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Review Articles

Regulation of Insulin Secretion by Means of Insulinoma Cell Lines

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The use of primary B cells in biochemical and molecular research is limited by the availability of pancreatic endocrine tissue. Numerous investigators have attempted to establsh an insulin-secreting cell line that retains normal regulation of insulin secretion. The most widely used insulin — secreting cell lines are RIN, HIT, MIN 6 and INS — 1. Insulin — secreting cell lines represent a potential source of transplantable tissue to overcome the limited availability of primary islets.

Key words: B cell, cell line, immortalization, insulin.

The transplantation of insulin — producing tissues in patients with diabetes (insulin — dependent — type1) offers an approach that is much more physiological to the restoration of the progressive diabetic neurovascular complications. While in the 1970s [2,14] the isolation and transplantation of Langerhans islets was only possible in experimental animals with diabetes the early 1990s gave rise to several teams working on diabetes treatment projects for transplantation of isolated pancreatic islets [11, 28, 29]. The recipients of these transplants maintain normal glucose levels without insulin therapy years end [16]. The limited opportunities for ensuring human donor pancreatic islets enhance the studies of a number of authors in search of means. For stimulating the proliferation of insulin producing endocrine cell types.

Insulinoma cell lines — definition and classification

Investigations of the differentiation and function of the B cells and elucidation of abnormalities associated with B cell dysfunction determine the necessity for establishment of insulinoma cell lines.

The insulinoma cell line is a cell line capable to grow in cultures and to synthesize and secrete insulin. These are spontaneously occurring and induced tumor lines even can be other than B cells [12]. To overcome the limited availability of primary B cells some authors have attempted to immortalize B cells and establish a stable insulin secreting cell line.

Insulin secreting cell lines can be classified according to the technique used for transformation of the original tissue:

Insulinomas

Naturally occurring insulinomas

Despite numerous attempts, derivation of stable cell lines from spontaneous insulinomas of human or animal origin is exclusively difficult [3, 26]. In principle naturally occurring B-cell tumors dedifferentiate very rapidly in vitro and lose their ability to synthesize and secrete insulin.

Induced insulinomas

Radiation-induced insulinomas — The first insulin-secreting cell line, the RIN cell line, was established in 1973 by Chick et al [4]. X-ray-irradiated rats developed pancreatic tumors 1,5 years after irradiation. The tumors were excised, sliced and transplanted under the skin of 6-week-old rats. Tumors were then propagated in vivo by serial transplantations into RIN rats or BAlb/C mice [8]. Endocrine cells were then purified from the tumors and seeded on a hepatocyte matrix. After several passages the cells begin to adhere spontaneously to the culture vessel. In 1992 [1] have derived INS-1 from parental RINm5f after the following procedure: In a co-culture of lymphocytes and RIN cells in the presence of 2-mercaptethanol free-floating cell aggregates were formed which appeared to be morphologically different from the parental cells. They gave origin to INS-cell line.

Virus-induced insulinomas

The first observation on the development of pancreatic tumors after injection of rats with a BK-virus suspension and investigation of the oncogenicity of the BK-virus and isolation of In-111 come from Uchida et al. [27].

Chemically-induced insulinomas

Insulinomas are obtained by administration of nicotinamid and streptozotocin after that they are serially transplanted in vivo, isolated and propagated in culture [5, 15].

BK- virus- transformation

By this method isolated rat Langerhans islets are transformed in vitro by incubation in the presence of BK virus. The problem that arises is that transformed cells do not secrete insulin after 50 days [10].

Morphological and functional characterization of insulin-secreting cell lines

Morphological characterization

Most insulin-secreting cell lines consist of several cell populations. Immuno-histochemically and with confocal microscopy it is demonstrated that BTC-6 cells contain insulin, glucagon and somatostatin. A colocalization of insulin and glucagon in the same cells is observed, but somatostatin — containing cells appear morphologically different [19]. HIT-T15 also contain the three hormones , but in this case most glucagon -containing cells do not contain insulin [13]. Probably synthesis of glucagon and somatostatin by transformed B-cells might represent a stage of dedifferentiation of all three hormones because the three hormones show a transiently coexpression in progenitor cells during pancreatic development. Most insulin-secreting cells tend to associate in clusters. Usually somatostatin- secreting cells segregate at the periphery, similarly to primary islets of Langerhans [1, 24, 19]. Electron microscopic analysis of insulin-secreting cells shows typical of the the B cell numerous secretory granules resembling those of primary B cells [19, 9].

Functional characterization

• Insulin content — Intracellular insulin content in insulinoma cell lines is lower than that of primary B cells by one to three orders of magnitude [1, 24, 9, 30]. Suppression of the decrease in insulin content of HIT-T15 and BTC-6 cells by a chronic culture in 0,8 mM glucose is shown [20, 23].
• Glucose-induced insulin secretion — Among the described cell lines only a few react to changes in glucose concentration. Regulation of insulin secretion by glucose is assessed by static incubation in the presence of increasing glucose concentrations. We must note that insulin release from all cell lines is lower than that of native islets.

Moreover, the magnitude of insulin secretion above the medium level is greatly diminished. Some of of RIN cell line subclones can retain the ability to secrete insulin in response to glucose [21]. HIT cells secrete insulin in response to glucose with a half-maximal stimulatory concentration of ~ 1,5 mM. Obviously differences found in the literature clearly suggest that assessment of glucose-induced insulin secretion in static incubation highly depends on the techniques used by investigators [6].

The transformed insulin-secreting cells lose some of the functional characteristics of the primary B cells. The described changes in the functional characteristics (reduction in intracellular insulin content, alteration in glucose-sensing, coexpression of glucagon and insulin) represent dedifferentiation of the cells.

Possibilities for application of insulin-secreting cell lines

Investigation on the molecular regulation mechanisms of B cell function and abnormalities associated with B cell dysfunction require the use of insulin-secreting cell lines. For example B cell lines are especially appropriate for the study of glucose-signalling pathways, ion channels, sulfonylurea receptor and regulation of synthesis and secretion of insulin. Investigation of gene regulation at the transcriptional level is extremely difficult in primary islets. Transfection of islets using traditional approaches are of a limited success: calcium-phosphate precipitation, electroporation of lipofectinusually provides a transfection efficiency of 1 to 10 per cent [25, 17, 7]. Alternatively, transfection of monolayered transformed cells is easier and more efficient. Moreover, the possibility for long-term cultures allow chronic exposure of HIT-T15 cells [21, 18] and BTC-6 cells [23] to high glucose concentrations. Another possibility for application of insulin-secreting cell lines is as a source of tissue for therapeutic applications. The availability of transplantable human islet tissue for the transplantation and for the treatment of diabetes mellitus is limited. Xenotransplantation of large animal islets encapsulated in a perm-selective membrane for protection from immune rejection could overcome the shortage of human tissue [22]. However, large-scale application of this approach is limited by the cost and inconsistency of large animal islet isolation and purification. Alternatively, transformed B cells could provide unlimited amounts of transplantable tissue of constant quality.

Although insulin-secreting cell lines are not so perfect as primary B cells in terms of regulation of insulin secretion, they represent an extremely valuable tool for studies of B cell function as well as dysfunction. The use of transformed insulin-secreting cell lines as transplantable tissues could solve the problem with limitations of primary islets and tissue rejection. It is reasonable to speculate that recent progress in cellular engineering and molecular manipulation will render possibilities for modification and stabilization of cell lines, so that their functional properties can closely mimic those of native B cells.

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