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INDIVIDUALIZATION OF CANCER TREATMENT: CONTRIBUTION OF OMICS TECHNOLOGIES TO CANCER DIAGNOSTIC

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Cancer is a disease, which explicitly illustrates success, failures and challenges of the modern biomedical research. Technology development has been the driving force of improvements in the cancer treatment. Introduction into clinical practice of genomics, RNA profiling and proteomics technologies have provided a basis for development of novel diagnostic, drugs and treatments. In this chapter, contributions of OMICs technologies to personalization of cancer diagnostic and treatment are discussed. The focus is on technologies that showed capacity to deliver diagnostic that may be used in the clinic as routine tests. Three clinical cases are presented to illustrate already available individualized cancer diagnostic.

Key words: personalized cancer medicine, genomics, transcriptomics, proteomics, metabolomics, diagnostic.

Why OMICS technologies are needed for treatment of cancer?

Cancer still kills people. It was easier to send a man in the outer space or to the Moon, than to improve survival of patients having advanced and metastatic cancers. This highlights complexity of cancer as a disease, which is apparently much higher than to build and launch a space rocket. Carcinogenic transformation of cells is accepted as the main cause of cancer [1–4]. Carcinogenic transformation is defined as a number of changes in the cell physiology, which lead to expansion of malignant cells in the body, corrupting the normal physiology, and ultimately killing the person.

The key conclusion of more than 50 years of intense studies is that the collected knowledge has not reached the critical mass required to find cure against cancer. The oncogenes and tumor suppressor model has been a great step forward [1–4], but today is clear that carcinogenic transformation of cells is the result of interaction of hundreds molecules. Out of the hundreds of these cancer-promoting genes, RNAs, proteins and metabolites many are the same as they are in the normal cells. It is their corrupted activity, mis-localization, and misplaced interactions that make them tumor-promoting (Fig. 1). This confusion has only underlined complexity of cancer.

A solution to the complexity problem has been proposed by introduction of technologies for comprehensive study of carcinogenesis. These technologies focused on studies of genomic DNA (genomics), RNAs (transcrip-

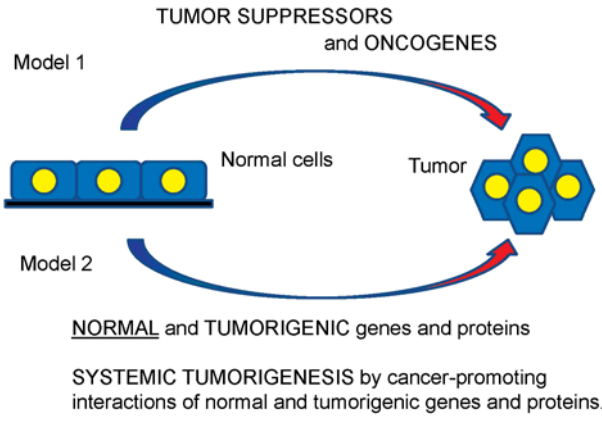

Fig. 1. **Two models of tumorigenesis**
The first model explains tumorigenesis by involvement of tumor suppressors and oncogenes (upper part). The second model explains tumorigenesis as a systemic effect of normal and tumorigenic genes and proteins (lower part). The difference is that the systemic tumorigenesis model postulates that even normal genes and proteins may contribute to tumorigenesis, if their interaction networks shift from the normal physiological reactions to the disease promotion.

tomics), proteins (proteomics) and metabolites (metabolomics) [5–7]. Historically, introduction of nucleotide microarrays to study expression of RNA was the first strong contribution to the comprehensive exploration of carcinogenesis [8]. Development of the microarrays was possible due to successes of technologies for synthesis of oligonucleotides and production of cDNA on a large scale and in automated way. A chip-printing technology was another component of the success. Development of sequencing technologies, especially of massive parallel sequencing, has given boost to comprehensive studies of genome for the clinical diagnostic [9]. Comprehensive studies of the proteome are still waiting for a wide use of intact protein analysis technology. Current technologies of mass spectrometry, 2D gel and other electrophoresis, or liquid chromatography are not providing quality that is required for full description of the human proteome [10, 11]. However, the situation may change with introduction of ZP-technology [12]. The least developed of the OMICs technologies is metabolomics. The high variability of physico-chemical and structural properties of the metabolites makes it challenging to detect and identify all metabolites by a single technology. Despite all shortcomings, OMICs studies have become essential for success in treatment of cancer, due to their ability to a comprehensive analysis. Therefore, there is no alternative to development of fast, reliable, informative and cost-efficient OMICs technologies for diagnostic and treatment of cancer.

Genomics

Since the discoveries that the genomic DNA carries hereditary information, and is the white-print of the most of the living creatures, study of genes, or genomics, has been a subject of intense developments. This excitement was translated in a slogan that «cancer is the disease of genes». The slogan's correctness is questionable today, as the non-genomic mechanisms may have a strong impact on tumorigenesis. How many genes do humans have? What is the structure of these genes, as introns and exons? What type and how many mutations are in the genome of a given patient? What are epigenetic changes in the genes? All these questions have importance for understanding of carcinogenesis, and subsequently for treatment of cancer.

In this section are discussed technologies for studies of genome, which may have a value for clinical applications (Fig. 2). These technologies have been developed to the extent that they may be applied in the clinic for diagnostic, selection of treatment and monitoring of response of a patient.

Massive parallel sequencing (MPS/NGS)

The excellent research on biochemistry of DNA paved the way to development of DNA sequencing techniques. The first generation

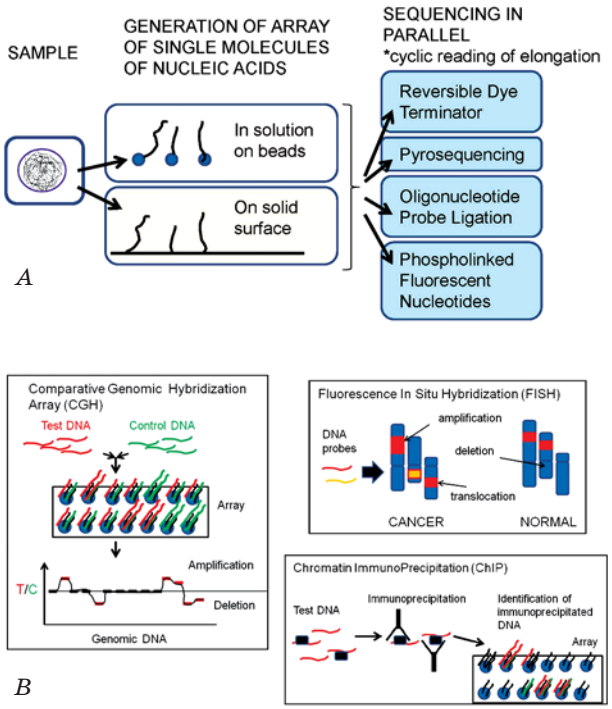

Fig. 2. **Presentation of genomics and transcriptomics technologies that may be used in clinical diagnostic:**

A — Massive parallel sequencing technologies. Two main parts of MPS are indicated. The first is preparation of nucleic acids for sequencing reactions. This is done by preparing single molecule suspensions, or by anchoring nucleic acids to a solid surface. The second part is enzymatic reactions to read the sequence. The reactions include polymerase or ligase reactions, and the sequence reading is by detecting incorporation of defined nucleotides which are added to the reaction in cycles.

B — Schematic presentation of CGH array, FISH and ChIP technologies. Amplifications or deletions of genomic DNA are detected by CGH arrays as increased or decreased relative signal of the test DNA in comparison to the control normal DNA. Binding of specific DNA probes to the chromosomal regions in FISH assays indicate regions of amplifications, deletions and translocations. For ChIP, immunoprecipitation of specific regions of DNA allows detection and profiling of only these regions, for example detection of transcriptionally active regions, or DNA regions interacting with specific proteins, or epigenetically silenced regions.

DNA sequencing methods are Maxam-Gilbert fragmentation and Sanger's dideoxy base-termination techniques [13, 14]. However, these techniques in their original forms were too cumbersome for being used in the clinical practice. The step toward clinic was by introduction of automated sequencers [15]. Automation allowed to detect gene mutations of the clinical importance. However, it was still far from a comprehensive analysis of the whole genome of a patient in the routine clinical practice. Faster and affordable methods were needed, and they come with development of massive parallel sequencing (MPS), known also as the next generation sequencing (NGS).

MPS is based on parallel sequencing of short fragments of DNA, which are then aligned to produce gene sequences (Fig. 2, *A*). The size of sequenced fragments is from 30 to 700 bases, depending on the sequencing method and instrumentation [16, 17]. This relatively short length of the sequenced fragments imposes limitations on the quality of definition of the complete genes sequence.

MPS has been used successfully for analysis of mutations in genes, with the emphasis on the exon analysis. Focus on exons allows generation of data which could be used in clinical diagnostics within relatively short assay time. As an example, the full exon sequencing and detection of the mutation profile of a tumor cells may be completed within 30 days [18, 19]. The second example is the contribution of MPS to profiling of mutations in different sub-types of cancer, providing insights into molecular heterogeneity of tumors [19]. Understanding this heterogeneity is essential for development of personalized treatment of patients. The expectation is that MPS will become a standard and routine examination of cancer patients. The nearest years will show whether this expectation will indeed be realized in better treatment of patients.

CGH, PCR, FISH and ChIP tests

Pre-MPS era had given rise to a number of methods to assess structure and mutations of the genes (Fig. 2, *B*). Comparative Genomic Hybridization Array (CGH) was used to detect gene aberrations on the whole genome level [20]. However, the resolution power of CGH arrays has been in the range of 5 kB to 0.2 kB, and variations in the gene structure have been the core information delivered by CGH [20].

Fluorescence in situ hybridization (FISH) is used to detect rearrangements of selected genes, e.g. deletions, amplifications and translocations [21, 22]. Clinical application of FISH is limited by its low number of monitored DNA fragments, and relatively large work-load for performing the test. Multiplexing FISH by using different probes with different detection wavelength, and use of nano-devices to minimize and automate the test are 2 developments which make FISH still useful in the clinical diagnostic [21, 22].

Polymerase-chain reaction (PCR)-based analysis of the genomic DNA is used less and less in the clinical diagnostic. The niche for PCR has become analysis of pre-selected mutations [23]. However, PCR is more used for analysis of RNAs than genomic DNA. Chromatin Immuno-Precipitation (ChIP) has proven the high informative value in studies of chromatin re-arrangements and methylation of the genomic DNA [24]. Therefore, the unique information which may be delivered by ChIP tests is the profile of epigenetic changes in the genome. On the other hand, complexity of the ChIP tests limits its clinical applications.

Complexity and low automation level of the CGH array, FISH, PCR, and ChIP tests are major hinders for their use in routine clinical diagnostic (Fig. 2, *B*). Cost efficiency of these assays is also lower, as compared to tests with the recent developments of MPS. Therefore, each of the genome profiling technologies will have their niches. MPS will with high probability dominate the whole genome profiling, while CGH, FISH, PCR and ChIP tests will focus on selected genes and genome areas.

Transcriptomics

Historically, mRNA profiling by expression arrays has been the first true OMICs technology. The ground of this technology was laid by excellent works on the biochemistry of oligonucleotides and generation of cDNA. PCR-based analysis of mRNA expression was competing with the RNA expression arrays, but PCR was inferior due to the higher complexity and lower robustness. mRNA profiling has also been proposed for the clinical diagnostic. However, arrival of the massive parallel sequencing technologies has given the real boost to RNA profiling by providing flexibility, speed and additional information about mRNA, microRNAs and long non-coding RNA, e.g. expression and mutations [25].

Massive Parallel Sequencing of RNAs

MPS technologies used for profiling of RNAs are similar to those used for profiling genomic DNA, but the focus is on mRNA,

siRNA/miRs, and lncRNA. The difference is only in preparation of samples for analysis [26–29]. RNAs are more sensitive to degradation, located in nuclei and cytoplasm, and have different sizes, as compared to the genomic DNA. These features make challenging MPS of RNAs, as variability in quality of the samples would be reflected in discrepancies of produced data. Despite the challenges, information delivered by MPS of RNAs allows better insight into molecular activities in the tumors. A number of examples confirmed value of RNA MPS for making clinical decisions [30, 31]. For example, MPS sequencing of RNAs in tamoxifen-resistant breast cancer cells identified 1728 RNAs associated with the resistance. This number of the affected RNAs indicates that the acquisition of the tamoxifen resistance is a complex process, with involvement of many activities. On the other side, this study opens for better monitoring of the resistance, and the most important, it provides the basis for selection of more efficient treatment by combined blocking of the key RNA-related regulators of the resistance [31].

Expression arrays

RNA expression arrays are undergoing evaluation of their use in the clinical diagnostic. Only 5 years ago, RNA expression arrays were at the leading edge of entering clinical diagnostic. The limitation at that time was not in the technology itself, but in applicability of the generated information for diagnostic and making decision about treatment. While measuring expression of RNA provided large volumes of information for research purpose, this information was difficult to translate into diagnostic and prognostic values. The reasons were discrepancies between mRNA expression and expression and activity of the corresponding proteins. Another critical limitation was not sufficient robustness of the arrays. As an example, RNA microarrays from different suppliers could produce different detection values for the same RNAs [32].

The niche for RNA expression arrays is changing from the all-gene coverage approach to measuring a set of RNAs of importance for specific type of cancer or a set of cancer drugs. Such arrays are combined now with dedicated systems biology tools to extract disease-relevant information. For example, the arrays have been used to identify long non-coding RNAs associated with breast cancer [33]. MicroRNA arrays are another novel niche-approach that may be the way to discover cancer-associated microRNAs [34].

PCR-based analysis

PCR-based RNA analysis is in the situation similar to RNA expression arrays. Notably, PCR-bases analysis is not anymore considered for a comprehensive full-transcriptome screening of RNA expression. PCR-based analysis is currently used for measuring defined sets of up to 100 different RNAs, predominantly mRNAs. For example, focused analysis of expression of the key genes involved in acute myeloid leukemia unveiled 19 up-regulated and 25 down-regulated genes [35]. An important advantage of the PCR-based assays is their technical simplicity. Such assays may be used even in a small size laboratory, and for the low cost. Introduction of companion diagnostic into clinical practice also contributes to the niche-development of PCR-based tests. As examples, PCR-based tests of mutations in BRAF, EGFR, BCR-ABL, PDGFRs and MEK1 genes are proposed to the clinic as companion diagnostic of drugs acting on these kinases [36, 37].

Thus, MPS technologies have begun to dominate a comprehensive RNAs profiling, while RNA expression arrays and PCR-bases assays are specializing in measurements of pre-selected sets of RNAs. It has to be notes that the recent developments of systemic analysis tools have strongly contributed to extraction of information useful for clinical diagnostic, prognostic and selection of treatment.

Proteomics

All known anti-cancer drugs act directly on proteins or require proteins for their activity [38]. This makes proteomics essential for diagnostic and selection of treatment. Early approaches of monitoring expression of the drug targets have shown their positive contribution to cancer diagnostic and treatment. Such approaches are currently a standard of routine diagnostic, and are applied overwhelmingly by using immunohistochemistry (IHC). Measurements of the expression of Her2, EGFR, ER, PgR in tumors by IHC are standards when an oncologist has to decide about use of Herceptin, Iressa, or antihormonal therapy [39]. The drawback of such approaches is in the limited number of measurements, as a single assay measures only one protein. This is why a comprehensive profiling of the whole proteome has been expected to improve diagnostic. As the minimum, the clinical oncologists need to evaluate a drug target itself, and all proteins which may affect drug

efficacy [40]. This requires to measure in a single assay up to thousands of proteins. This is why proteomics experiences strong pressure to deliver good diagnostic tools.

Proteomics technologies may be classified on the basis of what they study, e.g. proteins, peptides or epitopes, how they separate the analytes, and what they detect to identify proteins. Studying proteins or their products peptides, or protein epitopes, are the main cut-off points for deliverables of the proteomics technologies (Fig. 3). Peptidomics approaches by the Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) have recently been popular [41, 42]. However, LC-MS/MS is not capable to detect and identify the intact proteins and their isoforms. Therefore, there were too low correspondence of claimed identification of proteins, and the real impact of these results on the clinical diagnostic [42]. 2D gel electrophoresis (2D-GE) has been the technology to study intact proteins [43–45]. However, 2D-GE has limited protein separation capacity. Novel intact-protein proteomics techniques have been under development [12, 43]. The protein arrays are considered of being closest to enter routine clinical diagnostic. Proteomics has also other technologies for separation and identification of proteins, which are too laborious to be used in clinic. Therefore, in this section I focus on proteomics technologies which may have an impact on the clinical diagnostic, intact-protein proteomics, peptidomics and protein arrays (Fig. 3).

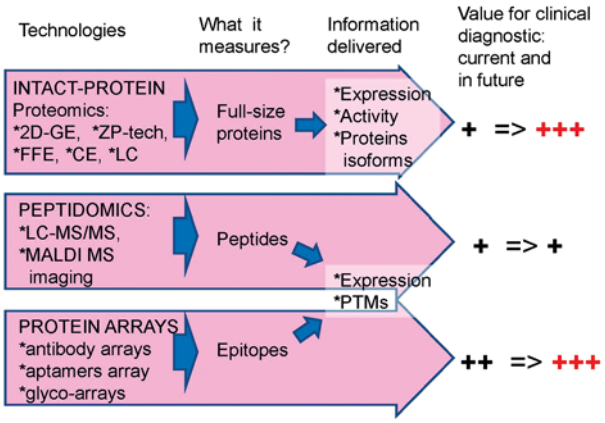

Fig. 3. **Proteomics technologies that may be used in clinical diagnostic**

Proteomics technologies to study intact proteins, peptides and protein epitopes are indicated. What these technologies measure, and type of delivered information are mentioned. Pluses indicate current and expected in the nearest years levels of application of the technologies in the routine clinical diagnostic.

Intact-protein proteomics

The key feature of the intact-protein proteomics is that the studied analytes are proteins. Therefore intact-protein proteomics is the most informative profiling of the proteome among all proteomics technologies. Despite such importance, technologies of the intact-protein proteomics have not had capacities to describe the whole proteome. Variability of physico-chemical properties of proteins has been the main barrier.

2D-GE has been the most common technique of the intact-protein proteomics. Classical 2D gels may separate up to 5,000 proteins in one gel. pH zoom-in gels were reported to separate between 7,000 and 10,000 proteins [43–46]. Recently developed ZP-technology may be the breakthrough of the intact-protein proteomics, with its capacity to separate more than 20,000 proteins in a single run [12]. Separated proteins have to be identified, and today the best identification technique is mass spectrometry. MALDI TOF mass spectrometry has been successfully used in combination with 2D gels.

2D-GE and ZP-technology have been used for cancer diagnostic in the clinic (see examples in the section #6 below). However, these techniques are rather labor intensive, and are not enough well automated for routine use in a clinical diagnostic laboratory. The use of these techniques has been in the translational cancer research, and in managing a limited number of patients. To be suitable for use in the routine clinical diagnostic at a large scale, an instrument with fully automated manipulations would be required. For the moment, all attempts to create such an instrument have not been successful.

Other techniques employed in the intact-protein proteomics are free-flow electrophoresis, capillary electrophoresis and liquid chromatography [46–49]. However, none of these techniques is capable to detect all proteins of the human proteome, and provide sufficient separation of even those proteins that were detected. Significant efforts have been invested in development of protein mass spectrometry. However, mass spectrometry is unlikely to succeed in analysis of full-length proteins due to limitations of the ion optics and physical properties of sub-atomic particles. Therefore, significant developments have to be made to prove that these techniques have a future in the clinical diagnostics.

The intact-protein proteomics has another advantage over other proteomics technologies, as it allows unbiased analysis of protein activities.

For example, it allows detection of all kinases which may be inhibited by a tested kinase-inhibiting drug [50]. The comprehensiveness of the tests means that it would be analyzed not only intended target of the drug, but also all kinases in the tested tumor. This provides information about efficacy of the drug, and about potential off-target and side-effects. Among other activities which can be tested by the intact-protein proteomics, there are acetylation, ubiquitylation, PARylation, proteolysis, and glycosylation [51]. Taking into account introduction in the clinical practice drugs affecting these post-translational modifications, the diagnostic value of the intact-protein proteomics is going to increase.

Peptide-based proteomics

Peptide-based proteomics is strongly associated with use of mass spectrometry [41, 42]. The focus of mass spectrometry on peptides is because of 2 limitations. The first limitation is due to biochemistry of proteins, and the second is due to the physics of ions and sub-atomic particles. The biological limitation is because of the enormous complexity of the mass spectrum of an intact protein. Many combinations of isotopes distribution in an intact protein, and multiple charge ionizations of the protein make it challenging to obtain a well defined protein spectrum of the high resolution. On top of that, if there is a mixture of proteins with the molecular masses in the same range, separation of these proteins by mass spectrometry would be very difficult to achieve. The physical limitation is imposed by changing mass values of proteins in different conformations and as polymers. Atomic interactions change the total energy of the protein as compared to the sum of masses of the amino acids. The energy change is relatively low for peptides, and does not have a significant effect on the mass value of the peptide. However, for the molecules of mass higher than 20,000 daltons, the change of energy may be in the range of 900 MeV. This may be translated in the mass change corresponding to more than 0.7 dalton, as 1 atomic mass unit is equal to 931,49 MeV. Such uncertainty of the mass of large molecules makes irrelevant applications of the high resolution mass spectrometry. What is the reason to rely on the resolution of less than 0.001 dalton, if the uncertainty of masses due to the large size of the intact proteins is of more than 0.7 dalton?

However, mass spectrometry has a potential to be used in the clinical diagnostic. The niche of mass spectrometry-based diagnostic is in measurements of a limited set of peptides, which then would serve as biomarkers. The types of peptides to be measured define type of mass spectrometry technology. For example, MALDI TOF mass spectrometry is used to detect peptides ionized from a tumor section [52]. While detection of the peptides flying in the instrument is rather robust, the challenge is to control ionization of the peptides. Variations in ionization are due to differences in composition of the tissue in its different areas, thickness of the section, and slightest differences in application of the matrix. Variable ionization efficiency generates false differences in distribution of the measured peptides in the tissue. Proposed solutions with using internal controls may help to overcome the ionization problem, but it will complicate the technology. Such a complication would have a negative impact on the clinical applications of MALDI MS imaging. Other issues of MALDI MS imaging are technical problems of the size of the laser beam, speed of the spectra acquisition, and processing of the collected data [52]. All together, it makes that MALDI MS imaging is not yet ready to enter routine clinical diagnostic.

However, when peptides are the biomarkers, mass spectrometry may be the method of choice. As an example, detection of peptides in brain may have a diagnostic value [53]. With controlled sample preparation methods and internal MS standards, mass spectrometers may become standard equipment in the clinical laboratory. In 2013, the cost of a good quality mass spectrometer is affordable for specialized diagnostic laboratories. Another important initiative is generation of the Peptide Atlas, which provides peptides that may be used as internal standards [54]. When such peptides added to the samples, it provides a reference of the quality of analyte detection, and for the quantification of the marker peptide in the sample. Thus, peptide-based proteomics is dominated by mass spectrometry, and may have use in diagnostic based on detection of peptides as markers.

Protein arrays

Among all proteomics technologies, protein arrays are the most advanced on the way into routine clinical use. Protein arrays measuring expression of a defined set of proteins are the most probable candidates for the routine diagnostic [55–57]. The protein array technology is well developed, and it has been extensively tested. To generate a protein array, it is required to select name of proteins

and epitopes to be detected. Generation of capturing agents, e.g. primary antibodies, detection reactions, e.g. secondary antibody and a signal generation system, and selection of a format for the array — all these steps can be done with the already available and robust methods.

Thus, the strong side of protein arrays is the well established technologies and knowledge of the targets to be measured. The weak side is the need to ensure stability and specificity of the capturing molecules, e.g. antibodies. Another weak side of the protein arrays is the lack of unbiased analysis. With the arrays, one gets an answer only to questions about pre-defined proteins. If there is an off-target effect, it would be difficult to identify it with the arrays. Therefore, protein arrays may be a great tool for companion diagnostic, when measurements of up to 100 targets are required for diagnostic.

Metabolomics: mass spectrometry and nuclear magnetic resonance spectrometry

Metabolome represents very diverse set of chemical compounds in the human body. Nucleic acids and proteins have single polymeric structures of nucleotides or amino acids, respectively, even if the monomers may be quite diverse. However, metabolites are much more diverse as chemical structures. Metabolites are different types of polymers, e.g. glycans and lipids, and non-polymeric low molecular weight organic and inorganic molecules [58, 59]. This chemical diversity of metabolites creates a big challenge for their comprehensive analysis.

On the other side, metabolomics holds promise of being of the high importance for diagnostics. Robust metabolome profiling of patients would contribute to prediction of drug pharmacokinetics, and response of the body to treatments. Metabolic syndrome and metabolic insufficiency are among the most serious complications in treatment of cancer. Metabolome profiling would provide a tool to predict response to treatments, and therefore has its role in the clinical diagnostic.

Nuclear magnetic resonance (NMR) and mass spectrometry are two techniques which dominate studies of the metabolites [58–60]. However, none of these techniques have yet capacity to be incorporated in the routine clinical practice. NMR has the best efficiency in identification of metabolites, but sensitivity has not been sufficient for application of NMR to small quantities of clinical samples. Notably, the quantities of metabolites in clinical samples are estimated at the picomolar level, while NMR requires micromolar quantities [58–69]. Mass spectrometry has higher sensitivity, but the drawback is in identification of ionized metabolites. Ionization degree and stability, or rather instability, of metabolites upon ionization limits coverage and identification of metabolites, and therefore application of mass spectrometry [58, 60]. The physical principles of NMR and MS, and the physico-chemical features of metabolites suggest that NMR may take the lead. This is because physics of NMR allows enhancing sensitivity, and it may pave the way to the breakthrough in metabolome-based diagnostic.

Integration of OMICs technologies in personalized cancer diagnostic: examples of applications

Clinical OMICs technologies are at different stages of development, and therefore deliver results of different quality. For diagnostics, the quality is defined by the type of measured analytes, comprehensiveness of the coverage of all analytes, sensitivity of detection, suitability of primary data for an analysis, and ability to deliver results to support diagnostic and selection of treatment. In this section, I would like to give examples of how OMICs technologies may help in management of patients.

When a patient arrives to a Hospital, decision has to be made about type of diagnostics to be applied. The samples for diagnostics may be blood and/or tumor biopsy as a surgically resected tumor or as a needle biopsy. The blood is used for preparation of circulating tumor cells (CTC), immunological tests, and use of the blood cells for mutation analysis of selected genes. The tumor material is used for preparation of an organ culture, primary tumor cells, and for biochemical tests.

Functional Molecular Diagnostics (FMDx) evaluates responsiveness of individual patient's tumors to different drugs by testing responsiveness of the living tumor samples in organ culture (Organ Culture FMDx), testing targets and modulators of the drugs' action (Functional Biochemical Assays), and by unbiased testing of the tumor's proteome profile (Proteomics FMDx). These assays measure in a real time how the patients' tumor may respond to different drugs before the patient is offered treatment, and whether the tumor is of an aggressive type (Fig. 4).

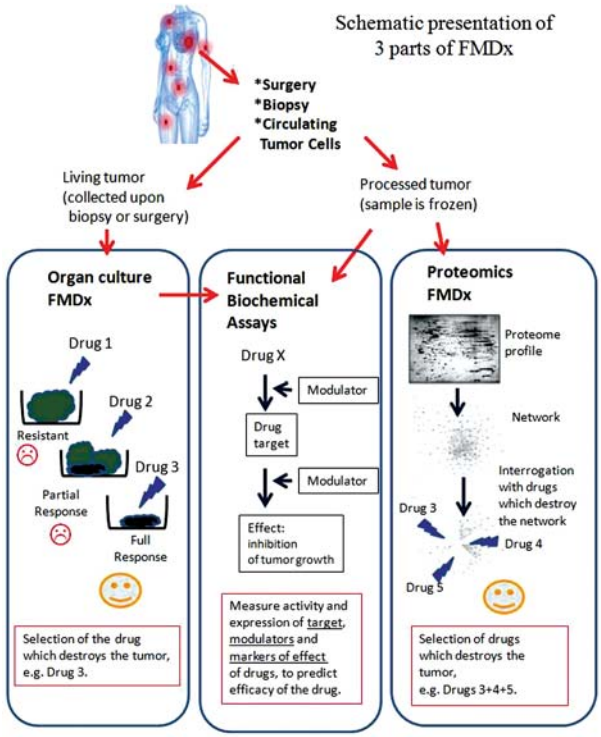

Fig. 4. **Overview of Functional Molecular Diagnostic**

The 3 components of FMDx are shown. The components are Organ Culture FMDx, Functional Biochemical Assays and Proteomics FMDx. Organ Culture FMDx is performed with living tumors, and evaluates sensitivity to drugs. Functional Biochemical Assays are used to evaluate mechanisms and efficacy of the drugs. Proteomics FMDx allow unbiased analysis of the molecular profile of the tumor, including diagnostic, prediction of tumor aggressiveness, and selection of drugs acting on the tumor.

In this section, examples of diagnostic with use of OMICs technologies are discribed. The Functional Molecular Diagnostic (FMDx) was developed to help oncologists in diagnostic and selection of treatment. FMDx consists of a number of tests to evaluate a molecular profile of a tumor, predict development of the disease, and select treatment tailored to the patient. The presented here examples describe 3 clinical cases. For the reason of the patients' integrity, no personal details are provided. The descriptions are to illustrate how OMICs technologies were integrated in the diagnostic efforts.

Case 1. Personalization of treatment with Proteomics FMDx

A lump was detected in both breasts of a woman. The lumps were removed surgically. No spreading to the lymph nodes, and no metastases were detected. The question of the oncologists was whether these 2 tumors were related, i.e. primary tumor in one breast and metastasis in the other, what is prediction of aggressive development of the disease, and which drugs would be most suitable for this woman.

The aliquots of the lumps were prepared for the proteomics study, by extracting proteins and performing proteome profiling (Fig. 5). 2D gel electrophoresis and mass spectrometry were used for generation of the proteome profiles of the tumors. The intact-protein proteomics was applied, which allowed detection of multiple isoforms of the tumor proteins. MALDI mass spectrometry was used to identify proteins, and the systems biology was used to build a network of relations between the tumor-related proteins. The network topology analysis and exploration of functional domains represented by the network, indicated that the tumors were of the similar profile. It means that the tumors in both breasts had the same origin, suggesting metastasis. However, the proteome profiles showed that the tumors were not of the aggressive type, and therefore unlikely that the disease would relapse. Immunohistochemistry tests did not provide conclusive basis for selection of chemotherapy or adjuvant therapies. Therefore, the results of Proteomics FMDx ensured oncologists that the most efficient treatment would be by applying tamoxifen, and restrain from chemo- and other adjuvant therapies. The woman has been regularly monitored for recurrence. Thus, for this patient, combination of the proteomics and systems biology, allowed to conclude about prognosis of the disease development, select the most suitable treatment, and preserve quality of life.

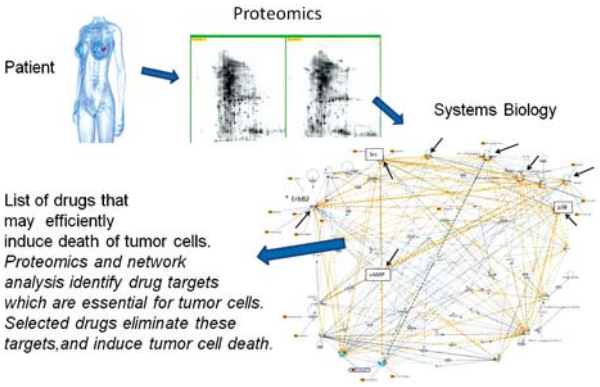

Fig. 5. **Workflow of Proteomics FMDx**

The tumor sample from the patient is subjected to proteome profiling, which then is analyzed by the systems biology tools. Systemic analysis identifies regulatory mechanisms deregulated in the tumor, and identify drugs which would have a tumor-eliminating effect.

Case 2. Personalization of treatment with Organ Culture FMDx

A patient was diagnosed with metastatic pancreatic cancer. Surgery was not applicable, due to multiple metastases. The oncologist required information about efficiency of drugs which were considered for treating this patient.

Biopsies of the primary tumor and metastases were collected. Organ culture samples were prepared immediately after resection of the tumors. Organ culture samples were prepared by using FMDx proprietary technique, and were exposed to drugs. The oncologist was interested in response to 5-fluorouracyl, oxaliplatin and gemcitabine. Two additional drugs were also tested. The first is an inhibitor of EGF receptor kinase, Iressa, and the second drug is an inhibitor of TGFβ type I receptor kinase, SB431542. During incubation of the tumor tissues with the drugs, it was observed very strong effect of 5-fluorouracil, significant but partial response to oxaliplatin and gemcitabine. Iressa and SB431542 both showed an cell-killing effect, but the response was partial (Fig. 6, *A*). In parallel, Functional Biochemical Assays were applied to the tumor samples. These tests showed significantly enhanced activity of pro-mitogenic kinase Erk1/2, as compared to the Erk1/2 activity in the control carcinoma cells (Fig. 6, *B*). Other biochemical tests showed that EGF and TGFβ signaling pathways were active, but at the levels comparable to the control carcinoma cells. The biochemical tests indicate that inhibitors of Erk1/2 pathway, e.g. MEK1 or Raf inhibitors, may have a strong inhibitory impact on the tumor growth for this patient. The tests confirmed also that Iressa and SB431542 indeed may have tumor-suppressing effect. Thus, the Organ Culture FMDx and biochemical tests provided the oncologist with information about efficacy of the tested drug, and indicated that the combination of chemotherapy with adjuvant therapy may be beneficial for the patient.

Organ Culture FMDx delivers informative results during the first 2 weeks of culturing. Longer culturing leads to changes in structure of the tumor tissue, and changes in physiology of tumor cells. These changes affect pattern of the cell response to the drugs. However, longer culturing allows obtaining primary culture of tumor cells which detach from the tumor tissue. These primary culture cells may be further used for research purposes. For this patient, a culture of primary cells was obtained. Thus, Organ Culture FMDx delivered information about sensitivity of the tumors of the patient to the selected drugs.

Case 3. Generation of individualized cancer vaccine

There is a strong confidence among oncologists that as long as the vital functions of a body are not compromised, there is a chance of curing even advanced cancer. One of the curation strategies is deciphering of the molecular profile of the tumor, and finding combination of drugs which may kill this tumor. These strategies are mentioned in descriptions of the cases #1 and #2. The case #3 describes the strategy to employ immunological protective mechanisms of the patient's body.

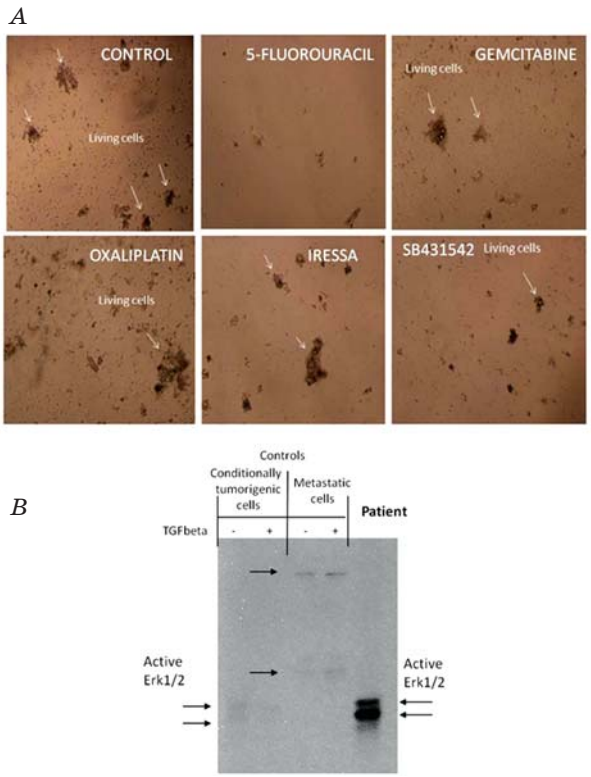

Fig. 6. **Organ Culture FMDx**

Organ Cultures are prepared from the tumor biopsy. *A* — Images of the organ culture cultured in a medium only (Control), or incubated with drugs as indicated. Arrows show clusters of living cells. Organ cultures were incubated with the drugs for 48 h. Note presence of living cells in cultures incubated with gemcitabine, oxaliplatin, Iressa and SB431542.

B —Functional Biochemical Assay with the same tumor samples as in (*A*). The assay was to evaluate activity of Erk1/2 kinase. Note strongly enhanced Erk1/2 signal in the tumor sample, as compared to conditionally tumorigenic and the metastatic control samples. Note that the Erk1/2 signal in the tumor corresponds to the Erk1/2 characteristic in the non-aggressive tumor cells. This assay indicates that the drugs inhibiting Erk1/2 may be beneficial for this patient.

The idea of the individualized cancer vaccine is based on the fact that the body is capable to recognize the tumor epitopes, but the response is too weak to remove the tumor. Therefore, boosting of the anti-tumor immunity is required. Currently, the first type of the anti-tumor vaccines is based on identification of tumor-recognizing lymphocytes, which are then expanded in vitro, and injected in the patient. The second type of vaccines is based on identification of tumor antigens which stimulated immunological response, even though weak and not sufficient to eliminate the tumor. These antigens are then purified, and used to boost the anti-tumor immune response.

The presented here case is an example of how such an antigen-based vaccine could be developed (Fig. 7). The patient described in this case could not be subjected to biopsy, due to the weak general condition and numerous metastases. Therefore, a blood sample was taken, and plasma was prepared. The plasma was used to detect tumor-specific antigens. The antigen array was prepared by 2D-GE, and transferring of separated proteins from the gels on the membranes. The transferred tumor proteins were probed with the patient's plasma, and with plasma of healthy individuals. To discriminate tumor and non-tumor antigens further, protein arrays from the non-tumor samples were prepared, and probed with plasma from the patient and from healthy individuals. All these tests allowed identification of 2 strong tumor-specific antigens (Fig. 7, *A*). These antigens were then tested as a scratch-test on the patient. As expected, the antigens showed immunological reactions, with one of the antigens showing reaction justifying use of this antigen for development of the individualized vaccine (Fig. 7, *B*). This selected antigen was prepared in quantities required for vaccination. The antigens were purified, and were subjected to tests of chemical purity, toxicity and sterility. The last test before application of such a vaccine is the scratch-test of responsiveness on the patient. The result was considered positive when there was observed a swelling and redness response after 2 days. Thus, application of proteomics allows generation of truly individual cancer vaccine.

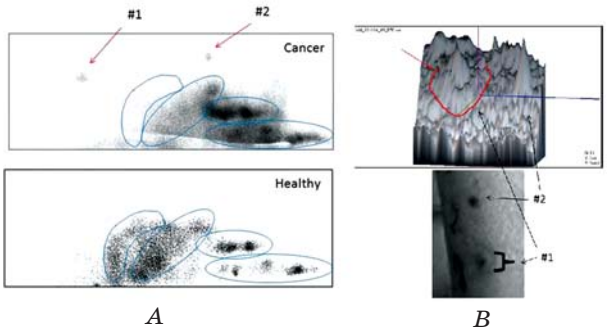

Fig. 7. **Development of individualized vaccine as part of FMDx:**

A — Proteomics is an important part in identification of tumor-specific antigens. Upper panel shows proteins separated in a 2D gel, and recognized by the antibodies of the patient. Lower panel shows the same proteins immunoblotted with antibodies from a healthy individual. #1 and #2 indicate proteins recognized as immunogenic tumor-related antigens.

B — Identified immunogenic tumor-related antigens were purified, and prepared as a vaccine. The scratch test with the vaccine is shown. The scratch test is performed before applying the vaccine, as part of controls. Note that the antigen #1 produced immunological response. The area of the skin is shown in the lower part of the image, and the computer-assisted analysis of the swelling is indicated in the upper part as the area marked by the red line.

Prospective

Complexity of cancer requires comprehensive evaluation of tumors and patients. Therefore, OMICs technologies have come to stay. We may expect significant improvements in quality of results delivered by profiling of genome, transcriptome, proteome and metabolome of a cancer patient and her/his tumors. Cost efficiency of OMICs technologies will allow performing such profiling on every patient. As an example, FMDx is already affordable for routine use in the cancer clinics.

Developments of OMICs technologies are promoted by the combination of biochemistry, cell and molecular biology, engineering, physics, chemistry and mathematics. When in 1961 Yurij Gagarin opened the era of manned space flights that was the result of collaboration between many different professionals. The same is valid for cancer — to make a breakthrough and find cure of cancer, a single discovery is not enough. It has to be a combined effort. And the biggest challenge is to create such an organization which would focus on fighting cancer with understanding complexity of this disease and hosting diverse expertise.

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REFERENCES

1. *Hanahan D., Weinberg R. A.* Hallmarks of cancer: the next generation // Cell. — 2011. — V. 144, N 5. — P. 646–674.
2. *Nature* Milestones in Cancer / Ed. A. Farrell, E. Hutchinson, B. Marte, N. McCarthy // Nat. Rev. Cancer. — 2006. — V. 6. — P. S7–S23.
3. *Vogelstein B., Kinzler K. W.* Cancer genes and the pathways they control // Nat. Med. — 2004. — V. 10. — P. 789 799.
4. *Waliszewski P.* Complexity, dynamic cellular network, and tumorigenesis // Pol. J. Pathol. — 1997. — V. 48, N 4. — P. 235–241.
5. *Casado-Vela J., Cebrián A., Gymez del Pulgar M. T., Lacal J. C.* Approaches for the study of cancer: towards the integration of genomics, proteomics and metabolomics // Clin. Transl. Oncol. — 2011. — V. 13, N 9. — P. 617–628.
6. *Garay J. P., Gray J. W.* Omics and therapy — a basis for precision medicine // Mol. Oncol. — 2012. — V. 6, N 2. — P. 128–139.
7. *Benjamin D. I., Cravatt B. F., Nomura D. K.* Global profiling strategies for mapping dysregulated metabolic pathways in cancer // Cell. Metab. — 2012. — V. 16, N 5. — P. 565–577.
8. *Grant G. M., Fortney A., Gorreta F. et al.* Microarrays in cancer research // Anticancer Res. — 2004. — V. 24, N 2A. — P. 441–448.
9. *Gullapalli R. R., Lyons-Weiler M., Petrosko P. et al.* Clinical integration of next-generation sequencing technology // Clin. Lab. Med. — 2012. — V. 32, N 4. — P. 585–599.
10. *Tangrea M. A., Wallis B. S., Gillespie J. W. et al.* Novel proteomic approaches for tissue analysis // Expert. Rev. Proteom. — 2004. — V. 1, N 2. — P. 185–192.
11. *Hanash S.* Disease proteomics // Nature. — 2003. — V. 422, N 6928. — P. 226–232.
12. *Souchelnytskyi N., Souchelnytskyi S.* Three-dimensional Hp/pI/Mr separation of more than 20,000 intact cellular proteins // Nature Methods. — 2013. — Under revision.
13. *Maxam A. M., Gilbert W.* A new method for sequencing DNA // Proc. Natl. Acad. Sci. USA. — 1977. — V. 74, N 2. — P. 560–564.
14. *Sanger F., Nicklen S., Coulson A. R.* DNA sequencing with chain-terminating inhibitors // Ibid. — 1977. — V. 74, N 12. — P. 5463–5467.
15. *Slatko B. E., Kieleczawa J., Ju J. et al.* «First generation» automated DNA sequencing technology // Curr. Protoc. Mol. Biol. — 2011. — Chapter 7:Unit7.2.
16. *Metzker M. L.* Sequencing technologies — the next generation // Nat. Rev. Genet. — 2010. — V. 11, N 1. — P. 31–46.
17. *Voelkerding K. V., Dames S., Durtschi J. D.* Diagnostic Next Generation Sequencing // J. Mol. Diagn. — 2010. — V. 12, N 5. — P. 539–551.
18. *Meldrum C., Doyle M. A., Tothill R. W.* Next-generation sequencing for cancer diagnostics: a practical perspective // Clin. Biochem. Rev. — 2011. — V. 32, N 4. — P. 177–195.
19. *Cancer* Genome Atlas Network. Comprehensive molecular portraits of human breast tumours // Nature. — 2012. — V. 490, N 7418. — P. 61–70.
20. *Shinawi M., Cheung S. W.* The array CGH and its clinical applications // Drug. Discov. Today. — 2008. — V. 13, N 17–18. — P. 760–770.
21. *Tsuchiya K. D.* Fluorescence in situ hybridization // Clin. Lab. Med. — 2011. — V. 31, N 4. — P. 525–542.
22. *Jain K. K.* Current status of fluorescent in-situ hybridisation // Med. Device Technol. — 2004. — V. 15, N 4. — P. 14–17.
23. *Ren H., Francis W., Boys A. et al.* BAC-based PCR fragment microarray: high-resolution detection of chromosomal deletion and duplication breakpoints // Human Mutation. — 2005. — V. 25, N 5. — P. 476–82.
24. *Zecchini V., Mills I. G.* Putting chromatin immunoprecipitation into context // J. Cell Biochem. — 2009 — V. 107, N 1. — P. 19–29.
25. *Saxena A., Carninci P.* Whole transcriptome analysis: what are we still missing? // Wiley Interdiscip. Rev. Syst. Biol. Med. — 2011. — V. 3, N 5. — P. 527–543.
26. *Samuel N., Hudson T. J.* Translating genomics to the clinic: implications of cancer heterogeneity // Clin. Chem. — 2013. — V. 59, N 1. — P. 127–137.
27. *Hawkins R. D., Hon G. C., Ren B.* Next-generation genomics: an integrative approach // Nat. Rev. Genet. — 2010. — V. 11, N 7. — P. 476–486.
28. *Gullapalli R. R., Lyons-Weiler M., Petrosko P. et al.* Clinical integration of next-generation sequencing technology // Clin. Lab. Med. — 2012. — V. 32, N 4. — P. 585–599.
29. *Desmedt C., Voet T., Sotiriou C., Campbell P. J.* Next-generation sequencing in breast cancer: first take home messages // Curr. Opin. Oncol. — 2012. — V. 24, N 6. — P. 597–604.
30. *Sandoval J., Esteller M.* Cancer epigenomics: beyond genomics // Curr. Opin. Genet. Dev. — 2012. — V. 22, N 1. — P. 50–55.
31. *Huber-Keener K. J., Liu X., Wang Z. et al.* Differential gene expression in tamoxifen-resistant breast cancer cells revealed by a new analytical model of RNA-Seq data // PLoS One. — 2012. — V. 7, N 7. — P. e41333.
32. *Humphery-Smith I., Cordwell S. J., Blackstock W. P.* Proteome research: complementarity and limitations with respect to the RNA and DNA worlds // Electrophoresis. — 1997. — V. 18, N 8. — P. 1217–1242.

33. *Jiang Z., Zhou Y., Devarajan K. et al.* Identifying putative breast cancer-associated long intergenic non-coding RNA loci by high density SNP array analysis // Front Genet. — 2012. — V. 3. — P. 299.
34. *Giovannetti E., van der Velde A., Funel N. et al.* High-throughput microRNA (miRNAs) arrays unravel the prognostic role of MiR-211 in pancreatic cancer // PLoS One. — 2012. — V. 7, N 11. — P. e49145.
35. *Yan-Fang T., Dong W., Li P. et al.* Analyzing the gene expression profile of pediatric acute myeloid leukemia with real-time PCR arrays // Cancer Cell Int. — 2012. — V. 12, N 1. — P. 40.
36. *Cheng S., Koch W. H., Wu L.* Co-development of a companion diagnostic for targeted cancer therapy // Nat. Biotechnol. — 2012. — V. 29, N 6. — P. 682–688.
37. *Ong F. S., Das K., Wang J. et al.* Personalized medicine and pharmacogenetic biomarkers: progress in molecular oncology testing // Expert. Rev. Mol. Diagn. — 2012. — V. 12, N 6. — P. 593–602.
38. *Curigliano G., Locatelli M., Fumagalli L. et al.* Targeting the subtypes of breast cancer: rethinking investigational drugs // Expert Opin. Investig. Drugs. — 2012. — V. 21, N 2. — P. 191–204.
39. *McCourt C. M., Boyle D., James J., Salto-Tellez M.* Immunohistochemistry in the era of personalised medicine // J. Clin. Pathol. — 2013. — V. 66, N 1. — P. 58–61.
40. *Souchelnytskyi S.* Current status and challenges of personalized treatment of cancer: View inspired by the workshop // Exp. Oncol. — 2011. — V. 33, N 3. — P. 166–169.
41. *Aebersold R., Mann M.* Mass spectrometry-based proteomics // Nature. — 2003. — V. 422. — N 6928. — P. 198–207.
42. *Van den Ouweland J. M., Kema I. P.* The role of liquid chromatography-tandem mass spectrometry in the clinical laboratory // J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. — 2012. — V. 883–884. — P. 18–32.
43. *Conrotto P., Souchelnytskyi S.* Proteomic approaches in biological and medical sciences: principles and applications // Exp. Oncol. — 2008. — V. 30, N 3. — P. 171–180.
44. *Görg A., Weiss W., Dunn M. J.* Current two-dimensional electrophoresis technology for proteomics // Proteomics. — 2004. — V. 4, N 12. — P. 3665–3685.
45. *Lypez J. L.* Two-dimensional electrophoresis in proteome expression analysis // J. Chromatogr. B. — 2007. — V. 849, N 1–2. — P. 190–202.
46. *Stasyk T., Huber L. A.* Zooming in: fractionation strategies in proteomics // Proteomics. — 2004. — V. 4. — P. 3704–3716.
47. *Islinger M., Eckerskorn C., Vųlkl A.* Free-flow electrophoresis in the proteomic era: a technique in flux // Electrophoresis. — 2010. — V. 31, N 11. — P. 1754–1763.
48. *Righetti P. G., Sebastiano R., Citterio A.* Capillary electrophoresis and isoelectric focusing in peptide and protein analysis // Proteomics. — 2013. — V. 13, N 2. — P. 325–340.
49. *Xie F., Smith R. D., Shen Y.* Advanced proteomic liquid chromatography // J. Chromatogr. A. — 2012. — V. 1261. — P. 78–90.
50. *H S Lu C., Liu K., Tan L. P., Yao S. Q.* Current chemical biology tools for studying protein phosphorylation and dephosphorylation // Chemistry. — 2012. — V. 18, N 1. — P. 28–39.
51. *Nodwell M. B., Sieber S. A.* ABPP methodology: introduction and overview // Top. Curr. Chem. — 2012. — V. 324. — P. 1–41.
52. *Schwamborn K.* Imaging mass spectrometry in biomarker discovery and validation // J. Proteomics. — 2012. — V. 75, N 16. — P. 4990–4998.
53. *Van Eeckhaut A., Maes K., Aourz N. et al.* The absolute quantification of endogenous levels of brain neuropeptides in vivo using LC-MS/MS // Bioanalysis. — 2011. — V. 3, N 11. — P. 1271–1285.
54. *Deutsch E. W., Lam H., Aebersold R.* PeptideAtlas: a resource for target selection for emerging targeted proteomics workflows // EMBO Rep. — 2008. — V. 9, N 5. — P. 429–434.
55. *Lee J. R., Magee D. M., Gaster R. S. et al.* Emerging protein array technologies for proteomics // Expert. Rev. Proteomics. — 2013. — V. 10, N 1. — P. 65–75.
56. *Sanchez-Carbayo M.* Antibody microarrays as tools for biomarker discovery // Methods Mol. Biol. — 2011. — V. 785. — P. 159–182.
57. *Carlsson A., Wingren C., Ingvarsson J. et al.* Serum proteome profiling of metastatic breast cancer using recombinant antibody microarrays // Eur. J. Cancer. — 2008. — V. 44, N 3. — P. 472–480.
58. *Bowen B. P., Northen T. R.* Dealing with the unknown: metabolomics and metabolite atlases //J. Am. Soc. Mass. Spectrom. — 2010. — V. 21, N 9. — P. 1471–1476.
59. *O'Connell T. M.* Recent advances in metabolomics in oncology // Bioanalysis. — 2012. — V. 4, N 4. — P. 431–451.
60. *Zhou W., Liotta L. A., Petricoin E. F.* Cancer metabolism: what we can learn from proteomic analysis by mass spectrometry // Cancer Gen. Proteom. — 2012. — V. 9, N 6. — P. 373–381.

ІНДИВІДУАЛЬНИЙ ПІДХІД У ЛІКУВАННІ РАКУ: ВНЕСОК OMIC-ТЕХНОЛОГІЙ У ДІАГНОСТИКУ ЗАХВОРЮВАННЯ

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Рак — хвороба, яка є показовою ілюстрацією успіхів, невдач і перспектив сучасних біомедичних досліджень. Розвиток відповідних технологій став вирішальним чинником поліпшення якості лікування раку. Впровадження в клінічну практику досягнень технологій геноміки, РНК-профілювання та протеоміки забезпечило основу для розроблення нових діагностичних засобів, лікарських препаратів і методів лікування.

У статті обговорено внесок OMIC-технологій у персоналізацію діагностики та лікування цього захворювання. Акцент зроблено на технологіях, які показали можливість відповідного доставлення діагностичних засобів, що може бути використано в клініці для простих діагностичних тестів. Як ілюстрацію наведено три клінічних випадки з використанням доступних на сьогодні методів персоналізованої діагностики раку.

Ключові слова: персоналізоване лікування раку, діагностика, геноміка, транскриптоміка, протеоміка, метаболоміка.

ИНДИВИДУАЛЬНЫЙ ПОДХОД В ЛЕЧЕНИИ РАКА: ВКЛАД OMIC-ТЕХНОЛОГИЙ В ДИАГНОСТИКУ ЗАБОЛЕВАНИЯ

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Рак — болезнь, которая является показательной иллюстрацией успехов, неудач и перспектив современных биомедицинских исследований. Развитие соответствующих технологий стало решающим фактором улучшения качества лечения рака. Внедрение в клиническую практику достижений технологий геномики, РНК-профилирования и протеомики обеспечило основу для разработки новых диагностических средств, лекарственных препаратов и методов лечения.

В статье обсуждается вклад OMIC-технологий в персонализацию диагностики и лечения этого заболевания. Акцент сделан на технологиях, показавших возможность доставки диагностических средств, что может быть использовано в клинике для простых диагностических тестов. В качестве иллюстрации приведены три клинических случая с использованием доступных на сегодняшний день методов персонализированной диагностики рака.

Ключевые слова: персонализированное лечение рака, диагностика, геномика, транскриптомика, протеомика, метаболомика.