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（Summary）

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（要　旨）

The liver acts as the first barrier to the spread of fungi present in intestine. However, in alcoholic steatohepatitis (ASH) and non-alcoholic steatohepatitis (NASH) patients, these fungi are known to invade gastrointestinal mucosa to reach the liver and cause severe fungal infections, causing hepatic injury and cancer. Transglutaminase 2 (TG2) catalyzes Ca^{2+}-dependent protein crosslinking reactions and plays a key role in cell growth, differentiation, and apoptosis. Nuclear transglutaminase (TG) 2 activity plays an important role in ethanol- and free fatty acid- induced liver injury via crosslinking and silencing Sp1 transcription factor thus decreasing expression of c-Met essential for survival of hepatic cells. In this study, by investigating the cellular activity of TG2 in human hepatic cell line (HC cells) following co-incubation with *Candida* species, we explored the hypothesis that these fungi might induce the nuclear activity of TG2 in hepatic cells leading to hepatic cell death.

It was observed that incubation of human liver cell with *C. albicans* and *C. glabrata* increased cellular TG activity, TG2 mRNA and nuclear accumulation of TG2. The increased TG activity was inhibited by TG inhibitors cystamine and R283. ZDON and phenosafranin significantly inhibited cell death following activation of caspase-3 in FLC-7 cells. This suggests fungi *C. albicans* and *C. glabrata* enhances cellular TG2 activity in host cells and leads to cell death via activation of caspase-3 pathway.

Several virulence factors of *C. albicans* were evaluated in order to identify the factor(s) responsible for induction of cellular TG activity. For this, HC cells were co-cultured with heat-inactivated *C. albicans*, hyphae form of *C. albicans*, *C. albicans* with delayed hyphae development and *C. albicans* mutant cells with defect in adhesion and biofilm formation such Caecm33, Cabgl2, Cafad2. In addition, HC cells were also co-cultured with *C. albicans* cells in presence of 5 μm Ebelactone B, a lipase inhibitor. The results suggested that the increase in TG activity in HC cells requires viable fungi cells and is not related to structural component of fungal cell wall, polymorphic form of the *C. albicans*, its ability to form biofilm, hyphae development, and lipase secreted by *C. albicans*. Next, HC cells were co-cultured with living fungus plated in dialysis membrane (≤ 10kDa) or fungus-cultivated media to evaluate the necessity of direct contact of these fungi. Enhancement of TG activity in HC cells was observed in living fungus plated in dialysis membrane but not in the fungus-cultivated media. This result suggested that certain soluble factor(s), which might be very unstable in nature, was secreted into the culture medium by *C. albicans* and caused increased cellular TG activity.

Further, to verify whether the responsible factor is reactive oxygen species (ROS) or not, effect of Terbinafine (a free radical interceptor) in fungus-HC co-culture and exogenously added H_2O_2 in HC cells were

evaluated. While addition of Terbinafine did not showed increase in TG activity in the fungus cultured HC cells, exogenously added H_2O_2 increased TG activity in HC cells. These results suggested that ROS might be the responsible factor. Next, chemi-luminescence probe Lucigenin, fluorescent probe CM-H2DCFDA and Electron Paramagnetic Resonance (ESR) spectroscopy analysis were used to verify and identify the involvement of ROS. The result showed a higher amount of ROS identified as •OH in the conditioned medium co-incubated with HC cells and *C. albicans* /*C. glabrata*.

Next, the role of fungus derived ROS (identified as •OH) was clarified by both gain of- and loss of-function. For this HC cells were exogenously treated with H_2O_2 in the presence of NAC and a NOX1- deleted mutant in *C. glabrata*. Reproducible enhancement of cellular TG activity by exogenously added H_2O_2 were observed, while the ROS scavenger NAC blocked these effects. Furthermore, a mutation in NOX1 in *C. glabrata* abrogated the capacity to enhance cellular TG activity in *C. glabrata*. To investigate the role of TG2 in fungus-derived ROS in hepatic cell death, primary hepatocytes from $TG2^{+/+}$ and $TG^{-/-}$ mice were incubated with pathogenic fungi and the TG activity along with caspase-3 activation were analyzed. Increased cellular TG activity and caspase-3 activation were observed in co-culture of *C. albicans*/*C. glabrata* and $TG2^{+/+}$ hepatocytes but not with $TG2^{-/-}$ hepatocytes and $TG2^{+/+}$ hepatocytes treated with *Cgnox1/ C. utilis/ S. cerevisiae* and NAC. This indicated that fungi-derived ROS was able to increase cellular TG activity, especially TG2, and increases caspase-3 activation, leading to cell apoptosis.

The study highlights an association of ROS-produced by fungi like *C. albicans* and *C. glabrata* in close proximity to host cells with enhanced nuclear TG2 activity in hepatic cells leading to apoptosis, illustrating the impact of ROS-generating pathogens in inducing and/or exacerbating nuclear TG2-related liver injuries, which may explain a molecular mechanism of hepatic injury seen in ASH/NASH patients.