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Analysis artefacts of the INS-IGF2 fusion transcript

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Abstract

Background: In gene expression analysis, overlapping genes, splice variants, and fusion transcripts are potential sources of data analysis artefacts, depending on how the observed intensity is assigned to one, or more genes. We here exemplify this by an in-depth analysis of the INS-IGF2 fusion transcript, which has recently been reported to be among the highest expressed transcripts in human pancreatic beta cells and its protein indicated as a novel autoantigen in Type 1 Diabetes.

Results: Through RNA sequencing and variant specific qPCR analyses we demonstrate that the true abundance of INS-IGF2 is >20,000 fold lower than INS in human beta cells, and we suggest an explanation to the nature of the artefacts which have previously led to overestimation of the gene expression level in selected studies. We reinvestigated the previous reported findings of detection of INS-IGF2 using antibodies both in Western blotting and immunohistochemistry. We found that the one available commercial antibody (BO1P) raised against recombinant INS-IGF2 show strong cross-reaction to native proinsulin, and we did not detect INS-IGF2 protein in the human beta cell line EndoC-βH11. Furthermore, using highly sensitive proteomics analysis we could not demonstrate INS-IGF2 protein in samples of human islets nor in EndoC-βH11.

Conclusions: Sequence features, such as fusion transcripts spanning multiple genes can lead to unexpected results in gene expression analysis, and care must be taken in generating and interpreting the results. For the specific case of INS-IGF2 we conclude that the abundance of the fusion transcript/protein is exceedingly lower than previously reported, and that current immuno-reagents available for detecting INS-IGF2 protein have a strong cross-reaction to native human proinsulin. Finally, we were unable to detect INS-IGF2 protein by proteomics analysis.

Keywords: Insulin, INS-IGF2, Beta cell, Fusion-transcript, Gene expression, Proteomics, Antibody, Bioinformatics, Analysis artefact, Pancreatic islets, Diabetes

Background

Gene expression analysis artefacts

As part of our research on pancreatic beta cell biology, our group has been investigating the transcripts originating from the insulin loci using both microarrays and RNA-sequencing platforms. On several occasions we have noticed issues with the way overlapping transcripts

are handled in gene expression analysis pipelines, and this sparked the interest for looking further into the underlying reasons for the problems. Our initial observations were as follows:

In DNA microarray analysis different results will be obtained for the genes in the genomic region around INS (Ensembl: ENSG0000002546-47) depending on the mapping scheme used. In extreme cases INS itself will disappear from the analysis and all its signal would instead be mapped to INS-IGF2 (Ensembl: ENSG00000129965). The reason for this artefact turned out to be that certain widely used enhanced remapping schemes for Affymetrix probes (including the popular BrainArray CDF

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