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Discovery and Validation of a Novel Set of Putative Progression Markers in Well-Differentiated Primary Pancreatic Endocrine Carcinomas

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Background: Molecular mechanisms of progression in pancreatic endocrine tumors/carcinomas (PETs/PECAs) are not fully understood. We have identified a novel set of potential molecular markers of progression in these clinically unpredictable neoplasms.

Materials and Methods: Five clinically-localized primary (CLP)-PETs from 5 patients (mean age 66; 3M/2F) and 6 well-differentiated (WD) metastatic primary (MP)-PECAs from 6 other patients (mean age 59, 3M/3F) were macro dissected to achieve 80-98% viable tumor for RNA extraction and run on Affymetrix U133 2.0 gene chip. The data were RMA normalized and differentially expressed genes in MP-PECAs vs. CLP-PETs were identified by t-test. This gene set was further refined by excluding those with a significant frequency of Type I errors and enforcing a median 2-fold change between these two groups. Genes satisfying these criteria were grouped into functional categories based on GO annotation and for a correlative analysis to select 'putative progression genes' for further validation on the original frozen PETs/PECAs and also on independent test sets of archival MP-PECAs and CLP-PETs by real-time PCR using micro fluidics cards (ABI).

Results: 217 transcripts were differentially expressed between MP-PECAs and CLP-PETs, using p-value <0.05 and fold-change values >1.5/>2/>4/>8 (217/94/19/1 gene respectively). Among those with a fold-change >2, several exhibited a high level of reliability, based on similar patterns of differential expression for multiple probe sets targeting the same mRNA. Among our 85 'putative progression genes' from the original frozen tumors, we validated under-expression of RUNX1T1, DRD1IP, ISL1, ETV1 and GCG and over-expression of TMPRSS6, SERPINA1, SSTR5, SMURF1 and CD24 on independent test sets of archival MP-PECAs relative to CLP-PETs.

Conclusion: We have discovered a novel set of 'putative progression genes' in sporadic primary WD-pancreatic endocrine carcinomas. Further validation of these candidate genes will support their role as potential prognostic markers in primary pancreatic endocrine tumor tissues.