The role of clinical, pathological and molecular characteristics in colorectal cancer management

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Introduction

Pathogenesis of Colorectal Cancer

General

Colorectal cancer (CRC) is the second leading cause of cancer death in the Western world. The WHO estimates that 945.000 new cases occur yearly, with 492.000 deaths [1,2]. Colorectal cancer is placed third after lung and breast cancer. The overall five year survival is 60% and up to 50% of all patients will develop metastases[3,4]. Metastases in distant organs are responsible for the majority of CRC deaths. The common localizations of distant metastases are liver, lung and the intra-abdominal space. Of all patients who die of advanced colorectal cancer, ~85% have colorectal liver metastases (CLM). The etiology of CRC and the sequential adenoma-carcinoma-metastasis process is relatively well understood, the specificity and characteristics for site of metastasis however, is much less comprehended and subject of this thesis.

The intestinal tract

The intestinal tract can be divided into the small bowel, the colon and rectum. Both the small bowel and the colon are covered with a layer of serosa, a layer of smooth muscle, a layer of connective tissue (stroma) and an inner absorptive and secretory epithelial ring (the mucosa). Most of the rectum lacks of serosa.

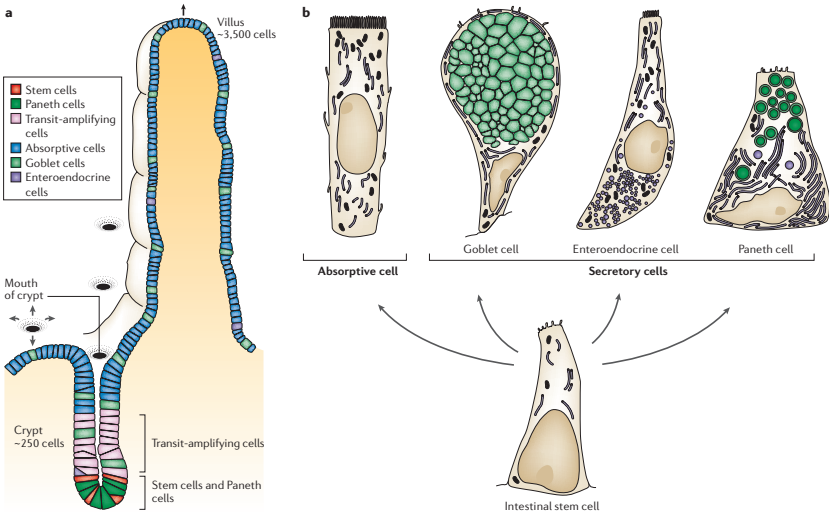
The majority of cancers in the gastro-intestinal tract originate from the epithelial layer, a single layer of differentiated cells. These differentiated cells originate from the crypts of Lieberkühn, located at the connective tissue of the intestinal tract. Each crypt contains several pluripotent stem cells that are able to differentiate into four specific cell types[5-7]; the absorptive cells or enterocytes (90%), mucus producing goblet cells, enteroendocrine secretory cells (secreting hormones) and Paneth cells (secreting antimicrobial peptides and enzymes) (Figure 1). In the small intestine these differentiated cells migrate to the surface and form villi; finger-shaped luminal protrusions (Figure 1). The function of these villi is to increase the exchange interface of the small intestine.

In contrast to the small intestine, colon epithelium has no villi, but consists of large crypts with several thousand differentiated cells produced out of 1-10 stem cells. Stem cells give rise to the transit-amplifying (TA) cells; an intermediate cell population with the aim to transform into a differentiated cell population

(Figure 1)[6,8,9]. The stem cells are slowly dividing from the base of the crypt in contrast to the differentiated cells which divide rapidly and travel to the surface within 5 days to undergo apoptosis[6].

This process of cell proliferation, differentiation and migration is carefully controlled by the epithelial–mesenchymal transition (EMT) process. Mutations in pathways organizing the EMT (consequently; cell proliferation, differentiation and migration) give rise to the development of CRC [9].

Figure 1, Cell types of the Intestinal Tract Epithelial layer lining the lumen[10]

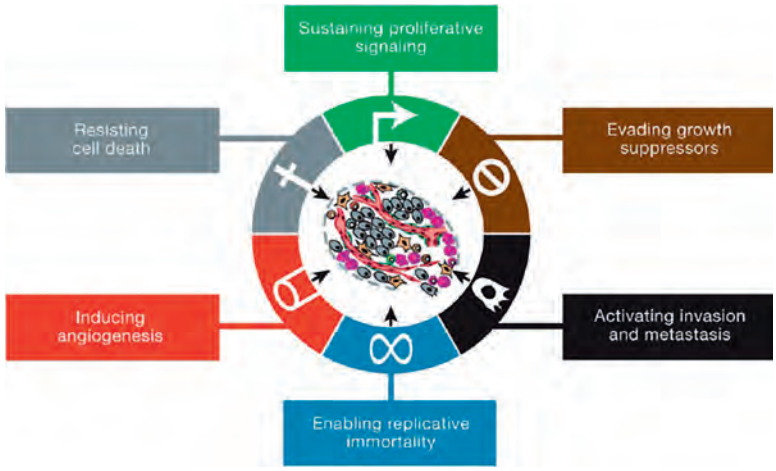


Carcinogenesis

Hallmarks of Cancer

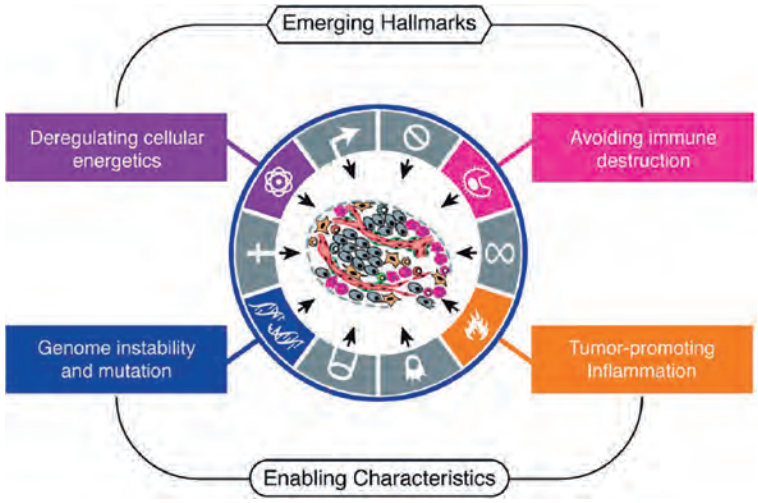
The last decade's research has generated a wealth of knowledge of mechanisms involved in the occurrence of human cancer. Hanahan and Weinberg proposed that cancer may have the following six common traits: self-sufficiency in cell growth signalling; insensitivity to growth-inhibitory (antigrowth) signals; invasion and metastasis; unlimited replicative potential; sustained angiogenesis; evasion of apoptosis (Figure 2). They suggest that these six capabilities are shared in common by most types of human cancers.[11]

Figure 2, The Hallmarks of Cancer[12]



In 2011, Hanahan and Weinberg added two additional hallmarks of their model; the enabling and the emerging hallmarks. The enabling hallmark defined as genomic instability in cancer cells, which generates mutations in chromosomes and the inflammatory state of cells, driven by the immune system; and the emerging hallmark, reprogramming of the cellular energy metabolism in order to support continuous cell growth, and secondly the evasion of cancer cells from attack and elimination by immune cells (Figure 3)[12].

Figure 3, Emerging Hallmarks and Enabling Characteristics[12]

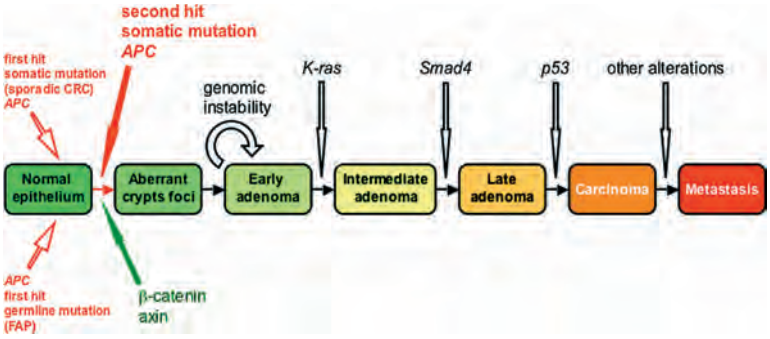


Colorectal Carcinogenesis

The hallmarks described by Hanahan and Weinberg also apply to the development of cancer in the intestinal tract. The occurrence of CRC is either 'sporadic' (85%), as a part of a hereditary cancer syndrome (<10%), or against the background of inflammatory bowel disease (2%)[1].

It is believed that CRC arises and progresses through the adenoma–carcinoma sequence. The transition from adenoma to carcinoma may take up to several decades and follows a well-defined path of phenotypically distinguishable stages, each characterized by distinct mutations in oncogenes and tumour suppressor genes, largely following the hallmarks of cancer paradigm. This multistep tumourigenesis is determined by gatekeeper and caretaker pathways described by Vogelstein et al. depicted and summarized into the so-called "Vogelgram" (Figure 4) [13].

Figure 4, Vogelgram: Genetic model of colorectal carcinogenesis[14]



The process of cell proliferation, differentiation and migration; the epithelial–mesenchymal transition (EMT) process, is managed by signals from multiple pathways like the hedgehog, platelet-derived growth factor (PDGF), bone morphogenetic protein (BMP) and the Wnt, Notch and Eph/ephrin pathways[10]. An accumulation of mutations in genes affected in these pathways is known as genetic instability and can induce tumourigenesis. Genetic instability in CRC can be explained by three destabilizing pathways: The chromosomal instability (CIN) pathway, the micro satellite instability (MSI) pathway and the CpG island methylator phenotype (CIMP) pathway[15,16].

The most common CIN pathway in CRC is characterized by allelic losses, chromosomal amplifications and translocations (70% of CRC)[17,18]. Gains and losses of whole or large portions of chromosomes leading to aneuploidy and mutations occurring in specific tumour suppressor genes and oncogenes will activate oncogenetic pathways essential for CRC development.

Second most frequently affected pathway in CRC (15%) is the MSI pathway[19]. Microsatellites are repetitive sequences distributed throughout the whole genome. These sequences are prone for mutations, mainly because DNA polymerases cannot bind DNA efficiently during DNA synthesis. Tumours affected with MSI are characterized by DNA sequence changes, with small frame shift mutations throughout the whole genome. These changes in the DNA, including the repetitive microsatellite sequences are a result of inadequate repair caused by a mutation in one of the DNA mismatch repair genes (*MLH1*, *MSH2*, *MSH3*, *MSH6*, *PSM2* and *MGMT*), responsible for the surveillance and correction of errors induced after DNA replication. Mismatch repair genes are part of a DNA integrity checkpoint; every newly synthesized DNA strand is checked for defects and if necessary mismatch strands will be repaired by these genes. Insertions or deletions in the non-coding microsatellites indicate that coding regions are affected as well and thus form a marker of microsatellite instability.
The third pathway is the CIMP pathway. In this pathway several changes in genes occur, without changes in the DNA sequence, by methylation of CpG islands. The CpG island is often located at the promoter and transcription start site of the gene. Methylation is a physiologic tool to regulate cell function by activating or de-activation specific genes. Depending on the function of the gene, hypo- or hyper methylation can result in dysfunction of the cell mechanism and cause tumour induction.
Because the definitions of the CIN, MSI and CIMP pathways are not mutually exclusive, a tumour can occasionally exhibit features of multiple pathways. For example methylation of the *MLH1* gene (CIMP pathway) results in silencing this mismatch repair gene and cause microsatellite instability.
Jass took the molecular information as described by Vogelstein et al together with clinicopathological characteristics and described the development of CRC as a multi-pathway disease, comprising dissimilar subgroups with particular clinical, pathological and molecular features[15].

Jass classified CRC into five (molecular) subtypes (Table 1);

1. CIMP-high, methylation of *MLH1*, *BRAF* mutation, chromosomally stable, MSI-H, origin in serrated polyps, known generally as sporadic MSI-H (12%).
2. CIMP-high, partial methylation of *MLH1*, *BRAF* mutation, chromosomally stable, MSS or MSI-L, origin in serrated polyps (8%).
3. CIMP-low, *KRAS* mutation, MGMT methylation, chromosomal instability, MSS or MSI-L, origin in adenomas or serrated polyps (20%).
4. CIMP-negative, chromosomal instability, mainly MSS, origin in adenomas (may be sporadic, FAP associated or MUTYH (formerly MYH) polyposis associated (57%).
5. Lynch syndrome, CIMP-negative, *BRAF* mutation negative, chromosomally stable, MSI-H, origin in adenomas (3%)

Table 1 Molecular, Clinical and Morphological features of five colorectal cancer groups20

Feature	Group 1	Group 2	Group 3	Group 4	Group 5
MSI status	H	S/L	S/L	S	H
Methylation	+++	+++	++	+/–	+/–
Ploidy	Dip > An	Dip > An	An > Dip	An > Dip	Dip > An
APC	+/–	+/–	+	+++	++
KRAS	–	+	+++	++	++
BRAF	+++	++	–	–	–
TP53	–	+	++	+++	+
Location	R > L	R > L	L > R	L > R	R > L
Gender	F > M	F > M	M > F	M > F	M > F
Precursor	SP	SP	SP/AD	AD	AD
Serration	+++	+++	+	+/–	+/–
Mucinous	+++	+++	+	+	++
Dirty necrosis	+	+	?	+++	+
Poor differentiation	+++	+++	+	+	++
Circumscribed	+++	+	?	++	++
Tumour budding	+/–	+	?	+++	+
Lymphocytes	+++	+	?	+	+++

MSI, microsatellite instability; H, high; S, stable; L, low; Dip, diploid; An, aneuploid; Serration, serrated morphology; SP, serrated polyp; AD, adenoma; Circumscribed, circumscribed invasive margin.

Migration and invasion of colorectal cancer cells

The transformation of stem cells into differentiated cells is characterized by cell proliferation, differentiation and migration and vulnerable to errors, especially with a dysfunctional EMT. Important pathways involved in the transformation are the;

- Wnt-β-catenin signalling pathway,
- transforming growth factor (TGF)-β /bone morphogenetic protein (BMP)/Smad4 pathway,
- PI(3)K signalling pathway and the EGFR-mitogen-activated protein kinase (MAPK).

These pathways are well studied in the pathogenesis of CRC and of interest for clinical implementations in the management of CRC.

- *The Wnt-β-catenin signalling pathway*

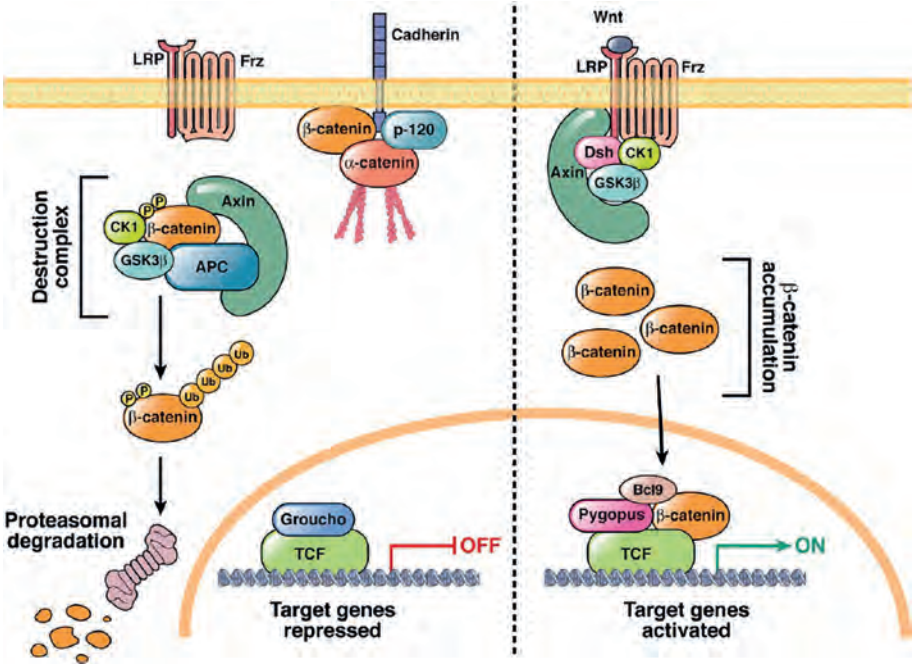
The majority of CRC shows activation of the Wnt-β-catenin signalling pathway, mostly due to mutations in the *APC* gene. Mutation of the *APC* gene (>70% of sporadic CRC), the gate keeper of the adenoma-carcinoma sequence, results in activation of the Wingless/Wnt signalling pathway and induction of chromosomal instability[21]. An essential role of *APC* is the binding with β-catenin. Without a proper function of *APC*, β-catenin will accumulate in the cell and activate transcription factors (TCF/LEF family) in the nucleolus (Figure 5). Deregulation of the transcriptional factors will affect the balance of proliferation and differentiation of the intestinal stem cells in the crypt-villus axis, leading to an unrestricted cell growth. This disruption not only occurs trough mutations in the *APC* gene but also as result from mutations in β-catenin[22,23]. Despite the fundamental role of the Wnt-β-catenin signalling pathway in CRC development, so far, there is no clinical use for *APC* or β-catenin mutations in diagnostic, prognostic or predictive markers[24].

- *The transforming growth factor (TGF)- β /bone morphogenetic protein (BMP)/ Smad4 pathway*

The transforming growth factor (TGF)- β /bone morphogenetic protein (BMP)/ Smad4 pathway is a tumour-suppressor pathway that is frequently mutated in CRC and plays a role in the differentiation and migration of stem cells in the intestinal tract. In CRC, TGF- β receptor type II (TβRII) is mutated in >55% of cases, *BMPRI/RII* is mutated in >70% of cases and *Smad4* mutations occur in 20%

to 30% of cases[26-30]. Smad4 protein plays an essential role in the mediation of the TGF-beta intracellular signalling pathway which suppresses tumour growth and dedifferentiation[31]. *Smad4* is located on chromosome 18q, in the region frequently deleted in CRC. There is evidence that LOH of 18q (loss of *Smad4*; 18q21) is related with poor outcome in advanced CRC, development of lymph-node and CLM [28,32-34]. Furthermore, reduced levels of SMAD4 protein expression are associated with poor prognosis with fluoroucil-based (5-FU) chemotherapy[34,35].

Figure 5, The Wnt-β-catenin signalling pathway in inactive (left) and active (right) constellation 25



- *The PI(3)K signalling pathway and the EGFR-mitogen-activated protein kinase (MAPK)*

In over 50% of CRCs the PI(3)K signalling pathway is affected. This pathway plays a central role in tumourigenesis by regulating cell growth, differentiation and apoptosis[36-38]. In this pathway, *PIK3CA, KRAS* and *BRAF* genes are frequently activated by mutations [36,39-41] with frequencies of 10-30%[36,40,42-46], 30-40% [13,39,47] and 5-22%[39,48], respectively. Mutations in any one of these three genes will activate the PI(3)K signalling pathway and increases the transcription of different oncogenes, such as *C-MYC, CREB, NF-kB* and others[42], resulting in unrestricted cell growth.

There is a potential role of this signalling pathway in predicting survival [40,42] and several molecular studies have revealed that *KRAS* mutation status predicts sensitivity to EGFR-targeted drugs[49].

The EGFR is a transmembrane glycoprotein that signals through the PI(3)K pathway, but also interacts with the Wnt signalling pathway. EGFR expression is found in 8% to 97% primary CRC[50-52]. Unfortunately, there are discordant reports in the relation of EGFR expression in primary CRC and poor survival[53]. Some evidence shows that high EGFR expressions in metastatic lymph nodes is more accurate in predicting survival than in primary or metastatic tissues[54]. EGFR and the PI(3)K signalling pathway provide opportunities for targeted drug treatment as is discussed below.

Clinical Management of Colorectal Cancer

Clinical and pathological staging and characteristics of CRC

So far, the pathological assessment of CRC is of foremost importance for the determination of 1) local extent of disease, 2) whether tumour free resection margins were achieved by the surgical procedure (not only for clinical management but also as a quality indicator for surgery), 3) for the choice of adjuvant treatment and 4) defining prognosis in the individual patient. The most widely used staging system is the pathological T (Tumour), N (Node), and M (Metastasis) (pTNM) staging system, published by the International Union Against Cancer [55]. This system includes the stratification of bowel wall involvement and peritoneal serosa of the colorectal tumour, taking into account the number of involved regional lymph nodes and the presence of distant metastasis (Table 2a)[55]. Table 2b shows the 5-year survival rates of CRC based on the pTNM and various stages[56]. Because of the difficulties in reproducibility of the modifications in the TNM sixth (2003) and seventh (2010) edition, the Netherlands has chosen to use the fifth TNM edition (1997) for staging CRC [56-58].

Table 2a Pathological TNM Classification55.

pTNM CLASSIFICATION OF COLORECTAL TUMOURS
pT Primary tumour
pTX Primary tumour cannot be assessed
. pT0 No evidence of primary tumour
. pT1 Tumour invades submucosa
. pT2 Tumour invades muscularis propria
. pT3 Tumour invades through muscularis propria into subserosa or non-peritonealised pericolic or perirectal tissues
. pT4 Tumour directly invades other organs (pT4a) and/or involves the visceral peritoneum (pT4b)
pN Regional lymph nodes
pNX Regional lymph nodes cannot be assessed
. pN0 No regional lymph node metastasis
. pN1 Metastasis in 1 to 3 regional lymph nodes
. pN2 Metastasis in 4 or more regional lymph nodes
pM Distant metastasis
pMX Distant metastasis cannot be assessed
. pM0 No distant metastasis
. pM1 Distant metastasis

Table 2b, 5-year survival CRC in relation to pTNM, stage and Dukes' Classification56.

Stage	T	N	M	5-Year survival	Dukes' Classification
AJCC fifth edition					
I	T1 or T2	N0	M0	93,20%	A
II	T3 or T4	N0	M0	82,50%	B
III	Any T	N1	M0	59,50%	C
IV	Any T	Any N	M1	8,10%	D

The depth of the tumour invasion is described by the T stage; starting as a T1 tumour (invasion in the mucosa) to T4 tumours (invasion of the serosa or adjacent structures). The N status defines the tumour metastasis in regional lymph nodes; N0 (no lymph nodes with tumour), N1 (1-3 Lymph nodes are involved with metastasis), N2 (4 or more Lymph nodes involved). Lymph node metastasis is considered as one of the most important prognostic factors[59]. Patients with an early stage CRC, without presence of lymph node metastasis (Dukes A and B, TNM stage I and II) have a 5-year survival rate of 80%-90%, while patients with advanced CRC with regional lymph node disease (Dukes C, TNM stage III) have a 5-year survival rate of 60%. Furthermore, patients with distant metastatic disease (Dukes D, TNM stage IV) have a 5-year survival rate of less than 8%[56,56,60,61].

The total number of lymph nodes to be identified for accurate staging is an area of debate. Guidelines in the US recommend the identification of at least 12 lymph nodes whereas in the Netherlands 10 lymph nodes are recommended. Several studies showed significant survival advantage when more lymph nodes are evaluated[62-68]. The examination of fewer lymph nodes may be related to incomplete oncologic surgical resection (increasing the risk of local recurrence), inadequate inspection of the pathologist, obesity, neoadjuvant (chemo therapy; radiation therapy or combination of both) therapy, hospital volume, MSI, location of the tumour and variation in patient anatomy[69-73]. In a large study of lymph node examinations, tumours with prominent lymphocytic infiltration were easier to find due to reactive enlargement. Patients with these characteristics showed an survival advantage due to the number of identified lymph nodes and possibly as a reflection of the immune response to the tumour [68]. The improved survival, associated with infiltration of lymphocytes, might reflect the presence of a good systemic immunosurveillance mechanism resulting in tumour suppression [74,75]. Furthermore, the lymph node ratio, defined as the ratio of the number of positive nodes over the total number of examined nodes, has recently shown to be of independent prognostic value in stage III patients[76,77].

Contrary to patients with colon cancer, patients with rectal cancer are frequently treated neoadjuvantly with (chemo)radiation therapy. Although, in these patients the number of retrieved lymph nodes is lower due to the (chemo) radiation therapy there is still prognostic value[76,78-83].

Besides the Dukes and TNM staging systems, several other pathologic and clinical features have been identified that are associated with increased risk for systemic recurrence and thus worse survival. The most important factors are emergency presentation, bowel perforation, poorly differentiated tumour, depth of tumour invasion, adjacent organ involvement (T4), lymphovasculair invasion, perineural invasion and an elevated carcinoembryonic antigen (CEA)[84-86]. The presence of one these factors will result in worse outcome. CRC population detection programmes reduce the prevalence of some of these clinical features. While, these screening programs will focus on the general population (age > 50 years) there is also a need for focussed surveillance of high risk patients with hereditary syndromes.

Hereditary syndromes

Approximately 30% of all CRC are an inherited form of the disease and 3-5% of CRC occurs in the context of well-defined, hereditary colon cancer syndromes[18]. Lynch syndrome (previously called hereditary nonpolyposis colorectal cancer: (HNPCC) and familial adenomatous polyposis (FAP) represent the most common hereditary syndromes associated with CRC, followed by other less common diseases including attenuated FAP, and MUTYH-associated polyposis (MAP), juvenile polyposis (JPS) and Peutz–Jeghers syndrome PJS) and hyperplastic polyposis (HPP). Except for MAP and HPP, all these syndromes are autosomal dominant disorders with their own risk in developing cancer and clinical manifestation.

Lynch syndrome is the result of germline mutations in the genes involved in the mismatch repair system (*MLH1, MSH2, MSH6, PSM2*). Patients with Lynch syndrome have a life-time risk of 50-80% for developing CRC[87]. To determine if a patient is prone to be carrier of the Lynch syndrome, evaluation with the Bethesda Guidelines needs to be performed[88]. Patients with the Lynch syndrome require an intensified surveillance not only for CRC but also for extra colonic manifestations of Lynch associated tumours[89].

Familial adenomatous polyposis (FAP) is the second-most common inherited form of CRC and the result of germline mutations in the *APC* gene. Mutation of the *APC* gene, the gate keeper of the adenoma-carcinoma sequence, results in activation of the Wingless/Wnt signalling pathway and induction of chromosomal instability[21]. This entity is characterized by forming hundreds to thousands of colonic adenomas with an increased risk of developing extra colonic cancer (e.g., duodenal, pancreatic, thyroid cancer and desmoids tumours). The occurrence of colonic adenomas will start in early adolescence and finally, if untreated, result in the development of CRC. The average age of developing CRC is at age 35 and 95% of the patients with FAP have CRC at age 50. Therefore preventive proctocolectomy is advocated. Timing for surgery should be late teens or early twenties[90].

Surgical and (neo)-adjuvant treatment

The cornerstone for CRC treatment is surgery. For tumours located in the colon and the rectum, the goal is to perform a radical resection. The surgical approach can be laparoscopic or via an open procedure. In the beginning there was concern about the oncological radicality of laparoscopic surgery for CRC.

The main concerns were about irradical resections and port site metastases. For that reason, many surgeons preferred the conventional open approach. However, several randomized trials showed that laparoscopic surgery is safe in CRC treatment with the benefit of reduced pain, shorter duration of ileus, faster recovery, better pulmonary function, less fatigue, lower peri-operative mortality and a better quality of life[91-96]. However, laparoscopic colon resection remains a challenging technique with a longer operation time and conversion rates that international vary between 2%-40%[95,97,98]. Data from the Dutch Surgical Colorectal Audit reported that in the Netherlands 42% of the patients with colon cancer and 45% of the patients with rectal cancer were treated with a laparoscopic procedure wherein 15% was converted to an open procedure[99]. Conversion is associated with poorer results in terms of worse peri-operative outcome and worse disease-free survival[100,101]. The cause for conversion may be due to the type of procedure performed, high BMI (BMI greater than 28.5 kg/m2 is associated with a 2.2-fold increase of conversion), intra abdominal abscesses or fistulas and surgeon seniority[102].

In rectal cancer the resection has to include the total excision of the mesorectum (TME). In this procedure the rectum is resected together with the mesorectum and mesorectal fascia. This TME procedure resulted in a decrease of 50% local recurrence rate compared with conventional surgery (respectively, 11% and 27% at 5 years)[103,104]. The most important prognostic factors that influence local recurrence rates after resection of rectal cancer is defined by the circumferential resection margin (CRM), radical surgery (R0), the stage of the tumour and number of involved lymph nodes [105-108]. Therefore, preoperative assessment of the chance for radical resection and clear CRM is of pivotal importance. This can be done by endorectal ultrasound (EUS), CT-scan or MRI[109,110]. For the T-stage of the tumour, EUS is accurate in differentiating T1 from T2 tumours but performs less in staging T3 and T4 tumours. The disadvantage of EUS is that EUS is operator dependent and cannot appreciate the mesorectal fascia (MRF) involvement. The performance of MRI and CT is less subject to the skills of the operator. The MRI is in many respects superior to the CT scan, especially for determining the MRF involvement. The only advantage of CT would be that it allows local and distant staging in a single examination but, especially for the low rectal tumours, the accuracy is moderate. Identifying nodal disease remains difficult in the pre-operative assessment. Lymph nodes with a diameter of 10 mm or more are almost always malignant but many lymph nodes affected

with metastases are smaller than 5 mm. High-resolution MR images are able to identify nodes <5 mm but differentiation between malignant or benign nodes is difficult. Ultrasmall superparamagnetic particles of iron oxide (USPIO) showing high sensitivity and specificity in defining nodal involvement but are so far not FDA- or EMEA-approved[111,112].

Depending on the local extent of the tumour in relation to the mesorectal fascia and the number of suspected lymph nodes patients will be offered neoadjuvant radiotherapy (RT) nowadays increasingly in combination with chemotherapy. The Dutch TME trial showed that preoperative radiotherapy resulted in significantly lower local recurrence rates compared with the TME alone group (5% and 11%, respectively). The same trial did not show differences in overall survival[113,114].

Combining RT with chemotherapy (CRT) will result in a increased radiosensitivity and as a result enhances the antitumour activity of RT. Unfortunately, (C)RT does not benefit DFS or OS at five years and has to be balanced against the morbidity of the treatment (higher risk of faecal incontinence, sexual dysfunction, bowel dysfunction, wound healing disorders)[115-119]. Therefore, pre-operative assessment of the tumour needs to be done to define which patients will benefit most from neo-adjuvant treatment. For *high risk rectal cancer* (T1-3 with a MRF <1mm, or T4, and/or the risk of 4 ore more positive lympnodes within the mesorectum or positive lympnodes outside the mesorectum) this will result in a treatment with CRT. For *low risk rectal cancer* (T1-3N0, extramural invasion ≤5 mm, distant MRF ≥1mm) in a TME resection alone without neo-adjuvant treatment and for the *intermediate risk rectal cancer* (cT1-3N1 or cT3N0 with extramural invasion >5 mm, distant MRF ≥1mm) short-course radiotherapy (5X5 Gy)[120]. After neo-adjuvant treatment an open or laparoscopic rectum (extralevator) TME resection will follow[121]. Although the great advantage of this TME procedure, rectum surgery is associated with high morbidity rates (~30%). Leakage of the anastomosis is a real problem in rectal surgery, especially for tumours close to the anus, and is reported in 1-19%[122-126]. Therefore, there is a need for new treatment modalities that could reduce morbidity in rectal cancer treatment.

After critical patient selection and with the assumption that an adequate resection margin can be achieved, patients with low risk rectal cancer; a tumour confined to the mucosa of the rectum (T1), could be prevented from major surgery with a Transanal Endoscopic Micro resection (TEM) or Single-incision laparoscopic surgery TEM procedure (SILSTEM)[127-130]. An ongoing study in the

Netherlands (CARTS-study) investigates the role of rectum saving surgery for distal rectal cancer with higher T-stage. This CARTS-study is a prospective multicenter clinical trial, investigating patients with a clinical T1-3 N0 M0 rectal adenocarcinoma below 10 cm from the anal verge. These patients will receive neoadjuvant CRT therapy (25 fractions of 2 Gy with concurrent capecitabine) followed by TEM procedure 8 - 10 weeks after the end of the preoperative treatment. Patients with lymphangioinvasion, an incomplete resected ypT1 (<2 mm margin), an inconclusive resection margin, an ypT2 or ypT3 tumour after TEM will subsequently undergo TME surgery to remove the rectum within 4 weeks [131]. The objectives of the study are to determine the number of patients with a (near) complete pathological response after chemoradiation therapy and TEM, the local recurrence rate and quality of life[132].
In contrast with cancer located in the colon, there is debate whether patients with rectal cancer benefit from adjuvant chemotherapy[133].
Depending on the stage in which cancer located in the colon is discovered, adjuvant treatment with 5-fluorouracil, leucovorin, and oxaliplatin or irinotecan are known to be beneficial in patients with stage III disease. The risk of death for stage III tumours will be reduced by 33%, resulting in a 10–13% absolute improvement in survival[134-136]. Although chemotherapy after surgery is standard for patients with stage III colon cancer, the role of adjuvant therapy for stage II colon cancer remains controversial. There is debate whether stage II patients benefit enough of adjuvant chemotherapy[137-139]. Currently, the American Society of Clinical Oncology recommend only high risk stage II patients, defined as patients with one or more of the following characteristics; an emergency presentation, poorly differentiated tumour, depth of tumour invasion, adjacent organ involvement (T4), fewer than 10-12 lymph nodes sampling[85,86,140], to offer adjuvant chemotherapy.

Targeted therapies have more recently become recommended as first line or subsequent treatment for metastatic CRC. Bevacizumab is a monoclonal antibody therapy that targets the vascular endothelial growth factor (VEGF) thereby blocking blood vessel formation. Cetuximab and Panitumumab, are both monoclonal antibodies targeting the Epidermal Growth Factor Receptor (EGFR). The combination of these targeted drugs with the regular chemotherapeutic drugs results in a considerable improvement of survival in patients with colorectal cancer metastases, with a median survival up to 20.3 months[141-145]. In 4

out of 10 CRC patients, however, EGFR targeted drugs are not effective due to a down stream mutation in the *KRAS* gene[49]. Based on these findings, the European Medicines Agency (EMEA) and the U.S. Food and Drug Administration (FDA) have placed restrictions on the usage of EGFR-targeted drugs and only approved for CRC metastatic patients with wild-type *KRAS* tumours.

Distant metastases

In addition to lymph node metastases, lymphangioinvasion, perineural invasion and pericolonic tumour deposits, CRC can develop distant metastases by haematogenous spread of tumour cells into the bloodstream.

Despite the (neo-)adjuvant treatment of CRC, 50% of all patients will develop distant metastases[3]. These distant metastases can occur synchronic or metachronic. Synchronous metastases are defined as metastases that occur simultaneous with the primary tumour whereas metachronous metastases occur after the diagnosis of the primary tumour. Untreated, these patients have a median survival of approximately 10 months and a 5-year survival rate of less than 5%[146,147]. Distant metastases are responsible for the great majority of CRC deaths, mainly due to liver, lung and peritoneal metastases[148].

About 50% of patients with stage III and 20% of patients with stage II disease will develop colorectal liver metastasis (CLM). Of all patients who die of advanced CRC, 60-70% have developed CLM. Even with the use of targeted drugs, the overall survival in patients with non-resectable CLM is only 2-years and late detection of CLM could be fatal. In 15-25% of patients with CLM, partial hepatic resection is a potentially curative treatment option[149]. In these patients a 5-year survival of up to 60% can be achieved and up to 20% of this population will still be alive after 10 years[150-155]. However, this survival benefit has to be balanced against the procedure related morbidity rates of 15% to 35% and mortality rates of 1% to 4%, respectively[156].

Eligibility for hepatic surgery depends on the likelihood that all metastases are resectable while an adequate liver reserve can be maintained[157]. Furthermore, there should be no extrahepatic disease, with the possible exception of few resectable lung metastases[153]. These patients may benefit from pulmonary metastasectomy with a 5-year survival rate of 27-50%[158-161]. Unfortunately, only

15-30% will be eligible for liver resection and even in this group, two-thirds of patients will develop a recurrence despite optimal metastasectomy [153,155,162,163]. In patients with resectable CLM, perioperative combination chemotherapy with 5-fluorouracil/leucovorin/oxaliplatin (FOLFOX regimen) improves the 3-year progression-free survival from 28.1% to 36.2% compared to surgery alone and seems to benefit most when CEA levels are elevated and in patients with unaffected performance status[164].

So far, surgical resection of CLM remains the only chance for cure. However, a large proportion of patients with CLM are unable to undergo a complete surgical resection. These patients are offered to be treated with liver-directed therapies like radio frequent ablation (RFA), cryoablation, hepatic artery infusion and stereotactic radiotherapy. All these therapies have aided in prolonging survival in patients with CLM. A recent study comparing non-resectable colorectal liver metastases between systemic treatment or systemic treatment plus RFA (±resection), showed no significantly difference in 30-month overall survival (57.6% and 61.7% respectively). However, the median progression-free survival was significantly improved with 7 months in the RFA plus systemic treatment group[165].

Following CLM, a second preferential site of distant metastases are pulmonary located. Most of these pulmonary metastases are in combination with CLM. Only 10% of pulmonary metastases are isolated lung metastases. Isolated lung metastases are metastases confined to the lung without other distant metastases. The incidence of isolated pulmonary metastases is higher in rectal cancer compared with colon cancer, with an incidence up to 12% and 6% respectively. The explanation for this finding is that a rectal tumour spread directly into the systemic circulation via the inferior and middle rectal veins, bypassing the portal venous system[166]. The 5-year survival rate after resection pulmonary metastases range from 40-63[161]%.

A third preferential site of metastases is the peritoneal cavity. Peritoneal Metastases (PM) is uniformly seen as a fatal condition. However, in the last decade survival has improved due to aggressive cytoreductive surgery in combination with hyperthermic intraperitoneal chemotherapy (HIPEC). Patients with PM from colorectal origin who underwent complete cytoreduction in combination

with HIPEC showed 5-year survival rates of 22–49%[167,168]. Peritoneal metastases of appendix tumours showed an even better outcome, up to 85% 10-year survival[169,170].

Several predictors for outcome after HIPEC treatment, such as the completeness of cytoreduction, the number of affected intra-abdominal tumour regions and the histological characteristics of the PM, have been described[171-174]. In many studies peritoneal dissemination from appendiceal neoplasm's is reported as a specific entity. However, this condition ranges from borderline malignancy to true colon like carcinoma and may thus be closely related PM from colorectal origin. Other studies focused only on PM originating from colon and rectal cancers including both mucinous and non-mucinous PM and thereby excluding tumours with a primary appendix lesion[168,172,174-176]. Conversely, studies focused on the clinical diagnosis such as the clinical entity 'pseudomyxoma peritonei', a disease with an ongoing discussion on the definitions of origin, histopathology and proper treatment. Therefore, an internationally accepted histopathological classification is needed to compare outcome of specialized treatment regimes like the HIPEC procedure.

Follow-up

Eligibility for surgery for metastases of liver, lung or PM is depending on the extensiveness at the time they are discovered. Approximately 30-50% of all patients with CRC develop metachronous metastases. These metastases mostly occur within 3 years after surgery[177]. A 2007 Cochrane report presents strong evidence that intensive surveillance is life-saving and appropriate for CRC patients[20]. It is supposed that the survival gain (7-13%) for patients managed with intensive follow-up after primary tumour treatment, is a result of earlier detection in which further curative treatment is possible[20,178,179]. Between 35% to 47% of patients who experience recurrences after primary CRC resection can be treated with secondary curative-intent surgery when followed intensively after primary surgery. Thirty-six percent of all patients with a recurrence undergo secondary surgery with a curative intent. These patients have a median survival between 36 and 51months[180,181]. Intensive follow-up programs include: frequent medical check-ups, CEA determinations, imaging the thorax and abdomen and colonoscopy.

Increase in CEA levels is often the first signal of recurrence. Subsequent elevation of CEA in a post operative patient is indicative for tumour recurrence

or distant metastases, mainly due to CLM[166]. Approximately 75% of the patients with local recurrence or distant metastases have elevated CEA levels[182]. Therefore, several guidelines (American Society of Colon and Rectal Surgeons (ASCRS), ASCO, National Comprehensive Cancer Network (NCCN), European Society for Medical Oncology (ESMO)) recommend CEA determination every 3–6 months for 3 years and every 6–12 months at years 4 and 5 after surgery[179,183]. Furthermore, CT scan of chest and abdomen every 6–12 months for the first 3 years can be considered in patients who are at higher risk for recurrence. As far as liver imaging is concerned, CT scan has been shown to be more sensitive than ultrasonography (0.67 compared with 0.43), but contrast enhancement ultrasound scan can significantly increase the sensitivity of ultrasonography[179].
Complete visualization with (CT)-colonoscopy of the colon to identify synchronous lesions is recommended before curative resection. If not possible (e.g. acute presentation due to obstruction, perforation) a colonoscopy should be performed within 3–6 months after resection. One year after surgery and thereafter every 3–5 years a colonoscopy must be performed to detect recurrence or pre-malignant lesions.
All these screening and follow up modalities are designed to improve the prognosis by early detection of primary or recurrent CRC. In the Netherlands, a screening program performed with a immunochemical faecal occult blood tests (iFOBT), will be established in 2013 to discover primary CRC in an early stage. iFOBT has demonstrated mortality benefit in several studies although the accuracy is low in detecting adenomas at an early stage[184-189]. A recent systemic review and meta-analysis shows that flexible sigmoidoscopy (FS) as screening modality between average risk 55 and 64 years results in a reduction of CRC incidence of approximately 32% and CRC-related mortality by 50%[190]. Because the right sided colon is only screened in case of a left sided adenoma, the protective effect of FS is limited for proximal CRC[190].

Molecular Biomarkers for the management of CRC

Prognostic and Predictive Markers

Selecting the optimal treatment strategy for patients with stage II CRC is still a clinical challenge. The majority (80%) of these patients is cured by surgery alone and do not need adjuvant chemotherapy. Patients who are classified as high

risk, based on pathological risk features, are the only group of stage II patients who currently receive adjuvant chemotherapy. This results in under treatment of the patients in the clinical-pathological low risk group and over treatment for the high risk patients, as these predictions are not that precise. Therefore, there is a strong need for new, clinically validated molecular tests which can provide more accurate, quantitative recurrence risk information to guide treatment decision-making for individual stage II colon cancer patients. Several studies have now described prognostic gene expression profiles for CRC patients[9,191-197]. For example the new prognosis signature ColoPrint, which distinguishes low from high risk patients using gene expression analysis, including the validation of this signature in an independent dataset. This gene expression profile was able to predict prognosis of stage II and III patients better than the conventional recommended clinical-pathological risk factors[198]. The use of these gene expression profile in clinical setting will provide more accurate information on the risk of recurrence compared to the use of conventional clinico-pathological criteria alone and can facilitate the selection of low risk patients who can be spared chemotherapy.

KRAS mutation testing is currently performed as part of EGFR targeted therapy, Cetuximab and Panitumumab eligibility for metastatic CRC patients as mutated *KRAS* prevents therapeutic benefit [46].

Prediction colorectal liver metastases

Clinical pathological assessment of CRC is used for determination of local extent of disease, choice for adjuvant treatment and defining prognosis in the individual patient. However, this conventional clinical pathologic classification does not provide information on predicted site of metastases.

Gene expression profiles have been described for breast cancer that predict site specific recurrence e.g., bone and lung metastases[199-201]. However, gene expression or genomic profiles in CRC that predict site specific recurrence have not been well studied.

In this thesis we describe the research that investigated molecular biomarkers in defined groups of primary colorectal tumours to determine markers for site specific metastases.

Selection of patients for specialized surgery

Patients to select for hepatic surgery

One of the challenges in CRC management lies in the early detection and treatment of CLM. Several clinical pathologic and molecular models predict outcome for individual patients with CLM. Unfortunately, these models do not predict extra hepatic recurrence after CLM resection. Knowledge of likelihood for extra-hepatic metastases after CLM surgery may limit hepatic resections to those who are not likely to develop extra-hepatic metastases. Therefore, we investigated whether genomic aberrations in primary CRC could aid to identify these patients who will develop extra hepatic recurrence after CLM resection.

Patients to select for HIPEC surgery

In this thesis we investigated whether a classification system that identifies patients who could benefit from HIPEC surgery is feasible for both colorectal and appendiceal tumours. We investigated whether a standardized histological classification of PM from appendiceal and colorectal origin can more precisely predict survival and thus help to tailor therapy in the future and select patients most suitable for the HIPEC treatment.

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