Energy balance and colorectal cancer risk

SUMMARY

Energy balance-related factors have been associated with risk of colorectal cancer. Even though the mechanism behind these associations is currently unknown, Warburg-effect activation via PI3K/Akt signaling is one of the proposed mechanisms. The Warburg-effect is a metabolic phenotype characterized by increased aerobic glycolysis and is considered an important step in carcinogenesis.

In this thesis, we investigated potential involvement of the Warburg-effect in the etiological pathway between energy balance and colorectal cancer risk using a molecular pathological epidemiology (MPE) approach. We investigated associations of energy balance-related factors in adulthood (i.e. body mass index [BMI]; lower body clothing size, as a proxy for waist circumference; physical activity) and early in life (i.e. height; energy restriction proxies of exposure to the Dutch Hunger Winter, World War II, and the Dutch Economic Depression; BMI at age 20 years) with colorectal cancer risk in relation to the estimated presence of the Warburg-effect in the tumor. The presence of the Warburg-effect was estimated by establishing Warburg-subtypes based on the immunohistochemical (IHC) expression of six proteins involved in different levels of the Warburg-effect (PTEN, P53, GLUT1, PKM2, LDHA, MCT4). Furthermore, we investigated subgroups of colorectal cancer based on mutations in oncogenes that have been associated with the upstream regulation of the Warburg-effect (*KRAS*, *PIK3CA*, and *BRAF* mutations) as well as mismatch repair (MMR) status, hereafter referred to as subgroups of molecular features. In addition, we aimed to investigate whether non-pathologists can generate valid and reproducible IHC scoring results.

All studies presented in this thesis were conducted using data from the Netherlands Cohort Study (NLCS). The NLCS is a large prospective cohort study that was initiated in 1986, and included 120,852 subjects aged 55-69 years at baseline. Information on energy balance-related factors and other cancer risk factors were collected through a mailed, self-administered questionnaire at baseline. A case-cohort approach was used, in which cases were derived from the entire cohort, whereas person-years at risk for the entire cohort were estimated from a subcohort (n=5,000) randomly sampled at baseline. Cancer cases from the total cohort were identified via record linkage with the Netherlands Cancer Registry and the Dutch Pathology Registry, PALGA, covering 20.3 years of follow-up for the current thesis. After excluding cases and subcohort members with a history of cancer (except skin cancer) at baseline, a total of 4,597 incident colorectal cancer cases and 4,774 subcohort members were available.

For the Rainbow-TMA project (2012-2017), formalin-fixed paraffin-embedded (FFPE) tissue blocks from primary tumor and matched normal tissue were requested from 3,872 incident colorectal cancer cases. From these blocks, three tumor cores per case were sampled and combined into tissue microarrays (TMAs). In total, tumor tissue of 2,694 colorectal cancer cases was successfully assembled in 78 TMAs. For the Warburg-subtypes, TMAs were subjected to IHC in order to establish expression levels of six proteins involved in the Warburg-effect (PTEN, P53, GLUT1, PKM2, LDHA, MCT4).

All IHC stained TMAs were scored by three non-pathologist assessors and a random 10% was additionally scored by an experienced pathologist. The expression levels were combined into a pathway-based sum score and categorized into three Warburg-subtypes (Warburg-low, -moderate, -high). For subgroups of molecular features, two slices were cut from FFPE tissue blocks containing primary tumor. DNA was isolated from these tissue slices. Then, tumor DNA was screened for *KRAS*, *PIK3CA*, and *BRAF* mutations. In addition, MMR status was assessed using IHC staining of MLH1 and MSH2 on TMAs. These molecular features were investigated individually (*KRAS* mutations [$KRAS_{mut}$]; $PIK3CA_{mut}$; $BRAF_{mut}$; MMR deficiency [dMMR]) as well as combined (all-wild-type+MMR-proficient [pMMR]; any-mutation/dMMR).

After exclusion of cases and subcohort members with incomplete covariate data, 3,911 subcohort members were available for analyses, 1,972 colorectal cancer cases with complete IHC expression data for Warburg-subtypes (Chapters 3 and 4), and 1,934 cases with complete data on molecular features (Chapters 5 and 6). Multivariable Cox regression analyses were used to estimate associations of energy balance-related factors with Warburg-subtypes and with subgroups of molecular features in colorectal cancer.

In Chapter 2, we investigated whether non-pathologists can generate valid and reproducible IHC scoring results. This was done by assessing interobserver agreement between trained non-pathologists and an experienced pathologist and by assessing intraobserver agreement within non-pathologists. We found that trained non-pathologists can generate reproducible IHC scoring results that are similar to those of an experienced pathologist. Combining the scores of at least two non-pathologist assessors yielded optimal results.

In Chapter 3, we investigated associations of adult energy balance-related factors with Warburg-subtypes in colorectal cancer. We found that measures of adiposity (i.e. BMI and clothing size) were associated with an increased risk of Warburg-moderate and Warburg-high colon cancer in men, and with Warburg-low and Warburg-high colon cancer in women. Furthermore, we observed that measures of physical activity were mainly associated with a decreased risk of Warburg-low and Warburg-moderate colon cancer, both in men and women.

In Chapter 4, we investigated associations of early-life energy balance-related factors with Warburg-subtypes in colorectal cancer. We found that height was positively associated with colon cancer in men, regardless of Warburg-subtypes, and with Warburg-low colon and Warburg-low and -moderate rectal cancer in women. We did not observe clear patterns across associations of early-life energy restriction proxies with Warburg-subtypes. A high adolescent BMI was associated with an increased risk of Warburg-high colon cancer in men, and Warburg-moderate rectal cancer in women.

In Chapter 5, we investigated associations of adult energy balance-related factors with risk of colorectal cancer subgroups of molecular features. We found that adiposity

measures in women were only associated with an increased risk of $KRAS_{mut}$ colon cancer, whereas in men they were associated with all subgroups of molecular features. Furthermore, we observed that non-occupational physical activity was associated with any-mutation/dMMR colon cancer in both men and women, but not with all-wild-type+pMMR colon cancer.

In Chapter 6, we investigated associations of early-life energy balance-related factors with risk of colorectal cancer subgroups of molecular features. We found that height was associated with an increased risk of any-mutation/dMMR, but not all-wild-type+pMMR, colorectal cancer, with the exception of men with rectal cancer. Again, results on early-life energy restriction proxies in relation to risk of subgroups based on molecular features did not show clear patterns. A high adolescent BMI in men seemed to be mainly associated with an increased risk of $KRAS_{mut}$ colon cancer, and in women with $BRAF_{mut}$ and dMMR colon cancer.

Chapter 7 concludes this thesis with a summary of the main findings, interpretation of study results, a discussion of methodological considerations, and recommendations for future research. Overall, the results presented in this thesis seem to indicate a role for the Warburg-effect in the etiological pathway between adiposity and colon cancer, but not rectal cancer, both for adolescent and adult adiposity. A role for the Warburg-effect is not indicated by our results for associations of physical activity or adult-attained height with colorectal cancer risk. We did not observe clear patterns for the three energy restriction proxies and therefore do not draw a conclusion regarding early-life energy restriction. Since we were the first to investigate associations of energy balance-related factors with risk of colorectal cancer in relation to the estimated presence of the Warburg-effect in the tumor, confirmation in additional large prospective MPE studies is necessary.