ABSTRACT

Cancer remains one of the most challenging health issues worldwide, despite considerable progress in oncology research. Immunotherapy, which leverages the body's own immune system to fight cancer, has emerged as one of the most promising strategies for effective cancer treatment. Among immune cells, Natural Killer (NK) cells have drawn special attention due to their inherent capacity to identify and eliminate tumor cells directly, without prior sensitization. However, therapies based directly on NK cells have several limitations, such as challenges in storage, limited penetration into solid tumors, risk of graft rejection, and potential for severe side effects.

Recently, a promising alternative has emerged: extracellular vesicles (EVs) derived from NK cells. EVs are tiny, membrane-enclosed particles naturally secreted by nearly all cell types, including immune cells. These nanoparticles serve as messengers, transferring proteins, lipids, and genetic material between cells. NK cell-derived EVs (NK-EVs) retain many advantages of their parental cells, such as selective recognition and killing of cancer cells, while offering additional benefits. Importantly, NK-EVs have lower immunogenicity, enhanced stability, can be more easily stored and transported, and exhibit better penetration into solid tumor tissues compared to whole NK cells. These unique features make them an attractive platform for developing safer and more effective cancer treatments. Despite this potential, the clinical use of NK-EVs faces significant limitations, including rapid clearance from the bloodstream, lack of precise tumor targeting, and poor ability to effectively deliver therapeutic molecules into cancer cells. To address these limitations, the present project proposes a comprehensive strategy involving genetic and chemical modifications of NK-EVs, with the aim of greatly enhancing their therapeutic effectiveness against cancer.

The project aims first to engineer NK cells to produce EVs loaded with increased amounts of cancer-killing cargo. Subsequently, NK-EVs will be modified to include specialized proteins on their surfaces, enhancing their ability to deliver therapeutic content directly into the interior of cancer cells. Additionally, specific targeting molecules – such as antibodies and peptides recognizing common tumor receptors (EGFR or FGFR), frequently overexpressed in breast, lung, and pancreatic cancers, will be introduced to enable precise tumor targeting. Finally, NK-EVs will undergo surface modifications to extend their circulation time, including the addition of protective coatings and the incorporation of “don’t eat me” signals to prevent premature clearance by the immune system. Engineered EVs will undergo rigorous validation in laboratory experiments to ensure they exhibit enhanced tumor targeting, efficient cytoplasmic cargo delivery, and effective killing of cancer cells. These properties will subsequently be confirmed in animal tumor models – first in immunocompetent mice with tumors engineered to express human EGFR or FGFR, and subsequently validated in clinically relevant human tumor xenograft models in immunodeficient mice. These comprehensive studies will demonstrate therapeutic proof-of-concept, biodistribution, safety, and efficacy in realistic cancer scenarios. Finally, recognizing the potential of combination therapies in oncology, the project will evaluate the synergistic effects of engineered NK-EVs with clinically established immune checkpoint inhibitors (such as anti-PD-1 antibodies). This combination therapy approach aims to enhance the body's own immune response, potentially leading to complete tumor regression and durable anti-cancer immunity.

This project addresses a crucial medical need for more effective, selective, and safer cancer treatments. By overcoming current limitations through strategic NK-EV modifications, this research could significantly advance cancer immunotherapy, offering new therapeutic options for difficult-to-treat solid tumors and substantially improving patient outcomes.