

学位論文抄録

Suppression of GANP causes DNA damage and cell death
in p53-insufficient cholangiocarcinoma cells

(p53不活性型胆管癌細胞においてGANP発現抑制はDNA障害と細胞死を引き起こす)

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Abstract of the Thesis

Background and Purpose: Cancer is associated with mutations of various tumor suppressor and/or oncogenic molecules. Cholangiocarcinoma (CCA) is a typical inflammation-associated tumor that often accompanies with DNA damages of critical molecules such as p53, therefore eventually displaying highly malignant trait. Recent studies demonstrated that the inflammation to cholangiocytes causes the high-level expression of an activation-induced cytidine-deaminase (AID) and its binding protein germinal center-associated nuclear protein (GANP) that are both involved in *immunoglobulin V-region* gene somatic hypermutation in B-cells. For prevention of CCA development, we attempted to treat the various CCA cell lines established from the patient samples by knockdown of GANP. Interestingly, GANP-knockdown effectively caused the cell death of p53-insufficient CCAs. Here, we studied the effect of GANP-knockdown on malignant CCAs and details of molecular mechanism in the induction of apoptosis.

Methods: *Samples*, CCA cell lines (KKU100, M156, M213 and M214) are from patient tumors obtained and established previously in Khon Kaen University. MMNK-1 is a cholangiocyte line wild type-p53. HeLa is a cervical cancer cell line with inactivated p53. *siRNA treatment*, Cells were treated with *ganp* siRNA (*siGanp*) compared with control siRNA (*siCtrl*). The knockdown efficiency was confirmed by Real time RT-PCR and western blot analysis. *p53 abnormality*, p53 mutation was determined by DNA sequencing and the western blot analysis. *Cell damage analyses*, Cell cycle change and DNA damage were determined by BrdU- and alkaline comet-assays. Cell death was quantified by trypan blue staining, AnnexinV/PI staining, mitochondrial membrane potential ($\Delta\Psi_m$), and activation of caspases. Caspase-dependent apoptosis was determined using a pan-caspases inhibitor (z-VAD-fmk). *Electron microscopic (EM) analysis*, Apoptotic and necrotic cells were observed. *Tumor growth in vivo*, Tumor growth was measured after transplantation into Balb/c-Rag-2/Jak3 double-deficient mice. p53-insufficient M213 cells were treated by *siGanp*.

Results: *Cell death* *siGanp* significantly induced cell death in M156, M213, M214 and HeLa but not in MMNK-1 and KKU100. M156, M213 and M214 showed the deletion of DNA binding- and oligomerization-domains. *siGanp* also caused the cell death of the p53-knockdowned MMNK-1. *DNA damage response* *siGanp* decreased the S-phase cells with G2/M arrest specifically in p53-insufficient tumors by inducing DNA damages measured by comet assay. *siGanp* induced activation of apoptosis-associated caspase 3, 8, 9 and poly (ADP-ribose) polymerase (PARP) indicating that both intrinsic- and extrinsic-pathways are activated. z-VAD-fmk couldn't completely block the *siGanp*-induced cell death indicating the interaction of both caspase-dependent and -independent mechanisms. EM analysis showed apoptotic and necrotic cell death. *In vivo treatment* *siGanp* efficiently prevented the tumor growth *in vivo* with the histological findings of reduced tumor cellularity and the fibrosis.

Discussion and Conclusion: *Role of GANP in DNA damage* Here the results indicate that GANP is essential for preventing the DNA damage particularly under the insufficient cell cycle checkpoint function. *siGANP* treatment induces the strong cell death reaction causing the immediate DNA damage as measured by comet assay and fully activates caspase-dependent and independent-apoptosis pathways, resulting in cell apoptosis and necrosis. As a possible molecular basis underlying this reaction, the mRNA export function of GANP might be critical for preventing the DNA damages in actively transcribed genes during cell proliferation. p53-insufficient tumors are highly malignant and resistant to the regular cancer therapy. *siGanp* is selectively effective to the p53-insufficient tumors with only a minimum effect on the p53 wild-type normal cells and tissues. *siGanp* might be an efficient and selective treatment of p53-insufficient CCA patients.