Functional characterization of a novel mutation in PRKCA, the major driver of Chordoid gliomas

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Chordoid gliomas (ChG) are a rare low-grade brain tumour, believed to be derived from Tanycytes. An analysis previously identified a novel mutation present in all ChGs: PRKCA p.D463H (Rosenberg S. et al. 2018, Nat Commun 9(1):2371). This mutation involves a D463H amino acid substitution at the kinase domain of the Protein kinase C alpha (PKCα) and represents the hallmark of ChG. PKCα is a serine/threonine kinase, widely expressed, that carries out the regulation of various functions. The D463H mutation affects a critical residue of the kinase domain of PKCα, suggesting that such change may modify substrate affinity and specificity. Following the purification of the mutated form of PKCα, we have shown it maintains activity by kinase assay using the Promega ADP-Glo activity assay. To study the effect of the mutation on the specificity of the kinase, we designed a peptide array comprising of 384 unique peptides corresponding to phosphorylation sites. To identify if there is a change in substrates between the wild type and mutant, the peptide array analysis will reveal candidate substrates of PKCαD463H. These candidates will be explored in a context that is representative of ChGs, for this we are establishing the PKCαD463H mutation in immortalized astrocytes using a CRISPR/Cas9 approach, as well as primary Tanycyte culture. These models will allow us to study the effect of the mutation on cellular processes, involved in the tumorigenesis. We are also exploring the effect of the D463H mutation on the tertiary structure of the protein and therefore its stability, by producing constructs by mutagenesis and then characterising their sensibility to co-factors again in the Promega ADP-Glo system. The aim of the project is to identify novel and biologically relevant PKCαD463H substrate candidates, which will demonstrate the involvement and the role of this mutated kinase in cellular functions implicated in the development of ChGs.