

## ANTITUMOR DRUGS REVEAL THAT EUKARYOTIC ELONGATION FACTORS Eef1B $\gamma$ AND eEF1B $\delta$ ARE PHOSPHORYLATED DURING MITOSIS

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Eukaryotic elongation factor 1 (eEF1) is a macromolecular complex formed by two different components, eEF1A and eEF1B, both implicated in the transference of the amino acids from the aminoacyl-tRNA to the ribosome site A. eEF1B complex is formed by three proteins, eEF1B $\alpha$ , eEF1B $\delta$  and eEF1B $\gamma$ . Specifically, eEF1B $\alpha$  and eEF1B $\delta$  have guanine nucleotide exchange activity required to activate eEF1A.

Numerous antitumor drugs target different mitotic mechanisms to induce cell death in cancer cells. Then, microtubule interfering agents (many of them such as paclitaxel, docetaxel and vincristine in clinical use) inhibit microtubule dynamics and blocks mitosis. This microtubule dynamics blockage affects processes other than mitosis. For this reason other drugs targeting specific mitotic proteins such as Aurora A, Aurora B, polo like kinase 1 (PLK1), kinesin spindle protein (KSP) and centromeric protein E (CENPE) are being investigated.

2D-PAGE and PRO-Q diamond staining showed that paclitaxel treatment phosphorylates eEF1B $\gamma$  in HeLa cells (*Prado M.A. y cols., Proteomics, 2007, Vol.7, 3299-3304*). A dephosphorylation assay with  $\lambda$ -protein phosphatase confirmed this post-translational modification of eEF1B $\gamma$  and revealed that eEF1B $\delta$  is also phosphorylated after paclitaxel treatment. Moreover, treatment with other antimetabolic agents such as docetaxel and STLC (a KSP inhibitor) induce these phosphorylations of elongation factors. We demonstrate that these post-translational modifications depend on mitotic events; specifically it occurs during normal mitosis in HeLa cells. Analysis of eEF1B $\gamma$  sequence and MALDI-ToF analysis after digestion with endoproteinase Glu-C of the protein suggest that CDK1 phosphorylates eEF1B $\gamma$  in Thr230. In summary, we demonstrate that eEF1B $\gamma$  and eEF1B $\delta$  are phosphorylated by taxanes and KSP inhibitor treatments and during normal mitosis.