Molecular pathology selected abstracts

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Emerging landscape of circular RNAs in healthy hearts and cardiomyopathy

January 2021—Circular RNAs are a novel class of recently discovered RNA with emerging roles in gene regulation, homeostasis, and disease. They are generated by ligation of the distal ends to form a circular product and originate from parental-coding genes and noncoding regions of the genome. Circular RNAs (circRNAs) are widespread in the plant and animal kingdoms and conserved in multiple species. Recent literature suggests that they inhibit micro RNA (miRNA), an important class of RNAs that regulates gene expression by binding to messenger RNAs (mRNA). Therefore, the post-transcriptional regulation of gene expression by RNAs has expanded to include a circRNA–miRNA–mRNA regulatory network. The ability to identify, test, and correlate this regulatory network with human disease may provide insights into pathogenesis and novel therapies directed at gene regulators. Although much is now known about the genes and miRNAs associated with heart failure, these alterations are only identified in a fraction of patients, suggesting that there are other molecular and nonmolecular factors related to the disease. The authors conducted a study in which they provided a genomic landscape of circRNAs from healthy hearts and hearts with dilated cardiomyopathy. They performed a stringent in silico analysis of publicly available datasets, correlated circRNA with mRNA expression data, and identified miRNAs associated with heart failure as likely targets of circRNA inhibition. The authors obtained primary RNA sequence data from five healthy control subjects and five dilated cardiomyopathy patients to extract gene-expression data and identify circRNAs. They identified from the 10 cases more than 20,000 circRNAs, originating from all chromosomes and with two-thirds originating from exons. Approximately 42 percent of the genes producing circRNAs expressed one circRNA. The number of circRNAs generated per gene correlated with the number of exons. For example, two of the largest genes—*TTN* and *RYR2*—produced the most circRNAs. The circRNAs identified were cross-referenced to previously identified cardiac circRNAs from three prior independent studies and four public circRNA databases. They showed congruence in approximately 80 percent of cases. The authors demonstrated the presence of distinct circRNAs that were associated with normal heart or dilated cardiomyopathy, or both. Of the most abundant circRNAs identified in normal human hearts, half were conserved in mouse and rat hearts, most were identified in two prior studies, and most had expression levels that were highly correlated with their host parental mRNA expression levels. When compared with normal hearts, approximately 400 circRNAs from hearts with dilated cardiomyopathy demonstrated differentially expressed circRNAs from approximately 300 unique host genes, with a predominant downregulation of circRNAs. Although most of the differentially expressed circRNAs correlated strongly with their parental gene mRNA expression, some were discordant. Pathway analysis showed that most of the parental genes were associated with cardiomyopathies, including arrhythmogenic right ventricular cardiomyopathy, hypertrophic cardiomyopathy, and dilated cardiomyopathy. This indicated that most of the differentially expressed circRNAs are from already established heart disease-related loci. To evaluate the circRNA–miRNA–mRNA regulatory axis, the most abundant circRNAs identified in this study were analyzed for their potential to function as a competitive inhibitor, or molecular sponge, of miRNAs. One hundred and forty-two circRNAs were predicted to have putative binding sites for at least 34 of 50 miRNAs that have established roles in heart disease. In addition, more than 16,000 putative target genes of these miRNAs were identified, including approximately 600 upregulated and approximately 300 downregulated genes in hearts with dilated cardiomyopathy. For example, the novel cardiac read-through circRNA circSCAF8_e4:TIAM2_e2, which is downregulated in hearts with dilated cardiomyopathy, was characterized with putative miRNA binding sites to multiple miRNAs, including miR-17, that have been shown to be

induced in diseased mouse and rat hearts. The downstream targets of miR-17, including *SCN2B, F2R, KCNB1*, and *EGLN3*, were downregulated in hearts with dilated cardiomyopathy, as revealed by expression analysis of linear RNAs, likely through the unleashed miR-17 that resulted from the downregulation of circSCAF8_e4:TIAM2_e2. This study provides a refined database of conserved and novel circRNAs expressed in healthy hearts and those with dilated cardiomyopathy, and it sheds light on gene regulatory mechanisms that may be targets of future therapy.

Dong K, He X, Su H, et al. Genomic analysis of circular RNAs in heart. *BMC Med Genomics*. 2020:13;167. https://doi.org/10.1186/s12920-020-00817-7

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Cell-of-origin identification of high-grade serous carcinoma

Whole genome DNA methylation signatures provide a molecular fingerprint of specific epigenetic changes that can be used to distinguish between tumor types and cell types of origin. It can be challenging to identify the cell of origin of a primary or metastatic tumor using conventional histomorphology and IHC techniques, yet this is critical for clinically managing the cancer. Recently, there has been a paradigm shift in the medical community's understanding of the cell of origin of high-grade serous carcinoma (HGSC). In most cases, these common and deadly tumors likely arise not from the ovarian epithelium but from the distal fallopian tube fimbriae. The authors took a novel approach to investigate the cell of origin of HGSC by subtyping the tumor based on DNA methylation profiles. They showed differences in prognosis based on cell of origin, identified transcriptional variations underlying the biology of the disease, and provided insights into the tumor microenvironment that may better inform future targeted therapies. The cell of origin of HGSC cases was determined by first establishing the tissue-specific DNA methylation patterns in primary non-neoplastic ovarian surface epithelia (OSE) and fimbrial (FI) epithelia. These reference methylation profiles were then compared with the retained tissue-of-origin DNA methylation imprints of HGSC by applying a cell-of-origin–specific DNA methylation print (OriPrint), a publicly available bioinformatics resource to analyze cell type-specific DNA methylation patterns. As expected, the tissue-specific DNA methylation patterns of HGSC cases aligned with ovarian (OSE-like) or fimbrial (FI-like) methylation patterns, supporting the notion that HGSC can arise from either tissue type. Gene-expression profiling of HGSC demonstrated distinct differences between OSE-like and FI-like tumor subtypes. Even though both subtypes were equally prone to recurrence, the OSE-like cases demonstrated worse prognosis than the FI-like cases, as determined by five-year overall survival. Gene-mutation analysis of the subtypes demonstrated no difference in the frequency of mutations between the two. However, FI-like cases showed higher genomic instability, which may result in patients with this type of tumor responding better to platinum therapy. Furthermore, survival outcomes were independent of *BRCA1/2* mutations, as these mutations were present in both OSE-like and FI-like HGSC cases, with no difference in frequency between the groups. The authors also examined the tumor-immune microenvironment of HGSC and showed that OSE-like tumors, but not FI-like tumors, induce an immunosuppressive state. Transcriptional signatures and functional assays demonstrated an immunomodulatory phenotype in OSE-like tumors, with increased memory resting T cells and M2 polarized macrophages, increased survivability, and active cell-to-cell signaling as compared with the FI-like tumors. Furthermore, IHC of HGSC formalin-fixed paraffin-embedded tumor samples demonstrated an increase in tumor-associated CD8-positive T cells and increased protein expression of immunosuppressive cytokines/chemokines in the supernatants of primary tumor cell cultures. By correlating DNA methylation and gene-expression data from OSE-like and FI-like HGSC tumors, the authors identified 38 genes that were differentially expressed and differentially methylated between the two groups. They further investigated the *PAX8* gene, a defining marker of HGSC. Significantly higher *PAX8* promoter methylation was seen in the OSE-like cases compared with the FI-like cases, with a corresponding decrease in *PAX8* RNA expression. PAX8 IHC staining of formalin-fixed paraffin-embedded tissue from HGSC cases (N=142) was not entirely specific for either group, as the majority of OSE-like and FI-like tumors demonstrated weak/moderate PAX8 IHC staining. However, FI-like cases were enriched for high levels of PAX8 tissue staining. In summary, the authors demonstrated the capability of bioinformatics to capture retained cell-of-origin imprinted epigenetic markers, analogous to a molecular fingerprint, to resolve the cell-type origins of HGSC. This more definitive cell-type-of-origin designation will be of value not only to categorize this common tumor into distinct prognostic subgroups but,

hopefully, to inform the development of cell type-specific, rationally designed targeted therapies to change the course of this fatal disease.

Lo Riso P, Villa CE, Gasparoni G, et al. A cell-of-origin epigenetic tracer reveals clinically distinct subtypes of high-grade serous ovarian cancer. *Genome Med*. 2020;12:94. https://doi.org/10.1186/s13073-020-00786-7

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