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COMPARING THE BEHAVIOUR OF HUMAN AND RODENT BETA CELL LINES FOR *IN VITRO* DIABETES MELLITUS STUDY

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Abstract

For the past 30 years, considerable effort has been made to generate useful rodent and human insulin-secreting cell lines to understand the complexity and functionality of the pancreatic beta cells. Since then, numerous types of research have been carried out using these cell lines to assess the pathology of diabetes mellitus. Among them, the cell lines INS-1, MIN6, 1.1B4 and EndoC-βH1 are the most widely used due to their stability and appropriate response to glucose stimulation. With multiple choices of cell lines available, there is a question of which cell line can be the best cell model for *in vitro* pancreatic beta cell study. The characterisation of each of these cell lines has already been vigorously studied, but there is still the need to understand how rodent and human cell lines differ from each other in order to choose the most appropriate cell model for a specific study. In this review, we explore the differences between human and rodent insulin-secreting cell lines in terms of their culturing condition, glucose-stimulated insulin secretion and reaction toward oxidative stress. Together, it is hoped that these comparisons could provide new perspectives for researchers in dealing with the available insulin-secreting cell lines for their *in vitro* diabetic studies.

INTRODUCTION

According to a report by the International Diabetes Federation, an astonishing number of 537 million people globally were found to live with diabetes in 2021 [1]. This number will continue to rise in the next 10 years, making this disease a major global concern. Pancreatic beta cells play a huge part in defining the causes of diabetes mellitus as it functions primarily to secrete insulin, the key factor to maintaining normoglycemic condition. In type 1 diabetes, insulin production is reduced due to autoimmune destruction of beta cells. In contrast, type 2 diabetes (T2D) is mainly caused by lifestyle-induced insulin resistance, in which the action of insulin is no longer effective toward body tissues [1]. Recently, however, increasing evidence suggests that insulin resistance may not be the main culprit for T2D development. Instead, insulin hypersecretion is shown to cause more harm towards the progression of this disease, or at least it works in synergy with insulin resistance [2,3]. Either way, this leads to a condition called hyperinsulinemia. In order to compensate for increasing metabolic demands, the insulin-secreting beta cells are able to make adaptive responses to multiple aspects such as increasing beta cell mass and proliferation as well as enhancing their functions such as increasing insulin secretion [4]. Over time, however, the cells become overworked, and these changes trigger oxidative stress, activate pathways to apoptosis, induce beta cell exhaustion, and promote beta cell loss. Ultimately, the functions of beta cells are disrupted and thus worsening the prediabetic condition [4].

This is further proved by the T2D genome-wide association studies where a majority of mutated transcriptional genes found are proteins that are responsible for beta cell dysfunctions rather than insulin resistance [5,6]. For instance, the mutated gene hepatic nuclear factor 1β (*HNF1B*) was identified for reduced beta cell development and metabolism, eukaryotic translation initiation factor 2 alpha kinase 3 (*EIF2AK3*) for increased endoplasmic reticulum stress, hepatic nuclear factor 4α (*HNF4A*) for increased beta cell apoptosis, and calcium binding and coiled-coil domain 2 (*CALCOCO2*) for loss of insulin content [5,7]. Furthermore, several examples of improved beta cell function can be seen after insulin secretion was suppressed in humans [8,9] and mice [10] where improved insulin sensitivity, better pulsation of insulin release, and lower glycaemia were reported.

Over the years, many *in vitro* works involving the study of beta cells have been done to understand the complexity and functionality of these cells, particularly on insulin secretion and cell apoptosis [11–14]. It is impossible to completely replicate functioning pancreatic beta cells that includes its surrounding regulation systems, so the usage of human and rodent islets in such studies is valuable as they do provide similar architectural characteristics of native tissues [15]. However, the process of obtaining, maintaining, and working on such islets is rather difficult. The islet isolation by itself is already an intricate process where it requires highly skilful techniques to ensure the islets can maintain their functions and viability [16]. Once obtained, these islets are only available for limited number of passages due to telomere shortening and cellular senescence [17]. In addition, limited availability of suitable islet donors is also a main concern for human islets [18], whereas the usage of animal islets will have to sacrifice animal models, which may bring up ethical concerns.

Hence, various human and rodent insulin-secreting beta cell lines were generated for the past 30 years to overcome these limitations [19–22]. The many advantages of using these cell lines over islets in diabetic studies include the easiness to obtain and culture the cells, stable proliferation rate, and the genes can easily be manipulated for specific works [23]. Since then, the characterisation and physiological functions of these cell lines were critically analysed, studied, and compared [24–26]. However, the behaviour of the cells during culture and *in vitro* assays have yet to be compared, especially the differences between rodent and human cell lines. Therefore, in this review, we aim to compare these differences in four aspects: culturing environment, passage stability, glucose-stimulated insulin secretion (GSIS), and oxidative stress-induced cell death (apoptosis).

HISTORY OF INSULIN-SECRETING CELL LINES

Rodent Insulin-Secreting Cell Lines

The effort to establish a valid and useful pancreatic beta cell model began in 1977 when a transplantable insulinoma was discovered from the propagation of an insulin-producing pancreatic tumour [20]. From there, a continuous cell line named RIN (specifically termed RIN-r and RIN-m which was derived from rat and mice transplant, respectively) managed to be developed through irradiation, making it the first beta cell model to exist [27]. While this cell secretes insulin, the level of secretion fluctuates inconsistently due to the simultaneous secretion of somatostatin, an inhibitor of insulin secretion, by the cell. Besides, the cell possesses different physiological characteristics to native beta cells such as having different glucose transport properties, slower glucose transport compared to normal β-cells as well as abnormal glucose-phosphorylating enzymes expression, and is insensitive towards glucose [28–30].

Since then, various methods and transformations have been done over the years to generate several numbers of better insulin-releasing cell lines. Improvisations were made to these cell lines to eliminate existing limitations while also ensuring that the standard regulation of insulin secretion is maintained. Just like RIN-r and RIN-m, several other beta cell lines had been formed by inducing irradiation towards pancreatic tissues, such as CRI-G1 [31] and INS-1 [19] that were derived from rats. However, CRI-G1 did not respond well to the normal physiological range of glucose concentration [31]. On the other hand, INS-1 was reported to have high content of insulin while still retaining proper functions of normal beta cells [19]. Apart from irradiation, another common method used to generate beta cell lines is by expressing the simian-vacuolating virus 40 (SV-40) antigen oncogene from the insulin gene promoter in transgenic mouse. Examples of the cell lines produced from this method include MIN6 [32], βTC [33], βhC [34] and NIT-1[35] derived from the *Mus musculus* species. Through this method, only MIN6 cells are considered successful as the rest of the cell lines generated from the SV40 transgenic mouse were also unable to be responsive towards the normal physiological range of glucose concentrations [26].

Apart from this, other methods to generate functioning beta cell lines were also explored. For instance, induction of insulinoma with the BK virus produced the cell line IN-111, but it has little to no responsiveness towards physiological glucose concentration [36]. On the other hand, McClenaghan and his co-workers managed to engineer a working beta cell line through electrofusion. The RINm5F cell line was combined with a normal rat pancreatic beta cell forming a cell line named BRIN-BD11, in which GLUT-2 was evidently expressed and some of its characteristics, namely glucokinase activity and insulin content were reported to behave in a similar pattern with normal rat islets [37].

Human Insulin-Secreting Cell Lines

While the usage of rodent cell lines in diabetes research has brought many beneficial findings and knowledge, the functions and characteristics of rodent cell lines are thought to provide major differences to that of human tissues, making it difficult for researchers to predict correct responses for diabetes studies. There is a possibility of immune rejection as well should the cells were to be implanted in the future for cell therapy purposes [24]. Hence, advances on human beta cell lines were made along the way starting with the CM cell line, derived from the insulinoma of a 66 year old patient back in 1980. The cell managed to proliferate over 70 passages [38]. However, no hormone secretion by the cell was reported, despite having characteristics of a secretory cell, making it unsuitable for further *in vitro* beta cell study [39].

Similar to rodent cell lines, several human beta cell lines were also generated from the transfection of the SV40 antigen and other oncogenes with purified human beta cells, namely TRM-1 [40], BetaLOX5 [41], NAKT-15 [42] and the EndoC-βH variants [22,43,44]. These cell lines successfully secreted insulin and had appropriate sensitivity towards glucose stimulation. However, the level of insulin content in these cells were reported to be relatively low compared to primary islets, even after modifications had been done on EndoC-βH2 and EndoC-βH3 [43,44].

Due to the success of generating BRIN-BD11 from electrofusion, the same group attempted to produce a new human beta cell line with the same concept, which is by fusing two human cells together: freshly isolated human pancreatic beta cells and the immortal human PANC-1 epithelial cell line. Three cell lines were generated, namely 1.1B4, 1.4E7 and 1.1E7 [21]. Among these three, 1.1B4 showed the best result as it retained proper mechanism of insulin secretion even after extended passages as well as having more sensitivity towards glucose compared to the other two.

The challenge in creating a new and useful beta cell line lies in the ability of the cell model to secrete insulin in response to glucose stimulation within the physiological range as well as having genotype and tissue markers that are similar to its parental tissues. Looking back, many attempts and developments have been made since the last 30 years [19,27,31,45]. Majority of the generated cell lines such as INS-1, MIN6, BRIN-BD11, and EndoC-βH1 are able to secrete insulin yet still managed to maintain the important beta cell characteristics. However, problems arise when majority of these generated cell lines have much lower insulin contents in the cells compared to that of primary islets and/or the cells have low sensitivity towards glucose stimulation [46]. Hence, more innovations are needed to improvise these limitations, so, valid and dependable research on future diabetes mellitus studies can be ensured.

Nevertheless, the creations of these beta cell lines have greatly contributed in many diabetes-related discoveries. Marafie et al. utilised the INS-1 cell line in discovering the interplay of the free fatty acid receptor 1 (FFAR1) receptor with the mTOR, Akt, and IRS-1 signalling pathways in palmitate-induced beta cell lipotoxicity [47]. The MIN6 cells were used in identifying novel agonists of the FFAR2 receptor that have the ability to potentiate insulin secretion [48]. The effect of *Cornus officinal*, a plant that is commonly used as a traditional Chinese medicine, on minimising β-cells loss was meanwhile characterised and analysed on the 1.1B4 cells [49]. Recently, Reeder et al. discovered that the maternal serum released by the placental cells were able to increase the proliferation of EndoC-βH1 beta cells 20% better than fetal bovine serum. This discovery is in line with the association made between pregnancy and increased beta cell mass [50]. Additionally, it was reported in MIN6 and mouse islet that the loss of G-protein coupled receptor 43 (GPR43) caused an increase of insulin secretion and glucose tolerance [51]. Similarly, the same increase was also reported in GPR43-deficient mice in another study [52].

Taken together, such findings indicate that these cell lines are widely accepted, and can serve as a good cellular model for *in vitro* pancreatic beta cells studies. Additionally, this venture has opened up many research possibilities, and provided fast and reliable findings that bring tremendous benefits.

COMPARING RODENT AND HUMAN INSULIN-SECRETING CELL LINES

With a lot of cell lines generated, be it human or rodent, one question stands: Which cell line serves as the best cell model to conduct *in vitro* diabetes mellitus researches?

Culturing Human and Rodent Beta Cells

Knowing the culturing condition of a cell is important as different cells may require different growth medium for a stable propagation [53]. INS-1, BRIN-BD11, and 1.1B4 are cultured specifically with RPMI-1640 along with 10% fetal bovine serum (FBS) or fetal calf serum (FCS) supplementation [19,21,37]. One key disadvantage of culturing INS-1 is that it requires β-mercaptoethanol in the culture media to maintain stable cell propagation and ensure functioning beta cell characteristics [19]. B-mercaptoethanol is known as a toxic compound that can induce irreversible protein denaturation [26]. MIN6 and EndoC-βH1 meanwhile are cultured in Dulbecco's Modified Eagle Medium (DMEM) and also require β-mercaptoethanol. Whereas MIN6 requires 15% FBS supplement and 4.5 g/L glucose [32], EndoC-βH1 is supplemented with 2% (v/v) bovine serum albumin and 1 g/L glucose instead [22]. However, we find that culturing EndoC-βH1 can be quite expensive compared to the other cells as it requires many other reagents to ensure cell stability such as nicotinamide, transferrin and selenite. Furthermore, it is vital for this cell line to be cultured on Matrigel-fibronectin flasks/plates to allow optimum cell adhesion [22], thus further increasing the cost.

It is also important to note that MIN6 is chronically exposed to high glucose concentration during culture. Hence, there may be alterations in the glucose-stimulated insulin secretion and overall responsiveness towards glucose such as having increased glucokinase activity, GLUT2 affinity, and glucose utilization [54–56].

Although it may seem rather trivial, culturing and working on these beta cells at the right passage number is important as these cells can lose their important functional characteristics over a certain passage. INS-1E, a variant of the parental INS-1, has been proven to be stable over 2-years of continuous culture as well as more than 100 passages, with remarkable stability noted between passage 40 to 100 as the cells passaging below 40 and above 100 has lesser insulin amount [57]. The passage stability in long culture shown by INS-1E is similar to its parental INS-1 and clonal INS-1 832/13 where both of them remained stable at passage 80 and above [19,47,58]. As for BRIN-BD11, the cells were reported to remain stable at passage 50, where it retained its morphological appearance, insulin content, and insulin secretion capabilities [37]. In a MIN6 cell study where passage 30-40 was categorized as low passage (LP) and passage 60-70 as high passage (HP), HP MIN6 cells was observed to have a stark metabolic profile difference to LP cells such as having severely impaired insulin secretion, along with reduced frequency of insulin granules, concentration of intracellular ATP, and expression of proinsulin mRNA [59]. These differences may be attributed to significant changes in almost 1000 genes expressions, such as reduced *Glut1, Gck, Pfk, Srebp1c, Ucp2, Sirt3* and *Nampt* expressions. These mRNA transcripts are responsible in encoding glucose transporters, catalysing TCA cycle and oxidative phosphorylation, lipid synthesis and also mitochondrial fatty acid oxidation. Ultimately, this causes impaired glucose uptake, glucose oxidation reduction, and failure for intracellular ATP to be increased, which is an important component in stimulating insulin secretion [59,60]. Recently, a new MIN6 clone named MIN6-CB4 was established where its GSIS was reported to remain stable after 7-month of continuous culture as well as having significantly higher insulin granules than the parental cells, but the passages used in the experiments are of the low passages (ranging from 4-21 passages) with no report on higher passages [61].

As for human beta cell lines, EndoC-βH1 was reported to be stable for 80 passages at least [22], but a 2018 finding revealed that the glucose transporters 1 – 4 (GLUT 1-4) were only expressed in low passaged EndoC-βH1 [46]. In 1.1B4, there was no significant differences reported between cells in passage 17 and passage 40 [21]. However, nothing has been reported yet for passage above 40, as all researches on 1.1B4 had only been using passage 15-40.

From our observation, we find that there is no major difference in terms of culturing condition between human and rodent cells as they require almost the same media and supplementation. Meanwhile, rodent beta cell lines seem to have better passage stability especially in long-term continuous culture compared to human beta cell lines. This observation, however, is based on the limited report available on the long-term stability of both EndoC-βH1 and 1.1B4 cells, and may be proven otherwise with more studies done in the future.

Glucose-Stimulated Insulin Secretion

One major key factor to acknowledge the functionality of a beta cell is their ability to secrete insulin to glucose stimulation, and is majorly tested through the GSIS assay. Comparing available insulin-secreting cell lines with human islets, the total insulin content inside the cell lines were found to be in the range of only 0.008–17% from the total insulin content of human beta cells (as seen in Table 1). While it is inevitable for these cell lines to be inferior to the actual beta cells characteristics in terms of their insulin content, their responsiveness towards glucose stimulation on the other hand can be considered on-par (based on the percentage of insulin secreted over insulin content as shown in Table 1). It needs to be noted however that insulin content and secretion may decrease with prolonged culture, and these values only generally reflect the insulin contents and secretion without considering the passage numbers.

The insulin content of INS-1 cells whether at low or high passage remains stable at the average of 8.92 μg/106 cells. However, only 2.2-fold increment of secreted insulin was reported after 3-20 mM glucose exposure [19]. Its clonal cell lines INS-1E and INS-1 832/13 on the other hand reported an increase of glucose-stimulated insulin secretion up to 6.2-fold and 10-fold, respectively, indicating improvements on the generation of β-cell lines [57,58]. Meanwhile, both high and low passages of clonal MIN6 named MIN6-cl4 secreted insulin 8- and 40-fold better than their parental cells [62]. As for human β-cell lines, the 1.1B4 cells reported an increase of 2.3-fold insulin secretion following 16.7 mM glucose stimulation [21], whereas the EndoC-βH1 reported a 3-fold increment after 15 mM glucose stimulation [22]. Interestingly, the fold increment of insulin secretion reported from these cell lines are almost similar and comparable to that of rat islets, where their GSIS-secretory response was found to be on the range of 4.2- to 12.3-fold of increment [57]. When stimulated with the same concentration of glucose, INS-1E was shown to secrete insulin 2-fold higher than 1.1B4 cells [63]. In another study, MIN6 showed the highest secretory response at 3-fold after 16.7 mM glucose exposure, compared to ~2.5-fold of INS-1 and 1.7-fold of βTC3 cells [64]. The same study also reported that 16.7 mM

Table 1. Approximate comparison of total insulin content and insulin secretion in different cell lines

Cell Type	Insulin content (ng/10^6 cells)	Insulin secretion (% of cell content)[a]	Reference
INS-1	8920	Low glucose: 4.15	[19]
		High glucose: 11.78	
MIN6	5792	Low glucose: 2.48	[109]
		High glucose: 9.9	
BRIN-BD11	76.8	Low glucose: 2.6	[37]
		High Glucose: 3.9	
1.1B4	1.5 - 4.08	Low glucose: 0.09	[21,86]
		High glucose: 0.15–5.56	
EndoC-βH1	1000	Low glucose: 4.0	[46]
		High glucose 6.6	
Human islet (6 studies)	50000	Low glucose: 0.9–2.7	[46]
		High glucose: 4.3–8.9	

[a]The approximate percentage of insulin secretion was calculated by dividing the numerical value of insulin secretion with the insulin content of the cell (for normalisation) as provided by each respective studies

glucose exposure only induce 2-fold of insulin secretion in purified rat beta cells, indicating that these insulin-secreting cell lines were again comparable to the purified cells [64]. Conversely however, Krause et al. showed the normal and expected superior pattern of islets (5.2-fold increased) over BRIN-BD11 and 1.1B4 cell lines (2.6- and 1.5-fold increased) insulin secreting capability [65]. In majority of studies however, the glucose-responsiveness and insulin secretion in these cell lines were not directly compared to those of rodent and human islets in their respective studies. So, while it is proven that these beta cell lines are appropriately responsive towards glucose stimulation, its comparison against islets' glucose responsiveness remains controversial.

Together with glucose stimulation, incretin such as glucagon-like peptide 1 (GLP-1) can also further increase the secretion of insulin in human body. It has been previously established that the GLP-1 receptor (GLP-1R) are expressed in INS-1E, MIN6, 1.1B4, and EndoC-βH1 cells [46,66,67]. Rather than using GLP-1 itself as the incretin, most GSIS studies prefer to use exendin-4, a GLP-1R agonist, instead due to its better potency, longer shelf-life and is already used as blood glucose lowering-drug in clinical practice [68].

The addition of exendin-4 along with high glucose (ranging from 11.2–20 mM) increased the insulin secretion in INS-1E, MIN6, 1.1B4, and EndoC-βH1 by 1.6-,1.8-,1.8-, and 1.8-fold, respectively [46,66,67]. This shows that incretin almost equally affects both human and rodent cell lines on a similar manner in stimulating insulin secretion, except for one difference: EndoC-βH1 required 100 nM of exendine-4 concentration when the other cell lines only needed 10 nM to exert equal stimulatory effect.

The physiological concentration of blood glucose to stimulate insulin secretion in human falls under the range of 8–15 mM [69]. As shown in Table 1, the responsiveness of these insulin-secreting cell lines towards glucose managed to follow the physiological range accordingly. Generally, a functioning beta cell should have only a slight increase of insulin secretion at basal glucose concentration (3 or 5.5 mM), begin to spike up the secretion when high glucose concentration is administered (usually at 16.7 mM) and start to decrease with higher glucose concentration (18 mM and above) [19]. This is in agreement with the GSIS assay result done on INS-1 [70] and 1.1B4 cells [71], in which exposure to 18 mM and 25 mM glucose, respectively, caused significant decrease to insulin secretion. Reversible insensitivity towards glucose can happen when beta cells are acutely exposed to high glucose, whereas severe exposure will either cause the cells to be exhausted and lose its functions (decrease in insulin content, insulin secretion and glucokinase activity) in the long run; a phenomenon known as glucose toxicity [72].

Oxidative Stress-Induced Cell Damage

Beta cell failure to secrete enough insulin to meet the demand from the high blood glucose concentration can also be attributed to the reduction of beta cell mass; a situation commonly caused by apoptosis or beta cell death. When beta cells are exposed to oxidative stress, either from glucose toxicity, or reactive oxygen species (ROS) build-ups, the cells will shrink, leaving only apoptotic bodies behind [13]. Hence, numerous studies have been carried out to find alternatives that can assert protective effects towards beta cells and prevent this programmed beta cell death from happening. To confirm the effectiveness of these alternatives, the response of insulin-secreting cell lines towards glucolipotoxicity- and cytokine-induced cell damage and apoptosis needed to be assessed first. The level of cell apoptosis is usually evaluated with the activity of caspase-3/7, a well-known apoptosis mediator, through the Caspase 3/7 assay kits [73].

As mentioned previously, high glucose concentration can harm the cells by inducing oxidative stress towards the cells. This is proven when high glucose (25 mM) treatment on 1.1B4 cells significantly increased the caspase 3/7 activity after 72 h exposure [74], as also reported in MIN6 cells [75] and INS-1 cells [76]. However, we noticed that the rate of glucotoxicity effect on human beta cell line is slower than in rodent cell line. In INS-1 for instance, the cells only took 24 h of high glucose exposure to show a significant increase in caspase-3 activity, whereas 1.1B4 cells needed 48 h or even 72 h of high glucose exposure [71,77]. The authors did not highlight and explain this discrepancy, but this occurrence might have happened due to the slow development of ROS build-ups in monolayer human beta cell lines, as this also happened on EndoC-βH1 cells [78].

Interestingly, in a study where EndoC-βH1 was grown in richer DMEM/F12 culture medium, the effect of 24 h treatment of high glucose (22 mM) alone did not significantly affect EndoC-βH1 cell death, but the cell death significantly increased when it is combined with 1.5 and 2.0 mM of palmitate for 24 h, but not for lower palmitate concentrations [79]. Consistent with this finding, 72 h treatment with high glucose (20 mM) and 0.1-0.7 mM of palmitate also did not affect EndoC-βH1 cell death [46]. According to a 2010 study, human cell lines were less resistant towards palmitate compared to rodent cell line, where the lethal dose (LD50) of palmitate for 1.1B4 was 576 ± 49 μM while the LD50 for MIN6 and 1NS-1 were 993 ± 123 μM and 1860 ± 540 μM, respectively [80]. Contradicting result on MIN6 and INS-1 cells however was reported by Lai et al. where INS-1 was reported to be less resistant towards 500 μM of palmitate by almost 60-fold of apoptosis compared to MIN6 in just 16 hours of treatment [81]. Accordingly, the maximal expression of ER stress marker C/EBP homologous protein (CHOP) in INS-1 could be observed from only 6 hours of palmitate treatment, while MIN6 required 16 hours of treatment before the protein could be expressed [81]. Additionally, prolonged palmitate exposure also did not affect the morphology of MIN6 cells, similar to the behaviour of human islets [81]. Similar finding was also reported by Dhayal et al. where palmitate exposure induced approximately only 33% cell death in MIN6 cells but 70–80% cell death in INS-1 and BRIN-BD11 cells [82]. This is believed to be caused by the presence of stearoyl-coenzyme A desaturase-1 (SCD1) in MIN6 cells and human islets (and absent in INS-1) [81], which helps in converting toxic saturated fatty acid into a less toxic unsaturated form [83]. It is also discovered that MIN6 is protected against lipid-induced apoptosis by the ER chaperone glucose regulatory protein 78 (GRP78), which was overexpressed in MIN6 compared to INS-1 [84], through its ability in enhancing protein folding [85].

Eighteen-hour treatment of cytokine cocktail increased the percentage of apoptotic 1.1B4 cells to around 45% while its caspase 3/7 activity was increased 2-fold over control [86]. As for EndoC-βH1 cells, the cytokines treatment increased the caspase 3/7 activity by 3-fold [46]. In comparison with rodent cell lines, the rate for cytokine to induced damaged to the cell lines were three times slower in EndoC-βH1 than RINm5F and INS-1E [78,87,88]. The activation of NFκB, an important transcription factor to cytokine-induced beta cell death, was also found to be milder than that of the rodent cell line [78], which was also reported on a 1.1B4 cells study [86]. Similarly, MIN6 seemed to be more affected by the damaging effects of cytokines compared to the EndoC-βH1 cells and human islets [89].

From our point of view, human origin beta cell lines seem to be more resistant towards the damaging effect of cytokines compared to rodent cell lines. However, unlike human islets, human beta cell line EndoC-βH1 is reported to lack the ability to generate endogenous nitric oxide in response to cytokines, whereas similar response towards cytokines is observed between rodent beta cell lines with rodent islets [90]. So, focusing on this beta cell death area of study, human beta cell lines are seemingly unable to replicate appropriate responses towards oxidative stress that are similar to the actual human beta cells. Hence, many improvements still need to be done on the cell line to address this issue.

HETEROGENEITY OF INSULIN-SECRETING BETA CELL LINES

Recent advances in technology have allowed us to understand that beta cells exist heterogeneously, consisting of multiple subpopulations of different characteristics and properties. Dorrell et al. demonstrated the presence of at least four beta cells subpopulation in human pancreatic islets, which they named β1 (largest subpopulation), β2, β3, and β4 (least subpopulation) that differ according to their expression of cell surface markers. These beta cells subpopulations also have different insulin release kinetics where the subtype β1 secreted the least insulin and β4 has the highest insulin secretion [91]. Consistent with this finding, distinct beta cell subpopulations were also discovered by Bader et al. in rodent islets where different insulin release kinetics between subtypes were again reported [92]. Apart from the β1-4 subgroups, several other beta cells subpopulations were also discovered such as the “extreme” beta cells located at the centre of the pancreatic islets with higher contents of ribosomal and proinsulin [93] and the “hub” beta cells that act as a pacemaker to the rest of the beta cells in orchestrating insulin response towards glucose [94].

Considering that the discovery of beta cells heterogeneity and subpopulations are very recent, available reports on the heterogeneity of available insulin-secreting cell lines are scarce. Nevertheless, Acosta-Montalvo et al. confirmed the heterogeneity of INS-1 cells based on the existence of an immature INS-1 subpopulation that co-expressed insulin and glucagon proteins, considering that no alpha cell markers were expressed [95]. In a different study, heterogenous expression of insulin and pancreatic duodenal homeobox-1

(*Pdx1*) gene were reported between the beta cells in human and mouse islets where some of these beta cells expressed only insulin, some only expressed *Pdx1* and some other beta cells expressed much lower insulin level compared to the rest [96]. According to Szabat et al., similar heterogeneity profiles was also reported in the insulin-secreting MIN6 and INS-1 cell lines [96]. Although no study on beta cell heterogeneity were carried out on the human 1.1B4 cell line, the other human EndoC-βH1 cell lines was found to be quite homogenous based on the single-cell qPCR gene profiling carried out by Tsonkova et al. [46]. Despite that, however, there were some varying protein expressions (such as HDAC9) observed between the beta cells in the cell line that were also observed in heterogenous human islets [91]. Further analysis by using spectral karyotyping (SKY) later confirmed the heterogeneity of this EndoC-βH1 cell line due to the varying chromosomal profiles that exist between individual cells in the EndoC-βH1 population [97].

The topic of beta cells heterogeneity still requires extensive research, even at the islets level. Therefore, we believe that in due time, there will be many more future discoveries on the heterogeneity of these existing beta cell lines that would hopefully mimic those of human and rodent islets.

LIMITATION IN CULTURING RODENT AND HUMAN BETA CELLS

There are clear pros and cons to each of the cell lines reviewed here, so to pick out one particular cell line that is considered the best in all aspects is rather impossible. Furthermore, considering that different parameters and environments were applied in every research study, such comparisons are hard to make. A systematic comparison between all cells can be made in the future under the same parameters and environment should such finding is to be deemed important. The comparison between proteome and transcriptional landscape between these two cell model categories should also be done so a full understanding of these differences can be achieved.

Rather than being cultured as monolayer, we find that growing these cells as pseudoislets (three-dimensional islet-like structure) gave better results overall. Depending on the cell type, pseudoislets can be formed by either seeding the cells on non-adherent culture plates with or without orbital shaking, on gelatin-coated plates, by centrifugation to allow cell aggregation and many other methods [98]. Spelios et al. came up with a new method of generating pseudoislet, which is by coculturing EndoC-βH1 cells with murine pancreatic islet endothelial cell line MS1 [99]. This pseudoislets can be useful model system for the study of GLP-1 signalling pathway due to its better responsiveness to glucose and GLP-1 analogs compared to its monolayers. The exact method was also done on the βTC3 insulinomas, where the pseudoislets produced better insulin production and glucose responsiveness [100]. The possibility of utilising other non-beta cells in generating pseudoislets were also explored in order to best mimic the functionality of human and rodent islets, such as the hepatic stellate cells [101], neuroblastoma [102], and mesenchymal stem cells [103].

The Ca^{2+} signalling in pseudoislets MIN6 and INS-1E were reported to improve, thus further increasing GSIS in these cells better than monolayers [104,105]. Pseudoislets 1.1B4 also exhibited better resistance towards cytotoxic agents with 2-fold increase in LD50, as well as upregulation of genes involved [106]. When the cells are grown in the formation of pseudoislets, cell-to-cell communication is established, in which there will be better cellular regulation and signalling among cells. In contrast, beta cells uncoupling resulted in reduction of cellular functions, which could explain the findings in this review [107]. A protein profiling of MIN6 monolayer and pseudoislets were carried out where the expression of 488 proteins were significantly enhanced in the pseudoislets. In addition, a total of 11 signalling pathways were also significantly enhanced, namely glycolysis, oxidative phosphorylation, TCA cycle, tight junction, gap junction, adherens junction, actin cytoskeleton regulation, lysosome, ribosome, spliceosome, and proteasome [104]. Similarly, the expression of GPR40, GPR55, and GPR119 were also significantly increased as much as 20,000-fold in MIN6 pseudoislets compared to its monolayer form [108]. This finding is very useful as these GPCRs have been some of the crucial targets used by researchers in potentiating GSIS in beta cells to combat metabolic diseases.

It is undeniable that human and rodent islets are the gold standard for beta cell studies and are much superior in many aspects, but from our point of view, pseudoislets from beta cell lines could solve many problems (limited donor availability, animal scarification, cellular senescence, etc.) that researchers are facing during their ex vivo studies.

CONCLUSION

Based on our observation, rodent cell lines seem to have many advantages over human cell lines in terms of their passage stability and better responses toward glucose-stimulated insulin secretion and cytokine damage. This could be attributed to the fact that rodent cell lines have been studied for decades. The generation of human beta cell lines meanwhile is still fairly new, and a lot of developments can still be done to make it better. Nevertheless, the generations of these insulin-secreting cell lines have brought many developments and useful knowledge in regard to beta cell and diabetes mellitus studies. However, the characteristics of both human and rodent cells still bear striking differences to the native beta cells, which may hinder complete understanding for this area of study. While the formation of pseudoislets from the existing cell lines has been a powerful model system for *in vitro* studies, a better beta cell model is still needed, either from a healthy human pancreas or stem

cell-derived. From what we can see, the possibility is promising.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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