Cancer cell metabolism and colorectal cancer survival

CHAPTER 8

Impact

Colorectal cancer (CRC) is the world's third most common cancer, with more than 1.9 million newly diagnosed patients in 2020[1]. The global incidence of CRC has more than doubled over the past 30 years[2]. Its incidence is expected to increase even further in the coming years to 3.2 million new CRC cases in 2040[3], as more and more countries adopt a so-called "western" lifestyle (e.g., high consumption of animal fats, processed meats, low physical activity)[4, 5]. Even though screening and treatment has improved significantly over the past few decades[6, 7], CRC is still the second most deadly cancer worldwide, accounting for more than 900,000 deaths in 2020[1]. In addition, CRC places a significant economic burden on populations and healthcare systems[8], estimated to be around €19.1 billion in Europe in 2015[9].

Disease stage at diagnosis, as assessed by the American Joint Committee on Cancer (AJCC)[10] and the Union for International Cancer Control (UICC)[11] tumor-node-metastasis (TNM) staging system, remains the most important determinant of prognosis and guides clinical management in patients with CRC[12]. In the Netherlands, five-year survival for TNM stage I is 95% and drops to only 12% for TNM stage IV CRC patients[13]. However, the survival of individual CRC patients diagnosed with the same disease stage may differ substantially, often due to the heterogeneous nature of the disease[12]. Furthermore, it has been described that under-treatment or over-treatment of some patient groups may arise when using the TNM staging system for treatment allocation[14]. Hence, there remains an urgent clinical need to identify novel prognostic and/or predictive biomarkers in CRC[15]. However, despite the great interest and immense amount of research invested in the development of additional prognostic and/or predictive markers[12, 16-20], only few markers have been implemented in clinical practice to date[12, 21].

Metabolic reprogramming is a recognized hallmark of cancer cells[22-24]. The most commonly known metabolic abnormality in cancer cells is the so-called Warburg-effect (named after Dr. Otto H. Warburg, who was the first to describe the altered metabolism of cancer cells in the 1920s[25]), a phenomenon characterized by increased glycolysis even in the presence of oxygen[24]. It has been proposed that the Warburg-effect promotes the malignant potential of cancer cells[26], thereby potentially affecting patient prognosis and response to therapy[27-34]. However, to date, the evidence is very limited and results are inconsistent[27]. This inconsistency in results may be explained by the fact that previous prognostic studies mostly focused on investigating a single protein involved in the Warburg-effect, while this pathway is much more complicated. The predictive value of the Warburg-effect has mostly been investigated in *in vitro* cell culture studies[29]. To the best of our knowledge, only one study to date has investigated the association between response to 5-fluorouracil (5-FU) therapy and the expression of proteins related to metabolism in human tissue samples[34]. However, this was investigated in a (retrospective) case-control design, which is prone to various biases (e.g., selection bias, confounding bias).

Targeting the Warburg-effect has become a major area of focus in the development of new anti-cancer drugs[35], as inhibition of the Warburg-effect may reduce tumor cell proliferation and metastasis[36]. Various inhibitors of glycolytic enzymes and transporters (e.g., GLUT, PKM2, LDHA, MCT1) are currently in (pre)clinical development[37, 38]. Unfortunately, there has been little clinical success to date[37, 38]. It has been proposed that this lack of clinical success may result from the limited knowledge on the metabolic pathways involved in CRC, as no metabolic profiling is currently performed before initiation of therapy[38]. Even though the Warburg-effect is a common phenomenon observed a variety of cancers, it is not a universal trait of all tumor cells[38, 39].

The principal aim of this thesis was to examine whether Warburg-subtyping, based on the estimated presence of the Warburg-effect in cancer cells, has prognostic value and can be used to predict survival benefit from adjuvant therapy in CRC. Our research provides evidence that Warburg-subtyping may have prognostic value in CRC, independent of known prognostic factors such as TNM stage (**Chapter 3**)[40]. Furthermore, our research indicates that of all subgroups based on molecular characteristics that have been associated with the presence of the Warburg-effect (mutations in *RAS, BRAF, PIK3CA,* and *MET*, as well as MMR deficiency), especially *BRAF*-mutated proficient (p)MMR CRC, *KRAS*-mutated pMMR CRC, and deficient (d)MMR CRCs were related to the Warburg-high subtype in our patient series (**Chapter 3-4**). In addition, our results suggest that Warburg-subtyping may provide additional prognostic information in patient subgroups with *KRAS*-mutated pMMR CRC or *BRAF*-mutated pMMR or dMMR CRC (**Chapter 4**)[41]. Furthermore, our research indicates that associations between adult BMI, weight change since age 20 years, energy restriction during childhood and adolescence, and potentially adult-attained height and survival in CRC differ according to Warburg-subtype (**Chapter 5**). Lastly, our research provides evidence that Warburg-subtyping may predict survival benefit from adjuvant (chemo)therapy in CRC patients (**Chapter 6**).

As we were the first to investigate the potential prognostic and/or predictive value of Warburg-subtyping in a large population-based series of CRC patients, validation of the current findings in other cohort studies is required. Nevertheless, our results indicate that Warburg-subtyping may have prognostic value and may be used to predict survival benefit from adjuvant (chemo)therapy in CRC, independent of known clinical factors such as TNM stage. Although caution is warranted in drawing conclusions based on the results presented in this thesis alone, our results are promising and indicate that Warburg-subtyping may in the future be used for risk stratification of CRC patients, and the design and tailoring of Warburg-targeted therapies. This may, in the future, potentially improve the survival of CRC patients.

Next to the potential future clinical impact of our Warburg-subtyping, our research also impacts academic endeavors. As mentioned before, many previous studies have investigated the prognostic value of the Warburg-effect in CRC using one (or multiple) immunohistochemistry (IHC) markers, showing conflicting results. In addition, the predictive value of the Warburg-effect in tumor cells has, to the best of our knowledge, mostly been investigated in *in vitro* cell culture studies[29], with the exception of one (retrospective) case-control study[34]. In this thesis, we have developed and described a transparent and comprehensive methodology for Warburg-subtyping on formalin-fixed paraffin-embedded (FFPE) tissue samples of CRC patients. Using a pathway-based sum score, based on the IHC expression of six glycolytic proteins and transcriptional regulators involved in different steps of the Warburg-effect pathway, we attempted to capture the presence of the Warburg-effect in tumor cells in a large population-based series of CRC patients (n = 2,347). Furthermore, we have attempted to increase the reproducibility of the current results as well as to enhance the applicability of our methodology in future research by (i) optimizing and describing all used IHC staining and scoring protocols (**Chapter 2-3**), (ii) evaluating and describing the validity and reproducibility of IHC scoring results (**Chapter 2**), (iii) describing how to combine the IHC scores on core-level to patient-level scores (**Chapter 3-6**), and (iv) by developing and describing a comprehensive and transparent way of Warburg-subtyping based on a pathway-based sum score and pre-defined cut-off values. (**Chapter 3**).

Knowledge transfer

To ensure knowledge transfer, the scientific research summarized in this thesis has been, or will be, shared with fellow researchers through publication in open access, international peer-reviewed scientific journals. In addition, our results were presented at various national and international conferences and symposia covering a broad audience consisting of both pathologists and epidemiologists. Presentations were given at the virtual annual meeting of the American Association of Cancer Research (2021), the online Dutch Epidemiological Conference (WEON) (2021), the virtual conference of the Pathological Society of Great Britain and Ireland (PathSoc, Manchester Pathology) (2021), the Science Day of the Maastricht University Medical Centre+ (2021), and the online GROW Science Day of Maastricht University (2021).

Conclusion

In this thesis, we aimed to investigate whether Warburg-subtyping, based on the estimated presence of the Warburg-effect in cancer cells, has prognostic value and can be used to predict survival benefit from adjuvant therapy in CRC. Altogether, the results presented in this thesis suggest that Warburg-subtyping has prognostic value in CRC, independent of known prognostic factors such as TNM stage, and may be used to predict survival benefit from adjuvant (chemo)therapy. As we were the first to investigate this in a large

population-based series of CRC patients, validation of the results presented in this thesis is necessary. Nevertheless, our results are promising and may suggest that Warburg-subtyping could in the future be used for risk stratification of CRC patients and the design and tailoring of Warburg-targeted therapies, thereby potentially (eventually) improving the survival of CRC patients.

REFERENCES

1. Ferlay J, Ervik M, Lam F, et al. Global Cancer Observatory: Cancer Today. Volume 2021: Lyon, France: International Agency for Research on Cancer, 2020.
2. Sharma R, Abbasi-Kangevari M, Abd-Rabu R, et al. Global, regional, and national burden of colorectal cancer and its risk factors, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. The Lancet Gastroenterology & Hepatology 2022.
3. Morgan E, Arnold M, Gini A, et al. Global burden of colorectal cancer in 2020 and 2040: incidence and mortality estimates from GLOBOCAN. Gut 2022.
4. Dekker E, Tanis PJ, Vleugels JL, et al. Pure-AMC. Lancet 2019;394:1467-80.
5. Sawicki T, Ruszkowska M, Danielewicz A, et al. A review of colorectal cancer in terms of epidemiology, risk factors, development, symptoms and diagnosis. Cancers 2021;13:2025.
6. Araghi M, Soerjomataram I, Jenkins M, et al. Global trends in colorectal cancer mortality: projections to the year 2035. International journal of cancer 2019;144:2992-3000.
7. Schreuders EH, Ruco A, Rabeneck L, et al. Colorectal cancer screening: a global overview of existing programmes. Gut 2015;64:1637-1649.
8. Bhimani N, Wong GY, Molloy C, et al. Cost of colorectal cancer by treatment type from different health economic perspectives: A systematic review. European Journal of Surgical Oncology 2022.
9. Henderson RH, French D, Maughan T, et al. The economic burden of colorectal cancer across Europe: a population-based cost-of-illness study. The Lancet Gastroenterology & Hepatology 2021;6:709-722.
10. Edition S, Edge S, Byrd D. AJCC cancer staging manual. AJCC cancer staging manual 2017.
11. UfIC C. UICC and the TNM Classification of Malignant Tumours. 2021.
12. Marks K, West N, Morris E, et al. Clinicopathological, genomic and immunological factors in colorectal cancer prognosis. Journal of British Surgery 2018;105:e99-e109.
13. (IKNL) IkN. NKR Cijfers. Volume 2022: IKNL, 2022.
14. Reimers MS, Zeestraten EC, Kuppen PJ, et al. Biomarkers in precision therapy in colorectal cancer. Gastroenterology report 2013;1:166-183.
15. Lee JK, Chan AT. Molecular prognostic and predictive markers in colorectal cancer: current status. Current colorectal cancer reports 2011;7:136-144.
16. Lugli A, Kirsch R, Ajioka Y, et al. Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016. Modern pathology 2017;30:1299-1311.
17. Fridman WH, Sautès-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. Nature Reviews Cancer 2012;12:298-306.
18. Mesker WE, Junggeburt J, Szuhai K, et al. The carcinoma–stromal ratio of colon carcinoma is an independent factor for survival compared to lymph node status and tumor stage. Analytical Cellular Pathology 2007;29:387-398.
19. Li MX, Liu XM, Zhang XF, et al. Prognostic role of neutrophil-to-lymphocyte ratio in colorectal cancer: A systematic review and meta-analysis. International journal of cancer 2014;134:2403-2413.
20. Guinney J, Dienstmann R, Wang X, et al. The consensus molecular subtypes of colorectal cancer. Nature medicine 2015;21:1350-1356.

21. Specialisten FM. Richtlijnendatabase. URL: https://richtlijnendatabase.nl/richtlijn/colorectaal_carcinoom_crc/gemetastaseerd_colorectaalcarcinoom_crc.html 2020.
22. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. cell 2011;144:646-674.
23. Hanahan D. Hallmarks of cancer: new dimensions. Cancer discovery 2022;12:31-46.
24. Wu W, Zhao S. Metabolic changes in cancer: beyond the Warburg effect. Acta biochimica et biophysica Sinica 2013;45:18-26.
25. Warburg O. The metabolism of carcinoma cells. The Journal of Cancer Research 1925;9:148-163.
26. Lu J, Tan M, Cai Q. The Warburg effect in tumor progression: mitochondrial oxidative metabolism as an anti-metastasis mechanism. Cancer letters 2015;356:156-164.
27. Yu M, Chen S, Hong W, et al. Prognostic role of glycolysis for cancer outcome: evidence from 86 studies. J Cancer Res Clin Oncol 2019;145:967-999.
28. Dong S, Liang S, Cheng Z, et al. ROS/PI3K/Akt and Wnt/β-catenin signalings activate HIF-1α-induced metabolic reprogramming to impart 5-fluorouracil resistance in colorectal cancer. Journal of Experimental & Clinical Cancer Research 2022;41:1-27.
29. Liu C, Jin Y, Fan Z. The Mechanism of Warburg Effect-Induced Chemoresistance in Cancer. Frontiers in oncology 2021:3408.
30. Zaal EA, Berkers CR. The influence of metabolism on drug response in cancer. Frontiers in oncology 2018;8:500.
31. Desbats MA, Giacomini I, Prayer-Galetti T, et al. Metabolic plasticity in chemotherapy resistance. Frontiers in oncology 2020;10:281.
32. Zhong J-T, Zhou S-H. Warburg effect, hexokinase-II, and radioresistance of laryngeal carcinoma. Oncotarget 2017;8:14133.
33. Morandi A, Indraccolo S. Linking metabolic reprogramming to therapy resistance in cancer. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer 2017;1868:1-6.
34. Kitazawa M, Hatta T, Sasaki Y, et al. Promotion of the Warburg effect is associated with poor benefit from adjuvant chemotherapy in colorectal cancer. Cancer science 2020;111:658.
35. Li Y, Wang Y, Liu Z, et al. Atractylenolide I Induces Apoptosis and Suppresses Glycolysis by Blocking the JAK2/STAT3 Signaling Pathway in Colorectal Cancer Cells. Front Pharmacol 2020;11:273.
36. Nenkov M, Ma Y, Gassler N, et al. Metabolic Reprogramming of Colorectal Cancer Cells and the Microenvironment: Implication for Therapy. Int J Mol Sci 2021;22.
37. Le A. The Heterogeneity of Cancer Metabolism: Springer Nature, 2021.
38. Vanhove K, Graulus G-J, Mesotten L, et al. The metabolic landscape of lung cancer: new insights in a disturbed glucose metabolism. Frontiers in oncology 2019;9:1215.
39. Correa PN, Correa AN. A unitarian biochemical and bioenergetic theory of adaptive oncogenesis: from hypoxia and energy starvation (aerobic and ambipolar) to the roles of HIF-1, IGF-I, and vitamins C and D. Journal of Biophysics, Hematology and Oncology 2010;1:1-93.
40. Offermans K, Jenniskens JC, Simons CC, et al. Expression of proteins associated with the Warburg-effect and survival in colorectal cancer. The Journal of Pathology: Clinical Research 2021.
41. Offermans K, Jenniskens JC, Simons CC, et al. Association between mutational subgroups, Warburg-subtypes, and survival in patients with colorectal cancer. Cancer Medicine 2022.