**Vismodegib-Treatment Alters Basal Cell Carcinoma Cells Nuclear Size and DNA**

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**Objective**: An abnormal signaling of the Sonic Hedgehog (SHh) pathway has been linked to the development of numerous basal cell carcinomas (BCCs) in Gorlin Syndrome patients. SHh-targeted therapy with the SMO inhibitor vismodegib reduces the manifestation of BCC tumors in these patients. We studied the effect of vismodegib on the nuclei of human BCC cells, using fluorescence in situ hybridization (FISH).

**Methods**: BCC cells (ATCC TE-354.T) were treated with 10μM vismodegib or DMSO (control) for 10 days, and fixed with 4% paraformaldehyde. FISH was performed using an α-satellite probe for the X chromosome, and >600 DAPI-stained nuclei were imaged for each growth condition using an Olympus-BX50 microscope. Nuclear measurements were conducted using ImageJ, and the number of X-chromosome signals per nucleus was recorded. Statistical tests were used to determine associations between nuclear size, X-chromosome signals and culture condition. *p*-values <0.05 were considered significant.

**Results**: BCC cells exhibited small, medium, large, and kidney bean-shaped nuclei that were different in their sizes (p<0.005) in control and vismodegib-treated cultures. The nuclei exhibited a range of 2-10 X-chromosome signals. The majority of small nuclei exhibited 2 signals, medium and kidney bean-shaped nuclei mainly presented 2 or 4 signals, and the majority of large nuclei showed 4 or 8 signals. There was a significant association (p<0.001) between the number of X-chromosome signals and nuclear size within each culture condition. There was also a significant increase (p<0.04) in the number of kidney-bean shaped nuclei with 2 X-chromosome signals in vismodegib-treated cultures.

**Conclusion**: Chemotherapy treatment modalities may result in the restoration of normalized nuclear architecture in neoplastic cells. Our study of cultured vismodegib-treated BCC cells demonstrated a reduction in nuclear size and tendency for normalization of the number of X chromosomes per cell. It is likely that similar changes in vivo result in the apparent efficacy of this SHh-targeted treatment modality.

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