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Inherited variability in a master regulator polymorphism (rs4846126) associates with survival in 5-FU treated colorectal cancer patients

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ABSTRACT

Background: Treatment with 5-fluorouracil (5-FU) is known to improve survival in many cancers including colorectal cancer. Response to the treatment, overall survival and recurrence show inter-individual variation.
Methods: In this study we employed a strategy to search eQTL variants influencing the expression of a large number of genes. We identified four single nucleotide polymorphisms, defined as master regulators of transcription, and genotyped them in a set of 218 colorectal cancer patients undergoing adjuvant 5-FU based therapy.
Results: Our results showed that the minor allele variant of the rs4846126 polymorphism was associated with poor overall and progression-free survival. Patients that were homozygous for the variant allele showed an over two fold increased risk of death (HR 2.20 95%CI 1.05–4.60) and progression (HR 2.88, 95% 1.47–5.63). The integration of external information from publicly available gene expression repositories suggested that the rs4846126 polymorphism deserves further investigation. This variant potentially regulates the gene expression of 273 genes with some of them possibly associated to the patient's response to 5-FU treatment or colorectal cancer.
Conclusions: Present results show that mining of public data repositories in combination with own data can be a fruitful approach to identify markers that affect therapy outcome. In particular, a genetic screen of master regulators may help in order to search for the polymorphisms involved in treatment response in cancer patients.

1. Introduction

Colorectal cancer (CRC) remains a major health burden with particularly high incidence reported in the Western countries. Worldwide, it is the second most frequent diagnosed cancer with an estimated 1.2 million new cases reported in 2008 [1]. Despite, major strides made in the treatment, the disease remains associated with a high rate of mortality. The malignant disease of colon and rectum is the second leading cause of cancer-related deaths in the developed world with an estimated 680,000 deaths recorded in 2008 [1].

Treatment with 5-fluorouracil (5-FU), a drug developed in 1957, is known to improve survival in various cancers, with largest impact reported in CRC. Though surgery remains the major treatment in localized CRC, in node positive (stage III) disease, administration of adjuvant 5-FU reduces the risk of death by 30% [2,3]. The toxicity associated with 5-FU is ascribed to incorporation of fluoronucleotides in RNA and DNA and to the inhibition of enzyme thymidylate synthase. Overall response rate to 5-FU in advanced

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CRC is limited to 10–15%. The combination of 5-FU with irinotecan and oxaliplatin has been reported to improve survival rates considerably [4,5].

Response to 5-FU treatment, overall survival and related toxicity are crucially associated with large inter-individual variations [6]. Previous studies have shown survival differences in CRC patients treated with 5-FU depending on individual genotypes in the gene encoding methylene tetrahydrofolate reductase (*MTHFR*) [4,7,8]. In order to identify further genetic variants that can influence treatment response, we employed a strategy to search expression quantitative trait loci (eQTL) variants influencing the expression of a large number of genes [9]. We hypothesized that those genetic polymorphisms that influence expression of al large number of genes will affect the drug metabolic pathways and consequently influence the treatment outcome. Within that context, we identified four single nucleotide polymorphisms (SNPs) *a priori* defined as "master regulators of transcription" (for more details see [9]) and genotyped a set of 218 patients with CRC and undergoing 5-FU chemotherapy to evaluate their role in the response to 5-FU-based treatment and survival.

2. Materials and methods

2.1. Study population

The study population comprised of 1098 CRC patients recruited between September 2003 and October 2010 in nine different oncology departments of various hospitals in the Czech Republic (3 hospitals in Prague, 1 in Benesov, Brno, Liberec, Ples, Pribram, Usti nad Labem, and Zlin, respectively). The population has been previously described and used to investigate the effect of genetic susceptibility on disease risk and response to treatment [10–13]. Seven hundreds and twenty-nine cases were followed up on August, 31st 2011. Finally, another 76 CRC cases were excluded because of incomplete clinical information. All subjects were informed and provided written consent to participate in the study and to approve the use of their biological samples for genetic analyses, according to the Helsinki declaration. The study design was approved by the Ethics Committee of the Institute of Experimental Medicine, Prague, Czech Republic.

For all subjects, clinical data at the time of diagnosis, including location of the tumour, UICC (International Union Against Cancer) tumour-node-metastasis (TNM) stage system, grade and adjuvant chemotherapy treatment were collected, as well as information about distant metastasis, relapse and date of death. Since 186 patients had incomplete clinical information, they were excluded from the analyses. Two hundred and nineteen CRC cases received a 5-FU-based adjuvant regimen as first-line postoperative therapy. The therapy consisted of either a Mayo regimen, delivered as a bolus infusion of 5-FU (425 mg/m^2) and leucovorin (10 mg/m^2) for 5 days every 4 weeks six times or a simplified DeGramond regimen which consisted of a 2 h intravenous (i.v.) infusion of leucovorin (200 mg/m^2), then a 5-FU i.v. bolus (400 mg/m^2) followed by a 46 h 5-FU continuous i.v. infusion (2400–3000 mg/m^2). Three hundred twenty-four subjects did not receive any adjuvant chemotherapy after surgery. In this study, the outcome variables measured were overall survival (OS, time from operation till death or censorship) and progression free survival (PFS, time interval from operation till progression, death or censorship).

2.2. Selection of SNPs

Studies investigating the influence of genetic variation on gene expression (until January 2012) were retrieved by using the search keywords: "gene expression variation", "eQTL study", "expression quantitative loci mapping", "master regulators of transcription", "disease-associated expression polymorphisms" in Pubmed. Selection criteria for the inclusion in the present investigation were: (a) the availability of a complete list of SNPs associated with differential expression of genes and (b) studies performed exclusively on human cell lines (Fig. 1). Reviews were excluded.

The information about the association between the SNPs retrieved from the selected publications and gene expression levels was obtained from the SCAN database (http://www.scandb.org/newinterface/about.html). The SCAN database provided the number of differentially transcribed genes in lymphoblastoid cell lines (LCL) from individuals of Caucasian origin for each SNP. An arbitrary threshold of a minimum of 40 genes regulated by a single genetic variant was used to define a "master regulator of transcription" [9].

From the selected publications, we obtained a list of 478 SNPs that were tested in the SCAN database [14]. Four SNPs could be identified as "master regulators", according to our initial criteria. The rs668413, rs754553, rs4846126 and rs10735234 polymorphisms, in fact, were reported to significantly regulate the expression of 45, 133, 273, and 54 genes, respectively (Supplementary Table S1).

Supplementary Table 1 can be found, in the online version, at http://dx.doi.org/10.1016/j.mrfmmm.2014.05.007.

2.3. SNP genotyping

Genomic DNA was isolated from peripheral blood lymphocytes, using standard procedures. Genotyping of the four selected SNPs was carried out by using allelic discrimination method (KBioscience, Hoddesdon, UK). Duplicate samples (5%) and no-template controls in each plate were used as quality control tests.

2.4. Statistical analysis

A Chi-square test with 1 degree of freedom, with a type-I error rate equal to $\alpha = 0.05$ was used to verify whether genotypes were in Hardy–Weinberg equilibrium. Chi-squared tests were also used to investigate the possible relationship between tumour characteristics at diagnosis and genotype. Survival time was measured from the date of diagnosis to two different events: death by any cause for overall survival (OS); recurrence, metastasis, or death by any cause for progression-free survival (PFS). Survival times were censored for patients who did not experience the investigated event, for example patients alive at last contact. The association between patient survival and polymorphisms was first assessed by log-rank tests and univariate survival analysis, and represented by Kaplan–Meier curves. In order to account for the influence of possible confounders, hazard ratios (HRs) and 95% confidence intervals (CIs) were also estimated relying on a multiple Cox proportional hazard regression model. Possible confounder characteristics were identified based on a stepwise model selection. Significant variables at the 10% level (score test) entered the model, and they were not removed if significant at the 10% level. Statistical analyses were conducted using R (http://www.r-project.org/) and the SAS statistical package (Version 9.1, SAS Institute Inc., Cary, NC, USA). The present observational study has an exploratory nature. No adjustment was made for multiple testing and test results surpassing a 5% confidence level were interpreted as statistically significant.

2.5. Gene set enrichment analysis

In order to identify a gene expression signature related to 5-FU chemotherapy we compared our original gene list of 273 genes coregulated by rs4846126 with another study [15]. Sixty genes in common among the two lists were obtained. The list of those significant genes were tested for over-representation using a WEB-based