Cell-Cycle Changes in Vismodegib-Treated Fibroblasts are Time- and Microenvironment-Context Dependent

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Objective: Recurrent odontogenic keratocysts and basal cell carcinomas (BCC) are characteristic craniofacial and dermatologic pathologies of Gorlin syndrome. The abnormal proliferation of BCC cells is attributed to the upregulated and constitutive activity of the SMO receptor in the Sonic Hedgehog (SHh) pathway. While systemic chemotherapy with vismodegib, a small molecule SMO inhibitor, lessens BCC tumor appearance, its effects on surrounding stromal fibroblasts that reside within the tumor microenvironment is less understood. We investigated the effects of BCC cells, vismodegib treatment, and duration of exposure on the cell-cycle of fibroblasts.

Methods: Human dermal fibroblasts were grown in monocultures and in co-culture with BCC cells. In co-culture, the two cell types were physically separated but environmentally connected through the use of an insert and tray system. Cultures were treated with DMSO (control) or clinically-relevant10nM vismodegib for four or ten days. Thereafter, cells were trypsinized, fixed with 70% ethanol, treated with PI/RNAse solution, and analyzed by flow cytometry (BD FACSAria II). Multiple statistical analysis tests were conducted, and *p*-values <0.05 were considered significant.

Results: Comparison of fibroblast monocultures and BCC/fibroblast co-cultures revealed the additive effects of BCC cells, vismodegib, and duration of exposure on the cell-cycle of fibroblasts. Compared to control cultures, vismodegib treatment in the presence of BCC cells resulted in a significant increase in the percentage of fibroblasts arrested at G_0/G_1 phase after four days exposure and ten days exposure ($p<0.05$). Simultaneously, there was a reduction in the percentage of fibroblasts in the S phase and, to a lesser extent, in the M phase of the cell-cycle. Ten-day treatment was found to have more impact than four-day treatment ($p<0.05$).

Conclusion: BCC cells and vismodegib independently influence the cell-cycle of fibroblasts, and have an additive inhibitory effect on fibroblast proliferation when present together in cultures. This effect intensified with increased treatment duration.

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