

Creation of the mathematical model for the prognostic analysis of human pluripotent stem cells based on their morphological portrait

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The ability of human pluripotent stem cells (hPSCs) for unlimited proliferation and self-renewal promotes their application in the fields of regenerative medicine, new drugs creation, *in vitro* human diseases modeling, cell banks development. The ‘quality control’ of the colony requires information on how morphological parameters of hPSCs are associated with pluripotency maintenance. We analyzed ten morphological parameters extracted from the phase-contrast images of hESC line (H9), control induced pluripotent stem cell line (iPSC AD3), and iPSC CaSR. Each colony was classified, by visual analysis, according to its potential for pluripotency and clonality maintenance, thus defining the colony phenotype as control parameter. Mathematical analysis of the variance of the morphological parameters in groups with different phenotype allows us to select parameters that were potentially informative in determining the phenotype. Features such as Area, Feret’s diameter, Compactness, and Shape factor are among the most prominent under various conditions. These parameters were used as predictors in the phenotype classification model, which was trained to produce high classification accuracy (79 % for colony parameters and 90 % for cellular parameters). Further, we performed qRT-PCR analysis for the expression of 14 pluripotency genes in groups of hPSCs with the same morphological portrait. By addressing the expression variance of the selected genes, we analyzed which ones can be described as the most informative for the separation of different morphological phenotype. Models of phenotype and clonality classification based on these genes expression predict the phenotype and clonality with 100 % accuracy based on the expression of only four genes (*SALL4*, *NANOG*, *REX1*, and *SOX2*). Our results indicate the fundamental possibility of constructing a morphological portrait of a colony informative for the automatic identification of the phenotype and link this portrait to expression of the pluripotency markers.

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