

Answering Reviewers

Q1. When rat drink water containing 4NQO, cancer will develop in 28 weeks, but where is it located, upper, middle, or lower? 2.Histologically, it is SCC, but how about adenocarcinoma? 3.When esophagoduodenostomy is performed in rats after total gastrectomy, SCC and ADC occur in 40-50 weeks due to bile acid reflux. The above also causes cancer, but please explain the difference in esophageal carcinogenesis between oral administration of 4NQO and esophagoduodenostomy after total gastrectomy

AQ: We appreciate the valuable comments deeply. **(1)** In the current study, the tumor is located mainly in the middle and lower part of the esophagus.

(2) Prior to commencing the study, we conducted a thorough literature search and discovered that esophageal cancer induced by 4NQO is predominantly squamous cell carcinoma. As a result, 4NQO has become a popular choice for researching esophageal squamous carcinoma. This study verifies the pathological type of 4NQO-induced esophageal cancer using immunohistochemistry. The results show abnormal expression of marker proteins for squamous cell carcinoma such as CK5 and p40, confirming the pathological type as squamous cell carcinoma. The possibility of an adenocarcinoma type at the gastro-esophageal junction requires further exploration in the future.

(3) Esophageal cancers induced by the two types of animal modeling differ in the following aspects: First, the time required for mold formation differs: 4NQO takes about 24-28 weeks to induce esophageal carcinogenesis, whereas esophagoduodenostomy after total gastrectomy takes longer to induce esophageal carcinogenesis. Second, the pathologic types of esophageal cancer induced by the two modalities differ. According to PMID8813264, esophageal cancer induced by 4NQO is squamous cell carcinoma, while esophagoduodenostomy after total gastrectomy predominantly induces

adenocarcinoma. As mentioned in PMID10839958, it can also result in both squamous cell carcinoma and adenocarcinoma. The location of esophageal cancer induced by the two modalities differs. Esophageal cancer induced by 4NQO is mainly located in the middle and lower esophagus, while esophageal cancer induced by esophagoduodenostomy after total gastrectomy is mainly located in the lower esophagus and the gastroesophageal junction.

It is worth noting that the incidence of esophageal adenocarcinoma induced by carcinogen combined with esophagoduodenostomy has been reported to reach 87% (PMID: 8813264). The most common modeling modality for esophageal squamous cell carcinoma is carcinogen induction, such as 4-nitroquinoline 1-oxide (4NQO), N-nitrosomethylbenzylamine (NMBA), methyl-n-amyl nitrosamine (MNAN), etc. Adenocarcinomas, on the other hand, are mainly induced by esophagoduodenostomy, but this type of modeling still requires further study. Thank you very much again.