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REVIEW ARTICLE

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Barriers to antibody therapy in solid tumors, and their solutions

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Abstract

Antibody drugs have become the mainstream of cancer treatment due to advances in cancer biology and Ab engineering. However, several barriers to Ab therapy have also been identified. These include various mechanisms for Ab drug resistance, such as heterogeneity of antigen expression in tumor cells and reduction in antitumor immunity due to expression diversity, polymorphism of Fc receptors (FcR) in effector cells, and reduced function of effector cells. Countermeasures to each resistance mechanism are being investigated. This review focuses on barriers that impede the delivery of Ab drugs due to features of the solid tumor microenvironment. Unlike hematological malignancies, in which the target tumor cells are in blood vessels, clinical solid tumors contain cancer stroma, which interferes with the delivery of Ab drugs. In addition, the cancer mass itself interferes with the penetration of Ab drugs. In this article, I will consider the etiology of cancer stroma and propose a new Ab drug development strategy for solid cancer treatment centering on cancer stromal targeting (CAST) therapy using anti-insoluble fibrin Ab-drug conjugate (ADC), which can overcome the cancer stroma barrier. The recent success of ADCs, chimeric antigen receptor T cells (CAR-Ts), and Bi-specific Abs is changing the category of Ab drugs from molecular-targeted drugs based on growth signal inhibition to cancer-specific targeted therapies. Therefore, at the end of this review, I argue that it is time to reorient the concept of Ab drug development.

KEYWORDS

ADC, antibody, antibody drug resistance, blood coagulation, cancer specificity, cancer stroma, CAST therapy, EPR effect, insoluble fibrin

1 | INTRODUCTION

The history of Ab drug development began with the development of a hybridoma methodology for the production of mAbs by Milstein and Kohler.[1] With the subsequent development of Ab engineering, Ab drugs have become the mainstream in cancer treatment today. Antibody therapy acts through a wide range of mechanisms, such as inhibition of growth factor receptors, angiogenesis factors, and immune checkpoints, neutralization of other target antigens, and enhancement of antitumor effects of effector cells such as natural killer (NK) cells and macrophages.[2,3]

In addition, clinical development of armed Abs bound to anticancer drugs or radiation nuclides has progressed, and some therapies have already been launched.[4,5] Antibody-drug conjugates (ADCs) with anticancer drugs and toxins have been studied since the 1980s, but until recently none had been approved. In the 2000s, gemtuzumab ozogamicin with calicheamicin conjugated to anti-CD33 Ab was approved for acute myeloid leukemia,[6] trastuzumab emtansine

with DM1 conjugated to anti-human epidermal growth factor receptor 2 (HER2) Ab was approved for metastatic breast cancer,[7] and brentuximab vedotin with monomethyl auristatin E (MMAE) conjugated to anti-CD30 Ab was approved for lymphoma.[8] Subsequently, we have witnessed an explosion in ADC development. Recently, trastuzumab deruxtecan, an anti-HER2 Ab conjugated to a camptothecin derivative, was approved for breast and gastric cancers and has received a great deal of attention.[9] Radioimmunotherapy (RIT), which uses a complex of radioisotopes and mAbs, initially focused on β-ray nuclide-binding RIT, but research on α-ray nuclide-binding Ab has also been active.[5,10,11] Conventional radiation therapy is a local treatment in which the tumor site is identified and intensively irradiated, whereas RIT is given intravenously and can target tumors that have spread throughout the body.

Recently, triggered by an initial approval for melanoma, immune checkpoint-inhibiting mAbs have been approved for various cancers and have had a large impact on cancer treatment.[12] In the category of modified mAbs, chimeric antigen receptor T cell (CAR-T) therapy was approved for hematological malignancies,[13] and bispecific Abs are also under development.[14]

However, barriers to these Ab therapies are becoming clear. Antibody drug resistance arises through multiple mechanisms, such as heterogeneity of antigen expression in tumor cells and reduced antitumor immunity due to expression diversity and polymorphism of FcR, although countermeasures have been investigated for each.[2] This review focuses on the lesser-known barrier that impedes the delivery of Ab drugs due to features of the solid tumor microenvironment.

2 | PATHOPHYSIOLOGY OF SOLID TUMORS RELATED TO ANTIBODY DELIVERY

2.1 | Immunoglobulin G selectively accumulates in solid tumors through the enhanced permeability retention effect

In solid tumors, there is an increase in tumor neovascularization, no corresponding increase in the lymphatic recovery system, and a marked increase in local vascular permeability. Utilizing these pathological properties, macromolecular substances that do not ordinarily leak out of normal blood vessels can leak easily from tumor blood vessels, and macromolecules that leak locally into the cancer tissue stay in place for a long time because of a lack of efficient lymphatic drainage. As a result, highly stable macromolecular substances that are not captured by the reticuloendothelial systems in the body and are not filtered from the renal glomerulus can accumulate selectively in cancer tissue. Collectively, these ideas are termed the enhanced permeability retention (EPR) effect[15] (Figure 1). The first paper on the EPR effect revealed the accumulation of various high-molecular-weight proteins in tumors; among

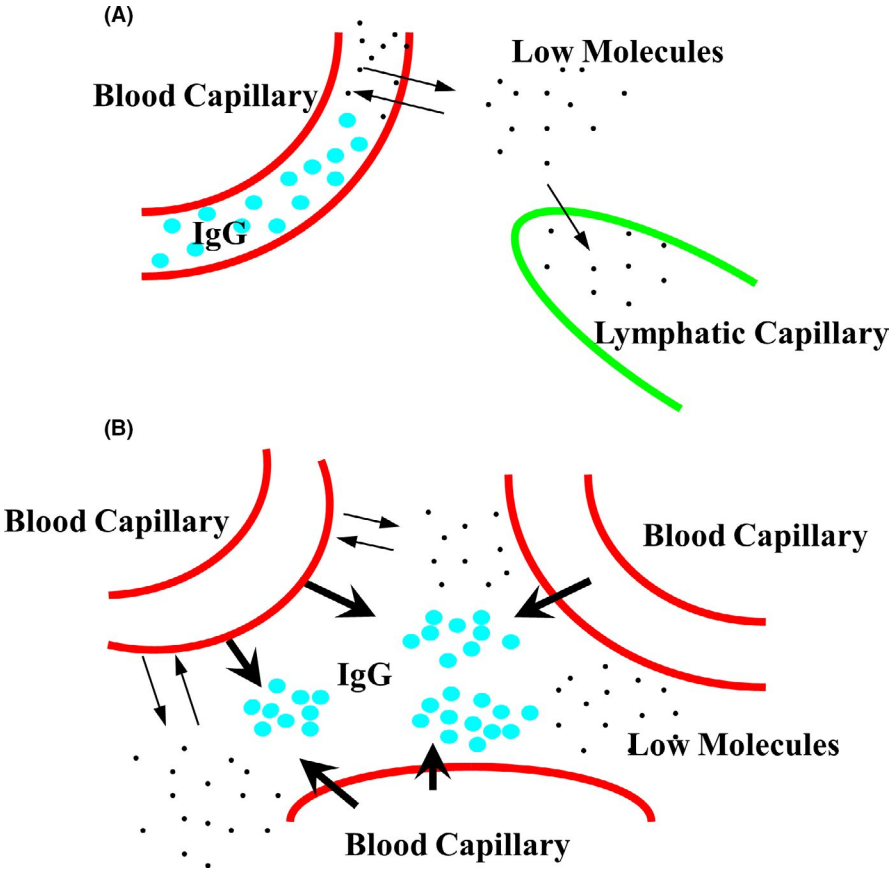

FIGURE 1 Diagram of the enhanced permeability retention (EPR) effect. (A) Small molecules easily leak from normal vessels, but macromolecules (including IgG) are too large to pass through normal vessel walls. (B) Even macromolecules can extravasate from tumor vessels and be retained in the tumor tissue for long periods of time due to the EPR effect (modified from Matsumura [2012]26)

them, IgG accumulated most efficiently. That is, IgG retains not only active targeting based on the antigen-Ab reaction, but also passive targeting ability. Animal experiments have contributed to the development of drug delivery systems (DDSs) for several payloads such as anticancer agents and nucleic acids, and these approaches have been accepted worldwide.[16,17] However, some clinical problems remain, and DDSs based on the EPR are not fully functional.[18] It has become clear that the most important mechanism underlying the EPR effect is tumor vascular hyperpermeability caused by the production of vascular permeability factors associated with cancer-induced hypercoagulation of blood. This increase in blood coagulation results in formation of cancer stroma, which interferes with the EPR effect.[19]

2.2 | Formation of cancer stroma due to increased blood coagulation in cancer tissue

In regard to cancer-induced hypercoagulation, the 19th-century French doctor Trousseau first reported an association between gastric cancer and thrombophlebitis in the extremities.[20] Hypercoagulation in cancer is also related to the production of tumor vascular permeability factors, which are important for EPR effects. We elucidated the mechanism by which vascular permeability factor kinins are produced in association with hypercoagulation of the intrinsic blood coagulation system.[21] Dvorak revealed that production of vascular endothelial growth factor (VEGF) is enhanced by extrinsic hypercoagulation.[22] Tissue factor (TF), which triggers extrinsic coagulation, is expressed on the surface of many human cancer cells, and tumor vascular endothelial cells are also positive for this marker.[23,24] Most importantly, cancer is not just a swelling, but grows while infiltrating and destroying its surroundings. If cancer clusters erode adjacent normal or tumor vessels, microscopic hemorrhage could occur at any place and any time within or adjacent to cancer tissues, and fibrin clots will form immediately in situ to stop the bleeding. The fibrin clots are subsequently replaced by collagen in a process similar to normal wound healing or other nonmalignant diseases. Unlike injuries, bleeding, fibrin, and collagen formation continue to occur and remain asymptomatic throughout the cancerous tissue as long as the cancer survives and grows in the body. As a result, the cancer stroma is rich in fibrin and collagen. This phenomenon becomes more pronounced as the cancer becomes more invasive.[25,26] Of course, fibrin is formed not only in malignant tumors but also at sites of injury, as well as during attacks of myocardial infarction, cerebral infarction, acute pancreatitis, and rheumatoid arthritis. In these nonmalignant diseases, fibrin is formed only at the onset and during the acute exacerbation period, and symptoms such as pain are always associated. After the extreme period, fibrin disappears and is replaced by

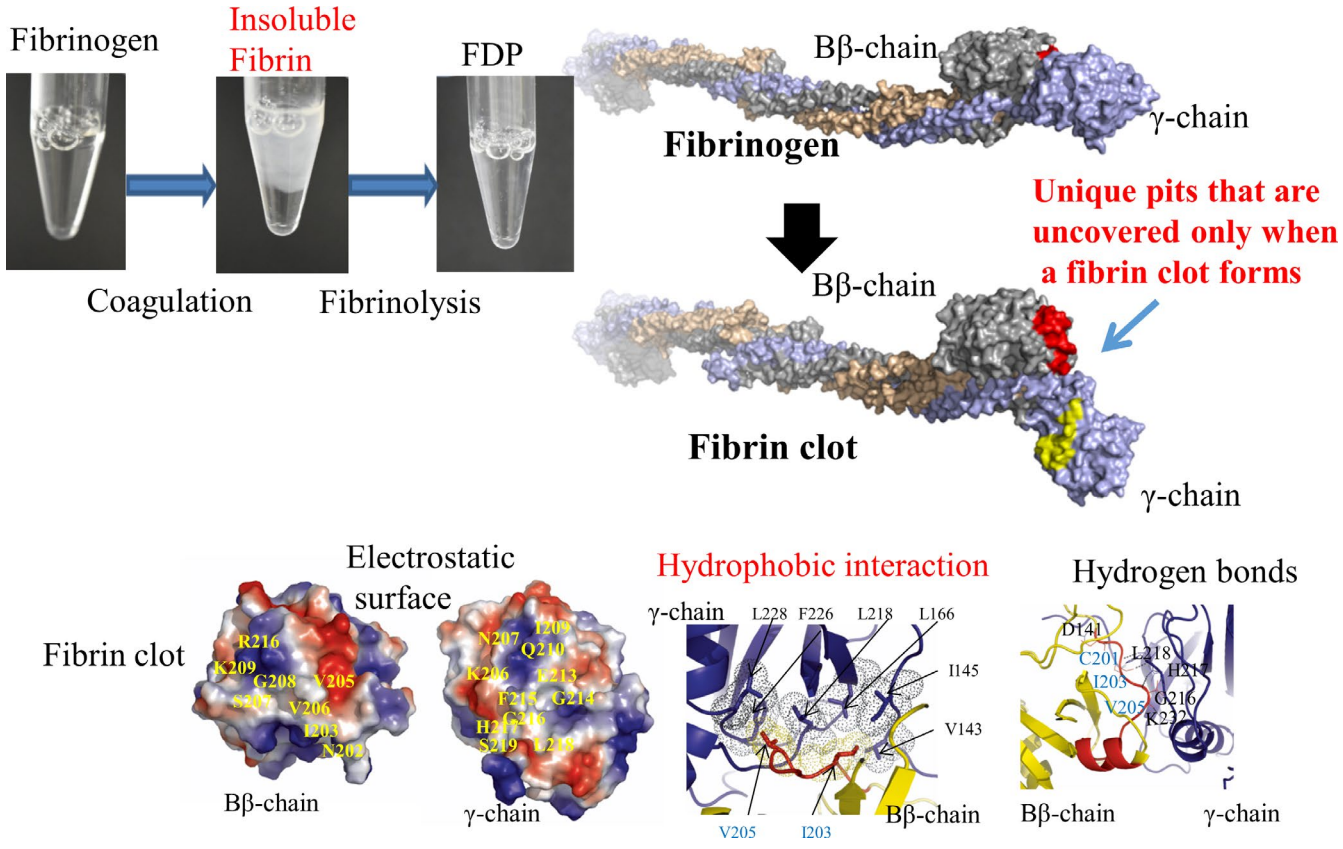

FIGURE 2 Structural change from fibrinogen to fibrin clot, and discovery of a unique pit in the fibrin clot. The epitope in the pits is a hydrophobic region on the β chain; in the soluble state, this region interacts closely with its counterpart region on the γ chain (modified from Hisada et al [2013]25). FDP, fibrin degradation product

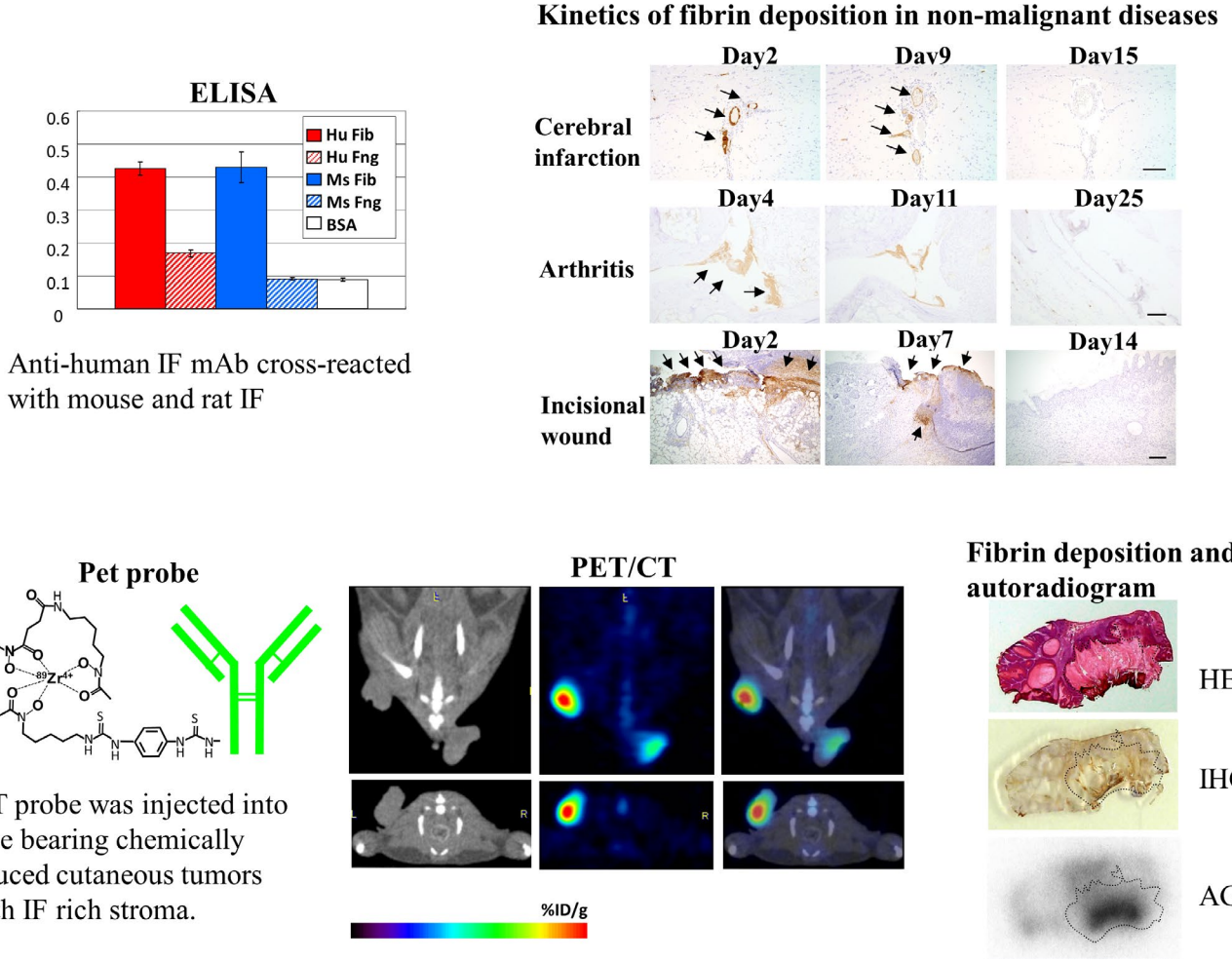

FIGURE 3 Kinetics of fibrin deposition in several nonmalignant disease models and PET/computed tomography (CT) with anti-insoluble fibrin (IF) mAb probe in spontaneous tumor models. Anti-IF mAb cross-reacted with mouse (Ms) or rat fibrin (Fib) clots. Immunohistochemistry (IHC) indicated that fibrin clot formation occurred only in the acute phase of nonmalignant diseases, and these clots virtually disappeared within a few weeks and were substituted by collagen in the late phase. Radiolabeled anti-IF mAb (PET probe) was injected into mice bearing chemically induced cutaneous tumors with abundant IF-rich stroma. The PET/CT scans show clear and specific accumulation in tumors (modified from Hisada et al [2013][25]). AG, autoradiography ; BSA, bovine serum albumin ; Fng, fibrinogen; Hu, human

collagen. Thus, we can conclude that asymptomatic and persistent fibrin formation is cancer-specific.[26]

3 | OVERCOMING BARRIERS THAT IMPEDE ANTIBODY DELIVERY

3.1 | Cancer stromal targeting (CAST) therapy

Solid cancers are nourished by tumor blood vessels present in the tumor stroma, and the drug also leaks from the tumor blood vessels, attacking the cancer cells. Clinical solid tumors are rich in cancer stroma, as mentioned above, and even high-molecular-weight drugs that leak from blood vessels must penetrate this dense stroma to reach the cancer cells. Therefore, cancer stroma is a barrier to macromolecular drugs, including Ab-based therapeutics. Among various interstitial components such as collagen, fibronectin, and proteoglycan in the cancer stroma, we focused on insoluble fibrin (IF), the final product of blood coagulation, as a lesion-specific molecule. Insoluble fibrin ground in liquid nitrogen was suspended in PBS and given as an immunogen in the abdominal cavity of mice, hybridomas were screened on fibrinogen plates and IF plates, and ultimately IF-specific Ab-producing clones were successfully established.[27] Subsequent analysis revealed that the epitope was on the β chain, which lines an indented structure that is exposed only when IF is formed.[25] In addition, in fibrinogen and soluble fibrin, the epitope site is closed by a hydrophobic bond between the β and the γ chains. Therefore, our anti-IF Ab, unlike previously produced anti-IF Abs, recognizes only IF and does not bind to the soluble precursor fibrinogen or fibrin degradation product (FDP; Figure 2). Fortunately, the amino acid sequences of the epitope are completely conserved from fish to humans, allowing experimental results in mice to be extrapolated to humans.[25] Positron emission tomography/computed tomography demonstrated that radiolabeled IF Abs selectively accumulate in chemically induced

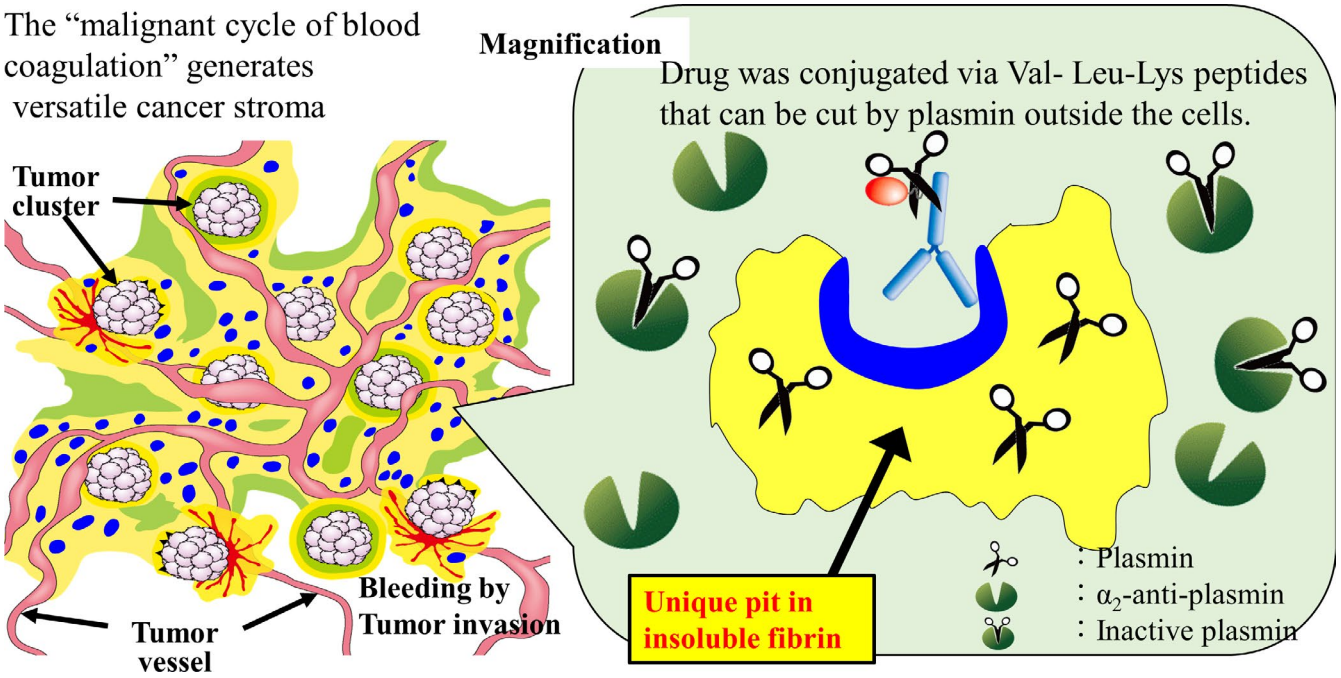

FIGURE 4 Diagram of cancer stromal targeting therapy (anti-insoluble fibrin (IF)-drug conjugate). The Ab-drug conjugate (ADC) selectively accumulates in tumor tissues due to the enhanced permeability retention (EPR) effect, binds to the specific pits in the fibrin clot, and creates a scaffold from which effective sustained release of free anticancer agent (ACA) occurs. Free ACA is only released when the ADC is bound to epitopes in insoluble fibrin because plasmin is active only on IF and is neutralized by endogenous α2-plasmin inhibitor circulating in the blood. The free ACA can easily reach the tumor (modified from Fuchigami et al [2018]28)

spontaneous tumors with remarkable fibrin deposition and abundant interstitial tissue, as in clinical human cancers (Figure 3).[25]

Based on these data, we prepared an ADC in which an anticancer drug was bound to an IF mAb. To enable therapeutic use of this ADC, the peptide in the linker moiety contained an amino acid sequence, Val-Leu-Lys, that is specifically cleaved by plasmin, which is activated only on IF. Moreover, this ADC can reach cancer tissues efficiently because anti-IF mAb is not neutralized by fibrinogen or FDP in the bloodstream due to its unique properties. The anti-IF ADC leaked into the cancer stroma due to the EPR effect, bound to IF, and the anticancer drug was released with plasmin. Because the released anticancer drug is a small molecule, it easily penetrates the stroma, is evenly distributed in solid cancer tissues, and efficiently damages cancer cells. The released anticancer drug also acted on tumor blood vessels.[28] Because plasmin is completely neutralized by plasmin inhibitors in vivo, except on IF, the release of anticancer drugs from the ADC occurs only on IF in the cancer stroma.[28] This treatment method was named cancer stromal targeting (CAST) therapy (Figure 4).[19,26,29] Thus far, more than 10 clones of anti-IF Abs have been established. Immunostaining revealed that clone 102-10 of the original Ab was nonspecifically bound, but a comparative study revealed that clone 1101 had higher specificity and sensitivity. In the case of ordinary ADC, the anticancer agent (ACA) is conjugated to the Ab by a particular linker that is cut by cathepsin in the lysosome following the internalization of the ADC by target cancer cells. In contrast, ADC classified as CAST therapy is targeted to IF in the cancer stroma, and the ACA is conjugated by Val-Leu-Lys peptides that can be cut by plasmin outside the cancer cells; subsequently, the released free ACA is internalized to cancer cells. Therefore, we are considering adding a more hydrophobic camptothecin-based ACA to the anti-IF Ab to increase internalization of the released ACA by cancer cells, as more hydrophobic ACA would be more efficiently internalized by cancer cells by hydrophobic interactions between the ACA and cancer cell membrane.

3.2 | Overcoming binding-site barriers in solid cancer

Tissue factor is a trigger protein for extrinsic blood coagulation.[30] As described above, vascular destruction, bleeding, blood coagulation, necrosis, and interstitial formation occur continuously in the cancer tissue, and TF is expressed in the cancer stroma. Tissue factor is actually expressed not only on the interstitium but also on the surface of many cancer cells, and higher expression is correlated with worse prognosis.[31,32] Therefore, it makes sense to target TF as an ADC. A clinical trial of an anti-TF ADC, tisotumab vedotin, is now underway.[33] We also established several anti-TF Abs, selected IgG clones 1084 and 1849, and prepared MMAE-added ADCs for both.[34] In surface plasmon resonance analysis, the dissociation constant (K_D) value of clone 1084 was approximately 65 times higher than that of 1849; that is, 1849 has clearly higher affinity. Although the association rate constant (K_a) of both clones are almost identical, the dissociation

SPR analysis

	K_a (1/Ms × 10^4)	K_d (1/s × 10^{-4})	K_D (nM) (=Kd/Ka)
1084ADC	2.61 ± 0.87	8.25 ± 0.64	34.96 ± 0.47
1849ADC	4.20 ± 1.94	0.21 ± 0.09	0.54 ± 0.19

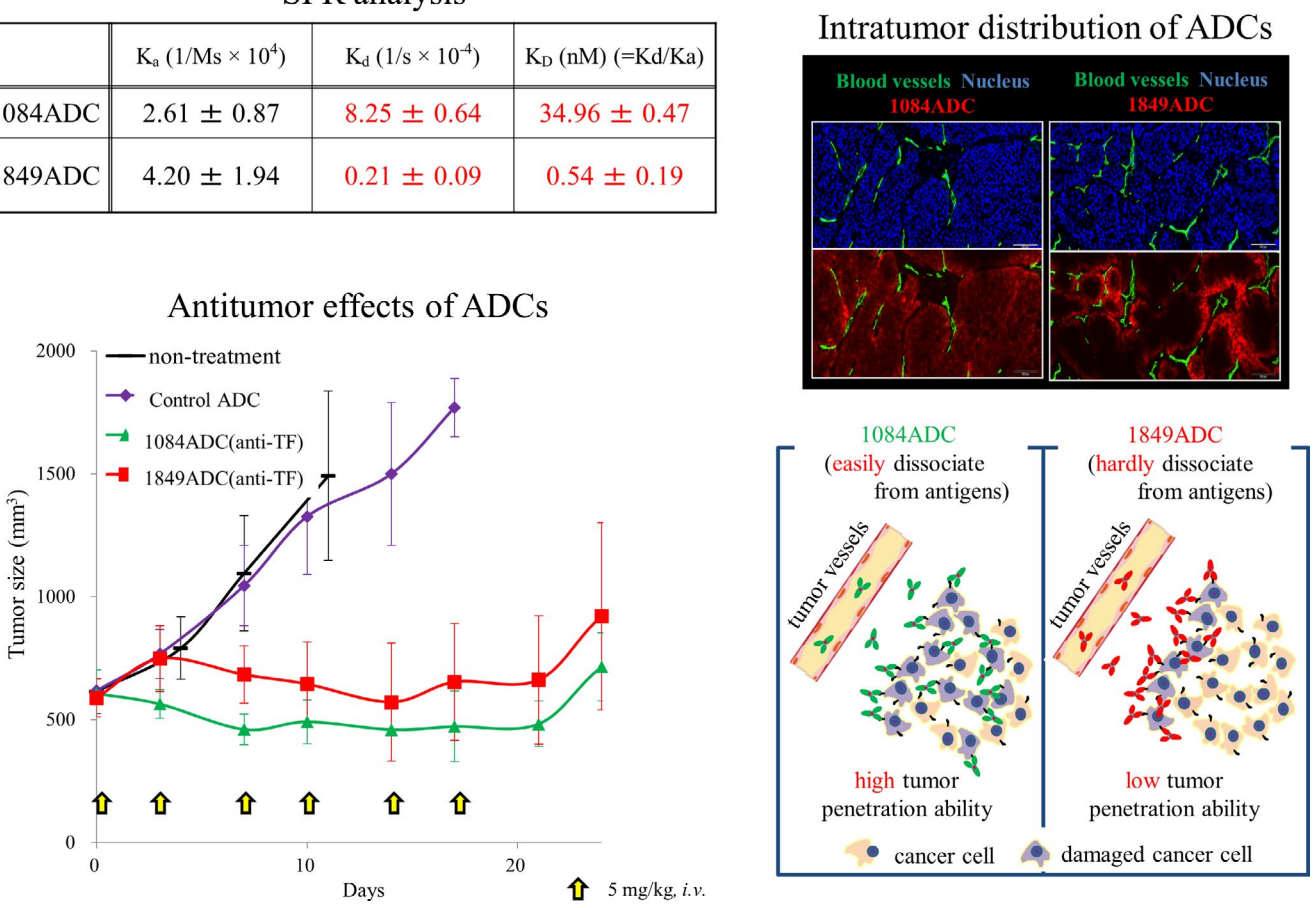

FIGURE 5 Binding site barrier. An Ab-drug conjugate (ADC) with higher affinity does not always exert higher antitumor activity. Penetration efficiency should be more seriously considered in clinical practice. Immunofluorescence staining revealed that 1084ADC was distributed evenly in all BxPC3 tumors, confirming the efficient penetration of 1084ADC into the central region of the tumors. In contrast, 1849ADC was mainly localized near blood vessels (modified from Tsumura et al [2018]34). K_a, association rate constant; K_d, dissociation rate constant; K_D, dissociation constant; SPR, surface plasmon resonance; TF, tissue factor

rate constant (K_d) value of 1084 was 39-fold greater than that of 1849. In other words, 1084 has the same ability to bind the antigen as 1849, but once bound, 1084 can be easily separated from the antigen. In in vitro studies, ADCs based on 1084 and 1849 had comparable internalization efficiencies and cell-killing effects. However, in an antitumor effect study in which experiments were started with a large tumor size (600 mm^3) in vivo, the antitumor effect of the 1084 ADC was significantly higher than that of the 1849 ADC.[34] From the standpoint of affinity, this result is the exact opposite of what was expected, but it is presumed that the difference in effect is due to the difference in dissociation rate constants. That is, 1084 ADCs and 1849 ADCs bind to antigens in the same way, but 1084 ADCs dissociate more easily than 1849 ADCs, resulting in more efficient penetration through the tumor tissues. In fact, 1084 was evenly distributed throughout the tumor tissue 3 hours after administration, whereas 1849 was mainly present around tumor blood vessels (Figure 5). These results suggested that the dissociation rate constant of IgG may affect the antitumor effect of ADCs.[34]

If the target cancer is leukemia in a blood vessel, K_D could reign supreme, but for solid tumors, tissue penetration ability due to efficient dissociation could be more important.

4 | FUTURE DIRECTIONS OF CAST THERAPY

We reported CAST therapy after many years of DDS research.[19,26,27] Later, Gebleux et al[35] from ETH in Switzerland and Szot et al[36] from NCI in the United States reported strategies targeting the cancer stroma; however, these approaches have not been clinically developed. Therefore, we are rushing to determine Ab clones, linker structures, and additional anticancer agents, with the aim of clinically developing anti-IF Ab ADCs. Following several non-GMP procedures, good manufacturing practice (GMP) production of Abs and ADCs and good laboratory practice (GLP) toxicity testing will be initiated, and companion diagnostics should be developed at the same time. However, unlike normal surface antigens of cancer cells, IF is

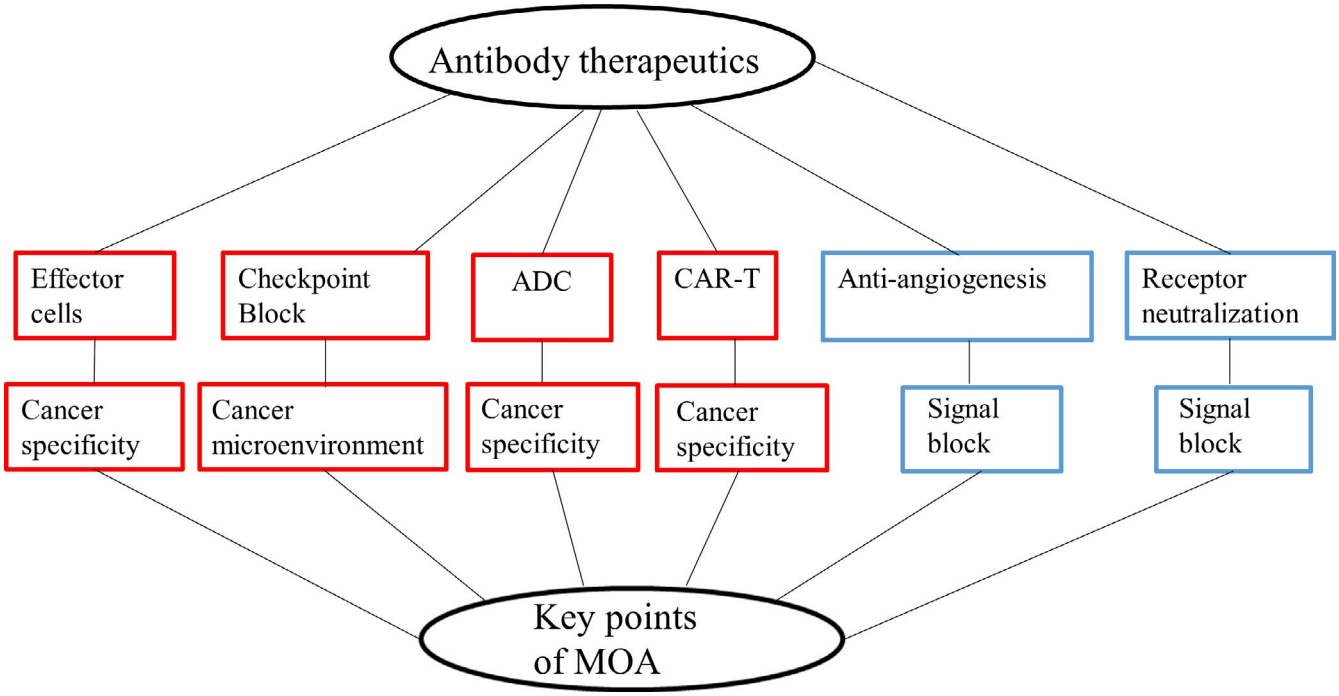

FIGURE 6 Various modes of action of Ab therapeutics. Typical modes of action (MOA) for Ab therapy were selected. The Abs shown in blue boxes act primarily by inhibiting cancer growth signals. Cancer specificity is of the utmost importance for the Ab drugs shown in red boxes. ADC, Ab-drug conjugate; CAR-T, chimeric antigen receptor T cell

irregularly distributed in the interstitium; thus, it could be difficult to determine therapeutic indications by conventional immunohistochemical staining. Therefore, we are investigating methods for quantifying D-dimer in blood released from cancer tissues.[37]

Recent work showed that the combined use of an immune checkpoint inhibitory Ab and an anticancer drug is useful, and such combined use has become the standard treatment in clinical practice.[38] Our CAST therapy can selectively attack suppressive T cells even in the interstitium of a cancer that has spread throughout the body; hence, we consider it to be a more effective combination partner.

Furthermore, hypercoagulation is related not only to cancer but also to blood diseases, infectious diseases, and organ transplantation.[39] Accordingly, we anticipate that the range of applications of this anti-IF mAb will expand.[40,41]

5 | A NEW IDEA OF CANCER TREATMENT BASED ON THE MECHANISM OF ACTION OF ANTIBODY THERAPY

Antibody drugs for the past 20 years have generally been classified as molecularly targeted agents. For example, the epidermal growth factor receptor (EGFR) Ab binds to EGFR on the surface of cancer cells, neutralizes its function, stops the intracellular growth signal, and exerts a cell-killing effect. Consequently, anti-EGFR mAbs have become standard treatments for colorectal cancer. However, EGFR is strongly expressed in normal skin and normal mucosa and, unlike hematological malignancies, growth signals for solid tumors are identical to those of normal mitotic epithelial cells. In clinical practice, anti-EGFR Ab therapy is a useful treatment because its antitumor effect outweighs adverse events. I believe that the usefulness of this treatment is exactly due to the EPR effect in cancer tissues.

With the recent success of CAR-T and ADC, Ab development strategies have changed significantly, largely because CAR-T and ADC do not need to neutralize growth signals. Instead, cancer specificity is essential in the new tactics for Ab therapeutics. Cancer specificity is also essential for bispecific Abs, which are being actively studied (Figure 6).

6 | CONCLUSION

In this review, we described Ab drugs from the perspective of DDS. We also clarified that its effect is diminished as a result of various barriers, even though Ab drugs accumulate selectively in the tumor. As with CAST therapy, which we emphasized here, future development of Ab drugs will take into account not only the molecular theory of cancer cells but also the dynamic pathophysiology such as blood coagulation that occurs in cancer tissues.

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CONFLICT OF INTEREST

Yasuhiro Matsumura is a cofounder, shareholder, and Board Member of RIN Institute Inc., the company that owns the anti-insoluble fibrin Ab.

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REFERENCES

1. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature*.` 1975;256:495-497.
2. Shah A, Rauth S, Aithal A, et al. The current landscape of antibody-based therapies in solid malignancies. *Theranostics*. 2021;11:1493-1512.
3. Lu R-M, Hwang Y-C, Liu I-J, et al. Development of therapeutic antibodies for the treatment of diseases. *J Biomed Sci*. 2020;27:1-30.
4. Drago J, Modi S, Chandarlapaty S. Unlocking the potential of antibody-drug conjugates for cancer therapy. *Nat Rev Clin Oncol*. 2021;18:327-344.
5. Kairemo KJ. Radioimmunotherapy of solid cancer: A review. *Acta Oncol*. 1996;35:343-355.
6. Castaigne S, Pautas C, Terré C, et al. Effect of gemtuzumab ozogamicin on survival of adult patients with de-novo acute myeloid leukaemia (ALFA-0701): a randomised, open-label, phase 3 study. *Lancet*. 2012;379:1508-1516.
7. Verma S, Miles D, Gianni L, et al. Trastuzumab Emtansine for HER2-positive advanced breast cancer. *N Engl J Med*. 2012;367:1783-1791.
8. Younes A, Bartlett NL, Leonard JP, et al. Brentuximab vedotin (SGN-35) for relapsed CD30-positive lymphomas. *N Engl J Med*. 2010;363:1812-1821.
9. Doi T, Shitara K, Naito Y, et al. Safety, pharmacokinetics, and antitumour activity of trastuzumab deruxtecan (DS-8201), a HER2-targeting antibody-drug conjugate, in patients with advanced breast and gastric or gastro-oesophageal tumours: a phase 1 dose-escalation study. *Lancet Oncol*. 2017;18:1512-1522.
10. Gill MR, Falzone N, Du Y, Vallis KA. Targeted radionuclide therapy in combined-modality regimens. *Lancet Oncol*. 2017;18:414-423.
11. Takashima H, Koga Y, Manabe S, et al. Radioimmunotherapy with an 211At-labeled anti-tissue factor antibody protected by sodium ascorbate. *Cancer Sci* 2021;112(5):1975-1986.
12. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer*. 2012;12:252-264.
13. Schuster SJ, Bishop MR, Tam CS, et al. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. *New Engl J Med*. 2019;380:45-56.
14. Krishnamurthy A, Jimeno A. Bispecific antibodies for cancer therapy: A review. *Pharmacol Ther*. 2018;185:122-134.
15. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. *Cancer Res*. 1986;46:6387-6392.
16. Duncan R. The dawning era of polymer therapeutics. *Nat Rev*. 2003;2:347-360.
17. Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer B. Nanocarriers as an emerging platform for cancer therapy. *Nat Nanotech*. 2007;2:751-760.
18. Matsumura Y, Gotoh M, Muro K, et al. Phase I and Pharmacokinetic Study of MCC-465, a doxorubicin (DXR) encapsulated in PEG-immunoliposome, in patients with metastatic stomach cancer. *Annals Oncol*. 2004;15:517-525.
19. Matsumura Y. Cancer stromal targeting therapy to overcome the pitfall of EPR effect. *Adv Drug Deliv Rev*. 2020;154:142-150.
20. Trousseau A. Pegmasia alba dolens, vol 3. Balliere JB et Fils, Paris, 1865.
21. Matsumura Y, Kimura M, Yamamoto T, Maeda H. Involvement of the kinin-generating cascade and enhanced vascular permeability in tumor tissue Jpn. *J Cancer Res*. 1988;79:1327-1334.
22. Dvorak HF. Vascular permeability factor/vascular endothelial growth factor: a critical cytokine in tumor angiogenesis and a potential target for diagnosis and therapy. *J Clin Oncol*. 2002;20(21):4368-4380.
23. Stein PD, Beemath A, Meyers FA, Skaf E, Sanchez J, Olson RE. Incidence of venous thromboembolism in patients hospitalized with cancer. *Am J Med*. 2006;119:60-68.
24. Saito Y, Hashimoto Y, Kuroda J-I, et al. The inhibition of pancreatic cancer invasion-metastasis cascade in both cellular signal and blood coagulation cascade of tissue factor by its neutralisation antibody. *Euro J Cancer*. 2011;47:2230-2239.
25. Hisada Y, Yasunaga M, Hanaoka S, et al. Discovery of an uncovered region in fibrin clots and its clinical significance. *Sci Rep*. 2013;3:2604-2610.
26. Matsumura Y. Cancer stromal targeting (CAST) therapy. *Adv Drug Deliv Rev*. 2012;64:710-719.
27. Yasunaga M, Manabe S, Matsumura Y. New concept of cytotoxic immunoconjugate therapy targeting cancer-induced fibrin clots. *Cancer Sci*. 2011;102:1396-1402.
28. Fuchigami H, Manabe S, Yasunaga M, Matsumura Y. Chemotherapy payload of anti-insoluble fibrin antibody-drug conjugate is released specifically upon binding to fibrin. *Sci Rep*. 2018;8:14211.
29. Matsumura Y. Principle of CAST strategy. In: Matsumura Y, Tarin D, eds. *Cancer Drug Delivery Systems based on the Tumor Microenvironment*. Tokyo: Springer; 2020:255-268.
30. van den Berg YW, Osanto S, Reitsma PH, Versteeg HH. The relationship between tissue factor and cancer progression: insights from bench and bedside. *Blood*. 2012;119:924-932.
31. Breij ECW, de Goeij BECG, Verploegen S, et al. An antibody-drug conjugate that targets tissue factor exhibits potent therapeutic activity against a broad range of solid tumors. *Cancer Res*. 2014;74:1214-1226.
32. Koga Y, Manabe S, Aihara Y, et al. Antitumor effect of antitissue factor antibody-MMAE conjugate in human pancreatic tumor xenografts. *Int J Cancer*. 2015;137:1457-1466.
33. Bono JS, Concin N, Hong DS, et al. Tisotumab vedotin in patients with advanced or metastatic solid tumours (InnovaTV 201): a first-in-human, multicenter phase 1–2 trial. *Lancet Oncol*. 2019;20:383-393.
34. Tsumura R, Manabe S, Takashima H, Koga Y, Yasunaga M, Matsumura Y. Influence of the dissociation rate constant on the intra-tumor distribution of antibody-drug conjugate against tissue factor. *J Control Release*. 2018;284:49-56.
35. Gebleux R, Stringhini M, Casanova R, Soltermann A, Neri D. Non-internalizing antibody-drug conjugates display potent anticancer activity upon proteolytic release of mono Methyl auristatin E in the sub-endothelial extracellular matrix. *Int J Cancer*. 2018;140:1670-1679.

36. Szot C, Saha S, Zhang XM, et al. Tumor strom-targeting antibody-drug conjugate triggers localized anticancer drug release. *J Clin Invest*. 2018;128:2927-2943.
37. Beer JH, Haeberli A, Vogt A, et al. Coagulation markers predict survival in cancer patients. *Thromb Haemost*. 2002;88:745-749.
38. Gandhi L, Rodríguez-Abreu D, Gadgeel S, et al. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. *N Engl J Med*. 2018;378:2078-2092.
39. In Colman RW, Marder VJ, Clowes AW, George JN, Goldhaber SZ, eds. *Hemostasis and Thrombosis*. Philadelphia: Lippincott Williams & Wilkins; 2006.
40. Prince R, Bologna L, Manetti M, et al. Targeting anticoagulant protein S to improve hemostasis in hemophilia. *Blood*. 2018;131:1360-1371.
41. Calzavarini S, Prince-Eladnani R, Saller F, et al. Platelet protein S limits venous but not arterial thrombosis propensity by controlling coagulation in the thrombus. *Blood*. 2020;135:1969-1982.

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