Diplomarbeit

Analysis of germline polymorphisms in the HIF1A gene to predict CRC risk and relapse

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Abstract

Das kolorektale Karzinom ist eine der am häufigsten vorkommenden Tumorentitäten. Die Inzidenz in Österreich liegt bei ungefähr 33 Neuerkrankungen pro Jahr. Das Risiko, an einem bösartigen Tumor des Gastrointestinaltraktes zu erkranken, steigt mit zunehmendem Alter und hängt von Einflussfaktoren wie unter anderem Umwelt und Genetik ab. Letzterer wurde in den vergangen Jahren besondere Aufmerksamkeit geschenkt – unter anderem dem Hypoxie-induzierbaren Faktor alpha (HIF1A) Gen auf Chromosom 14q. Dieses trägt zwei missense-Mutationen – P582S (C1772T) und A588T (G1790A). In der vorliegenden Arbeit soll die Rolle dieser beiden Einzelnukleotidpolymorphismen in Bezug auf das krankheitsfreie Überleben nach kolorektalem Karzinom und das Erkrankungsrisiko an einem kolorektalen Karzinom untersucht werden. Dabei wurden 381 Patienten mit einem histologisch verifizierten kolorektalen Karzinom mittels TaqMan genotypisiert und einer Kontrollgruppe von 2156 gesunden Individuen gegenübergestellt. Dabei konnte festgestellt werden, dass keine Korrelation zwischen den beiden Polymorphismen und dem krankheitsfreien Überleben und dem Erkrankungsrisiko besteht. In einer explorativen Analyse konnte jedoch gezeigt werden, dass das HIF1A 588T Allel mit der Tumorgröße und Tumorlokalisation assoziiert ist. Des Weiteren konnte ein signifikanter Zusammenhang zwischen Karnofsky-Index, Tumorgröße, klinischem Stadium, Anzahl der metastasierten Lymphknoten und der Verabreichung einer 5-FU hältigen Chemotherapie mit dem krankheitsfreien Überleben gezeigt werden.

Colorectal carcinoma is one of the most common cancers. The incidence in Austria is about 33 new cases per year. The risk of developing cancer of the gastrointestinal tract is increased in individuals of higher age and influenced by factors like environment and genetic. In the last few years various genetic factors have been examined, including the hypoxia inducible factor alpha (HIF1A) gene on chromosome 14q. Two missense mutations are situated on this gene region – P582S (C1772T) and A588T (G1790A). In this diploma thesis the role of these single nucleotide polymorphisms (SNPs) regarding the risk of developing colorectal cancer (CRC) and furthermore the association between these SNPs and

DFS shall be examined. Therefore 381 patients with histologically confirmed CRC were genotyped with TaqMan and compared with a control group of 2156 healthy individuals. We found that neither a correlation between these SNPs and disease-free survival nor an association between both SNPs and the risk of developing CRC exists. However, in an exploratory analysis, the HIF1A 588T allele was associated with tumor localization and tumor size. Furthermore, a significant correlation could be found for the Karnofsky index, tumour size, clinical stage, number of metastatic lymph nodes and the treatment with 5-FU chemotherapy with disease-free survival.

1. Introduction

Although significant progress in the treatment of colorectal carcinoma has been made in the past years, this disease still remains serious. Due to the high incidence rate of CRC, the identification of risk factors and the course of the disease are of fundamental importance. Foremost the inter-individual differences in tumour development and tumour progression have to be investigated, in order to improve screening and individual treatment. Since several risk factors like lifestyle, or predisposing diseases could already be detected, modern investigations focus on the genetic causes of this disease. Genetic polymorphisms are responsible for inter-individual differences and may influence the development and progression of colorectal carcinomas, respectively cancer in common.

CRC represents one of the most common malignancies in Austria and it is mostly found among patients of higher age. Although many factors favouring the colorectal tumour genesis are still unknown, it seems sure that a multi-level process in which the normal gastrointestinal mucosa subsequently becomes an invasive carcinoma, after having changed into an adenoma and a dysplasia. Vogelstein et al. showed that several changes or damages of the normal DNA lead to malignancy, such as point-mutations in the K-ras protooncogene, hypomethylation of the DNA, loss of the DNA in the area of the APC-gene on chromosome 5 and in the area of the DCC-gene on chromosome 18 and last but not least loss of the allele on chromosome 17, leading to functional loss of the tumour suppressor gene p53.

About 25 per cent of colorectal carcinomas are associated with a positive family history, therefore a hereditary predisposition seems obvious. Hereditary disposition for CRC are given in several diseases of the colon, like in familial adenomatous polyposis, hereditary non-polyposis CRC (Lynch-Syndrome), Peutz-Jeghers-Syndrom, familial juvenile polyposis, Cowden-Syndrome and hereditary mixed polyposis syndrome.

Hypoxia-inducible factor-1 (HIF1) is a transcription factor found in mammalian cells that plays an essential role in cellular and systemic homeostatic responses to hypoxia (Wang GL, 1995). Northern blot and Western blot analyses indicated that HIF1 mRNAs and proteins are induced in cells exposed to 1% oxygen and

decrease rapidly upon return of the cells to 20% oxygen (Hogenesch JB, 1997). HIF1 plays a key role in the cellular response to hypoxia, including the regulation of genes involved in energy metabolism, angiogenesis, and apoptosis. The alpha subunits of HIF are rapidly degraded by the proteasome under normal conditions but are stabilized by hypoxia. Maxwell et al. could show an interaction between the von Hippel-Lindau tumour suppressor gene product VHL and the HIF1 regulation (Maxwell PH, 1999). HIF1 activity is controlled by the oxygen-regulated expression of the HIF1A subunit.

An involvement of HIF1 in human disease pathophysiology, including myocardial ischemia, cerebral ischemia, retinal ischemia, pulmonary hypertension, preeclampsia, intrauterine growth retardation, and cancer could be shown by Semenza (Semenza, 2000). Two common single nucleotide polymorphisms (SNPs) within the oxygen-dependent degradation domain of the HIF1A gene have been described: P582S (C1772T; rs115494650) and A588T (G1790A; rs11549467). Both SNPs have been related to an increased trans-activation capacity of HIF1A under normoxic and hypoxic conditions in vitro. In this diploma thesis the role of these specific SNP will be investigated. On the one hand the association between the SNPs and disease-free survival of colorectal carcinoma patients should be examined and on the other hand a correlation between these SNPs and the overall risk of developing a colorectal carcinoma should be investigated.

2. Anatomy of the Large Intestine

The Large Intestine is about 1.5 metres long and consists of Appendix vermiformis, Caecum, Colon, Rectum and Canalis analis. The Colon forms a kind of frame for small bowel on the backside of the peritoneal cave. As a result of this some segments are located intraperitoneal, whereas some others are retroperitoneal.

Distal of the Ostium ileale (Valva ileocaecalis/Bauhin-Klappe), which forms the border to the Ileum, the Coecum begins, which is approximately seven centimetres long. Proximal of the Bauhin-Klappe the Colon starts. It is divided into four segments – Colon ascendens, Colon transversum, Colon descendens and Colon sigmoideum. At the level of the second/ third vertebra sacralis the Colon sigmoideum merges into the Rectum (Bob & Bob, 2007).

The vasculary course is of fundamental importance due to the metastatic behaviour of CRC. Therefore a short overview of the major vessels, arterially as well as venously, shall be given.

2.1. Vascularisation of the Large Intestine

2.1.1. Arterial vascularisation

Basically the Large Intestine is supplied by two main arterial vessels, firstly the A. mesenterica superior and secondly the A. mesenterica inferior. The Caecum, the Colon ascendens and the Colon transversum are supported by the A. mesenterica superior, which is divided into the A. iliocolica, the A. colica dextra and the A. colica media. Distal of the left colon flexure (Flexura coli sinistra), the Colon descendens, the Colon sigmoideum and most of the Rectum are nourished by the A. mesenterica inferior, splitting into A. colica sinistra, Aa. sigmoidea and A. rectalis superior. In the area of the Flexura coli sinistra, the A. colica media, as a vessel branch of the A. mesenterica superior and the A. colica sinistra as a vessel branch of the A. mesenterica inferior form an anastomosis, the so called Riolan Anastomosis.

2.1.2. Venous vascularisation

The Venous drainage of the Caecum, the Colon ascendens and the Colon transversum goes through the V. mesenterica superior into the V. portae. The blood of the Colon descendens, the Colon sigmoideum and partially of the Rectum runs off to the V.mesenterica inferior. However, a part of the Rectums' blood flows via the V. interna pudenda interna to the V. cava inferior.

2.1.3. Lymphatic vascularisation

The entire lymph of the Large Intestine goes to the Truncus instestinalis. To be more precise, there are the Nll. ileocolici, in the area between the Ileum and the Colon ascendens, and the Nll. colii dextri, medii, sinistri and sigmoidei in the proximity of colon, which end up in the Nll. Mesntericii inferiores and superiors (Waldeyer, 2003).

3. Epidemiolgy of CRC

CRC represents one of the most common malignancies in Austria. Among men it is the third most common malignancy, after prostate cancer and lung cancer, whereas CRC in women comes second after breast cancer (Statistik, 2009). Colorectal carcinoma mostly occurs among patients of higher age – the highest mortality rate in Austria in 2008 could be found among men between the age of 75 and 80 and women in the age group of 85 and higher (Statistik, 2009). Considering the incidence rates between 1983 and 2007, it is striking that, except for some annual fluctuations, the total number of new cases of CRC remained quite steadily. In 1983 4487 new cases of CRC, and in 2007 4462 cases could be recorded in Austria.

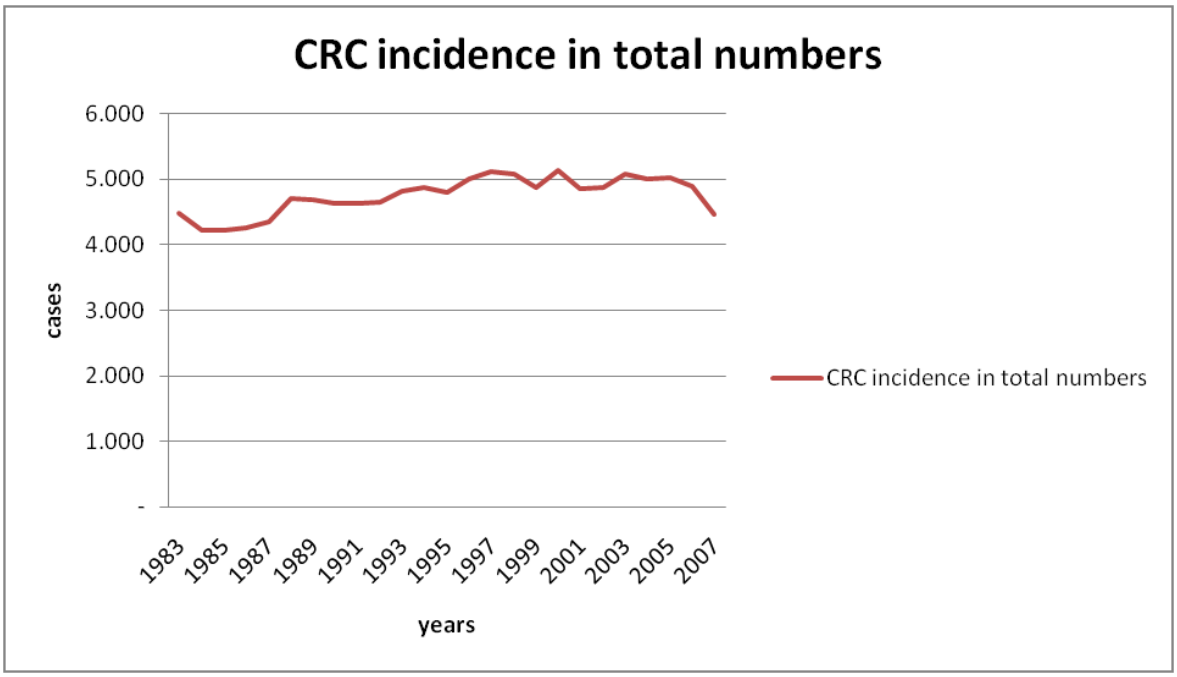

Image 1 (CRC incidence in total numbers in Austria)

: [Quelle: Statistik Austria; Österreichisches Krebsregister (Stand 27.8.2009)]

By contrast, in the total number of deaths caused by CRC since 1983 a slight decrease could be recorded, referring to 2688 deaths caused by CRC in 1983 to 2210 deaths in 2007.

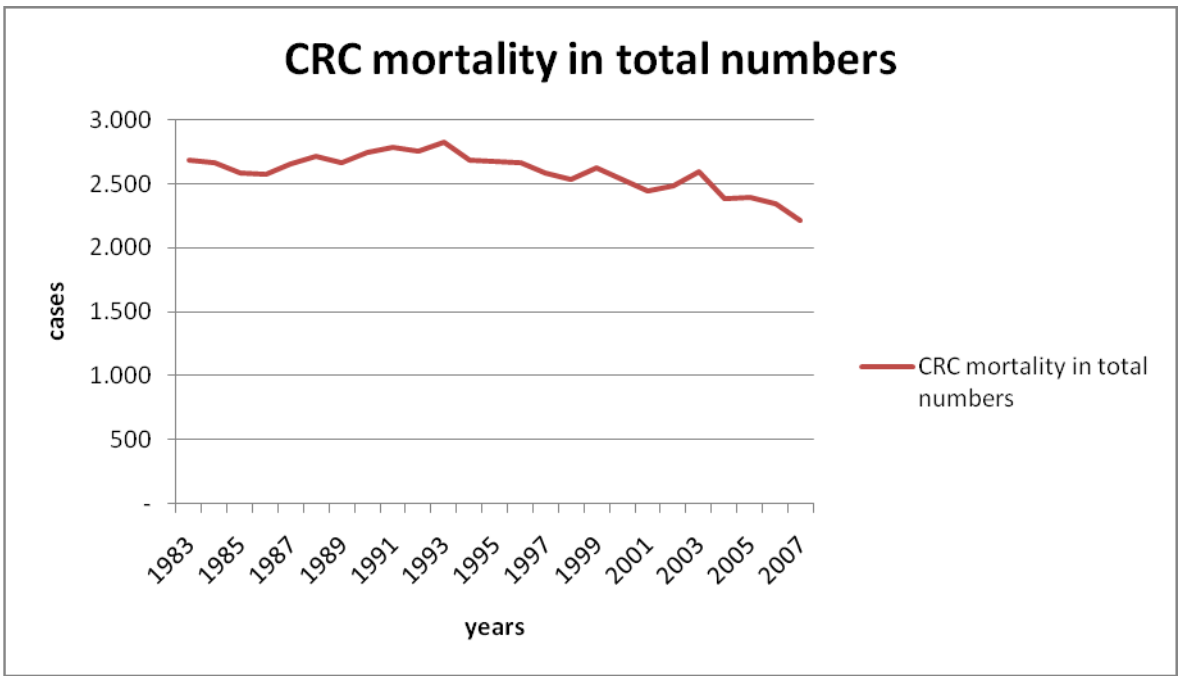

Image 2 (CRC mortality in total numbers in Austria)

: [Quelle: Statistik Austria; Österreichische Todesursachenstatistik (Stand 02.11.2009)]

However, the frequency of colorectal carcinomas varies in different areas. It is more common in developed countries, with a westernized lifestyle, such as The United States, Europe, New Zealand and Australia. In poor regions, such as South America and Africa, CRC is found less often (G.Layer & J.F.Riemann, 2007). As a result it is pretty likely that this correlates with the environmental exposure surroundings and the duration of residence in this environment (Vladimír Janout, 2000). Even in a small country like Austria a gradient of the CRC's incidence from east to west can be found (Hauser, 2004). Defining East Austria as Burgenland, Vienna, Lower Austria, Styria and Carinthia and West Austria as Upper Austria, Salzburg, Tirol and Vorarlberg a mean incidence of 32.9 cases per 100000 people in East Austria to a mean incidence of 29.8 cases per 100000 people a year in West Austria can be observed (including both sexes) [Quelle: Statistik Austria: Krebsinzidenz Jahresurchschnitt (2005/2007)].

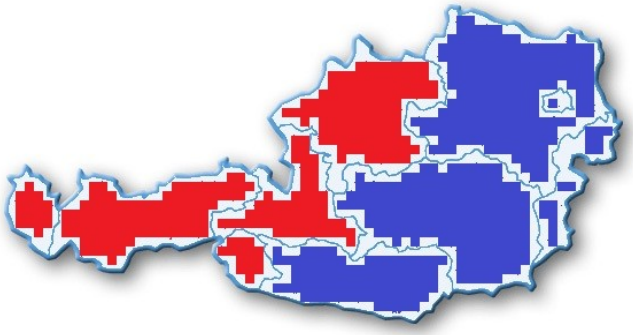

Image 3 (Cancer incidence East- and West Austria)

4. Aetiology and Risk factors

Colorectal carcinomas develop on the adenoma-carcinoma sequence of polyps of the gastrointestinal mucosa. This adenoma-carcinoma sequence is a multi-level process in which the normal gastrointestinal mucosa changes into adenomas followed by dysplasia and an invasive carcinoma. Vogelstein et al. showed that several changes or damages of the normal DNA lead to malignancy, such as point-mutations in the K-ras protooncogene, hypomethylation of the DNA, loss of the DNA in the area of the APC-gene on chromosome 5 and in the area of the DCC-gene on chromosome 18 and last but not least loss of the allele on chromosome 17, leading to functional loss of the tumour suppressor gene p53 (Longo, 2005).

Risk factors favouring the development of CRC are lifestyle, predisposing diseases and genetic factors (Herold, 2006).

4.1. Lifestyle

Some lifestyle factors seem to correlate with the incidence of CRC. Among those are nutritional behaviour, physical activity, smoking habits, alcohol consumption, folic acid, a high calcium intake and some medical treatment.

4.1.1. Nutritional behaviour

The lifetime risk to develop CRC correlates with the calorie consumption per head, the intake of red meat, animal fats and a high blood cholesterol level. Regional differences in the incidence of a CRC do not seem to be a result of genetic factors but rather a consequence of the environment and nutrition. Migrants from regions with a low risk of developing CRC quickly accommodate to the rate of disease in their immigration country. Moreover, westernized “malnutrition” leads to an increase of the incidence of CRC even in countries with a generally low risk. For example, an age-adjusted rate of colorectal carcinoma in Japan per 100000 people was 12.6 for males and 8.7 for females in 1974. The corresponding rate in 1991 was 42.5 and 25.6 (Nakaji, 1998). One reason for the high incidence rate in 1991 could be the increased intake of animal fats which results in a higher ratio of

anaerobic bacteria in the intestine. High calorie food also leads to adiposity, followed by insulin resistance that provokes an increased blood level of the Insulin-like Growth Factor Typ 1 (IGF-1). IGF-1 is suspected to stimulate mucosal changes in the intestine (Longo, 2005). Furthermore, observations showed a protective effect for an increased fibre intake. Riboli et al. even found that an approximate doubling of the total fibre intake could reduce the risk of CRC by 40% (Riboli, 2003). However, the role of a dietary fibre intake is still discussed quite controversially. While the EPIC study showed the benefit of dietary fibre nutrition (30g/day), (Bingham, 2003) this correlation couldn't be observed in the Nurses' Health Study (Fuchs, 1999).

4.1.2. Physical activity

Various studies could show the protective effect of a moderate physical work (30 to 60 minutes per day) (Friedenreich, 2002). Basically people with a high BMI are two times more likely to develop a CRC but it is uncertain, whether the increase of the risk results from the overweight, the high calories intake or the poor physical work (Giacosa, 1997).

4.1.3. Smoking habits

It has been frequently demonstrated that smoking raises the risk of developing CRC (Giovannucci, 2001). Reid et al. showed that long-term abuse of cigarettes increases the prevalence of multiple and larger adenomas. An increased risk of adenoma recurrence could be shown within smokers for a period of over 35 years (Reid, 2003).

4.1.4. Alcohol intake

A growing body of evidence shows that alcohol intake is a risk factor for CRC. Recent molecular observations strongly support a role for alcohol acting through disruptions in one-carbon metabolism. Above all the combination between high alcohol consumption and low folate intake seems to be a fatal mix of risk factors (Giovannucci, 2004).

4.1.5. Folic acid and calcium intake

A higher intake of food containing folacin and calcium could be associated with a lower risk of developing CRC. However, it is still not clear whether the risk reduction is achieved by folate (Konings, 2002) or calcium (Wu, 2002) itself or by other ingredients contained in food which is rich in folacin and calcium.

4.1.6. Medical treatment

Chan et al. could show a protective effect of acetylsalicylic acid (ASS) for CRC. However these results couldn't be confirmed in other studies. As a result there are no general recommendations for ASS as a primary prevention (Chan, 2004). Furthermore, hormone replacement therapy (HRT) has shown some protective effects (Johnson, 2009) but because of other well known side effects HRT should not be used to minimize the CRC risk (Schmiegl, 2008). A rare risk factor for CRC is pelvic irradiation (Tsunoda, 1997).

4.2. Predisposing diseases

CRC occurs more often in patients with predisposing diseases like ulcerative colitis, Crohn's disease, streptococcal bovis bacteraemia and ureteral sigmoid ostomy.

4.2.1. Chronic inflammatory bowel diseases

Colorectal carcinomas appear more often in patients suffering from chronic inflammatory bowel diseases. During the first ten years of the disease no appreciable aggravation of risk can be found. However, after 10 years of disease the risk is increased about one per cent per annum. The enormous difficulty concerning chronic inflammatory bowel diseases and CRC is the diagnosis. Crohn's disease and ulcerative colitis are similar to each other regarding

symptoms. Bloody diarrhoea, abdominal pain, obstructions and irregular faeces can appear in both diseases (Longo, 2005).

4.2.2. Streptococcus bovis bacteraemia

CRC often occur with streptococcus bovis bacteraemia. Patients with an endocarditis or septicaemia caused by this pathogen have a high incidence of developing intestinal tumours. However, the reason is still unknown.

4.2.3. Ureteral sigmoid ostomy.

Patients who received an ureteral sigmoid ostomy have a five to ten per cent higher risk to develop CRC after duration of more than 15 years. Carcinomas develop almost always distal of the ureteral entry into the large intestine where the mucosa has to deal with urine and faeces.

4.3. Genetic factors

About 25 per cent of colorectal carcinomas are associated with a positive family history. Hereditary disposition for CRC is given in several diseases of the colon. These are: the familial adenomatous polyposis (FAP), the hereditary non-polyposis CRC (Lynch-Syndrome; HNPCC), the Peutz-Jeghers-Syndrom, the familial juvenile polyposis, the Cowden-Syndrome and the hereditary mixed polyposis syndrome, among which FAP and HNPCC are certainly the most common.

4.3.1. Familial adenomatous polyposis

Per definition there have to be more than 100 adenomas in the whole large bowel. It is a rare disease with an incidence of about 1.3 cases per 1 000 000 individuals, equal in females and males. Nearly 70 per cent of FAP are transmitted autosomal dominant whereas 20 to 30 per cent are caused by sporadic mutations of the APC-gene. This gene is located on chromosome 5q21-5q22. Since 1987, when the APC gene was found, more than 800 different germline mutations have been discovered, being able to damage the APC coded protein. In addition to that, some allelic variations of FAP are known, the so called Gardner Syndrome, Turcot

Syndrome, Oldfield Syndrome and the hereditary flat adenoma syndrome. Except for the hereditary flat adenoma syndrome, all other manifestation patterns show more than 100 polyps in the gastrointestinal tract, mostly appearing at the age of 30 years. Most of the adenomas are located in the lower part of the intestine. After approximate 30 years, nearly 100 per cent of diseased persons develop CRC. But FAP-patients can also develop extracolic tumours, like osteomas, mostly located at the mandible, cutaneous fibromas, astrocytomas and carcinomas of the thyroid gland. In more than 80 per cent patients with FAP show a hypertrophy of the retinal pigment (H.F.Otto, 2004). Children of FAP patients have a 50 per cent probability to inherit the disease. Therefore, molecular screening should be offered to the children at risk. Children having been tested positive for the disease should undergo a yearly sigmoidoscopy or colonoscopy starting at the age of about ten years (Longo, 2005).

4.3.2. Lynch-Syndrome

Hereditary non-polyposis CRC is transmitted autosomal dominant. Basically there are two forms of the Lynch-Syndrome, Lynch I and Lynch II, which are associated with extraintestinal tumours, such as an ovarian carcinoma or endometrial carcinoma. To diagnose HNPCC several requirements have to be confirmed: three or more relatives have to developed CRC with one of them being a first-grade relative and one having developed CRC prior to the age of 50 years. Furthermore CRC has to affect two generations and FAP has to be barred. (criteria of Amsterdam – further criteria Amsterdam II and Bethesda) Contrary to FAP most carcinomas in HNPCC appear in the proximal colon. The average age of disease is under 50 years. Subforms of HNPCC are the Turcot-Syndrome and the Muir-Torre-Syndrome. Nearly 10 per cent of all CRCs are based on mutations on HNPCC gene. Although HNPCC's penetrance is not as high as in FAP about 90 per cent of men and 70 per cent of women develop CRC until the age of 70 years. The genetic cause of HNPCC is due to dysfunctions of DNA repair mechanisms. Among those mutations in gene hMSH2 on chromosome 2p16 and hMLH1 on chromosome 3p13.3 are the most relevant. Further affected genes can be hMMS1, hPMS2 or hMSH6/GTBP (H.F.Otto, 2004).

4.3.3. Peutz-Jeghers-Syndrome

The Peutz-Jeghers-Syndrome is a rare autosomal dominant transmitted disease with a grade of heredity of about 90 per cent. Characteristics are a muco-cutaneous pigment malformation and colorectal polyposis. In 1998 the gene STK11, responsible for the Peutz-Jeghers-Syndrome was discovered on chromosome 19p. It encodes for a serin-threonin-kinase (STK), which probably is responsible for the regulation of cell differentiation and signal transduction. A problematic factor of this disease is the high percentage of invaginations of the colon, followed by a number of deaths at an early age (Harrison, 2005).

4.3.4. Familial juvenile polyposis

Familial juvenile polyposis is a highly rare disease that mostly manifests at the age of 4 to 5 years. The aetiology is still unknown. About 30 per cent of patients with familial juvenile polyposis show a germline mutation in the SMAD4/DPC4 gene (Harrison, 2005).

4.3.5. Cowden-Syndrome

Characteristics of the Cowden-Syndrome are hamartomas of the oropharyngeal mucosa as well as hands and feet, breast cancer, goitres of the thyroid gland, cancer of the thyroid and gastrointestinal polyps. The causing mutation lies on the PTEN-gene on chromosome 10 (Harrison, 2005).

4.3.6. Hereditary mixed polyposis syndrome (HMPS)

Hereditary mixed polyposis syndrome (HMPS) is characterized by atypical juvenile polyps, colonic adenomas, and colorectal carcinomas (WHITELAW, 1997).

5. Morphology of CRC

For the localisation of CRC age seems to be an important factor. Colorectal carcinomas in young patients are predominantly located in the right colon, whereas in older patients (>40a) rectum and sigmoid are preferably affected (Savas, 2007). However, about 60 per cent of all carcinomas are located in the rectum. Another 20 to 25 per cent can be found in the sigmoid (H.F.Otto, 2004). Carcinomas may grow exophytic with predominantly intraluminal growth, endophytic/ulcerative with predominantly intramural growth, diffusely infiltrative with subtle endophytic growth or annular with circumferential involvement of the colorectal wall. Overlaps of these types frequently occur. Furthermore, carcinomas of the proximal colon usually grow more exophytic and bulky while those of the distal colon are more often endophytic and annular.

5.1. Histhopathology

The colorectal carcinoma is defined as to break through the muscularis mucosae into the submucosa. Pre-stages of CRC are high grade intraepithelial neoplasia and intramucosal neoplasia. A majority of colorectal carcinomas are gland forming, with variability in size and configuration of the glandular structures. Two classifications of tumours of the colon and rectum are of tremendous importance – firstly the WHO classification of tumours of the colon and the rectum and secondly the TNM classification of tumours of the colon and rectum.

WHO histological classification of tumours of the colon and rectum

- Epithelial tumours
    - Adenoma
        - Tubular
        - Villous
        - Tubulovillous
        - Serrated
    - Intraepithelial neoplasia (dysplasia) associated with chronic inflammatory diseases
        - Low-grade glandular intraepithelial neoplasia
        - High-grade glandular intraepithelial neoplasia

    - Carcinoma
        - Adenocarcinoma
        - Mucinous adenocarcinoma
        - Signet-ring cell carcinoma
        - Small cell carcinoma
        - Squamous cell carcinoma
        - Adenosquamous carcinoma
        - Medullary carcinoma
        - Undifferentiated carcinoma

    - Carcinoid (well differentiated endocrine neoplasm)
        - EC-cell, serotonin-producing neoplasm
        - L-cell, glucagon-like peptide and PP/PYY producing tumour
        - Others
    - Mixed carcinoid-adenocarcinoma
    - Others
- Non-epithelial tumours
    - Lipoma
    - Leiomyoma
    - Gastrointestinal stromal tumour
    - Leiomyosarcoma
    - Angiosarcoma
    - Kaposi sarcoma
    - Malignant melanoma
    - Others
    - Malignant lymphomas
        - Marginal zone B-cell lymphoma of MALT Type
        - Mantle cell lymphoma
        - Diffuse large B-cell lymphoma
        - Burkitt lymphoma
        - Burkitt-like /atypical Burkitt-lymphoma
        - Others
- Secondary tumours

- Polyps
    - Hyperplastic (metaplastic)
    - Peutz-Jeghers
    - Juvenile

T – Primary Tumour

TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis	Carcinoma in situ: intraepithelial or invasion of lamina propria
T1	Tumour invades submucosa
T2	Tumour invades muscularis propria
T3	Tumour invades through muscularis propria into subserosa or into non-peritonealized pericolic or perirectal tissues
T4	Tumour directly invades other organs or structures and/or perforates visceral peritoneum

N – Regional Lymph Nodes

NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in 1 to 3 regional lymph nodes
N2	Metastasis in 4 or more regional lymph nodes

M – Distant Metastasis

MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

5.1.1. Mucinous adenocarcinoma

More than 50 per cent of the lesion has to be composed of mucin to define the tumour as a mucinous adenocarcinoma. This histopathological type is characterized by a cluster of extracellular mucin that contains malignant epithelium, like acinar structures, strips of cells or single cells.

5.1.2. Signet-ring cell carcinoma

The typical signet-ring cell shows a big mucin vacuole, that displaces the nucleus to the cell border. More than 50 per cent of tumour cells must have the feature of intracytoplasmatic mucin to be able to speak of a signet-ring cell carcinoma.

5.1.3. Adenosquamous carcinoma

In this kind of tumour, both – squamous carcinoma and adenocarcinoma – appear. The subtypes can exist as separate areas or mixed.

5.1.4. Medullary carcinoma

In comparison with other types this histopathological subtype has a rather good prognosis. However it is a quite rare type and characterized by sheets of malignant cells with vesicular nuclei, prominent nucleoli and abundant pink cytoplasm exhibiting prominent infiltration by intraepithelial lymphocytes.

5.1.5. Undifferentiated carcinoma

In this histopathological type no well-defined differentiation can be made. As a result, this kind of tumour can show a broad variability of histological features.

5.1.6. Other variants

Other rare variants of colorectal carcinomas are the spindle cell carcinoma or the carcinosarcoma.

6. Grading of colorectal carcinoma

Basically colorectal carcinomas can be divided into low grade and high grade lesions. The most important factor for grading CRCs is to determine the extent of glandular appearances. Consequently they should be divided into well, moderately, poorly and undifferentiated lesions. The percentage of the tumour showing gland like structures is used to define the grade. Grade 1 or well-differentiated tumours show more than 95 per cent of glandular structures. In grade 2 or moderately-differentiated lesions about 50 to 95 percent exhibit glandular structures. Poorly-differentiated or grade 3 adenocarcinomas only have 5 to 50 per cent of glandular structures. In undifferentiated or grade 4 carcinomas less than 5 percent show glandular growth (WHO).

7. Molecular genetics

Most colorectal carcinomas are expected to begin in a colorectal epithelial cell with inactivation of the APC suppressor gene, caused by mutation (Breivik, 1999). As a result of this it comes to an interference with E-cadherin homeostasis and dysregulation of the gene transcription happens. Further genetic mutations, such as activation of proto-oncogenes like c-myc and ras occur, and suppressor genes are inactivated (Kinzler, 1998). During tumour progression the genes on chromosome 18 (Thiagalingam S, 1996) and the TP53 gene on chromosome 17 are inactivated (Levine, 1997). The mutated TP53 gene product, in turn, cannot regulate normally a serious of genes regulated by wild-type p53, including p21WAF1/CIP1 cyclin-dependent kinase inhibitor which complexes with proliferating cell nuclear antigen, and genes resulting in apotosis, including BAX (Stanley R. Hamilton, 2000, S. 116).

The loss of genomic stability in CRC is caused by several causes, such as:

7.1. Chromosomal instability

The most common type of genomic instability in CRC is chromosomal instability (C. Lengauer, 1997). Tumour suppressor genes like APC, P53 or SMAD family member 4 are often inactivated by chromosomal instability. Ampflification of gene copy number or gene rearrangement normally does not play an important role in CRC (Leary RJ, 2008).

7.2. Aberrant DNA Methylation

Silencing of genes, mostly mediated by aberrant DNA methylation, is another mechanism of gene inactivation in patients with CRC (Issa, 2004). In the normal genome, cytosine methylation occurs in areas of repetitive DNA sequences outside of exons. By contrast, there is a modest global depletion of cytosine methylation in the CRC genome but also a considerable acquisition of aberrant methylation within certain promoter-associated CpG islands. This aberrant promoter-associated methylation can induce epigenetic silencing of gene expression.

Among the loci that can undergo aberrant methylation in CRC, a subgroup seems to become aberrantly methylated as a group, a phenomenon called CpG island

methylator phenotype (CIMP, or CIMP-high). The phenomenon is observed in about 15% of CRCs and is present in nearly all such tumours with aberrant methylation of MLH1. A third pattern of aberrant methylation is exemplified by exon 1 of the gene encoding vimentin. Although this locus is not expressed by normal colon mucosa or CRC, it is aberrantly methylated in 53 to 83% of patients with CRC in a pattern that is independent of CIMP (Sanford D. Markowitz, 2009).

7.3. Growth factors

The activation of growth factors is high in CRC. Several pathways can be affected by irritation:

- Prostaglandin signalling
- Epidermal growth factor
- Vascular endothelial growth factor

Inflammation or mitogene-associated up-regulation of COX-2 can cause an abnormal response of the prostaglandin pathway. Prostaglandin E2 activity can also be increased by loss of 15-prostaglandin dehydrogenase (15-PGDH), the rate-limiting enzyme in catalyzing degradation of prostaglandin. Inhibition of COX-2 by nonsteroidal antiinflammatory drugs prevents the development of new adenomas (Baron, 2003).

The epidermal growth factor (EGF) is a protein that has trophic effects on intestinal cells. Clinical studies have supported an important role for signaling through the EGF receptor (EGFR) in a subgroup of CRCs (David Cunningham, 2004). EGFR mediates signaling by activating the MAPK and PI3K signaling cascades. Recent clinical data have shown that advanced CRC with tumour-promoting mutations of these pathways — including activating mutations in KRAS, BRAF, and the p110 subunit of PI3K do not respond to anti-EGFR therapy.

The vascular endothelial growth factor (VEGF) that is produced in states of injury or during the growth of normal tissue, drives the production of new blood vessels. Clinical studies have suggested a role for angiogenic pathways in the growth of CRC.

7.4. Microsatellite instability (MSI)

"*MSI is defined as a change of any length due to either insertation or deletion of repeating units, in a microsatellite within a tumour when compared to normal tissue*." (Stanley R. Hamilton, 2000, S. 116-117) Some CRCs are marked by numerous nucleotide changes in unstable repeated sequences in tumour DNA, the so called microsatellite instability (MSI) (Jiricny, 1998). Five microsatellites should be used as a reference standard: BAT25, BAT26, D5S346, D2S123 and D17S250. When two or more markers show MSI one speaks of high frequency MSI whereas if only one marker is affected, it involves too low frequently microsatellite instability (MSI-L) About 15 percent of colorectal carcinomas are represented by MSI-H.

A kind of environmental factor regarding angiogenesis or tumour growth is hypoxia. An important role in several genes play adaption to hypoxia, which are all activated by HIF (hypoxia-inducible factor) (Imamura, 2009).

7.5. HIF

Hypoxia-inducible factor-1 (HIF-1) is a family member of transcription factors that controls a variety of embryonic and physiologic events and plays a significant role in the oxygen homeostasis. It is situated on chromosome 14q21 – 14q24.

HIF-1 is a heterodimeric protein that is composed of 826 amino acids and 2 subunits, HIF1A and HIF1B, both of which are members of the basic-helix-loop-helix-Per-Arnt-Sim (PAS) family of proteins (Wang, 1995). HIF1A is the oxygen-regulated component and determines HIF1 activity, while HIF1B is constitutively expressed (Imamura, 2009). The N-terminal part of the protein is responsible for the attachment to DNA, whereas the C-terminal part of the protein moderates either the breakdown or the activation of HIF1A. HIF1A's activity is mediated by several modifications, like hydroxylation, acetylation or phosphorylation. The hydroxylation proceeds within the oxygen dependent degeneration domain (ODDD). HIF1A inactivation, as well as activation underlies complex processes in both normoxic and hypoxic situations. The inactivation of HIF1A under normoxic conditions can be amplified by several substances, like insulin or EGF, (epidermal

growth factor) (Jiang, 2001) IGF1-1 (insulin like growth factor) (Rosenzweig, 2004) or some interleukines, for example IL 8 (interleukine 8) (Kim, 2006). HIF1A is degraded via the ubiquitin-proteosome degradation system (Jaakkola P, 2001). This process is mediated by the von Hippel-Lindau tumour suppressor protein (pVHL), which predominantely targets the minimal N-terminal trans-activation domain (N-TAD) within the ODDD (Maxwell PH, 1999). In case of hypoxia the hydroxylation and acetylation of HIF1A is blocked (Bárdos, 2004) with the consequential effect of increased activity of HIF1A related genes. HIF1A plays an essential role in embryogenesis as well as in malignant transformation of normal cells in many different kinds of human tumours (Zhong, 1999). HIF1A mediates among others the generation of VEGF (vascular endothelial growth factor) that leads to angiogenesis. Therefore it is not surprising that loss of HIF-1 results in slower growth of tumours and transformed cell lines (Goda, 2003).

Two common single nucleotide polymorphisms (SNPs) within the oxygen-dependent degradation domain of the HIF1A gene have been described: P582S (C1772T; rs115494650) and A588T (G1790A; rs11549467). Both SNPs have been related to increased trans-activation capacity of HIF1A under normoxic and hypoxic conditions in vitro (Tanimoto, 2003). The influence of HIF1A SNPs on the risk to develop cancer has been investigated in several trials. However, the role of these SNPs in CRC is still unclear. In the present study, we have therefore investigated the association between HIF1A P582S and A588T polymorphisms and the development of CRC and disease-free survival of CRC.

7.6. Single nucleotide polymorphisms

SNP are a type of genetic variation, in which one nucleotide is found at a given position in a specific gene in some individuals, whereas a different nucleotide is found in others. However, SNPs occur approximately once every 100 to 300

bases. In normal cells, there is always a 50% ratio of the two alleles for any SNP. This happens due to the fact that one allele comes from the mother whereas the other one is inherited from the father. Nonetheless in tumour cell lines the ratio can be shifted due to chromosomal changes (Vogelstein, 2002).

SNPs' frequency differ from different ethnic groups – some polymorphisms are more common in europeans whereas others are more frequent in Africans, and so on. However, until now – about 57 million different SNPs are known (National Center for Biotechnology Information).

On the one hand SNPs can be single nucleotide changes, like A/G (adenosine to guanine) and on the other hand SNPs can be small insertions or deletions. This may cause diseases or have no impact. Moreover SNPs give us a valuable insight into inheritance patterns.

8. Clinical basics of CRC

During the last decades remarkable progress in diagnostic and therapeutic possibilities in CRC has been made. However, CRC is still a frequent and life-threatening disease.

8.1. Clinical presentation

General symptoms of CRC show a considerable degree of variability and vary according to their anatomic localisation. Since the consistency of the faeces of the ileum is quite liquid, tumours in colon ascendens can grow relatively quickly without generating any symptoms like obstruction or faecal conspicuities. Lesions of the right colon nonetheless often grow ulcerative and lead to chronic occult bleedings. Therefore the first symptoms of these patients are fatigue or ischaemic symptoms, such as angina pectoris, loss of power or dyspnoea. A diagnostic blood count often shows a hypochromatic microcytic anaemia as a result of a chronic loss of iron. Due to sporadic bleeding of the tumour, several tests for occult blood in faeces can be negative. During the passage through the large intestine the faeces becomes more and more harder. Therefore more distal tumours, for example in colon descendens or sigmoid tumours often lead to obstruction symptoms, such as abdominal spasms and pains. Far advanced carcinomas even can perforate the bowel, leading to an escape of the faeces into the abdominal cavity followed by severe infection and inflammation. Carcinomas of rectum or sigmoid often manifest as bloody faeces or the so called “pencilfaeces” (Bleistiftstuhl) (Longo, 2005).

8.2. Staging

Tumour spreading usually happens continuously along, or through the intestinal wall into peri-colic fat tissue or other near organs. The most important prognostic factors are penetration depth into the intestinal wall, infiltration of regional lymph nodes and the state of metastasis. Former classifications, like the Duke-classification are now replaced by the TNM classification of International Union against Cancer.

The TNM classification includes on the one hand clinical and on the other hand histopathological criteria. T stands for the infiltration depth of the tumour, N means lymph-node involvement and M describes whether distant metastases occur or not. As already mentioned, colorectal carcinomas are evaluated by the UICC classification and divided into 4 stages. In stage one all superficial tumours that do not infiltrate the subserosa and show now lymph node metastasis are categorized. (T1/T2N0M0) Tumours that infiltrate the subserosa (T3) or other organs (T4) but do not show any lymph node metastasis are included in UICC stage II. (T3/T4N0M0) UICC stage III is defined as appearance of regional lymph node metastasis. (TxN1/N2M0) In UICC stage IV metastasis occurs in distant organs, like liver, lung or bones (TxNxM1) (Longo, 2005).

If there is a justified guess that a patient suffers from a colorectal carcinoma, several basic examinations should be carried out. A detailed and exactly carried out examination is of an enormous importance due to the subsequent consequences regarding therapeutic management. A basic examination includes digital rectal examination, a colonoscopy of the entire large intestine with biopsy, an abdominal ultrasound, thorax radiography and CEA measurement.

8.3. Basic examinations

8.3.1. Digital rectal examination

A digital rectal examination is usually carried out to proof the function of the anal sphincter and could further on be used in cases with very distal rectal carcinomas to make a rough estimate whether the sphincter could be left unchanged or has to be removed in a surgical therapy.

8.3.2. Colonoscopy

The colonoscopy is an obligate pre-therapeutic examination. The biopsy control while colonoscopy is the only diagnostic option to proof the malignancy of a tumour. It is used to determine the tumour's histology and its spread in the intestinal wall. Furthermore it is very important to check the whole large bowel

because in about 5 percent of all colorectal carcinomas more than one carcinoma appears. However in some cases a colonoscopy of the entire large intestine is not possible. In those cases an alternative radiologic exploration type, such as a barium enema examination can be taken into consideration (Chen, 2000).

8.3.3. Abdominal ultrasound

Abdominal ultrasound has a lot of positive aspects. On the one hand it is quite cheap and on the other hand it is easily available nearly everywhere. The ultrasound's key task is to give a general overview over the abdominal status. Pathologies, like ascites or organ-related tumours can be found in sonography.

8.3.4. Thorax radiography

The main reason of thorax radiography is to eliminate the guess of lung metastasis. In case of pathologic results further, more exact examinations should be carried out.

8.3.5. CEA measurement

The carcinoembryonic antigen (CEA) is a glycoprotein found in many cell types, predominantly in gastrointestinal, bladder and cervix cells. It is a sensitive but non-specific tumour-marker, useful for monitoring the treatment of tumours. In case of high CEA level before treatment, the level should decreased to normal value in case of successful therapy. Especially in tumours of the gastrointestinal tract but also in lung or breast cancer CEA is used to record tumour progression. There is a strong correlation between the tumour-mass and the serum CEA level. In healthy individuals the reference region is from 1,5 to 5 µg/l in serum. In smokers or patients with liver diseases it can be higher (Pschyrembel, 2007).
CEA was first characterized by Gold and Freedman in 1965 (GOLD P, 1965). In different studies the role of CEA is discussed quite controversially. However, apparently the preoperative CEA-value has some prognostic relevance, therefore it should be measured preoperatively (MAS Chapman, 1998).

8.4. Further examinations

In case of pathologies found in basic examinations, respectively to specify the tumour development, some other accurate examinations could be helpful, such as abdominal, thoracic and pelvic CT or MRT,or rectoscopy and endosonography.

8.4.1. CT or MRT

The efficacy of preoperative CT or MRT in CRC patients of stage I or II couldn't be assigned despite uncertain outcomes in primary investigations. However, in cases of stage III or IV carcinomas (organ exceeding tumours), CT or MRT results are expressive. Suspect results in thorax radiography also should be verified through thorax CT (Schmiegl, 2008).

8.4.2. Rectoscopy

In distal carcinomas of the rectum the distance of tumour to linea dentata can be defined, which is very important for further surgical therapy.

8.4.3. Endosonography

To define the local tumour-growth an endoscopy should be carried out, a very exact examination to see the depth of carcinomas infiltration. This is of tremendous importance as to decide which kind of therapy to target.

8.5. Screening

The screening plays an essential role for the prognosis and therapeutic outcome of CRC patients. In general treatment of CRC – like in any other tumour – a quick identification of the carcinoma is essential. There are several screening methods for an asymptomatic population:

- FOBT (fecal occult blood test)
- Immunological testing methods
- Sigmoidoscopy
- Colonoscopy
- CT-colonography
- MRT – colonography
- Molecular tests

However, recommendations for screening depend on risk factors and age. Patients who reject a colonoscopy should at least be tested by FOBT on a yearly basis.

8.5.1. FOBT

The traditional guaiac smear test is used to detect occult blood in faeces. In the guaiac smear test three consecutive bowel movements should be collected and applied to the test card. Subsequently some hydrogen peroxide is put on the test card. If there is any haemoglobin in faeces it reacts with the H2O2 and the colour turns blue. Towler B et al. showed a reduction of 23 percent of CRC mortality by using FOBT (Towler, 1998). The advantages of FOBT are the low cost, the simple operability and the early detection of bleeding CRCs. Disadvantages are the addiction of FOBT test results to nutritional behaviour and medical treatment regarding false positive test results.

8.5.2. Immunological test

Up to now, immunological tests are no proper alternative to traditional FOBT (Schmiegl, 2008).

8.5.3. Molecular screening

Although explorations of DNA mutations in faeces show good potential, until now no sufficient results regarding asymptomatic population are available. Furthermore, this kind of test is rather complicated and expensive.

8.5.4. Endoscopic Testing

Sigmoidoscopy and colonoscopy show the highest sensitivity and specificity rates concerning the discovery of colorectal carcinomas. Reductions of mortality of about 60 to 80 percent could be shown in several studies (Atkin, 2002). Asymptomatic persons should do their first colonoscopy at the age of 50, due to a raising incidence of CRC at this age (WINAWER, 1997). If the results of the examinations are negative an interval of 10 years between 2 examinations can be recommended at present.

In patients with chronic inflammatory bowel diseases, such as ulcerative colitis a colonoscopy of the whole large intestine is recommended every year – on the condition, that the disease exists for more than 8 years. On the contrary in Crohn's disease no general references are given, so the examination methods have to be an individual decision, similar to other diseases, like the Peutz-Jeghers Syndrome. Nonetheless it is of enormous importance to carry out the colonoscopy or any other examination during a non-inflammatory period in order to simplify the diagnosis, respectively the differential diagnosis. In patients with genetic predispositions of their relatives clear recommendations are given. First grade relatives of patients with FAP should undergo a genetic exploration – not later than at the age of 10. In negative tested persons a separate examination is not necessary. However, positive tested persons should do a yearly recto-sigmoidoscopy, provided that no adenoma can be found. Beginning at the time when an adenoma is found, a colonoscopy should be done every year (J E King, 2000). Relatives, respectively persons at risk of HNPCC should be examined genetically as well – provided that the molecular variant is known in the index-patient. Furthermore a yearly colonoscopy should be carried out every year from the age of 25 onwards, principally 5 years before the index-patient's age of disease (Schmiegl, 2008). Järvinen et al. could show a reduction of mortality and incidence for more than 60 percent by examining risk patients every three years

(JÄRVINEN, 2000). Moreover, female patients should also be offered a gynaecological examination, starting at the age of 25 because of the correlation between HNPCC and gynaecological tumours. The routine method for discovering colorectal carcinomas is the colonoscopy. Sensitivity as well as specificity are high, although both values strongly depend on the examinations' quality. Important factors of quality are that the colonoscopy reaches the caecum, that there are no or few faeces in the large intestine and an accurate assessment of the intestinal mucosa.

9. Metastasis of colorectal carcinoma

There are three main ways of metastatic behaviour in colorectal carcinomas. Firstly they can spread continuously to peri-colic, respectively peri-rectal tissues. Secondly, CRCs can infiltrate the lymphatic vessels and lastly they can grow invasive into blood vessels. Referring to the taken place or kind of metastasis the target organ of metastasis distinguishes.

9.1. Infiltration per continuitatem

To be able to grow invasive, normal cells need several requirements. In normal tissue, cells are interlinked by cell adhesion molecules, like cadherins, integrins, selectines or proteoglycans. When a normal benign cell becomes malignant, tumour cell lose their cell-cell contact and invade the surrounding tissue. In order to do so, tumour cells have to break intercellular junctions. Normally cadherins play an important role in such intercellular complexe. A loss of the cadherins' functions' often happens during transformation of a benign tumour into a malignant one. (for example: Loss of E-cadherin in lobular breast cancer) Moreover cells need to migrate through the surrounding stroma to reach other regions. This usually functions via integrins. Normally the primary tumour of colon or rectum does not reach another organ in this way, however local infiltration often happens into peri-colic fat-tissue.

9.2. Lymphatic metastasis

Infiltration of lymphatic vessels leads to further dissemination of the tumour. The main metastatic ways are the lymph nodes and vessels along the A. mesenterica superior in case of the tumour situated in Caecum or Colon ascendens and along the A. mesenterica inferior when the tumour is situated in colon descendens. Tumours of colon transversum are able to follow both pathways because of the anastomosis riolani.

9.3. Haematogenous metastasis

The entire blood volume of the large intestine flows via the V. portae into the liver. As a result of this the first next organ is the liver – the most frequent organ of colon carcinoma. However, carcinomas of the deep rectum follow another way referring to blood drainage. Via the V. interna pudenda the blood runs directly into the V. cava. In this case the first next organ mostly affected is the lung.

10. Therapeutic strategies

In the last years considerable progress concerning therapeutic possibilities has been made for CRC treatment. Better surgical capabilities, better aimed radiation and chemotherapy and of course new therapeutic substances have increased the performance regarding cure and prolongation of life time.

Generally there are four important factors in the oncologic therapy – surgery, radiation, chemotherapy and biological therapy. In common all these modalities are combined to reach a maximum of therapeutic success. To be more precise surgery and radiation have a local effect whereas chemotherapy and biological therapy operate systematically. However, the used therapeutic strategy strongly depends on the clinical stage and tumour localization.

10.1. Surgery

The complete tumour resection is the main factor in the therapy of the colorectal carcinoma. During surgery the whole abdominal cave, especially the liver, the diaphragm and the whole large intestine should be examined thoroughly. As a function of tumour localization different parts of the large bowel have to be removed. Carcinomas of the caecum and the colon ascendens are eliminated by right hemi-colectomy including the A. colica dextra and the A. ileocolica. Moreover the right part of the greater omentum will be resected. Carcinomas of the right flexure are treated by an enlarged right hemi-colectomy. In this case the A. colica media, the ligamentum gastrocolicum, the A./V. gastroepiploica dextra and the greater omentum are resected. Tumours of the colon transversum have to be removed by transversectomy including the A. colica media. Carcinomas of the left flexure, the colon descendens and the sigmoid are treated by ectomy of the distal colon transversum, the colon descendens and the sigmoid including the ligation of A. colica media, A.colica sinistra, respectively the A. mesenterica inferior. Resection of the rectum should be done in case of a rectal carcinoma. If possible, surgery should be done by obtaining consistence. Of course the regional lymph-nodes of the nutritional vessels have to be removed too. The histopathological

examination of the resected tumour is imperative. Following information should be given by the pathologist.

- Histological type of tumour
- Infiltration depth of tumour
- Infiltration status of the regional lymph nodes
- Number of examined lymph nodes
- Grading
- Distance to healthy tissue
- Resection of the whole tumour tissue

This information is very important to make a proper staging and consecutively offer an effective therapy. The number of examined lymph nodes should be at least more than twelve (Wong, 1999).

10.2. Radiation

The main therapeutic effect of high energetic rays mostly arises from two mechanisms. One the one hand the tissue, respectively the single cell – more precisely – the DNA is damaged directly through the rays and one the other hand the interaction between water molecules and the spouts leads to the genesis of free radicals which stimulate the destruction of the DNA. To put it simply, the correlation between the dose of radiation and the cell destruction is linear as well as exponential. The linear component is made from a chromosomal DNA helix fracture, caused by single hits, whereas the exponential part arises from multiple hits. The main effect of radiation therapy consequently can be expected in the phase of cell proliferation. However, there are several other important factors which influence the effect of radiation, such as hypoxia or expression of oncogenes, for example ras. But also TNF or Il 1 as well as BFGF or PDGF-ß interact in radiated tissue und play an important role in the efficiency of therapy, and thus also in the peculiarity of side effects (Harrison, 2005).

10.2.1. Side effects

Regarding the anatomic localization of colon and rectum, side effects can occur mostly in the pelvic cave, above all in the bladder, the vagina and of course the primary organ itself. The most common side-effects are gastrointestinal bleedings, diarrhoea, emesis and spasmodic pain caused by irradiation of the rectum. The bladder's damage leads to dysuria and high frequent micturition, the irradiation of vagina to colpitis. Although the radiation usually just affects a small part of the body systemic side effects occur. Some of them are fatigue, loss of appetite, nausea and emesis.

10.3. Chemotherapy

Several therapeutic substances are used to target colorectal tumours. The most common regimens are:

- FOLFOX
    - Folinic acid
    - Fluorouracil
    - Oxaliplatin
- FOLFIRI
    - Folinic acid
    - Fluorouracil
    - Irinotecan
- XELOX
    - Capezitabin
    - Oxaliplatin
- Biologicals
    - Bevacizumab
    - Cetuximab
    - Panitumumab

10.3.1. Folinic acid (cancer care ontario, 2009)

Leucovorin calcium (folinic acid) is a reduced form of folic acid. It is used in combination with fluoruracil in tumour therapy. It concurs by binding to the enzyme thymidylate synthetase which leads to a decreased intracellular level of thymidylate. By doing this leucovorin improves the activity, respectively the efficiency of fluoruracil. Folinic acid is rapidly absorbed and is distributed to all tissues, especially to the liver apart from some inactive metabolites. It is rapidly and extensively converted into 5-methyltetrahydrofolate. The higher part of the elimination runs via urine, nearly 90 per cent of the given dose. The rest is excreted by faeces. Possible side effects are:

- Hand foot syndrome (in combination with fluorouracil)
- Stomatitis (in combination with fluorouracil)
- Myelosuppression (in combination with fluorouracil)
- Hypersensitivity
- Seizures, syncopes

10.3.2. Fluorouracil (cancer care otario, 2007)

Fluorouracil was developed based on the observation that tumour cells utilized the base pair uracil for DNA synthesis more efficiently than normal cells of the intestinal mucosa. The active form, fluorouridine monophosphate, inhibits DNA synthesis by inhibiting thymidylate synthetase and the normal production of thymidine. Effects on RNA occur especially with bolus application. The oral absorption strongly varies, however it distributes passively into the whole body water. The elimination primarily runs through the respiration tract and partly through the liver and the kidney. Adverse effects are

- Photophobia
- Alopecia
- Rash
- Photosensitivity
- Nausea and vomitig
- Diarrhea
- Myelosuppression

- Hand foot syndrome.

10.3.3. Oxaliplatin (cancer care ontario, 2009)

„*Oxaliplatin is an alkylating agent, belonging to a new class of platinum agent, consisting of platinum complexed to oxalate and diaminocyclohexane complex. Platinum complexes are formed intracellularly and inhibit DNA synthesis through covalent binding of DNA molecules to form intrastrand and interstrand DNA cross-links. Oxaliplatin differs molecularly, from other platinums (cisplatin and carboplatin), by its bulky diaminocyclohexane carrier ligand that most likely accounts for both its efficacy and lack of cross-resistance with other platinum compounds. Cytotoxicity is cell-cycle nonspecific. Oxaliplatin is a radiation-sensitizing agent*." (cancer care ontario, 2009)

Oxaliplatin can only be administered parenteral. The main part of the administered platinum is rapidely distributed into tissues, or eliminated in the urine. The metabolism is nonenzymatic but extensive. The bigger part of the platinum is excreted in the urine via renal elimination. The most common adverse effects are:

- Edema
- Anaphylaxis
- Rash
- Diarrhea
- Stomatitis
- Fatigue
- Neutropenia
- Liver function abnormalities
- Thromboembolism
- Alopecia
- Hand foot syndrome
- Constipation and abdominal pain
- Nausea and vomiting
- Infection
- Sensory neuropathy

10.3.4. Irinotecan

Irinotecan is a semi-synthetic derivative of camptothecin, that belongs to the class of Topoisomerase I inhibitors. Topoisomerase I is responsible for controlling and modifying DNA during replication and translation of genetic materials.

Irinotecan and its active metabolite bind to the topoisomerase-DNA complex, preventing religation of the single-strand breaks in the DNA molecule. The drug

and its active metabolite are believed to exert their cytotoxic effects during the S-phase of cell cycle (cancer care ontario, 2009).

In the body Irinotecan is converted into an active metabolite, the so called SN 38. The responsible enzyme for this conversation is the hepatic or gastrointestinal carboxylesterase. The peak plasma concentration of irinotecan and its activated form is reached immediately after the infusion of irinotecan and 0.5 to 2 hours later for SN 38. Although the entire cycle of elimination is still unknown, it seems that a part of the administered drug is eliminated via the biliary system. Some of the main adverse effects of irinotecan are:

- Alopecia
- Nausea and vomiting
- Constipation
- Stomatitis
- Increased bilirubin/liver enzymes
- Fatigue
- Dyspnea
- Diarrhea
- Abdominal pain
- Anorexia, weight loss
- Neutropenia
- Dizziness, headache
- Fever

10.3.5. Capecitabine

Capecitabine belongs to the fluoropyrimidine carbamate class and causes cell collapse via RNA and DNA related mechanisms. It is a precursor of fluorouracil, which can be administered orally. The conversion to 5FU is catalyzed by carboxyesterase, cytidine deaminase and thymidine phosphorylase. All this enzymes mainly occur in the liver but also in tumours.

After an oral absorption, Capezitabine is sufficiently metabolised in the liver. The elimination mainly runs through the kidney via urine. The main adverse effects of capecitabine are:

- Hand foot syndrome
- Nausea and vomiting
- Anorexia
- Hyperbilirubinemia
- Diarrhea
- Stomatitis
- Abdominal pain
- Paresthesia

- Eye irritation
- Fatigue

10.3.6. Bevacizumab

Bevacizumab is a recombinant humanized monoclonal antibody that binds to and neutralizes the biologic activity of human vascular endothelial growth factor (VEGF). Bevacizumab inhibits the binding of VEGF to its receptors, Flt-1 and KDR, on the surface of endothelial cells (cancer care ontario, 2009).
The elimination, respectively the metabolism of bevacizumab are produced by the reticuloendothelial system. The most severe side effects are:

- Hypertension
- Arterial or venous thromboembolism
- Headache
- Anxiety and confusion
- Peripheral sensory neuropathy
- Dermatitis
- Delayed healing
- Diarrhea
- Constipation
- Nausea and vomiting
- Proteinuria
- Nephritic syndrome
- fatigue
- Neutropenia
- Bleeding
- Metabolic dysfunction

10.3.7. Cetuximab

Cetuximab is a recombinant, human chimeric monoclonal antibody which binds specifically to the epidermal growth factor receptors (EGFR) and competitively inhibits the binding of epidermal growth factor (EGF) and other ligands. EGFR is expressed in normal tissues such as skin, mucosa and kidney as well as in common epithelial tumours such as NSCLC, CRC and head and neck tumours (cancer care ontario, 2009). However, in patients with CRC bearing mutated *K-ras* no benefit of cetuximab could be found, whereas patients with a tumour bearing wild-type *K-ras* benefit from cetuximab (Karapetis, 2008).

Similar to Bevacizumab, Cetuximab is also metabolised and eliminated via the reticuloendothelial system. The most common, adverse effects are:

- Infusion reactions
- Diarrhea
- Constipation
- Anorexia
- Hypomagnesemia
- Headache
- Acneiform rash
- Nausea and vomiting
- Abdominal pain
- Dyspnea
- Fatigue
- Fever

10.3.8. Panitumumab

Panitumumab is a recombinant, fully humanized IgG2 monoclonal antibody. It binds competitively to the extracellular domain of epidermal growth factor receptor (EGFR) on normal and tumour cells, and thus inhibits ligand-induced receptor autophosphorylation. EGFR activation leads to cell proliferation, differentiation, cell survival, angiogenesis, and invasion/metastases. The KRAS gene encodes a protein involved in signal transduction. Patients with mutated KRAS colorectal tumours do not seem to benefit from the EGFR monoclonal antibody inhibitor therapy. In clinical trials Panitumumab has shown an improvement in the progression free survival (cancer care ontario, 2009).
The most commen, respectively severe advers effects are:

- Rash
- Anorexia
- Constipation
- Nausea and vomiting
- Cough
- Paronchia
- Abdominal pain
- Diarrhoea
- Dyspnoea
- Fatigue

10.3.9. Adjuvant therapy

Adjuvant therapy means that the whole tumour could be removed (R0) and subsequently the patient receives different kinds of therapeutic modalities to

improve the chances for cure. The number of metastatic lymph nodes is of significant importance regarding adjuvant therapy. For example, patients in stadium I (UICC) do not need any further adjuvant therapy. Patients in stadium II can receive an adjuvant therapy, whereas the absolute benefit – means the improvement of the survival rate – of an adjuvant therapy rests with three to six percent (Group, 2007). Patients in stadium III should undergo an adjuvant therapy because of a documented benefit (Gill, 2004). However, final decision could only be made taking into account the patient's comorbidities (Schmiegl, 2008). In general, adjuvant therapy always has to consider the therapy's side effects regarding efficiency and benefits for the patients.

10.3.9.1. Therapy in patients UICC stadium I

Patients in UICC stadium I of colon cancer do not need a further therapy after the accurate ectomy of the tumour. Also patients with rectal carcinoma in UICC stadium I do not need any further therapy (Schmiegl, 2008).

10.3.9.2. Therapy in patients UICC stadium II

10.3.9.2.1. Therapy in patients UICC stadium II of colon cancer

Patients in UICC stadium II should be treated cautiously and by mutual consent. If these patients receive chemotherapy, the usage of fluoropyrimidines is recommended (Schmiegl, 2008). Oral application is possible, for example 5-FU prodrug, the so called Capecitabine 2 times a day 1250 mg/m² body surface for fourteen days every three weeks. This cycle should be done 8 times. Several other application methods can be offered, whereby the infusion bolus leads to much more side effects than a long time infusion. Poplin et al. could show that the therapy should start at least within eight weeks after the surgical elimination of the tumour (Poplin, 2005). In several other studies the optimal duration of chemotherapy could be determined as 6 months (André, 2003).

10.3.9.2.2. Therapy in patients UICC stadium II of rectal cancer

UICC stadium II patients strongly profit by neoadjuvant chemoradiotherapy (Group C. C., 2001). Moreover, UICC stadium II patients as well as stadium III patients have to undergo adjuvant chemotherapy, in order to reduce the risk of tumour recurrence. The treatment normally starts four to eight weeks after surgery. (Rödel, 2004; NIH consensus conference 1990)

10.3.9.3. Therapy in patients in UICC stadium III

10.3.9.3.1. Therapy in patients UICC stadium III of colon cancer

For both, tumour recurrence and survival, a significant reduction treating CRC patients with 5-FU and folinic acid could be proofed by Francini or O'Connell (Francini, 1994) (O'Connell, 1997). Combination of fluoropyrimidines with oxaliplatin shows a further benefit in therapy of CRC. This could be shown in the MOSAIC trial. DFS time could be increased by 7.5 percent whereas the overall survival could be increased by 4.4 percent compared to single therapy with 5-FU (André, 2004).

10.3.9.3.2. Therapy in patients UICC stadium III of rectal cancer

Patients in situation of UICC stadium III do not differ from patients in UICC stadium II, regarding therapeutic consequences.

10.3.9.4. Therapy in patients in UICC stadium IV

Surgery is a possible option for metastases that are resectable. In patients who are not operable, either because of the patients' preconditions or because of the number or location of the metastases itself, a systemic chemotherapy or chemoimmunetherapy should be offered. Furthermore, in the palliative situation the quality of life, an efficient pain therapy, psychosocial and psycho-oncological care is crucial.

11. Prognostic factors

Generally the prognosis of patients with CRC strongly depends on the stage of disease at the time of diagnosis (Harrison, 2005). Important prognostic factors are the number of evaluated lymph-nodes, perforation, obstruction, vascular and perineural invasion and histopathological grading. Molecular features, however, keep on producing promising data which is believed to refine future preventive and early intervention strategies on an individual basis. In order to gain deeper insight into their importance the following study was carried out

12. Materials and methods

12.1. Subjects

Between May 2008 and June 2009, 381 patients with histologically confirmed CRC have been recruited at the Division of Clinical Oncology, Department of Internal Medicine, Medical University Graz, Austria. All patients had a detailed interview for medical history to evaluate family history of CRC, cigarette smoking habits, alcohol consumption and the regular use of NSAIDs or hormone replacement therapy. The presence of pre-existing diseases (HNPCC, FAP, chronic inflammatory bowel disease) was evaluated by medical records and the Division of Clinical Oncology. Medical history, pre-existing diseases, clinical data (age at diagnosis, sex, body mass index (BMI), Karnofsky-Index (KI)) and tumour data (tumour size, lymph node involvement, clinical stage, histopathological grade, tumour location (rectum, rectosigmoid, colon) have been documented in an EXCEL file and transformed into a SPSS data file. 2156 controls have been recruited from outpatients admitted to the Department of Internal Medicine, Medical University Graz. To be eligible as controls, subjects had to be free of cancer diagnosis in their medical history. The study was performed according to the Austrian Gene Technology Act and has been approved by the Ethical Committee of the Medical University Graz. All participants gave written informed consent. All subjects were Caucasian.

12.2. Genotyping

The genomic DNA was isolated by a fully automated procedure on a GeneMole instrument (Mole AS, Lysaker, Norway). HIF1A genotypes were determined by a 5'-nuclease assay (TaqMan). Primers and probes were designed and manufactured using the "Assayby-Design" of the Applied Biosystems custom service. (Applera, Austria) For analysis of the HIF1A P582S polymorphism, sequences of primers and probes were forward primer TTCCAGTTACGTTCCTTCGATCAG, reverse primer CTTTGAGGACTTGCGCT-TTCAG, 582P probe VIC-ACTGCTTTCTAATGGTGACAA-NFQ, and 582S probe FAM-ACTGCTTTCTAATGATGACAA-NFQ. The A588T polymorphism was analyzed using forward primer 588F GTTACGTTCCTTCGATCAGTTGTCA, reverse primer 588R CTTTGAGGACTTGCGCTTTCAG, 588A probe VIC-

CAGTTCCGCCAAGCC-NFQ, and 588T probe FAM-CAGTTCCACAAGCC-NFQ. Polymerase chain reaction (PCR) was performed in a Primus 96 plus thermal cycler (MWG Biotech AG, Germany). Fluorescence was measured in a Lambda Fluoro 320 Plus plate reader (MWG Biotech AG, Germany) using excitations/ emissions filters of 485 nm/ 530 nm for FAM-labeled probes and 530 nm/ 572 nm for VIC-labeled probes. Data was exported into EXCEL format and analyzed as scatter plot. For each sample of reactions, one negative control containing water instead of DNA was added. The technicians responsible for genotyping were blinded for case/control status.

Function	mRNA				Protein		
	mRNA to Chr	Accession	Position	Allele change	Accession	Position	Residue change
missense	+	NM_001530.3	2148	CCA ⇒ TCA	NP_001521.1	582	P [Pro] ⇒ S [Ser]
missense	+	NM_181054.2	2148	CCA ⇒ TCA	NP_851397.1	582	P [Pro] ⇒ S [Ser]

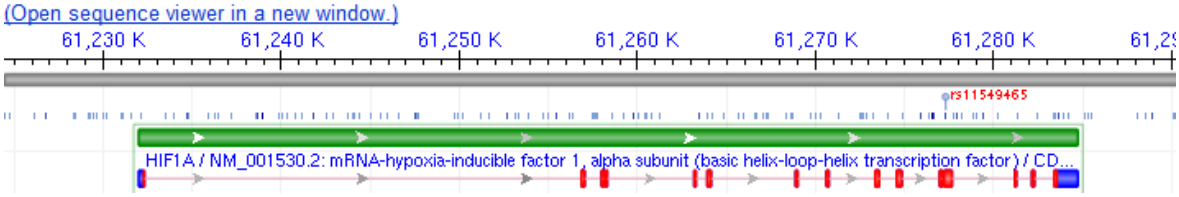

Image 4 (C1772T – Allele change from C to T, missense mutation)

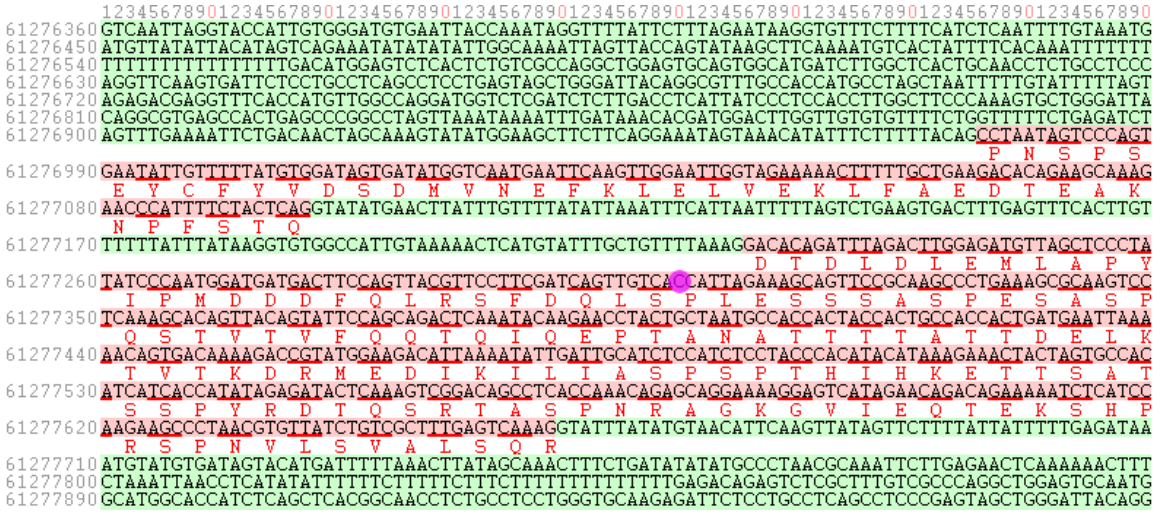

Image 5 (Polymorphism C1772T – rs11549465)

Function	mRNA				Protein		
	mRNA to Chr	Accession	Position	Allele change	Accession	Position	Residue change
missense	+	NM_001530.3	2166	GCA ⇒ ACA	NP_001521.1	588	A [Ala] ⇒ T [Thr]
missense	+	NM_181054.2	2166	GCA ⇒ ACA	NP_851397.1	588	A [Ala] ⇒ T [Thr]

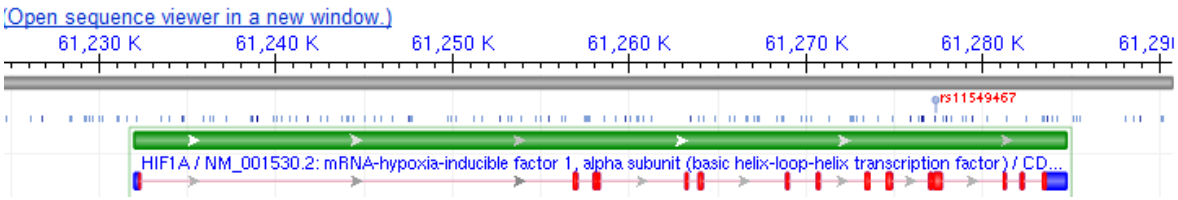

Image 6 (A588T – Allele change from G to A, missense mutation)

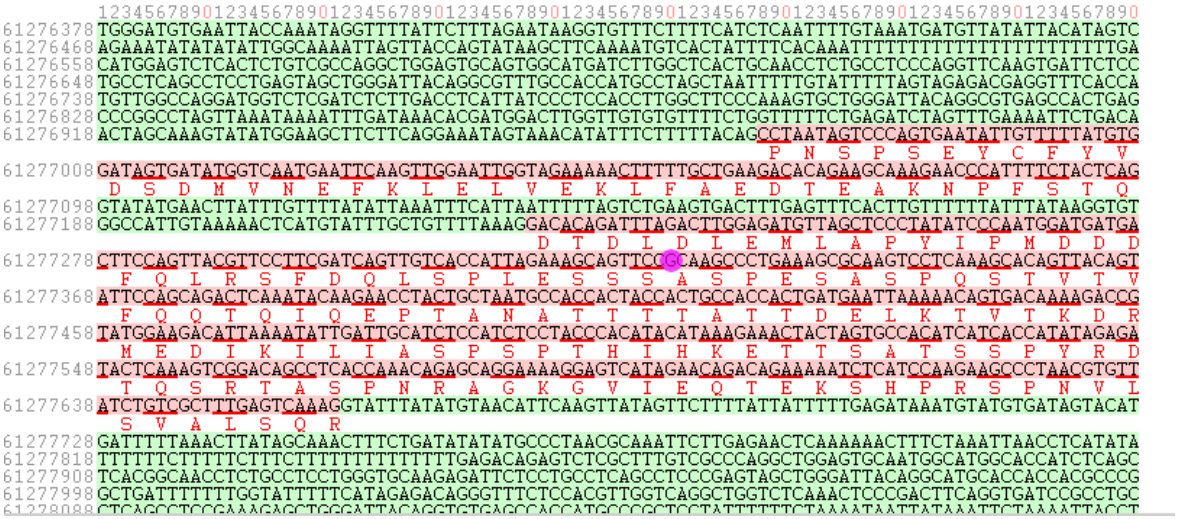

Image 7 (Polymorphism A588T – rs11549467)

12.3. Statistical analyses and Results

12.3.1. Prognosis

The primary outcome was disease-free survival. The endpoints included cancer recurrence/metastasis or death caused by CRC. In our study 45 patients were excluded for evaluation of disease-free survival because of secondary tumours. Follow up information was available for all patients. Follow-up was censored at 5 years. The genotypes were coded assuming an allele dose-effect (wildtype=0, heterozygous carrier of the mutated allele=1, homozygous carrier of the mutated allele=2). DFS curves were generated by the Kaplan-Meier method and verified by the log-rank test. Cox's proportional hazards regression analysis was used for univarate and multivariate analyses of prognostic values. SNPs were re-evaluated in a model adjusted for known CRC prognostic factors, which included age at

diagnosis, Karnofsky-Index, tumour-size, histological grade, number of lymph nodes evaluated after resection, number of metastatic lymph nodes, clinical stage (according to the AJCC TNM-classification) and application of 5-FU based chemotherapy. Differences were considered significant when a P value < 0,05 was obtained. All analysis were performed using the SPSS 14.0 statistical software package (SPSS Inc., Sunnyvale,USA).

Frequency data for clinical and tumour biological factors and therapy modalities are described in Table 1.

Table 1: Clinical, tumour biological and treatment characteristics

Age (years)	65 (median)	33-82 (range)
Sex		
Men	198	58.9%
Women	138	41.1%
Karnofsky-Index		
100	167	49.7%
90-80	153	45.5%
≤70	10	3%
Unknown	6	1.8%
Tumour		
Colon	212	63.1%
Rectum	123	36.6%
Unknown	1	0.3%
Tumour size		
T1	12	3.6%
T2	30	8.9%
T3	218	64.9%
T4	63	18.8%
Unknown	13	3.9%
Evaluated lymph nodes	18 (median)	0-69 (range)

Lymph node involvement		
N0	114	33.9%
N1	128	38.1%
N2	75	22.3%
Unknown	19	5.7%
Primarily metastasized		
M0	226	67.3%
M1	68	20.2%
Grade		
G1	15	4.5%
G2	231	68.8%
G3	84	25%
Unknown	6	1.8%
Stage		
I	15	4.5%
II	92	27.4%
III	156	46.4%
IV	67	19.9%
Unknown	6	1.8%
5-FU based chemotherapy	201	59.8%

Mean follow-up time was 41.7 months (median 31, range 3-208 months) and the mean DFS (disease free-survival) time was 30.9 months (median 20, range 0-200 month).

Disease-free survival of CRC patients after diagnosis stratified by genotypes of HIF-1α C1772T (C/T heterozygous and T/T homozygous genotypes are combined)

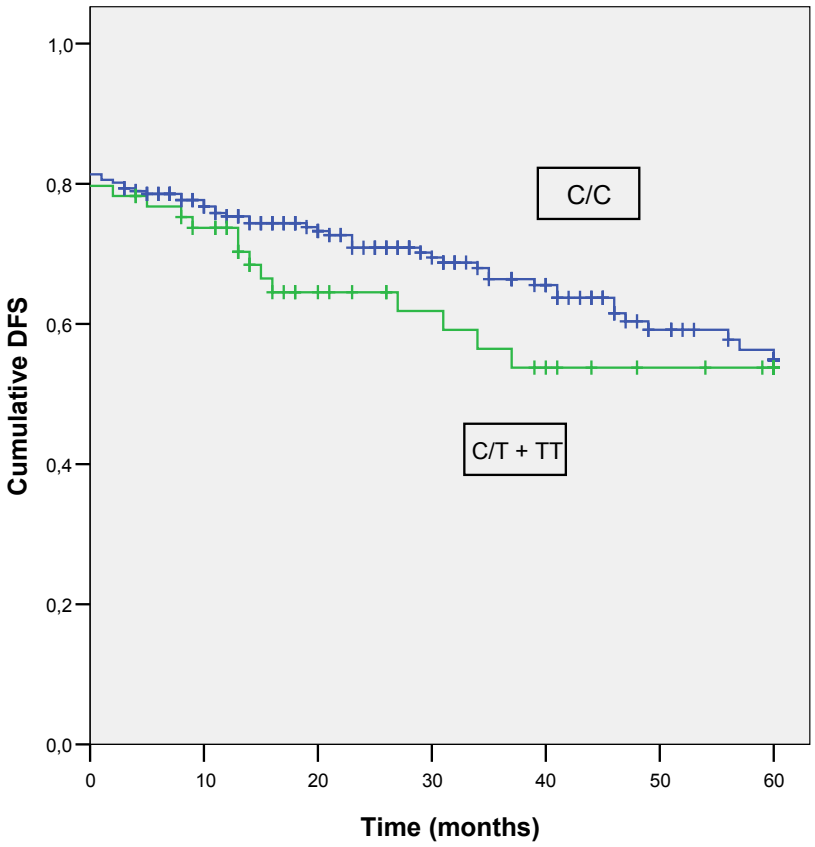

Image 8 (Disease free survival I)

Disease-free survival of CRC patients after diagnosis stratified by genotypes of HIF-1α G1790A (G/A heterozygous and A/A homozygous genotypes are combined)

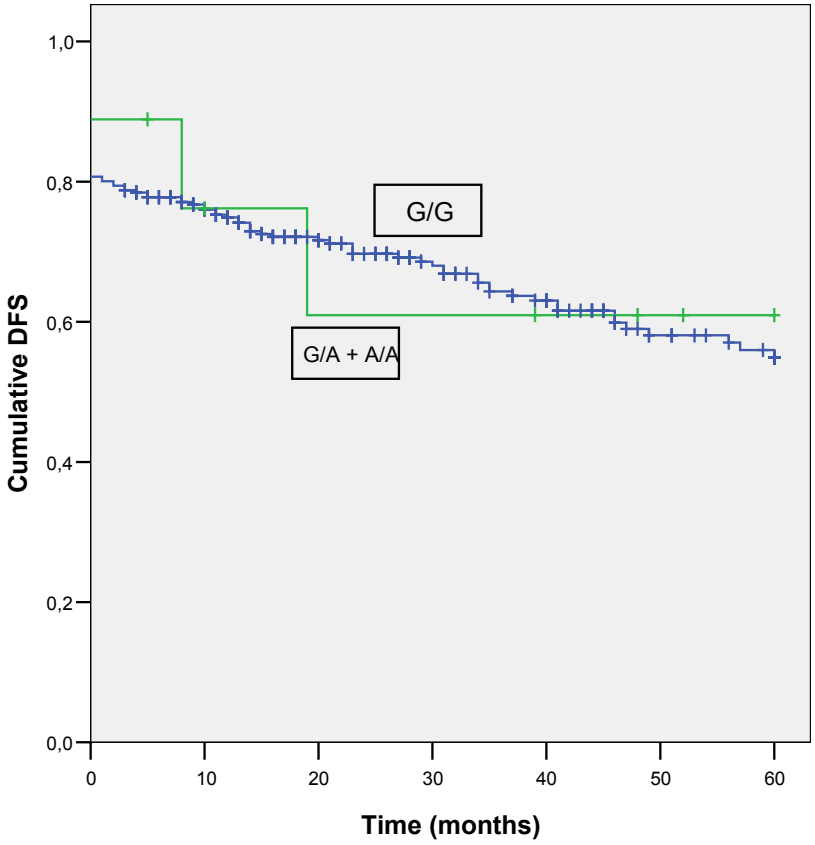

Image 9 (Disease free survival II)

We combined the C/T heterozygous and T/T homozygous and the G/A heterozygous and A/A homozygous genotypes because of the small number of the T/T and A/A genotypes. In our study population we observed a statistical significant association between DFS and Karnofsky-Index, tumour size, clinical stage, number of metastatic lymph nodes and application of 5-FU based chemotherapy.

Univariate Cox' model (disease-free survival)

Table 2: Univariate Cox' model (disease-free survival)

Variable	RR	95%CI	p-value
C1772T	1.185	0.768-1.827	0.443
G1790A	0.921	0.292-2.901	0.888
Age at diagnosis	0.997	0.979-1.014	0.707
pT	2.089	1.495-2.918	**0.001**
Evaluated N	0.991	0.971-1.012	0.414
Positive N	1.069	1.031-1.108	**<0.001**
Histologic grading	1.265	0.876-1.825	0.210
Clinical stage	5.030	3.543-7.140	**<0.001**
5-FU based CTX	0.279	0.159-0.489	**<0.001**
Karnofsky-Index	0.970	0.0951-0.989	**0.002**

*

However, no association was found between HIF1A C1772T (P = 0,443, risk ratio or recurrence [RR] = 1,185, 95% confidence interval [CI] =0,768 to 1,827) and HIF1A G1790A (P = 0,888, RR =0,921, 95% CI = 0,292 to 2,901) polymorphisms and DFS in univariate and multivariate Cox-regression analysis including known CRC prognostic factors.

12.3.2. Risk

Continuous variables were analyzed by t-test and presented as means ± standard deviation (SD). Categorial variables are presented as per centages and were compared by chi-square test. Odds ratios (OR) and 95% confidence intervals (CI) were determined by logistic regression analysis, with genotypes coded assuming an allele dose-effect (wildtype genotype=0, heterozygous carrier of the mutated allele=1, homozygous carrier for the mutated allele=2). The criteria for statistical significance was $p<0.05$. Hardy-Weinberg equilibrium of genotypes was analyzed using the HW Diagnostics software (Fox Chase Cancer Center, 1999). SPSS for

* RR: relative risk; CI: confidence interval; pT: primary tumour size according to AJCC-TNM system; evaluated N: number of lymph nodes evaluated after resection; positive N: number of metastatic lymph nodes; CTX: chemotherapy

windows (release 15.0.1; SPSS, Inc) was used for statistical analyses. Medical history data of 381 CRC patients are summarized in Table 3.

Table 3: Medical history data of CRC patients

BMI		
<18.5	6	1.6
18.5-25	225	59.1
26-30	105	27.6
>30	42	11.0
Unknown	3	0.8
IBD*		
Yes	4	1
No	376	98.0
unknown	1	0.3
Alcohol		
Yes	20	5.2
No	347	91.1
unknown	14	3.7
Cigarette smoking		
Yes	104	27.3
no	258	67.7
unknown	19	5.0
Regular use of NSAD		
Yes	63	16.5
no	316	82.9
unknown	2	0.5
Genetik risk **		
yes	5	1.3
No	372	97.6
unknown	4	1.0
Family history***		
yes	51	13.4
No	287	75.3
unknown	43	11.3

†

The control group comprised 1209 (56.1%) women and 947 (43.9%) men with a mean age of 58.15 ± 17.646 years. Genotyping was successful in 368 patients for HIF1A P582S, in 367 patients for HIF1A A588T. Due to the small number of homozygous mutant variants we combined the HIF1A P582S heterozygous and

† Abbreviations: BMI: Body mass index, IBD: inflammatory bowel disease; NSADs: nonsteroidal anti-inflammatory drugs;
*ulcerative colitis or Crohn's disease
**familial adenomatous polyposis (FAP) or hereditary nonpolyposis colorectal cancer (HNPCC)
***affected first degree relative

homozygous mutant and the HIF1A A588T heterozygous and homozygous mutant variants. Genotypes did not deviate from the Hardy-Weinberg equilibrium among patients and controls and showed no sign of linkage disequilibrium. Distribution of the HIF1A P582S and A588T genotypes was not significantly different in patients and controls (Table 4).

Table 4: HIF1A genotypes in CRC and controls

Genotype		CRC patients	Control subjects	p-value
HIF1-A 582	PP	291 (76.4%)	1773 (82.2%)	0.147
	PS/SS	77 (20.2%)	383 (17.8%)	
HIF1-A 588	AA	356 (93.4%)	2080 (96.5%)	0.608
	AT/TT	11 (2.9%)	76 (3.5%)	

The Crude odds ratio (OR) of the 582S allele for CRC risk was 1.225 (95% CI 0.931–1.612, p=0.147) and 0.846 (95% CI 0.445–1.607, p=0.609) for the 588T allele. In a multivariate logistic regression analysis including age and sex neither the HIF1A 582S nor the 588T variant was significantly associated with CRC (Table 5).

Table 5: CRC risk in multivariate logistic regression

Risk factor	Odds ratio	95 % CI	p-value
HIF1A 582S (number of allels)	1.204	0.911 – 1.592	0.193
HIF1A 588T (number of allels)	0.851	0.444 – 1.631	0.626
Age at diagnosis (years)	1.025	1.017 – 1.032	<0.001
Sex (male/female)	0.556	0.443 – 0.698	<0.001

Clinico-pathological features of CRC in relation to HIF1A genotypes are summarized in table 6.

Table 6: HIF genotypes and CRC risk factors

	HIF1A 582			**HIF1A 588**		
Risk factors						
	PP	**PS/SS**	**p value**	**AA**	**AT/TT**	**p value**
age at diagnosis(years)						
< = 40	5	0	0.554	5	0	0.862
41 – 60	86	23		104	4	
61 – 80	191	50		234	7	
> 80	4	9		13	0	
sex						
Male	167	49	0.322	208	8	0.342
female	124	28		148	3	
Localisation						
Colon	170	42	0.830	208	3	**0.016**
rectum	103	27		122	8	
Tumour size						
T1	11	3	0.709	14	0	**0.003**
T2	28	5		32	1	
T3	190	46		227	8	
T4	54	18		71	1	
Tx	2	0		1	1	
Lymph node involvement						
N0	106	21	0.309	122	3	0.828
N1	111	26		133	5	
N2	63	21		81	3	
Metastasis						
M0	196	47	0.530	236	6	0.928
M1	58	17		73	2	
Stage						
I	13	3	0.832	16	0	0.878
II	84	20		99	3	
III	135	33		163	6	
IV	56	18		72	2	

Data were analyzed by chi2 test for 2x3 tables and ANOVA. Tumour location and tumour size (T) were significantly associated with the presence of the HIF1A 588T allele.

13. Discussion

The study's aim was to evaluate the association between HIF1A polymorphisms and disease free-survival, respectively the risk of developing CRC. Neither a correlation between both SNPs and disease free-survival nor an association with the risk of developing a CRC could be found. However, a significant correlation between patients carrying the HIF1A 588T allele and clinical-pathological features and an association between known prognostic factors and DFS could be shown.

Under normoxic conditions as well as under hypoxic conditions both HIF1A polymorphisms show a higher transcription activity. Since HIF1A is activated by a multiple-step pathway several hypotheses concerning the increased trans-activation may be conceivable. Because of the location of the substituted amino acids within or near N-TAD and the interaction with the pVHL, the alteration of protein stability of the variant proteins may be one of the possible mechanisms for the enhancement of trans-activation capacity.

Various recent studies suggest that HIF1A SNPs play an essential role in inter-individual tumour progression as well as in tumour susceptibility. Tanimoto et al. found in 2003 that HIF-1α polymorphisms show an increased number of micro-vessels and a higher disease stage among head and neck squamous cell carcinoma patients (Tanimoto K, 2003). Moreover, Ollerenshaw et al. showed a correlation between the renal cancer phenotype and the presence of polymorphisms in the HIF1A gene (Ollerenshaw M, 2004). Similiar conclusions could be made by Ling et al., who found an association between HIF1A polymorphisms and the tumour size and the rate of lymph node metastasis in esophageal squamous cell carcinoma (Ling TS, 2005).
An increased risk for developing a colorectal carcinoma in case of mutation in HIF1A could be shown for gastric cancer (Li K, 2009) and breast cancer (Naidu R, 2009). In contrast Apaydin et al. could not find a correlation between HIF1A gene variants and breast cancer susceptibility (Apaydin I, 2008). Other studies, which

do not suspect a higher cancer risk in case of HIF1A polymorphisms, were carried out by Li et al. (Li H, 2007) or Ling et al. (Ling TS, 2005).

Despite the fact that studies of prognostic significance concerning HIF1A polymorphisms are rare, an unfavourable prognosis could be shown for oral squamous cell carcinoma (Muñoz-Guerra MF, 2009) and bladder cancer (Nadaoka J, 2008).

In the present analyses both SNPs were not associated with CRC susceptibility. This result is similar to that reported in two previous studies investigating the role of HIF1A SNPs in CRC (Kuwai T, 2004) (Fransén K, 2006). Our findings do not argue against a role of HIF-1 itself in the pathogenesis of CRC, but solely suggest that HIF1A polymorphisms have no major effect on the CRC risk. However, we found a significant association of the rare HIF1A 588T allele and clinical-pathological features. The HIF1A 588T allele was found more often in patients with tumours of bigger size. This finding is consistent with the results of Tanimoto et al. who investigated patients with head and neck squamous cell carcinomas and found larger tumours in patients carrying the HIF1A 588T allele (Tanimoto K, 2003). In CRC, Fransen et al showed that patients carrying one or more polymorphic alleles in either the HIF1A P582S or the A588T polymorphisms display a significant higher risk for the development of ulcerative CRCs (Fransén K, 2006). Moreover, in our analyses the HIF1A 588T allele was associated with tumour location. However, the mechanistic background for this correlation is unclear. The variable results of the studies investigating HIF1A SNPs and CRC prognosis are not necessarily in contradiction to our findings, as the etiology and progression of malignant diseases and CRC in particular is multifarious.

The strengths of the study are its relatively high number of participants as well as the clinically validated phenotypes. Some limitations of our study have to be taken into account. The control subjects were not age and sex matched to the CRC patients. But HIF1A polymorphisms are not associated with sex and are constant during life. So it is unlikely that the negative risk finding of our study is due to the lower age and female dominance of the control group. Frequencies of gene variants have been shown to vary between populations of different ethnic origin. Thus, our findings may not necessarily apply to ethnicities other than Caucasian. Finally, no information about the medical history or presence of preexisting

diseases was available for the control group. To summarize, we found that HIF1A P582S and A588T SNPs do not influence CRC risk.

14. Conclusion

CRC is a clinically heterogeneous disease, and it is generally accepted that the different clinical courses of patients with clinically and histopathologically similar tumours are due to molecular differences. Therefore, detailed molecular analysis could yield prognostic information. The TNM staging system and conventional clinicopathologic factors have led clinical decisions for decades. However, current staging methods remain suboptimal. Patients with CRC who are at low risk of recurrence could be spared the toxicity of systemic treatment if clearly distinguished, while others at high risk of disease recurrence could get maximal benefit if therapy matched the molecular genetic profile. Areas of cancer research such as single-nucleotide polymorphism keep on producing promising data which is believed to refine future preventive and early intervention strategies on an individual basis.

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