Novel insights into circular RNA regulation in arsenic-exposure-induced lung cancer

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Lung cancer is the leading cause of cancer-related deaths worldwide and a major global public health problem that seriously threatens patients’ lives and health. Arsenic exposure is considered one of the main causes of lung cancer occurrence.1 However, the detailed mechanisms of arsenic exposure involved in the initiation and development of lung cancer are still largely unknown. A new class of regulatory non-coding RNAs called circular RNA (circRNA) exhibits distinct cellular roles in a variety of cancer types. In an article in Molecular Therapy – Oncolytics, Xiaofei Li et al. performed RNA sequencing and qPCR assays using an arsenic-induced malignantly transformed cell model (BEAS-2B-As) and multiple lung cancer cell lines and discovered that circBRWD1 was significantly downregulated in these cells. Functional assays demonstrated that circBRWD1 knockdown facilitated cell viability and proliferation, suppressed cell apoptosis, and accelerated the G0/G1 phase transition in BEAS-2B-As cells. Mechanistically, downregulation of circBRWD1 induced by arsenic exposure resulted in enhanced stability of the mRNA (c-JUN, c-MYC, and CDK6) to which it directly bound, increasing its expression.2 Overall, the results of this study provide the first evidence that circRNAs are involved in the progression of arsenic-exposure-induced lung cancer by altering downstream target gene expression via directly binding to their mRNAs.

circRNAs are mainly generated from precursor mRNAs via a back-splicing process and play vital roles in cancer initiation and progression. An increasing number of circRNAs have been found to be aberrantly expressed in lung cancer tissues and cell lines. They are involved in the regulation of lung cancer progression by targeting cancer-related signaling pathways and/or modulating the expression of genes involved in cell proliferation, apoptosis, metastasis, epithelial-to-mesenchymal transition, and stemness, as well as resistance to therapy.3 circRNAs regulate the expression of their target genes through distinct mechanisms, such as facilitating gene transcription by interacting with the RNA polymerase II complex, altering alternative splicing events, and sponging microRNAs (miRNAs).4 However, thus far, no study has investigated the function and mechanism of circRNAs in the progression of lung cancer caused by arsenic exposure.

In their study, Xiaofei Li et al.3 first performed RNA sequencing to analyze the expression pattern of circRNAs using an arsenic-induced malignantly transformed cell model. They discovered that 2,248 circRNAs were upregulated, whereas 2,354 circRNAs were downregulated, in BEAS-2B-As cells compared with BEAS-2B control cells. To further understand the biological functions of these circRNAs, the researchers conducted pathway enrichment analysis on the differentially expressed genes. The data showed that these differentially expressed circRNAs are strongly linked to multiple signaling pathways that regulate cancer occurrence and development. Next, the team attempted to identify circRNA with the most significantly different expression using qPCR. The authors demonstrated that circBRWD1 was the most significantly downregulated circRNA in BEAS-2B-As cells. Moreover, they discovered that circBRWD1 is involved in cancer-related pathways, indicating its potential role in lung cancer progression. To validate the role of circBRWD1 in arsenic-induced lung cancer, CCK-8, EdU, and flow cytometry assays were performed. The results showed that circBRWD1 modulated cell processes by regulating cell viability, apoptosis, and cell-cycle progression. Together, these data confirm that circBRWD1 participates in the arsenic-induced malignant transformation of lung cells.

Currently, the best understood regulatory mechanism of circRNA is to indirectly alter the expression of mRNA by acting as a miRNA sponge.5 However, whether circRNAs modulate mRNAs by directly binding to them remains largely unknown. By performing an RNA sequencing analysis, the authors discovered that there were 3,508 upregulated mRNAs and 207 downregulated mRNAs in BEAS-2B-As cells compared with BEAS-2B cells. Therefore, they proposed that circBRWD1 might regulate the expression of some of these genes. Li and colleagues performed bioinformatics analysis and qPCR experiments to identify potential downstream genes. The results showed that circBRWD1 can negatively regulate the expression of c-JUN, c-MYC, and CDK6. Moreover, circBRWD1 also exhibited the potential to directly bind to mRNA. Dual luciferase reporter and RNA antisense purification assays were performed to validate this hypothesis. The authors found that circBRWD1 affected the stability of c-JUN, c-MYC, and CDK6 mRNA by directly binding to them. Then, they used EdU assays and flow cytometry assays to validate the underlying mechanism affecting the cell cycle and proliferation of BEAS-2B-As cells, and the results showed that low expression of circBRWD1 mediated cell-cycle progression and facilitated proliferation in lung cancer cells.

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One of the main concerns is the clinical implication of circBRWD1 in the treatment of arsenic-exposure-induced lung cancer. circBRWD1 was the most significantly downregulated circRNA in BEAS-2B-As cells, and it played a vital role in the progression of arsenic-exposure-induced lung cancer. These data strongly suggest that circBRWD1 possesses great potential to be a valuable diagnostic and prognostic biomarker, as well as therapeutic target, in the treatment of arsenic-exposure-induced lung cancer. This should be validated using clinical trials with large patient cohorts in future studies. In addition, circRNAs can be involved in cancer progression by multiple mechanisms, such as targeting cancer-related signaling pathways and serving as miRNA sponges to regulate specific gene expression. In the newly accepted article by Li et al., circBRWD1 was found to regulate the expression of its specific downstream gene by altering the stability of the target mRNAs via directly binding to them.² It will be interesting in the future to investigate whether circBRWD1 can also participate in the regulation of arsenic-exposure-induced lung cancer progression through other mechanisms.

In summary, the study by Li et al. has added an important layer to the understanding of circRNAs in regulating gene expression during the progression of arsenic-exposure-induced lung cancer (Figure 1). Their results not only clarified the mechanism by which affecting the mRNA stability and expression of c-JUN, c-MYC, and CDK.

Figure 1. Role of circBRWD1 in arsenic-exposure-induced malignant transformation

The expression of circBRWD1 was decreased in cells treated with NaAsO₂. circBRWD1 downregulation resulted in increased protein levels of c-JUN, c-MYC, and CDK6 by enhancing their mRNA stability via directly binding to them, thereby accelerating the G0/G1 phase transition in human bronchial epithelial cells and eventually facilitating the malignant transformation of cells.
circBRWD1 mediates arsenic-exposure-induced lung cancer but also provided a new direction for the investigation of circRNAs in regulating gene expression. The exciting findings from this study lay important groundwork for future research on circRNAs in lung cancer progression and other major diseases. The results will also help develop new therapeutic strategies based on circRNAs for patients with cancer.

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AUTHOR CONTRIBUTIONS
X.A. and Y.L. designed, drafted, and wrote the manuscript. Both authors read and approved the final manuscript.

DECLARATION OF INTERESTS
The authors declare that they have no competing interests.

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