Synopsis of Original Research Paper

Application of BiFC system to detect cellular stress that induces skin aging and cancers

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Cdc25B is a cell cycle regulator that activates CDK/cyclin complex that leads to mitotic entry. Recently, we found that Cdc25B is degraded by cellular stresses that activate stress-activated MAP kinases p38 and JNK and that such stresses cell cycle retardation. We also indicated that the Cdc25B degradation is mediated by phosphorylation of Cdc25B by such kinases followed by ubiquitylation by $SCF^{\beta TrCP}$. We identified that the responsible sequence in Cdc25B for the interaction with $SCF^{\beta TrCP}$ is in N-terminal 175 amino acids. Using such Cdc25B-derived peptide, we established a fluorescent protein-based bio-imaging system that enables us to detect the stress-specific interaction between the Cdc25B-peptide and βTrCP1, a substrate-recognition component in $SCF^{\beta TrCP}$. For this purpose, we applied the bimolecular fluorescence (BiFC) method. We made two fusion constructs; N-terminal GFP fragment was fused to N-terminal Cdc25B fragment and N-terminal deletion construct of βTrCP1 was fused to C-terminal GFP fragment. Such two constructs were further fused to make one artificial gene. Although such GFP-derived fragments do not emit green fluorescence when they are separated each other, the green fluorescence can be detected when two GFP-derived fragments were reconstituted through Cdc25B-βTrCP1 interaction that is induced by cellular stress to phosphorylate Cdc25B. We were not able to detect green fluorescence not only co-transfection of the Cdc25B-βTrCP1 Bi-FC probe with JNK1 but also after application of non-genotoxic stress, such as anisomycin and NaCl to probe-transfected cells. Furthermore, such stress-derived green fluorescence was detected by flow cytometry-based methods, where we used a tetracycline-controlled expression cell lines.