ONDERZOEKS PROJECTEN STAGIAIRES PATHOLOGIE

Titel project: The role of MYC-regulated long non-coding RNAs in Burkitt lymphoma

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Begeleidende analist: Jasper Koerts

Korte theoretische beschrijving:
Burkitt lymphoma (BL) is a highly aggressive B-cell lymphoma that mostly affects children and young adults. A chromosomal translocation at the *myc* locus is the hallmark for BL and results in overexpression of the oncogenic transcription factor MYC. This causes deregulation of MYC target genes, ultimately inducing cellular growth, blockade of cell differentiation and transformation. Apart from protein-coding genes, also non-coding RNAs have been identified as MYC targets. These include microRNAs and long non-coding (lnc)RNAs. LncRNAs function in normal cellular homeostasis on chromatin structure, transcription or posttranscriptional regulation. Aberrant expression of lncRNAs has been reported to be critically involved in various aspects of tumorigenesis. We identified MYC-regulated lncRNAs using the MYC inducible B-cell lymphoma cell line P493-6. In addition, we determined which lncRNAs are differentially expressed between BL cell lines and normal germinal center (GC) B-cells and between primary cases of BL (with high MYC levels) in comparison to chronic lymphocytic leukemia (CLL, low MYC levels). Based on these data, we identified 22 lncRNAs that are induced by MYC, upregulated in BL compared to normal GC B-cells and expressed at higher levels in primary BL as compared to CLL. In this project we want to unravel the role of these MYC-regulated lncRNAs in BL.

Korte praktische beschrijving:
To explore the role of MYC-induced lncRNAs in BL first we will determine which candidate lncRNAs affect growth of BL cells. To this end lncRNAs will be downregulated and we will asses the effect of lncRNA downregulation on cell growth. Based on the severity of lncRNA knockdown-induced phenotype we will select up to 3 lncRNAs for further functional study. First, we will determine whether the mechanism underlying the observed effect on cell growth is related to apoptosis or proliferation. Then, we will further characterize candidate lncRNAs to fully understand their function in BL pathophysiology. We will analyze their subcellular localization (nuclear or cytoplasmic), as this is an important determinant of lncRNA function. We will also aim to solve the secondary structure of lncRNAs and to identify their binding partners (DNA, RNA and protein).Target gene levels will be manipulated in phenotype copy and rescue experiments to confirm the relevance of the interaction for the observed phenotype. Finally, we we will confirm expression pattern of the lncRNAs and their binding partners in a cohort of BL patients and controls

Technieken:
cell culture, cloning, gene downregulation and overexpression, DNA/RNA isolation, quantitative RT-PCR, transfection, CRISPR-Cas9 system, flow cytometry, RNA-FISH, chemical probing of RNA secondary structure (SHAPE), RNA immunoprecipitation

Gevraagd wordt: A student interested in molecular biology techniques

Duur van de stage: minimaal 6 months

Informatie:

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