Examining p27 Expression and Quiescence in Clobetasol-Exposed UMSCV-4 Vulvar Cancer Cells

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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 often treated with the ultra-potent corticosteroid, clobetasol propionate. This treatment may, in turn, be associated with vulvar carcinogenesis. Our previous findings suggest that initial clobetasol exposure can result in a state of quiescence in UMSCV-4 cells and that long term clobetasol exposure selects for cell subpopulations that are unable to re-enter quiescence upon clobetasol re-exposure [5]. Quiescence is a temporary removal from the cell cycle and can be thought of as a dormant state in which cells are not actively dividing [2]. There is growing evidence suggesting that quiescence may play a role in allowing cancer cells to contribute to the recurrence of the cancer months or years after treatment [3]. There are several cell cycle inhibitors that may indicate quiescence, including p16, p21, p53, and p27 [4]. Preliminary studies in our lab suggest that p16, p21, and p53 do not play a clear role in clobetasol-induced quiescence of UMSCV-4 cells. The role of p27 in quiescence of UMSCV-4 cells also does not appear to be straightforward, and further studies confirming these results and exploring other signaling pathways related to quiescence are necessary.

MATERIALS & METHODS

Cells were examined for changes in mRNA expression of key markers indicative of entering a state of quiescence via RT-PCR. 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 +EtOH) 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 (same concentration as would be found for the clobetasol containing medium) for 10 days
- ❏ Clob10d: Treated with clobetasol for 10 days
- ❏ Clob 6d-4d: Treated with clobetasol for 6 days and changed to ethanol containing medium for 4 days
- ❏ Cells from each group were subsequently harvested and tested using protocols described in each figure.

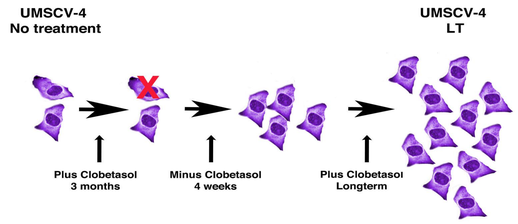

Figure 1. Re-exposure of UMSCV-4 cells to clobetasol results in resistance to the growth inhibition effects of clobetasol. This model represents the process that led to generation of the long-term, clobetasol "resistant" UMSCV-4 cells. Untreated UMSCV-4 cells were exposed to clobetasol for 3 months during 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 in medium not containing ethanol or clobetasol. 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.

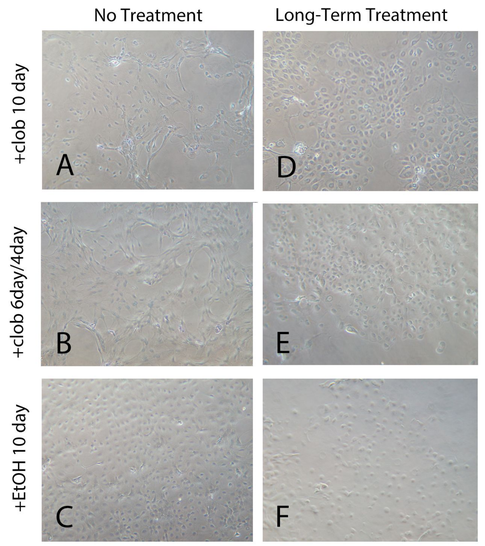

Figure 2. UMSCV-4 untreated cells (no treatment) show a decrease in proliferation and changes in morphology when treated with clobetasol while the long-term cells (see Figure 1) showed little change in growth rate or morphology. UMSCV-4 untreated cells (NT) or long-term (LT) were exposed to clob for 10 days (+clob 10 day, panels A and D) or to clob for 6 days followed by treatment with ethanol for a subsequent 4 days (+clob 6 day, 4 day, panels B and E) or to vehicle alone for the full 10 days (+EtOH, panels C and F). Clob treatment of the untreated cells caused the cell morphology to become more neuronal-like and many of the cells became vacuolated (panel A). The growth rate also decreased [5]. When the cells were treated for 6 days and then removed from clob the cells did not recover their normal morphology (panel B) but several patches of mitotic cells began to appear at day 4 post treatment [5]. Cells that were grown in the presence of vehicle (+EtOH, panel C) did not show any morphological changes nor growth arrest. The LT cells grew at basically the same rates whether in clob for 10 days (panel D), clob for 4 days followed by EtOH only (panel E) or in EtOH for the full 10 days (panel F). Total RNA was extracted from these cells and used for subsequent RT-PCR as described in subsequent figures.

RESULTS

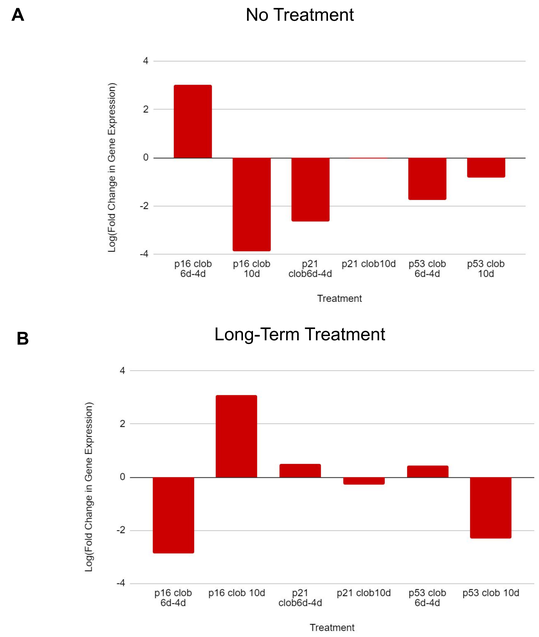

Figure 3. Cell cycle inhibitors p16, p21, and p53 do not appear to play a direct role in clobetasol-induced UMSCV-4 cell quiescence. A) RT-PCR gene expression analysis of p16, p21, and p53 in clobetasol treated NT cells. **B**) RT-PCR gene expression analysis of p16, p21, and p53 in clobetasol treated LT cells. We would expect the levels of the aforementioned cell cycle inhibitors to be upregulated upon initial clobetasol exposure in NT cells that typically enter quiescence, and downregulated in LT cells that are typically unable to re-enter quiescence. Initial results do not demonstrate this relationship or any other clear relationship between the regulation of these inhibitors and clobetasol exposure.

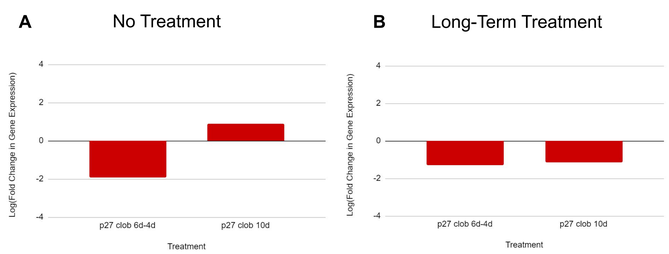

Figure 4. Cell cycle inhibitor, p27, does not appear to play a direct role in clobetasol-induced quiescence in UMSCV-4 cells, although it is slightly upregulated after 10 days exposure to clobetasol in NT cells. A) RT-PCR gene expression analysis of p27 in clobetasol treated NT cells. **B**) RT-PCR gene expression analysis of p27 in clobetasol treated LT cells. We would expect the levels of the p27 to be upregulated upon initial clobetasol exposure in NT cells that typically enter quiescence, and downregulated in LT cells that are typically unable to re-enter quiescence. Initial results do not demonstrate this relationship or any other clear relationship between the regulation of these inhibitors and clobetasol exposure.

CONCLUSIONS

- ❏ We would expect NT cells exposed to clobetasol to upregulate cell cycle inhibitors, since these cells are temporarily growth arrested when exposed to clobetasol [5]. There does not appear to be a clear correlation between the upregulation of the cell cycle inhibitors, p16, p21, p53, and p27 upon initial clobetasol exposure in NT cells.
- ❏ We would expect LT cells exposed to clobetasol to downregulate cell cycle inhibitors, as we have previously demonstrated these cells appear unable to re-enter quiescence [5]. There does not appear to be a clear correlation between the downregulation of the cell cycle inhibitors, p16, p21, p53, and p27 upon clobetasol re-exposure in LT cells.
- ❏ It is possible that the aforementioned cell cycle inhibitors do not play a role in UMSCV-4 cell quiescence, as previous studies have demonstrated quiescence can relieve the requirement for Notch signaling in certain cell types [6]. Quiescence could also relieve the requirement for p16, p21, p53, and p27 inhibitors, and this could explain our otherwise unexpected results.

FURTHER STUDIES

- ❏ Further research will focus on repeating RT-PCR examining p16, p21, p53, and p27 expression, as we need to have a larger number of trials in order to determine statistical significance of our results.
- ❏ Further research will study the role of Notch signaling in clobetasol treated NT and LT cells. If our results are consistent with previous studies examining Notch signaling in quiescent cells, it is possible that quiescence relieves the requirement for both Notch signaling and the cell cycle inhibitors we studied [6].

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