Mutational profiling of glioblastoma
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Summary
Glioblastoma is the most common and the most malignant brain tumor in human, with a dismal prognosis. Despite an intensive treatment based on surgical resection, radiotherapy and chemotherapy with temozolomide, the average survival is only 15 months. For this reason, the development of new therapies is very important.

In general, the new anticancer therapies, developed the last decade, are ‘targeted’ therapies as the proteins and signaling cascades, which are activated in cancer cells, are specifically inhibited. A group of proteins, important for this aim, is formed by ~550 kinases, a family of proteins with enzyme activity which can place a phosphate group on other proteins or lipids. By this phosphorylation, the target protein or lipid is activated or, less frequently, inactivated. Because of this key function, kinases form an important factor in signaling cascades in cells, and therefore also in cancer cells. Because most of the kinases work activating, they form good targets to inhibit. The inhibition of kinases is possible by means of kinase inhibitors or antibodies which can inhibit the phosphorylation by a specific kinase. In several types of cancer good results have been achieved already.

To be able to develop specific therapies, it is necessary to unravel the etiology of cancer. Not only kinases, but also other proteins which are encoded by oncogenes, tumor suppressor genes and DNA repair genes, are important in the development of tumors. Changes in activity of kinases and other proteins can rely on several mechanisms, such as amplification, overexpression or mutation of that gene. In cancer, relatively many mutations are found in kinase genes. Mutations can lead to permanently activated kinases, which, in turn, cause constant activation of the signal transduction cascades regulated by them. An interesting paradox arises, which can be employed for effective therapy: the tumor needs the permanently activated kinase for its growth, and as a result, the cancer cells become dependent (‘oncogene addiction’). Inhibition of the kinase then forms a threat to the cancer cell (‘the Achilles heel of the cancer cell’).

For the identification of deregulated genes in tumors several approaches are possible, among which mutation analysis of tumor DNA. This can be done very systematically and on a large scale. For a limited number of tumor types this has been carried out for the family of kinases. Mutation analysis by polymerase chain reactions (PCR) is an expensive but robust technique. Since mutations are digital and relatively rare, mutations have been frequently located in a cancer gene. On the basis of the type and the location (the functional domain) of the mutation, it may be possible to forecast the impact of the mutation on the activation of the protein encoded by the mutated gene. Moreover, mutations can be predictors of the response to a treatment (predictive factor), the prognosis of the patient (prognostic factor) and, last but not least, mutations can pinpoint mutated genes as therapeutic targets.
The aim of the research described in this thesis is the identification of mutations in glioblastoma, specifically focused at finding somatic mutations, which can play a role in the development of the tumor, or can form a predictive, prognostic factor and/or therapeutic target in glioblastoma. In this thesis various mutation analysis studies are described that focus on the identification of genetic mutations in glioblastoma and other tumor types.

In Chapter one a short general introduction is given concerning the genetic causes of cancer and the different forms of mutations are explained.

In Chapter two an overview of mutation profiling of tumors and the possibilities that have been provided for the use of ‘targeted’ therapies in the clinic is given. Mutations appear to have a predictive value for the response of a patient/tumor to an inhibitor. For several types of tumors nowadays the histological tumor classification is extended with mutation profiles and/or expression profiles of tumors, on the basis of which is decided what treatment a patient receives. The hitherto performed mutation analyses of some tumor types have provided a genetic overview. This overview can be considered as a landscape with mountains and hills. The genes which are frequently mutated are indicated as ‘mountains’. In contrast, genes which are mutated sporadically are seen as ‘hills’. The mutations in the ‘mountain’ genes have already been validated for a large part and appear causative. For this reason they are called ‘drivers’. In contrast, most of the ‘hill’ type of mutations have not been validated yet, and thus it is impossible to make a distinction between the ‘driver’ and the so-called ‘passenger’ mutations. These last mutations are epiphenomena in the tumorigenesis, but are not of causal importance. For a limited number of tumor types, large-scale analyses have been performed. To be able to identify also the low frequent mutations (‘hills’), it is necessary to perform mutational profiling of a large number of samples of all tumor types. The identified mutations must be validated before the importance of the found mutations can be interpreted. Until now, this part of the research lags behind the mutation analyses which are taking place.

In Chapter three a large-scale mutation analysis of 37 genes, among which 34 kinases in 113 glioblastoma tumors and 19 high-grade astrocytoma cell lines, is described. Important findings are that 59% of the tumors have a somatic mutation in PTEN or TP53 and that most glioblastomas have an activating mutation in the PI3K-AKT pathway. Mutations in PIK3CA and PTEN occur ‘mutually exclusive’. This means that none of the examined tumors had a mutation in PIK3CA and PTEN, but that either PIK3CA or PTEN were mutated. Also a number of other somatic mutations were found in the genes EPHA3, BRAF, FLT3, NRAS, RPS6KC1 and TGFRB2. These mutations have been found in a small number of glioblastomas, but could be important ‘driver’ mutations for these patients. Functional validation of these mutations is necessary before possible patient-centered therapies can be developed.
Following two extensive mutation analyses in colon and breast cancer, in which a large number of novel mutated ‘cancer candidate genes’ (CAN genes) was identified,\(^1,2\) a number of these genes was examined for genetic alterations in glioblastoma, melanoma and pancreas ductal adenocarcinoma (PDAC). The findings are described in Chapter four and Chapter five. In Chapter four 19 CAN genes were analyzed for mutations in glioblastoma, melanoma and PDAC tumors. Eight novel somatic mutations were found in the genes which encode for EPHA3, MLL3, TECTA, FBXW7 and OBSCN, and which were not described before in cancer. In addition, a germline nucleotide variant was found in OBSCN. It is very interesting that this change was already described as a somatic mutation. We can speculate that in analogy with other oncogenes (for example RET) and tumor suppressor genes (for example TP53) in several types of cancer, germline mutations in OBSCN may form a genetic predisposition for glioblastoma. As not all examined genes were found to contain mutations, every tumor may have its own mutated CAN genes. Since the somatic mutations, which were found, were not described before, this suggests that the mutation profiles of CAN genes, which are mutated in several tumor types, is specific for every tumor type.

In a comprehensive mutation analysis of 18.191 genes in 11 colon and 11 breast cancer tumors, mutations were identified in 1.718 genes.\(^2\) The mutated genes were reflected as a genomic landscape.\(^2\) In Chapter five, 27 of these genes, belonging to the ‘hill’ type, were selected, and analyzed for DNA changes in 26 glioblastoma, 24 melanoma and 12 PDAC tumors. The question was whether the mutated ‘hill’ genes would be shared by different tumor types. If so, this would pinpoint new therapeutic targets in glioblastoma, melanoma and PDAC. The candidate genes were selected because of (a) considerable mutation frequency in breast and/or colon cancer; and of the following criteria; (b) CAN gene type status in both tumors; (c) the possibility to ‘inhibit’ enzymatic activity; and (d) known to be involved in oncogenic signaling cascades. Novel somatic mutations were found in four of the breast/colon ‘hill’ CAN genes: SMAD4, MYO18B, NAV3 and MMP2 in melanoma and pancreas cancer. These results confirmed the conclusions described in Chapter three. Every tumor has its own mutated CAN genes, only a limited number of CAN genes was found to be mutated in several tumor types, and the mutation profiles of shared CAN genes can be tumor-specific. This indicates that tumor-specific genome-wide mutation analyses are necessary for every tumor type, to identify mutated ‘hill’ genes for every tumor type.

AKT1 is a kinase in the, for glioblastoma important, PI3K-AKT pathway. In breast, colon and ovarian cancer, a recurrent mutation (E17K) was identified, which results in constitutive activation of AKT1 and which induces leukemia in mice.\(^3\) In Chapter six a mutation analysis of AKT1\(^{E17K}\) in 764 tumors belonging to seven cancer types, among which glioblastoma, is described. Mutations were found in breast, colon and lung cancer, but not in glioblastoma. Within the breast tumors, AKT1 mutations were seen ‘mutually exclusive’ with respect to PIK3CA mutations. AKT1\(^{E17K}\) mutations only occurred in ductal and lobular histotypes of breast cancer. The mutation pattern of AKT1 in solid tumors suggests a tissue-specific pattern.
Since the E17K mutation in AKT1 was not found in glioblastoma, other domains of AKT1 were analyzed for somatic mutations in glioblastoma, which can ensure an activating role of AKT1 in the PI3K-AKT signaling cascade. In Chapter seven the mutation analysis of the coding sequence of the entire AKT1 gene in 109 glioblastoma tumors is described. In none of these tumors a somatic mutation was found in AKT1. This suggests that the oncogenic deregulation of the PI3K-AKT pathway does not rely on AKT1 mutations in glioblastoma.

In Chapter eight PLXNB1 mutations in cancer are discussed. Recently, a study was published, which reported that almost half of the primary prostate cancers contain a somatic mutation in PLXNB1. As these results were not confirmed in our set of 15 primary prostate cancers, we suspected that the reported changes were brought on by deamination of cytosines or adenines. Particularly in small quantities of DNA, PCR errors can originate. By repeating the PCR it should become evident that these findings concern PCR artifacts. Based on our results, we think that the mutation frequency of PLXNB1 in prostate cancers must be doubted and possibly reconsidered.

Very recently, new hotspot mutations were found in glioblastoma in the gene isocitrate dehydrogenase 1 (IDH1). This concerned recurrent mutations of the evolutionary strongly conserved amino acid R132. In Chapter nine the mutation analysis of IDH1 in 672 tumors, belonging to eleven different tumor types and 84 cell lines, is described. IDH1 mutations in our set of tumors were found specifically in the group of high-grade gliomas. IDH1R132 mutations were present in 20% of glioblastomas, and especially within the group of secondary glioblastomas. In addition to the already reported R132H and R132S mutations, three new somatic mutations were identified (R132C, R132G, and R132L), which also change amino acid IDH1R132. Strikingly, no mutations were found in other tumor types. This suggests that IDH1 plays a specific role in the development of gliomas.

The specificity of IDH1 mutations in high-grade gliomas brought us to examine the preceding tumors of secondary glioblastoma, the low-grade gliomas and other brain tumors, in Chapter ten, for mutations in IDH1. Mutations were found in high frequencies in low-grade gliomas. Mutations in IDH1 seem to be a very early event in gliomagenesis. IDH1 belongs to a family of five isocitrate dehydrogenases, of which 3 are NAD+-dependent and 2 NADP+ dependent, among which IDH1. The activity of NAD+- and the NADP+-dependent IDHs was examined by means of enzyme histochemistry in sections of glioblastoma tumors. The activity of the NADP+-dependent IDHs, but not the activity of the NAD+-dependent IDHs and NADP+-dependent G6PDH, was found to correlate with the presence of IDH1R132 mutations. The NADP+-dependent IDH activity was strongly diminished in IDH1R132-mutated glioblastomas. This suggests that IDH1 mutations are inactivating ex vivo. By multivariate analysis, IDH1R132 mutations were identified as an independent strong prognostic factor for the survival of glioblastoma patients.
Beside mutations, specifically addressed in this thesis, other changes in genes, transcripts and proteins play a role in cancer. By the developments in techniques which have occurred in the previous decades, enormous progress has been achieved in identifying these changes, and nowadays a lot of these analyses are performed genome-wide. In Chapter eleven an overview is given of the molecular changes which have been found in glioblastoma; these are discussed at the (epi)genetic, transcript and protein level. The meaning of some of these changes as prognostic and predictive factors is highlighted. Also the possibility of some of these identified changes as a target for new therapies is described.

Finally, the results of this thesis are discussed in Chapter twelve. Mutation profiling has already proved its usefulness, looking at the clinical consequences which have emerged. Not only sequencing of well-known hotspots, but genome-wide analyses (including not-encoding DNA and regulatory elements) in large numbers of all tumor types is necessary. Functional validation of the mutations described until now is urgently necessary. This could provide an answer to the question why mutations occur tissue-specific. In the future faster and cheaper sequencing techniques will make it possible that every tumor will be analyzed for mutations and that every patient receives a therapy based on the mutational profile of the individual’s tumor.

Summarized, new somatic and germline mutations have been identified in their associated genes in glioblastoma and other tumor types. These mutations may, after functional validation, provide new insights in the etiology of cancer and, in particular, glioblastoma. In the future, these mutations can possibly contribute to the development of new therapies.
References