



Commentary

A Clinically Distinct and ‘Atypical’ Subgroup of Head and Neck Cancers Positive for a CpG Island Methylator Phenotype

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The current era of genomic medicine has greatly increased our understanding of the inter-connectedness of genetic, epigenetic, transcriptomic and proteomic profiles of human health and disease, none more evident than in human cancer. While human cancer genomics has primarily focused on genetic mutations and copy number alterations, epigenetic changes, namely DNA methylation and chromatin modifications are highly prevalent in every cancer type, occur more frequently than genetic alterations in human cancers (Schuebel et al., 2007) and can result in gene expression modulation. Human cancers are characterized by global DNA hypomethylation of repetitive elements and CpG-poor regions together with gene-specific, promoter CpG island DNA hypermethylation. DNA methylation changes may result in tumor suppressor gene silencing and the activation of oncogenes. This aspect of cancer epigenetics is compelling, as unlike somatic mutations and copy number alterations, cancer-specific DNA methylation changes can be reversed by treatment with DNA methylation inhibitors, such as 5-aza-cytidine (5-Aza-CR) and 5-aza-2'-deoxycytidine (5-Aza-CdR), which have been used in treatment of human cancers (reviewed in Raynal and Issa (2016)).

DNA methylation-based subgroups of human cancers have been identified that show clinical utility. Evidence of a CpG Island Methylator Phenotype (CIMP) was first described in colorectal cancer as DNA hypermethylation of a subset of CpG islands in a subset of tumors (reviewed in Weisenberger (2014)). CIMP status is also identified in glioblastoma, gastric cancer, endometrial and breast cancers (reviewed in Weisenberger (2014)). Colorectal CIMP was further shown to strongly correlate with a point mutation in the *BRAF* gene, older patient age and female gender (Weisenberger et al., 2006). Overall, CIMPs generally do not present with a consistent genomic profile, suggesting that CIMPs can result from multiple and unique sets of aberrations (Weisenberger, 2014).

In the accompanying report, Brennan and colleagues (Brennan et al., 2017-in this issue) describe a CIMP for head and neck squamous cell carcinomas (HNSCCs) after meta analyses of TCGA DNA methylation and gene expression datasets (The Cancer Genome Atlas Network, 2015). There are five HNSCC subgroups based on human papillomavirus infection (HPV +), CIMP status and smoking history: 1) NSD1-smoking,

2) Stem-like smoking, 3) CIMP-Atypical, 4) Non-CIMP-Atypical and 5) HPV +.

The HNSCC CIMP subgroup, similar to HPV + HNSCC tumors and CIMPs of other tumor types, displays only minor copy number alterations. CIMP tumors did show moderate enrichment of *CASP8* mutations, and to a lesser extent, *HRAS* mutations, as compared to the other HNSCC subgroups, also described by TCGA (The Cancer Genome Atlas Network, 2015), suggesting that the NF-κB and EGFR signaling pathways, respectively, may be targeted by specific therapeutic strategies that inhibit these pathways. However, further research will be required to investigate specific signaling pathways altered in CIMP HNSCC patients.

The CIMP-Atypical subgroup is molecularly distinct and has translational and clinical importance. The ‘Atypical’ nomenclature reflects that this subtype is overrepresented for patients that do not have history of smoking, alcohol and other risk factors that are associated with HNSCC patients in the non-Atypical subgroups. Atypical CIMP patients are also of older age, much like colorectal CIMP patients, however, patient outcome differences between CIMP and the other HNSCC subgroups have not been determined. An analysis of gene expression modulation in CIMP-Atypical tumors shows strong enrichment of immuno-responsive genes activated by Type I and Type II interferons (IFNs). Moreover, the CIMP tumors also showed increased levels of CD8 + T cells, another marker for immune response. These data indicate that chronic inflammation may be a driving force for these patients.

The immune-based signature of CIMP-Atypical tumors is also important with respect to precision medicine based approaches for HNSCC treatments. In general, HNSCC patients have poor outcome and diminished anti-tumor immune response (Economopoulou et al., 2016). The activation of IFN-related genes in patients with CIMP-Atypical tumors suggests that these patients may benefit from anti-inflammatory and immune-based therapeutics, however, patient survival and response to therapy of CIMP-Atypical patients compared to other HNSCC subgroups remain to be determined.

The DNA hypermethylation status of the CIMP-Atypical subgroup is intriguing, since these patients may respond to epigenetic therapies. Recent evidence of treating cancer cells with 5-Aza-CdR resulted in activation of tumor suppressor genes, reduction in oncogene expression, and activation of endogenous retroviral elements (ERVs) that stimulate an immune response (Chiappinelli et al., 2015; Roulois et al., 2015). Moreover, the addition of vitamin C, a required co-factor for TET enzyme based DNA demethylation provides a synergistic effect of 5-Aza-CdR

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treatment (Liu et al., 2016). Most cancer patients are vitamin C deficient, and therefore, combining DNA methylation inhibitors with vitamin C may prove efficacious for CIMP-positive HNSCC patients. Pre-clinical studies and clinical trials of these drug combinations are necessary to determine clinical utility, however, epigenetic therapies may provide promise for a subset of HNSCC patients.

Taken together, these findings provide strong evidence for a unique subset of HNSCC patients defined by DNA methylation profiles. Further research is required to determine the connection between CIMP and *CASP8* mutations, however, the correlations between individual CIMPs and specific somatic gene mutations across several cancer types are striking.

Disclosure

Daniel J. Weisenberger is a consultant for Zymo Research Corporation (Irvine, CA USA), a company that distributes DNA methylation-related products. Zymo Research does not have an interest in Dr. Weisenberger's research or the writing of this manuscript.

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