lncRNAs potentially implicated in inflammation related anemia: back and forth between bench and R.

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Résumé

Anemia, characterized by decreased erythrocyte and hemoglobin production, occurs in chronic inflammatory diseases and cancer, affecting more than 30% of cancer patients. Chronic inflammation is one of the main causes and overproduction of pro-inflammatory cytokine TNFα has been incriminated in this process. It has been shown to affect molecular mechanisms of erythropoiesis, including transcription factor and regulatory RNA activities [1]. Based on our recent studies involving microRNAs, we hypothesized that also long non-coding RNAs (lncRNAs) could be targeted by TNFα, contributing to inhibition of erythropoiesis. Long noncoding RNAs have been shown to play a role at several levels of gene expression. Those regulators are involved in transcriptional gene silencing by regulating chromatin structure, RNA processing and by controlling post-transcriptional regulatory steps [2]. To support our hypothesis and discover potential lncRNAs implicated in TNFα-related anemia, we set up a microarray analysis in erythroid differentiation inducible TF-1 cells. The experimental design compared cells treated with EPO (human recombinant erythropoietin, triggering differentiation), or GM-CSF (basal condition) to cells co-treated with TNFα. Our results will describe the analysis pipeline, alternating from bench to computer, from microarray data to finding candidates, confirming them and going further using public data.

Statistical analysis of our microarray dataset revealed that many probes were regulated by both EPO and TNFα, and interestingly, TNFα counteracted the effect of EPO for 137 probes, meaning that those could be part of the process preventing erythropoiesis under inflammation. A smaller number of candidates were selected for bench confirmation and further experiments. To select them, we used two strategies based on correlations, first with known actors of inflammation and erythroid differentiation, and second we computed a sliding correlation with sequence neighbors to find cluster of co-regulated probes. One of the most relevant candidates was ENST00000517927. This lncRNAs was up-regulated about 20 times by TNFα but that no modulation by EPO occurred. In addition, its sequence contained sequences of two microRNAs, miR3142 and miR146a, the second being associated to inflammation by targeting its effector NF-B. Experiments confirmed the link between this candidate and inflammation in several cell lines, including hematopoietic stem/progenitor

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cells. As very little was known about miR3142, we used public data to show that microRNA was expressed, and that its expression was correlated to our candidate expression.Potential targets of those microRNAs were retrieved and annotations allowed to further select those targets associated with inflammation or differentiation for bench confirmation. Our perspectives include bench analysis of miR3142 and its targets.

Références

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