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RAPID COMMUNICATION

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Deep learning-based identification of sinoatrial node-like pacemaker cells from SHOX2/HCN4 double-positive cells differentiated from human iPS cells

Takayuki Wakimizu MSc[1] | Junpei Naito MSc[2] | Manabu Ishida MSc[2,3] | Yasutaka Kurata MD, PhD[4] | Motokazu Tsuneto PhD[5] | Yasuaki Shirayoshi PhD[5] | Ichiro Hisatome MD, PhD[6]

[1]Division of Regenerative Medicine and Therapeutics, Department of Genetic Medicine and Regenerative Therapeutics, Tottori University Graduate School of Medical Science, Yonago, Japan

[2]ERISA Corporation, Matsue, Japan

[3]Department of Neurology, Shimane University, Izumo, Japan

[4]Department of Physiology II, Kanazawa Medical University, Uchinada, Japan

[5]Genomic Medicine and Regenerative Therapy, Division of Regenerative Medicine and Therapeutics, Faculty of Medicine, Tottori University, Yonago, Japan

[6]Department of Cardiology, National Hospital Organization, Yonago Medical Center, Yonago, Japan

Correspondence
Takayuki Wakimizu, Division of Regenerative Medicine and Therapeutics, Department of Genetic Medicine and Regenerative Therapeutics, Tottori University Graduate School of Medical Science, 86 Nishi-cho, Yonago, Tottori 683-8503, Japan.
Email: taka.11.26.1993@gmail.com

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Abstract

Background: Cardiomyocytes derived from human iPS cells (hiPSCs) include cells showing SAN- and non-SAN-type spontaneous APs.

Objectives: To examine whether the deep learning technology could identify hiPSC-derived SAN-like cells showing SAN-type-APs by their shape.

Methods: We acquired phase-contrast images for hiPSC-derived SHOX2/HCN4 double-positive SAN-like and non-SAN-like cells and made a VGG16-based CNN model to classify an input image as SAN-like or non-SAN-like cell, compared to human discriminability.

Results: All parameter values such as accuracy, recall, specificity, and precision obtained from the trained CNN model were higher than those of human classification.

Conclusions: Deep learning technology could identify hiPSC-derived SAN-like cells with considerable accuracy.

KEYWORDS
automaticity, CNN model, deep learning, human iPS cells, SAN-like cells

1 | INTRODUCTION

Sinoatrial node (SAN) cells exhibit automaticity.[1] The dysfunction of SAN cells causes bradyarrhythmia. Because of the medical shortcomings of artificial pacemakers, the biological pacemaker has been proposed.[2] We reported a novel method to select SAN-like cells from cardiomyocytes derived from gene-manipulated dual reporter human iPS cells (hiPSCs) targeting two SAN-specific genes, SHOX2 (short stature homeobox 2) and HCN4 (hyperpolarization-activated cyclic nucleotide-gated K^+ channel 4), as markers, which enable us to enrich SAN-like pacemaker cells.[3] However, because of the requirement of genetic manipulation, it is difficult to apply this method for the development of biological pacemakers.

It has been reported that the size of cardiac cells and their intracellular organelles are closely related to their physiological properties.[4] The human SAN cell shows spindle shape, and is much smaller than atrial and ventricular myocytes; they have specific action potentials (APs).[5] AP parameters are most useful for the identification of each cell type in cardiomyocytes.[6] Deep learning technology in convolutional neural networks (CNNs) is advancing to identify certain cells through feature values of cell shape. It has been reported to be applied to the identification of hiPSC-derived endothelial cells[7] and quality control of hiPSC-derived cardiac cells.[8] In the present study, we have established the deep learning technology to identify SAN-like cells which exhibit SAN-type-APs from SHOX2/HCN4 double-positive cardiomyocytes differentiated from hiPSCs.

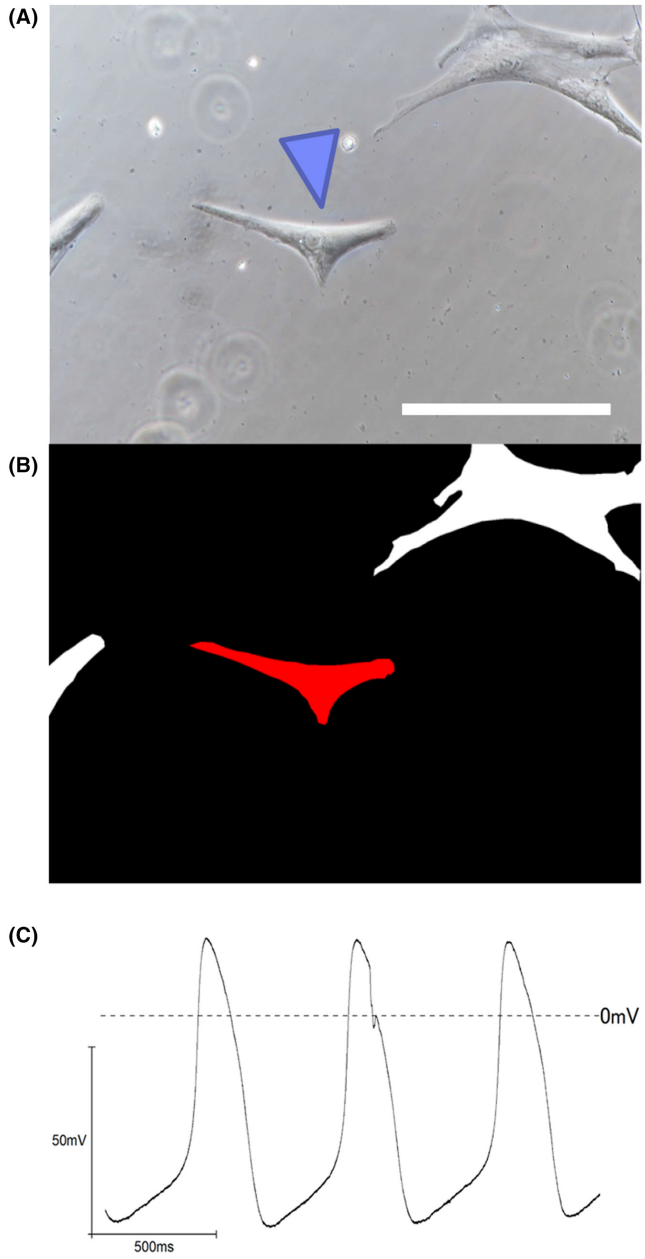


FIGURE 1 Annotation of a phase-contrast image of a hiPSC-derived SAN-like cell showing SAN-type APs. (A) A phase contrast image of the SAN-like cell as indicated by the arrowhead. (B) Annotation of the SAN-like cell colored in red using visual object tagging tool. (C) SAN-type spontaneous APs recorded from the SAN-like cell in the image.

2 | METHODS

2.1 | Measurement of APs of dual reporter hiPSC-derived cardiomyocytes

Cardiac differentiation of dual reporter hiPSCs and AP measurement were performed using the protocol reported previously.[3] "SAN-like cell" was defined as the cell showing SAN-type-APs, according to the criteria described by Ma et al.[9]

(A)

All data	SAN-like cell	non-SAN-like cell
85	42	43

(B)

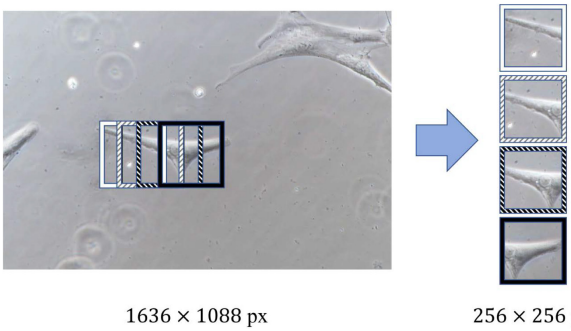


FIGURE 2 Distribution of SAN-like and non-SAN-like cells and extraction of small fraction images from the image of an annotated cell. (A) Total number of images (All data) and numbers of images labeled as SAN-like cell (SAN data) and non-SAN-like cell (non-SAN data). (B) Cutout of the small images (*right*) including the cell fragment (256×256 pixel) from the square areas on the larger image (*left*) of the annotated cell shown in Figure 1 (1636×1088 pixel). The frame lines of the left and right images correspond to each other.

2.2 | Preparation of datasets for training and evaluation of a neural network model

Eighty-five phase-contrast images for hiPSC-derived SHOX2/HCN4 double-positive cardiomyocytes of which electrophysiological properties had been determined were acquired and saved as 1636×1088 pixel gray scale images. Each image contained several cells (Figure 1A) and at least one cell out of them has been identified and annotated (Figure 1B) as "SAN-like cell" having SAN-type APs (Figure 1C) or "non-SAN-like cell" with those of ventricular or atrial-type APs. To generate datasets for training and evaluation of deep learning algorithms, we conducted data augmentation, which is a technique of artificially increasing training data by creating modified copies of a dataset using existing data.[10] First, images were randomly split, 80% and 20% of them were assigned to the training set and the validation set, respectively, so that both sets included SAN-like and non-SAN-like cells (Figure 2A). And then in order to increase images for each training and validation set, 256×256-pixel small fraction images were cut out and saved from top left to bottom right, with 50-pixel stride, so as to cover the entire original image (Figure 2B). Finally, small fraction images which did not include any cell were excluded. The resultant 5415 small fraction images were used as input blocks for training of our deep neural network.

2.3 | Establishment and evaluation of a deep neural network model

To classify a cell in an input image into the SAN-like cell group or the non-SAN-like cell group, we used VGG16-based CNN model,

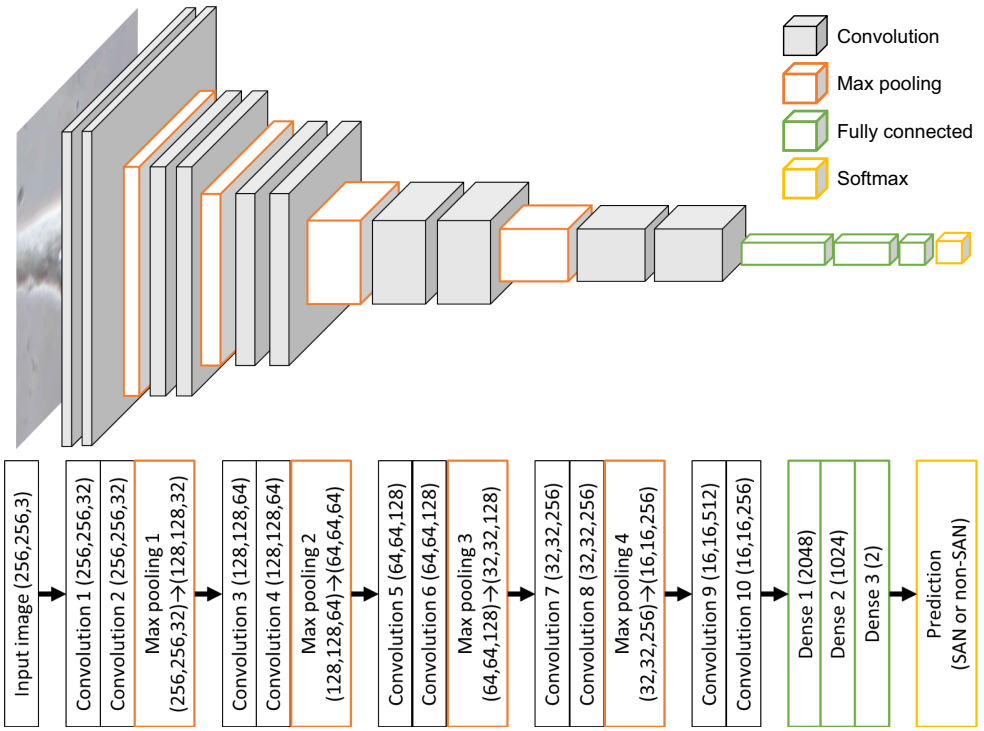
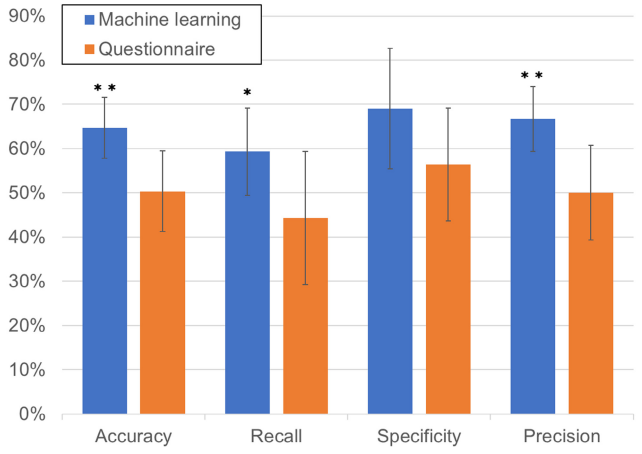


FIGURE 3 Network structure of VGG16-based CNN model. VGG16-based CNN model includes an upstream feature extractor convolutional networks followed by a downstream classifier fully connected networks, which consist of 10 convolutional layers, four max pooling layers and three fully connected layers. Each convolutional layer is connected to rectified linear units for activation. Max pooling layers are used after convolutional layers to reduce the feature size and summarize the feature. Softmax activation function is used in the output layer. Color image data consisting of 256×256 pixels with three channels (red, green, blue) are inputted. As convolutional networks' output is an image of 256×256 pixels with the same width and height, represented by a 32-channel feature map through 1st convolution (convolution 1, 2) followed by applying Max pooling (Max pooling 1) to reduce to 128×128 pixels, is an image of 128×128 pixels represented by a 64-channel feature map through 2nd convolution (convolution 3, 4) followed by applying Max pooling (Max pooling 2) to reduce to 64×64 pixels, is an image of 64×64 pixels represented by a 128-channel feature map through 3rd convolution (convolution 5, 6) followed by applying Max pooling (Max pooling 3) to reduce to 32×32 pixels, is an image of 32×32 pixels represented by a 256-channel feature map through 4th convolution (convolution 7, 8) followed by applying Max pooling (Max pooling 4) to reduce to 16×16 pixels, and finally is an image of 16×16 pixels represented by a 256-channel feature map through 5th convolution (convolution 9, 10), respectively. As fully connected layers, the 1st dense layer compresses the input 65 536-dimensional feature vector into a 2048-dimensional feature space and passes it to a 1024-dimensional feature space through the 2nd dense layer. The final dense layer uses the softmax activation function, which is suitable for classification tasks, to output the probabilities of belonging to the two classes (SAN or non-SAN). To train the network, categorical cross-entropy function is used as a loss function and adaptive moment estimation function is used as an optimizer with a batch number of 32 and a maximum training epoch number of 100.[10]



	Accuracy	Recall	Specificity	Precision
Machine learning	0.64 ± 0.06	0.59 ± 0.09	0.69 ± 0.19	0.66 ± 0.07
Questionnaire	0.50 ± 0.09	0.44 ± 0.12	0.56 ± 0.12	0.50 ± 0.10

FIGURE 4 Performance of the VGG16-based CNN model evaluated using testing data. Accuracy, recall, specificity, and precision of classification data from the model (Machine learning; $n = 5$) were compared with those from the questionnaire for volunteers (Questionnaire; $n = 14$). Statistical significance was determined using the Mann–Whitney U test for two-group comparisons (*$p < .05$; **$p < .01$). Error bars in the bar graph indicate the standard deviation (SD) and the values of Mean ± SD are given in the lower table.

which contains 10 convolutional layers, four max pooling layers, and three fully connected layers.[10] The network structure is shown in Figure 3. The training process was conducted using the TensorFlow/Keras framework on a personal computer (NEC, Tokyo, Japan) with a Core-i9-9820X-CPU (Intel, Santa Clara, CA, USA), 32 GB memory, and GeForce-GTX1080Ti-GPU (NVIDIA, Santa Clara, CA, USA). *Accuracy*, *precision*, *recall*, and *specificity* of CNN model classification were estimated using evaluation data. These parameter values were compared with those estimated by the questionnaire for volunteers ($n = 14$); they took the test to determine whether a cell in image data is classified into the SAN-like cell using the same pictures of cells as for testing the CNN model.

3 | RESULTS

SHOX2-mCherry/HCN4-EGFP double-positive cells sorted by FACS included cells showing SAN-, atrial-, and ventricular-like spontaneous APs. We evaluated our VGG16-based CNN model based on four parameters, *accuracy*, *recall*, *specificity*, and *precision*, of identification data on SAN-like cells with SAN-type APs. To assess the performance of the post-training CNN model, we performed a 5-fold cross-validation using the testing dataset. It takes 0.01–0.02 s to analyze one 256 × 256-pixel picture and around 1 s for one cell image analysis to determine cell type. As shown in Figure 4, the averaged values of *accuracy*, *recall*, and *precision* obtained by the trained CNN model were significantly greater than those obtained from the questionnaire. Matthews correlation coefficient (MCC) is the geometric mean of the regression coefficients of the problem and is robust against imbalanced classes. MCC ranges from +1 to −1, and the prediction is random when MCC is 0.[11] The value of MCC for the CNN model identification was 0.29 ± 0.14, suggesting that our CNN model has greater predictive performance than random selection.

4 | CONCLUSIONS

In this study, we tried establishing a method for automated identification of hiPSC-derived SHOX2/HCN4 double-positive SAN-like cells showing AP waveform characteristics of SAN cells (SAN-type-AP).[3] We found that after training, our CNN model could distinguish SAN-like cells among hiPSC-derived SHOX2/HCN4 double-positive cardiomyocytes by the feature value of cell shape more accurately than human discrimination or random selection as estimated by MCC. This is the first report showing that SAN-like cells differentiated from hiPSCs harboring the SAN-specific marker genes SHOX2 and HCN4 could possess the feature value of their shape as SAN cells. Deep learning technology could identify SAN-like cells exhibiting SAN-type-APs by their shape with considerable accuracy. When we develop the way to identify SAN-like pacemaker cells differentiated from hiPSCs, we could utilize them as biological pacemakers for implantation in the heart of bradyarrhythmia patients.

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CONFLICT OF INTEREST STATEMENT

Authors declare no conflict of interests for this article.

ORCID

Takayuki Wakimizu https://orcid.org/0009-0003-8785-2731

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