Emerging technologies for the genomic analysis of cancer

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Abstract

Cancer-cell survival, growth and metastatic potential are directed by dominant molecular signalling patterns, the components of which have been shown to be qualitatively different from their normal tissue counterparts. These signalling patterns can now be further distinguished by quantitative assessment, either at a single point in time or at intervals. This commentary will focus on the emergence of proteomic analysis which, in conjunction with the genomic expression data, is an evolving technology that one day will enable personalized therapeutic strategies that are differentially targeted against cancer.

Introduction and context

Alterations in the six essential functions defining the neoplastic gene expression pattern collectively dictate malignant growth. These functions include self sufficiency, insensitivity to growth inhibition including immune 'escape', circumvention of programmed cell death, unlimited replicative potential, sustained angiogenesis, and local and metastatic invasiveness [1]. Targeted therapeutics currently being used are directed against derivatives of amplified genes and/or over-expressed protein kinases in malignant cells involving one or more of these core functions. Fifteen molecular targeting therapies (Herceptin, Tykerb, Gleevec, Tasigna, Rituxan, Bexxar, Avastin, Tarceva, Iressa, Vectibix, Erbitux, Velcade, Sutent, Nexavar and Sprycel) have already been approved by the US Food & Drug Administration (FDA) for cancer treatment. Cancer functions through a robust network with both adaptive pliability and functional redundancy, which (with the exception of chelates such as Bexxar) buffers the effect of any single gene/target modification on the malignant process [with some rare exceptions, such as chronic myeloid leukaemia (CML)] [2]. For example, a number of agents targeting epidermal growth factor receptor (EGFR) have entered the clinic (Tarceva, Iressa, Vectibix, and Erbitux) primarily for use in epithelial tumors. It has recently been shown that EGFR inhibition will induce upregulation of insulin-like growth factor 1 receptor (IGF-1R) resulting in a regulatory shift of Akt from the EGFR pathway to the IGF pathway. A reciprocal activation also occurs with IGF-1R inhibition [3]. Mathematical analyses of targeting strategies (such as antivirals and targeted therapies) of a variety of biological systems suggest that a disruption of at least three key biorelevant nodes will result in network disarray. These data include modelling resistance in CML [4], HIV viral escape following RNA interference (RNAi) therapeutics [5], and the effectiveness of RNAi at targeting Coxsackie virus [6]. A large number of potential therapeutic targets exist and the list continues to expand. Many are undergoing preclinical and clinical testing with a variety of target specific agents (monoclonal antibodies, small molecules, antisense constructs, ribozymes and RNAi technology) [7]. Unfortunately, given the potential for targeted therapeutic development and the availability of technology to assess genomic networks relevant to cancer-cell function, there is a discrepancy between the ability to identify presumptive targets and their actual biological relevance and integrated target sensitivity (the converse of robustness). The necessity to more effectively interrogate and quantify system functions, which would enable the

pursuit of predictable, biorelevant, low-morbidity personalized therapeutics, has also become more glaring.

Based on our understanding of spatial distribution, kinetics, and post-translational modifications, proteins are thought to be the direct effectors of cellular behavior rather than their DNA and intermediary mRNA templates. Characterization of protein expression provides the most proximate assessment of cellular functional activity. Proteins assemble themselves into complex small-world networks composed of functional modules with key regulatory hubs and interconnecting, informational, 'bottleneck' hubs [8], through a variety of protein-protein interactions. This protein-network based approach has recently been used in the analysis of breast cancer metastases [9]. We believe that characterization of these interactions and *in silico* prediction of outcomes from the reasoned and deliberate disruption of these events will provide the basis for defining novel and more effective target-complexes for drug therapy [10]. Gene mutation, gene loss, and gene duplication or amplification can result in absent, defective or overexpressed proteins. These proteins realign within the cellular protein network in a 'degenerative' pattern resulting in an 'oncopathologic' hostile takeover [11]. Although correlations of genomic patterns with survival have been demonstrated in a variety of cancers, it remains undetermined which of these anomalies are pathogenic and which are not. Yet, we believe using new technologies, it is feasible to reduce the finite but unwieldy number of overexpressed proteins in malignant tissue into a manageable subset of candidate target-complexes against which potentially effective multi-target therapies can be constructed [12].

Major recent advances

Newer technology platforms, such as yeast two-hybrid screens, forward-phase and reverse-phase protein arrays, and protein chips, combined with emerging bioinformatic analytic technologies, help define how proteins interact with each other and will enable us to elucidate and simulate functional modules within networks, regulatory motifs and informational cross-talk linkages [13].

The field of microarray technology has also rapidly evolved. Over the last 5 years, it has become possible to simultaneously analyze integrity and/or expression level of hundreds of thousands of genes within days. Microarray technology can be used to examine the integrity of genome and gene expression levels; genomic DNA analysis identifies the genes that have been mutated, deleted or amplified, whereas RNA analysis reveals differences in transcription and RNA processing.

Brown and Botstein [14] first reported the utilization of microarray technology to examine gene expression patterns in human malignancy in 1999. However, testing of clinical samples in the past generally utilized admixed tissue containing endothelial cells, stromal tissue, and hematopoietic cells, in addition to cancer cells. As a consequence, many of the reported gene expression patterns of malignant cells are likely to be confounded by these less than homogenous specimens. This potentially confounding factor is now being addressed with the routine implementation of laser capture microdissection (LCM). With this technique, malignant cells can be selectively dissected and captured so that only a morphologically homogeneous population of cells is investigated [15]. However, only a relatively small amount of material is retrieved with the LCM process, so the captured RNA requires amplification. As a result, an unbalanced amplification of transcripts can lead to misinterpretation of cancer gene expression levels. Recent improvements in amplification technology are addressing this concern.

Gene transcript levels often show poor correlation with protein levels, and clearly cannot predict post-transcriptional regulatory changes (for example, splicing) and post-translational modifications (for example, phosphorylation, sulphation, and glycosylation) of the encoded proteins. Proteomic technologies such as two-dimensional difference gel electrophoresis (2D-DIGE), bead capture, micro-enzyme-linked immunosorbent assays (micro-ELISA), and liquid chromatography aligned with tandem mass spectroscopy, are currently employed for drug discovery and biomarker identification [16]. Recent technologies quantifying mRNA and protein signalling patterns have in fact been successfully used to demonstrate that a finite number of genes are unique to each individual cancer and that their identification visualizes critical pathways or networks which can be utilized for therapeutic targeting [17]. RNAi technology is rapidly approaching clinical evaluation in oncology. Early trials in primates indicate that its safety and potency (as measured by knockdown of mRNA and protein levels) is far greater than small molecules or antisense constructs targeting the same gene products [18].

Future directions

With these tools a mere step away from the bedside [19], the challenge is now to identify a systems-dependent target-complex as the network's fragile site (its Achilles' heel) so as to allow for the production of a therapy that disarms the malignant 'hostile takeover'. Therapeutic targeting based on unique individual dynamic genomic/proteomic constructs, differentially expressed, as opposed

to traditional non-discriminative chemotherapy agents, can now be envisioned. It is probable that future RNAi-based gene therapy for cancer can be prescribed based on the integrated mRNA proteomic expression profile of each individual's tumors. The accumulation of data from fresh tissue obtained from the patient's tumors (preferably, primary and metastatic sites), the employment of LCM with refined amplification technology, and the incorporation of quantitative genomic/proteomic methodologies together with sophisticated modelling techniques provide a means for automated construction of probabilistic network models based on 'real-world' clinical data. This will enable the rapid discovery and characterization of tumor-specific oncogenic pathways that are responsive to each individual patient's needs within a realistic time frame. Advances in genomic sequencing technology, developed through the Human Genome Project, and high throughput SNP (single nucleotide polymorphism) analysis have resulted in the elucidation of consensus coding sequences in a variety of human tumor types [20,21] allowing for an increased depth of understanding of intratumoral interactions such as synergy between amplified and mutated genes in breast cancer [for example, TGFβ (transforming growth factor beta), Wnt (wingless-type pathways), and FGF (fibroblast growth factor)] [22].

Quantitative molecular profiling promises to change the way oncology is practiced by enabling us to: (1) reformat tumor classification using biomolecular parameters; (2) individualize therapeutics; and (3) establish 'early' assessment of therapeutic efficacy using biorelevant markers rather than volume-based endpoints. Furthermore, it will also enable a systems biological approach to the understanding of the malignant process using mathematical methods capable of virtualizing intracellular signalling pathways, intercellular communications, and cellular-environmental interactions.

Abbreviations

2D-DIGE, two-dimensional difference gel electrophoresis; CML, chromic myeloid leukaemia; EGFR, epidermal growth factor receptor; ELISA, enzyme-linked immunosorbent assays; FDA, Food & Drug Administration; FGF, fibroblast growth factor; IGF, insulin-like growth factor; IGF-1R, insulin-like growth factor 1 receptor; LCM, laser capture microdissection; RNAi, RNA interference; SNP, single nucleotide polymorphism; TGFβ, transforming growth factor beta; Wnt, wingless-type.

Competing interests

JN declares that he is co-founder of Gradalis, Inc, a RNA interference development company. NS declares that he is the Scientific Officer of Gradalis, Inc.

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