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 human malignancies, 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 resulted from *ARID1A*-deficiency in different cellular contexts.

In order to achieve this goal, we have generated stably-transduced cell lines for a doxycycline-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.

INTRODUCTION

Major subclasses of mammalian SWI/SNF complex

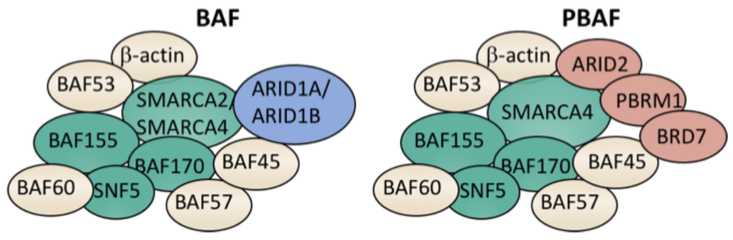


Figure 1. SWI/SNF complexes are composed of evolutionarily conserved core subunits (green) and variant subunits (yellow). ARID1A and ARID1B (blue) are unique to BAF complexes, whereas ARID2, PBRM1 and BRD7 (red) are only part of PBAF complexes. Imagen adapted from *Wilson and Roberts* (2011)[1].

Mutational profile of the variant subunits ARID1A and ARID1B in human cancer

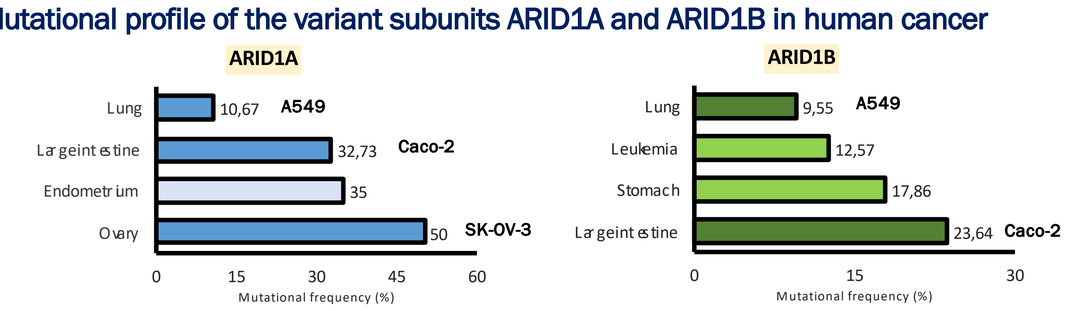


Figure 2. Reported mutational frequencies of ARID1A and ARID1B in different tumour types[1,2,3]. The human cancer cell lines selected for this master's thesis are indicated next to the corresponding tumour tissue.

METHODOLOGY

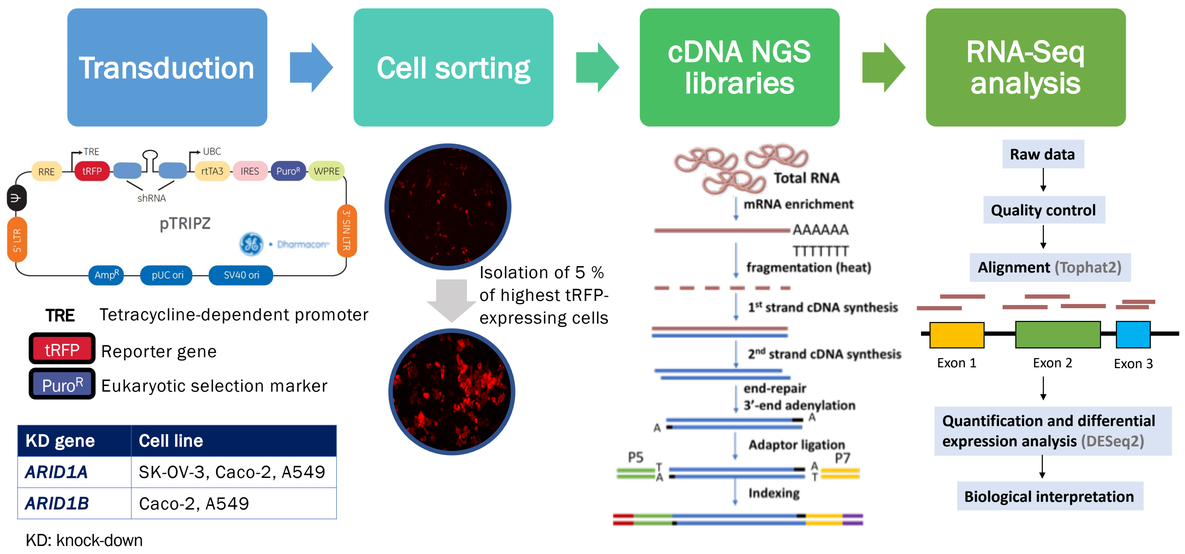
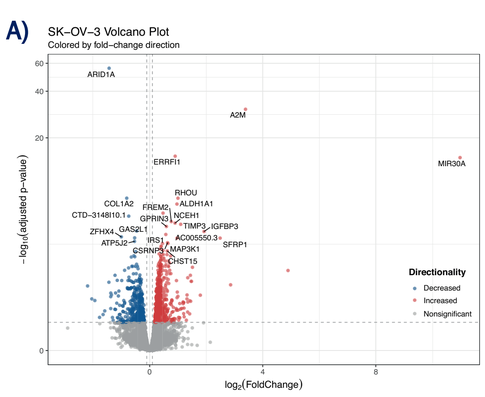


Figure 3. Workflow schematic representation. 1) SK-OV-3, Caco-2 and A549 cell lines were transduced with pTRIPZ constructs harbouring shRNAs targeting different subunits of the SWI/SNF complex. **2)** Once selected, stable cell lines were sorted according to tRFP expression. **3)** After inducing the 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 characterize the transcriptional alterations after *ARID1A*-downregulation in different cellular contexts.

RESULTS

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



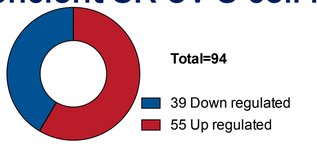


Figure 4. Differentially expressed genes in *ARID1A*-deficient SK-OV-3 cells.

A) Volcano plot representing the -log10(adjusted p-value) versus log2(FoldChange) of expressed genes in SK-OV-3 cells after *ARID1A*-downregulation. Dysregulated genes (absolute log2(FoldChange)>0.5 and adjusted p-value <0.05) are represented in red (up regulated) or blue (down regulated), whereas not significantly altered genes are shown in grey.

B) Doughnut chart representing the number of up and down regulated genes after *ARID1A* knock-down.

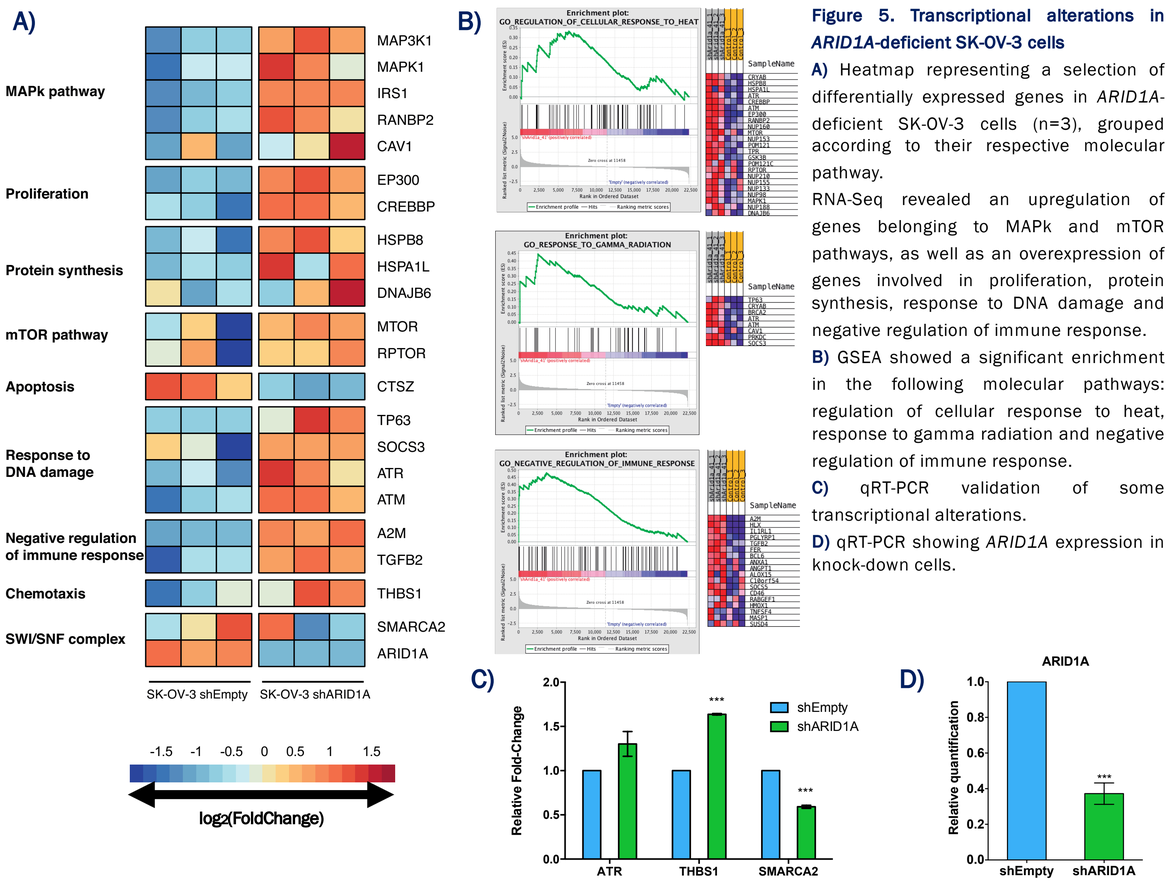


Figure 5. Transcriptional alterations in *ARID1A*-deficient SK-OV-3 cells

A) Heatmap representing a selection of differentially expressed genes in *ARID1A*-deficient SK-OV-3 cells (n=3), grouped according to their respective molecular pathway.

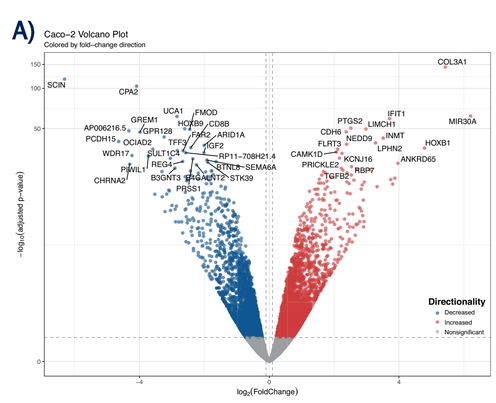
RNA-Seq revealed an upregulation of genes belonging to MAPk and mTOR pathways, as well as an overexpression of genes involved in proliferation, protein synthesis, response to DNA damage and negative regulation of immune response.

B) GSEA showed a significant enrichment in the following molecular pathways: regulation of cellular response to heat, response to gamma radiation and negative regulation of immune response.

C) qRT-PCR validation of some transcriptional alterations.

D) qRT-PCR showing *ARID1A* expression in knock-down cells.

Transcriptional alterations in *ARID1A*-deficient Caco-2 cell line



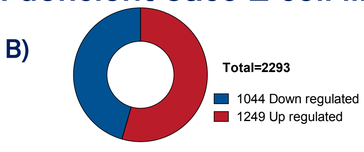


Figure 6. Differentially expressed genes in *ARID1A*-deficient Caco-2 cells.

A) Volcano plot representing the -log10(adjusted p-value) versus log2(FoldChange) of expressed genes in Caco-2 cells after *ARID1A*-downregulation. Dysregulated genes (absolute log2(FoldChange)>0.5 and adjusted p-value <0.05) are represented in red (up regulated) or blue (down regulated), whereas not significantly altered genes are shown in grey.

B) Doughnut chart representing the number of up and down regulated genes after *ARID1A* knock-down.

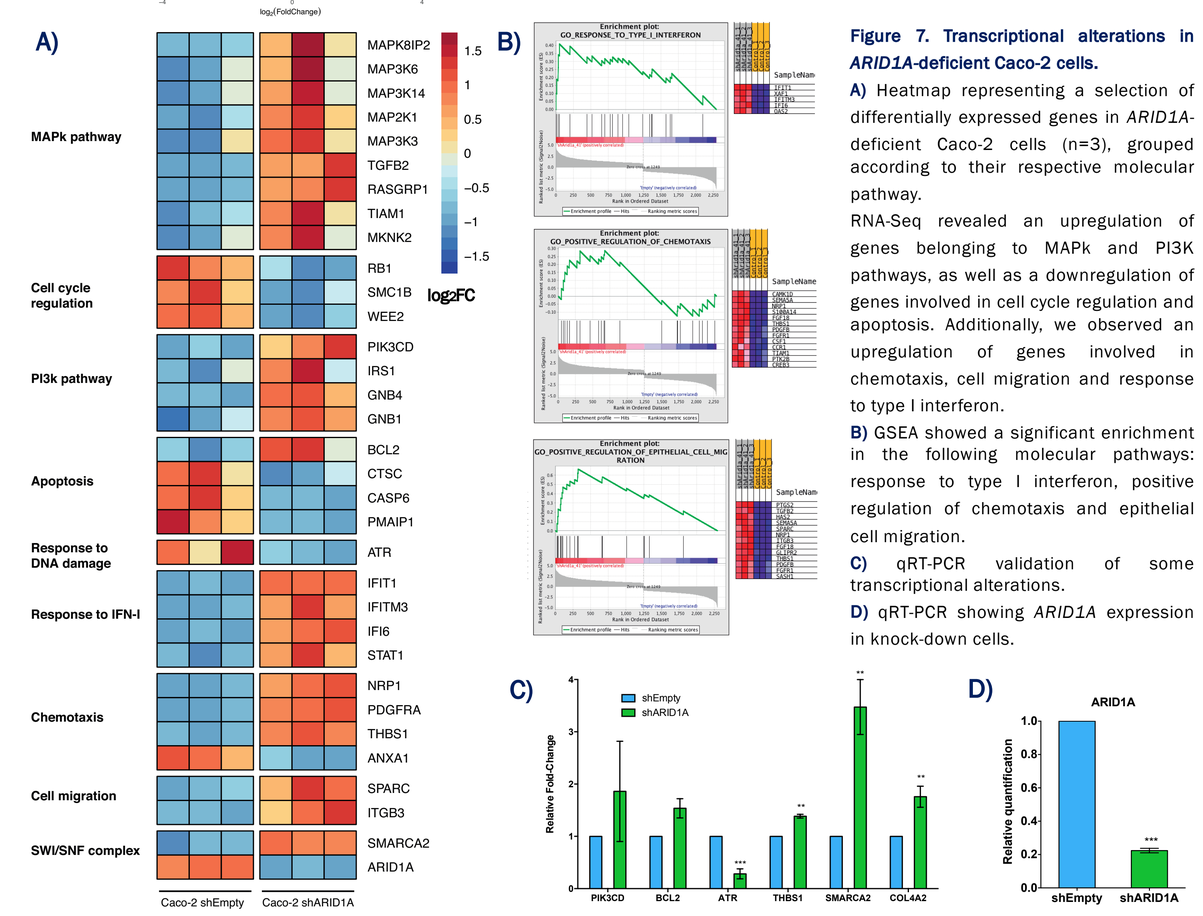


Figure 7. Transcriptional alterations in *ARID1A*-deficient Caco-2 cells.

A) Heatmap representing a selection of differentially expressed genes in *ARID1A*-deficient Caco-2 cells (n=3), grouped according to their respective molecular pathway.

RNA-Seq revealed an upregulation of genes belonging to MAPk and PI3K pathways, as well as a downregulation of genes involved in cell cycle regulation and apoptosis. Additionally, we observed an upregulation of genes involved in chemotaxis, cell migration and response to type I interferon.

B) GSEA showed a significant enrichment in the following molecular pathways: response to type I interferon, positive regulation of chemotaxis and epithelial cell migration.

C) qRT-PCR validation of some transcriptional alterations.

D) qRT-PCR showing *ARID1A* expression in knock-down cells.

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 with 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 T-cell 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 knock-down at protein level
- ✓ Study of the potential synthetic lethality among subunits of SWI/SNF complex and between this complex and canonical pathways dysregulated in cancer

References

1-Wilson, B. G. & Roberts, C. W. M. SWI/SNF nucleosome remodellers and cancer. *Nat. Rev. Cancer* **11**, 481–492 (2011).

2-Kadoch, C. et al. Proteomic and bioinformatic analysis of mammalian SWI/SNF complexes identifies extensive roles in human malignancy. *Nat. Genet.* **45**, 592–601 (2013).

3-COSMIC. Cell Lines Project v85. https://cancer.sanger.ac.uk/cell_lines