

Expression of ZNF292 Linear and Circular RNAs in Rat MtT Pituitary Cell Lines is Related to Somatotroph Differentiation

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ZNF292 is a complex protein of ≈ 3000 amino acids with 16 zinc finger DNA binding domains. First discovered as a Growth Hormone (GH) transcription activator, ZNF292 was then found to play a role in the initiation of certain cancers, in specific autism spectrum disorder types, and to produce high levels of unique circular RNAs (circRNAs). In the rat, we have found that ZNF292 circRNAs are present in multiple related structures containing differing combinations of exons 1-7, thus increasing in sizes like “babushka” or nested Russian dolls. To begin to understand the functions of these ZNF292 circRNAs, we assayed expression in four rat MtT cell lines that model stages of pituitary somatotroph development. These cell lines have specific GH levels: MtT/E are empty; MtT/Se have estrogen stimulated GH expression; MtT/SM somatomammotrophs have both GH and PRL; and MtT/S somatotrophs produce only GH. Rat GH3 somatomammotroph cells were also assayed for comparison. Thus, the defined stages of pituitary development in these cells are an excellent model for evaluating linear or circular ZNF292 RNA changes. All cell lines were grown according to standard conditions, then harvested and total RNA was extracted (RNeasy kit, Qiagen) and used in qRT-PCR assays where both RT and PCR were performed in each well (Luna kit, NEB). Primers were designed in MacVector: RPL19 as a normalization control, and GH, linear, and circular ZNF292 primers targeted selected exons. Standards for quantitation used purified total rat RNA (ThermoFisher). After qRT-PCR, all data was normalized to the amount of RPL19 in each sample, then average RNA levels calculated for each cell line using Excel and GraphPad Prism. We found that GH RNA rose from 0% in MtT/E and MtT/Se cells to 157% in MtT/SM and 275% in MtT/S cells. Linear ZNF292 was increased to 219% in MtT/Se cells, then declined to 110% in MtT/S and 37% in MtT/SM cells. Circular ZNF292 was expressed in MtT/E cells at 26%, increased to 184% in MtT/Se cells and fell to $\approx 100\%$ in MtT/SM and MtT/S cells. GH3 cell expression was similar in pattern to MtT/SM cells but reduced in relative abundance. Statistical analysis with two-way ANOVA indicated significant differences. First, these data confirmed that GH RNA levels correlate with stages of somatotroph differentiation in these cell lines. Second, ZNF292 linear RNA expression increases prior to the onset of GH expression, agreeing with the function of linear ZNF292 in initiating GH transcription. Third, ZNF292 circRNAs have a developmental pattern of expression that is higher in somatomammotroph cells. Expression of other RNAs central to pituitary development will be used to evaluate whether ZNF292 circRNA levels specifically relate to GH status or another criterion of cellular differentiation.

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