Photosynthetic Algae/Insulinoma Cell Fusion Creating Self-Sustaining Insulin Producer

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Introduction
Insulin is a hormone that regulates blood sugar concentration. In the approximately 300 million diabetic patients worldwide, utilization and production of insulin is substantially, and in the case of Diabetes mellitus type I patients, completely, impaired [1]. These patients require the direct pumping of exogenous insulin into the blood. There are currently two methods of exogenous insulin production: extraction from other mammals, such as cattle, and genetic translation of bacteria with the human genes necessary for insulin production. The former has become increasingly uncommon due to patients’ allergic reactions to the insulin produced by artificially dissimilar animals, and the latter method is inefficient in that it requires the use of complex protocols in order to generate sufficient quantities of insulin [2].

The purpose of this study was to create a new method for the efficient in vitro production of exogenous insulin. The basis of this method is the creation of a low maintenance, plant-animal cell hybrid that produces insulin while remaining self-sustaining via photosynthesis. The unicellular, photosynthetic green-algae, Chlorella keissleri, was fused with rat insulinoma RIN 5F cells, or with primary cultured rat pancreatic islet cells to create cell hybrids, referred to as Modified Insulin Production (MIP) cells. It was hypothesized that successful fusion of algae and insulinoma cells would lead to an efficient, insensitive approach to in vitro insulin production using plant-animal cell hybrids containing the biochemical properties of each cell type. Prior to fusion, the rigid cell walls of the algae cells were removed by mechanical disintegration with a sterile scalpel, after which cells were cultured twice each week.

Results and Discussion
The inter-kingdom fusion process for exogenous insulin production was hypothesized to work effectively if algae cell walls were successfully degenerated, and if PEG acted as a strong fusion agent, inducing RIN 5F to ingest C. keissleri. The fusion process was used to show that the combination of two significantly different cell types can yield a new cell type that retains the biochemical properties of each cell type. The immortal, insulin-producing cell line, RIN 5F, derived from rat pancreatic tumor, was used as the foundation of the MIP cell line, and one of few known cell lines to readily produce insulin in vitro. The unicellular photosynthetic green-algae C. keissleri was selected as the fusion partner due to its photosynthetic nature and low maintenance requirements for culture. The UCSF Plant Science Laboratory was approached in an attempt to create a hybrid cell that produces useable quantities of exogenous insulin and remain self-sufficient and viable via photosynthesis.

Materials and Methods

Cell Culture
Rat pancreatic insulinoma cells (RIN 5F) were cultured in Dulbecco’s Modified Eagle’s Medium: Nutrient F-12 (DMEM-12) supplemented with 1% penicillin/streptomycin and 10% fetal bovine serum, and incubated at 37°C and 5% CO2. Cells were sub-cultured twice each week. Primary rat pancreatic islet cells were isolated from 600 g rats by sterile mechanical methods directly from extracted rat pancreatic tissue. RIN 5F were cultured in DMEM/F12 medium, while 5F cells were cultured in DMEM-12 medium. 5F cells plus 0.125 mg/ml PEG solution was added to the cell mixture. The flask was incubated overnight prior to light microscopy analysis.

Microscopy
Transmission electron microscopy was used to obtained high resolution cross-section images of algae cells. Samples were fixed by pelleting at 3000 g for 5 minutes, fixation in 2% glutaraldehyde, 1% osmium tetroxide in 0.1M sodium cacodylate buffer at pH 7.4, dehydration in a graded series of acetone, embedding in Epon 812, sectioning (100nm), and staining with uranyl acetate and lead citrate. Cells were observed at 80Kv in a JEOL 1010 transmission electron microscope.

Results

**Insulin Production**

RIN 5F and MIP cell fusions were induced. 24 hours after PEG was introduced to the algae/insulinoma fusion, light microscopy showed membrane interaction between C. keissleri and RIN 5F cells (Figure 1). Media samples were taken from the MIP cell fusions and from the RIN 5F control flask each day for one week following fusion, and samples were analyzed by ELISA.

**Insulin Concentration Over Time**

It was determined that the MIP hybrid cells produced insulin in quantities consistent with insulin produced by RIN 5F cells alone (Figure 6). Furthermore, the amount of insulin produced by the MIP hybrids was comparable to that produced by a similarly sized sample of bacteria by current bacterial transfection methods of exogenous insulin production according to the NIH (Figure 7). It is important to note that, with any additional culturing or exchange of media, the inter-kingdom fusions continued to produce consistent quantities of insulin, approximately 45 ng/mL, for 14 days after fusion was induced. This suggests that during this time, the cells remained viable and that the photosynthetic products of the algae in the MIP fusion was sustaining the hybrid cells. The initial RIN 5F/C. keissleri fusion process was expanded by hybridization between the same algae species and primary cell line cells cultured directly from a extracted rat pancreas. Fusion in this case was determined via scanning electron microscopy, and suggested a more superficial interaction between the membranes of the algae and plant cell, which can be used to test for insulin secretion in media by this cell hybrid to yet be performed.

**Insulin secretion**

Different cell fusion cell processes represents the potential for the effective, insensitive production of essential cellular bio-products. In this study, insulin was the bio-product measured due to the properties of the photosynthetic components of the hybrid cells. However, altering the cell types used in the plant-animal fusion process also gives insight into inter-kingdom, multi-cellular hybrid formation, an emerging technique that has the potential to yield remarkable results in mass production of useable bio-products.

References


Acknowledgments

• Kathleen A. Donovan, Bergen County Academies
• Bergen County Board of Chosen Freeholders
• Bergen County Technical Schools
• Bergen County Technical School District

Presented at Microscopy and Microanalysis 2014 August 3 – 7, Hartford CT
Paper Number: 327 Poster Number: 317