Epigenetic regulation of gene expression in the vascular endothelium

Akademisk avhandling

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av

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Institutionen för kliniska vetenskaper
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I Larsson P, Ulfhammer E, Magnusson M, Bergh N, Lunke S, El-Osta A, Medcalf RL, Svensson PA, Karlsson L, Jern S. Role of Histone Acetylation in the Stimulatory Effect of Valproic Acid on Vascular Endothelial Tissue-Type Plasminogen Activator Expression.
PLoS One. February 2012;7(2):e31573.

II Magnusson M, Lu EX, Larsson P, Ulfhammer E, Bergh N, Carén H, Jern S. Dynamic Enhancer Methylation - A Previously Unrecognized Switch for Tissue-Type Plasminogen Activator Expression.
PLoS One. October 28, 2015;10(10):e0141805.

III Magnusson M, Larsson P, Lu EX, Bergh N, Carén H, Jern S. Rapid and specific hypomethylation of enhancers in endothelial cells during adaptation to cell culturing.
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ABSTRACT

Epigenetic mechanisms, such as DNA methylation and histone modifications, constitute one way for a cell or an organism to respond to changes in the surrounding environment. While histone modifications are recognized to be quite dynamic, DNA methylation has been considered a more stable, or long-term, modification.

Ischaemic heart disease and stroke are major causes of morbidity and mortality in the Western world. In the majority of cases, these conditions are caused by intra-arterial clot formation, which can occur because the components of the haemostatic system are out of balance. This can be caused by either genetic or life-style issues. With this thesis, I have focused on epigenetic regulation of genes in endothelial cells, specifically the *PLAT* gene which encodes the key fibrinolytic enzyme tissue-type plasminogen activator (t-PA).

In Study I, we found that the expression from *PLAT* was induced when endothelial cells were treated with the histone deacetylase inhibitor valproic acid (VPA), and that this indeed was associated with increased acetylation levels around the t-PA promoter. In patients, a defective t-PA expression results in an increased risk of suffering from myocardial infarctions, and the findings in Study I open up for a new possible treatment regimen.

In Study II and III, we used sub-culturing of primary human umbilical vein endothelial cells (HUVECs) as a model of environmental challenge to study how this affects the DNA methylation level, around the t-PA gene (Study II) as well as genome-wide (Study III). In Study II, we found that the DNA methylation level decreased in the t-PA enhancer, but not in the promoter nor in the region immediately upstream of the promoter. This enhancer demethylation was in strong negative correlation with an increase in t-PA gene expression. Thus, methylation in the t-PA enhancer may constitute a previously unrecognized way to regulate the expression of this essential fibrinolytic enzyme.

In Study III, we went on to examine how sub-culturing of HUVECs changed the genome-wide methylation level. We discovered that to passage 4, almost 2% of the investigated sites showed dynamic methylation, mostly displaying decreasing levels. The majority of the differentially methylated sites (DMSs) were annotated as “enhancer”. In addition, we found that several gene ontology terms were highly enriched for among the genes with DMSs situated in their enhancers. Taken together, this indicates that the demethylation process was not random, and that it occurred quite fast.

We suggest that the fibrinolytic enzyme t-PA is dynamically regulated on a transcriptional level by both histone acetylation and DNA methylation, which is important in order for the production of this key enzyme to be able to be rapidly modified locally. We also propose that DNA methylation in endothelial cells is more dynamic than previously recognized, as high levels rapidly can be erased.

Keywords: epigenetics, histone acetylation, DNA methylation, t-PA, *PLAT*, HUVECs, valproic acid, gene expression, enhancers

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