UNVEILING THE ROLE OF CD81/TSPAN28 IN COLON CANCER: INSIGHTS INTO PROLIFERATION, CELL CYCLE PROGRESSION, AND MOTILITY

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Background

- Tetraspanins (TSPANs) is a family of 33 conserved transmembrane proteins that interact with proteins at the cell membrane.
- They modulate diverse biological processes under physiological conditions or cancer.
- CD81 (TSPAN28) belongs to the TSPAN family and is shown to regulate cell growth, differentiation, and motility.
- Its role in carcinogenesis is context-dependent, functioning as either a tumour promoter or suppressor.
- CD81's contribution to colon cancer remains unclear.

Objective

This study aims to investigate the molecular mechanisms by which CD81 influences colon cancer cell behaviour *in vitro*.

Methods

- CD81 knockdown (CD81KD) was achieved in DLD1 colon cancer cells using shRNA in pSiren retroviral vectors.
- Cell proliferation was monitored via real-time imaging using the IncuCyte ZOOM system.
- Cell motility was assessed through wound healing assays.
- Tumorigenic potential was evaluated using an anchorage-independent growth assay.
- RT-qPCR was used to analyze the effects of CD81 on key cell cycle regulators (E2F1, CCND1, and CDC6).

Conclusions

- CD81 plays a crucial role in colon **cancer progression** *in vitro* by enhancing cell proliferation, motility and colony formation.
- The observed downregulation of E2F1 and CDC6 following CD81KD suggests a potential mechanism by which CD81 influences cell cycle progression.

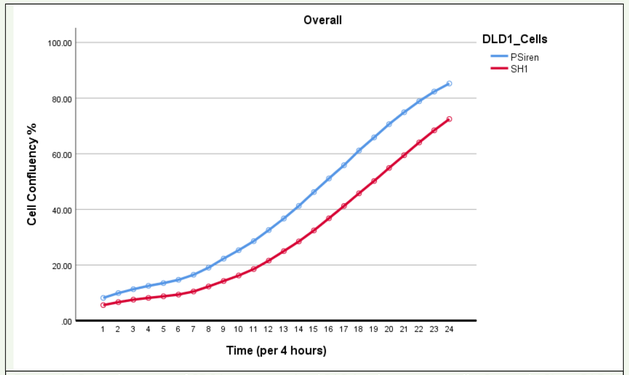

Figure 1. *Growth curves of CD81-compromised DLD1 colon cancer cells*. Control pSiren DLD1 cells (blue), and CD81^{KD} DLD1 cells (red) (n=26 wells examined per cell line). Repeated Measure ANOVA **p=0.049**

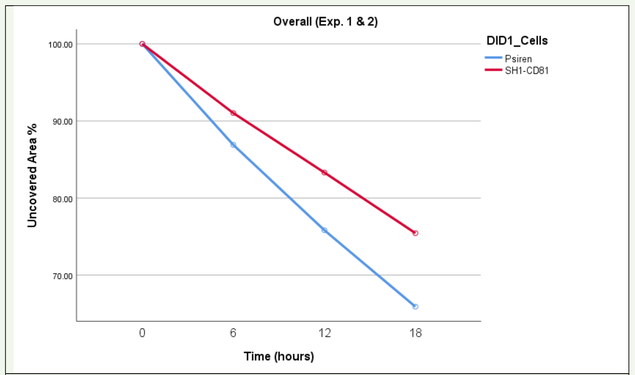

Figure 2. *Wound closure rate of CD81-compromised DLD1 colon cancer cells*. Control pSiren DLD1 cells (blue) (n=20), and CD81^{KD} DLD1 cells (red) (n=36). Repeated Measure ANOVA **p<0.001**

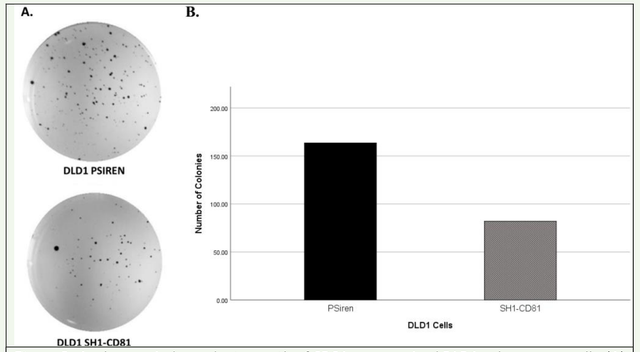

Figure 3. *Anchorage independent growth of CD81-compomised DLD1 colon cancer cells*. (A) Colony formation in agar. (B) Mean colony number. Control pSiren DLD1 cells (n=82 colonies), and CD81^{KD} DLD1 cells (n=163 colonies) (t-test ***p = 0.002***)

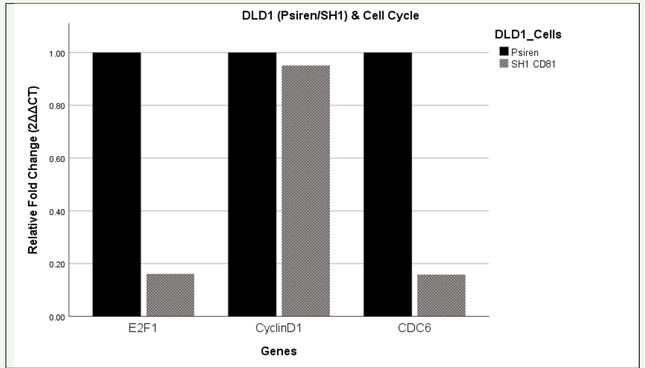

Figure 4. *Effect of CD81 on expression of cell cycle genes E2F1, Cyclin D1 and CDC6 on DLD1 colon cancer cells*. Control pSiren CD81^{KD} DLD1 colon cancer cells were analysed for the expression of *CcnD1*, *CDC6* and *E2F1* genes by RT-qPCR. E2F1: 2ΔΔCT 0.161, ***p<0.001***; CDC6: 2ΔΔCT 0.158, ***p<0.001***.

Results

Compared to control DLD1 colon cancer cells, knockdown of CD81 (CD81^{KD}) resulted in significantly reduced:

- Cell proliferation (p = 0.049)
- Cell motility (p < 0.001)
- Anchorage-independent colony formation (p = 0.002)
- Expression of cell cycle genes E2F1 & CDC6 (p < 0.001)

Acknowledgements

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Conflicts of Interest None

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