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Mediation of sex steroid effects on colorectal cancer risk and survival: Influence of genetic variation and estrogen receptor beta expression

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An increasing body of evidence points to an involvement of sex steroids in the development and progression of colorectal cancer (CRC) and the use of menopausal hormone therapy (MHT) has been associated with a lower risk for CRC. Variants in genes related to sex hormone metabolism, transport and signaling potentially influence genetic susceptibility towards CRC. CRC risk associated with the use of MHT could also be modified by genetic polymorphisms in hormone related genes. The estrogen receptor beta (ERβ) is the primarily expressed estrogen receptor in the large intestine and might partly mediate the preventive effects of estrogens. Loss of ERβ expression in neoplastic colorectal tissue has been associated with advanced cancer stages and poorer differentiation. It has not yet been studied whether the ERβ status of CRC tumors modifies the association between the use of MHT and CRC risk and it is unclear if the expression of ERβ in colorectal tumors is associated with CRC survival.

The overall objective aims of the present thesis were:

- To investigate the association of polymorphisms in hormone related genes with the risk for CRC, separately in men and women.
- To explore the potential genetic modification of CRC risk associated with MHT use.
- To assess whether the ERβ status of tumors modifies CRC risk associated with MHT use.
- To evaluate the association between the expression of ERβ and tumor characteristics.
- To investigate the prognostic value of tumoral ERβ expression.

The thesis is based on a population-based case-control study on CRC (DACHS). Overall 51 variants in 19 candidate genes were selected, including single nucleotide polymorphisms (SNPs), copy number variations and microsatellite polymorphisms. They were genotyped in 1,798 cases (746 women and 1,052 men) and 1,810 controls (732 women and 1,078 men). Comprehensive information on CRC risk and preventive factors as well as on MHT use was collected by face-to-face interviews. Cases were followed up and data on cancer treatment, new concomitant diagnoses and cancer recurrences was collected. Information on the vital status and date of death was obtained from population registries and the cause of death was verified by death certificates. ERβ expression was measured in samples from 1,262 cases via immunohistochemistry and scored independently by two pathologists. Multivariate logistic regression models were used to assess odds ratios (ORs) and 95% confidence intervals (CIs) for CRC risk associated with genetic polymorphisms. Effect modification of MHT use by genetic polymorphisms was evaluated among 685 postmenopausal female cases and 684 corresponding controls by using multiplicative interaction terms and log likelihood ratio tests. Multinomial logistic regression was employed to investigate CRC risk specific for the tumor

localization as well as ERβ status. The association with overall survival (OS) as well as disease specific survival (DSS) was assessed in 1,143 cases using multivariate Cox proportional hazard models to calculate hazard ratios (HRs).

Significant allele dose-response associations were observed with the SNPs rs1255998 and rs928554 in *ESR2*, rs605059 in *HSD17B1*, and rs2229109 in *ABCB1* in women (p trend = 0.004, 0.05, 0.03 and 0.05, respectively) and with rs1045642 and rs9282564 in *ABCB1*, and rs6259 in *SHBG* in men (p trend = 0.01, 0.03, and 0.02, respectively). Also, the CA(n) repeat polymorphism in the *ESR2* gene was associated with CRC risk in men (p trend = 0.01). The G allele of rs1255998 in *ESR2* showed the most significant association with risk for CRC in women, with a per-allele OR of 0.68 (95% CI 0.52 - 0.88). This finding was replicated in an independent study from North Germany (OR = 0.84, 95% CI 0.71 - 1.04), yielding a per-allele OR of 0.80 (95% CI 0.69 - 0.93, p trend = 0.003) in the pooled sample.

CRC risk associated with ever MHT use as well as with duration was significantly modified by rs1202168 in *ABCB1* (p interaction = 0.04). The MHT associated risk reduction was not significant in homozygous non-carriers (OR ever use = 0.84, 95% CI 0.53 - 1.34; OR per 5 year duration = 0.94, 95% CI 0.83 - 1.08), while homozygous carriers of the minor T allele had a 57% lower risk with ever use of MHT and a 22% lower risk per 5 years of MHT use. Significant effect modification was also observed for rs910416 in *ESR1* (p interaction = 0.03 for ever use and 0.07 for duration of use), whereby the decreased risk was attenuated in homozygous carriers of the minor C allele.

The association of current MHT use as well as duration of use with a reduced risk for CRC was stronger for ERβ positive disease than for ERβ negative disease. The opposite was observed for ever use of estrogen monotherapy. However, the p value for effect heterogeneity was not significant in any case. Compared to a high ERβ expression, lack of ERβ was significantly associated with advanced cancer stages and larger tumor extent. In multivariate analysis, ERβ negativity was associated with an increased HR for death (HR = 1.61, p = 0.02) as well as death attributed to CRC (HR = 1.54, p = 0.06). The associations were even stronger when restricting to patients with stage I-III disease (overall survival HR = 2.20, $p < 0.01$, disease specific survival HR = 2.38, p = 0.02, respectively).

In conclusion, these findings implicate a role of *ESR2* in the risk for developing CRC and suggest that the *HSD17B1*, *ABCB1*, and *SHBG* genes may contribute to sex steroid-mediated effects on CRC development. The present work provided also first evidence that polymorphisms in genes related to estrogen transport and signaling may modify MHT associated CRC risk. The observed CRC risk associated with MHT by ERβ status suggests that the preventive effect of MHT is in part mediated by ERβ. Previous findings regarding the association between loss of ERβ expression and advanced cancer stages were confirmed. A new finding was that low tumoral ERβ expression is independently associated with poorer survival, which supports an important role of ERβ in the progression of CRC. Due to the clinical relevance of the findings, further independent studies are warranted.