J Cancer Sci Clin Ther 2020; 4 (3): 365-368 *DOI: 10.26502/jcsct.5079078*

Review Article

Neuropilin-1, A Rising Star in Cancer Immunotherapy

Qianqian Gao*#, Qumiao Xu#

#Authors contributed equally

BGI-Shenzhen, Shenzhen, China

***Corresponding Author:** Qianqian Gao, BGI-Shenzhen, Shenzhen 518083, China, E-mail: gauk_g@163.com

Received: 07 August 2020; **Accepted:** 25 August 2020; **Published**: 01 September 2020

Citation: Qianqian Gao, Qumiao Xu. Neuropilin-1, A Rising Star in Cancer Immunotherapy. Journal of Cancer Science and Clinical Therapeutics 4 (2020): 365-368.

Keywords: NRP1; PD-L1; Siglec-15; Memory checkpoint; Combinatorial therapy

1. Introduction

Neuropilin-1 (NRP1), one of neuropilins (NRPs) in addition to neuropilin-2 (NRP2), is a highly conserved membrane receptor in vertebrates. Initially identified for its role in neuronal development, NRP1 was subsequently found expressed on various cell types. It participates in a broad range of physiological activities from embryonic vessel formation to adaptive immune responses [1, 2].

Upregulated expression of NRPs on tumor cells was correlated with tumor progression, likely through enhancing tumor angiogenesis and tumor survival [1]. NRP1 expression on various immune cells has been reported, but its function in the immune system under normal and pathological conditions remains to be fully characterized.

2. The Role of NRP1 in CD4+ T Cells

NRP1 was essential in establishing the immunosuppressive function of murine $CD4^+CD25^+Foxp3^+$ regulatory T cells (T_{reg}s). Deletion of NRP1 in $CD4^+$ T cells led to increased experimental autoimmune encephalitis (EAE) severity, due to preferential differentiation into Th17 effector cells and impaired functionality of T_{reg}s [3]. On another hand, ablation of NRP1 in $CD4^+$ T cells inhibited tumor infiltration of T_{reg}s, resulting in enhanced $CD8^+$ T cell activation and reduced tumor growth. The results suggested that NRP1 might serve as a potential target for cancer immunotherapy [4]. Nonetheless, the functional role of NRP1 on the anti-tumor activity of $CD8^+$ T cells has been much less addressed.

3. The New Function of NRP1 as a Memory Checkpoint Molecule in $CD8^+$ T Cells

NRP1 was recently identified as a unique and novel "immune memory checkpoint" in $CD8^+$ T cells [5]. NRP1 deletion in $CD8^+$ T cells did not affect primary tumor growth. Instead, growth of re-challenged tumor was inhibited maybe through promoting effector-to-memory transition of $CD8^+$ T cells by loss of NRP1. The mechanism is different from that of other inhibitory checkpoint molecules, such as PD-1, CTLA4, and LAG3, which mainly influence the development and function of effector T cells. Loss of NRP1 affected development of T cell memory in response to tumor re-challenge, while production and maintenance of $CD8^+$ T cell memory play a crucial role in long-term host survival [6].

As a result, NPR1 loss holds great potential to provide durable anti-tumor benefits. Canonical checkpoint molecules include PD-1, CTLA-4, LAG-3, TIM-3, TIGIT, and 2B4. The expression of these T cell inhibitory receptors is limited to the activated T cells and terminally differentiated effector memory T cells, while their cognate ligands are expressed on activated antigen-presenting cells (APC) or epithelial cells. Their interaction leads to the restriction of effector T cell (T_{eff}) function to balance immunity and tolerance [6]. Application of inhibitors of these checkpoint molecules results in transient reinvigoration of T_{eff} but often with tumor relapse, indicating a lack of durable T cell memory post checkpoint inhibitor (CPI) therapy. Good news is that NRP1 deficiency promotes development of tumor-specific memory T cells (T_{mem}) with long-term antitumor activity, which could probably compensate for the shortcoming of conventional CPI therapy. Indeed, NRP1 loss significantly improved sensitivity to anti-PD-1 immunotherapy, and combinatorial blockade of NRP1 and PD-1 achieved a greater antitumor effect than either blockade alone [5]. Furthermore, one clinical trial administrated with anti-NRP1 (ASP1948) and anti-PD-1 antibodies is ongoing (NCT03565445). It can be expected that combinatorial inhibition of NRP1 and other inhibitory checkpoint molecules, not limited to PD-(L)1, will enhance tumor clearance. Recently, Siglec-15 was identified as an immune suppressor, and targeting Siglec-15 by genetic deletion or antibody blockade inhibited tumor growth [7].

Siglec-15 is normally expressed on some myeloid cells, while its expression is upregulated on human cancer cells and tumor-associated myeloid cells. Importantly, expression of Siglec-15 is mutually exclusive to PD-L1 and suppressed by IFNγ, which is in contrast to PD-L1 expression induced by IFNγ. Consistently, double blockade of Siglec-15 and PD-1 further repressed tumor growth compared to single blockade [7]. Siglec-15 functions like a ligand in much the same way as PD-L1 on cancer cells or tumor stroma, and engages an unknown inhibitory receptor on T cells. The complementary expression of PD-L1 and Siglec-15, and the functional complementation of NRP1 and PD-1 make it highly possible that double blockade of NRP1&PD-1, and NRP1&Siglec-15 will be efficient for treating most types of human cancer.

4. NRP1 Blockade Combined with CAR-T or TCR-T Cell Therapy to Prevent Tumor Relapse

Genetically engineered T cells expressing chimeric antigen receptor (CAR) or T-cell receptor (TCR) that recognizes and binds a tumor antigen have shown great potential in treating cancer patients [8]. CAR-T cell therapy has shown impressive efficacy in treating hematological malignancies, while TCR-T cell therapy has yielded remarkable results for solid tumors. CAR-T or TCR-T cell therapy can be a good combination for CPI therapy since only a fraction of patients are sensitive to the CPI and few experience durable benefits in clinical [9]. Therapeutic response of patients to

CPI therapy correlated positively with tumor mutation burden (TMB) and T cell infiltration in tumors. Combined application with CAR-T or TCR-T cell therapy could increase the counts of infiltrated T cells, which might in turn promote the clinical benefit of CPI therapy. On the other hand, a major challenge of CAR-T or TCR-T cell therapy is T cell exhaustion resulting in loss of memory precursors and weak persistence. This phenomenon has been shown to associate with the immunosuppressive tumor microenvironment (TME).

Tumor cells often increase the expression of PD-L1, which could induce apoptosis of antigen-specific T cells when binding to PD-1 [8]. Multiple inhibitors of suppressive immune checkpoint molecules have been developed to enhance the clinical efficacy of T cell therapy. Alternatively, genes encoding for inhibitory checkpoint molecules have been deleted by gene-editing technologies to protect T cells from TME [10]. Due to the critical role of NRP1 in memory differentiation of T cells, CAR-T or TCR-T cells could benefit from NRP1 loss to develop sustainable and functional memory T cell pools, which could improve T cell persistence and achieve long-term tumor remission. Moreover, targeting NRP1&PD-1 or NRP1&Siglec-15 simultaneously in CAR-T or TCR-T cells might further prevent tumor recurrence or relapse.

5. Remaining Questions and Strategies to Target NRP1

Leclerc M et al. [11] showed that NRP1 defined a subset of $CD8^+$ tumor-infiltrating lymphocytes (TILs) enriched with antigen-specific cells, which exhibited high expression of inhibitory molecules, including PD-1, TIM-3, LAG-3, and CTLA-4. It was shown that pre-incubation with anti-NRP1 antibody increased the migration of $NRP1^+$ T cells and *in-vitro* lysis of autologous tumor cells. In mice grafted with melanoma, NRP1 blockade inhibited tumor growth and combination with anti-PD-1 antibody further improved the anti-tumor efficacy. The data supported the role of NRP1 as an immune inhibitory molecule and its potential as a target for combinatorial therapy. Nevertheless, the direct effect of NRP1 blockade did not match completely with the results from Liu et al. [5], which demonstrated that NRP1 deficiency had no effect on primary tumor control. This discrepancy might be possibly explained by the use of different study systems. Liu et al. applied deletion of NRP1 specifically in $CD8^+$ T cells, while Leclerc M et al. used an antibody to block NRP1 with no discrimination on cell types. Tumor suppression by anti-NRP1 antibody might have resulted from mixed mechanisms, such as increased migration of $CD8^+$ cytotoxic cells to the tumor site and decreased infiltration of T_{reg}s. The most appropriate way to target NRP1 is still not clear. As discussed above, indiscriminate blockade of NRP1 might have benefits from broad targeting of a variety of cell types expressing NRP1.

However, considering the multifunctional role of NRP1 under normal conditions, it might be of advantage to perform local blockade in the tumor site or gene-editing in specialized cell subsets. Furthermore, whether the role of NRP1 as an immune checkpoint for developing T cell memory is restricted to $CD8^+$ T cells, or it also works in the same way in $CD4^+$ helper cells and $CD4^+$ cytotoxic cells awaits future studies. Finally, since current studies were mainly conducted in mouse models, it is of high interest and importance to investigate the effect of NRP1 blockade or deletion in human T cells, e.g., using humanized mice or adoptive transfer of $NRP^{-/-}$human T cells to immunodeficient mice. The ongoing clinical trial (NCT03565445) will provide outstanding insights on the safety and efficacy of blocking NRP1 alone as well as NRP1 and PD-1 simultaneously, and guide the future design of NRP1-targeted immunotherapy. In summary, the

recent discovery of NRP1 as a T cell memory immune checkpoint opens a new path to develop cancer immunethe-rapies with better persistence. Targeting NRP1, in combination with other immunotherapies, such as PD-1/Siglec-15 inhibition, CAR-T or TCR-T cell therapy is promising to provide durable anti-tumor effects and protection from tumor relaps.

Acknowledgements

We sincerely thank the support provided by China National GeneBank.

Author Contributions

Q. G. and Q. X. wrote and revised the manuscript.

Declaration of Interests

The authors declare no competing financial interest.

References

1. Klagsbrun M, Takashima S, Mamluk R. The role of neuropilin in vascular and tumor biology. Adv Exp Med Biol 515 (2002): 33-48.
2. Roy S, Bag A K, Singh R K, et al. Multifaceted Role of Neuropilins in the Immune System: Potential Targets for Immunotherapy. Front Immunol 8 (2017): 1228.
3. Solomon B D, Mueller C, Chae W J, et al. Neuropilin-1 attenuates autoreactivity in experimental autoimmune encephalomyelitis. Proc Natl Acad Sci U S A 108 (2011): 2040-2045.
4. Hansen W, Hutzler M, Abel S, et al. Neuropilin 1 deficiency on CD4+Foxp3+ regulatory T cells impairs mouse melanoma growth. J Exp Med 209 (2012): 2001-2016.
5. Liu C, Somasundaram A, Manne S, et al. Neuropilin-1 is a T cell memory checkpoint limiting long-term antitumor immunity. Nat Immunol (2020).
6. Reading J L, Galvez-Cancino F, Swanton C, et al. The function and dysfunction of memory CD8(+) T cells in tumor immunity. Immunol Rev 283 (2018): 194-212.
7. Wang J, Sun J, Liu L N, et al. Siglec-15 as an immune suppressor and potential target for normalization cancer immunotherapy. Nat Med 25 (2019): 656-666.
8. Zhang J, Wang L. The Emerging World of TCR-T Cell Trials Against Cancer: A Systematic Review. Technol Cancer Res Treat 18 (2019): 1533033819831068.
9. Sharma Padmanee. Immune Checkpoint Therapy and the Search for Predictive Biomarkers. The Cancer Journal. 22 (2016): 68-72.
10. Gao Q, Dong X, Xu Q, et al. Therapeutic potential of CRISPR/Cas9 gene editing in engineered T-cell therapy. Cancer Med 8 (2019): 4254-4264.
11. Leclerc M, Voilin E, Gros G, et al. Regulation of antitumour CD8 T-cell immunity and checkpoint blockade immunotherapy by Neuropilin-1. Nat Commun 10 (2019): 3345.