Module II: Molecular and cellular phenotype

• Discuss the history of the gene. When was the gene discovered? How was the gene cloned? (Be brief.)

• Discuss the cellular phenotype. What cells or tissues are affected, and how? What is the normal role of the gene in the cell (be specific)? How do mutations lead to disease (be specific)? Does the type of mutation affect the phenotype of the disorder?

• Discuss model systems. Describe one or two of the main model systems (animals, bacteria, yeast, etc). Is the wild-type function of the gene in the animal model similar to the wild-type function of the human gene?

• Molecular Biology. Does the gene product contain any functional domains? How does the sequences from the model organisms compare with the human protein sequence?

History of the CFTR chase

1985 - Tsui and Buchwald traced the mutation to chromosome 7 by following markers (linked genes that were inherited with the disease).

1989 - The gene was cloned by Riordan et al. via chromosome “walking”

1990 - Rich et al. took mutant CF epithelial cells in culture, expressed wild-type CFTR, and showed that the defect in chloride ion transport was corrected.
Normal role of the gene in the cell?

CFTR protein: cystic fibrosis transmembrane conductance regulator

• ATP-binding cassette (ABC) transporter
• The CFTR protein consists of 2 similar motifs each with membrane-association domain, and a domain believed to be involved in ATP binding (regulation)
• CFTR is a chloride channel, regulates the rate of transepithelial salt and water transport.
• CFTR also regulates other ion transport pathways (e.g., sodium, bicarbonate)

Source: OMIM, GeneCards, http://wsrv.clas.virginia.edu/~rjh9u/cfsciam.html (figure)

What cells or tissues are affected, and how?

• CFTR is expressed in epithelia that line the lungs, pancreas, sweat glands, and small intestine.
• Defective bicarbonate transport affects pH
  – Normal tissues secrete alkaline fluids
  – CF tissues secrete acidic fluids
• Acidic fluids lead to secretion of thick, sticky mucus
• Effects of increased mucus:
  – Clogged ducts and tubes in pancreas and lung
  – Increased bacterial binding and infection
  – Inflammation and decreased function of organs
  – Pancreatic digestive enzyme transport blocked
  – Malabsorption of nutrients in intestine

How do mutations lead to disease?

- ΔF508 leads to protein degradation in the ER--no cell surface expression
- Other mutations permit protein to be properly localized, but impair function (e.g., defective activation, defective chloride transport).
- Severity of phenotype varies with amount of CFTR channel function
  - Depends on nature of mutation

Model systems
Model systems are used to study diseases and disease genes. For a model to be useful, the disease gene should have a similar function in the model as in humans and should produce a similar phenotype when mutated.

Mouse Model: CFTR null mice and delF508 mice
- Defects in ion permeability of airway and intestinal epithelia
- Human CFTR gene can be introduced into these mice
  - found to ameliorate intestinal abnormalities
  - could this work for humans?
- Mutant mice do not show the same lung and pancreas pathologies as human CF patients

Source: OMIM

Animal models
• Large animal model--sheep?
  - sheep CFTR cDNA is cloned and sequenced
  - Human CFTR sequence more homologous to sheep
  - 94% aa similarity vs. 88% for mouse/human
  - Expression patterns are very similar
    • Location of CFTR expression (tissue types)
    • Developmental pattern of CFTR expression
  - Now that sheep have been cloned--could a sheep model be possible?
Molecular Biology

- Some close homologs of human CFTR:
  - Chimpanzee (99% aa identity)
  - Sheep (90%)
  - Cow (90%)
  - Mouse (78%)

- CFTR contains two transmembrane domains and two nucleotide binding domains

- Phe508 is in a highly-conserved nucleotide binding domain

Source: EntrezProtein, BLASTp

Alignment of human CFTR with animal homologs

Phenylalanine 508

human 481 KIKHSGRISFCSCQFQ5WMPGTTIKNIIFGVSDEYRYR5VIKACQLEEDSKFAEKNDIV
chimpanzee 481 KIKHSGRISFCSCQFQ5WMPGTTIKNIIFGVSDEYRYR5VIKACQLEEDSKFAEKNDIV
sheep 480 KIKHSGRISFCSCQFQ5WMPGTTIKNIIFGVSDEYRYR5VIKACQLEEDSKFAEKNDIV
cow 480 KIKHSGRISFCSCQFQ5WMPGTTIKNIIFGVSDEYRYR5VIKACQLEEDSKFAEKNDIV
mouse 481 IIKHSGRISFCSCQFQ5WMPGTTIKNIIFGVSDEYRYKSVVQACQLEEDSKFAEKNDIV

Source: BLASTp, ClustalX, BoxShade
Useful resources

- Links under “HGDRP page,” “Useful Links”
- OMIM
  - Gene function
  - Cloning history
  - Genotype/phenotype correlations (info on mutations)
  - Animal models
  - Links to research articles (accessible through Biolabs library)
- GeneCards - concise database of human genes
- EntrezGene - summary of gene with links to other NCBI sites
- See handout on “Finding Homologs and Obtaining Alignment Data”