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COMMENTARY

Hodgkin Lymphoma Cells Have a Specific Long Noncoding RNA Expression Pattern

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Since their discovery in the late 1990s, the study on long noncoding RNAs (lncRNAs; RNAs longer than 200 nucleotides that do not code for proteins, but regulate the function of proteins) has seen a rising interest. Indeed, the application of sensitive techniques, like microarray and next-generation sequencing, to whole genome and transcriptome revealed that at least 75% of genome is actively transcribed into noncoding RNAs.¹ Due to the ability of lncRNAs to regulate gene expression, there has been a focus on studying their roles in cancer development, progression, and as tumor biomarkers.

Increasing evidence shows that many of the genomic alterations implicated in different cancers (such as single base substitutions, copy-number alterations, or translocations) are located within lncRNA transcripts determining their over-expression or down-regulation in cancer cells.^{2,3} For example, tumor-specific expression and aberrant expression of lncRNAs compared with normal tissue has been identified in prostate cancer (PCA3 and PCGEM1),^{4,5} lung cancer (MALAT1),⁶ breast cancer (HOTAIR),⁷ and colon cancer (PINT).⁸

In this issue of *The American Journal of Pathology*, a novel study by Tayari et al⁹ compared the expression profiles of lncRNAs in normal B-cell subsets [naïve, germinal center (GC), memory B-cells] and Hodgkin lymphoma (HL) cells. The study identified distinct expression patterns of lncRNAs among the normal B-cells and between normal B-cells and HL cells. In addition, a tumor-specific expression pattern was identified for three lncRNAs in primary HL tissues and a potential *cis*-regulatory role was observed for 110 differentially expressed lncRNA-messenger RNA pairs localized within a region of 60 kb.

HL is a B-cell–derived cancer originating from GC B-cells and is characterized by two histological types: classical Hodgkin lymphoma (cHL), which accounts for 95% of all cases, and the less common (5%) nodular lymphocyte

predominant Hodgkin lymphoma. HL accounts for approximately 11% of all malignant lymphomas.¹⁰ Although the advances in therapeutic strategies have increased the 5-year survival rate to 86% (National Cancer Institute, <http://seer.cancer.gov/statfacts/html/hodg.html>, last accessed June 13, 2016) with a reduction of more than 60% mortality rate,¹¹ around 20% of patients that relapse or have progressive disease are still destined to die.^{12–14} Therefore, the discovery of novel biomarkers able to identify patients at risk to develop refractory or relapsing disease is essential, as is the development of targeted therapies for them. The recent advances in molecular biology and genetics can help us understand the normal B-cell development and the molecular oncogenic pathways that drive the transformation of normal B-cells to develop HL. Although the role of microRNAs in normal B-cell and HL development has been extensively studied, very little is known about the functions of lncRNAs in B-cell lymphopoiesis and HL development. Recently, the lncRNA expression pattern was analyzed in 11 distinct flow-sorted B-cell subsets ranging from pre-B to plasma cells in bone marrow and tonsil tissue. Specific lncRNA expression was associated with distinct stages of B-cell development.¹⁵ It is important to

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note that high levels of genomic instability are necessary during the development of mature B-cells, particularly during the class switch recombination and somatic hypermutation catalyzed by the DNA cytidine deaminase activation-induced deaminase.¹⁶ This fact suggests that during this physiological process, accidental genetic alterations, like translocation, amplification, deletion, and mutation, might occur and induce a consequent aberrant expression of coding genes and non-coding RNAs that normally regulate the B-cell leading to the development of transformed HL cells. Indeed, molecular pathways that regulate normal B-cell development are also involved in pathogenesis of lymphomas, and several malignant lymphomas originate from distinct stages of normal B-cell development.¹⁷

The study by Tayari et al⁹ gave insight toward the identification of a specific lncRNA expression pattern for normal B-cells as well as for HL B-cells. It was shown that there is a dynamic regulation of lncRNA and mRNA expression during the normal B-cell maturation with an expression change of 251 lncRNA loci during the transition from naïve to GC-B cells and a reversion toward levels comparable to naïve B-cells during the differentiation to memory B-cells. The gene expression profile revealed that naïve and memory B-cells had highly similar lncRNA and mRNA expression patterns, whereas GC-B cells had significantly distinct patterns. Interestingly, when compared with the normal B-cell subsets (naïve, GC-, and memory B-cells), the lncRNA and mRNA expression pattern of the five cHL cell lines was similar to that of the GC-B cells and distinct from naïve and mature B-cells, confirming the GC-B cell origin of the cHL tumor cells. Further analysis revealed a significant differential expression pattern for 475 lncRNA loci between cHL and GC-B cells. These results indicate that lncRNA expression undergoes significant changes during normal B-cell maturation steps and a prominent deregulated lncRNA expression occurs during transformation to cHL. The understanding and characterization of the role of lncRNAs during the process of GC-B cell transformation to cHL may hold importance, especially in identifying the possible function of lncRNAs in the regulation of B cell interaction with the tumor microenvironment. Indeed, in the cHL tumor microenvironment the malignant cells are significantly outnumbered by reactive cells including lymphocytes, macrophages, eosinophils, neutrophils, plasma cells, and fibroblasts. The cytokine milieu present in the tumor microenvironment provides proliferation and anti-apoptotic stimuli to cHL cells that drive the expansion and survival of transformed cells.¹⁸ The NF- κ B, the Janus kinase—STAT, and the tyrosine kinase receptors represent the major signaling pathways that are activated in the HL microenvironment. Recently, it was reported that lncRNAs are involved in the regulation of inflammatory signaling and can be transcriptionally induced by pro-inflammatory cytokines [eg, tumor necrosis factor- α —NF- κ B axis].^{19–21}

Thus, lncRNAs may play a significant role in the regulation of pro-survival and proliferation signaling and consequently they can serve as a novel molecular target to disrupt the cross talk between cHL cells and the surrounding

tumor microenvironment. The study by Tayari et al⁹ reported a distinct cluster of lncRNAs between cHL and normal GC-B cells. The cluster was composed of 745 differentially-expressed lncRNA loci and it would be interesting to study if this lncRNA cluster may be involved in the regulation of tumor microenvironment signaling.

It was found that 59 out of 475 lncRNAs that were differentially expressed between GC-B cells and cHL were in close vicinity to differentially expressed mRNA, implying a potential *cis*-regulatory function of those lncRNAs. In this regard, an analysis on allele-specific regulation and loss/gain of function would provide more evidence of the *cis*-regulatory effect of the differentially expressed lncRNA. In view of this evidence, deeper investigation on differentially expressed lncRNA-mRNA pairs may provide further advance in the understanding of pathogenic pathways from GC-B cells to cHL with particular focus on the cross talk between tumor cells and the surrounding tumor microenvironment. Particularly, because the inflammatory milieu promotes tumor cell growth and survival, the identification of the signaling pathways regulated by the differentially expressed lncRNAs could aid the discovery of new targets and the development of novel therapeutic strategies. In this context, it is interesting to note that *SLC20A1*, one of the candidate *cis*-regulated protein-coding genes, suppresses tumor necrosis factor-induced apoptosis and c-Jun NH2-terminal kinase activity regulating cell death in Hela cells.²² The use of antisense oligonucleotides, which showed an increased and more effective ability to suppress nuclear lncRNAs,²³ may represent a useful method to further evaluate the individual functions of the differentially expressed lncRNA identified in the study by Tayari et al.⁹

As mentioned earlier, another clinical application of lncRNAs is their potential use as biomarkers. Several lncRNAs were reported as promising diagnostic and predictive biomarkers for breast, prostate, lung, kidney, and colon cancer.^{24,25} The study by Tayari et al⁹ proposed the use of lncRNAs as tumor biomarkers for cHL. The expression of three lncRNAs that were differentially up-regulated in cHL cells (FLJ42351, LINC00116, and LINC00461) was further evaluated in 24 different cancer types. The Cancer Genome Atlas RNA-seq analysis revealed low expression levels of FLJ42351 and LINC00116 without significant differences across the different cancer tissues. High levels of LINC00461 were found in glioma tumors. The expression of the three lncRNAs was further validated by RNA-FISH in five randomly selected cHL cases from the pathology files of University Medical Center Groningen. Due to their specific tumor cell expression patterns, the study by Tayari and colleagues⁹ proposed these three lncRNAs as diagnostic biomarkers for cHL. This discovery may represent a novel approach for the specific identification of transformed B-cells in lymphoma tissue. However, since only five cHL cases were tested, further validation is required.

An interesting advancement in the development of lncRNA-based biomarkers for cHL would be to extend the lncRNA profiling approach to a larger cohort of patient samples and evaluate the ability of specific lncRNA

expression patterns to identify patients at risk to develop refractory or relapsing disease. As mentioned above, around 20% of HL patients develop refractory or relapsing disease and have a poor clinical outcome.

lncRNAs can regulate cytokine signaling pathways. Thus, it would be intriguing to evaluate whether or not lncRNAs can play a role in the selection of tumor cells responsible for the refractory or relapsing disease. Particularly, because of their ability to regulate cytokine signaling, the lncRNA profiling might help us understand the regulation of the pro-survival and growth signals responsible for the expansion of treatment-resistant cHL tumor cells. Therefore, lncRNA profiling may be a useful tool to predict which patients are at risk of treatment failure and accordingly need more effective therapeutic treatments.

The study by Tayari et al⁹ has provided a strong rationale to investigate the role of differentially-expressed lncRNAs in the normal B-cell biology and between cHL and GC-B cells. The study also suggests an investigation of the mechanisms involved in the pathogenesis of HL, with a particular focus on the putative *cis*-regulatory ability of these lncRNAs. Additional investigations will help to evaluate the possible clinical applications of lncRNAs as diagnostic and predictive biomarker and target for novel therapeutic treatment of HL.

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