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Biological Behaviour of Craniopharyngiomas

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Short Title: Biology of Craniopharyngioma

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Abstract

Jacob Erdheim (1874-1937) first described craniopharyngioma (CP) as “hypophyseal duct tumours” and postulated the existence of two tumour types based on their histological features: (1) an aggressive type showing similarities to adamantinomas (tumours of the jaw) and (2) a more benign form characterised by the presence of papillary structures. More than a century later, these initial observations have been confirmed; based on their distinct genetic, epigenetic and histological features, the WHO classify CPs as two types, papillary (PCP) and adamantinomatous (ACP). Considerable knowledge has been generated on the biology of CPs in the last 20 years. Mutations in *CTNNB1* (encoding β-catenin) are prevalent in ACP, whilst PCPs frequently harbour mutations in *BRAF* (*p.BRAF-V600E*). The consequence of these mutations is the activation of either the WNT/β-catenin (ACP) or the MAPK/ERK (PCP) pathways. Murine models support a critical role of these mutations in tumour formation and have provided important insights into tumour pathogenesis, mostly in ACP. A critical role for cellular senescence has been uncovered in murine models of ACP, with relevance to the human tumours. Several gene profiling studies of human and murine ACP tumours have identified potential targetable pathways, and novel therapeutic agents are being used in clinical and preclinical research, in some cases with excellent results. In this review, we will present the accumulated knowledge on the biological features of these tumours and summarise how these advances are being translated into potential novel treatments.

Introduction

Craniopharyngiomas (CPs) are benign tumours (WHO grade 1) that develop in the sellar region, which is an anatomical structure limited ventrally by the cranial base, dorsally by the dorsum sella, (with the suprasellar cistern and optic chiasm immediately superior to this), laterally by the cavernous sinuses and carotid arteries and caudally by the brain stem. CPs were first described by the Viennese pathologist Jacob Erdheim in a 200 page-long paper publis. Despite their benign histological nature, CPs can be clinically challenging due to their location and their tendency to invade surrounding structures such as the pituitary gland, hypothalamus and visual pathways. Current treatments are surgery followed by radiotherapy, but these modalities are not always curative and can often contribute to further damage. Overall, CPs are associated with a high degree of morbidity, leading to poor quality of life, and increased mortality o.

There are two types of CPs: (1) The adamantinomatous form (ACP) is the most frequent pituitary tumour in children and shows a bimodal peak of distribution (5-15 years in the childhood-onset ACP and 45-60 years in the adult-onset ACP); (2) The papillary form (PCP, which is mostly an adult tumour (peak at 40-45 years). Research from the last 10 years has demonstrated that these two tumour types represent distinct identities each with specific genetics, epigenetics and pathological features. In this minireview, we will discuss the main features that differentiate ACP and PCP, and elaborate how the biological differences have helped identify novel targeted treatments. Further readings are recommended to cover more detailed pathological and clinical descriptions (e.g. (4, 6-11).

Pathology of craniopharyngioma

ACPs are tumours that usually contain solid as well as cystic components. The solid part of the tumour comprises the epithelial tumour cells, which are highly heterogeneous and include the palisading epithelium (PE), stellate reticulum (SR) and groups of cells forming whorl-like (WL) structures (12) (**Fig. 1**). The PE and SR form finger-like protrusions near the invasive front, which usually contain a string of cell whorls inside (13). Surrounding the epithelial tumour, ACPs often contain glial reactive tissue, mostly comprising astrocytes and immune cells. The proportions of tumour epithelium and glial reactive tissue can vary considerably between ACP samples, for instance, some tumour samples may contain mostly tumour epithelium with little or no reactive glial tissue, whilst others may be comprised mostly of glial tissue with little epithelial component (14). Other histopathological features include calcification, which can be observed by computerised tomography (CT) scans, and the presence of nodules of wet keratin (containing cells without visible nuclei). Both

of these features help establish a diagnosis of ACP. ACP tumours can hold one or several cysts filled with a dark fluid commonly referred to as 'machine oil', which is rich in lipids and inflammatory mediators.

PCPs are solid epithelial tumours, characterised by the presence of a well-differentiated non-keratinising squamous epithelium (SE) supported by fibrovascular cores (FCs) (**Fig. 1**). FCs are tubular structures that contain stroma and blood vessels, lined by a well-defined pseudostratified epithelium (PSE) (**Fig. 1**). PCPs are rarely cystic and do not show calcification.

Genetic and epigenetic alterations in craniopharyngioma

ACP

Mutations in *CTNNB1* were first reported in ACP by Sekine et al (2002) (15). This finding has been subsequently recapitulated in many independent studies and *CTNNB1* mutations have been identified in between 16-100% of the tumours analysed (16). These mutations, which affect mostly the amino acids encoded by exon 3 of *CTNNB1*, are predicted to result in the expression of a degradation-resistant form of the protein leading to the activation of the WNT/β-catenin pathway (17). Failure to identify the mutation in all ACP samples has led to the speculation that other genetic mutations may underlie ACP tumourigenesis. Indeed, coexisting mutations in *p.BRAF-V600E* and *CTNNB1* have been identified in one ACP tumour (18). Sanger sequencing of specific cell populations has furthered controversy on whether the mutations are clonal or present only in some but not all the epithelial tumour cells (15, 19, 20). Recently, laser capture microdissection was combined with *T*agged-*Am*plicon Deep *Seq*uencing (TAm-Seq), an ultrasensitive approach that detects very low mutant allelic frequencies, to screen 22 ACP tumour samples. *CTNNB1* mutations were identified in all samples including those with very low mutant allelic frequencies (John Apps and Martinez-Barbera, Neuropathology and Applied Neurobiology, in press). These data suggest that failure to identify *CTNNB1* mutations in a low proportion of ACP tumours may be due to the lower sensitivity of the sequencing technology used in previous studies (e.g. Sanger sequencing, single-strand conformation polymorphism analysis, exome sequencing and targeted next generation sequencing). Therefore, if there are other oncogenic mutations in human ACP, these are likely to be rare compared with *CTNNB1* mutations.

Murine studies have confirmed that *CTNNB1* mutations are oncogenic drivers, i.e. capable of initiating and sustaining tumourigenesis. The expression of a functionally equivalent form of stabilised β-catenin in either pituitary embryonic precursors or SOX2+ adult stem cells result in

formation of ACP-like tumours in mice (21, 22). These tumours resemble some of the histological and radiological features of human ACP (23), but do not calcify or show wet keratin. A common characteristic in mouse and human ACP is that nucleo-cytoplasmic accumulation of β-catenin occur only in sporadic cells, frequently organised forming cell clusters that overlap with the epithelial whorls previously described or dispersed throughout the tumour as single cells (**Fig. 1**) (24). The reasons why protein accumulation occurs only in a small fraction of the cells, despite the presence of the *CTNNB1* mutation throughout the tumour remain unknown. These cell clusters, showing nucleo-cytoplasmic-accumulation of β-catenin, are not present in PCP or any other pituitary tumour (25). As well as histologically, gene expression profiling has demonstrated that mouse and human clusters are equivalent molecular structures (14). Moreover, the pattern of gene expression in the clusters resembles the 'enamel knot', a critical signaling centre that controls epithelial and mesenchymal interactions during tooth development. These similar molecular signatures have provided a molecular paradigm that explains the histological similarities between ACP and tooth development and tumours of the teeth, which have been reported for over a century (2, 26).

ACPs and PCPs have a low mutation rate (nonsynonymous mutation rate of 0.9 per Mb), which is expected in benign grade I tumours (27). They have stable genomes and gains or losses of large chromosomal regions are rare. In one study, more focal losses and gains of unknown function were identified (28). The methylomes are different between ACP and PCP tumours, a feature that facilitates molecular diagnosis (29, 30), but the functional significance of distinct epigenetic landscapes remains unknown.

PCP

PCPs are likely to be driven by mutations in *BRAF*, specifically *p.BRAF-V600E*. This mutation has been identified in the vast majority of PCP tumours analysed and the expression of the mutant protein confirmed by immunohistochemistry using an anti-BRAF-V600E antibody (**Fig. 1**) (27, 31). Although this mutation is predicted to result in the activation of the MAPK/ERK pathway in all the tumour cells, immunohistochemistry against phospho-ERK1/2 (pERK1/2), a read-out of active MAPK/ERK pathway, has revealed that only a small proportion of epithelial cells lining the fibrovascular cores activate this pathway, despite the expression of BRAF-V600E throughout the tumour (31). In this study, these pERK1/2+ cells were shown to express the pituitary stem/progenitor markers SOX2 and SOX9, suggesting that these lining cells may represent tumour stem cells. Moreover, the vast majority of the Ki67+ proliferative cells are contained within the SOX2/SOX9+ compartment around the

fibrovascular cores. Mouse models expressing the *p.BRAF-V600E* mutation have been generated, but perinatal lethality has prevented assessment of the potential tumourigenic effect (31). Nonetheless, close examination of these murine models has revealed that the expression of this oncogenic driver in early pituitary precursors leads to the expansion of SOX2/SOX9+ stem cells, which are highly proliferative and show impaired differentiation. Together, the mouse and human studies suggest a likely tumourigenic mechanism, by which the activation of the MAPK/ERK pathway within SOX2/SOX9 stem cells may lead to tumour formation.

Cellular senescence in ACP tumourigenesis

Molecular profiling and immunohistochemistry analyses have revealed that the cluster cells in both mouse and human ACP contain senescent cells. Senescence is defined as a cellular state that is characterised by a permanent cell cycle arrest due to the expression of cell cycle inhibitors (e.g. p16 and p21) (32, 33). Senescence is induced by several stressors that cause DNA damage, among them radiotherapy, chemotherapy and oncogenic signalling. Despite the fact that senescent cells are unable to re-enter cell cycle (except if cell cycle arrest pathways are inactivated by genetic or epigenetic mechanisms), these cells are metabolically very active and secrete a plethora of growth factors and inflammatory mediators referred to as the senescence-associated secretory phenotype (SASP) (34). A bulk of research has shown that senescent cells underlie several aging-related diseases or even contribute to organismal aging through SASP activities (35). In cancer, senescent cells are a double-edged sword that can prevent expansion of cells harbouring DNA damage cell autonomously but also promote tumour expansion and progression to malignancy in a cell non-autonomous manner (34, 36).

Studies of the ACP mouse models have provided insights into the role of senescent cluster cells in initiating tumour formation. Initial experiments, in which SOX2+ pituitary stem cells were targeted to express oncogenic β-catenin and simultaneously a fluorescent reporter (e.g. yellow fluorescent protein, YFP), demonstrated that these stem cells are the cell of origin of the β-catenin-accumulating cell clusters, but not of the tumours, which are derived from a different cell lineage (21). Based on these results, a model of paracrine tumourigenesis was proposed, in which the cluster cells may be able to induce tumour formation in a paracrine manner, but the underpinning mechanisms were not understood (**Fig. 2**). More recently, it has been shown that mouse and human clusters contain senescent cells with an activated SASP, and that the attenuation of the senescent/SASP response in

murine cluster cells either genetically or in aged mice, result in a significant reduction in tumour-inducing potential (37, 38).

From biology to novel therapies

The significant increase in knowledge of tumour biology that has accumulated over the last few years has led to the identification of novel targetable pathways in both PCP and ACP. The presence of *p.BRAF-V600E* mutations in PCP patients has provided a molecular rationale for the use of MAPK/ERK pathway inhibitors in these patients. Although the pERK1/2+ cells are just a minority of the tumour cells in PCP tumours (31), the inhibition of the MAPK/ERK pathway using BRAF-V600E or MEK inhibitors, alone or in combination, has given excellent results in the patients (39, 40). The success in these small studies has led to a clinical trial in BRAF-V600E positive PCP patients using a combination of vemurafenib (BRAF-V60E inhibitor) and cobimetinib (a MEK inhibitor) (ClinicalTrials.gov Identifier: NCT03224767).

In ACP tumours, however, the identification of *CTNNB1* mutations leading to the activation of the WNT/β-catenin pathway has not been translated into novel targeted treatments, due to the difficulty of targeting this pathway without causing unacceptable toxicity. However, gene profiling has revealed other potential targetable pathways downstream of the WNT/β-catenin pathway. Inflammatory mediators (e.g. IL6, IL1) have been identified both in the solid and cystic tumour compartments, suggesting a critical role of these factors in ACP pathogenesis (14, 41, 42). Supporting this hypothesis, two patients have been treated with tocilizumab, an IL6 inhibitor, leading to a discreet improvement of disease management (43). Sonic hedgehog, a signaling factor with critical roles during development, was found to be upregulated in mouse and human ACP (44) and further confirmed in other studies (14, 30, 42, 45). The activation of the SHH pathway can be targeted with several inhibitors, including vismodegib, a clinical approved drug that is used against other human cancers (e.g. medulloblastoma). Unfortunately, preclinical data in vitro and in the ACP mouse models as well as patient-derived xenograft mice, have shown that vismodegib treatment lead to increased tumour cell proliferation, premature tumourigenesis and reduced mouse survival (46).

A recent study has revealed that the MAPK/ERK pathway is activated in human and mouse ACP tumours, as evidenced by the expression of p-ERK1/2 (14). Since ACPs do not carry mutations in MAPK pathway components, these data suggest that the pathway is activated in a paracrine manner. Indeed, cluster cells express many ligands known to signal through this pathway such as fibroblast growth factors (FGFs), epithelial growth factor (EGF) and platelet-derived growth factor (PDFG) (14,

44). Interestingly, the inhibition of the MAPK/ERK pathway using the MEK inhibitor trametinib has been shown to result in reduced proliferation and increased apoptosis in both mouse and human ACP tumours in vitro (14). There is currently an open clinical trial of single agent Tocilizumab (IL-6R inhibition; ClinicalTrials.gov # NCT03970226) and other multicenter trials are in development.

Conclusion

ACP and PCP are relatively simple tumours carrying mutations in either in *CTNNB1* or *BRAF* (*p.BRAF-V600E*), respectively. At the cellular level, senescence has been identified as a potentially pro-tumourigenic mechanism that may initiate ACP tumourigenesis in mice and promote growth and invasion in human ACP. The accumulated knowledge on the biology of these tumours is being translated in clinical and preclinical trials testing novel targeted therapies. It is likely these studies will provide efficacious medical treatments against these aggressive tumours.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

This paper was written by both authors.

Figure Legends

Fig. 1. Histological features of human craniopharyngioma.

Top panel. Haematoxylin & eosin staining and immunohistochemistry against β-catenin on human ACP histological sections. Human ACPs are heterogenous tumours containing tumour epithelia (TE) and glial reactive tissue (GRT). Closer examination of the tumour epithelia identifies cells grouped in whorl-like structures (WL), which are surrounded by large cells with empty cytoplasm (stellate reticulum, SR) and a pseudostratified palisading epithelial layer (PE). Immunohistochemistry shows that nucleo-cytoplasmic accumulation of β-catenin occurs mostly in the whorl-like structures (WL).

Bottom panel. Haematoxylin & eosin staining and immunohistochemistry against BRAF-V600E of human PCP histological sections. Human PCPs contain large sheets of squamous epithelia (SE) surrounding by fibrovascular cores (FC), which provide support to the tumour cells. FCs are lined by a pseudostratified epithelium (PSE). Immunohistochemistry shows the expression of BRAF-V600E throughout the squamous epithelium, but not in the fibrovascular cores.

Fig. 2. Schematic showing a working model for the role of the β-catenin-accumulating cell clusters in mouse and human ACP.

Top panel. Expression of oncogenic β-catenin in SOX2+ pituitary stem cells (both embryonic and postnatal) results in the formation of β-catenin-accumulating cell clusters, which contain senescent cells (oncogene-induced senescence). Senescent cluster cells activate a senescence-associated secretory phenotype (SASP), which leads to the synthesis and secretion of a plethora of active peptides, some of which are included in the box. The persistent activity of the SASP factors on surrounding cells eventually causes cell transformation of a cell not of the SOX2 cell lineage (purple cell) and subsequent tumour development in a paracrine manner.

Bottom panel. The human tumour depicted in the schematic derives from a three-dimensional reconstruction of a micro-CT-imaged human ACP sample, in which the glial reactive tissue has not been rendered. Purple indicates the stellate reticulum and cells oft he palisading epithelium, and green represents the β-catenin-accumulating cell clusters. Note the presence of finger-like protrusions of tumour cells, which project away from a tumour epithelium mass, containing a string of clusters inside. These human clusters are molecularly analogous to the mouse clusters and share a signature of senescence and SASP. The model proposes that the SASP activities underlie tumour growth and invasive behaviour by promoting epithelial remodeling and proliferation.

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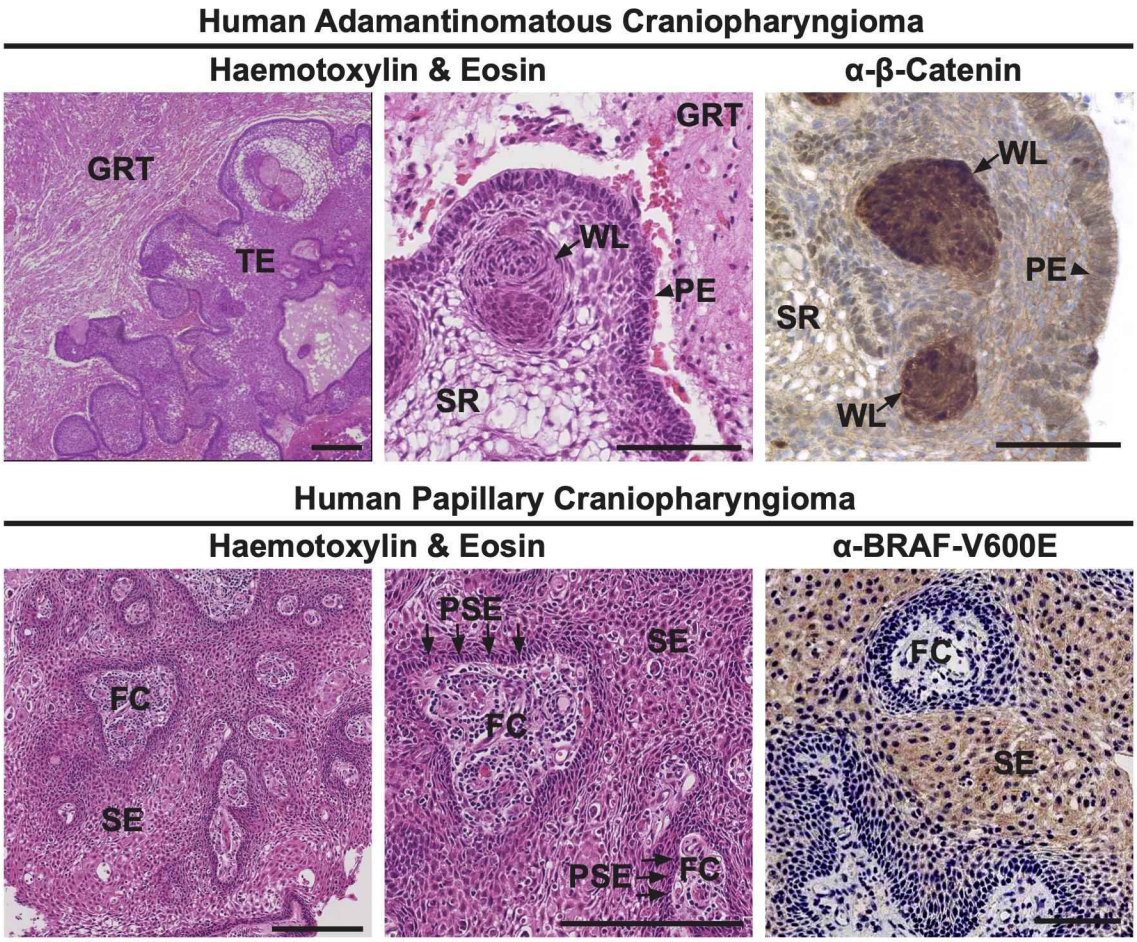
Human Adamantinomatous Craniopharyngioma
Haemotoxylin & Eosin
α-β-Catenin
GRT
TE
GRT
WL
PE
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WL
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WL
Human Papillary Craniopharyngioma
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α-BRAF-V600E
FC
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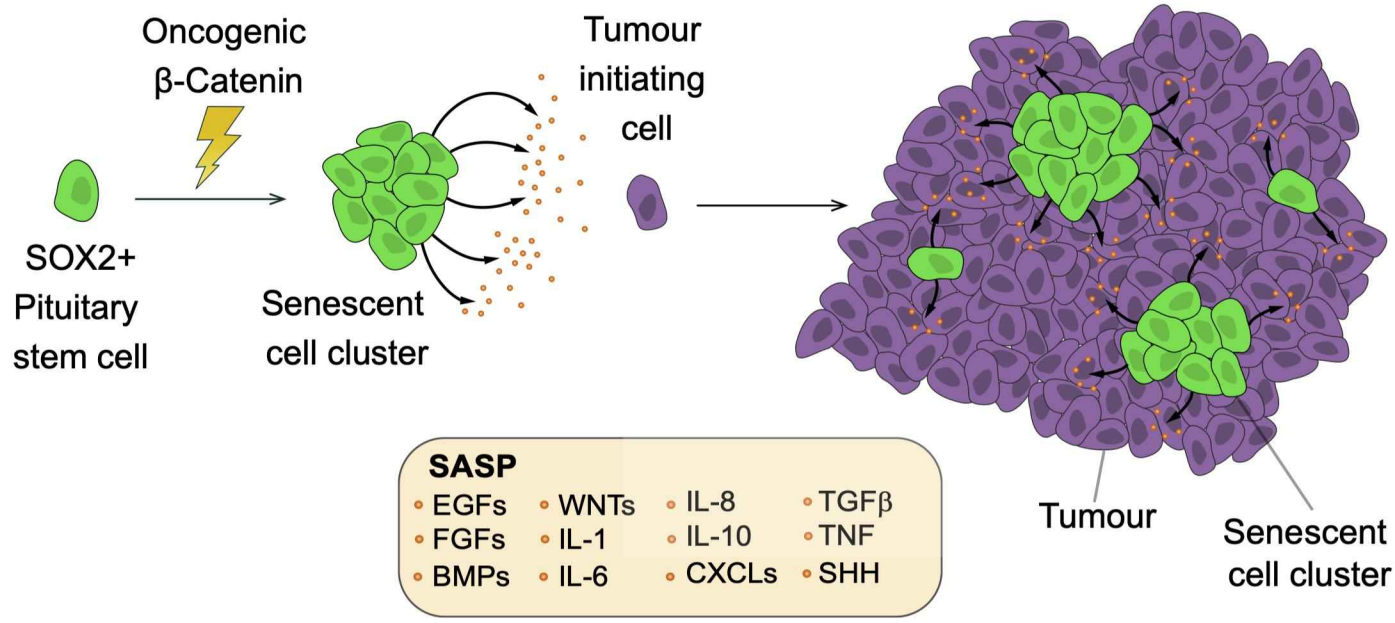
Mouse ACP



Human ACP