GATA6 and GATA4 CRISPR Cas-9 and shRNA Technology to Investigate Human Gastric Development and Disease Using Human Organoid Model Systems

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Gastric cancer is one of the most common causes of cancer related deaths, yet little is known about the molecular drivers behind the progression of normal stomach to gastric cancer. GATA4 and GATA6 are transcription factors critical for differentiation and development of the murine gastrointestinal tract. Additionally, GATA4 and GATA6 have been shown to be over-expressed or depleted in several cases of gastric adenocarcinoma in humans. These data provide evidence that GATA4 and GATA6 may be important during human gastric development. Here, shRNA, CRISPR/cas-9 and drug inducible over-expression techniques combined with the differentiation of human induced pluripotent stem cells (hiPSC's) into human gastric organoids (hGOs) were utilized to build tools to study GATA factors during human stomach development. When GATA4 levels are depleted by 70% using an shRNA targeted against GATA4, spheroid generation was disrupted during the differentiation of hiPSC's into hGOs. To study if complete loss of GATA4 alters human gastric development, the generation of GATA4 null iPSC's is currently underway. GATA4 levels can be titrated with doxycycline when G418 resistant hiPSC clones were isolated after infection with a doxycycline inducible GATA4 lentivirus. Additionally, GATA6 null CRISPR rescue hiPSC's were successfully differentiated into early gastric spheroids showing GATA6 is not required for the differentiation of hiPSC derived gastric spheroids. Lastly, glands were isolated from normal human stomach and grown into hGOs that display characteristics of human stomach. Tools generated in this study will allow for the role of GATA4 and GATA6 to be defined during human gastric development.

Awards Won:

National Anti-Vivisection Society: First Award of $10,000

National Taiwan Science Education Center: Taiwan International Science Fair Special Award is a trip to participate in the Taiwan International Science Fair