ABSTRACT

Natural Killer (NK) cells are the key lymphocytes in solid tumors as these are not Human Leukocyte Antigen (HLA) restricted, unlike cytotoxic T-cells. Its activity is regulated by the germline-encoded Killer cell immunoglobulin-like receptors (KIRs) upon interaction with its cognate HLA class I ligands. In terms of the quantity and variety of KIR-HLA genotypes, these two separate loci, KIR and HLA class I, segregate independently. Recent research has demonstrated how different KIR-HLA interactions can affect NK cell reactivity, which in turn can affect susceptibility/resistance to a range of ailments, including cancer. We have earlier reported KIR2DL1+-HLA-C2+ genotype was found to be a heritable risk factor in OSCC predisposing to OSCC at a younger age and tumor resident NK cells had lower cytolytic activity. The present study is a follow-up from the above findings in which we have explored further whether (a) high-affinity binding alleles of *KIR2DL1* and its copy number variation could explain the association of KIR2DL1-HLAC2 with HNSCC and (b) if NK Cell phenotype accounts for its anergy in tumor tissue.

Polymerase chain reaction (PCR)-sequence-specific primer (SSP) approach was used to type the *KIR2DLI/S1* genes as well as five KIR2DL1 alleles in individuals and SSP-real-time PCR was used for HLA class I ligand genotyping. Real-time quantitative reverse transcriptase-PCR was used for the cytokine expression study and copy number variation assay. The first phase of the study signified that the populations of North-East India, in particular Assam, are distinct in terms of genetic makeup, evolutionary and migratory history from North Indian populations which have mainly Indo-European origins, and the South-Indian populations which have Dravidian origins as the Phylogenetic tree based upon KIR2DL1-HLAC2 combined genotype and KIR2DS1 gene frequencies was mapped in the node consisting of

South-East Asian countries. In addition, the KIR2DL1*003 allele was seen at the highest frequency of 79% in all the 429 participants in the study. In the second phase of the study, it was found that the KIR2DL1*003-HLA-C2 genotype was more frequent in HNSCC patients and was positively associated with HNSCC and the odds of this genotype in HNSCC patients were nearly 2 times (p=0.0152; OR=1.9, 95% CI 1.118–2.534). In contrast, the frequency of the KIR2DL1*003-HLA-C1 genotype was higher in healthy controls and was negatively associated with HNSCC. Interestingly, the combined genotype of KIR2DL1*003+HLAC2+ in the younger age group patients was positively associated with the early onset of the disease where the median age was 46 and the range 26-55 yrs. Notably, the odds of the disease at a younger age with the KIR2DL1*003+HLAC2+ genotype increased by 2.0 folds (p=0.0008 OR=2.0, 95% CI 1.157–2.363). Patients with KIR2DL1*003-HLA-C2 had a higher copy number of the *KIR2DL1* gene as compared to healthy controls (p< 0.0001). Similarly, healthy participants with the KIR2DL1*003-HLA-C1 genotype were seen to have a higher copy number of the *KIR2DL1* gene (p < 0.0001).In addition, the cytokine expression study suggested an immunosuppressive microenvironment in HNSCC patients favouring the proliferation of suppressive immune cells. The study's concluding section refuted the notion that the recruitment of decidual NK cells in the tumor microenvironment was the cause of the non-cytolytic property of tumor NK cells in the study population. To sum up, our results indicated that immune surveillance in HNSCC was hampered by greater affinity binding alleles combined with increased CNV of inhibitory KIR2DL1, and this raises the prospect of using KIR-HLA allotype as prognostic indicators.