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INVESTIGATING THE STATUS OF THE MITOTIC CHECKPOINT AND ITS RELATION TO MITOTIC CHECKPOINT COMPLEX-ASSOCIATED PROTEINS

Anti-mitotic drugs such as Nocodazole (Nz) and Taxol are commonly utilized in the initial chemotherapeutic regimen used to treat various cancers. However, such drugs are less successful when used to treat recurrent disease, as cells tend to develop a resistance to previously used drugs. Previous studies in the Yen laboratory have revealed a relationship between the development of such drug sensitivity and the integrity of the mitotic checkpoint and strength of mitotic arrest. The mitotic checkpoint ensures proper chromosomal segregation prior to the completion of mitosis and contains several proteins that are specific to mitosis and are thus ideal candidate targets for anti-mitotic drug development.

The primary goal of the project was to identify the strength of the mitotic checkpoint and to quantify the levels of the mitotic checkpoint complex protein BubR1 in various cancer cell lines (ovarian, leukemic, and pancreatic). Protein lysates were created from cell lines treated with Nz and Taxol, and Western blots were performed in order to identify BubR1 and other related protein levels. The cell cycle profile was also analyzed via FACS analysis. Various siRNA transfections were performed in order to investigate the effects of BubR1 knockdown. Following experimentation, cell lines were categorized according to their strength of mitotic arrest (strongly arrested, partially compromised, weakly arrested). It was found that the level of BubR1 correlates with the strength of arrest, with high levels of BubR1 correlating with a strong mitotic arrest and resulting in cell death and low levels of BubR1 correlating with a weak mitotic arrest and polyploidy. Thus, cellular fate (death or polyploidy) following treatment with anti-mitotic drugs seems to depend on the levels of BubR1.

Additionally, indirect immunofluorescence (IF) was performed in order to visualize mitotic checkpoint proteins in real time by the use of video microscopy. A method of image analysis was developed by comparing the pixel intensity to the background pixel intensity in order to quantify and compare levels of staining under various cellular conditions. Mouse xenograph samples were stained utilizing similar methods in order to investigate in vivo situations. Lastly, video microscopy was utilized to investigate the real-time effects of various pre-treatments and anti-mitotic treatments on cellular fate.

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