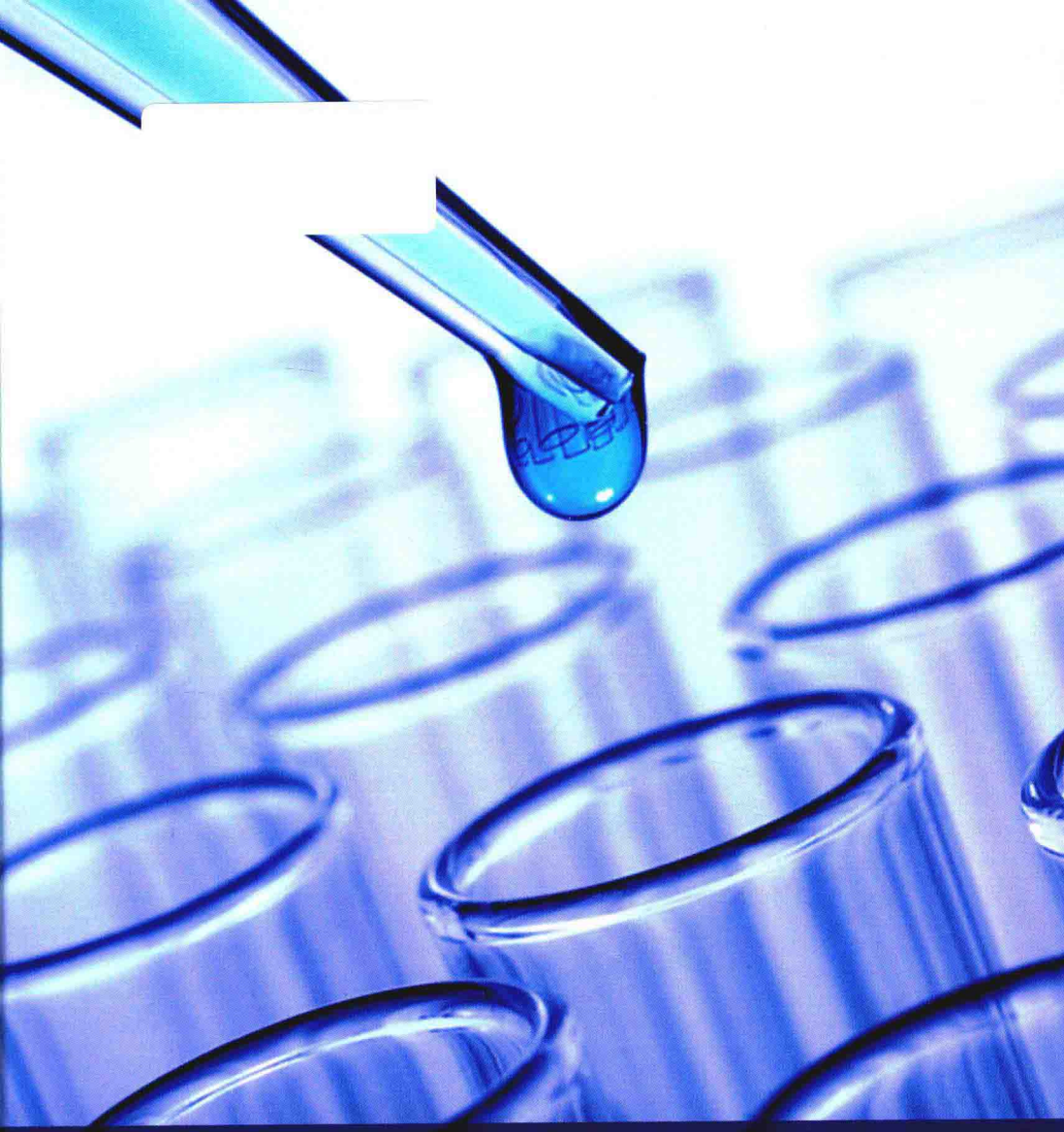

Biomarker

Theory and Concepts

Zach Henderson

Biomarker: Theory and Concepts

Edited by **Zach Henderson**

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Preface

Over the recent decade, advancements and applications have progressed exponentially. This has led to the increased interest in this field and projects are being conducted to enhance knowledge. The main objective of this book is to present some of the critical challenges and provide insights into possible solutions. This book will answer the varied questions that arise in the field and also provide an increased scope for furthering studies.

This book is a detailed and comprehensive medium helping students and researchers to understand the theory and concepts of biomarkers. Biomarkers or biological markers are used as an indicator of a person's health state or condition by scientists or medical professionals. If a biomarker can detect basic symptoms of a disease, differentiate between similar diseases, identify the stage of advancement, work on an easy-to-perform and inexpensive system of diagnosis and is easily accessible to the body; then it is considered to be the ideal biomarker.

I hope that this book, with its visionary approach, will be a valuable addition and will promote interest among readers. Each of the authors has provided their extraordinary competence in their specific fields by providing different perspectives as they come from diverse nations and regions. I thank them for their contributions.

Editor

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Biomarkers in Gastrointestinal Cancer: Focus on Colon, Pancreatic and Gastric Cancer

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1. Introduction

Personalized cancer medicine based on genetic profiling of individual tumors is regarded as the treatment strategy of the future. The targeted drugs for the treatment of cancer have rapidly developed. However, our understanding (at the molecular level) of the precise role that potential targets have in tumorigenesis, and the survival dependence of tumors on these components, has not progressed at the same rate (De Roock et al., 2011). Since patient selection for therapy remains problematic, there has been an increasing interest in biomarkers of cancer risk in predicting future patterns of disease. In the broadest sense, a biomarker is any biological, chemical, or biophysical indicator of an underlying biological process. From a medical perspective, a biomarker is a physiological characteristic that is indicative of health and disease. A cancer biomarker has been defined as "a molecular, cellular, tissue, or process-based alteration that provides indication of current, or more importantly, future behavior of cancer" (Hayes et al., 1996). Cancer biomarkers are employed across the entire healthcare spectrum from the cancer biological research laboratory to patient monitoring in the clinic. Clinical applications include disease risk stratification, chemoprevention, disease screening, diagnosis and prognosis/prediction, treatment planning and monitoring, and posttreatment surveillance. Cancer biomarkers have contributed greatly to our current understanding of the heterogeneous nature of specific cancers and have led to improvements in treatment outcomes. However, full adoption of cancer biomarkers in the clinic has been slow to date, and only a limited number of cancer biomarker products are currently in routine use (http://www.insightpharmareports.com/reports_report.aspx?r=559&id=78452). Two primary challenges in developing cancer biomarkers are the discovery of candidate markers and the validation of those candidates for specific uses. The discovery process depends on the technologies available, and their sensitivity and specificity, to investigate the complex biochemistry of health and disease in order to identify differences that can be detected consistently in diverse populations. The validation process is also arduous and costly, often

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requiring collection of or access to many patient samples with extensive clinical annotation and long-term follow-up. In addition, a biomarker must be validated for each specific application for which it will be used. There must be convincing evidence that a surrogate endpoint accurately predicts the clinical endpoint of interest or in the case of screening, a test must have sufficient sensitivity, specificity, and positive predictive value to accurately identify a disease in the general population (US National Academy Press, Institute of Medicine (U.S.). Committee on Developing Biomarker-Based Tools for Cancer Screening, Diagnosis, and Treatment, 2007). Rapidly growing insights in the molecular biology of cancer and recent developments in gene sequencing, global gene expression profiling or genome wide analysis have led to high expectations for the identification, validation and assessment of cancer biomarkers alongside the established "standards of care" for cancer diagnosis and treatment.

In this review, the most promising biomarkers in gastrointestinal cancer are discussed, focusing on the epidermal growth factor receptor (EGFR)-pathway in colon cancer, the serum biomarkers, the glucose transporter (GLUT) receptors, and human equilibrative nucleoside transporter 1 in pancreatic cancer and HER2 in gastric tumors.

2. Colon cancer

2.1 Introduction

Colorectal cancer (CRC) is a major public health problem. CRC results from the cumulative effects of sequential genetic alterations, leading to a progressive and irreversible loss of normal control of cell growth and differentiation. Treatment of CRC consists of complete surgical removal of the primary tumor and the regional lymph nodes. Despite improvements in surgical techniques, dosing and scheduling of adjuvant and neo-adjuvant systemic therapy, five year survival for early stage colorectal cancer, i.e. without invasion or lymph node metastases, is about 90%, but this falls of to 65% for tumors with regional spread and to 10% for late stage disease in which the cancer has metastasized to distant sites (Deschoolmeester et al., 2010). Currently, the tumor-node-metastasis (TNM) stage is the only proven prognostic marker to aid in the identification of patients with aggressive disease (Tejpar et al., 2010). However, its predictive value is limited because even the outcome within each stage group is not homogeneous (Deschoolmeester et al., 2010). CRC should be regarded as a heterogeneous disease defined by different activating mutations in receptor tyrosine kinases (RTKs), or activating or loss of function mutations in downstream components of the RTK-activated intracellular pathways, some of which could occur in the same tumor. The efficacy of targeted drugs is therefore linked to the specific molecular alterations in the tumor (De Roock et al., 2011). The availability and application of various treatment modalities in CRC has resulted in the elucidation of prognostic and predictive biomarkers that will improve outcome through patient classification and selection for specific therapies. A prognostic biomarker provides information about the patient's overall outcome, regardless of therapy, whereas a predictive marker gives information about the effect of a particular therapeutic intervention (Tejpar et al., 2010). Consequently, in recent years a huge amount of research has been devoted to the study of new biological prognostic/predictive markers as recently reviewed by our group (Deschoolmeester et al., 2010). Several criteria must be met to ensure a biomarker is clinically useful. In addition, the biomarker needs to be tested and validated in a large cohort of randomized patients.

Although hundreds of these markers have been proposed in the last 2 to 3 decades, the current reality is that no molecular marker, other than the *KRAS* gene in the case of epidermal growth factor receptor (EGFR)-targeted therapy for metastatic disease, has made it into clinical practice (Duffy & Crown, 2008)(De Roock et al., 2009).

EGFR is a receptor tyrosine kinase belonging to the HER-family. When activated, EGFR phosphorylates and activates other intracellular proteins that affect cell signaling pathways, (Harding & Burtness, 2005) cellular proliferation, and control of apoptosis and angiogenesis (Figure 1) (Tedesco et al., 2004)(Harding & Burtness, 2005). EGFR has been implicated in colorectal tumorigenesis, tumor progression, and metastasis, as reviewed in Lockhart and Berlin (Lockhart et al., 2005)(Ng & Zhu, 2008). Overexpression of EGFR has been described in up to 65%–70% of human colon tumors and has been associated with the progression of CRC to a more advanced stage (Ng & Zhu, 2008). Therefore, EGFR not only represents a possible prognostic marker in the adjuvant setting of primary tumors but primarily a rational molecular target for a new class of anticancer agents, especially in the setting of metastatic CRC (mCRC) (Scartozzi et al., 2006a)(Scartozzi et al., 2006b)(Overman & Hoff, 2007).

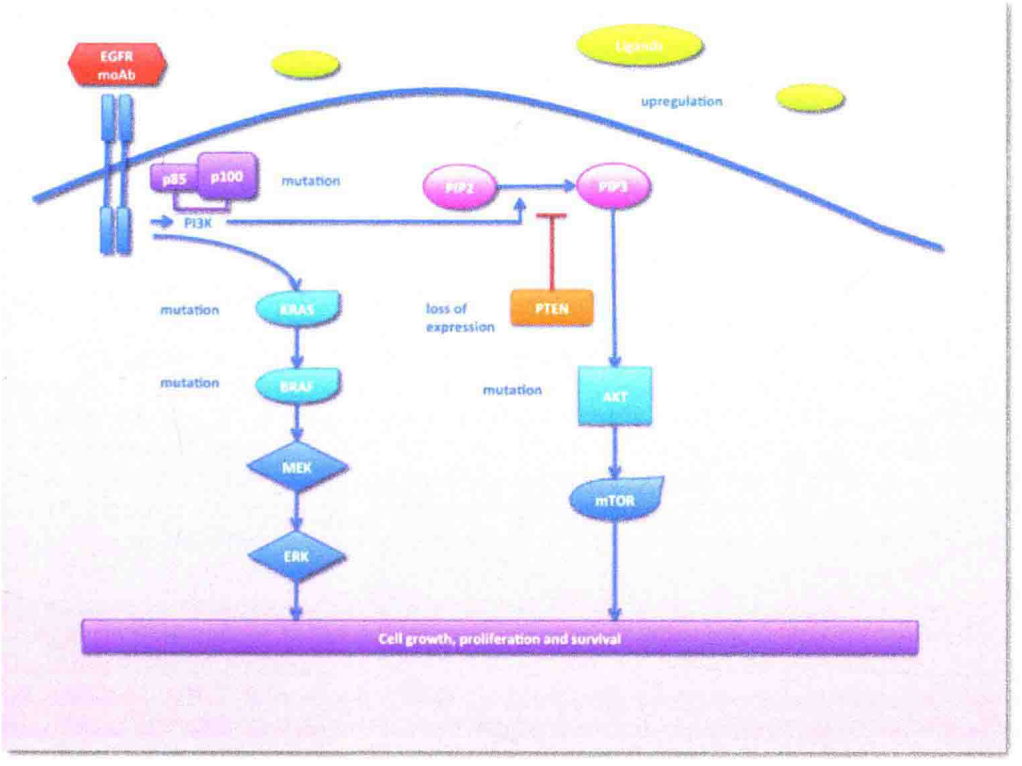

Fig. 1. EGFR signaling pathways and its main transduction pathways.

In preclinical studies, it was found that the inhibition of EGFRs had antitumor activity, and available data suggests synergy with both chemotherapy and radiotherapy (Rivera et al.,

2008). EGFR signaling can be targeted by either monoclonal antibodies (moAb) (cetuximab and panitumumab) or tyrosine kinase inhibitors (TKIs). Cetuximab (a mouse chimeric IgG1) and panitumumab (a fully human IgG2) block ligand induced EGFR tyrosine kinase activation, thereby probably preventing downstream activation of phosphatidylinositol 3-kinase (PI3K)/AKT and RAS/MAPK (mitogen activated protein) signaling pathways, resulting in inhibition of cellular proliferation and induction of apoptosis (Deschoolmeester et al., 2010). Nowadays, anti-EGFR targeted therapy is undergoing extensive clinical evaluation as single agents and in combination with chemotherapy for the treatment of recurrent or first-line mCRC (as reviewed by (Deschoolmeester et al., 2010)). Results of these studies have demonstrated a manageable and acceptable toxicity profile and a promising level of activity. Initially, these therapies were given to unselected populations, but novel insights based on the independent reanalysis of eight randomized trials suggested that these therapies would be effective only in wild type *KRAS* populations (Allegra et al., 2009). Based on these results, the recommended use of these drugs was amended by both the European Medicine Agency (EMEA) and the U.S. Food and Drug Administration (FDA), with important differences, however. The FDA issued a recommendation in 2009 against the use of these drugs in patients with tumors mutated in codon 12 or 13 of *KRAS*, but a label change of the drugs will require additional validation of a single mutation detection assay and reassessment of all randomized trials using this assay. In Europe, the EMEA changed the approval of these drugs for use in wild-type *KRAS* populations only. This has important implications because the exact mutations to be tested are not specified nor is the methodology (see further below) (Bellon et al., 2011).

2.2 KRAS

KRAS belongs to the *RAS* family of genes (*KRAS*, *NRAS* and *HRAS*) that encode guanosine-5'-triphosphate (GTP)-binding proteins. *KRAS* is an important effector of ligand-bound EGFR, mainly, but not exclusively through *BRAF* and *MAPK* axis. KRAS can also activate PI3K through direct interaction with its catalytic subunit (Figure 1) (De Roock et al., 2011). Mutations in the *KRAS* gene are found in 30-40% of CRC and these mutations disable the GTPase activity, causing tumor-associated KRAS to accumulate in the active GTP-bound conformation. About 85-90% of these mutations occur in codons 12 and 13 while the remaining mutations occur in codon 61 (5%) and 146 (5%). The most frequent types of mutations detected are glycine to aspartate on codon 12 (p.G12D, 36.0%), glycine to valine on codon 12 (pG12V, 21.8%), and glycine to aspartate on codon 13 (p.G13D, 18.8%) (Neumann et al., 2009). Several retrospective studies (single-group and randomized clinical trials, summarized by Allegra and colleagues (Allegra et al., 2009)) confirmed the finding by Lievre and colleagues (Lièvre et al., 2006) that mutant *KRAS* is a predictor of resistance to EGFR moAb. This discovery led to the first practical implication of personalized medicine in mCRC. All patients with mCRC are now profiled for seven mutations in *KRAS* codons 12 and 13 before receiving cetuximab or panitumumab (De Roock et al., 2011). However, the picture is not that simple. There is growing evidence for the existence of a whole orchestra of variables and mutations that influence the responsiveness to an anti-EFGR treatment and their role is not fully understood. A European consortium study showed that codon 61 mutations had an adverse effect similar to codon 12 mutations, whereas codon 146 mutations did not affect cetuximab efficacy. Codon 146 mutations co-occurred with other *KRAS* mutations, an additional indication that this might not be an important oncogenic

codon (De Roock et al., 2010b). In vitro data also suggest that *KRAS* codon 13 mutations have a weaker transforming activity than codon 12 mutations and some reports also suggest that some of these patients do respond to cetuximab (Koch et al., 2011). Based on these findings, de Roock and colleagues performed a thorough retrospective subgroup analysis in a pooled data set of 579 patients with chemotherapy-refractory CRC. Their data puzzles the picture of the negative predictive value of a *KRAS* mutation, because patients with the p.G13D mutation seem to respond to cetuximab therapy, in contrast to other *KRAS* mutated tumors, albeit with a lower response rate than those with *KRAS* wild type tumors. The prolonged progression-free and overall survival of patients with p.G13D-mutated tumors in comparison with those with other *KRAS*- mutated tumors may not be due to a real reduction in tumor burden but to a delay in progression. A possible explanation of this clinical observation is that p.G13D mutant tumors do not undergo apoptosis (cytotoxic effect) on EGFR inhibition, but proliferation is inhibited (cytostatic effect). However, prospective randomized trials are needed before conclusions about potential beneficial effects of cetuximab in p.G13D-mutated chemotherapy refractory metastatic colorectal cancer should be inferred (De Roock et al., 2010a).

Furthermore, mutations in the *KRAS* gene can be detected by several different molecular methods and no gold standard methodology is currently available. Because the correctness of the *KRAS* test results is of utmost importance for good patient care, a quality control scheme was set up to (a) assess the performance of *KRAS* testing in Europe, (b) provide remedial measures if necessary, and (c) ensure uniform performance over time by repeated testing rounds. In total, 59 laboratories from eight different European countries participated in the regional *KRAS* external quality assessment (EQA) scheme in 2009. Only 70% of laboratories correctly identified the *KRAS* mutational status in all 10 samples. Genotyping mistakes can be the result of several reasons. A very important issue is the starting material and the type of fixative used. Another important issue in *KRAS* genotyping is the method used for testing. The TheraScreen®DxS kit is considered to be the gold standard for *KRAS* testing in Europe for diagnostic use. However, in this EQA scheme, several mistakes were made using this kit. In addition, the kit is designed to detect only one mutation in a sample, and therefore the mutation scoring ignores possible double mutations, interpreting it as crosstalk. Furthermore, there was a very high variability among laboratories in the estimation of the percentage of tumor cells in H&E stained paraffin sections and the general quality of the reports received in the context of this EQA scheme were very poor. Incomplete or inaccurate exams lead to incorrect diagnoses and can have important consequences for a patient. Therefore, further development of the *KRAS* EQA scheme aims to provide a baseline picture of the accuracy and reliability of the analysis of the *KRAS* test, to identify areas of particular difficulty in testing procedures and to provide a mechanism for improvement for the participating laboratories (Bellon et al., 2011).

In addition, up to 50-65% of patients with *KRAS* wild-type tumors are resistant to EGFR moAb therapies. Therefore the quest for predictive markers continues. Genetic alterations in other EGFR effectors, acting downstream of *KRAS* together with alternative *KRAS* mutations (in codon 61 and 146) could drive primary resistance to anti-EGFR therapy and are currently investigated (Sartore-Bianchi et al., 2009a)(Molinari et al., 2009)(Souglakos et al., 2009)(Laurent-Puig et al., 2009)(Meriggi et al., 2009)(Prenen et al., 2009)(Loupakis et al., 2009a)(Loupakis et al., 2009b)(Perrone et al., 2009)(De Roock et al., 2011). Moreover, Sartore-

Bianchi et al., described that when expression of PTEN and mutation of *KRAS, BRAF* and *PIK3CA* are concomitantly ascertained, up to 70% of mCRC patients unlikely to respond to anti-EGFR therapies can be identified (Sartore-Bianchi et al., 2009a).

It is unclear to what extent the effects of mutant *KRAS* are the same for other RTK-targeted therapies. It is possible that *KRAS*-mutant tumors are not dependent on any RTK upstream component, and therefore will not respond to drugs targeting these RTKs. Alternatively, it might be that *KRAS* mutations confer only part of the survival advantage needed for tumor cells, and therefore will still benefit from RTK inhibition. Moreover, to define CRC as *KRAS* mutant versus *KRAS* wild-type probably underestimates additional heterogeneity found within both populations (De Roock et al., 2011).

2.3 BRAF

BRAF, a member of the *RAF* gene family (*ARAF*, *BRAF* and *CRAF*), encodes a serine-threonine protein kinase, downstream of activated KRAS, and initiates a mitogenic kinase cascade leading to cell proliferation (Figure 1). Activating mutations of *BRAF* have been reported in 5–15% of CRC and >95% of all known mutations involve a thymine to adenine transversion in nucleotide 1799, which leads to a substitution of valine by glutamic acid at amino acid residue 600 (V600E), which results in an upregulation of the ERK signaling pathway independently of *KRAS* mutation (Nash et al., 2010)(Barault et al., 2008)(Oliveira et al., 2004). In addition, the V600E mutation could have additional functions, since *KRAS* and *BRAF* mutations seems to be mutually exclusive in CRC, with very rare exceptions, suggesting they occur in different tumor types and have different outcomes. Moreover, *BRAF* mutations are associated with sporadic microsatellite instability (MSI), CpG island methylator phenotype (CIMP) and right sided tumors, whereas mutant *KRAS* are not (De Roock et al., 2011)(Dasari & Messersmith, 2010).

BRAF mutation status appears to be a valid negative prognostic marker for CRC in the adjuvant and metastatic setting, as demonstrate in the PETACC-3 (Roth et al., 2010), the CRYSTAL (Van Cutsem et al., 2011) and other studies (Yokota et al., 2011)(Park et al., 2011). The presence of CIMP-high appears to eliminate, at least in part, the adverse effect of *BRAF* mutations, whereas the good prognosis associated with MSI-high was abrogated in the presence of a *BRAF* mutation (Ogino et al., 2009a). In contrast, Samowitz et al. (Samowitz et al., 2005) and Roth et al. (Roth et al., 2010) found that *BRAF* mutations were associated with a significantly poorer survival in MSS tumors, but had no effect on the excellent prognosis of MSI-high tumors. Therefore, it has been postulated that it is not the *BRAF* mutation itself which confers a poor prognosis but rather that the mutation has different effects depending on the type of genetic pathway in which it is produced (Barault et al., 2008).

In addition, the currently available data suggest that the *BRAF* V600E mutation confers resistance to EGFR moAb in patients with chemotherapy-refractory *KRAS* wild-type mCRC and might be used as an additional predictive factor in this setting (Siena et al., 2009)(Laurent-Puig et al., 2009)(Sartore-Bianchi et al., 2009a)(Di Nicolantonio et al., 2008)(Tol et al., 2009).

Furthermore, the treatment of *KRAS*-mutated CRC with a selective *BRAF* inhibitor could be an interesting approach since *BRAF* is an important effector downstream of *KRAS* in the ERK signaling pathway. Phase II clinical trials are currently ongoing with the combination of sorafinib (*BRAF* inhibitor) with either FOLFOX, FOLFIRI or cetuximab.

2.4 PIK3CA

The PI3Ks are a family of lipid kinases grouped into three classes with different structure and substrate preferences. Class 1 phosphatidylinositol 3-kinases (PIK3) are heterodimeric proteins composed of a p85 regulatory subunit and one of several p110 catalytic subunits. Among several isoforms of the catalytic subunits, only the α-type, PIK3CA, has been shown to harbor oncogenic mutations or amplifications in its gene in human malignancies (Ogino et al., 2009b)(Jang et al., 2010). Activation of class I PI3K is initiated when a growth factor binds to its cognate RTK, which includes members of the ERBB-family, platelet-derived growth-factor receptor (PDGFR) and the insulin and the insulin-like growth-factor 1 receptors (IGF1R) (De Roock et al., 2011). Activated PIK3CA will phosphorylate phosphatidyl-inositol-4,5-biphospate (PIP2) to produce phosphatidyl-inositol-3,4,5-triphospate (PIP3) which localizes the serine threonine kinase Akt to the cell membrane where it becomes activated (Figure 1). Activated Akt phosphorylates downstream protein effectors and amplifies the signaling cascade, enhancing cell proliferation and survival (Ogino et al., 2009b). Based on the current data, it seems that *PIK3CA* mutation frequency in CRC is probably between 15 and 25% (Dasari & Messersmith, 2010). More than 80% of *PIK3CA* mutations in CRC occur in exon 9 (60-65%) or exon 20 (20-25%). Mutation in *PIK3CA* can co-occur with *KRAS* and *BRAF* mutations. A European consortium recently suggested that only *PIK3CA* exon-20 mutations are associated with a lack of cetuximab activity in *KRAS* wild-type tumors (De Roock et al., 2010b). However, because of the low frequency of this mutation, these data require confirmation in large patient population studies. In contrast, *PIK3CA* exon-9 mutations are associated with *KRAS* mutations and do not have an independent effect on cetuximab efficacy (De Roock et al., 2010b). The apparent difference between exon-9 and exon-20 mutations could explain the conflicting data regarding *PIK3CA* mutations reported by Sartore-Bianchi and colleagues (Sartore-Bianchi et al., 2009b) (lack of response to cetuximab and more exon-20 mutations) and Prenen and colleagues (Prenen et al., 2009) (no correlation). *PIK3CA* mutations as a whole were associated with shorter cancer specific survival in a series of surgically resectable CRC, but exon-9 and exon-20 were not studied separately (Ogino et al., 2009b)(Kato et al., 2007). More studies on large patient populations are needed to establish the prognostic role of *PIK3CA* exon-9 and exon-20 mutations.

2.5 PTEN

PI3K-initiated signaling is inhibited by phosphatase tensin homologue (PTEN). The PTEN protein acts as a phospholipid phosphatase with PIP3 as a substrate. PIP3 is an important lipid second messenger that provides docking sites for multiple downstream components, including AKT, which is activated by phosphorylation and inhibited by PTEN (Figure 1). Since PTEN protein is a negative regulator of the AKT signaling pathway, inactivation of PTEN, which is a common event in human malignancies, facilitates cell proliferation and apoptosis (Sawai et al., 2008)(Goel et al., 2004). PTEN activity may be lost trough various mechanisms, including mutations, deletions, silencing, allelic losses at chromosome 10q23 or hypermethylation of the *PTEN* promoter region (especially in MSI-high CRC). Therefore, ascertainment of PTEN status is usually done on protein level and the recorded frequency of loss of PTEN expression varies from 19% to 36% in CRC. Data on the loss of PTEN are not concordant between primary and metastatic tumors (De Roock et al., 2011)(Dasari & Messersmith, 2010). In addition, PTEN loss in metastatic tumors predicted lack of response

to cetuximab and PTEN null metastasis had shorter progression free survival, which was even more significant in *KRAS* wild-type patients. In sharp contrast, the PTEN analysis on the primary tumor did not reveal any predictive or prognostic information. Although the relative low concordance rate between the primary and metastatic tumors for PTEN expression could be secondary to selection of clonal populations during metastasis, it could be the subjective nature of immunohistochemistry testing with significant method and observer variability. This consideration and the possible need to analyze PTEN from metastatic tumors may limit the role of PTEN as biomarker in CRC (Dasari & Messersmith, 2010).

2.6 Conclusion

In summary, both MAPK and PI3K pathways are stimulated by EGFR, with important implication for EGFR targeted therapy and future drug development. Current American Society of Clinical Oncology (ASCO) guidelines recommend testing only for *KRAS* mutations in codon 12 and codon 13, in patients being considered for EGFR moAb therapy (Dasari & Messersmith, 2010). However, evidence shows that other molecular alterations, such as *BRAF*, *PIK3CA* (exon-20) mutations or loss of PTEN expression, could preclude response to EGFR moAb. The subjective nature of PTEN assessment, however, is a significant challenge. In addition, new drugs are being developed against numerous targets in these pathways, and many are in early clinical stages. Finally, a better understanding of the functional interactions within RTK-activated intracellular pathways is essential to target the individual tumor and to deliver more effective medical treatment to patients with mCRC. Furthermore, the ability of the cancer cell to develop drug resistance via new mutations or alternative signaling pathways also needs to be addressed by combination therapy, and, if possible, analysis of tumor tissue upon progression (Dasari & Messersmith, 2010)(De Roock et al., 2011).

3. Pancreatic cancer

3.1 Introduction

Pancreatic cancer has the worst prognosis of all gastrointestinal malignancies with the mortality approaching the incidence (Buxbaum & Eloubeidi, 2010)(Bünger et al., 2011). Late clinical presentation, intrinsic biological aggressiveness, and resistance to conventional chemotherapy and radiotherapy represent the predominant reasons for its poor prognosis (Pizzi et al., 2009). This demonstrates an urgent demand for improved screening tools for early detection (Buxbaum & Eloubeidi, 2010)(Bünger et al., 2011). While surveillance is performed in individuals with genetic syndromes, hereditary pancreatitis, and a strong family history there are no clear guidelines for those with clinical risk factors like diabetes mellitus, tobacco use, and chronic pancreatitis (Buxbaum & Eloubeidi, 2010). Pancreatic ductal adenocarcinoma is the most commonly diagnosed pancreatic neoplasm, and reported to be the forth or fifth leading cause of cancer death in Western countries. Diagnosis of pancreatic cancer at early stages is crucial because successful surgical resection remains the only possibility of cure (Ansari et al., 2011). Only 10-30% of pancreatic tumor patients are operated on with curative intent. The expected 5-year survival rate of R0 resected patients with additional adjuvant chemotherapy is about 4-26%. In contrast, for the remaining patients who present with unresectable UICC stage III and IV carcinomas, no curative

therapy is available. These patients have median survival times of 8-12 months (stage III) and 5-8 months (stage IV), respectively (Bünger et al., 2011). In addition, early-stage pancreatic cancer is usually clinically silent, and symptoms only become apparent after the tumor invades surrounding tissues or metastasis to distant organs. Therefore, most persons who present with symptoms attributable to pancreatic cancer have advanced disease (Vincent et al., 2011).

The Holy Grail for pancreatic cancer investigators is to identify early markers, which predict the development of pancreatic cancer, uncover early resectable disease, and guide therapy (Buxbaum & Eloubeidi, 2010).

Potential molecular markers are sought in the pancreatic tissue, juice as well as other body fluids including serum and urine. An important consideration is that pancreatic tumor cells and secreted molecules are found in markedly higher concentrations in the pancreas and pancreatic juice compared to serum. Additionally, molecules and proteins in the serum are overwhelmed by high concentrations of albumin, transferrin, and immunoglobulins (Buxbaum & Eloubeidi, 2010).

Both hypothesis driven and high throughput searches for molecular markers to predict disease, early diagnosis, and treatment response are underway. Challenges include differentiation of cancer from chronic inflammatory disease of the pancreas and achieving reproducible results among diverse patients. Minimally invasive methods including endoscopic ultrasound guided fine needle aspiration (EUS-FNA) to acquire tissue may facilitate these important efforts (Buxbaum & Eloubeidi, 2010). This method enabled not only accurate diagnosis, but also the collection of cancer tissue before surgery or chemotherapy even in inoperable cases. Evaluation of the expression status of multiple molecules within the FNA specimen will lead to the establishment of individualized therapeutic strategies based on the prediction of prognosis or response to chemotherapy (Hamada & Shimosegawa, 2011).

3.2 Serum biomarkers

Improved screening for early diagnosis is essential in order to increase the rate of curatively resectable carcinomas, thereby ameliorating patient's prognosis. In present clinical practice, screening for pancreatic cancer is based on state-of-the-art imaging or even invasive diagnostics. A relatively non-invasive, cost efficient possibility could be provided by the measurement of disease-specific markers in peripheral blood. A wide range of serum markers has been reported to be elevated in pancreatic cancer patients since the eighties. Despite these many markers or their combinations with high diagnostic potential for pancreatic cancer screening, none of them have achieved the levels of sensitivity and specificity necessary to be recommended as a screening tool for asymptomatic patients in the general population (Bünger et al., 2011)(Xu et al., 2011). Only a few markers have shown promising results in recent studies with CA19-9 being the most widely investigated and evaluated single marker (Bünger et al., 2011).

3.2.1 CA19-9

The best-established marker is CA19-9, which is a sialylated Lewis antigen of the MUC1 protein with an overall sensitivity ranging from 41 to 86% and specificity from 33 to 100%