इम्यूनोथेरेप्यूटिक्स का विकास

16

ट्यूमर के माइक्रोएनवायरनमेंट की जांच के माध्यम से नए इम्यूनोथेरेप्यूटिक्स की पहचान

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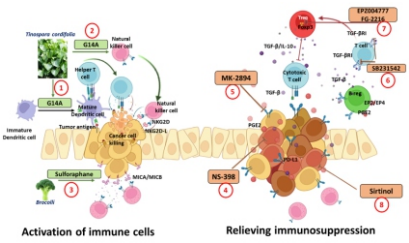

इम्यूनोथेरेपीटिक्स

सारांश

कैंसर प्रतिरक्षा निगरानी में प्रतिरक्षा प्रणाली की भागीदारी लगातार विकसित हो रही है। कैंसर की प्रगति के प्रारंभिक चरणों के दौरान, अन्योन्यक्रिया करने वाली प्रतिरक्षा कोशिकाएं सक्रिय होती हैं, जिसमें विभिन्न प्रकार की कोशिकाएं और साइटोकिन्स ट्यूमर-रोधी गुणधर्म प्रदर्शित होते हैं। हमारे शोध से पता चला है कि G1-4A, टिनोस्पोरा कॉर्डिफोलिया से प्राप्त एक पॉलीसेकेराइड, डेंड्राइटिक कोशिकाओं और प्राकृतिक घातक कोशिकाओं को सक्रिय करके प्रभावी रूप से एंटी-ट्यूमर प्रतिरक्षा को बढ़ाता है। ब्रोकोली से प्राप्त एक अन्य यौगिक, सल्फोराफेन, ट्यूमर कोशिकाओं पर कुछ सतह रिसेप्टर्स को बढ़ाता है जो प्राकृतिक घातक कोशिकाओं को उनका पता लगाने में मदद करता है और ट्यूमर कोशिकाओं को मारने के लिए अधिक अतिसंवेदनशील बनाता है। कैंसर बढ़ने से बचने के चरण के दौरान, कैंसर कोशिकाएं सक्रिय दमन को नियोजित करके प्रतिरक्षा प्रणाली द्वारा पता लगाने से बचती हैं। घुलनशील मध्यस्थ जैसे प्रोस्टाग्लैंडिन और परिवर्तनशील विकास कारक-β(TGF-β), साथ ही नियामक T कोशिकाएं, कैंसर सूक्ष्म वातावरण में प्रतिरक्षा दमन में योगदान देती हैं। हमने पहचान की है कि NS-398, साइक्लोऑक्सीजिनेज-2 का एक अवरोधक, SB-431542, विकास कारक- रिसेप्टर (TGF- R) सिग्नलिंग को बदलने का एक अवरोधक; और EPZ004777 और FG-2216, विनियामक कोशिकाओं के एपिजेनेटिक अवरोधक, ट्यूमर प्रेरित प्रतिरक्षा दमन को रोक सकते हैं और प्रतिरक्षा सक्षम कोशिकाओं को बहाल कर सकते हैं। अपने प्रतिरक्षात्मक गुणों के कारण, G1-4A, NS-398 एवं SB431542 जैसे अणु पूर्व-नैदानिक मॉडल में ट्यूमर के भार को कम करने में सक्षम थे और इनका उपयोग प्रतिरक्षात्मक चिकित्सा के रूप में किया जा सकता है।

Development of Immunotherapeutics

16

Identification of Novel Immunotherapeutics Through Tumor Microenvironment Investigations

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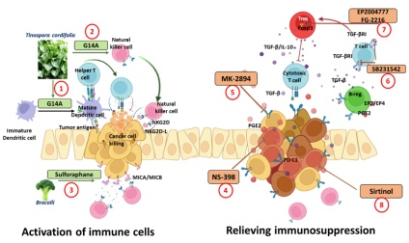

Immunotherapeutics

ABSTRACT

The immune system's involvement in cancer immunosurveillance is constantly evolving. During the early stages of cancer progression, various immune cells interact actively, with diverse cell types and cytokines exhibiting anti-tumor properties. Our research has shown that G1-4A, a polysaccharide derived from *Tinospora cordifolia*, effectively boosts anti-tumor immunity by activating dendritic cells and natural killer cells. Another compound, sulforaphane, derived from broccoli, increases certain surface receptors on tumor cells that help natural killer cells detect them and make tumor cells more susceptible to killing. During the escape stage of cancer progression, the cancer cells elude detection by the immune system by employing active suppression. Soluble mediators such as prostaglandins and transforming growth factor -β (TGF-β), as well as regulatory T cells, contribute to immunosuppression in the cancer microenvironment. Our studies have identified several compounds, including NS-398, an inhibitor of cyclooxygenase-2, SB-431542, an inhibitor of transforming growth factor-β receptor (TGF-βR) signaling; and EPZ004777 and FG-2216, epigenetic inhibitors of T regulatory cells, can prevent tumor induced immunosuppression and restore immunocompetent cells. Due to their immunomodulatory properties, molecules such as G1-4A, NS-398, and Sb431542 have demonstrated the ability to reduce tumor burden in pre-clinical models and may be used as immunotherapeutics.

KEYWORDS: *Tumor microenvironment, Immunotherapeutics, NS-398, G1-4A, SB431542, EPZ004777, FG 2216, Sulforaphane*

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Introduction

The capacity of all somatic cells to divide into two daughter cells is limited. This is known as 'Hayflick's limit' and is due to the shortening of telomeres, which are repetitive DNA sequences present at the ends of linear chromosomes. When cells exceed Hayflick's limit and divide excessively, it can result in uncontrolled cell proliferation or cancer. Although uncontrolled cell proliferation is the initial cause of cancer, as it grows, a number of changes take place, including immune cell infiltration, interaction with nearby fibroblasts, blood vessel development (angiogenesis), resulting in the formation of tumor microenvironment (TME). The way the many components of tumor microenvironment interact impacts how the cancer grows, spreads to other organs, and responds to treatment. The TME of different cancers varies and can be categorized as: (a) 'hot' tumors with numerous lymphocytes and other immune cells infiltrating them (b) 'excluded' or suppressed tumors with immune cells present only at the periphery (c) ignored or 'cold' tumors with no immune recognition at all. The relationship between cancer cells and immune cells is always changing, even in 'hot' tumors. Smaller tumors allow the infiltrating immune cells to be active, identify modified cells and destroy them through activities of cytotoxic T cells or natural killer cells. The immune system gets the upper hand during this phase, which is referred to as the 'elimination' stage, and can destroy the tumor cells with the help of cytotoxic T cells, natural killer cells, and mediators such as tumor necrosis factor (TNF)-α, interferon-γ, interleukins-6 and 12, etc. At this stage, the use of immunomodulators that stimulate the various immune cells that can help with tumor cell detection and destruction will be helpful. As the tumor advances, in addition to secreting various substances that actively suppress the immune system, the tumor actively exploits the growth factors and cytokines produced by immune cells to support its growth. Important mediators in the micro environment that cause immunosuppression include soluble mediators like prostaglandins, transforming growth factor-β (TGF-β) and cells such as T regulatory cells. Therefore, at this point, cancer treatment will benefit from the use of drugs that can prevent the immunosuppressive effects. Abrogation of immunosuppressive T regulatory cells is crucial for the treatment of 'excluded' tumors and enable the immune cells to infiltrate the tumor microenvironment.

Materials and Methods

Dendritic cells, natural killer cells, and cytotoxic T cells were isolated from the mouse spleen for *in vitro* treatments. T regulatory cells and B regulatory cells were generated *in vitro* using appropriate cytokine cocktail treatments. *In vivo* experiments were carried out in syngeneic tumor models. Expression of various markers was analyzed using flow cytometry or Western blot. Cytokines were monitored by ELISA, or ELISpot. Cytotoxicity assays were conducted using fluorometric or flow cytometry-based methods.

Results and Discussion

Immunomodulators that activate immune cells

Dendritic cells are the most effective antigen presenting cells, and are crucial for the development of an adaptive immune response against cancer cells. These cells interact with the cancer cells, process the cancer antigen, and activate the effector T cells, thereby becoming more immunogenic. Microbial products like lipopolysaccharide (LPS) help DC to mature and become more immunogenic. Immunomodulators, particularly those derived from natural resources, are being extensively studied for the treatment of a number of diseases, including cancer. Native to India, *Tinospora cordifolia*, commonly referred to as 'Guduchi', is a climbing shrub that has

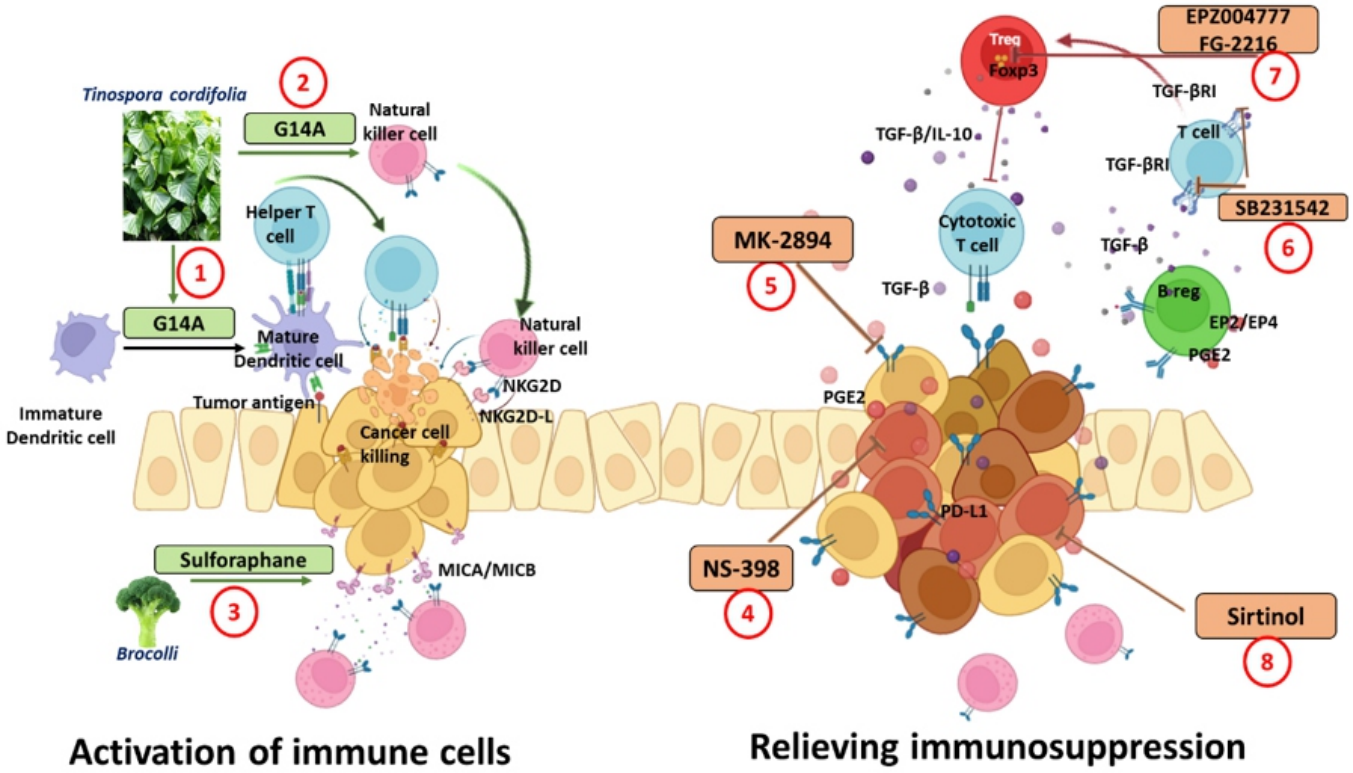

Fig.1: Immunotherapeutics with differing modes of action according to the changing tumor microenvironment: (1) G1-4A, a polysaccharide isolated from Tinospora cordifolia, is efficient in enhancing anti-tumor immunity by activating dendritic cells and (2) natural killer cells. (3) Sulforaphane (derived from broccoli) upregulates tumor cell surface molecules, that aid in detection by natural killer cells and making tumor cells more susceptible to killing. (4) cyclooxygenase-2 inhibitor NS-398; (5) EP4 antagonist MK2894 (6) transforming growth factor -β receptor (TGF-βR) inhibitor SB-431542; and (7) epigenetic inhibitors of T regulatory cells, EPZ004777 and FG-2216 (8) Sirtinol (a class III histone deacetylase inhibitor) prevent tumor induced immunosuppression and restore immunocompetent cells.

long been utilized in Ayurvedic medicine. We examined how the polysaccharide G1-4A, isolated from *Tinospora cordifolia*, affected the functional and phenotypic development of murine bone marrow derived dendritic cells (BMDC) and whether it may be used as an adjuvant in immunotherapy. G1-4A induced the maturation of dendritic cells, making them more immunogenic. These G1-4A treated DC in turn activated cytotoxic T cells that could destroy cancer cells. Administration of the tumor lysate pulsed G1-4A-treated DC reduced tumor burden in both therapeutic and preventative tumor challenge experiments in a mouse lymphoma model [1]. DCs also have cytotoxic ability against tumor cells in addition to their role as antigen presenting cells. We discovered that BMDC matured in the presence of G1-4A, [mBMDC (G1-4A)] killed tumor cells several times more effectively via a mechanism mediated by nitric oxide [2]. According to these results, G1-4A treated mBMDC matures and develops a killer phenotype and therefore may be a good nontoxic maturation agent for use in DC based immunotherapy. In BALB/c mice, immunomodulation with repeated doses of *T. cordifolia* polysaccharide-rich extract (PRE) and the purified polysaccharide G1-4A had comparable effects indicating that PRE can also be used as a viable immunotherapeutic adjuvant in place of G1-4A therapy [3].

Additionally, G1-4A treatment improved NK cell phenotypic and functional activation. NK cells were directly activated by G1-4A in (a) CD11c-depleted splenic cells and (b) purified NKp46^{+} cells. Co-culturing NK cells with bone marrow derived DC matured with G1-4A or splenic DC isolated from G1-4A-treated mice demonstrated DC crosstalk-mediated NK cell activation. In summary, G1-4A treatment has the potential to be employed as an immunotherapeutic drug [4] due to its varied effects on DCs and NK cells. Natural killer group 2, member D (NKG2D) is a receptor that is expressed on the surface of natural killer cells. When this receptor of NK cells interacts with the NKG2D ligand on tumor cells, it can result in tumor cell killing. We found that sulforaphane (SFN), a molecule obtained from broccoli, increased expression of these NKG2D ligands, hence boosting the sensitivity of tumors to NK cell-mediated death, highlighting the immunotherapeutic potential of SFN for application in cancer therapy [5].

Immunomodulators that relieve tumor induced immunosuppression

The relationship between tumor cells and the immune system is a significant factor affecting the cancer progression. Although many of the soluble molecules that mediate immunosuppression in the microenvironment are known, the mechanism by which the tumor affects the distal progenitors is not known. We discovered that the prostanoids produced by the tumor cells alter the development of the distal progenitor cells, by preventing the synthesis of transcription factor Zbtb46, which is specific to classical dendritic cell (cDC) lineage. The COX-2 inhibitor NS-398 reduced tumor induced phenotypic impairment of DC *in vitro*. Tumor-bearing mice treated with NS-398 developed immunocompetent DC and had lowered tumor burden. The conclusion that these effects were due to immunomodulation was reinforced by the lack of such an effect in immunodeficient SCID mice. These findings indicate that COX-2 inhibitors could be useful in cancer immunotherapy and that Zbtb46 expression is a marker of immunocompetent DC [6]. We also elucidated through in silico, *in vitro* and *in vivo* experiments that the PGE2 induced DC dysfunction was mediated through EP4/miR365/IL-6/pSTAT3 signaling. The treatment of mice with NS-398 or EP4 antagonist MK 2894 restored DC function and decreased tumor burden. This effect of EP4 antagonist was abrogated upon *in vivo* depletion of CD11c cells, indicating the crucial role of PGE2 signaling in DCs in tumor progression [7].

Developing immunotherapy methods for soft tissue sarcomas (STS) requires an understanding of the immunological milieu, which is relatively unknown. We demonstrated that the mouse fibrosarcoma evoked the development of B regulatory cells with CD19^{+}CD25^{+}PD-L1^{hi} phenotype that secreted TGF-β. These Bregs suppressed the proliferation of B-depleted T cells in a co-culture system. Treatment with SB431542, a small-molecule inhibitor of TGF-β receptor type I restored T cell responses. This T cell restoratory effect of SB431542 was observed in tumor bearing mice (TBM) along with considerable decrease in tumor burden. Our findings suggest that tumor-induced Breg suppresses immunity via a TGF-β mediated mechanism. Immunotherapy drugs targeting the Breg-Treg axis can thus have potential applications in soft tissue sarcomas [8].

The long-term stability of immunosuppressive regulatory T cells that plays a crucial role in immunological tolerance and homeostasis depends on epigenetic modifications. Identification of small-molecule modulators of Treg differentiation has several applications such as treatment of cancer, autoimmune diseases etc. To identify inhibitors that can potentially affect the generation of TGF-β1 induced T regulatory (iTreg) cells, we screened 160 compounds from an epigenetic chemical library. Two molecules, EPZ004777 and FG-2216 consistently reversed TGF-β1 iTregs without changing TGF-β downstream signaling in terms of (a) the development of naïve T cells into Treg cells, (b) the expression Foxp3 target genes, and (c) the suppressive function of Treg cells. We have thus identified the drugs EPZ004777 and FG-2216 as effective epigenetic modulators that reverse TGF-β1 induced T regulatory cells and could be used to treat a variety of immunological diseases [9]. Additionally, we demonstrated that another epigenetic drug, a class III histone deacetylase inhibitor sirtinol restored the immune microenvironment and inhibited epithelial mesenchymal transition and metastasis of 4T1 breast cancer [10].

Conclusion

The immune landscape of different malignancies might differ and even within a single malignancy, aside from inter-individual differences, it may evolve in tandem with cancer progression in a single individual. Immunotherapeutics, like molecular targeted therapies, must therefore be tailored to the tumor's immune infiltration and activation status. Therefore, immunomodulators which either activate immune cells or those that relieve tumor induced immunosuppression may be useful adjuvants in cancer treatment depending on the immune status of the cancer.

References

[1] V. K. Pandey, B. S. Shankar, K. B. Sainis, G1-4 A, an arabinogalactan polysaccharide isolated from Tinospora cordifolia increases dendritic cell immunogenicity in a murine lymphoma model, International Immunopharmacology, 2012, 14, 641–649.

[2] V. K. Pandey, P. J. Amin and B. S. Shankar, G1-4A, a polysaccharide from Tinospora cordifolia induces peroxynitrite dependent killer dendritic cell (KDC) phenotype, International Immunopharmacology, 2014, 23, 480–488.

[3] K. Premkumar, P. J. Amin, V. K. Pandey, P. Yadav and B. S. Shankar, Repeated dose treatment of Tinospora cordifolia polysaccharide rich extract activates splenic antigen presenting cells with no associated organ toxicity, Phytomed. Plus, 2021, 1, 100065.

[4] P. J. Amin and B. S. Shankar, Arabinogalactan G1-4A isolated from Tinospora cordifolia induces PKC/mTOR mediated direct activation of Natural Killer cells and through Dendritic Cell Cross-talk, BBA General Subjects, 2023, 1867, 130312.

[5] P. J. Amin and B. S. Shankar, Sulforaphane induces ROS mediated induction of NKG2D ligands in human cancer cell lines and enhances susceptibility to NK cell mediated lysis, Life Sciences, 2015, 126, 19–27.

[6] V. K. Pandey, P. J Amin and B. S. Shankar, COX-2 inhibitor prevents tumor induced down regulation of classical DC lineage specific transcription factor Zbtb46 resulting in immunocompetent DC and decreased tumor burden, Immunology Letters, 2017, 184, 23–33.

[7] V. K. Pandey, K. Premkumar, P. Kundu, B. S. Shankar, PGE2 induced miR365/IL-6/STAT3 signaling mediates dendritic cell dysfunction in cancer, Life Sci., 2024, 350, 122751.

[8] K. Premkumar and B. S. Shankar, TGF-βR inhibitor SB431542 restores immune suppression induced by regulatory B–T cell axis and decreases tumour burden in murine fibrosarcoma, Cancer Immunol Immunother., 2021, 70,153-168.

[9] K. Premkumar and B. S. Shankar, Identification of EPZ004777 and FG2216 as inhibitors of TGF-β1 induced Treg cells by screening a library of epigenetic compounds, Life Sciences, 2022, 301, 120643.

[10] S. Satam, N. Palekar, K. Premkumar, B.S. Shankar, Sirtinol, a SIRT1 inhibitor inhibits the EMT and metastasis of 4T1 breast cancer cells and impacts the tumor microenvironment, Immunopharmacology and Immunotoxicology, 2024, 46, 829-842.