Cancer Genetics 208 (2015) 55–61

Cancer Genetics

Expression of Kit and Etv1 in restricted brain regions supports a brain-cell progenitor as an origin for cranial germinomas

Chris Tan*, Paul Scotting
Queen's Medical Centre Medical School, Nottingham, United Kingdom

Mismigrating germ-cell progenitors have historically been accepted as the cell of origin for central nervous system (CNS) germinomas. However, an alternative hypothesis suggests that CNS germinomas arise from a brain-cell progenitor. Germinomas often acquire Kit signaling pathway mutations, and there is evidence for an oncogenic relationship between KIT and the ETV1 transcription factor. KIT appears to be necessary to stabilize ETV1, and ETV1 then activates oncogenesis-associated genes. ETV1 expression is not increased by KIT, so ETV1 already needs to be expressed in order for KIT to have an oncogenic function. Therefore, if brain-cell progenitors are the cell of origin for germinomas, those cells would already need to coexpress ETV1 and KIT. We examined Kit and Etv1 in situ hybridization data from the Allen Brain Atlas, for mouse brain tissue at various stages of development. Both Kit and Etv1 were expressed in the regions where germinomas most commonly arise, and in the medulla oblongata. All human cases of germinomas correlated to the regions where ETV1 and KIT are coexpressed. We therefore postulate that germinomas in the brain share a similar mechanism with other KIT-driven cancers, which supports the hypothesis that germinomas arise from a brain-cell progenitor.

Keywords Germ cell tumor, germinoma, medulla oblongata, KIT, ETV

Germinomas are a subtype of central nervous system (CNS) germ-cell tumor (GCT). These tumors are reported to occur predominately in the ventral midline of the brain, and are only rarely reported in the lateral hemispheres. Specifically, germinomas most frequently form in the pineal or suprasellar/hypothalamic regions. In the West, germinomas have a peak incidence in teenagers at around 10–14 years of age (1).

Germinomas are histologically identical to "seminomatous" tumors, occurring at various locations throughout the body. The tumors within this category are largely grouped by their identical histology and protein markers.

Almost all germinomas express high levels of the proto-oncogene *KIT* (also known as mast/stem cell growth factor receptor), in addition to possessing an activating mutation in the *KIT* coding sequence (2). The KIT protein is a tyrosine kinase receptor. The binding of KIT-ligand (also known as Steel) causes two monomers of KIT to dimerize to activate the tyrosine kinase function (3). The activating mutation in the *KIT* gene causes the KIT protein to become constitutively active; in other words, it does not require the usual ligand (Steel) for activation of its tyrosine kinase function. Therefore, KIT continues to signal downstream targets such as the transcription factor ETV1. A study of gastric cancers in mice revealed that the activation of Etv1 function in cells with endogenous Etv1 expression is necessary to cause a cascade of events that eventually leads to a gastrointestinal stromal tumor (GIST) (4). Kit phosphorylates several proteins, and one of those paths leads to the stabilization of Etv1.

The cell of origin for GISTs is derived from a subset of interstitial cells of Cajal (ICC). Chi et al. reported high expression of *Etv1* in these cells, and showed that activation of the KIT pathway triggered the formation of cancer (4). The inhibition of Kit caused a rapid decrease in the amount of Etv1 protein but did not change the level of *Etv1* RNA, which supports the hypothesis that Kit stabilizes the Etv1 protein.

Received July 2, 2014; received in revised form December 24, 2014; accepted December 27, 2014.
* Corresponding author.
E-mail address: ccl.tan@outlook.com

2210-7762/$ - see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cancergen.2014.12.007

Furthermore, the overexpression of endogenous Kit lacking an activating mutation did not have a significant effect on the stability of Etv1.

Etv1 is a transcription factor that promotes the expression of genes related to proliferation and a reduction in apoptosis. Importantly, Chi et al. showed that Kit does not increase the expression level of Etv1 (4); therefore, endogenous Etv1 expression was required in the progenitor cells that formed GISTs. Indeed, a key finding by Chi et al. was that the inhibition of Etv1 prevented tumor formation in cells expressing a mutated form of Kit (4). Etv1 is therefore required for Kit-driven tumor formation. Equally, Etv1 overexpression was shown to have little effect on proliferation, confirming that it is the stabilization of the protein that is important, rather than the amount of mRNA. Since KIT is both mutated and overexpressed in most cases of human germinoma, ETV1 might also be essential to oncogenesis in this tumor type.

The cell of origin for CNS germinomas is contentious. There are two established hypotheses: (1) germinomas arise from a germ-cell progenitor that became misplaced during embryogenesis; or (2) they arise from a brain-cell progenitor (reviewed by Scotting et al. (5), Tan et al. (6), and Oosterhuis et al. (7)). The latter model predicts that the similarity of these tumors results from a similar molecular etiology, rather than from having a shared cell of origin.

ETV1 expression is a prerequisite for the induction of GISTs when KIT is activated (4). If the same relationship applies to germinomas and these are derived from brain-cell progenitors, then ETV1 would be predicted to be expressed in cells in the brain where KIT-positive germinomas occur. We therefore set out to investigate whether Kit and Etv1 might be normally expressed in regions where germinomas form.

Materials and methods

In situ hybridization from the Allen Brain Atlas

The Allen Brain Atlas (8) was used to analyze mouse tissue sections of several different ages and planes, stained for *Etv1* and *Kit* expression. Our lab independently confirmed some of these expression patterns using different *Kit* and *Etv1* probes (data not shown).

Criteria for literature review

The literature was reviewed for cases of germinoma in the CNS to determine the locations where germinomas occur. Cases in which germinomas were mixed with other subtypes were not included in the results.

There were no restrictions on the age of the patient, language of the publication, or access to publication. For inclusion in the results, there must have been a diagnostic scan (e.g., MRI or CT), confirmation of markers, or biopsy; only cases reported between 1990 and 2013 were included.

The main sources of peer-reviewed case reports were PubMed (9) and Web of Knowledge (10). The search terms used were: “germinoma,” “germ cell tumor,” “intracranial,” “central nervous system,” “brain,” “hindbrain,” “medulla oblongata,” and “head,” in various combinations. In all, 23 articles were included; these articles represented 167 cases in total. Each report of a case that matched the criteria was assessed for references to other articles.

ETV1 expression in germinomas

Array data (.CEL) files from Palmer et al. (11) were processed using the statistical package R as described in the reference above. These data were then processed in Excel, and a one-tailed (right tail) Welch *t* test was performed for each gene, comparing seminoma samples to yolk-sac tumor (YST) samples (a P value of <0.05 indicated significantly higher expression in seminoma samples versus YST samples). All the Palmer data is from pediatric patients and is from a range of intracranial and extracranial locations. This comparison was used since our model suggests that ETV1 is important in all germinomas.

The ideal control would be healthy brain tissue, but this would require a biopsy of brains of healthy children of the same age and region as germinoma formation. YSTs arise in the same areas as germinomas and are presumed to be from the same cell of origin regardless of the mechanism of tumor formation. Therefore, comparing YSTs to germinomas allowed for the detection of expression levels in germinomas.

Results

Expression analysis of *Etv1* and *Kit* in the CNS

Histological sections of mouse brain probed for *Etv1* or *Kit* expression by in situ hybridization were collated from the Allen Brain Atlas. The in situ data revealed three main brain regions where *Kit* and *Etv1* had overlapping expression and therefore were coexpressed: the medial habenula (the pineal region), the periventricular thalamic nucleus (approximately the thalamic region), and the medulla oblongata region (at the posterior of the brain near the brain stem) (Figure 1).

The medial habenula is part of the pineal stalk and shows strong and discrete staining for *Etv1* from stage E15.5 through to adulthood (Figure 1). *Etv1* is also expressed in the thalamic region, and this pattern is consistent from E15.5 through to adulthood for *Etv1* (Figure 1).

Kit was broadly expressed across many brain regions. Higher magnification revealed that *Kit* expression was strong in individual cells, but these cells are diffusely distributed, including in the pineal and thalamic areas (Figure 1).

A striking region where both *Kit* and *Etv1* expression was also seen was the hindbrain. Specifically, *Kit* and *Etv1* produced a strong signal in the medulla oblongata, beginning at E15.5 (Figure 2).

Review of CNS germinoma locations

Germinomas are restricted to the ventral midline

It is commonly stated that germinomas arise in the pineal and suprasellar regions, and more rarely in the third-ventricle and basal ganglia regions (12). Therefore, we conducted a literature search and meta-analysis of CNS germinoma locations.