iPSC SAFEGUARD

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ABSTRACT

Since the technology of iPSCs(induced Pluripotent stem Cells) are widely concerned these several years, its high potential risks of tumorigencity has become the largest hurdle for clinical trial. This summer we created a new device, which is composed of miRNA122 targets, suicide gene, and Tet-off system, to try to minimize this problem. With our device, only normal differentiated hepatocytes can survive, while other types of cells, including hepatomas and undifferentiated cells, will automatically commit suicide without effecting other normal cells around.

WHY LIVER

Considering iPSC can be differentiated into many types of cells, we need a specific type to be our model to carry out our new device., Hhere, our choice is liver cell. Recent statistics from WHO by 98 countries show that liver transplants demand is increasing straightly these years. In this March, scientists have for the first time created a functional human liver from iPSCs. This gave us a hope that in the future the patients could be transplanted with the liver from their own iPSCs and no longer need to take any medicine in their lives. Based on this promising technology of inducing iPSC to liver cell, to minimize the potential tumorigenicity risks of iPSCs before clinicle application is very urgent .

TET-OFF SYSTEM

Tet-off system is the switch of our whole device to accurately turn on and off our killing system. The TRE is located upstream of the promoter called PminCMV, which is silent in the absence of activation. tTA binds the TRE—and thereby activates transcription of Gene X. After Dox are added, they immediately combine to the tTA protein, thus change its comformation, pull it down from TRE and make Pminicmv return back to state of inactivation.

We have got three generations of PminCMV, each of them has different expression level at ON/OFF state.

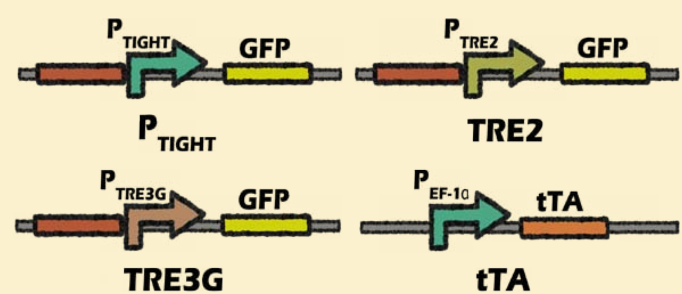


Figure 1. Plasmids of three generations of tet-off system.

RESULT:

1. To find the best PminCMV, whose leaky expression should not be too high to kill cells, we firstly tested the leaky expression of each generation of PminCMV. pTight turned out to be the one with lowest leaky expression.

Figure 2. The leaky expression of different TRE.

2. We then tested the expression of both ON and OFF states of the whole tet-off system, using pTight as the PminCMV. The GFP expression showed Tet-off system worked as expected.

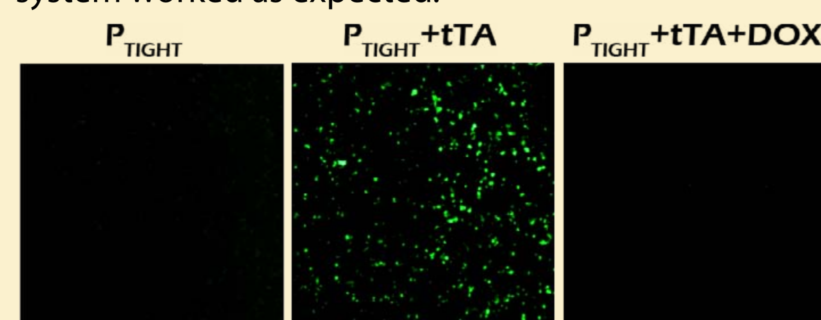


Figure 3. The expression of both ON and OFF states of tet-off system.

3. We proved that the leaky expression of TRE3G in mouse iPSC is low enough for iPSCs to survive.

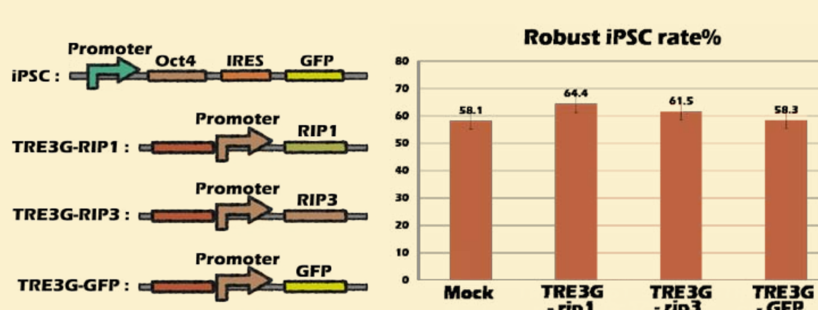


Figure 4. The leaky expression of TRE3G in mouse iPSCs.

MIRNA122

miRNA122 is a liver-specific small RNA whose function is to suppress gene expression either by degrading the target mRNA or by blocking its translation. Because its sperific high expression only in normal liver cells, we use it to be our intrinsic sensor to distinguish liver cells from other types of cancer cells.

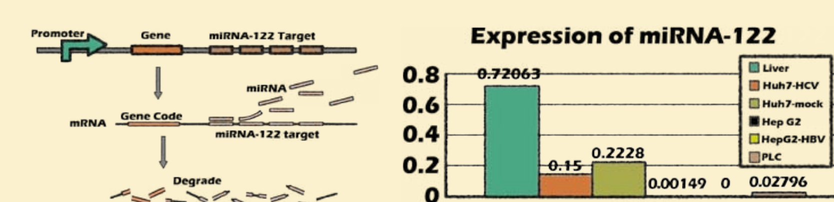


Figure 5. Left: The expression of miRNA-122 in different liver cell lines. Right: The mechanism of miRNA suppression.

RESULT:

1. We verified that the expression level of miR122 is high enough in mouse liver cells (Hepatocytes) to suppress the expression of gene of interests.

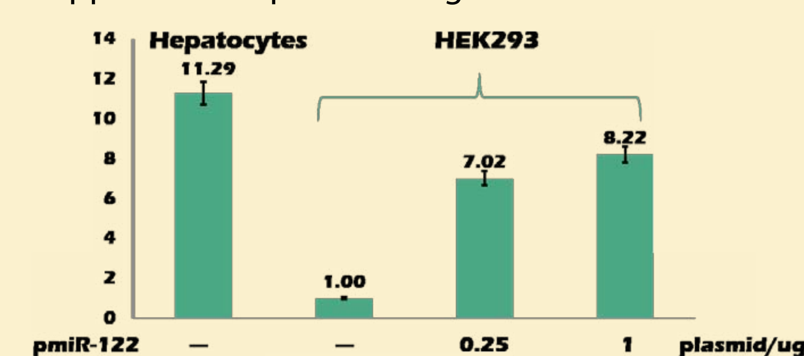


Figure 6. MiRNA122 level in Hepatocytes and HEK 293T cell line w/ or w/o the transfection of miRNA122 by q-PCR. p miR-122: the plasmid containing miRNA122.

2. We successfully proved and quantitatively characterizated the suppression efficiency of miRNA122.

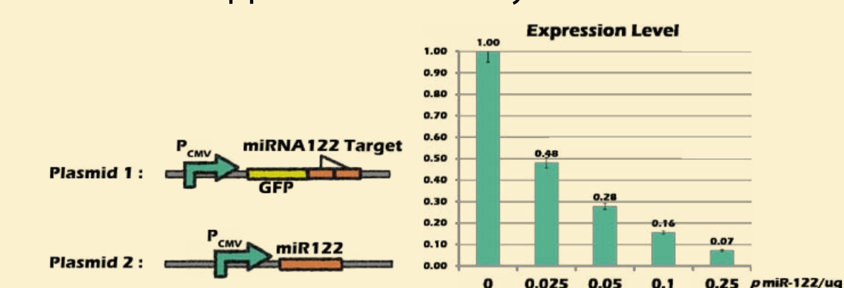


Figure 7. Left is the two plasmids transfected into cells. Right is tThe suppression efficiency of miRNA122 tested by Western-blot. All cells were tranfected with the same concentration of plasmid of GFP-target and the increasing gradient of plasmid of miRNA122. The expreiment was done in HEK 293T cell line, which is a type of cell with low level of miRNA122.

3. We tested the supression effiency of miRNA122 with different copy number and taget-site and built a model.

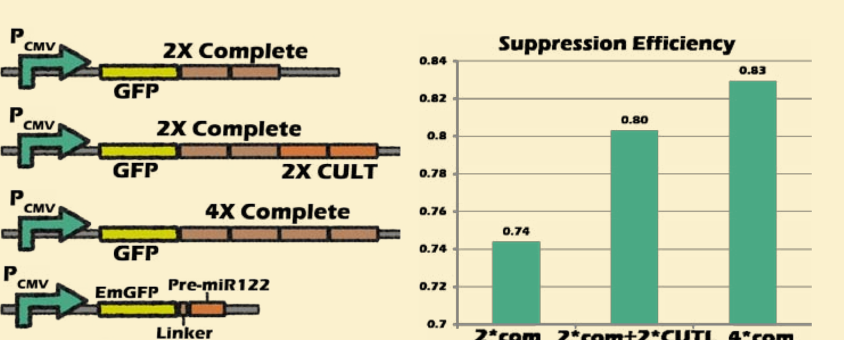
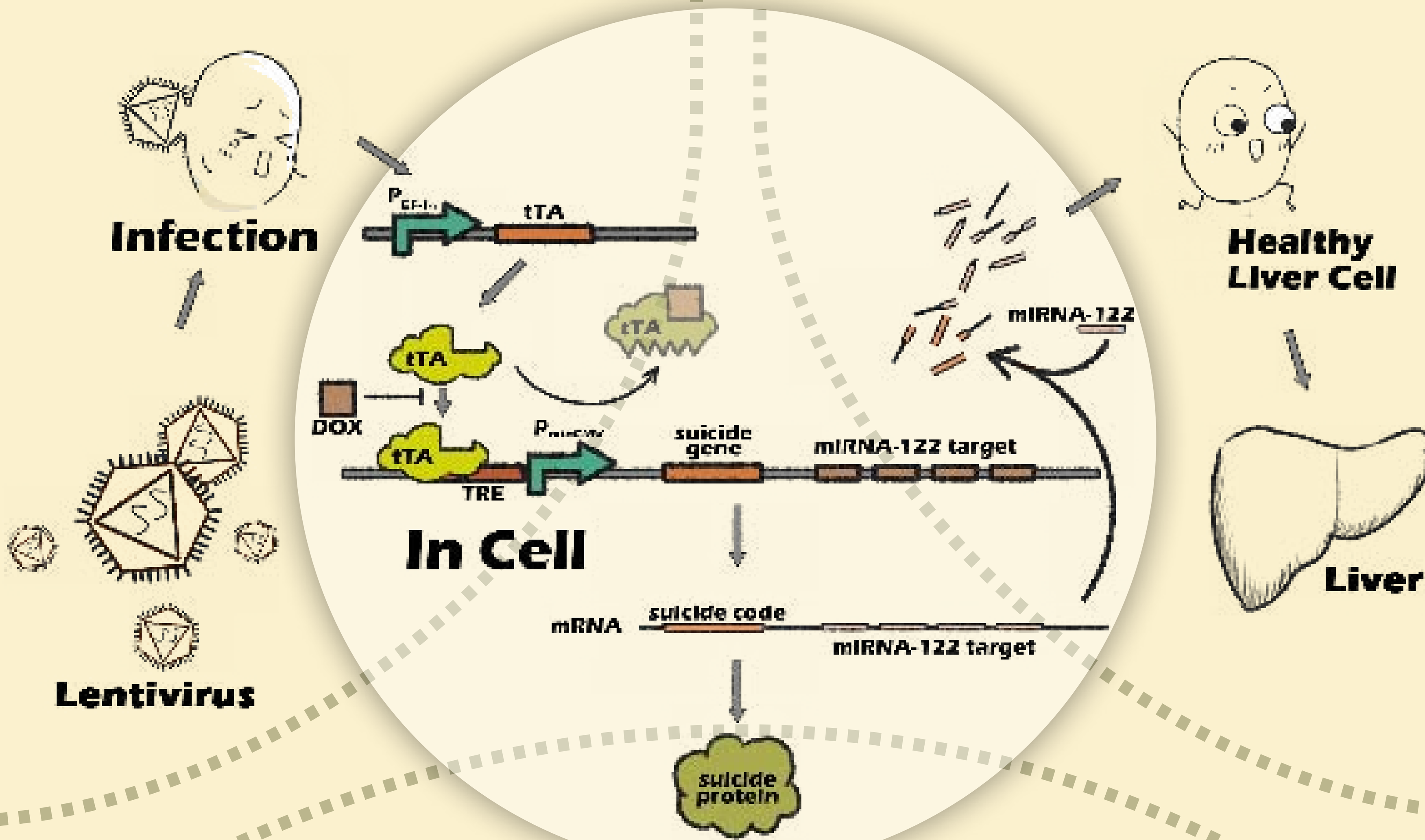


Figure 8 . The supprssion efficiency of miRNA with different copy number and taget-site. Com and CUTL represent two different sequences of target that has different binding energy. we tried different constructions,like duplication,quadruplication or combining different sequences. In figure we can see that the efficiency is promoted by increasing the copy number or replace CUTL with the Com sequence.



Death Cell

DELIVERY SYSTEM

Lentivirus is viral-based gene delivery system that can stably deliver genes or RNAi into primary cells or cell lines with up to 100% efficiency. To achieve safeguarding the liver derived from iPSCs in the long term, our device is delivered by lentivirus into cell genome.

Lentivirus was firstly packaged with two packaging plasmids and the plasmid of our genes in HEK 293T. After 48h, intact lentivirus was collected and concentrated into a extremely high multiplicity of infection. The last step is cell infection.

RESULT:

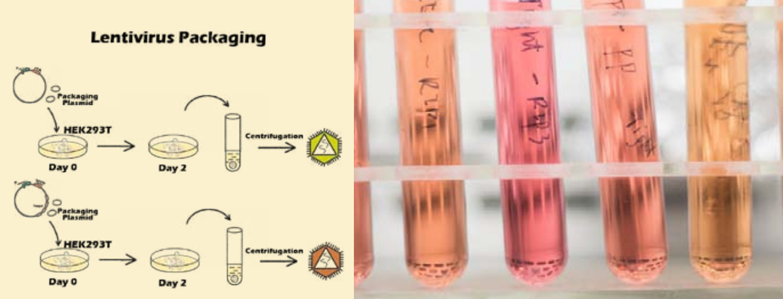


Figure 12. Left: the process of lentivirus packaging. Right: the photos of the lentivurs after concentrated.

We have successfully infected the iPSCs and proved that both our suicide genes and the TRE3G worked as expected (see result in suicide gene and Tet-off system).

SUICIDE GENE

Suicide gene is the performer of cell death. In stead of using medicine to kill cells, which may cause side effect to cells around, here we want to use the suicide gene to trigger the cell death. Literally there are many genes which have been reported to have important roles in cell death. our candidates are hBax, hBax mutant, delta TK, caspase 3, rip1, rip3, and apoptin.

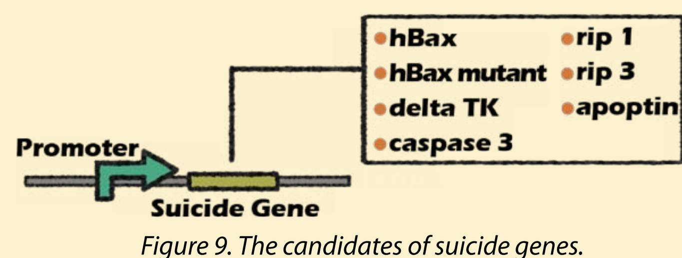


Figure 9. The candidates of suicide genes.

RESULT:

Figure 10. The cell death rate in Hep G2 cell line.

Figure 11. The survival rates of suicide gene in iPSCs. All cells are infected by lentivirus containing suicide genes.

1. 1. rip1, rip3 and apoptin are proved to be the best suicide genes in Hep G2 cell line(a type of liver cancer cell).
2. 2. rip1, rip3 and apoptin are proved to have obvious cell death rates in iPSCs.

IN PROGRESS

1. To test the long-term performance of our device in different cells, we have infected HepG2 cell lines and iPSCs with lentivirus containing our device and the cell lines are still under resistance screening.
2. After screening, we will inject iPSCs with our device into naked mouse to test the performance in vivo.
3. We have successfully isolated and cultured the primary mouse liver cells, which is a type of cell very hard to passage and tranfect. We are still trying to transfect and infect it test our device in liver cells.
4. We have tested the different suppression efficiency of miRNA122 with different target-sites and different copy number, and we are trying to build models to figure out the best parameters.

HUMAN PRACTICE

We carried out several human practice activities including a campus popularization, TEDx talk, popularizing iPSCs with medical workers and patients in the Provincial Hospital of Traditional Chinese Medicine in Guangdong. What's more, we had a lot of interactions with Guangdong international life science foundation (GDILSF), who then provided us an opportunity to visit Guangzhou institutes of biomedicine and health (GIBH). Besides, we have writen several popular science articles about iPSCs and regenerate medicine field, which are ready to be published on our local newspaper.

ACHIEVEMENT

1. We created a new device to safeguard iPSCs and the differentiated liver cells which derived from them, and successfully proved that each parts of our design (miRNA122 target, suicide gene, and tet-off system) work as expected in both iPSCs and HepG2 cell lines. We have built both iPSCs and HepG2 cell lines stably expressing our device.
2. We have submitted 9 pieces of biobricks to the iGEM Registry and have improved the function of an existing BioBrick Part (BBa_K1061006).
3. We have carried out several human practice activities.
4. We have helped SCUT iGEM team to debug one of their promoter T7 constructs.
5. We have implications for the iPSCs safety in regenerate medicine field. Actually our design itself is a novel approach we used to minimize the potential tumorigenicity risks of iPSCs and accelerate the development of moving iPSC-based therapies to the patient bedside.