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Relevance of estrogen-receptor beta for risk and prognosis of colorectal cancer

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Colorectal cancer is the third most common cancer type worldwide. Incidence rates for colorectal cancer are higher in men than in women, and evidence from observational and experimental studies suggests that this difference could be due to differential exposure to sex-hormones, especially to estrogen. This hypothesis is supported by observational studies reporting that postmenopausal women using menopausal hormone therapy have a reduced risk for colorectal cancer. In contrast to this, studies examining endogenous estrogen exposure using reproductive factors and colorectal cancer risk reported inconsistent results. Estrogen exerts its effects on colon cells predominantly through the nuclear receptor estrogen-receptor β, the primarily expressed estrogen-receptor in the colon, which has mainly anti-proliferative and apoptotic effects. The expression of estrogen-receptor β decreases during carcinogenesis, and patients with estrogen-receptor β positive tumors have better survival rates compared to patients with estrogen-receptor β negative tumors. Mechanisms underlying the relationship between estrogen-receptor β expression and prognosis of colorectal cancer are currently not well understood. To shed light on the role of estrogen and estrogen-receptor β in colorectal cancer development, this thesis focused on the following aspects:

- Firstly, a Mendelian randomization analysis was used to test for a causal effect of age at menarche and age at menopause as surrogates for endogenous estrogen exposure on colorectal cancer risk.
- Secondly, DNA methylation was investigated as a possible mechanism behind colorectal cancer risk reduction associated with menopausal hormone therapy as a source of exogenous estrogen exposure. So the aim was to identify genes showing differential methylation according to use of menopausal hormone therapy before colorectal cancer diagnosis and to explore whether the effect is modified by estrogen-receptor β expression status.
- Thirdly, DNA methylation was explored as possible mechanisms explaining reported differences in survival between estrogen-receptor β positive and estrogen-receptor β negative tumors. Therefore, it was investigated whether estrogen-receptor β -expression status in the colorectal tumor is associated with differences in DNA-methylation of the estrogen-receptor β promotor region and it was aimed to identify genes showing differential methylation according to estrogen-receptor β expression status using whole-genome DNA-methylation profiles.

For the Mendelian randomization analysis, the study population consisted of 12,944 women diagnosed with colorectal cancer and 10,741 women without colorectal cancer from three large consortia: the GECCO Consortium (Genetics and Epidemiology of Colorectal Cancer Consortium), CORECT (Colorectal cancer transdisciplinary study) and CCFR (Colon Cancer Family Registry). Weighted genetic risk scores based on 358 single nucleotide polymorphisms associated with age at menarche

and 51 single nucleotide polymorphisms associated with age at menopause were used to assess the association with colorectal cancer using logistic regression. Sensitivity analyses were conducted to address pleiotropy using summery statistics methods (weighted median estimator and Mendelian randomization-Egger regression) and to account for possible confounding by body mass index.
To investigate differences in DNA-methylation according to estrogen-receptor β expression, two independent tumor sample sets of colorectal cancer patients recruited in 2003-2010 to the German DACHS study (Darmkrebs: Chancen der Verhütung durch Screening) were used (discovery cohort n=917, replication cohort n=907). To explore whether methylation is associated to use of menopausal hormone therapy, data of postmenopausal female DACHS-patients, recruited between 2003-2010, was used (discovery cohort n=379, replication cohort n=413). Epigenome-wide DNA-methylation profiling was done using Illumina HumanMethylation450k BeadChip arrays. Estrogen-receptor β expression was measured using immunohistochemistry. Use of menopausal hormone therapy was self-reported. Differentially methylated sites and regions were determined using limma in the R-package RnBeads.

In the Mendelian randomization study, genetically predicted yearly increment in age at menarche (odds ratio 0.98, 95% confidence interval: 0.95, 1.02) and in age at menopause (odds ratio 0.98, 95% confidence interval: 0.95, 1.01) were both not associated with colorectal cancer risk. The sensitivity analysis accounting for confounding by body mass index and for horizontal pleiotropy yielded similar results. The analysis of differential methylation according to estrogen-receptor β expression yielded 1220 differentially methylated sites (adjusted p-value <0.05) comparing tumors with negative expression (score 0) with tumors of positive expression (score 1 and 2 combined). None of those sites was found significant in the replication set. Comparing tumors of negative expression (score 0) of estrogen-receptor β with tumors of high expression (score 2), 2904 CpGs have been found significant in the discovery analysis of which 403 could be replicated. In the analysis of menopausal hormone therapy use and differential methylation, one CpG site was associated to current use and one with ever use of menopausal hormone therapy in the discovery analysis, both of which could not be replicated. In a combined dataset, stratified analyses found 342 differentially methylated CpGs in current hormone users with estrogen-receptor β positive tumors and no differences in the other strata. A dataset for replication was not available.

For endogenous estrogen exposure, this thesis does not support a causal relationship between genetically predicted age at menarche, age at menopause and colorectal cancer risk. Methylation analyses according to estrogen-receptor β expression status detected genes formerly reported to be involved in carcinogenesis like CD36, HK1 or LRP5. These genes need to be further investigated using mechanistic studies. So, DNA methylation seems to be involved in the mechanisms explaining differences in survival associated with estrogen-receptor β expression. No robust methylation differences were found among ever/current and never users of menopausal hormone therapy. Methylation changes found in the discovery analysis influence genes (like MIR25, PTPRF, CtBP1) in a way supporting the risk reducing effect of hormone therapy on colorectal cancer risk. Stratified analyses point towards current MHT use having an influence on DNA methylation only in estrogen-receptor β positive tumors. Differentially methylated genes and the role of estrogen-receptor β expression need to be investigated in further studies.