A promising future for hypothalamic dysfunction in craniopharyngioma

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Adamantinomatous craniopharyngioma (ACP) is an epithelial tumour caused by mutations in *CTNNB1* (gene encoding beta-catenin), leading to the activation of the WNT/beta-catenin signalling pathway. These tumours usually comprise solid components and cysts, the latter containing a lipid-enriched fluid and inflammatory mediators. Although histologically benign, these tumours can behave aggressively in the clinic. This is due to their tendency to invade nearby structures such as optic pathways, the pituitary gland and hypothalamus, resulting in loss of function. In addition to profound neuroendocrine (e.g. hypopituitarism and diabetes insipidus) and visual impairment, survivors experience symptoms related to hypothalamic dysfunction, such as obesity, disturbed circadian rhythm (e.g. daytime sleepiness), behavioural changes and impaired regulation of thirst, body temperature, heart rate and/or blood pressure. Although survival is high, these defects lead to significant morbidity and poor quality of life for the majority of patients [1,2].

Despite the profound effect it has on patients, little is known about the mechanisms underlying hypothalamic dysfunction in ACP. Hypothalamic defects are often attributed to direct tissue invasion or damage from surgery and radiotherapy [3,4]. In this issue, Wang et al. [5] describe a set of elegant and extensive studies combining analyses on human ACP with detailed functional characterisation of a new hypothalamic injury mouse model to investigate the pathogenesis of the hypothalamic defects in ACP. A main conclusion of this research is that lipid droplets can diffuse from the tumour epithelium into the nearby hypothalamic structures, where they can induce senescence and interfere with hypothalamic neural stem cell differentiation. Therefore, the authors have uncovered that hypothalamic damage can be induced by the tumour in cells that are not in direct contact to the tumour.

They first reveal that areas of the hypothalamus adjacent to the invasive front are deprived of neurons and oligodendrocytes but enriched in undifferentiated cells expressing SOX2 (hypothalamic neural stem cells, SOX2+ htNSCs) and activated microglia. These defects are not observed in other brain tumours such as papillary craniopharyngioma, meningioma and hypothalamic glioma, suggesting a specific ACP defect and more extensive

local tissue damage than can be attributed by direct invasion. Epithelial senescent tumour cells have been shown to participate in the pathogenesis of ACP [6], and further characterisation by immunohistochemistry and RNA-seq reveal that these SOX2+ htNSCs express markers of senescence and are enriched for senescence/SASP signatures. Injection of cystic fluid obtained from human ACP into the hypothalamus of wild-type mice partially phenocopy the hypothalamic defects observed in human ACP, e.g. accumulation of SOX2+ htNSCs and lack of neuronal and oligodendrocyte differentiation, which can be reversed by inhibition of the NFκB pathway. Moreover, these mice show weight gain and display memory loss.

Why are SOX2 htNSCs induced to become senescent? As cystic fluid is rich in lipids, the authors investigate their potential role in this phenotype. This analysis reveals that lipid droplets (LDs) accumulate throughout the tumour epithelia as well as in the adjacent SOX2+ htNSCs. LD accumulation is mediated by the receptor CD36, and using murine SOX2+ htNSCs cultures, the authors show that LD accumulation causes damage to the DNA and mitochondria, thus explaining the senescent phenotype observed. Moreover, their molecular analyses of ACP tumour cells show the enrichment for pathways involved in lipid metabolism, including cholesterol biosynthesis, cholesterol efflux and triglyceride metabolism. Together, these findings suggest that ACP tumour cells' metabolic state leads to high lipid concentration within both the tumour cells and cystic fluid, as well as in the adjacent hypothalamic region.

Having characterised this phenotype, the authors aim to identify new interventions to diminish hypothalamic damage. From the molecular studies, the integrated stress response (IRS), a critical pathway that modulates protein synthesis in response to stress to maintain cellular homeostasis, is found to be significantly upregulated in SOX2+ htNSCs when compared with control htNSCs. Culture of SOX2+ htNSCs in the presence of ISR inhibitors is able to prevent senescence induction and improve neuronal differentiation. Finally, the authors show that oxytocin, a hypothalamic peptide able to improve hypothalamic function in

ACP patients, and whose receptor is expressed in htNSCs and OXT neurons, can also inhibit the ISR and reduce the senescence response in SOX2+ htNSCs in vitro. These findings are further corroborated in vivo in the hypothalamic injury model the authors have developed.

To conclude, the authors analyse the integrity of the brain blood barrier in regions adjacent to the ACP tumour to reveal a lack of integrity and increased permeability, resulting in a significant infiltration of macrophages and T cells, which they hypothesise could also contribute to the hypothalamic dysfunction. To test this hypothesis, they use their murine model of hypothalamic injury, to demonstrate that injection of cystic fluid also results in blood-brain barrier (BBB) disruption and macrophage infiltration, which can be reversed by OXT treatment. Restored BBB function is associated with normalisation of numbers of NPY and proopiomelanocortin neurons, which normally participate in the control of metabolic homeostasis, energy balance and appetite. Not surprisingly, OXT treatment also reduces obesity in mice injected with cystic fluid.

This is a truly impressive piece of research that not only reveals important insights into the pathogenesis of hypothalamic dysfunction in ACP patients, but also provides potential ways to prevent it. OXT is currently being tested in ACP patients, and the authors have revealed the mechanisms by which OXT can reduce weight gain and improve behavioural defects. In addition, they have identified potential new interventions, e.g. preventing lipid accumulation by inhibition of specific lipid metabolic pathways, lipid intake or ISR, which merit further investigation. For example, other important sequelae to craniopharyngioma, such as vasculopathy and visual impairment [1,7] may also be caused or partially caused, by similar mechanisms to those uncovered in Wang et al.. The novel insights provided by Wang et al. will be very informative to develop novel therapies in the near future.

Declaration: The text is the sole product of the authors and no third party had input or gave support to its writing.

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