IDENTIFICATION OF MOLECULAR TARGETS OF SWI/SNF ALTERATIONS IN CANCER DEVELOPMENT

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ABSTRACT

SWI/SNF chromatin remodelling complex has been described to be altered in nearly 20% of all human tumour types, which places it among the most broadly mutated molecular systems in human cancer, just after TP53. However, the molecular mechanism underlying its involvement in tumour progression remains elusive. Among the different subunits of the complex, ARID1A has been identified as one of the most frequently mutated genes in several malignant histologies, such as gynaecological and intestinal tumours. Therefore, the main purpose of this Master’s thesis is to get a further insight into the transcriptional alterations resulting from ARID1A-deficiency in different cellular contexts.

In order to achieve this goal, we have generated stably-transduced cell lines for a dosyicyte-inducible vector that directs the expression of different shRNAs targeting ARID1A in different human cancer cell lines. After the verification of an effective ARID1A knock-down, RNA-Seq experiments revealed both shared and tissue specific molecular pathways altered in the different cell lines. Among them, it should be highlighted an upregulation of genes belonging to proliferative pathways, as well as a downregulation of genes involved in apoptosis, which suggests an augmentation in their oncogenic capacities. What is more, there was also an upregulation of genes involved in cell migration, which might imply a potential increase in their metastatic capacities. Finally, gene set enrichment analysis showed a significant upregulation of genes related to the immune response. These results might help to clarify the molecular pathways underlying the role of ARID1A alteration in tumour progression and they could also suggest new therapeutic opportunities for SWI/SNF-deficient tumours.

RESULTS

Transcriptional alterations in ARID1A-deficient SK-OV-3 cell line

Figure 4. Differently expressed genes in ARID1A-deficient SK-OV-3 cells. A) Volcano plot representing the log(adjusted p-value) versus log(FoldChange) of expressed genes in SK-OV-3 cells after ARID1A knock-down. Upregulated genes (absolute log(FoldChange)>0.5 and adjusted p-value <0.05) are represented in red upregulated or blue (downregulated), whereas not significantly altered genes are shown in grey. B) Bar chart representing the number of up and down regulated genes after ARID1A knock-down.

CONCLUDING REMARKS

1. We have generated stably-transduced SK-OV-3, Caco-2 and A549 cell lines that inducibly express shRNAs for ARID1A and ARID1B, efficiently repressing the expression of both genes.

2. ARID1A knock-down in different cell lines results in the alteration of both shared and tissue specific molecular pathways.

3. ARID1A deficiency in both SK-OV-3 and Caco-2 cells is accompanied by an upregulation of genes associated with proliferation and a downregulation of genes involved in apoptosis.

4. ARID1A deficiency in SK-OV-3 cells is accompanied with an upregulation of genes involved in DNA repair, which suggests an increase in genomic instability.

5. ARID1A deficiency in Caco-2 cells correlates with an overexpression of genes involved in chemotaxis and cell migration, which might imply a potential increase in their metastatic capacities.

6. ARID1A deficiency in SK-OV-3 and Caco-2 cells alters the expression of genes associated with different sensitivities to immunotherapy, which could bring new therapeutic opportunities for cancer patients.

ON-GOING WORK

- Proliferation assays on ARID1A deficient cell lines
- Characterization of the effect of ARID1A deficiency on DNA repair mechanisms
- Co-culture of ARID1A deficient cell lines with Tcell and NK cell lines to study their sensitivities to immunotherapy
- Invasion and migration assays on ARID1A deficient Caco-2 cells
- RNA-Seq analysis of ARID1A and ARID1B deficient A549 cell lines
- Verification of ARID1A knockdown at protein level
- Study of the potential synthetic lethality among subunits of SWI/SNF complex and between this complex and canonical pathways deregulated in cancer

METHODOLOGY

- Transduction
- Cell sorting
- eDNA NGS libraries
- RNA-seq analysis

Figure 3. Workflow schematic. 1) SK-OV-3, Caco-2 and A549 cell lines were transduced with pTRIPZ constructs harbouring shRNAs targeting different subunits of the SWI/SNF complex. 2) Since selected stable cell lines were sorted according to shFP expression. 3) After inducing the downregulation of ARID1A or ARID1B for at least 5 days, RNA-Seq libraries were prepared and sequenced. 4) Finally, RNA-seq results were analysed to characterise the transcriptional alterations after ARID1A downregulation in different cellular contexts.

REFERENCES