Characterizing the role of long non-coding RNAs as epigenetic regulators in disease model

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ABSTRACT

Long non-coding RNAs (lncRNAs) are a class of biological molecules which are transcribed from DNA but are not translated into any protein. LncRNAs have been identified as critical players in gene regulation. Misregulation of lncRNAs has been considered as one of the underlying causes for cancer pathogenesis and in other human diseases. In the current thesis, I have addressed the epigenetic roles of lncRNAs in regulating gene expression in cell line based and disease model systems.

We investigated the functional role of lncRNAs in the maintenance of active chromatin by sequencing lncRNAs associated with active chromatin enriched with H3K4me2 and WDR5. We identified 209 lncRNAs to be commonly enriched in H3K4me2 and WDR5 pulldown chromatin fractions and we named them as active chromatin associated RNAs (active CARs). Interestingly, 41% of active CARs mapped to divergent transcription units having transcription factor genes as their partner. CARs were found to regulate the expression of partner protein coding genes at the transcriptional level by recruiting WDR5 to maintain the active histone marks H3K4me2/H3K4me3 at these promoters. Depletion of active CARs results in reduced WDR5and H3K4me2/H3K4me3 occupancy at these promoters. However, in absence of WDR5, we found the levels of H3K4me2 to remain unchanged at divergent promoters. Taken together our findings indicates that, conversion of H3K4me2 to H3K4me3 is mediated via active CARs-WDR5 interaction at the active divergent promoter, whereas, the maintenance of H3K4me2 marks appears to be WDR5 independent.

Additionally, we used transcriptome profiling approach, to identify lncRNAs that are differentially expressed between low- and high- risk neuroblastoma tumours. We report *NBAT-1* lncRNA as an independent prognostic biomarker in predicting clinical outcome of neuroblastoma patients. The expression profile analysis showed *NBAT-1* to be lowly expressed in high-risk tumours relative to low-risk tumours. Using cell line and mouse models we characterized *NBAT-1* as a tumour suppressor lncRNA which regulates gene expression by interaction with PRC2 repressive chromatin complex. *NBAT-1* lncRNA promotes differentiation and acts as a tumour suppressor by epigenetic regulation of genes to inhibit cell proliferation and invasion.

Thirdly, we sought to study genomic imprinting in a disease model. Genomic imprinting is an epigenetic regulation of gene expression in a parent of origin-specific manner. Studies in mouse have identified *Kncq1* imprinted domain to be epigenetically regulated by a 91kb long lncRNA *Kcnq1ot1* which is expressed from the paternal chromosome to silence imprinted genes in *cis*. Using BW-syndrome human disease model, we identified a maternal 11p15.5 micro duplication which included the 5′ 20 kb of the non-coding *KCNQ1OT1* gene. Its maternal transmission was associated with ICR2 hypomethylation and familial BWS phenotype. Normally ICR2 is methylated to repress *KCNQ1OT1*, thereby allowing maternal copies of the imprinted genes including growth inhibitor *CDKN1C* to be expressed. We demonstrated that this duplicated maternal *KCNQ1OT1* RNA also interacts with chromatin through its most 5′ 20 kb sequence to silence *CDKN1C*. This provides a mechanism for biallelic silencing of *CDKN1C* which contributes to the BWS disease phenotype.

In summary, by ChRIP-seq, RNA expression profiling in tumours and human patient-derived cell line based model systems, we have uncovered new roles of lncRNA in epigenetic gene regulation.

Keywords: Long non-coding RNA, Epigenetics, Active Chromatin, Genomic Imprinting, Neuroblastoma, Beckwith-Wiedemann Syndrome.

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Akademisk avhandling

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Av

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Avhandlingen baseras på följande delarbeten:

I. H3K4me2 and WDR5 enriched chromatin interacting long non-coding RNAs maintain transcriptionally competent chromatin at divergent transcriptional units. **Mishra K**, Subhash S, Akhade VS, Kanduri M, Mondal T and Kanduri C (manuscript).

II. The Risk-Associated Long Noncoding RNA NBAT-1 Controls Neuroblastoma Progression by Regulating Cell Proliferation and Neuronal Differentiation. Pandey GK, Mitra S, Subhash S, Hertwig F, Kanduri M, **Mishra K**, Fransson S, Ganeshram A, Mondal T, Bandaru S, Ostensson M, Akyürek LM, Abrahamsson J, Pfeifer S, Larsson E, Shi L, Peng Z, Fischer M, Martinsson T, Hedborg F, Kogner P, Kanduri C. ***Cancer Cell,*** 2014 Nov 10; 26(5):722-37. doi: 10.1016/j.ccell.2014.09.014.

III. The *KCNQ1OT1* imprinting control region and non-coding RNA: new properties derived from the study of Beckwith–Wiedemann syndrome and Silver–Russell syndrome cases. Chiesa N, De Crescenzo A, **Mishra K**, et al. ***Human Molecular Genetics***. 2012; 21(1):10-25. doi:10.1093/hmg/ddr419.

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