Human ZNF292 Circular RNA Structure and Expression in Colorectal Cancer Cell Lines

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ZNF292 is a gene encoding a zinc finger protein regulating growth hormone expression and tumor formation. Additionally, it has been shown that variants in ZNF292 are responsible for neurodevelopmental disorders resulting in autism spectrum disorders. Finally, ZNF292 in humans has been shown to produce an abundant circular RNA (circRNA) in endothelial and cancer cells that is regulated by environmental conditions. We wished to determine whether circRNA production in the colorectal cancer cell lines CaCo-2 and HCT116 responds to chemotherapy treatment. Rather than RNA seq analysis, we examined circZNF292 expression using RT-PCR. Amplification employed primers that flanked the unique circular junction of hZNF292 circRNA but would not yield products from linear ZFN292. These primers were first used to perform PCR amplification on RNA isolated from untreated CaCo-2 and HCT116 cells. After reverse transcription using random hexamers, linear and circular ZNF292 primer sets used in previous studies each produced a single product band of predicted size. However, after amplification with the newly-designed circular-specific primers, there were multiple products of increasing molecular weights suggesting that several circZNF292 RNAs exist. To confirm that the products were from circRNA, RNase R treatment to remove all linear RNA was done prior to reverse transcription. Both the published primers and our newly-designed circular primers gave products after RNase R treatment, confirming the circular origin of the products. Importantly, the multiple product sizes were again present after removal of linear RNA. Product bands are being sequenced to determine the specific splicing sites that give rise to this family of related circRNAs. Further investigation will concentrate on the expression of circular ZNF292 RNA after chemotherapy and whether individual species vary independently. Although there is little information about the role of circRNA in cellular regulation, these findings expand our understanding of how these unique structures are formed to determine how they modulate cellular functions.

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