Integrating DNA and RNA sequencing analysis to describe somatic alterations and expression in the HLA gene loci

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Introduction: Next generation sequencing analysis of the human leukocyte antigen (HLA) genes, encoding the major histocompatibility complex (MHC), has remained computationally complex due to the highly polymorphic and diverse nature of the HLA genes. Advances in graph-based genome alignment have promoted the ability to deconvolve reads associated with HLA genes and increase the resolution of analysis of individual alleles. This is especially relevant in the context of immunotherapy, where loss of specific HLA alleles may confer resistance to therapy.
Methods: We established a workflow that incorporates HISAT2 for alignment and HISAT-genotype for Class I and II HLA haplotyping to describe the balanced presence and expression of HLA alleles. In addition, realignment of tumor DNA/RNA to matched normal alleles was used to perform somatic variant calling in matched tumor-normal DNA, as well as assess the allelic balance within each heterozygous gene. This analysis was deployed across blood, melanoma tumor biopsies, including baseline, on-treatment, and relapse timepoints, and melanoma cell lines.
Results: The HLA haplotypes, including MHC Class I and II genes, detected in both tumor and cell line DNA and RNA were highly consistent with those detected in matched normal DNA. Copy number imbalances in the HLA gene loci often span the entire gene complex on chromosome 6 and results in differential expression of HLA alleles at the RNA level. Allelic balance was associated with increased expression of inflammatory processes and interferon signaling. Allelic imbalance was not associated with response to anti-PD-1 therapy, supporting previous studies describing loss of either allele as an immunoediting event during tumorigenesis.
Conclusions: HLA gene allelic imbalance can be evaluated at both the DNA and RNA level, and is relevant with respect to tumor evolution and immunotherapeutic response. In cases without allelic imbalance, the haplotypes detected using this analysis approach in tumor DNA and RNA are highly consistent with those detected in matched normal DNA. However, allelic imbalance is supported by both tumor DNA and RNA, and this event can be evaluated by independent data types.