
Background
The human leukocyte antigen (HLA) genotype of an individual defines the repertoire of peptides which can be presented to T cells. In the tumor microenvironment, neoantigen presentation can be abrogated by alterations of HLA class I molecules, which have a direct impact in immune surveillance. This study aims to elucidate the impact of hereditary homozygosity and imbalanced expression of HLA-I loci on the repertoire of immunogenic peptides that are presented in esophago-gastric adenocarcinoma patients (EGA).

Materials and Methods
High-resolution clinical-grade HLA- genotyping was performed using the NGS method (n=80). Whole exome sequencing (WES) was done on tumor and blood (n=39) to call for somatic mutations. The amount of potential high affinity binders derived from 10 tumour-associated antigens (TAAs) were estimated in EGA and non-synonymous mutations obtained from WES were determined using an in-silico approach for HLA-binding (IEDB.org). Gene expression profiling was done with RNAseq (n=39). Whole RNAseq data was used to detect imbalanced or loosened expression of HLA-A/B/C alleles (n=19).

Results
We compared the frequency of HLA homozygosity in EGA patients to an HLA-matched reference population (35% vs. 19%, corresponding to an odds ratio (OR) of 2.282 (95% CI 1.442-3.615, p<0.001)). We then aimed to estimate the influence of HLA-homozygosity in the context of tumor immune surveillance. Predictions by IEDB analysis resource tool indeed showed a reduced repertoire of high and moderate-affinity MHC-binders (both TAA-derived and mutation-derived peptides) in the homozygous cohort. Our findings demonstrate a reduced amount of potentially immunogenic peptides in EGA patients with HLA-homozygosity for at least one locus, which may result in impaired cancer immunosurveillance. In line with this observation, artificial restoration of the genotype of homozygous patients to a heterozygous genotype, resulted in a set of predicted good-binding peptides of comparable size to the heterozygous cohort. While 35% of EGA patients showed germline homozygosity of HLA-I loci, quantification of allele-specific expression of HLA-I revealed altered expression in 9 out of 12 heterozygous patients (75%). Of these patients showed complete loss of heterozygosity, whereas the others had altered expression of one or two HLA-I molecules. The allelic imbalance was significantly higher in heterozygous compared to homozygous were only 2 patients showed altered expression of one HLA-I molecule (28.6%, p=0.0240). None of the patients with allelic imbalance carried genetic mutations associated with HLA-I genes, underlying epigenetic regulation.

Conclusions
The high frequency of genomic HLA-I homozygosity observed in the EGA cohort suggests that limitation of neoantigen presentation might have a role during EGA development and may reflect an increased cancer risk for these patients. Moreover, the results herein highlight that despite having a complete set of HLA-I alleles on a genomic level, the majority of EGA patients carry allelic imbalance that limit the repertoire of neoantigens for presentation to immune cells.

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The role of IDO1 and cellular senescence in the patient-derived head and neck tumor slice culture – A versatile tool to study oncolytic virus action

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Background Glioblastoma is the most common primary brain tumor in adults with a median overall survival between twelve to fifteen months.1,2 The prognosis is worse for patients older than 65 years of age, which leads to secretion of proinflammatory cytokines and increased levels of the immunosuppressive enzyme indoleamine-2,3-dioxygenase 1 (IDO1).3 Increased levels of IDO1 at an advanced age may attenuate the efficacy of immunotherapy.

Materials and Methods One hundred and seven mice between 79 and 92 weeks of age with differential Ido1 expression were intracranially injected with syngeneic murine GL261 cells and treated with PD-1 monoclonal antibodies and radiotherapy. The mice have been monitored for ninety days. U87 glioblastoma cells were cultured and treated with interferon-γ and NU223612.

Results PD-1 blockade and irradiation were less effective when Ido1 expression was preserved. Mouse lines with Ido1 knock-out in dendritic and endothelial cells had better survival. There was a higher senescent cell burden within the brain tumor than the extra-tumoral tissue in mice reaching the humane endpoint. In U87 glioblastoma cells, interferon-γ induced upregulation of PD-L1 and IDO1 in a similar pattern. IDO1 degradation resulted in concomitantly lower levels of PD-L1.

Conclusions Taken together, these results suggest that the treatment protocol with PD-1 pathway blockade plus radiotherapy should be combined with IDO1 inhibitors and potentially with senolytics to achieve a better therapeutic outcome in the elderly population with glioblastoma.