Pilocytic astrocytoma (PA) is the most common primary brain tumor in children, and various signaling pathways have been implicated in its biology. The Notch signaling pathway has been found to play a role in development, stem cell biology and the pathogenesis of several cancers but its role in PA has not been investigated. We studied alterations in Notch signaling components in clinical PA tumor tissue, to identify much needed therapeutic targets. In this study, we found Notch pathway members to be overexpressed at the mRNA (NOTCH1, NOTCH2, HEY1, HEY2) and protein (HES1) level in PA at various anatomical sites compared to non-neoplastic brain. The changes were not associated with specific BRAF alterations. Inhibiting the Notch pathway in pediatric low grade astrocytoma-derived cell lines (Res 186 and Res 259) using either RNA interference or a gamma secretase inhibitor resulted in a significant, but variable reduction in cell growth and migration. This study suggests a role for Notch signaling in the context of pediatric low grade astrocytoma tumorigenesis, and that Notch signaling is a viable pathway to target therapeutically.