Clobetasol-induced quiescence in the vulvar carcinoma cell line, UMSCV-4 can be overcome by repeated removal and re-exposure to this ultrapotent corticosteroid

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INTRODUCTION

Vulvar cancer is rare, mostly afflicting women aged 60 and older [1]. The cancer is often preceded by a common vulvar rash, Lichen sclerosis, that is usually treated with the ultra-potent corticosteroid, clobetasol propionate. This treatment may, in turn, be associated with vulvar carcinogenesis.

Quiescence, a common characteristic of stem cells, is a reversible state of growth arrest. Our results suggest that clobetasol is causing UMSCV-4 vulvar carcinoma cells to enter quiescence, and may allow them to evade the standard treatments that target rapidly proliferating populations. Furthermore, when these cells are removed from clobetasol and re-exposed, they proliferate at higher levels. This suggests that the initial clobetasol treatment selects for cells that are now unable to enter quiescence when re-exposed to clobetasol.

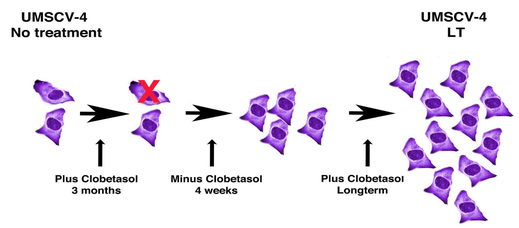

Figure 1. Re-exposure of UMSCV-4 cells to clobetasol results in resistance to the growth inhibition effects of clobetasol. This model represents the process that led to generation of the long-term, clobetasol resistant UMSCV-4 cells. Untreated UMSCV-4 cells were exposed to clobetasol for 3 months during which time the clobetasol medium was refreshed periodically to account for evaporation but the cells were not removed or passaged. After 3 months the clobetasol was removed and cells were allowed to "recover" for 4 weeks. Note that mitotic cells were visible within 3 days of clobetasol removal. After 4 weeks proliferating cells were re-exposed to clobetasol. These cells did not experience the growth arrest seen when originally exposed to clobetasol. This population of cells is referred to as UMSCV-4 LT.

MATERIALS & METHODS

Cell death assays (trypan blue) and immunofluorescence detection of the proliferation markers Ki67 as well as BrDU incorporation were used to determine rates of proliferation in the clobetasol treated UMSCV-4 cells. In addition, cells were examined for changes in mRNA expression of key markers indicative of entering a state of quiescence. Clobetasol was diluted in 95% ethanol (10^{-7} M final concentration) and UMSCV-4 cells were cultured in the presence (+clob) or absence of clobetasol (-clob) for these experiments. UMSCV-4 LT cell populations were generated as described in Figure 1.

The basic procedure for examination of clobetasol effects on UMSCV-4 NT and UMSCV-4 LT cells was as follows

- ❑ EtOH 10d: Ethanol treated for 10 days
- ❑ Clob10d: Treated with clobetasol for 10 days
- ❑ Clob 6d-4d: Treated with clobetasol for 6 days and changed to ethanol medium for 4 days

Cells from each group were subsequently harvested and tested using protocols described in each figure..

RESULTS

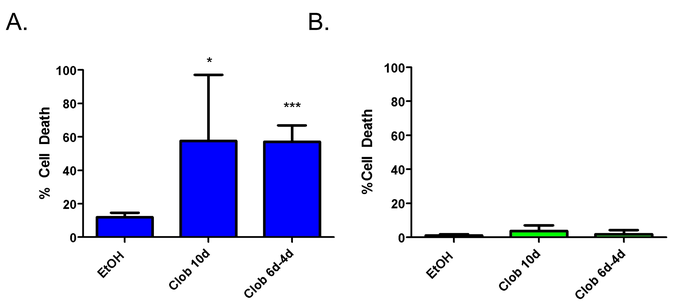

Figure 2. Clobetasol treated NT cells (A), but not LT cells (B), experienced relatively higher cell death when compared to controls grown in ethanol. Percentage of cell death was measured using a trypan blue exclusion assay in UMSCV-4 cells exposed to medium containing EtOH in the control group, or clobetasol. Two groups of SCV4 cells were examined: long term cells grown in clobetasol for >3 months and no treatment cells grown in normal medium. Cells with long term exposure to clobetasol (panel A) exhibited considerably lower rates of death when grown with clobetasol compared to the no treatment cells (panel B) (* = Significance).

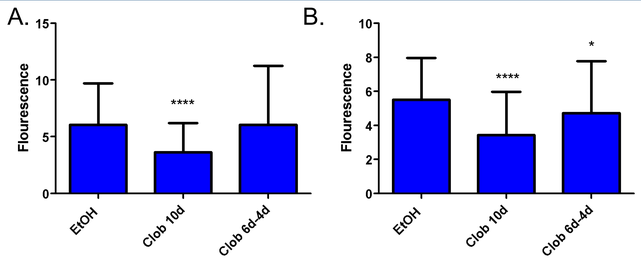

Figure 3. Clobetasol treated NT cells had lower proliferation rates relative to controls. BrdU incorporation is a measure for relative DNA replication amounts and corresponds to proliferation rate. Ki67 is a proliferation associated protein that, as with BrdU, is detected with immunofluorescence (Figure 3A &3B) and Image J [3]. Clob10d cells had lower BrdU incorporation and Ki67 relative to ethanol treated cells (control). When removed from clobetasol, Clob6d-4d, cells had proliferation rates increase to levels similar to controls.

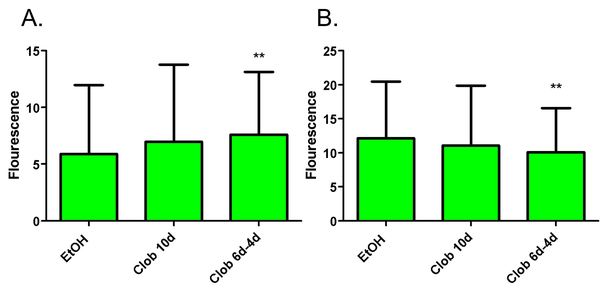

Figure 4. Clobetasol treated LT cells proliferate at similar rates as controls. LT Clob10d cells had similar BrdU incorporation (panel A) and Ki67 levels (panel B) relative to Ethanol treated cells (control). When removed from clobetasol, Clob6d-4d, cells had higher proliferation rates than controls.

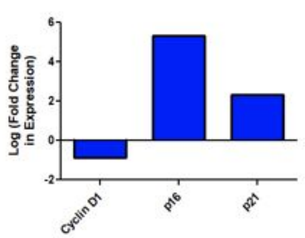

Figure 5. Clobetasol treated NT Cells express lower levels of proliferation associated proteins and higher inhibitory proteins. RT-qPCR gene expression analysis shows increased levels of p16 and p21 Cdk inhibitors. Additionally, Cyclin D1 is decreased. Cdk2, Cdk4 and Cyclin E were increased, however this may be related to quiescence progression.

CONCLUSIONS

- ❑ Clobetasol treatment lead to increased cell death in UMSCV-4 cell subpopulations that were not previously exposed to clobetasol (NT).
- ❑ Clobetasol treatment lead to decreased proliferation in UMSCV-4 cell subpopulations that were not previously exposed to Clob (NT). This could be reversed within 4 days if cells were removed from Clob after 6 days treatment.
- ❑ UMSCV-4 cells that were treated for 3 months with clobetasol, then removed, and re-exposed (LT) showed similar proliferation rates when treated with clobetasol, ethanol, or clobetasol short term.
- ❑ NT cells had upregulation of cell cycle inhibitors, p16 and p21 while downregulation of cyclin D1 after 10 days of clobetasol treatment, indicating a negative change in the proliferation
- ❑ Long term exposure to clobetasol may select for cells that are resistant to the antiproliferative effects of clobetasol.

FURTHER STUDIES

- ❑ Studies are now aimed at defining the growth rate of surviving cells post initial clobetasol treatment.
- ❑ Further research will focus on repeating RT-qPCR experiments and trypan blue exclusion assays on NT cells and LT cells to gather more definitive data.

REFERENCES

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2. Cheung, T. and Rando, T. Molecular regulation of stem cell quiescence. Nature reviews: Molecular cell biology. 2013;14(6):329-40.
3. Image J Free Cell Imaging Software (NIH)

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