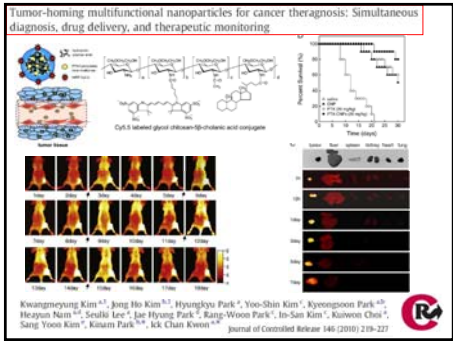
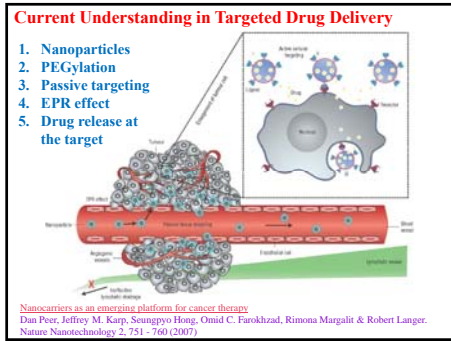
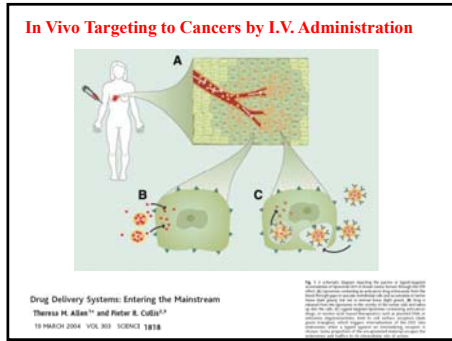
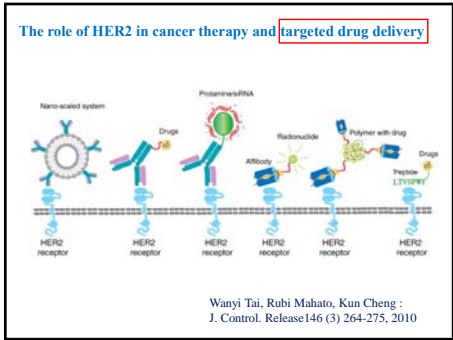
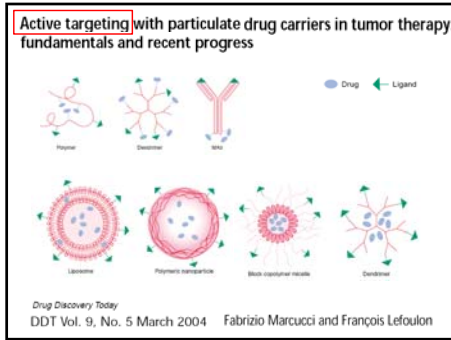
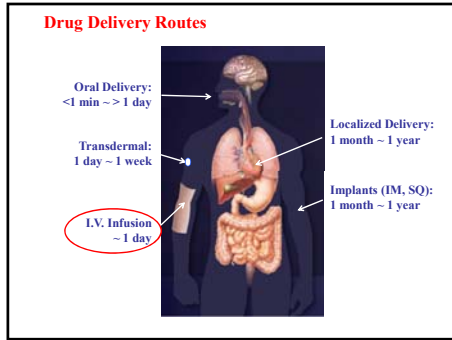
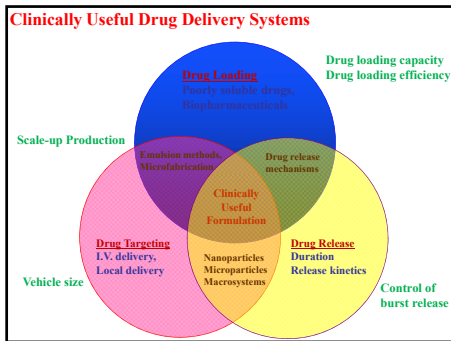
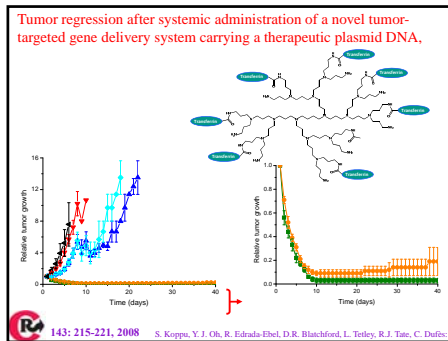


Targeted Drug Delivery: Myth, Reality, & Possibility





With all these nanotechnology-based drug delivery systems, why can't we cure cancer now?

Drug targeting and tumor heterogeneity

4. A new paradigm in drug targeting to solid tumors

Perspective by You Han Bae

EPR effect is the single most commonly used term to explain drug targeting. The porosity and pore size of tumor blood vessels has made all kinds of nanocarriers attractive for tumor targeting therapy, imaging, and diagnosis. The porosity is developed in tumors to timely supply enough nutrients to the newly developing area. However, when the tumor stops growing or when its mass starts reducing by any therapy, does the porosity still remain unchanged and thus allow continuous intervention with nanocarriers? It can be postulated that when a growing tumor changes its phase to shrinking, the blood vessel may change its mode from a rapid to a normal supply with a matured structure [6]. In this case, what will be the role of nanocarriers?

It is now the time to re-think tumor specific targeting approaches and cancer nanotechnology, a long-lasting paradigm. Current targeting approaches may need a paradigm shift. The key words in the new paradigm are 'heterogeneity' and 'dynamic state' of cancer cells and tumor physiology.

JCR 133 (2009) 2-3

Targeted Drug Delivery

1. **Systemic Targeting** Does current biochemical targeting really work?

Biological targeting
Ag-Ab, ligand-receptor interactions

Locally-activated targeting
Self-regulated targeting
Externally-activated targeting

Trojan targeting

2. **Intracellular Targeting**

Why Can't We Cure Cancer by Biochemical Targeting?

1. Passive targeting first (Is this really targeting?)
2. Extended blood circulation by PEGylation?
3. Fraction of intact nanoparticles at the target site? (Blood interaction with nanoparticles)
4. Is animal model realistic?

Enhanced Permeation & Retention (EPR)

J. Med. Chem. 1988, 31, 455-461

Conjugation of Poly(styrene-co-maleic acid) Derivatives to the Antitumor Protein Neocarzinostatin: Pronounced Improvements in Pharmacological Properties

Hiroshi Maeda,* Minoru Ueda,* Tetsuo Moriguchi,* and Tsutomu Matsumoto*

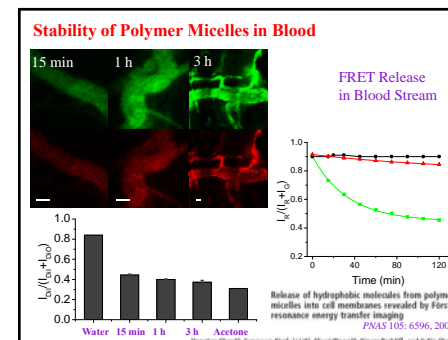
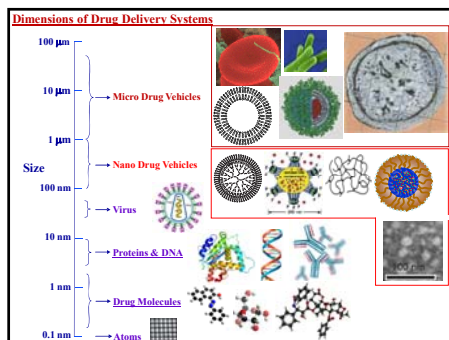
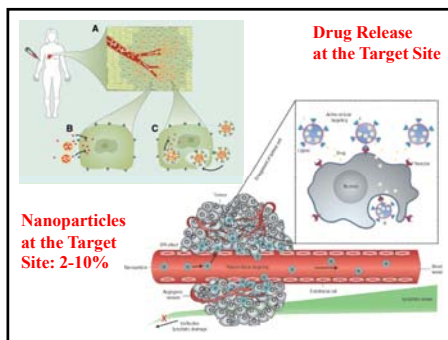
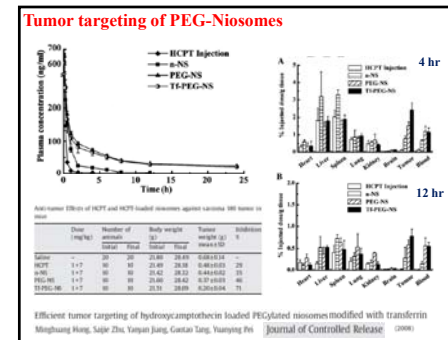
Department of Chemistry, Graduate School, Kyoto University, Kyoto 606-8501, Japan

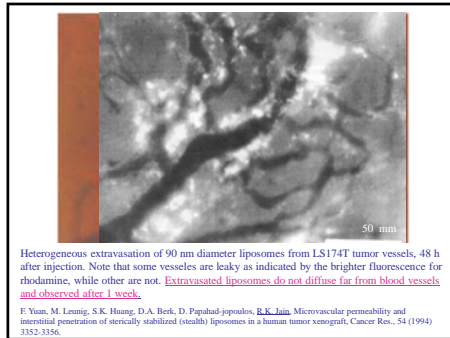
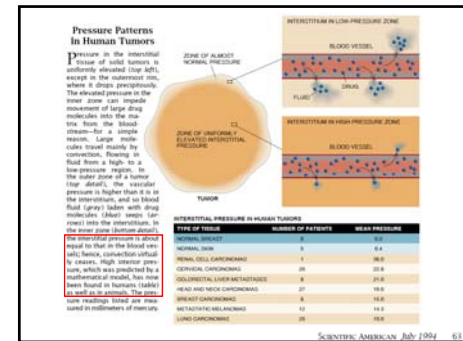
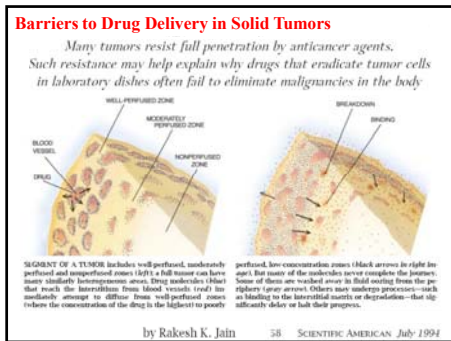
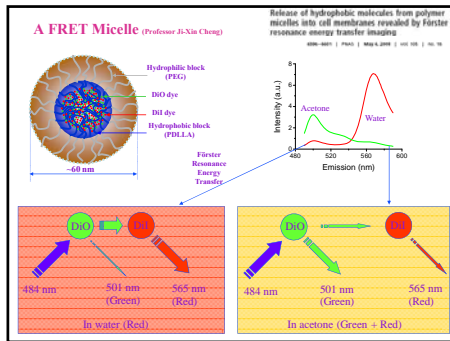
A New Concept for Macromolecular Therapeutics in Cancer Chemotherapy: Mechanism of Tumor-tropic Accumulation of Proteins and the Antitumor Agent Smancs*

Yasuhiko Matsumura and Hiroshi Maeda*

Department of Chemistry, Graduate School, Kyoto University, Kyoto 606-8501, Japan

We have reported that a copolymer of styrene and maleic acid conjugated to the antitumor protein NCS, which we designated as smancs (1, 2), accumulated more effectively in the tumor than normal tissues as measured by biological activity (3). Furthermore, its pronounced lymphotropic character and hence antilymphatic metastasis have been noted (4, 5).





Enhanced Permeation & Retention (EPR)

A New Concept for Macromolecular Therapeutics in Cancer Chemotherapy: Mechanism of Tumorotropic Accumulation of Proteins and the Antitumor Agent Smacns¹

If one injects small molecules, which can traverse barriers freely, into the tumor or the normal tissue, they will immediately disappear from the tumor or the normal tissue by diffusion, primarily into blood capillaries. Macromolecules contrast sharply with the small molecules, as shown in Figs. 2 and 3. Normal tissue (represented by the skin), however, carried the Evans blue-albumin complex rapidly compared with the tumor tissue (Fig. 3). Thus, both the anatomical difference and the molecular size and property of the drug appear to be critical for selective tumor targeting.

Macromolecules and lipids in the interstitial space are known to be recovered via lymphatics in normal tissues (22). The present findings demonstrated that a clear contrast exists in the tumor tissue which has a highly enhanced leakiness and no lymphatic clearance.

Yasuhiro Matsumura and Hiroshi Maeda² CANCER RESEARCH 46, 6387-6392, December 1986

Folate-Liposome-Mediated Antisense Oligodeoxynucleotide Targeting to Cancer Cells: Evaluation in Vitro and in Vivo

Christopher P. Leamon,¹ Scott R. Cooper,² and Gregory E. Hardin²

Chemical form of the folate moiety (pterate) | Bioorganic Chem 2003, 14, 738-747

The objective of this study was to investigate the use of folate-targeted liposomes for the delivery of encapsulated oligonucleotides to folate receptor (FR)-positive tumor cells in vitro and in vivo. This project involved the synthesis and biological evaluation of many folate-PEG-lipid conjugates, where the chemical form of the folate moiety (pterate) and the length of the PEG linker chain were varied widely. Folate-targeted oligonucleotide-containing liposomes were prepared using conventional methods, and the extent of cell uptake was evaluated using, among others, the FR positive KB cell line. Oligonucleotide-loaded folate-targeted liposomes were found to rapidly associate with the KB cells, and saturation was typically reached within the first hour of incubation at 37 °C. Nearly 100,000 liposomes per cell were bound or internalized at saturation. Importantly, cell association was blocked by a large excess folic acid, thus reflecting the FR-specific nature of the cell interaction. Full targeting potential was achieved with PEG linkers as low as 1000 in molecular weight, and pterates bearing glycine or γ -aminobutyryl residues juxtaposed to the pterate acid moiety were also effective for targeting, provided that a terminal cysteine moiety was present at the distal end of the PEG chain. For added hydrophilicity. When tested in vivo, folate-targeted liposomes were found to deliver ~1.8 fold more oligonucleotide to the livers of nude mice relative to the nontargeted PEG-containing formulations. However, no improvement in KB tumor uptake was observed. We conclude from these results that folate liposomes can effectively deliver oligonucleotides into folate receptor-bearing cells in vitro, but additional barriers exist in vivo that prevent or decrease effective tumor uptake and retention.

Perspective

Drug targeting and tumor heterogeneity

You Han Bae

3. Is active targeting really effective?

Most scientists working in this area love to use **cell-specificity** induced by short-range interactions in the interaction pairs of antigen/mAb, ligand/receptor, aptamer/counter part, and a peptide selected from phase display method/counterpart, etc. Specific interactions accompany the words of 'target cell-unique expression' or 'over-expression' when compared with other organs/cells. However, there are seldom quantitative analyses of such specific interactions for active endocytosis in clinical settings. Numerous unanswered questions persist in this regard. For example, what fraction of tumor cells from patients expresses a specific antigen or receptor? What is the expression level in each individual cell? Is the expression level sufficient for active endocytosis? How does the binding affinity between a carrier and a target cell influence the internalization? Is this a matter of kinetics? After endocytosis, does any recycling occur before the release of the payload? If so, what degree?

133 (2009) 2-3

Targeted Drug Delivery

- Systemic Targeting**
 - Key to the success of drug delivery systems
 - Biochemical targeting
 - Ag-Ab, ligand-receptor interactions
 - Locally-activated targeting
 - Self-regulated targeting
 - Externally-activated targeting
 - Trojan targeting
- Intracellular Targeting**
 - Occurs only after systemic targeting

Reality of Targeted Drug Delivery

- Systemic Targeting**
 - Fact: Distribution throughout the body by blood circulation
 - Current magic bullet: PEGylated Nanoparticles having EPR Effect
- Intracellular Targeting**
 - Still elusive


Paul Ehrlich's Magic Bullet

Selective targeting to a bacterium without affecting other organisms.

Selective killing

A drug that **goes straight** to their intended cell-structural targets to treat disease.

interacts with

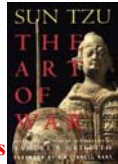


Nobel Prize 1908

The Art of War (孫子兵法)
(Sun Zu Bing Fa)

知己知彼 百戰百勝

The Art of War against Diseases



Know the properties of the nanoparticles in vitro and in vivo.

Know the properties (heterogeneity and dynamic states) of the body.

Properties of Nanoparticles In Vitro & In Vivo

In vitro properties

- Drug loading & release
- Cellular interactions

In vivo behavior

- Stability (Interaction with blood components)
- Delivery to the target site

In vitro – in vivo correlation

Properties of the Body

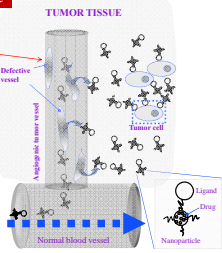
Targeting: Tumor stem cells protecting themselves from drug treatment.

Heterogeneity & Dynamic State

Will the EPR effect remain the same as the tumor mass reduces or tumor stops growing?

Extrapolation of in vitro results to in vivo

Translation of small animal data into human (Unexpected responses by human body)



TUMOR TISSUE

Defective vessel

Normal blood vessel

Nanoparticle

Ligand

Particles of various sizes and their known impacts on bioactivity

Particle Size	Bioactivity
25 nm	Large pores present in normal tissue endothelium.
50 nm	Average size of polymer micelles without loaded drugs.
100 nm	The size of drug-loaded polymeric micelles.
150 nm	Particle extravasation in liver is proposed to be <150 nm.
200 nm	Nanoparticles <200 nm have significantly longer circulation time due to low uptake by the reticuloendothelial system (RES).
350 nm	The pore cutoff size of porous blood vessels in tumors is known to be 380-780 nm. An upper limit for EPR effect.
500 nm	The maximum size of nanoparticles allowing penetration through cell membranes is known to be 500 nm.
1 μm	Particles below 2 μm were taken up by Peyer's patches and then migrated to mesenteric lymph nodes.
5 μm	The upper limit for rigid particles circulating the smallest capillaries.
50 μm	Useful size of microspheres for intramuscular injection.

Heterogeneity of particle sizes

DNA Drugs Come of Age

After years of false starts, a new generation of vaccines and medicines for HIV, influenza and other ailments (Dissmore) is now in clinical trials. BY PATRICIA F. HENDERSON AND DANIEL S. WEISBERG



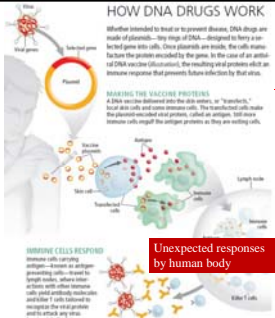
KEY CONCEPTS

- Vaccine and medicine concepts
- DNA-based vaccines
- Plasmid-based vaccines
- Plasmid-based medicines
- Plasmid-based vaccines
- Plasmid-based medicines
- Plasmid-based vaccines
- Plasmid-based medicines

HOW DNA DRUGS WORK

Extrapolation of in vitro results to in vivo

Unexpected responses by human body



Immune cells carrying antigens—either an antigen-presenting cell, or a T helper cell, or a T killer cell—adhere to the antigen and then migrate to the lymph nodes, where they meet with other immune cells and coordinate an immune response to the antigen and its effects on the body.

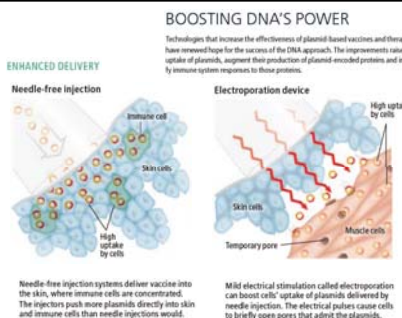
As the trial progressed, though, a disturbing difference between the two groups began to emerge: people who got the vaccine were not better protected than those who received the placebo, and eventually they appeared to be more vulnerable to being infected by HIV. An early

BOOSTING DNA'S POWER

Enhanced Delivery

Needle-free injection

Electroporation device



Needle-free injection systems deliver vaccine into the skin, where immune cells are concentrated. The injectors push more plasmids directly into skin and immune cells than needle injections would.

Mild electrical stimulation called electroporation can boost cells' uptake of plasmids delivered by needle injection. The electrical pulses cause cells to briefly open pores that admit the plasmids.

Properties of the Body

Translation of small animal data into human (Unexpected responses by human body)

Human clinical studies: Long-term & Costly
Not affordable for screening

New animal models: Represent human conditions more accurately.

Drug targeting and tumor heterogeneity

Perspective by You Han Bae

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JCR
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