A New Hepato Stella System To Treat Glioblastoma

CPU _ GlioFighter

ABSTRACT: Given the recurrent nature and clinical challenges of gliomas, we have developed a self-assembly RNAi system (Hepato Stella System) by taking a synthetic biology approach. This system exhibits prominent advantages compared to conventional RNAi-based treatments. The uniform and quality-controllable circulatasomes (Nano Liposo Gleam Carrier Particles) are produced by hepatocyte cells, containing live pharmaceuticals with safe features. The circulatasomes can efficiently across the blood-brain barrier to precisely achieve effective concentrations in targeted cells. The design principle of this self-assembly RNAi system is to create adjustable plug-and-play genetic circuits by coding and inserting various sequence combinations. The combinational and randomly programmed genetic circuits can be targeted delivery for secreting small nucleic acid molecules (siRNA) to specifically interfere the patients with different genetic backgrounds. Moreover, multi-site and multi-dimensional combination therapies for both primary and secondary brain tumors can be accomplished by this newly developed system. The implementation of this RNAi system opens a new avenue for precision medicine, further advancing the concept of personalized treatments for possible pan-cancer therapy.

BACKGROUND:

The standard treatment for newly diagnosed glioblastoma (GBM) is surgical resection followed by radiation therapy, supplemented by multimodal temozolomide. At present, the standard treatment for GBM is called “Stupp protocol”, which includes as complete surgical resection as possible, followed by fractional radiotherapy of 60 Gy, combined with synchronous temozolomide chemotherapy, followed by 6 months of

adjuvant chemotherapy [1]. This treatment regimen can only gradually extend the median survival of patients from 2.5 months to 14.6 months. This option has the following limitations:

(1) Tumor heterogeneity: GBM has significant genomic and cellular heterogeneity, including PTEN, TP53, EGFR and other mutations. This heterogeneity leads to limited targeted effectiveness of therapy [2] [4].

(2) Blood-brain Barrier (BBB) limitation: Although temozolomide is one of the few drugs that can penetrate BBB, its efficacy is affected by the methylation status of MGMT promoter. Patients with unmethylated MGMT promoters usually show drug resistance and poor treatment response [3] [7].

(3) Glioma stem cells (GSCs): GSCs are one of the main causes of tumor recurrence. These cells are resistant to chemotherapy and radiotherapy and further drive tumor progression after recurrence [3] [5].

Recurrent brain gliomas are almost universal, and treatment options for recurrent GBM include surgical resection [6], directed radiation therapy (SRS) [10], systemic application of the DNA alylation agent Lomustine [6] [9], and systemic application of the anti-angiogenic monoclonal antibody bevacizumab [3] [8]. Each of these therapies has shown some efficacy against recurrent tumors, although the efficacy is limited and inconsistent, but all are associated with severe treatment-related toxicity .

To effectively address the challenges in the treatment of glioblastoma multiforme (GBM), we hope that RNA interference (RNAi), enabled by small interfering RNA (siRNA), will serve as an emerging therapeutic strategy. This method can accurately regulate tumor genes at the level of messenger RNA (mRNA) or protein, and has higher specificity and therapeutic efficiency compared with traditional chemotherapy [11]. However, the roll-out of siRNA therapy faces some significant challenges. First of all, naked siRNA has low stability and is easily degraded by nucleases in serum, thus reducing its efficiency.

Effectiveness in vivo [12]. Secondly, the physical properties of siRNA make it difficult to penetrate the blood-brain barrier (BBB) and the cell membrane of target cells, which limits the entry of most therapeutic siRNA into target cells in the brain for effective

treatment [13]. To address these issues, in vivo siRNA delivery is primarily achieved through cationic charged nanoparticle delivery systems, including lipids, inorganic nanoparticles, and mixtures. In the context of our proposal, we mainly selected lipid nanoparticles that have been widely used in siRNA delivery studies due to their biocompatibility and ability to efficiently package siRNA. Some studies have shown that lipid nanoparticles can successfully deliver siRNA to liver tissue and promote gene silencing [14].

OBJECTIVES:

Ⅰ. Building a delivery system that combines efficacy, safety, autonomy, and targeting.

Ⅱ. In the treatment of brain glioma, the right target is found to achieve high inhibition.

DESIGN PROCESS:

Concept Introduction

Synthetic biology is an interdisciplinary research domain that focuses on devising and fabricating novel biological components, functions, and systems. It aims to create controllable mechanisms, biological logics, and production systems that do not occur naturally. With a wide range of applications, the medical and healthcare field stands as the largest market for synthetic biology. As pharmacy students, we are perpetually concerned about the development of the global pharmaceutical industry. In view of the severe situation of glioma, we have developed the Hepato Stella System (HSS). The term "Hepato" is derived from a root related to the liver, while "Stella", meaning "star" in Latin, symbolizes that this system can shine brightly like a star and exhibit remarkable therapeutic effects.

We metaphorically compare the human body to an expansive galaxy, with each organ and cell within it being regarded as a planetary body. Exosomes, functioning as the "rocket ships" for communication among these planetary bodies, play a vital role. The HSS is capable of modifying the endogenous cells in the human liver (which are

originally like ordinary planetary bodies) into "Galactic Factory Cells", which serve as "factories" for producing specific "Life Rockets". The so - called "Life Rockets" are essentially exosomes. However, the specific "Life Rockets" generated by "Galactic Factory Cells" can encapsulate exogenous "cosmic heavy elements" - drugs necessary for glioma treatment, thus better facilitating "astral development", that is, treating human glioma.

"Galactic Factory Cells" utilize Nano Liposo Gleam Carrier Particles (NLGCP) to selectively transport and package exogenous specific gene "heavy elements" - small interfering ribonucleic acid (siRNA) into "Life Rockets". After being launched, the "Life Rockets" containing gene "heavy elements" can leverage the body's own circulatory system to target and deliver these gene "heavy elements" to specific "astral bodies" (gliomas). At the initial design stage of the HSS, through genetic modification at the organic level, instructions can be incorporated into the system, enabling the launched "Life Rockets" to be equipped with a "Starlight Navigation Tool" (targeting protein). In other words, this special tool is embedded on the outer shell of the "Life Rockets". This tool can perform precise navigation, bind to specific targets on the blood - brain barrier in the brain, and guide the "Life Rockets" to pass through the blood - brain barrier smoothly and enter the brain. Subsequently, the "Starlight Navigation Tool" accurately locates the "Life Rockets" to specific "astral bodies" (glioma cells). Once the outer shell of the "Life Rockets" ruptures, the gene "heavy elements" are automatically released to seek out and match their target genes, thereby disrupting the gene sequences that promote glioma growth and interfering with the expression of these genes, achieving targeted treatment of glioma.

This process fully demonstrates the regulatory, dynamic, hierarchical, and relatively stable characteristics of synthetic biology within living organisms. The entire treatment process is like a natural and highly efficient life - coordinated operation. The HSS can not only design personalized gene "heavy elements" (siRNA) for different patients but also utilize "Galactic Factory Cells" (liver endogenous cells) to continuously and autonomously produce "Life Rockets" containing gene "heavy elements". Moreover, this system does not rely on foreign materials that may trigger immune rejection and side

effects, maintaining the homeostasis within the human body. It paves a new way to conquer glioma in a green, safe, and efficient manner and also holds potential value in ensuring supply chain security.

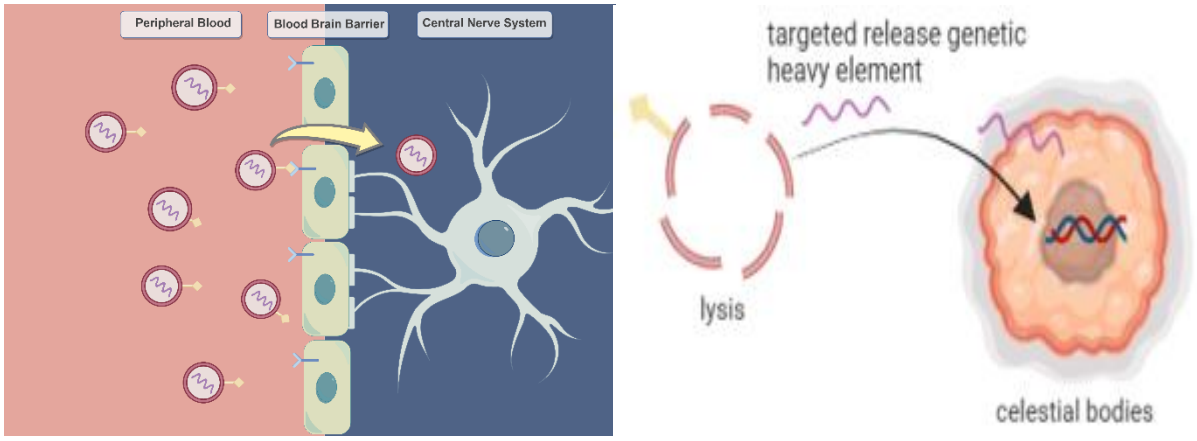

Figure 1. Cross the blood-brain barrier and function

Specific Design

After successfully designing this highly effective treatment system, we have screened a target that is highly expressed in glioma - Bcl2 - L12. Proteins of the Bcl2 family play a crucial role in the regulation of cell apoptosis. As a member of this family, Bcl2 - L12 regulates the survival and proliferation of tumor cells by influencing the apoptosis signaling pathway within cells. Structurally, it possesses unique protein domains that are essential for its functionality. Inside the cell, Bcl2 - L12 is mainly located on the membranes of organelles such as mitochondria. In terms of function, apart from its significant role in apoptosis regulation, it is also associated with processes such as autophagy. During the process of tumorigenesis and development, Bcl2 - L12 often shows abnormal expression. In many tumor cells, its expression level is significantly elevated. By inhibiting the activation of apoptosis - related proteases and other mechanisms, it promotes the survival and proliferation of tumor cells, enabling tumor cells to resist apoptosis - inducing stimuli from chemotherapeutic drugs, radiotherapy, etc., thus leading to tumor drug resistance and increased treatment difficulty. Therefore, interfering with the expression of Bcl2 - L12 using gene "heavy elements" (siRNA) can effectively inhibit the growth of glioma and achieve the therapeutic goal.

The HSS is constructed in vitro. Its main component is an organic molecule (RNA)

sequence. Based on this sequence structure, we use genetic modification technology to modify it, allowing us to insert the required "starlight information molecules". For example, the "navigation" tool mentioned earlier is synthesized under the guidance of the inserted sequence. To enable this sequence to function, we add a promoter sequence at the front of the sequence as an activation signal. When the sequence reaches the "Galactic Factory" cells, the activation signal takes effect first, awakening the entire sequence from its dormant state and enabling it to start working.

This synthetic system consists of three modules. Through the coordinated operation of high - precision genetic modification and the activation signal, it achieves personalized customization and enables efficient industrial production. It ensures the stability of the production chain, significantly reduces off - target effects, and guarantees the specificity and safety of the system.

How the HSS enters the liver (the "Galactic Factory Cells") in the body from the outside is a crucial issue. To address this, we adopt bio - information carrier technology and use Nano Liposo Gleam Carrier Particles (NLGCP) as bio - information transport carriers for encapsulation. These carriers can encapsulate the organic (RNA) sequence bound to the gene "heavy elements". These carrier particles have high biocompatibility and targeting ability and can stably transmit core information within the body. We can administer the drug via intravenous injection, injecting the encapsulated core sequence into the body. Subsequently, the sequence and its encapsulation will reach the liver through the body's blood circulatory system and start to function after entering stem cells. The main function of our lipid nanoparticles is to ensure that the core sequence is not degraded by the body before

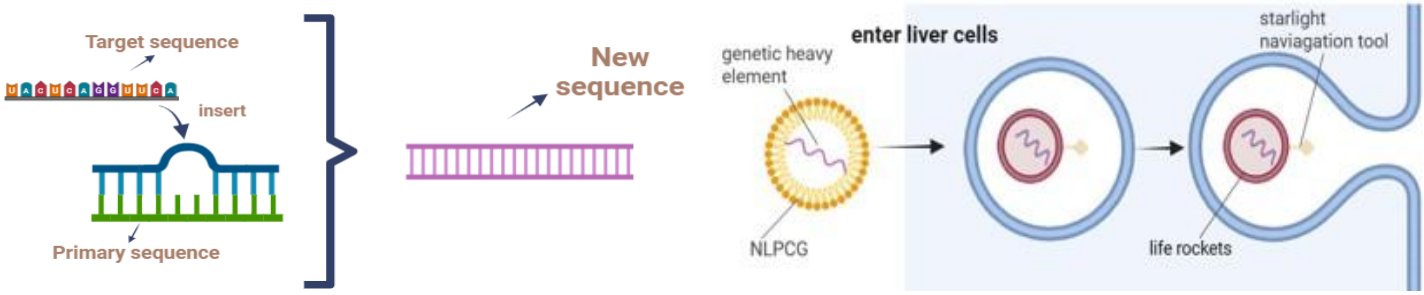

Figure 2. Genetic heavy elements enter liver cells by NLGCP

RESEARCH VERIFICATION:

To validate the effectiveness of the system, we first conducted in vitro functional verification using experimental cells, including HEK293T [15]and U87MG [16]cells.

In Vitro Experiments

HEK293T cells are a widely used cell line known for their ease of transfection and high expression characteristics, making them popular in transgenic research. [1]We introduced the complete system into HEK293T cells and assessed the silencing effect of the target protein after 24 hours. By analyzing the expression levels of the corresponding protein, we can confirm whether the system has functioned within the cells. In this experiment, HEK293T cells served both as the chassis cells and the target cells.

After co-culturing with HEK293T cells for 48 hours, we collected the cell culture supernatant and extracted specific exosomes through high-speed centrifugation. The exosomes were then resuspended in PBS to obtain purified and enriched specific exosomes. We utilized transmission electron microscopy to observe the physical characteristics (such as morphology and size) of these exosomes, confirming their normal secretion without changes in morphology or size.

U87MG cells are a human glioblastoma cell line commonly used in neuroscience and tumor research, providing important information regarding exosome responses. [2]Next, we co-cultured the collected specific exosomes with U87MG cells, adding the exosomes at a ratio of 1:10. After 48 hours of culture, we assessed the expression levels of the target protein in U87MG cells.

In Vivo Functional Studies

siRNA can effectively enter target cells through exosomes and regulate gene expression.[3] We first injected the system into normal mice, and 48 hours later, we extracted specific exosomes from the mice's plasma and purified them using density gradient centrifugation for further analysis. We examined whether the system could efficiently load siRNA into specific exosomes and detected the presence of siRNA within the exosomes.

To verify whether the exosomes could reach the brain, we used existing eGFP transgenic mice[17]. We designed siRNA targeting the brain that silences the eGFP gene, and by analyzing the fluorescence intensity in the mouse brain, we confirmed that the exosomes successfully penetrated the blood-brain barrier and interfered with the target.

Additionally, we injected the system into diseased mice. To verify whether the liver serves as the initial assembly site for the exosomes, we isolated primary liver cells from the mice at different time points and evaluated the siRNA levels extracted from the

culture medium of the primary liver cells. We also collected major organs from the mice, including the heart, kidneys, liver, and lungs, to analyze the siRNA concentrations in these organs, ensuring that the exosomes could reach these sites.

Verification of Therapeutic Effects

Ⅰ. Record the body weight of the mice daily.

Ⅱ. Analyze the expression of silenced proteins and mRNA in the animals.

Ⅲ. Conduct histological analysis of the affected tissues.

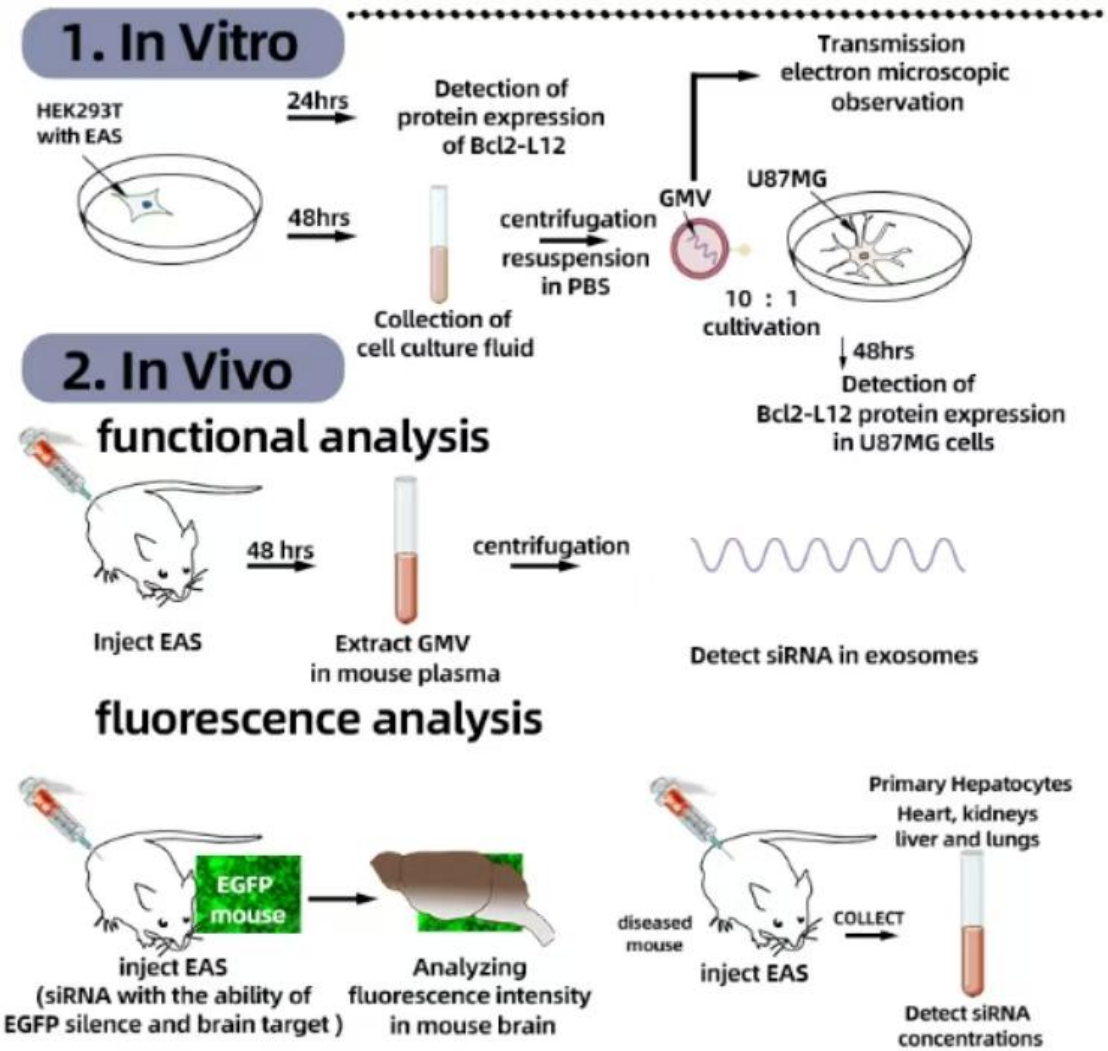

Figure 3. Verification in vitro and vivo

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