Adult Primary Pineal Alveolar Rhabdomyosarcoma with FOXO1 Gene Rearrangement and OLIG2 Expression: A Rare Case Report and Literature Review

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Abstract
Alveolar rhabdomyosarcoma is a common malignant soft tissue tumor in child and adolescents. Intracranial alveolar rhabdomyosarcoma in adults is rare, especially in the pineal region. We present a case of primary alveolar rhabdomyosarcoma of the pineal gland in a 36-year-old Chinese male with a chief complaint of dizziness, headache and a loss of balance when walking. Imaging identified a space-occupying mass in the pineal region with obstructive hydrocephalus. An endoscopic-assisted pineal mass resection was performed. Pathology revealed a solid, sheet-like growth of medium-sized, round or oval cells with map-like necrosis and some rhabdomyoblasts. The tumor cells were diffusely positive for desmin, myogenin, MyoD1, ALK, and OLIG2. Fluorescence in situ hybridization (FISH) detected FOXO1 gene rearrangement. This rare case is presented to expand the knowledge of pineal gland tumors and alert us to pay attention to the differential diagnosis of OLIG2-positive round-cell tumors of the central nervous system.

Keywords
rhabdomyosarcoma, alveolar rhabdomyosarcoma, pineal gland, OLIG2, FOXO1

Introduction
Although rhabdomyosarcoma is a relatively common malignant soft tissue tumor in children and adolescents, it is rare in adults. The common primary sites of adult rhabdomyosarcoma are the extremities, head-neck region and the genitourinary tract. Primary intracranial rhabdomyosarcomas in adults, especially those originating in the pineal gland, are extremely rare. Until now, only three cases of primary pineal rhabdomyosarcoma in adults have been reported in the literature. According to the fifth edition of the 2020 World Health Organization (WHO) classification of soft tissue and bone tumors, rhabdomyosarcoma is classified into four subtypes: embryonal rhabdomyosarcoma, alveolar rhabdomyosarcoma, pleomorphic rhabdomyosarcoma and spindle cell rhabdomyosarcoma. Embryonal rhabdomyosarcoma is classified into four subtypes: embryonal rhabdomyosarcoma, alveolar rhabdomyosarcoma, embryonal rhabdomyosarcoma, and embryonal rhabdomyosarcoma. Two most common subtypes, which have considerable differences in their clinical manifestations and molecular features. Furthermore, the specific histologic subtype is correlated to prognosis and survival in both pediatric and adult patients. Due to the complexity of the morphological appearance of rhabdomyosarcoma, the classical histopathological examination alone is sometimes not sufficient to accurately determine the rhabdomyosarcoma subtype. Therefore, immunohistochemical and molecular analysis emerged as complementary diagnosis tools. In a children’s oncology study, approximately 80% of alveolar rhabdomyosarcoma patients were characterized by the presence of PAX3/7-FOXO1 gene fusion, which suggested the diagnostic value of PAX3/7-FOXO1 fusion status in alveolar rhabdomyosarcoma. The majority of histologically diagnosed alveolar rhabdomyosarcomas contain characteristic fusion gene PAX3/7-FOXO1, and rare cases show alternative novel gene fusion such as PAX3-NC/2. A recent study revealed the occurrence ...
of PAX3-NCOA2 fusion gene in primary pineal alveolar rhabdomyosarcoma in a 12 year old boy. A similar case in the posterior III ventricular/pineal region just near the pineal gland has been confirmed by PAX3-NCOA2 fusion in an adult. However, none of the previously published cases of primary pineal rhabdomyosarcoma in adult has been subtyped by the presence of certain fusion genes.

Here we report a rare case of primary alveolar rhabdomyosarcoma of the pineal gland in a 36-year-old Chinese male. To our knowledge, this is the fourth case of primary pineal rhabdomyosarcoma in adults. Furthermore, our present study provided the first evidence of coexistence of FOXO1 gene rearrangement and the expression of OLIG2, which is highly correlated to PAX3/7-FOXO1 fusion gene, in adult primary pineal alveolar rhabdomyosarcoma.

**Clinical Summery**

A 36-year-old Chinese male was admitted to the Department of Neurosurgery of Affiliated Hangzhou First People’s Hospital Zhejiang University School of Medicine with a chief complaint of dizziness for three months and headache accompanied by loss of balance when walking for one month. After admission, enhanced magnetic resonance imaging (MRI) of the brain revealed a space-occupying mass in the pineal region with obstructive hydrocephalus (Figure 1). He received ventriculo-peritoneal shunt immediately to relieve the symptoms of acute hydrocephalus. 14 days later an endoscopic-assisted pineal mass resection was performed. A 2 cm × 1 cm × 1 cm mass with grayish, red soft tissue was completely removed and sampled for pathological examination. Hematoxylin and eosin (H&E)-stained sections showed a solid, sheet-like growth of neoplastic cells, accompanied by map-like necrosis and abundant thin-walled vessels (Figure 2A). The neoplastic cells were mainly medium-sized, round or oval, and had pale pink or clear cytoplasm and well-defined cell borders. There were some scattered bigger cells with abundant pink cytoplasm and eccentric nuclei, resembling rhabdomyoblasts. A few multinucleated large rhabdomyoblasts could be seen (Figure 2B). In the neoplastic clusters, the atypical cells had a higher nuclear/cytoplasmic ratio, hyperchromatic and pleomorphic nuclei, and more mitotic figures (Figure 2C). Spindle-shaped tumor cells with pink or clear cytoplasm, arranged in a weaving pattern, were also observed (Figure 2D). On immunohistochemical staining sections tumor cells showed diffuse and strong cytoplasmic positivity for desmin (Figure 2E), diffuse and strong nuclear positivity for myogenin (Figure 2F) and MyoD1, diffuse and moderate to strong nuclear positivity for OLIG2 (Figure 2G) and INI-1 (Figure 2I); diffuse but weak to moderate cytoplasmic positivity for ALK (clone number D5F3, Figure 2H); partial and weak membranous positivity for CD99 and cytoplasmic positivity for S100; but negative for cytokeratin, EMA, SMA, GFAP, H3K27M, synaptophysin and chromogranin A, NUT, BCOR, SALL4, OCT4 and CD34. The Ki-67 index was 30-35% in hot spots. Fluorescence in situ hybridization (FISH) detection with the FOXO1 break-apart probe was positive (Figure 2J). No 1p/19q deletion or IDH1/2 gene amplification was detected. FISH detection with the EWSR1 break-apart probe was negative. Taken together, the histopathological features, immunohistochemical and molecular analysis support a final diagnosis of adult primary pineal alveolar rhabdomyosarcoma. The patient received six cycles of temozolomide therapy after the surgery. During the first eight months after surgery, no evidence of recurrence or metastasis was found.

**Figure 1.** Enhanced magnetic resonance imaging of the primary tumor. (A) Axial contrast-enhanced T1-weighted image shows slight enhanced lesion in the pineal region (yellow circle). (B) Axial T2-weighted image shows quasi-circular isointense lesion in the pineal region (yellow circle), with strip-like hyperintensities inside, but the main body is not obviously enhanced.
Figure 2. Pathologic findings of the primary tumor. (A) The tumor grows in a solid sheet-like pattern, accompanied by multifocal map-like necrosis and hemorrhage. A small number of gravel-shaped calcifications can be seen at the peripheral areas. H&E staining, 10× magnification. (B) The neoplastic cells are relatively uniform with slight atypia. Medium-sized round or oval cells are adherent to each other with clear cell borders. The cytoplasm is stained light pink or clear and bright. The nuclei are round, with fine chromatin and tiny nucleoli. Large rhabdomyoblast-like cells with eccentric nuclei and abundant pink-stained cytoplasm and one large multinucleated rhabdomyoblast-like cell can be seen. H&E staining, 400× magnification. (C) An area with higher cell density and atypia. The atypical cells have higher nuclear/cytoplasmic ratio, hyperchromatic and pleomorphic nuclei and more mitotic figures. H&E staining, 200× magnification. (D) An area of short spindle-shaped tumor cells with pink or clear cytoplasm arranged in a weave or storiform pattern. H&E staining, 400× magnification. (E) Desmin immunohistochemical staining shows diffuse and strong cytoplasmic positivity in tumor cells. Rhabdomyoblasts are more strongly positive. 400× magnification. (F) Myogenin immunohistochemical staining shows diffuse and strong nuclear positivity in tumor cells. Rhabdomyoblasts and multinucleated rhabdomyoblasts are also strongly positive. 200× magnification. (G) OLIG2 immunohistochemical staining shows diffuse and moderate to strong cytoplasmic positivity in most of the tumor cells. 400× magnification. (H) ALK immunohistochemical staining shows diffuse and strong cytoplasmic positivity in tumor cells. 400× magnification. (I) INI-1 immunohistochemical staining shows diffuse and moderate to strong nuclear positivity in tumor cells. 400× magnification. (J) FISH detection with the FOXO1 break-apart probe shows separated red and green signals in some tumor cells.
by using any imaging techniques. However, the latest MRI scanning of the head nine months after surgery revealed local recurrence, and computed tomography of the abdomen detected multiple peritoneal metastasis which was confirmed by fine-needle aspiration (Figure 3). Unfortunately, the patient died just one year after surgery.

Discussion

Primary rhabdomyosarcoma occurring in the adult pineal region is an extremely rare and aggressive malignancy. Prior to our study, only three cases of primary pineal rhabdomyosarcoma and one in the posterior III ventricular/pineal region were reported in adults. Table 1 summarized the demographic characteristics, morphological features, immunohistochemical analysis, diagnosis, treatment and survival of these patients. The first three patients all presented with typical symptoms of brain tumors. The morphological and immunohistochemical findings of the tumor cells favored a diagnosis of primary pineal gland rhabdomyosarcoma. The disease progressed so rapidly that all the patients survived only 4-6 months from the time of diagnosis. It is to be noted that none of these cases was subtyped as alveolar rhabdomyosarcoma and it

Figure 3. Imaging and pathology of recurrent and metastatic tumors 9 months after surgery. (A) Enhanced magnetic resonance imaging of the head reveals a nodular foci of abnormal enhancement in the pineal region (yellow circle), indicating local recurrence. (B) Enhanced computed tomography of the abdomen shows multiple nodules of abnormal enhancement in the right paracolic sulcus area (yellow circles). (C) Fine-needle aspiration of the abdominal masses reveals a small cell tumor with similar morphology to primary tumors of the pineal gland. H&E staining, 100× magnification. (D) MyoD1 immunohistochemical staining shows diffuse and strong nuclear positivity in tumor cells. 100× magnification.
Table 1. Summary of the Clinical and Pathological Features of Reported Primary Pineal rhabdomyosarcoma in Adults.

| Case     | Age | Sex | Location                     | Morphology                                                                 | Diffusely positive IHC staining markers     | Fusion gene                          | Tumor type                                 | Post-op treatment | Post-op        | Survival (months) |
|----------|-----|-----|-------------------------------|----------------------------------------------------------------------------|---------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------|----------------|------------------|
| Laul (2015) | 33  | F   | Pineal gland                  | solid sheet-like; loose round cells with rhabdomyoblasts                  | Desmin, Myogenin, MyoD1                    | None-done                                | rhabdomyosarcoma with alveolar morphology | Chemotherapy      | Intra-cranial failure | 5, D            |
| Scull (2016) | 43  | F   | Pineal gland                  | solid sheet-like; spindle cells with rhabdomyoblasts                      | Desmin, Myogenin, MSA                      | None-done                                | rhabdomyosarcoma                           | None              | Rapid local recurrence | 4, D            |
| Pandey (2020) | 44  | M   | Pineal gland                  | Solid sheet-like; round cells                                            | Desmin, Myogenin                           | None-done                                | rhabdomyosarcoma                           | Chemotherapy + Radiation | Rapid local recurrence | 6, D            |
| Present case | 36  | M   | Pineal gland                  | Solid sheet-like; adhesive round cells with rhabdomyoblasts; focal spindle cells; some with clear cytoplasm | Desmin, Myogenin, MyoD1, OLIG2, ALK        | FOXO1-PAX3/PAX7                           | alveolar rhabdomyosarcoma                  | Chemotherapy      | Local recurrence with multiple peritoneal metastases | 12, D           |
| Jour (2019) | 22  | F   | Posterior III ventricular/pineal region | solid sheet-like; spindle and round cells, no rhabdomyoblasts          | Desmin, Myogenin                           | PAX3-NCOA2                                | alveolar rhabdomyosarcoma                  | None              | Recurrence with spinal metastases       | 14, D           |

Abbreviations: IHC, immunohistochemistry; MSA, muscle-specific actin; D, dead.
remains unknown whether there is a rhabdomyosarcoma associated gene fusion. Recently, a genomically confirmed primary alveolar rhabdomyosarcoma was reported in a young female with a lesion in the posterior III ventricular/pineal region. An in frame PAX3-NCOA2 fusion was also revealed in this case. In comparison, our present case is the first primary pineal alveolar rhabdomyosarcoma with PAX3/7-FOXO1 gene fusion in adults.

The typical morphological feature of alveolar rhabdomyosarcoma is an alveolar pattern with the presence of discohesive round cells arranged in nests separated by fibrovascular septa, accompanied by variable number of rhabdomyoblasts. The rare solid variant of alveolar rhabdomyosarcoma usually appears as a completely solid sheet-like growth lack of fibrovascular septa, which is seen in the present case and the previously reported ones in Table 1. To distinguish it from other small round cell malignancies, the immunohistochemical and molecular methods are utilized for an accurate diagnosis. The myogenin-associated proteins myogenin, MyoD1 and desmin, are considered as the sensitive and specific markers for rhabdomyosarcoma. In the present study, the immunohistochemical staining showed that the neoplastic cells had strong and diffuse nuclear reactivity for myogenin and MyoD1, as well as diffuse cytoplasmic reactivity for desmin.

At the molecular level, the most common translocation characteristic of alveolar rhabdomyosarcoma is PAX3/7-FOXO1 fusion gene, which is seen in the majority of the alveolar rhabdomyosarcoma cases. Furthermore, the presence of PAX3/7-FOXO1 fusion appears to predict a worse outcome. However, most of the data are restricted in the pediatric patients. The gene fusion status and its clinical significance are not well defined in adults. In addition, PAX3/7-FOXO1 fusion-negative alveolar rhabdomyosarcoma could be true negative or carrying novel fusion genes such as PAX3-NCOA1/2. Indeed, there are two recently reported cases showing a PAX3-NCOA2 fusion in alveolar rhabdomyosarcoma in the pineal region. FISH assay detected FOXO1 rearrangement in our study, confirming a common PAX3/7-FOXO1 fusion pattern.

Although PAX3/7-FOXO1 fusion status is an important molecular marker and prognostic indicator of alveolar rhabdomyosarcoma, the traditional immunohistochemistry still remains a more widely used method than FISH or RT-PCR. Microarray analysis revealed high expression of several genes that are associated with PAX3/7-FOXO1 fusion. Immunohistochemical assay are applied to detect the surrogate markers as gene fusion-positive indicators. OLIG2 appears to be a such novel immunohistochemical marker. It is a transcription factor that is required for appropriate development of motor neurons and oligodendrocytes. Immunohistochemical staining of OLIG2 is widely used in diagnosis of glial neoplasms and “neuronal neoplasms”, including oligodendroglioma, astrocytoma, and ependymoma, medulloblastoma, central neurocytoma and neuroepithelial tumors. The tumor cells in the present case did not express GFAP but diffusely expressed OLIG2. It was difficult to argue against the diagnosis of glial tumors, especially oligodendroglioma. Subsequent molecular testing showed that there was no deletion of 1p/19q and no mutations in the IDH1/2 gene, ruling out the diagnosis of glial cell tumors. A recent study found that OLIG2 expression was positive in 7 cases from 45 rhabdomyosarcoma tumors, which was associated with the presence of PAX3/7-FOXO1 rearrangement. Similar results were shown by Jour et al. that OLIG2 expression was positive in 27 cases from 28 PAX3/7-FOXO1 fusion-positive rhabdomyosarcoma specimens, while in 3 cases from 45 PAX3/7-FOXO1 fusion-negative rhabdomyosarcoma. Immunohistochemical staining also detected OLIG2 expression in our present study. These results indicate that OLIG2 may serve as a surrogate marker for PAX3/7-FOXO1 fusion status, making it possible to predict a fusion status by a standard immunohistochemical analysis. Interestingly, a recent study also found OLIG2 expression in the pineal gland of a 12-year-old boy with PAX3-NCOA2 fusion-positive alveolar rhabdomyosarcoma. It is speculated that since PAX3 is an important transcription factor in the development of the central nervous system, the related fusion genes might activate a variety of neurodevelopment-related genes, including OLIG2, leading to an up-regulation of OLIG2 expression.

Conclusion
We present the first case of primary alveolar rhabdomyosarcoma with OLIG2 expression and confirmed by FOXO1 gene rearrangement in adult pineal gland. An integrated utilization of the histopathological examination, immunohistochemical and molecular analysis facilitates an accurate diagnosis and subclassification of rhabdomyosarcoma. This rare case can expand the knowledge of pineal gland tumors and alert us to pay attention to the differential diagnosis of OLIG2 positive round cell tumors of the central nervous system.

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Ethical Approval
All procedures were in accordance with the ethical standards of institutional review board of Affiliated Hangzhou First People’s Hospital Zhejiang University School of Medicine (reference number: Non-registered clinical studies ZN-20211110-0107-01).

Informed Consent
Oral informed consent was obtained from the patient’s wife for publication of this case, including clinical information and radiologic and pathologic images.

Trial Registration
Not applicable since this report does not contain any clinical trials.

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