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Genetics and pharmacogenomics of diffuse gliomas

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ABSTRACT

Rapidly evolving techniques for analysis of the genome provide new opportunities for cancer therapy. For diffuse gliomas this has resulted in molecular markers with potential for personalized therapy. Some drugs that utilize pharmacogenomics are currently being tested in clinical trials. In melanoma, lung-, breast-, gastric- and colorectal carcinoma several molecular markers are already being clinically implemented for diagnosis and treatment. These insights can serve as a background for the promise and limitations that pharmacogenomics has for diffuse gliomas. Better molecular characterization of diffuse gliomas, including analysis of the molecular underpinnings of drug efficacy in clinical trials, is urgently needed. We foresee exciting developments in the upcoming years with clinical benefit for the patients.

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1. Introduction

The role of the genetic code, of a patient or a tumor, has been appreciated for a long time as an important factor in efficacy of pharmaceutical agents. Although the terms pharmacogenetics and — genomics are used interchangeably in literature (Pirmohamed, 2001), we refer to pharmacogenetics as the constitutional chromosomal variations and mutations that can influence metabolism of drugs. Pythagoras already noted in 510 B.C. that ingestion of fava beans was lethal to only some individuals; if the fava beans had been eaten for medical purposes, we would categorize this congenital trait here as pharmacogenetics. Pharmacogenomics is the discipline that comprises all therapies which exploit information of somatic, molecular alterations of the cancer genome. It has gained enormous interest in the past decades and is the focus of this review. Specific molecular alterations can serve as markers for survival, called prognostic marker or for response to treatment (predictive). In personalized therapy a tumor is tested for the presence or absence of such a marker which enables the clinician to decide on the right timing of treatment and, when indicated, to prescribe a drug that targets this specific alteration. Drugs are often designed to target pathways in which these alterations act. Not all pathways are well-defined and 'accidental' discoveries may also result in the identification of a relation between a molecular alteration and increased sensitivity to a specific agent, while the biological interaction is not (yet) explained. In other scenarios, a drug is known to target molecular features, but the specific marker needed to select patients that are sensitive to this treatment has not been identified. We still consider these drugs to have pharmacogenomic features, but true personalized therapy is performed when prescribing drugs on the basis of genomic information of the tumor of an individual patient (companion diagnostics).

In recent years, several drugs with pharmacogenomic features have been designed, most of which belong to the family of kinase inhibitors. More than 500 kinases have been identified; among other functions, these cellular proteins mediate transduction of signals that influence proliferation, migration and survival of (cancer) cells (Manning et al., 2002). Although the success rate of agents that target these kinases varies, they are promising compounds as the knowledge on pathways involved in tumorigenesis rapidly increases. At present, the possible therapeutic effects of drugs targeting these kinases have already changed experimental approaches considerably. There is a diversity in markers for tumor (sub)types; in addition to classic genomic alterations, markers can be of epigenetic or non-coding RNA origin.

2. Pharmacogenomics: boosted by the new generation of laboratory techniques

When genomic applications in clinical care are discussed, a short description on the technical aspects is required to comprehend the rapidly growing potential as well as the limitations of the genetic code as a diagnostic tool. In the 1950s, after the fundamental discovery that normal human cells contain 46 chromosomes (Tjio & Levan, 1956), chromosomal alterations were detected using karyotyping. Karyotyping enables identification of gross chromosomal abnormalities in diseases such as cancer and congenital disorders. A limitation of this early cytogenetic technique was the need for chromosomes in metaphase (dividing cells) and the inability to detect small aberrations. It took 20 more years before substantial improvement was achieved through the introduction of fluorescence in situ hybridization (FISH) (Van Prooijen-Knegt et al., 1982). FISH uses chromosome region-specific probes with a fluorescent signal. These probes hybridize to the complementary DNA-sequence in the sample, and then the presence and number of DNA molecules in the specific region in the tumor can be analyzed. However, with this technique only one or few chromosomal locations could be studied in one experiment. In the 1990s comparative genomic hybridization (CGH) was developed which offered a much higher spatial resolution compared to karyotyping for the analysis of chromosomal aberrations on a genome-wide basis. With CGH, signal of fluorescently labeled patient-tumor DNA (e.g. red) is compared to fluorescently labeled normal human DNA (e.g. green). The ratio of these signals was translated to copy number changes for each location yielding a resolution of 5–10 Mb (Kallioniemi et al., 1994; Oostlander et al., 2004). The technique was soon replaced by array CGH (Snijders et al., 2001) to yield ever increasing resolution (Ylstra et al., 2006). Array CGH was still limited to assessment of numerical copy number changes alone, omitting detection of balanced translocations and (point) mutations (Smeets et al., 2011).

Since 1977, mutations in genes of interest have been analyzed by Sanger sequencing. Often, these techniques were used in complementary fashion; array CGH to detect chromosomal aberrations, while genes within regions of interest were analyzed one-by-one by sequencing analysis. Next-generation sequencing (NGS), or massively parallel sequencing (MPS) is the new kid on the block in genome research. This technique allows high resolution analysis of the entire genome of multiple samples in one experiment whilst yielding chromosomal translocations, copy number measurements and point mutations. MPS has shown to work robust in many laboratories on DNA isolated from routinely collected clinical material (usually formalin fixed paraffin embedded, (FFPE)) (Kerick et al., 2011; Smeets et al., 2011). A major challenge of this technique is to cope with the enormous volume and complexity of data it provides. The constant and dramatic drop in costs for this technique (www.genome.gov/sequencingcosts, 2012) combined with the development of application to FFPE samples are now making MPS accessible for implementation in the routine diagnostic setting (Fig. 1).

3. Molecular markers that influence clinical decision-making in medical oncology

Cancer researchers and scientists at pharmaceutical companies are currently interested in the development of companion diagnostics: the detection of markers for clinical or therapeutic decision-making. Ideally, this enables unequivocal stratification of patients and selection of the proper medication and dosage. The presence of several markers already influences clinical decision-making in medical oncology, e.g. ERBB2(17q21-22) amplification in breast cancer, EGFR(7p11) mutations in non-small cell lung carcinoma (NSCLC), KRAS(12p12) mutations in colorectal carcinoma (CRC), c-KIT(4q12) in gastro-intestinal tumors (GIST), and BRAF(7q34) mutation in melanoma (Tran et al., 2012). Here, we highlight the corresponding agents, because their discoveries are prime examples of personalized therapies with pharmacogenomics features.

With respect to breast cancer, the ERBB2 gene encoding HER2 (human epidermal growth factor Receptor 2) is amplified in 20% to 30% of all breast tumors. The monoclonal antibody trastuzumab inhibits proliferation of HER2 positive cancer cells by selectively blocking the receptor and is in use for early and metastatic breast cancer. Trastuzumab was approved by FDA in 1998 and is since 2010 also in use for gastric cancers showing HER2 overexpression (Slamon et al., 2001; Romond et al., 2005; Bang et al., 2010). It took seven more years before gefitinib, a 'small molecule' EGFR tyrosine kinase inhibitor (EGFR-TKI) was introduced for treatment of advanced NSCLC with EGFR-mutation (Mok et al., 2009). In CRC, KRAS mutation is highly predictive for response to antibodies such as panitumumab or cetuximab. Only patients with EGFR-mutated tumors not bearing KRAS mutation seem to benefit from treatment with these agents (Amado et al., 2008). Another example of pharmacogenomics in personalized cancer therapy is the small molecule kinase inhibitor imatinib which is effective in chronic myeloid leukemia (CML) patients with a BCR-ABL translocation (Druker et al., 1996). Due to high homology between binding sites, c-Kit mutations in GIST can also be targeted by imatininb. Recurrence-free survival in GIST-patients improved significantly after the introduction of imatinib (Joensuu, 2002; Dematteo et al., 2009). More recently