[S3-1] [4/19/2002(Fri) 10:00-10:25/Hall A]

A Budding Yeast Model to Identify Inhibitors of the Mammalian Polo-Like Kinase as an Anticancer Drug

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Since the *polo* gene of *Drosophila* has been first isolated a decade ago, a family of the essential gene has been identified in various organisms. Whereas a single polo-like kinase gene(Plk) has been identified in the yeasts and *Drosophila*, the higher vertebrates have multiple plk genes of which Plk1 is most similar to *Drosophila polo*. Two other subclasses of plks, Snk/hSnk (Plk2) and Fnk/Prk (Plk3), have been described in mouse and human, respectively. A large body of evidences has indicated that the subfamily of polo-like kinases (Plks) regulates multiple stages of mitotic progression such as centrosome maturation, Cdc2 activation, and activation of anaphase promoting complex (APC). In addition to a highly conserved amino-terminal catalytic domain, these members are characterized by the presence of a distinctly conserved region, termed the polo box. M phase at the cell cycle is a highly orchestrated process that requires precise regulation of various biochemical steps and cellular events for faithful separation of genetic and cellular materials into two dividing cells. Deregulation of this process causes mitotic catastrophe, leading to cell death. At the end of normal cell cycle, cytokinesis commences to complete the cell division process. Thus, coordination of the completion of mitosis with timely initiation of cytokinesis is critical to ensure completion of mitotic events prior to cell division.

In *Saccharomyces cerevisiae*, the mammalian polo kinase homologue Cdc5 has been shown to function in a pathway leading to the degradation of mitotic cyclin Clb2, thereby permitting mitotic exit. However, it was unclear whether it functions in cytokinesis. We have demonstrated that the polo-box is essential for the subcellular localization of Cdc5 at spindle poles and cytokinetic neck-filaments, which is critical for cytokinesis. Whereas higher amount of Cdc5 activity upon overexpression was enough to induce nascent septal structures within elongated bud, depletion of Cdc5 function leads to a cell cycle arrest at a previously unidentified cytokinesis step, suggesting a positive role of Cdc5 in cytokinesis step. In addition, overexpression of the C-terminal domain of Cdc5 (*cdc5 ΔN*), but not the corresponding polo-box mutant, resulted in connected cells without interfering with nuclear division cycle. These cells shared cytoplasm and possessed aberrant septin rings, subcellular structures essential for

cytokinesis. Provision of additional copies of endogenous *CDC5* remedied this abnormal phenotype, indicating a dominant-negative inhibition of cytokinesis. The cell biological and genetic analyses with temperature-sensitive CDC5 mutants revealed a delayed septin disassembly and reassembly in chained cell. These findings suggest that Cdc5 coordinates mitotic exit with cytokinesis by participating in both APC activation and a polo-box-dependent cytokinetic pathway, and support the notion that polo kinases play multiple roles in regulating mitotic events and cytokinesis in various eukaryotic organisms. It has been reported that the mammalian Plk1 is a functional homolog of Cdc5 in terms of genetic complementation, localization, and mitotic functions. Since unregulated polo activity has been suggested to the development of cancers in humans and the polo-box is an unique and essential domain for polo kinases, inhibition of polo kinases by a dominant-negative polo-box domain or by specific polo-box inhibitors may yield a strategy to control highly proliferating malignant cells.