Lecture 17: Drug targeting

Last time: Intracellular drug delivery

Today: Drug targeting

Reading: T.J. Wickham, 'Ligand-directed targeting of genes to the site of disease,' *Nat. Med*. **9**(1) 135-139 (2003)

Drug Targeting

Applications of drug targeting[1]

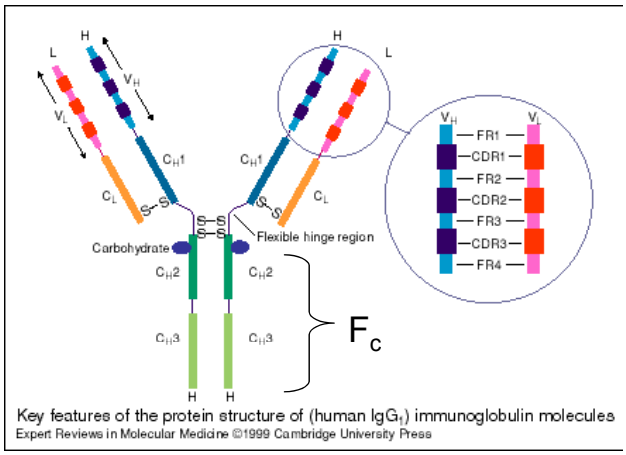
- delivery of toxic drugs to tumors
  - highly toxic drugs that are too dangerous to deliver in a systemic manner
    - e.g. potent radionuclides, cellular toxins
    - allow smaller doses to be used
- delivery of DNA vectors to target cell type for genetic corrections
- targeting to vasculature
  - cancer treatment
    - target to neovasculature forming around tumors[2]
  - pulmonary, cardiovascular, and inflammatory diseases
- targeting to pathogen-infected cells
  - infected cells undergo changes in cell-surface molecule expression
- crossing blood-brain barrier[3]

Application	Cellular target	Molecular target	Targeting ligand	Ligand type
Anti-cancer therapy	Various tumor cells	Folate receptor EGF receptor	Folate EGF	Protein ligand for target receptor preferentially expressed on target cells
	Neovascular tissue	B-FN (fibronectin isoform)	anti-B-FN antibody	antibody against fibronectin isoform only expressed during embryonic development and in aggressive tumors
Anti-cancer therapy, pulmonary, cardiovascular, and inflammatory diseases	Endothelial cells	E-selectin P-selectin	sialyl Lewis^X receptor	receptor expressed at sites of inflammation
Anti-cancer therapy (leukemias and B cell lymphomas)	Transformed B lymphocytes	CD20	Anti-CD20 antibody	Antibody against target cell-surface protein unique to target class of cells (e.g. B cells)
Anti-cancer therapy (T cell lymphomas)	Transformed T lymphocytes	IL-2Rα (interleukin-2 receptor a chain	Anti-IL-2Rα antibody	Antibody against target cell-surface protein not expressed on normal resting cells

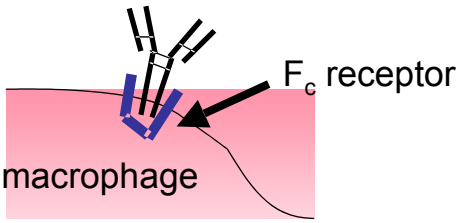
Targeting Approaches[4]

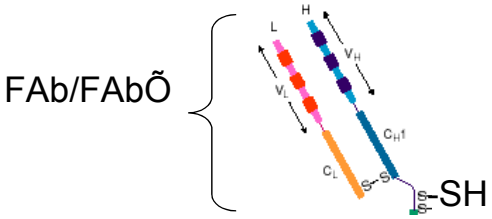
Targeted delivery

- ***receptor-ligand-based targeting***
  - general cell surface receptor-ligand pairs
    - guide drug to target based on unique or over-expressed receptor on target cell type
    - folate receptor
      - over-expressed in 95% of non-mucinous ovarian carcinomas[5]
  - antibody targeting
    - pros
      - high affinity (~1 nM K_D- typical half-life at 37°C?)
      - high specificity
    - cons
      - need to be 'humanized' to avoid rapid opsonization
        - only variable region of mouse antibody need be retained for antigen recognition
      - need to consider possible F_c receptor binding
        - *F_c = 'fragment crystallizable'*
      - use of FAb fragments as an alternative
    - source of Ab 3D animation: http://digilander.libero.it/danielefocosi/immunity.html#But,%20on%20the%20contrary%20of%20TcR,%20other%20BcR%20isotypes

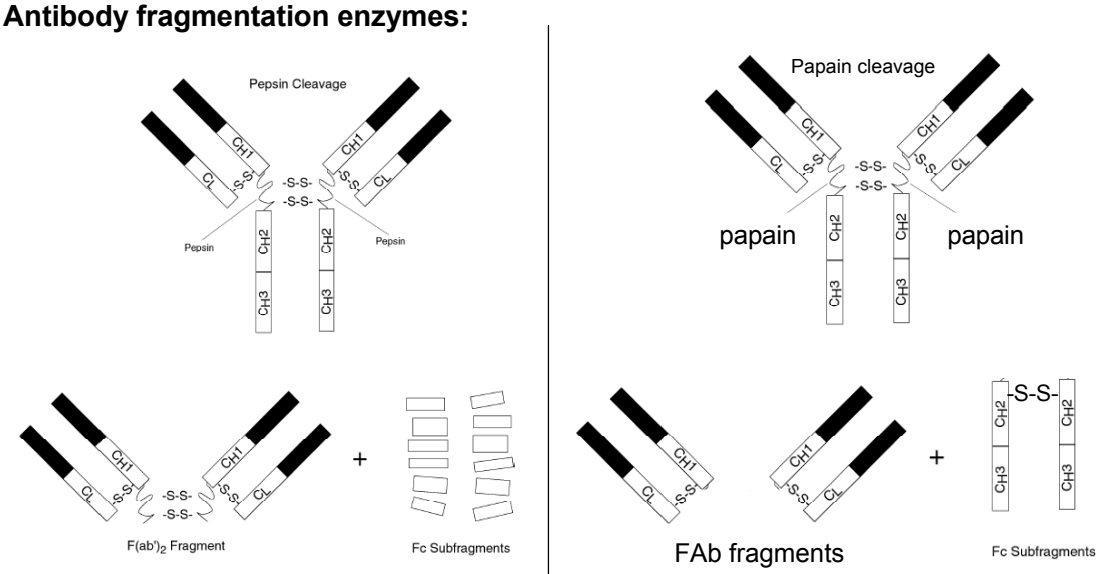


Key features of the protein structure of (human IgG_1) immunoglobulin molecules
Expert Reviews in Molecular Medicine ©1999 Cambridge University Press



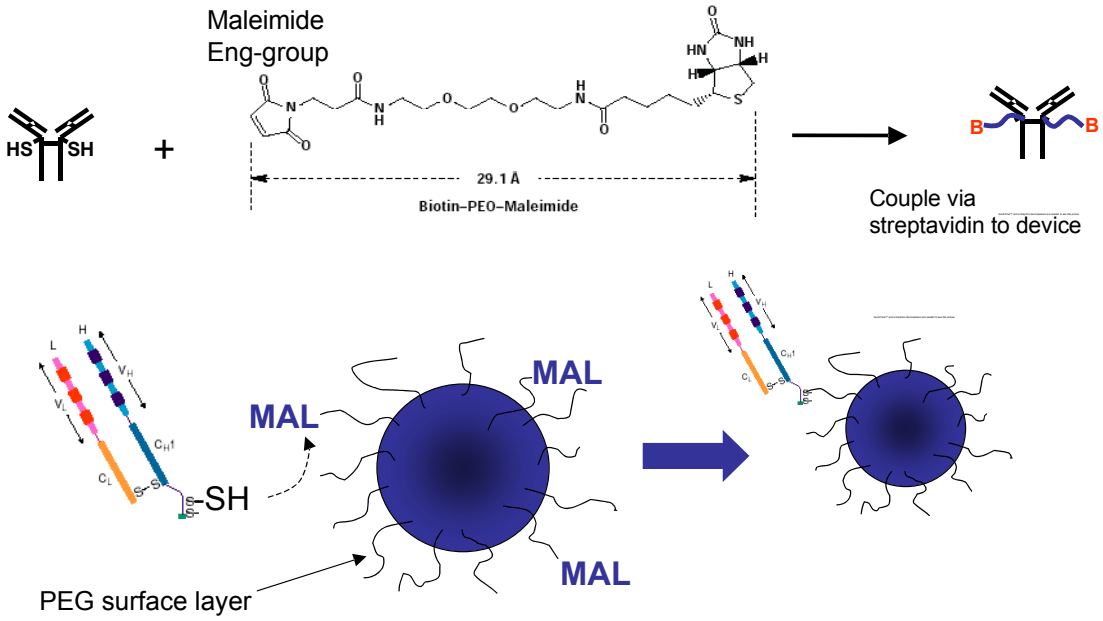


Antibody fragmentation enzymes:

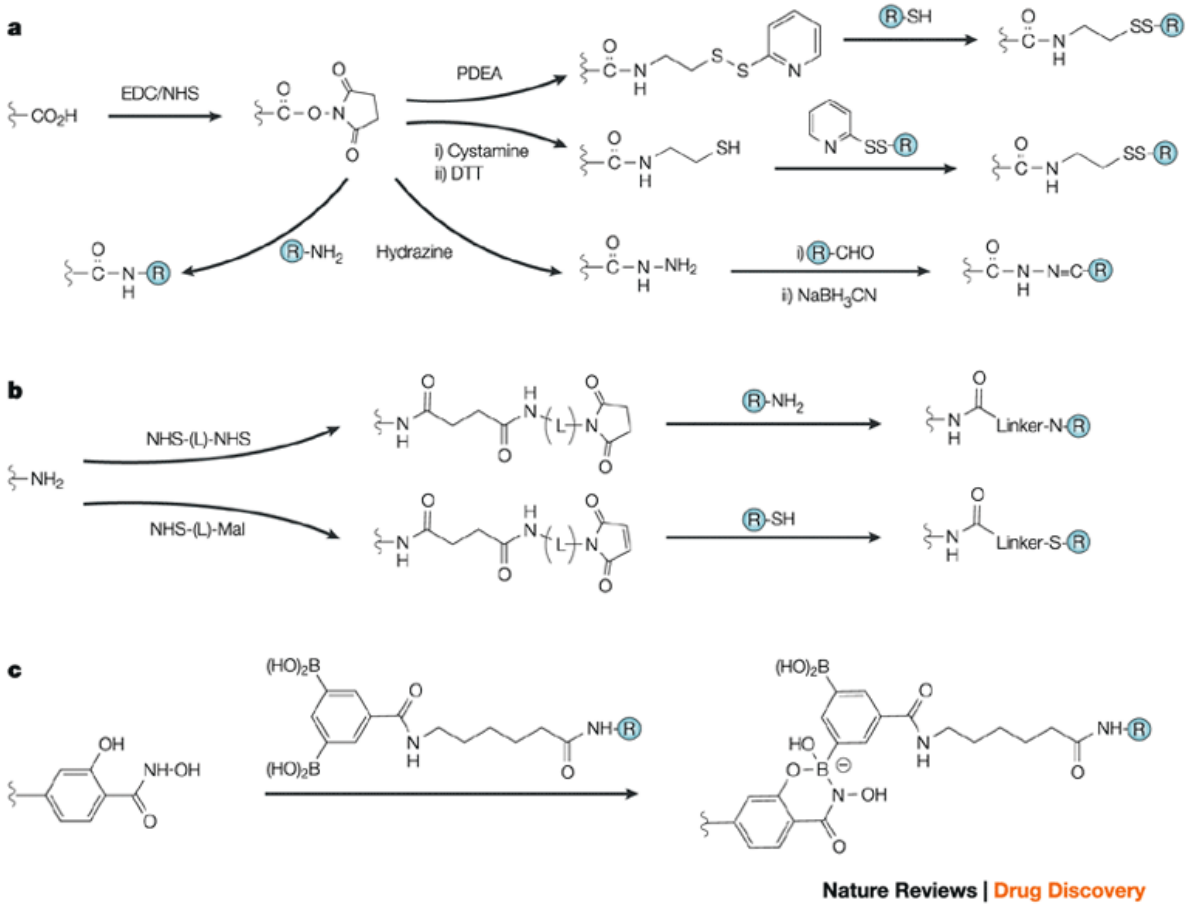


(Pierce Chemical Co.)

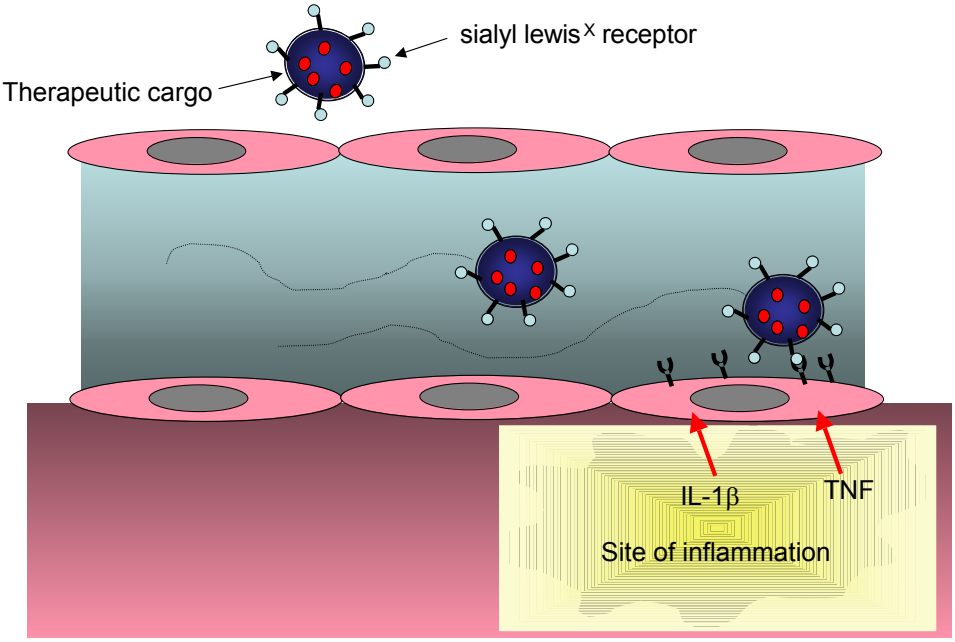
- Utility of antibody fragments:
  - Lack Fc region; reduced binding to phagocytic FcR-bearing macrophages and other phagocytes
  - Reduced immunogenicity for non-humanized antibodies
  - FAb allows production of monovalent binding molecule
    - Bivalent binding can trigger unwanted signaling cascades (e.g. EGFR)
  - Unique chemical sites introduced at opened hinge region in FAb' or $F(Ab')_2$



- Maleimide reaction with thiol creates stable thioether linkage:
  - Source of graphic: http://www.nature.com/nrd/journal/v1/n7/slideshow/nrd838_bx1.html



- *Example: targeting to vasculature*
  - Inflammatory signals delivered from peripheral tissues to endothelial cells induce upregulation of 'threat' signals on the surface of these cells within the lumen of blood vessels
    - Cytokine signals such as IL-1β, TNF
    - Chronic inflammation: upregulation of E-selectin
    - Acute inflammation: upregulation of P-selectin
      - Used to direct neutrophils and monocytes to sites of inflammation

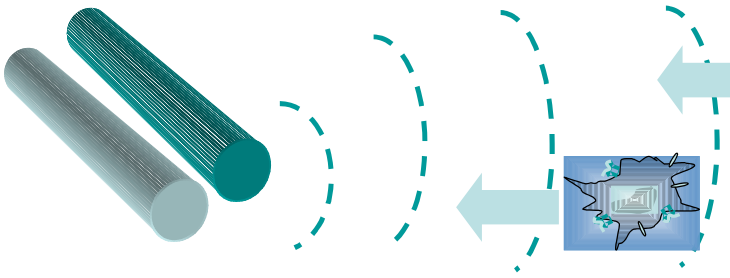


- antibody-based targeting

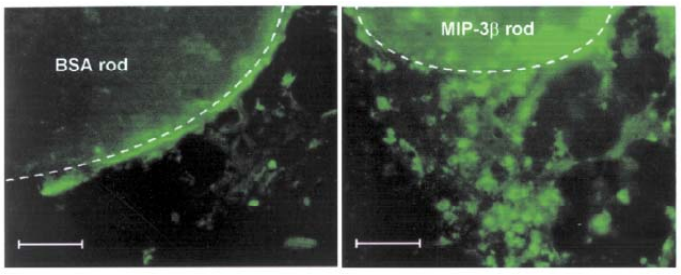
Targeted Activation
- local activation of a conjugate by action of enzymes or cellular environment
- example of cathepsin-sensitive linkages

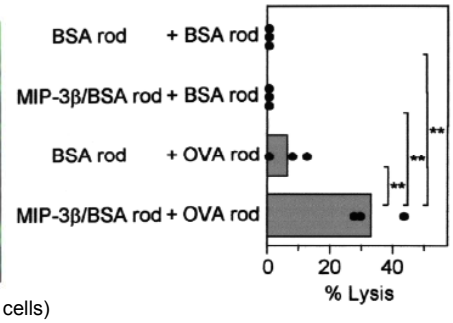
'Reverse' targeting[6]
- attraction of target cells to carrier

Attraction of target cells to device via chemotaxis:



Dendritic cells
~1 per 100 cells in most tissues





Tissue sections stained for MHC class II (expressed by antigen-presenting cells)

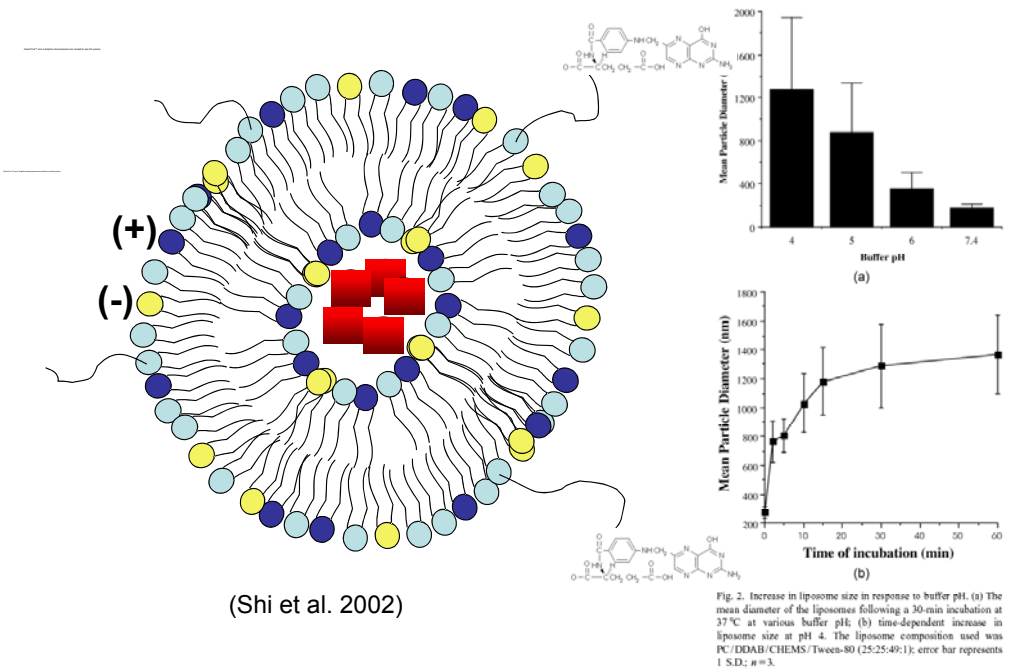
(Kumamoto et al. 2002)

Issues in Drug Targeting

- **'collateral damage'**
  - how unique is target? Is it expressed in normal tissues
    - often ratio of drug delivery tumor:normal tissue is not high enough
    - In certain cases, elimination of healthy cells is acceptable
      - E.g. hematopoietic system (T cells, B cells) can be replenished by bone marrow transplant
    - Many times the normal tissue from which tumors are derived *cannot* be safely destroyed
- **Tumor and viral escape**
  - Loss of target antigen expression due to rapid mutations (antigen-loss variants)
- **Immunological response to targeting agent**
  - Early studies used mouse antibodies for targeting
    - Low efficacy due to very short half-life and development of anti-sera

Integrating targeting, activation, and intracellular delivery

- Example of targeted delivery to cytosol by functionalized pH-sensitive liposomal carriers
  - Shi et al. 2002[5]
- Objective: intracellular delivery of a cellular toxin to tumors
  - Target receptor also triggers receptor-mediated endocytosis



(Shi et al. 2002)

Fig. 2. Increase in liposome size in response to buffer pH. (a) The mean diameter of the liposomes following a 30-min incubation at 37 °C at various buffer pH; (b) time-dependent increase in liposome size at pH 4. The liposome composition used was PC/DDAB/CHEMS/Tween-80 (25:25:49:1); error bar represents 1 S.D.; $n = 3$.

  - Charge neutralization at low pH drives irreversible aggregation of particles at low pH (membrane fusion on particle aggregation)
    - Rapid aggregation at 37°C

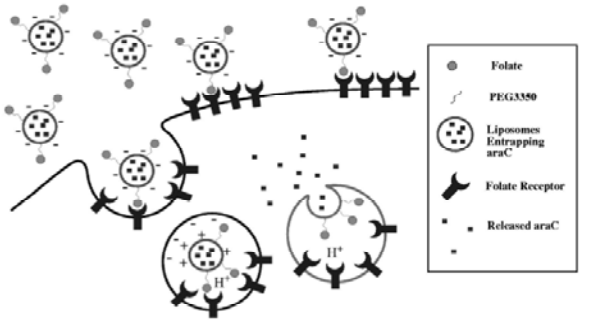


Fig. 6. Possible mechanism of intracellular araC delivery by FR-targeted cationic lipid-based pH-sensitive liposomes. At first, the folate-derivatized liposomes are taken into the cell via binding to the FRs on the plasma membrane and FR-mediated endocytosis. This is followed by acidification of the endosome, which results in protonation of the anionic lipid component and generation of a net positive surface charge on the liposomes. Finally, the electrostatic interactions between the liposomal and endosomal membranes result in bilayer fusion and the cytosolic delivery of the encapsulated araC.

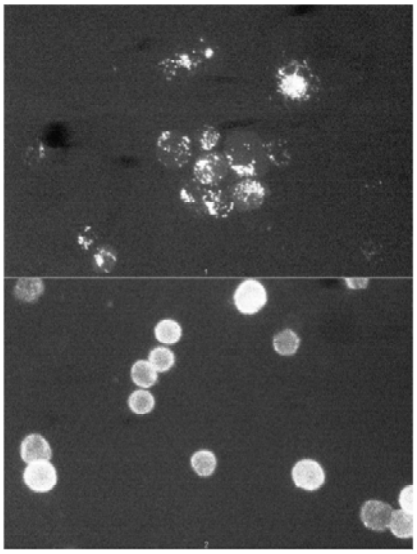


Fig. 4. Fluorescence micrograph of KB cells treated with FR-targeted calcein-containing liposomes. (Upper panel) Cells treated with FR-targeted non-pH-sensitive liposomes composed of PC/CHEMS/f-PEG-PE (60:40:0.1). (Lower panel) Cells treated with FR-targeted pH-sensitive liposomes composed of egg PC/DDAB/CHEMS/Tween-80/f-PEG-PE (25:25:49:1:0.1).

- Liposomes are electrostatically stabilized at neutral pH
  - Change in net surface charge leads to membrane fusion within endosomes
  - AraC = cytosine-β-D-arabinofuranoside
    - Cytotoxic agent for anti-tumor therapy

References

1. Eniola, A. O. & Hammer, D. A. Artificial polymeric cells for targeted drug delivery. *J Control Release* **87**, 15-22 (2003).
2. Halin, C. et al. Enhancement of the antitumor activity of interleukin-12 by targeted delivery to neovasculature. *Nat Biotechnol* **20**, 264-9 (2002).
3. Pardridge, W. M. Drug and gene targeting to the brain with molecular Trojan horses. *Nat Rev Drug Discov* **1**, 131-9 (2002).
4. Wickham, T. J. Ligand-directed targeting of genes to the site of disease. *Nat Med* **9**, 135-9 (2003).
5. Shi, G., Guo, W., Stephenson, S. M. & Lee, R. J. Efficient intracellular drug and gene delivery using folate receptor-targeted pH-sensitive liposomes composed of cationic/anionic lipid combinations. *J Control Release* **80**, 309-19 (2002).
6. Kumamoto, T. et al. Induction of tumor-specific protective immunity by in situ Langerhans cell vaccine. *Nat Biotechnol* **20**, 64-9 (2002).