Ruprecht-Karls-Universität Heidelberg
Medizinische Fakultät Mannheim
Dissertations-Kurzfassung

Effects of Gastrointestinal Cancer Cell Secretion on Ion Channel Functions of Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes

Autor: Rujia Zhong
Institut / Klinik: I. Medizinischen Klinik
Doktorvater: Prof. Dr. M. Borggrefe

Background - Cardiovascular disease (CVD) and cancer remain the two most common causes of mortality in developed countries. It is believed that cancer may interact with the host organs both in the microenvironment and at a distant anatomic site. Gastrointestinal (GI) cancers are a group of highly aggressive malignancies with heavy cancer-related mortalities. More and more evidences suggest a correlation between cancer and arrhythmias. However, there are no experimental studies investigating the effects of cancer cell secretion on ion channel function in human cardiomyocytes. DNA methylation, which is mainly established and maintained by DNA methyltransferases (DNMTs) by adding methyl groups to DNA molecules, is one of the mechanisms of epigenetic regulations. Recent researches suggest that DNA methylation can respond to external stimuli and is related to the functional status of cells in cardiovascular disease. Human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have been demonstrated to recapitulate the physiological and pathological features of human cardiomyocytes and therefore are useful for studies on human cardiac diseases.
Purposes - The study was designed to assess the possible effects of gastrointestinal cancer cell secretion on cardiac ion channel functions and explore the underlying epigenetic mechanism by using hiPSC-CMs.
Methods - The hiPSC-CMs generated from human skin fibroblasts of a healthy donor were treated by GI cancer cell (AGS or SW480 cells, cultured for 8 days) medium in different concentrations (the cancer cell secretion groups) or the same medium without cancer cell culture (medium control) for 48 hours, and hiPSC-CMs without any treatment were taken as control group. Then qPCR, patch-clamp, western blotting, immunostaining, dot blotting, bisulfite sequence and overexpression of the ten-eleven translocation (TET) family enzymes were used for the study.
Results - Patch clamp recordings of action potentials (AP) exhibited that the maximum depolarization velocity (V_{max}) and the action potential amplitude (APA) were reduced in cancer cell secretion groups compared with control. The action potential duration at 10% repolarization (APD10) prolonged in cancer cell secretion groups. In ion channel current measurements, peak Na^+ current (I_{Na}) was significantly reduced in cancer cell secretion groups, in agreement with the V_{max} and APA reduction. The transient outward current (I_{to}) was decreased in presence of cancer cell secretion compared with control group, consistent with the APD10 prolongation. Both the late Na^+ and the slowly activating delayed rectifier K^+ current (I_{Ks}) were significantly increased. qPCR results showed that the expression of SCN5A (Na^+ channel, $Na_v1.5$) and KCND3 (I_{to}, $K_v4.3$) were decreased, while SCN10A (Na^+ channel, $Na_v1.8$) and KCNQ1 (I_{Ks}, $K_v7.1$) were increased by cancer cell secretion. Furthermore, the changes of protein expression level of ion channels determined by western blotting and immunofluorescence, respectively, were in consistent with the results of qPCR and current measurements. The whole genome DNA methylation level detected by 5-mC antibody was increased in cancer cell secretion groups, along with increased protein levels of DNMT3A and DNMT3B, but no differences of 5-hmC level were observed. After overexpression of TET1 enzyme that is responsible for DNA demethylation, DNA methylation level of 5-mC of the cancer cell secretion groups was decreased and both the current and protein expression level of I_{to} and I_{Ks} channels were rescued.
Conclusions – Gastrointestinal cancer cell secretion can induce ion channel dysfunctions and subsequently abnormal action potentials, which may contribute to occurrence of arrhythmias in cancer patients. The ion channel dysfunctions might result from DNA methylation of ion channel genes.
Key words – Gastrointestinal cancer cell secretion; hiPSC; cardiomyocyte; electrophysiology; ion channel; DNA methylation.