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Li-Fraumeni Syndrome in Breast Cancer: Development of Theranostic Probes on Variation of P53

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Abstract

Li-Fraumeni Syndrome (LFS) is a hereditary condition characterized by a high predisposition to various cancers due to mutations in the p53 protein. Effectively targeting and treating this syndrome requires early detection and personalized treatment according to one's specific nucleotide sequence. With the use of various forms of theranostics, LFS can be treated, increasing life expectancy; however, there is no absolute cure that could immediately end this syndrome. This paper delves into three different theranostic approaches: Gendicine (rAd-p53), Phesgo, and gold nanoshell poly(D, L-lactic-co-glycolic acid) nanoparticles (PLGA NPs). While each approach has limitations, PLGA nanoparticles exhibit advantages that outweigh their drawbacks, making it an attractive solution. This proposal will describe improvements to PLGA nanoparticles, which are a promising area of research for the development of new LFS treatments.

1. Introduction

Li-Fraumeni Syndrome (LFS), first described in 1969 by Frederick Li and Joseph Fraumeni, is an inherited genetic condition that makes individuals predisposed to specific types of cancer. LFS is associated with high risks for a diverse spectrum of childhood- and adult-onset malignancies; survivors are at increased risk for multiple primary cancers.

The syndrome is most commonly caused by mutations in the TP53 gene, which encodes the tumor suppressor protein p53. Germline mutations in the TP53 tumor suppressor gene are identified in 70-90% of families meeting the classic criteria of LFS. These genes are usually inherited in an autosomal dominant manner from a parent with the condition, which implies the possibility that the cancer risk can be passed down from generation to generation.

The p53 protein plays a crucial role in cellular response to various stimuli, including DNA damage, nutrient deprivation, hypoxia, and hyperproliferative signals. It attenuates cell proliferation, thus preventing tumor formation. Germline mutations in p53 occur in a high

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proportion of individuals with the LFS, which confers an increased risk of five types of cancer: adrenocortical carcinomas, central nervous system tumors, soft-tissue sarcomas, osteosarcomas, and breast cancer. These cancers occur in age-related phases: adrenocortical carcinomas during the childhood phase (age 0-15 years), osteosarcomas during the childhood-to-young adulthood transition phase, and breast cancer and soft-tissue sarcomas predominantly during the early adulthood phase (age 16-50).

Among women with germline TP53 mutations, breast cancer is the most common tumor. Female TP53 variant carriers have an excessively high risk of developing breast cancer before 31. By using information mainly from familial cases, the cumulative cancer incidence of germline disease-causing TP53 variants was estimated to be 73-100% by age 70, with risks close to 100% in women. Moreover, the risk of breast cancer in women with LFS is approximately 49% by age 60, with significant risk before age 40.

Early diagnosis and effective treatment of cancers are crucial for a beneficial prognosis; therefore, many novel technologies are being developed for the early detection of primary tumors for effective breast cancer management. Furthermore, for the development of more specific, individualized therapies not only for cancers but for various diseases, the term “theranostics” emerged in clinics. Theranostics is a combination of therapeutic and diagnostic agents within a single platform. In recent studies, new technologies such as nanotechnology have offered an opportunity to draw diagnosis and therapy closer.

Ongoing research on TP53 and LFS has developed various diagnostic probes to detect TP53 mutations and LFS from a person’s genome. However, there are no cures for LFS and breast cancer that are caused by LFS currently available. Despite this situation, numerous scientific institutions are developing theranostic and therapeutic agents. This proposal will focus on numerous approaches that could treat Li-Fraumeni Syndrome and potentially improve the most suitable approach.

2. Gendicine: rAd-p53

Gendicine, also known as rAd-p53 or a recombinant adenovirus gene therapy, is an engineered adenoviral vector directly transporting the genetic material to a specific cell. Inside this vector exists a wild-type p53 gene; by replacing the harmful and viral genes with functional p53, the virus will effectively navigate toward the targeted cells, in this case, cells with malfunctioning p53 genes. When this treatment is administered to a patient through an intra-tumoral injection, ultimately, the capsid will enter directly into the tumor tissue and implement the wild-type genome, expressing functional p53 protein and inhibiting the development of the tumor. Gendicine, therefore, renders the cell to regain its ability to regulate the cell cycle or signal apoptosis, preventing the development and distribution of cancer in different parts of the patient's body.

Gendicine stands out with its precision, offering a tailored treatment engineered according to the patient's nucleotide sequence. This approach, housed within the circular-shaped membrane capsid, boasts a high gene transfer efficiency and close to 100% transduction efficiency (Qi et al., 2023). The tailored method not only enhances the safety and controllability of the variable but also reduces adverse side effects such as inflammation, fever, and other immune system-related symptoms, providing a reassuring sense of efficacy.

Another compelling reason Gendicine is a preferred choice in gene therapy is its potential to work in synergy with conventional therapies. When combined with chemotherapy, radiotherapy, and biologically targeted therapy, rAd-p53 can enhance the effectiveness of these

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traditional cancer treatments. This is promising because Gendicine is not just a standalone solution but a valuable addition to the arsenal against cancer.

At first glance, these plausible aspects of recombinant human adenovirus gene therapy seem to be the ultimate solution, but limitations exist. One critical point is that cells can gain resistance to this type of therapy. The cells could gain resistance for numerous reasons. They would produce innate immune responses since they would recognize the therapy as a viral attack, as this therapy uses a bio-vector based on adenovirus. The innate immune system would recognize adenoviruses and activate cytokines, natural killer cells, and other guardian components in the cell. In addition to the inherent characteristics of the immune system, it may also adapt to the virus even though the viral aspects of the cell are removed. This includes the production of neutralizing antibodies that bind to the virus and prevent it from infecting neighboring cells, ultimately killing the adenoviral proteins.

Another way the immune system of the cell prevents the adenoviral vector from coming in is by downregulating the receptors and increasing the endosomal tensions. Adenoviruses enter the cell by binding to specific receptors, often called coxsackievirus and adenovirus receptor CAR. Likewise, adenoviral vectors pursue the same pathway. The cells can downregulate these receptors, reducing the virus entry and binding rate. Even if the virus enters the cell by passing through the membrane, the main door, it still needs to pass the next door, called an endosome, a membrane organelle encompassing substances that have gone through endocytosis. Cells can enhance endosomal retention or degradation pathways, preventing the virus from delivering its genetic material. The factors above and countless others exist regarding how cells could gain resistance, blocking the use of rAd-p53.

The periodic repetition of this treatment is another quarrel oncologists and biochemists are fighting for. Adenoviral vectors don't integrate directly into the host genome; therefore, the wild-type p53 cannot be replicated by the cell's own will and only produces "transient transgene expression" (Kajon et al., 2019). Moreover, to maintain an optimal p-53 concentration within the tumor cell to prevent p53-MDM2 interaction and dilution of the concentration in the blood vessels, direct injection of wild-type p-53 is required for better efficacy. However, looking at the problem addressed in the paragraphs above, we can see a dilemma with this treatment: this therapy requires the scientists to inject multiple times over time for better efficacy; however, an overdose of this approach would enable the cells to gain resistance over some time.

When it comes to treating breast cancer in Li-Fraumeni Syndrome, the potential of rAd-p53 is reassuring. Once injected directly into the cancer cells, the wild-type p53 gene is transcribed and translated to produce a functional p53 protein. This protein enables the cell to perform its normal tumor-suppressing functions, inducing the expression of p21, a cyclin-dependent kinase inhibitor. This induction leads to cell cycle arrest at the G1 phase, a crucial step in preventing the proliferation and distribution of cancer cells from the breast to other major organs in the patient's body.

Considering the inadequate number of case studies and the lack of research conducted on the use of recombinant human adenovirus gene therapy, specifically on breast cancer, this treatment is unsuitable for treating Li-Fraumeni Syndrome at the moment. Although this therapeutic agent stands as a valid treatment for reactivating p53 protein, better alternatives exist specifically for breast cancer in Li-Fraumeni Syndrome.

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3. Phesgo

Phesgo also known as pertuzumab, trastuzumab, and hyaluronidase, is a drug used to treat patients with early-stage HER-2 positive breast cancer. HER2-positive breast cancer is characterized by the overexpression of the human epidermal growth factor receptor 2 (HER2) protein. The drug is a fixed-dose combination, meaning that it is a combination of other drugs (pertuzumab, trastuzumab, and hyaluronidase–zzxf). It is delivered through a subcutaneous injection in the thigh. The side effects of the Phesgo include anemia, nausea, diarrhea, and hair loss

The components pertuzumab and trastuzumab are monoclonal antibodies, while hyaluronidase–zzxf is an enzyme that helps the body absorb the two former drugs. Pertuzumab and trastuzumab both target the HER2 protein and inhibit the growth of cancer cells. Pertuzumab works by inhibiting the activation downstream of cell-signaling by inhibiting HER2 dimerization and activation. More specifically, it inhibits receptor dimerization, which then inhibits receptor phosphorylation, and the activation of downstream cell signaling.

On the other hand, trastuzumab's mechanism is binding to the HER2 domain IV and triggering antibody-dependent cell-mediated cytotoxicity (ADCC), where the body's immune system and white blood cells kill the targeted cancer cell. However, this is extracellular action of triggering ADCC is suspected not to be the only mechanism. Evidence shows that it is effective in fighting cancer cells through intracellular mechanisms by inhibiting of HER2-mediate cell signaling, inhibition of HER2 cleavage, and angiogenesis, and finally, inhibition of DNA damage repair. However, these intracellular mechanisms are supported by limited data and are not as well supported as the data supporting trastuzumab's role in ADCC.

There is also a synergistic effect between the two drugs, pertuzumab and trastuzumab. Currently, in the literature, three significant mechanisms are proposed for this synergy. The first is that the two antibodies function differently and have different mechanisms for targeting and affecting cancer cells. Pertuzumab works by inhibiting the signaling pathways that help cell proliferation and survival, while trastuzumab works by triggering ADCC. These two approaches complement each other and are more effective than simply using only one of the drugs at a higher dose. Experiments have verified these results. The second proposed synergistic mechanism is the composition-independent inhibitory effects of the two antibodies in a wide range of HER2/HER3 compositions. Finally, the third proposed synergistic mechanism is cooperative interactions between the antibodies. The latter two proposed mechanisms have not been experimentally validated but were supported with computational methods.

4. Gold nanoshelled poly(D,L-lactic-co-glycolic acid) nanoparticles (PLGA NPs) carrying Anti-P53 antibody

5.1. Current state of Au@PGLA-NPs with Anti-P53 Antibody

In recent studies, gold nanoparticles (GNPs) have been suggested as a unique ultrasound/CT/MRI contrast agent. GNPs demonstrate excellent size and shape control for ample permeability, easy synthesis, and functionalization with self-assembled monolayers (SAMs) that present diagnostic and radiotherapeutic capabilities (Gao et al., 2021). Therefore,

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various biomedical fields have adopted this technology, exhibiting tremendous potential as a basis for clinical application. (Figure 1)

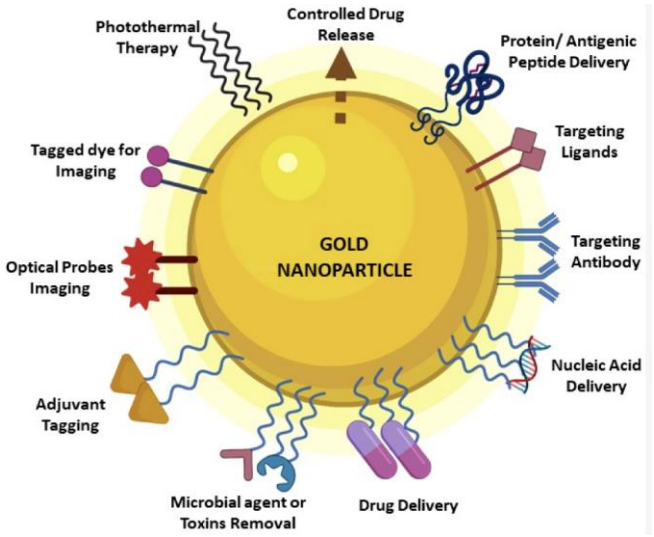

Figure 1

Representation of various potential uses of GNPs in the biomedical field. In theranostic applications, optical probing, photothermal therapy, and targeting antibodies are noteworthy features of GNPs. Adapted from 'Efficacy and Immune Response Elicited by Gold Nanoparticle-Based Nanovaccines against Infectious Diseases,' by Sengupta, A., Azharuddin, M., & Hinkula, J. (2022). Vaccines, 10(4), 505.

Upon Breast Cancer, not enough research has been done. In the latest research, the Gold-Plated poly (D, L-lactic-co-glycolic acid) nanoparticles (PLGA NP) have been applied in theranostics targeting of near-infrared (NIR) photothermal therapy (PTT). Composed of lipids, proteins, and polymers, PLGA is biodegradable and biocompatible (Xu et al., 2017).

Their diagnostic schematics focused on the characteristics of Gold (Au). Applied with Fluorescein isothiocyanate (FITC), Au nanoparticles revealed bright green fluorescence when conjugated with MCF-7s. The anti-P53 antibody attachment further suggested a high specificity of its binding to tumor cells (MCF-7 genes), according to the results drawn by the laser scanning confocal microscope (LCSM) and flow cytometer (FCM). In ultrasound imaging, the signaling of Au nanoparticles was concentration-dependent but demonstrated to be a suitable contrasting agent even under low concentration. Furthermore, Au nanoparticles carried a maximum absorption peak at 522 nm around a range of 500 to 900 nm, allowing it to become a photo absorber for NIR photothermal ablation therapy (Xu, 2017).

Au Nanoparticle in Photothermal therapy was effective, exhibiting excellent optical and electronic properties. Further, when testing the cell viabilities via MTT assay, PLGA NPs presented no impact on the cell itself. However, under irradiation of NIR laser, less than 20% of the MCF-7 cells remained viable. In vitro conditions, the biocompatibility of PLGA NPs presented minimal cytotoxicity, ensuring safety for ultrasound imaging and clinical cancer therapeutics (Xu, 2017).

However, the limitations are present in such a theranostic approach. In diagnostics, the efficacy of ultrasound is restricted by individual breast density and is highly user-dependent (Rebolj et al., 2018). Relatively, ultrasounds often cannot detect microcalcifications and minor

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abnormalities in tissues, such as ductal carcinoma in situ, which are crucial in breast cancer diagnostics (Izumori et al., 2010). Conventional PTT requires sub-50 degrees Celsius of heat in therapeutics, which causes collateral damage. While there have not been any vivo tests of PLGA NPs, potential side effects must be the focal point in evaluating the effects of applying PTT in current research (Gao et al., 2021).

Moreover, the current sizing of Au Nanoparticles imposes diverse problems as well. Though still serving sufficient time for ultrasonography beyond 8 minutes for prolonged evaluation of the pathogenesis, the current sizing of Au@PLGA NPs fails to remain in our system for an extended period (Xu, 2017). Additionally, accumulating Au@PLGA NPs may present unknown cytotoxicity over a prolonged period. Therefore, a solution must be found to resolve the problems of permeability, clearance, and circulation period. The current state of Au@PLGA-NPs lags in various manners, necessitating overall improvements for practical application.

5. Proposal

The evaluation of Phesgo, Gendicine, and Au@PLGA-NPs suggests restrictive application, short efficacy, and ineffective theranostics. However, despite the diverse array of limitations, we concluded that Au nanoparticles still possess the most significant potential once the problems are addressed, which would include tangible solutions that can effectively resolve its drawbacks. By applying magnetic resonance imaging (MRI), 2nm NP sizing, sheddable PEG (H−(O−CH2−CH2)n−OH) synthesis, and Mn+2 induced indocyanine Green Nano coordination polymers (Mn-ICG@pHis-PEG), the current limitations of Au@PLGA-NP can be solved.

MRI eliminates the user-dependent maneuverability. While MR imaging requires highly skilled technical support in acquiring and interpreting images, the absence of a sensitive transducer option will allow MRI probes to produce optimal images. Especially in the case of breast cancer, MRI provides high-resolution imaging around the soft tissues in visualizing subtle anatomical details in breast cancer (Morris, 2002). Conclusively, MRI suggests high sensitivity in tumor detection, treatment response monitoring, and evaluation of Axillary Lymph Nodes, which are crucial for therapeutic analysis (Kuijs, 2015). Despite the conventional price and set limitations of its frequent application, MRI is a superior imaging agent over ultrasound in breast cancer diagnostics.

In molecular structures, synthesizing a sheddable PEG chain of sub 2nm Au NP can significantly enhance the permeability and circulation of the nanoparticles. (Figure 2)

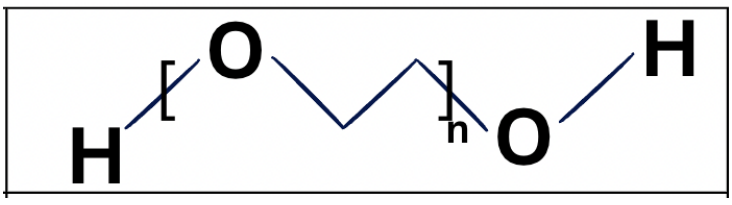

Figure 2

Molecular structure of Polyethylene glycol chain(PEG)(H−(O−CH2−CH2)n−OH)

According to Venkatachalam and Rennke (1978), the epithelium lining of the kidney contains filtration slits of 4-6 nm in width. For adequate circulation and clearance through urine, GNPs

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smaller than this filtration—ideally 2nm considering clearance through major organs, including lymph nodes and skin—must be created (Qui et al., 2012). Accordingly, the application of PEG can also aid with these attributes. Highly hydrophilic, PEGylation can increase the solubility of Au@PLGA NPs, enhancing the dispersibility in our circulatory system. Consequently, PEGylation reduces immunogenicity by its shielding effect, increasing the circulation time in our body.

The synthesis of sheddibility could minimize the cytotoxicity for various reasons. First, non-shreddable were found to create anti-PEG antibodies over time, inhibiting the subsequent doses to experience poor circulation (Yang et al., 2015). Second, the prolonged presence of PEG may present binding interference with the p53 antibody, reducing its specificity and efficacy of targeting (Li et al., 2010). Lastly, this may present a chronic presence in our system, where the NPs never end up leaving our system, which may present unknown effects on our biological system over extended periods (Yang, 2015). Therefore, the PEG chain on Au@PLGA NPs must be sheddable to minimize its cytotoxicity.

Further, applying complementary nanoparticles, Mn-ICG@pHis-PEG, could significantly lower the lethal temperature for tumor cells in near-infrared (NIR) photothermal therapy (Deng et al., 2021). In controlled conditions, the presence of Heat Shock Protein (HSP) was found to protect the tumor cells from laser irradiation, requiring a high level of heat to tear down this barrier (Yang et al., 2017). However, in the presence of Mn-ICG@pHis-PEG, the synthesis of HSPs can be inhibited, reducing the temperature needed to terminate cancer cells. Significantly reducing the NIR exposure can minimize collateral damage for prolonged therapeutics. (Figure 3)

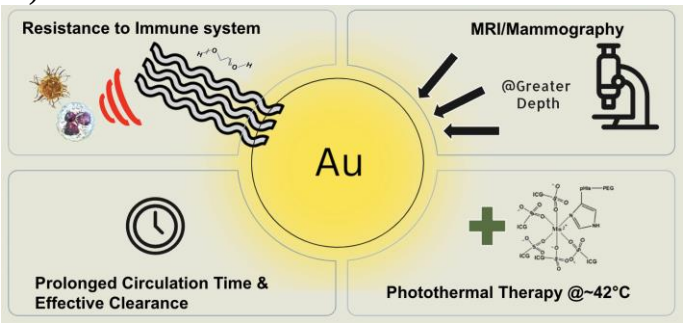

Figure 3

Expected outcome and refinements on existing GNPs synthesizing the sub ~2mn size and PEG chains, using MRI, and applying alongside Mn Nanoparticles.

6. Conclusion

Utilizing theranostics and therapeutic agents such as recombinant adenovirus gene therapy, Phesgo, and gold nanoparticles have all demonstrated promising results in the patients. Unfortunately, these approaches have signs of challenges and drawbacks that should be overcome to treat more patients. Starting with Gendicine, there are still insufficient case studies and clinical trials regarding rAd-p53, specifically on breast cancer. Thus, it is mainly challenging for oncologists and physicians to utilize this approach. Furthermore, this therapy requires engineering a virus. The immune system would respond by down-regulating the CAR receptors and increasing endosomal tensions. In addition to this immune system, this therapeutic agent requires multiple injections over a while because one unique characteristic of adenovirus is that they do not integrate into the host genome, which allows the cell only to

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express the gene temporarily. This presents the dilemma of this theranostic since the cells could gain resistance; however, periodic injections are required.

Phesgo, a promising approach, is particularly effective for those already compatible with specific cancer medications. While its current target is specific, it holds the potential to be accessible to a broader patient population, not limited by the need for the HER 2-Positive gene. Biochemists can focus on nullifying or eradicating the side effects of this medication, thereby expanding its reach and impact.

Considering the challenges of low permeability, collateral damage, poor clearance, and short circulation time, our fellow scholars have proposed innovative solutions. These include implementing PEGylation stealth technology, inhibiting heat shock protein (HSP), and resizing the molecule into two mn. These improvements hold the potential to significantly enhance permeability and clearance, resist immune response, and minimize collateral damage, inspiring us to continue our research and development efforts.

With the continuation of further research and clinical trials in the biomedical engineering field, gene therapy treatments can advance into the following stages. This progress holds the potential to save millions of vital lives on Earth, instilling a sense of hope and optimism in our professional community.

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