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Bile acids at neutral and acidic pH induce apoptosis and gene cleavages in nasopharyngeal epithelial cells: implications in chromosome rearrangement

Sang-Nee Tan and Sai-Peng Sim*

Abstract

Background: Chronic rhinosinusitis (CRS) increases the risk of developing nasopharyngeal carcinoma (NPC) while nasopharyngeal reflux is known to be one of the major aetiological factors of CRS. Bile acid (BA), the component of gastric duodenal contents, has been recognised as a carcinogen. BA-induced apoptosis was suggested to be involved in human malignancies. Cells have the potential and tendency to survive apoptosis. However, cells that evade apoptosis upon erroneous DNA repair may carry chromosome rearrangements. Apoptotic nuclease, caspase-activated deoxyribonuclease (CAD) has been implicated in mediating translocation in leukaemia. We hypothesised that BA-induced apoptosis may cause chromosome breaks mediated by CAD leading to chromosome rearrangement in NPC. This study targeted the *AF9* gene located at 9p22 because 9p22 is one of the most common deletion sites in NPC.

Methods: We tested the ability of BA at neutral and acidic pH in inducing phosphatidylserine (PS) externalisation, reactive oxygen species (ROS) production, mitochondrial membrane potential (MMP) disruption, and caspase 3/7 activity in normal nasopharyngeal epithelial (NP69) and NPC (TWO4) cells. Inverse-PCR (IPCR) was employed to detect *AF9* gene cleavages. To investigate the role of CAD in mediating these cleavages, caspase inhibition was performed. IPCR bands representing *AF9* cleaved fragments were sequenced.

Results: BA-treated cells showed higher levels of PS externalisation, ROS production, MMP loss and caspase 3/7 activity than untreated control cells. The effect of BA in the induction of these intracellular events was enhanced by acid. BA at neutral and acidic pH also induced significant cleavage of the *AF9* gene. These BA-induced gene cleavages were inhibited by Z-DEVD-FMK, a caspase-3 inhibitor. Intriguingly, a few chromosome breaks were identified within the *AF9* region that was previously reported to participate in reciprocal translocation between the mixed lineage leukaemia (*MLL*) and *AF9* genes in an acute lymphoblastic leukaemia (ALL) patient.

Conclusions: These findings suggest a role for BA-induced apoptosis in mediating chromosome rearrangements in NPC. In addition, CAD may be a key player in chromosome cleavages mediated by BA-induced apoptosis. Persistent exposure of sinonasal tract to gastric duodenal refluxate may increase genomic instability in surviving cells.

Keywords: CRS, NPC, Nasopharyngeal reflux, BA, Apoptosis, AF9, CAD

* Correspondence: spsim@unimas.my
Department of Paraclinical Sciences, Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak, Sarawak, Malaysia