

Genomics and Proteomics

The Potential Role of Oral Diagnostics

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ABSTRACT: Advances in genomics and proteomics increasingly contribute to the understanding of signal transduction pathways that control growth, differentiation, and death of cells. Since defects in these processes may result in the expression of inherited and/or acquired disease, the identification of candidate disease genes and modifier genes by parallel use of genotyping together with an integrated study of gene expression and metabolite levels is instrumental for future health care. This approach, called systems biology, aims to recognize early onset of disease, institute preventive treatment, and identify new molecular targets for novel drugs in cancer, cardiovascular and metabolomic disease (e.g., diabetes), and neurodegenerative disorders. Gene interaction networks have recently been demonstrated, in which hub genes, that is, genes that show the highest level of interactions with other genes, play a special role. Hub genes, often chromatin regulators, may act as modifier genes (genes that modify the effect of other genes) in multiple mechanistically unrelated genetic diseases in humans. In addition, it has been shown that small metabolites such as hormones and cytokines, or proteins/enzymes such as C reactive protein (C-RP) and matrix metalloproteinase (MMP), reflect disease status in case of oral cancer, asthma, or periodontal and cardiac disease. Many of these molecular targets, as well as pathogen-specific DNA and RNA sequences, can be measured in oral fluids, providing a unique opportunity to develop novel noninvasive diagnostic tests. Efforts so far concentrate on the use of lab-on-a-chip technology in combination with novel reporters and microsensor arrays to measure multianalytes in oral fluids. Handheld devices that perform sensitive detection of multiple analytes in oral fluid will be obtainable in the near future.

KEYWORDS: oral fluid; diagnosis; genomics; proteomics; metabolomics; point-of-care testing; infectious diseases; cancer; diabetes; hub genes

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GENOMICS, PROTEOMICS, AND METABOLOMICS

Achievements of genomics and the knowledge of the sequence of the human genome have inspired numerous *-omics* disciplines such as proteomics, glycomics, and metabolomics, mainly aiming to understand the signaling pathways that allow cells to divide, differentiate, and die in a controlled manner.¹ A new discipline has been coined called *systems biology* of which the central strategy is devoted to identify candidate disease genes and modifier genes in a unique set of population and clinical cohorts by parallel use of genotyping and an integrated study of gene expression and metabolite levels. Of the latest *-omics* disciplines, it is anticipated that metabolomics will provide essential information about various chronic and multifactorial diseases such as cardiovascular disease, diabetes, and diseases of the central nervous system. In the past decade the sensitivity of bioassays was spectacularly improved.² Often in the past, only high-concentration compounds that reflect homeostasis and housekeeping gene products could be measured in body fluids. However, now steroids, neurotransmitters, and trace elements can also be studied because of advances in analytical techniques such as mass spectroscopy,³ chromatography, electrophoresis, or fluorescence-based detection technologies.

The sequencing of the human genome and many other species will identify major components of the proteome and result in a map of the major metabolites and their function. The key question is how this enormous amount of information will be amalgamated in order to understand the signal transduction pathways that allow cells to function normally, to divide and die.

TOWARD UNDERSTANDING SIGNALING PATHWAYS

Despite the large number of scientific papers published to date on signal transduction pathways, the major pathways are not well understood. This situation exists for several reasons: some of the published results are inevitably incorrect; it is almost certain that all the major regulatory mechanisms have not yet been described (e.g., the recent discovery of the regulatory role of micro RNAs); the majority of papers are mainly descriptive (e.g., “substance A helps B to get C”) and lack detailed quantitative kinetic information (e.g., “how much of A and B is needed, how fast is the reaction, and how much C is produced”). In a noteworthy paper “Can a Biologist Fix a Radio? or, What I Learned While Studying Apoptosis,” Lazebnik⁴ makes a comparison between the views of a biologist and an electrical engineer, in particular with respect to signal transduction. An oversimplified statement of the different views is that cutting a wire in the radio stops the music, whereas that is seldom the case in biology. True quantitative analysis of pathways is essential and currently possible on account of the combined technical progress of advanced analytical instrumentation and computer power, so that analysis of the signaling systems

in an integrated approach including extremely complex interconnectivity of the individual systems is possible. As an example, it has become clear that many genetic diseases are very complex in the sense that disease susceptibility is not necessarily caused by a single genetic lesion but often arises from the combined effect of mutation in multiple genes, suggesting genetic interactions.

THE CONCEPT OF HUB GENES

Recently, Lehner *et al.* used RNA interference techniques to systematically study 65,000 pairs of genes in *Caenorhabditis elegans* for their ability to interact and found about 350 genetic interactions between genes in signaling pathways that are mutated in human disease.⁵ Note that a large part of the molecular machinery in *C. elegans* is conserved in humans. Interestingly, a subset of genes called “hub genes” showed a high degree of connectivity with other genes. They all encoded chromatin regulators and it was proposed by the authors that these hub genes could act as modifier genes in multiple genetic diseases that are mechanistically unrelated. It is unlikely that this observation and hypothesis are the last ones to occur. It took two decades to understand the role of micro RNAs.⁶ Their presence and regulatory role implied the end of a central dogma, namely that noncoding sequences have no function; note that 98% of the transcriptional output in humans is noncoding RNA. Further studies of “junk” DNA, noncoding RNA sequences, and deeper insight in post-translational modifications likely will reveal new regulatory mechanisms. Thus, it leaves biologists with conflicting thoughts: on the one hand there is great excitement about any major discovery since completion of the sequencing of the human genome, while on the other hand there is the frustrating idea that “nothing is certain as yet” and the feeling that we only see the tip of the iceberg, to use an old cliché. In that dynamic uncertainty, it is not easy to identify the gene products and analytes that are diagnostically relevant in case of important diseases. To make the situation even more complex, it has become known that many key molecules have both promoting and repressing function, which implies that a single analyte measurement, without studying its effects on other analytes, may not be meaningful in many cases. Nevertheless, it remains a great challenge to identify the key modifier genes and their products, since that knowledge could drive new diagnostic platforms that recognize early disease onset, allow for the institution of preventive treatment, and identify new molecular mechanisms for novel drug development.

THE ROLE OF ORAL DIAGNOSTICS

The potential role of oral diagnostics has been clearly identified elsewhere. In short, there is convincing evidence that the various kinds of oral fluid contain

many of the analytes that reflect normal function or disease status, as is the case in blood, cerebrospinal fluid, or urine. Second, technologies have emerged with high sensitivity that allow many important analytes to be routinely measured. Oral fluids are therefore an ideal target for a new generation of noninvasive diagnostic tools. This was recognized by the National Institutes of Health (NIH) and an NIH/NICDR grant program was started in 2002 called "Development of Technologies for Saliva/Oral Fluid Based Diagnostics." Seven groups were funded by that program and it is of interest to analyze their achievements in a bird's eye approach. In terms of technology, many groups use microfluidics and the latest lab-on-a-chip achievements. Furthermore, surface plasmon resonance (SPR) imaging platforms and novel nanosensors and microsensor arrays were developed, some allowing the measurements of hundreds of analytes. In terms of reporter molecules, electroluminescence and up-converting phosphor technology (UPT) are used next to conventional fluorescent reporters.

The analytes chosen by these seven groups basically cover the entire spectrum from viral/bacterial nucleic acids, including 16 S ribosomal genes, to mRNA transcripts, proteins and small metabolites, including hormones and cations. The targeted diseases are periodontal disease, viral and bacterial infections, renal disease, asthma, cardiac disease, and oral cancer. The analytes measured include C-reactive protein (C-RP), matrix metalloproteinases (MMPs), cytokines/chemokines, and tumor necrosis factor (TNF). Interestingly, the group of cytokines (IL-6, IL-8) and the MMPs are considered indicative of different diseases. For instance, cytokine levels are informative for periodontal disease, asthma, and oral cancer, while MMPs can process many bioactive mediators such as cytokines, growth factors, their receptors, and specific matrix protein anchors for these molecules.⁷ MMPs also play a role in development, wound healing, learning, aging, and in diseases such as cancer. The processing of bioactive mediators can lead to profound alterations in cell behavior that result in shedding of cell surface molecules, activation, and inactivation of signaling molecules that occasionally convert agonists to antagonists.

From a conceptual point of view, mechanistically cytokines and MMP function in a manner parallel to the hub genes in that they reflect a complex "biostate" that is altered in case of periodontitis and asthma (both inflammatory diseases) and also in cancer.

STRATEGY AND APPROACH IN ORAL DIAGNOSTICS

With ongoing achievements in proteomics, metabolomics, and new classes of important biomolecules undoubtedly yet to be discovered, paralleled by further improvement of key analytical technologies, a main strategy for the development of oral diagnostic devices can be designed. The three key issues are: (1) performance of measurements of multiple analytes in order to

profile the status of a (group) of diseases; (2) utilization of a generic approach in the design of detection platforms that can be easily adapted for the detection of different target molecules; note that this is achievable with easily substituted specific bioreagents such as antibodies and nucleic acid probes; and (3) the need to achieve the highest possible level of sensitivity since levels of target analytes may be orders of magnitude lower in saliva than in other body fluids, and newly discovered analytes are unlikely to occur in high concentration.

Lastly, diagnostics need to anticipate developments in future health care such as personalized medicine, aiming at early prediction and intervention.⁸ It also needs to be noted that multinational electronics companies invest in the development of micro devices used for *in vivo* remote sensing. A noninvasive oral fluid test provides considerable advantages compared to a blood test. In Western societies the major target diseases are changing. Next to cancer there are increasing efforts to develop new diagnostic tests and therapeutics for cardiovascular diseases, metabolic disorders such as diabetes, and neurodegenerative disorders such as Alzheimer's and Parkinson's disease. Diagnostics for mental disorders are still in a relatively preliminary phase, despite their enormous socioeconomic impact. In contrast, in developing countries, infectious disease affecting more than a billion people remains the number one target disease and especially in this environment, hand-held oral diagnostic devices that are robust and easy to use at low operational costs can create a major step forward in basic health care.

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