Title:
CD44v9-positive cancer stem cell is developed from CAPZA1 over-expressing cell induced by oxidative stress.

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Introduction:
It is known that CD44 variant 9 (CD44v9) is a splicing variant of cancer stem cell marker and CD44v9 expression is strongly associated with the recurrence of early gastric cancer (Br. J. Cancer 109:379-386, 2013). However, the mechanism of CD44v9 expression in H. pylori-infected gastric mucosa has not been clarified. Although translocated Helicobacter pylori-derived CagA is usually degraded by autophagy, it specifically accumulates in CD44v9-positive cancer stem cells (Cell Host Microbe 12:764-777, 2012). We recently reported that CAPZA1 functions as a negative regulator of the autophagy, and thereby translocated CagA accumulates in CAPZA1-overexpressing cells (Autophagy 15:242-258, 2019). The present study was conducted to examine the generating mechanisms of CAPZA1-overexpressing cells that lead to the production of CD44v9-positive cancer stem cells.

Methods:
CAPZA1 expression levels in gastric mucosa were evaluated using H. pylori-infected Mongolian gerbils. Lipid peroxidation and protein carbonylation in the H. pylori-infected mucosa were evaluated on the basis of malondialdehyde and protein carbonyl levels, respectively. Expression mechanisms of CAPZA1 were examined by western blotting and chromatin immunoprecipitation. CD44v9 expressions were evaluated by western blotting and immunohistochemistry.

Results:
In CAPZA1-overexpressing cells infected with H. pylori, CD44v9 expression was enhanced due to accumulation of CagA oncoprotein. CD44v9-expressing cells were detected among cells strongly stained for CAPZA1 in H. pylori-infected gastric mucosa of Mongolian gerbils and human gastric cancer tissues. Moreover, CAPZA1-overexpressing cells infected with H. pylori exhibited enhanced expression of Sal-like protein 4 (SALL4) and Krüppel-like factor 5 (KLF5), which encode reprogramming factors. Our findings show that CD44v9-expressing cancer stem cells arise from CAPZA1-overexpressing cells following CagA accumulation. We subsequently examined the induction mechanisms of CAPZA1 expression. The levels of CAPZA1 expression in the gastric mucosa of H. pylori-infected Mongolian gerbils were significantly higher than that in uninfected gastric mucosa. In H. pylori-infected gastric mucosa, a significant linear correlation was observed between CAPZA1 expression and lipid peroxidation (r = 0.614, p <0.005). CAPZA1 expressions in AGS cells were increased in the dose-dependent manner by the treatment with H₂O₂ or Di-tert-butyl peroxide. Such increased expression of CAPZA1 was abolished by treatment with N-Acetyl-L-cysteine. These results show that CAPZA1 expression is enhanced by oxidative stress stimulus.

Conclusions:
CAPZA1-overexpressing cells behave as progenitor cells for CD44v9-positive cancer stem cells. Oxidative stress in H. pylori-infected gastric mucosa could increase CAPZA1 expression.

Key words: Oxidative stress, CAPZA1, CD44v9 cancer-stem cells