

The Effect of Engineering Methods on the Efficacy of Therapeutic Exosomes

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Abstract. Exosomes have been brought to attention in recent years with the rapid development of cell therapy and biotechnology. Exosomes are small extracellular vesicles that facilitate intercellular communication and play a crucial role in various physiological and pathological processes. They have been proven to be less harmful in comparison to other vector counterparts. With engineering methods, the efficacy of exosome therapy can be further amplified. This literature review aims to overlook the current state of knowledge on engineering methods used to enhance the efficacy of therapeutic exosomes. By incorporating knowledge from previous studies, this article attempts to discover a direct influence of engineering methods on the efficacy of exosome therapy in main three aspects: enhanced ligand-gated targeting, larger production yield, and a larger degree of freedom to deliver different mediums. The following literature will mainly focus on engineering advantages for treating different types of cancer.

Keywords: Exosome engineering, Cancer therapy, Exosome production yield, Scalability, Function enhancement.

1. Introduction

Exosomes are extracellular vesicles generated by all types of cells. In recent years, exosome has been widely recognized as carriers of biomarkers in extracellular space. Depending on the origins and distinct characteristics of exosomes, they can play significant roles in cell-to-cell communication and transportation of cellular cargo. While cell therapy has gained a lot of attention in recent years with the modern advancement of biotechnology and stem cell therapies. In gene therapy, various types of vectors (such as adenovirus and lentivirus) allow researchers to integrate genes or cellular components into the cell. In doing so, however, some vectors have been proven to bear toxicity or to potentially instigate rejection by the immune system [1] (Syyam, et al.). To cope with such deflection in various vectors, therapies using exosomes derived from the body itself are becoming increasingly popular. Therapies using exosome as vector not only minimizes the rejection by the immune system but also results in better targeting control. The major dilemma in the current development of exosome therapy becomes the lack of standardization in exosome isolation and analysis methods as well as the difficulty in obtaining exosomes in large production yields [2] (Doyle and Wang). With engineering methods like CRISPR, scientists are now able to modify cellular ligands or pathways such that the clinical usage of exosomes have even enhanced targeting and production yields. Previous studies have suggested several examples of engineering exosome to serve particular therapeutic purposes. For example, Lianxiang Huang and his colleagues created an “in situ dendritic cell vaccine” by loading immunogenic cell death factors into engineered breast cancer-derived exosomes [3] (Huang et al.). With many similar yet distinctive research made, this literature review attempts to outline and categorize studies about engineering methods on therapeutic exosomes. Overall, modern engineering methods promote the efficacy of therapeutic exosomes for treating cancers in mainly three aspects: enhanced targeting, enlarged production yields, and less hostility to the patient’s body.

2. Advantages of Engineering Exosomes

2.1. Enhanced Targeting

Since many present exosomes used for treating cancer engage with ligand-gated pathways, the targeting ligands can be engineered onto the surface of exosomes or onto cancer cells to enhance the delivery of therapeutic molecules. The enhanced targeting can be exemplified with the application of nano-bioconjugation and function enhancement.

Nie, Weidong, et al. (2019) [4] pointed how native exosomes fail to induce sufficient effects in vivo and simple act as drug delivery vehicles. In addition, the researchers synthesized nano-bioconjugates (azide-modified exosomes derived from M1 macrophages conjugated with dibenzocyclooctyne-modified antibodies of CD47 and SIRP α through PH-sensitive linkers) for cancer therapy. The synthesized nano-bioconjugates exhibit enhanced active targeting of tumors through the recognition between aCD47 and CD47 on the tumor cell surface. Moreover, the nano-bioconjugates can be cleaved in the acidic tumor micro environment to release aSIRP α and aCD47 to improve phagocytosis of macrophages.

2.2. Function enhancement

Kojima, Ryosuke, et al. (2018) [5] used genetic engineering to introduce a targeting peptide on the surface of exosomes, enhancing their specificity for cancer cells. The engineered exosomes, which were derived from dendritic cells and displayed a brain metastasis-targeting peptide, exhibited improved therapeutic efficacy in a mouse model of brain metastasis.

Shi, Sixiang et al. (2019) [6] presents one of the first instances of using in vivo positron emission tomography (PET) to noninvasively track exosomes that have been modified with polyethylene glycol (PEG) and radiolabeled with copper-64. The modification with PEG not only improved the behavior of the exosomes in the body and increased their accumulation in tumors, but also helped to decrease their premature removal by the liver, indicating potential for better therapeutic delivery effectiveness and safety in future research. Engineering Methods for Increasing Exosome Production:

The efficient production of exosomes is crucial for their use in cancer therapy. In the current phase of therapeutic exosome studies, the production of exosome from natural sources remains within a low amount. In recent studies, however, strategies have been employed to increase exosome production, including optimizing cell culture conditions and employing genetic or pharmacological manipulation.

2.3. Standardizing/optimizing cell culture conditions

Watson, Daniel C., et al. (2018) [7] showed that the use of a serum-free supplement led to a 3-fold increase in exosome yield compared to standard culture conditions, without altering the exosome size, morphology, or surface marker expression. Since the lack of standardization in exosome isolation and analysis was a major dilemma in previous studies, this optimization of cell culture conditions could contribute to large-scale exosome production for cancer therapy.

2.4. Genetic and pharmacological manipulation

Mendt, Mayela et al. (2018) [8] used genetic manipulation to generate mesenchymal stem cell-derived exosomes with enhanced therapeutic potential. The authors overexpressed the CD63 protein in parent cells, resulting in a 10-fold increase in exosome production. The overexpression of CD63 also led to enhanced exosome cargo loading and improved therapeutic efficacy in a mouse model of pancreatic cancer.

Debbi, Lior et al. (2022) [9] reviewed current methods of boosting exosome secretion. The authors discovered that the secretion of exosomes was increased up to two orders of magnitude by employing external inducers, molecular interference, physical signals, and environmental factors.

3. Engineering Methods for Modifying Exosome Content

Modifying the content of exosomes is crucial to enhance their therapeutic efficacy. The freedom to modify the content of exosomes also allows researchers to vary the effect and target of delivery when treating different diseases. This section provides insights into different approaches for modifying exosome cargo.

3.1. Genetic engineering of parent cells

O'Brien, Kevin, et al. (2020) [10] employed genetic engineering to produce exosomes for the targeted delivery of small interfering RNA (siRNA) to cancer cells. By overexpressing a specific siRNA in parent cells, the authors successfully generated exosomes carrying the siRNA, which effectively silenced the target gene in recipient cells both *in vitro* and *in vivo*.

Cheng, Zhe et al. [11] summarized the major exploration of the potential of modifying extracellular vesicles for cancer treatment, with focus on loading them with a variety of therapeutic agents such as nucleic acids, small molecules, and proteins. For example, researchers have utilized EVs to deliver nucleic acids, and have demonstrated that engineered EVs can enhance the cytotoxicity of drugs like doxorubicin, reducing its accumulation in the heart. The engineered exosomes, which were loaded with a tumor-suppressive miRNA, exhibited enhanced therapeutic efficacy in a mouse model of pancreatic cancer. Also, certain types of EVs, like exosomes, have been used as delivery vehicles for siRNAs and microRNAs (miRNAs) involved in RNA interference, which is being evaluated for targeting oncogenic mRNAs.

3.2. Post-isolation loading of therapeutic cargo

Kim, Min Sung, et al. (2017) [12] developed a method for engineering exosomes to enable efficient intracellular delivery of therapeutic agents. The authors utilized a membrane permeabilization technique to load exosomes with various cargos, including small molecules, proteins, and nucleic acids. The engineered exosomes demonstrated efficient delivery and release of cargo in recipient cells, both *in vitro* and *in vivo*.

Hao, Ming, et al. (2022) [13] stated that methods such as coincubation, genetic engineering, electroporation, ultrasound, and artificial synthesis of engineered stem cell exosomes endow engineered stem cell exosomes with the potential to cargo nucleic acids, proteins, and small molecules. These methods gave exosomes anti-inflammatory and cell proliferation regulatory abilities and eventually promoted the efficient soft tissue repair of patients after trauma.

4. Conclusion

The engineering methods discussed in this review highlight the potential of exosomes as versatile therapeutic agents for cancer therapy that can be tailored to specific applications. Enhancing targeting capabilities ensures that exosomes deliver their cargo to the desired cancer cells, minimizing off-target effects and maximizing therapeutic outcomes. Increasing exosome production allows for the generation of large quantities of exosomes for therapeutic applications, addressing the scalability challenge prominent in the present phase of exosome research. Lastly, modifying exosome content enables the loading of specific therapeutic molecules, including small molecules, proteins, and nucleic acids, improving the overall efficacy of exosome-based therapies for cancer treatment.

Despite the numerous advancements in engineering therapeutic exosomes for cancer therapy, several challenges remain. For instance, the scalability of exosome production is still a significant hurdle, as it often requires large-scale cell culture, which can be expensive and labor-intensive. Although many methods mentioned above can enlarge the production of exosomes with engineering methods, many of these methods remain costly and laborious. Developing cheaper and more accessible methods remain a necessity for the mass production and usage of exosome for clinical usage. Furthermore, although various approaches have been developed for modifying exosome content, achieving efficient loading of therapeutic molecules remains a challenge. Additionally,

ensuring the safety and biocompatibility of engineered exosomes is crucial for their clinical translation.

In addition to the three main aspects discussed earlier that outline the benefits of engineering methods on therapeutic exosomes, enhancing immune modulation and improving the stability and circulation time of therapeutic exosomes are also crucial for their effective use in cancer therapy. By engineering exosomes derived from immune cells or loading them with immune-stimulating molecules, researchers have successfully developed exosome-based cancer therapeutics with potent immunomodulatory properties. Moreover, modifying the surface of exosomes or incorporating protective agents can help to improve their stability and circulation time in the bloodstream, further enhancing their therapeutic potential.

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