Tumor stromal barrier and cancer stromal targeting therapy

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Abstract

Antibody drug conjugates (ADCs) are effective for tumors with no or little stroma, such as malignant lymphoma or breast cancer. However, in refractory cancers (pancreatic cancer or scirrhous gastric cancer) forming hypovascular and stroma-rich tumors, the penetration of monoclonal antibodies (mAbs) into the cells is impeded (stromal barrier), which leads to failure of conventional cell-targeting ADCs. To overcome this, we developed cancer stromal targeting (CAST) therapy using anti-collagen IV or anti-fibrin mAbs. These stroma-targeting ADCs selectively extravasated from leaky tumor vessels and bound to collagen IV or fibrin on the tumor stroma, from which effective sustained release of the payload drug occurred. The released drug subsequently diffused through the tumor tissue, causing marked arrest of tumor growth associated with damage to tumor vessels and death of cancer cells. In terms of the pathological findings after treatment, empty sleeves collagen IV-positive and CD31-negative remnant ring structures) were observed in the destroyed vessels. This review highlights the tumor stromal barrier and the development of CAST therapy. Insights into the pharmacokinetics and efficacy of antibodies or ADCs may also be informative to understand the pathophysiological role of the tumor microcirculation involved in the stromal barrier. [MVRC 6(1): 2-8, 2013]

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Introduction

Although many monoclonal antibodies (mAbs) have already been approved for the treatment of cancer, they are usually used in combination with anticancer agents (ACAs) because of their limited anti-tumor activity when used alone\(^1\). Antibody-drug conjugates (ADCs), the next generation of therapeutic antibodies, are a promising strategy to enhance the cytotoxic effect\(^2-4\). Conventional ADCs depend on enzymatic cleavage following internalization into the cytoplasm or lysosome. Most human solid tumors, however, possess abundant stroma, which hinders the distribution of ADCs (stromal barrier). Moreover, the process of cell uptake is disturbed by the stromal barrier. Therefore, this barrier limits the effectiveness of ADCs, regardless of the internalization ability. The heterogeneity of tumor cells also prevents the development of ADC therapy based on a cell-specific antigen\(^5-8\).

To overcome these issues, we created a unique type of ADC, namely, cancer stromal targeting (CAST) therapy, in which stroma-targeting mAbs (anti-collagen IV or anti-fibrin mAbs) were conjugated to cytotoxic ACAs\(^6-8\). Our ADC bound to collagen IV or fibrin in the stroma, from which sustained release of ACAs and their distribution throughout the tumor occurred; this had a strong anti-tumor effect against stroma-rich tumors compared with that of conventional ADCs\(^5-8\).

In this article, we initially outline the tumor stromal barrier and conventional ADCs, followed by an introduction and discussion of CAST therapy.

Physiological and pathophysiological roles of stroma

Organ or tissue consists of not only parenchymal cells but also stroma. Stroma has various components such as fibroblasts, blood vessels, and nerve or extracellular matrix proteins, for example, collagen, fibrin and fibronectin (Fig. 1A). As a result, stroma exhibits various biological activities\(^9-12\). In embryogenesis, stroma can induce organogenesis of the pancreas, liver or lung (Fig. 1B). Regarding tissue remodeling, vasculogenesis by endothelial progenitors is promoted by collagen IV and VEGF, but the tube formation is insufficient, whereas well-organized vascular tube formation occurs in the presence of stroma cells (Fig. 1C). Upon inflammation, stroma blocks the overreaction of immune cells and terminates inflammatory process (Fig. 1D). Stroma also acts in host defense within the reproductive system (Fig. 1E).

Fig. 1. Physiological and pathophysiological roles of stroma. (A) Parenchymal cells and stroma. (B) Pancreas organogenesis induced by stroma. (C) VEGF and collagen IV promote insufficient vasculogenesis (upper panel). Well-organized vascular tube formation occurred in the presence of stroma cells (lower panel). (D) Stroma suppresses overreaction of the inflammation. (E) Stroma defends the reproductive system against autoimmune attack. (F) Hematoxylin-eosin staining of malignant lymphoma (ML) (upper panel) and pancreatic cancer (PC) (middle panel). Immunostaining of stromal collagen IV (brown) in PC. Cancer cells were stained by eosin (blue) (lower panel).
In malignant tissues, stroma promotes the growth, survival, invasion or metastasis of tumor cells. It is thus increasingly important to understand the role of the stroma in tumorigenesis. There are two distinct types of tumor tissue: those with little stroma, for example, malignant lymphoma (ML), and those with dense stroma, for example, pancreatic cancer (PC) (Fig. 1F), and the latter is strongly associated with the treatment-resistant phenotype.

Antibody drug conjugate and stromal barrier

Recently, several ADCs have been approved for oncological treatment. These antibody therapies are effective for ML or breast cancer (BC), but not for refractory cancers such as PC or scirrhous gastric cancer (SGC). Although many researchers have investigated the molecular mechanism behind enhanced anti-apoptotic effect, the abundance of drug-efflux transporters or the existence of natural chemoresistant cancer stem cells, in order to solve this question, the issue of the tumor stromal barrier has been almost entirely overlooked. We speculated that dense stroma prevents antibody distribution within tumor tissue, which would be one of the reasons for therapeutic resistance. Therefore, in a previous study, we evaluated antibody using an in vivo imaging system. We used two types of tumor model: ML and PC. Anti-CD20 mAb and anti-EpCAM mAb were also used as specific mAbs against ML and PC, respectively. In whole body imaging, both non-specific mAbs and specific mAbs accumulated in the tumor by an enhanced permeability and retention (EPR) effect as passive targeting on Day 3 after injection (Fig. 2A). High-molecular-weight agents including mAbs were shown to extravasate from leaky tumor vessels but not normal tissues. Moreover, they remained at the site for a long time because of the lack of effective lymphatic drainage, which impeded the efficient clearance of mAbs accumulated in solid tumor tissues. On Day 7, although non-specific mAbs disappeared from the tumor, specific mAbs still accumulated there by utilizing their specific antigen-binding ability (Fig. 2A). We considered mAbs to be one of the ideal drug delivery system (DDS) carriers because IgG ranging in size from 10 to 20 nm can utilize the EPR effect. Moreover, specific mAbs were able to stay longer by the EPR effect plus active targeting.

We then examined the mAb distribution within tumor tissue. Extravasated anti-CD20 mAbs were distributed throughout the whole tumor in ML, whereas the distribution of anti-EpCAM mAbs was restricted to the tumor margin adjacent to vessels in PC. A very small amount of mAb reached the central area within the tumor (Fig. 2B). This phenomenon can be explained by the stromal barrier. Although some authors reported that mAbs extravasted from leaky tumor vessels were able to reach the tumor cells sufficiently, they used hypervascular and stroma-less tumor models. We therefore considered these models were close to the ML rather than PC. On the other hand, genetic engineered mouse (GEM) models have come to be used for the studying human cancer. The pancreatic tumors observed in the model displayed pathophysiological features similar to human pancreatic cancers. By using this model, it was shown that the dense stromal was able to prevent drug delivery into the tumor cells. We speculate that stromal barrier involves three factors: 1) mechanical interference by stromal cells and extracellular proteins, 2) pharmacological disturbance depending on low convection and diffusion, and 3) a long distance from vessels to tumor cells. We need to further investigate the mechanism of the stromal barrier to elucidate this hypothesis.
Conventional ADCs

In the 1980s, the development of anti-tumor-cell-specific mAbs carrying a cytotoxic drug, known as missile therapy and expected to be a magic bullet for cancer treatment, was actively pursued. However, the induction of human anti-mouse antibody (HAMA) response was a major impediment to the success of antibody-related therapies. In addition, most of the linkers connecting the cytotoxic drug to an mAb were unstable and easily degraded in the body. Fortunately, recent progress in antibody engineering and linker technology has overcome these problems. Chimeric or humanized mAb reduces the rate of HAMA reactions. In addition, linkers that are stable enough in the body to last until the ADC reaches the tumor cells, and also sufficiently cleavable to allow effective drug release within cells, have been successfully developed (Fig. 3A and B). Recently, the clinical benefit of newly developed anti-HER2 mAb-DM1 has been demonstrated in the treatment of BC. However, there is less stroma in BC than in refractory solid tumors such as PC or SGC. Moreover, most BCs are hypervascular tumors, in which the stromal barrier may be weakened. Furthermore, the treatment is limited to HER2-overexpressing tumors. Most human refractory solid tumors, however, possess hypovascularity and dense stroma that hinders the distribution of ADCs. (Fig. 3B). ADCs can release drug by enzymatic cleavage after internalization into a cell and delivery to the lysosome. However, the stromal barrier in solid tumor prevents ADCs from reaching the cells. Unlike the limited cases of HER2-overexpressing BC, in most cases, heterogeneity of the tumor cells prevents the development of ADC therapy.
based on a cell-specific antigen. It seems that conventional cell-targeting ADC therapy is effective for hematological malignancy (ML) or hypervascular solid tumors having little stroma (BC), but not for high-stromal hypovascular solid tumors (PC).

**CAST therapy**

To overcome the limitations of conventional ADCs, we created CAST therapy, which utilizes abundant stroma as a scaffold for drug delivery (Fig. 3A and C). It was composed of ACAs conjugated to stroma (collagen IV or fibrin)-targeting mAb via an ester-bond linker. Our stroma-targeting ADCs were shown to reach tumor passively by the EPR effect. Moreover, they bound to the tumor stroma, from which effective sustained release of ACAs occurred. The released ACAs could be distributed throughout the tumor because the ester bond could gradually be cut by hydrolysis (non-enzymatically) outside of the cells. Polyethylene glycol (PEG) adjacent to the ester bond protected the degradation of the bond in the blood before the tumor was reached. ACAs released from the ADCs were shown to damage both tumor cells and tumor vessels, which resulted in the arrest of tumor growth. We focused on two stromal components, fibrin and collagen, the levels of which are generally high in tumors. In non-malignant diseases such as cerebrovascular disease, cardiac infarction, traumatic wound and rheumatoid arthritis, fibrin formation occurs at onset or during the active phase. At 7 days after onset, fibrin disappears and is replaced by collagen. A contracted collagenous scar is then formed as the final step of healing. On the other hand, in malignant tumor, bleeding, fibrin formation and collagen replacement as a malignant cycle of blood coagulation are reproduced as long as tumor cells exist and invade the adjacent vasculature.

To confirm the specificity of fibrin clot formation, anti-fibrin-specific mAbs were administered to mouse bearing both tumor and traumatic wound. The anti-fibrin mAbs disap-

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**Fig. 4.** Tumor specificity of anti-fibrin mAb. Biodistribution of Alexa 647-labeled anti-fibrin mAb (Red) and Alexa 555-labeled anti-collagen-1 mAb in mouse bearing a traumatic wound (left columns) and tumor (right columns) on days 1, 3 and 7 after the injection. OI: optical image.

**Fig. 5.** Anti-collagen IV ADC. (A) Anti-tumor effect of anti-collagen IV ADC (Coll.4) was compared with control (saline), non-specific mAb ADC (NS) and anti-EpCAM ADC (EpCAM). SN-38 is used as the payload. (B) Damaged tumor vasculature immunostained by CD31 (red), collagen IV (green) and DAPI (blue) is shown. Arrow indicates CD31-negative, collagen IV-positive ring structure. Scale bar, 20 μm.
appeared from healing traumatic wound, whereas they still accumulated in the non-healing tumor (Fig. 4).

**Anti-tumor activity of CAST therapy**

We used a PC xenograft model to examine the anti-tumor effect of anti-collagen ADC compared with anti-CD20 ADC as non-specific targeting, anti-EpCAM ADC as specific cell targeting or saline as a control. Anti-collagen IV ADC exerted the most potent antitumor activity among them7, 8) (Fig. 5A). Destroyed tumor vasculature that involved empty sleeves of basement membrane was observed7, 8) (Fig. 5B). There was no hepatotoxicity, nephrotoxicity or bone marrow toxicity in the treated mice. In addition, no autoimmune disease-like adverse effects such as arthritis were observed upon the administration of anti-collagen IV mAb, whereas anti-collagen II mAb combined with lipopolysaccharide caused severe arthritis7, 8). There were three issues in the xenograft model: (1) artificial stromal formation (low host reaction because of immunodeficiency, chimera status of mouse & human), (2) no early event of carcinogenesis and (3) rapid growth (leading to overestimation of the drug efficacy because clinical human cancer grows slowly). Therefore, we next used a mouse model of chemically induced skin cancer as a spontaneous tumor for the evaluation of anti-fibrin ADC6) (Fig. 6A). Anti-fibrin ADC showed strong anti-tumor activity against this fibrin-rich tumor6) (Fig. 6B and 6C). By in vivo fluorescence endomicroscopy, destroyed tumor vasculature was observed after the treatment6) (Fig. 6D).

**Proof of concept study and future prospects**

We are now conducting two proof of concept (POC) studies: 1) The pathophysiological specificity of fibrin deposition in malignant diseases has been demonstrated in the immunohistological examination of various human tissues and animal disease models, and 2) the specificity of anti-fibrin mAb has also been validated in an immunoPET/CT imaging study by a third research group (Hisada et al., unpublished data).

In this review, we have described the tumor stromal barrier and the development of CAST therapy. Besides the stromal barrier, microcirculation, inflammation or blood coagulation affects the pharmacokinetics and efficacy of antibody or ADC in the tumor microenvironment. Among these factors, microcirculation is very important because (1) it is the root between blood vessels and tumor cells (the stromal barrier limit microcirculation), and (2) it regulates tumor growth and metastasis (changes in the microcirculation context have direct effects on the efficacy of the drug). Therefore, we have to understand the mechanism of microcirculation-regulation by further investigation and identify methods of manipulation to develop innovative ADCs including CAST therapy.

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None.

References