Experimental Therapeutics I

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Specific Topics for Today

- Preclinical and clinical testing
- Gene therapy
  - Nonviral vectors
  - Methods of delivery and targeting
  - Genetic information delivered
    - Protein-coding sequences
    - RNA interference
- Oncolytic viruses

References

- **Weinberg, chapter 16**
- **Cancer treatment resource:**
  - Canadian Cancer Society: http://www.cancer.ca
  - (U Penn): http://www.oncolink.org/
  - National Cancer Institute (USA); http://www.cancer.gov/cancerinformation
- **Canadian cancer clinical trials:**
  - Canadian Partnership Against Cancer: http://www.canadiancancertrials.ca/
- **Gene therapy:**
  - Gene therapy clinical trials database**:** http://www.abedia.com/wiley/index.html/
- **RNA interference:**
  - http://www.rnaiweb.com/
  - http://www.protocol-online.org/prot/Molecular_Biology/RNA/RNA_Interference__RNAi_/
- **Oncolytic viruses:**
  - Melcher et al., (2011) Mol Ther. 19:1008-16.
  - Parato et al., (2005) Nature Rev. Cancer 5: 965-976

Testing Experimental Cancer Therapies

Experimental Therapies: Preclinical Cancer Research

- *Preclinical* stage: To assess agent in tissue culture and in animal models
- NCI's Developmental Therapeutics Program (DTP):
  - >400,000 drugs in repository
  - ~80,000 compounds screened since 1990

DTP Screening Process

- Preliminary screening:
  - 3 human tumor cell lines, one drug dose, 48 hour treatment
  - If growth inhibited in ≥ one cell line → test in full panel of human tumor lines *in vitro*
- Large scale *in vitro* screen in human tumors:
  - Panel of 60 human tumor cell lines, 5 doses, 48 hours
  - If drug is promising (kills preferentially ≥one tumor cell line, has unique mechanism of action, or works at low concentration) → testing in mice
  - ~ 2,500 compounds/yr tested at NCI
  - ~ 2 percent of those screened are recommended for testing in mice

Drug Discovery at NCI-Fact Sheet

Issues with Testing in Tissue Culture Models

- Are cell lines representative of human tumors?
  - NCI panel more aggressive than average clinical samples
  - Cell lines less heterogeneous than tumors
  - Tumor environment/stromal cell component missing

Testing in Animal Models

- Efficacy (how well does it work?): human tumor cell line xenografts in nude (immunocompromised) mice
- Drug properties: stability, uptake, excretion, activity profile
- Toxicity: effects on normal cells and tissues (two species of animals)

Problems with Animal Models

- Drug activities in mice not always predictive for humans
- Regression of tumors in animal models not always predictive of results with human cancers:
  - Model tumors (human xenografts or mouse tumors) grow much faster than typical human cancers
  - No (or limited) immune component in xenograft models
  - Mouse tumors equivalent to human tumors not always available
  - Tumor environment in models very different from that in patients

Clinical Cancer Research

- Clinical testing for Investigational New Drugs (IND): To assess agent in patients
  - Phase I, II, III for experimental agents
  - IV for approved treatments
- Currently, 659 cancer trials recruiting patients in Canada (119 current trials at the Cross Cancer Institute)

Clinical Testing

- **Phase I**: **Safety and toxicity**
  - Small numbers of patients (~30)
  - Measures side effects
  - Starts at low doses, increases incrementally up to the maximum tolerable dose
  - May measure some readout of anti-cancer activity, but not statistically significant

Phase II/III Clinical Testing

- **Phase II: Efficacy (how well does it work?)**
  - Agents that pass phase I
  - Larger numbers of patients (~50-100)
  - Indications (type of tumor, stage of progression, etc) considered
  - Efficacy and side effects measured

- **Phase III: Comparison with current standard of care**
  - Larger groups of patients (100s to 1000s)
  - Patients usually randomized
  - Efficacy and side effects measured
  - Statistical significance

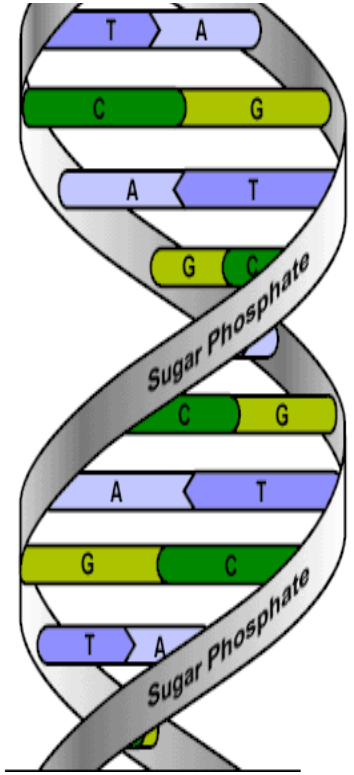
Approval

- After successful phase III trials
- Health Canada (Food and Drug Administration in US) approval for a specific indication
- ~15 years (~$1 billion) from preclinical to approval: new fast-tracking can speed this up

Novel/Experimental Cancer Therapies

- Targeted low molecular weight drugs
- Biological agents:
  - Monoclonal antibodies targeting
    - antigens on surface of tumor cells (e.g. HER2): goal is immune clearance of tumor cells
    - secreted proteins (e.g., VEGF): goal is inactivation
  - Proteins for
    - immunotherapies (cytokines): to induce an anti-tumor immune response
    - anti-angiogenic therapies (endostatin): to block nutrient and oxygen supply to tumor
  - Gene therapy
  - Virotherapy

Gene Therapy



What is Gene Therapy?

- Transfer of genetic information to recipient (cell/tissue/organism) in order to treat a disease or its symptoms

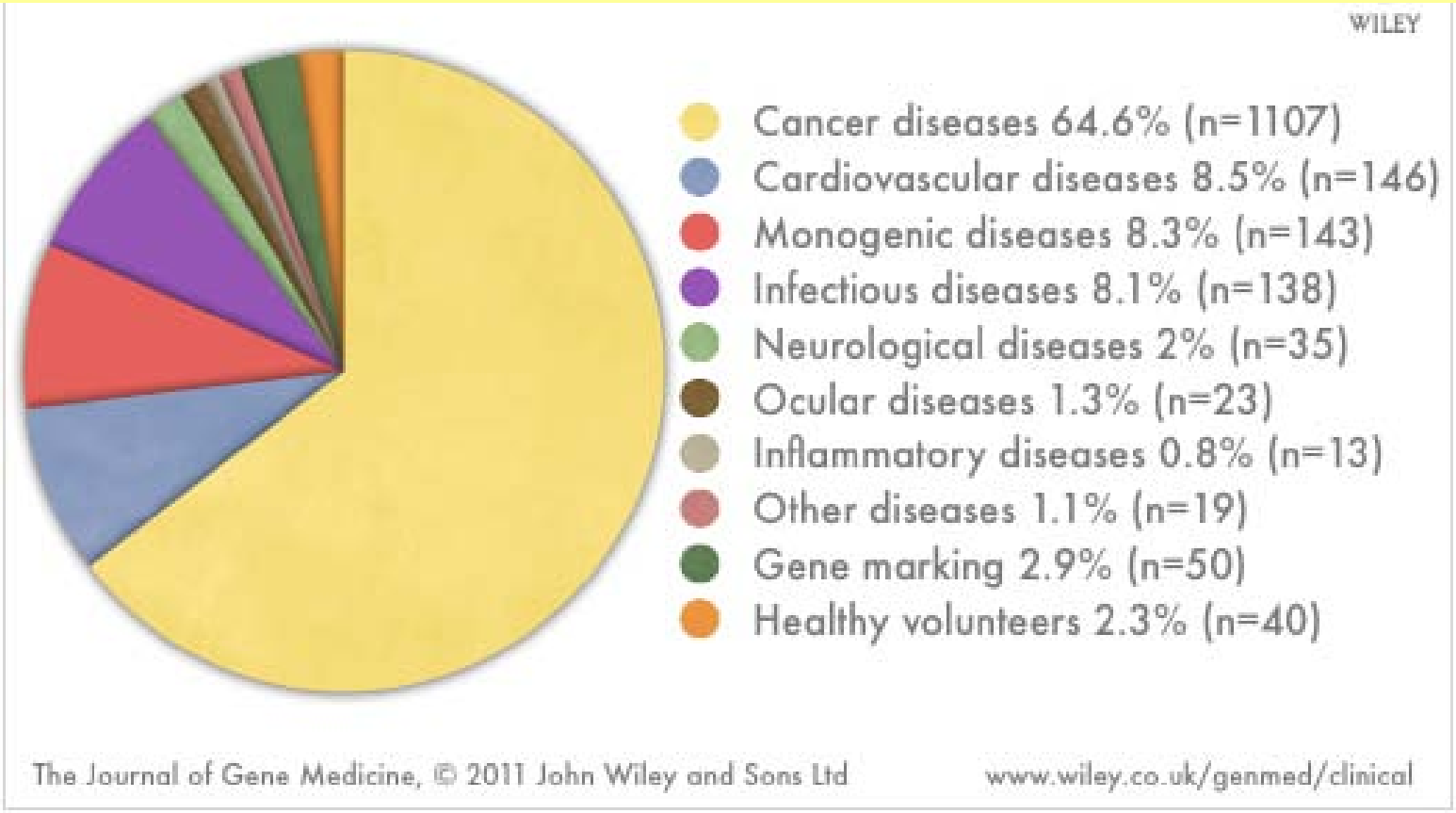
When is Gene Therapy Applicable?

- When the therapeutic gene encodes an intracellular or transmembrane protein
- For local protein or RNA delivery
- (Cancer vaccines to deliver antigens)

- No shortage of potential gene targets in cancer

➢ **Challenge is delivery**

Gene Therapy Clinical Trials by Disease & by Phase

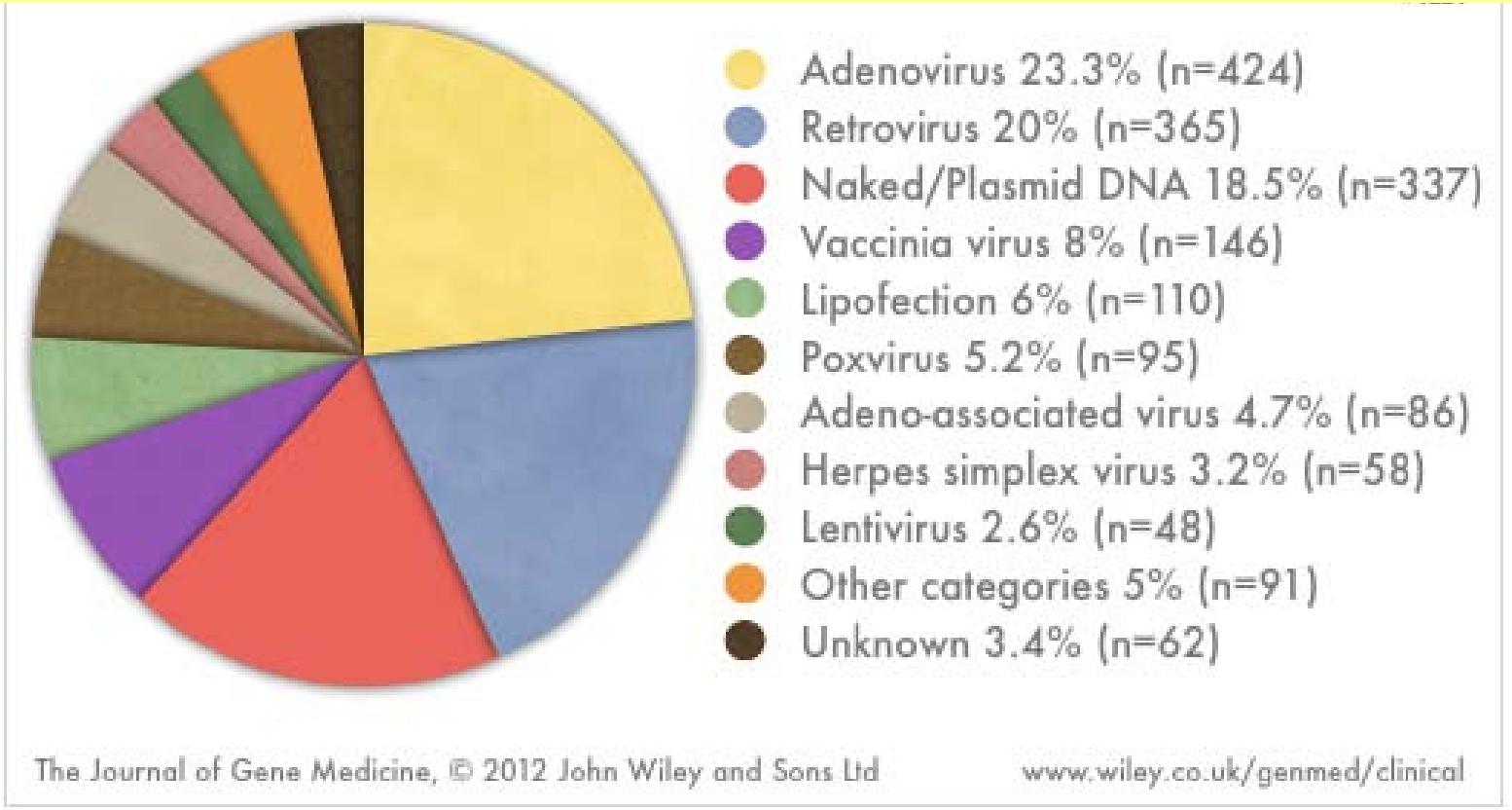


Phase I: 60%; Phase I/II: 19%
Phase II: 16%; Phase III: 3.4%

Components of Gene Therapy Vectors

- Delivery vehicle (*e.g.,* virus, liposome)
- Regulatory elements controlling the transgene (*e.g*., tissue-specific promoter/enhancer)
- Transgene (*e.g*., p53)

Vectors Used in Gene Therapy Clinical Trials



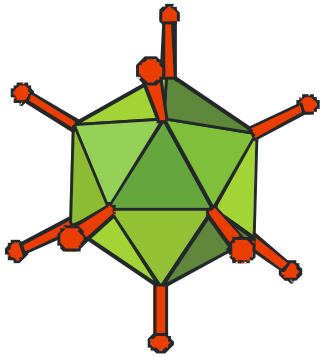
Viral vs. Non-viral Vectors

Viral vectors

- Selected by nature for highly efficient gene transfer
- Replication-defective or replication-competent

Nonviral vectors

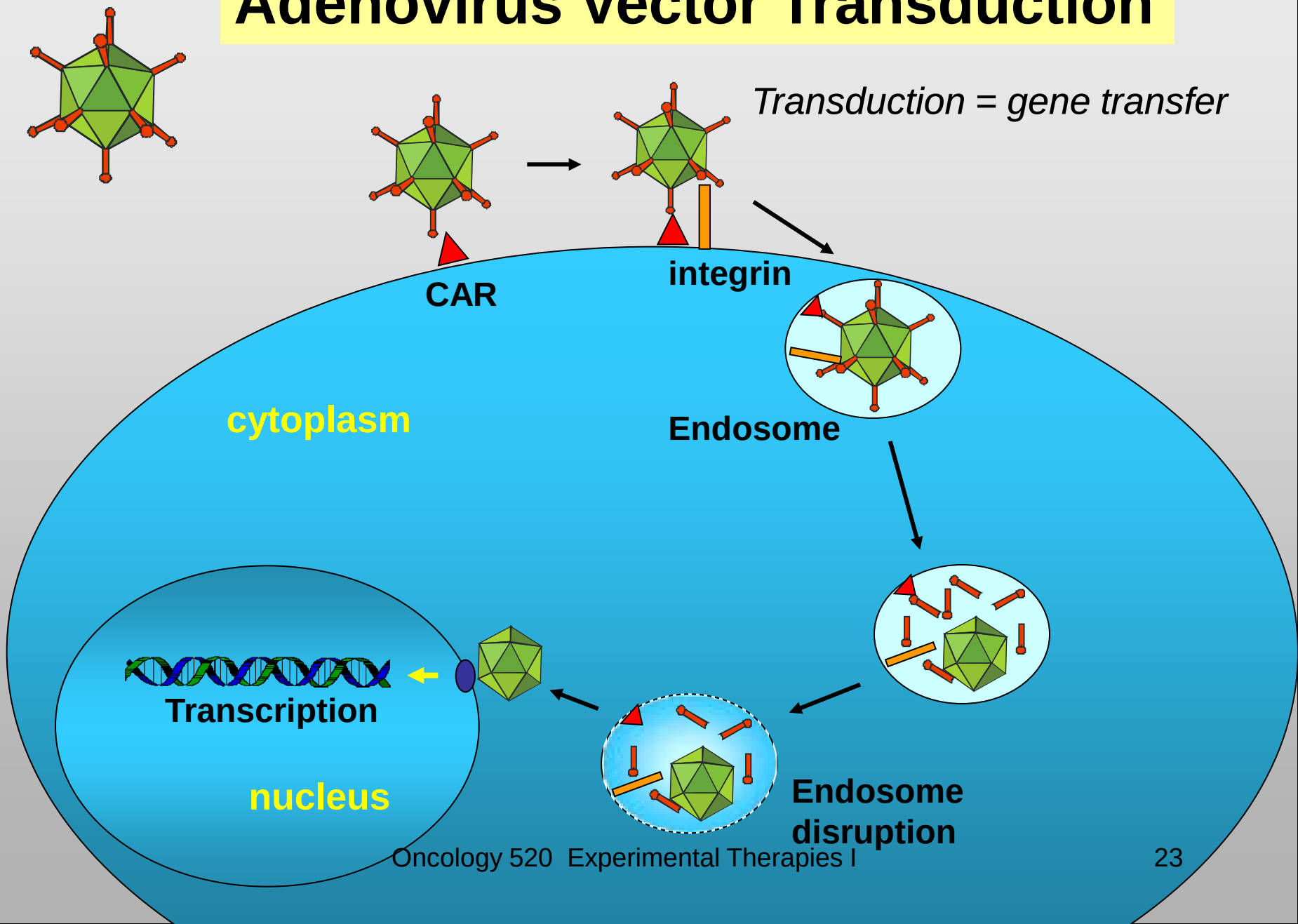
- Higher degree of safety (no revertants or recombinants)
- Usually cheaper to make
- Fewer limitations on size of gene transferred



Adenovirus Properties

- Medium size (36 kilobase) double-stranded DNA virus
- Infects a wide variety of cell types from many different species
- Infects both proliferating and quiescent cells
- Can evoke a strong immune response
- Viral genome does not integrate into host genome
  - no insertional mutagenesis
  - Transient expression

Adenovirus Vector Transduction



Retroviral Vector Properties

- RNA virus, ~8 kb genome
- Engineered to infect a variety of cell types from different species
- Viral genome integrates into host genome
  - usually only in proliferating cells (lentivirus is exception)
  - long term expression is possible
  - potential for insertional mutagenesis
- No immunity induced against transduced cells

Retroviral Vector Transduction

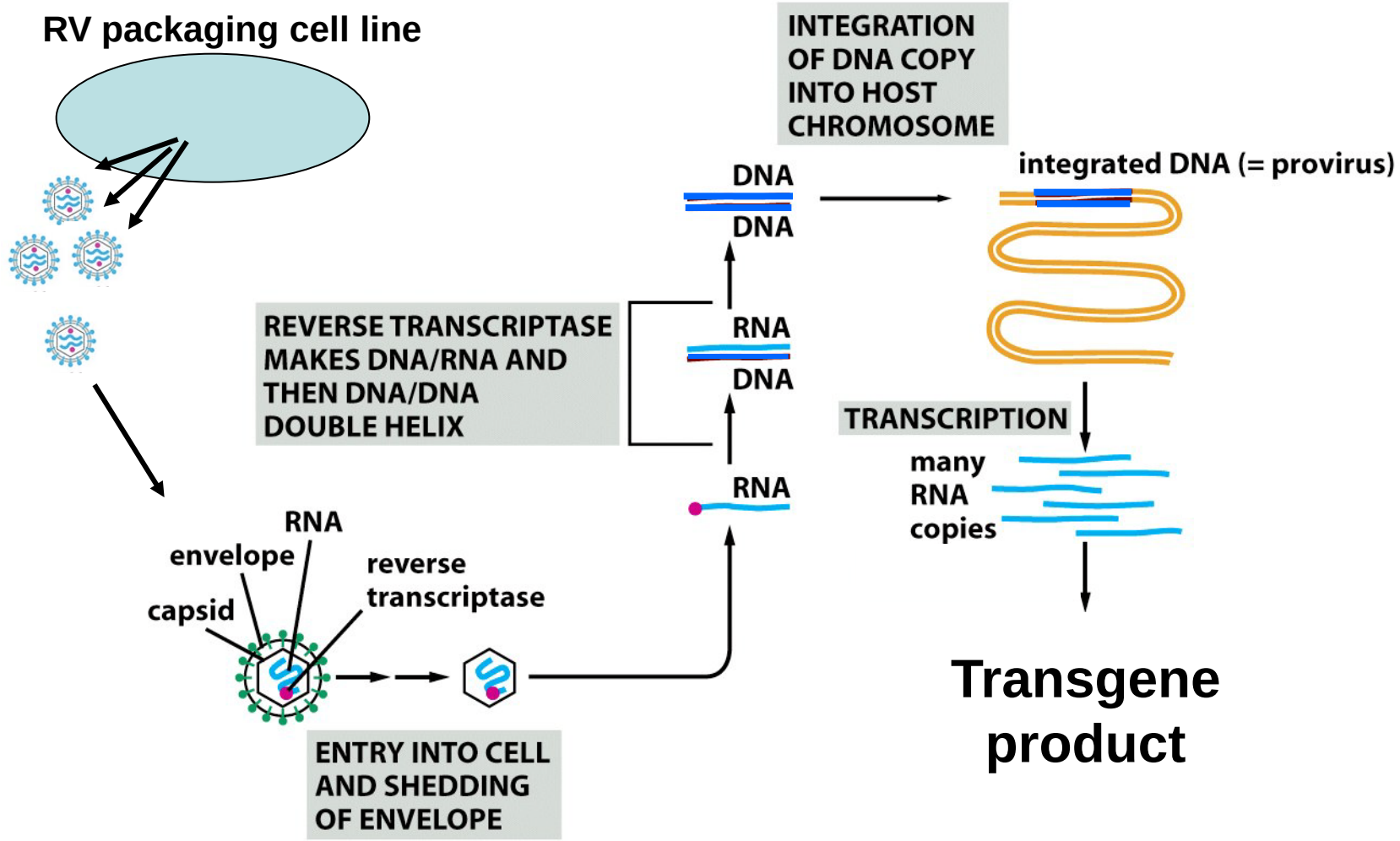
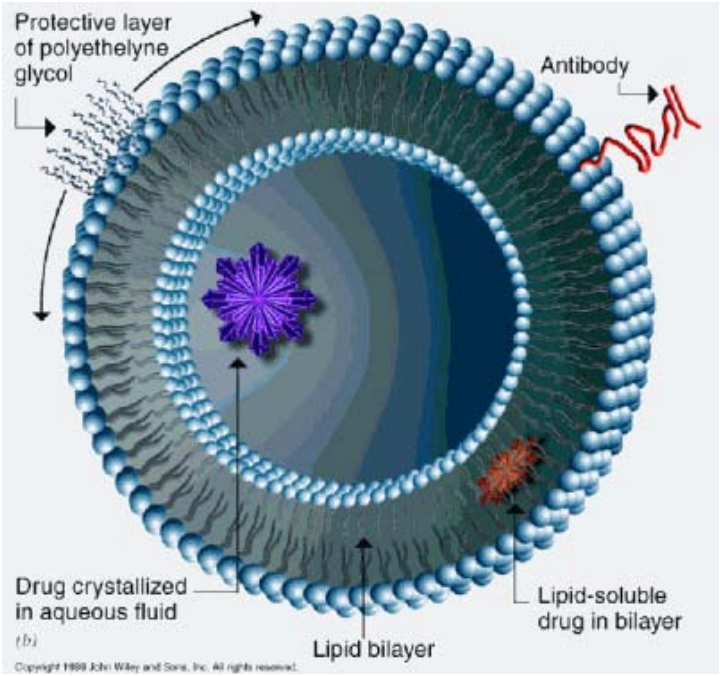


Figure 3-17 The Biology of Cancer (© Garland Science 2007)

Nonviral Delivery Systems

- Nanoparticles:
  - *Liposomes*: lipid spheres that can harbor DNA, RNA or other molecules
  - Nucleic acids can be encapsulated in non-lipid coats (amino acid polymers)
- Naked DNA transfer
  - *In vivo* or *ex vivo*



Liposome structure

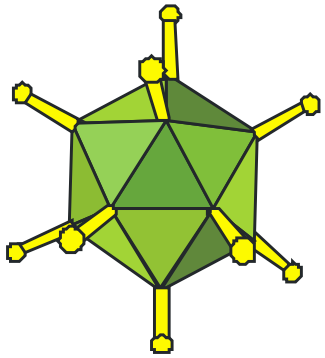
Vector Targeting

To improve therapeutic index (minimize damage to normal tissues)

- Transductional targeting: modification of delivery vehicle so that vector enters only certain cells
- Transcriptional targeting: modification of regulatory DNA or RNA sequences in vector so that gene is activated only in certain cells

Transductional Targeting

Adenovirus



Retrovirus

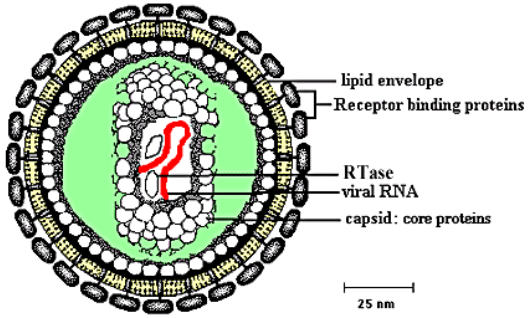
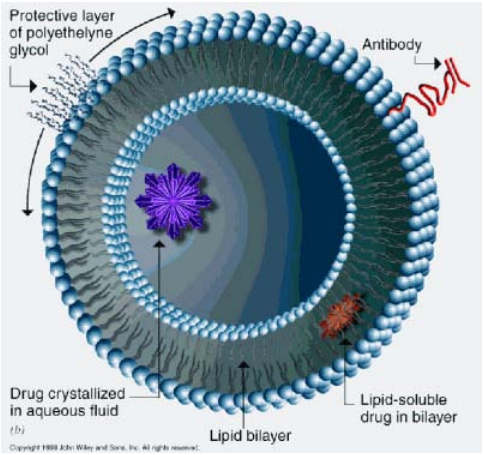


Diagram of a Retrovirus

Liposomes

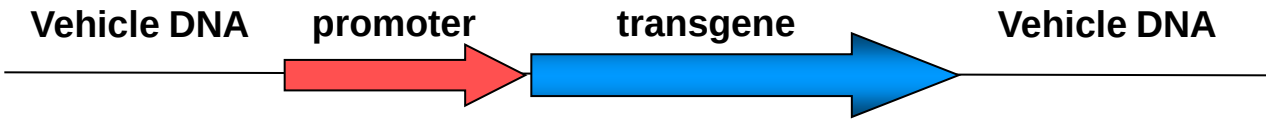


Modify viral genes:

- Modify capsid proteins genetically
- Replace envelope gene with an alternate envelope gene during vector production

Or make non-genetic modifications:

- Chemically attach (or embed) antibodies, ligands or other targeting molecules to surface of vehicle



Transcriptional Targeting to Tumors

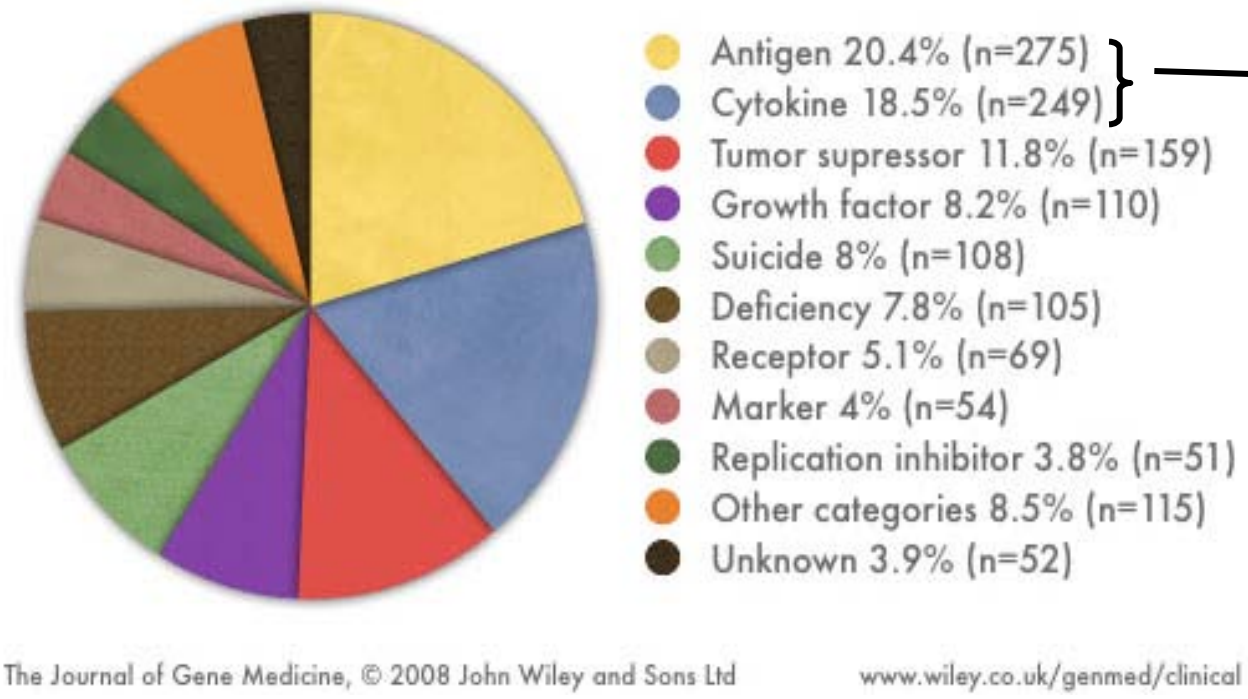
Transcriptional targeting: mRNA only produced in specific targeted cells

Transgene transcription made specific by :

- Promoters of genes expressed in tissues from which the cancer originates (e.g., prostate-specific antigen)
- Promoters of genes expressed in metastatic cancers (osteocalcin: expressed in bone mets of prostate cancer)
- Promoters of genes expressed by tumor cells specifically (telomerase)
- Promoters of genes induced by conditions at the tumor site (e.g., hypoxia-inducible genes)

Promoters = regulatory DNA sequences that control gene expression

Types of Genes Transferred in Clinical Trials



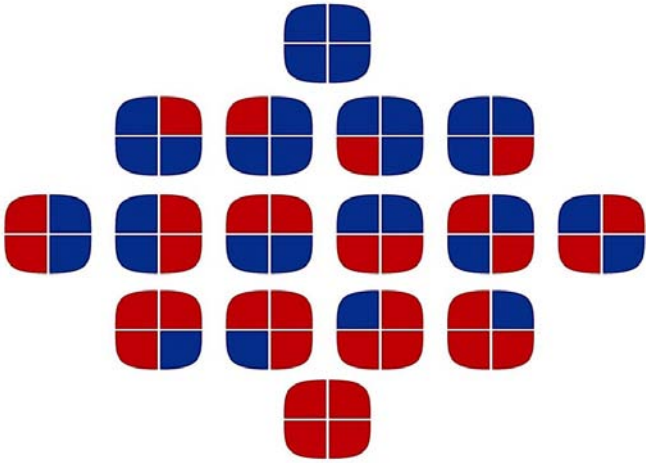
Strategies for Cancer Gene Therapy

Strategies that target specific processes involved in tumor development:

- Expression of tumor suppressor genes or other genes that prevent tumor growth or progression
- Inhibition of oncogenes or other genes that promote tumor growth or progression

➢ Hundreds of candidates genes

Replacement of p53



- Tumor suppressor
- Induces cell cycle arrest and apoptosis
- Most commonly mutated gene yet identified in human cancers
- Over-expression of p53 induces cell cycle arrest (but not apoptosis) in normal cells

➢ Potentially good candidate for gene therapy

p53 Gene Therapy

- Phase III trial for head and neck cancer end stage patients: survival increased with superior safety for Adp53 compared to methotrexate
- Clinical trial with Adp53 plus radiation or chemotherapy for non-small cell lung carcinoma (NSCLC):
  - clinical response in $\geq$ half of patients (biopsies 3 months post-treatment show no evidence of tumor in 70% of patients)
  - evidence of apoptosis induction at the tumor site
- Not yet approved by US FDA

Adp53 Gene Therapy Approval in China (2003)

- China has approved Gendicine (Adp53) for commercialization (the first gene therapy vector approved) based on primary response rates in NSCLC

Adp53 Clinical Trial for Ovarian Cancer

Phase III trial of Adenovirus-p53 plus chemotherapy in ovarian cancer patients

- ➢ No therapeutic benefit

Possible reasons for failure:

- Impairment of molecules “downstream” of p53 (77% of ovarian cancers are impaired in activation of caspase-9 and caspase-3)
- Some p53 mutations are dominant-negative
- Pre-existing immunity to Ad
- Low/variable level of Ad receptor on primary tumor

Strategies for Cancer Gene Therapy (II)

Strategies that target specific processes involved in tumor development:

- Replacement of tumor suppressor genes or other genes that prevent tumor growth or progression
- Inhibition of oncogenes or other genes that promote tumor growth or progression

➢ Hundreds of candidates genes

Inhibition of Genes that Promote Tumor Growth or Progression

The Nobel Prize in Physiology or Medicine 2006

"for their discovery of RNA interference - gene silencing by double-stranded RNA"

Photo: L. Cicero/Stanford

Andrew Z. Fire

Photo: R. Carlin/UMMAS

Craig C. Mello

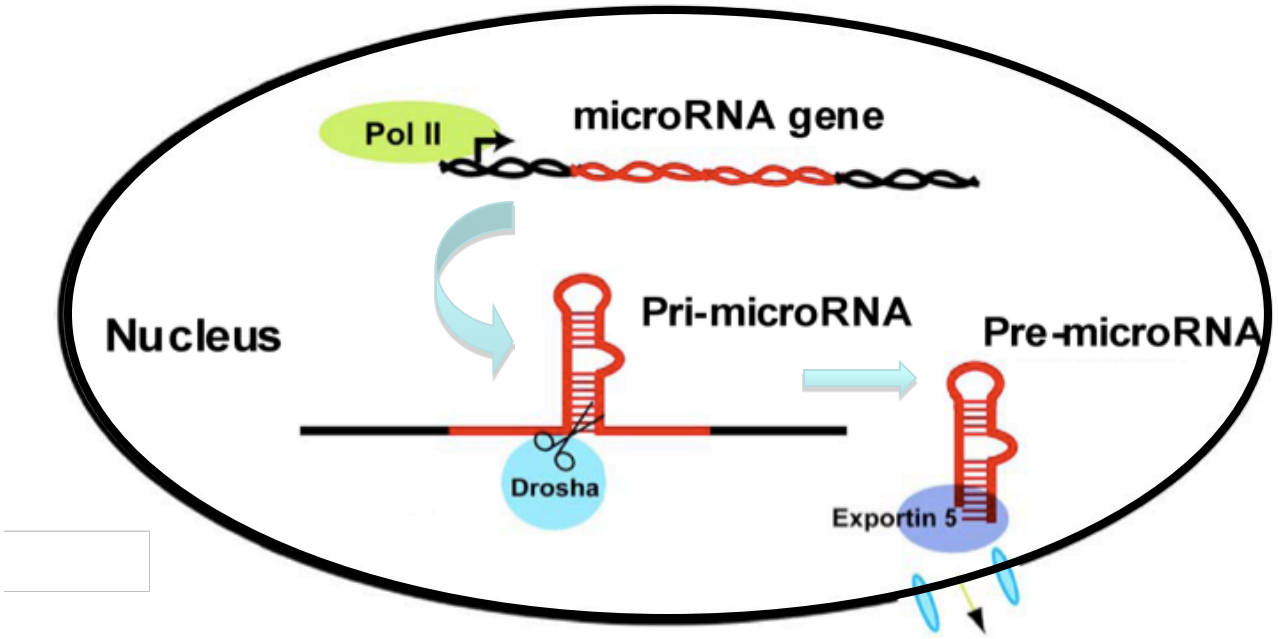
RNA interference:

- naturally occurring process
- sequence-specific inhibition of gene expression
- mediated by small double-stranded RNAs

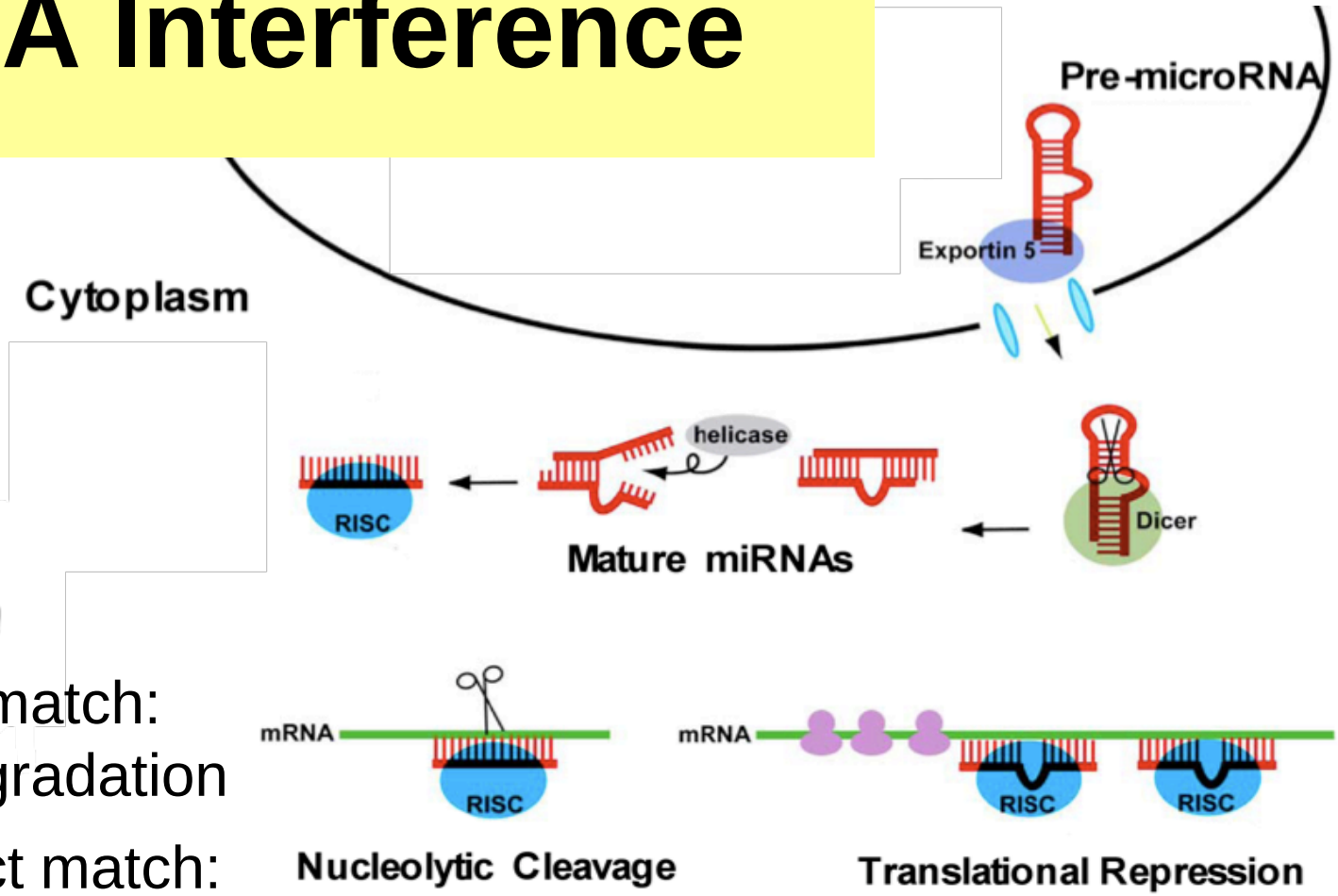
RNA Interference in Mammals

Role of RNAi: to regulate gene expression

MicroRNA Processing:



RNA Interference



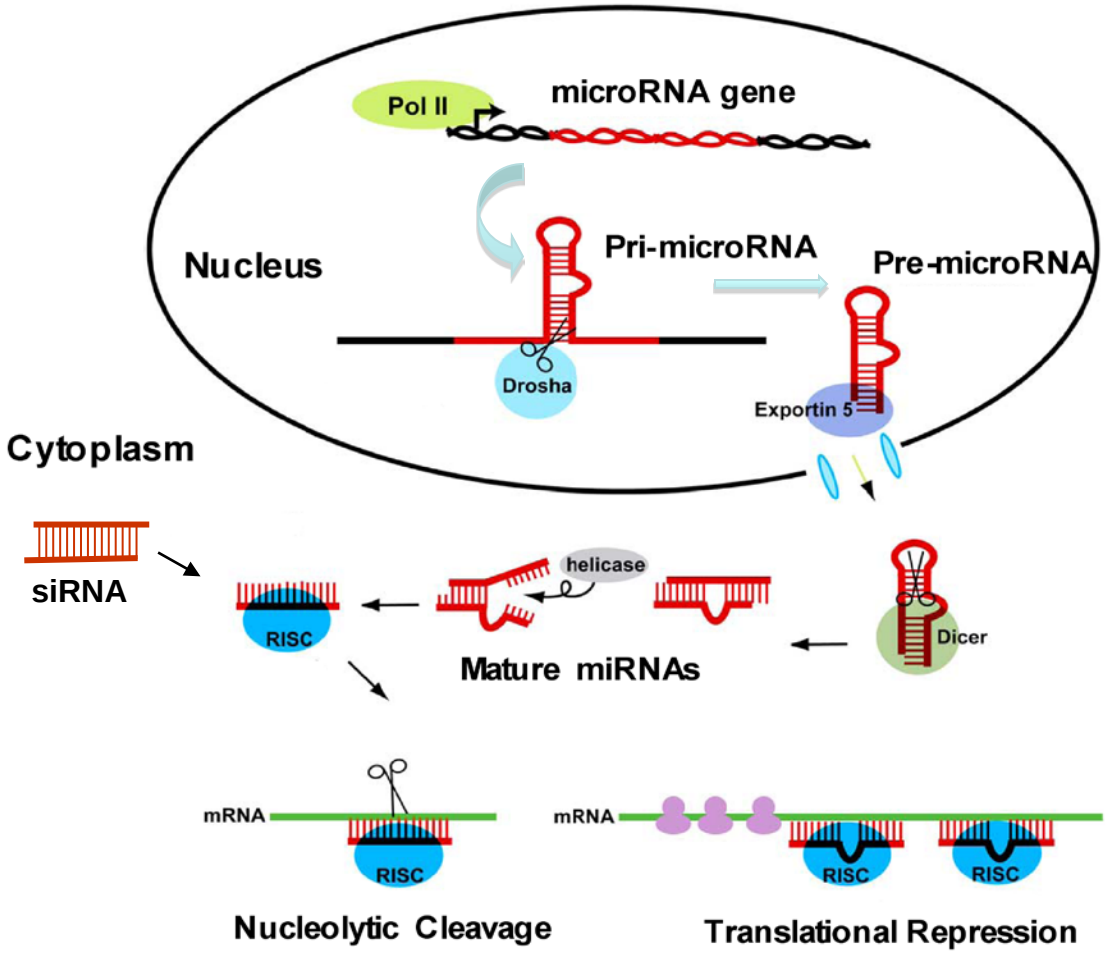
- Perfect match: mRNA degradation
- Imperfect match: blocks translation (protein synthesis)

RISC: RNA-Induced Silencing Complex

Hammond (2005) *FEBS Letters* **579**: 5822-5829

Experimental "Knockdown" of Gene Expression

- Synthetic double-stranded short interfering RNA (siRNA)
- Engineered miRNA genes produce short hairpin RNA (shRNA)



Hammond (2005) *FEBS Letters* **579**: 5822-5829

Inducing RNA Interference as a Cancer Therapy

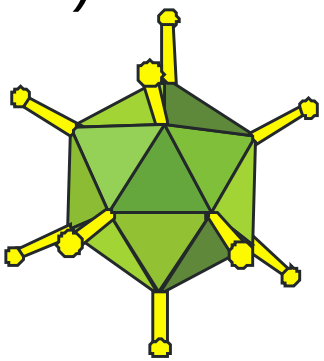
- ➢ *To reduce expression of genes involved in cancer progression*
- Advantage of si/shRNA as a therapeutic: high specificity based on gene sequence
- Challenges similar to other gene therapy approaches: delivery, stability, safety
- Additional challenge: incomplete knockdown

Targets of si/shRNAs

- Expression of a variety of targets has been inhibited by engineered si/shRNAs in experimental cancer studies
  - Anti-apoptotic proteins
  - Signaling molecules
  - Telomerase
  - HPV E6 & E7
  - MDR-1
  - Many, many more

Oncolytic Viruses

Oncolytic virus: Virus that undergoes a productive lytic infection in tumor cells (infects, replicates and packages the viral genome, then lyses host cell)



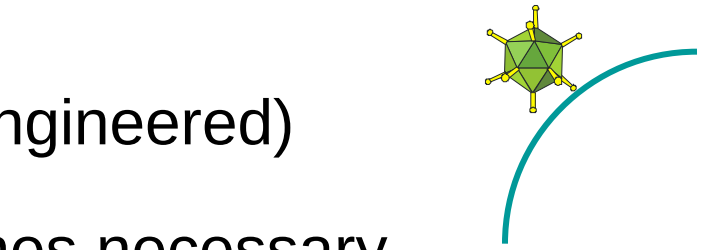
History

- **1956 (NCI) studies:** cancer patients treated with wild-type lytic viruses
- 20/30 cervical carcinoma patients treated with Ad had clinical response (low)
- Abandoned due to potential safety issues and chemotherapy alternatives
- Revisited after technology available to make viruses more tumor-selective and more robust

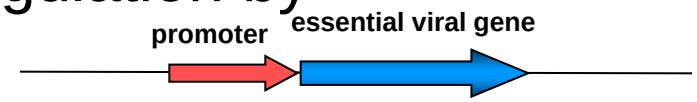
Ideal Properties of Virus for Development as Oncolytic Agent

- Tumor-selective
- Safe (therapeutic virus and possible revertants)
- Replicates rapidly
- Spreads to adjacent cells (overcomes delivery problems)
- Spreads throughout the host (reaches metastatic sites)
- Evades the host immune response
- Can be manipulated genetically (to enhance above properties, or to “arm” the virus)

Mechanisms for Tumor-Selective Replication of Oncolytic Viruses



- Selective cell entry (natural or engineered)
- Selective expression of viral genes necessary for replication (transcriptional regulation by tissue-specific promoters)



- Selective replication dependent on pathways that are dysregulated in tumor cells

Pathways that Could Limit Virus Replication in Normal Cells

- Cell cycle control (many viruses require host cell replication machinery)
- Control of signaling pathways (e.g., ras, Akt activation leads to replication of some viruses)
- P53, pRb and apoptosis induction
- Interferon response induction

➢ Viruses that dysregulate these pathways can replicate in normal cells

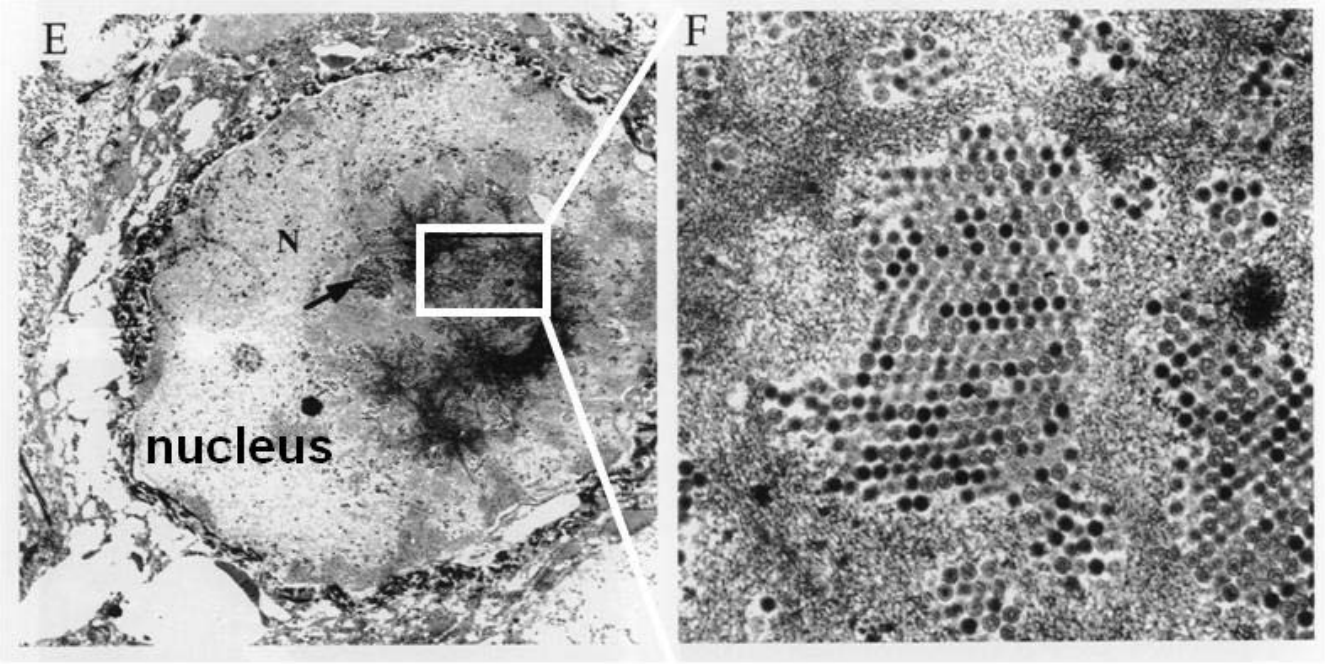
Oncolytic Virus Replication in Cancer Cells

- Cancer cells carry mutations in these control pathways (p53, pRb, etc.)
- Viral genes involved in dysregulation of p53, pRb, etc, are redundant in cancer cells
- Deletion of these viral genes should have no effect on virus replication in tumor cells, but virus replication is blocked in normal cells

Examples of Oncolytic Viruses in Clinical Trials

- Adenoviruses (ONYX-015: viral E1B gene deletion; other Ads: prostate-specific promoters controlling E1A and/or E1B)
- Reovirus (requires activated ras)
- Newcastle Disease Virus (requires interferon-defective cells)
- Vaccinia (multiple deletions)
- Herpes simplex virus (Phase III trial for glioblastoma)
- Measles virus (CD46 receptor over-expressed on tumor cells)
- Vesicular stomatitis virus (VSV) (interferon-defective cells)

ONYX-015 (Ad) Replicates in Human Tumors



From D. Kirn (1999), in “Gene Therapy of Cancer” (E. Lattime and S. Gerson, eds.) Academic Press, San Diego, CA, USA, pp. 235-250.

ONYX-015 (Ad) Clinical Results

- Well tolerated
- Anti-viral antibodies did **NOT** block anti-cancer activity after intra-tumoral injection
- Potential synergism with chemotherapy

➢ H101, similar to ONYX-015 approved for head and neck cancer in China in Nov 2005

Current Status of Oncolytic Viruses

- Oncolytic viruses: tumor-selective (tumor cell or tumor vasculature), safe, replicate rapidly and spread to adjacent cells
- Oncolytic viruses elicit anti-tumor immune response, particularly when “armed” with immunomodulatory genes
- Balance between anti-viral response (limiting virus spread) and anti-tumor response is important

Summary

- Novel cancer treatments include immunotherapies, anti-angiogenic therapies, small molecular weight drugs, gene therapies and viruses
- Novel cancer agents undergo preclinical and clinical testing
- Each type of gene therapy vector has its own advantages and disadvantages
- Gene therapy agents act by
  - inducing anti-tumor immunity
  - activating tumor suppressor pathways (e.g., p53) or other cytotoxic pathways
  - inhibiting tumorigenic pathways (using RNA interference)
- Oncolytic viruses, either naturally or through genetic engineering, should preferentially replicate in and kill tumor cells: safety and efficacy in patients under evaluation