DIRNANO

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Modifying the antigen to advance the vaccine efficacy; a new asset to our arsenal against cancer

In recent years, the field of vaccines has witnessed remarkable progress, revolutionizing the way we combat infectious diseases and improve public health. [1]
Traditional vaccines have played a crucial role in preventing numerous illnesses by training our immune systems to recognize and respond to specific pathogens. [1.2] However, a new frontier has emerged with the advent of nanovaccines, harnessing the power of nanotechnology to enhance vaccine efficacy and tailor immune responses. [3]
One key aspect of this progress lies in antigen development, where researchers have been focusing on designing and optimizing antigens to elicit robust immune reactions and broaden vaccine coverage. [4]

Importance of the antigen and the platform in vaccines formulation

Antigens are the key components of vaccines that stimulate our immune system to recognize and target specific pathogens. In the past, the development of antigens primarily relied on live attenuated or inactivated pathogens, as well as subunit vaccines containing purified components. [4,5] However, with advancements in molecular biology and biotechnology, scientists are now able to engineer antigens with precision and explore novel strategies to improve their effectiveness. [4] This has led to the development of recombinant antigens, where specific genes encoding pathogen proteins are inserted into expression systems to produce large quantities of pure and well-characterized antigens. [3,4,5] Such approaches allow for greater control over antigen structure, function, and presentation, enabling the design of vaccines with improved immunogenicity and safety profiles.

Nanovaccines have emerged as a promising avenue for vaccine development,

offering unique advantages over traditional vaccine formulations. [3]
By leveraging nanotechnology, scientists can engineer nanoscale carriers, such as liposomes, nanoparticles, or viral-like particles, to deliver antigens more efficiently to immune cells and tissues. [4,5,6] These carriers can enhance antigen stability, protect against degradation, and facilitate controlled release, thereby optimizing the immune response. [3,5,6] Additionally, nanovaccines enable the incorporation of multiple antigens or adjuvants into a single formulation, promoting a broader immune response and potentially providing protection against diverse strains or even multiple pathogens. [6,7] The precise control and modulation of immune responses offered by nanovaccines hold great potential for tackling challenging infectious diseases and improving vaccination strategies. [3,5,6,7]

Approaches and strategies to modify the antigen

Modifying the antigen by itself can be a challenging process, including the tailoring of the amino acid sequence to increase its immunogenicity and stability while maintaining a structure identical or the closest possible to the initial. [4,5]
This process can become even more challenging when the targeting condition is cancer since, in this case, the targeting antigen would be a self-antigen, raising two main challenges: directing the immune response only towards cancer and not any other organ or tissue and generating an immune response towards to a self-antigen by itself. [5]
Researchers tackled these issues by focusing on certain proteins met with different post-translational modifications ***(Figure 1.)*** in the membranes of healthy and cancer cells, the mucins. [8,9,10]
More specifically, these glycoproteins when found in the membrane of healthy cells their proteinic backbone is heavily glycosylated; the density of the glycan units does not allow any interaction between the protein and the components of the immune system, while the opposite is true for the cancer cells, their proteinic backbone is under glycosylated or completely naked, enabling these interactions. [11,12,13]

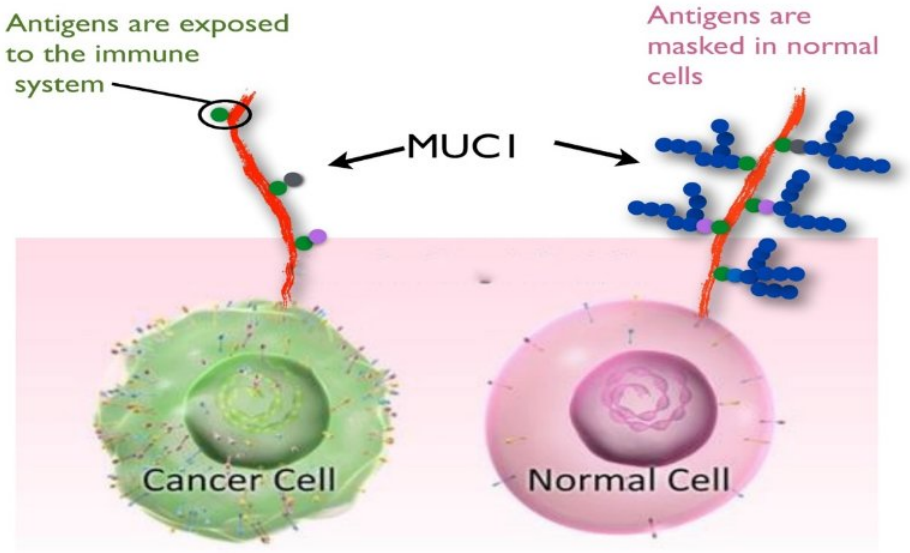

Figure 1. Schematic depiction of a healthy (shown in pink) and cancer (shown in green) cell; bearing a mucin glycoprotein with different glycosylation patterns. In the healthy cell, the mucin is heavily glycosylated, resulting in a protective coating blocking any interaction with the components of the immune system.
In the cancer cell, the mucin is underglycosylated or not glycosylated at all, exposing its proteinic backbone and making it vulnerable to the immune system. Glycosylation units are shown as colorful balls on the proteinic backbone of the mucins; each ball represents one glycosylation unit.

Our contribution

The project aims to develop an antigen library bearing different modifications. The antigen candidates will then undergo an initial screening phase, checking their stability, binding affinity towards well-characterized antibodies (crystal structure already known), and biophysical studies. [9,10]
The most promising candidates will be implemented in vaccines. To further address this issue, the project aims not only to modify the Mucin-1 but also to modify the vaccine formulation.
In collaboration with other partners of the consortium *(group of Dr. Roberto Fiammengo, University of Verona)*, we are developing innovative cancer vaccines by mounting the modified antigen on different nanoparticle-based platforms, having greater success with PEGylated gold nanoparticles.
Finally, the vaccine candidates will be used in preclinical studies to evaluate their performance; preliminary results are already available – the manuscript has been submitted but not accepted yet.

Future perspectives & applications

The future perspectives and applications of cancer nanovaccines featuring unnatural antigens are poised to transform cancer treatment in remarkable ways. For instance, these nanovaccines could be tailored to target specific types of cancer by incorporating antigens uniquely expressed by those tumors. Additionally, they hold potential for combination therapies, where nanovaccines could be used in conjunction with immune checkpoint inhibitors or targeted therapies to enhance treatment outcomes. Furthermore, the ability to precisely engineer the composition of unnatural antigens allows for personalized vaccines, designed to activate an individual patient's immune system against their specific cancer profile. As research progresses, we can anticipate the development of nanovaccines with improved safety, efficacy, and delivery systems, enabling more effective immunotherapeutic interventions and bringing us closer to a future where cancer can be effectively controlled and potentially eradicated. [3,14]

Conclusion

Summarizing, the progress in vaccines and the emergence of nanovaccines have transformed the field of immunization. Antigen development has played a pivotal role in this advancement, allowing scientists to design and optimize antigens to elicit robust immune responses. Meanwhile, nanovaccines have leveraged nanotechnology to enhance vaccine efficacy, improve antigen delivery, and broaden immune responses. As research continues to unfold in these areas, we can anticipate even more innovative approaches to vaccine development that will contribute to the prevention and control of infectious diseases, ultimately leading to improved global health outcomes.

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News in the DIRNANO consortium:

1. *Margarita Kislukhina (ESR11)* attended the *EUTOPIA Doctoral Summer School* (19/06-23/06/2023) in *FCT NOVA*, Lisbon, Portugal. The summer school focused not only on the importance of the interdisciplinary approach to modern, complex topics of scientific research but also granted the chance to the student to develop an EU research proposal over the course of the workshop, highlighting the importance of a multidisciplinary approach to the scientific problems, and promoting the out-of-the-box thinking – summing up a unique experience for the attendees.

2. *Carlos Pavón Regaña (ESR05)* attended the *Macrogiovani 2023* conference (21/06-23/06/2023), organized by *Associazione Italiana di Scienza e Tecnologia delle Macromolecole (AIM)* , in Catania, Italy. He joined the event with an oral presentation, sharing the latest results of his current project: Tuning Dispersity of Polymer Brushes to Modulate Their Interfacial Properties, which granted him the best oral presentation award!

3. *Rita Ribeiro (ESR08)* and *Ander Eguskiza Bilbao (ESR09)* attended the *Gordon Research Conference in Cancer Nanotechnology* (11/06-16/06/2023) and the associated seminar in Waterville Valley, United States. The fellows had the opportunity to present their latest results in two poster sessions, firstly in the seminar and later in the conference, and to thoroughly discuss their work with experts in the field. It was an outstanding and enriching experience, filled with insightful talks and plenty of time for networking.

4. *Haritha Isukapatla (ESR 10)* just started her secondment at *Molecular Horizon*, under the supervision of *Dr. Sara Tortorella* working on the effects of cholesterol analogues on lipid metabolism and the QSAR analogy.

5. *Foivos Sokratis Lazaris (ESR14)* just published a science communication blog at *The Marie Curie Alumni Association Blog*, a peer-reviewed science

communication journal, with the title: *“Cancer Vaccines: a distant utopia or an immediate future?”*.

6. *Michele Tomaz* just started her secondment at *Lipocoat BV*, under the supervision of *Prof. Pascal Jonkheijm.*

References

1. Excler, JL., Saville, M., Berkley, S. et al. *Nat Med* **2021**, 27, 591–600.
2. Riedel, S. *Proc (Bayl Univ Med Cent).* **2005**,1,21-5.
3. Cordeiro, AS., Patil-Sen, Y., Shivkumar, M., et al. *Pharmaceutics* **2021**, 12, 2091.
4. Kacen, A., Javitt, A., Kramer, M.P. *Nat Biotechnol* **2023**,41, 239–251.
5. Chaplin, D.D. *J Allergy Clin Immunol.* **2010**, 125,3-23.
6. D’Amico, C., Fontana, F. *Drug Deliv. and Transl. Res.* **2021**,11, 353–372.
7. Afzal, O., Altamimi, A.S.A. *Nanomaterials (Basel). 2022 Dec* **24**,4494.
8. Gao, T., Cen, Q., Lei, H. *Biomedicine & Pharmacotherapy* **2020**, 132, 110888.
9. Asín, A., García-Martín, F., Corzana, F., et al. *Curr Med Chem.* **2022,** 7, 1258-1270.
10. Martínez-Sáez, N., Peregrina, JM., Corzana, F., et al. *Chem Sci.* **2016**, 3, 2294-2301.
11. Pihno and Reis *Nat. Rev. Cancer* **2015**, 15, 540–555.
12. Hollingsworth, M., Swanson, B. *Nat. Rev. Cancer.* **2004**, 4, 45–60.
13. Nath, S., Mukherjee, P. *Trends Mol Med.* **2014**, 6, 332-42.
14. Pippa, N., Gazouli, M., Pispas, S. *Vaccines (Basel)* **2021**, 6, 558.