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CURRENT STATUS AND CHALLENGES OF PERSONALIZED TREATMENT OF CANCER: VIEW INSPIRED BY THE WORKSHOP

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INTRODUCTION

Cancer still ravages human lives. Survival rates of patients with advanced and metastatic cancers are in the range of 50 % (*http://www.who.int/cancer*). This is the ultimate proclamation that we need to improve diagnostics, treatment and monitoring of cancer patients. These improvements are dependent on advances of fundamental and translational cancer research.

The Ukrainian-Swedish workshop "New approaches in diagnostics and treatment of cancer" was the great opportunity to discuss some of the recent achievements. Variety of the research projects presented during the workshop was a good illustration of tackling the cancer problems from different directions. An important theme was highlighting the complexity of cancer, whether it is about mechanisms of tumorigenesis or novel treatments.

COMPLEXITY OF CANCER: REVISION OF THE ONCOGENES AND TUMOR SUPPRESSORS MODEL

Almost every day there is a publication describing a mechanism or a gene or a protein with explicit pro- or anti-tumorigenic activity. This raises a question about what exactly triggers growth of a tumor. Apparently, the classical "oncogenes and tumor suppressors" model of tumorigenesis may not be correct anymore. Today we have more than 100 genes, RNAs and proteins that showed a strong impact on breast cancer initiation and development. Majority of these components are not classical oncogenes or tumor suppressors (Fig. 1). The list of regulators with a strong impact on breast tumorigenesis includes scaffold proteins (BRCA1, BRCA2, BRCA3), transcriptional factors (p53, eIEF-4E, c-myc), components of signaling pathways by EGF, TGFb, FGF, VEGF families (receptors and intracellular signaling molecules), steroid-dependent signaling, telomerase, cell cycle regulators, DNA damage response pathways, and regulators of metabolic response [1, 2]. The cancer-related processes include cell proliferation, immortalization, cell death, migration, invasiveness, ability to form metastasis, to interact with non-malignant cells, ability to build a tumor, and escape of a tumor being recognized by the organism as a foreign body.

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Abbreviations: BRCA1 – Breast Cancer susceptibility gene-1; EGF – epidermal growth factor; MALDI – matrix-assisted laser desorption/ionization; TGFb – transforming growth factor-b.

Complexity of tumorigenesis leads to conclusions, that the number of molecular cancer profiles may be significantly higher than is assumed today, and definitely much higher than the 5 molecular profiles predicted by a cohort study using mRNA microarrays [3]. A molecular cancer profile is defined as a set of molecules and activities that lead to initiation and growth of a tumor. As the same output, e.g. enhanced growth, may be achieved by changes in activities of different molecules, e.g. cyclins, CDKs, or CDK inhibitors, the number of combination of changed molecules may be higher than 100. Taking into account a combination of different cellular processes, such as cell proliferation, death, migration, invasiveness, immune tolerance, etc, the number of molecular cancer profiles may be as high as 100.000.

Regulators with strong impact on breast tumorigenesis:

HER2	p53	Ras	p27	PI3K	BRCA1
AKT	BRCA2	EIF-4E	CHK2	Cyclin D1	
ATM	Cyclin E		PTEN	C-myc	Rb

Regulatory processes and molecules strongly affecting tumorigenesis:

Tyrosine Kinase receptor signaling (EOF, FGF, VEGF, PDGF) Serine/Threonine kinase receptor signaling (TGFbeta) Steroids (estrogen), Phosphatases, Metabolism regulators

Tumor-associated cells:

Immune response cells Fibroblasts, Endothelial cells, Adipocytes

Fig. 1. Tumorigenesis is regulated on different levels by a number of proteins and genes that are not typical oncogenes or tumor suppressors. Selected genes, RNAs and proteins reported to have a strong impact on breast tumorigenesis are shown. Regulatory processes are also mentioned; specific components are not listed due to space limitation. Tumor-associated cells of importance for breast tumorigenesis are listed

SCIENTIFIC, MANAGERIAL AND TECHNOLOGICAL LIMITATIONS FOR CONTROLLING MOLECULAR CANCER PROFILES

The high number of potent regulators of tumorigenesis raises the next question: Can we detect all molecular cancer profiles? To my opinion, we do not. The problem is in scientific, managerial and technological limitations.

The scientific limitations are in our incomplete knowledge of tumorigenesis. Constant reporting of new mechanisms of regulation of tumor growth is the best proof that a lot still has to be discovered. Especially pronounced this limitation is when to analyze studies of cancer proteome. Proteins are the main working entities of cells. To have a mutated oncogene does not mean that the person will get a cancer. The oncogene has to be expressed as a malfunctional protein to have an impact on cell functions. Thus, understanding

of cancer proteome is of importance. Our observations and reports by others show that most of proteins have post-translational modifications (PTMs). The estimate is that more than 90% of proteins have at least one PTM. Today are known more than 300 PTMs of proteins that may create huge numbers of protein isoforms (*www.abrf.org/index.cfm/dm.home?AvgMass=all*). As an example, Fig. 2 shows a typical pattern of isoforms of a single protein with different patterns of O-phosphorylation. That means that the variability of proteins may be very large.

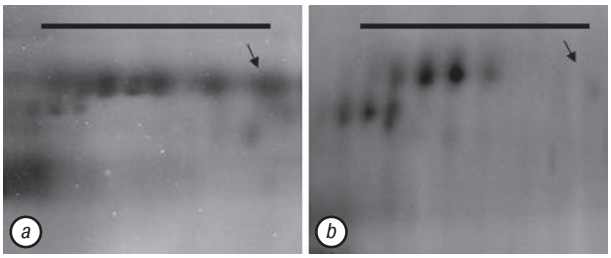

Fig. 2. Multiple isoforms of a same protein: impact of post-translational modifications. Images show inserts of 2D gels from tumor biopsies of patients that responded (*a*) or was resistant to a treatment (*b*). Arrows indicate a changed isoform. Lanes indicate the cluster of isoforms of the same protein with different pI. Proteins are enriched for O-phosphorylation

Modern physics, chemistry and biology indicate that our knowledge of biological systems is far from being complete. As an example, discovery that DNA may react on a spin moment suggests that DNA may have information-sensing capacity previously known only in physical systems [4]. For proteins, such phenomena have not been even studied. Another example is the lack of studies of N- and C-phosphorylation of proteins, despite that up to 50% of phosphoryl groups bound to proteins are N- or C-bound. This gap in signaling studies is most probably due to the lack of chemical methods to preserve, detect and analyze these PTMs. Even more astonishing example of how little we know is the fact that more than 30% of predicted by DNA or RNA sequencing proteins have not been cloned and studied. Thus, we are still scratching on the surface of biological mechanisms that govern cellular functions.

Domination of cohort-based approach in cancer studies is another hinder for individualization of anti-cancer treatment. Cohort-based approach, by definition, disregards individual difference between patients, and focuses on features common for a group of patients. As the focus is on specific genes, RNAs or proteins, and as exact components of the same regulatory process may be different in different patients, the cancer-related components will be disregarded in a cohort study as individual variations. To overcome this limitation, we proposed meta-data analysis that would preserve individual features [5]. Meta-data analysis is based on evaluation of cancer-related changes on the individual level, as a case-by-case study. The primary dataset of individual changes is then subjected to systemic analysis to unveil affected regulatory processes. And then these processes and affected proteins, genes and RNAs are compared between different patients. Application of meta-data analysis has been shown to be more informative in unveiling cancer-related changes on the individual level. This individualization made possible selection of drugs that would be suitable for the patient. In other words, cohort-based approach would propose that "if you have a marker/kinase A expressed, you may benefit from drug X, with the rate of success 70%". Meta-data individualized approach would propose that "you have expression and activity of a kinase B, thus you will benefit from a drug that inhibits kinase B". The difference is between a possibility ("may" for cohort) and more secured assessment ("will" for individualized meta-data). Meta-data approach provides individualization of assessment of molecular mechanisms governing tumorigenesis in a given patient. This allows truly individual tailoring of drugs and treatments.

Managerial limitations may originate due to the human psychology. Majority of scientists follow each other, and only few dare to take unexplored paths. Management of academic research does not promote high risk projects, even if such a risky project may be a breakthrough. Granting agencies prefer "safe cards" of established research fields. The joke that "new Albert Einstein would never get a grant today" is correct in the most of countries. As long as a citation index of a journal in which you publish your paper would have stronger impact on your granting success than the quality of your research, managerial issues would hamper scientific progress.

Technological limitations are going hand-in-hand with scientific ones. Many of the biological mechanisms are not studied because the lack of technologies to discover them. In application to proteomics, technologies for comprehensive study of proteomes are not available and are under development. The progress in proteomics has been due to implementation of two-dimensional gel electrophoresis (2D-GE) and mass spectrometry (MS). Other electrophoretic and chromatographic techniques have not yet proven their superiority to 2D-GE and MS. However, 2D-GE has limited protein separation capacity, as it may allow detection up to 3.000 proteins in a single 2D gel. MS is today the most sensitive technology for identification of proteins, with identification of picomolar quantities of proteins. Femto- and attomolar levels of detection have been claimed, but they are rather exceptions than the routine performance of MS instruments. The main limitation of MS is its intrinsic inability to handle molecules of Mr more than 10.000 Da. Mass loss in larger proteins due to intramolecular interactions, isotopic composition and multiple ionization make informative MS analysis of proteins with Mr higher than 20.000 Da very difficult, if not an impossible task. What to expect? New technologies are under way, with 3-tech for separation of proteins, and nanosequencing for identification of the proteins being two examples.

Similar situations are valid for studies of genome, transcriptome and metabolome. Deep sequencing technologies apparently make it possible to explore genome and transcriptome within reasonable quality,

time and efforts [6]. However, even deep sequencing technologies require amplification of targets and very extensive puzzling of information from short sequences. Metabolomics is in infancy, as the most informative technology, nuclear magnetic resonance (NMR), is only reaching required sensitivity to detect and identify molecules in at least nanomolar range.

Clear definition of what exactly scientific, managerial and technological limitations are is essential for finding clinical solutions. These limitations are interdependent, and progress in solving of one type of limitations stimulates progress in solving others.

WHAT CAN WE DO TODAY? AN EXAMPLE.

In clinics today, decision about selection of breast cancer treatment is based on imaging information (mammography, CT-scans, X-ray examinations), lymphnode status, metastasis detection, histo-pathological description of a biopsy (histological description of a tumor, differentiation status, mitotic index, inflammation and necrosis areas, etc), on immunohistochemical evaluation of expression of Her2/neu, EGFR, ERa/ERb, PgR, E-cadherin, p53, VEGF and VEGF receptors. Here I would like to present an example of personalized approach to anti-cancer treatment that is applied in my laboratory and that was reported at the Workshop (Fig. 3). This approach is based on an individual profiling of tumor proteomes, systemic analysis of cancer-related changes, building of an Individual Dynamic Response Network (Ind-DRNet), prediction of a set of drugs that would have tumor-killing impact, and transfer recommendations to an oncologist who will make final selection of a treatment.

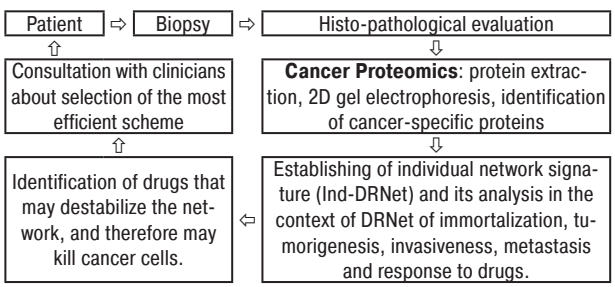

Fig. 3. The workflow of personalized treatment with application of proteomics technologies. The main steps in personalized assessment of a patient are indicated and briefly described

Proposed Ind-DRNet approach incorporates histo-pathological and biochemical diagnostics currently used in clinic. The main application of Ind-DRNets is to cancer cases when surgery is performed. That allows obtaining a biopsy for proteome profiling. When a patient underwent surgery, standard histo-pathological and biochemical tests are performed. Then, a biopsy is used for proteome profiling in which tumor and adjacent histologicaly normal tissue are compared. Extracted proteins are separated by 2D-GE, and image analysis is used to detect cancer-related proteins that are then identified by mass spectrometry. To our experience, the number of cancer-related proteins is rather large and significantly different for different patients. Identified proteins are then used for building a patient-specific network that describes cancer-specific changes in a tumor of the patient. The networks provide also information about drug targets and applications of drugs to destroy the given tumor. An important value of the network is a possibility to predict whether certain drugs would kill the tumor or would just slow tumor growth. The network shows also which drugs may compensate and block each other and become less efficient, which drugs would have an additive effect, and which drugs would synergize in their anti-cancer action.

When considering use of a certain drug, an oncologist would have to consider whether drug target is expressed and is active, specificity, pharmacokinetics/pharmacodynamics of the drug, possible off-target effects, compensatory mechanism that can block action of the drug, and any systemic effects of a combined use of the drug with other therapeutics. This is a lot to think about. Can clinicians be helped? Here I present 2 examples of how proteomics can help with analysis of specificity (1st example) and clinical application (2nd example) of the type I TGFβ receptor (TβR-I) kinase inhibitor. This inhibitor is a prototype of a drug in clinical trials. These examples were also presented during the Workshop.

TβR-I inhibitor, an imidazole-based compound SB431542, was claimed to be “highly specific” [7]. However, in clinical trials the drug showed side effects on cardio-vascular system. Our proteomics-based analysis showed that more than 20 kinases could be inhibited by this compound. Why these kinases were not detected earlier, before clinical trials? The answer is that the company used traditional approach, with more than 40 kinases tested. However, the human kinome consists of more than 400 kinases [8]. Thus, missing to perform unbiased test of all kinases may be quite expensive, when off-targets would lead to drug-prohibiting side effects.

When inhibition of TGFβ signaling would be beneficial? An answer to this question can be provided by proteomics and immunohistochemistry (IHC) (Fig. 4). Proteome profiling and systemic analysis followed by building of Ind-DRNets for 4 patients showed that 2 patients (#6 and #47) have over-activated TGFβ signaling in tumors [5]. Validation tests of TβR-I activity on its substrate Smad2 showed by IHC that cases #6 and #45 had enhanced Smad2 phosphorylation. This conclusion indicated that the patient #1 would not benefit from TβR-I inhibition (no TGFβ signaling over-activation as by proteomics and IHC), while patient #6 may have a positive response (over-activation by proteomics and IHC). What about patients #45 and #47? Both showed indications that TGFb signaling may be over-active, but 2 tests gave not fully complementary results. In this situation, the network analysis allowed to make a decision. Ind-DRNets of these patients showed that the TβR-I inhibition may be beneficial if at the same time inhibition of steroid-dependent and EGF family-dependent signaling would be done for patient #45. For patient #47, TβR-I inhibition would have to be combined with inhibition of inflammatory

responses [5]. Therefore, the use of TβR-I inhibitors would be tailored to each of the patients.

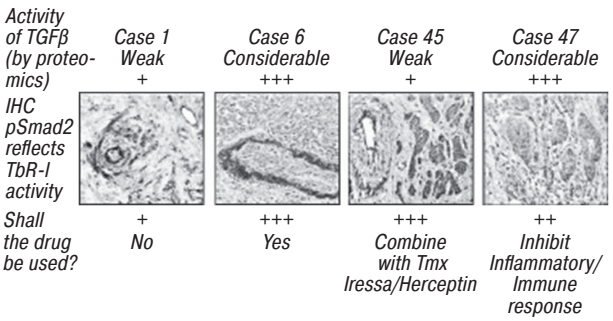

Fig. 4. Selection of patients who would respond to treatment with TβR-I kinase inhibitor. Results of proteome profiling of tumor biopsies and immunohistochemistry staining for activated/phosphorylated Smad2 are indicated. IHC images for phosphorylated Smad2 staining are shown. In the lower part, there are recommendations for use of the TβR-I kinase inhibitor

OUTLOOK

Cancer can be treated. It is only a matter of time when cancer will not be anymore a terminal disease. Two main parallel roads are ahead. The first is continued progress of fundamental and translational research. The second is understanding that biomedicine requires help from other fields, such as mathematics, computer sciences, chemistry, physics and engineering.

However, even today we have accumulated sufficient knowledge and experience to help patients. Early detection, improved diagnostics and especially tailoring of drugs to patients are realistic, and are used in clinics. We need further development of technologies for a single-molecule sequencing, comprehensive separation of proteins and metabolites, and introduction of high throughput assays for testing individual patients. These developments would be complemented by novel non-invasive diagnostic tools. The workshop highlighted many of the tasks to be solved, and also showed how solutions are developing.

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