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Functional variants in *NLRs* and *NLR* pathway-related genes in colorectal cancer risk, survival and response to treatment.

Fach: Molekulargenetische Epidemiologie
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Colorectal cancer **(**CRC) is the third most common cancer and the fourth leading cause of cancer-related death worldwide. Chronic inflammation and immune evasion are key drivers of CRC. Pattern recognition receptors (PRRs), such as Nod-like receptors (NLRs) and Toll-like receptors (TLRs), are crucial components of the intestinal innate immune system. Pathogen or endogenous damage associated molecular patterns (PAMPs or DAMPs) trigger the activation of these molecules, which in turn activate sub-sequential cell signaling pathways, including nuclear factor-kB (NF-kB), mitogen-activated protein kinases (MAPKs), and type I interferon (IFN) response. Particularly, IFN signaling pathways present anti-tumoral potential prompting strong CD8+ T cell responses, through induction of the MHC class I transactivator, NLRC5. Physiologically, an up-regulation of *PD-L1* expression is required to control the CD8+ cell expansion. However, abnormal *PD-L1* expression enables the cancer cells to evade the immune system. Furthermore, given its involvement in the IFN pathway, *NLRC5* might also exert a role in the 5-fluorouracil (5-FU)-based therapy. Elimination of myeloid-derived suppressor cells (MDSCs) is one of the consequences of the 5-FU treatment, leading to elevated IFNγ secretion with subsequent *NLRC5* expression and CD8+ T cell activation.

A case-control study in a Czech population of 1424 cases and 1114 controls was carried out to evaluate the single and synergic effects of potentially functional variants in *NLRs* and *NLR* pathway-related genes on CRC risk and a case-only study in a smaller sample set of 589 cases was performed to estimate their influence on the survival of CRC. A subset of 232 patients who received 5-FU-based therapy was used to assess the effect of *NLRC5* variants on survival of CRC patients after 5-FU-based therapy.

SNPs within *NLRs* and *NLR* pathway-related genes were selected using *in silico* bioinformatic tools such as UCSC browser, HaploReg, Regulome DB, Gtex Portal and microRNA binding site prediction tools. Genotyping was performed using allelic discrimination methods. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using logistic regression. Hazard ratios (HRs) and 95% CIs were estimated using Cox regression model. Survival curves were derived using the Kaplan-Meier (K-M) method.

A nominal association was observed between 3 SNPs and CRC risk: 2 SNPs, rs72960018 and rs9352000 mapping within *MB21D1* and a third one, rs13153461 within *TMEM173*. Given that these 2 genes encode proteins that interact with each other, we further evaluated the cumulative effect of the 3 associated SNPs, which revealed a 3-fold increased CRC risk in carriers of 5-6 risk alleles compared to those with 0-2 risk alleles (OR = 2.98, 95%CI: 1.35 –6.56).

Additionally, we reported a marginal association between rectal cancer risk and two *NLRC5* SNPs, rs1684575 and rs3751710.

To better understand the genetic etiology of CRC, we studied the interplay between the variants within *NLRC5, PD-L1* and the previously genotyped *IFNGR1* and *IFNGR2* variants. Overall we obtained 24 pair-wise interactions, of which 13 were below the threshold for the false discovery rate (FDR) calculated at an arbitrary level q*<0.10. The interaction *IFNGR2* rs1059293-*NLRC5* rs289747 (P<0.0001) survived also the stringent Bonferroni correction.

In addition, two *NLRC5* SNPs showed a significant association with the survival outcome. All patients and metastasis-free patients at the time of diagnosis (pM0) who were homozygous carriers of the minor allele of rs27194 had a decreased overall survival (OS) as well as event-free survival (EFS) under recessive model (OS_{all}: HR 1.92, 95% CI 1.25-2.93 and OS_{pM0}: HR 2.31, 95% CI 1.30-4.13, EFS_{pM0}: HR 2.04, 95% CI 1.17-3.54, respectively); overall survival was also decreased for all patients and for patients with no distant metastasis at the time of diagnosis who carried at least one minor allele of rs289747 (HR 1.32, 95% CI 1.02-1.70 and HR 1.73, 95% CI 1.21-2.47, respectively). Additionally, when we tested the survival in the smaller set of CRC patients, who underwent 5-FU-based therapy, one polymorphism, *NLRC5* rs12445252, presented association with OS_{all}, OS_{pM0} and EFS_{pM0}, according to the dosage of the minor allele T (HR: 1.99, 95% CI: 1.36-2.92, HR: 2.37, 95% CI: 1.55-3.63, HR: 1.64, 95% CI: 1.14-2.35, respectively), supported by the Kaplan-Meier analysis.

Our data suggest that epistatic interactions and high number of risk alleles may play important roles in the onset of CRC, deviating from additivity in their effect and generating differences among individuals, thus offering novel biological hints. Additionally, survival analyses showed that few SNPs within *NLRC5* significantly associated with the survival outcome of all CRC patients and one SNP specifically in CRC patients who underwent 5-FU-based chemotherapy, suggesting that these SNPs may serve as candidate prognostic markers of clinical outcome of CRC.