

COMMENTARY

Recombinant human granulocyte macrophage colony stimulating factor (hGM-CSF): Possibility of nanoparticle-mediated delivery in cancer immunotherapy

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ABSTRACT

Most of the cancer treatment strategies from chemotherapy to radiotherapy render cancer cells apoptotic and these apoptotic cancer cells accumulate at the tumor sites. The accumulation of apoptotic cancer cells often result in inflammation and autoimmune responses causing serious health implications. Macrophages, which are effective immune combatants, can help in the clearance of these deleterious occupants. Granulocyte macrophage colony stimulating factor (GM-CSF) is a key cytokine, modulator of immune system and responsible for growth and differentiation of granulocytes and macrophages. In this regard, supply of recombinant GM-CSF can enhance the capability of macrophages for clearance of apoptotic cancer cells. However, delivery of the cytokine *in vivo* can suffer from certain disadvantages like faster depletion, less stability and low targeting efficiency. We believe that the stability and sustained release of GM-CSF can be improved through its encapsulation inside appropriately designed nanoparticles.

ARTICLE HISTORY

Received 7 July 2016
Accepted 7 July 2016

KEYWORDS

apoptosis; cancer immunotherapy; granulocyte macrophage colony stimulating factor; macrophages; nanoparticles; sustained release





Cytokines

Cytokines are glycosylated or non-glycosylated proteins or polypeptides secreted by cells in response to a stimulus which brings about a change in the behavior of the target cell.^{1,2} Members of the cytokine family are interleukins (ILs) (sub family constituting 33 ILs), tumor necrosis factors (TNFs) (sub family of 20 members), colony stimulating factors (CSFs) (including granulocyte-CSF (G-CSF), granulocyte macrophage-CSF (GM-CSF), macrophage-CSF (M-CSF)), interferons (IFNs), chemokines and growth factors (such as epidermal-derived growth factors (EGF), platelet-derived growth factors (PDGF), fibroblast growth factors (FGF), insulin like growth factors (IGF), transforming growth factors (TGF) and haematopoietic growth factors (HGF)).¹ Cytokines play a key role in immune surveillance and are involved in activation of immune cells. Additionally, they aid in promoting hematopoiesis, inflammatory responses and some cytostatic, cytotoxic and anti-viral effects.

Cancer immunotherapy

In addition to the conventional approaches including surgery, radiotherapy and chemotherapy, other

advanced techniques such as endocrine therapy, targeted therapy, anti-angiogenic therapy, virotherapy and immunotherapy are emerging as viable strategies for treating cancer.³ Among these, cancer immunotherapy exploits the potential of immune cells to bring about an effective clearance of cancer cells.⁴ Currently, there are 3 types of immunotherapy strategies available for treating cancer. These include innate immunotherapy that involves activation of IL 2 through various feedback mechanisms, systemic delivery of TNF α , or use of adjuvants like lipopolysaccharides (LPS) and CpG-containing oligodeoxynucleotides recognized by toll-like receptors (TLRs) to produce pro-inflammatory cytokines. Humoral immunotherapy employs monoclonal antibodies (mAb) which can target IL 2 receptors in T-cell leukemias and lymphomas, prevent vital survival signals or initiate apoptosis in cancer cells and use of cancer vaccines to stimulate patient's own cancer reactive B-cell population.⁴ The third strategy is the cellular immunotherapy involving the use of factors like GM-CSF, modulated to secrete at the site of tumor to elicit local accumulation of dendritic cells, by

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Commentary on: Vanitha S, Goswami U, Chaubey N, Ghosh SS, Sanpui P. Functional characterization of recombinant human granulocyte colony stimulating factor (hGMCSF) immobilized onto silica nanoparticles. *Biotechnology Letters*. 2016 Feb 1; 38(2):243-9.

blocking negative regulators of T-cell proliferation such as CTLA-4 using mAb or adoptive therapy to replenish normal cell population by transplanting haematopoietic stem cells (HSCs).⁴

GM-CSF in cancer immunotherapy

GM-CSF is an important cytokine responsible for differentiation and proliferation of granulocytes and macrophages from their precursors. Additionally, it plays a vital role in promoting growth and differentiation of dendritic cells which acts as sentinels for developing key immunogenic responses.^{2,5} First human GM-CSF cloning was reported using mouse GM-CSF cDNA library derived from the mRNA libraries of HUT-102 and mitogen stimulated T-lymphocyte.⁶ The gene for GM-CSF has shown to be localized at chromosomal region 5q23-q31. Human GM-CSF protein is a 144 amino acid long peptide having molecular weight of around 16 kDa.⁶ Recombinant GM-CSF is a FDA approved cytokine for treating immunodeficiency of neutropenia, acute myeloid leukemia, myeloid reconstitution and bone marrow transplantation.^{7,8}

In order to exploit GM-CSF as tumor vaccines, GM-CSF genes can be transduced into tumor cells and subsequently be released at the tumor site. This in turn can trigger tumor-specific immune response. These GM-CSF transduced tumor vaccines have been tested in various pre-clinical and clinical settings and proven to be effective in melanoma cells.^{9,10} Genetically modified tumor cells secreting GM-CSF, studied for its effect in cancer immunotherapy in mice and human, showed consistent profile in the activation of immune response.¹¹ GM-CSF expressing mouse and human colorectal tumor cells have shown significant anti-tumor activity in both immune dependent and independent manner.¹² In a study, administration of GM-CSF to patients suffering from prostate cancer showed a significant anti-prostate cancer activity with significant reduction in the PSA levels in patients.¹³ Role of GM-CSF protein as an adjuvant in chemotherapy has been demonstrated in a study in which comparative effects of G-CSF and GM-CSF primed with peripheral blood progenitor cell after high-dose chemotherapy, for eliminating absolute period of acute leukopenia.¹⁴ In a clinical study, patients with stage III high-risk melanoma showed high survival rate on treatment with GM-CSF as an adjuvant after surgical

resection.¹⁵ In another study, anti-tumor activity of 2 different cytokines, GM-CSF, IL-12 separately and in combination was studied in a comparative manner.¹⁶

Potential of nanoparticles (NPs) for controlled release of GM-CSF

Due to recent advancement in nanotechnology, different types of NPs have emerged as smart drug delivery vehicles.¹⁷ Encapsulation of various drugs including chemotherapeutic one inside these NPs present the possibility of controlled release of drug with additional benefits such as prevention of premature degradation or elimination of drugs, increased bio-availability of drugs, decreased toxicity to healthy cells/tissues and even spatio-temporal control of drug-release.¹⁸ There are also reports of NP-mediated delivery a number of therapeutically important proteins and peptides.^{19,20} In this regard, possibility of using NPs for delivery and/or sustained release of cytokines such as GM-CSF seems promising.

Chemotherapies, used to combat cancer, aims at killing cancer cells through activation of apoptotic pathways.^{21,22} Thus, at the end of treatment, there is a vast accumulation of apoptotic cell bodies. Eventual leakage of several cytotoxic agents from these apoptotic bodies can damage local cells through inflammation and autoimmunity.^{23,24} Hence, it becomes imperative to evoke rapid clearance of apoptotic cells from the body. Our hypothesis, in this line of thought, is that sustained release of recombinant GM-CSF from appropriately designed NPs would help in enhanced proliferation of macrophages eventually resulting in fast and improved clearance of apoptotic cancer cells generated due to conventional chemotherapies.

We have recently explored the prospect of immobilizing human GM-CSF (hGM-CSF) onto silica NPs in order to improve applicability of the cytokine in potential therapeutic applications.² For this, we first cloned hGM-CSF gene into in *Escherichia coli*, expressed the cytokine as a GST-tagged protein and established efficient purification protocol for the recombinant cytokine.^{2,25} On the other hand, non-porous and spherical silica NPs of ca. 130 nm were prepared by the Stober's process.²⁶ The cytokine was immobilized on these silica NPs by simple adsorption. The cytokine immobilized on the silica NPs demonstrated a linear release profile and interestingly retained structural integrity as probed by circular

dichroism spectroscopy. Most importantly, the proliferation of murine macrophages (RAW 264.7) was enhanced by 50% (at 600 ng hGM-CSF/ μ g of silica NPs).² However, future experiments with human macrophage cell lines are needed for evaluating the potential of our system and is the focus of our current research.

Moreover, mesoporous silica NPs will be more effective and should be explored in this regard as they possess large surface area and pore volume for maximum adsorption of hGM-CSF.²⁷ Additionally, suitable electrostatic interactions with the cytokine, adjustment of pore diameters and mesoporous structure of the silica NPs would allow control over hGM-CSF loading as well as fine tuning of release kinetics. Different polymeric matrices including hydrogel also offer potential option for sustained release of cytokines including hGM-CSF.²⁸ To this end, encapsulation of various cytokine such as IL-2, IFN- α , IL-12 and IL-18 in polymeric NPs has already been demonstrated.²⁹ However, protein denaturation during encapsulation remains a critical issue that needs to be addressed by optimizing encapsulation parameters for hGM-CSF for future applications.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

Financial supports from the Department of Biotechnology Program Support (BT/PR13560/COE/34/44/2015), and the Department of Electronics and Information Technology (No. 5 (9)/2012-NANO (Vol. II)), Government of India are acknowledged. P.S. acknowledges financial supports from the Department of Science and Technology, Government of India (SB/FTP/ETA-0122/2014).

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