Biomarkers in colorectal cancer

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Chapter 1

General introduction and thesis outline

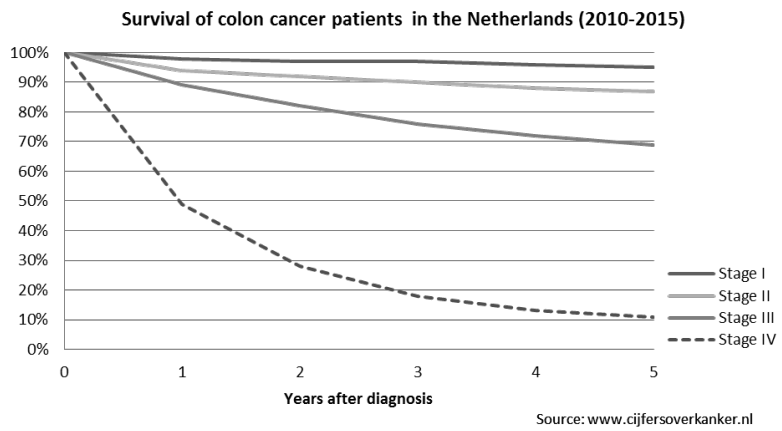
COLORECTAL CANCER INCIDENCE, TREATMENT AND PROGNOSIS

Colorectal cancer (CRC) is the second most common diagnosed cancer in females and the third most common cancer in males and accounts for the second cause of cancer death in Europe with an estimated incidence of 447.000 new CRC cases and 215.000 deaths, in 2012 [1]. As shown in figure 1, the estimation of the prognosis is mainly determined by conventional staging such as, tumour, node and metastasis (TNM) classification. Despite a continuous improvement of the TNM classification, outcome among patients with the same tumour stage varies significantly [2]. Consequently, it could be stated that adequate individualized assessment could not be accomplished with conventional classification. Furthermore, evidence is accumulating that rectal tumours differ from colon tumours [3,4], resulting in a separation of colon and rectal cancer regarding biology and treatment.

In general, treatment of CRC employs a multidisciplinary approach, though surgery remains the cornerstone of curative treatment for non-metastasized CRC in most cases. Prior to surgery, clinical staging is performed by a combination of endoscopy, CT (Computerized Tomography) and in rectal cancer also MRI (Magnetic Resonance Imaging). For colon cancer stage III and high risk stage II, surgery is followed by adjuvant chemotherapy [5-7]. Still, approximately 20% of the patients with stage I-III colon cancer, develop metastatic disease within 5 years [8]. For rectal cancer, important advances have been made in treatment with the implementation of total mesorectal excision (TME) [9], combined with preoperative (chemo)radiotherapy and the ability to more accurately stage rectal cancer with MRI. However, approximately 30% of the patients with rectal cancer treated with a curative intent will develop distant metastasis [10-12]. Adjuvant chemotherapy was thought to prevent distant metastasis by eliminating micrometastases and circulating tumour cells. However, the advantageous effect of adjuvant chemotherapy for patients with stage II/III rectal cancer, treated with preoperative (chemo)radiotherapy and TME surgery, is not accepted as a standard in according to ESMO guidelines [13]. In the PROCTOR-SCRIPT trial, a multicentre randomized phase III trial, patients were randomized between adjuvant chemotherapy or observation in patients with (y)pTNM stage II-III rectal cancer treated with preoperative (chemo)radiotherapy and TME surgery. This study showed no survival benefit for patients with (y)pTNM stage II-III rectal cancer treated with adjuvant chemotherapy compared to observation [14]. In order to provide robust and stable evidence for the use of adjuvant chemotherapy in patients with locally advanced rectal cancer, a meta-analysis based on individual patient data was performed in this thesis. Furthermore, accumulating evidence suggests a more important role for preoperative chemo radiation therapy (CRT) compared with postoperative CRT in rectal cancer patients [11,15]. Trials with intensified preoperative treatment, such as the RAPIDO trial, are in progress, and results

are awaited [16]. Furthermore, in patients with locally advanced rectal cancer receiving preoperative chemo radiation a complete pathological response has been observed in 20-30%, consequently there is an emerging role for watch-and-wait strategies as introduced by Habr-Gama [17]. However, more data and long term outcomes are needed before this organ-preservation strategy could be incorporated safely. Therefore, The European Registration of Cancer Care, and the Cahmpalimaud foundation has initiated the International Watch and Wait Database to collected uniform data [18].

Currently, in contrast to colon cancer for rectal cancer, there are no molecular markers for rectal cancer that evaluate, whether a patient benefit from preoperative treatment, predict response to (chemo)radiotherapy or whether a tumour will metastasize.



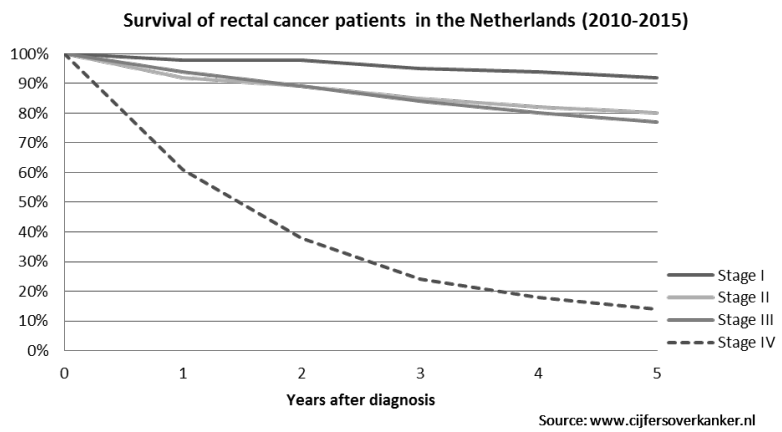


Figure 1: Survival curves. Upper panel shows survival of colon cancer patients stratified by disease stage. Lower panel shows survival of rectal cancer patients stratified by disease stage.

PROGNOSTIC AND PREDICTIVE BIOMARKERS IN CRC

Treatment choices are mainly influenced by the TNM classification, which provides an estimation of the clinical prognosis and creates uniformity in the diagnosis of oncologic diseases [19,20]. Although, outcome among patients with the same tumour stage differs [2]. Insight into the biological diversity of CRCs in relation to clinical features is needed, to ultimately find the right equilibrium in the treatment to avoid mortality and morbidity and prevent over- and under treatment.

Biomarkers are biological entities that can be measured, for example in blood or tumour tissue, to be used as indicators of pathological processes. Investigating biomarkers that reflect tumour growth and metastatic potential can provide information on the clinical outcome, based on the underlying biological mechanism. The detection of prognostic and predictive biomarkers has become a crucial part of CRC research. Despite encouraging preliminary data, so far the use of biomarkers in clinical practice is very limited. In CRC, only a few biomarkers are used in daily clinical practice, such as *RAS/RAF* and microsatellite status. For example, it has been demonstrated that *RAS* mutations were found in patients who were resistant to monoclonal antibodies targeting the epidermal growth factor receptor [21]. A second well known example is microsatellite instability (MSI), which is without doubt the single most informative genetic characteristic in early stage colon cancer. In contrast to colon cancer, the implications of a MSI tumour located in the rectum remain undefined. Besides the fact that MSI is a hallmark of hereditary non-polyposis colorectal cancer (HNPCC), MSI is found in approximately 15% of the sporadic CRC tumours [22]. In addition, accumulating evidence advocates that deficient mismatch repair mechanism, especially in early-stage colon cancer, is associated with a clinical prognostic advantage [23-25], in comparison with microsatellite stable (MSS) colon tumours. In contrast, an adverse prognostic effect of MSI was observed in metastatic CRC [26]. On the predictive value of MSI regarding the response to 5-fluorouracil (5-FU), although with conflicting results, [25,27-29], accumulating preclinical and clinical evidence reports a resistance to 5-fluorouracil (5-FU), in patients with deficient MMR tumours [24,25,30,31].

SOURCES OF BIOMARKERS IN CRC

Tumour-immune interactions

Molecular mechanisms responsible for tumour genesis are likely to influence clinical outcome. In 2000, it has been proposed that six biological alterations must be acquired during the multistep development of cancer [32]. These well-known six hallmarks of cancer consist of: sustaining proliferative signalling, activating tissue invasion, evading growth

suppression and metastasis, enabling replicative immortality, inducing angiogenesis, and resisting apoptosis. After the recognition of the importance of tumour microenvironment, additional hallmarks were added in 2011 of which evasion of the immune recognition was one of these emerging hallmarks [33]. The concept of tumour immune-editing, in order to escape the host defence immunity is currently widely accepted [34]. A well described mechanism, in the escape from the host immune recognition and destruction is complete loss or downregulation of classical HLA class I molecules. Downregulation or complete loss of HLA class I diminishes tumour-associated antigen presentation on the cell membrane. Consequently, cytotoxic T-cells recognition and destruction of tumour cells is minimized [35,36]. Another mechanism in escaping the anti-tumour immune response, could be *de novo* expression of non-classical HLA class I proteins on the cell surface, such as HLA-G a molecule with important immunomodulatory properties. HLA-G is rarely expressed in non-pathological conditions, other than in immune privileged sites, such as placenta tissue, where it is involved in fetal immune tolerance towards the maternal immune system [37,38]. Alternative splicing of the primary HLA-G transcript has been described in literature, resulting in seven HLA-G isoforms: four membrane bound (HLA-G1, G2, G3 and G4) and three soluble isoforms (HLA-G5, G6 and G7) [39]. Furthermore, HLA-G expression in a *de novo* matter has been reported in human tumour cells, including CRC [40-42]. The influence of the tumour-driven *de novo* expression of HLA-G in escaping immune surveillance is by interaction with inhibitory receptors on T lymphocytes and natural killer (NK) cells [40], in other words HLA-G functions as an immune checkpoint inhibiting antitumor responses. This could explain why expression of the HLA-G protein on tumour cells might be associated with higher tumour grade and adverse prognosis [43]. Therefore, HLA-G has been proposed as a potential target for immunotherapy strategies [44]. However, it should be noted that discrepancies among and within different tumour types were reported. For, CRC, HLA-G expression, detected with immunohistochemistry (IHC), varies from 20-72% [44,45]. IHC is a widely accepted technique, although remains controversial in detecting HLA-G protein expression [46,47]. To firmly evaluate HLA-G protein expression additional molecular and biochemical analysis are essential. Thereby, HLA-G protein expression should be investigated in patient derived samples, rather than (cancer) cell lines. In this thesis, HLA-G expression was intensively investigated in both CRC cell lines and patient derived samples. Moreover, different biochemical techniques to detect HLA-G were used and results will be compared in order to firmly evaluate whether or not results obtained by IHC will be reliable and if HLA-G indeed plays an important role in CRC.

Tumour genetics and epigenetics

Currently, the first genetic biomarkers are used clinically, such as *RAS/RAF* and MSI status. In the current guidelines it has been recommended to determine *RAS* and *BRAF*

mutation status in patients with irresistible CRC metastasis . Since patient with *BRAF* mutated tumours do not benefit from anti-epidermal growth factor receptor (EGFR) treatment [48]. In addition, in patients with wild-type *RAS/BRAF* metastasized colon cancer, anti-EGFR therapy is only recommended in colon cancer patients with left-sided primary tumours [49,50].

As earlier mentioned, approximately 15% of the sporadic stage II-III CRC has MSI [22] and in addition MSI tumours have distinct features such as a more proximal localization, higher grade, a mucinous histology with tumour infiltrating lymphocytes and the presence of a *BRAF* mutation. Furthermore, a prognostic advantage has been observed for MSI tumours [23-25]. Currently, accumulating evidence illustrates the significance of determining MSI in colon cancer. Besides a prognostic effect, MSI status is predictive of response to adjuvant chemotherapy. For example, in patients with high-risk stage II colon cancer with MSI tumours, no beneficial effect of adjuvant chemotherapy has been observed, indicating that these patients should not treated with 5-FU-based adjuvant chemotherapy [25,51,52]. Therefore, the routine screening for deficient mismatch repair (MMR) mechanisms in patients with newly diagnosed CRC has been supported by the guidelines from American Society of Clinical oncology (ASCO), the European Society for Medical Oncology (ESMO) and has been implemented in the most recent edition of the TNM staging classification (eight edition, 2017) [53-55]. In contrast to colon cancer, the role of a MSI in rectal cancer remain undefined. The long-term prognosis of MSI in sporadic rectal cancers has not been well-established in large patient cohorts, although it may be highly relevant to enable the implementation of personalized treatment strategies driven by biomarkers. Furthermore, an increased radio-sensitivity in MSI tumours has been suggested based on *in vitro* experiments and in small patient series [56,57]. Accordingly, the clinical significance of MSI in rectal cancer needs to be evaluated in a large rectal cancer cohort.

Interestingly, MMR germline mutations are found in patients with HNPCC, although in sporadic CRC MSI results most frequently from inactivation of the *MLH1* gene by hypermethylation of CpG islands in the promoter region [58,59]. This example illustrates the important role of epigenetics in carcinogenesis. Epigenetics has become a recent focus in cancer research. Besides hypermethylation, genome-wide DNA hypomethylation is an crucial epigenetic alteration in cancer too. In CRC, hypomethylation is considered as an early event in the carcinogenesis and thereby contributing to genomic instability [60,61]. To indirectly measure global hypomethylation, the methylation status of long interspersed nucleotide element (LINE-1) repeats can be used as surrogate marker [62]. LINE-1 repeats make-up approximately 17% of the human genome and are present on most of the chromosomes [63]. Tumour LINE-1 methylation status is intensively

studied and a decrease of LINE-1 methylation has been observed in almost all human malignancies [64,65]. LINE-1 hypomethylation in CRC is thought to be associated with an adverse prognosis, which suggests a role for LINE-1 as prognostic biomarker [66]. In the current available literature tumour LINE-1 methylation status has been related to clinical outcome in CRC. However, LINE-1 methylation status was predominantly investigated in study cohorts consisting of rectal and colon cancer patients together. Compelling evidence illustrates that rectal cancers biologically differ significantly from colon cancer [3,4]. For rectal cancer it has been demonstrated by Benard *et al.* that LINE-1 hypomethylation was associated with an unfavourable survival and higher tumour recurrence rates, in early stage [67]. Large patient studies on exclusively patients with early stage colon cancer are not available in the current literature. Therefore, studies investigating the prognostic role of tumour LINE-1 methylation level in stage II colon cancer specifically are needed. In this thesis we aimed to investigate the prognostic role of tumour LINE-1 methylation level in stage II colon cancer, in order to identify high-risk patient to ultimately avoid over-, or under treatment.

OUTLINE OF THE THESIS

Potential relevant biomarkers can be found at different levels in tumour development and disease progression. This thesis is divided into three overarching parts. Colorectal cancer was studied from a population-based perspective (part I) to a molecular level, detailed as protein expression (part II) and (epi)genetics (part III), as indicated in Figure 2. In part I the use of adjuvant chemotherapy in patients with locally advanced rectal cancer, who underwent resection after preoperative (chemo)radiotherapy, was evaluated in a meta-analysis based on individual patient data. Since four randomized controlled trials individually did not end the ongoing debate about the role of adjuvant chemotherapy [14,68-70]. In part II the ability by tumour cells to evade the immune recognition was studied, especially the role of the non-classical HLA class I molecule HLA-G was studied in detail. In part III, an epigenetic biomarker, LINE-1 methylation level, was studied in a dedicated stage II colon cohort. In addition, an established genetic biomarker for colon cancer, MSI, was studied in a large rectal cancer cohort.

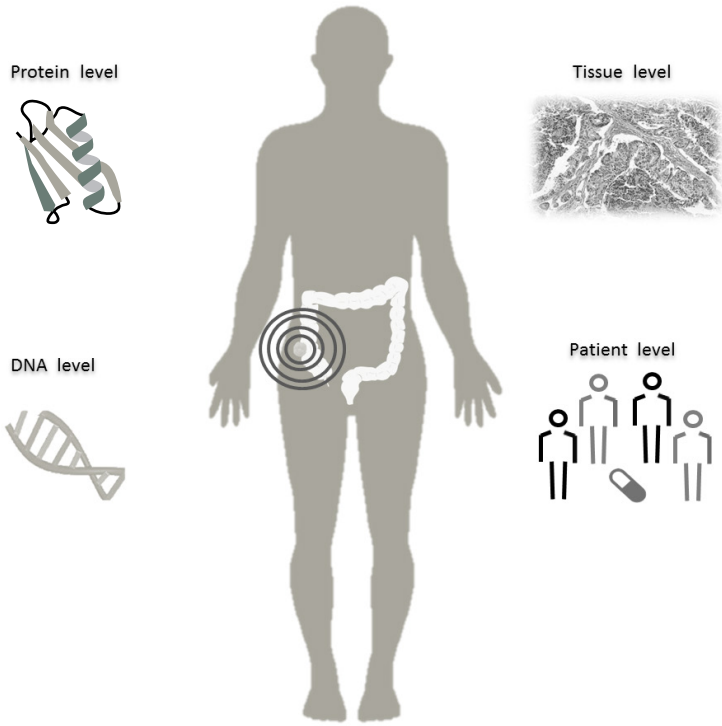


Figure 2: Global overview of different levels involved in colorectal cancer formation and disease progression investigated in this thesis.

REFERENCES

1. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, et al. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. Eur J Cancer 2013;49:1374-403.
2. Puppa G, Sonzogni A, Colombari R, Pelosi G. TNM staging system of colorectal carcinoma: a critical appraisal of challenging issues. Arch Pathol Lab Med 2010;134:837-52.
3. Kapiteijn E, Liefers GJ, Los LC, et al. Mechanisms of oncogenesis in colon versus rectal cancer. J Pathol 2001;195:171-8.
4. Li JN, Zhao L, Wu J, et al. Differences in gene expression profiles and carcinogenesis pathways between colon and rectal cancer. J Dig Dis 2012;13:24-32.
5. Andre T, Boni C, Navarro M, et al. Improved overall survival with oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment in stage II or III colon cancer in the MOSAIC trial. J Clin Oncol 2009;27:3109-16.
6. Figueredo A, Coombes ME, Mukherjee S. Adjuvant therapy for completely resected stage II colon cancer. Cochrane Database Syst Rev 2008:CD005390.
7. Moertel CG, Fleming TR, Macdonald JS, et al. Levamisole and fluorouracil for adjuvant therapy of resected colon carcinoma. N Engl J Med 1990;322:352-8.
8. Elferink MA, de Jong KP, Klaase JM, Siemerink EJ, de Wilt JH. Metachronous metastases from colorectal cancer: a population-based study in North-East Netherlands. Int J Colorectal Dis 2015;30:205-12.
9. Kapiteijn E, Marijnen CA, Nagtegaal ID, et al. Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer. N Engl J Med 2001;345:638-46.
10. Engelen SM, Maas M, Lahaye MJ, et al. Modern multidisciplinary treatment of rectal cancer based on staging with magnetic resonance imaging leads to excellent local control, but distant control remains a challenge. Eur J Cancer 2013;49:2311-20.
11. Sauer R, Liersch T, Merkel S, et al. Preoperative versus postoperative chemoradiotherapy for locally advanced rectal cancer: results of the German CAO/ARO/AIO-94 randomized phase III trial after a median follow-up of 11 years. J Clin Oncol 2012;30:1926-33.
12. van Gijn W, Marijnen CA, Nagtegaal ID, et al. Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer: 12-year follow-up of the multicentre, randomised controlled TME trial. Lancet Oncol 2011;12:575-82.
13. Glynne-Jones R, Wyrwicz L, Tiret E, et al. Rectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2017;28:iv22-iv40.
14. Breugom AJ, van Gijn W, Muller EW, et al. Adjuvant chemotherapy for rectal cancer patients treated with preoperative (chemo)radiotherapy and total mesorectal excision: a Dutch Colorectal Cancer Group (DCCG) randomized phase III trial. Ann Oncol 2015;26:696-701.
15. Park JH, Yoon SM, Yu CS, Kim JH, Kim TW, Kim JC. Randomized phase 3 trial comparing preoperative and postoperative chemoradiotherapy with capecitabine for locally advanced rectal cancer. Cancer 2011;117:3703-12.
16. Nilsson PJ, van Etten B, Hospers GA, et al. Short-course radiotherapy followed by neo-adjuvant chemotherapy in locally advanced rectal cancer--the RAPIDO trial. BMC Cancer 2013;13:279.

17. Habr-Gama A, de Souza PM, Ribeiro U, Jr., et al. Low rectal cancer: impact of radiation and chemotherapy on surgical treatment. Dis Colon Rectum 1998;41:1087-96.
18. Beets GL, Figueiredo NL, Habr-Gama A, van de Velde CJ. A new paradigm for rectal cancer: Organ preservation: Introducing the International Watch & Wait Database (IWWD). Eur J Surg Oncol 2015;41:1562-4.
19. Gospodarowicz MK, Miller D, Groome PA, Greene FL, Logan PA, Sobin LH. The process for continuous improvement of the TNM classification. Cancer 2004;100:1-5.
20. Greene FL, Sobin LH. The staging of cancer: a retrospective and prospective appraisal. CA Cancer J Clin 2008;58:180-90.
21. Diaz LA, Jr., Williams RT, Wu J, et al. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature 2012;486:537-40.
22. Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology 2010;138:2073-87 e3.
23. Guastadisegni C, Colafranceschi M, Ottini L, Dogliotti E. Microsatellite instability as a marker of prognosis and response to therapy: a meta-analysis of colorectal cancer survival data. Eur J Cancer 2010;46:2788-98.
24. Hutchins G, Southward K, Handley K, et al. Value of mismatch repair, KRAS, and BRAF mutations in predicting recurrence and benefits from chemotherapy in colorectal cancer. J Clin Oncol 2011;29:1261-70.
25. Sargent DJ, Marsoni S, Monges G, et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. J Clin Oncol 2010;28:3219-26.
26. Venderbosch S, Nagtegaal ID, Maughan TS, et al. Mismatch repair status and BRAF mutation status in metastatic colorectal cancer patients: a pooled analysis of the CAIRO, CAIRO2, COIN, and FOCUS studies. Clin Cancer Res 2014;20:5322-30.
27. Des Guetz G, Schischmanoff O, Nicolas P, Perret GY, Morere JF, Uzzan B. Does microsatellite instability predict the efficacy of adjuvant chemotherapy in colorectal cancer? A systematic review with meta-analysis. Eur J Cancer 2009;45:1890-6.
28. Ribic CM, Sargent DJ, Moore MJ, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med 2003;349:247-57.
29. de Vos tot Nederveen Cappel WH, Meulenbeld HJ, Kleibeuker JH, et al. Survival after adjuvant 5-FU treatment for stage III colon cancer in hereditary nonpolyposis colorectal cancer. Int J Cancer 2004;109:468-71.
30. Elsaleh H, Joseph D, Grieu F, Zeps N, Spry N, Iacopetta B. Association of tumour site and sex with survival benefit from adjuvant chemotherapy in colorectal cancer. Lancet 2000;355:1745-50.
31. Elsaleh H, Shannon B, Iacopetta B. Microsatellite instability as a molecular marker for very good survival in colorectal cancer patients receiving adjuvant chemotherapy. Gastroenterology 2001;120:1309-10.
32. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57-70.
33. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646-74.
34. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science 2011;331:1565-70.
35. Algarra I, Garcia-Lora A, Cabrera T, Ruiz-Cabello F, Garrido F. The selection of tumor variants with altered expression of classical and nonclassical MHC class I molecules: implications for tumor immune escape. Cancer Immunol Immunother 2004;53:904-10.
36. Atkins D, Breuckmann A, Schmahl GE, et al. MHC class I antigen processing pathway defects, ras mutations

and disease stage in colorectal carcinoma. Int J Cancer 2004;109:265-73.

37. Ellis SA, Palmer MS, McMichael AJ. Human trophoblast and the choriocarcinoma cell line BeWo express a truncated HLA Class I molecule. J Immunol 1990;144:731-5.
38. Roussev RG, Coulam CB. HLA-G and its role in implantation (review). J Assist Reprod Genet 2007;24:288-95.
39. Paul P, Cabestre FA, Ibrahim EC, et al. Identification of HLA-G7 as a new splice variant of the HLA-G mRNA and expression of soluble HLA-G5, -G6, and -G7 transcripts in human transfected cells. Hum Immunol 2000;61:1138-49.
40. Carosella ED, Moreau P, Lemaoult J, Rouas-Freiss N. HLA-G: from biology to clinical benefits. Trends Immunol 2008;29:125-32.
41. Fukushima Y, Oshika Y, Nakamura M, et al. Increased expression of human histocompatibility leukocyte antigen-G in colorectal cancer cells. Int J Mol Med 1998;2:349-51.
42. Hansel DE, Rahman A, Wilentz RE, et al. HLA-G upregulation in pre-malignant and malignant lesions of the gastrointestinal tract. Int J Gastrointest Cancer 2005;35:15-23.
43. Rouas-Freiss N, Moreau P, LeMaoult J, Carosella ED. The dual role of HLA-G in cancer. J Immunol Res 2014;2014:359748.
44. Guo ZY, Lv YG, Wang L, et al. Predictive value of HLA-G and HLA-E in the prognosis of colorectal cancer patients. Cell Immunol 2015;293:10-6.
45. Zeestraten EC, Reimers MS, Saadatmand S, et al. Combined analysis of HLA class I, HLA-E and HLA-G predicts prognosis in colon cancer patients. Br J Cancer 2014;110:459-68.
46. Apps R, Gardner L, Moffett A. A critical look at HLA-G. Trends Immunol 2008;29:313-21.
47. Polakova K, Kuba D, Russ G. The 4H84 monoclonal antibody detecting beta2m free nonclassical HLA-G molecules also binds to free heavy chains of classical HLA class I antigens present on activated lymphocytes. Hum Immunol 2004;65:157-62.
48. Dahabreh IJ, Terasawa T, Castaldi PJ, Trikalinos TA. Systematic review: Anti-epidermal growth factor receptor treatment effect modification by KRAS mutations in advanced colorectal cancer. Ann Intern Med 2011;154:37-49.
49. Arnold D, Lueza B, Douillard JY, et al. Prognostic and predictive value of primary tumour side in patients with RAS wild-type metastatic colorectal cancer treated with chemotherapy and EGFR directed antibodies in six randomized trials. Ann Oncol 2017;28:1713-29.
50. Boeckx N, Koukakis R, Op de Beeck K, et al. Primary tumor sidedness has an impact on prognosis and treatment outcome in metastatic colorectal cancer: results from two randomized first-line panitumumab studies. Ann Oncol 2017;28:1862-8.
51. Des Guetz G, Uzzan B, Nicolas P, Schischmanoff O, Perret GY, Morere JF. Microsatellite instability does not predict the efficacy of chemotherapy in metastatic colorectal cancer. A systematic review and meta-analysis. Anticancer Res 2009;29:1615-20.
52. Sinicrope FA, Foster NR, Thibodeau SN, et al. DNA mismatch repair status and colon cancer recurrence and survival in clinical trials of 5-fluorouracil-based adjuvant therapy. J Natl Cancer Inst 2011;103:863-75.
53. Balmana J, Balaguer F, Cervantes A, Arnold D, Group EGW. Familial risk-colorectal cancer: ESMO Clinical Practice Guidelines. Ann Oncol 2013;24 Suppl 6:vi73-80.

54. Stoffel EM, Mangu PB, Gruber SB, et al. Hereditary colorectal cancer syndromes: American Society of Clinical Oncology Clinical Practice Guideline endorsement of the familial risk-colorectal cancer: European Society for Medical Oncology Clinical Practice Guidelines. J Clin Oncol 2015;33:209-17.
55. Jessup JM GR, Asare EA, et al.. Colon and Rectum. In: AJCC Cancer Staging Manual, 8th, Amin MB. (Ed), AJCC, Chicago 2017. p.251.
56. Davis TW, Wilson-Van Patten C, Meyers M, et al. Defective expression of the DNA mismatch repair protein, MLH1, alters G2-M cell cycle checkpoint arrest following ionizing radiation. Cancer Res 1998;58:767-78.
57. Franchitto A, Pichierri P, Piergentili R, Crescenzi M, Bignami M, Palitti F. The mammalian mismatch repair protein MSH2 is required for correct MRE11 and RAD51 relocalization and for efficient cell cycle arrest induced by ionizing radiation in G2 phase. Oncogene 2003;22:2110-20.
58. Weisenberger DJ, Siegmund KD, Campan M, et al. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. Nat Genet 2006;38:787-93.
59. Cunningham JM, Kim CY, Christensen ER, et al. The frequency of hereditary defective mismatch repair in a prospective series of unselected colorectal carcinomas. Am J Hum Genet 2001;69:780-90.
60. Goelz SE, Vogelstein B, Hamilton SR, Feinberg AP. Hypomethylation of DNA from benign and malignant human colon neoplasms. Science 1985;228:187-90.
61. Jones PA, Baylin SB. The epigenomics of cancer. Cell 2007;128:683-92.
62. Yang AS, Estecio MR, Doshi K, Kondo Y, Tajara EH, Issa JP. A simple method for estimating global DNA methylation using bisulfite PCR of repetitive DNA elements. Nucleic Acids Res 2004;32:e38.
63. Lander ES, Linton LM, Birren B, et al. Initial sequencing and analysis of the human genome. Nature 2001;409:860-921.
64. Portela A, Esteller M. Epigenetic modifications and human disease. Nat Biotechnol 2010;28:1057-68.
65. Barchitta M, Quattrocchi A, Maugeri A, Vinciguerra M, Agodi A. LINE-1 hypomethylation in blood and tissue samples as an epigenetic marker for cancer risk: a systematic review and meta-analysis. PLoS One 2014;9:e109478.
66. Ogino S, Nosho K, Kirkner GJ, et al. A cohort study of tumoral LINE-1 hypomethylation and prognosis in colon cancer. J Natl Cancer Inst 2008;100:1734-8.
67. Benard A, van de Velde CJ, Lessard L, et al. Epigenetic status of LINE-1 predicts clinical outcome in early-stage rectal cancer. Br J Cancer 2013;109:3073-83.
68. Bosset JF, Calais G, Mineur L, et al. Fluorouracil-based adjuvant chemotherapy after preoperative chemoradiotherapy in rectal cancer: long-term results of the EORTC 22921 randomised study. Lancet Oncol 2014;15:184-90.
69. Glynne-Jones R, Counsell N, Quirke P, et al. Chronicle: results of a randomised phase III trial in locally advanced rectal cancer after neoadjuvant chemoradiation randomising postoperative adjuvant capecitabine plus oxaliplatin (XELOX) versus control. Ann Oncol 2014;25:1356-62.
70. Sainato A, Cernusco Luna Nunzia V, Valentini V, et al. No benefit of adjuvant Fluorouracil Leucovorin chemotherapy after neoadjuvant chemoradiotherapy in locally advanced cancer of the rectum (LARC): Long term results of a randomized trial (I-CNR-RT). Radiother Oncol 2014;113:223-9.