spectroscopy in two crucial ways, however.   First, currently used photosensitizers, *e.g.*, Laserphyrin® and Photofrin®, do not satisfy the spectroscopic requirements.   HeNe lasers emit light only at 635 nm, whereas these photosensitizers being used in the clinical setting can be excited by light irradiation within the range of 380-430 nm but not at 635 nm, and thus no significant generation of singlet oxygen occurs.

The second issue is that the currently used photosensitizing agents have molecular weights less than 1,000 and will be distributed indiscriminately throughout the body, almost evenly in all tissues and organs *in vivo*, after i.v. infusion, *i.e.*, without any tumor selectivity (see Fig. 2B).[55)]   Therefore, illumination of a patient, or exposure of the patient to ambient daylight, may result in damage to any exposed surface skin, but no tumor cells are killed because no significant accumulation of the sensitizer occurs in the tumor.

1-2-7. Adverse effects of cancer chemotherapy.   The most serious problem in conventional cancer chemotherapy is the occurrence of severe adverse effects, primarily inducing systemic toxicity, including bone marrow suppression; kidney, liver, cardiac, and peripheral neuronal toxicity; diarrhea; bleeding; and immunological suppression.   Quantifying and pinpointing the causes of the toxicity, particularly at the molecular level, and thus eliminating adverse effects such as

anorexia, fatigue or weakness, diarrhea, alopecia, discomfort, pain, and others are

rather difficult.   However, these effects are the main reason for the lower quality of

life and morbidity of the patients.   These adverse effects are attributed to systemic

and non-selective drug delivery and other multiple causes.   However, a few

rational symptomatic treatments, *e.g.*, erythropoietin and neutrophil growth factors,

can now control such adverse effects as hematopoietic suppression and bone marrow

suppression including erythrocytopenia, leukocytopenia, and neutropenia.

Whatever the symptomatic treatments, palliative care is similarly important,

particularly at the end stage of disease.   Under these circumstances, development

and use of truly tumor-targeted drugs are urgently needed, as described later.

1-2-8. Economic issues: poor response rates, prohibitive costs, and problems

with the health insurance system. Another problem in cancer chemotherapy involves

the cost of recent molecular target drugs: they are quite expensive, but in many cases no

satisfactory survival benefits have been reported (*e.g.*, Refs. 10-26).   Many of these

drugs may cost US$100,000 per year, or up to one third of a million dollars per course of

treatment. Other nanomedicine-type anticancer agents such as Doxil® and Avastin®

(bevacizumab) would cost about US$5,000 per injection, or about 10 times of the price of

the parent drugs (DOX and paclitaxel, respectively), without a significant survival

benefit (*e.g.*, Refs. 22-24).   The media in the United States and United Kingdom frequently report on this issue.[17]-[26]   One advantage of using macromolecular drugs such as Doxil® is said to be a more tolerable toxicity compared with the parent drug (DOX).   However, more than half of personal bankruptcy filings each year in the United States are reportedly due to the high cost of medical care, including drugs (Time, March 4, 2013).

In the Japanese National Health Insurance System, all patients are eligible to receive government-approved medications and treatments.   However, patients must pay all medical and hospital costs of any new unapproved medicines.   That is, patients who use just one additional unapproved medication lose all the privilege of receiving the Japanese National Insurance System benefits, even health care procedures that are vitally needed. In contrast, some or many very costly approved drugs yield no substantial survival benefits as discussed above. I believe that such approved drugs should remain available to individual patients who want them but only if the patients pay the cost, so as to prevent increasing the huge debt of the Japanese National Health Insurance System, with the remaining cost of treatment covered by the insurance.

In this regard, government and industrial resources should more vigorously support research efforts to reduce medical costs and increase therapeutic efficacy.

2. Solutions to tumor-selective drug development: The EPR effect and sound rationales for drug design

In 1986, Yasuhiro Matsumura (then a graduate student) and I discovered a novel phenomenon in cancer chemotherapy, which we named the EPR effect of macromolecular drugs in solid tumors. Since then, the EPR effect has been widely cited—more than 15,000 times by 2012, after its first publication in *Cancer Research*[27]—and it is becoming a gold standard in cancer drug design, despite inadequate development as discussed in Section 1-2-4.

The EPR effect in solid tumors in general results from a number of causes. First, tumor vasculature is architecturally defective, *e.g.*, it frequently lacks a smooth muscle layer (or pericytes), shows irregular stretching, and has large gap openings; thus, tumor blood vessels are much leakier than normal blood vessels. Macromolecular drugs therefore selectively leak out of blood vessels in tumor tissues, *i.e.*, a drug with a molecular size larger than 40 kDa can leak out into the tumor interstitium. Also, because of insufficient lymphatic clearance, these drugs are retained in tumor tissues for a very long time, *i.e.*, days to weeks[27]-[31] (Fig. 2). This EPR effect was not observed in normal tissues or organs unless they had some lesion or inflammation. [27]-[31] Healthy,

normal tissue will therefore be protected from the toxic effects of macromolecular drugs, or so-called nanomedicines, containing cytotoxic active ingredients.

We found that biocompatible polymers conjugated with active drugs are ideal for tumor-selective targeting and delivery of drugs.　We first prepared a polymer-conjugated anticancer drug that we named SMANCS, with　SMA as the polymer consist of styrene-co-maleic acid and neocarzinostatin (NCS) as the drug, in 1979.[32] SMANCS was approved by the Japanese FDA in 1993 and has been used as a drug for hepatoma.　It can be dissolved in the oily contrast agent Lipiodol® and is administered via the tumor-feeding artery via a catheter under X-ray monitoring. We achieved highly selective delivery of SMANCS to tumors (tumor-to-blood ratio = 2,000:1), and more important, the drug was not delivered to normal tissues.[30],[33]-[35]　However, this method of arterial administration required a technical skills involving angiography, which is a rather advanced procedure compared with conventional i.v. infusion.　In addition, for use, SMANCS necessitated mixing with Lipiodol® at the bedside.　These requirements, together with a small sales volume, meant that SMANCS was less lucrative for pharmaceutical companies and thus attained only limited popularity. However, this strategy may eventually stimulate a new field of cancer therapy with arterial infusion of nanomedicines.　In fact, this method proved highly effective against

advanced primary and metastatic liver cancers, cancers of the gallbladder, pancreas, and kidney, and lung cancer of all types, even at stage IV.[30),34)-36)]

3. Problems with the EPR effect for tumor-selective drug delivery

The EPR concept is the first, most important step for tumor-selective drug delivery.[37)] Although numerous researchers confirmed the EPR effect by using various rodent tumors implanted in non-orthotopic sites, one can argue its validity in metastatic tumors, spontaneous primary tumors, and human tumors in general. These issues are discussed elsewhere in this article.

The issue of the heterogeneity of the EPR effect in general is also important. Tumors, not only rodent tumors but also human tumors, manifest many differences in size, stage, and pathology. When a tumor reaches a diameter larger than 1.0 cm, it frequently has areas of necrosis as well as thrombogenic, hypoxic, or coagulative areas or some necrotic tissue; obviously, these areas do not exhibit the EPR effect.[16),31),36)] To make these inert areas more responsive to the EPR effect, the EPR effect can be augmented, as described in Section 4, which produces a more homogeneous EPR effect in tumor tissues and hence better drug delivery. Therefore, one can overcome the problem of the heterogeneity of the EPR effect to a great extent.[31),36),38)]

We demonstrated augmentation of the EPR effect by using vascular effectors such as nitroglycerin (NG) and angiotensin I-converting enzyme inhibitors, both of which are widely used non-toxic drugs (cf. Tables 1 and 2).[29)-31),35),36),39),40)] Furthermore, we[39),41)] recently reported on a second step in achieving tumor selectivity by using an environment-sensitive bond cleavage in the setting of the low pH of tumor tissue: the conjugate was cleaved at the linker, a hydrazone bond or an ester bond, and released free active drug near the tumor cells.[41),42)] Free drugs such as THP can thereby easily reach tumor cell membranes by diffusion and attach to receptors or transporters (nucleotide transporters), which efficiently take the drugs into the cells. This system is upregulated more in tumor cells than in normal cells.[42)] Another drug, DOX encapsulated in STEALTH liposomes (Doxil®), also exhibits the EPR effect. The plasma levels and tumor concentrations of Doxil® far exceeded those of the free drug DOX—as much as 11 times higher—in AIDS patients with Kaposi sarcoma[43)] (see Fig. 1).

Our new drug conjugates will utilize this property, thereby minimizing toxicity or even achieving zero toxicity. We can thus accomplish the best targeting of drugs to tumors by means of three mechanisms: (i) the EPR effect, (ii) release of active free drug under the lower pH conditions in the vicinity of tumor tissues, and (iii) rapid

intracellular uptake of released drugs by means of transporters (*e.g.*, pirarubicin and

zinc protoporphyrin, or ZnPP, as described later).   Endocytic uptake of microparticles

or nanomedicines is also believed to occur much faster in dividing tumor cells than

non-dividing normal cell.[44),45)]   If macromolecular conjugates are taken up into cells via

such endocytosis, the conjugates would rapidly release free drugs because the lysosomal

or endosomal pH value is much lower than pH 6, and because hydrolytic enzymes in the

subcellular compartment would facilitate hydrolytic cleavage of these chemical bonds in

the cells.

4. Augmentation of the EPR effect for tumor delivery

We previously discussed the importance of the EPR effect for drug delivery to

solid tumors.[28)-31),38),39),46),47)]   As described earlier, the reasons for the EPR effect are

multiple and include defective vascular architecture and excessive production of

vascular mediators (Table 1), as occurs in inflammation.   These mediators, such as

nitric oxide (NO), bradykinin, and prostaglandins, induce the tight junctions of

endothelial cells in blood vessels in tumors and normal tissues to open (Tables 1 and

2).   It is interesting that the EPR effect can be augmented 2- to 3-fold by

administration of widely used non-toxic drugs, *e.g.*, nitroglycerin (NG) and

angiotensin I-converting enzyme inhibitor (ACEI); the latter inhibits bradykinin

degradation, with the consequences being elevated local bradykinin levels and an

enhanced EPR effect.[30),31),36),38),39),46)-48)]

Many solid tumor tissues have suppressed blood flow, similar to that in

infarcted cardiac tissue, and become hypoxic.   When NG is applied to the skin of

tumor-bearing animals by using an ointment, NG will become nitrite (NO_2^-) and

will then be converted to NO in hypoxic tumor tissue (Fig. 3), which induces the

EPR effect and enhances tumor-selective blood flow 2- to 3-fold, as well as

improving drug delivery (Fig. 3C,D) (*e.g.*, Refs. 31,36,39,46,48).   As discussed

earlier, angiotensin II-induced high blood pressure, increasing from 100-110 mmHg

to 150-160 mmHg, can also enhance tumor-selective drug delivery and reduce drug

toxicity.[30),31),38),49)] Such augmentation of drug delivery to tumors is possible only by

using nanomedicines because of their long retention in tumor tissues.

5. The EPR effect in metastatic cancer and outlook for polymer-conjugated

candidate drug 1.

The EPR effect has been studied mostly in primary tumors or implanted tumors, so

whether it would occur in metastatic tumors was not clear.   In fact, we did observe a

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similar EPR effect in metastatic liver and lung cancers in rodents, as shown in Figure 4.

Figure 4 clearly demonstrates selective accumulation of a macromolecular drug

(Evans blue-albumin, a putative 70-kDa drug) in metastatic nodules in the lung.

Conventional low-MW drugs are frequently ineffective for metastatic tumors, when

patients reach to stage III or IV, which may be attributed to most often metastatic

cancers.   We see an evidence of clear uptake of Evans blue-albumin or other polymer

conjugate by EPR effect[49] in even small metastatic tumor nodules in the lung and the

liver, even less than 1 mm or so[49], e. Therefore, EPR effect-based macromolecular

chemotherapy can be applied similarly to treatment of metastatic tumors and a

complete eradication was seen on day **50** after treatment of metastatic lung cancer in

mice (Fig. 4B).   This finding, if indeed the EPR effect operates in metastatic tumors

with macromolecular drugs it will be a great advance in the history of cancer

chemotherapy.

In the clinical setting, surgeons can remove most of the primary or visible tumors,

but removing numerous metastatic tumors spread throughout the entire body is most

formidable, because many of the metastatic tumors are frequently invisible. They also

do not respond to chemotherapy.

In relation to clinical setting, we observed clear uptake of drug in the metastatic

tumor in the liver from stomach cancer. In this case macromolecular SMANCS in

Lipiodol, lipid contrast agent, was infused via the intra-hepatic arterial infusion and

analyses by X-ray CT scan[30),35)].

Our new polymer conjugates (candidate drug 2), described in the next section, with

an apparent size of about 70 kDa with pirarubicin, were effective for treatment of

metastatic tumors. Mice with colon 26 tumors implanted in the dorsal skin were all

cured, and all metastatic tumor nodules disappeared (Fig. 4B) using SMA polymer

conjugate of pirarubicin. It was also found in similarly effective treatment in another

metastatic tumor model—MoCR (dimethylhydrazine-induced colon cancer in CBA mice)

implanted in the spleen and metastasized to the liver[49)].

6. Photodynamic therapy (PDT) using macromolecular candidate drug 2

As demonstrated in Figure 2 and discussed in Section 1-2-6, conventional

low-MW photosensitizer such as Laserphyrin® used in PDT has no tumor

selectivity and distributed in the body almost evenly. This means least antitumor

effect and adverse effect on the skin even under ambient light. To take advantage of

the EPR effect of macromolecular drugs (photosensitizers) and tumor delivery, we

have synthesized polymer-conjugated ZnPP (P-ZnPP) (Fig. 6) and obtained

remarkable results using conventional light irradiation.   This theranostic

treatment can detect fluorescence of even minute tumor nodules (Fig. 7A) and

metastatic liver cancers (not shown).   Thus, it would also be applicable to

simultaneous tumor detection and tumor regression by means of intracavitary light

irradiation with use of an endoscope. This method will be a completely non-toxic

therapeutic technique.

Our polymer conjugated ZnPP (P-ZnPP) (Fig. 6) has a number of advantages.

First, P-ZnPP demonstrates the EPR effect and accumulates selectively in tumor

tissues[41] (Fig. 2A, cf. Fig. 2B).   Light irradiation of any site on the body will thus affect

only tumor tissues. The tumor distribution of P-ZnPP was similar to that of P-THP in

mice (Fig. 5), and as seen   as fluorescence in breast cancer in rats (Fig. 7A).

The second advantage of using P-ZnPP is therefore that we can utilize an

endoscope or similar light source with a xenon light or LED that can excite any

photosensitizers at the wavelength range from 400 to 700 nm, especially at range of

ZnPP: 400-440 nm.   Our P-ZnPP has in fact demonstrated light absorption at about

410-435 nm and generates singlet oxygen, thus effectively inhibiting tumor growth by

means of endoscopic light.   In fact, our *in vivo* rodent experiments showed complete

eradication of chemically induced breast cancer after only one i.v. drug injection at 20-30

mg/kg followed by illumination two to four times (Fig. 7B,C).   One problem with

current PDT is that, in most cases HeNe lasers are used which emit 633-nm light and

can penetrate tissue better than low wave length light, but it can not excite

photosensitizers. However, a conventional flush light and endoscopic light (400-700 nm)

can penetrate human tissues (or hands), as one can observe in the darkness: a

substantial amount ($\sim$0.1%) of light can penetrate the tissue and can generate $^1O_2$ to

kill tumor cells effectively (Fig. 7B,C).

7. Conclusion

I have analyzed the causes of therapeutic failures in current cancer chemotherapy

against solid tumor with a particular focus on molecular target drugs, antibody drugs,

and nanomedicines, as well as immunotherapy. Major causes of the inefficient

therapeutic effect of these modalities are believed to be genetic diversity of human solid

tumors for molecular target drugs or peptide vaccines, and inadequate consideration of

spectroscopic properties in the case of PDT. I also analyzed currently used PDT and

discovered that it is theoretically unsound and has problems with tumor-selective

delivery of photosensitizers (*i.e.*, the low-MW photosensitizers have no tumor selectivity

and no EPR effect) and difficulties with the spectroscopic conditions, which are totally

incorrect.   Our novel polymer-based PDT using fluorescent nanoprobes is thus

completely different from the currently used PDT, and I believe that our therapeutic

approach will revolutionize cancer treatment. In contrast to using currently available

therapeutic modalities, I and my team have been working on macromolecular

therapeutics, in which macromolecular agents are designed to accumulate in the tumor

selectively by means of the EPR effect and release the active principle in the tumor

environment, where the pH value is 1-1.5 units lower than that in normal tissues or

where active proteases (or esterases) are present.   This acidic pH and hydrolytic

enzymes will facilitate spontaneous cleavage of the cross-linking chemical bond, such as

hydrazone and some ester bonds, between polymers and drugs. The result is more

effective release of free drugs in the vicinity of tumor cells and thereby improved

therapeutic efficacy.   I discuss tumor proteases with susceptible peptide linkers and

other bonds in this context as well.

In addition, EPR effect-dependent tumor-selective drug delivery can be augmented

2- to 3-fold by modulating vascular permeability factors that contribute to the EPR

effect by using NG, angiotensin I-converting enzyme inhibitors, or angiotensin

II-induced high blood pressure (*e.g.*, 110 mmHg $\rightarrow$ 150 mmHg).   NG generates NO_2^-

in vivo, which is converted to NO in hypoxic tumor tissues.   This EPR-enhancing effect

achieved by NO-releasing agents, or angiotensin I-converting enzyme inhibitors, occurs

selectively in tumor tissues, which ensures greater effectiveness and fewer side effects

with our polymer-conjugated drugs.

In our preclinical studies using P-THP and P-ZnPP, autochthonous chemically

induced tumors or various implanted tumors in rodents, some of which had metastatic

tumors, were completely eradicated at doses far below the maximum tolerable dose

P-ZnPP with light irradiation.   Similarly, we further observed tumor-selective

accumulation of polymer ZnPP conjugate with a fluorescence detection system. These

findings will stimulate development of new imaging and detection strategies using

polymer conjugates.

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