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Exosomes: Role and Potential in Cancer and Treatment

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Abstract. Exosomes are very important and can also be used in cancer. Exosomes are a kind of natural nanometer sized vesicle, which is produced and secreted by nearly all kinds of eukaryotic cells through endosome way. They all have the role of messengers, carrying biochemical material such as proteins and lipids, and various forms of nucleic acids (mRNA, miRNA, circRNAs) and small molecules to transport biological chemicals back and forth for cell-to-cell communication but also play a role in physiology and pathology. Exosomes are produced by various kinds of cells such as MSCs,immune cells like lymphocyte,tumor cell and hepatic cell and so on.The different kind of cell determines the function of the exosomes. Like exosome originated from tumors will go to tumor environment but exosomes originated from stem cell has low toxicity and is easy to mix with other exosome from stem cells. We give a brief account on the role of exosome as diagnostic biomarker and drugs carrier and immune therapy regulator here: Problems about having enough production process, complex components involved in purifying process and not very effective target during translating to clinics are stated in the paper. It then went on to discuss how these problems would be overcome via engineering modifications.

Keywords: Exosomes, Tumor treatment, Targeted delivery, Surface ligand modification

1. Introduction

Exosome is a kind of small vesicle. The size range is about 30 - 150nm, and it comes out from endosome of eukaryote cells. Exosome's formation process starts when the early endosomes are matured and converted into MVBs. The MVB can be released into the outside through the plamsa membrane, or it is still in the cell for some other intracellular compartments. Exosome has lots of different bioactive molecules including some proteins (both cell-surface and heat shock protein), lipids (like phosphatidylcholine and cholesterol) ,nucleic acids (mRNA,micro RNA,circulating RNA) Exosomes are secreted by a variety of different cell types like mesenchymal stem cells, lymphocytes, cancer cells and so on [1]. Exosomes contain similar components of the cell since it is based off its parent cell so it plays a role in both normal biological functions and also with disease. As there is more research emerging on this topic, it is becoming clear that tumor-expressed exosomes (TDEs) are very useful in predicting the progression of a disease, being used as a target for treatment purposes and even being used to deliver drugs. The creation of exosomes takes place in 4 key stages: First is the formation of a depression in the Plasma membrane which forms an Early Endosome. The next stage is for Inward budding to take place inside the MVB, causing Intraluminal

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Vesicles. After that happens is when the Acidification of the MVB causes it to Mature. Then finally it is when the fully developed MVB fuses with the Plasma Membrane and Releases the Exosomes. And MVB's can go into the lysosome and be degraded so that won't be an exosome for release [2].

Exosome has very good potential as a drug delivery system because of the unique nature of its biology in tumor microenvironment (TME) [3]. The first main benefit of exosome as a drug delivery vehicle is that it has a natural membrane structure similar to those found in human cells and delivers exosome drugs to human body without our immune system recognising the drug, so little or no toxicity will be associated with exosome-based drugs. With similar biological and chemical composition, natural exosome and human cells share similar slow toxicity or complete compatibility with humans [4]. Exosome is an excellent carrier system because it has natural targeting properties, mediated by the presence of membrane proteins such as integrins and CD47 that bind to specific receptors on cancer cells [5, 6]; therefore, when an exosome hits a cancer cell, that binding helps it get into the TME and accumulate in cancer tissues. The third benefit of exosome is that its nanosized dimension allows for very good penetration capacity of exosomes across tissues. Furthermore, it is able to enters the outer or external cell structures (dense) of various types of tumors and penetrate into the inner (center) of cancer cells. This would likely increase the spread of the drug inside the single tumor. However, there are two main challenges: natural targeting accuracy and large amount of drug loaded on exosome. Therefore, it is important to do surface modification of exosome using specific peptides/antibody for the purpose of enhancing tumour targeting ability of exosomes. composite structure of exosomes may also be manipulated by using/exposing certain chemicals or chemically to increase drug load [7].

2. The advantages and problems of exosome drug carriers

Exosomes, as natural nanosized EVs, provide both advantages and unique challenges for drug delivery. The viability of Exosomes drug delivery vehicles is mainly that they are biocompatible with the human body and can bypass the immune system without any reaction. For instance, CD47 found on the Exosome binds with the SIRP Alpha receptor located on the Macrophage cell and signals the Macrophage cell not to attack them; thus, the time of systemically- circulating drug increases [5, 6]. This property is also responsible for homotypic targeting, i.e., the parent Tumour shows a tendency towards homing on its own derived Exosomes. The generation technology can be used to increase the targeting capability to deliver the drug to specific loci. Also, the lipid bilayer significantly protects the substances within the exosome; including nucleic acids, proteins, and small molecules like drugs, by preventing them from being broken down by enzymes [8]. In terms of stability, exosomes are much more stable than other traditional artificial carriers such as liposomal or polymeric nanoparticles; they exhibit a blood circulating half-life of approximately 12–24 h and they can easily cross physiological barriers [9]. The clinical application of exosome, however, still has many issues: One major issue is how to produce enough. The predominant means of recovering cell culture-grown exosomes continues to require isolation of the exosomes from culture supernatant, where methodologies such as ultracentrifugation and size exclusion chromatography are inefficient, expensive, and provide high levels of batch-to-batch variability [10]. It is very challenging to establish standards and obtain the regulatory agency's approval due to the varying sizes of exosomes, the protein content in them have, and the other differences in the contents of exosomes. Moreover, a priority is to raise the efficiency of drug loading since the efficiency of passive loading is less than 10% (usually the encapsulation), whereas active loading, which includes electroporation and sonication, increases the efficiency but is typically accompanied by cell membrane and cargo leakage [11, 12]. Last but not least, there are certain concerns regarding safety;

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the exosomes that tumors express have been proven to participate in tumor development and metastasis, thus not properly cleared or modified, they may pose a risk of oncogenic introduction through the transfer of tumor supportive material. So moving forward into the future, it will be critical to develop a way of manufacturing exosome facilities which is scalable, as well as repeatable. We also need to develop a process to load drugs into exosomes with minimal damage to their structure and to demonstrate that we can safely use exosomes in humans before we can start using them for treating people.

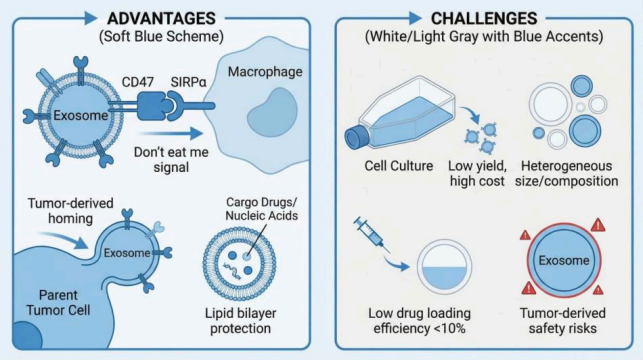

Figure 1. Advantages &challenges

3. Exosome surface modification techniques

In addition to exosome technology, surface modification of exosomes has become a critical component in achieving targeted delivery and has developed tremendously over the past several years [13]. In terms of Genetic Engineering, the research done by Alvarez- Evriti was extremely significant as they were able to pass exosomes through the Blood Brain Barrier successfully by fusing the neuron targeting Peptide RVG to the Extracellular domain of the Exosome membrane protein Lamp2b and pass it through [14]. This is true in Alzheimers model showing that it is possible to deliver siRNA into the central nervous system. But it also has some restrictions, like fluctuating amounts of the fusion protein being made, possibly changing the usual jobs of real exosome outer proteins, and different degrees of getting changed based on which other cells are there. So in order to achieve this, they went about making changes on the linker area for both what sort of flexibility was there along with being more responsive when it came to proteases around 3x faster. This resulted in how much target peptide got displayed per membrane surface. Moreover they used their own promoter to make the expression, which greatly reduced interference with the native exosome production [15]. Regarding the chemical modifications, it is notable that the Smyth group created a DBCO – azide coupling system based on click chemistry. They put some azide groups onto the exosome membrane phospho- lipid, then did a good and exact addition of cRGD cyclic peptide conjugation (60 - 70%) In the breast cancer model, it is noted that the modified exosomes can specifically recognize tumors over expressed avb3 integrin which in turn increase the doxorubicin's tumor accumultion efficency by 4.2 fold [16]. But still this method is having issues like it binds in a nonspecific way to serum proteins as well and can also damage the membrane when coupled. In a more recent work, it is mentioned that this too was faced and resolved by improvement through release upon tumor micro environment response due to the introduction of pHsensitive hydrazone bond linkers. Regarding concern membrane fusion technology, Kooijman et.al used a new kind of anchoring which is Hydrophobic Anchoring (PDP-PE) molecule so that they could display EGFR Nanobody directly on Exosome Surface [17]. Regarding the glioblastoma model, they have shown a

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better capability of going from the blood stream into your brain and then hit the tumor as well [18]. But it is usually limited because its unstable when you stick it in the membrane, and also it might mess with the fluidity of the membrane. For addressing above challenge, group from chen had come-up with some special form of an Amphiphilic Peptide bridging system which act as artificual transcemnber domain and showed up to 40 % increase in anchoring strengh however membrande protein still maintained their latera mobility [19].

Although it has become evident that single-targeted approaches do not work and are ineffective, the Multi-Target Cooperative Model provides a new opportunity. Wei produces an exosome type with an attached CD3-targeted peptide along with a her2 nanobody, which will offer additional cytolytic weapons when combined with the human T cell(the study used mice). The combination of these two made CAR-T cells five times more efficient at killing cancer cells than before, and they almost eliminated Cytokine Release Syndrome [20]. The issues associated with multi-target therapies are related to the complex production process, complicated QA, and high chance of antigenicity.

With regard to additional research related to the surface modifications of exosomes, we have identified three areas that should be addressed. The first area is the development of a process that uses computer-based intelligence for altering the surface of an exosome so that we can develop exosomes that possess a known level of surface modification. The second important element will involve utilizing a system for multiple-assessment of engineered exosomes to enable researchers to determine what an engineered exosome can do for them. The third thing that we are trying to do is find ways to make modifications that are responsive to light and magnetic fields. Along with finding new methods of modifying exosomes, another major area that needs to be addressed is that many of these new technologies (such as the creation of smart delivery systems that are activated once they reach their intended site) have a lot of potential; however, we have not yet determined what their long-term metabolic fate or safety profiles is contained within the body (i.e., after the application of engineered modifications). Therefore, thorough and long term research needs to be conducted in order to determine the biodistribution, catabolic pathways, and the possibility of non-purpose or aggregate toxicities. Clinical translation may proceed with confidence only if we possess this basic level of knowledge regarding the mechanism of action. Exosomes appear to be effective delivery vehicles, but, because of their inherent biocompatibility, and because they come from cells, they also appear to be a promising biologic of the next generation.

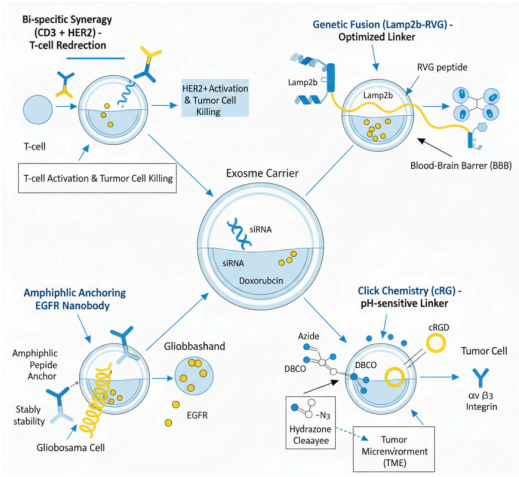

Figure 2. Surface modification methods

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4. Application of exosomes, targeted delivery of sRNA and anti -cancer drugs

As a form of nature's exosome, it has certain advantages to deliver anticancer drugs and sRNAs [21]: This is how the research path goes in this field, from delivering single type of chemo medicine and later on careful transportation of gene silencers to combined effort with other form of treatment such as immune. Concerning the realm of chemotherapeutic drug delivery, at first it was mostly focused on using exosomes to improve the pharmacokinetics of traditional drugs [22]. It has been found that hydrophobic substances such as doxorubicin and paclitaxel could also successfully be loaded into exosomes with methods like electroporation, sonication, co-incubation etc. Qiao et al. in an early work proved that homologous exosome which were derived from tumor cells can obviously enhance the amount of drugs like doxorubicin which was encapsulated up to 15.8% as well as EPR effect and homologous targeting [23]. As a result, there was about 3.7 times more drug accumulated at tumor sites and the cardiotoxicity was greatly decreased [4]: From a kinetic perspective, we also studied the excretion as follows: it is shown that the intelligent release behavior in the acidic tumor microenvironment (pH 6.5) exhibited by this drug delivery system can be observed; after 24h, about 78% of the drug was released compared to only ~35% under normal physiological conditions [24]. It also is a very important finding that will lay good groundwork to use exosomes in clinic, carry drug.

Due to the success of exosomes as vehicles for the delivery of chemotherapy drugs, researchers have begun investigating the use of exosomes to deliver gene therapy drugs (i.e., small interfering RNA (siRNA)). Exosomes protect nucleic acid-based therapeutics from degradation by nucleases that are present in serum and selectively deliver nucleic acid-based therapeutics to specific cells. In a recent study, researchers proposed the use of exosomes to deliver siRNA targeted against the mutated KrasG12D gene. This approach was able to silence 72% of KrasG12D oncogene expression in pancreatic cancer cells, and it resulted in a 61% decrease in tumour volume [22]. The use of genetic engineering allowed us to express the GE11 peptide on the exosomal surface, thus providing a means to more effectively target cells that expressed a high concentration of epidermal growth factor receptor on their surfaces [25]. The next step in the process involved silencing MYC oncogene expression in a liver cancer model. They subsequently used cholesterol molecules to encase exosome, substantially increased the retention time of siRNA in the mouse model. This approach represents a major shift in how exosomes can be used, from their traditional role in delivering drugs during chemotherapy to their potential use in targeted gene delivery.

Recently, researchers have begun to think about exosomes as "trucks," and in fact, they are currently working on ways to use them for immunotherapies. In experiments, exosomes containing PAK4 siRNA were administered in combination with an anti-PD-1 antibody in treating the TME in mice. For melanoma, the primary difficulty with treatment is not just to inhibit oncogene expression, but also to improve the efficiency of CD8+ cytotoxic T lymphocytes (CTLs) in carrying out antitumor activities. The proportion of CD8+ (cytolytic) T cells (i.e., a fundamental component of the anti-tumor response) was increased to 45% in relation to the previous level (38%), and in comparison to their virtually completely depleted state (~100%) previously [26]. This improvement in infiltration was beyond that of the ~45% levels achieved with either monotherapy regimens individually. The combined exosome and siRNA immunotherapy operates through co-delivery of siRNA via exosomes, resulting in two types of immunomodulation; first, it has an effect on intrinsic immune signalling pathways in the tumor, and second, it also relieves the suppression of the T-cell response, allowing for clearance of the tumor by the T-cells. This paradigm can be considered a state-of-the-art signal of exosome-based therapeutics research, as it focuses on the transition from single modality to multifaceted therapeutics. However, there still exist many fundamental

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translational barriers. Encapsulation efficiency of hydrophilic products was to poor. This required the development of advanced load protocols, among other things. The production and safety rules of exosomes were very different. In addition, there should be thorough investigation of the safety profile of combination regimens. Therefore, future research should concentrate on achieving three interrelated objectives: (1) developing next-generation cargo loading systems; (2) establishing standardized and robust quality control measures; and (3) utilizing an integrated multi-omics approach to identify the exact molecular mechanisms behind exosome-mediated antitumor responses, for the purpose of expediting clinical development and application. Exosomes have far evolved from their original "passive" role as a delivery vehicle to a new role as an active device with multiple therapeutic functions. The continued development of exosomes is providing an expanding range of new approaches to biological systems and providing the potential for many new opportunities for therapeutic intervention through the use of exosomes as a source of therapeutics.

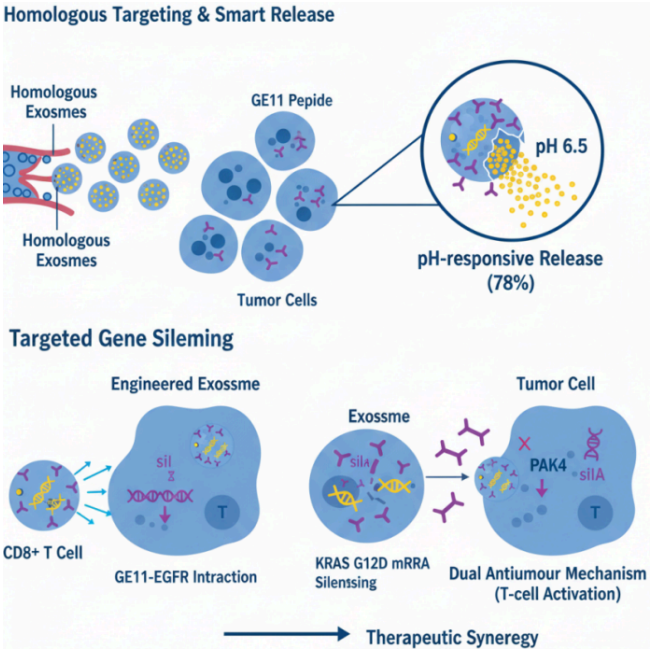

Figure 3. Targeted delivery & smart release

5. Clinical transformation and the outlook

Exosomes have rapidly developed into a new type drug delivery system, and have had several clinical breakthroughs in the last few years, with many more in the advanced trial stage. One example of this is clinical trial NCT03608631, which is using MSC-derived exosomes to deliver a therapeutic KRAS G12D siRNA to patients with advanced pancreatic cancer [27]. There are some examples of exosome therapeutics in clinical trials having demonstrated exemplary safety profiles, and additional have shown their potential to be used for the delivery of therapeutics to tumours. Regarding trial NCT01294072, paclitaxel is loaded into an exosome derived from a tumour cell that has a more favourable pharmacokinetic profile and is a much more well behaved exosome in nature [28]. A group of 33 patients with non-small cell lung cancer was then injected with these drugs [22]. It has been demonstrated in this type of research that exosomes can be used as drug delivery systems. However, major issue continue to persist when manufacturing exosomes on an industrial scale to produce large amounts of exosomes, and producing exosomes with acceptable quality.

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Microfluidics have been tested in overcoming the aforementioned challenges by offering a highly effective method of isolating and purifying exosome populations. Engineered cell factories will also create higher exosome yields that are standardized by overexpressing Rab27a or Alix [29]. After this is accomplished, exosome yields will be higher overall and by batch; therefore, it is essential for us to develop a production system that complies with GMP and protocols for testing long-term toxicity as exosome-based therapeutics have sufficient proof for quality assurance and safety when utilized within clinics.

In the future, neoantigen-loaded exosome personalised vaccines may be the most promising avenue of cancer immunotherapy research. Incorporating both the patient-specific neoantigen along with immunostimulatory adjuvants into exosomes would lead to generation of an entirely new and very specific type of anti-tumour immune response directed towards your body's tumour. Studies conducted in the past have also demonstrated that the use of these exosome vaccines, when used in tandem with immune checkpoint blockade, resulted in complete regression of tumours and generation of durable immunological memory.

Most importantly, the multi-omic guidance has already pushed Exosome Technology to a higher level of precision and sophistication. It helps me use proteomics to find exosome membrane connections (protein) and use ML to help make the best target compound choices and help find the right linker. The sequencing of our tumor cells at different locations using single-cell sequencing technology is giving us a much greater ability to actually see what a molecule has the potential to do for you (exmolecule) [30]. Once we combine all these technologies and obtain better outcomes and solutions, Exosomes can advance from a research model to being a treatment option and, therefore, the very next generation of personalized cancer treatments.

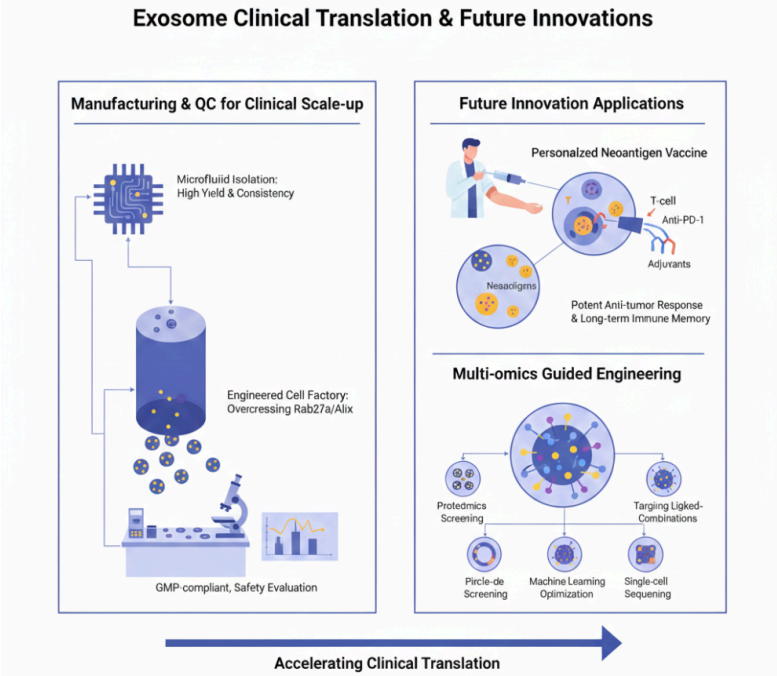

Figure 4. Translation, innovation in the future

6. Conclusion

Exosomes have been acknowledged as endogenously sourced nanocarriers inside humans, and more and more people believe it will be an important part of creating future medicines. It is because they

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are having lessimmunogenic and good biocompatible with natural targeting ability. Exosome has a great advantage that it can greatly increase the amount of drugs accumulated in tumor-specific tissue but does not cause immune clearance or toxicity in the body by using different engineering methods. And there is genetically engineered target of peptides, chemicals or conjugates that may respond upon stimulus as well asmembrane protein targeted anchorings with something powerful all together when the therapeutic moment has come

However, a wide range of issues have contributed to the delay experienced in transitioning the use of Exosome-Based Systems from basic research to clinical applications. Resolving these issues requires more in-depth collaborative efforts across multiple disciplines, including Material Science, Nanomedicine, Cancer Biology, and Artificial Intelligence, for instance, Materials Science is developing new, bioinspired materials for modification, along with innovative, stimuli-responsive components. Cancer Biology will provide accurate, innovative characterization of tumor microenvironment to give us an understanding of how to design our exosomes. Biomanufacturing and microfluidics potentially can solve some of the large-scale production problems of exosomes. In addition to that, I'm also anticipating that I will be able to uniquely modify and tailor the exosomes for you using multi-omics data through AI. With the way things are going to the future and the development of the technology of the multimodal functional integration, we believe the exosome will not be the typical type of drug used for the diseases but will be converted to an integrated multifunctional theranostic platform, such as molecular diagnostic, targeted drug delivery, immune modulation and monitoring of the therapeutic response. Complex systems will transform all oncology and neurodisease, tissue regeneration, and anti-infective drugs. It will radically redefine the personalized medicine concept in all areas.

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