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rasH2 mouse-derived esophageal organoids show higher susceptibilities to 4-NQO without S9 than those with S9 in an *ex vivo* carcinogenesis model

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Background and Purpose

It was reported that rasH2 mice showed high susceptibility to tongue and esophageal carcinogenesis caused by 4-nitroquinoline 1-oxide (4-NQO) in drinking water (Miyamoto S *et al.*, 2008). Therefore, this study examined the carcinogenic potential of 4-NQO in an *ex vivo* model using rasH2 mouse-derived esophageal organoids to determine the carcinogenic mechanisms at the cellular level.

Materials and Methods

Animals ; rasH2, a hemizygous transgenic mouse carrying the human proto-oncogene c-*HRAS*, and non-Tg (wild type) male mice (5-7-week-old)

Pretreatment of esophagus ; incubation in Dispase II at 37°C for 45 min, followed by epithelial peeling and incubation in trypsin for 10 min.

Medium composition in Advanced DMEM/F12 medium ;

HEPES	10 mM	NAC	1 mM
N2 Supplement	1×	B-27™ Supplement	1×
mEGF	100 ng/mL	Noggin	100 ng/mL
R-Spondin	100 ng/mL	Y-27632	10 μM

Chemical treatment ; chemicals were mixed in the medium after passaging at each concentration on day 1, followed by washing with PBS and overlaying with Matrigel™ on day 2 (Figure 1).

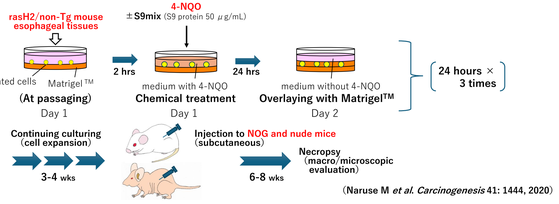

Figure 1 Schematic illustration of the *ex vivo* chemical carcinogenesis model using mouse tissue-derived organoids

Selection of concentrations of 4-NQO; cell viability assays of rasH2 mouse- and wild type mouse-derived esophageal organoids were performed using Real-time Glo™.

Results

(1) Diminished cytotoxicities under conditions of S9(+) in the both rasH2 and non-Tg mice-derived organoids (Figure 2)

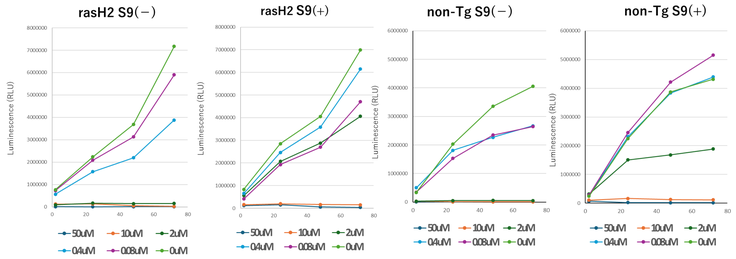

Figure 2 Cell viabilities of 4-NQO-treated esophageal organoids under S9(+) and S9(-) conditions

(2) No tumor induction in non-Tg mouse-derived esophageal organoids treated with 4-NQO under S9 (–) and S9 (+) conditions (Figure 3)

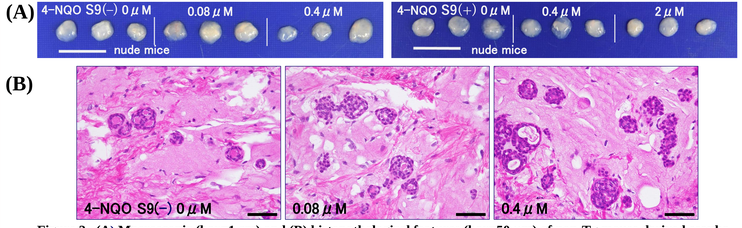

Figure 3 (A) Macroscopic (bar=1 cm) and (B) histopathological features (bar=50 μm) of non-Tg mouse derived esophageal organoids treated with 4-NQO under S9(+) and S9(–) conditions (subcutaneous tissues in nude mice)

(3) No tumor induction in rasH2 mouse-derived esophageal organoids treated with 4-NQO under S9 (+) conditions

Macroscopic and histopathological features of rasH2 mouse-derived organoids treated with 4-NQO under S9(+) conditions were similar to those of non-Tg mouse-derived ones as above.

Results (cont.)

(4) Induction of papillomas/carcinomas in rasH2 mouse-derived esophageal organoids treated with 4-NQO under S9 (–) conditions (Tables 1 and 2, Figure 4)

Table 1 Volumes of subcutaneous nodules of rasH2 mouse-derived esophageal organoids treated with 4-NQO under S9(–) conditions (mean ± s.d., mm³)

4-NQO conc. (μM) S9(–)	N *1	NOG mouse	*P* value *3	N *2	nude mouse	*P* value *3
0 (cont.)	4	15.4 ± 5.2	0.232	6	10.5 ± 5.9	0.013
0.08	4	15.9 ± 2.4		3	12.0 ± 6.0	
0.4	4	55.3 ± 31.2		6	216.4 ± 218.2	(0.026 vs. cont.)

*1 The number of NOG mice was set to a total 4/group due to an accidental death.
*2 The number of nude mice in 0 and 0.4 μM groups were set to 6 (3+3)/group for checking reproducibility.
*3 Kruskal-Wallis test followed by Bonferroni post-hoc test were performed.

Table 2 Incidences of tumors induced from rasH2 mouse-derived esophageal organoids treated with 4-NQO under S9(–) conditions

4-NQO conc. (μM) S9(–)	NOG mouse				nude mouse			
	N	papil.	carci.	*P* value *1	N	papil.	carci.	*P* value *1
0 (cont.)	4	0	0		6	0	0	
0.08	4	0	0	0.006	3	0	0	0.0004
0.4	4	4	3		6	6	5	

*1 Fisher's exact test was performed for the incidences of papilloma+carcinomas.

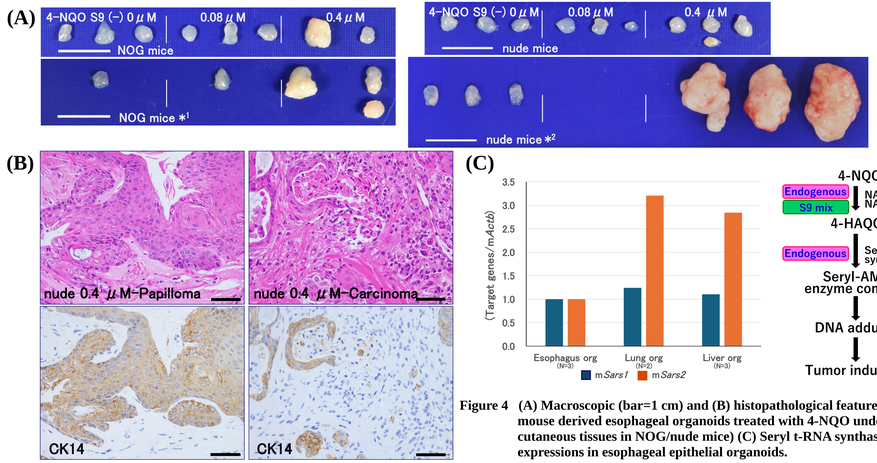

Figure 4 (A) Macroscopic (bar=1 cm) and (B) histopathological features (bar=50 μm) of rasH2 mouse derived esophageal organoids treated with 4-NQO under S9(–) conditions (subcutaneous tissues in NOG/nude mice) (C) Seryl t-RNA synthase gene (m*Sars1*/m*Sars2*) expressions in esophageal epithelial organoids.

Conclusion

The combination of intracellular metabolic modification of 4-NQO via seryl-tRNA synthase and inserted *c-HRAS* gene expressions in the esophageal epithelia was suggested to contribute the tumor induction; while extracellular S9 negatively worked.