Detection of active fraction of glycogen synthase kinase 3β in cancer cells by nonradioisotopic *in vitro* kinase assay.

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Glycogen synthase kinase 3β(GSK3β) is a well-known marker and potential therapeutic target in non-insulin-dependent diabetes mellitus and Alzheimer's disease. Our recent demonstration that GSK3β has a previously unrecognized role in colorectal cancer facilitates the development of a nonradioisotopic *in vitro* kinase assay (NRIKA) for detecting GSK3β activity in gastrointestinal cancer cells. The NRIKA uses a sequential combination of immunoprecipitations to isolate GSK3β in sample cells' lysates, and an in vitro kinase reaction that uses recombinant β-catenin protein (substrate) and nonradioisotopic ATP, followed by immunoblotting to detect β-catenin phosphorylated in serine 33, 37 and/or threonine 41 residues. The NRIKA detected higher expression of active GSK3β in stomach, colon, pancreas and liver cancer cell lines than in human embryonic kidney cells (HEK293) considered nonneoplastic. Inhibition of cancer cell-derived GSK3β activity by GSK3β inhibitors (SB-216763, AR-A014418) was detected by the NRIKA. GSK3β inhibition attenuated survival and proliferation and induced apoptosis in all types of cancer cells but not in HEK293. These findings supported the idea that the pathologic roles of GSK3β are definite and common in various types of cancer. The NRIKA provides a basis for evolving a high-throughput tool for testing substances for GSK3β inhibition, and for screening and identifying novel GSK3β inhibitors with a view to discovering drugs for treatment of cancer as well as non-insulin-dependent diabetes mellitus and Alzheimer's disease.

[Reference]

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