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Distinct contributions of APC10 mutations and Cep63 to aneuploidy and cancer

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Background

Genetic instability is a hallmark of cancer and a driver of tumorigenesis through increased rates of mutations and genetic variability within tumor cells. The fidelity of chromosomal distribution during mitosis is maintained by the evolutionary conserved spindle assembly checkpoint (SAC). The SAC ensures the accurate segregation of DNA into daughter cells by inhibiting the anaphase promoting complex/Cyclosome (APC/C) when improperly attached chromosomes are detected. The APC/C is an E3 ubiquitin ligase that initiates chromosome segregation. Mutations in Anaphase Promoting Complex Subunit 10 (APC10) were identified by whole exome sequencing of BRCA-1/2-negative familial breast cancer patients. Our aim was to perform a functional analysis on the impact of APC10 mutations on mitosis progression and chromosomal stability in vitro.

Cep63 is a constitutive centrosomal protein whose mutations lead to centrosomal amplification and chromosomal instability. The overexpression of Cep63 in tumor cells had been correlated with delayed tumor stages and bad disease prognosis. In a study by our laboratory, Cep63 had been found to be necessary for the initiation of mitosis through the recruitment of Cdk1 to the centrosomes. A recent study has questioned our study as well as the results of more than 200 studies based on the use of nonspecific Cdk1 antibodies. Our aim was to revise the results produced by our laboratory through using Cdk1-specific antibodies to reinforce the phenotype analysis of Cep63.

Methods

We used tagged wild-type and mutant APC10 nucleotide sequences for the creation of stable cell lines through stable transfection techniques. Biochemical techniques for the purpose of functional analysis included immunofluorescence microscopy, Western Blotting, and flow cytometry. For the Cep63 part of our study, U2OS cells were depleted from Cep63 using siRNA transfection techniques. Cells were then immunostained using Cdk1 antibodies of different specificities and observed by immunofluorescence microscopy. For an objective quantification of centrosome signals, confocal microscopy was used.

Results

Stable cell lines were successfully created conditionally expressing either wild-type or mutant APC10. Microscopic examination of both cell lines upon overexpression did not reveal a special phenotype. Centrosome overduplication as well as mitotic aberrations were not detected microscopically. Aberration of cell cycle phases upon overexpression of wild-type or

mutant APC10 were not observed. The expression of APC/C substrates already known to be involved in tumorigenesis was not significantly altered.

When staining control U2OS cells for Cdk1using nonspecific antibodies, Cdk1 centrosomal signal was strong throughout cell cycle phases. The strong signal was diminished significantly upon using Cdk1-specific antibodies. Examining cells in late G2 cells has shown a significant decrease of Cdk1 centrosomal signal in response to Cep63 depletion.

Conclusions

In this study we focused on specific outcomes that could arise as a result of APC10 mutations. Aneuploidy, spindle assembly abnormalities and cell cycle aberrations were not observed in our set of experiments. A further possible study using APC10 mouse models could be the next step to exploit the benefits of in vivo functional analysis.

Our findings of diminished centrosomal Cdk1 signal upon using Cdk1-specific antibodies compared to nonspecific antibodies were attributed to the fact that Cdk1 localizes on the centrosomes in late G2. The use of Cdk1 specific antibodies has validated the previously published data about Cep63 phenotype and pathophysiologic role.