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Minireview

Genomics as a basis for precision medicine

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Summary. Precision medicine, also known as genome-based medicine and personalized medicine, uses knowledge of the molecular basis of a disease in order to individualize treatment for each patient. The development of novel, powerful, high-throughput technologies has enabled better insight into the genomic, epigenomic, transcriptomic and proteomic landscape of many diseases, resulting in the application of personalized medicine approaches in healthcare. Research in the field of biomedicine in Serbia has followed the modern trends and has made a great contribution to the implementation of genomics in Serbian clinical practice. This is a review of the state of the art of scientific achievements and their application, which have paved the way for personalized medicine in Serbia.

Keywords: genomics, hematological malignancy, molecular diagnosis, molecular-targeted therapy, personalized medicine, pharmacogenomics, stem cell therapy.

Introduction

Although medicine always aimed to be personalized, a true implementation of personalized medicine in healthcare practice has only recently started. The fascinating progress of molecular genetics has strongly contributed to this great achievement of modern medicine. Personalized medicine, also known as genome-based medicine and precision medicine, uses knowledge of the molecular basis of a disease in order to individualize treatment for each patient. The development of novel, powerful, high-throughput technologies has enabled better insight into the genomic, epigenomic, transcriptomic and proteomic landscape of many diseases, resulting in the application of personalized medicine approaches in their treatment.

One of the most important achievements of personalized medicine is the discovery of novel diagnostic molecular markers. Furthermore, numerous newly discovered molecular markers have contributed to a more precise classification of patients into distinct prognostic groups, leading to specific, more successful treatment protocols. The development of pharmacogenomic platforms and the application of molecular-targeted therapy have led to the individualization of therapy, tailoring it to the genetic profile of a disease in each patient. The ultimate goal of genome-based medicine is to develop gene therapy that will cure or prevent a disease by targeting disease-causing molecular defects. Additionally, cellular and tissue therapies have opened new possibilities for the personalized treatment of many patients. There is no doubt that we are getting closer to full implementation of personalized medicine in everyday clinical practice.

Genomics in the service of the diagnosis of rare diseases

The majority of rare diseases (~80%) are genetic and therefore identification of a specific gene defect in a patient is important. For over ten years, PCR-based techniques, Sanger DNA sequencing and multiplex ligation-dependent probe amplification (MLPA) have been used for the complete molecular diagnosis of monogenic diseases. Thus, the first data on molecular genetic characteristics of thalassemia (*HBB* gene), phenylketonuria (*PAH* gene), tetrahydrobiopterin deficiency (*PTS* gene) and congenital adrenal hyperplasia (*CYP21A2*gene) were reported for various populations, including the Serbian population (Stojiljković et al. 2006; Radmilović et al. 2010; Milačić et al. 2015).

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Recently, high-throughput targeted resequencing methodology based on a simultaneous analysis of all clinically relevant genes was introduced into routine diagnostic practice. Clinical exome sequencing enabled the diagnosis of genetically heterogeneous diseases, such as glycogen storage diseases, organic acidurias or mitochondriopathies. Using next-generation sequencing (NGS) methodology, it became possible to precisely identify a genetic defect in one gene among a dozen of suspected genes. Moreover, different diseases with partially overlapping clinical manifestations are being easily and accurately diagnosed (Skakić et al. 2017).

As a consequence of NGS application, identification of novel genetic variants has become a frequent event. To assess the pathogenic effect of all genetic variants detected for the first time, computational, expressional and/or functional analysis needs to be performed (Stojiljkovic et al. 2009, 2016; Skakic et al. 2017). Detailed functional characterization contributes to the unambiguous diagnostic interpretation of novel genetic variants worldwide. Furthermore, given that patients with particular genotypes are rare, it is important to report clinical observations about variants' effect on patients' phenotypes in order to contribute to the general knowledge about monogenic diseases.

In order to explain genotype-phenotype inconsistencies present in monogenic disorders such as phenylketonuria, non-coding gene regions should be investigated. Successful identification of several novel transcriptional regulators located in the promoter, intron and 3' region of the *PAH* gene shed new light on the fine regulation of *PAH* expression and contributed to a better understanding of PKU phenotype complexity (Stojiljkovic et al. 2010; Klaassen et al. 2017).

National databases that collect allele frequencies of clinically relevant genetic variations could be used to study gene flow among populations, human demographic history and most importantly, to optimize genetic testing in the country. The Serbian National Database was developed as a part of the FINDbase worldwide database to hold information specific to the Serbian population and serve as a valuable source of information for genetic specialists and medical doctors in Serbia as well as in other countries in southeastern Europe (Viennas et al. 2016).

An approach that combines Sanger and next-generation sequencing leads to timely and accurate genetic testing, sets definite diagnoses and enables rapid implementation of optimal therapy for patients with rare and other genetic diseases.

Personalized medicine in hematological malignancies: molecular markers for prognosis and follow-up of the disease

Application of the principles of personalized medicine is especially useful in the treatment of various types of cancer. Individual cancer is characterized by its distinctive and unique genetic and epigenetic profile. The study of the genetic basis of cancer contributes not only to a better understanding of its etiology, but it also greatly influences common clinical practice. Nowadays, standard clinical protocols for the treatment of oncological patients cannot be applied without the detection of specific genetic markers, representing either important diagnostic/prognostic indictors, or targets for the implementation of specific therapeutics. Hematological malignancies represented the ideal context for the implementation of personalized medicine programs.

Hematological malignancies are cancers of blood-forming tissues (bone marrow or lymph nodes). There are three main types of blood cancers: leukemia, lymphoma and multiple myeloma. Hematological cancers are rare diseases affecting predominantly the middle-aged and older population. Acute lymphoblastic leukemia (ALL) is the most common type of cancer in children. On the other hand, acute myeloid leukemia (AML) is very common in adults, whereas in children it is detected in about 15% of cases of all acute leukemia.

Leukemia is a genetic disease of somatic cells that are characterized by unlimited proliferation and/or impaired differentiation of progenitor cells, leading to the accumulation of immature blast cells in the bone marrow and peripheral blood, and resulting in hematopoietic failure.

The application of personalized medicine in the treatment of leukemia first started through the application of molecular methods for identifying the pretreatment karyotype of leukemia patients. Pretreatment karyotype is the most reliable tool used for risk stratification and the categorization of patients into certain risk groups, and entails the use of the appropriate treatment protocol. The most common genetic aberrations found in leukemia are translocations, and their detection is essential for precise risk stratification. For example, in childhood ALL there are four major risk-stratifying translocations (*BCR/ABL*, *MLL/AF4*, *TEL/AML1* and *E2A/PBX1*) whose presence allows the use of contemporary protocols, ensuring high-remission rates and long-term free survival (Lazic et al. 2010).

By using sensitive methods like reverse transcriptase polymerase chain reaction (RT-PCR) analysis, it is possible to detect not only common fusions, but also fusions that are less frequent (such as rare *NUP98/HOXC13* fusion, derived from t(11;12)(p15;q13) translocation), and every such case can provide new insight in the etiology of the disease itself (Tošić et al. 2009).

It is important to emphasize that common disease-causing translocations have been used as targets against which new, molecular-based therapies have been designed.

Contrary to ALL, AML patients are predominately characterized by the presence of normal karyotype (AML-NK). This cohort of AML patients is characterized as patients with intermediate risk. As a result of this imprecise classification, the overall survival of adult and pediatric AML-NK patients is only 30% and 60%, respectively. However, with

the application of new technologies (not only PCR, but DNA sequencing as well), new genetic markers with predictive values were detected. Some of these markers are mutations in fms-related tyrosine kinase-3 (*FLT3*), nucleophosmin (*NPM1)* and CCAAT/enhancer-binding protein alpha gene (*CEBPA*). Additional data on the mutational status of these genes contributed significantly to allocating the leukemia patients into the appropriate risk groups (Colovic et al. 2007; Krstovski et al. 2010; Kuzmanovic et al. 2012).

In chronic lymphocytic leukemia (CLL), the malignancy of mature B lymphocytes, the most powerful independent prognostic factor, is the somatic hypermutation status of rearranged immunoglobulin heavy variable (*IGHV*) genes. In around 50% of cases, the immunoglobulin heavy chain (*IGH*) rearrangements of CLL clones carry somatic hypermutations, and these patients suffer from more aggressive disease with shorter time to first treatment and overall survival than patients with unmutated *IGH* rearrangements (Karan-Djurasevic et al. 2012). The prognostic value of *IGHV* mutational status, besides its consistent association with the clinical behavior of CLL, lies in the fact that it does not change over time, and that it can predict the course of the disease at the time of diagnosis as well as at any other stage (i.e. regardless of the tumor burden).

Furthermore, the configuration of clonotypic *IGH* rearrangement and, hence, the structure of the B cell receptor (BCR) expressed on the surface of CLL cells, provides additional prognostic information, particularly in cases expressing so-called "stereotyped BCRs" (Agathangelidis et al. 2012). The structure and somatic hypermutation pattern of the BCR of the malignant clone represents its specific genetic signature. As a result of the enormous progress in CLL immunogenetic research in recent years, it is plausible that, in addition to *IGHV* mutational status, the information about the clonotypic BCR will soon be included into the risk-stratification systems and, ultimately, contribute to the tailoring of individualized treatment modalities.

To overcome the extreme clinical variability of CLL and improve individual patients' prognostication, especially in early-stage disease, other cellular and molecular prognostic markers have also been identified, some of which have been introduced into routine clinical practice (cytogenetic aberrations, CD38 and ZAP-70 expression, *TP53* mutations) (Antić et al. 2011). Finally, the recent genomic profiling studies of CLL patients, based on next-generation sequencing methodology, have identified several recurrently mutated genes in CLL (e.g. *NOTCH1*, *SF3B1*, *MYD88*, *BIRC3*, *NFKBIE*, *TP53*, *ATM*), some of which exert prognostic relevance even independently of *IGHV* mutational status (Baliakas et al. 2015).

Like no other technical innovation before, the application of NGS for the detection of somatic mutations has contributed to better understanding the biology of cancer in general, and hematological malignancies, as well. The application of NGS technology in the analysis of various types of leukemia has enabled the detection of previously unknown mutations, such as *IDH1* and *IDH2* mutations, which are very frequent in AML (as much as 20%), and which have a significant prognostic role (Virijević et al. 2016). Another interesting finding obtained through NGS technology is that acute leukemias are characterized by a very low number of somatic mutations, only 3-5 per patient (Marjanovic et al. 2016). The somatic mutation number is low even in comparison with other hematological malignancies. For example, the number of somatic mutations detected in rare primary diffuse large B cell lymphoma of central nervous system (DLBCL CNS) was up to 46 per patient (Todorović Balint et al. 2016). This has led to the conclusion that the epigenetic component must be considered as an important factor in the pathogenesis of leukemia.

Gene expression profiling (GEP) represents a technology that is very useful for identifying gene expression signatures associated with distinct types of leukemia. Almost every type of leukemia (acute or chronic) has its own gene expression profile. For example, acute promyelocytic leukemia (APL), characterized by translocation t(15;17), has a unique profile. It stands also for AML-NK. It has been shown that distinct GEP has a predictive value for relapse in AML-NK patients (Hackl et al. 2015). Numerous studies have revealed that certain genomic and transcriptomic profiles are associated with the pathogenesis of leukemia, but more importantly, that they have excellent value for accurate diagnosis and prediction of disease outcome. These new molecular markers have taken on an important role in everyday clinical practice and the treatment of hematological malignancies. For instance, in AML-NK, in addition to genomic profile, the analysis of the expression level of some individual genes, such as *WT1*, *BAALC*, *MN1*, and *EVI1*, is recommended due to their significant prognostic impact (Marjanovic et al. 2017). In another type of hematological malignancy, in multiple myeloma, the expression of the cereblon gene (*CBN*) can influence prognosis in patients treated with modern immunomodulatory drugs (Bila et al. 2016). All these findings have led to a deeper insight into the genomic and transcriptomic landscape of different hematological malignancies and, consequently, to the discovery of novel prognostic markers and innovative therapeutic strategies, resulting in better survival of patients with hematological malignancies.

Pharmacogenomics

Pharmacogenomics is a major cornerstone of personalized medicine. Pharmacogenomics identify genomic and clinical information in order to predict the response to treatment of a person (Pavlovic 2009) and establish guidelines for using therapeutics according to the individual's genomic, epigenomic and transcriptomic profile (Mizzi et al. 2017). It is the tailoring of drug treatments to people's genetic makeup.

Pharmacogenomics completely changed the old therapeutic paradigms of "one dose fits all patients" and "trial-and-error" prescription, into a novel, personalized concept of "matching the right therapeutic and the right dose to the specific genetic signature of the patient".

Before administering certain drugs to a patient, it is mandatory to perform defined pharmacogenomic testing. For some drugs, pharmacogenomic analysis is recommended but for most drugs, the testing used today is only informative. Several genomic, epigenomic, transcriptomic and proteomic markers have already been introduced in routine diagnostic, prognostic and therapeutic protocols for many diseases (Georgitsi et al. 2011; Stojiljkovic et al. 2011).

One of the first clinically recognized pharmacogenomic markers, now mandatory for testing prior to administering immunosuppressive therapy (6-mercaptopurine, azathioprine and thioguanine), is a variation in the *TPMT* gene (thiopurine S-methyltransferase) (Pavlovic and Zukic 2010; Pavlovic et al. 2012). Mercaptopurine drugs are used in the treatment of various diseases and the doses of these drugs are routinely adjusted according to the patient's *TPMT* genotype in childhood ALL, inflammatory bowel disease (IBD), autoimmune diseases and in transplantation medicine (Jojić et al. 2003; Dokmanovic et al. 2006, 2008). Furthermore, the variable number of tandem repeats (VNTR) in the *TPMT* promoter is a novel and promising pharmacogenomic marker (Zukić et al. 2010). Testing the number and type of the repeats, VNTR architecture can be potentially used as a pharmacogenomic marker to predict toxicity due to 6-MP treatment in childhood ALL patients (Kotur et al. 2012). The *TPMT* promoter VNTR region has the most benefit as a pharmacogenomic biomarker at the very beginning of the maintenance therapy for pediatric ALL patients (Kotur et al. 2015). The key drug in the intensification phase in therapeutic protocols for children with ALL is an antifolate chemotherapeutic agent, methotrexate. Implementation of pharmacogenomic testing for pharmacogenomic markers involved in methotrexate metabolism in current protocols will lead to the optimization of pediatric ALL therapy (Lazić et al. 2017). Glucocorticoid drugs, due to their ability to induce apoptosis, are a central component of many chemotherapy protocols, including protocols used for the treatment of leukemias and IBD. Several pharmacogenomic and pharmacotranscriptomic markers have been intensively studied in order to avoid drug dependence, drug resistance, treatment-related side effects and to predict the outcome of glucocorticoid therapy in pediatric IBD patients (Lucafòet al. 2017). The therapeutic response to the drug etanercept, a TNF-α blocker, in patients with rheumatoid arthritis (RA) was assessed in order to increase treatment efficacy. RA patients who are genetically low TNF-α and IL-6 producers may be the most suitable candidates for anti-TNF-α therapy (Jančić et al. 2013).

Population pharmacogenomic research has shown that the study of pharmacogenomic markers in various populations is of great importance. Comprehensive data repositories that record the prevalence of clinically relevant genomic variants in populations worldwide, including pharmacogenomic biomarkers, are valuable tools that can be exploited not only to develop guidelines for medical prioritization, but most importantly, to facilitate the integration of pharmacogenomics into healthcare systems and to support preemptive pharmacogenomic testing (Viennas et al. 2016). The microattribution approach was implemented in the assessment of the pharmacogenomic biomarkers allelic spectrum in 18 European populations. There are significant interpopulation differences in pharmacogenomic biomarker allele frequencies. The establishment of particular population-specific or European genotyping panels could be used preemptively, prior to or at the point of taking the required medication (Mizzi et al. 2017). This would be beneficial because a reduction in healthcare expenditure will be realized, but also a better quality of treatment of patients and, most importantly, better quality of patients' lives will be achieved.

Molecular-targeted therapy

Molecular-targeted therapy is the best example of accurate, causal therapy since the disease-causing molecular defect is a target of a drug. Most molecular-targeted therapies are used in the treatment of cancer. They have contributed to the fact that several cancers are becoming chronic diseases.

Most common translocations detected in hematological malignancies have been used as targets of novel molecular-based therapies. Such is the case of the Philadelphia chromosome (t(9;22)(q34;q11)), or *BCR/ABL* fusion, that is prevalently found among chronic myeloid leukemia (CML) patients (in 95% of cases), but is also present in ALL (in 30%), and very rarely in some other hematological disorders (Colovic et al. 2004). Imatinibmesylate is one of the first success stories of molecular-targeted therapy (Druker et al. 1996). It was designed as a specific inhibitor of the aberrant tyrosine-kinase protein, encoded by *BCR/ABL* fusion. Imatinibmesylate specifically binds the ATP-binding site of the aberrant tyrosine kinase, blocks it and prevents its activation. Thus it prevents uncontrolled proliferation of leukemic cells and introduces other homeostasis preservation mechanisms, such as apoptosis. Due to the application of imatinibmesylate in the treatment of CML and ALL patients, the cure rate, as well as overall survival rate, have been immensely improved. Another success story is noted in the treatment of a specific type of AML patients (AML-M3), or APL patients. APL patients are characterized by the presence of *PML/RARA* fusion (t(15;17)(q22;q21)) in more than 85% of cases. This *PML/RARA* fusion encodes the aberrant transcriptional factor that inactivates all RARA-dependent genes, causing differentiation blockage. It was found that *all-trans* retinoid acid (ATRA), a substance belonging to the group of vitamin

A-related compounds (retinoids), triggers PML/RARA degradation, thereby activating the differentiation process and also causing proliferation inhibition (Degos 1992). Imatinibmesylate and ATRA are so far the most successful examples of molecular-targeted therapies, not only in leukemias but in all cancers.

In recent years, there have been many examples of the development of molecular therapeutics specific for missense, splice site and in-frame stop genetic variants. Due to these novel therapeutic strategies, the identification of gene variants has become increasingly important for the implementation of individualized treatment. It has been shown that carriers of certain variants should be treated with compatible molecular therapeutics (e.g. Kuvan for missense *PAH* gene variants). This molecular-specific therapy could become an important approach in future for enhancing the quality of life and improving the life-time of PKU-affected individuals (Stojiljkovic et al. 2014).

Gene therapy

Gene therapy, introducing genetic material into a cell to fight or prevent disease, had its beginnings over thirty years ago and is still considered very controversial in many scientific communities (Pavlovic et al. 2014). One of the first attempts at this therapeutic gene transfer technology was gene therapy of β-thalassemia syndrome, which arose as an answer to the many limitations of bone marrow transplantation as the only definitive cure for this monogenic disease. The goal of this gene therapy is the substitution of a defective or missing protein by introducing an intact, undamaged copy of the faulty gene (β-globin gene) or by induction of a gene that modifies the effect the defected gene has on a cell (gene modifiers) (Pavlovic et al. 2015). β-thalassemia modifiers represent promising therapeutic genes and include genes within (γ-globin genes) and outside globin gene loci (*KLF1*).

Among the most potent modifier genes considered for gene therapy of β-thalassemia syndromes are γ-globin genes (*HBG1*, *HBG2*). Persistent expression of these fetal globin genes, which leads to increased production of fetal hemoglobin (HbF:$_2\gamma_2$), could ameliorate the severity of the β-thalassemia phenotype as γ-globin polypeptide chains compensate for the lack of functional β-globin chains. It is well documented that variants within the γ-globin gene promoter can cause its overexpression and that some of them exert their effect under conditions of erythropoietic stress characteristic for β-thalassemia patients (Kollia et al. 2008; Ugrin et al. 2016).

In the recent years, *KLF1* emerged as a potential gene target for gene therapy of β-thalassemia. Namely, experimental evidence suggests that variants within the *KLF1* regulatory region can lead to reduced *KLF1* transcription and thus to increased expression of fetal globin genes though the regulation of *BCL11A*, a known γ-globin gene repressor (Giardine et al. 2011; Radmilovic et al. 2013).

Gene therapy is one of the most promising approaches for the treatment of β-thalassemia patients. Understanding the mechanisms that govern human fetal globin gene switching will bring us closer to gene manipulation as a permanent cure for β-thalassemia syndromes and individualized therapy as a therapy of the future.

Stem cell and tissue therapy in personalized medicine

Stem cells (SCs) are undifferentiated cells with almost unlimited potency for self-renewal and differentiation in various cell types. Not long ago it was discovered that all the organs of our bodies have a pool of SCs serving for repopulation of the cells of the organ. These cells have certain features making them interesting not only for research but also for the treatment of injuries and various diseases. The fact that everyone has their own pool of SCs (which can be isolated and propagated *in vitro* up to a therapeutic number at any moment during the lifespan) offered a specific personalized approach in regenerative medicine. Such individualized therapy overcomes most of the ethical and health issues that were previously connected to the application of SCs, such as graft versus host disease.

Various genetic and epigenetic factors are specific for SCs and make them different from other cell types. The fundamental discovery that a cocktail of four genes is capable of converting differentiated adult fibroblast into pluripotent stem cell presented a completely new perspective in research and medicine (Takahashi and Yamanaka 2006). SCs have a potential to differentiate into various cell types. SC differentiation is driven by specific molecules; either these factors are excreted by the cells in the specific microenvironment where they are injected, or the factors are added *in vitro* before injection. SCs are capable not only of making specific cells but, if grown in scaffolds, constructing complete organs. This is a goal of tissue therapy, which is a promising prospect in stem cell research with personalized approach.

Among adult SCs, there is a specific population called mesenchymal stem cells (MSCs). MSCs are isolated from various tissues, such as the umbilical cord, tendon, dental pulp, etc. They are of mesodermal origin, but thanks to their plasticity, they are able to differentiate into various cell types, not only of mesodermal origin (neural, endothelial). They are easy to isolate using a minimal invasive procedure, which makes them very attractive for regenerative medicine. Currently, there are more than 50 clinical trials conducted to explore their potential in the treatment of various diseases. In one of these trials, the effects of usage of autologous MSCs isolated from the adipose tissue of patients with knee and hip osteoarthritis was studied. The results showed an improvement in all scores that were scanned during the one-year follow-up period (Spasovki V, unpublished data). None of

the patients had side effects and the quality of their everyday life greatly improved. None of therapies available at this moment show such efficacy and maintenance of improvement.

Nowadays, there are many diseases that can be treated using autologous SCs: hematopoietic diseases, osteoarthritis, different skin defects arising from diseases as well as from burns and other injuries. As our knowledge about the features of SCs grows, it seems that treatment possibilities are almost unlimited. Individualized therapy, which is becoming the most specific trend in medicine, is something that stem cell therapy fulfils in all aspects. This is also why this kind of therapy is the therapy of the future.

Conclusion

Genomics and other -omics, like all other great breakthroughs in science, have been taken up with enthusiasm, but have also provoked skeptical responses among researchers and healthcare practitioners. The dilemma will soon be solved since the full integration of innovative personalized treatment into clinical practice is expected in next decade. Until then, intensive bench research continues in order to make its way to the patient's bedside.

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