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Zmiany epigenetyczne w żywych komórkach — modelowanie procesu metylacji DNA

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Summary

Epigenetic changes in living cells — modeling the DNA methylation process

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Epigenetic modifications, which include DNA methylation, may contribute to the occurrence and progression of many diseases, including cancer. Methylation, unlike genetic modifications, is a change that does not depend on the DNA sequence, and the epigenome largely remains stable throughout an organism's life. DNA methylation occurs in every living cell and is a reversible epigenetic change, but for this process to make sense, the reverse reaction—demethylation—must also occur.

Methylation involves the attachment of a methyl group to a nitrogenous base, usually cytosine, resulting in the formation of 5–methylcytosine, and this process is catalyzed by enzymes from the DNA methyltransferase group, DNMT1. During demethylation, 5–mC is oxidized to 5–hydroxymethylcytosine, which is an intermediate product. 5–hmC is further converted to 5–formylcytosine and then to 5–carboxycytosine. Enzymes from the TET family are responsible for demethylation: TET1, TET2, TET3. 5–fC and 5–caC are recognized by DNA repair systems, replacing the damaged site with unmethylated cytosine. 5–hmC can be deaminated to 5–hydroxyuracil, which like uracil, can be recognized by SMUG and TDG enzymes, leading to the substitution of cytosine in their place.

In this study, a mathematical model of cytosine methylation and the demethylation of its derivative forms was developed based on data from biological experiments. For model construction, did not employ real concentrations of particular enzymes participating in the reactions but instead, approximated protein levels from the levels of their transcripts as well as the quantities of deoxynucleosides— nucleosides in which the nitrogenous base is connected to deoxyribose.

To be able to calculate the real values of flows between groups of modified cytidines, one needs to know the values of parameters characterizing the action of participating enzymes and the levels of modified cytidines in each group. The model parameters were estimated using the non-negative least squares (NLLS) method. The predictive ability of the model was assessed using leave-one-out cross-validation.

It is not precisely known which of the TET proteins act at which stage of the oxidation of 5-methylcytosine to 5-carboxycytosine (via 5–hydroxymethylcytosine and 5–formylcytosine). In this thesis, a model was selected to explain the role and function of TET proteins.

To find out the role of TETs in different flows, a series of models have been created with all possible combinations of TET1, 2, and 3 actions in different reactions of cytidine

modification, from the full model structure to one where only one TET is working at a given stage. The structure was defined by a 9-element vector of zeros and/or ones. There are 343 possible versions of such models.

According to the model simulations, the best result, indicating the closest fit to the experimental data, was obtained when TET1 and TET2 were active between 5–mC and 5–hmC, with TET3 being excluded, while TET1 and TET3 were active between 5–hmC and 5–fC. However, the performance index of the best model does not differ significantly from that of the second-best and subsequent models. It would be difficult to rely on a single best model, so instead of looking only at the single model structure with the lowest performance index, the next 10 model structures were also considered. Based on these models, the conclusion was drawn that during the oxidation of 5-mdC to 5-hmdC TET3 does not exhibit enzymatic activity; during the oxidation of 5-hmdC to 5-fdC TET2 does not exhibit enzymatic activity; whereas between 5-fdC and 5-cadC, TET1 may be replaced by either TET3 or TET2.

The mathematical model was solved numerically using the ODE function, based on the estimated model parameters, to investigate the impact of changes in TET enzyme levels on the model.

The proposed type of modeling and especially, the way of fitting the models to some experimental data, may provide us with a tool for studies of not only the action of TET enzymes but also other modifications and cellular reactions. Model simulations show that by inactivating one of the TET enzymes in one particular reaction (for example, by specific modification of protein participants in the complex), it is possible to change the level of a single modification while maintaining the levels of other modifications in the cell.