Do PKC and CDK-1 Use α/β -Tubulin as a Molecular Toggle Switch In Breast Cancer?

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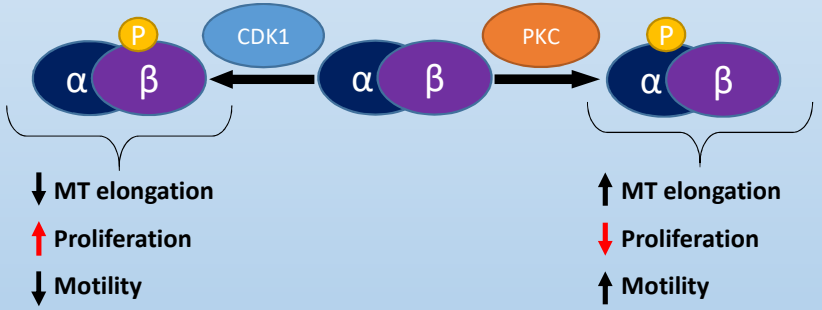
Introduction: The Rotenberg Lab has found reciprocity between cell motility and cell proliferation in human breast cells. Protein kinase C (PKC) was shown to increase cell motility (an indicator of metastatic potential) and to decrease cell proliferation in human breast cells (Sun and Rotenberg, 1999). Thereafter, PKC was discovered to phosphorylate α-tubulin at residue Ser-165. Phosphomimetic mutants S165D mimicking phosphorylation at Ser-165 showed increased microtubule elongation, highly increased cell motility, and decreased proliferation (Abeyweera et al., 2009). Inversely, S165N mutants with blocked phosphorylation at Ser-165 showed decreased motility and increased proliferation. Recently, phosphorylation by PKC was shown to affect the alignment of catalytic residue Glu-254 with the exchangeable GTP of β-tubulin, thereby impairing GTP hydrolysis (Maddula et al., 2022). The presence of the GTP cap prevents microtubule depolymerization and promotes elongation, thus enhancing motility.

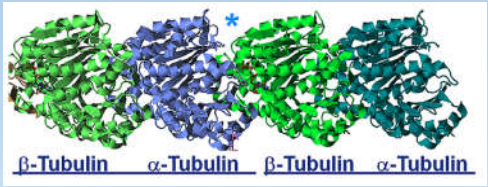
Cyclin-dependent kinase 1 (CDK-1) is involved in regulating the cell cycle by promoting the transition of the G_2/M phase into mitosis. CDK-1 phosphorylates β-tubulin at residue Ser-172 which was proposed to interfere with GDP binding on β-tubulin, and therefore impairing the incorporation of tubulin into microtubules (Fourest-Lieuvin et al., 2006). This leaves free α/ β -tubulin dimers in the cytoplasm available to form spindle fibers for mitosis, thus enhancing proliferation.

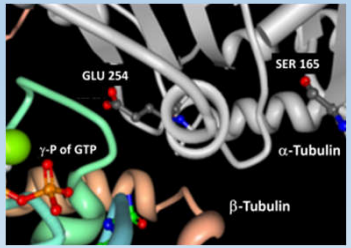
PKC and CDK-1 activity inversely affect cell proliferation. It is possible that α/β-tubulin operates as a switch that is regulated by phosphorylation events at the individual α/β subunit, thus resulting in altered microtubule dynamics and cell behavior.
It probes for further investigation to determine whether phosphorylation of α-tubulin by PKC precludes phosphorylation of β-tubulin by CDK-1.

Methods: MDA-MB-231 human breast cancer cells will be treated with and without PKC activator diacylglycerol (DAG) and incubated for 30 minutes. Cell lysates will be prepared and divided into two equal samples. Immunoprecipitation of each sample will be preformed with α-tubulin and β-tubulin antibodies. SDS-PAGE will be done with the pelleted samples in duplicate on two gels, followed by Western Blot gel transfer. Each blot will be cut in half for a total of four replicates. Two replicates will be probed with a primary antibody that recognizes the phosphorylated consensus site of each protein kinase (PKC substrates AB, CDK-1 substrates AB). As controls, the remaining replicates will be probed with either α-tubulin-specific or β-tubulin-specific antibodies. Following chemiluminescence, bands corresponding to α- or β-tubulin will be located at 50-55 kDa.

Discussion: If the results are to support the model shown on the right, it is expected that the DAG-treated cells will show a stronger signal corresponding to α-tubulin on the PKC probed blot, indicating greater phosphorylation by PKC. Conversely, it is also expected that DAG-treated cells will show a weaker signal corresponding to β-tubulin on the CDK-1 probed blot, indicating reduced phosphorylation by CDK-1.







References

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