Lecture XII –
Gene therapy for cancer
Figure 1 | Cancer gene therapy and immunotherapy trials currently listed as open by the US Recombinant Advisory Committee. Over half of all gene-therapy-based protocols in the United States (113 currently open) are aimed at boosting the immune response to tumour antigens. Trials in melanoma alone account for 54% of immunotherapy trials. Delivery of the tumour-suppressor gene TP53 accounts for the next largest group, followed by suicide gene delivery, in which viral vectors deliver enzymes that activate prodrugs to toxic products that kill tumour cells and their neighbours. Most of these use herpes simplex virus thymidine kinase (HSV-TK), which activates the prodrug ganciclovir. Chemoprotection is an indirect approach in which bone marrow cells are infected with viruses that protect them from the toxic effects of chemotherapy, by expressing drug-resistance genes.
Aim of cancer gene therapy

Effective killing of most (if not all) cancer cells without serious damage to normal cells and tissues
Cancer gene therapy

1. Direct attack on tumor cells
   a) transfer of tumor suppressor gene
   b) inhibition of oncogenes
   c) suicide genes
   d) oncolytic viruses (replication-competent viruses)

2. Harnessing immune response to tumor antigens

3. Chemoprotection

4. Anti-angiogenic therapy
p53 - the guardian of the genome

Cellular Stress

DNA damage, hypoxia, Expression of oncogene (E1A, Myc)

Inactive p53 → Stabilization → Active p53

Regulation of target genes

GADD45, p21WAF1, MDM2, Bax, IGF-BP3, Fas, DR5

DNA repair, Cell cycle arrest, Regulation of p53 → APOPTOSIS
p53-induced cell-cycle arrest in response to DNA damage.

The normally unstable p53 protein is stabilized by damaged DNA, so its concentration increases. Acting as a transcription factor, p53 induces expression of p21^{CIP}, a cyclin-kinase inhibitor that inhibits all Cdk1-, Cdk2-, Cdk4-, and Cdk6-cyclin complexes. Binding of p21^{CIP} to these Cdk-cyclin complexes leads to cell cycle arrest in G_1 and G_2.
Ionizing radiation increases the cellular level of p53. This stimulates p21 expression, which inhibits cyclin/CDK activity. This maintains Rb in its hypophosphorylated, growth inhibitory state. Additionally, p53 stimulates GADD45 expression, which binds PCNA and blocks DNA synthesis.
Transcription factor E2F

Quiescence

- cdk
- Cyclin A
- Rb
- E2F

TTTCGCGC

silent

- c-myc
- c-myb
- cdc2 kinase
- PCNA

Proliferation

- cdk
- Cyclin A
- Rb
- E2F

TTTCGCGC

transactivate

- c-myc
- c-myb
- cdc2 kinase
- PCNA

Wild-type p53 activates transcription of p21 and GADD45

P53 mutations found in tumor disrupt transcription

The ability of p53 to stimulate transcription is essential for tumor suppression
p53 and gene therapy of cancer
Head and Neck Cancer

Overview
Head and neck cancer is the term given to a variety of malignant tumors that develop in the
• oral cavity (mouth);
• pharynx (throat);
• paranasal sinuses (small hollow spaces around the nose lined with cells that secrete mucus);
• nasal cavity (airway just behind the nose);
• larynx ("Adam's apple" or voice box); and
• salivary glands (parotid, submandibular, sublingual glands that secrete saliva).

Many authorities also include skin tumors of the face and neck and tumors of the cervical lymph nodes.

Excluding superficial skin cancers, but including cancer of the larynx and thyroid, it is conservatively estimated that about 60,000 people are diagnosed with head and neck cancer annually - about 5% of all cancers diagnosed in the United States. There are more than half a million survivors of oral, head, and neck cancer living in the United States today.
Squamous cell carcinoma

Review of SCC

- SCC is a malignant neoplasm of stratified squamous epithelium that is capable of locally destructive growth and distant metastasis.

M. Katz, R. Willden
Review of SCC

- SCC is the most common malignant neoplasm of the oral cavity
- SCC represents 90% of all oral cancers
- Most common on lower lip, lateral borders of tongue, and floor of mouth
- Incidence increases with age. Most after 40 years
- Survival rate is 50% long term
First officially registered therapeutic nucleic acid

Gendicine (SiBiono GeneTech, Chiny)
Adenoviral vector with a correct p53 gene

Efficient in patients with head and neck cancers

Appears 3 x more efficient than radiotherapy alone

Registered on 16. X. 2003, after 5 years of clinical trials
Advexin – adenoviral vector expressing p53 gene

effective in patients with head and neck cancer

Alfredo and Elena Gonzalvo enjoyed visiting relatives recently in the Philippines.

Research nurse supervisor Marcelo Dolormente helps Bernis Teaters celebrate her fifth anniversary after gene therapy.
Examples of the most advanced clinical trials of cancer gene therapy

<table>
<thead>
<tr>
<th>Company or research institute</th>
<th>Indication</th>
<th>Delivered gene</th>
<th>Vector</th>
<th>Phase of clinical development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shenzhen SiBiono Gene Technologies (Shenzhen, China)</td>
<td>HNSCC</td>
<td>Tumor protein p53</td>
<td>Adenovirus</td>
<td>Approved</td>
</tr>
<tr>
<td>Shanghai Sunway Biotech (Shanghai, China)</td>
<td>HNSCC</td>
<td>HAdv5 oncolytic virus</td>
<td>Adenovirus</td>
<td>Phase 3</td>
</tr>
<tr>
<td>AnGes MG (Osaka, Japan)</td>
<td>Arteriosclerosis obliterans</td>
<td>Hepatocyte growth factor</td>
<td>Plasmid</td>
<td>Phase 2</td>
</tr>
<tr>
<td>GenVec, Inc. (Gaithersburg, MD, USA)</td>
<td>Pancreatic, esophageal and rectal cancers</td>
<td>Human tumor necrosis factor ?</td>
<td>Adenovirus</td>
<td>Phase 2</td>
</tr>
<tr>
<td>Introgen (Austin, TX, USA)</td>
<td>Head and neck, lung, breast, esophageal, ovarian, bladder, brain, prostate and bronchoalveolar cancers</td>
<td>Tumor protein p53</td>
<td>Adenovirus</td>
<td>Phases 1–3</td>
</tr>
<tr>
<td>Transgene (Strasbourg, France)</td>
<td>Cervical cancer</td>
<td>Human papilloma virus type 16 E6 and E7 antigens and interleukin 2</td>
<td>Vaccinia virus</td>
<td>Phase 2</td>
</tr>
<tr>
<td>Transgene (Strasbourg, France)</td>
<td>Breast, lung, prostate and renal cancers</td>
<td>Human mucin 1 antigen and interleukin 2</td>
<td>Vaccinia virus</td>
<td>Phase 2</td>
</tr>
</tbody>
</table>

Nature Biotechnology, January 2004
Cancer gene therapy

1. Direct attack on tumor cells
   a) transfer of tumor suppressor gene
   b) inhibition of oncogenes
      - antisense therapy
      - ribozymes
   c) suicide genes
   d) oncolytic viruses (replication-competent viruses)
2. Harnessing immune response to tumor antigens
3. Chemoprotection
4. Anti-angiogenic therapy
Cancer gene therapy – inhibition of oncogenes

Quiescence

Proliferation

Antisense or ribozyme gene therapy in cancer

Diagram showing:
- Viral vector
- Normal cell: no effect
- Tumour cell
- Antisense or ribosome-encoding gene
- Onc or Chemo- or radiotherapy
- No bystander effect
- Arrest
- Apoptosis
Blocking oncogenes in tumors

Sis - growth factor
 erbB-2
 abl
 ras
 jun
 myc
DNA decoys for cancer gene therapy

Will they be effective?

Remeber the story of Edifoligide
When Bad Gene Transfer Is Good...
Cancer gene therapy

1. Direct attack on tumor cells
   a) transfer of tumor suppressor gene
   b) inhibition of oncogenes
      - antisense therapy
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   c) suicide genes
      d) oncolytic viruses (replication-competent viruses)

2. Harnessing immune response to tumor antigens

3. Chemoprotection

4. Anti-angiogenic therapy
Suicide gene therapy - pro-drug activation

The objective of pro-drug activation therapy is to express an activating enzyme within the tumor, which will then activate a systemically delivered, inactive pro-drug at the target site only.
Bystander effect (efekt sąsiedztwa)
## Enzyme-prodrug combination for suicide gene therapy

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Prodrug</th>
<th>Product</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-tk</td>
<td>ganciclovir</td>
<td>ganciclovir triphosphate</td>
<td>blocks DNA synthesis</td>
</tr>
<tr>
<td>cytosine deaminase</td>
<td>5-fluorocytosine</td>
<td>5-fluorouracil (5-FU)</td>
<td>blocks DNA and RNA synthesis (pyrimidine antagonist)</td>
</tr>
<tr>
<td>cytochrome P450</td>
<td>cyclophosphamide</td>
<td>phosphoramidemustard</td>
<td>DNA alkylating agent; blocks DNA synthesis</td>
</tr>
</tbody>
</table>
1. Glioblastoma multiforme - Infiltrative; rapid-growing; occurs: most frequently in mid-aged; apt to involve both cerebral hemispheres via the corpus callosum; Average Survival: 1 year
Glioblastoma multiforme

Department of Neurosurgery, University of Kuopio, A.I. Virtanen Institute, Finland.

Malignant glioma is a devastating brain tumor with no effective treatment. This randomised, controlled study involved 36 patients with operable primary or recurrent malignant glioma. Seventeen patients were randomized to receive AdvHSV-tk gene therapy (3 x 10(10) pfu) by local injection into the wound bed after tumor resection, followed by intravenous ganciclovir (GCV), 5 mg/kg twice daily for 14 days. The control group of 19 patients received standard care consisting of radical excision followed by radiotherapy in those patients with primary tumors. The primary end-point was survival as defined by death or surgery for recurrence. Secondary end-points were all-cause mortality and tumour progression as determined by MRI. Overall safety and quality of life were also assessed. Findings were also compared with historical controls (n = 36) from the same unit over 2 years preceding the study. AdvHSV-tk treatment produced a clinically and statistically significant increase in mean survival from 39.0 +/- 19.7 (SD) to 70.6 +/- 52.9 weeks (P = 0.0095, log-rank regression vs. randomized controls). The median survival time increased from 37.7 to 62.4 weeks. Six patients had increased anti-adenovirus antibody titers, without adverse effects. The treatment was well tolerated. It is concluded that AdvHSV-tk gene therapy with GCV is a potential new treatment for operable primary or recurrent high-grade glioma.
AdvHSV-tk gene therapy with intravenous ganciclovir improves survival in human malignant glioma: a randomised, controlled study
Cancer gene therapy

1. Direct attack on tumor cells
   a) transfer of tumor suppressor gene
   b) inhibition of oncogenes
      - antisense therapy
      - ribozymes
   c) suicide genes
   d) oncolytic viruses (replication-competent viruses)

2. Harnessing immune response to tumor antigens

3. Anti-angiogenic therapy
Replication competent viruses

Naturally occurring viruses

• autonomously replicating parvoviruses
• human reoviruses
• vesicular stomatitis virus
• Newcastle disease virus

Engineered oncotropic viruses

• conditionally replicating adenoviruses (such as ONYX-15)
Oncolytic viruses

Lyse only tumor cells,
Adenovirus ONYX-015, with deletion in E1B gene, may amplify only in cells with mutated p53 gene

E1B 55K protein binds to p53 and blocks its function - wild type virus can amplify in normal cells

In normal cells p53 is active, and lack of E1B prevents inactivation of p53
Cancer gene therapy

1. Direct attack on tumor cells
   a) transfer of tumor suppressor gene
   b) inhibition of oncogenes
      - antisense therapy
      - ribozymes
   c) suicide genes
   d) oncolytic viruses (replication-competent viruses)

2. Harnessing immune response to tumor antigens
   - overexpression of cytokines genes
   - tumor vaccines

3. Anti-angiogenic therapy
Genetically modified tumor vaccines

- Immune system may play a role in controlling tumor growth and development
- However, antigens present on tumor cells are not sufficient to boost immune response

hence

Modification of tumor cells - i.e., overexpressing certain genes, may stimulate immune system to respond to tumor cells
Strategy of genetically modified tumor vaccines

1. Isolate tumor cells from a patient - however, it is often not possible to use autologous cells

2. Alternative - culture other tumor cells - eg, cell line of the same type - i.e. allogeneic cell line

3. Transduce such cells with vector - eg. Retroviral vector harboring cytokine gene

4. Inject such modified cells into patients

5. Antigens present on allogeneic tumor cells stimulate immune system, which respond to the same antigens present on patient’s tumor
   Cytokines enhance the response
TABLE 3  Cytokine gene therapy trials

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Vector</th>
<th>Malignancy</th>
<th>Immune response</th>
<th>Clinical response</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>IL-12</td>
<td>Vaccinia</td>
<td>Mesothelioma</td>
<td>T cell infiltrate</td>
<td>0/6</td>
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<tr>
<td>GM-CSF</td>
<td>Retrovirus</td>
<td>Melanoma</td>
<td>Infiltrate at vaccine site</td>
<td>1/5 CR</td>
<td>79</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Retrovirus</td>
<td>Melanoma</td>
<td>Antibodies to tumor antigens</td>
<td>5/8 SD</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3/8 CR/PR</td>
<td></td>
</tr>
</tbody>
</table>

CR, complete response; PR, partial response; SD, stable disease.

**IL-6 and sIL-6R**  retrovirus  melanoma (allogeneic)

A. Mackiewicz et al., - Poznań

_Wadhwa et al., Ann Rev Med. 2002_
How to starve tumor to death...

Anti-angiogenic gene therapy
Physiological angiogenesis in adults is restricted to placenta, uterus, hair growth, and wound healing.
In healthy organism the action of activators and inhibitors of angiogenesis is balanced.

**Inhibitors:**
- Thrombospondin-1
- The statins: Angiostatin
- Endostatin
- Canstatin
- Tumstatin

**Activators:**
- VEGFs
- FGFs
- PDGFB
- EGF
- LPA

*Nature Review Cancer*
Tumor growth is dependent on angiogenesis.
Anti-angiogenic therapy
RNA interference

- dsRNA
- Dicer
- siRNA (small interfering RNA)
- RISC (RNA-induced silencing complex)
- 5’ mRNA
- 3’ mRNA
degradacja mRNA
siRNA against mRNA VEGF

Acuity Pharmaceuticals

Normal Macula

Dry AMD: Drusen formation under the Macula

Wet AMD: Macula with abnormal blood vessels

Carla McCullough, a 79-year-old woman in Cleveland, Ohio - 9 November 2004 - first injection of siRNA
Strategies of antiangiogenic gene therapy

Approaches of Antiangiogenic Gene Therapy

Suppression of Angiogenic Activator
- Inhibiting Angiogenic Growth Factors
  - VEGF Antisense/Ribozyme
  - FGF2 Antisense/Ribozyme

Interfering with Signaling of the Endothelium
- Dominant-Negative VEGFR2
- Soluble VEGFR1
- Soluble Tie2 Receptor
- VEGFR1 Ribozyme*

Enhancement of Angiogenic Inhibitor
- p53*
- TSP-1
- TSP-2
- Angiostatin
- Endostatin
- IL-12*
- TIMPs

* : Currently in clinical trial
Gene therapy of cancer: anti-cancer vs anti-angiogenic

**Anti-cancer cells** - introduction of genes that:
1. Permit tumor cells to express toxic molecules
2. Prevent or correct genetic defects
3. Increase the immunogenecity of tumor cells
4. Increase the sensitivity of tumor cells to drugs

**Anti-angiogenic - anti-endothelial cells**: introduction of genes that:
1. Specifically target tumor endothelial cells
2. Block tumor-derived angiogenic activity

*They may be employed together or separately*
Drawbacks of anti-cancer gene therapy

1. Limited access to tumor cells
2. Heterogeneity of tumor cells
3. Emergency of resistant tumor cells
4. Toxicity

Advantages of anti-angiogenic gene therapy

1. Little or no toxicity
2. Does not require that therapeutic agent enter any tumor cells nor cross the blood brain barrier
3. Acts independently of tumor cell heterogeneity and tumor type
4. Does not induce acquired drug resistance
Gene Types Transferred in Gene Therapy Clinical Trials

- Cytokine 25% (n=274)
- Antigen 15% (n=162)
- Tumor suppressor 12% (n=132)
- Suicide 7.6% (n=82)
- Deficiency 6.5% (n=70)
- Drug resistance 5.2% (n=56)
- Replication inhibitor 3.7% (n=40)
- Receptor 3.1% (n=33)
- Others 18% (n=183)
- N/C 4.1% (n=44)
Summary
Problems of gene therapy

1. Efficacy vs safety
   - vector choice
   - target choice

2. Bioethical problems?
   a) embryonic progenitor cells in gene therapy – so called therapeutic cloning (experimental nuclear transfer)
   b) gene therapy of germ line
   c) „cosmetic” gene therapy/gene doping
   d) quality of life vs gene therapy
   e) costs of gene therapy

3. Commercialisation – is it really a problem?
**Protocols by vector**

- Retrovirus (n=212) 35.8%
- Adenovirus (n=164) 27.7%
- Lipofection (n=77) 13%
- Naked/Plasmid DNA (n=55) 9.3%
- Pox virus (n=37) 6.2%
- Adeno-associated virus (n=13) 2.2%
- RNA transfer (n=5) 0.8%
- Gene gun (n=5) 0.8%
- Herpes simplex virus (n=3) 0.5%
- N/C (n=22) 3.7%

*The Journal of Gene Medicine, © John Wiley & Sons 2001 www.wiley.co.uk/genmed*

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**Vectors Used in Gene Therapy Clinical Trials**

- Retrovirus 25% (n=272)
- Adenovirus 25% (n=271)
- Naked/Plasmid DNA 16% (n=174)
- Lipofection 8.6% (n=93)
- Pox virus 7.1% (n=76)
- Vaccinia virus 6.3% (n=67)
- Herpes simplex virus 3.3% (n=36)
- Adeno-associated virus 3.1% (n=33)
- RNA transfer 1.2% (n=13)
- Others 2.1% (n=21)
- N/C 3.9% (n=42)

*The Journal of Gene Medicine, © 2005 John Wiley and Sons Ltd www.wiley.co.uk/genmed/clinical*
Gene therapy clinical trials

Number of Gene Therapy Clinical Trials
Approved Worldwide 1989-July 2005

<table>
<thead>
<tr>
<th>Year</th>
<th>Trials</th>
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<td>2005</td>
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<td>2004</td>
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<td>N/C</td>
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</table>
Gene Therapy’s Fall and Rise (Again)

http://www.the-scientist.com/yr2004/sep/research_040927.html

The Scientist, Sept 27, 2004
Gene therapy can cure the disease not only in experimental animals but also in human - SCID trials.

Gene therapy will never be a simple method effective in treatment of all imaginable diseases.
Treatment of diseases by means of gene therapy is a fascinating idea, but scientists have to work a lot before it will be fully implemented and before all hopes connected with it will be realized.

Theodore Friedmann, 1997

The difficulty is making it work..."

Ronald Crystal
"It's too easy to forget the incredible progress that has been made and to say, 'Well, why haven't we cured all these diseases?'" says Daniel Salomon, an associate professor at The Scripps Research Institute in La Jolla, Calif. "But when you're pioneering a new field, there are going to be advances and setbacks that are going to frustrate both the scientists and the public.

*Society cannot expect the immediate success of every approach and scientists and physicians may not generate hype and promise effective treatment when it is not yet available.*
THANK YOU FOR YOUR ATTENTION

GOOD LUCK AT EXAM!
Young-Joon Surh, Ph.D.
Chief and Professor
National Research Laboratory of Molecular Carcinogenesis and Chemoprevention
College of Pharmacy, Seoul National University

Redox-Sensitive Transcription Factors as Molecular Targets for Chemoprevention and Cytoprotection

Monday, 23rd January, 12.00 am, room D107
Spring semester course

Molecular mechanisms of angiogenesis

First lecture

Monday, 20th February 2006

3 – 5 pm, room D104

7 lectures (15 hours)

30 h practical course (limited number of participants!)