Expression of Programmed Cell Death-L1 (PD-L1) Protein and Mismatch Repair Mutations in Orbital Tumours-a Pilot Study

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Background

Programmed cell death protein 1 (PD-1) is part of a signaling pathway for T cell activation and plays an important role in tumor progression by altering the status of immune surveillance. Programmed cell death ligand 1 (PD-L1) may be expressed by tumor cells and interacts with the PD-1 receptor on lymphocytes, a coupling known as an immune checkpoint. PD-1/PD-L1 inhibitors act by blocking the interaction of immune checkpoints, resulting in reinvigoration of T cell activity that may lead to an effective immune response. DNA mismatch repair (MMR) deficiency is a form of genetic instability in cancer characterized by failure to repair DNA replication-associated errors. A defective MMR system leads to the persistence of mutations across the genome, particularly in regions of repetitive DNA.

Objective

The aims of this study

- To determine PD-L1 expression by immunohistochemistry (IHC)
- To examine the presence of Mismatch Repair (MMR) mutation by Next Generation Sequencing (NGS) in specific orbital tumors.

Methods

We reviewed surgical specimens from patients with rhabdomyosarcoma, adenoid cystic carcinoma (ACC), pleomorphic adenoma (PA; a benign tumors control) and biopsy negative tissue from orbital tumors used as a control group. Immunohistochemistry was performed on Formalin fixed paraffin embedded (FFPE) tissue using a PD-L1 antibody, and DNA was extracted for targeted gene panel next generation sequencing of the MMR genes PMS2, MLH1, MSH6 and MSH2.

Results

The study included 17 orbital specimens. Scattered membrane PD-L1 staining was noted in 3/6 rhabdomyosarcoma specimens without an accompanying lymphocytic infiltrate. PD-L1 immunostaining was absent in 3/3 ACC, and 5/6 PA specimens. PD-L1 immunostaining was not detected in 2/2 control specimens. 4/17 samples shared the same pathogenic mutation in the MLH1 gene, including 3/6 rhabdomyosarcoma and 1/3 ACC tumor samples. 1/6 PA samples had a pathogenic frameshift mutation in MSH6.

Table 1. Summary of demographic data, type of orbital tissue involved with PD-L1 expression, and MMR sequence analysis.

Tissue Type	Sex	Age	PD-L1 Expression with TPS	Gene Name	Protein Change
Rhabdomyosarcoma: Embryonal	M	14	0.7	-	-
Rhabdomyosarcoma: Embryonal	M	6	0.5	MLH1	p.Lys195Ter
Rhabdomyosarcoma: Embryonal	M	7	1.0	-	-
Rhabdomyosarcoma: Alveolar	M	9	0.0	MLH1	p.Lys195Ter
Rhabdomyosarcoma: Alveolar	M	12	0.0	-	-
Rhabdomyosarcoma: Alveolar	M	1	0.0	MLH1	p.Lys195Ter
Cribriform Adenoid Cystic Carcinoma	F	32	0.0	-	-
Cribriform Adenoid Cystic Carcinoma	M	30	0.0	-	-
Cribriform Adenoid Cystic Carcinoma	F	37	0.0	MLH1	p.Lys195Ter
Pleomorphic Adenoma	M	43	0.0	MSH6	p.Ala40fs
Pleomorphic Adenoma	M	58	0.0	-	-
Pleomorphic Adenoma	M	35	0.0	-	-
Pleomorphic Adenoma	F	62	0.8	-	-
Pleomorphic Adenoma	M	35	0.0	-	-
Pleomorphic Adenoma	F	36	0.0	-	-
Control : Normal Lacrimal gland	F	65	0.0	-	-
Control : Orbital dense connective tissue	F	61	0.0	-	-

Conclusion

Our study demonstrated scattered, non-quantifiable or absent PD-L1 staining in a limited sample of orbital tumors suggesting that PD-1/PD-L1 inhibitor therapy may not be useful in treatment of malignant orbital tumors such as rhabdomyosarcoma and ACC when refractory to conventional therapy. Only a small subset of primary orbital tumors were associated with pathogenic mutations in MMR genes. Results of our pilot study suggest that the PD-L1/MMR axis, as analyzed here, might not play a major role in the pathogenesis of primary orbital tumors. Further testing of additional MMR genes, and future studies analyzing PD-L1 expression during treatments might unveil so far undiscovered functions of the PD-L1/MMR axis in primary orbital tumor pathology

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