LSC401 Project Research Methods
Unit 1
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Wu, H.-T. et al. (2022a) 'Sucralose, a non-nutritive artificial sweetener exacerbates high fat diet-induced hepatic steatosis through taste receptor type 1 member 3', Frontiers in Nutrition, 9. doi:10.3389/fnut.2022.823723.

Reason why I chose this paper

I have chosen this specific paper for my dissertation as the paper focuses on analyzing lipid accumulation and sweeteners, which happens to be the main topic of my dissertation. Worldwide, 537 million adults have diabetes (primarily type 2), projected to reach 783 million by 2045. Similarly, non-alcoholic fatty liver disease (NAFLD), an ectopic lipid accumulation in the liver is the most common chronic liver disease globally, affecting 25% of the population (Eguchi et al., 2020). Due to the significant impact of metabolic diseases, finding effective treatment is crucial. Lifestyle changes are the main approaches, including losing weight, modifying diet, and increasing exercise (Kim. et al., 2019). Consequently, high-intensity sweeteners with low-calorie content, including saccharin and sucralose, are used to regulate body weight to further reduce the prevalence of metabolic disorders (Bian et al., 2017). In this paper, they are investigating the effects of sucralose on hepatic steatosis, using HepG2 cells and high-fat-diet-fed mice. They conducted their initial investigations using cell culture and further validated their findings using a more complex in vivo model to comprehend the true relevance of their findings in a physiological context (Wu, H.-T. et al., 2022). I am interested in examining the methodology used regarding cell culture in the paper to gain a better understanding of key methodologies in my research area.

Theoretical foundation of methodological framework

Cells are grown in a controlled environment outside of their natural habitat in a process known as cell culture. This method has allowed researchers to employ cells as a crucial model system for comprehending physiological mechanisms in the human body (Segeritz & Vallier, 2017). Cell culture has several benefits, including the ability to obtain consistent and reproducible results using a batch of clonal cells. Cell lines can be propagated infinitely, ensuring reliable and consistent outcomes during experiments. Additionally, cell techniques are scalable, which means that large quantities of cells can be generated for high-throughput screenings. Another advantage of cell culture can provide precise control over experimental conditions. The versatility of the cell culture system allows it to be tailored to mimic conditions in vivo, making it possible to study the mechanisms of diseases. This control goes beyond regulating factors such as nutrient composition, growth factors, pH, or temperature, and includes the manipulation of experimental variables. This involves exposing cells to specific treatments, drugs, or interventions with accuracy and reproducibility (Segeritz & Vallier, 2017).

There are two types of cells commonly used in laboratories: primary cells and established cell lines. Primary cells are derived directly from the tissues of an organism and can grow and divide under the right conditions. However, they have a finite lifespan (senescence) because each time the cells divide, a portion of their DNA telomeres is lost until they reach a point when they can no longer divide, known as senescence. Nevertheless, certain cell lines, known as established cell lines, attain immortality through a phenomenon termed transformation. This process may happen spontaneously or can be induced chemically or virally (Thermo Fisher Scientific Inc., no date). Carcinogens are substances used

to induce genetic mutations in primary cells, leading to their transformation into immortalized cell lines. These tumour promoters disrupt the usual control mechanisms of cell division and growth, giving the cells immortality and the ability to proliferate continuously. Examples of such chemical treatments include toxins and mutagens that modify DNA repair mechanisms, stopping them and promoting cell growth. In addition, some viruses can integrate essential genes into the host cell's genome, disrupting normal cellular functions and causing cell transformation. When introduced into primary cells, viral oncogenes can take over the functions of the cell machinery, causing uncontrolled cell division and blocking processes that normally limit the life of the cell. Consequently, this leads to cellular immortalization and the formation of continuous cell lines (Reddel, 2000).

Cells that are grown in culture can take on one of two forms - they can either grow as monolayers or float freely in the culture media. Adhering cultures are formed by cells that grow as monolayers, while suspension cultures are formed by free-floating cells. To ensure that the cells remain alive, it is crucial to maintain the conditions as close to the physiological conditions as possible. Cells in this experiment are kept in T75 flasks and placed in an incubator at 37ºC and 5% CO2. Cell culture temperature depends on host body temperature, typically 36–37°C for human and mammalian cells. Moreover, supplements are added to the culture media to provide the necessary nutrients, such as amino acids, inorganic salts, and vitamins. Choosing the best media for the kind of cell and transfection technique is crucial in transfection research since different cell types have highly particular requirements for their medium, serum, and supplements. The published literature typically contains information on choosing the right medium for a particular cell type and transfection technique. If the right medium for a sort of cell is unknown, empirical research is needed. Ultimately, regarding CO2, the growth medium maintains culture pH and buffers cells against pH changes, typically with organic or CO2–HCO3– buffers. Atmospheric CO2 fluctuations affect medium pH, requiring exogenous CO2 for CO2–HCO3– buffered media, especially in open dishes or dense cultures. CO2 levels of 4–10% are common, per medium-specific recommendations (Thermo Fisher Scientific Inc., no date).

Finally, the knockdown technique involves reducing gene expression through gene-specific mRNA degradation, blocking gene expression post-transcription. RNA interference (RNAi) technology is commonly used for this purpose. After gene silencing, cellular behaviours are examined, gaining more accurate insights into the roles of genes in different biological processes. Cell culture is essential for knockdown techniques due to its controlled environment and the ability to efficiently deliver reagents. Moreover, cells can be maintained under specific conditions, such as serum-free media, for knockdown optimization. This facilitates the observation of the effects in cellular behaviour of reducing the presence and activity of a specific gene (Han, H., 2018).

Methodology chosen

Selecting an appropriate cell line for an experiment is crucial to ensure its success, as different cell lines can present different characteristics and responses to experimental manipulation. In this case, HepG2 cells, which are derived from hepatocellular carcinoma, were used. HepG2 is an immortal cell line established in 1975 from the liver tissue of a 15-year-old Caucasian male from Argentina with well-differentiated hepatocellular carcinoma. The selection of this specific cell line was based on various factors. Firstly, HepG2 cells are readily available commercially, making them easily accessible to researchers. Additionally, these cells are quite easy to culture in the laboratory and can be maintained in standard cell culture conditions using common growth media. Lastly, HepG2 cells were chosen specifically for this experiment due to their hepatocyte-like properties. They express many liver-specific functions, such as the synthesis of proteins, including albumin and clotting factors, as well as the metabolism of drugs and food additives (Arzumanian et al., 2021).

In the 2D monoculture model, steatosis induction is commonly achieved by adding a mixture of unsaturated and saturated fatty acids to the culture medium, often oleic acid (OA) or palmitic acid (PA). This reflects typical dietary conditions and mimics NAFLD-NASH indicators. This model, favoured

for its affordability, simplicity, and suitability for high-throughput screening, has limitations based on the cell sources used. For instance, while hepatocyte-like cells from human embryonic stem cells (hESC) retained important features, such as the ability to form 5-hydroxy-methylcytosine (5hmC), which is rapidly lost in HepG2 cultures, facilitating the study of epigenetic dysregulation in NAFLD pathogenesis. Additionally, human pluripotent stem cell (hPSC)-derived models were used to investigate mitochondrial metabolism changes in early steatosis. However, monoculture models have proved valuable in exploring metabolic, fibrotic pathways, drug efficacy, and hepatic toxicity (Jimenez Ramos et al., 2022).

A key aspect of cell culture research involves designing a defined cellular environment where individual variables can be manipulated to observe cellular responses. To achieve this, the in vitro cellular environment is often simplified, relying on a single cell type cultured in a monolayer. While this may not fully capture the cellular interactions found in vivo, such as those between different cell types and extracellular matrices, animal studies are required for that purpose. Nevertheless, in vitro models offer advantages, including reduced ethical concerns, lower costs and time, greater control over experimental variables, and the ability to study specific cellular processes in isolation (Thermo Fisher Scientific Inc., no date).

In this paper, the authors use lentiviral vectors that contain short hairpin RNA (shRNA) to silence the expression of the gene T1R3 in HepG2 cells. These lentiviral vectors were made by transfecting HEK293T cells with the lentiviral transfer plasmid as well as pMD2G and psPAX2 plasmids (Wu, H.-T. et al., 2022). The majority of current methods for producing lentiviral vectors typically include cotransfection, preferably using HEK293T or HEK293 cells. HEK293T cells are preferred because their SV40 T-antigen enhances efficiency in vector production. Additionally, HEK293T cells exhibit faster cell growth and higher transfection efficiency compared to HEK293 and HepG2 cells (Merten et al., 2016). The particles that resulted from the cultures were used to infect HepG2 cells along with puromycin. This culture was used to confirm the role of T1R3 in lipogenesis induced by sucralose. Lipid synthesis genes decreased in HepG2 cells when T1R3 was silenced, suggesting that sucrose may bind to T1R3 to trigger downstream signaling and further stimulate hepatic lipogenesis (Wu, H.-T. et al., 2022). The advantages of gene knockdown over other techniques lie in its ability to analyze the effects of gene silencing without permanently altering the genome. This offers reversibility, cost-effectiveness, and flexibility. The temporary and specific suppression of gene expression allows for targeted investigation of gene function and its association with pathways and cellular behaviors (Mocellin & Provenzano, 2016). In this study, gene knockdown was chosen to confirm the role of T1R3 in sucralose-induced lipogenesis. By silencing the gene, researchers could observe how the expression of other proteins and the accumulation of lipids in response to sucralose were affected by the absence of taste receptor type 1 member 3 (Wu, H.-T. et al., 2022).

Application of methodologies in the paper

There were several methodologies that were performed in this study apart from the previously mentioned. These include western blot analysis, and the determination of hepatic triglyceride content was performed using a triglyceride colorimetric assay kit, along with the measurement of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in the liver. It was observed that the paper lacked necessary controls and had limited replicates. It is important to have appropriate replication to measure the reproducibility and reliability of results. The study used ImageJ software to quantify the relative signal intensity of proteins, which could have introduced variability depending on the analysis parameters and user's settings. To ensure accurate quantification of protein expression levels and reduce variability, it is recommended to standardize the analysis protocols. Additionally, even though commercially available assay kits were used, the performance of these kits could vary between laboratories and batches. To ensure accuracy, it is important to standardize the measurements

against reference samples (Wu, H.-T. et al., 2022; Thermo Fisher Scientific Inc., no date).

The researchers used cell culture to examine the lipid content, measure cellular triglyceride, and detect reactive oxygen species generation. However, this paper lacks any negative controls, making it difficult to evaluate the specificity of lipid accumulation and triglyceride measurement. The methodology also fails to provide details on the statistical analysis of the results. It would be better to include information about the statistical tests that were used to compare the different treatment groups, which would strengthen the interpretation of the findings. Additionally, the paper does not report whether the concentration of sucralose and oleic acid is the final concentration in the cell culture medium or stock solutions. Moreover, while the methodology focuses on ROS generation, it would be helpful to assess the cell viability under different treatment conditions to provide important context for the ROS generation results (Wu, H.-T. et al., 2022).

Furthermore, the paper lacks detailed information on specific culture conditions such as cell culture media composition, passage techniques, and supplementation. While it provides adequate information on chemical doses, it fails to include timecourse data on the progression of lipid accumulation. These details are essential for accurately replicating the study (Wu, H.-T. et al., 2022). While the use of oleic acid and palmitic acid to induce lipid accumulation is common in cell culture models of hepatic steatosis, the paper does not explain the rationale behind this choice or the duration of treatment (Arzumanian et al., 2021). Additionally, the study only assesses lipid accumulation and cellular responses at a single time point (48 hours after treatment). Considering the dynamic nature of lipid metabolism and cellular behavior, exploring longer time periods or repeated exposures could offer valuable insights (Wu, H.-T. et al., 2022).

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