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I read with interest the recent report by Caulfield et al. In this elegant piece of work, the authors performed RNA-seq and whole exome sequencing (WES) on tumors from a small cohort of subjects that presented insulin-dependent diabetes after treatment by immune checkpoint inhibitors (ICIs), which is a rare but life-altering immune-related adverse effect (irAE) with undetermined underlying mechanisms. Their RNA-seq analyses suggested possible roles of tumorous ORM1, PLG, and G6PC gene expression, and their WES data highlighted a missense germline variant of the NLRC5 gene, the NLRC5 Pro191Leu (hg38, chr16:57025515C>T; rs74439742 in the dbSNP database), which was significantly enriched in patients that developed ICI-induced diabetes mellitus (ICI-DM). These findings, though based on data from a very limited number of patients (n=13), provided interesting clues about the possible mechanisms and biomarkers of ICI-DM.1

Despite the striking over-representation of the NLRC5 Pro191Leu variant in the ICI-DM patients compared with the general population (of European ancestry), this protein-coding mutation itself is more likely to be a non-causal marker given its predicted benign effect by multiple tools including Polyphen and SIFT, as shown in the gnomAD database (https://www.gnomad-sg.org). Nevertheless, this does not necessarily mean no causal role of the NLRC5 gene in ICI-DM, for the following reasons. First, as also discussed in Caulfield et al, the NLRC5 gene has been experimentally shown to play an important role in the development of IFNα-induced autoimmunity against pancreatic β cells, through not only transcriptional activation of HLA class I and antigen presentation-related genes, but also generation of β cell neoantigens by cross-talk with alternative splicing.2 Second, although the NLRC5 Pro191Leu amino acid change may confer non-significant impact on the function of NLRC5 protein, this variant is indeed significantly associated with the NLRC5 gene expression level in blood, as shown in the eQTLGen database (https://eqtlgen.org; rs74439742 T vs C, n=13 100, Z-score=−5.1, p=2.8×10−7, FDR=8.6×10−4). This cis-eQTL relationship suggest a potential causal role of NLRC5 gene in the development of ICI-DM, though further studies are needed for validation. On the other hand, no NLRC5 genetic variants have been linked with type 1 diabetes (T1DM) at genomewide significance in GWAS studies so far, per queries in the GWAS Catalog (https://www.ebi.ac.uk/gwas) and IEU-GWAS (https://gwas.mrcieu.ac.uk) databases, which is consistent with the report by Caulfield et al that no significant difference in the allele-frequency of NLRC5 Pro191Leu was noted between the T1DM subjects and the controls in previously published T1DM datasets and the Type 1 Diabetes Genetics Consortium database.1 However, interestingly, summary statistics-based Mendelian randomization (SMR) analyses suggest a possible cause-and-effect relationship between NLRC5 and T1DM, as provided in the eQTLGen database (β_SM=−0.40, P_SM=3.3×10−5).

NLRC5, also known as MHC class I transactivator, has been widely recognized as a key transcriptional activator of MHC class I and antigen presentation genes. It has recently been shown to play crucial roles in cancer immune surveillance, and a recent study by the Kobayashi lab suggested the translational significance of NLRC5 gene expression in ICI-based immunotherapy in melanoma patients.2 Interestingly, strong coexpression is observed between NLRC5 and PDCD1/CD274/CTLA4 in tumors of a number of ICI-relevant cancer types, including melanoma, lung cancer, bladder cancer, breast cancer, colorectal cancer, and head-and-neck cancer, among others, per queries for TCGA.
data in the cBioPortal database (https://www.cbioportal.org; eg, TCGA BLCA, n=404: \textit{NLRC5} with \textit{PDCD1}, Spearman’s \textit{r}=0.78, \textit{p}=2.9×10^{-54}; \textit{NLRC5} with \textit{CD274}, Spearman’s \textit{r}=0.65, \textit{p}=2.2×10^{-48}; \textit{NLRC5} with \textit{CTLA4}, Spearman’s \textit{r}=0.76, \textit{p}=6.1×10^{-84}). Considering the contributing effects exerted by genetic variants in anticancer immunity and ICI-response, \textit{NLRC5} variants could be potential biomarkers for ICI responsivenes and efficacy, similar as the previously identified marker SNPs associated with \textit{CTLA4}, \textit{PDCD1}, and \textit{CD274} genes.\textsuperscript{3,4} On the other hand, per query in the GTEx database (https://www.gtexportal.org/), although immune-related tissue types present the highest expression level of \textit{NLRC5} (ie, spleen and whole blood, median TPM, 63.98 and 35.93, respectively), it is also widely expressed in many other tissue types, and at a high level in lung, small intestine, and skin (median TPM, 24.63, 22.05, and 19.15, respectively). Therefore, if \textit{NLRC5} does play a role in irAE and with possible effects conferred by its germline variants, presumably ICI-DM is not the only form of manifestation. Indeed, as reviewed by Wang et al, \textit{NLRC5} is also implicated in a number of immune-related disorders, such as rheumatoid arthritis, inflammatory liver injury, and fibrosis in the liver, heart, and kidney.\textsuperscript{5}

Taken together, current data by Caulfield et al and others suggest possible implications of \textit{NLRC5} in ICI-based cancer immunotherapy, with regard to both clinical efficacy and adverse effects. Further studies are warranted to test and validate the clinical utility of \textit{NLRC5} variants as potential pharmacogenomic markers for ICI-treatment efficacy and safety. Common germline polymorphisms located in \textit{NLRC5}-related regulatory regions and/or demonstrate significant correlation with \textit{NLRC5} gene expression or methylation could be candidates of interest.

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