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Pancreatic *Reg* And A Conserved Bioactive Fragment Are Mitogenic Through The MAPK P38 Pathway

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Introduction

Pancreatic *reg* proteins are exocrine proteins which are mitogenic to pancreatic ductal and β -cells (1,2). Using multiple alignment sequence comparison of the *reg* proteins with known mitogenic activity, we identified an amino acid sequence (RegP) highly conserved between rat, mouse and human *reg*'s (3), and postulated that it is the bioactive fragment. This sequence contained an 'WIGL' amino acid motif, considered important in calcium-dependant lectins. We also postulated that the action of *reg* would be through activation of mitogen-activated protein kinases (MAPK).

Material and Methods

Reg was isolated from human pancreatic juice, and RegP synthesized. Rat ARIP ductal cells (7.5×10^3 per well) were cultured in DMEM + 1% serum replacement media and inoculated with protein, and mitogenesis assayed at 48 hours using a tetrazolium assay. A 24 hr time course of phosphorylation of the mitogen-activated kinases (MAPK) P44–42 (ERK1/2), P38 and SAPK/JNK was assessed on 10ug of cellular protein by Western analysis using antibodies directed against the phosphorylated forms of the MAPKs.

Results

In Figure 1, data is expressed as Mean $\pm$ SEM O.D. units (n=3 for each point). Both *reg* and RegP were mitogenic to ARIP cells in a dose related fashion. While it was also active on cells, it did not affect AR42J acinar cells or rat islets (not shown). Two random amino acid β -peptides were not active.

Western analysis of cellular protein showed activation of MAPK P38 in the first 24 hours (Figure 2); P44–42 and SAPK/JNK were not activated.

Conclusion

The pancreatic *reg* proteins have been identified in human, rat, mouse, hamster and cow, and are subdivided into three types, I, II and III (3). The sequence homologies between the *reg* proteins range between 41–68% (3,4). They are found in the pancreas, small intestine and brain. *Reg*'s I (also known as Pancreatic Stone Protein) and III (Pancreatitis Associated Protein) are expressed in the pancreas, the former constitutively and the latter after induction of pancreatitis. Both are mitogenic to ductal and β -cells (1), suggesting involvement during pancreatic regeneration.

Reg I protein is mitogenic to pancreatic ductal cells via activation of the MAPK P38 pathway. A highly conserved amino acid portion of this calcium-dependant lectin which

confers this proliferative activity, also via phosphorylation of P38. *Reg* is therefore involved in the expansion of the ductal population of the pancreas, and the new bioactive peptide can be used therapeutically to induce proliferation of these and other pancreatic-derived cells.

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Figure 1. Mitogenesis on ARIP Cells

* $p < 0.05$ compared to controls, ANOVA

Figure 2. Western Analysis of P38 in ARIP Cells
Reg= human Reg protein, RegP=peptide (10 nM)