

Targeting Achaete-scute Complex-like1 (ASCL1): A Regulator of Carcinoid Growth and Phenotype

Yash Somnay, BS¹; Jake Eide, BS¹; Renata Jaskula-Sztul, PhD¹; April Harrison, BS¹;
Kevin Simon, BS¹; Selvi Kunnimalaiyaan, MS¹; Herbert Chen, MD, FACS¹;
Muthusamy Kunnimalaiyaan, PhD¹

¹Endocrine Surgery Research Laboratories (ESRL), Department of Surgery, University of Wisconsin School of Medicine and Public Health 3028 Wisconsin Institutes for Medical Research, 1111 Highland Avenue, Madison, WI, 53705

Background: Achaete-scute complex-like1 (ASCL1), a basic helix-loop-helix transcription factor of early neuroendocrine development, is abundantly expressed among neuroendocrine tumors, including carcinoids, but absent in normal adult tissues. We've reported that Raf-1 or Notch pathway activation, or AKT pathway inhibition suppresses carcinoid growth and hormone production. Importantly, this correlates with significantly reduced ASCL1 levels. Thus, we sought to determine firstly, ASCL1's role in carcinoid proliferation and bioactive hormone production, and secondly, its expression following treatment with select small-molecule compounds.

Methods: Human pancreatic BON, and pulmonary H727 carcinoid cells were transfected with either ASCL1-siRNA to suppress ASCL1, or non-specific no target siRNA as a negative control. The effect of siRNA was assessed by Western analysis for ASCL1. BON and H727 were also treated for 4 days with MK-2206, a novel small-molecule compound developed by Merck® for treating solid tumors, after which ASCL1 levels were determined. Levels of bioactive hormones chromogranin A and synaptophysin, and cell cycle arrest markers were also assessed. Methylthiazolyldiphenyl-tetrazolium bromide (MTT) rapid colorimetric assay measured cell viability.

Results: ASCL1-siRNA treatment suppressed ASCL1 expression in both cell lines, while non-specific no target siRNA showed no effect. ASCL1 depletion reduced cell proliferation and levels of chromogranin A and synaptophysin. Notably, cell viability gradually recovered as ASCL1-siRNA-induced suppression diminished and ASCL1 expression was restored. ASCL1 depletion increased p21 and p27 and reduced cyclin B1 and D1 expression, suggesting cell cycle arrest. Furthermore, ASCL1 expression declined following MK-2206 treatment, also inhibiting chromogranin A expression and cell proliferation.

Conclusions: ASCL1-knockdown experiments suggest ASCL1 is necessary for carcinoid bioactivity and viability. Furthermore, agents like MK-2206 may be effective for treating carcinoids given their ability to reduce ASCL1 expression and inhibit growth and tumor marker expression. Understanding the regulatory role of ASCL1 in carcinoid pathogenesis and clinical course may help identify molecular targets for novel treatments.