A functional analysis of variants associated with therapy-induced second malignancies after Hodgkin lymphoma identified by genome-wide scan.

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Survivors of Hodgkin lymphoma (HL) are susceptible to radiation-induced second malignant neoplasms (SMNs). In a genome-wide association study (GWAS) of patients treated for HL who did or did not develop SMNs, we identified and validated two SMN-associated single nucleotide polymorphisms (SNPs) at 6q21, intergenic between PRDM1 and ATG5 [rs4946728: P = 1.04x10^{-9}, OR = 3.21 (95% CI = 2.37-6.42), and rs1040411: P = 4.24x10^{-8}, OR = 2.43 (95% CI = 1.76-3.34)]. Recently, it was demonstrated that disease-associated SNPs are more likely to be expression quantitative trait loci (eQTLs), SNPs that regulate gene expression, than are randomly chosen SNPs matched for their population allele frequencies. Indeed, we found that the 1000 SNPs most associated with SMNs are significantly enriched for eQTLs (P = 0.01). Exploring the processes regulated by SMN-associated SNPs can inform the mechanism by which SMNs result in patients treated with radiation therapy. As an initial investigation of the effect of these SNPs on gene expression, we studied the effect of the validated 6q21 haplotype (comprised of rs4946728 and rs1040411) on global gene expression in HapMap lymphoblastoid cell lines (LCLs). Gene set enrichment analysis of genes differentially expressed (log2>0.05) between cell lines carrying either the risk or protective haplotype revealed that carriage of the risk-associated haplotype was associated with increased expression of transmembrane proteins (enrichment P = 2.1x10^{-13}) and immune response proteins (enrichment P = 1.2x10^{-6}). Because the 6q21 haplotype is in close physical proximity to ATG5 and PRDM1, we investigated its functional consequence on expression of these genes. We discovered the risk-associated haplotype was significantly associated with lower levels of PRDM1 mRNA (P = 0.04) but not ATG5 mRNA. As exposure to radiation is the primary etiologic factor for SMNs, we assessed the effect of the risk haplotype on protein levels of PRDM1 and ATG5 in six LCLs (three with the risk haplotype and three with the protective haplotype) following 10Gy of gamma irradiation (IR). PRDM1 protein levels were significantly lower in LCLs carrying the risk-associated haplotype in the absence of IR. In all lines, PRDM1 levels increased following radiation exposure, but this effect was significantly attenuated in presence of the risk haplotype. In sum, these data suggest that SNPs associated with SMNs following HL are enriched for SNPs that regulate gene expression. We demonstrate that the validated risk alleles at 6q21 are associated with differences in PRDM1 mRNA and protein levels and response to radiation. These observations suggest a model in which PRDM1 may be a key regulator of the radiation-response that protects against the emergence of SMNs.

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