Current Status of Gene Therapy for Pancreatic Cancer

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Pancreatic adenocarcinoma remains a widespread and difficult disease to treat. Surgical resection offers the possibility of cure in a select few. However, most patients are not eligible, and conventional chemotherapy and radiation remain largely ineffective. Despite this, our understanding of the pathogenesis of the disease has advanced considerably over the past few decades. These findings provide the basis for the development of much needed new therapeutic modalities. Specifically, the application of new recombinant DNA technology and techniques to pancreatic cancer has yielded promising results. This paper reviews our current understanding of the molecular biology of pancreatic adenocarcinoma and its applications to the field of gene therapy. (Curr Surg 61:84-92. © 2004 by the Association of Program Directors in Surgery.)

KEY WORDS: pancreas, oncology, gene, and therapy

INTRODUCTION

In the United States, 30,000 new cases of pancreatic cancer are diagnosed annually. The disease is responsible for 29,000 deaths a year. The incidence has slowly been on the rise over the past 4 decades, which ranks it as the fifth leading cause of cancer death in Western society. Nearly 92% of all patients with pancreatic cancer are dead at 2 years. Overall, it accounts for 2% of all newly diagnosed cancers but for 5% of all cancer deaths in the United States. Most (95%) of these cancers are adenocarcinomas originating from the ductal cells of the exocrine pancreas. At present, only surgical resection provides the opportunity for a long-term disease-free state. Despite imaging advances that provide for more accurate diagnosis and staging, disease detection at an early stage remains poor. Although cigarette smoking and various other risk factors have been identified, no screening modalities are currently available. Because of the insidious and aggressive natural history of the disease, most patients have local or metastatic spread at the time of presentation that precludes a resection. Less than 10% of cases constitute candidates for surgical resection at the time of diagnosis.[1]

Pancreatic cancer is highly resistant to the currently available chemotherapy and radiation protocols. New agents such as gemcitabine, which has become the standard for locally advanced or metastatic disease, appear to provide a clinical benefit without much improvement in median survival.[2] Overall, the median survival from diagnosis is less than 3 to 5 months.[3]

The prognosis for patients with pancreatic cancer remains grave. The overall 5- year survival rate from the time of diagnosis is less than 5%. Therefore, new modalities in the treatment of this disease are required. Gene therapy has proved promising in many cancers and constitutes 1 of these modalities. We review the current status of gene therapy as applied to pancreatic cancer in light of our present understanding of the molecular basis of the disease.

HISTORY OF GENE THERAPY

The modern understanding of genetic therapeutics may be said to have begun with the description of the structure of DNA by Watson and Crick in 1953. Progress quickly followed over the ensuing decades with the unraveling of the genetic code. The discovery of restriction endonucleases allowed for the development of the molecular technology to clone and transfer specific DNA sequences.

In 1980, the first human trial in gene therapy was carried out. Investigators harvested bone marrow stem cells from thalassemia patients and, ex vivo, attempted to transfer into them the human-β-globin gene. The altered stem cells were then reinfused into the patients. Controversy surrounding the trial resulted in the ad hoc creation of various committees intended to monitor and regulate the newly evolved field of Gene Therapy.

During the decade of the 1980s, much work was carried out that allowed for improved efficiency in the delivery of specific DNA sequences. In 1990, the first approved human trial in gene therapy was carried out.[4] Investigators used a retroviral vector to transfer a normal copy of the adenosine deaminase gene into the peripheral lymphocytes of patients with the deficiency. Since this landmark study, other diseases have been

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approved for gene replacement protocols. Perhaps most notable among these are cystic fibrosis, alpha-1-antitrypsin deficiency, hemophilia, and Gaucher's disease.

Soon enough the paradigm was broadened from single gene defects resulting in blood dyscrasias and inborn errors of metabolism. The molecular technology was applied to more complicated problems such as the diverse cancer states. Recently, there have been reports in the literature on phase I trials administering ONYX-015 in patients with pancreatic carcinoma.[5,6] Currently, the National Cancer Institute lists on its website 17 active trials in gene therapy and 83 closed ones.

MOLECULAR BIOLOGY

Many advances have been made over the past 2 decades in the characterization of the molecular alterations that take place in pancreatic cancer. These may be classified as alterations in oncogenes, tumor suppressor genes, or growth factors.

Oncogenes

Certain genes exhibit increased biologic activity as a result of mutation and are termed oncogenes. The oncogene most commonly detected in human cancers is the ras gene. Not surprisingly, the ras gene is also the most important oncogene identified to date in pancreatic cancer. It comprises 3 families, H-ras, K-ras, and N-ras. Of these, the K-ras family is responsible for almost all of the pancreatic cancer mutations, with mutations in the other families occurring only rarely.[7] The gene encodes p21, a membrane-associated guanine nucleotide binding signal transduction protein that regulates many cellular functions, including cell growth, proliferation, and differentiation.[8] Studies suggest that K-ras, which is located on chromosome 12p13, is mutated in up to 95% of pancreatic adenocarcinomas.[9] These mutations, thought to be an early event in the pathogenesis of pancreatic carcinoma, are point mutations. They are usually located in codon 12, although mutations in codons 13 and 61 have also been reported.[10] These mutations in the K-ras gene result in a constitutively active GDP-bound product that promotes increased signal transduction and uncontrolled growth.[11]

Other oncogenes occasionally mentioned in the literature of pancreatic cancer include c-erbB-2 and c-myc.[12]

Tumor Suppressor Genes

Inactivation of this class of genes results in the elimination of vital negative regulators of cell proliferation allowing for uncontrolled growth. A growing number of tumor suppressor genes have been identified in the pathogenesis of pancreatic cancer: p53, members of the INK4 family, and DPC4/SMAD4.

The p53 tumor suppressor gene is located on chromosome 17p. It encodes a 53-kD nuclear phosphoprotein that acts as a transcription factor capable of modulating the expression of an array of genes involved in critical functions, including cell cycle regulation, arrest, apoptosis, differentiation, DNA surveillance, and repair.[13] p53 is thought to be mutated in anywhere between 40% and 100% of cases and constitutes the most commonly mutated tumor suppressor gene in pancreatic cancer.[14-17] These are mostly point mutations occurring in exons 5-9.[18]

The p16 tumor suppressor gene was the first member to be identified in the INK4 family of cyclin-dependent kinase (CDK) inhibitors. It is located on chromosome 9p and has been implicated in a variety of tumors, including pancreatic cancer. The p16 gene product normally binds to the cyclin-CDK4 complex and prevents it from phosphorylating the retinoblastoma protein (RB). In its nonphosphorylated state, the RB protein arrests the cell cycle at the G1/S checkpoint. It does so by forming a complex with E2F and, by sequestering it, preventing it from acting as a transcription factor that allows for the progression of the cell cycle into the S phase. Loss of p16 activity results in no inhibition at the level of the cyclin-CDK4 complex and allows for uncontrolled growth. Studies suggest that p16 activity is lost in about 40% of pancreatic cancers by homozygous deletion.[19]

The p15 tumor suppressor gene, also a member of the INK4 family, is located next to p16 on chromosome 9p. In certain pancreatic carcinoma cell lines, it has been reported to be codeleted with p16 by a common mechanism.[20]

The deleted in pancreatic cancer, locus 4 gene (DPC4/SMAD4), is located on chromosome 18q21. It encodes a protein thought to be critical in the signal transduction of the TGF-β superfamily of cell surface receptors. It is inactivated mostly by homozygous deletion in 30% to 50% of cases of pancreatic cancer.[21,22] One study reports that all pancreatic cancer cases with the DPC4 inactivation curiously also had the p16 deletion, but not vice versa.[23] Such a finding lends support to the multistep paradigm of carcinogenesis popularized in colon cancer.

Growth Factors

Various growth factors and their receptors have been implicated as modifying the level of aggression of pancreatic cancer and influencing the clinical course of the disease. In this section, we address the roles of the fibroblast growth factors (FGF), the tumor growth factor-β (TGF-β) superfamily of ligands and receptors, and the epidermal growth factor receptor (EGFR) and ligands.

The FGF family consists of 19 homologous polypeptide growth factors that participate in a host of essential cell functions, including cell differentiation during tissue repair, mitogenesis, and angiogenesis. FGF-1-5 and 7 have been found to be overexpressed in certain human pancreatic cancer cell lines.[24,25] Furthermore, most human pancreatic cancers have been shown to overexpress 1 (FGFR-1β) of the high affinity transmembrane tyrosine kinase receptors that function as signal molecules to mediate the effects of FGF.[26,27]

Upon binding a ligand, the EGF receptor homo- and het-

erodimerizes. It then transphosphorylates tyrosine residues located on its intracellular domain, which allows for signal transmission via 1 of various cascades. These include the phosphatidyl-inositol-3 kinase, the ras, raf, and MAP kinases. Which one is employed depends on the cell type, the types of receptors in the heterodimer, and the nature of the ligand. Activated MAP kinases then translocate to the nucleus where they induce oncogenes such as fos and jun, leading to cell proliferation. Some studies have reported increased levels of EGFR as well as closely related receptors such as HER 2 and HER 3 in human pancreatic cancer.[28,29] Furthermore, it is also known that several ligands for the EGF receptor such as EGF, tumor growth factor-α (TGF-α), and heparin binding EGF-like growth factor (HB-EGF) are also overexpressed in pancreatic cancer.[30,31] It has been proposed that this receptor-ligand system plays a role in pancreatic carcinogenesis via autocrine and paracrine mechanisms.[32]

It has been reported that human pancreatic cancers overexpress all 3 isoforms of the TGF-β receptor and, furthermore, that this is associated with a worse prognosis.[33] Upon ligand activation, the TGF-β type II receptor (TGF-β RII) heterodimerizes with a TGF-β type I receptor (TGF-β RI), which under normal circumstances will not bind ligand without TGF-β RII. TGF-β RII then transphosphorylates the serine rich domain of the TGF-β RI, thereby activating it. Once activated, the TGF-β RI phosphorylates the intracytoplasmic proteins Smad 2 and 3. These latter 2 then form a complex with Smad 4, which translocates to the nucleus and serves as a transcriptional activator.[34] Members of the same family, Smad 6 and Smad 7, are known to inhibit the phosphorylation of Smad 2 and Smad 3 by TGF-β RI, thus preventing the interaction with Smad 4 and the complex's translocation to the nucleus. Smad 6 and Smad 7 have been shown to be overexpressed in pancreatic cancer.[35,36] Although epithelial cell growth is normally inhibited by TGF-β signaling, it is thought pancreatic cancer cells have become unresponsive to these signals. Furthermore, some investigators suggest that in such a context, TGF-β signaling works to increase metastasis, stimulate angiogenesis, and elude immune surveillance.[37]

Other growth factors that may play a role in pancreatic cancer include insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF).[38]

In summary, greater understanding of the molecular biology of pancreatic cancer has allowed for the emergence of novel therapeutic modalities such as gene therapy.

DELIVERY SYSTEMS

At present, there are 3 commonly used approaches to achieve the desired gene delivery: viral vectors, nonviral vectors, and physical methods. When considering a viral vector, various attributes directly influence its usefulness. Specifically, an ideal agent would have to be able to infect its target in vivo with a certain degree of stability, which would allow for survival of the delivery process as well as amplification. Furthermore, the agent should be specific as well, leaving normal tissue preferentially unharmed. With this paradigm in mind, several points in the process present themselves as logical steps subject to manipulation: attachment, entry, replication, packaging, and immunomodulation.

Retroviruses

Retroviruses are enveloped viruses whose genome is contained in the form of single-stranded RNA. The genomic material codes for long terminal repeats (LTRs), gag, pol, env, and psi genes. The gag and pol genes code for core proteins, an integrase, a protease, and reverse transcriptase. The env gene codes for envelope proteins and determines the specificity of cell type that can be infected. The psi gene encodes for a packaging signal. After infection of a target cell and release of the viral contents into the cytoplasm, reverse transcriptase transcribes the RNA into DNA, which then randomly integrates into the host genome. It is customary to use replication incompetent retroviral vectors in order to prevent undesired recombinants. Briefly, the gag, pol, and env genes are deleted and replaced by a gene of interest. This allows for a considerable cloning capacity of about 8 kb. Also, it lowers the viral immunogenicity. The proteins coded for by the deleted genes are supplied in trans by a packaging cell line, yielding an infectious nonreplicative retroviral particle.[39]

Gene delivery to pancreatic cancer cells has been accomplished with retroviral vectors such as the Moloney murine leukemia virus.[18]

There are several limitations when dealing with retroviruses. Perhaps most importantly, active replication of the target cell is necessary for viral integration to occur. Because at any given time the fraction of actively dividing tumor cells is small, the efficiency of gene transfer is considerably decreased.[40] Furthermore, the highest titers that may be achieved when manufacturing the virus are on the order of 10 e 8 particles/ml. This is substantially lower than may be obtained with other systems. Finally, in order to better target different tissue types, the retroviral cell receptor needs to be better characterized. There have been reports of improving the specificity of cells induced by modifying the env gene. Also, packaging cell lines have been engineered that attempt to target the vector by a variety of means.[41,42]

Lentiviruses

Lentiviruses constitute an interesting vector because as opposed to retroviruses, lentiviruses do replicate in quiescent cells. Like retroviruses, however, integration of the gene of interest into the viral genome occurs at random.

Adenoviruses

Adenoviruses possess an icosahedral protein shell enclosing a linear double-stranded DNA genome approximately 36 kb

long. There are at least 46 serotypes known, the more commonly used for gene therapy being type 5. The virus attaches to its target cell via the Coxsackie-adenovirus receptor (CAR). This results in receptor-mediated uptake of the virus into endosomes from which it escapes into the cytoplasm. The viral DNA then gets delivered into the nucleus where it remains as an episome and does not integrate in the host genome. Here the viral DNA gets transcribed and translated as well as replicated. With all of the nuclear material and structural proteins available, assembly can then take place followed by cell lysis and release of progeny.

Initial adenoviral vectors were engineered for gene therapy by deleting the E1A region and rendering them replication incompetent. The desired gene of interest can then be fitted in its place, allowing for about 7.5 kb of foreign DNA to be inserted. The virus can be propagated in cells engineered to complement the E1 deficiency.

Several advantages to adenoviral vectors endorse their use. They can consistently be propagated in a stable fashion to high titers (up to 10 e 13 particles/ml) that permit its use in vivo. They are able to infect a broad array of both replicating and quiescent target cells with high transduction. There is no disruptive random integration into the host genome as with the vectors described above. They do, however, seem to elicit a dose-dependent host immune response that prevents a second dosing. Furthermore, expression of the gene of interest is transient. It may be that these drawbacks are used in an advantageous fashion such as increasing tumor immunogenicity.

A recent development has been the engineering of conditionally replicative adenoviruses (CRAD) for cancer therapy. These adenoviral vectors retain most of their E1A region, which allows for replication. The partial deletion is complemented only by tumor cells. Alternatively, a tumor-specific promoter may be used to selectively drive the E1A region and allow for replication only within the context of the tumor cells. Theoretically, the vector remains replication incompetent in normal cells that are unable to complement it. Furthermore, it allows for lateral spread within the cancerous tissue, conferring specificity. This strategy has been used successfully in pancreatic cancer in vitro and within the context of a murine model.[43]

Other recent developments include the modification of the adenoviral envelope fibers in order to achieve enhanced tropism and attachment in cells such as pancreas ductal cells, which normally are thought to be CAR receptor deficient.[44-46]

Adeno-Associated Viruses (AAVs)

Adeno-associated viruses are small, nonenveloped, single-stranded DNA parvoviruses that, in order to replicate successfully, require coinfection with an adenovirus or a herpesvirus. The wild-type version usually integrates into the host genome specifically on to chromosome 19. This specificity is, however, lost in engineered vectors. The AAV genome codes for 2 palindromic inverted terminal repeats in between which may be found the cap and rep genes. These latter 2 code for structural and viral replication proteins, respectively. When engineering a vector, the gene of interest is inserted in the place of the cap and rep genes, which are deleted. Space is limited, and only about 4.5 kb of foreign genetic material may be inserted. When producing the vector, it is propagated along with a helper virus and a packaging cell line capable of supplying the cap and rep gene products. Of note, high concentrations of rep proteins are toxic to both helper viruses and the packaging cell line, which makes it difficult to achieve high titers.

Despite its limitations, AAVs possess several advantages, including their ability to infect both replicating and quiescent cells. Furthermore, they are safe because they elicit a minimal immunogenic response in humans. They have a reasonable range of tropism for various cell types and are able to target various different kinds of tissues. Long-term transgene expression in transduced tissue has been reported.[47] These vectors have been used successfully in the context of pancreatic cancer cell lines.[48,49]

Liposomes

Liposomes are spherical synthetic lipid bilayers that mimic biological membranes. DNA is bound by polycationic lipids as a result of electrostatic interaction. This allows for fusion of the liposome with the target cell membrane, endocytosis, and delivery of the DNA into the cytoplasm. Delivery into the nucleus is more difficult and accounts for transient transgene expression. Although safe and easy to manufacture, this system is plagued by lack of targeting specificity.

Liposomes have been used in a rat model to successfully deliver a reporter gene to pancreatic tissue after local artery and pancreatic duct infusion. Limited transgene expression was detected for 28 days after administration.[50]

Naked DNA

Physical transfer of genetic material may be achieved by mechanical methods. Naked DNA may be complexed to gold particles, accelerated to high speeds, and then bombarded on the target tissue, in effect injecting the foreign DNA. Although straightforward and safe, this method is limited by very low efficiency of transduction.[51]

ANTICANCER GENE THERAPY STRATEGIES

Antisense Approaches

This strategy seeks to prevent the transcription, translation, or processing of certain cancer-associated genes. Antisense oligonucleotides are short sequences of deoxynucleotides that bind in a complementary fashion to specific DNA or RNA sequences. After binding the target, it is thought that gene expression is hampered by various mechanisms: translational arrest or inhibition after binding mRNA or rRNA, transcription arrest after binding single stranded stretches of DNA, interference of RNA transportation within the different cell compartments,

and interference with RNA splicing.[52] The approach, although ingenious, is plagued by a variety of problems. Delivery of oligonucleotides is limited and nonspecific. Furthermore, once delivered, these molecules are unstable in vivo. One study delivered an antisense oligonucleotide to amphiregullin to a pancreatic cancer cell line. This resulted in a decrease of the concentration of amphiregullin released into the medium. Amphiregullin is a ligand of EGFR, which is overexpressed in pancreatic cancer. The result was a dose-dependent inhibition in cell growth.[53] Similarly, an antisense nucleotide to gastrin mRNA has been shown to inhibit the growth of a pancreatic cancer cell line.[54]

Antisense RNA constitutes an extension of the previous approach. Antisense RNA may be delivered efficiently in a large molar excess via a viral vector and, once expressed, binds to the target mRNA in a complementary fashion much as described above. This system has been shown useful to inhibit the oncogene activity of c-myc and K-ras expression.[55] One limitation with this approach has been the degradation of RNA by nucleases.

One final approach in this category has been the use of ribozymes. These are small catalytic RNA molecules with endonuclease activity that may be manipulated to target specific RNA molecules. They too are difficult to specifically deliver exogenously, although they may be incorporated into a viral vector.

Tumor Suppressor Replacement

Given our understanding of the molecular basis of pancreatic cancer, an obvious strategy is the replacement of defective tumor suppressor genes. As discussed above, prime candidates for this approach include p53, p16, and Smad4/DPC4. Indeed, all 3 have been the subject of experiments with various results.

The human wild-type p53 gene has successfully been transduced into pancreatic cancer cell lines by means of adenoviral and retroviral vectors, which results in growth inhibition and induction of apoptosis.[56-59] Similar reports have followed after transduction of wild-type p16 into various pancreatic cancer cell lines that possess a functional retinoblastoma gene.[60,61] Interestingly, different results have been obtained upon transduction of Smad4 into pancreatic cancer cell lines. Although in vitro studies failed to show inhibition of pancreatic cancer cell growth, transduction of a murine subcutaneous xenograft pancreatic tumor model did.[62]

Drug Sensitivity Genes

Also known as suicide gene therapy or gene-directed enzyme prodrug therapy, this approach attempts to selectively transduce tumor cells with a gene, which, when expressed, will convert a systemically administered nontoxic prodrug into a toxic metabolite. High concentrations of the toxic metabolite will be achieved in the tumor while leaving normal tissue unharmed. The classic paradigm for this approach has been the herpes simplex thymidine kinase/ganciclovir system (HSVTK). [63] Another commonly used system is the toxin gene cytosine deaminase, which converts 5FC to the antimetabolite 5FU.

An interesting phenomenon that has been described with this approach is that of the bystander effect. It involves the killing of nontransduced tumor cells and is thought to be related to the uptake of toxic metabolites via intercellular communication paths such as gap junctions.[64,65]

Immunotherapy

This field attempts to circumvent the fact that pancreatic tumor cells have a low immunogenicity and escape surveillance by the host. In using recombinant DNA technology, the aim is to augment the level of immunogenicity of tumor cells in an attempt to engage the host immune system. Various strategies have been adopted. One such strategy is to genetically modify tumor cells such that they express cytokines that attract antigen presenting cells to the tumor site. Another approach has been to transduce tumor cells such that they express costimulatory surface molecules or cytokines that attract and activate killer T cells. Pancreatic cancer cell lines have been transduced with IL 2, IL 4, IL 6, IL12, IL15, and TNF-α, resulting in an inhibition of tumor growth.[66,67] One study pulsed professional antigen presenting cells (APCs) with synthetic mutant ras peptides and injected these into patients with pancreatic cancer exhibiting the same mutation.[68] There was evidence of enhanced T cell responses in some subjects. This study suggested the possibility of transducing APC with vectors coding for specific tumor antigens. Upon expression, the antigen would be presented to T cells, resulting in a heightened immune response.[69]

Vaccines constitute another form of immunotherapy. Because of their low immunogenicity, any attempt at vaccination using unmodified pancreatic tumor cells has little, if any, response. One approach to circumvent this problem has been to use allogeneic vaccine pancreas cells that have been genetically altered to express GM-CSF in order to recruit APCs and killer T cells.[70] Yet another approach in development consists of using known cancer-associated antigens delivered by recombinant viral vectors that also contain other immune stimulatory genes in order to produce an antigen-based vaccine.[9]

Monoclonal antibodies have been used in an attempt to administer passive immunotherapy in murine pancreatic cancer models as well as phase I and II trials.[10,11]

Tissue Inhibitors of Matrix Metalloproteinases (TIMP)

Pancreatic cancer cells are known to overexpress certain matrix metalloproteinases (MMP) responsible for degrading the basement membrane.[71] It is thought that degradation of the basement membrane allows for ease in the development of metastases. Thus, increased expression of MMPs might contribute to the aggressive nature of pancreatic cancer in developing local invasion and early metastases. Following this line of reasoning, 1 group transduced a pancreatic cancer cell line with a vector coding for the TIMP-1. The group noted an attenuation of

tumor growth and a decreased level of implantation, metastasis, and angiogenesis.[72-75]

CURRENT STATUS OF GENE THERAPY IN PANCREATIC CANCER

Current review of the National Cancer Institute's website reveals 17 active gene therapy trials and 83 closed ones. Specifically within the context of pancreatic cancer, there are no closed gene therapy trials listed. There is, however, 1 active gene therapy trial listed for pancreatic cancer: a phase II randomized study of intratumoral adenovirus 5-tumor necrosis factor alpha with fluorouracil and radiotherapy in patients with unresectable locally advanced pancreatic cancer.

CONCLUSIONS

Pancreatic cancer remains a highly aggressive disease with a dismal prognosis. Currently, the only modality that provides the possibility of a cure is surgical resection. However, only a small, highly select subpopulation of patients are eligible. Conventional chemotherapy and radiation remain largely ineffective. Over the past decades, great strides have been made in the characterization of the molecular biology of pancreatic cancer. This has facilitated the development of new therapeutic and investigational modalities, which make use of recombinant DNA technology. However, it remains clear that the greatest advance in the treatment of pancreatic cancer will come from improved early detection and diagnosis. Methods of screening individuals at risk for early pancreatic cancer are much needed. Finally, it seems that in the future, the best results will be yielded by a multimodality approach based on surgical resection that also incorporates neoadjuvant and adjuvant therapy.

GLOSSARY

Amino Acid: A set of 20 different molecules that comprise the basic building blocks of proteins.

Codon: The unit of the universal genetic code. Each codon is coded for by a 3-nucleotide sequence of mRNA and in turn codes for an amino acid.

DNA: Deoxyribonucleic acid. The molecule that codes for genetic information, located within the nucleus (of eukaryotic cells). It is a double-stranded nucleic acid held together by weak bonds between base pairs of nucleotides. Nucleotides are the building blocks of DNA and are in turn comprised by 1 of 4 bases [adenine (A), thymine (T), guanine (G), and cytosine (C)] joined to a backbone of sugar (deoxyribose) and phosphate, which lend it its polarity. The strands are annealed to each other in an antiparrallel, complementary fashion, and they are wound up in a double helix configuration. The pairing of bases can only occur between the following base pairs: a "C" and a "G" or between an "A" and a "T." Chromosomes are in essence strands of DNA. Complementary DNA (cDNA) is the DNA equivalent of spliced RNA, created with a certain polymerase (reverse transcriptase) and used in amplifying DNA sequences by PCR.

Gene: A particular sequence of nucleotides that codes for a certain molecule, usually a protein. Not all of the DNA material within the gene necessarily codes for the protein. In eukaryotic genes, DNA contains introns that are noncoding intervening sequences of DNA. These get trancribed into an RNA molecule but are then excised by the process of splicing the RNA into messenger RNA (mRNA). Conversely, exons code for a sequence of nucleotides; all of which will comprise the spliced mRNA.

Mutation: A class of events that result in a change of genetic structure. This class includes point mutations in which 1 nucleotide is substituted for another. Point mutations may be classified as silent, missense, or nonsense mutations depending on the kind of change that occurs. Another class occurs when a base pair is added or deleted, resulting in a frameshift mutation.

Oncogene: A class of gene that exhibits increased biologic activity as a result of a mutation.

Promoter: A regulatory element upstream of a particular gene that is recognized by RNA polymerase as the site at which to begin transcription of that particular gene.

Protein: A class of molecules comprising amino acids and responsible for a wide variety of cell functions.

RNA: Ribonucleic acid. Single-stranded nucleic acid composed of a linear polymer of nucleotides (base, ribose sugar, phosphate group), which codes for amino acids and acts as an intermediary, transcribing DNA so that it may be translated into proteins. There are a variety of types of RNA with different functions, including messenger RNA (mRNA), ribosomal RNA (rRNA), and transfer (tRNA). RNA uses uracil as a base instead of thymine.

Plasmid: A circular DNA molecule capable of self-replication within a host cell.

Polymerase: A class of enzymes that assemble complementary strands of DNA or RNA from free nucleotides by using a template as reference. Polymerases always read the template in the 5′ to 3′ direction.

Ribosome: A class of molecule that assembles proteins from amino acids by decoding mRNA according to the genetic code.

Transcription: The process by which a single DNA strand is used as a template by RNA polymerase in order to synthesize a complementary strand of RNA. Genetic material is always read in the 5′ to 3′ direction.

Translation: The process by which a strand of mRNA is decoded into a chain of amino acids by the ribosomal apparatus.

Tumor suppressor gene: A class of gene that, when inactivated, results in the elimination of vital negative regulators of cell proliferation allowing for uncontrolled growth.

Vector: A DNA molecule capable of replicating itself within a host cell.

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