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Methods and approaches to disease mechanisms using systems kinomics

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ABSTRACT

All cellular functions, ranging from regular cell maintenance and homeostasis, specialized functions specific to cellular types, or generating responses due to external stimulus, are mediated by proteins within the cell. Regulation of these proteins allows the cell to alter its behavior under different circumstances. A major mechanism of protein regulation is utilizing protein kinases and phosphatases; enzymes that catalyze the transfer of phosphates between substrates [1]. Proteins involved in phosphate signaling are well studied and include kinases and phosphatases that catalyze opposing reactions regulating both structure and function of the cell. Kinomics is the study of kinases, phosphatases and their targets, and has been used to study the functional changes in numerous diseases and infectious diseases with aims to delineate the cellular functions affected. Identifying the phosphate signaling pathways changed by certain diseases or infections can lead to novel therapeutic targets. However, a daunting 518 putative protein kinase genes have been identified [2], indicating that this protein family is very large and complex. Identifying which enzymes are specific to a particular disease can be a laborious task. In this review, we will provide information on large-scale systems biology methodologies that allow global screening of the kinome to more efficiently identify which kinase pathways are pertinent for further study.

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1. Introduction

There is enormous variability in the complexity of living organisms. Small simple viruses may contain fewer than a dozen genes on a genome consisting of a few kilo-bases that encode up to a dozen proteins. Significantly more complex eukaryotic organisms possess genomes in the mega-base range that, with alternative splicing and various possible post-translational modifications, may encode upwards of millions of protein permutations. For many decades, much research effort went into either understanding the simpler organisms, or trying to delineate a few molecules within more complex systems. With advances in whole genome sequencing, bioinformatics and instrumentation, it has been possible for more than a decade to assess, both quantitatively and simultaneously, changes in the levels of total mRNA expression and in levels of thousands of proteins. Despite these advances, cellular regulation is more often determined by protein post translation modifications than by absolute quantity. This review will focus on one of the largest and best-studied subsets of proteins, which are proteins involved in kinase signaling. This field of “kinomics” encompasses kinases, kinase targets and antagonistic phosphatases [1].

The development of genomics and proteomics tools has made it possible to create large amounts of information about many processes that occur throughout a cell or tissue in response to a stimulus. The first such technologies - microarrays and quantitative proteomics - were revolutionary in their ability to simultaneously measure thousands of genes and proteins within a single experiment. This ability to globally assess the state of a cell or tissue has since expanded and evolved into numerous other techniques that have been adapted to allow more high-throughput analyses. In an effort to probe even deeper into the cellular proteome, tools have been developed to detect and isolate specific subsets of proteins that might not otherwise be detected. Examples of these protein subsets include those with post-translational modifications (e.g. phosphorylation, ubiquitination, lipidation) and localizations in response to different stimuli. Similarly, different classes of enzymes (e.g. kinases, proteases, hydrolases) can be probed for their activity levels in response to various conditions.

Kinase signaling is a powerful and central cellular mechanism that mediates signal transduction events and is involved in a wide range of nearly all cellular processes including, but not limited to, the control of cell cycle progression, transcriptional regulation, cell transformation, proliferation, differentiation, and apoptosis. Given its central role in cellular function, aberrant regulation of kinase signaling can profoundly affect homeostasis and has been found to be involved in many disease states including insulin resistance [3,4], autoimmunity [5,6], viral infection [7,8], and oncogenesis [9,10]. Hence, assessing the kinome can provide insight into complex pathological processes across a wide array of diseases and has also been a well-studied target for therapeutics. It is therefore not surprisingly that many approved pharmaceuticals target kinases in an effort to restore homeostatic cell signaling events, and that efforts have been made to explore repurposing these drugs for other diseases [11–14]. Notably, kinase signaling may also be exploited clinically as a diagnostic tool and will be discussed below.

In this current review, we provide an overview of some of the popular high-throughput methodologies, analysis tools and databases that are commonly used in kinase signaling studies and how they may be used to understand particular disease processes in virology, cancer and clinical diagnostics. Some important areas of current research include the purification and characterization of protein kinases (both natural and recombinant), the elucidation of biological functions and ligands of kinases and the development of specific kinase inhibitors.

2. High throughput systems methodologies for studying the kinome

2.1. Nucleic acid-based approaches

2.1.1. siRNA

siRNAs, or small interfering RNAs, are regulators of expression and function of genes [15]. Double stranded precursors are cleaved by dicer proteins into short fragments. The siRNA consists of a guide strand that is assembled into a RISC-loading complex which binds to dsRNA, cutting it into a single stranded functional siRNA. This RISC complex will recognize a complementary mRNA strand and cleaves this strand at a single site, releasing the fragments, which are ready to cleave more mRNA. The resulting decrease in mRNA in the cell leads to a decrease in expression of the gene. By using this endogenous host mechanism, siRNA fragments can be transfected into the cell for targeted gene knockdown.

RNAi panel screening is a high throughput method using siRNAs to determine the effects of multiple genes in a specific experimental system. siRNA panels can be purchased against target genes of choice in each well of multiwall plates from different companies. Some panels are specific for particular cellular functional pathways, such as Qiagen's SureSilencing® siRNA arrays (http://www.sabiosciences.com/pathwaymagazine/pathways9/suresilencing-sirna-arrays.php), which are predesigned to target main cellular genes in a biological or functional pathway.

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