UAEU جامعة الإمارات العربية المتحدة United Arab Emirates University

The College of Graduate Studies and the College of Medicine & Health Sciences Cordially Invite You to a

PhD Dissertation Defense

Entitled

ESTABLISHMENT OF ORGANOIDS FROM GASTRIC STEM CELLS AND THEIR USE TO STUDY THE ROLE OF ARYL-HYDROCARBON RECEPTORS

by

Shakila Afroz Taleb

Faculty Advisor

Dr. Sherif Karam, Department of Anatomy

College of Medicine & Health Sciences

Date & Venue

5:00 PM

Sunday, 3 May 2020

Medical Education Blue Room, A Block, CMHS

Abstract

The stem cells are powerful tools for disease modeling, drug testing, and tissue engineering for regenerative medicine. However, isolation and culture of organ-specific stem cells are challenging tasks. Therefore, the biological features of many adult stem cells are not well studied and their involvement in the development of cancer is still controversial. Some immortal stem cell lines have been established and used as an alternative to study features of organ-specific stem cells. The ability to grow cells in a scaffold-free, three-dimensional (3D) model system that mimics in vivo conditions would help in revealing more and more properties of stem cells. In this study, two types of 3D culture models were established to define specific properties of gastric stem cells. In the first organoid model, the hanging drop method was used to grow an immortalized mouse gastric epithelial progenitor/stem cells with molecular and morphological features similar to those of stomach stem cells. Within a day, the cells formed a small cluster. When transferred onto the surface of agarose, each cell cluster developed into a spherical organoid which was possible to maintain for several months. The second type of organoid was developed from incipient gastric glands freshly isolated from neonatal mouse stomach using the matrigel. Organoids were developed within a day and were maintained for up to 10 days. The stem cell contribution and cellular dynamics during formation of these organoids were defined using bromodeoxyuridine method. Organoids were further characterized by using calcein and propidium iodide labeling, lectin histochemistry, immunohistochemistry and quantitative reverse-transcription polymerase chain reactions (qRT-PCR). Evidences of differentiation into gastric mucus-producing epithelial cells were detected. To use the gastric organoid model for investigating the role of aryl-hydrocarbon receptors (AhR), their expression levels were first tested using immunohistochemistry. Data revealed that some cells of gastric organoids expressed AhR which were further supported by qRT-PCR. These findings correlated with the cellular expression of AhR in stomach tissues of mice, rats and humans. Immunolabeled cells were located in the middle of gastric glands where dividing stem cells are located and showed both cytoplasmic and nuclear expression. To activate AhR, two-day-old organoids were incubated with 0.1 and 1.0 nM of dioxin for two days. Results revealed upregulation of cytochrome P450 which indicated activation of AhR. There was also upregulation of Oct4 expression which suggested enhancement of self-renewal of gastric stem cells. These findings were supported by upregulation of both AhR and Oct4 in human gastric precancerous and cancer tissues. In conclusion, Gastric organoids were established and used to demonstrate role of AhR on gastric stem cell self-renewal and suggest their possible use as a diagnostic and/or therapeutic target for gastric cancer.

Keywords: Stem cells, Cell proliferation, Cell differentiation, Stomach, 3D culture, Organoids, Aryl-hydrocarbon receptors.