DON'T RUN, MELANOMA!

Shenzhen_SFLS

SUMMARY

Melanoma is one of the most serious types of skin cancer with a high potential for metastasis and a low survival rate [1,2]. It has been reported that about 60% of melanomas contain a mutation in the v-raf murine sarcoma viral oncogene homolog B (BRAF), and V600E (1799T>A) mutation in BRAF is the main type of mutations in the cancer tissues, which plays a critical role in carcinogenesis of melanoma . In the present study, we designed a synthetic device base on the CRISPR/CAS9 system to specifically disrupt the mutant BRAF in melanoma cells. Our results showed that the system significantly inhibited the proliferation and migration, and induced apoptosis in the two cell lines, which suggested that we could target a specific oncogene and achieve personalized therapy for different types of cancer by simply changing the sequence of a sgRNA.

DESIGN

We designed a synthetic device based on the CRISPR/Cas9 system that can accurately identify and kill melanoma cells with BRAF mutations. This device includes two parts: a CRISPR/Cas9 system and a regulatory system.

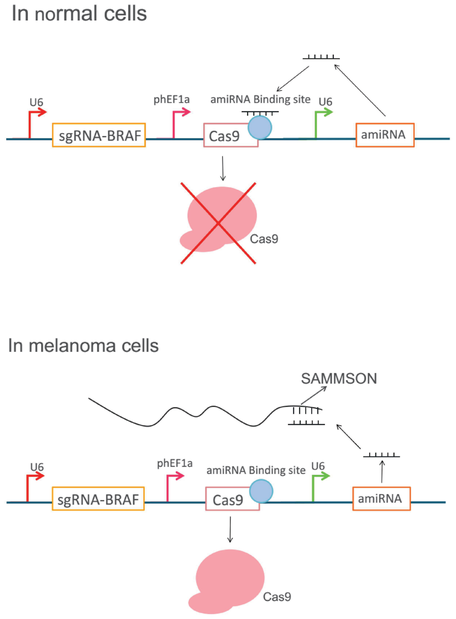
CRISPR/Cas9 System

Our sgRNA was designed for specifically binding to mutant BRAF V600E gene in melanoma cells.

Regulatory System

The regulatory system includes an artificial microRNA (amiRNA) which can specifically bind to SAMMSON and an artificial complementary sequence after Cas9 as the binding site of amiRNA. Thus, the CRISPR/Cas9 system can be regulated.

As the figures shows, in normal cells where SAMMSON has no expression, the amiRNA will bind to the binding site after Cas9, and prohibit the translation of Cas9. In contrast, in melanoma cells, the highly expressed SAMMSON binds to the amiRNA and the inhibition of the amiRNA on the CRISPR/Cas9 system will be released, which results in the activation of the CRISPR/Cas9 system cleaving the mutant BRAF V600E gene.



SAMMSON: Survival Associated Mitochondrial Melanoma Specific Oncogenic Non-Coding RNA (SAMMSON) is a long non-coding RNA (lncRNA) which is specifically expressed in melanoma cells.[3]

MODELING

Part one:

Our modeling group used a scoring method called CFD (cutting fequency determination) to predict sgRNA off-target probability. We found 7 sequences of high probability of off-target effect.

	GTGATTTTGGTCTAGCTACA**GAG**	Score 100%
Seq1	TTGATTCTGGTCTAGCTACT**TCG**	Score 23.87%
Seq2	TAGATTTTGGTCTAGATGCA**TGG**	Score 17.76%
Seq3	CGGATTGTGGTCTAGCTACC**TGG**	Score 17.39%
Seq4	GGCATTTTGTCTAGCTACA**TGG**	Score 15.26%
Seq5	TTAAAAATGGTCTAGCTACA**TGG**	Score 11.55%
Seq6	GCAAAATTGGTCTAGCTACA**GGG**	Score 08.89%
Seq7	GCGATTGTGGTCTAGCTACC**TGG**	Score 06.82%

Part two:

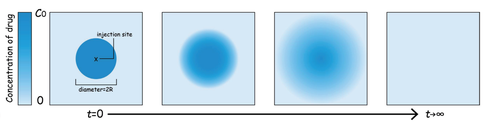
We developed a model to describe the drug release in tumor tissue. The goal of this model is to take control of the drug by adjusting initial concentration and dosage.

$$\frac{\partial C}{\partial t} = D\Delta C - kC$$

$$V = \frac{4}{3}\pi R^3$$

$$\begin{cases} C(x,0) = C_0, x\epsilon[-R,R] & \text{I} \\ C(x,\infty) = 0 & \text{II} \\ C(\infty,t) = 0 & \text{III} \end{cases}$$

Unfortunately, we failed to gain experiment data of lentivirus vector due to the limited time. However, the values of parameters of liposomal drug have been determined, and liposomal drug release simulation is shown below.



DEMONSTRATE

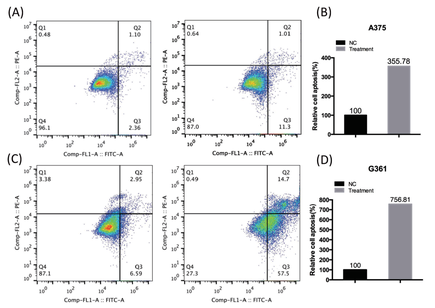


Fig.1 Cell apoptosis was induced after infection with plasmid and detected by flow cytometry analysis. Apoptosis of infected cells A375 (A) and G361 (C) was measured by flow cytometry. The cell apoptotic rate was significantly increased in the Treatment group in A375 and G361 cells (A-D).

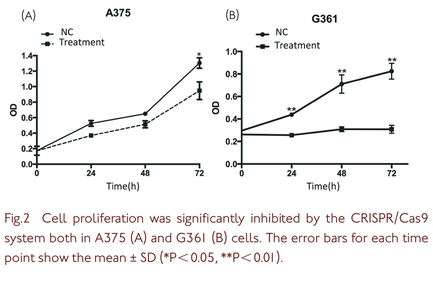


Fig.2 Cell proliferation was significantly inhibited by the CRISPR/Cas9 system both in A375 (A) and G361 (B) cells. The error bars for each time point show the mean ± SD (*P<0.05, **P<0.01).

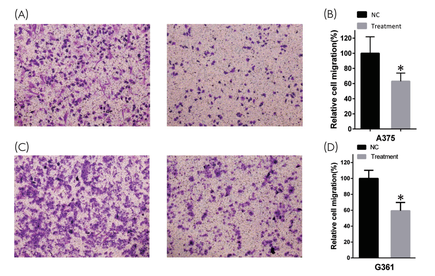


Fig.3 Cell migration was restrained by the CRISPR/Cas9 system. Migration of the infected cells A375 and G361 were measured by the Transwell assay. Cell migration was significantly suppressed in the treatment group in A375 (A) and G361 (C) cells. Each experiment was performed on at least three independent occasions. Error bars show mean ± SD (*P< 0.05).

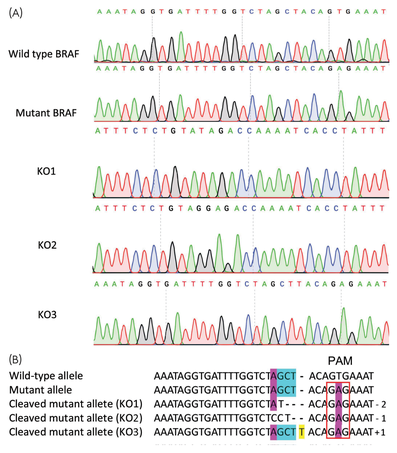


Fig 4. The mutated BRAF gene was specifically cleaved in Melanoma cells.A, the Chromatogram of DNA sequencing. B,DNA sequence comparison. Our CRISPR/CAS9 system specifically cleaved the mutated BRAF gene in melanoma cells, resulting in insertion, deletion and frameshift mutation on the gene.

HUMAN PRACTICE

The future plan

In the future, we would like to put the regulatory system and CRISPR/Cas9 system together and to test whether the Cas9 protein is only expressed and works in melanoma cells.

Next, we will pack the vectors containing the amiRNA, amiRNA binding-site, Cas9 and sgRNA into lentivirus in order to increase the transfection efficiency and use them to deliver the device into the target cells in the functional experiments.

Finally, we also want to sequence the whole genome of the transfected cell and see whether we can reduce the off-target effects on the melanoma cells by the system.

Gold Standard

1 Interviewing with professors and doctors
2 Questionnaire about Cancer and CRISPR /Cas 9 system
3 Helped by the instructors from SIAT_SCIE and Peking University
4 Attending meet- ups in SUSTech_Shenzhen , SZU_China and SIAT-SCIE.

Silver Standard

1 Delivering speeches for students and the public.
2 Popularization of iGEM, synthetic biology and melanoma on the Internet.

Reference & Attribution

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011; 61:69–90.
[2] Balch CM, Gershenwald JE, Soong SJ, Thompson JF, Atkins MB, Byrd DR, et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol. 2009; 27:6199–6206.
[3] Leucci E, Vendramin R, Spinazzi M, et al. Melanoma addiction to the long non-coding RNA SAMMSON. Nature, 2016, 531(7595):518-522.

Laboratory support: Shenzhen PKU-HKUST Medical Center
Thanks to our instructors: Prof. Yaoting Gui, Prof. Fangting Zhang, Mr. Chengle Zhuang, Dr. Xiaomin Luo, Mr. Xinbo Huang, Mr. Minghua Li
Thanks to our sponsors: Shenzhen Book City, NOVA