

Персонализированный подход в гастроэнтерологии и гепатологии: достижения 2016 года

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Последние достижения клеточной и молекулярной биологии позволили сделать существенный рывок в понимании патогенеза многих заболеваний, их диагностики, лечения и профилактики. С помощью современных методов молекулярного, генетического, эпигенетического, микробиологического и биохимического анализа, в том числе в рамках полного исследования генома человека, мы получили возможность выявлять диагностически значимые точечные мутации или полиморфизм отдельных нуклеотидов. С помощью высокопроизводительного анализа нуклеотидных последовательностей стало возможным проведение одновременного исследования тысяч генов (ДНК) или молекул, связанных с генами (РНК, белки), что позволяет определить индивидуальный генетический профиль больного («генетическую подпись») или оценить индивидуальные особенности микробиома, включая его патогенный потенциал. Подобная информация позволяет определить индивидуальную предрасположенность к заболеваниям, более точно установить прогноз болезни и определить эффективность выбранной стратегии лечения («персонализированная медицина»). Все указанные методы уже сегодня дают возможность улучшить диагностику, лечение и профилактику многих заболеваний человека.

Ключевые слова: структура человеческого генома, полногеномное ассоциативное сканирование, проект по исследованию микробиома человека, профиль генной экспрессии, индивидуализированная противоопухолевая терапия, блокада иммунных контрольных точек.

Molecular and cell biology have resulted in major advances in our understanding of disease pathogenesis as well as in novel strategies for the diagnosis, therapy and prevention of human diseases. Based on modern molecular, genetic, epigenetic microbiologic and biochemical analyses it is, on the one hand, possible to identify disease-related point mutations and single nucleotide polymorphisms in the context of *genome-wide association analyses* (GWAS). On the other hand, using high throughput array and other technologies, it is possible to simultaneously analyze thousands of genes (DNA) or gene products (RNA and proteins), resulting in an individual gene or gene expression profile ('signature') or to characterize the individual microbiome and its pathogenetic potential. Such data increasingly allow to define the individual disease predisposition or risk and to predict disease prognosis as well as the efficacy of therapeutic strategies in the individual patient ('personalized medicine'). All these aspects have greatly contributed to the recent advances in the diagnosis, treatment and prevention of human diseases.

Key words: human genome organization, genome-wide association studies, human microbiome project, array/ signature analyses, personalized tumor therapy, immune checkpoint inhibition.

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Introduction

The basic aspects of molecular and cell biology are not only integral part of biomedical research but are also increasingly translated into patient's care. The genetic material of all living organisms is made up of DNA that in its entirety makes up the individual's genome. In recent years, DNA sequencing, including whole genome sequencing (WGS), and omics analyses have identified genetic markers and signatures which allow to predict the individual disposition/risk for a specific disease, its prognosis and natural course as well as the response to therapy.

Three major global consortia have been launched and in part completed during the last decades. All of them continuously transform basic biomedical research as well as its translation into clinical and personalized medicine with a tremendous potential for the diagnosis, treatment and prevention of human diseases. Based on recent discoveries in basic sciences and their evaluation in randomized clinical trials (RCTs), clinically relevant novel strategies increasingly enter clinical practice as 'personalized medicine'.

Human Genome Organization

The international human genome organization (HUGO) project established the complete sequence of the human genome more than ten years ago [1, 2]. In order to utilize the sequence information from the

HUGO project for research as well as for clinical applications and to define the function(s) of newly identified genes, collectively termed 'functional genomics', strategies were developed to globally analyze genomic DNA sequences as well as their cell-, tissue- or organ-specific expression profile. Using chips, so-called 'microarrays' (Fig. 1), thousands or ten thousands of single-stranded DNA species, reverse transcribed RNA (cDNA) or oligonucleotides of known sequence provide a global gene (genomics), gene expression (transcriptomics, proteomics) or metabolite (metabolomics) profile («signature») that is characteristic for the disease of individual patient.

Genome-wide Association Studies

In 2005 the international haplotype map (HapMap) project was initiated to identify, based on genome-wide association studies (GWAS) in ethnically different populations, single nucleotide polymorphisms (SNPs) and their association with specific human diseases and individual phenotypic characteristics [3, 4]. Through GWAS an increasing number of gene loci have been identified that are associated with individual phenotypic traits, such as hair or eye color, height, body mass index and others as well as with the individual predisposition to develop a specific disease (Fig. 2) [3, 4]. Examples in the field of gastroenterology are inflammatory bowel diseases [5, 6]. Furthermore, polymorphism in the apolipoprotein C3 gene has

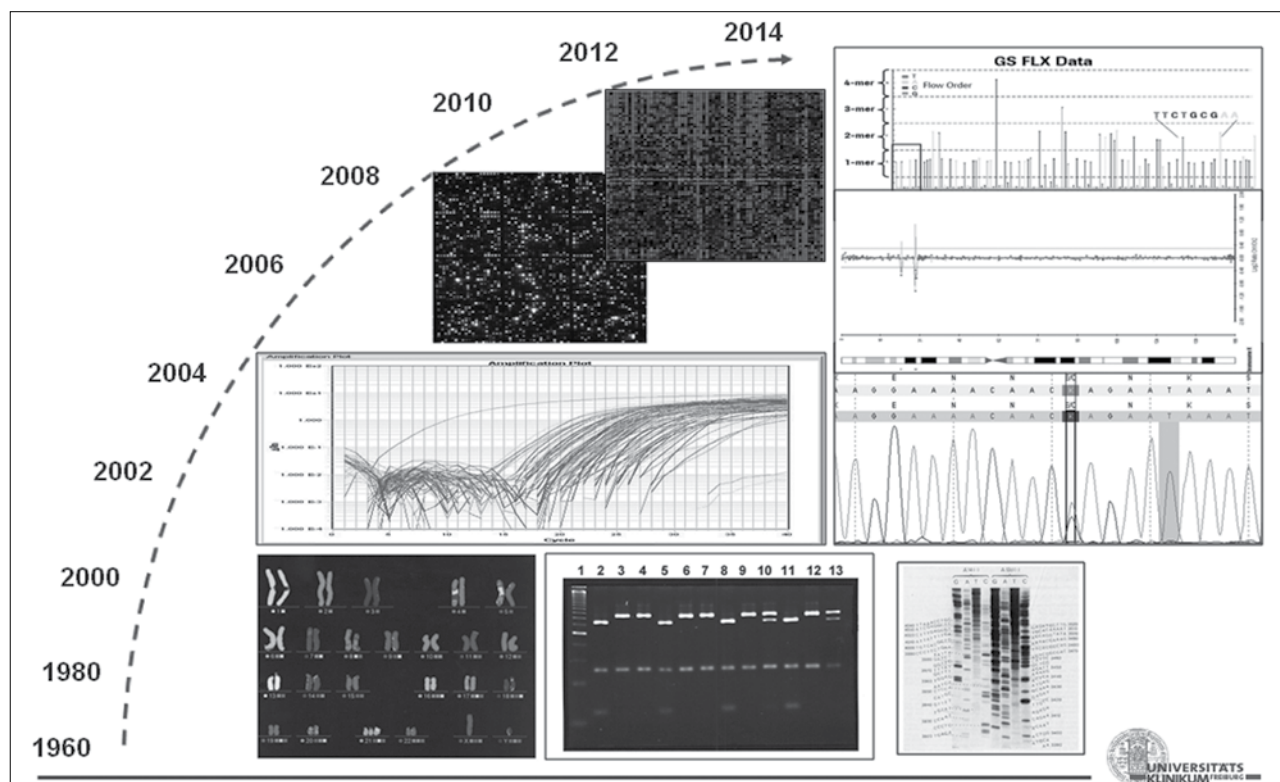


Fig. 1: Molecular and cell biology analyses: 1960s — up to date.

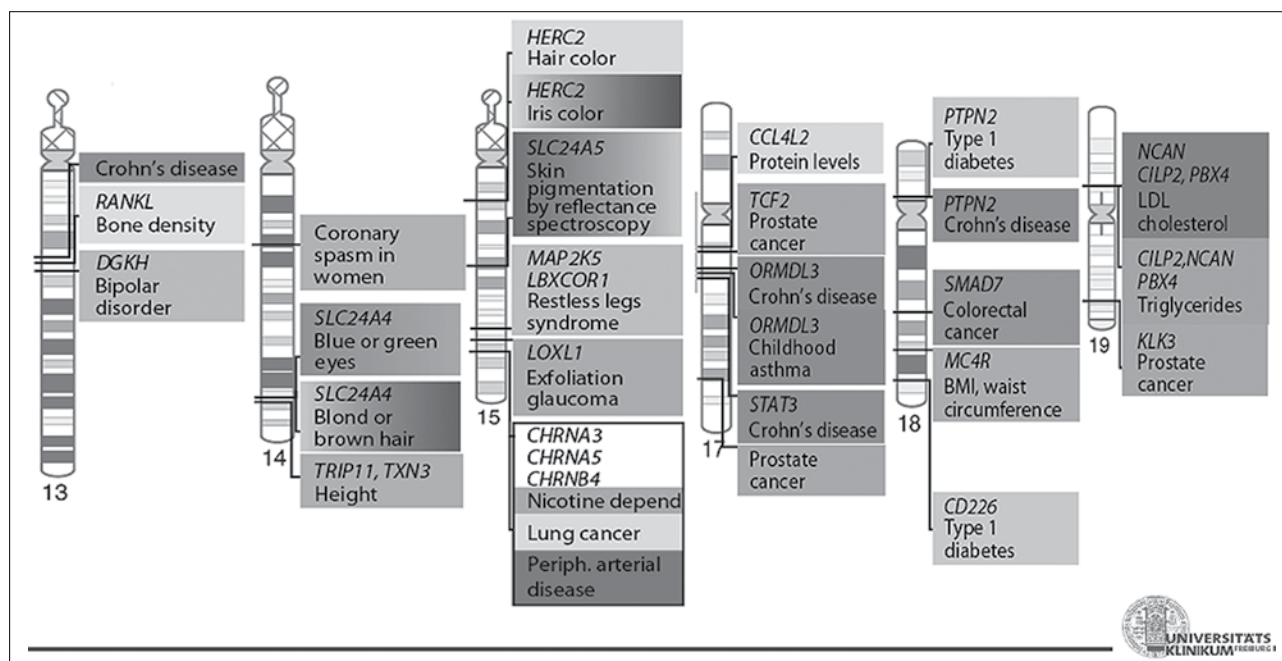


Fig. 2: HapMap for chromosomes 13–15 and 17–19 [3].

recently been found to be associated with non-alcoholic steatohepatitis (NASH) and insulin resistance [7]. For hepatocellular carcinoma (HCC), a G/G polymorphism in the epidermal growth factor gene (8) and other variants were found to be associated with an increased tumor risk [9, 10]. Further, genetic variants are associated with other liver diseases [11, 12], benign recurrent intrahepatic cholestasis (BRIC), gallstone formation [13], drug-induced liver injury (DILI), and cryptogenic cirrhosis.

Taken together, GWAS allow an increasingly better understanding of disease pathogenesis and more accurate assessment of the individual risk to develop a specific disease. Clinically, this may eventually translate into improvements in disease prevention, early diagnosis and therapy. It should be cautioned, however, that the contribution of a defined SNP to the risk assessment for a given disease must be carefully weighed against established clinical parameters. Despite the tremendous potential of GWAS, the clinical relevance of SNPs for the prediction of individual traits or disease risks needs to be carefully evaluated [14, 15].

Human Microbiome Project

A third global consortium, the human microbiome project (HMP), was established in 2007. The HMP and the 'Metagenomics of the Human Intestinal Tract (Meta-HIT) Consortium Europe' aim at the sequencing of all microbes (eukaryotes, archaea, bacteria, viruses) that inhabit specific body sites, such as the mouth cavity, throat and

airways, stomach and intestine, urogenital system and skin (Fig. 3). Important factors for the composition of the intestinal microbiome are diet [16, 17], lifestyle and exposure to certain pharmaceuticals. In this context it appears that the administration of low-dose penicillin early in life has lasting effects on the body mass index (obesity) through alteration of the intestinal microbiome [18, 19]. Recent evidence further suggests that human genetic variation also influences the abundance of specific members for example of the intestinal microbiome [20].

Recent data clearly demonstrate that specific members of the microbiomes are associated with human health as well as human diseases [21, 25]. Clinical examples are a causal relationship between the gut microbiome and kwashiorkor [26], obesity [27], the obesity-associated HCC [28], metabolic syndrome [29], immune responses [30], inflammatory bowel diseases [31, 32] and colorectal cancer [33] (Fig. 4).

In addition, the intestinal microbiome plays a central role in drug metabolism, e.g., of sulfasalazine, levodopa and irinotecan. Taken together, the emerging data suggest that the detailed characterization of the human microbiome composition, function and variation across different body sites will reveal important commensal host-to-microbe as well as microbe-to-microbe interactions that may play a role in human health and disease with diagnostic as well as preventive and therapeutic implications, e.g., by fecal microbiota transplantation [34, 35].

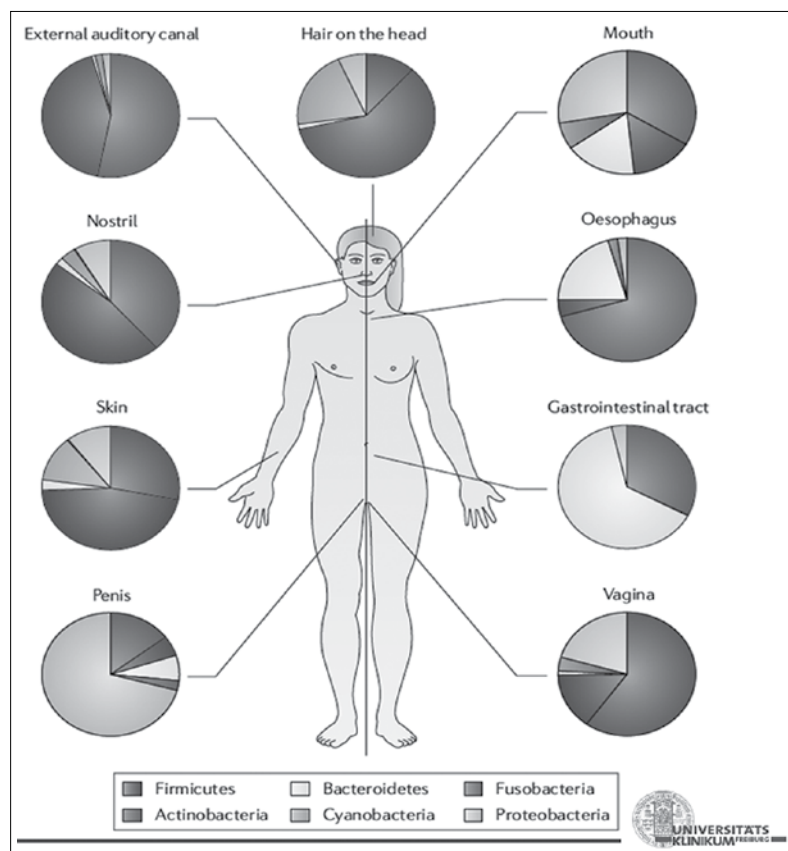


Fig. 3: Different microbiomes in healthy individuals [22].

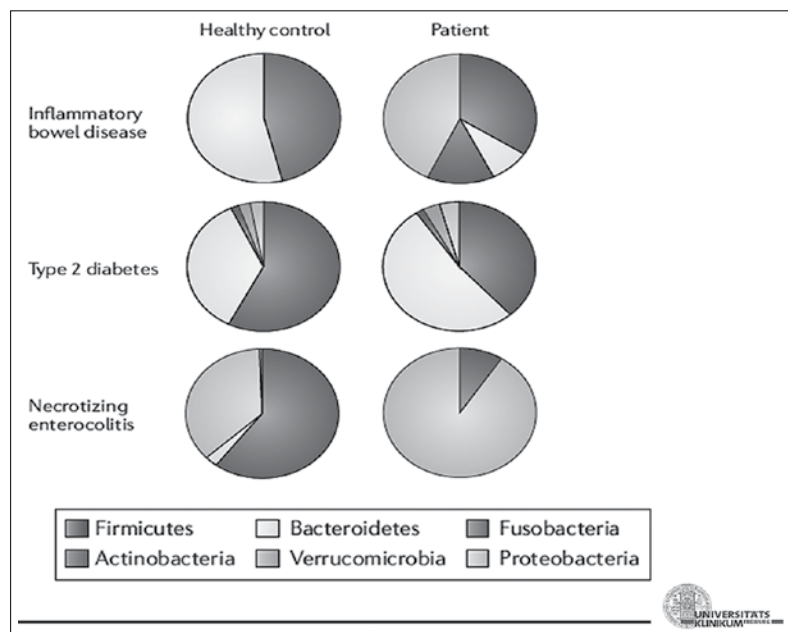


Fig. 4: The intestinal microbiome in healthy individuals and patients [22].

Molecularly Stratified Personalized Tumor Therapy

One of the major objectives of the translation of discoveries in basic biomedical sciences into clinical applications ('translational medicine') is

the increasingly individualized therapy of patients with gastrointestinal and hepatobiliary tumors ('personalized tumor therapy'). Studies addressing the predictive power of these analyses for different tumor entities are presently under way [36, 37].

In the following, clinical examples for the targeted therapy of selected malignant gastrointestinal tumors (GITs) with monoclonal antibodies (mabs) and low molecular weight tyrosine kinase inhibitors (TKI, nibs) alone or in combination with conventional chemotherapeutic agents [38] as well as by the newly emerging concept of cancer immunotherapy through immune checkpoint inhibition [39] are presented to highlight the recent developments in these areas. Overall, these strategies significantly improved overall or recurrence-free patient survival compared to conventional chemotherapies.

Colorectal cancer. For patients with advanced or metastasized colorectal cancer (CRC), for example, the mabs: bevacizumab, cetuximab and panitumumab have already been introduced into clinical practice [40, 41]. In this context, it was shown that only patients with wild-type KRAS or wild-type NRAS tumors will benefit from anti-epidermal growth factor receptor (EGFR) therapies with cetuximab or panitumumab [42-46]. Therefore, the determination of the KRAS and NRAS status (exons 2-4) before therapy allows to predict whether the patient will potentially benefit from these drugs [47-50]. CRC patients with BRAF mutations (V600E) have only a minor benefit from anti-EGF strategies but may benefit from the EGFR inhibitor panitumumab, the BRAF inhibitor dabrafenib and the MEK inhibitor trametinib [51]. Further, patients with wild-type RAS, BRAF and P13-Kinase genes do not respond to anti-EGFR antibodies. Some of these patients overexpressing HER2, however, may benefit from the double targeting with trastuzumab (anti-HER2) and lapatinib (anti-EGFR)

[38, 52]. Interestingly, in patients not qualifying for treatment with oxaliplatin or irinotecan, the combination of capecitabine and bevacizumab was shown to be superior to capecitabine monotherapy [53].

An interesting new development in personalized oncology is the possibility to perform mutation analyses not only in tumor tissue but also in circulating tumor cells (CTC) or circulating tumor DNA (ctDNA) ('liquid biopsy') [54, 55]. These analyses allow to monitor therapy and to identify newly emerging therapy resistant tumor cell clones [56, 57].

A novel concept in the treatment of malignant diseases is the inhibition of immune checkpoints. In this context, the anti-CTLA-4 antibodies ipilimumab and temilimumab, and the anti-PD1 antibodies nivolumab, pembrolizumab and others have been already introduced in the treatment of patients with malignant melanoma and lung cancer [58, 59]. Among patients with advanced CRCs, those with a defect of mismatch repair (MMR) system, characteristic for patients with hereditary CRCs or hereditary non-polyposis colorectal cancer (HNPCC), seem to benefit most from immune checkpoint inhibition. Results from randomized clinical trials are expected in the near future.

Gastric cancer. The medical treatment of locally advanced or metastasized gastric cancer is still a major challenge. Apart from resection of gastric cancer and extensive lymph node dissection, neoadjuvant chemotherapies as well as radiochemotherapy have been evaluated in clinical trials. In this context the molecular classification of the tumor increasingly allows to subclassify this malignancy with respect to the prognosis and the efficacy of targeted therapy. Only patients with human epidermal growth factor receptor-2 (HER2) positive metastasized gastric cancer benefit from treatment with trastuzumab (60), similar to patients with HER2-positive breast cancer. Also, ramucirumab as an anti-VEGF/ VEGFR strategy has recently been shown to prolong patient survival [61, 62]. Several other targeted therapies are being presently studied in randomized clinical trials.

Pancreatic cancer. As compared to gemcitabine monotherapy, combination therapies, such as oxaliplatin, 5-fluorouracil plus folinic acid (OFF), nanoliposomal irinotecan, 5-fluorouracil plus folinic acid [63] or gemcitabine plus nab-paclitaxel [64] have been shown to result in prolonged

overall or recurrence-free survival. Several novel therapeutic strategies, such as the modulation of epigenetic alterations by the inhibition of histone deacetylases or methyltransferases as well as of DNA methyltransferases, the inhibition of immune checkpoints or the therapeutic vaccination [65] are expected to result in an improved survival of patients with locally advanced or metastasizing pancreatic cancer.

Gastrointestinal stroma tumors. The treatment of unresectable or metastasized gastrointestinal stroma tumors (GISTs) has been revolutionized by the finding of the mutational activation of the KIT proto-oncogene in about 80% and the mutational activation of the related TK receptor platelet-derived growth factor receptor alpha (PDGFRA) in some KIT-negative tumors. This led to effective systemic therapies with small molecule inhibitors of receptor TKs, such as imatinib [66], sunitinib [67] and regorafenib [68]. Other TK inhibitors are presently in clinical evaluation (e.g., sorafenib, nilotinib, dasatinib, ponatinib and pazopanib).

Conclusions and perspectives

Recent advances in cell and molecular biology resulted in an increasingly detailed understanding of the pathogenesis of gastrointestinal and liver diseases. With the rapid development of novel molecular, genetic/epigenetic, microbiological and biochemical analyses it is now possible to identify, on the one hand, disease-related point mutations and SNPs. On the other hand, based on array technologies, thousands of genes, RNA species, proteins or metabolites can be analyzed simultaneously to yield a disease-specific profile ("signature") that are relevant for diagnosis, prediction of disease prognosis and efficacy of different therapeutic strategies at the individual level.

Taken together, basic biomedical research has made major advances in recent years and holds the promise to increasingly provide individual diagnostic, preventive as well as therapeutic options for patients with inherited or acquired, malignant or non-malignant diseases.

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