
Background: Genome-wide association studies (GWAS) are designed to provide novel insights on candidate genes involved in the pathophysiology of cancer and outcome of therapy. We have previously conducted a GWAS in 294 genetic European advanced pancreatic cancer patients treated with gemcitabine in CALGB80303, a phase III randomized clinical trial (Innocenti et al., Clin Cancer Res, 2012;18:577). In the present study, we aim to identify novel genes associated with overall survival (OS) by replicating the most significant single-nucleotide polymorphisms (SNPs) for OS in CALGB80303 in a cohort of pancreatic cancer patients from the Mayo Clinic. We also aim to provide the mechanistic basis for the replicated associations.

Methods: We have selected the top 300 most statistically significant SNPs for OS in CALGB 80303, and tested their association in 408 genetic European advanced pancreatic cancer patients from the Mayo Clinic. The characteristics of the patients in CALGB 80303 are described in our previous publication. Half of the Mayo Clinic patients had locally advanced disease (and half with metastatic disease), about 30% received prior radiation, and about 60% received gemcitabine. They have been genotyped for about 550,000 SNPs using the same Illumina platform of CALGB80303, and the association with OS was tested using a Cox proportional hazard regression model from date of diagnosis to death or last-follow-up.

Results: In the Mayo Clinic patients, we selected 10 SNPs with an effect on OS concordant with CALGB80303 (p<0.05), and 4 of them where located in genes: VDR, CMYA5, C7orf58, CAMK4. Because of the supportive biology of vitamin D and cancer, here we report the results for VDR, the gene coding for the vitamin D receptor. A SNP in VDR (rs2853564) associated with OS in the Mayo Clinic patients (HR 0.82 (95 % CI 0.71-0.95), p=0.0077) and CALGB80303 (HR 0.74 (0.63-0.87), p=0.0002). Patients with 2 variant alleles of rs2853564 have longer median OS survival (10.5 and 8.9 months in the Mayo Clinic and CALGB80303 patients, respectively) than patients with 1 (8.34 and 5.9 months) or 0 (6.6 and 5.7 months) alleles. Using the replication p value as a feature selection to direct translational science, we performed mechanistic studies on VDR. Bioinformatic prediction of the functionality of VDR SNPs indicates that rs2853564 (or SNPs in high linkage disequilibrium with it) might increase VDR expression. In luciferase assays in two cell lines, we have confirmed that the minor allele of rs2853564 increases luciferase expression by 22% (p<0.05) and 37% (p<0.05) in HEK293 and PANC-1 cells, respectively. PANC-1 cells exposed to calcitriol, the hormonally active form of vitamin D, were up to 38% (p=0.0005) less viable than PANC-1 cells which had a siRNA-mediated knockdown of VDR expression.
Conclusions: Efforts of external replications of GWAS, when combined with mechanistic functional studies, are able to direct reverse translational science. In this study, VDR has been selected for further investigation in pancreatic cancer. Calcitriol has several VDR-mediated antitumor properties, and is synergic with gemcitabine in human pancreatic cancer models (Yu et al., Cell Cycle 2010;9:3022). This study proposes that VDR germline variants may modulate VDR expression, resulting in differences in the behavior of pancreatic cancer. The putative prognostic role of VDR germline SNPs should be replicated in additional cohorts of advanced pancreatic cancer patients.