The Impact of Gene Therapy on Dentistry

- Commonly refers to any clinical application of the transfer of a foreign gene
- Initially, gene therapy was associated with either the correction of inherited genetic disorders or the treatment of life-threatening conditions
<table>
<thead>
<tr>
<th>Title/Protocol approved</th>
<th>Institution</th>
<th>Approval date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment of advanced cancer patients using interleukin-2 and tumor-infiltrating lymphocytes</td>
<td>National Cancer Institute</td>
<td>3/2/89</td>
</tr>
<tr>
<td>Ex vivo gene therapy of severe combined immune deficiency</td>
<td>National Cancer Institute</td>
<td>9/6/90</td>
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<tr>
<td>Ex vivo gene therapy of familial hypercholesterolemia</td>
<td>University of Michigan/University of Pennsylvania</td>
<td>11/14/91</td>
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<td>Immunotherapy of malignancy by in vivo gene transfer into tumors</td>
<td>University of Michigan</td>
<td>4/17/92</td>
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<tr>
<td>Gene therapy for the treatment of brain tumors</td>
<td>National Institute of Neurological Disorders and Stroke</td>
<td>8/14/92</td>
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<tr>
<td>A phase I study of a recombinant adenovirus carrying the cDNA of the normal cystic fibrosis transmembrane conductance regulator gene</td>
<td>National Heart, Lung and Blood Institute</td>
<td>4/16/93</td>
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<td>Gene therapy for cystic fibrosis</td>
<td>University of Iowa</td>
<td>4/16/93</td>
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<tr>
<td>A phase I trial of HIV-1 env-transduced autologous fibroblasts</td>
<td>University of Southern California</td>
<td>9/3/93</td>
</tr>
<tr>
<td>Gene therapy for advanced colorectal carcinoma</td>
<td>Mayo Clinic</td>
<td>4/19/04</td>
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<tr>
<td>A phase I study of direct gene transfer for metastatic renal cell carcinoma</td>
<td>University of Chicago</td>
<td>4/19/04</td>
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*Adapted from listing in Human Gene Therapy 1996;5:1067-74.
The Development of a Science

- Gene transfer is possible due to incredible progress in molecular biology
- Seminal advances in past 50 years shown in next slide
Major Scientific Advances

Tools of molecular biology
- Reverse transcriptase
- Restriction endonucleases

- 1944: Avery, et al.; DNA as genetic material
- 1953: Watson and Crick; Double helix
- 1964: Nirenberg, et al.; Genetic code
- 1970-73: Baltimore, Tamin; Nathans, Smith; Reverse transcriptase; restriction endonuclease
- 1989-90: Anderson, Blaise, Rosenberg; Human gene therapy
General Principles of Gene Transfer

- Typical mammalian gene
- Many modular elements
  - Coding regions
  - Regulatory elements
- Enzymatic tools enable researcher to modify and rearrange elements
Major Technological Challenges

- Designing the correct genetic architecture
- Choice of promoter is critical for obtaining stable, high level expression of a foreign gene
- Early experiments in gene transfer employed viral promoters that acted promiscuously
- Not all promoters are equal
- Current promoters are tissue-specific, more stable gene expression
Methods of Gene Transfer

- Two general methods of gene transfer into cells
  - Viral
  - Non-viral
Viral Methods

- Greater safety risk
- Nature’s way of efficiently transferring genes
- Many viruses could be used. Only a few are actually employed
  - retroviruses
  - Adeno-associated viruses
  - herpesviruses
  - Selection criteria: tissue target, desired stability of gene expression, size of gene
Non-Viral Methods

- Safety
- Less efficient mechanisms for gene transfer
- Two promising methods
  - Liposomes (bags of lipid containing DNA)
  - Macromolecular conjugates (negatively charged DNA mixed with large positively charged molecules linked to a specific cell ligand)
- Capable of transferring large genes, but expression is transient
- Less risk for inflammatory or immune reactions
### METHODS OF GENE TRANSFER

<table>
<thead>
<tr>
<th></th>
<th>VIRAL</th>
<th>NON-VIRAL</th>
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<tbody>
<tr>
<td></td>
<td>Retro-viruses</td>
<td>Adeno-viruses</td>
</tr>
<tr>
<td>Infects non-dividing cells</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Accommodates gene of &quot;reasonable&quot; size</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Effects stable gene integration</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Is in clinical use presently</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Has cell-type specificity</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

Table 2
Uses of Gene Transfer

**Two clinical applications**
- Therapy
  - Correction of an inherited or acquired defect
- Therapeutics
  - Production of biomolecules with pharmacologic functions

**Gene transfer can be accomplished two ways**
- *In vivo*
- *Ex vivo*
Applying Gene Therapy to Oral Cancer

- Gene therapy for treatment of oral cancer and precancerous lesions (E.J. Shillitoe, Univ. Texas Dental Branch)
- Reasoned that therapy is likely to be more effective focused on targets expressed only in cancer cells
- Targeted human papillomaviruses, present in many oral neoplasms
Human Papilloma Viruses

- DNA viruses with an affinity for epithelium
- HPV types 16 and 18 can transform normal keratinocytes *in vitro* into an immortal, malignant-like phenotype
- Requires expression of two HPV genes
  - E6 and E7
- Other factors such as trauma, or an environmental irritant is needed for tumor development
**HPV Gene Therapy Strategy**

- Used molecules called ribozymes to disrupt the function of E6/E7.
- Ribozymes are a class of RNA molecules that can act as enzymes:
  - Cleave the RNA molecules at defined sites
  - Cut mRNA transcripts of E6/E7
- No message, no protein
- Recently placed DNA encoding ribozymes in a replication-deficient adenovirus vector.
Gene Transfer to Oral Mucosal Keratinocytes

- Studies led by L.B. Taichman (Dept. of Oral Biology and Pathology, SUNY at Stony Brook)
- Grow keratinocytes in sheets *in vitro* and return to donor (e.g. burn patients)
- No specific oral disease targeted yet, but method has considerable promise
- Process on next slide used to transfer foreign genes into both epidermal and oral keratinocytes
Gene Transfer to Salivary Glands (NIDR)

- Bruce J. Baum and Brian C. O’Connell
- Easy target for *in vivo* gene transfer because of anatomic location
- Initial studies examined feasibility of using replication-deficient recombinant adenovirus vectors to transfer foreign genes into rat salivary glands *in vivo*
Gene Transfer into Rat Salivary Glands (con’td.)

- Vectors could infect \textit{in vitro}
- Administered vectors to cannulated ducts through the duct orifice via retrograde injection
- All major rat salivary glands (parotid, submandibular, and sublingual) could be infected by adenoviruses
- Histology showed that both acinar and ductal epithelial cells could act as recipients for gene transfer
Repair of Irreversibly Damaged Acinar Cells

- Two situations result in acinar cell damage
  - Therapeutic irradiation of head and neck
  - Sjögren’s syndrome (an autoimmune exocrinopathy)
- Goal was to convert surviving ductal cells into acinar-like cells that secrete salt and fluid
- An example of “organ engineering”: Changing the basic function of a cell type
- Adenoviral-mediated transfer of aquaporin-1 into rat salivary gland
Figure 5

GENE THERAPY
Transfer of genes to correct
an inherited or acquired defect

Acinus
destroyed by
irradiation

Duct
(water-impermeable)

No saliva
secreted

Vector containing
gene(s) of interest

Duct “made”
water-permeable

“Saliva”
secreted
Gene Therapeutics

- Use normally functioning salivary gland to deliver biopharmaceuticals
- Feasibility has been demonstrated by transferring \textit{in vivo}, gene for human \(\alpha_1\)-antitrypsin (liver protein) into rat submandibular glands.
- Other candidates: histatin, \textit{P. gingivalis} fimbrillin (local immunization to make sIgA)
Figure 6

GENE THERAPEUTICS
Transfer of genes that encode biopharmaceuticals to functioning glands for use in upper gastrointestinal tract

Acinus

Duct

"Normal" saliva

Vector containing gene encoding molecule of interest (bio)

Saliva containing "biopharmaceutical"
The Future of Gene Transfer and its Impact on Dentistry

- Now accepted a feasible by the general biomedical community
- No longer considered an esoteric exercise with practical application
- Numerous articles on gene transfer in mainstream journals (e.g., NEJM).
- Not a panacea for all clinical problems
Current Tools are Crude

- Vectors available for gene transfer have problems
  - Transient and inflammatory nature of adenovirus use
  - Low titers
  - Mutagenic potential (safety concerns)

- Biotechnical Industry is addressing shortcomings
  - Tremendous commercial potential

- Treatments appear heroic, mechanics of gene transfer are mundane
Conclusion

- Initially, gene transfer approaches will not be used for routine care
  - Refractory to conventional treatment (high risk for periodontal disease or caries)
- Envision scenarios in which gene transfer is applied to periodontal bone loss, oral ulcers, delayed tooth eruption
- Biology is changing rapidly and will dramatically impact on the way dentistry is practiced.
Excellent References
