Genomic Investigation of Dedifferentiated Liposarcoma Suggests a Role for Therapeutic Targeting of the Tumor Epigenome

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Summary: A comprehensive genetic analysis of dedifferentiated liposarcomas suggests that epigenetic modifications are common and may alter the differentiation capacity in these tumors. Furthermore, these data suggest that treatment strategies aimed at altering histone acetylation and/or DNA methylation are worthy of further study.

Cancer Discovery; 1(7); 555–6. ©2011 AACR.

Commentary on Taylor et al., p. 587 (2).

Dedifferentiated liposarcomas pose a particular therapeutic challenge because to date there is no convincing evidence that any modality other than surgical removal affects the ultimate outcome of these tumors (1). In this issue of Cancer Discovery, Taylor and colleagues (2) address the genomics of dedifferentiated liposarcoma (DLPS) by using an integrated second-generation sequencing approach to investigate DNA and RNA structural rearrangements, DNA sequence changes, and DNA methylation in DLPS samples from 2 patients. This approach points out both the importance and power of second-generation genomic technologies that can determine structural changes and copy number alterations as well as epigenetic alterations.

However, this approach likewise points out the difficulty in applying such robust technology where the challenge will be to interpret the plethora of data in a way that will lead to new biologic insights and hopefully imply new therapeutic options. This approach will be a particular challenge in relatively rare tumors such as liposarcomas, where literally thousands of events are observed, and conclusions drawn from relatively rare recurrent events can only be confirmed when very large sample sets are available for confirmatory studies.

Here, consistent with previous studies, the authors found large numbers of structural rearrangements (355 and 543 in each case) that mainly were associated with regions of DNA amplification, including the well-known 12q amplification involving MDM2, HMGA2, and CDK4 found in DLPS and its precursors (3). Structural alterations in HMGA2 in one tumor suggest the possibility that the loss of microRNA binding sites in the 3’UTR, as described in tumors with HMGA2 translocations, may contribute to increasing expression of HMGA2 in this case beyond the effect of copy number alone (4). With genomes so scrambled, it is inevitable that some breakpoints lead to rearrangements affecting genes, and indeed, multiple rearrangements generating possible fusion transcripts were observed, including several that are predicted to generate in-frame fusions. None of these were shared between the 2 cases. Much larger studies will be required to determine whether these are passenger or driver events. However, given the overall genome complexity of these tumors, it is likely that the majority represent collateral damage without necessarily having a major functional role in tumor growth.

At the level of small mutations (nucleotide substitutions, insertions, and deletions) a relatively small number of changes (8 and 13) were observed and verified in the 2 cases. This number is relatively low compared with tumors with high exposure to carcinogens, such as melanoma. The prevalence of mutations in 4 genes (HDAC1, MAPKAP1, PTPN9, and DAZAP2) in 96 liposarcomas called particular attention to HDAC1, which exhibited mutations in 8.3% of tumors.

Mutations in genes that regulate epigenetic pathways at the DNA and chromatin levels have emerged as an increasingly important phenomenon in several tumor types. The discovery of this class of mutations is leading to profound mechanistic insights with significant therapeutic implications because of the potential for small molecule targeting of enzymes that modify the epigenome. Mutations in HDAC1 have not been previously reported in cancer, but truncating mutations in HDAC2 have been observed in tumors with microsatellite instability (5). The significance of HDAC1 mutations in DLPS remains to be fully defined at the biochemical level. The potential role of these mutations in affecting DLPS tumor growth and differentiation certainly justifies further investigation.

Epigenetic modifications and the DNA methylation level also were investigated by the use of a sequencing strategy that is based on affinity enrichment of 5-methylcytosine containing DNA fragments. By comparing DLPS to matched normal adipose tissue, Taylor and colleagues (2) were able to identify 833 differentially methylated regions affecting the promoters of 677 genes. Of course, not all of these genes are necessarily deregulated by these events, but significant tumor-specific promoter methylation associated with decreased expression was found in KLF4 and CEBPA, which code for 2 important transcription factors with a well-established connection to adipocyte differentiation. KLF4 regulates CEBPA, and loss of expression of these factors in tumors would be expected to impair differentiation.
Consistent with this view, in a set of normal adipose tissue samples, well-differentiated LPS, and DLPS, CEBPA methylation was limited to DLPS samples. This observation suggested the possibility that treatment with demethylating agents might affect CEBPA expression and DLPS cell growth. Indeed, treatment with decitabine led to the restoration of CEBPA expression in a DLPS cell line (DDLS8817), an effect that was augmented by concurrent treatment with the HDAC inhibitor SAHA. This effect was accompanied by increased expression of adipocyte markers. Xenograft growth was also reduced in mice, suggesting a potential new approach to DLPS therapy.

The DLPS tumor methylation data led to a further discovery connecting epigenetic alterations with tumor growth. Taylor and colleagues (2) identified tumor-specific hypermethylation in proximity to a set of microRNAs, notably miR-193b, and established that decreased expression of this small RNA was DLPS specific in comparison with normal adipose tissue and well-differentiated LPS. Predicted targets of miR-193b behaved as expected, with increased expression in DLPS. The biologic consequences of this alteration remain to be explored.

In summary, Taylor and colleagues (2) have provided us with an early look at the potential power of applying second-generation sequencing technology to develop new insights into understanding biologic underpinnings of a specific malignancy. Of particular note are the implications suggesting methylation alterations that might contribute to the dedifferentiated state of some tumors, as well as the hint of a potentially important mutation that could lead to changes in histone acetylation, with both findings suggesting an important epigenetic contribution to liposarcomas discovered by genomic analyses. Likewise, these data also point out the need for the ability to follow these early observations with confirmatory studies of very large sample sizes, and this should provide a wake-up call to the entire oncology community. The future need for large, annotated tumor collections will be substantial and necessary if these types of early observations are ultimately going to be game-changing.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Received November 7, 2011; accepted November 8, 2011; published online December 14, 2011.

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_Cancer Discovery_ 2011;1:555-556.

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