**Impact of Vismodegib-Treatment on Cell-Cycle of Basal Cell Carcinoma Cells**

Babak Senfi, Tatiana Mendez, Matthew A. Evers, Sarah Pagni, Janet M. Cowan, Addy Alt-Holland

**Objective:** Two of the clinical manifestations of Gorlin Syndrome are the development of multiple odontogenic keratocysts and basal cell carcinoma (BCC) tumors. BCC tumors are associated with abnormal activity of the Sonic Hedgehog (SHh) pathway, a key pathway in cell growth and proliferation. Patients treated with vismodegib, a SHh inhibitor, show improvement of their symptoms. We examined the effect of vismodegib on the cell cycle and proliferation of cultured BCC cells to better understand its role in treatment.

**Methods:** Cultures of human BCC cells (ATCC TE 354.T) were grown for 3-4 weeks, treated with 10nM-10μM Vismodegib or DMSO (control) for additional 10 days, and imaged using an Axiovert Zeiss microscope. Thereafter, trypsinized cells were with 70% ethanol, treated with Propidium-Iodide/RNAase solution and analyzed using BD FACSAria II Flow Cytometer. Parallel cultures were fixed with 4% paraformaldehyde and immunostained with anti-proliferating cell nuclear antigen (PCNA) antibodies, as a marker of proliferation. DAPI-stained nuclei were imaged using an Olympus BX50 microscope. *T*-tests were used for statistical analysis and *p*<0.05 was considered significant.

**Results:** All BCC cultures exhibited a variety of cell shapes and sizes. Flow cytometry analysis of triplicates of 10,000-30,000 BCC cells from control and vismodegib-treated cultures revealed that 70-80% of cells were at the G0/G1 phase, 5-8% were at the S phase, and 12-15% were at the M phase of the cell cycle in all culture conditions. However, there were significant differences (p<0.001) in the percentages of cells in these phases. As expected, few nuclei demonstrated positive PCNA immunostaining.

**Conclusion:** In vitro, a small percentage of BCC cells demonstrated cell proliferation, consistent with the observed slow growth of these cultures. Exposure of cultures to 10nM-10μM vismodegib for 10 days did not significantly impact BCC cell cycle. Experiments are ongoing to study the effects of longer treatment duration on BCC cell proliferation.

This study was funded in part by the “Michael J. Rainen Foundation.”