TARGETING A NOVEL CHIMERIC RNA, A-B, AS A NON-TRADITIONAL THERAPEUTIC IN COLORECTAL CANCER

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Background Colorectal cancer (CRC) is the second-leading cause of cancer-related deaths worldwide, affecting over 100,000 men and women in the United States every year. There is an urgent and unmet need for early biomarker discovery and the identification of novel therapeutic targets in CRC. Chimeric RNAs and their protein products, including BCR-ABL in chronic myelogenous leukemia and EML4-ALK in lung cancer, have been well-established as ideal biomarkers and drug targets for malignant cancers. Using RNA-Sequencing data from The Cancer Genome Atlas (TCGA) database, we've identified a chimeric RNA, A-B, which is present in nearly 50% of CRC samples but absent in non-cancer colon tissue. We've found this chimeric RNA results from cis-splicing between adjacent genes, producing a novel chimeric protein. This protein includes a splice variant of the 5' gene, which alters the reading frame of the 3' gene, making a completely novel peptide sequence which may be immunogenic. The protein is predicted to be a transmembrane protein with the novel peptide sequence on the extracellular surface, making it an ideal target for antibody therapeutics.

Methods We generated an antibody to the chimeric peptide sequence and have validated its use for immunoblotting, immunoprecipitation, and immunohistochemistry. We used NetMHCpan4.1 to predict binding of peptides from the novel protein to common HLA alleles. We've tested the immunogenicity of peptides by presenting monocyte-derived dendritic cells with peptides and co-culturing peptide-loaded DCs with autologous T cells from healthy donors. We used junction-specific siRNAs to knockdown A-B in CRC cell lines.

Results The novel A-B protein is expressed in patient CRC samples. Interestingly, we've found the protein localizes in a speckled nuclear pattern in a subset of patients with adenocarcinoma but is localized to the membrane and cytoplasm of adenomas. We've found co-culture with peptides from A-B increases autologous T cell proliferation and IFNg secretion. Overexpression of this chimeric RNA resulted in increased cell proliferation, while chimeric junction-specific siRNA-mediated knockdown led to a reduction in cell proliferation.

Conclusions Chimeric RNA A-B is expressed in a large proportion of colorectal cancers. This protein-coding RNA contains a splicing event which shifts the reading frame, making a completely novel peptide sequence which is not expressed in normal tissue. This peptide may be immunogenic and we are currently investigating the clinical relevance and therapeutic potential of this chimeric RNA in CRC, as well as the hypothesis that neoantigens are created from the novel peptide, which could be used for cancer vaccines.

Ethics Approval Healthy donor material was studied following informed consent, and with University of Virginia Health Sciences Institutional Review Board (IRB#230116) approval.

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