Future Perspectives of Personalized Oncology

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ABSTRACT

Based upon an individual’s molecular make-up, personalized molecular medicine provides information regarding the origin of disease, its treatment and progression, while personalized molecular pharmacology advises on drug prescription and patient response to it, thus ensuring drug effectiveness and preventing drug toxicity or lack of response. Interindividual differences in drug responses are mostly due to structural variation in parts of genome, e.g. in genes participating in drug metabolism, transport or targeting. However, a wide variety of diseases and accompanying health conditions, including patient’s therapy or drug response, also have epigenetic or epigenomic etiology. High priority for personalized oncologic research stems from inter/intraindividual tumor heterogeneity provoked by gradual acquisition of multiple random, or programmed mutations and rearrangements as well as epigenetic alterations or by stochastic fluctuations in cell components, all in tight feedback interaction with tumor’s environmental or therapy conditions. Natural selection subsequently shapes inter/intraindividual tumor heterogeneity by promoting clonal expansion of cells that have acquired advantageous mutations for tumor population. Hence, the main rationale of personalized molecular oncology should focus on treating disease by relying on relevant structure and state of patient’s whole molecular network (genome/transcriptome/RNome/proteome/metabolome/metabonome) in interaction with its unique environmental conditions, thus implying right therapy for the right patient at the right dose and time. The future of personalized oncology should therefore rely on the methods of systems biology applied in cytology and pathology in order to develop and utilize the efficient and effective diagnostic, prognostic and predictive biomarkers, consequently providing the molecular information on tumor origin, its potential for metastasis, adequate therapy, tumor specific therapy responsiveness, and the probability of its recurrence.

Key words: personalized medicine, cancer, personalized molecular oncology, systems biology, biomarker

Introduction

Personalized medicine implies preventing, diagnosing and treating disease in each patient on the basis of an individual’s biology and respective disease pathology. Personalized medical treatment is concerned with therapy safety and efficacy as well as with optimized nutrition regimes and lifestyle management and it is tailored considering patient’s unique clinical data such as family history, demographic, and environmental factors, all relying on patient’s unique molecular network make-up. Hence, variability in interpopulation or interindividually disease susceptibility and progression as well as in individual’s therapy response may depend on respective genome/transcriptome/RNome/proteome/lipidome/metabolome/metabonome state and structure.

There are several types of structural variations in human genome: large-scale genomic variation ascribed to differences in copy numbers, covering about 20 percent of the length of the human genome; small-scale variation caused by a difference in one out of about 500 nucleotides between the genomes of two randomly selected individuals, or 6 million single nucleotide polymorphisms (SNPs); and variants in simple sequence repeats, in larger segmental duplications and in sequences containing transposable elements, all together accounting for about 60% of the length of the human genome. In addition, variations of human molecular networks might be attributed to the activity of epigenetic mechanisms such as DNA methylation; acetylation, phosphorylation, ubiquitylation, and sumoylation of histones; protein degradation; alternations in RNA transcription, RNA splicing, RNA stability and transposon and viral activ-
ity. Such a plethora of human molecular variations points to the significant plasticity due to molecular network instability induced also by environmental or dietary conditions and cell to cell and cell to matrix interactions, and fixed by evolutionary selection pressure.

In the area of drug response, personalized molecular medicine overlaps with personalized molecular pharmacology, the field studying how individual molecular networks or their parts affect drug response and how drug affects individual’s molecular networks. For example, personal pharmacogenomics studies the effects of an individual genome or its parts (e.g., specific mutations in genes encoding drug metabolizing, drug transporting, or drug targeting enzymes) on efficiency and safety of drugs. It also studies the side-effects of a drug on the respective genome and its derivatives. Hence, pharmacogenomic evaluation may improve patient’s lifestyle, save both life and unnecessary expenses and prevent adverse drug reactions by prescribing selected drugs and determining their precise dosing, while avoiding lack of drug response. Personalized medicine thus relies on translating the science of molecular pharmacology, limited by complexity of disease processes and drug therapies, into clinical practice.

Malignant neoplasms are the leading cause of death among men and women under 85 years of age in the United States. Oncogenesis is initiated and promoted or simply accompanied by the accumulation of numerous random or programmed somatic mutations or rearrangements and epigenetic alterations, which might be triggered by proficient or irregular intracellular molecular maintenance systems or left unrepaired due to their inefficiency. Both kinds of multiple and sequential alterations responsible for oncogenesis, i.e., those occurring in tumor oncogenes and those in tumor suppressor genes, may be provoked and acted upon by environmental conditions and interactions (e.g., carcinogenic agents and cell-cell or cell-matrix interactions) or may arise from and be driven by stochastic fluctuations in configuration, expression (e.g., gene and protein expression) and in other epigenomic states of intracellular molecular networks. In the process of carcinogenesis, there are two classes of molecular alterations, the «driver» class that confers growth advantages of the respective tumor cells (e.g., mutations occurring in «driver genes»), while the «passenger» class of alterations occurring in tumor cells, is considered neutral to the cancer cell fitness. Oncology research is focused on discerning between these two classes of alterations. However, since tumor cell population is a dynamic structure constantly under selection pressure, these two classes of alterations might be the subject of sequential, temporal and spacial changes resulting in tumor interindividual and intraindividual heterogeneity.

Standard tumor diagnostic procedures are currently based on a combination of morphological and immunocytochemical or immunohistological methods closely connected to clinical data. This strategy, in most cases, provides sound information on tumor tissue origin, tumor type, stage and grade as well as on the surgery success of tumor removal. However, personalized molecular oncology should be growingly applied in cancer diagnosis, prognosis and treatment due to disease complexity arising from interindividual and intraindividual tumor heterogeneity, both depending on intricate feedback relationships among tumor’s somatic alterations, individual molecular network maintenance systems, stochastic fluctuations in cellular components, tumor’s environmental conditions, and on degree and type of mutagenic and cancerogenic exposure and susceptibility to it, all acted upon and shaped by evolutionary selective pressure.

**Rationale of Personalized Molecular Oncology**

**Interindividual tumor heterogeneity**

Interestingly, although pancreatic cancers contain an average of 63 gene alterations (considering only point mutations, deletions and amplifications in protein-coding regions) only 12 cellular signaling pathways were shown to be genetically altered in 67–100% of 24 pancreatic cancers surveyed, hence those alterations appear to occur in a limited number of «driver pathways», i.e., the signaling pathways. However, the pathway components, i.e., specific genes containing the alterations, are largely different in each individual tumor. Breast and colorectal cancers contain respectively a median of 84 and 76 protein-coding genes altered by point nonsilent mutation, and a median of 24 and 9 protein-coding genes altered by copy number changes with the number of potential driver mutations per tumor averaging 15. Only 25 out of 40 putative cancer genes among the alterations discovered in the total of 11 colorectal and 11 breast cancers and chosen to be reanalyzed further in 96 patients with colorectal cancers, were found to be mutated in one or more of those patients, while majority of those 25 genes were mutated in 5% or fewer of the patients, meaning that a large number of mutated cancer driver genes occur very rarely. In addition, studying exons and splice junctions of 518 protein kinase genes (the most commonly found genes among known cancer genes) in 210 diverse human cancer types, the total of 921 different base substitutions was revealed, out of which 158 were expected to be drivers, placed in the total of 119 genes, and confined to only 66 cancer samples.

Hence, pathway components altered in any individual tumor (e.g., by gene mutations) vary widely among individuals, as well as the sites and types of their alterations (e.g., type of somatic mutation). Identical alterations may lead to different clinical manifestations in different individuals, contributing to tumor heterogeneity and resulting in variation in diagnosis, prognosis, potential treatment and treatment outcome of two seemingly identical tumors.

**Intraindividual tumor heterogeneity (clonal heterogeneity)**

As for intraindividual tumor heterogeneity, widely confirmed in cancer research, it contributes further to
the cancer complexity. Primary tumor tissue should be continuously scrutinized due to incidental acquisition of additional molecular alterations also provoked by stochastic intracellular fluctuations or by stressful tumor environmental conditions. Some tumor-acquired alterations might be transient in nature and provoked by stressful conditions imposed by drug therapy. For example, tumor cells from non-small cell lung cancer succeed to survive due to transient «drug-tolerant» state developed during drug infliction, while being devoid of drug for some time, tumor cell population reverts to «drug-sensitive» state. Such reversible «drug-tolerant» state has been developed by subpopulation of cells as a response to a lethal drug exposure. On the other hand, advantageous proliferation of cells containing heritable mutation(s) responsible for therapy resistance should be predicted by detecting the cell or subpopulation carrying respective mutation. It is therefore obvious that both heritable and transient therapy-resistance states should be the subject of diagnostic personalized molecular oncology.

In addition to primary tumors, both disseminated and metastatic tumor cells may also possess the clonal heterogeneity character either in comparison to primary tumor or between themselves. Individual cells from breast cancer patient showed different abilities to metastasize specific organs of immunodeficient mice depending on molecular signatures of respective cancer cell. Potentially metastatic cells primarily infiltrate different organs (via systemic circulation), but not necessarily all of them colonize respective organs, which depends on the set of acquired molecular alterations (mainly in genes encoding proteins that influence the interaction of tumor cells with the invaded microenvironment) as well as on the microenvironmental conditions. The difference between tumor suppressor and metastasis suppressor genes was documented with special emphasis on metastatic colonization suppressors.

Premalignant cells may disseminate during the early stages of tumor progression and subsequently may acquire alterations in the invaded tissue, resulting in even greater tumor clonal heterogeneity. Evidences of parallel clonal evolution of primary and metastatic tumor population stem from the existence of metastatic tumors with unknown primary origin, which constitute a significant fraction of clinical cases.

Hence, intraindividual tumor heterogeneity detection requires personalized molecular oncology to be applied not only to tumor of an individual but also to its specific subpopulations in specific microenvironments and at specific tumor stages. Again, this places special emphasis on sensitive diagnostic and prognostic methods provided by molecular oncology.

**Cancer Treatment and Prognosis**

Based on detection of tumor inter/intraindividual heterogeneity and on deduced respective diagnosis, personalized oncology should deal with cancer prognosis and adequate individually tailored cancer therapy. The research of personalized molecular oncology should therefore be performed on the following topics: individual cancer risk assessment and susceptibility based on individual germline molecular information; type and extent of individual exposure to mutagens, carcinogens or other environmental agents; impact of respective exposure on individual molecular network state and structure; individual susceptibility to primary and metastatic tumors upon exposure to environmental agents; and individual repair potential of cellular maintenance systems deduced from their state and structure. The above information could be obtained from the analysis of individual molecular network signatures, which should be compared to well documented and established database of different types of molecular network signatures either from cells belonging to the carcinogen-exposed persons or from tumor cells belonging to individuals that developed different classes of cancer type and subtype. Both molecular data (on carcinogen-exposed individual and on cancer patient) should be accompanied by detailed clinical information on respective individuals. Hence, molecular network signatures (e.g. spectra of somatic mutations) can yield insights into potential carcinogens and other environmental exposures as well as on the potential of the host cell maintenance (repair) systems.

Therapy responsiveness relies on molecular make-up, microenvironmental conditions of the patient’s tumor and other cells interacting with tumor, all three components being unique for each individual. Hence, different therapies may need to be tailored for the primary tumor, disseminating seed and the evolving metastatic subpopulation of the same individual. It is important to determine not only the type of subpopulation alterations but also the frequency of cells in respective heterogeneous subpopulation, if one wants the personal cancer therapy to be efficiently tailored (e.g. in relation to the extent of drug sensitivity and patient’s response to it). In this process tumor development and putative therapy response should be predicted on the basis of early tumor analysis.

Drug responsiveness depends on subpopulation’s or individual’s molecular germline polymorphisms. An example of variation in drug targets directly influencing drug response is represented by thymidylate synthase (TYMS) over expressed gene variant that is responsible for poorer survival of respective patient carriers (that have developed metastasis) due to increased resistance to 5-fluorouracil, inhibitor of TYMS. On the other hand, an example of variation in drug metabolizing enzyme indirectly influencing drug response is represented by the existence of over 40 different polymorphic types of dihydropteridine dehydrogenase (DPYD) that metabolize 5-fluorouracil (implicated in treatment of colon cancer) into its inactive form. Hence, decreased DPYD activity can lead to the accumulation of 5-fluorouracil resulting in severe toxicities. It should be noted that pharmaceutical drugs including chemotherapy induce persistent epigenetic changes, resulting in adverse drug side-effects that might de-
pend on patient’s susceptibility and hence, should also be the subject of personalized oncology. In addition, side-effects might impact on parts of genome implicated in tumorigenesis (e.g. hypermethylation inflicted by tumor therapy may contribute in silencing of tumor suppressors genes), thus requiring continuous personalized cancer therapy surveillance.

It has been shown that three of the common chemotherapeutic agents applied to culture cells are more toxic to healthy progenitor brain cells than to the cancer cell lines they intended to treat\textsuperscript{26}. Side effects of chemotherapeutics also include increased incidence of cancers secondary to those being treated\textsuperscript{27}. Hence, cancer therapy should aim for targeted treatment based on personal oncology that treats the cancer tissue by evading detrimental effects on healthy tissue\textsuperscript{28}, all depending on individual molecular network signatures of both tumor and healthy tissue.

As for different potential of cancer recurrence, the rare intracellular alterations that confer a competitive advantage may take a long time to achieve clonal dominance. This process depends on the colonization potential in the respective tumor invaded sites, also requiring personal molecular oncology approach.

**Molecular Systems Biology in Oncology**

It is now an accepted fact that origin and progression of complex diseases, such as cancer, are caused by combined actions of individual’s multiple genes or proteins or metabolic pathways interacting with environmental factors and an individual’s lifestyle. Hence, the future of personalized molecular medicine including personalized cancer prediction, treatment and prognosis undoubtedly lies in systems biology. This biology is based on the analysis of integrated action of regulatory networks at many levels of biological organization, from subcellular level through cell, tissue, and organ, up to the whole organism\textsuperscript{29}. Different tools of molecular medicine range from evaluation of interindividual differences in single genes (i.e. gene sequencing, genotyping and detection of gene methylation) to the whole-genome sequencing and application of microarrays, i.e. simple collections of individual probes, that might determine DNA structure and its copy number; protein structure and its interaction with other macromolecules, as well as the expression of entire sets of genes, small non-coding RNAs, proteins, lipids and metabolic pathways\textsuperscript{30}. Molecular systems biology as applied in oncology thus implies different -OMIC methods such as genomics, transcriptomics, proteomics, or metabolomics/nomics serving in tumor diagnosis and classification as well as in malignant disease prognosis and treatment. For example, functional genomics goes beyond identification of variants and regions associated with tumor phenotypes; it involves the analysis of the whole of genome network\textsuperscript{31}. For the time being sequencing is the primary tool for cancer genome exploration, since in order to design and construct microarrays for genomic research one should sequence and target alteration (e.g. mutation) repertoire participating in tumor origin and development. For example, in comparative genomic hybridization method, DNA from tumor and respective normal cells is differentially labeled with fluorescent probes and hybridized to genomic oligonucleotide microarrays, e.g. to SNP (single nucleotide polymorphism) array that may provide the information on genome variations with resolution difference up to the single nucleotide; on the copy number variations and on loss of heterozygosity\textsuperscript{32}. In order to synthesize SNP array one should know the whole repertoire of variants appearing in respective cancer that is under analysis. Such information could be provided by deep sequencing of the many different cases of the respective cancer.

High-throughput sequencing may focus on exome capturing by array hybridization and further sequencing on a parallel sequencing platform\textsuperscript{33,34}. The whole exome sequencing aims to detect cancer specific mutations in genes belonging to pathway candidates for therapeutic activity. However, out of 22,910 point mutations discovered by sequencing of lung cancer cell line that has been exposed to tobacco smoking, only 134 were in protein-coding regions\textsuperscript{35} which implies necessity to supplement the mutation spectra of exon sequences by the rest of the genome. The first comprehensive catalogue of somatic mutations from an individual human cancer genome is completed on the basis of sequencing the genomes of a malignant melanoma and a lymphoblastoid cell line from the same person (COLO-829)\textsuperscript{36}. However, the comparison should be extended to germ line molecular make-up in order to distinguish somatic mutation potentially involved in tumorigenesis and carcinogenesis. Furthermore, considering lowering of the whole-genome sequencing expenses, the sequencing of all tumor stages should begin to be implemented. By using massively parallel sequencing technology and applying it to small-cell lung cancer cell line, molecular signatures of tobacco exposure were revealed (pointing on the specific carcinogens present in cigarette smoke) together with molecular signatures of DNA repair systems\textsuperscript{37} of the respective small-cell lung cancer cell line. Importantly, the ability of sequencing mixtures of cells with high sensitivity by catching the information from the single cell among tumor cell population, offers a possibility of increasing our knowledge of malignant lesions in situ, in that way obviating the need for tissue microdissection.

Another whole genome study i.e. flow cytometry analysis (FACS) may reveal ploidy status of the cell and may single out cells instead of microdissection method. In gene expression profiling by using cDNA arrays for analysis of transcription of thousands of genes or the entire transcriptome, information is provided on amplification or reduction of expression of genes of interest. One of the first classifications of human cancer on the basis of gene expression profiles was successfully performed for diffuse large B-cell lymphoma\textsuperscript{38}. For example, breast cancer may be profiled into four distinct molecular subtypes by applying gene expression arrays\textsuperscript{39} that are even commer-
cially available as prognostic markers. Another more informative and sensitive method of quantifying and, in addition, mapping transcriptomes (i.e. discovering new exons or genes) is performed by direct ultra-high-throughput sequencing of cDNA, referred to as RNA-Seq. On the other hand, in exploration of detection of DNA methylation (i.e. widespread epigenetic modification), a novel method based on polymerase-incorporated fluorescently labeled nucleotides promises to lower the time and expenses of bisulphite conversion-based sequencing.

Next step in personalized oncology research should integrate information obtained from the full cataloguing of somatic mutation with expression and epigenetic profiles of the same cancer cases and should be correlated with clinical features. In that way, classification of tumor and early prediction of drug action in tissue would be enabled by performing microarray analysis.

However, DNA and gene expression arrays may provide only limited data on molecular network state and structure due to epigenetic alterations including post-translational protein modifications. Cellular internal and environmental factors as well as communication and interaction among cells can alter the activity level or function of a protein. Protein-protein interactions, post-translational modifications and interaction between protein and DNA, RNA or other macromolecules can all shift the activity of a protein from what would have been predicted by its level of transcription. Proteomics tools are based on high-performance combinations of chromatography and mass spectrometry. The study of proteomics can generally be divided into two categories: characterization of protein expression and characterization of protein function. Expression proteomics evaluates the cellular production of proteins and exploits the differential expression and post-translational modifications of proteins between healthy and diseased states. Functional proteomics, on the other hand, studies the interaction of proteins to determine how the given protein accomplishes its specific cellular task. By detecting the function of aberrant or over expressed proteins one can target cancer therapy. In addition, functional proteomics explores the response of proteins to molecular targeted therapy in order to determine the efficiency of the targeted therapy. Mapping of protein-protein interactions can be useful for detecting new proteins that are involved in cancer as well as novel oncogens, or can provide evidence of common downstream events shared by two distinct signaling networks aiming to develop therapeutics directed against these pathway targets.

Proteomic profiling with protein microarrays has been applied in order to determine levels and activation states of key signaling proteins with the aim of reclassifying human tumors. Protein microarray is a series of immobilized spots, each containing a bait molecule such as an antibody, a nucleic acid, a drug, or a recombinant protein or peptide, to which the patient’s tissue or fluid is applied. If proteins from the sample bind to the bait molecule, they may be detected using antibody probes. Reverse-phase protein microarrays, in which the patient’s sample is fixed to the slide, and the probe is applied to the microarray, have demonstrated improved reproducibility and analytical sensitivity vs. conventional protein and DNA microarrays. Recent advances in proteomics research of cancer are described in review.

Metabolomics analysis provides quantitative description of all low-molecular-weight components of endogenous metabolites in a specified biological sample, characterized by species-specific environmental and physiological conditions. In addition to using chromatography and mass spectrometry, metabolomics is also taking advantage of nuclear magnetic resonance to analyze complex sets of metabolites in body fluids including urine, plasma and tissues in order to reflect biochemical profiles of the whole organism (host genetic factors) and regulation of functions that reflect normal and disease states, as well as to study interactions with the gut microbial flora and environmental factors. By profiling the metabolomic alterations of prostate cancer progression sarcosine was suggested as a potential biomarker while the members of sarcosine metabolic pathway as a potential therapeutic targets. Science of metabonomics, on the other hand, deals with understanding metabolic changes of a complete system that are caused by interactions, e.g. gene-environment interactions in their broadest sense or extended genome and parasitic interactions. Availability of increasingly powerful high-throughput technologies, computational tools and integrated knowledge bases, has provided a possibility of establishing new links among genes/proteins, their biological functions and a wide range of human diseases, thus providing molecular description of tumor pathophysiology.

The resected and fine needle-aspirated tissue is the major determinant of all downstream therapy in molecular medicine. On the contrary, culturing the cells may lead to preferential outgrowth of selected tumor cell subpopulations, thus changing the representation of the original tumor. However, iatrogenic variables such as surgical manipulation, intraoperative drug delivery, and pathological handling of molecular profile providers that reflect the biology of the resected tissue, are important factors in preservation of tissue integrity and quality of molecular networks. Postoperative tissue ischemia time, for example, has been shown to alter gene and protein expression profiles within minutes following the surgical excision in colectomy specimens and prostatectomy specimens. Until they are fixed or frozen, biospecimens are viable and capable of reacting to physiological factors such as changes in temperature, perfusion, oxygenation, and other physiological and biochemical variables, both pre- and intraoperatively, as well as postoperatively.

**Conclusion**

Oncology relies on determining molecular biomarkers that are involved in disease depiction, prognosis and treatment. Biomarkers can be grouped into three major classes: diagnostic, prognostic and predictive ones. Prog-
nostic and predictive markers, however, may inform on and predict the course of a disease or malignancy respectively and both should be involved in decision of the treatment choice. The future of personalized oncology belongs to personalized useful persons, i.e., the first diagnostic biomarker set (consisting of several single biomarkers specific for detecting colorectal and breast cancer-specific translocations) obtained by whole-genome cancer research using massively parallel sequencing, identifies residual and recurrent solid tumors with the sensitivity of detection of mutated DNA present in blood at levels lower than 0.001%.46

One can expect a growing trend in launching new drugs with diagnostic markers in order to improve the treatment outcome of individual patients. This could therefore shift the focus of healthcare industry from therapeutic to diagnostics. Unfortunately, at the moment, pharmaceutical companies are generally more inclined to apply whole-genome research in development of new drugs with pharmacodynamic tests that might limit the use of their drugs in the clinic. However, it is obvious that the advantages of personalized molecular oncology are so numerous that the future of cancer research definitely belongs to it. Being the most reliable cancer-diagnostic disciplines, cytology and pathology should participate in this challenge of deciding about the best therapy for every individual.

References


BUĐUĆNOST INDIVIDUALNE ONKOLOGIJE

S AŽETAK

Individualna molekularna medicina pruža dijagnostičke informacije o nastanku i napredovanju bolesti te o njezinom odgovoru na lijekove. Individualna molekularna onkologija pruža dijagnostičke informacije o potencijalnim lijekovima i pacijentova odgovoru na njih te na osnovi proučavanja individualnog molekularnog sadržaja i njegove strukture. Individualna molekularna onkologija pruža informacije o potencijalnim lijekovima i pacijentova odgovoru na njih te na osnovi proučavanja individualnog molekularnog sadržaja i njegove strukture. Individualna molekularna onkologija pruža informacije o potencijalnim lijekovima i pacijentova odgovoru na njih te na osnovi proučavanja individualnog molekularnog sadržaja i njegove strukture.

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