Genome-Wide Copy Number Variation Analysis of a Granular Cell Tumor of the Sellar Region: Literature Review

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Abstract
Granular Cell Tumor (GCT) of the sellar region is rare tumor with indolent course, occurring in 50-60 year-age group. We report a case of 60-year-old Thai man presented with visual disturbance, with a suprasellar mass by corresponding MRI imaging. Microscopic sections show sheets of polygonal cells with granular eosinophilic cytoplasm and eccentric nuclei. Periodic Acid-Schiff (PAS) stain shows coarse granular eosinophilic cytoplasm in neoplastic cells, corresponding with lysosomes. The tumor is immunopositive for S-100, Thyroid Transcription Factor-1 (TTF-1) and Galectin-3, but negative for Epithelial membrane antigen (EMA), Glial Fibrillary Acidic Protein (GFAP), Cytokeratin (AE1/AE3) and Synaptophysin, confirming a diagnosis of GCT of the sellar region. Due to rarity of molecular information of this tumor, we review the literature and present the first, to our knowledge, whole-genome characterization of this tumor. There are Copy Number Variations (CNV), including gains on chromosome arms 1p, 2p, 5q, 10q, 11q, 12q, 16q, loss on 3q while most of the p and q arms of chromosome X reveal loss and LOH. While this study includes only one case, it could be the first step to understanding this rare tumor.

Keywords: Granular cell tumor, pituicytoma, spindle cell oncocytoma, copy number variation

Introduction
Granular Cell Tumor (GCT) of the sellar region is a relatively rare tumor, with a female predominance (>2:1). The peak incidence is in the 50-60 year-age group [1]. WHO classification of central nervous system tumors designates this tumor as grade I. Most of the cases are asymptomatic and have been found in postmortem [2,3]. Here we describe a symptomatic case of granular cell tumor, presented with visual deficits secondary to compression of optic chiasm, correspond with the genetic alteration.
(Free thyroxin; T4 0.58 ng/dl, thyroid stimulating hormone; TSH 2.76 uIU/ml). However, decreased levels of morning cortisol levels (6.88 ug/dl). A preliminary diagnosis of pituicytoma was suggested, with other differential diagnoses of mesenchymal tumors, such as meningioma, hemangiopericytoma. The patient underwent partial resection of mass via right-sided pterional craniotomy on the 5th day after admission.

**Histopathology**

Multiple pieces of firm, light brown tissues were examined, measuring 1x1x1 cm in total. Microscopically, the lesion consisted of polygonal tumor cells with abundant granular eosinophilic cytoplasm. The nuclei were eccentrically placed, with centrally located nucleoli (Figure 1). Perivascular chronic inflammation was noted. Mitotic figures and necrosis were absent. Periodic Acid-Schiff (PAS) staining with diastase digestion highlighted intracytoplasmic granules in the tumor cells. Immunohistochemical study demonstrated positivity to Thyroid Transcription Factor-1 (TTF-1), Beta-catenin, Galectin-3, Vimentin, S-100 protein (Figure 1). Epithelial Membrane Antigen (EMA), Glial Fibrillary Acidic Protein (GFAP), Cytokeratin (AE1/AE3), Synaptophysin, and Smooth Muscle Actin (SMA) were negative. The pathological diagnosis was granular cell tumor of the sellar region (WHO Grade I).

**Figure 1:** Sheets of granular cell tumor with lymphocytic aggregation (A; Hematoxylin and eosin; 200X magnification). Neoplastic polygonal cells with granular eosinophilic cytoplasm (B; Hematoxylin and eosin; 400X magnification, C; PAS with diastase; 200X magnification). Positive immunohistochemical staining for S100 (D; 200X magnification), TTF-1 (E; 200X magnification), Galectin-3 (F; 200X magnification).
Postoperatively, the patient recovered well. The postoperative imaging revealed residual tumor, measuring 1 x 0.7 x 0.6 cm, with pressure effect to bilateral hypothalamus. At follow up 6 months later, the patient was stable and ophthalmological findings were similar to the previous examination. He was recommended for second operation. The patients accepted and wanted to visit another hospital.

**SNP Array Analysis**

Formalin Fixed, Paraffin Embedded (FFPE) tissue was sectioned. Archived hematoxylin and eosin-stained tissue slides were evaluated by a Pathologist (ST) for the area with at least 70% tumor cells for manual macrodissection using a needle tip or scalpel. A minimum amount of 200 ng of DNA extracted from FFPE tissue was quantified by the Qubit® 2.0 Fluorometer (ThermoFisher Scientific, Waltham, MA) and qualified using the Infinium FFPE QC Kit before being processed with the HumanCytoSNP FFPE-12 v2.1 DNA Analysis BeadChip (Illumina, San Diego, CA), according to the manufacturer’s instructions. SNP array was performed with the HumanCytoSNP FFPE-12 v2.1 DNA Analysis BeadChip (Illumina, San Diego, CA), according to the manufacturer’s instructions. This array contains approximately 299,140 SNP markers spanning the entire genome with an average probe spacing of 72 kb.

The data were analyzed using GenomeStudio Data Analysis Software v. 2011.1 (Illumina, San Diego, CA) and Nexus Copy Number v9.0 (BioDiscovery, Inc., El Segundo, CA) using the reference human genome hg19/GRCh37. Somatic CNV calls were identified by comparing to the germline CNVs in Thai CNV database [4]. Two CNVs at each genomic region were compared and defined as identical when more than 60% of the total length of both CNVs reciprocally overlapped. Data analysis identified 22 regions with CNVs (Table 1).

**Chromosomal gains** were detected for Chromosomes 1, 2, 5, 10, 11, 12 and 16. The gain of chromosome 12 was composed of several segments. Chromosome 3 showed losses of parts of long arm and chromosome X had a copy loss of most of the p and q arms coupled with Loss of Heterozygosity (LOH).

| Chro | Start  | End    | Length   | Cytoband      | Event | % of CNV overlap with Thai CNV |
|------|--------|--------|----------|---------------|-------|-----------------------------|
| 1    | 17,996,004 | 21,436,940 | 3,440,937 | p36.13 - p36.12 | gain  | 0                           |
| 2    | 234,793,741 | 239,004,043 | 4,210,303 | q37.1 - q37.3 | gain  | 1.15                        |
| 3    | 128,145,543 | 143,530,641 | 15,385,099 | q21.3 - q24 | LOH   | 4.16                        |
| 5    | 167,348,970 | 174,425,954 | 7,076,985 | q34 - q35.2  | gain  | 0.18                        |
| 10   | 122,460,628 | 132,862,139 | 10,401,511 | q26.12 - q26.3 | gain  | 0.49                        |
| 11   | 126,446,367 | 133,878,051 | 7,431,685 | q24.2 - q25  | gain  | 3.38                        |
| 12   | 105,592,271 | 108,980,816 | 3,388,546 | q23.3        | gain  | 0                           |
| 12   | 116,808,477 | 120,455,612 | 3,647,136 | q24.22 - q24.23 | gain  | 0.18                       |
| 16   | 9,864,754 | 56,980,187 | 7,115,434 | q12.1 - q13  | gain  | 6.84                        |
| X    | 25,585,883 | 59,072,369 | 33,486,486 | p21.3 - p11.1 | loss  | 0                           |
| X    | 65,125,757 | 129,842,114 | 64,716,358 | q12 - q26.1  | loss  | 0                           |
| X    | 2,674,629 | 59,072,369 | 56,397,741 | p22.33 - p11.1 | LOH   | 0                           |
| X    | 65,125,757 | 152,075,812 | 86,950,056 | q12 - q28    | LOH   | 0                           |

Table 1: Copy number variations identified by genome-wide SNP array analysis.

**Gene Ontology and Pathway Enrichment Analyses**

Gene ontology and pathway enrichment were analysed using Web-based gene set analysis toolkit (WebGestalt: http://www.webgestalt.org/). Pathway enrichment was performed using five embedded databases in WebGestalt including KEGG, Panther, Reactome, Wikipathway, and Wikipathwaycancer. Metal ion homeostasis and transport, fatty acid metabolism and transport are among the statistically significantly altered pathways in Granular cell tumor of the sellar region.
Discussion

GCT of the sellar region is low-grade tumor with slow progression. Clinical presentation of this tumor may include visual deficits, panhypopituitarism, galactorrhea, amenorrhea, diabetes insipidus, neuropsychological changes [5]. One study showed that 47% of symptomatic GCT patients had a mixed suprasellar and intrasellar lesion [6]. MRI studies of this tumor usually show a circumscribed nodule with both homogeneous/heterogeneous enhancement. The other differential diagnoses were craniopharyngioma and meningioma, but those tumors show calcification and dural attachment, respectively [7]. Previous decades, there was confusing nomenclature for sellar/suprasellar tumors, which were GCT, including pituicytoma (PC), spindle cell oncocytoma (SCO), choristoma, infundibuloma, granular cell myoblastoma. In 2011, Covington et al, reviewed 145 cases of these tumors by MRI imaging [6]. They developed criteria for classifying these tumors into three entities (GCT, PC, SCO), considering anatomic location, configuration and enhancement characteristics.

For example, the PC was purely intrasellar while the others were not, and the imaging character of SCO cannot be separated from normal pituitary gland [6]. The microscopic findings of GCT are aggregation of plump polygonal cells with abundant eosinophilic cytoplasm and eccentric nuclei. The nuclei are inconspicuous. The mitotic index is very low. PAS staining of cytoplasmic granules is resistant to diastase digestion. The immunohistochemical studied revealed variably positivity for S100 protein CD68, alpha-1-antitrypsin, alpha-1-antichymotrypsin, GFAP are negative in most tumor. Cytokeratins, chromogranin-A, synaptophysin, desmin, SMA, and the pituitary hormones are negative. In the past, it was postulated that the sellar GCT shared a similar histogenesis to extracranial GCT, as the tumor cells express S100 protein, suggesting that the cell of origin was Schwann cells [8]. Because no Schwann cells in the neurohypophysis, Busy et al, hypothesized sellar GCTs develop from specialized astrocyte, called “Pituicytes” [9].

There are five morphological variants of pituicytes, including major cells, dark cells, granular cells, ependymal cells, and oncocytic cells. A GCT is thought to originate from granular pituicytes. The same authors also found that GCT, PC and SCO show TTF-1 nuclear positive immunoreactivity, suggesting that these tumors may have a similar histogenesis but demonstrate a different morphology(GCT are lysosome-rich while SCO are mitochondrion-rich) [10]. With the current nomenclature, we can call these tumors “TTF-1 - positive tumors of sellar region”. Recently, Chamberlain et al. demonstrated that GCTs have strong nuclear expression of Translocation Factor E-3 (TFE-3). The authors hypothesize that aberrant nuclear TFE-3 accumulation may induced cytoplasmic accumulation of lysosomes in GCT [11]. Some authors have proposed that Galectin-3 is selectively found in PC and SCO [12].

Shibuya studied non-neuroendocrine sellar tumors, and demonstrated that most of PC (7/8 cases) and all SCO (21/21 cases) showed positive immunostaining with Galectin-3, but none of 2 GCT cases were positive. The author proposed that the GCT was a disease entity with uniform clinical and histological characteristics [13]. In our case, the neoplastic granular cells showed diffuse positive staining for Galectin-3. Our result was supported by Mete et al, who revealed that the Galectin-3 showed variably positivity in GCT [14]. However, the significance of staining of Galectin-3, as well as Annexin A1, Bcl-2, Alpha-crystallin B were not reliable in these tumors [15,16]. In 2013, Mete et al. analyzed the status of IDH1 R132H and BRAF V600E alterations, which are usually found in low-grade glial neoplasms. The study revealed no evidence of IDH1-R132H mutation, BRAF V600E mutation, or BRAF-KIAA fusion in PC, SCO, and GCT [14].

Recently, there have been studies reporting about genetic profile of these sellar tumors. Miller et al. demonstrated 3 cases of SCO with MAPK alteration. They performed whole exome sequencing and found that all 3 cases showed activating mutation in HRAS c.182A > G p.(Q61R), but none showed copy number imbalance [17]. Phillips et al, demonstrated copy number imbalances, including losses on chromosome arms 1p, 14q and 22q and gains on 5p [19]. Unfortunately, molecular profile of GCT is lacking [18]. Our study exhibits gain on chromosome arms 1p, 2p, 5q, 10q, 11q, 12q, 16q, and loss on 3q. There is whole arm loss of the X chromosome. The copy number changes in our case are distinct from those in the SCO. Whether these CNVs mediate the morphological differences between a SCO and a GCT remain to be seen. While high grade tumors often have many CNVs that reflect their high proliferation index, both SCO and GCT are indolent making these alterations less like to be random alterations from unfettered proliferation.

Conclusion

GCT of sellar region is a benign tumor and has slow progression, originated from pituicytes in posterior pituitary. This tumor characterized by lysosomal-rich cytoplasm, which is positive to PAS and other lysosomal markers, and express nuclear TTF-1 immunoreactivity. The treatment of choice is surgery. Radiotherapy possibly is an adjuvant treatment, but there are no evidences of prolong survival by radiation nowadays.[19] In this single case, the CNV profile appears distinct from that of SCO. Whether this is a consistent finding remains to be determined with evaluation of additional cases.

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