wild type for IDH1/2), consistent with previous reports (Amaya et al. 2011; Arai et al. 2012). Analysis of DNA methylation at gene promoters was performed by selecting differentially methylated CpGs at 1000 to +500 base pairs (bp) of each transcription start site. A total of 12,236 CpGs were found to be differentially methylated. The group of genes that were promoter DNA-hyper- methylated in IDH mutants was analyzed by Data base for Annotation, Visualization, and Integrated Discovery (DAVID) for functional relevance. The top 150 enriched functional categories involved various organism and cellular developmental processes. The most significantly hypermethylated genes in IDH mutant samples include line age specification regulators such as retinoic acid receptor A (RARA), platelet-derived growth factor receptor a (PDGFRA), and BCL6 co-repressor (BCOR). KEGG pathway analysis shows the top ten differentially regulated pathways in Table 2.

Discussion: 1) IDH1 and IDH2 mutations are genetic signatures in half of chondrosarcomas and are functional, associated with elevated 2HG. 2) Most common genetic mutations involving genes of the signal transduction pathways do not seem to play a role in the biology of chondrosarcoma. 3) Comprehensive genome-wide DNA methylation is extensive in IDH mutant chondrosarcoma suggesting that IDH mutations are associated with epigenetic dysregulation of genes. Many important
pathways are affected by these changes. 4) Mutant IDH is a potential target for chondrosarcoma where few other systemic treatment options are currently available.

Significance: Targeting the altered intermediary metabolism is a novel strategy to treat chondrosarcoma.

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