Antibody-mediated molecular-targeted therapy for adult T-cell leukemia: Recent progress and future challenges in the treatment of cancers

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The role of the secreted matricellular molecule osteopontin (OPN) and its receptor integrins in the pathogenesis of adult T-cell leukemia (ATL) and the possible applications of an anti-OPN monoclonal antibody (mAb) for ATL immunotherapy in NOD/Shi-scid, IL-2Rγnull (NOG) mice were investigated. Subcutaneous inoculation of ATL cell lines into NOG mice led to increased plasma levels of OPN, correlating well with metastasis of the inoculated cells and survival time. This result suggested that the xenograft NOG mouse model could be a useful system for in vivo assessment of the physiological role of OPN in ATL pathogenesis. Intraperitoneal administration of an anti-OPN mAb resulted in the inhibition of tumor growth, tumor invasion, and metastasis. In addition, the mAb treatment led to reduction in the number of fibroblasts expressing fibroblast activation protein. We have shown here a novel mAb-mediated therapeutic strategy targeting the interaction between OPN from stromal cells and integrins on the tumors of ATL patients. In this editorial research highlight, we also comment on the recent progress in the development of mAbs and their advanced counterparts, the antibody-drug conjugate, for the treatment of cancers.

Keywords: Adult T-cell leukemia; Osteopontin; Integrin; Cancer-associated fibroblasts; Tumor microenvironment; Monoclonal antibody; Antibody-drug conjugate


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Introduction

Adult T-cell leukemia (ATL) is caused by human T-cell leukemia virus type I (HTLV-I) and is a highly aggressive CD4⁺ T-cell leukemia characterized by clonal integration of HTLV-I in leukemic cells [¹]. As ATL cells are frequently resistant to chemotherapeutic agents, several treatment strategies based on clinical subtypes have been employed [²]. However, these strategies, including combinations of various chemotherapeutic agents, have met with limited success in the treatment of ATL, thereby warranting further improvements.
In the past decades, several attractive antigens have been investigated for application in molecular-targeted therapy of ATL cells. CD25, also known as IL-2 receptor (IL-2R) α-chain, is an example of a well-tested antigen expressed on ATL cells [3]. Additionally, CD2, CD30, CD52, CD70, CD71 (transferrin receptor), and CD122 (IL-2Rβ) are also highly expressed on the ATL cell surface, and monoclonal antibodies (mAbs) against these cell surface antigens have been evaluated as potential therapeutic targets [3]. Despite vast progress in the field of antibody development, only a few mAbs have been shown to exert their expected effects by binding to tumor-specific antigens in humans, and subsequently approved in the market. On the other hand, ATL cell invasion and metastasis is frequently observed in an early phase of disease progression, notably in the skin, as well as in the liver, lung and lymph nodes [4]. Thus, prevention of such invasion and metastasis could be a possible therapeutic strategy to prolong survival time. The chemokine receptor CCR4 is known to be highly expressed on ATL cells, and skin metastasis of ATL tumors depends, at least partially, on the preference of CCR4 [5]. Based on these findings, the anti-CCR4 mAb mogamulizumab, which showed significant anti-tumor activity against ATL cells in the NOD/Shi-scid, IL-2Rγnull (NOG) mice via enhanced antibody-dependent cell-mediated cytotoxicity (ADCC) [6], has been developed and subsequently approved for the
treatment of relapsed or refractory ATL in Japan [7]. Although mogamulizumab was effective in the treatment of ATL, severe skin rashes were observed as an adverse reaction [8], necessitating the development of other therapeutic agents.

A matricellular molecule osteopontin and its receptor integrin as a possible target for antibody-mediated ATL therapy

Recent studies highlight a growing interest in the targeting of molecules present on tissues that support tumor growth and metastasis, the so-called tumor microenvironment [9]. These non-tumor tissues, unlike cancer cells, have a stable genome that is less likely to undergo somatic mutations, thereby reducing the mutation-induced resistance against mAbs and inhibitors [10]. Based on this emerging concept, we primarily focused on one of the stromal factors, a matricellular molecule osteopontin (OPN), in the extracellular matrix of the tumor microenvironment for the treatment of ATL. OPN physiologically interacts with αvβ1, αvβ3, αvβ5, and αβ1 integrins via a classical cell-binding motif, the RGD sequence within the OPN molecule, or with αβ1 and αβ1 integrins via a SVVYGLR sequence within the OPN molecule [11]. The interactions of OPN are involved in the progression of various diseases including cancers, and OPN thus serves as a critical regulator of oncosogenesis [12-15]. Changan-Yasutan et al. reported that increase in plasma OPN levels in ATL patients was significantly correlated with poor prognosis [16], and plasma levels of OPN could be a prognostic marker for ATL. We assessed OPN production in the supernatant of ATL-derived cell lines and HTLV-I-transformed T cell lines, and observed little secretion of OPN from these cell lines into the supernatant [17]. On the other hand, we detected increased OPN expression in CD68-positive macrophages and fibroblasts expressing fibroblast activation protein (FAP), known as cancer-associated fibroblasts (CAFs) [18, 19], within the lymph nodes of ATL patients by immunohistochemical staining. This finding strongly implicated non-tumor stromal cells as the primary source of OPN production [17]. In addition, we also demonstrated that CD4^+CD25^+ T cells obtained from ATL patients expressed αβ1, αβ5, and αβ1 integrins, and the standard form of CD44 (CD44^std) as well as its variant form 6 (CD44v6) on the cell surface [17].

Treatment with anti-OPN mAbs exerts a positive therapeutic effect in ATL tumor-bearing immunodeficient mice

To verify the function of mesenchymal stroma-derived OPN and its interactions with integrins in ATL tumorigenesis in vivo, we utilized the NOG mouse, which has been widely used in the analysis of ATL and other tumors [20]. Subcutaneous inoculation of ATL cell lines into NOG mice led to increased plasma levels of OPN, which significantly correlated with invasion of the inoculated cells and survival time (Figure 1A) [17]. More importantly, treatment of tumor-bearing NOG mice with the SVVYGLR motif-recognizing anti-OPN mAb resulted in the inhibition of tumor growth, tumor invasion, and metastasis (Figure 1B) [17]. Interestingly, we also found a reduction in the number of FAP-positive CAFs in primary tumor tissues following treatment with the SVVYGLR motif-recognizing anti-OPN mAb. This indicated that OPN is required for the recruitment of FAP-positive fibroblasts to the tumor (Figure 1B) [17]. To further investigate the biological roles of CAF-derived OPN on ATL tumorigenesis and tumor metastasis in vivo, we subcutaneously inoculated mouse embryonic fibroblasts (MEFs) isolated from wild-type (WT) or OPN knockout (KO) mice, together with ATL-derived cell lines (TL-OmI cells) into the NOG mice [17]. Significantly lower tumor growth was observed in TL-OmI-injected mice co-inoculated with OPN KO MEFs than in mice co-inoculated with WT MEFs. Although the plasma OPN levels in TL-OmI/OPN KO MEF-inoculated mice were lower than those in TL-OmI/WT MEF-inoculated mice, they were still significantly higher than those in mice inoculated with TL-OmI alone, indicating that other factor(s) are involved in OPN production. Metastasis of inoculated cells significantly correlated with tumor growth. Collectively, the data indicated that OPN regulated primary tumor growth by recruitment of CAFs and by mediating tumor metastasis via its binding to αβ1 and/or αβ1 integrins. Further investigation on how other stromal cells such as tumor-associated macrophages and endothelial cells are involved in ATL pathogenesis should be performed in the ATL xenograft model.

Recent progress and future challenges of antibody development for cancer immunotherapy

While we primarily focused on OPN derived from non-tumor tissue cells and highlighted the therapeutic effects of the anti-OPN mAb on ATL tumor development, we also detected the expression of αβ integrin and CD44v6 in primary ATL cells; thus, it should be of value to evaluate the anti-tumor activity of the anti-αβ integrin mAb and anti-CD44v6 mAb in combination with anti-OPN mAb in the ATL xenograft model. Meanwhile, the representative immune check point molecules, programmed cell death-1 (PD-1) and its ligand PD-L1, are also expressed in ATL cells [21, 22], although the contribution of PD-1/PD-L1 signaling to the pathogenesis of ATL is largely unknown. The “immune checkpoint blockade therapy” involves interruption of immunosuppressive functions that transduce co-inhibitory
signals via PD-1/PD-L1 to immune cells such as T cells in the tumor microenvironment. Several challenges associated with the development of anti-PD-1 mAbs (pembrolizumab, nivolumab, pidilizumab, and MEDI0680) and anti-PD-L1 mAbs (MPDL3280A, MEDI4736, BMS-936559, and MSB0010718C) have been addressed in recent years [23]; it would be of great interest to investigate if the PD-1/PD-L1 interaction could regulate ATL progression and if the interaction could be targeted to augment the therapeutic effects of anti-PD-1 or anti-PD-L1 mAbs.

Impact of antibody-drug conjugates (ADCs) as molecular-targeted therapeutics: conjugation chemistry to clinical application

Although mAbs exert their effects on tumor cells by several mechanisms (e.g. agonistic interaction, ADCC, and complement-dependent cytotoxicity), these cytotoxic effects are largely inefficient. Thus, a new class of antibody drugs, in particular, antibody-drug conjugates (ADCs), has been evaluated in the past few years. The ADCs consist of a tumor-specific mAb attached to a potent cytotoxic drug (payload) via a stable or cleavable linker [24]. Currently, brentuximab vedotin and trastuzumab emtansine (T-DM1) are approved by the Food and Drug Administration (FDA) and are available in the market [25]. While brentuximab vedotin has been mainly used for relapsed Hodgkin’s lymphoma and anaplastic large cell lymphoma, it also exerted anti-tumor effects on ATL cells in vitro and in vivo [26]. Collectively, ADC-mediated therapy targeting the molecules involved in not only tumor growth but also in tumor invasion and metastasis would be a promising therapeutic approach for effective management of ATL.

Mechanism of action of an ADC is rather simple. When some portion of the ADC administered intravenously localizes to a tumor and binds to a target antigen on the cell-surface of the tumor cell, the complex should be internalized into the cell. Internalized vesicles fuse with other vesicles and enter the endosome-lysosome pathway. In the lysosome, proteases in mild acidic environment digest the mAb to release free payloads, which then cross the lysosome membrane to enter the cytoplasm and/or the nucleus where they bind to the target molecule, leading to cell death [27].

Even if the target antigen is overexpressed, treatment with a mAb is not being beneficial for patients due to resistance in which upregulation or downregulation of downstream signaling pathway or altering pathway [28]. However, an ADC is effective when it is internalized into target cells and decomposed to release cytotoxic payloads whatever the downstream signaling pathway do not work well. Clinical efficacy of unconjugated mAbs and ADCs has been compared. Objective response rate of ADCs is always much higher than that of the corresponding unconjugated mAbs against hematological and metastatic breast cancers [29].

For successful application of an ADC to a target cancer, following requirements should be met: (i) Payload: Since only a small percentage of the ADC administered is known to bind the target antigens on the surface of the tumor cells [30] and low amounts of drugs would be internalized, therefore, potency of the drugs is required in the picomolar range. Now a dolastatin analog and a maytansin analog (microtubulin binder) and calicheamicin (DNA damaging agent) have been employed in clinical ADCs. Tasidotin (a dolastatin analog), cryptophycin (a cyanobacteria toxin), eribulin (a tublin polymerization inhibitor), adozelesin, bizelesin, and pyrrolobenzodiazepine dimer (DNA-minor-groove binders and DNA-alkylating agents) have also extensively been studied as next-generation payloads [31]. (ii) Linker and payload release: The linker should be stable during circulation in serum so that the payload is not released systemically to cause off-target toxicity. After internalization in the target cells, the payload have to be released in an active form. Therefore, a stable linker would be ideal if the payload having the linker shows as potent as a mother compound. But chemical modification (linker attachment) of payloads are sometimes largely reduced their activity and time-consuming chemical works to find out the positions where the potency is not diminished are required. In such cases, a cleavable linker, which is activated by cleaving the linker in appropriate chemical or enzymatic stimuli, should be carefully designed to show sufficient cytotoxicity to kill the target cells without producing off-target toxicity. (iii) Payload-loading: Previously it was reported that the therapeutic index is maximum for 2-4 drugs per antibody (DAR) [32] and this might be caused by instability of the mAb structure and hydrophobicity of the payload. However, quite recently, reducing hydrophobicity [33] and site-specific conjugation [34] of the payload largely improve therapeutic index even with higher DAR than 2-4 DAR have been reported.

Concluding remarks

We have proposed that a xenograft NOG mouse model can be a useful system to assess the physiological roles of OPN and integrins in ATL pathogenesis. Using this xenograft model, we found that stromal cell-derived, but not tumor-derived OPN levels, increased during the course of tumor development. In particular, we have presented a novel OPN mAb-mediated immunotherapeutic strategy targeting the interaction of host fibroblast-derived OPN with integrins on tumor cells in ATL patients. We are currently developing a mAb and its single-chain fragment variable (scFv) in
addition to our original ADCs for cancer immunotherapy. Further investigation towards the identification of other attractive tumor-associated antigens by tumor oncologists and new conjugation techniques of mAb/linker/cytotoxins by medicinal chemists will allow innovative drug development with the potential of overcoming therapy-resistant tumors. We believe that our progress and future advances will contribute to the development of attractive strategies for cancer immunotherapy in ATL and for several other solid tumors.

Conflicting interests

The authors have declared that no conflict of interests exist.

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