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Review

Macromolecular therapeutics in cancer treatment: The EPR effect and beyond

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ABSTRACT

In this review, I have discussed various issues of the cancer drug targeting primarily related to the EPR (enhanced permeability and retention) effect, which utilized nanomedicine or macromolecular drugs. The content goes back to the development of the first polymer–protein conjugate anticancer agent SMANCS and development of the arterial infusion in Lipiodol formulation into the tumor feeding artery (hepatic artery for hepatoma). The brief account on the EPR effect and its definition, factors involved, heterogeneity, and various methods of augmentation of the EPR effect, which showed remarkably improved clinical outcomes are also discussed. Various obstacles involved in drug developments and commercialization are also discussed through my personal experience and recollections.

Contents

1. Introduction: from the past to the present

From the time of the hypothetical concept of the *magic bullet* proposed by Paul Ehrlich at the end of the 19th century, almost 40–50 years elapsed before the appearance of practical clinical drugs such as sulfonamide and penicillin for the control of microbial infections. However, more than 100 years have passed since Ehrlich's concept to achieve advances in cancer treatment. The field of cancer chemotherapy began when, in 1943 during World War II in Bari, Italy, nitrogen mustard gas, a chemical warfare agent, was accidentally found to have antileukemic activity. However, research aimed at discovering acceptable, effective anticancer agents has not achieved its goal. Only in the last 10 years have we developed promising agents such as imatinib (Glivec), which is quite effective against chronic myelogenous leukemia. Although SMANCS (discussed below) was such a candidate, interest in it has been declining because of business

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reasons. Thus, antitumor drugs that would be effective against a wide range of solid tumors are not available, and ideal drugs that act against solid tumors do not yet exist. In fact, the overall cancer mortality rate has not changed very much in the past 50 years [1,2], whereas the mortality rate in patients with bacterial infections has dropped dramatically since antibiotics became available.

One of the fundamental difficulties in cancer therapy lies in the great genetic diversification seen in human cancers, even in individual patients in which the process of genetic alteration of patients' tumor would have progressed in the past 10–30 years by the time when the patient tumor was diagnosed. In the past 10–20 years, so-called molecular target drugs became popular as the least toxic but promising therapeutics. This trend, despite its being fashionable, is now rather problematic. In fact, these agents, such as the well-known example of molecular target drug, rofecoxib (Vioxx®, though it is not anticancer drug), with more than US$ 10 billion being paid as compensation for unexpected adverse effects, are not miracle drugs. The highly diverse genetic mutations in individual cancer patients, which were just mentioned, make the drug development strategy of utilizing a single molecular target, based on a tumor-specific molecular epitope or target enzyme, very difficult if not completely impossible [3–6].

Other concerns about molecular target anticancer agents include not only poor efficacy but also the cost of drugs to patients. Some drugs, for example, cost far more than interferon or other anticytokine agents. Many molecular target drugs for cancer cost almost US$ 10,000 per dose, or more than US$ 100,000 per year, yet the expected prolongation of life is a few weeks more than the expected survival time of 3 or 5 years for control subjects not receiving the drugs. One can find such information in various references [e.g., 7–12].

With regard to a new modality with potentially less cost, the drug-targeting method based on the enhanced permeability and retention (EPR) effect has a more universal application for solid tumors, so lower costs seem possible, with greater therapeutic effects on more types of tumors and fewer adverse effects. These reasons provide a good rationale for pursuing this method and encouraging such drug development. As this symposium issue will show, anticancer drugs that are more generally effective against solid tumors should be developed, and investigation of the EPR effect, which is based macromolecular therapeutics, will lead to ideal candidates. (Other examples, e.g., that of F. Kratz et al., will also appear in this special issue). Tables 1 and 2 summarize the definition of the EPR effect and the factors involved in solid tumors and inflammation.

One can, of course, argue that the heterogeneity of the EPR effect reduces its universal validity. The EPR effect is not perfect or effective for all solid tumors, because tumors of different patients vary greatly in actual clinical settings. For example, tumor diameters can be less than 1 cm to larger than 10 cm; and tumors can be highly hypoxic to normoxic, can have different pathological classes, are genetically diverse, can have partial or extensive necrosis, can have occluded or compressed vascular systems with or without blood coagulation in or around the tumor mass, and so on. This heterogeneity can be overcome in a number of ways. For instance, modulating the patient's hydrodynamic state by systemic (i.v.) infusion of angiotensin II leads to higher blood pressure on the laminar side and more effectively pushes a drug into the tumor interstitium. Section 3 in this article demonstrates the proof of this method in the clinical settings. We have also developed easier methods utilizing various vascular mediators, as given in Table 3.

Table 1
Profiles of the EPR effect.

Characteristics	Comments
Molecular size	Above 40 kDa; 800 kDa still shows an active EPR effect
Biocompatibility	No coagulation, no interaction with blood components and blood vessels, no cell lysis, no RES[a] clearance (e.g., macrophages). Protease bound protease-inhibitor is cleared in a few min even though biocompatible macromolecules.
Time required to achieve EPR effects	More than several hours in circulation in mice[b]. The trend can be seen even initial 30 min.
Drug retention time of macromolecular drugs in tumor	Mostly days to weeks[a], in great contrast to passive targeting of low MW drugs which is only few minutes[b].
pH (isoelectric point)/surface change	Weakly acidic to weakly cationic. Polycationic particles will disappear rapidly from circulation.

[a] Reticuloendothelial system, such as macrophages.
[b] Passive tumor targeting (visualization) can be observed by radiography with low MW contrast agents upon infusion into the artery. These images are visible for only a few minutes after the infusion, which is typical in passive targeting, but disappears in a few min. In contrast biocompatible nanomedicine exhibits prolonged tumor-retention period of days of weeks. Thus a great contrast to the passive targeting to the EPR effect.

Table 2
Factors and mediators involved in the EPR effect in cancer and inflammation, and their responsible enzymes or effectors.[a]

EPR effect-enhancing factors/ mediators	Enzymes responsible for factors	Comments: actions of enzymes and factors, or sources of factors
1. Bradykinin (kinin)	Kallikrein and other proteases, plasminogen activator produce bradykinin	Angiotensin I-converting enzyme (ACE) degrades kinin; ACE-inhibitor potentiates activity by blocking kinin degradation. Kinin induces NO synthase
2. Nitric oxide (NO)	Nitric oxide synthase (NOS), inducible isoform of NOS (iNOS)	Nitroglycerin, isosorbide dinitrate (ISDN, Nitrol®), and nitroprusside yield nitrate, and nitrite-reductase, which occurs in hypoxic tissue (tumor), generates NO in hypoxic tumors.
3. Prostaglandins (PGs)	Cyclooxygenase 2 (COX-2)	PGI_2 agonist/beraprost affect the EPR effect
4. Carbon monoxide (CO)	Heme oxygenase-1 (HO-1)	Hemin, NO, and ultraviolet light, and heat induce HO-1
5. Peroxynitrite ($ONOO^-$)	Generated by $NO + O_2^-$	Extremely rapid reaction
6. Matrix metalloproteinase (MMP), or collagenase (← proMMP)[b]	Procollagenase activation by $ONOO^-$	$ONOO^-$ activates pro-MMP[b] → MMP
7. Vascular endothelial growth factor (VEGF/VPF)	Nitric oxide synthase (NOS)	NO, endotoxin, and other cytokines can induce this VEGF
8. Tumor necrosis factor α (TNF-α) and TFG-β inhibitor	Cytokines, growth factor	Induces inflammation and normalization of tumor vasculature
9. Heat	Heat shock protein, HO-1 (HSP-32)	e.g. HO-1 and inflammation etc.

See text for detail.
[a] Above factors are most common mediators of inflammation and cancer that facilitate extravasation.
[b] proMMP: pro-matrix metalloproteinase (collagenase) is activated by $ONOO^-$ or by other proteases.

2. Our prototype polymer-conjugate drug, SMANCS, and a new strategy for intraarterial (i.a.) infusion: the ultimate tumor-targeted delivery

In 1979, we pioneered the development of the protein–polymer conjugate SMANCS, which is the antitumor protein drug neocarzinostatin (NCS) chemically conjugated with a synthetic copolymer of styrene–maleic acid copolymer (SMA) [13–15]. SMANCS exhibited unique properties compared with the parental NCS [14–18]. These properties included (i) prolongation of the plasma $t_{1/2}$ (by 20-fold); (ii) improved tumor-targeting capacity because of the EPR effect, i.e., a markedly higher (10- to 20-fold) intratumor concentration compared with the

Table 3
Strategies to overcome the heterogeneity of the EPR effect, and augmentation of the EPR effect to enhance tumor drug delivery.[a]

Methods[a]	Mechanism	Remarks
1. Use of angiotensin II-induced hypertension	Hydrodynamic; vasoconstriction induced hypertension → mechanical opening of endothelial cell-cell gaps passively.	Drug is infused into the tumor-feeding artery via cathether.
2. Use of angiotensin I-converting enzyme (ACE) inhibitor such as enalapril	Selectively elevates the kinin level only in tumors, by inhibiting kinin degradation by ACE-inhibitor, which occurs in the tumor tissue.	Given orally, very safe, clinically proven.
3. Use of nitroglycerin given topically by dermal patch, or by infusion via the tumor-feeding artery	Generates NO in hypoxic tumor issue selectively. See analogy to angina pectoris.	Nitroglycerin, isosorbide dinitrate (ISDN, Nitrol®), nitroprusside, and others; clinically proven (see text).
4. Use of prostaglandin (PG) I_2 analogue, beraprost sodium	PG agonist effect (with the $t_{1/2}$ more than 100 times longer in plasma) when given orally.	
5. Use of TGF-β-inhibitor	TGF-β is tumor growth and differentiation factor. Facilitate productive of extracellular matrix. The inhibitor counteracts to restore vascular maturation and normalization, which may be affected by vascular mediator.	Shown effective in the pancreatic cancer in vivo model.
6. Use induction of HO-1, or a CO generator (ruthenium tri carbonyl, CORM2[b])	Zn protoporphyrin or hemin–polymer conjugates induce HO-1 in tumors; use of CORM2 generates CO. See text.	No data available for in vivo therapeutic efficacy.

[a] These strategies will be effective only with nanoparticle or polymeric drugs.
[b] Carbon monoxide-releasing molecule.

concentration in plasma [15–21]; (iii) no immunogenicity [15]; and (iv) higher lipophilicity, which enabled solubilization and formulation with a lipid contrast agent (Lipiodol®) as a carrier (i.e., the SMANCS/Lipiodol formulation) [14,18,21–25]. This lipid formulation allowed truly selective tumor targeting and tumor delivery by infusion into the tumor-feeding artery via a catheter under X-ray guidance of angiographic technique as viewed on the monitoring screen [21–25]. A drug concentration in the tumor as much as 2000 times the concentration in blood (2000:1) can be achieved by using this method [22]. The EPR effect is now known to allow most macromolecular drugs to be selectively delivered to solid tumors, where they remain for very long periods, several weeks or months or even more [16,18,21–25]. This sustained drug activity will result in a marked therapeutic effect [18,22–24].

3. Advancements in tumor targeting with SMANCS/Lipiodol via the i.a. route

We have now extended the application of SMANCS/Lipiodol therapy, administered via i.a. infusion, to advanced, difficult-to-treat solid tumors such as massive and multiple metastatic liver cancers, bile duct carcinomas and cholangiocarcinomas, and pancreatic cancers and their metastatic nodules in the liver [24]. We also successfully treated massive renal cell cancer similarly, by infusion into the renal artery. Descriptions of these examples have been published [18,25]. In this article, we provide examples of such augmented drug delivery, by means of angiotensin II-induced high blood pressure, to advanced, difficult-to-treat tumors: pancreatic cancer with metastatic liver cancer (Fig. 1A and B), and metastatic liver cancer that had originated from gastric cancer, which had previously been removed (Fig. 1C and D).

For both cases, we infused SMANCS/Lipiodol i.a. under conditions of angiotensin II-induced high blood pressure (e.g., from 100 mm Hg to 150 mm Hg) [25]. The blood pressure of 150–160 mm Hg was achieved via slow i.v. infusion of 0.5 μg/ml angiotensin II, that is set in a 20 ml infusion syringe-pump. This method offers not only an improved therapeutic effect but also a diagnostic value, given the highly sensitive detection, by means of computed tomography (CT), of the tumor-selective uptake of Lipiodol, even in small tumor nodules with diameters of 3–5 mm. Another advantage of using angiotensin II-induced high blood pressure is application to more types of tumors that may be treated by this method. In fact almost all cases responded very well (25). Under normotension, as SMANCS/Lipiodol was originally used, the drug was most effective for primary liver cancer (hepatocellular carcinoma) but was less effective for metastatic liver cancer and cholangiocarcinoma. The reason for this difference may be poor drug delivery to the tumor because of the heterogeneity of the EPR effect. As Fig. 1 shows, the improved delivery method that utilizes angiotensin II-induced high blood pressure indeed makes SMANCS/Lipiodol highly effective. Another benefit of this method is the reduced time required for tumor regression (e.g., to achieve 50% of tumor volume), perhaps because of increased targeted drug delivery, and with less frequent drug administration needed.

4. Heterogeneity of the EPR effect, which hinders tumor delivery, and the method of circumventing this heterogeneous drug delivery [16–18]

Although the EPR effect offers the first step in the process of delivering drugs to tumor tissue or near tumor cells, solid tumors in clinical settings frequently have heterogeneous characteristics as described in Section 1, and some tumors impede drug access to tumors because of necrosis, fibrosis, clot formation, or interference by stromal tissue [16,17,26,27]. However, in the past several years, we have devised ways, by means of different techniques, to improve this process of drug delivery to such tumors, as described below and as shown in Table 3.

4.1. Induced hypertension by using a slow i.v. infusion of angiotensin II

Inducing hypertension via a slow i.v. infusion of angiotensin II [21,25,28,29] is more useful for macromolecular drugs and drug/Lipiodol formulations given during arterial infusion than for low-MW drugs. This method was briefly described above (Section 3). Low-MW drugs offer little advantage in this method, perhaps because of rapid diffusion or washout [29].

4.2. Using nitroglycerin or other nitric oxide (NO)-releasing agents

Nitroglycerin and other NO-releasing agents generate NO from NO_2^- selectively in hypoxic tumor tissue compared with normoxic tissues [30,31]. Thus, such nitro agents facilitate the EPR effect via local NO generation in tumors, with drug delivery enhanced 2- to 3-fold and an improved therapeutic effect. Yasuda et al. [32,33] and Siemens et al. [34] also demonstrated the beneficial effect of NO-releasing agents used in combination with conventional low-MW drugs. In this review, I describe clinical cases of bronchogenic lung cancer for which isosorbide dinitrate (ISDN), an NO-releasing agent, was administered 50–100 μg in 1–2 ml of physiological saline, bolus, via the bronchial artery immediately before SMANCS/Lipiodol infusion into the same bronchial artery (see Section 6).

4.3. Using an angiotensin-converting enzyme inhibitor (ACEI)

Solid tumors generate bradykinin, which would aid the EPR effect. ACEIs inhibit the degradation of bradykinin, thus raising the local bradykinin concentration in tumor tissue more than in other tissues in the body [16,17,26]. For example, use of the combination of an ACEI and hypertension improved monoclonal antibody delivery 2- to 3-fold

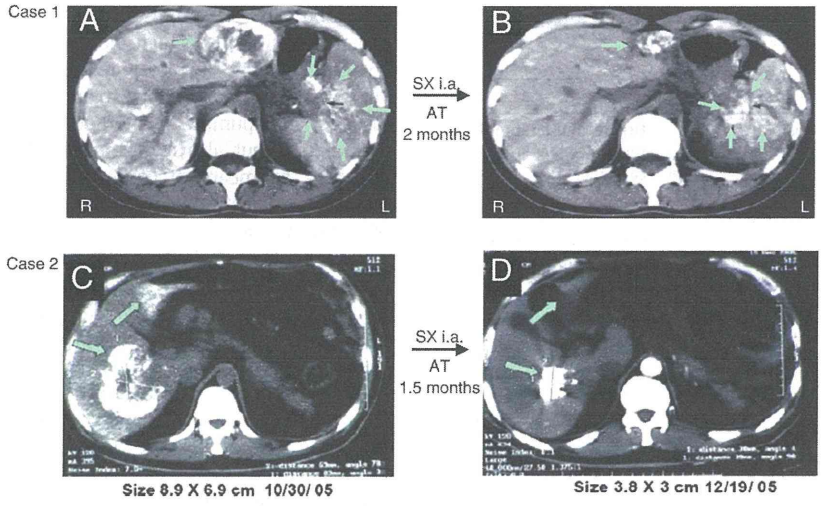

Fig. 1. Cases 1 and 2. Therapeutic effect of SMANCS/Lipiodol against advanced, difficult-to-treat cancers. The drug was infused via the tumor-feeding artery (hepatic artery) under angiotensin II-induced high blood pressure. A and C are abdominal CT scans 2 days after drug infusion. B and D are CT scans taken 2 and 1.5 months after drug infusion, respectively. Case 1. Pancreatic cancer with metastatic liver cancer (A) regressed significantly after 2 months. (B) Remarkable reduction in the size of the metastatic liver cancer (top, right). Case 2. Gastric cancer had metastasized to the liver (C). The two visible large tumor foci evidenced a marked size reduction after 1.5 months (D). White areas in the CT scans, other than bones, indicate where SMANCS/Lipiodol was selectively taken up by the tumors; other (normal) areas did not take up the drug (see the text for a description of the procedure). From Ref. [25].

in a xenograft mouse model of human gastric cancer [26,35]. This method was also validated effective by Dr. F. Kratz of Freiburg in different tumor model (personal communication).

4.4. Generating carbon monoxide (CO)

Fang et al. in our laboratory described the important role of heme oxygenase 1 (HO-1) in the EPR effect which is upregulated in most solid tumors; its product, CO, was also a factor influencing the EPR effect. CO has a physiological role similar to the vasodilator role of NO, so it will also have a key function in the EPR effect [36]. Thus, upregulation of HO-1 by HO-1 inducers such as pegylated hemin or similar agents, or CO-releasing agents (e.g., carbon monoxide-releasing molecule, CORM2), can facilitate the EPR effect [17,36].

5. Drug access to tumor cells and cellular drug uptake, followed by reaction of active drug with target molecules in tumor cells

Although nanoparticles can get to tumor tissues by means of the EPR effect, other issues complicating efficient drug uptake remain to be cleared. These issues include access of drugs to tumor cells and internalization of drugs, followed by release of the free or active drugs from macromolecular formulations composed of liposomes, micelles, or polymer conjugates such as polyethylene glycol (PEG), *N*-(2-hydroxyproply)methacrylamide (HPMA), or SMA. Achieving efficient drug uptake requires knowledge of tumor biology, such as targeting to unique receptors, making use of higher lipophilicity, and utilizing a unique ligand with high affinity to tumor cell receptors of a particular tumor. With regard to uptake of nanoparticle-drugs into tumor cells, in many cases cancer cells have more active endocytic uptake than do dormant normal cells.

Cellular drug uptake is more efficient with SMA micelles than with PEG-micelles [37 and our unpublished data]. For example, when we evaluated SMANCS and NCS, SMANCS was much more toxic to tumor cells than to normal cells [38]. Also, to kill 80% of the cells, NCS required more than 1 h at 30 nM, whereas SMANCS required only a few minutes at 15 nM [38]. Furthermore, a recent comparison of SMA-Zn protoporphyrin (PP) micelles and PEG–ZnPP micelles showed a more rapid cellular uptake for the former [37]. SMA–ZnPP micelles also demonstrated rapid uptake by tumor cells, with very quick disintegration of the micellar structure in the cells. Thus, release of free drug, ZnPP upon rapid endocytic uptake of SMA-ZnPP into tumor cells is anticipated [37]. Then, the free active drugs would be expected to react with target molecules in the cells (unpublished data).

6. Obstacles in drug development, drug promotion, and decision-making in business: dilemmas for science and business

6.1. Novel drug administration technique for use at the bedside

The first obstacle that we encountered in clinical drug development concerned the method of drug administration: the route via the tumor-feeding artery. In cardiology, the angiographic technique for imaging, which utilizes arterial infusion of a contrast agent to visualize an occluded artery and damaged tissue, is a routine practice in major hospitals. The same technique has been used much less frequently or very rarely in cancer treatment, although interventional radiologists utilize it, primarily for embolization of the tumor-feeding artery so as to achieve tumor necrosis, with limited effects [39].

In SMANCS/Lipiodol therapy, the lipid formulation of SMANCS is infused into the tumor-feeding artery—the hepatic artery for hepatoma, the renal artery for cancer of the kidney, and the bronchial artery for lung cancer or bronchogenic cancer (Figs. 1–3) [18,23,25]. This infusion occurs simultaneously with angiographic imaging of the tumor, with identification of the tumor-feeding artery. This technique requires adequate skill to manipulate the catheter under X-ray guidance, more skill than that needed for the commonly used i.v. infusion, and not every health care professional can perform such drug administration. Also, some pharmaceutical companies do not view such an elaborate method favorably, so it becomes a negative incentive for a business undertaking. However, this perception may be reversed when members of top management of a company carefully examine and investigate the positive clinical outcomes. For example, organizing a task force to promote this therapeutic modality using SMANCS/Lipiodol may encourage opening of a new market.

6.2. Market size: $n \times T$ dominates the corporate decision

The second obstacle to developing new candidate drugs, which is usually the first question that people ask us, is, how big will the market

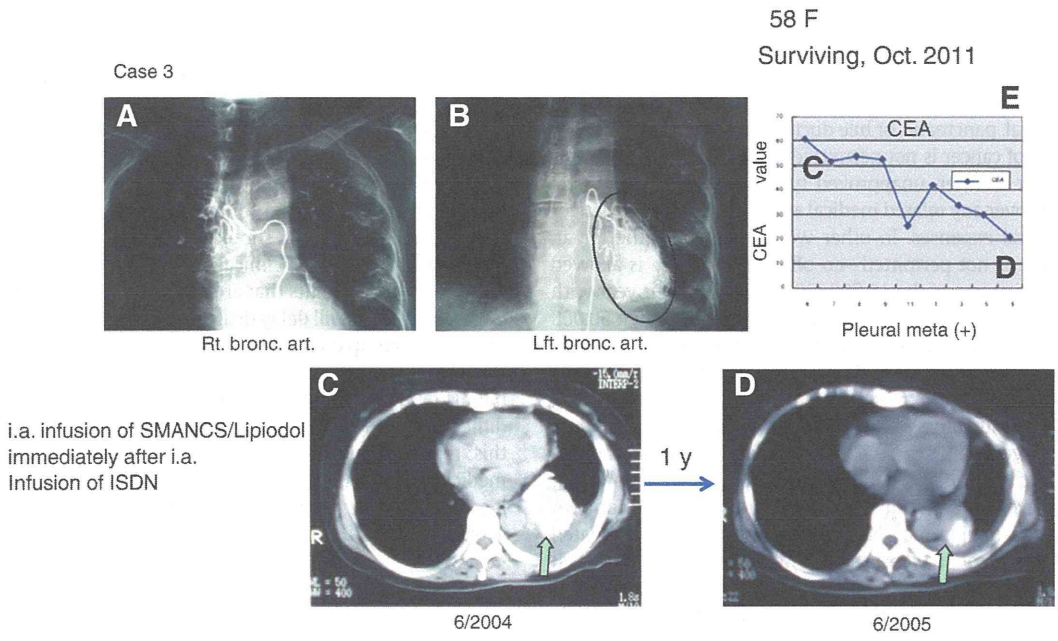

Fig. 2. Case 3. Lung cancer (adenocarcinoma). A and B are angiograms showing infusion of contrast agent into the right bronchial artery (A) and left bronchial artery (B). These X-ray images indicate that the tumor is fed by two different arteries. (C) Initial CT scan at the start of treatment (arrow shows tumor area). (D) CT scan showing considerable tumor regression (arrow) after 1 year. SMANCS/Lipiodol, about 0.5 ml (mg), was infused into each bronchial artery immediately after infusion of a microdose of Nitrol (10–50 μg/dose). (D) One year after treatment, the tumor in the pleural cavity is considerably smaller. White areas indicate remaining drug. (E) Graph showing the decrease in the tumor marker CEA (carcinoembryonic antigen); C and D in [E] correspond to the CT scans in C and D.

be? When we began the clinical application of SMANCS/Lipiodol to treat hepatoma in Japan in the 1980s, about 20,000 cases occurred per year, which by 2010 had increased to about 33,000. In the United States, the corresponding number in the 1980s was about 10,000, although it is much larger now, which was not a favorable size for drug development. In addition, not all of 10,000 patients would be using SMANCS so that actual market size would be far smaller.

Today in Japan, almost 500,000 new cancer cases arise annually. However, in contrast to the number of cancer patients, the number of patients in Japan with diabetes mellitus, hypertension, hypercholesterolemia, and osteoporosis, which are the primarily chronic diseases, exceeds several million. In addition, cancer patients require a much shorter period of drug administration compared with patients with those chronic diseases. Therefore, for anticancer drugs, the product of n (number of patients

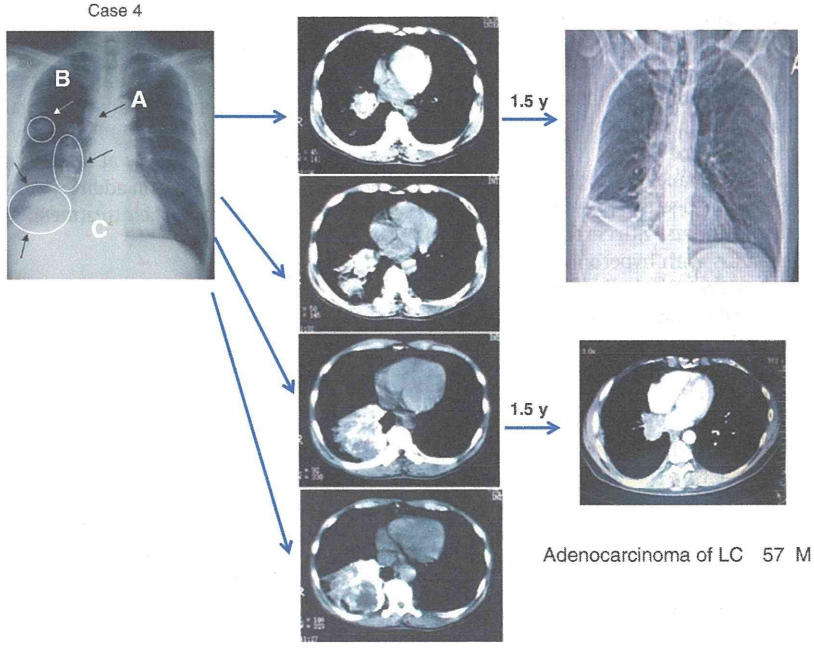

Fig. 3. Case 4. Lung adenocarcinoma. (Top left) Initial chest X-ray showing multiple tumors A, B, and C (circled areas, arrows). The patient underwent Nitrol infusion followed by SMANCS/Lipiodol infusion of 0.5 mg/0.5 ml into both bronchial arteries, as in Case 3. (Middle) CT scans from the subscapular to the lower pleural cavity at the time of initial infusion. (Right) Both chest X-ray (top) and CT scan (bottom) show remarkable tumor regression after 1.5 years; and the patient had no subjective complaints during this period.

eligible to use a drug for the approved tumor type) × T (time period during which the drug will be used) is far less than that for antihypertensive drugs, for instance. Furthermore, for any anticancer drug, a regulatory agency will approve its use by clinicians for only a specific type of cancer, e.g., brain, esophageal, pancreatic, or bile duct cancer individually, and its use for other types of cancer is not permitted. If a clinician in Japan prescribes the drug for another, unapproved type of cancer, the national insurance will not cover any related medical expenses, so that the patient must pay all medical expenses. In other words, use of a drug for an unapproved disease is not permitted—no off-label drug use is allowed in Japan. Therefore, in view of the 10,000–20,000 patients per year with many of these types of cancer in Japan, i.e., not much more than 50,000 patients per year, the market size (n × T), may be about 1/1000–1/10,000 for each cancer type, compared with, for example, that for statins (anticholesterol agents). Antihypercholesterolemic drugs, for instance, were obviously more lucrative because several million patients would use them for far longer than, say, 10–20 years. Many large pharmaceutical companies are therefore less enthusiastic about getting involved in anticancer drug development, which for them is not a high priority. A market size of half-a-million may be moderately interesting. A related issue concerns development of drugs for childhood cancer, in which there is not much interest. The development of orphan drugs also lags behind need, so society needs some system to support orphan drug development as well in Japan and elsewhere.

When SMANCS/Lipiodol was first demonstrated to be very effective against liver cancer, the market size in the United States was small, about 10,000, and the potential market for this drug was not so lucrative. The company that was developing SMANCS/Lipiodol in Japan also did little to promote the drug, regardless of its advantages—remarkable clinical benefits and very few adverse effects. Such decisions depend on the policies of each individual pharmaceutical company, and unfortunately, the executives of the company developing SMANCS/Lipiodol did not see the potential impact of this drug in Japan or elsewhere. In addition, this therapeutic strategy, which would have stimulated a paradigm change in solid tumor treatment as described earlier herein, would have stimulated the growth of a new market for use of this agent to treat other solid cancers.

With regard to the cost/benefit issue for cancer patients, many conventional anticancer drugs usually produce severe side effects but have marginal therapeutic efficacy and high costs. High drug prices mean that the drugs will be highly profitable and lucrative for the pharmaceutical companies. SMANCS/Lipiodol, however, is usually administered three to five times in the first year, and then two or three times the next year. This administration schedule means that the number of sales is quite small, which thus impedes drug development.

We have discovered very interesting therapeutic drugs or new modalities for treatment of cancer and other rare diseases [39–42], but making such drugs or modalities available for patients with chronic granulomatous disease [41] or fulminant hepatitis with hyperbilirubinemia [43] requires enthusiastic physicians, pharmaceutical scientists, and industrialists. In practice, development of such drugs is indeed quite difficult or almost, if not completely, impossible.

6.3. Regulation in drug development

The third obstacle to developing new drugs concerns regulatory agencies. SMANCS consists of two parts: NCS (protein) and a synthetic copolymer of SMA. Both parts are chemically conjugated, so the drug is a single chemical entity. For its administration, we developed a new formulation with the lipid contrast agent Lipiodol, as described above. However, a regulatory agency in Europe required all data related to toxicity, pharmacokinetics, and pharmacodynamics, as well as clinical data, to be provided separately for each component: NCS, Lipiodol, and SMA copolymer, (the latter two have no cytotoxic or anticancer activity). Our preclinical data in rodents showed that only the SMANCS in Lipiodol formulation demonstrated the far greater therapeutic benefit as well as diagnostic value. If a company had carried out such experiments for each separate component in humans, the cost would have been prohibitory, so no such experiments were done. In addition, my colleague physicians, with no reason to believe that clinical benefit would ensue, objected to doing such unethical clinical studies of humans, with legal actions following as the worst outcome.

Therefore, during the filing for approval process, regulatory agencies should require and examine only preclinical and clinical data of the drug being used. The agencies should be concerned with the formulation of the drug as used in the clinical setting, not each separate component. To conduct experiments that are not directly relevant to clinical practice or patient benefit will delay drug development and create great financial burdens on companies and society. In fact, cost reduction in drug development is now becoming a critical issue. Not only in the United States but also in Japan and Europe, huge national financial debts are causing difficulties, and medical expenditure is indeed partly responsible for this; reduction in medical cost is thus a requirement in every aspect of medical care, including drug development [7–10,44]. Therefore, imposing unreasonable requirements for filing for drug approval should be avoided [7,8].

7. Conclusion

In this article, I describe my personal experiences with the EPR effect and development of macromolecular therapeutics (SMANCS), including marketing issues. Essential focal points of development of such drugs involve the cost of the drug and its efficacy. Price setting is a complex issue: if a price is too low, a company will lose interest, but if it is too high, society will suffer. Furthermore, in the current arena of anticancer drug development, the need for a wide range of knowledge about cancer genomics and cancer biology is not fully appreciated. Drug development based on the EPR effect is certainly an important first step, but some problems still remain. Even after a drug is delivered to cancer tissue, it must be taken up by tumor cells, and free active drug must then be released and interact with target molecules. The case of SMA–ZnPP micelles serves as an example of such a drug in development. Also, the heterogeneity of the EPR effect must be overcome. I have addressed this heterogeneity and achieved realistic in vivo solutions that have no obvious adverse effects, and I will be excited when clinicians adopt these solutions. The enthusiasm of scientists as well as industry is by far the most important key for successful drug development.

I also discuss how regulatory agencies should act responsibly, with prudence and wisdom, not only with regard to safety issues, even when the remotest possibility of any harm may exist, but also with regard to economic burdens to society at large. Clinical efficacy is, of course, the most important issue, and in addition, patients should display a high degree of satisfaction with their treatment.

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EPR効果に基づく腫瘍のターゲッティングと蛍光イメージング

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1. はじめに

EPR効果は癌のDDSを考える上で最も重要なfindingと言われているが（1）、その発見25年になる昨年までの引用件数は、ほぼ累積1万件を超えているようである［図1］。

我々は1986年に別記の固型腫瘍におけるEPR効果を発見したが、それ以前に細菌感染局所（炎症部）において細菌の産生するプロテアーゼが宿主のもつプロテアーゼ・カスケード［Hageman factor→kallikrein→kininogen→bradykinin(kinin)］を活性化し、血管作動物質キニンを生成することによって血管透過性亢進する（enhanced permeability→浮腫形成）ことを見出していた。これを検出するために青色色素エバンスブルーを静注すると、キニンの生成部位（プロテアーゼ投与部皮膚局所）でのみ青色色素と結合したアルブミンの漏出をみた［図2A］。これを固型癌に応用してみると、アルブミンなどの高分子の血清タンパクは選択的に腫瘍部でのみ漏出し、さらに正常の皮膚と違って、その腫瘍部で漏出・蓄積したタンパクは1～2週間後でもまだそこに保持（retention）されることを見出した。これをEPR効果（enhanced permeability and retention effect）と命名した（2）。

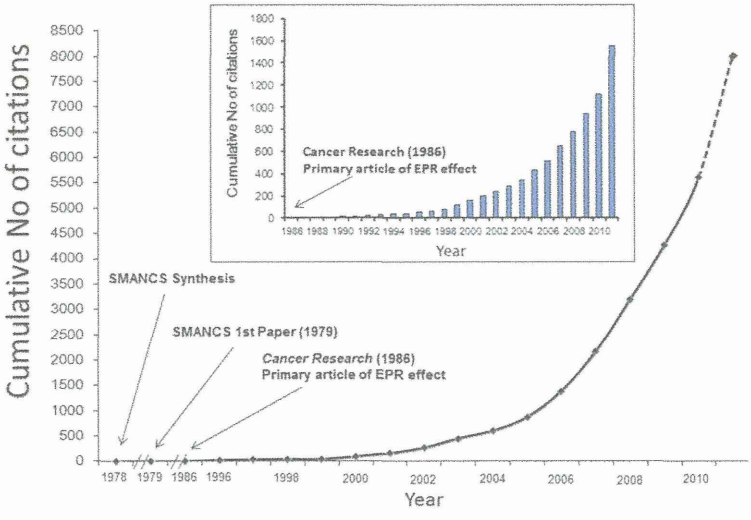

図1.　Cumulative citation numbers of the EPR effect

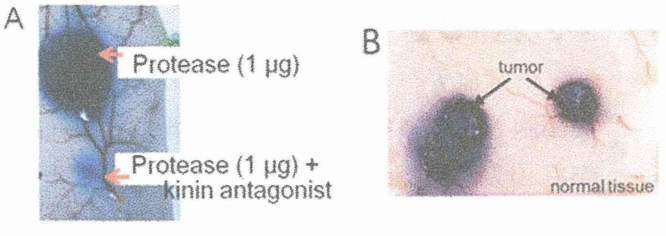

図2.　A shows extravasation of an Evans blue-albumin complex induced by intradermal injection of serratial protease (top), however when a kinin antagonist in addition to the protease (bottom) were injected similarly, the extravasation was inhibited almost completely (ref. 6,7).　B shows two tumor tissues exhibiting extravasation of the Evans blue-albumin complex only in tumor.　No Evans blue-albumin leakage occurred in the normal skin seen in the background.

これをEPR効果（enhanced permeability and retention effect）と命名した（2）。

これを生体親和性のある高分子HPMA（ヒドロキシプロピルメタアクリレートポリマー）で分子量を変えて調べてみると、血清タンパクと同じく、分子量約4万以上ではそのEPR効果を示したのである（3,4）。これら高分子の正常の炎症組織と癌組織での挙動の違いは、各々の局所での滞留性（時間）であるこ

とがわかった（2〜3 日 vs 数週、下記参照）。詳細は最近のレビューを参照されたい（3-7）。

2. EPR 効果の要因

2-1. 血管作動物質

もともと癌形成（発癌）の早期では血管形成が盛んで、VEGF などの細胞増生因子を癌細胞が分泌しているが、VEGF も内皮細胞の増殖促進作用の他に、血管透過性を高めている。我々はヒトおよび動物（マウス・ラット）で数多くの血管透過性増進因子を産出していることを明らかにしたが、そのなかでも上記のブラジキニン（8）、さらに一酸化窒素（誘導型 NO 合成酵素由来）は中心的なそのための血管作動因子である。その他にプロスタグランジンや最近では一酸化炭素（CO；ヘムオキシゲナーゼの作用による）も重要であることがわかってきた（6,7,9,11）[図 3、表 1]。これらは癌がマウスでは 1.5 g ぐらいまでは特に活発に産生されて、それ以上になると腫瘍 1 g 当たりになおすと減少する。注目すべきはそれらのメディエーターは正常血管にも作用することであり [図 3]、正常組織に転移した癌細胞も正常血管からの栄養の摂取を容易にしている。

2-2. 血管構築

正常血管は血管の最内腔側の内皮細胞とその外側をとりまく平滑筋作用（収縮・拡張作用）を示すペリサイトがあって、アンジオテンシン II（AT-II）、NO、キニン、その他の血管作動因子で血管の内径が制御されるが、腫瘍血管では例えば AT-II レセプターが欠損している、あるいはペリ

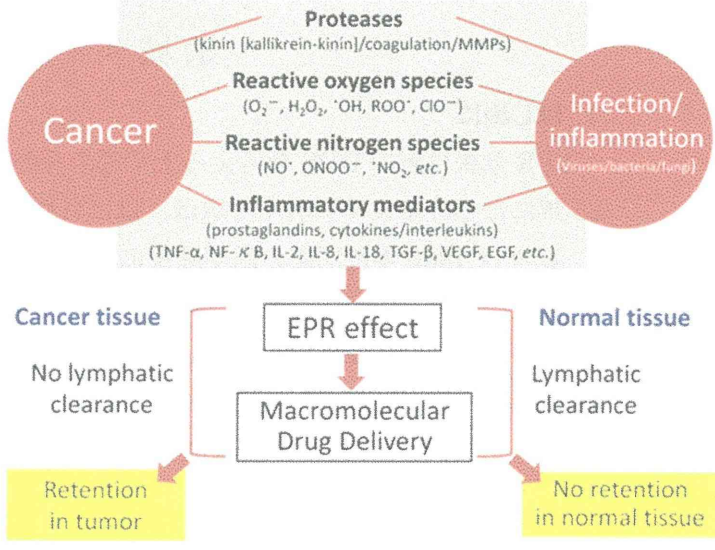

図 3. Various vascular mediators commonly found in inflammation and cancer that contribute to the EPR effect. These mediators also affect normal blood vessels. A major difference between the two pathological phenomena is that the slower clearance rate of extravasated macromolecules, *i.e.* a prolonged retention time, in tumor tissue compared with that in inflamed tissues.

表 1　Factors affecting the EPR effect of macromolecular drugs in solid tumors.[a]

Mediators	Responsible enzymes and mechanisms[b]
Bradykinin	Kallikrein/protease
NO	iNOS
VPF/VEGF	Involved in NO generation
Prostaglandins	Cyclooxygenase 1
Collagenase (MMPs)	Activated from proMMPs by peroxynitrite, or proteases
Peroxynitrite	$NO + O_2^{\cdot -}$
Carbon monoxide (CO)	Heme oxygenase (HO)-1
Induced hypertension	Using angiotensin II
Inflammatory cells and H_2O_2	Neutrophil/NADPH oxidase, etc
Transforming growth factor (TGF)-β inhibitor	
Tumor necrosis factor (TNF)-α	
Anticancer agents	
Heat	

[a]Extensive production of these vascular mediators that facilitate the extravasation from normal and tumor vessels. [b]The enzymes or mechanisms involved in each mediator are shown.