PLGA Foundation Grant Proposal
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Molecular Analysis of BRAF in WHO Grade II Fibrillary Pediatric Astrocytomas

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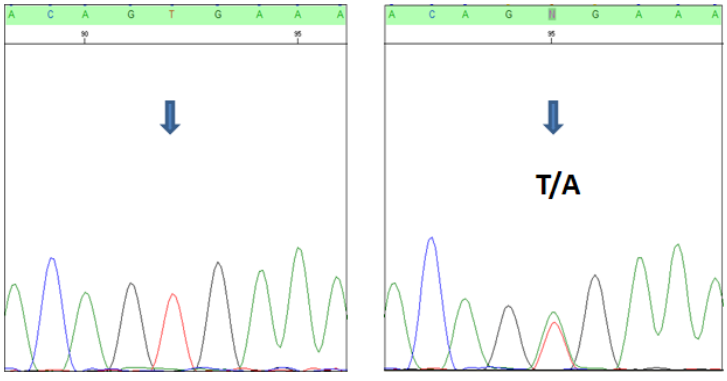
Introduction and Preliminary Data

Pediatric Low Grade Astrocytomas (PLGA) are a heterogeneous group of generally slow-growing glial neoplasms. Most common are the largely non-infiltrative pilocytic/pilomyxoid astrocytomas. Infiltrating WHO grade II fibrillary astrocytomas representing a second, less common type of PLGA (1). Relatively little is known about the genetic alterations that differentiate grade II fibrillary astrocytomas from other PLGA, or cause any of these neoplasms to form. However, the molecular biology and clinical behavior of grade II fibrillary astrocytomas arising in children seems to be different from that observed in adult cases.

We have recently identified a chromosomal change involving the BRAF oncogene in 17 of 25 pilocytic astrocytomas examined (2). This was a low-copy number gain 1.9 MB in size at chromosome 7q34. Similar gains were also recently described by the Lichter group in the Journal of Clinical Investigation (3), and the Gutmann group in Oncogene (4). The region of gain appears to terminate in the middle of the known oncogene *BRAF,* and Jones et al have recently shown that this results in expression of a constitutively active BRAF fusion protein (5). We have now confirmed expression of the same BRAF fusion protein identified by Jones and colleagues (unpublished data). We have also identified three activating point mutations in BRAF in pilocytic astrocytoma which do not contain the fusion, supporting this as a second mechanism of BRAF-ERK-MEK activation (see image below). These findings are of great potential clinical significance, as drugs which inhibit this cascade have already been developed, and could be used in patients with clinically problematic PLGA tumors.

It is not clear, however, if the BRAF gene duplications and mutations prevalent in pilocytic tumors also effect pediatric grade II fibrillary astrocytomas. In the recent paper from the Collins group, several pediatric tumors diagnosed as grade II infiltrating glioma (oligodendroglioma, astrocytoma, or mixed) did show the duplication. However, because these were cerebellar tumors, and the patients had uniformly good clinical outcomes, the authors suggested they might represent misdiagnosed pilocytic astrocytomas. We hope to directly address this



question by searching for gene fusions and/or point mutations involving BRAF in both snap frozen and paraffin-embedded pediatric grade II fibrillary astrocytomas and other low pediatric low grade gliomas which are not clearly piloid. We will also perform oligonucleotide array CGH analysis of all pediatric grade II fibrillary astrocytomas for which frozen material is available. The Specific Aims and methods we will employ are described below.

Aim 1 – Identify BRAF mutations and gene fusions in pediatric grade II fibrillary astrocytoma.

Tumor Bank: We will be using both frozen specimens and paraffin blocks for these studies. Only tumors arising in patients 18 years old or younger will be used. We have 4 cases of grade II PLGG with frozen tissue already in the Eberhart lab. Another 5 cases with frozen tissue are listed in the Pathology Department tumor bank, and we have requested these. To increase the number of cases available for analysis, we will also examine tumors with only paraffin blocks available. A review of pediatric low grade glioma diagnosed from 1998-2008 at Johns Hopkins Hospital, after excluding all pilocytic astrocytomas, resulted in 32 cases. In 20 of these, the lesion had at least a component of fibrillary astrocytoma (WHO grade II). We will review the slides for all of these cases, as some have mixed astrocytic-oligodendroglial features. Overall, we expect to successfully analyze 20-30 tumors for BRAF genetic changes.

Mutation Analysis: We will sequence the tumors for mutations activating BRAF, using DNA extracted either from frozen tissue or paraffin blocks.

Gene Fusion Analysis: We have validated primers which can detect the BRAF gene fusion product in cDNA. RNA will be extracted from frozen tissue or paraffin blocks and used to generate cDNA. Because the diagnostic fragment is small (100 bp or less), we believe this assay will work in RNA extracted from paraffin, although some optimization will no doubt be necessary, and it is possible that technical difficulties could make this problematic.

Aim 2 – Perform oligonucleotide array CGH analysis of pediatric grade II fibrillary astrocytoma.

We will use oligonucleoide array CGH to identify chromosomal alterations in grade II PLGA with frozen tissue available. As described above, we have already completed this for 25 pilocytic tumors using the Agilent 244K array, and we do not anticipate any technical difficulties. While this analysis will only be possible in a small number of cases with frozen tissue, the data can be combined with those from other centers to build a more complete picture of the molecular changes in these rare tumors.

References

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