ANTITUMOR DRUGS REVAL THAT EUKARYOTIC ELONGATION FACTORS Eef1Bγ AND eEF1Bδ 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, eEF1Bα, eEF1Bδ and eEF1Bγ. Specifically, eEF1Bα and eEF1Bδ 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 dinamics 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 eEF1Bγ in HeLa cells *(Prado M.A. y cols., Proteomics, 2007, Vol.7, 3299-3304)*. A dephosphorylation assay with λ-protein phosphatase confirmed this post-translational modification of eEF1Bγ and revealed that eEF1Bδ is also phosphorylated after paclitaxel treatment. Moreover, treatment with other antimitotic 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 eEF1Bγ sequence and MALDI-ToF analysis after digestion with endoproteinase Glu-C of the protein suggest that CDK1 phosphorylates eEF1Bγ in Thr230. In summary, we demonstrate that eEF1Bγ and eEF1Bδ are phosphorylated by taxanes and KSP inhibitor treatments and during normal mitosis.