EDITORIAL

Clinical Cancer Genome and Precision Medicine

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ABSTRACT Revolutionary sequencing technologies have changed biomedical research and life science exponentially. Revealing the whole landscape of causal somatic and inherited mutations underlying individual patient's cancer sample by whole-genome sequencing (WGS) and whole-exome sequencing (WES) can lead to not only a new mutations-based taxonomy of solid tumors (Stratton, *Science* 331:1553–1558, 2011). But also shapes a roadmap for precision medicine (Roychowdhury et al., *Sci Transl Med* 3:111ra121, 2011; Roukos, *Expert Rev Mol Diagn* 12:215–218, 2012; Mirnezami et al., *N Engl J Med* 366:489–491, 2012). This inevitable approach for personalized diagnostics in concert with free-falling genome sequencing costs raises now the question of applying next-generation sequencing (NGS) technology in the clinic. In the pragmatic clinical world and in contrast to innovative research, is NGS-based clinical evidence sufficient for decision-making on tailoring the best available treatment to the individual cancer patient?

TARGETED THERAPY

Surgery has evolved and been standardized to provide complete tumor resection (R0) in nonmetastatic stage and with or without radiotherapy leads to solid tumor locoregional control. Systemic adjuvant chemotherapy before and/or after an R0 resection has been the standard approach for controlling systemic micrometastatic disease, reducing recurrence rates and saving the lives of millions of patients. However, limited further oncological outcome by chemotherapy can be expected. Moreover, chemotherapy is associated with toxic adverse effects. Even more important

is the finding of a more recent WGS study using NGS that provides scientific evidence that although most primary tumor cells are sensitive and are killed by initial chemotherapy, a subclone of the founding clone survives, gains additional mutations, and is expanded at relapse. DNA-damaging chemotherapy itself contributes to this treatment failure.¹

It is not surprising, therefore, that recent and emerging R&D pharmaceutical and biomolecular diagnostic industry is shifted to signaling transduction-inhibitor drugs. Crucial cellular process including survival, growth, differentiation, proliferation, and apoptosis are regulated by signaling transduction from cell surface to the nucleus. Deregulation or amplification of this signal transmission by the accumulation of genetic and epigenetic changes and ultimately also gene expression deregulation, collectively result in major diseases such as cancer, diabetes, cardiovascular, neurodegenerative, and other multifactorial disorders. Inhibiting or restoring of deregulated signaling pathways and gene expression patterns in cancer cells is the main principle of signal transduction drug development. At present, 33 monoclonal antibodies (mAb) and small-molecules tyrosine kinase inhibitors (TKI) have been approved by the Food & Drug Administration for metastatic cancer and 1 only for clinical use in the adjuvant setting, and many others are in clinical development. Has this targeted therapy explosion resulted in true survival benefits and cure?

ADVANCES AND RESISTANCE

The first advance is the anti-human epidermal growth factor receptor 2 (HER2) humanized mAb trastuzumab, which improves overall survival in HER2-positive metastatic breast and gastric cancer and is a unique targeted drug approved in the adjuvant breast cancer setting. The second is the TKI vemurafenib (PLX4032) for metastatic melanoma with BRAF V600E mutation. However, even among selected patients on the basis of HER2 amplification

and protein overexpression or BRAF mutation, respectively, intrinsic or acquired resistance and recurrence or disease progression continue today to be a big challenge. For the vast majority of other targeted drugs, the results from phase-3 randomized trials are less promising. Either a progression-free survival of only improvement without any overall survival benefit or no treatment response has been reported.² For example, 2 phase-3 randomized trials by Baselga and colleagues^{3,4} published this year in NEJM for metastatic breast cancer demonstrated no overall survival benefit. Concretely, by adding the mammalian target of rapamycin (mTOR) inhibitor everolimus to endocrine therapy in hormone-receptor-positive patients or pertuzumab, another anti-HER2 mAb, to trastuzumab and chemotherapy in metastatic HER2-positive patients the progression-free survival benefit should be weighed against the side effects of these drugs. Most of approximately 150 current kinase-targeted drugs are in clinical development, and many more in various stages of preclinical development are in oncology indications; however, evidence from current phase-3 trials limits the expectations to temporal or no antitumor effect and no overall survival benefit.²

Emerging research and technology development is therefore primarily focused on 2 main directions based on NGS. First is to improve selection of patients for applying available targeted drugs based on substantial mutational landscape-based intratumor heterogeneity.⁵ Indeed, heterogeneity can explain resistance and thus a patient's biopsy-based personalized diagnostic may improve selection among the available signaling transduction inhibitors. Second, increasing understanding of dynamics of network biology can yield deeper insights into signaling pathway networks and development of next-generation drugs and biomarkers.^{6,7}

NGS AND PRECISION MEDICINE

The unprecedented powerful capacity of WGS and WES to identify mutation-causing cancer in individual patients based on NGS application in their tumor biopsies, the increasing validity of data by “sequence once read often” approach, and the dropping costs from \$5000–\$10,000 currently to “\$1000 genome” in 2013, raise the question of application of new sequencing technologies into clinic. Perhaps for no other technology and scientific field, such as with NGS and human genome sequencing that have so rapidly shifted research into clinical pilot studies and nationwide applications. Norway is the first country to incorporate NGS-based tumor sequencing for cataloging cancer mutations and potential personalized cancer therapeutics in a pilot study (<http://www.nature.com/news/norway-to-bring-cancer-gene-tests-to-the-clinic-1.9949>). Similarly in 2012, clinical sequencing programs were

launched in the United States, at Baylor College of Medicine, the University of California (UCLA), and the Mayo Clinic, for example. These pilot studies aim to reach personalized medicine by WGS and WES, particularly in cancer patients, by identifying the causal mutation's landscape underlying individual patients and thus provide specific ways to reach personalized therapeutic decisions (<http://www.nature.com/news/sequencing-set-to-alter-clinical-landscape-1.10032>). In addition, large-scale projects such as the Cancer Genome Atlas and the International Cancer Genome Consortiums and many individual academia and research centers have already been started for deep cancer genome sequencing that will provide multiscale databases and catalogs of all kinds of mutations—point mutations, copy-number variants, genomic rearrangements—for most cancer types. Altogether, the number of human whole genome sequences will rise from 30,000 by the end of 2011 to millions in a few years. New techniques have been developed for extracting both DNA and RNA simultaneously, allowing fast, inexpensive, and validated assessment of patient-specific structural variants and new mutations-based cancer taxonomy.⁸ This mutational completion is inevitably related to the greater challenge of the century in how to understand the complex structural-functional map of a personal cancer genome in a whole genome code-lifestyle spatiotemporal interactions network.⁹

CLINICAL GENOME

However, unlike the research world, huge challenges regarding the validity of genome sequencing and omics data derived from different platforms used, storage, analysis, and interpretation as well as regulatory, ethical, and financial aspects should be overcome to achieve clinical implementation and health-care improvement. Have we arrived at the destination of translation high-throughput technology data into the practical cancer genomic medicine? The “sequence everything” approach, the clonal mutational evolution at cancer relapse, and the DNA-binding proteins dynamic mapping using NGS in patient's cancer samples now shape new clinical dimensions in clinical personalized cancer medicine.^{1,10,11}

The “sequence everything” approach included NGS-based WGS, WES, and RNA-seq for transcriptome sequencing, and in addition gene expression profiling was studied in DNA and RNA extracted both from samples of 2 patients with metastatic solid cancer. Based on this integrative omics strategy, Roychowdhury et al. could provide assessments of all classes of genetic variants including point mutations, copy number alterations, and genomic rearrangements, as well as gene amplification and

overexpression.¹⁰ In their analysis and interpretation of the results, a multidisciplinary Sequencing Tumor Board identified several genetic alterations in each patient. Among all these genetic changes, the Board considers important for clinical trials the identified amplification of cyclin-dependent kinase 8 (CDK8) and thus CDK inhibitors or mitogen-activated or extracellular signal regulated protein kinase (MEK) in the first patient with colorectal cancer. The identified Ras mutation in the second patient with melanoma provides the rationale for phosphatidylinositol 3-kinase (PI3K) inhibitors. In summary, clinical trial of candidate drugs targeting CDK/MEK for patient 1 and PI3K pathways for patient 2 may lead to more precise personalized treatment.¹⁰ Sequencing the primary tumor and relapse genomes by NGS provides an unprecedented capacity for understanding the metastatic potential of some tumors. Ding et al. provide complementary information on clonal evolution to recently described mutation clonal analysis for breast and pancreatic cancer metastases.¹

Determining the mutational spectrum at the primary tumor and relapsed genomes from 8 patients with acute myeloid leukemia (AML) they found 2 major clonal evolution patterns during AML relapse. In the first, the founding clone in the primary tumor gained mutations and evolved into the relapse clone. In the second, a subclone of the founding clone survived initial therapy, gained additional mutations, and expanded at relapse.¹ Taken together, these clonal evolution data on breast and pancreatic metastatic cancer and AML suggest that some mutations and DNA-damage chemotherapy may contribute to clonal selection and initial therapeutic resistance. Given the wide patient-to-patient and even intratumor heterogeneity, tumor sequencing providing a whole picture of cancer-initiating and metastatic-causing mutations in individual patients can lead to personalized cancer medicine.

Chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq) provides a fascinating technology for transcriptome mapping at a genome-wide level. As estrogen receptor (ER) is the defining transcription factor and its target genes orchestrate growth and endocrine response, ER-binding events could be used to assess therapeutic response and prognosis. Shifting from cell-line models to clinical samples, Ross-Innes et al. for the first time mapped genome-wide ER-binding events by ChIP-seq, in frozen primary breast cancers samples from patients with good and poor clinical outcomes and in distant ER-positive metastases.¹¹ They found that unique ER-binding regions in primary tumors were associated with relapse and poor outcome. Unlike with the report by Ding et al. in AML and another breast cancer report on clonal evolution, Ross-Innes et al. found that the differential ER-binding program observed in tumors from patients with poor outcome was not due to the selection of a rare

subpopulation of cells, but was due to the FOXA1-mediated reprogramming of ER binding on a rapid timescale.^{1,11} This ChIP-seq-based transcription-factor mapping in primary tumor material therefore provides the potential for discovering novel biomarkers to predict therapeutic response and outcome in individual patients.

GRAND CHALLENGE

These 3 genomic studies represent excellent paradigms of how applying NGS techniques into cancer tissues and blood samples and of how combining these experimental omics data with patient's clinical data could lead to the translational genomic medicine. Figure 1a delineates the beginning of efforts to integrate high-throughput data into a clinical dimension toward precision cancer medicine. At the same time, however, these studies either provide diverse results on recurrence molecular mechanisms underlying therapeutic resistance or raise questions on the efficacy of proposed selection of mutations-targeting signaling pathway inhibitors.^{1,10,11} For example, which strategy should be given higher priority for performing large-scale genomic studies for the development of biomarkers with clinical robustness on predicting metastatic risk and therapy resistance? Should emphasis be given in the WGS sequencing approach for mutational landscape analysis of clonal evolution based on the concept of additional mutations at relapse, suggesting that some of these mutations may contribute to clonal selection and therapy resistance?¹ Is the ChIP-seq based transcriptome analysis and transcription-factor mapping in primary tumor samples a more appropriate strategy for discovering robust predictive biomarkers?¹¹ At present, no clear answer can be given while we are awaiting new genomic studies and data. The "sequence everything principle" by sequencing both coding (WES) and noncoding DNA (WGS) and RNA (RNA-seq) provides a comprehensive analysis of genome and transcriptome and cross-talk validation of causal mutations. However, the proposed selection of mutations-based or amplification-based single signaling pathways inhibitors such as CDK8, MEK, or PI3K does not consider the dynamics and complexity of intracellular physical and functional signal transduction interactions network and cell-cell connectivity.^{6,7,10}

Bioinformatics analysis of omics large quantity data represents another challenge. Even though the cost of WGS may be lowered to \$1,000/genome or even lower than \$500/genome in the next several years, the analysis of every single genome for real clinical or preclinical use would still cost several hundred thousand dollars. This is a real bottleneck preventing the expansion of new WGS technology to clinical use.

FIG. 1 Current and emerging clinical cancer genome for personalized treatment. **a** Current approach. Sequencing DNA alone or plus RNA or transcriptome mapping by ChIP-seq can identify either causal mutations or differential DNA-binding proteins regions that potentially can be used as predictive biomarkers for selecting efficient drug(s). **b** Future perspective. Mapping of intracellular physical protein connections and DNA-binding proteins and functional (gene) interactions provides a comprehensive picture of signaling transduction interactions networks. This can be a platform for discovering next-generation network-based drugs and biomarkers. *WGS* whole-genome sequencing, *WES* whole-exome sequencing, *TS* transcriptome sequencing, *RNA-seq* RN sequencing, *ChIP-seq* chromatin immunoprecipitation followed by high-throughput sequencing, *MS* mass spectrometry

Moreover, ethical and further legal issues are the other big challenges in the coming year, for example, the newly hot discussed topic about the patent of cancer gene mutations for diagnostics and therapeutics. Physicians and IRB members should be alarmed that sequencing data from patients need to be properly handled, and patients are supposed to be informed of it too when they sign ICF.

Another challenge follows a recent case involving premature use of omics-based tests in cancer clinical trials at Duke University; the National Cancer Institute requested

that the IOM establish a committee to recommend ways to strengthen omics-based test development and evaluation. It is urgent to develop a system to ensure that progress in omics test development and its application is grounded in solid scientific practice and is reproducible, resulting not only in improved health care but also in continued public trust.

DIFFERENTIAL SPATIOTEMPORAL SIGNALING NETWORK

Biological systems are highly dynamic, and the complexity of understanding and predicting the outcome of molecular networks still remain beyond the state-of-the-art. However, despite this complexity fascinating innovative research gives emphasis and priority on this field as both experimental high-throughput sequencing-based and array-based methods and evidence from clinical data, suggesting single pathways inhibitor limitations reveal the inevitability of measuring signaling transduction interactions networks and predicting cells circuits outcomes for discovering novel effective drugs and biomarkers.^{2,6,7,9,12}

Rapid advances in sequencing-based and array-based high-throughput technologies allowing substantial progress in systems computational biology, synthetic biology, mathematical networking models, and translational bioinformatics now shape a network of multidisciplinary coordination of researchers including biologists, chemists, physicists, bioinformaticians and clinicians that has evolved to provide a comprehensively differential mapping of physical and functional biologic circuits.¹³ Indeed, genome-wide mapping technologies, imaging techniques of the coordination of multiple interacting signaling activities in living cells, and 3D-genome resolution for mapping gene interactions at distant sites at chromosome level provide the basis of assembly for any class of physical interactions mapping. On the basis of these data, then computational and mathematical models would reveal the functional regulatory networks orchestrating chromatin remodeling and gene expression patterns. In the emerging clinical cancer genome, we begin to acquire new knowledge on genome structure and function and how causal mutations and epigenetic alterations deregulate the dynamics of biologic systems interactions network.^{6,7}

In the near future, progress in understanding how DNA-binding proteins at transcriptome level, noncoding RNAs including microRNAs, protein-protein interactions at interactome level, epigenome modifications, and their interactions, collectively will synthesize a highly dynamic molecular network. In cancer, a deregulation of this differential circuit in concert with structural genome alterations lead to a dysfunction of the expression patterns

of multiple functionally interacting genes.^{1,7–11} Targeting this network of physical connections (e.g., protein-protein interactions and DNA-binding proteins) and functional interactions (e.g., gene interactions) represents an emerging effort by academia and pharmaceutical industry for the next-generation of signal transduction drugs and biomarkers (Fig. 1b).^{2,7}

CONCLUSION

Despite the clinical success of trastuzumab and vemurafenib, modest or no efficacy due to resistance to all other signal transduction inhibitors for solid cancers treatment has been reported. Emerging biomedical research is focused either to develop robust biomarkers for better tailored treatment among the available drugs or to discover both novel biomarkers and targeted drugs for personalized cancer diagnostics and therapeutics. Powerful genome-wide mapping technologies and the new fields of systems-synthetic biology, translational bioinformatics, and computational mathematical strategies have rapidly evolved, shaping the new era of clinical cancer genome. The “sequence everything” concept including WGS, WES, as well RNA-seq and ChIP-seq techniques for transcriptome mapping of cancer cells from individual patient biopsies provide a clinical platform for precision cancer medicine. However, a plethora of challenges including omics data validation and interpretation should be overcome to achieve practical implementation in the clinic. In the future, deeper insights into regulatory molecular networks and understanding of how structural cancer variants affect genome function will provide the next generation of targeted drugs and biomarkers for precision cancer medicine and patient’s cure.

CONFLICT OF INTEREST The authors declare no conflict of interest.

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