Linear and Circular Human ZNF292 RNAs Decrease after Anti-Cancer Treatment of HCT116 Colorectal Cancer Cells

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ZNF292 is a gene that encodes for a large multifunctional zinc finger protein. ZNF292 has a role in Growth Hormone transcription, developmental disorders on the autism spectrum, and in the initiation of tumorigenesis. Cancer cells have revealed ZNF292 as a gene with unique features: it is present in both linear and circular RNA (circRNA) forms. Circular ZNF292 RNAs vary in size depending on the number of exons that are back-spliced together forming a nested set of babushkas or "Russian dolls" – larger forms add an exon to a smaller circle. To determine whether anti-cancer treatments change the expression of circRNA forms as well as the linear form of ZNF292, we performed quantitative Reverse Transcriptase Polymerase Chain Reaction (qRT-PCR) analysis. Primers used were designed to amplify only the specified form of ZNF292, either the linear form or one of four targeted circular forms. Control and flavone (3,5 dihydroxy-7-methoxyflavone)-treated cell lines were grown, harvested, and total RNA extracted. Then, samples were analyzed by qRT-PCR with specific ZNF292 primer sets for each product using a standard curve for comparisons. All results were normalized to actin levels in each sample prior to statistical analysis. When compared to untreated controls, two linear ZNF292 RNAs were each reduced to 52% of control levels ($p<0.05$). Of the four circRNA forms tested, only C1 was reduced to 74% compared to control ($p<0.05$). The three other circRNAs tested remained unchanged at 85%, 111%, and 89% compared to untreated cells. The finding of the reduction of linear form correlates with the role of ZNF292 as a "trunk-driver" in oncogenesis. Intriguingly, there was a distinct difference in expression between one circRNA and the other three forms. The functional roles of circRNAs are not fully understood, but the differential expression in response to anti-cancer treatments suggests distinct functions for each of the unique circular forms. We hypothesize that the circRNA form affected is involved in cancer progression and treatment with this flavone regulates cellular expression. Specific control of circular ZNF292 RNA expression provides a unique opportunity to explore the mechanisms behind the multiple forms of this gene.

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