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Introduction

The year 2003 marked a major milestone in the realm of genetics and molecular biology. This year marks the 50th anniversary of the discovery of the double-helical structure of DNA by Watson and Crick. This very same year, on April 14, 20 sequencing centers in 5 different countries declared the human genome project complete.

Since the human genome project began in 1990, the pace of change in the areas of molecular biology and genomics has been remarkable. One of the major surprises is that the number of human genes, once thought to be approximately 100,000, is actually around 30,000. Over 1,400 "disease genes" have now been identified. It is very easy for the clinician to be left behind.

This presentation aims to present a basic outline of molecular biology and genetics as it relates to the current practice of otolaryngology, with special attention paid to recent developments and likely therapeutic options in the near future.

Techniques

First, the "central dogma" of molecular biology: DNA is transcribed into RNA, which is then translated into protein.

There are four basic techniques which serve as the foundation for research in molecular biology: the Southern Blot, the Northern Blot, the Western Blot, and the polymerase chain reaction (PCR).

The Southern Blot is used to determine the sequence of an unknown DNA fragment via electrophoresis followed by hybridization with a known, radiolabeled DNA fragment.

The Northern Blot is used to determine the sequence of an unknown RNA fragment using similar techniques.

The Western Blot is used to detect specific proteins using electrophoresis followed by chromatography and a wash with a known, labeled antibody.

PCR involves the rapid amplification of an unknown DNA fragment into a massive quantity of copies. This process requires DNA Polymerase and a primer.

Fluorescence in situ hybridization (FISH) is a technique in which a probe (a specific strand of DNA capable of complementarily binding a DNA region of interest to locate a specific sequence of interest) is fluorescent-labeled. This method is useful in detecting deletions and additions on the chromosomal level, and has been applied clinically to Prader-Willi Syndrome and Angelman Syndrome.

For gene therapy to be successful, genes must be mapped (e.g., the human genome project). There are basically 2 approaches to mapping: functional cloning and positional cloning. In functional cloning, the goal is to find the disease's underlying protein defect, then find the gene that codes for that protein. This requires that the protein defect must be known, that the protein must be available in sufficient quantities, and that the protein must be capable of purification. Positional cloning, which is more commonly employed, does not require any specific knowledge about the protein product. This method depends upon the specific order of genes and markers within a chromosome.

Linkage analysis is used to determine the chromosomal region of a specific gene. This technique compares, through generations, the inheritance of a desired gene with the inheritance of DNA markers of known chromosomal locations. If a specific marker and a specific disease gene are present in the same chromosomal region, they will be inherited together unless recombination occurs. The closer together the gene and the marker are, the less likely recombination is to occur. The LOD (logarithm of the odds) score represents the chances that a particular marker is being inherited with a trait of interest. A LOD score >3 is generally accepted as evidence that a gene and marker are coinherited.

The types of genetic markers commonly used are microsatellites (repetitive DNA elements interspersed throughout the human genome) and single nucleotide polymorphisms (SNPs), which are single base changes scattered throughout the genome.

Once linkage analysis is complete, expressed sequence tags (ESTs) can then be employed. ESTs are complementary DNA fragments made from mRNA expressed in the tissue of interest that can then be hybridized to DNA from the chromosomal region of interest to identify specific candidate genes.

The introduction of microarrays had a dramatic effect on gene mapping, allowing for the monitoring of expression of thousands of genes simultaneously. (Terms used for this technology include DNA chip, DNA microarray, gene chip, and biochip.) The microarray is an orderly arrangement of nucleic acid samples allowing for the matching of known and unknown samples with each other based upon base-pairing rules. Fluorescence, or another method, is then used to detect hybridization, allowing for thousands of interactions at once. Data analysis for microarrays requires advanced computing systems.

Gene Therapy for Monogenic Disorders

Before tackling the subject of gene therapy, one must first talk about genes. Genomics is the study of the complete genetic set of an organism (which is no more or less important than proteomics – the study of the complete protein set of an organism). A gene is a segment of DNA that encodes for a specific mRNA via the process of transcription. Only approximately 10% of human DNA can be described as genes. The transcribed DNA strand is referred to as the “antisense” strand. The resulting mRNA has the same sequence as the “sense” DNA strand. Promoters are sequences of DNA that initiate transcription by signaling RNA polymerase.

The DNA in a single human cell measures approximately 1 meter in length (3 billion base pairs). To fit into a cell, the DNA wraps itself around histones (protein). The combination of DNA and a histone comprises a nucleosome. Nucleosomes, in turn, wrap around each other to form a chromosome.

Genetic disorders may be broken down into three categories: single gene disorders (e.g., cystic fibrosis, neurofibromatosis), chromosome disorders (e.g. Down Syndrome, Turner Syndrome), and multifactorial disorders (e.g., cleft lip and palate, gastroesophageal reflux, diabetes mellitus).

The first human gene therapy trial was approved in 1989, and the first clinical trial occurred in 1990 for the treatment of Severe Combined Immune Deficiency (SCID) – a single gene disorder characterized by Adenosine Deaminase deficiency. Therapy resulted in transient resolution of the disease. Presently, nearly 2/3 of all gene therapy protocols target cancer, and NOT monogenic diseases.

The key to success for gene therapy is to deliver the desired gene in sufficient quantity to target cells without adversely affecting normal host cells. There are basically 3 vector options: viral, nonviral, and naked DNA. The ideal vector specifically targets the desired cell population, maintains expression of the transduced gene for an adequate amount of time, and obtains the desired gene function.

Viral vectors used have included Lentiviruses (retrovirus), Adenovirus, Adeno-associated virus, Herpes virus, and Vaccinia virus.

Retroviruses represent very popular vectors (e.g., the Maloney Murine Leukemia Virus). These are RNA viruses that integrate into the host cell chromosome resulting in stable gene expression. Retroviruses only infect actively dividing cells and have an insertion capacity of approximately 10 kb. A retroviral vector works by binding a membrane target cell receptor, entering the cell, and then releasing its genetic material into the cytoplasm. Reverse transcription using virally encoded reverse transcriptase then results in a double-stranded DNA provirus which then inserts into the host cell chromosome in a stable but random manner retained during subsequent mitoses.

Retroviral vectors may be altered in a number of ways. Viral replication can be rendered incompetent by replacing functional viral genes with recombinant DNA. In addition, the viral

envelope may be altered to both broaden cell tropism and facilitate purification of viral particles from cell culture; this alteration is referred to as pseudotyping.

There are risks associated with the use of a retroviral vector. Insertional mutagenesis may occur if the transduced DNA is inserted into the regulatory region of a host cell chromosome, which could produce either activation of protooncogenes or inactivation of tumor suppressor genes. Homologous recombination, or recombination between vector genes and endogenous human retroviruses, may result in virus particles capable of replication.

Adenoviruses are relatively safe, producing only cold-like symptoms. These are DNA viruses which act by binding the cell, undergoing endocytosis, and forming an endosome which is subsequently disrupted releasing viral DNA into the cytoplasm. The DNA remains as a nonreplicating extrachromosomal episome, and is NOT incorporated into the host cell genome. The gene of interest and viral structural genes are then transcribed into RNA. The gene typically persists for only 7-42 days – which makes it adequate in therapy for neoplasia, but less desirable in therapy of inherited monogenic deficiencies. Adenovirus demonstrates a known tropism for cells of the upper aerodigestive tract. Its utility is limited by the virus's immunogenic properties; in fact, many patients have developed antibodies BEFORE initiating an attempt at gene therapy.

Adeno-associated virus is a single-stranded DNA parvovirus with no associated human disease. This requires the presence of a helper virus (e.g., Adenovirus or Herpes virus) to initiate replication. The viral genome is small (5 kb) and therefore not very useful clinically.

Herpes Simplex virus, like Adenovirus, is NOT integrated into the host cell genome, but DOES allow for the long-term expression of transgene in neuronal cells (which bodes well for the treatment of neurologic disorders). HSV is highly immunogenic, initiating inflammatory and cytotoxic reactions, which limit its usefulness in humans, but a mutant HSV (HSV-1716) has been found that is deficient in a specific neurovirulence factor, rendering it incapable of replicating in neuronal cells but capable of replicating within and lysing tumor cells.

Vaccinia and Pox viruses have the longest history of clinical use among humans, dating back to smallpox vaccines used in the 1700's. Again, these viruses do NOT integrate into the host cell chromosome. Though immunogenic, studies of revaccinated subjects have shown little or no residual immunity following the initial exposure. Thus, there is potential here.

Nonviral vectors and naked DNA enjoy the advantage of being noninfectious with minimal toxicity. However, cell targeting is nonspecific, transduction efficiency is low, and gene expression is transient, making nonviral vectors less useful *in vivo*. Examples of nonviral vectors include cationic liposome complexes, ballistic particles, plasmid DNA, calcium phosphate precipitation, and electroporation.

Cystic fibrosis (CF) arises from a mutation in the CFTR gene, affecting chloride ion transport in all cells of the body. However, most morbidity and mortality associated with the disease results from effects within the bronchopulmonary tree. Thus, the most basic goal of gene therapy in CF is to deliver a normal CFTR gene to the epithelial cells of the lower respiratory tract.

Adenoviruses have been the primary vector for gene delivery in trials because adenovirus demonstrates a tropism for respiratory epithelium. Major hurdles must still be overcome because, using the adenovirus vector, there is a need for frequent repeat dosing due to the short life span of airway epithelial cells. Furthermore, the host tends to develop neutralizing antiviral antibodies rendering this method of gene therapy ineffective. For gene therapy of CF to achieve success, one must increase vector efficiency while decreasing vector immunogenicity, which will likely necessitate the development of nonviral vectors.

Hereditary Hearing Impairment

Only 30% of hereditary hearing impairment (HHI) is syndromic, while the other 70% is nonsyndromic. 70-85% of nonsyndromic HHI is autosomal recessive, with autosomal dominant inheritance accounting for most of the remainder.

Most hearing loss associated with dominant genes is postlingual and progressive. In contrast, autosomal recessive hearing loss tends to be prelingual, nonprogressive, and severe to profound. Mutations in a single gene (GJB-2) account for approximately 50% of autosomal recessive hearing impairment. This is the gene that codes for the gap junction protein Connexin-26. In the presence of a Connexin-26 defect, there is near-total degeneration of hair cells with preservation of spiral ganglion neurons (thus, these patients are good candidates for cochlear implantation).

Syndromic deafness has also been studied at the genetic level. Usher Syndrome is an autosomal recessive disorder with the association of deafness and retinitis pigmentosa. There are 3 types of Usher Syndrome:

- Type I – there is a lack of vestibular function, profound deafness, and retinal degeneration beginning in childhood
- Type II – there is normal vestibular function, a lesser degree of hearing loss, and later onset of retinal degeneration
- Type III – there is progressive hearing loss and a variable onset of retinal degeneration with progressive decrease of vestibular function over time

Several loci have been associated with Usher Syndrome. The two best-known genes are MYO7A and Usherin. MYO7A encodes for Myosin VIIa; an MYO7A mutation can cause both Type I and Type III Usher Syndrome. An Usherin mutation is responsible for Type II-a Usher Syndrome.

Waardenburg Syndrome is an autosomal dominant disorder characterized by sensorineural hearing loss and pigmentary abnormalities. Waardenburg Syndrome has been divided into 4 types:

Type I – dystopia canthorum is present

Type II – dystopia canthorum is absent

Type III – Type I in addition to musculoskeletal abnormalities

Type IV – the combination of Waardenburg Syndrome and Hirschsprung Disease (aganglionic colon)

The PAX3 gene accounts for Types I and III. Type II has been associated with mutation of the Microphthalmia-associated Transcription Factor (MITF) gene. Type IV is associated with mutations of the gene encoding for SOX10. Gene counseling for Waardenburg Syndrome is complicated by the fact that there is a lack of genotype-phenotype correlation, such that the presence of a specific mutation does not guarantee a specific phenotype.

Pendred Syndrome is autosomal recessive disorder characterized by sensorineural hearing loss and euthyroid goiter. PDS, which encodes pendrin (a chloride and iodide transporter), has been identified as the causative gene.

Susceptibility to aminoglycoside-associated hearing loss has been associated with a mitochondrial DNA mutation. Mitochondrial DNA is inherited through the mother since sperm carries very little mitochondria. However, unlike X-linked disorders, both male and female offspring can be affected.

Branchio-Oto-Renal Syndrome has been associated with the EYA1 gene.

Of 28 pediatric otolaryngologists surveyed at the 1999 ASPO meeting, 71% stated that they offer genetic testing in all cases of congenital deafness. While the vast majority of normal hearing parents of deaf children reported a positive attitude toward genetic testing, 97% of parents tended to underestimate the risk of having another deaf child after genetic testing and counseling in one study. (7)

The chance of 2 deaf parents having a deaf child is approximately 10%. The recurrence risk for 2 normal hearing parents with a deaf child is 10-18%.

Approximately 1/3 of deaf children with normal-hearing parents test positive for a Connexin-26 mutation. 2/3 of normal-hearing siblings of a deaf child are carriers of a Connexin-26 mutation.

The current recommendation regarding carrier testing of children for genetic disease is that it should NOT be done based on the wishes of the parents. This decision should be left to the child once he or she is an adult. This recommendation is based on the idea that carrier status will affect reproductive decisions and is otherwise irrelevant.

In the case of Neurofibromatosis Type II, it is important to distinguish inherited disease from disease caused by a spontaneous mutation. In the former case, the mutation will be noted in both tumor cells and the blood. In the latter case, mutations will be present in tumor cells but NOT in the blood. Those with the spontaneous mutation (mosaics) are unlikely to have affected siblings or pass the disease on to their offspring. The opposite is true for the inherited form of the disease.

Tumor Biology and Immunology

According to Vogelstein, development of cancer involves a genetic progression requiring the sequential acquisition of *several* mutations. In head and neck cancer, the loss of 9p21 is the most common genetic error, and this occurs early in the progression to malignancy. The inactivation of p16, an inhibitor of cyclin and cyclin-dependent kinases, is also an important step. In addition, mutation of p53 or infection with HPV can result in the loss of apoptosis (HPV envelope protein E6 appears to degrade p53 gene product). In addition, there is frequently protooncogene overexpression (e.g., cyclin-D1, EGFR). Of note, histologically negative margins may still be genetically transformed and yet to express phenotypic changes, which may explain frequent locoregional recurrence.

Early detection of cancer, in general, is very important to successful therapy. One problem is that, in cancer, multiple genetic pathways are involved in producing numerous protein messengers that affect cell growth. As a result, the expression of antigens specific to tumor cells is inconsistent. Sidransky developed a panel of 21 microsatellite markers capable of detecting clonal genetic changes in cytologic specimens from cancer patients; 80% of different patient samples tested positive. The goal has been to find something equivalent to a PSA for head and neck carcinoma.

The opportunity for immunologic activity begins with an activated dendritic cell coming into contact with a virgin T cell. If the T cell contacts a tolerizing B cell instead, the result is no immunologic activity. In the newborn period, the number of dendritic cells is small, therefore a T cell is far more likely to contact a tolerizing B cell (hence the decreased immune response in newborns). Dendritic cells arise from the bone marrow and have a high expression of MHC II and adhesion molecules.

The dendritic cell-T cell interaction involves presentation of the antigen in the major histocompatibility complex (MHC) followed by activation of CD28 on the T cell and b7.1 (or b7.2) on the dendritic cell. If *both* of these conditions are not met, the antigen is considered non-foreign, resulting in immunologic tolerance.

Tumor cell antigens are antigens detected only on tumor cells and NOT on normal host cells. These antigens have been difficult to identify in head and neck cancer, as opposed to tumor-associated antigens found on both normal and malignant cells (e.g., Beta-2 microglobulin, keratin), but with qualitative and quantitative differences in expression. Most tumor antigens identified thus far have been from melanoma. Antigens recognized by CD8 cells have been far more successful than those recognized by CD4 cells. In addition many viruses associated with oncogenesis present proteins that can serve as targets, e.g., E6 and E7 (HPV), EBV epitopes (lymphoma), and HTLV-1 epitopes (T cell leukemia).

Large numbers of dendritic cells are present in the peripheral blood of patients with a variety of cancers. These cells (CD34+) are recruited by GM-CSF, which is produced by head and neck tumors. However, the tumor interferes with the maturation of these dendritic cells via the production of IL-10 and p15e, the conversion of CD34 to CD31+ endothelial cells (resulting in neovascularization of the tumor), and PGE2.

Three steps are required to trigger the anti-tumor immune response: costimulation of the T cell by both a specific antigen and a nonspecific signal from a dendritic cell, and before delivering the nonspecific signal, the dendritic cell must be activated by a danger signal from the injured cells.

The immunoglobulins primarily involved in the response to malignancy are IgG and IgA. IgG causes cytotoxicity via

1) complement fixation to IgG bound to tumor cells. However, circulating immune complexes in head and neck cancer patients can bind C1q and interfere with this complement pathway.

2) antibody-dependent cellular cytotoxicity. The Fab portion of the IgG binds the tumor cell, while the Fc portion binds to the effector cell (e.g., monocytes, macrophages, eosinophils, neutrophils, platelets).

The role of IgA is unclear. IgA may actually *protect* tumor cells by isolating them from cytotoxic mediators.

There are 3 kinds of lymphocytes: T cells, B cells, and NK cells. These lymphocytes account for 20% of all leukocytes circulating in the blood.

While T cells require antigen presenting cells (APCs) with MHC molecules to bind antigens, B cells can bind directly to antigens without MHCs. Activation of B cells by T cell dependent or T cell independent. In the case of T cell dependent activation, a T helper cell recognizes antigen and releases soluble mediators such as IL-2 and IL-4 that stimulate B cells.

NK cells (marked by CD16 and CD56) make up 10-15% of the circulating lymphocyte pool. NK cells demonstrate marked cytotoxic activity without the need for MHCs or prior antigen exposure. Activation of NK cells by IL-2 results in LAK (lymphokine activated killer) cells. These LAKs lyse tumor cells. Of note, Interferon alpha and Interferon gamma also increase the cytotoxicity of NK cells.

Cytokines are produced mostly by monocytes. If cytokines are produced by lymphocytes, they are termed 'lymphokines'. In the setting of cancer, cytokines regulate and activate other cellular effectors, exert growth and differentiation effects on tumor cells and surrounding tissues, and act directly as cytotoxins. Examples of cytokines include interferons, interleukins, and TGF beta.

There are 3 distinct subclasses of interferons: alpha, beta, and gamma. Alpha and beta comprise Type I interferons. These are produced in response to a virus or double-stranded RNA. Type I interferons are stable in exposure to acid. Alpha is produced by most leukocytes, while beta is produced by connective tissue cells.

Gamma comprises Type II interferon. These are produced early by T cells and large granular lymphocytes. Type II is labile at acidic pH. Type II interferon has more potent immunomodulatory effects than Type I, causing cytolysis, tumor cell differentiation, enhanced

NK cell function, activation of macrophages as NPCs, and enhanced antitumor effects of tumor necrosis factor (TNF)

The interferons have 7 major functions:

- 1) antiviral actions
- 2) antitumor cytotoxicity
- 3) inhibition of cell proliferation
- 4) gene activation
- 5) modulation of cell surface antigens
- 6) immune cell activation
- 7) stimulation of other cytokines

All of these functions occur as a result of direct binding of interferon to a specific target cell surface receptor.

Interleukins include all of the IL's and TNF alpha and beta. IL-1 causes enhanced T cell proliferation by increasing the production of IL-2, increasing B cell and antibody production, activating macrophages, and inducing intercellular adhesion molecules on host and tumor cells. IL-1b also results in osteoclast activation, which may play a role in bony metastasis.

IL-1 and TNF display a lot of redundancy in their actions, and they enhance each other's effects. TNF is produced by lymphocytes and monocytes in response to IL-1, TNF, and Interferon gamma. However, TNF has more profound anti-tumor cytotoxic effects than IL-1.

IL-2 is produced by activated T cells and functions to stimulate T cells, B cells, NK cell proliferation, lymphokine production, and the release of Interferon gamma, TGF beta, and IL-4. The major overall effect of IL-2 is the induction of cytotoxic anti-tumor activity.

TGF beta is an immunoregulatory peptide with INHIBITORY effects on the anti-tumor response.

Immunomodulation and Malignancy

There are 2 basic forms of immunotherapy in the setting of cancer: active immunotherapy and passive immunotherapy. Active immunotherapy involves the administration of agents to the tumor-bearing host to elicit an immune reaction designed to control or eradicate malignant disease. Passive immunotherapy (adoptive immunotherapy) involves the administration of externally stimulated immunologic components initially obtained from the patient being treated.

The goal of immunomodulation is to increase the immune response to tumor. A suitable application in the long run may be the prevention of cancer recurrence. Difficulties encountered with anti-tumor immunotherapy include

- 1) tumor antigens are difficult to identify and very heterogenous
- 2) tumor cells are capable of altering antigen expression
- 3) tumors produce immunosuppressive factors

One problem encountered thus far in attempts at immunomodulation has been that tumor cells are capable of escaping early immune detection because of a low level of expression of class I MHC antigens, therefore avoiding cytotoxic T-cell lysis.

The systemic administration of IL-2 results in significant morbidity. There has been some success with the direct injection of human IL-2 conjugated to a cationic liposome, inducing anti-tumor effects while circumventing known IL-2 toxicity.

An attempt has also been made to harvest NK cells from the host, expose those cells *ex vivo* to IL-2 to produce LAK cells, and then injecting LAK cells and IL-2 back into the host. This technique has resulted in marked regression of metastatic disease for melanoma, SCCA, renal cell carcinoma, and colorectal carcinoma. The same method of passive immunotherapy and tumor-infiltrating lymphocytes (TILs). Several trials have focused on TILs alone with little success. TILs have been found to increase focal expression of TNF-alpha in areas of disease, but no evidence of tumor response was found in a group of 39 patients. However, when used in combination with IL-2, TILs have been found 50-100% more potent than LAKs *in vitro*.

A different theoretical approach involves cytotoxic gene therapy, with the introduction of a gene that expresses toxin in a targeted cell. This is associated with a bystander effect, in which surrounding, non-transduced cells are also killed. The HSV thymidine kinase gene has been the most commonly used for this method. The primary focus has been the treatment of glioblastoma, but some success with human head and neck cancer has also been demonstrated. Another gene employed has been the cytosine deaminase gene, which converts 5-fluorocytosine to the highly toxic 5-fluoruracil, allowing for the targeted delivery of highly toxic doses of 5-FU.

Suppressor gene therapy represents another anti-tumor option. Mutations of p53, a tumor suppressor gene, occur in more than 1/2 of all squamous cell carcinoma (SCCA) of the head and neck. Wild-type p53 causes apoptosis and transient suppression of cell growth; these effects are lost in the setting of mutated p53 and cancer. Wild-type p53 with an adenovirus vector has resulted in increased survival among patients with advanced, recurrent SCCA of the head and neck. It has also been shown to increase tumor responsiveness to chemotherapy and radiation.

In antisense gene therapy, the goal is to introduce an RNA strand complementary to the gene or mRNA of interest to bind said gene or mRNA and block the production of its protein product. The targets in such cases are oncogenes, e.g., c-myc, c-for, insulin-like growth factor I, k-ras, and EGFR. This technology is still at an early stage.

Monoclonal Antibodies

Monoclonal antibodies (Mabs) directed at tumor associated antigens represent a means of targeting tumor cells. While Mabs are capable of stimulating an immune response in the host, they can also be linked to chemotherapeutic agents or radionuclides to enhance their cytotoxic effects. In experimental trials, Mabs have achieved success primarily with hematologic malignancies, but also with hepatoma, melanoma, ovarian carcinoma, and colorectal cancer.

In head and neck cancer, there have been 2 major groups of Mabs tried experimentally: Mabs targeting squamous associated antigens (e.g., E48, 174H.64) and Mabs targeting Epidermal Growth Factor Receptor (EGFR). In both cases, the Mabs interact not only with cancer cells, but also with normal host tissue cells. While EGFR Mabs have been found to sensitize tumor cells to chemotherapeutic agents, by themselves their effects are cytostatic rather than cytotoxic.

Radiolabeled Mabs have proven more effective against smaller tumors in mice. In addition, radiolabeled Mabs have proven more effective than Mabs tagged with chemotherapeutic agents in head and neck SCCA. This is because, unlike chemotherapeutic agents, radionuclides are capable of spreading their effects over several cell diameters.

Conclusion/A Few Random Terms that are Sort of Important

Gene therapy and immunomodulation in cancer remain in their infancy. To date, “good outcomes” have been considered a 30% complete response rate based upon the reports of most authors. The use of these modalities as adjuvant therapies may be a more realistic goal in the short term.

In summary, most of these modalities show much promise but little clinical usefulness at the present time. Meanwhile, the amount of information available to clinicians and researchers on the molecular level has grown dramatically over the past 10 to 15 years.

Germline mutation – a mutation occurring in a germline cell as opposed to a somatic cell. Only Germline mutations are heritable.

Mosaicism – the genome is normal at conception, but a mutation is acquired some time during embryonic development in some (but NOT all) cells of the body. Thus, the individual is at risk for disease, but NOT his siblings. Neurofibromatosis Type II is an example.

Oncogenes and Tumor Suppressor Genes – the genes felt to cause cancer. Oncogenes (e.g., RET, c-erb B2) are thought to facilitate malignant transformation and tumor growth, while tumor suppressor genes (e.g., P53, Bcl-2) block tumor growth and promote apoptosis.

Protooncogenes – normal growth genes which undergo a mutation to form oncogenes.

Transgene – when foreign DNA (gene of interest) is incorporated into the germline of an embryo, allowing the effects of the gene of interest to be studied for several generations. Transgenic mice have been useful in studying hereditary deafness.

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www.ornl.gov (for information on the human genome project)