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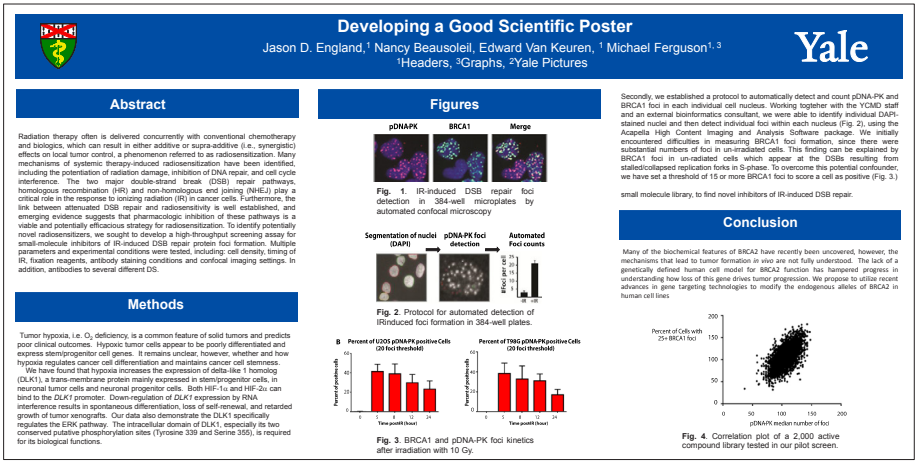

Developing a Good Scientific Poster

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[1]Headers, [3]Graphs, [2]Yale Pictures

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Abstract

Radiation therapy often is delivered concurrently with conventional chemotherapy and biologics, which can result in either additive or supra-additive (i.e., synergistic) effects on local tumor control, a phenomenon referred to as radiosensitization. Many mechanisms of systemic therapy-induced radiosensitization have been identified, including the potentiation of radiation damage, inhibition of DNA repair, and cell cycle interference. The two major double-strand break (DSB) repair pathways, homologous recombination (HR) and non-homologous end joining (NHEJ) play a critical role in the response to ionizing radiation (IR) in cancer cells. Furthermore, the link between attenuated DSB repair and radiosensitivity is well established, and emerging evidence suggests that pharmacologic inhibition of these pathways is a viable and potentially efficacious strategy for radiosensitization. To identify potentially novel radiosensitizers, we sought to develop a high-throughput screening assay for small-molecule inhibitors of IR-induced DSB repair protein foci formation. Multiple parameters and experimental conditions were tested, including: cell density, timing of IR, fixation reagents, antibody staining conditions and confocal imaging settings. In addition, antibodies to several different DS.

Methods

Tumor hypoxia, i.e. O_2 deficiency, is a common feature of solid tumors and predicts poor clinical outcomes. Hypoxic tumor cells appear to be poorly differentiated and express stem/progenitor cell genes. It remains unclear, however, whether and how hypoxia regulates cancer cell differentiation and maintains cancer cell stemness.

We have found that hypoxia increases the expression of delta-like 1 homolog (DLK1), a trans-membrane protein mainly expressed in stem/progenitor cells, in neuronal tumor cells and neuronal progenitor cells. Both HIF-1α and HIF-2α can bind to the *DLK1* promoter. Down-regulation of *DLK1* expression by RNA interference results in spontaneous differentiation, loss of self-renewal, and retarded growth of tumor xenografts. Our data also demonstrate the DLK1 specifically regulates the ERK pathway. The intracellular domain of DLK1, especially its two conserved putative phosphorylation sites (Tyrosine 339 and Serine 355), is required for its biological functions.

Figures

Fig. 1. IR-induced DSB repair foci detection in 384-well microplates by automated confocal microscopy

Fig. 2. Protocol for automated detection of IRinduced foci formation in 384-well plates.

Fig. 3. BRCA1 and pDNA-PK foci kinetics after irradiation with 10 Gy.

Secondly, we established a protocol to automatically detect and count pDNA-PK and BRCA1 foci in each individual cell nucleus. Working together with the YCMD staff and an external bioinformatics consultant, we were able to identify individual DAPI-stained nuclei and then detect individual foci within each nucleus (Fig. 2), using the Acapella High Content Imaging and Analysis Software package. We initially encountered difficulties in measuring BRCA1 foci formation, since there were substantial numbers of foci in un-irradiated cells. This finding can be explained by BRCA1 foci in un-radiated cells which appear at the DSBs resulting from stalled/collapsed replication forks in S-phase. To overcome this potential confounder, we have set a threshold of 15 or more BRCA1 foci to score a cell as positive (Fig. 3.)

small molecule library, to find novel inhibitors of IR-induced DSB repair.

Conclusion

Many of the biochemical features of BRCA2 have recently been uncovered, however, the mechanisms that lead to tumor formation *in vivo* are not fully understood. The lack of a genetically defined human cell model for BRCA2 function has hampered progress in understanding how loss of this gene drives tumor progression. We propose to utilize recent advances in gene targeting technologies to modify the endogenous alleles of BRCA2 in human cell lines

Fig. 4. Correlation plot of a 2,000 active compound library tested in our pilot screen.

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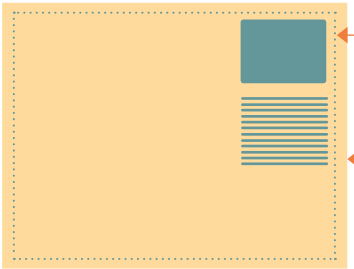

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- *To create a good Yale Blue color in PowerPoint (R=19, B=59, G=110)*

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