The Human Genome, Implications for Oral Health and Diseases, and Dental Education

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Abstract: We are living in an extraordinary time in human history punctuated by the convergence of major scientific and technological progress in the physical, chemical, and biological ways of knowing. Equally extraordinary are the sparkling intellectual developments at the interface between fields of study. One major example of an emerging influence on the future of oral health education is at the interface between the human genome, information technology, and biotechnology with miniaturizations (nanotechnology), suggesting new oral health professional competencies for a new century. A great deal has recently been learned from human and non-human genomics. Genome databases are being "mined" to prompt hypothesis-driven "post-genomic" or functional genomic science in microbial models such as *Candida albicans* related to oral candidiasis and in human genomics related to biological processes found in craniofacial, oral, and dental diseases and disorders. This growing body of knowledge is already providing the gene content of many oral microbial and human genomes and the knowledge of genetic variants or polymorphisms related to disease, disease progression, and disease response to therapeutics (pharmacogenomics). The knowledge base from human and non-human genomics, functional genomics, biotechnology, and associated information technologies is serving to revolutionize oral health promotion, risk assessment using biomarkers and disease prevention, diagnostics, treatments, and the full range of therapeutics for craniofacial, oral, and dental diseases and disorders. Education, training, and research opportunities are already transforming the curriculum and pedagogy for undergraduate science majors, predoctoral health professional programs, residency and specialty programs, and graduate programs within the health professions. In the words of Bob Dylan, "the times they are a-changing."

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"Without a doubt, this is the most important and most wondrous map ever produced by mankind," announced President Clinton as he described, at a White House ceremony on June 26 in the year 2000, that scientists had completed the first draft of the human genome, the blueprint of life that guides heredity and the biological processes of our minds and bodies.

Of course, it was only a first draft and much hard work lies ahead, but as a technological achievement and for its health and social implications, this was a profound milestone in the history of mankind. As Francis Collins, director of the National Human Genome Research Institute, one of the seventeen institutes that comprise the National Institutes of Health (NIH), observed at the White House event, "Researchers in just a few years will have trouble imagining how we studied human biology and disease without the genome sequence in front of us."

So how does this milestone affect the future of the oral health profession? In particular, how will the Human Genome Project (HGP) and related technology impact education, research, and oral health care practices? This review considers the human and microbial genomic achievements and their implications for oral health education. The review will cover eight dimensions: 1) a primer for the "new biology"; 2) genetics as a paradigm for studying human oral, dental, and craniofacial biology, diseases, and disorders; 3) the genomes of model organisms (from virus to dental educator); 4) microbial genomics (viral, bacterial, and yeast), oral infection, and systemic diseases; 5) the HGP and the cloning of disease and disorder genes; 6) single-nucleotide polymorphism (SNPs, pronounced "snips") maps for human genetic variations, pharmacogenomics, and molecular epidemiology; 7) circuits, molecular profiling, and oral cancer; and 8) a prospectus for oral health professional education in the twenty-first century.

The Human Genome Project and the "New Biology"

We live in a biosphere with multiple relationships with other living organisms ranging from prions and microbes (virus, bacteria, yeast) at the micro-

scopic level of organization, to insects, plants (e.g., *Arabidopsis thaliana* genome sequence),[1] animals, and humans at the macroscopic level. Common to all of these forms of life are the unifying genetic blueprints that serve to guide the genesis of each living creature. DNA (deoxyribonucleic acid) is a macromolecule composed of a linear array of deoxyribonucleotides; each nucleotide or base contains a nitrogenous base, a sugar, and a phosphate component.[2] Diseases and disorders can be associated with misspellings or genetic mutations of one or more nucleotides, and these mutations can be caused or induced by infectious microbes, environmental factors such as physical and chemical mutagens, genetic mutations and variations, or (more likely) combinations of these multiple factors.[3]

HGP is a large, coordinated, and multinational effort between public and private sectors to elucidate the genetic content and architecture of the human genome and, in parallel, that of infectious microbes, animal models, and plants with particular benefits for biopharmaceutical and nutritional advances.[4] The official beginning of the HGP in the United States was heralded on October 1, 1990. The term "human" in the name HGP is a misnomer since the project also supports non-human model organism genomics. Today, HGP includes the science and technology of microbial, plant, animal, and human genomics, an array of functional genomics and proteomics, bioinformatics, and education efforts to address the many ethical, social, economic, political, and philosophical dimensions associated with genomics in the twenty-first century (Table 1).

The haploid human genome consists of 3.2 billion nucleotide or base pairs (A, adenosine; T, thymidine; C, cytosine; and G, guanosine) within DNA that are distributed among twenty-three distinct chromosomes (twenty-two autosomes and one sex chromosome; either X or Y).[3] Within the vast array of bases are encoded approximately 45,000 regulatory or structural genes and the necessary elements that

Table 1. Human, animal, and microbial genomic information and databases

The reader is encouraged to access the expanding human, animal, plant and microbial genomic databases using the Internet:

Human Genome News http://www.ornl.gov/hgmis

National Coalition of Health Professional Education in Genetics http://www.nchpeg.org

American Society of Human Genetics http://www.faseb.org/genetics

Ethical, legal and social implications of genome research on privacy/confidentiality http://www.ornl.gov/hgmis/elsi/elsi.html

Craniofacial, dental and oral genetic diseases and disorders http://www.nidr.nih.gov/cranio/index.html

Genetic details for tooth formation http://bite-it.helsinki.fi.80/toothexp/

Human Genome Map compiled by the National Center for Biotechnology Information http://www.ncbi.nlm.nih.gov/genemap/

Online Mendelian Inheritance in Man (OMIM database) http://www.ncbi.nlm.nih.gov/omim/

GenBank, current status of human, animal, plant and microbial genomics http:www.ncbi.nlm.nih.gov/GenBank/GenBank Overview.html

Arachaea *and* Eubacteria *microbial genome projects sorted by taxonomic groups, present in GenBank, annotation in progress and "in progress"* http://www.ncbi.nlm.nih.gov/PMGifs/Genomes/bact.html

ORGANIZATIONS
National Institute of Dental and Craniofacial Research
Information Office
National Institutes of Health
Bethesda, Maryland 20892
1-301-496-4261
http://www.nidr.nih.gov

National Human Genome Research Institute
Information Office
National Institutes of Health
Bethesda, Maryland 20892
http://www.nhgri.nih.gov

control the regulation of genes throughout the lifespan of the organism.[4-6] In addition to the genomic information found within the nucleus of each human reproductive cell (sperm in testes and ova in ovaries) as well as the trillions of somatic cells (e.g., cartilage, bone, periodontal ligament, dental pulp, trigeminal ganglia, salivary glands, oral mucosa), genomic information is also encoded within genes located in the maternally inherited mitochondria termed the mitochondrial genome or mitDNA. Mutations in mitDNA are also associated with a number of human diseases and disorders.[4-6]

Analyzing the human genome involves the construction of various types of maps such as cytogenetic maps, genetic maps, and physical maps that reflect the features of DNA.[4] The highest resolution physical map is the complete DNA sequence map that provides the precise ordering of all 3.2 billion bases within the twenty-three haploid chromosomes.[4] The convergence of laser optical systems, information technology, miniaturization, and molecular biology has profoundly accelerated progress in this endeavor. The 3.2 billion bases were identified as of late June 26, 2000.[5] The recent 95.5 percent completion with 99.6 percent accuracy of the human genome sequence and annotation provides the foundation upon which to build an increasingly detailed description of oral, dental, and craniofacial diseases and disorders at the molecular level (see Table 1).[6] Following genomic sequencing of microbial, yeast, fruit fly, mouse, and human genomes, a diverse set of techniques have been developed to define, understand, and integrate the functions of human and microbial genes in health and disease.[5]

The comparison between bacteria and human genomes is intriguing. Most bacterial genomes (as an example) contain 1.8 million bases (the summation of all of the A, T, C, and G bases) and these are assembled into 1,740 discrete genes.[6] In contrast, the human genome contains 3.2 billion bases that are assembled into 35,000–45,000 discrete genes.[6] These human and non-human genome sequence databases have "evolved" essentially over the last ten years, and now serve as the cornerstone for technological progress in three "wet laboratory" research areas including gene mutagenesis in cells and animal models, nucleic acid hybridization technology, and protein chemistry. These three enabling technologies are promoting rapid progress in functional genomics or proteomics. Proteins make up essential constituents of cells, tissues, and organs and guide biochemical reactions as regulatory molecules (e.g., transcription factors, polypeptide growth factors, enzymes). The DNA sequence of a gene determines the amino acid sequence of the protein that the gene encodes. In some cases, a gene can produce as many as ten different proteins through a process of alternative splicing such as associated with enamel formation and amelogenin biosynthesis. Proteomics is the emerging field of science that defines the structure and function(s) of proteins. Proteomics is being used to address and solve a number of complex biological problems during development, health, and disease.

How do genes function? How do genes function in combinations? How do gene-gene and gene-environment interactions produce disease? What are the rules for the structure and function of proteins? What are the rules for protein-protein interactions? How do these circuits or networks function within biological systems? Which mechanisms resist mutation, and which mechanisms drive mutation in microbial, plant, animal, and human genomes? Integration of functional genomic data from these many types of experimentation will be key to developing a unified understanding of how the information encoded in the genome is interpreted in the clinical oral health community of this new era.

Today, we have the opportunity to compare the human set of genetic instructions with those in microbes, plants, and animals. These comparisons further provide analyses that may result in new models of thought to formulate a broader perspective in biology, diseases, and disorders. These comparisons in this "genome era" allow us to learn about human and non-human genomes, their evolution, organization, and rearrangements in health and disease. Moreover, these approaches provide for the development of innovative gene-based diagnostics, treatments, and therapeutics, and disclose how individual genetic variance or polymorphisms are reflected in drug responses and drug metabolism.[7,8] The "human genome era" heralds a call to action to reform dental education for the twenty-first century.[4-13]

Genetics As a Paradigm for Studying Human Oral, Dental, and Craniofacial Biology, Diseases, and Disorders

With the exception of trauma, essentially all diseases and disorders have a major genetic component. Human diseases and disorders may result from single gene mutations, but more commonly result from complex and multiple gene-gene and gene-environment interactions.[3-6,9-13] The study of genetics has traditionally been a formidable strategy to identify DNA sequence mutations (e.g., deletions, substitutions large and small) responsible for diseases or conditions vertically transmitted through the germ line by Mendelian inheritance patterns (e.g., autosomal dominant or recessive, X-linked dominant or recessive).[5,6] Genetic studies have revealed that often one base or nucleotide deletion or substitution (e.g., A, adenosine replaces C, cytosine) can be responsible for a disease.[4-6] Some of the documented examples of these types of genetic alterations include amelogenesis imperfecta, dentinogenesis imperfecta, osteogenesis imperfecta, Treacher-Collins syndrome, Pierre Robin syndrome, Crouzon syndrome, ectodermal dysplasia, achondrodysplasia, and Gorlin syndrome.[14-27] There are thousands of examples. Genetic variants or polymorphisms characterize non-disease human variations, diseases, host responses to trauma or pathogens and disease progression, pharmacogenomics, or individual host responses through drug metabolism, and host responses in the presence of disease progression to therapeutics.[5,7-8,24-27]

Human genetic studies often are initiated by identifying clinical phenotypes or traits, usually identified within disease or disorders that appear to cluster in a population, community, or family. For example, abnormal shaped and/or missing teeth, tooth size, discolored teeth, defective dental tissues (e.g., enamel, dentin, cementum, alveolar bone), abnormal quantity and/or quality of saliva, abnormal topographical features of tongue and oral mucosal tissues, responses to nutrients or nutrient deficiency, responses to drugs (pharmacogenomics), and manifestations of oral and/or systemic infections are considered clinical phenotypes. Of course, not all traits that appear multiple times in the same family or pedigree are "genetic" in origin, and possible contributions from "non-genetic" factors (like mutagens, carcinogens, teratogens, nutritional status, environmental insults) must always be considered.

The introduction of "positional cloning" improved the fidelity of the search for genetic causes of human diseases.[28-34] Positional cloning is a molecular biology strategy that tactically has been used to isolate hundreds of genes for numerous human single gene diseases or disorders (see www.nhgri.nih.gov/DIR/GTB/CLONE and www.nidcr.nih.gov). A number of single gene mutations have been identified for many of craniofacial, oral, and dental diseases and disorders (see www.nidcr.nih.gov). More common than Mendelian inherited human diseases and disorders are those caused by multiple gene mutations coupled to multiple environmental factors termed "human complex diseases." Particularly important oral-dental-craniofacial "complex diseases and disorders" include the large majority of cleft lip and palate and other craniofacial malformations, congenitally missing teeth, dental caries, severe malocclusions, head and neck cancers, periodontal diseases, oral candidiasis, temperomandibular joint diseases and disorders, neuropathies such as trigeminal neuralgia, autoimmune diseases and disorders such as fibromyalgia and Sjogren's syndrome, osteoporosis, osteoarthritis, and many others. Each of these complex diseases or disorders has a genetic etiology but their inheritance is complex (non-Mendelian) such that multiple gene-gene and gene-environment interactions develop the clinical phenotype or traits. Human complex diseases consist of multiple genes that are difficult to identify by positional cloning for several reasons. The major confounding issue is that there are often no strict correlations between genotype and clinical phenotype, and the common technical approaches have often not been able to identify large numbers of genes with varying levels of penetrance. These issues not only apply to considering the human genome but also to the microbial genomes associated with opportunistic pathogens within biofilms, plants, and animal genomes.

Studying the Genomes of Model Organisms

DNA is the major constituent of chromosomes that are localized to the nucleus of all somatic cells.[3] DNA is also a constituent of mitochondria, which are supramolecular organelles distributed in the cytoplasm of all eucaryotic cells (yeast to dental educators).[3-6] Mapping and sequencing the human genome (DNA within the twenty-three pairs of chromosomes) and the human mitochondrial genome (DNA within mitochondria) together will reveal all of the information required for the biological development of a human being.[5,6] However, the ability to interpret most of this information in terms of function will depend upon parallel studies of non-human organisms used extensively in research laboratories. In addition, oral-dental-craniofacial diseases and disorders are often associated with microbial opportunistic pathogens that interact with host biological processes such as immune responses, inflammation, vascularization, wound healing, repair, and regeneration.

The genomes of microbes such as *E. coli*, yeast (*S. cerevisiae*), nematode (*C. elegans*), and fruit fly (*Drosophila melangaster*) are approximately 1/600, 1/200, 1/30, and 1/20 the size of the human genome, respectively. In particular, the recent completion of the yeast genome and the development of associated technology such as DNA microarray for surveys of thousands of genes are rapidly changing biomedical research (see genome-www.stanford.edu/Saccharomyces). These microbial and invertebrate model genome knowledge bases have become a significant benchmark for all future genomics (Table 1). These emerging knowledge bases also reflect functional studies of individual or multiple and interacting genes in response to defined environmental factors such as pH, temperature, and a variety of putative teratogens, mutagens, and carcinogens. These emerging genomic databases and those being derived from zebra fish, frogs, birds, mice, rats, and primates are already being "mined" for insights into the molecular mechanisms that control the biological pathways related to etiology, pathogenesis, diagnosis, and treatment for many human diseases and disorders.[5,6]

Microbial Genomics, Oral Infection, and Systemic Diseases

The microbial ecology of biofilms found on tooth, mucosal, and implant surfaces offers extraordinary opportunities to investigate the community of pathogens associated with oral infection *per se*, as well as oral infections associated with human systemic diseases and disorders.[35-40] Oral microbial infections are associated with a number of systemic diseases including low-birth weight, premature babies, cardiovascular disease, cerebrovascular disease, osteoarthritis, a number of pulmonary diseases and disorders, and the management of type 1 diabetes.[35-40] Curiously, these often-commensal microorganisms (viral, bacterial, and fungal) may become virulent as a consequence of the host environment such as immunodeficiency, nutrition, other medical conditions, medications, and psychosocial stress factors. How commensal microbes become virulent is a critical and as yet unanswered question.

Microbial genome analysis provides molecular information into virulence, host adaptation, and evolution.[41-46] The Joint Genome Institute operated by the University of California for the U.S. Department of Energy reported the completion of high quality draft nucleic acid sequences of fifteen different microbial genomes in one month (see www.jgi.doe.gov/tempweb/News/news). As of February 28, 2001, forty-one microbial genomes had been completed, another thirteen were being annotated, and another eighty-nine microbial genomes were "in progress" (www.ncbi.nlm.nih.gov/PMGifs/Genomes/bact.html 02/28/01).

Functional genomics of oral microbial pathogens (virus, bacteria, fungi, or yeast) use DNA microarrays to investigate gene expression, gene-gene interactions, and multiple gene-environment interactions.[41-50] The microbial ecosystem termed biofilms that reside on various oral, dental, and implant surfaces offers a remarkable opportunity for ambitious studies of differential gene expression, gene polymorphisms and mutation, three-dimensional architecture and physiology of biofilms, com-

munity effects of microbes within biofilms under conditions of immune suppression or protein-calories malnutrition, microbial genome plasticity, identification and characterization of virulence factors, and drug action mechanisms, drug targets, and novel anti-microbial drug development.[7,8]

"Mining" through critical analyses of gene sequence databases can result in investigator-initiated, hypothesis-based studies to define the molecular pathways of pathogenesis. One fascinating approach is that using mutational analysis. The experimental approach requires the construction of defined microbial mutants that perhaps reside within a biofilms community. Using a technique called transposon mutagenesis or allelic replacement, it becomes technically feasible to specifically delete or add one or more genes to a specific genome such as *Lactobacillus acidophilus* or *Actinobacillus actinomycetemcomitans*. This is a powerful technical approach for determining numerous gene functions such as specific virulence in an oral pathogen. This technique enables studies to identify those genes responsible for virulence in many different bacteria or yeast pathogens. The approach is being pursued in bacterial as well as yeast models.[46-50] One of the recent innovations has coupled DNA tags or markers with allelic replacement. This experimental strategy is termed "signature-tagged allele replacement."[43-50]

Beyond microbial genomics are numerous opportunities to define protein-protein interactions that are key determinants towards understanding many significant biological processes. One strategy is to use the genomic database to establish protein interaction maps. This approach reveals specific discrete amino acid sequences or motifs within proteins that function in highly specific protein-protein interactions. During the last few years, interaction maps have been proposed for viral, bacterial, and yeast pathogens. The reader is encouraged to consider the recent interaction map produced for *Helicobacter pylori* as a model.[53]

Human Genome and the Cloning of Disease Genes

Thousands of genes are known to cause human diseases when present in a mutated form (see www3.ncbi.nlm.nih.gov/omim). The identification of oral, dental, and craniofacial disease or disorder genes has occurred using either functional or positional cloning strategies.[4-11,14-24,54] With functional cloning, the disease gene is isolated as a result of preexisting knowledge of the fundamental physiological defect such as the globin protein in beta-thalassemia, mutations in the carboxyl terminus of collagen associated with osteogenesis imperfecta, mutations in amelogenin associated with amelogenesis imperfecta, or mutations in fibroblast growth factor receptor 2 with a number of different craniofacial syndromes including Crouzon syndrome.[14-23] In contrast, with positional cloning the isolation of the unknown defective gene follows its position within the genome by genetic and/or physical mapping techniques (see www.nhgri.nih.gov/DIR/GTB/CLONE).

The major steps and sequence involved in positional cloning are pedigree collection with clinical phenotype determination and DNA collection from each family member, genetic mapping to specific regions of a specific chromosome, physical mapping and clone isolation, gene isolation, mutation, analysis and functional studies to determine how the candidate gene causes the disease or disorder. Functional and positional cloning have proven to be highly effective in identifying genetic mutations that cause relatively simple diseases or disorders.[12] The more complex human diseases require enabling technology that can assess thousands of different genetic mutations or polymorphisms and provide a more precise correlation between sequence variation and heritable phenotypes (http://www.ncbi.nlm.nih.gov/SNP).[5-7]

Single-Nucleotide Polymorphism (SNPs) Maps for Pharmacogenomics and Epidemiology

There are approximately three million differences or 0.1 percent variance between the DNA sequences of any two copies of the human genome—men versus women, or any two culturally different subpopulations.[5-6] In other words, all individuals share genome sequences that are 99.9 percent the same. Only 0.1 percent is responsible for all genetic diversity between individuals.[6] It is now evident that variations or polymorphisms in a single base or nucleotide within the genome (i.e., one of the 3.2 billion

bases) may be informative for the diagnosis of a disease.[3-7] The approximately three million different single nucleotide variance or polymorphism (SNPs) are physically distributed throughout the entire genome. SNPs are single nucleotide polymorphisms or one-letter variations in the DNA sequence. These variations in SNPs contribute to differences among individuals. The majority of SNPs may have no deleterious effects; others cause subtle differences in countless characteristics such as tooth size and shape, while others affect the risk for diseases or disorders and are associated with human complex diseases. The discovery of SNPs as markers or "tools" of choice for mapping complex disease gene loci is now emerging into diagnostics, pharmacogenomics to explain individual variations in responses to drug therapy, and even the epidemiology of diseases and disorders within populations.[5,7,51-52]

The number of SNPs required to cover the entire human genome is estimated to be several million. The "proof of principle" and technical feasibility were recently documented through an industry/government collaboration that has produced a SNP map of human chromosome 22.[51-52,55-56] A number of practical issues, such as patient sample size, SNP density and genome coverage, and data interpretation, are particularly important to consider when anticipating the application of pharmacogenomic information to clinical dental practice.[8,51-52,55-56]

Circuits, Molecular Profiling, and Oral Cancer

Today, we increasingly appreciate that head and neck cancers are relatively common. Oral and nasopharyngeal squamous cell carcinomas often are diagnosed at late stages of the disease progression; they usually then have a poor prognosis (50 percent) for survival after five years. These cancers often produce severe complications for patients and health care professionals.[54-57] Squamous cell carcinoma appears to reflect sequential and multiple mutations within complex pathways over time in response to multiple gene-environment interactions often associated with direct sunlight, alcohol consumption, chronic use of tobacco products, chronic infection, and immune and nutritional deficiencies.[57-60]

Combinations of hundreds to thousands of genes are required to provide housekeeping as well as unique functions in human cells. These genes are termed regulatory and structural genes. These genes encode proteins that in turn contain specific modules (sequence of amino acids), and within these modules are discrete motifs (short sequence of three to five amino acids) that provide the biological activity for the specific protein functions. Motifs provide functions such as enzymatic activities, calcium or zinc binding, affinity for specific cell surface carbohydrates, affinity to bind to extracellular matrix macromolecules (amelogenins, enamelins, dentin matrix proteins, cementum matrix proteins, fibronectins, laminins, collagens, proteoglycans) and immunoglobulins, and many other important biological properties. The biological problem of squamous cell carcinoma found in oral cancer patients likely demonstrates multiple mutations in discrete motifs that directly regulate cell division, cell adhesion, cell communication and/or programmed cell death or apoptosis. These new techniques provide an approach to unravel these possibilities and to focus upon candidate gene products for gene therapy or gene-mediated therapeutics.

Until now the clinical phenotype of human cancers has been identified, described, analyzed, ranked, and predicted using histopathology. Ironically, multiple patients with squamous cell carcinoma may provide biopsy samples that closely resemble one another, yet each patient may have very different neoplastic disease, responses to therapy, and clinical outcomes. More recently, a number of investigations have sought biomarkers to more accurately categorize and subcategorize tumors and to better understand the biology of the oral cancer neoplastic processes.[57-61] From these and other investigations evolved DNA array technology that enables molecular profiling of literally thousands of different genes. For example, two different human samples obtained by microdissection from an oral cancer tumor can be used for molecular profiling that compares transcriptional activity of thousands of regulatory and structural genes that reside in each of the tumor cell populations.[58-61] Further analyses can reveal housekeeping genes in common to both tissue samples, yet also reveal the number of unique patterns of gene expression associated with the neoplastic process (see Figure 1). Patterns of genes related to various pathways become candidates for subsequent efforts to improve diagnosis, prognosis, and response to drug therapy. This approach also can provide candidate pathways and genes for targeted drug therapy.

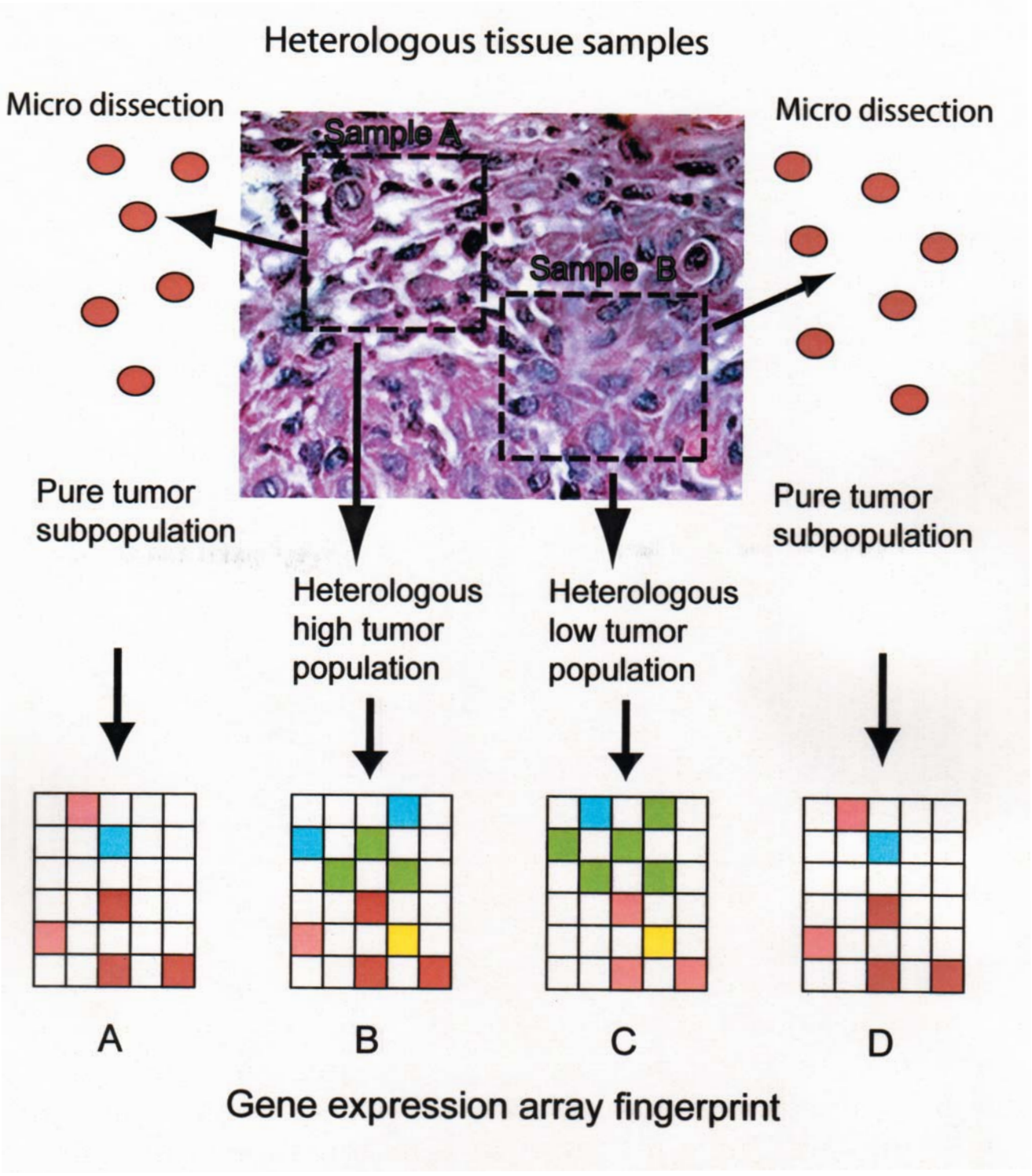

Figure 1. The induction, development and progression of squamous cell carcinoma is complex and represents multiple and sequential gene mutations, and multiple gene-gene and gene-environment interactions. In addition, human genetic variation produces additional variables related to the progression of the neoplastic process as well as the response of the individual to various types of chemotherapy. This illustration describes a tactical approach to obtain molecular profiles from discrete microdissected samples within heterologous tumors. [58-69]

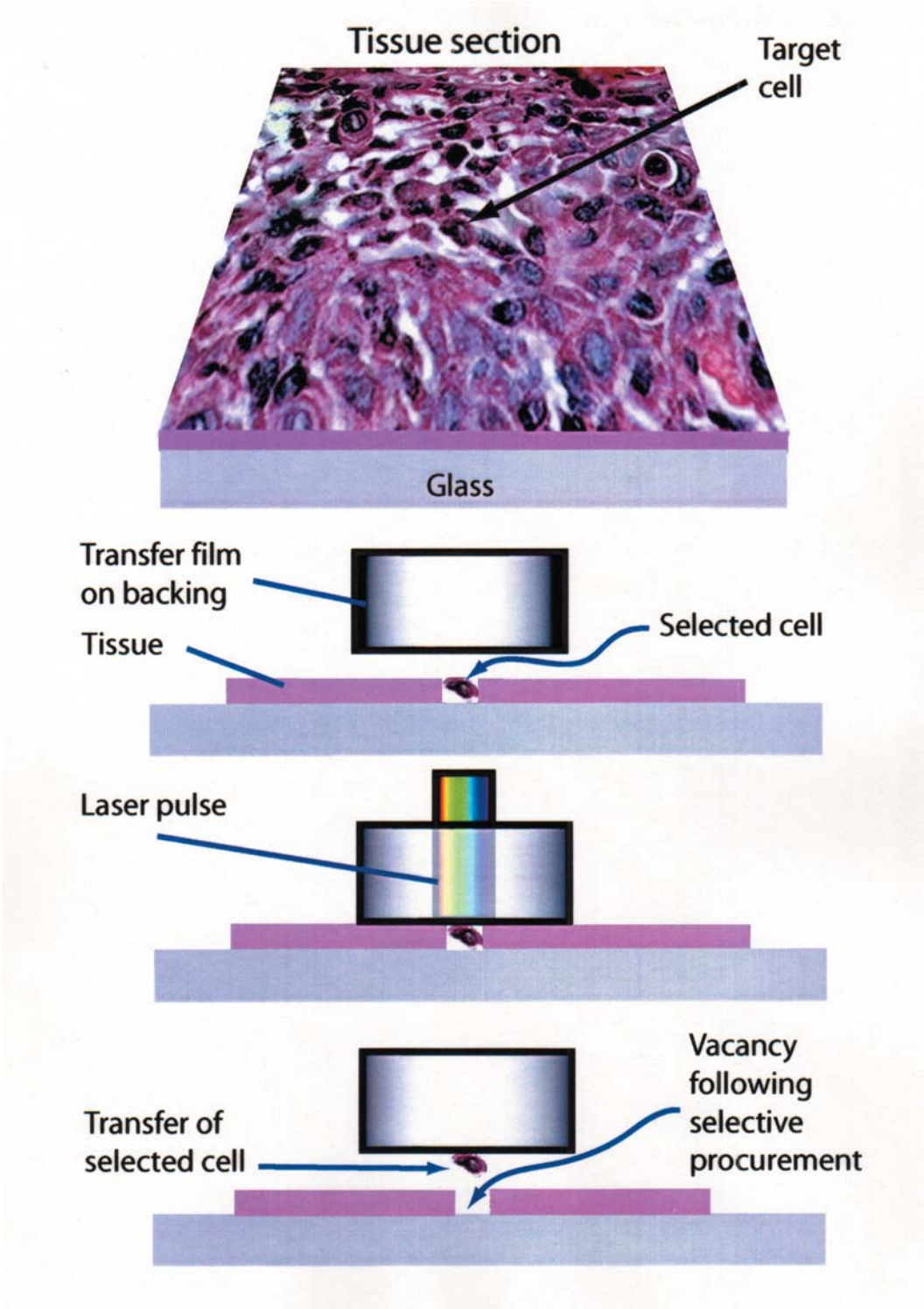

Figure 2. Laser capture microdissection is a new technology that enables rapid sampling from microscopic tissue samples representing stages in the induction, development, and progression of oral cancer. The initial histologic section can be either frozen, fixed or stained. A laser beam of approximately 7-8 micron diameter activates a polymer film in juxtaposition to the histologic section and enables the transfer of cells from the section to the polymer. Thereafter, the microdissected cells are used for DNA, RNA and/or protein profiling. The samples can then be compared within the tumor or between stages of the tumor or between patients presenting comparable tumors. [66-69]

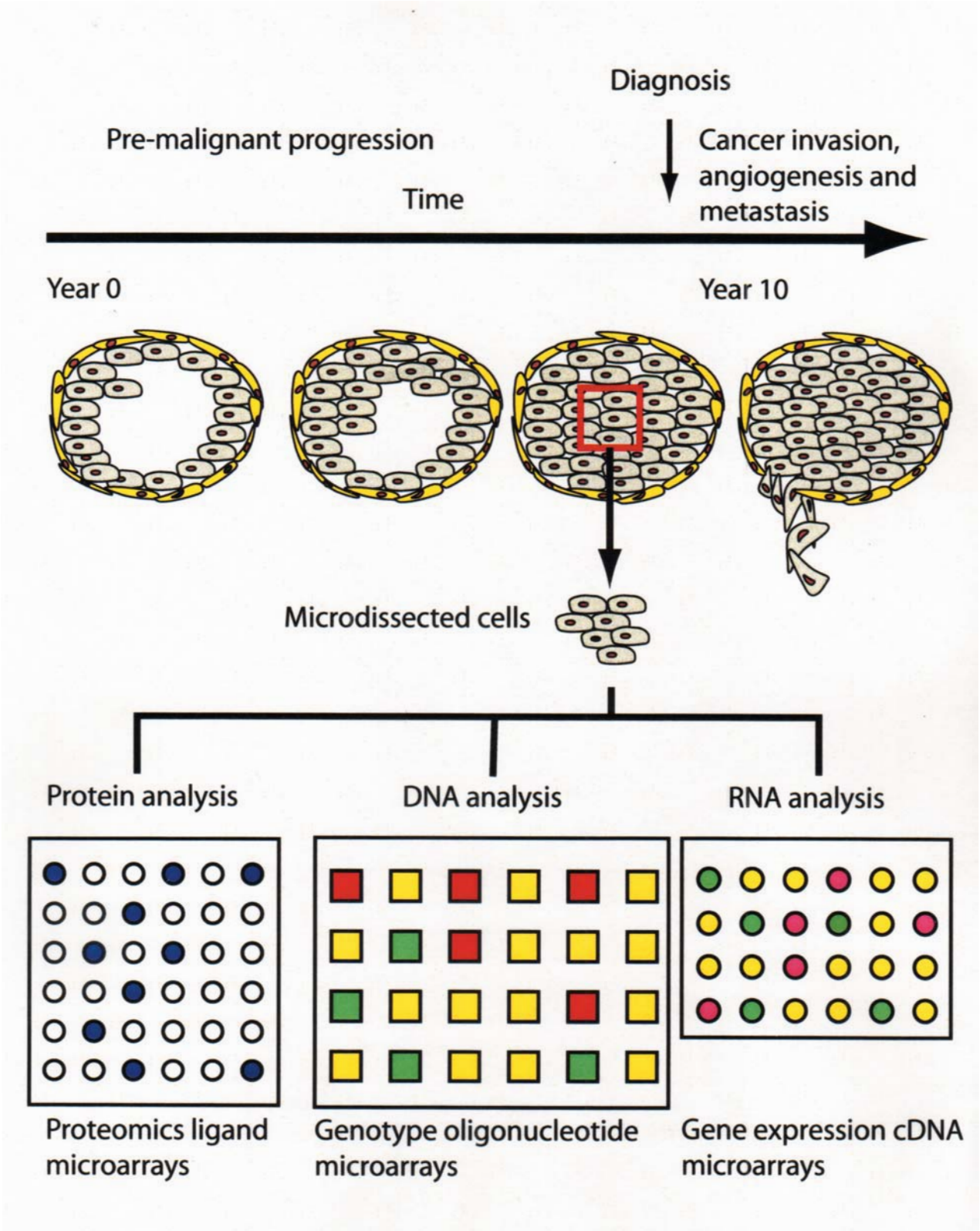

Figure 3. Molecular characterization of the pre-malignant progression of the neoplastic process in head and neck cancer. Microdissection coupled with DNA microarray technology provides rapid and comprehensive sampling of complex human neoplastic oral lesions. [57-59]

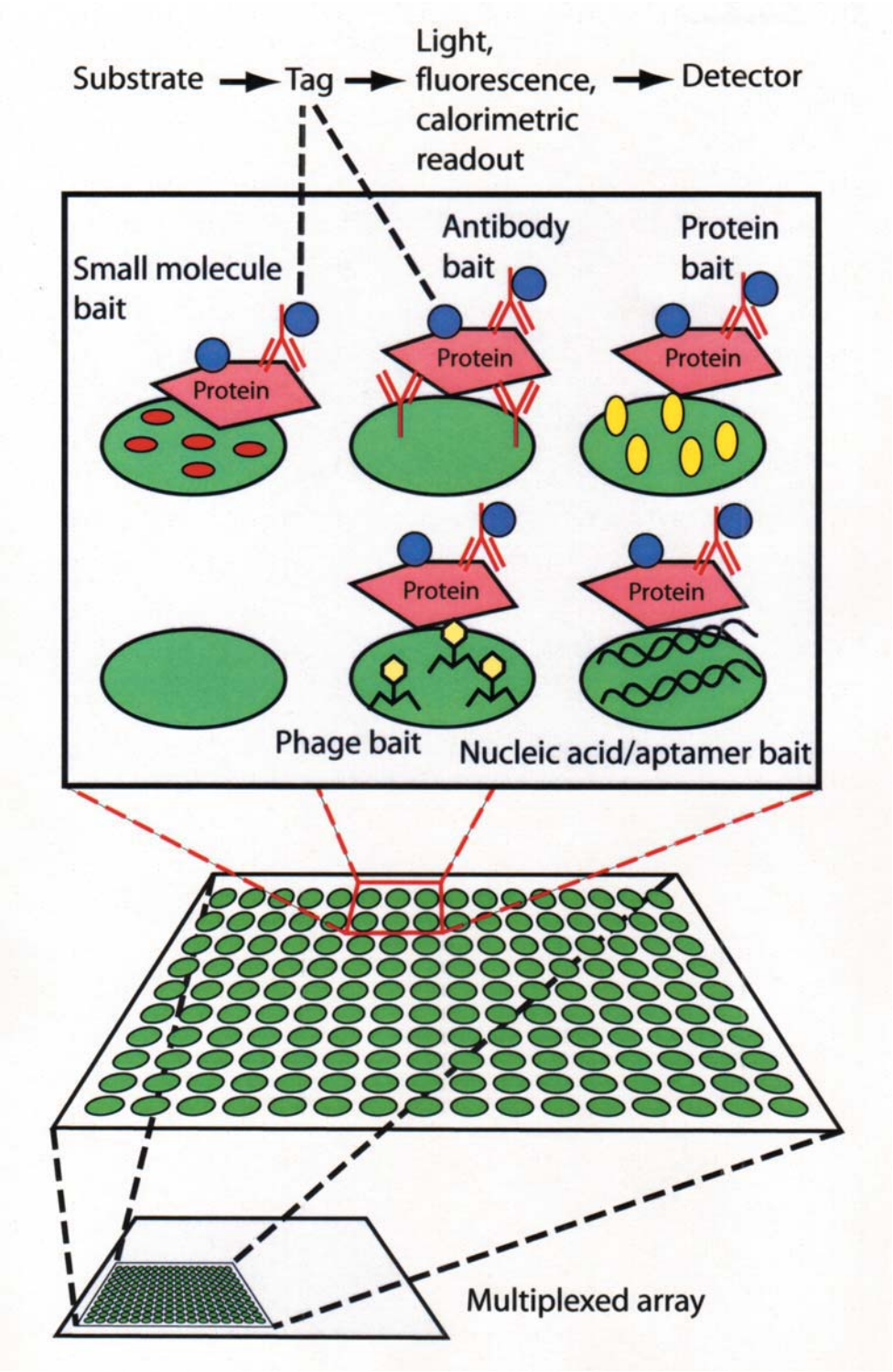

Figure 4. Proteomic arrays to survey hundreds or thousands of individual proteins can be achieved using immobilized antibodies or ligands in distinct locations within 96-well or larger platforms. The array of antibodies are incubated with these hundreds or thousands of proteins that are either directly or indirectly tagged with a reporter molecule using fluorescence. The resulting light that is generated is then captured as a signal for readout. The level of signal as each point of information becomes proportional to the concentration of the target protein in the original sample.[65]

One enabling technology for oral cancer investigations is laser capture microdissection.[62-69] This technology provides a methodology to isolate specific subpopulations of cells from tissue sections of oral tumors. The technique can literally isolate one selected cell from the visual field of light microscopy (see Figure 2). The strategy is to isolate candidate neoplastic cells to characterize their molecular uniqueness and/or stage of the neoplastic process in "head and neck" cancers including oral, pharyngeal, and laryngeal cancer.[57-61]

Another opportunity is to use DNA, RNA (ribonucleic acid), and protein analyses of laser-captured microdissection of pre-malignant progression of oral cancer (Figure 3). Invasive metastasis follows a protracted pre-malignant phase that can be observed from histopathology sections and light microscopy. The pre-malignant phase may take place over many years as shown in Figure 4. This tactical approach uses DNA analysis to identify which genes and how many genes have been mutated, deleted, or amplified at a specific place and time in the course of the neoplastic process.[69] RNA and protein arrays and analysis can demonstrate differences in transcription, protein synthesis, and post-translational modifications of proteins. The new "tools" enable surveys of 30,000 different gene products from each patient, and further enables the ability to identify genetic variance leading to unique individual diagnosis, treatments, response to treatments, and prognosis. These anticipated human genetic variations will result in new diagnostics, treatments, and innovative gene-based therapy.

The 35,000 to 45,000 human genes encode the information for producing proteins. Proteins function as structural or regulatory molecules during development, health, and disease. The bioactivity of individual proteins is derived from the uniqueness within the amino acid sequence and the folding properties of the molecule. Proteomics is the field of study that describes gaining an understanding of the full display or catalogue of protein structure, functions, and roles in complex pathways or circuits. Unlike DNA arrays that are based upon interactions between complementary DNA strands following base-pair rules of Watson and Crick,[2] protein arrays often use protein-antibody interactions that are informative with complex associations between epitopes on the target protein and the antigen-binding site on the antibody.[70-71] Figure 4 illustrates proteomic arrays that are made by immobilizing antibodies or ligands in discrete spots within 96-well format presentations. This technology is termed enzyme-linked immunoabsorbent assay (ELISA). Figure 4 further illustrates several approaches to visualization or the reporter molecules used in this approach for the detection of proteins. This method provides a technique to rapidly survey large numbers of candidate proteins.

The revolution in oral oncology is rapidly evolving through at least three phases. The first phase is gene discovery resulting in hundreds of genes associated or linked with oral cancers. The second phase is molecular fingerprinting that identifies multiple transcription profiles and proteins with functional roles in cells and tissues involved in squamous cell carcinoma. The third phase is the convergence of laser optics, informatics, and DNA and protein array technologies that correlate proteomic information into functional circuits, combinations, or pathways in epithelial, endothelial, and mesenchymal cells and tissues. The information "mined" from these various databases eventually will provide a molecular understanding of human variations and the individual characteristics for oral neoplastic diseases (see www.nci.nih.gov/cancer). Further analyses using SNPs, for example, will not only identify individual variations within disease comparisons, but will also identify human genetic variations as to how each of us responds to certain therapy. This can profoundly enhance our understanding of oral cancer—risk factors and chemoprevention, diagnostics, and innovative therapy disease!

Knowledge of the functional circuits or pathways through the entire pre-malignant progression and subsequent neoplastic alterations such as metastasis and apoptosis will provide the exquisite detail required for highly sensitive and specific therapeutics designed for individuals with unique SNP-based polymorphism detection. For example, extracellular signals or ligands bind to receptors and activate a signal transduction cascade of protein-protein interactions that elicit transcriptional responses and eventually biological processes within cells and/or tissues. A variety of post-translational modifications such as tyrosine or serine phosphorylations enables protein-protein interactions through functional motifs—discrete sequences of nucleic acids that encode highly functional instructions for biological activity. These motif-based interactions may be "rate-limiting" or serve as gate controls for a specific biological process. An example is a motif that encodes calcium-binding proteins. Mutations in se-

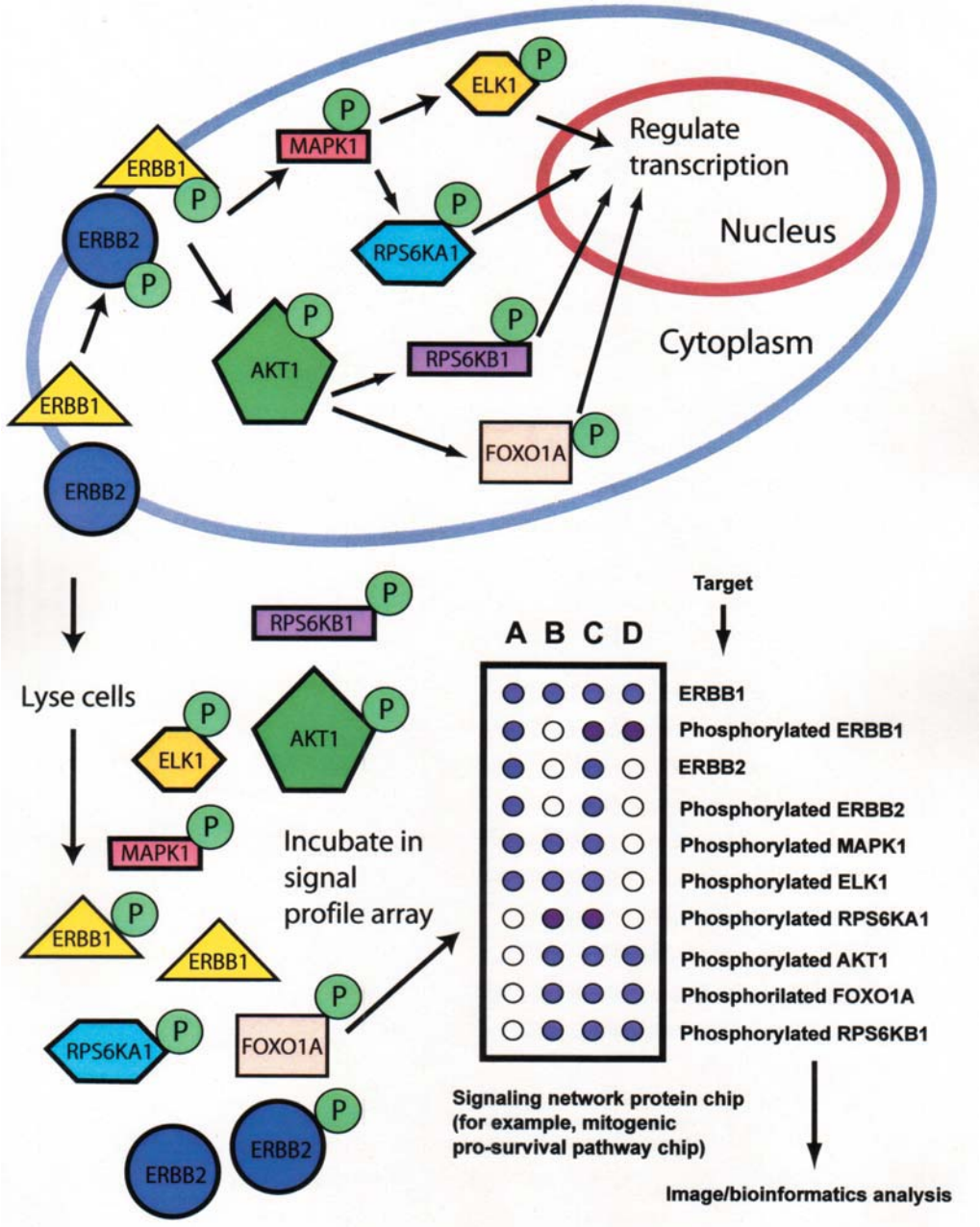

Figure 5. This diagram represents a proteomic array resulting from four samples (A-D) of human squamous cell carcinoma associated with oral cancer. In this hypothetical scheme, oral epithelial cells have become neoplastic and multiple interconnected pathways have been implicated in the disease progression.[72] *The proteomic array provides a profile of the mitogenic and housekeeping protein "targets" such as ERBB1 and Phosphorylated ERBB1; these proteins belong to epidermal growth factor receptor family (e.g. ERBB1 and ERBB2). The technique shown in figure 4 enables detection of phosphorylated versus non-phosphorylated proteins that are expressed in this EGF (epidermal growth factor) signal transduction process. Lysates from four patient samples are displayed (A, B, C and D). The pattern of signals detected from each patient lysate are shown as they might appear on a "protein chip." Abbreviations: MAPK1, mitogen-activated protein kinase; AKT1, murine thymoma viral oncogene homologue-1; ELK1, one of the ETS oncogene family members; RPS6KA1, a 90 kDa ribosomal protein S6 kinase; RPS6KB1, a 70 kDa ribosomal protein S6 kinase; and FOX01A, forkhead homologue-1.*

quences that encode significant motifs may be the primary source for the pre-malignant progression of oral cancers, and may serve as ideal targets for innovative drug designs in the future.

However, technology is required that enables consideration of multiple genes, RNAs (ribonucleic acids), and translation products (proteins) that are associated with the neoplastic process. Figure 5 provides a proteomic profile obtained from oral cancer squamous epithelial cells from four patients with oral cancer (A, B, C, and D), using a multiplexed array format as previously described in Figure 4.[72] The signaling network protein chip provides the comparison data for the four patients and indicates hypothetical result that might be informative for identification of candidates for gene therapy. This strategy is new and offers an approach that is rapid, sensitive, and specific for human genetic variance associated with squamous cell carcinoma.

Prospectus for Dental and Medical Education

The remarkable advances in microbial and human genomics require equally remarkable advances in dental and medical education: what we learn (curriculum revisions), how we learn (pedagogy), and reforms in clinical and cultural competencies to include bioinformatics, health disparities, human population genetics and pharmacogenetics based upon SNPs and molecular epidemiology, patient education and counseling based upon "the new biology," increased understanding of health outcomes and health services research, health promotion, and risk assessment to reduce the burden of disease. In tandem, we face the challenges of faculty and staff development and renewal. We also face the challenges of specialty and postgraduate education and possible changes in certification.

The reader is encouraged to access and evaluate the recommendations put forth by a recently sponsored Blue Ribbon Panel on the Future of Education and Training in Dental and Craniofacial Sciences (see www/nidcr.nih.gov). "Change" and "revision" are the operative terms for the coming decades of the twenty-first century.

Nested within "change" are changes in demography, patterns of disease, management of health care, access to health care, information technology, quality of life expectations, and innovations and discovery through biomedical research and technology. The recent Surgeon General's Report, *Oral Health in America*, serves as an excellent primer for "what is" and suggests "what could be."[73]

The study and application of human genetic variance during normal development, diseases, disorders, and responses to treatments and therapeutics herald a new era in oral health professional education. The potential impact of genomics on the future of education, science, and clinical practice includes understanding fundamental basics of diseases and disorders, targeting research to the fundamental root causes of disease processes, risk assessments for preclinical interventions, diagnostics, and tailoring treatment and therapeutics to individual risk and responses. This emerging knowledge base can result in clinical competencies for all health professionals including dental hygienists, dental technicians, general dentists, and dental specialists to enhance the quality of health care (see clinical competencies in HGP health professional coalition website, Table 1).

A cornerstone of American schools of dentistry and medicine in a major research-intensive university and public funding of scientific research has been the close link among science, education and training, patient care, and research. This is particularly evident when considering U.S. academic health science centers (dentistry, medicine, pharmacy, and nursing) and the significant advances in health-related technology. Another aspect of equipping educator scientists for the "new biology" is the need for university-private sector-government-nonprofit foundation partnerships to support infrastructure as well as operational costs in curriculum development and related informatics, predoctoral and postdoctoral education and training, continuing education programs, and computational and structural biology, molecular epidemiology, proteomics, and pharmacogenomics.

The impact of information technology has revolutionized our lives in many ways as low-cost informatics, computing, and the Internet have become broadly available to health care professionals and patients. These advances have already influenced patient management systems in dental and medical schools, clinics, and hospitals. Likewise, the human and non-human genome is leading to unifying theory in the biological sciences and is profoundly impacting oral biology, dental and craniofacial diseases and disorders, dental and medical sciences, education,

and clinical practice.[74-75] The rate and magnitude of change in the life sciences can not be underestimated as we ponder the future.

We are about to experience the completion of the human genome and a number of oral microbial pathogen genomes. We are entering the next phase of progress and experiencing the transformation from empirical solutions to scientific evidence serving to drive the design and fabrication of the next generation of personalized and more precise therapeutics for oral health care. Biomimetics, tissue engineering, biomaterials, gene-based diagnosis, and gene-mediated therapeutics herald the new century.[76-77] The integration of technology and information from gene to patient is the necessary precondition of transcending the limits of current oral health care. Our future is very bright.

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