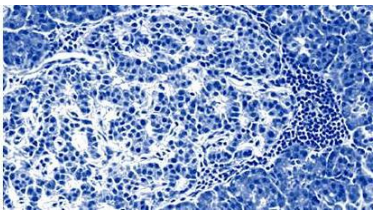
nPOD
Network for Pancreatic Organ Donors with Diabetes



Aberrant Methylation of the 11p15 Imprinted Region is Important for Insulin Expression and Cell Proliferation in Human Insulinomas

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PURPOSE

Diabetes results from loss or deficiency of insulin-producing beta cells. Restoring normal beta cell mass and function is key for reversing diabetes. We have previously shown that a rare and benign human pancreatic neuroendocrine tumor called insulinoma, holds the transcriptomic and genomic ‘recipe’ for inducing human beta cell replication. Our small pilot study revealed abnormal methylation patterns in the imprinted p15.5-p15.4 region of chromosome 11 in insulinomas. This region is also known to be imprinted and abnormally methylated in another disorder of expanded beta cell mass and function: the focal variant of congenital hyperinsulinism (FoCHI). The methylation abnormalities in our pilot study were relevant to beta cell proliferation and insulinoma pathogenesis. Therefore, we greatly expanded these studies.

METHODS

In this study, we report extensive, deep DNA methylome sequencing on an expanded beta cell cohort, and on the largest insulinoma cohort reported to date. More specifically, we describe deep methylome sequencing and detailed analysis of each of the ~30,000 CpG dinucleotides within the 11p15.5-15.4 region in a statistically meaningful cohort of five sets of normal human beta cells and an expanded set of 19 insulinomas. Further, we integrate and correlate the methylome results with additional large beta cell-relevant datasets.

SUMMARY OF RESULTS

We find abnormal, yet surprisingly consistent and recurrent methylation changes throughout the 11p15.5-15.4 region, predicting altered promoter and enhancer usage in human insulinomas vs. normal human beta cells. Our detailed bioinformatics analysis demonstrate that promoter methylation status has little effect on expression of genes in the target region. However, the abnormal methylation profile likely affects enhancers in their networks which

leads to alternative means to drive *INS* expression, and replaces the canonical *PDX1*-driven beta cell specification with a pathological, looping, distal enhancer-based form of transcriptional regulation. We also show that NFaT transcription factors, rather than the canonical *PDX1* enhancer complex, are predicted to drive *INS* transactivation in the context of the abnormal looping.

CONCLUSIONS

In conclusion, these findings strongly suggest that the aberrant methylation of 11p15.5-15.4 region leads to 3-D structural abnormalities in chromatin looping in insulinomas and they likely contribute to inappropriate insulin oversecretion and cell proliferation.