The Development of a CRISPR/Cas9 System with Nanoblades in Order to Study IBD-Related Genes in an *in vitro* Model

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Abstract

In an attempt to further understand the cellular mechanisms involved in Inflammatory Bowel Disease (IBD), this experiment studied the efficacy of a novel method of delivery of CRISPR/Cas9 known as “Nanoblades” into Intestinal Epithelial Cells (IECs) and Embryonic Stem Cells (ESCs) to target susceptible genes in IBD (XBP1, CX3CR1, and TAP1) and induce single nucleotide polymorphisms (SNPs). In order to efficiently transfer CRISPR/Cas9 ribonucleoproteins into IECs and ESCs *in vitro*, these Nanoblades delivered ribonucleoprotein cargo in a transient and rapid manner. In order to determine the effectiveness of Nanoblades in the target cells, a common form of retrovirus, Lentiviral Vectors (LVs), were used in comparison to Nanoblades. The results have indicated that Nanoblades induce SNPs in both IECs and ESCs more efficiently than LVs, opening a pathway to research in not only IBD, but also in other diseases that involve the study of primary cells. In future studies, in order to learn the significance of these SNPs in IBD, these ESCs will be differentiated into intestinal organoids, and the immune response will be analyzed. The use of this novel technology for a different application opened a gateway to research in IBD.

Introduction:

In 2015, an estimated 1.3% of all adults in the United States (3 million) reported being diagnosed with Inflammatory Bowel Disease (IBD) (Abraham et al., 2009). IBD represents a group of intestinal disorders (Crohn's disease and Ulcerative colitis) that cause prolonged inflammation of the gastrointestinal tract. If not treated properly, this inflammation can result in life threatening complications, such as anemia, malnutrition, liver disease, and even colon cancer (CDC). Both Crohn's Disease and Ulcerative colitis are characterized by intestinal epithelial injury, including mucosal erosion, ulceration, cryptitis, and crypt abscess formation (Garcia-Carbonell et al., 2018).

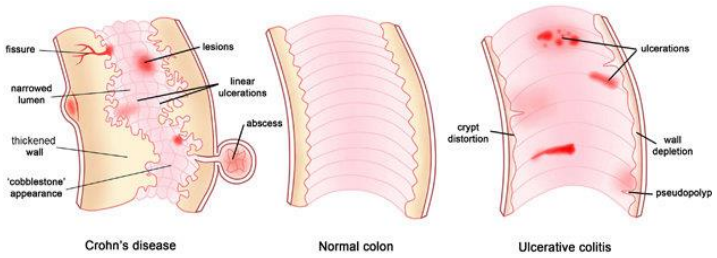

Figure 1: Diagram of the intestinal tract in the two main types of IBD: Crohn's Disease and Ulcerative colitis (Adapted from Wagnerova et al., 2013).

According to genome association studies, there are mutations in specific genes correlated to the development of IBD in humans (Abraham et al., 2009). In order to devise potential treatment possibilities for Crohn's Disease and Ulcerative Colitis, additional research is required to comprehend the underlying processes involved in the manifestation of IBD, including the role of the intestinal epithelium, and the role of various genetic factors. In order to study different genes and their involvement in IBD, the goal of this experiment was to deliver a CRISPR/Cas9 system into a primary cell line involved with IBD. Previously, success in delivery of CRISPR/Cas9 systems *ex vivo* by either transfection or transduction into primary cell lines has been limited (Mangeot et al., 2019). Therefore, in an attempt to further understand the processes and cellular mechanisms in IBD, this experiment studied the efficacy of a novel method of delivery of CRISPR/Cas9 known as "Nanoblades" into Intestinal Epithelial Cells and Embryonic Stem Cells (targeted primary cells). In these primary cells, the CRISPR/Cas9 system targeted the XBP1, CX3CR1, and TAP1 genes, which are susceptible genes in IBD.

Nanoblades, viral delivery vectors based on the Murine Leukemia Virus, deliver ribonucleoprotein cargo in a transient and rapid manner *in vivo* and *in vitro* without the delivery

of a transgene, unlike other delivery methods. Nanoblade preparation is relatively simple and inexpensive and can be easily implemented in any laboratory equipped for cellular biology.

The development of Nanoblades relies on the viral structural Gag polyprotein (a basic part of retroviruses), which multimerizes at the cell membrane and is sufficient, when expressed in cultured cells, to induce the release of Virus-like particles (VLPs) into the cell. When Gag is co-expressed together with a fusogenic viral envelope, pseudo-typed VLPs are produced that lack a viral genome but still retain their capacity to fuse with target cells and deliver the Gag protein into their cytoplasm (Mangeot et al., 2019). Figure 2 depicts the transfection of the producer cells, or HEK-293T Cells by plasmids, resulting in the production of Nanoblades.

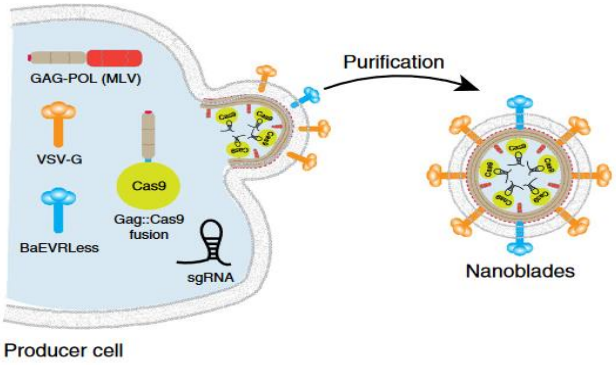

Figure 2:Scheme describing the MLV Gag::Cas9 fusion and the Nanoblade production protocol based on the transfection of HEK-293T cells by plasmids coding for Gag-Pol, Gag::Cas9, VSV-G, BaEVRLess, and the sgRNA (adapted from Mangeot et. al.,, 2019).

The intestinal epithelium at the interface between the intestinal microbiome and the lymphoid tissue associated with the gastrointestinal system plays a critical role in shaping the mucosal immune response (Abraham et al., 2009). Intestinal epithelial cells are part of a primary cell line that act as a physical barrier against excessive entry of bacteria and other antigens from the intestinal lumen into the circulation. In mouse models of IBD, several types of epithelial dysfunction have been shown to cause intestinal inflammation. These include defects in epithelial-cell development or proliferation, barrier function, cell-matrix adhesion, endoplasmic reticulum (ER) stress response, and epithelial restitution after injury (Abraham et al., 2009). This suggests that intestinal epithelial cells play a major role in the immune response in the intestine, and in the development of IBD. Therefore, this experiment focused on the use of Intestinal Epithelial Cells known as Caco2 Cells as target cells. Single-nucleotide polymorphisms (SNPs) were induced in these cells by the delivery of a CRISPR/Cas9 system at specific loci to further study the inflammatory response in IBD.

Nanoblades were also tested in other primary cells for genome editing. Targeting the same genes, Nanoblades were used in Embryonic Stem Cells (ESC's). ESC's represent a major interest in research and gene therapy due to their proliferative potential and their capacity to colonize (Mangeot, et al., 2019). By using Embryonic Stem Cells as the target cells, if

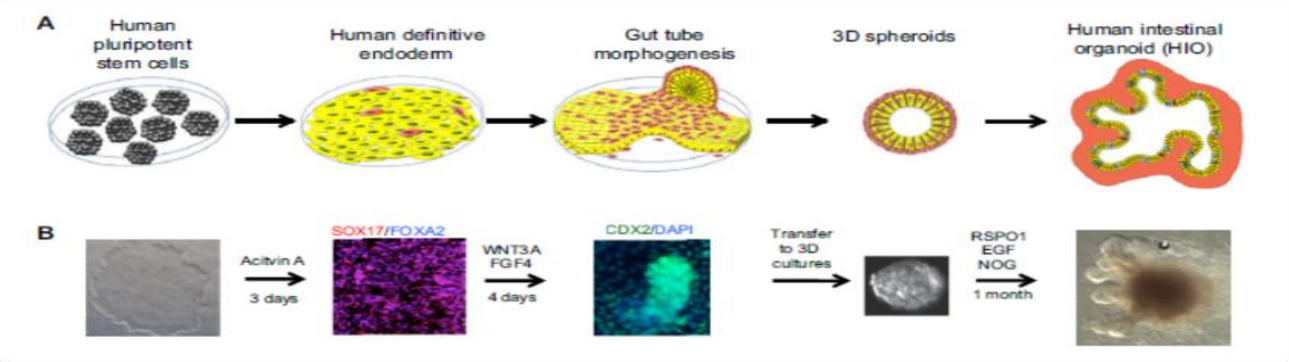

Figure 4: Diagram of the process of differentiation of ESC's into intestinal organoids with a primary cell line (Adapted from Wells et al., 2014)

transfection is successful, Embryonic Stem Cells can be differentiated into intestinal organoids, which will provide an accurate model of patients with IBD that can be studied in depth.

Consistent with predictions inferred from epidemiologic studies, genome association studies have identified genes associated solely with Crohn's disease and Ulcerative colitis, including the XBP1, CX3CR1, and TAP1 genes (Abraham et al., 2009). The XBP1 gene has been found to be vital in the ER stress response, which is necessary for the proper functioning of the immune system and in the cellular stress response. These pathways, known collectively as the unfolded protein response, are important for normal cellular homeostasis and organismal development and may also play key roles in the

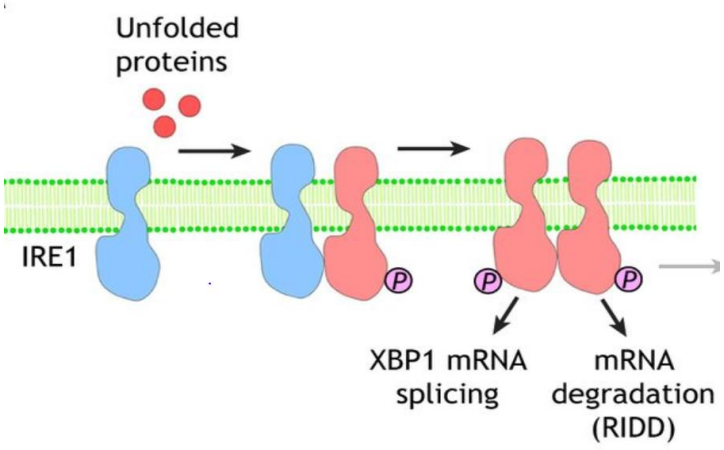

Figure 3: A schematic diagram of IRE1 signaling and the splicing of XBP1 mRNA in the ER Stress response (Adapted from Mitra et al., 2019).

pathogenesis of many diseases (Lin et. Al, 2013). When one of these pathways, known as the IRE (α and β) pathway, is activated, it results in the excision of 26 base pairs from the mRNA encoding the transcription factor X-box-binding protein 1 (XBP1) by an unconventional splicing event that generates XBP1s (Shaffer, et al., 2004) (Figure 4). In a previous study, it was found that induction of ER stress *in vivo* by cell-specific XBP1 deletion would cause organ-specific inflammation, providing an explanation for the initiation of proinflammatory diseases (Kaser et al., 2008). It was also found that intestinal inflammation was found to originate solely from XBP1 gene abnormalities in Intestinal Epithelial Cells (Kaser et al., 2008).

The Tap 1 gene is also known for its susceptibility in IBD, especially in Ulcerative colitis. Located within the Major Histocompatibility Complex (MHC) class-I region are transporter associated with antigen processing (TAP) genes which encode for proteins involved in the transfer of antigenic peptides through the endoplasmic reticulum prior to their association with MHC class-I molecules (Miller et al., 2015) (Figure 5). The function of these molecules is to display peptide fragments of proteins from within the cell to cytotoxic T cells; this will trigger an immediate response from the immune system against antigens displayed with the help of an MHC class I protein (Jongsma et al., 2013).

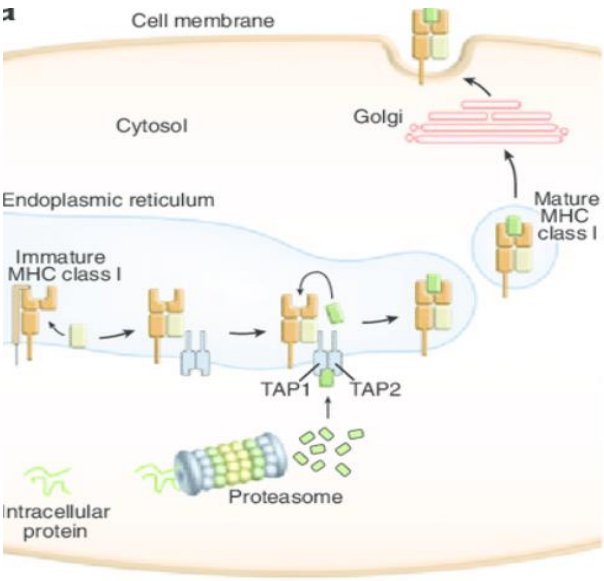

Figure 5: Diagram illustrating MHC class I antigen processing and antigen presentation pathways and the involvement of TAP1 (Adapted from Andersen et al., 2006).

According to a study conducted by Leonardi et al., CX3CR1+ mononuclear phagocytes (MNPs) have been identified as being essential for the initiation of innate and adaptive immune responses to intestinal fungi. CX3CR1+ MNPs express antifungal receptors and activate antifungal responses in a Syk-dependent manner. In this study genetic ablation of CX3CR1+ MNPs in mice led to changes in gut fungal communities and led to severe colitis that was rescued by antifungal treatment. In Crohn's disease patients, a missense mutation in the gene encoding CX3CR1 was identified and found to be associated with impaired antifungal responses. These results unravel a role of CX3CR1+ MNPs in mediating interactions between intestinal

microbiota and host immunity at steady state and during inflammatory disease (Leonardi et al., 2018).

Therefore, to further understand susceptible genes in IBD, the XBP1, TAP1, and CX3CR1 genes will be studied in depth in Intestinal Epithelial Cells (Caco2 cells) and in Intestinal Organoids derived from Embryonic Stem Cells using a novel delivery technology known as Nanoblades. Since there is a limited understanding of the causes of inflammation in IBD, by inducing mutations in these genes using efficient and novel technology, we may be able to further understand the processes involved in intestinal damage, IEC damage, and the development of Crohn's disease and Ulcerative colitis, which could potentially lead to future applications of these methods in Immunology.

Methods:

Target Cells:

The target cells used in this experiment were Caco2 Cells (Intestinal Epithelial Cells) and Embryonic Stem Cells. These two types of cells from primary cell lines are easy to maintain and are used for many avenues of scientific research, although they are known for their incompatibility with transfection, which poses a problem, since inducing mutations (SNPS) involves the transfection of virus-like particles that deliver a CRISPR/Cas9 system. Therefore, in an attempt to efficiently induce mutations in these human cells, both Nanoblades and Lentiviral Vectors (LVs) were used to target the genes XBP1, CX3CR1, and TAP1 to create random SNPs—random deletions across the gene sequence, usually in the beginning or middle of the gene's sequence. Both Nanoblades, a novel technology, and Lentiviral Vectors, a relatively common form of retrovirus used for gene editing in cells, were used in order to compare the efficacy of both types of editing in these target cells.

HEK-293T Cell Maintenance:

The HEK-293T-cells used to produce Nanoblades and Lentiviral Vectors in this project were maintained in a cell culture in petri dishes and were kept incubated. In order to maintain the cell culture, PBS was placed on the cells as a cleaning agent and Trypsin was put into the cell culture in order to dissociate the T cells the bottom of the plates. The cells were then divided onto separate plates, depending on the confluency, and Dulbecco's Modified Eagle Medium (DMEM) was added to support the growth of the cells. This process was repeated until the cells were at a sufficient confluency (approximately 80% or higher). This method was adapted from a protocol provided by the ATCC, where these cells were obtained from.

Creation of CRISPR/Cas9 Plasmids for both Nanoblades and LVs:

The guide RNAs for each gene targeted were selected in order to be incorporated onto the CRISPR/Cas9 plasmids. The sgRNA for XBP1 was sgRNA21, the sgRNA for CX3CR1 was sgRNA40, and the sgRNA for TAP1 was sgRNA33. The sgRNAs were annealed and inserted

onto pre-made CRISPR Cas-9 Plasmids (Figure 6). Next, they were transformed into bacteria and plated on agar plates. To make sure the ligation was successful, colonies of bacteria were collected randomly and digested to check if they were positive for the ligation of the sgRNA. A gel was run to check for colonies positive for the ligation, and this was determined by a specific band that was visible on the gel.

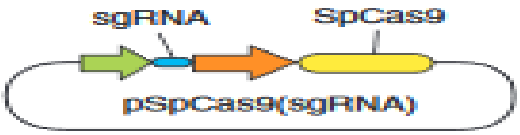

Figure 6: Depiction of the custom sgRNAs (light blue bars) for each target created (Adapted from F Ann Ran et al., 2013)

Nanoblade Production:

In order to create Nanoblades for this experiment, HEK-293T cells were transfected with plasmids coding for Gag::Cas9, Gag-Pro-Pol, a sgRNA for each gene, viral envelopes, and Calcium phosphate as a transfection reagent (Figure 7). These transfected plasmids would then be formed into vesicles inside these T-cells which were then released out of the cell membrane, resulting in the production of fusogenic VLPs being released in the culture medium, creating Nanoblades. This protocol was adapted from Mangeot et al.

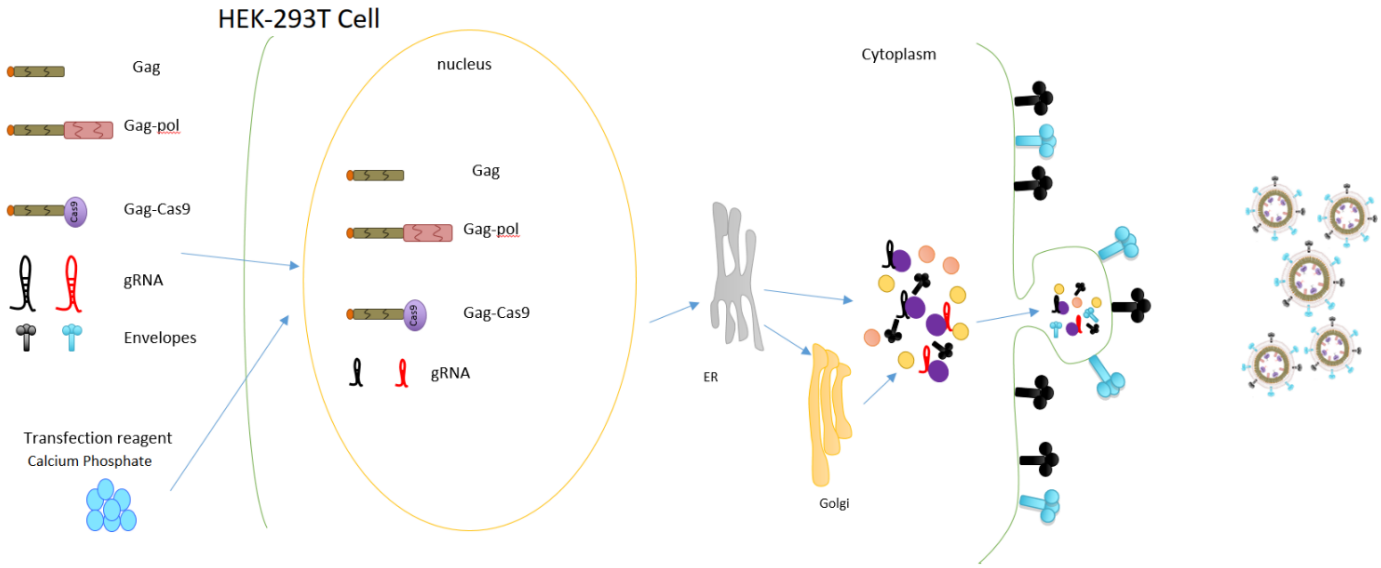

Figure 7: Nanoblade production with different plasmids in HEK-293T Cells (Adapted from Mangeot et al., 2019)

Lentiviral Vector Production:

Lentiviral Vectors represented a mode of comparison in this experiment. LV's are a common type of retrovirus used for transfection, but they do not have the transient mode of transport that the Nanoblades have. In order to create LV's in this experiment, HEK-293T cells were once again transfected with three separate transfection systems: a transfer vector plasmid which contains genetic sequences necessary for the vector to infect the target cell and for the transfer of the therapeutic (or reporter) gene, retro-transcriptase and integrase packaging plasmids (8.9), which are required for the packaging of structural proteins, and an envelope (VSV-G) to provide stability to the vector by bringing together the particles that were made by the packaging plasmid. Also transfected were plasmids of interest, which were the CRSPR/Cas9 plasmids annealed with different sgRNA depending on the gene, and Calcium phosphate as a transfection reagent (Figure 8). This protocol was taken from F Ann Ran et al., but it was adapted to fit this experiment.

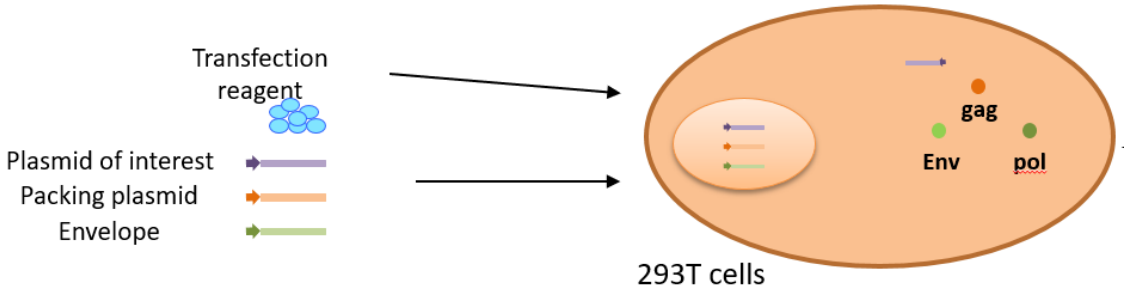

Figure 8: Diagram of the creation of Lentiviral Vectors with the necessary plasmids in HEK-293T Cells.

Transduction of HEK-293T Cells into Caco2 Cells:

By depositing the viruses created by the packaging cells and plasmids into cell media with Caco2 Cells, a transduction of the viruses into the Caco2 cells was conducted. Caco2 cells were set into 2x4 wells in order to be transduced. The wells were set up in the following way (Figure 9):

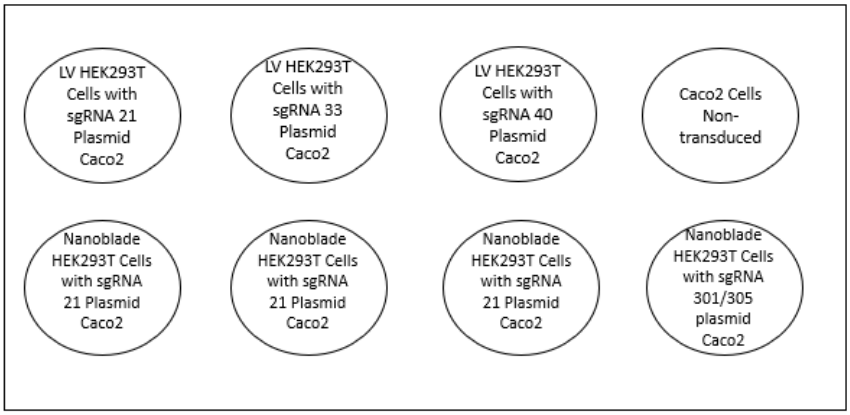

Figure 9: Diagram of the setup of the transduction with Caco2 Cells.

The first 3 wells in the first row were Caco2 Cells transduced with Lentiviral Vectors with the CRISPR/Cas9 systems of the different sgRNAs. The 4th well in the first row was made up of just Caco2 Cells and media, but no viruses, and these cells function as a control.

The second row of wells all represent Caco2 Cells transduced with the different Nanoblades for each sgRNA. For the last well, Nanoblades for the WASP gene, which were already created and tested to be effective, were used in order to have a functioning control for Nanoblades, since they were already found to induce SNPs.

Transduction of HEK-293T Cells into Embryonic Stem Cells:

For transduction of the Embryonic Stem Cells, the same procedures were used as the Caco2 Cells. The same setup in a 2x4 well was used as well, with LV's, a control, and Nanoblades (Figure 10).

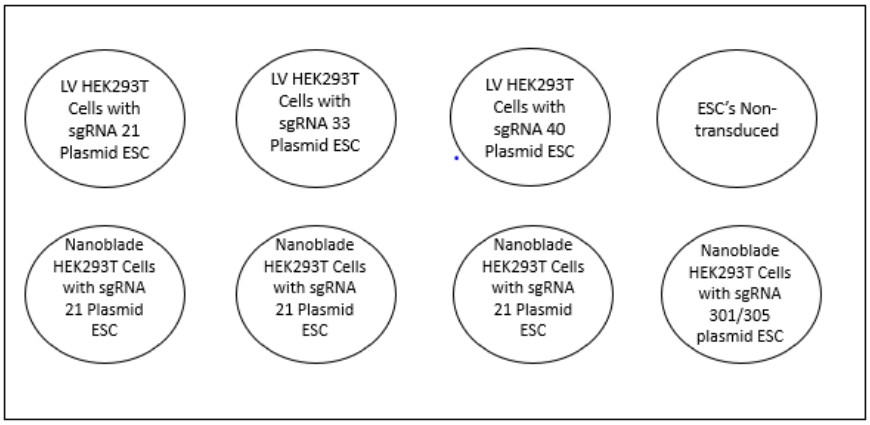

Figure 10: Diagram of the setup of the transduction of Embryonic Stem Cells.

Genomic Extraction:

After each transduction, a genome extraction was performed. The genomic extraction was conducted by following a standard protocol for human or animal tissue in cultured cells. that was provided by. In the protocol, it was required to lyse the sample of cells, bind the DNA, dry the silica membrane, and elute the highly pure DNA (Figure 11). After the genome extraction, a nanodrop quantification was conducted in order to determine the concentration of DNA obtained. The Nanodrop Quantification was performed using a spectrometer machine that measured the concentration of nucleic acids. After determining the concentrations of each sample, they were all diluted to 20 ng/uL in order to be used for the Polymerase Chain Reaction (PCR) and the SURVEYOR Nuclease Assay.

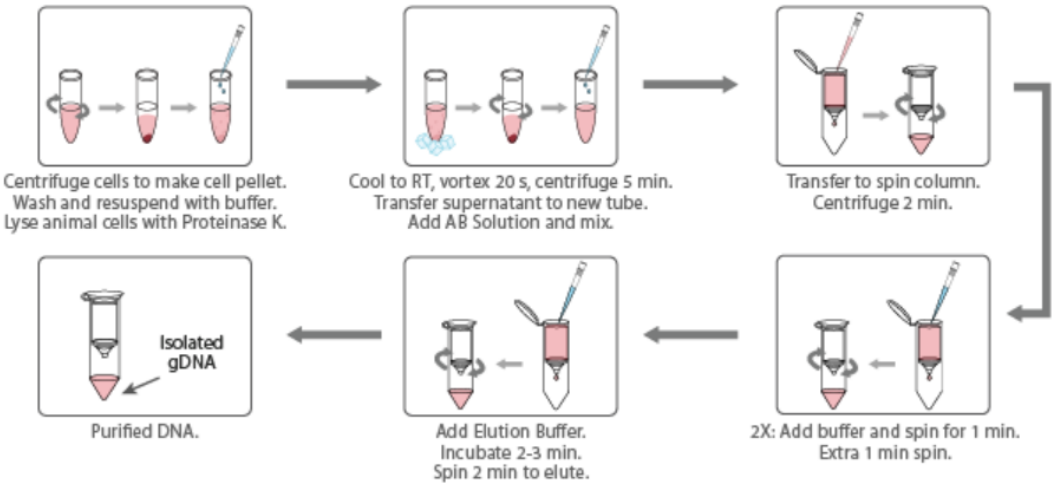

Figure 11: Genomic Extraction protocol for human cells (Adapted from genomic extraction kit).

Polymerase Chain Reaction (PCR) of the Genomic Extraction:

A PCR was conducted in order to determine if the WASp and CX3CR1 genes were transduced, and the percentage of transduction. Since two sgRNAs were required to create SNPs in both these genes, the cleavage would be visible in the PCR alone. On the other hand, the XBP1 gene and the TAP1 gene required the use of a SURVEYOR Nuclease Assay because they both required only one sgRNA in order to induce SNPs. Each gene was at a different temperature in order to have maximum efficiency.

SURVEYOR Nuclease Assay:

In cells that were transfected with a pair of sgRNAs to mediate a genomic (micro)deletion or inversion, indel mutations or SNPs can be detected by the SURVEYOR nuclease assay. This is an enzyme mismatch cleavage assay, or endonuclease, used to detect single base mismatches or small insertions or deletions (indels). In this experiment, the SURVEYOR was used in order to detect SNPs in Caco2 Cells and Embryonic Stem Cells. In order to detect SNPs, the SURVEYOR program requires the heating and separation of the two strands of DNA, which are then cooled and reannealed in order to detect mutations on one strand of the DNA, or SNP (Figure 12). In order to see the results from this assay, an agarose gel electrophoresis is performed in order to visually detect cleavage, or two bands of DNA. If cleavage is present, it is evident that there are SNPs in the targeted cells, and the delivery of the CRISPR/Cas9 system was successful.

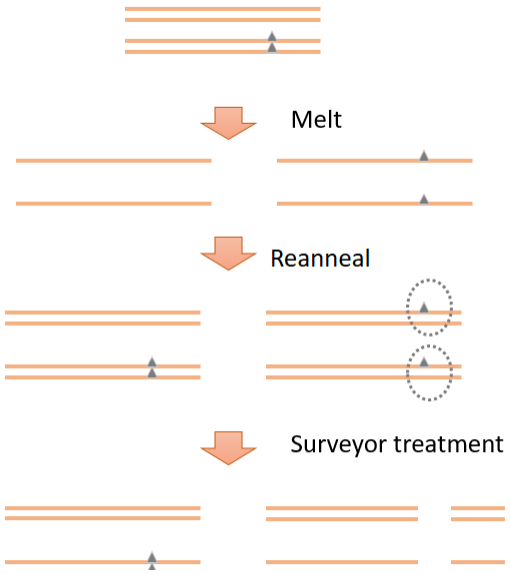

Figure 12: Diagram of the SURVEYOR Nuclease Assay, and how it detects SNPs in the genome (Adapted from F Ann Ran et al., 2013)

Results:

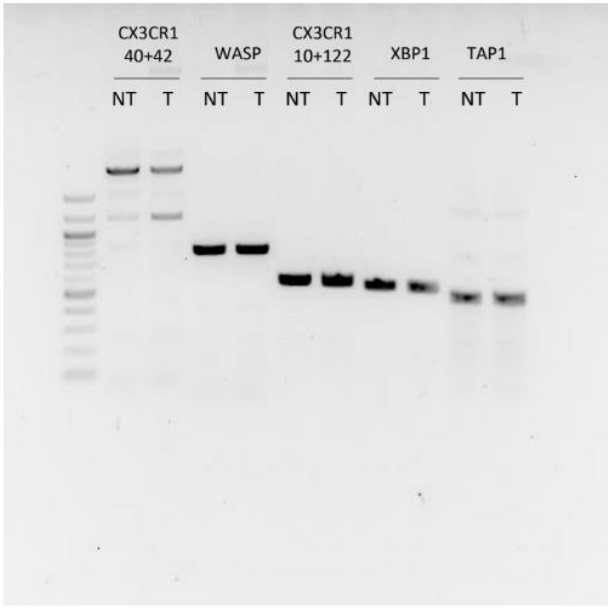

Figure 13: Trial 1- PCR of the transduction of Caco2 Cells. "NT" represents the non-transduced cells, while "T" represent the cells transduced with Nanoblades with sgRNA for CX3CR1, WASp, TAP1, and XBP1.

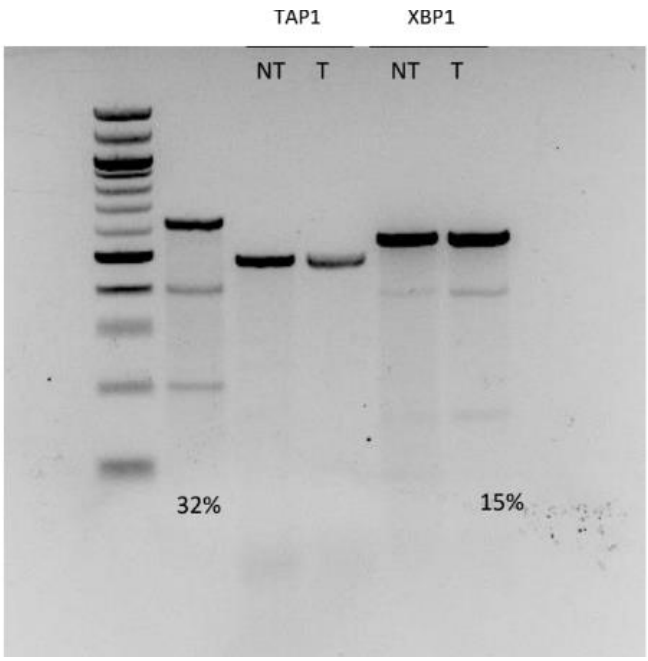

Figure 14: Trial 1- SURVEYOR Nuclease Assay of Transduction with Nanoblades and XBP1 and TAP1 genes. Cx3CR1 not included.

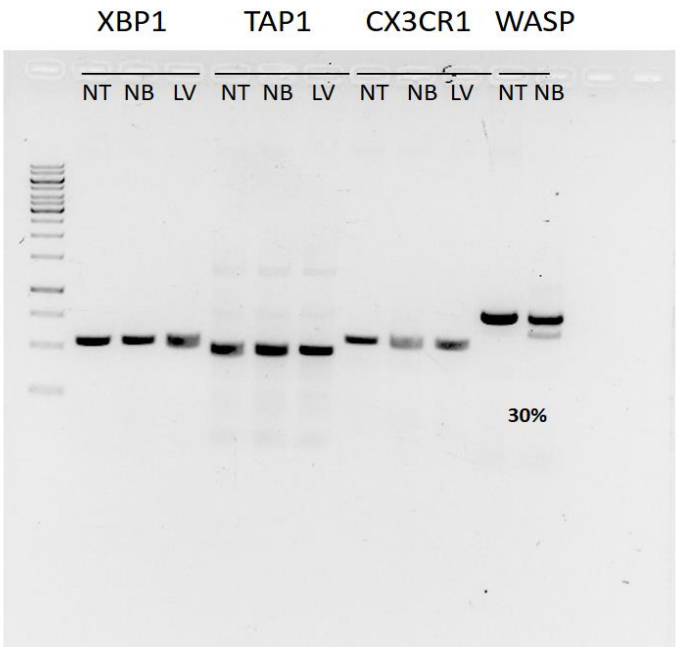

Figure 15: Trial 2- PCR of the Transduction of Caco2 Cells in XBP1, TAP1, and CX3CR1, and WASp. The "NT" represents the non-transduced cells, while the "NB" and "LV" represent the Nanoblade and Lentiviral Vector transductions, respectively. Since the WASp needs 2 sgRNAs in order to transduce, cleavage was visible on the PCR, and there was no need for Surveyor.

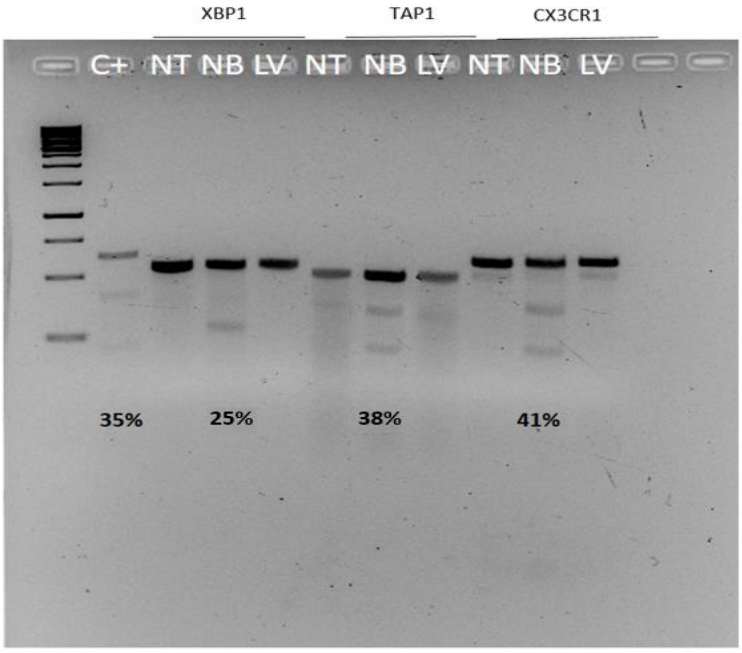

Figure 16: Trial 2- SURVEYOR Assay of the Transduction of Caco2 Cells in XBP1, TAP1, and CX3CR1. The "NT" represent the non-transduced cells, while the "NB" represent the cells transduced with Nanoblades and the "LV" represent the cells transduced with lentiviral vectors. WASp not assayed using SURVEYOR.

Table 1:

Percentage of Cleavage in XBP1, TAP1, and Cx3CR1 Genes in Caco2 Cells

	Lentivirus	Nanoblade
XBP1	0%	25%
TAP1	12%	38%
CX3CR1	0%	41%

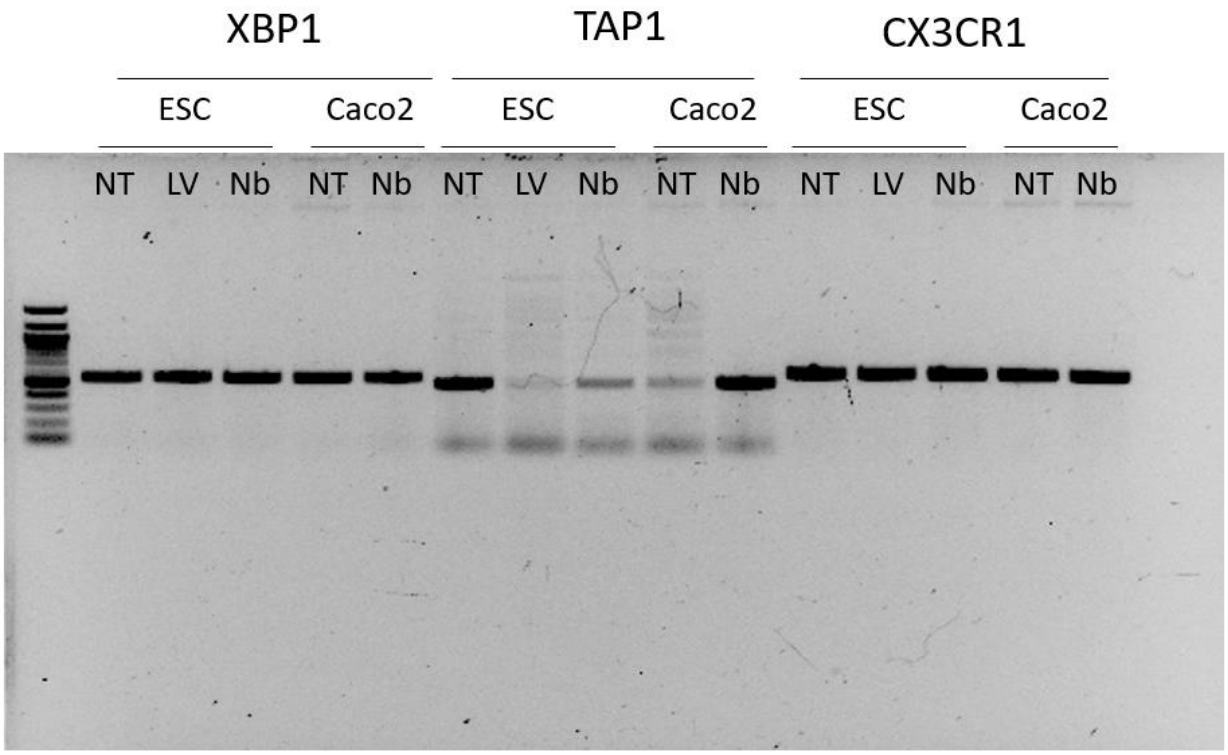

Figure 17: Trial 3- PCR of the transduction of Embryonic Stem Cells with Lentiviral Vectors and Nanoblades and Caco2 Cells with Nanoblades with the same genes. "NT" represents the non-transduced cells, "Nb" and "LV" represent the cells transduced with Nanoblades and Lentiviral Vectors, respectively.

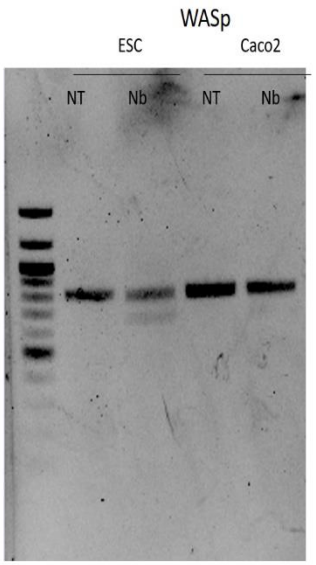

Figure 18: Trial 3- PCR of the transduction of Embryonic Stem Cells and Caco2 Cells with Nanoblades and Lentiviral Vectors with sgRNA of WASp gene

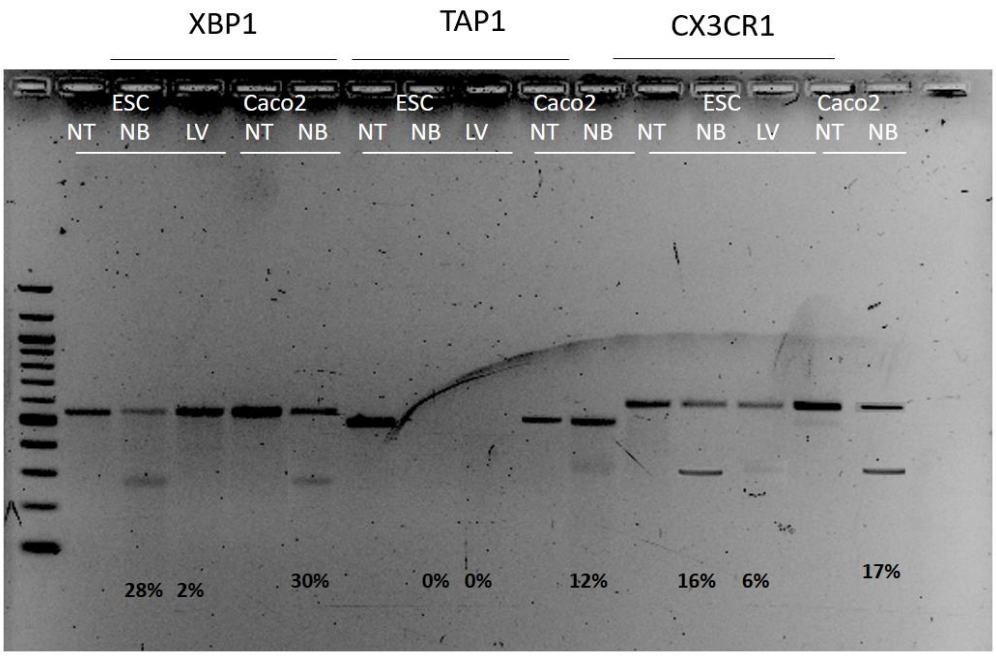

Figure 19: Trial 3- SURVEYOR Assay of the transduction of Embryonic Stem Cells and Caco2 Cells. "NT" represents the non-transduced cells, while "NB" and "LV" represent the cells transduced with Nanoblades Caco2 Cells, respectively. WASp not assayed using SURVEYOR.

Table 2:

Percentage of Cleavage in XBP1, TAP1, and CX3CR1 Genes in Embryonic Stem Cells

	Lentivirus	Nanoblade
XBP1	2%	28%
TAP1	0%	0%
CX3CR1	6%	16%

Discussion:

In order to study genetic associations in IBD, a novel form of gene editing called "Nanoblades" was used to deliver a CRISPR/Cas9 system into Intestinal Epithelial Cells and Embryonic Stem Cells. In this study, the genes studied were XBP1, TAP1, and CX3CR1. The sgRNA from these genes were ligated onto CRISPR/Cas9 plasmids and delivered into target cells using Nanoblades and Lentiviral Vectors in order to compare their efficiencies in gene editing in the targeted cells.

Figures 13 and 14 show the PCR and SURVEYOR Assay of the first trial, in which only Nanoblades were used in order to test their effectiveness. Figure 14 shows that only the Nanoblades for XBP1 in this trial were successful. The gel showed approximately 15% cleavage, which means that approximately 15% of the cells in the culture were transduced, and they now had SNPs. The TAP1 genes did not show any cleavage, and this could have been due to a number of reasons, but since the genomic band from the PCR was visible from TAP1, it was clear that the problem was in the transduction itself.

In order to display cleavage, it was necessary for two bands to be visible in one lane of the gel: the first band represents the DNA of the cells that were not transduced and did not have any SNPs. The second band represents the SNP in the targeted gene, meaning that the DNA is different for each strand, so they are at different sizes, and at different intervals on the gel. The XBP1 gene showed cleavage in the first trial, meaning the two bands were present and at the correct intervals, while TAP1 did not show these two bands, meaning SNPs were not created in that gene.

In this first trial, the PCR for the CX3CR1 gene for sgRNA 40+ 42 did not show a band at the correct interval (evident in Figure 13), indicating that the genomic DNA was not present or expressed. Therefore, it was not analyzed by the SURVEYOR Nuclease Assay. This could have been due to a number of reasons, the most likely reason being that the primers were not effective and had to be redesigned. For this reason, the primers for CX3CR1 were redesigned to make sure the band of DNA would appear on the PCR. In the next trial, CX3CR1 for sgRNA 40 did show genomic DNA on the PCR, and it was transduced successfully in Caco2 Cells (shown in Figures 15 and 16).

A gene known as WASp was also targeted using Nanoblades only in all the trials. In a previous study with Nanoblades, Mangeot et al. targeted the WASp gene in their Nanoblades, and as a result, we used the WASp Nanoblade in this experiment as a control. If the Nanoblades were not successful, using the Wasp gene transduction, it would be evident if the reason for the failure of the transduction was due to the Nanoblades or due to a previous inaccuracy in the methods.

The second trial in this experiment was conducted with both Nanoblades and Lentiviral Vectors in order to compare the efficiencies of a novel form of delivery (Nanoblades), and a fairly common form of delivery (Lentiviral Vectors) in Caco2 Cells (Figures 15 and 16). In this trial, after a PCR (shown in Figure 15), the WASp Gene showed approximately 30% transduction. Since the WASp gene transduction was successful, a prediction was made that the transduction was successful for the rest of the genes, since the methods administered were the same for all the genes involved. After the SURVEYOR Assay was conducted in this trial, cleavage was evident in the Nanoblades for all three genes studied, but not as much in the Lentiviral Vectors, supporting the hypothesis that this novel technology induces SNPs in target cells more efficiently. The Nanoblades for XBP1 showed 25% cleavage, the TAP1 gene showed 38% cleavage, and the CX3CR1 gene showed 41% cleavage, as opposed to the Lentiviral Vectors that barely showed any cleavage in all three genes (Table 1).

The third trial in this experiment was conducted on Embryonic Stem Cells (Figures 17, 18, and 19). After the transduction of the viruses and the genomic extraction, a PCR was run in order to determine the cleaving efficiency in the WASp gene (Figure 18) and to see if the DNA from all the cells is present (Figure 17). In the PCR of the WASp gene in the third trial (Figure 18), it was evident that the transduction in the Embryonic Stem Cells was successful, since two bands were visible for the WASp gene. The cleavage for the WASp gene was approximately 20%. Since the WASp gene transduction was successful, a prediction was once again made that the transduction was successful for the rest of the genes. The PCR also showed that the TAP1 genomic extraction was abnormal, since the band was not present at the correct interval, and this was most likely due to an inconsistency in the methods.

The SURVEYOR Assay for the third transduction (Figure 19) showed that the transduction was successful for all the genes except for TAP1, which was expected. It is evident

in this gel that the Nanoblades were more effective in their induction of SNPs, since the percentages of cleavage were high in the Nanoblades than in the Lentiviral Vectors (shown in Table 3). In the XBP1 and CX3CR1 genes, there was 28% and 16% cleavage due to the Nanoblades, respectively, while there was only 2% and 6% cleavage in the Lentiviral Vectors, respectively. This once again proves the hypothesis that Nanoblades are a better option when inducing SNPs in primary cell lines.

Overall, the results to this experiment proved that Nanoblades were a superior tool in inducing SNPs in primary cell lines, more specifically Caco2 Cells (Intestinal Epithelial Cells) and in Embryonic Stem Cells. Lentiviral Vectors were used as a comparison to Nanoblades, and Nanoblades proved to be the more effective method to gene editing for susceptible genes involved in the development of IBD and intestinal inflammation. This research opened a gateway to research using Nanoblades in not only IBD, but also in different diseases previously hindered because of the lack of effective technology for transfection of primary cells.

Conclusion:

My research discovered a new use for the novel technology of Nanoblades in research for IBD. In this project, Nanoblades were used to deliver a CRISPR/Cas9 system into target cells to induce single nucleotide polymorphisms (SNPs) in specific genes known to be susceptible factors in the development of IBD. The genes studied in this experiment were the XBP1, TAP1, and CX3CR1 genes: all genes susceptible in intestinal inflammation according to previous studies.

In order to determine if Nanoblades were effective in their induction of SNPs in the target cells, trials were conducted with Lentiviral Vectors as a comparison. The results to this experiment found that Nanoblades are statistically significantly more effective in inducing SNPs in Caco2 cells, and the also are more effective inducing SNPs in Embryonic Stem Cells. Both Embryonic Stem Cells and Intestinal Epithelial Cells are cell lines known for their unaccommodating manner in terms of transfection, and this issue was solved through the use of this novel technology.

Hence, future research will be conducted with these genes in IBD. The Embryonic Stem Cells that were transduced effectively in this experiment will be differentiated into intestinal organoids, and they will be analyzed in depth, by studying the immune response, as well as autophagy pathways (cell death pathways) in order to understand the immune response and its implications in the development of IBD.

This results of this experiment have implications in not only IBD studies, but in hundreds of thousands of diseases. Genetic factors can now be easily studied in different diseases, since Nanoblades have been shown to be an effective method to study Inflammatory Bowel Disease. This research can act as a steppingstone for research in many different diseases. This experiment proved that Nanoblades are the future of gene editing technologies, and in studying IBD.

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