Molecular Biology in Otolaryngology

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Grand Rounds Presentation
September 17, 2003
Introduction

- 50\textsuperscript{th} Anniversary of Watson & Crick
- Completion of human genome project
Techniques

- Central Dogma
  DNA-(Transcription)-RNA-(Translation)-Protein
- Southern Blot – DNA
- Northern Blot – RNA
- Western Blot – Protein
- PCR – DNA amplification
  – DNA Polymerase + Primer
Techniques

- FISH – Radiolabeled probe
- Gene mapping
  - Functional cloning: Find protein and work back
  - Positional cloning: Uses known sequences and markers
- Linkage Analysis – Localize chromosomal region based upon frequency of recombination
  - LOD score >3 suggests coinheritance
Techniques

- Expressed Sequence Tags (EST) – labeled DNA made from mRNA of interest
- Microarrays (DNA chip, gene chip, biochip) – allows for thousands of interactions at once
Gene Therapy

- Genomics vs. Proteomics
- Gene = segment of DNA that encodes for specific mRNA (10% of human DNA)
- Antisense = transcribed DNA strand
- Sense = DNA corresponding to mRNA produced
- DNA + Histone = Nucleosome
- Multiple Nucleosomes = Chromosome
Gene Therapy

- First clinical trial in 1990 for SCID (Adenosine Deaminase deficiency) – Transient resolution of disease
- 2/3 of all protocols target cancer, not monogenic disease
- Goals of vector
  Specifically target cell population
  Maintain expression of transduced gene
  Obtain desired gene function
Gene Therapy

3 vector options
- Viral
- Nonviral
- Naked DNA

Viral vectors
- Lentivirus (Retrovirus) – insertion into host DNA
- Adenovirus – upper aerodigestive tract
- Adeno-associated virus – requires helper virus
- Herpes virus – inflammation and cytotoxicity
- Vaccinia virus/Pox virus
Gene Therapy

- Nonviral vectors – noninfectious, minimal toxicity; nonspecific, low transduction efficiency, transient expression
  - Cationic liposome complexes
  - Ballistic particles
  - Plasmid DNA
  - Calcium phosphate precipitation
  - Electroporation
Gene Therapy

- Cystic Fibrosis
  - Goal: Deliver normal CFTR to lower respiratory tract
  - Adenovirus has been used in trials
  - Problems: Frequent redosing, acquired immunity to vector
Hereditary Hearing Impairment

- 30% syndromic, 70% nonsyndromic
- 70-85% of HHI nonsyndromic
- Autosomal dominant – postlingual, progressive
- Autosomal recessive – prelingual, nonprogressive, severe to profound
- GJB-2 mutation accounts for 50% of autosomal recessive HHI (Connexin-26): spiral ganglion neurons preserved
Hereditary Hearing Impairment

- **Usher Syndrome**
  - Type I = no vestibular function, profound deafness, early retinal degeneration (MYO7A)
  - Type 2 = normal vestibular function, lesser degree of hearing loss, late onset retinal degeneration (USHERIN)
  - Type 3 = Progressive hearing loss, progressive vestibular dysfunction, variable retinal degeneration (MYO7A)
Hereditary Hearing Impairment

Waardenburg Syndrome – lack of genotype/phenotype correlation
- Type 1 – dystopia canthorum present (PAX3)
- Type 2 – dystopia canthorum absent (MITF)
- Type 3 – Type 1 + musculoskeletal abnormalities (PAX3)
- Type 4 – Waardenburg Syndrome + Hirschsprung Disease (aganglionic colon) (SOX10)
Hereditary Hearing Impairment

- **Pendred Syndrome**
  - SNHL and euthyroid goiter
  - PDS gene encodes Pendrin (chloride and iodide transport)

- **Susceptibility to aminoglycoside-associated hearing loss**
  - Mitochondrial DNA mutation
Hereditary Hearing Impairment

- Playing the odds
  - 2 deaf parents – 10%
  - Normal hearing parents + deaf sibling – 10-18%
  - 1/3 of deaf children with normal hearing parents Connexin-26 +
  - 2/3 of normal hearing siblings of deaf child Connexin-26 +

- Testing for carrier status should not be done in children
Tumor Biology and Immunology

- Common H&N cancer mutations
  - Loss of 9p21
  - Inactivation of p16
  - P53 mutation/infection with HPV
  - Protooncogene overexpression

- Dendritic cell – MHC II and adhesion molecules; from bone marrow

- Activated dendritic cell + virgin T cell initiates response
Tumor Biology and Immunology

- Tolerizing B cell + virgin T cell produces no response
- Dendritic cell/T cell interaction
  - MHC+antigen+T cell
  - Activation of CD28 on T cell
  - Activation of b7.1 (b7.2) on Dendritic cell
- Tumor cell antigen vs. tumor-associated antigen: antigens recognized by CD8 cells more successful
### Tumor Biology and Immunology

<table>
<thead>
<tr>
<th>Antigen</th>
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<tbody>
<tr>
<td>MART-1 (Melan-A)</td>
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<tr>
<td>gp100 (pmel-17)</td>
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<tr>
<td>Tyrosinase</td>
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<tr>
<td>Tyrosinase related protein-1</td>
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<td>Tyrosinase related protein-2</td>
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<td>Melanocyte-stimulating hormone receptor</td>
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**I. Class I-restricted antigens recognized by CD8\(^+\) lymphocytes**

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<td>NY-ESO-1</td>
<td>54, 55</td>
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**Cancer-testes antigens**

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**Non-mutated shared antigens overexpressed on cancers**

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<td>α-Fetoprotein</td>
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<td>Telomerase catalytic protein</td>
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<td>G-250</td>
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<td>MUC-1</td>
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<tr>
<td>Carcinembryonic antigen</td>
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<tr>
<td>p53</td>
<td>67</td>
</tr>
<tr>
<td>Her-2/neu</td>
<td>68</td>
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**II. Class II-restricted antigens recognized by CD4\(^+\) lymphocytes**

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**Epitopes from non-mutated proteins**

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**Epitopes from mutated proteins**

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<td>Thiosphosphate isomerase</td>
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<td>CDC-27</td>
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<td>LDLR-FUT</td>
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Human cancer antigens restricted by HLA-A class I and recognized by CD8\(^+\) lymphocytes fall into four general categories. (1) Melanoma-melanocyte differentiation antigens are normal non-mutated proteins that are expressed exclusively on melanomas and on normal pigment-producing cells such as melanocytes. Lymphocytes that are reactive against these differentiation antigens can be found infiltrating into tumours. (2) Cancer-testes antigens can be widely expressed on a variety of epithelial tumours as well as on testis and placental tissue. (3) Mutated antigens represent normal proteins that contain mutations or translocations that give rise to unique epitopes. (4) Non-mutated shared antigens that are overexpressed on cancers. There is some evidence that overexpressed proteins, such as carcinoembryonic antigen, p53 and Her-2/neu, are tumour antigens, although evidence is controversial. As for antigens recognized by CD8\(^+\) cells, epitopes recognized by CD4\(^+\) cells are derived from both non-mutated and mutated proteins.
Tumor Biology and Immunology

- IgG and IgA principle antibodies in cancer response
- IgG: Complement fixation and antibody-dependent cellular cytotoxicity
- IgA: Role unclear; may protect tumor cells by blocking
Tumor Biology and Immunology

- T cells, B cells, and NK cells
- T cells require APCs
- B cells: T cell dependent (IL-2 and IL-4) or T cell independent
- NK cells activated by IL-2 = LAK cells
Cytokines: Interferons, Interleukins, and TGF beta

Interferons
- Type 1 = alpha and beta; respond to virus, double-stranded RNA; acid stable
- Type 2 = gamma; more active in immunomodulation/tumor response; acid labile
Tumor Biology and Immunology

- Interleukins
  - IL-1b activates osteoclasts; may play role in bony mets
  - TNF and IL-1 have similar effects; TNF has more anti-tumor cytotoxic effects

- TGF beta – inhibits anti-tumor response
Immunomodulation & Malignancy

- Active immunotherapy – elicits response in host
- Passive immunotherapy – administration of externally stimulated immunologic components
- Problems
  - Tumor antigens difficult to identify
  - Tumor cells capable of altering antigen expression
  - Tumors produce immunosuppressive factors
Immunomodulation & Malignancy
Immunomodulation & Malignancy

- **Active**
  Immunotherapy: IL-2 + cationic liposome

- **Passive**
  Immunotherapy: IL-2 + NK cells, IL-2 + Tumor Infiltrating Lymphocytes (TILs)
Monoclonal antibodies: can be linked to chemotherapeutic agents or radionuclides

- In H&N target either squamous associated antigens or EGFR
Conclusion

- “Good results” considered 30% complete response
- Frequently presented as potential adjuvant therapies
- Much promise, little practice