Master's Thesis

Implementation and Comparative Assessment of Diagnostic Cancer Gene Panels in the Molecular Pathology Laboratory

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by

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Abstract

Contents

1 Introduction

Cancer represents a huge burden for health care systems worldwide and is one of the leading death causes. Scientific discoveries in the last decade have had an enormous impact on our understanding of the underlying causes of cancer. The development of omics techniques, in combination with advanced computational power, has lead to an explosion of biological data. It has become clear that cancer is an incredibly complex malignancy, which is affected by genetic, environmental and behavioural factors. The research community is trying to interprete this vast amount of data with the goal to get a deeper understanding of cancer and to cure it eventually. In recent years, several drugs have been approved, which target proteins needed for cancer development, proliferation or metastasis. Molecular testing is employed to check whether these targeted drugs would be of benefit. In that regard, Next-Generation Sequencing (NGS) is an interesting method to gain deep insights into the genetic information of a tumor and to guide personalized therapy.

1.1 The cancer genome

DNA undergoes continuous damage. In normal cells, this damage is repaired without without errors. In cancer cells, the equilibrium between DNA damage and repair systems is dysbalanced [1]. Genetic and epigenetic alterations, in combination with several environmental factors, such as inflammation, enables the hallmarks of cancer [2]. These include replicative immortality, cell death resistance, ongoing proliferative signaling, invasion and metastasis, growth suppressor evasion, inducement of angiogenesis, energy metabolism reprogramming and immune destruction evasion.

Cancer progression is a process that recognizes basic Darwinian evolution principles [3] [4] [5] [6]. The population of cancer cells harbors heritable genetic variation. These mutations may be of germline origin or may occur through somatic processes. If the occurring mutations are non–deleterious, they can be passed on to the next generation of cells. The second process is natural selection. Each cell exhibits a unique combination of genetic and environmental perturbations. Cells are in competition for a variety of resources in their microenvironment, which include space, oxygen and nutrients. Eventually, cells with the best fitness, e.g. with the highest proliferative potential and the lowest death rate, are then selected through natural selection principles. These cells will outlast less fit cells. Additionally, these cells will continue to accumulate new mutations. This results in sequential waves of clonal expansion [3], leading to different subclones within the same tumor.

It is widely accepted that tumors accumulate somatic mutations during their progression in ma-

lignancy [8] [9]. DNA can be damaged by endogenous and environmental agents [10]. Carcinogenic substances produced by industry [11] [12] or present in tobacco smoke [13] are known to increase cancer risk. Cellular metabolic processes also produce DNA-damaging products that induce cancer, such as reactive oxygen species [14] [15]. Several cellular DNA repair systems have emerged. DNA lesions can escape these repair mechanisms if the damage happens in an inaccessible region of the DNA or if the DNA repair system is defective [16]. Also, the repair systems cannot cope with the rate of mutation if the frequency at which they occur becomes to important. These DNA lesions, if not repaired, then induce errors in the replication by DNA polymerases.

Also, the process of DNA replication has an intrinsic error rate. It has been estimated that DNA polymerase has error rates ranging from 10^{-4} to 10^{-6}. This is followed by mismatch repair, which corrects 90-99% of the replication errors, decreasing the overall error rate to 10^{-6} to 10^{-8} [10]. Additionally, several DNA polymerases exist, which differ in their error rates, which can be used interchangeabilly. DNA polymerase β has a much worse error rate than DNA polymerase δ or ϵ. There is evidence that that DNA β is increased in some tumors [17], resulting in increased mutagenesis.

Processes affecting chromosomal and microsatellite integrity instability contribute to genomic instability in cancer cells. **Chromosomal instability (CIN)** is the most common kind of instability in solid tumors [18]. Chromosome missegregation plays a crucial role in cancer adaptation [19]. Defects in proteins needed for chromosome segregation lead to chromosome missegregation. This leads to telomere dysfunction, faulty sister chromatid cohesion, loss of heterozygosity (LOH), hypo– or hyperactive spindle assembly checkpoint or defective centrosome duplication and aneuploidy. [18]. About 70% of solid tumors are aneuploid [22]. Another chromosomal instability process has been described recently: chromothripsis happens when chromosomes are fragmented [20] [21] [21]. The cell tries to repair the chromosomes, but this process is far from being perfect, leading to massive chromosomal rearrangements. The question whether CIN is a cause or consequence of tumor development remains unanswered.

Microsatellite instability (MSI) is caused by inactivation or loss of DNA mismatch repair [23]. Microsatellites are short DNA segments with tandem repeats. Microsatellite elongation or shortening is a consequence of defective or inactive DNA mismatch repair (MMR), which corrects base replication errors [18]. Germline mutations in MMR genes cause the Lynch syndrome (hereditary nonpolypsos colorectal cancer) [25]. Patients have an 80% lifetime risk to develop colon cancer. DNA polymerase has a higher error rate in repetitive regions. When MMR genes are inactivated or defective, the replication mistakes in microsatellites cannot be corrected: MSI is the consequence.

In some cancers, MSI can occur despite functional MMR through frameshift mutations at microsatellites. MSI is often associated with cancers harboring mutations in TGFβRII, EGFR, PTEN, and BAX, which contain such simple repeats [27].

Epigenetic changes

Blablabla

1.1.1 Molecular Profiling of Solid Tumors

The previously discussed cancer characteristics, heterogeneity and accumulation of mutations, becomes evident in the molecular profiling of solid tumors.

Lung cancer is the most common cancer worldwide, both in terms of new cases (1.8 million) and deaths (1.6 million). Smoking is a widely accepted risk factor, as chemical carcinogens in tobacco smoke induce several genetic mutations. Lung cancer can be divided into two histological subtypes: small-cell lung cancer (SCLL) and non-small cell lung cancer (NSCLC). Over the last decade, it has become clear that these subtypes can be classified into additional classes by the mutational status of recurrent driver mutations.

A combination of oncogenic triggers cause cells of the normal bronchial epithelium to proliferate, giving rise to meta–, hyper– and dysplastic epithelial lesions. Genomic events in early stages of lung cancer giving rise to atypical adenomatous hperplasia include LOH of 3p, $p16^{INK4a}$ or RB inactivation, as well as mutations in KRAS or in β–catenin. TP53 inactivation and LOH at 13q are believed to favor progression into the primary adenocarcinoma stage. After that stage, major chromosomic instability is often detected, giving rise to metastatic adenocarcinoma. These chromosomic events include LOH of 2q, 9p, 18q, and 22q. Additionally, the oncogene c-myc can be amplified.

Frequent mutations in NSCLC, which are of potential interest in a targeted anti-tumor chemotherapy, affect EGFR (10–35%), KRAS (15–25%), PTEN (4–8%), HER2 (2–4%), DDR2 (4%), PIK3CA (1–3%), BRAF (1–3%), AKT1 (1%), MEK (1%) and NRAS (1%). Additionally, rearrangement of ALK (3–7%), RET (1%) and ROS1 (1%) and amplifications of FGFR1 (20%) and MET (2–4%) are found recursively. These mutations are rarely observed together in the same tumor.

Melanoma develops from the malignant transformation of melanocytes in the basal epidermal layer of the skin. Exposure to UV light, immunosuppression, fair-skin and multiple nevi are risk factors. UV radiation causes cyclobutane pyrimidine dimers (CPDs). T–T, C–C or C–T dimers (UV fingerprints) are formed, leading to direct DNA damage. People diagnosed with rare genetic dis-

orders like xeroderma pigmentosum are at great risk. Traditionally, melanoma has been classified based on histological and pathological properties, such as the thickness of the tumor, ulceration or the anatomic location of the tumor.

Spontaneous mutations in BRAF and NRAS and epigenetic modulations of APC are believed to promote progression of normal epithelium into a dysplastic nevus. Mutations affecting genes PTEN and CDKN2A and altered gene expression of MGMT or RASSF1 then favor invasion of the dermis, which is underlying to the epidermis, in the radial growth phase. Genetic and epigenetic alterations in AKT, MTAP, APAF, CDH1 and CDH2 then result in the vertical growth phase, where the tumor invades surrounding tissues. Finally, loss of TP53 functionality and further epigenetic modulations of MTA2 and MAGE enable metastasis.

The occurrence of the different mutations differs by the anatomic location of the tumor, e.g. whether the specific body part is chronically exposed to the sun. Mutations frequently found in melanoma, which are potential targets for targeted therapy, occur on BRAF (37–50%), NRAS (13–25%), MEK (6–7%), NF1 (11.9%), CTNNB1 (2-4%), GNAQ (1.3%) and GNA11 (1.2%).

Colorectal cancer (CRC) 1.4 million cases are detected yearly with 694,000 deaths. CRC is one of the best studied cancers. The development of colorectal adenocarcinomas occurs over many years. Caused by the acquisition and accumulation of driver mutations, a normal colorectal epithelium can progress to adenoma, which develops into carcinoma, which can eventually mestastasize.

The molecular progression models in CRC are dependent on the underlying instability process (chromosomal vs. microsatellite). In CRCs, where CIN is the driving force, loss of the tumor suppressor gene APC is often causing the evolution from a normal to a hyperproliferative epithelium. Progression to adenoma stages are associated with DNA hypomethylation, KRAS activation and loss of 18q. Mutations in TGFβRII and PIK3CA and loss of TP53 by LOH at 17p then lead to the final carcinoma stage. In MSI CRCs, mutations and hypermethylations in MMR genes result in an hyperproliferative epithelium. BRAF mutations, followed by PIK3CA mutations, loss of TP53 and frameshift mutations affecting TGFβRII, BAX or IGF2R are associated with CRC progression towards the carcinoma stage.

Mutations recursively detected in CRC occur on KRAS (36–40%), SMAD4 (10–35%), PIK3CA (10–30%), BRAF (8–15%), PTEN (5–14%), NRAS (1–6%), and AKT1 (1–6%).

The mentioned molecular progression profiles are likely to be an oversimplification. These models are based on frequently found alterations in the respective cancers. Due to tumor heterogeneity, these alterations do not always have to be observed in the tumor, and the chronological appearance

of these alterations may vary from one tumor to another.

1.1.2 Driver and passenger mutations

Genomic instability in cancerous cells becomes a critical mechanism if it affects oncogenes or tumor suppressor genes, which have the potential to be causative tumor 'driver' mutations. The identification of driver mutations has been a central aim of cancer research. Mutations in at least 350 human genes are found recursively in cancer genomes and are believed to contribute to cancerogenesis [34]. These driver mutations are positively selected during cancer progression and confer a growth advantage to the cells harboring them. Many alterations found in cancer cells are passenger mutations, which occur subsequently or coincidentally to driver mutations. These mutations are defined to not contribute to the selective fitness of the cell, even though this conception has been challenged by stochastic tumor progression simulations [33]. Some studies have reported that cancer cells carry 40–80 somatic mutations, and only 5–15 of them are driver mutations [28].

Estimating the number of somatic driver and passenger mutations and the rate at which they occur is not well established [29]. Two tumors, even though histologically indistinguishable, might present different subsets of mutations [29] [30]. This observation has been defined as inter-tumor heterogeneity. Additionally, tumors present heterogeneity at the intra-tumor level [31]. Subclones of the tumor might present different mutations.

As mentioned, cancer progression is an evolutionary process. Chemotherapy creates a selective environment [32]. Initially, patients often respond to the therapy, but might then become resistant to the treatment. This is due to intra-tumor heterogeneity: a subclone of the tumor might have acquired a driver mutation that confers resistance to the treatment. Chemotherapy might kill a large part of the tumor cells, but actively selects for this resistant clone. Eventually, this clone will be the origin of relapses and another treatment option is lost.

Tumor suppressor genes protect a cell from entering the path to cancer. They comprise genes encoding for cell adhesion proteins, DNA repair proteins, proteins acting in apoptosis pathways, or cell cycle proteins. The action of these proteins inhibits metastasis, excessive cell survival or proliferation. Tumor suppressors mostly follow the two-hit hypothesis, which was first proposed by Knudson for the retinoblastoma protein (pRb): to inactivate the tumor-protecting role of tumor suppressors, two genetic events, often LOH in combination with silencing point mutations or silencing of both alleles by somatic events, are necessary to inactivate both alleles of the gene. Compared to

dominant oncogenes, tumor suppressor genes are often considered to be recessive. Alternatively, tumor progression can be influenced by functional haploinsufficiency of tumor suppressors. According to this conception, a disease state can emerge if a cell / organism has only one functional copy of a given gene and if it cannot produce enough of a gene product to establish a wild-type condition. APC and TP53 are amongst the best known tumor suppressors.

Adenomatous Polyposis Coli (APC) protein has binding sites for microtubules, cytoskeletal regulator proteins and Wnt signaling proteins (β–catenin, axin). Wnt signaling regulates cell migration, polarity, differentiation, adhesion and apoptosis. In the canonical Wnt signaling pathway, a destruction complex, including APC, leads to β–catenin phosphorylation, followed by ubiquitination, marking it for degradation in the proteasome. Once Wnt binds to the N-terminus of an activated surface receptor of the Frizzled family and a co-receptor of the LRP5/6 family, the destruction complex is inhibited. Consequently, β–catenin is no longer marked for degradation and can translocate to the nucleus, where it acts on gene expression of target genes. Loss or dysfunction of APC leads to β–catenin accumulation in the nucleus even in the absence of an extracellular stimulus.

TP53 is one of the master guardians of the genome. In normal situations, p53, the protein encoded by TP53, is targeted for ubiquitination and degradation in the proteasome. In case of cellular stress, p53 is no longer ubiquitinated. p53 can then stop the cell cycle at the G1/S and G2/M transitions, induce DNA repair, and induce apoptosis if the damage cannot be repaired. TP53 thereby maintains genomic stability. The importance of TP53 as tumor suppressor gene becomes evident in the autosomal dominant Li–Fraumeni syndrome. People suffering from this disorder inherit only one functional copy of TP53 and are likely to develop cancer in early ages. One mechanism by which p53 acts on cell-cycle arrest is by activating expression of p21. p21 binds to the G1/S transition complex and inhibits its activity, leading to cell-cycle arrest. Inactivation or mutation of TP53 is a crucial step in many cancers, leading to a loss of control over DNA stability.

Oncogenes comprise several GTPases, transcription factors, receptor tyrosine kinases and growth factors. Overexpressed or overactive versions of these proteins lead to increased mitogenic signals, causing increased cell growth or proliferation. Mutations in proto–oncogenes can cause a loss of regulation or overactive proteins. Gene duplications or other chromosomal alterations lead to increased protein synthesis. Other mechanisms of importance include post-transcriptional mechanisms as misregulation of protein expression or increase of mRNA / protein stability. Two important oncogenic pathways include the RAS–RAF–MAPK and PTEN–PI3K–AKT pathways. Epithelial Growth Factor Receptor (EGFR) is a cell surface tyrosine kinase receptor. Ligand-binding on EGFR can induce

both the RAS–RAF–MAPK and the PTEN–PI3K–AKT pathways. EGFR is anchored in the cytoplasmic membrane and is composed of an intracytoplasmic tyrosine kinase domain, a short hydrophobic transmembrane domain and an extracellular ligand-binding domain. Ligand binding causes a conformational change of the receptor, which leads to homo– or heterodimerization, followed by an auto– and cross–phosphorylation of key tyrosine residues on its cytoplasmic domain. This forms docking sites for cytoplasmic adaptor proteins that contain phosphotyrosine-binding and Src homology 2 domains.

Signaling through the PI3K–AKT pathway leads to cell growth, proliferation and survival. The signaling cascade is initiated by integrins, cytokine receptors, T and B cell receptors, G–protein coupled receptors receptor tyrosine kinases, such as EGFR. Activation of the receptor results in production of PIP3 by activation of PI3K. PIP3 is anchored in the cell membrane and acts as docking site for proteins containing PH domains, such as PDK1. PIP3-bound PDK1 partially activates Akt by phosphorylation. Full activation of Akt is achieved by phosphorylation of PDK1 by mTORC2. Activated Akt then acts on a variety of proteins necessary for protein synthesis, glucose metabolism, cell survival / death and proliferation. The phosphatases PP2A and PHLPP can dephosphorylate and thereby inactivate Akt. Additionally, PTEN dephosphorylates PIP3 and indirectly also inactivates Akt. Dysregulation of the PI3K–AKT has been associated with several human diseases including neurological diseases, diabetes and cancer. In cancer, inactivation of PTEN and kinase activity activating mutations on PI3K and Akt are found recursively, leading to enhanced signaling, leading to inhibition of apoptosis and increased proliferation.

In the RAS–RAF–MEK–ERK pathway, ligand binding on cell surface receptor tyrosine kinases activates the receptor. One of these receptors is the EGFR. GRB2 binds to Tyr1068 of EGFR through its SH2 domain and recruits SOS, a guanine nucleotide exchange factor. Grb2 and SOS then form a complex with the activated EGFR, which activates SOS. Activated SOS promotes recruition of Ras proteins to the activated EGFR. Through its GEF activity, SOS then induces GDP removal from Ras proteins, which can subsequently bind GTP and become active. Ras then recuits Raf proteins to the cell membrane and binds to their N-terminus. The activation of Raf, serine/threonine kinase proteins, is complex. In fact, Raf proteins are considered as gatekeepers of the RAS–RAF–MAPK pathway. In its inactive form, Raf is present in a 'closed' conformation, in which an autoinhibitory domain blocks the catalytic kinase domain. Recruitment to the cell membrane of Raf by Ras results in a conformational change, which disrupts the autoinhibitory interaction of Raf. Rafs then form homo– or heterodimers, which leads to partial activation by allostery. Transphorylative events, with optional phosphorylation by other kinases, such as PAK1, then fully activates Raf. Activated Raf can now bind

to MEKS, which are tyrosine/threonine kinases. MEKs then phosphorylate ERKSs, which are also serine/threonine kinase enzymes. ERKs then translocate to the cell nucleus, where they influence expression of target genes. RAS–RAF–MEK–ERK signaling promotes cell-cycle progression, cell differentiation, growth and survival.

1.2 Targeting the EGFR signaling pathway

1.2.1 Biological Role of EGFR in Solid Tumors

In normal cells, the tighly regulated EGFR signaling pathway drives cell-cycle progression, affects differentiation and migration and acts as a survival signal. EGFR ligand binding leads to an activation of several signaling cascades, such as the PI3K–AKT, RAS–RAF–MEK–ERK, JAK–STAT and NFkB pathways. The EGFR pathway is long known to be dysregulated in most solid tumors. EGFR levels have been demonstrated to be higher in CRC samples than in surrounding tissues. Also, more of EGFR's ligands EGF and TGFα are found in these locations. Increased EGFR mRNA levels in in vitro culture of human CRC cells has also linked EGFR overexpression to tumor progression. EGFR overexpression has been associated with poor prognosis, as this leads to a more aggressive progression.

It was suggested that EGFR overexpression results from epigenetic alterations, leading to enhanced gene expression. Gene amplification and oncogenic viruses may also cause EGFR overexpression.

http://annonc.oxfordjournals.org/content/16/1/102.full http://www.nature.com/onc/journal/v28/n1s/full/onc2009199a.

EGFR signaling as a survival signal Cells communicate with their environment. Without extracellular signals, a cell undergoes apoptosis. Signaling pathways induced by cell–matrix interactions, cell-cell interactions, and soluble survival factors act on a variety of genes and proteins.

Loss of matrix attachment leads to cell growth arrest and even cell death in normal epithelial cells, a process called anoikis. The cell–matrix interaction provides important spatial informations to the cell and acts as a safeguard against inappropriate proliferation and migration.

Activation of the EGFR signaling pathway allows protection of normal epithelial cells against anoikis in the suspended state. EGFR blockade sensitizes normal epithelial cells to apoptosis. This effect is much more pronounced in the suspended state than in the attached state. The redundancy of cell survival signals, makes normal epithelial cells relatively resistant to EGFR-blockade in their

normal microenvironment. MAPK activation in the RAS–RAF–MAPK–ERK pathway is required for high expression of Bcl–XL, an anti-apoptotic protein of the Bcl–2 family. Bcl–2 can be either pro– or anti–apoptotic and regulate liberation of cytochrome c from the mitochondria, which is essential in the apoptotic caspase pathway. Expression of pro–apoptotic Bcl–2 proteins (Bax, Bad, Bak) are not influenced by EGFR signaling. Additionally, EGFR signaling leads to post-transcriptional phosphorylation on the pro-apoptotic Bad protein, which is thereby functionally inactivated.

Tumor cells are often in transit or at sites with inadequate matrix composition. They are often provided with inadequate or missing cell–cell or cell–matrix interactions. They are thereby more dependent on survival signals propagated by soluble mediators, such as EGF or TGFα. This can counterbalanced by an upregulation of cell surface receptors that activate anti–apoptotic pathways. Consequently, tumor cells are relatively sensitive against blockade of EGFR (or other cell surface receptors. Due to inappropriate interactions, these cells heavily depend on survival signals propagated by soluble mediators, such as EGF or TGFα.

1.2.2 EGFR-targeted drugs

The observations that EGFR is recursively upregulated in many cancers and that EGFR is such an important mediator of not only cell-cycle progression and cell growth, but also cell survival, has lead to the development of agents that block this pathway. Pharmacologically, these agents can be classed by their mode of action: EGFR-targeted monoclonal antibodies and EGFR-specific tyrosine kinase inhibitors. Additionally, several proteins acting downstream of EGFR are recursively found to be mutated. This lead to the development of other targeted drugs, such as BRAF– or MEK–inhibitors. Table XXX shows a selection of FDA-approved cancer drugs that target components of the EGFR signaling pathway.

Anti–EGFR monoclonal antibodies bind to the extracellular domain of EGFR in its inactive state. They compete for receptor binding by occluding the ligand-binding domain. They thereby inhibit ligand-induced EGFR tyrosine kinase activation. The most popular anti–EGFR monoclonal antibody is cetuximab. Cetuximab binds to EGFR with a higher affinity than the natural ligands EGF or TGFα. Cetuximab binding induces internalization of EGFR, following by its degradation. Cetuximab also binds to EGFRvIII, a constantly active version of EGFR. Binding of cetuximab to EGFR blocks cell–cycle progression at the G0/G1 boundary.

EGFR-specific tyrosine kinase inhibitors comprise three classes that include first generation reversible EGFR-inhibitors (gefitinib, erlotinib), second generation irreversible inhibitors (afatinib, da-

Table 1: FDA-approved cancer drugs for solid tumor treatment that target the EGFR pathway

Agent	Target(s)	FDA-approved indication(s)
Afatinib (Gilotrif)	EGFR	NSCLC (with EGFR del19 or L858R)
Cetuximab (Erbitux)	EGFR	Colorectal cancer (KRAS WT)
Cobimetinib (Cotellic)	MEK	Melanoma (with BRAF V600E or V600K
Dabrafenib (Tafinlar)	BRAF	Melanoma (with BRAF V600 mutation)
Erlotinib (Tarceva)	EGFR	NSCLC
Gefitinib (Iressa)	EGFR	NSCLC (with EGFR del19 or L858R)
Necitumumab (Portrazza)	EGFR	Squamous NSCLC
Osimertinib (Tagrisso)	EGFR	NSCLC (with EGFR T790M)
Panitumumab (Vectibix)	EGFR	Colorectal cancer (KRAS WT)
Trametinib (Mekinist)	MEK	Melanoma (with BRAF V600)
Vemurafenib (Zelboraf)	BRAF	Melanoma (with BRAF V600)

comitinib, neratinib) and third generation mutant-selective inhibitors (brigatinib, osimertinib, rociletinib). Third class agents have a better sensitivity against mutated than wild-type EGFR and have been designed to further decrease treatment-associated side effects. Tyrosine kinase inhibitors are low molecular weight molecules that are mainly derived from quinazoline. These compounds bind to EGFR and block ligand-induced receptor phosphorylation by competing for the ATP– binding site. This results in inhibition of cell proliferation, cell–cycle arrest at the G0/G1 boundary and apoptosis.

1.2.3 Predictive markers

Wild-type status of EGFR, KRAS, NRAS and BRAF has been associated with increased sensitivity to anti-EGFR antibodies.

EGFR is a strong predictive biomarker for the success of the administration of EGFR-specific tyrosine kinase inhibitors. EGFR activating mutations are observed in 10–35% of NSCLC. In melanoma and CRC, these mutations are seldom. 90% of EGFR mutations are exon 19 deletions and exon 21 L585R (c.2573T$>$G) point mutations. Amongst EGFR-mutated NSCLC, EGFR L858R occurs with a frequency of 43%.

KRAS mutations in CRC are found with a frequency of 36–40%. Amongst these, the G12C variant is the most common (7.9%). Critical mutations in the KRAS gene include variants in codons 12, 13 and 61. These mutations lock KRAS in its GTP-bound state, resulting in a constantly active protein. This then leads to a constantly active signal transduction. Blocking EGFR in that case is useless, as KRAS acts downstream of EGFR. Several KRAS point mutations in codons 12, 13 and 61 have been shown to confer reduced sensitivity to EGFR-targeted monoclonal antibodies in CRC. The situation is similar in the case of the KRAS-isoform NRAS.

BRAF mutations are very common in melanoma (37–50%). Amongst BRAF-mutated melanomas, the V600E variant is found in 80–90% cases. This variant occurs in the activation segment of the BRAF kinase domain and results in increased kinase activity. BRAF V600E mutations have been associated with increased sensitivity to BRAF and MEK inhibitors.

1.3 Tumor DNA Sequencing

Cancer sequencing using next-generation sequencing (NGS) methods provides more information in less time compared to traditional single-gene and array-based approaches. With NGS, researchers can perform whole-genome studies, targeted gene profiling, tumor-normal comparisons, and more. NGS also offers the sensitivity to detect rare somatic variants, tumor subclones, and circulating DNA fragments.

the field of cancer genomics has been impacted most profoundly by the application of next-generation sequencing technology, which has tremendously accelerated the pace of discovery while dramatically reducing the cost of data production.

Many biological discoveries about cancer have been the product of a reductionist approach, which focuses on modeling phenomena with as few major actors and interactions as possible [1, 2]. This reductionist thinking led the initial theories on carcinogenesis to be centered on how many “hits” or genetic mutations were necessary for a tumor to develop. It was assumed that each type of cancer would progress through a similar, if not identical, process of genetic hits.

However, most cancers are genetically complex, and are better defined by the activation of signaling pathways rather than a defined set of mutations. The success of the Human Genome Project inspired similar projects looking at the genome in various cancers [4]. That success, along with the increased affordability and reliability of sequencing [5], has led to the integration of genome science into clinical practice.

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http://www.comprehensivegenomicprofiling.com/

1.3.1 Targeted NGS

profiling of mutational status of some genes of interest point mutations and small insertions deletions guide targeted cancer therapy

Target enrichment methods

1.3.2 Practical implications in the laboratory

FFPE (quantity, quality, C¿T) Tumor:normal Tumor heterogeneity Choice of instrument library preparation Bioinformatic pipeline + expertise to interprete data

1.4 Aims of the Thesis

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