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Commentary

A novel human tumoroid 3D model of sustained ACTH-secreting cell cultures to study critically needed therapies for Cushing's disease

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The pituitary gland is a master endocrine organ composed of distinct hormone-producing cell types that serve as sensors to integrate central and peripheral signals in order to fine-tune whole-body homeostasis and control multiple physiological functions including growth, reproduction, metabolism and stress [1]. Classically pituitary tumours have been considered rare and benign; however, they represent the most common intracranial neoplasms and are often aggressive. Moreover, these tumours are highly heterogeneous, displaying strikingly diverse clinical behaviours that parallel the different pituitary cell subtypes from which they arise [2]. Currently, the *in vitro* and *in vivo* pituitary tumour models available are highly limited and, in the case of the corticotroph tumours producing adrenocorticotropic hormone (ACTH), have been mainly restricted to ACTH-secreting murine cell lines (*ie,* AtT-20 and AtT-20/D16v-F2) and transgenic/PDX mouse models (eg, *Crh−120/+* and POMC-SV40, among others), respectively [3]. However, extrapolation of the findings derived from these cell-lines and transgenic/PDX mice should be considered with caution as these surrogate murine corticotroph tumour models have been useful models for Cushing's disease but they cannot recapitulate precisely human corticotroph tumours. In this sense, various laboratories have made significant efforts to generate useful data using primary cell cultures from fresh surgically resected human corticotroph tumour tissues to translate it to humans [4]. However, these primary cell cultures have known limitations (eg, low viability and maintenance over time, which also typically lose ACTH hormone production after 1-2 weeks), which clearly emphasized the need to generate novel human models of long-term pituitary tumour cultures [5,6].

In this article of EBioMedicine, Zhang et al. [7] developed a new sustained ACTH-secreting human corticotroph tumoroid cell culture model from surgically resected human tumours based on the identification of four major cell subpopulations derived from human corticotroph tumours [namely corticotroph tumour (73.6%), stromal (11.2%), progenitor (8.3%), and immune (6.8%) cells] as well as on the transcriptional shifts experienced by the cells after several passages. To do that, the authors characterized the transcriptional patterns of these cellular subpopulations at a single cell level (high-throughput scRNA-seq) and compared individualized paired consecutive culture passages using a microarray expression method. Then, they optimized the *in vitro* culture methods to prevent fibroblast overgrowth and to improve the viability of corticotroph tumour cells and their progenitors in order to develop sustained ACTH-secreting human corticotroph tumoroid cultures. Finally, the authors developed an *in vitro* 3D structure that maintains corticotroph tumour cell-cell and cell-extracellular matrix interactions mimicking the tumour microenvironment assembly with a defined medium using matrigel. All these analyses and conditions allowed the development of a 3D culture methodology for research use, able to maintain human corticotroph tumour ACTH secretion and propagate sufficient human corticotroph tumour cells and proliferation of the progenitor cell population over time [7].

From a clinical point of view, this elegant model provides a novel tool to gain a better understanding of the molecular pathogenesis of corticotroph tumours and to identify critically needed therapies for Cushing's disease, with more reproducible results to be applied in humans [eg, wide drug sensitivity screening, detailed mechanism(s) of action of therapeutic agents at the cellular and subcellular level] [7]. Hence, this model solves two challenging aspects to study this tumour type. First, the incidence of these tumours and, thenceforth, the tumour size (3-5mm), which results in insufficient fresh material to carry out robust research [8]. Furthermore, the optimization of this protocol reinforces the well-known inter-patient variability of corticotroph tumor [9], which could be helpful to stablish an *in vitro* personalized patient model looking for a precision medicine [10] for these disabling and often devastating tumours. Therefore, the findings presented in this study provide a novel strategy to improve basic and translational research in pituitary tumours, particularly, in the pathogenesis of corticotroph tumours. Remarkably, further optimization of this new tool would allow applying the two-step 3D culture to

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other types of pituitary tumour (ie, growth hormone-secreting cells), which also exhibit significant limitations in terms of human models.

Contributors

Both authors wrote this commissioned Commentary.

Declaration of competing interest

The authors declare no conflicts of interest.

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