Nanoparticle (NP)-Based Delivery Systems

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Nanoparticle (NP)-based delivery systems can be designed to take advantage of the aberrant vasculature, or an acidic or hypoxic tumor microenvironment (TME), to induce the release of therapeutic drugs directly in the TME, reducing off-target side effects. In the last twenty years, the discovery of novel biomaterials has dramatically impacted on the field of nanobiotechnology, such as, for example, the addition of novel stimuli-responsive polymers, which can be used to develop advanced nanostructures with the ability to improve the pharmacokinetic properties of many drugs used in oncology.

Keywords: nanoparticles ; nanomedicine ; immunotherapy ; adoptive cell therapy ; nanovaccines ; immunomodulation ; tumor microenvironment ; nanotechnology ; immune checkpoint ; PD-1

1. Introduction

The tumor microenvironment (TME) is a complex system composed of proliferating tumor cells, infiltrating immune cells, the extracellular matrix (ECM), blood vessels and a variety of associated cells. The multifaceted cellular compartments present in the TME cooperate in the maintenance of the necessary conditions for tumor development: (1) angiogenesis, to offer nutritional support for tumor growth, and (2) immunosuppression, to inhibit the adaptive immune response against cancer cells [1]. In particular, tumor infiltrating immune cells not only fail to exercise their anti-tumor effector function, but they are able to promote tumor growth, invasion and metastasis [2].

Recently, investigation of the molecular mechanisms behind the immunosuppressive state in the TME led to the discovery of immune checkpoint inhibitors (ICIs), which changed the paradigm of cancer treatment, giving rise to novel immunotherapeutic options able to induce a strong infiltration of active immune cells in the TME, with consequent control of tumor growth [3]. ICIs currently used in the clinical setting are monoclonal antibodies (mAb) able to block the activity of cytotoxic T-lymphocyte antigen-4 (CTLA-4) or programmed cell death protein 1 (PD-1), expressed by T cells. Both CTLA-4 and PD-1 are repressor molecules that de-activate T effector function [4]. These checkpoint proteins are essential to control the balance between self-tolerance and auto-immunity [5]. Currently, the anti CTL4 mAb, Ipilumab, is approved for the treatment of unresectable melanoma, advanced renal cell carcinoma and advanced colorectal cancer in combination with the anti PD-1 mAb Nivolumab [6][7][8]. Interestingly, Nivolumab is active as standalone treatment in melanoma and non-small cell lung carcinoma (NSCLC) [9]. Pembrolizumab is another anti PD-1 mAb, employed for the treatment of a wide variety of cancer types [10]. Overall, ICIs are particularly effective for the treatment of high mutational burden, mismatch repair-deficient or high microsatellite instability tumors, where many mutations are present, thus favoring the generation of anti-tumor immune responses against specific tumor associated neo-antigens [10].

Another type of novel immunotherapeutic treatment is adoptive cellular transfer (ACT). In this case, patient-derived immune cells are expanded ex vivo and re-infused into the body. ACT-based cancer immunotherapy treatments mainly involve the re-infusion of genetically modified T cells [11]. However, other cell types such as natural killer cells (NK) and macrophages have been explored [12][13]. T cell–based ACT can be divided into three sub-categories: (1) tumor-infiltrating lymphocytes (TILs), where patient derived T cells are simply expanded and re-infused; (2) T cell receptor (TCR) engineered cells, where a TCR that is able to identify a specific tumor antigen, is added into the genome of T cells; and (3) chimeric antigen receptors T cells (CAR-T), where T cells are modified with a single chain variable fragment (scFv) able to recognize neo-antigen epitopes in a major histocompatibility complex (MHC) independent manner [14].

Interestingly, in the vast majority of advanced tumors, the TME is characterized by acidosis and hypoxia [15]. These two characteristics derive from the altered metabolism of cancer cells, fueled by an enhanced glycolytic activity necessary to support active cell proliferation [16]. Glycolysis results in the production of lactic acid, which is excreted in the TME by cancer cells, causing acidification of the TME. On the other hand, a hypoxic TME is caused by aberrant vascularization and poor blood supply [17]. Tumors tend to become hypoxic as a consequence of their growth, which leads to a lower blood supply to the inner part of the tumor. This can give rise to necrosis and a perpetually inflamed state in tumors, which was initially described more than thirty years ago, as a “wound that does not heal” [18].

NP-based delivery systems can be designed to take advantage of the aberrant vasculature, the acidic or hypoxic TME, to induce the release of therapeutic drugs directly in the TME, reducing off-target side effects [19]. In the last twenty years, the discovery of novel biomaterials has dramatically impacted on the field of nanobiotechnology, such as, for example, the addition of novel stimuli-responsive polymers, which can be used to develop advanced nanostructures with the ability to improve the pharmacokinetic properties of many drugs used in oncology [20]. The application of nanotherapeutics to cancer therapy has already reached the clinical stage, with more than ten FDA-approved nanoformulations, mainly employed for the delivery of chemotherapeutics such as doxorubicin (DOX), daunorubicin, paclitaxel and irinotecan, among others [21]. In addition, nanovaccines designed for the co-delivery of antigen and adjuvants to antigen presenting cells (APCs), have also been recently deployed for COVID-19, opening novel avenues for the use of nucleic acids-loaded NP for cancer therapy in the near future [22].

2. NP-Based Delivery Systems for Cancer Therapy

Nanocarriers can be developed to mimic the characteristics of immunogenic pathogens and provide tumor associated antigens (TAA) to re-establish and sustain the ongoing anti-tumor immune response in the TME [23][24]. Other strategies rely on the delivery of immunomodulatory drugs in the TME to modify the activity of tumor infiltrating lymphocytes [25]. In other cases, NP are utilized to deliver chemotherapeutics to the TME to specifically kill tumor cells, with consequent releases of TAA able to support anti-tumor immunity [26].

NP can also be tailored to be stimuli-responsive to take advantage of the acid and hypoxic nature of the TME. This can be accomplished by incorporating stimuli-responsive compounds and polymers in nanostructures, with consequent release of their therapeutic payload in the TME [27][28][29]. Polymers composed of histidine, 4-vinyl pyridine, aspartic and methacrylic acid are some examples of pH-sensitive molecules widely included in NP formulations, while derivatives of nitrobenzil or azobenzene are incorporated as hypoxia-responsive elements [19]. In addition, NP can be made responsive to particular enzymes present in the TME such as metalloproteinases [30][31].

Another therapeutic strategy to impact the immunosuppression of the TME relies on depletion of TAMs. This can be achieved by a liposomal formulation of the bisphosphonate clodronate (CodroLip) leading to tumor growth reduction in a wide variety of mouse xenograft models [32][33][34]. Of note, CodroLip therapy has been used in pre-clinical mouse models to study the effect of macrophage depletion in many diseases, including cancer [35]. However, this treatment can induce severe side effects such as neutrophilia and anemia, and it was not recommended for human trials due to its high toxicity [36]. Lastly, there is little interest from the pharmaceutical industry in the development of clodronate (or any other bisphosphonate) nano formulations, since these drugs are considered “old”, therefore non-patentable.

In addition, NP can be tailored to extravasate into the lymphatic system to reach the lymph nodes directly. For this purpose, NP ranging from 10 to 100 nm have been shown to effectively reach lymph nodes after subcutaneous injection, while NP of a larger size are unable to drain effectively into the lymphatic system and are retained at the injection site [37] [38]. The incorporation of antigens in NP can be achieved by covalent linkage of a protein or peptide to components of the nanostructure. In addition, nucleic acids can be attached through electrostatic interactions to the surface of NP (similarly to siRNAs) and can be processed and translated by APCs into antigenic peptides. Moreover, DNA and mRNA-based cancer vaccines can be designed to include multiple antigens to further increase immunogenicity. These nanosystems have the advantage of more closely mimicking live infections by incorporating multiple antigenic epitopes and pathogen-derived immune-adjuvants, into one single nanostructure.

3. NP-Based Delivery Systems Designed to Improve ICI and ACT Immunotherapies

Immunotherapeutic strategies rely on the infiltration of activated CD8+ T cell in the TME to kill cancer cells. However, when the TME is highly immunosuppressive, T cells are unable to efficiently exert their function and can become anergic. Thus, acting on the immunosuppressive TME, while at the same time inducing a strong and specific anti-tumor immune response, is essential to achieve a strong durable response able to eradicate established tumors. Therapeutic nanovaccines have been recently employed to stimulate a *de novo* immune response against tumor neoantigens. However, as a standalone therapy, nanovaccines are not able to completely eradicate established tumors. To improve the efficacy of nanotherapeutics, many research groups have explored their use in combination with ICIs in pre-clinical models with great success.

The choice of TAA is a critical step in the development of nanovaccines. In fact, the impressive results obtained in pre-clinical models are mediated by the targeting of specific antigens, which are, in some cases, model antigens such as OVA. In these cases, the model antigen is overexpressed by tumor cells and cannot be considered as a surrogate of a

mutated or overexpressed self-antigen present in human tumors. To circumvent this problem, Xu and colleagues developed a nanoplatform able to include neoantigens from the surface of tumor cells. This strategy allows the integration of tumor extract from resected autologous tumors with a fluoropolymer-based NP and can effectively prevent post-operative tumor recurrence and tumor metastases in treated mice, if used in combination with anti PD-1 or anti CTLA-4 therapy [39].

Unfortunately, the clinical translation of CAR-T cells for the treatment of solid tumors showed only moderate success in clinical trials [40][41]. This is, in part, due to the low infiltration of the infused T cells in the TME, which then encounter multiple immunosuppressive signals able to reduce their anti-tumor function. To further stimulate the expansion and effectiveness of transduced T cells in vivo, different NP-based “backpack” strategies have been developed to deliver immunomodulating agents together with T cells in the TME. Protein nanogels targeted to CD45, which served as a stable, non-internalizing anchor, were employed to bind to T cells and slowly release an IL-15 superagonist complex in the TME to support T cell effector functions. This strategy improved the efficacy of CAR-T cells in B16F10 xenografts dramatically, leading to complete tumor eradication in 80% of treated mice, compared to only 20% in mice treated with standard CAR-T cells [42].

The efficacy of CAR-T cells can also be improved by the treatment with immunomodulatory NP prior to T cell transfusion. This strategy can support CAR-T cells homing to the tumor lesion, leading to an enhanced expansion and anti-tumor function in the TME. For example, 4T1-ROR1 tumor bearing mice treated with an integrin-targeted liposomes loaded with a combination of the PI3K inhibitor PI-3065, and the α-GalCer agonist 7DW8-5, showed enhanced efficacy of transplanted CAR-T cells which were able to eradicate tumors in 50% of treated mice, while NP and CAR-T cells alone were ineffective [43].

4. Drawbacks and Future Perspectives

In general, NP-based therapeutics show strong anti-tumor effects in pre-clinical models of cancer. Nonetheless, clinical trials have provided little evidence of efficacy, especially if NP are administered as a standalone treatment. This could be explained by the exaggerated intratumoral distribution of NP in xenograft models, which harbor a relatively well-developed tumor vasculature, enhancing the passive targeting of NP mediated by the EPR effect. Murine xenograft models are rapidly proliferating, in addition to being highly vascularized tumors, which is also very different from their human counterparts, characterized by a more complex stromal architecture and a higher stromal density. Therefore, NP-based therapeutics, which are effective in murine models of cancer, may encounter additional issues to achieve similar results in human solid tumors. Slow growing tumor models or transgenic mice models could be employed to better recapitulate the stromal architecture of human tumors, since NP accumulation can dramatically differ between patients due to intrinsic heterogeneity [44].

There is a strong rationale for the use of cancer nanovaccines in combination with other immunotherapies or immunomodulators. NP offer the optimal platform for combinational immunotherapy, as they are able to encapsulate multiple immunomodulators and/or neoantigens in biodegradable particles. The main argument for this combinatorial therapeutic approach relies on the reinvigoration of the adaptive immune response against tumor cells by the nanovaccines with a simultaneous and synergistic treatment aimed at reducing the local immunosuppression in the TME. Furthermore, pre-clinical evidence also supports this hypothesis for CAR-T cell therapy, where nanovaccines can be utilized to enhance the in vivo expansion of transplanted T cells to augment and prolong their anti-tumor activity [45]. In addition, NP-based therapeutic strategies aimed at activating, re-polarizing or depleting the myeloid cellular compartment within the TME have shown compelling evidence for synergy with both ICIs and CAR-T cells. Therefore, translation of NP-based therapeutics should be accompanied in clinical trials in combination with other immunotherapies or immunostimulators to attack advanced metastatic tumors from multiple and different angles, in order to limit the strong immunosuppressive role of the TME, and support cytotoxic T cell functions.

References

1. Hanahan, D.; Coussens, L.M. Accessories to the Crime: Functions of Cells Recruited to the Tumor Microenvironment. Cancer Cell 2012, 21, 309–322.
2. Baghban, R.; Roshangar, L.; Jahanban-Esfahlan, R.; Seidi, K.; Ebrahimi-Kalan, A.; Jaymand, M.; Kolahian, S.; Javaheri, T.; Zare, P. Tumor microenvironment complexity and therapeutic implications at a glance. Cell Commun. Signal. 2020, 18, 1–19.

3. Wei, S.C.; Duffy, C.R.; Allison, J.P. Fundamental mechanisms of immune checkpoint blockade therapy. Cancer Discov. 2018, 8, 1069–1086.
4. Vaddepally, R.K.; Kharel, P.; Pandey, R.; Garje, R.; Chandra, A.B. Review of indications of FDA-approved immune checkpoint inhibitors per NCCN guidelines with the level of evidence. Cancers 2020, 12, 738.
5. Keir, M.E.; Liang, S.C.; Guleria, I.; Latchman, Y.E.; Qipo, A.; Albacker, L.A.; Koulmanda, M.; Freeman, G.J.; Sayegh, M.H.; Sharpe, A.H. Tissue expression of PD-L1 mediates peripheral T cell tolerance. J. Exp. Med. 2006, 203, 883–895.
6. Larkin, J.; Chiarion-Sileni, V.; Gonzalez, R.; Grob, J.J.; Cowey, C.L.; Lao, C.D.; Schadendorf, D.; Dummer, R.; Smylie, M.; Rutkowski, P.; et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. N. Engl. J. Med. 2015, 373, 23–34.
7. Motzer, R.J.; Tannir, N.M.; McDermott, D.F.; Arén Frontera, O.; Melichar, B.; Choueiri, T.K.; Plimack, E.R.; Barthélémy, P.; Porta, C.; George, S.; et al. Nivolumab plus Ipilimumab versus Sunitinib in Advanced Renal-Cell Carcinoma. N. Engl. J. Med. 2018, 378, 1277–1290.
8. Overman, M.J.; McDermott, R.; Leach, J.L.; Lonardi, S.; Lenz, H.J.; Morse, M.A.; Desai, J.; Hill, A.; Axelson, M.; Moss, R.A.; et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (Check-Mate 142): An open-label, multicentre, phase 2 study. Lancet Oncol. 2017, 18, 1182–1191.
9. Topalian, S.L.; Hodi, F.S.; Brahmer, J.R.; Gettinger, S.N.; Smith, D.C.; McDermott, D.F.; Powderly, J.D.; Sosman, J.A.; Atkins, M.B.; Leming, P.D.; et al. Five-Year Survival and Correlates among Patients with Advanced Melanoma, Renal Cell Carcinoma, or Non-Small Cell Lung Cancer Treated with Nivolumab. JAMA Oncol. 2019, 5, 1411–1420.
10. Gaynor, N.; Crown, J.; Collins, D.M. Immune checkpoint inhibitors: Key trials and an emerging role in breast cancer. Semin. Cancer Biol. 2020.
11. Laskowski, T.; Rezvani, K. Adoptive cell therapy: Living drugs against cancer. J. Exp. Med. 2020, 217.
12. Morvan, M.G.; Lanier, L.L. NK cells and cancer: You can teach innate cells new tricks. Nat. Rev. Cancer 2016, 16, 7–19.
13. Anderson, N.R.; Minutolo, N.G.; Gill, S.; Klichinsky, M. Macrophage-based approaches for cancer immunotherapy. Cancer Res. 2020.
14. Leon, E.; Ranganathan, R.; Savoldo, B. Adoptive T cell therapy: Boosting the immune system to fight cancer. Semin. Immunol. 2020, 49.
15. Yang, L.V. Tumor microenvironment and metabolism. Int. J. Mol. Sci. 2017, 18, 2729.
16. Missiroli, S.; Perrone, M.; Genovese, I.; Pinton, P.; Giorgi, C. Cancer metabolism and mitochondria: Finding novel mechanisms to fight tumours. EBioMedicine 2020, 59.
17. Petrova, V.; Annicchiarico-Petruzzelli, M.; Melino, G.; Amelio, I. The hypoxic tumour microenvironment. Oncogenesis 2018, 7, 10.
18. Flier, J.S.; Underhill, L.H.; Dvorak, H.F. Tumors: Wounds That Do Not Heal. N. Engl. J. Med. 1986, 315, 1650–1659.
19. Thomas, R.G.; Surendran, S.P.; Jeong, Y.Y. Tumor Microenvironment-Stimuli Responsive Nanoparticles for Anticancer Therapy. Front. Mol. Biosci. 2020, 7.
20. Yang, F.; Shi, K.; Jia, Y.P.; Hao, Y.; Peng, J.R.; Qian, Z.Y. Advanced biomaterials for cancer immunotherapy. Acta Pharmacol. Sin. 2020, 41, 911–927.
21. Anselmo, A.C.; Mitragotri, S. Nanoparticles in the clinic: An update. Bioeng. Transl. Med. 2019, 4, e10143.
22. Kim, J.; Eygeris, Y.; Gupta, M.; Sahay, G. Self-assembled mRNA vaccines. Adv. Drug Deliv. Rev. 2021.
23. Donninger, H.; Li, C.; Eaton, J.W.; Yaddanapudi, K. Cancer Vaccines: Promising Therapeutics or an Unattainable Dream. Vaccines 2021, 9, 668.
24. Tay, B.Q.; Wright, Q.; Ladwa, R.; Perry, C.; Leggatt, G.; Simpson, F.; Wells, J.W.; Panizza, B.J.; Frazer, I.H.; Cruz, J.L.G. Evolution of cancer vaccines—Challenges, achievements, and future directions. Vaccines 2021, 9, 535.
25. Liu, Z.; Jiang, W.; Nam, J.; Moon, J.J.; Kim, B.Y.S. Immunomodulating Nanomedicine for Cancer Therapy. Nano Lett. 2018, 18, 6655–6659.
26. Hernández, Á.P.; Juanes-Velasco, P.; Landeira-Viñuela, A.; Bareke, H.; Montalvillo, E.; Góngora, R.; Fuentes, M. Restoring the immunity in the tumor microenvironment: Insights into immunogenic cell death in onco-therapies. Cancers 2021, 13, 2821.
27. Asrorov, A.M.; Gu, Z.; Li, F.; Liu, L.; Huang, Y. Biomimetic camouflage delivery strategies for cancer therapy. Nanoscale 2021, 13, 8693–8706.

28. Huang, R.; Luther, D.C.; Zhang, X.; Gupta, A.; Tufts, S.A.; Rotello, V.M. Engineering the Interface between Inorganic Nanoparticles and Biological Systems through Ligand Design. Nanomaterials 2021, 11, 1001.
29. Wang, A.; Han, X.; Qi, W.; Du, S.; Jiang, Z.; Tang, X. The Design of Abnormal Microenvironment Responsive MRI Nanoprobe and Its Application. Int. J. Mol. Sci. 2021, 22, 5147.
30. Yao, Q.; Kou, L.; Tu, Y.; Zhu, L. MMP-Responsive 'Smart' Drug Delivery and Tumor Targeting. Trends Pharmacol. Sci. 2018, 39, 766–781.
31. Vaghasiya, K.; Ray, E.; Sharma, A.; Katare, O.P.; Verma, R.K. Matrix Metalloproteinase-Responsive Mesoporous Silica Nanoparticles Cloaked with Cleavable Protein for "Self-Actuating" On-Demand Controlled Drug Delivery for Cancer Therapy. ACS Appl. Bio Mater. 2020, 3, 4987–4999.
32. Fritz, J.M.; Tennis, M.A.; Orlicky, D.J.; Lin, H.; Ju, C.; Redente, E.F.; Choo, K.S.; Staab, T.A.; Bouchard, R.J.; Merrick, D.T.; et al. Depletion of tumor-associated macrophages slows the growth of chemically induced mouse lung adenocarcinomas. Front. Immunol. 2014, 5, 587.
33. Zhang, W.; Zhu, X.D.; Sun, H.C.; Xiong, Y.Q.; Zhuang, P.Y.; Xu, H.X.; Kong, L.Q.; Wang, L.; Wu, W.Z.; Tang, Z.Y. Depletion of tumor-associated macrophages enhances the effect of sorafenib in metastatic liver cancer models by antimetastatic and antiangiogenic effects. Clin. Cancer Res. 2010, 16, 3420–3430.
34. Zeisberger, S.M.; Odermatt, B.; Marty, C.; Zehnder-Fjällman, A.H.M.; Ballmer-Hofer, K.; Schwendener, R.A. Clodronate-liposome-mediated depletion of tumour-associated macrophages: A new and highly effective antiangiogenic therapy approach. Br. J. Cancer 2006, 95, 272–281.
35. La-Beck, N.M.; Liu, X.; Shmeeda, H.; Shudde, C.; Gabizon, A.A. Repurposing amino-bisphosphonates by liposome formulation for a new role in cancer treatment. Semin. Cancer Biol. 2019.
36. Bader, J.E.; Enos, R.T.; Velázquez, K.T.; Carson, M.S.; Sougiannis, A.T.; McGuinness, O.P.; Robinson, C.M.; Murphy, E.A. Repeated clodronate-liposome treatment results in neutrophilia and is not effective in limiting obesity-linked metabolic impairments. Am. J. Physiol. Endocrinol. Metab. 2019, 316, E358–E372.
37. Howard, G.P.; Verma, G.; Ke, X.; Thayer, W.M.; Hamerly, T.; Baxter, V.K.; Lee, J.E.; Dinglasan, R.R.; Mao, H.Q. Critical size limit of biodegradable nanoparticles for enhanced lymph node trafficking and paracortex penetration. Nano Res. 2019, 12, 837–844.
38. Schudel, A.; Francis, D.M.; Thomas, S.N. Material design for lymph node drug delivery. Nat. Rev. Mater. 2019, 4, 415–428.
39. Xu, J.; Lv, J.; Zhuang, Q.; Yang, Z.; Cao, Z.; Xu, L.; Pei, P.; Wang, C.; Wu, H.; Dong, Z.; et al. A general strategy towards personalized nanovaccines based on fluoropolymers for post-surgical cancer immunotherapy. Nat. Nanotechnol. 2020, 15, 1043–1052.
40. Schaft, N. The landscape of car-t cell clinical trials against solid tumors—A comprehensive overview. Cancers 2020, 12, 2567.
41. Gou, L.; Gao, J.; Yang, H.; Gao, C. The landscape of CAR T-cell therapy in the United States and China: A comparative analysis. Int. J. Cancer 2019, 144, 2043–2050.
42. Tang, L.; Zheng, Y.; Melo, M.B.; Mabardi, L.; Castaño, A.P.; Xie, Y.Q.; Li, N.; Kudchodkar, S.B.; Wong, H.C.; Jeng, E.K.; et al. Enhancing T cell therapy through TCR-signaling-responsive nanoparticle drug delivery. Nat. Biotechnol. 2018, 36, 706–716.
43. Zhang, F.; Stephan, S.B.; Ene, C.I.; Smith, T.T.; Holland, E.C.; Stephan, M.T. Nanoparticles that reshape the tumor milieu create a therapeutic window for effective t-cell therapy in solid malignancies. Cancer Res. 2018, 78, 3718–3730.
44. Hare, J.I.; Lammers, T.; Ashford, M.B.; Puri, S.; Storm, G.; Barry, S.T. Challenges and strategies in anti-cancer nanomedicine development: An industry perspective. Adv. Drug Deliv. Rev. 2017, 108, 25–38.
45. Reinhard, K.; Rengstl, B.; Oehm, P.; Michel, K.; Billmeier, A.; Hayduk, N.; Klein, O.; Kuna, K.; Ouchan, Y.; Wöll, S.; et al. An RNA vaccine drives expansion and efficacy of claudin-CAR-T cells against solid tumors. Science 2020, 367, 446–453.