Short Communication

A scientometric study of chemical carcinogen-induced experimental oral carcinogenesis with emphasis on chemopreventive agents

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Abstract *Background/purpose:* 4-Nitroquinoline 1-oxide (4NQO)-induced tongue carcinoma and 7,12-dimethlybenz(a)anthracene (DMBA)-induced cheek pouch carcinoma are the most common and classical chemical carcinogen-induced animal models of oral carcinogenesis. The purpose of this study was to provide the research trends and characteristics of 4NQO- and DMBA-induced experimental oral carcinogenesis.

Materials and methods: The papers on both 4NQO- and DMBA-induced experimental oral carcinogenesis were published since 1962. All the eligible papers were retrieved on 12 May 2023 from the Scopus database.

Results: There were 506 and 349 papers on 4NQO- and DMBA-induced experimental oral carcinogenesis with 10,152 and 6306 citations, respectively. The common distinctive keywords such as rat, tongue neoplasms, drinking water, tumor microenvironment, and cyclooxygenase (COX)-2 were identified in the papers on 4NQO; and the common keywords such as hamster, cheek pouch, lipid peroxidation, glutathione, antioxidants, and topical drug administration

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were identified in the papers on DMBA. Importantly, 105 and 65 potential chemopreventive agents were identified from the papers on 4NQO and DMBA, respectively. Furthermore, 15 promising agents such as COX-2 inhibitor, curcumin, garlic were researched concurrently in both the two animal models.

Conclusion: This study for the first time reports the scientometric characteristics of 4NQO- and DMBA-induced experimental oral carcinogenesis. Importantly, we identify a valuable profile for oral cancer chemopreventive agents, which will aid researchers and investigators in studying oral cancer chemoprevention.

Introduction

Oral carcinogenesis is a multistep process characterized by initiation, promotion, and tumor progression, and usually undergoes clinical precancerous changes, mainly being oral leukoplakia and erythroplakia.[1] Histologically, this multistep process typically preceded by hyperplasia, various degrees of dysplasia, followed by oral squamous cell carcinoma (OSCC) development. Nowadays, the pathogenesis and potential therapeutic strategies of OSCC development remain to be elucidated and further research.[1] It is crucial that the use of suitable animal models mimics histological and biological processes of human oral carcinogenesis and makes use of them to develop the relevant prevention strategies.[2,3] Currently, OSCC development can be established in murine by tumor cell transplanted, chemical carcinogen-induced, and genetically engineered mouse models. As expected, each specific model system has its advantages and disadvantages that researchers need to consider in their preclinical studies.[2,3]

Chemical carcinogen-induced murine models have an obvious advantage with sequential stages of oral epithelial lesions resembling the dynamic multistage of human oral carcinogenesis.[4] Of chemical carcinogens, 4-nitroquinoline 1-oxide (4NQO)-induced tongue carcinoma and 7,12-dimethlybenz(a)anthracene (DMBA)-induced cheek pouch carcinoma are the most common and classical animal models of oral carcinogenesis.[5−7] The multiple stages associated with OSCC progression indicate that a prevention strategy that can delay, inhibit, or reverse carcinogenesis before it becomes an invasive carcinoma would be the most beneficial approach. Hundreds of studies on the 2 animal models have published and provided the relevant pathogenesis research and predictable preclinical strategies to mimic human oral carcinogenesis, which can be used to investigate the efficacy of novel chemical agents for OSCC chemoprevention.

Scientometrics is a useful tool that utilizes bibliometric and citation data to evaluate the academic influence of the literature within the designated area to guide research forward.[8−11] In this context, the objective of the current study is to provide the research trends and characteristics of 4NQO- and DMBA-induced experimental oral carcinogenesis and highlight the potential profile for oral cancer chemopreventive agents.

Materials and methods

As per the methodology described previously,[9,10] all the papers on 4NQO- and DMBA-induced experimental oral carcinogenesis were retrieved on 12 May 2023 from the Scopus database. According to the search strategy described in Supplementary Table S1, we used medical subject term "4NQO" or "DMBA" and the respective synonyms in the title/abstract to retrieve the relevant papers on oral cancer. There was no restriction in the search regarding publication year, type, and design of the papers. The papers on both 4NQO- and DMBA-induced experimental oral carcinogenesis were published since 1962. Titles and abstracts or full texts of the papers were screened and re-evaluated to confirm the eligible ones. Data search and extraction were performed independently by two investigators (X.H. and W.L.), and discrepancy of results was resolved in a consensus symposium. The scientometric characteristics of all the eligible papers were reviewed and recorded the following information: authorship, publication year, title, citation count, paper type, keywords, affiliation, and country/region of origin. The chemopreventive agents were retrieved in reviewing all the eligible papers. Descriptive statistics and associations were calculated for scientometric characteristics. The Bibliometrix Biblioshiny R-package software (https://www.bibliometrix.org/home/; K-Synth Srl Inc., Naples, Italy) was used to analyze the relevant scientometric data.

Results

Citation characteristics

With the search strategy algorithm, a total of 506 and 349 papers on 4NQO- and DMBA-induced experimental oral carcinogenesis were published until the time of the search, respectively. Fig. 1A illustrates the number and distribution of the paper types. The total count of citations was 10,152 and 6306 and the *h* index was 47 and 44 for the papers on 4NQO- and DMBA-induced model, respectively (Fig. 1B). To concretize the treads of academic influence, we evaluated the annual number of the papers and accumulated citations during 2007−2022. The annual number of the papers on 4NQO stably raised from 16 to 28 during 2007−2022; while the number of the papers on DMBA was ups and downs with
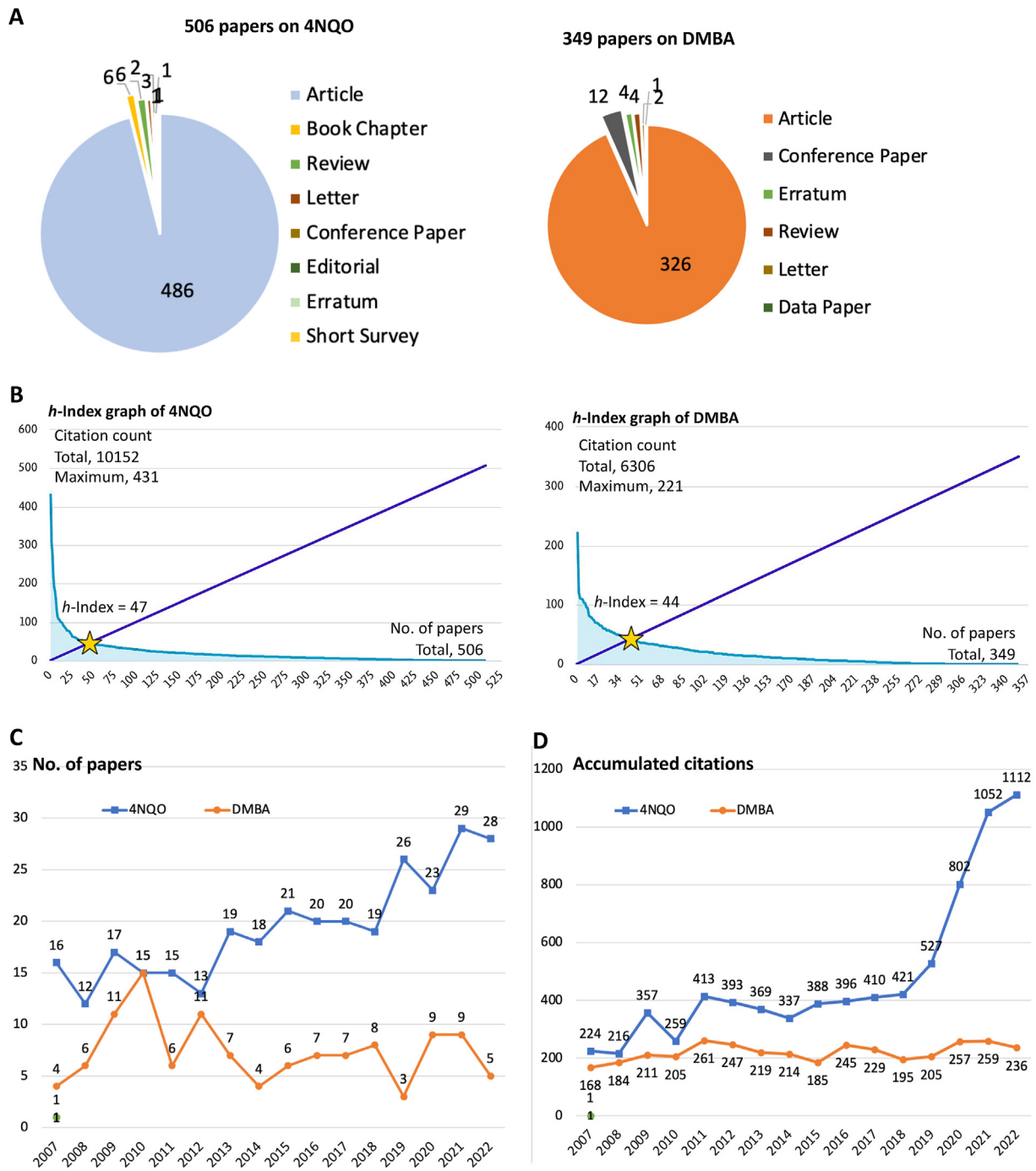
Figure 1 Citation characteristics of the papers on 4NQO- and DMBA-induced experimental oral carcinogenesis. (A) Document types and distribution of the papers. (B) The *h*-index graphs. (C) The annual number of the papers during 2007−2022. (D) The accumulated citations of the papers during 2007−2022.

the range of 4−15 during the period (Fig. 1C). Consistently, the accumulated citations of the papers on 4NQO stably increased from 224 to 527 and then rapidly increased up to 1112 during 2007−2022; while the accumulated citations of the papers on DMBA remained slow growth from 168 to 235 during the period (Fig. 1D). The detailed information on title, publication year, journal, citation count, authors, affiliation, keywords, and document types of all the papers on 4NQO- and DMBA-induced oral carcinogenesis are presented in Table S2 and Table S3, respectively. The cloud

graphs of journal of publication, contributing authors, institutions, and countries/regions of origin regarding the papers on 4NQO- and DMBA-induced oral carcinogenesis are shown in Fig. 2.

Research characteristics

All the keywords were automatically recognized in the order of highest to lowest frequency by the database. As
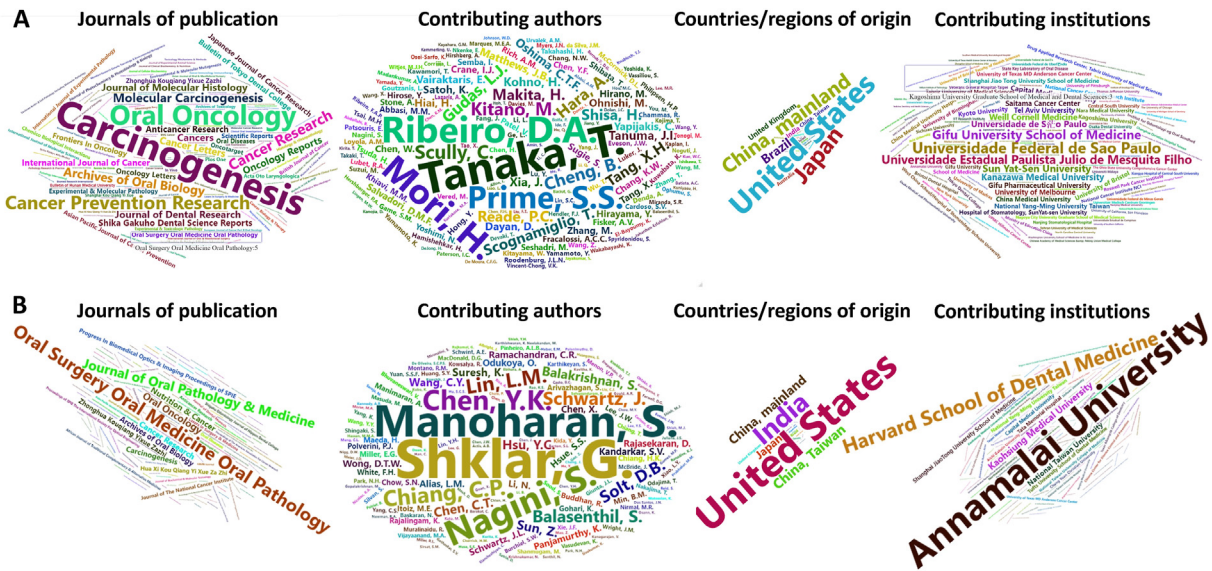
Figure 2 Cloud graphs of journal of publication, contributing authors, and countries/regions and institutions of origin in the papers on (A) 4NQO- and (B) DMBA-induced experimental oral carcinogenesis. For the papers on 4NQO, the journal with largest number is *Carcinogenesis* (n = 30), followed by *Journal of Oral Pathology & Medicine* (n = 28) and *Oral Oncology* (n = 22). The contributing author with largest number of papers is Tanaka, T. (n = 33), followed by Ribeiro, D.A. (n = 27) and Mori, H. (n = 26). The contributing institution and country of origin with the maximum number is Universidade Federal de São Paulo (n = 24) and United States (n = 122), followed by Gifu University School of Medicine (n = 20) and Japan (n = 96), respectively. For the papers on DMBA, the journal with largest number is *Oral Surgery Oral Medicine Oral Pathology* (n = 22), followed by *Journal of Oral Pathology & Medicine* (n = 16) and *Oral Oncology* (n = 12). The contributing author with largest number of papers is Shklar, G. (n = 29), followed by Manoharan, S. (n = 24) and Nagini, S. (n = 19). The contributing institution and country of origin with the maximum number is Annamalai University (n = 60) and United States (n = 106), followed by Harvard School of Dental Medicine (n = 32) and India (n = 71), respectively.

shown in Fig. 3A, the same keywords were observed from the papers on both 4NQO and DMBA, such as carcinogenesis, pathology, protein expression, metabolism, antineoplastic agent. In the papers on 4NQO, the distinctive keywords were rat, tongue neoplasms, drinking water, tumor microenvironment, cyclooxygenase (COX)-2, and so on. In the papers on DMBA, the distinctive keywords were hamster, cheek pouch, lipid peroxidation, glutathione, antioxidants, topical drug administration, and so on. It is noteworthy that the potential chemopreventive agents for oral carcinogenesis were profiled in this analysis. Of 506 papers on 4NQO and 349 ones on DMBA-induced oral carcinogenesis, 121 (23.9%) and 83 (23.8%) involved in research on chemopreventive agents, respectively.

A total of 105 and 65 potential chemopreventive agents were identified from 124 to 83 papers on 4NQO and DMBA, respectively (Fig. 3B). Of 105 agents researched in 4NQO-induced oral carcinogenesis model, the research with the maximum number was COX-2 inhibitor (8 studies), followed by curcumin (5 studies), garlic (4 studies), PD-1 agent (4 studies), black raspberry (3 studies), geraniol (3 studies), grape seed (3 studies). Of 65 agents researched in DMBA model, the research with the maximum number was beta-carotene (5 studies), followed by garlic (4 studies), withaferin-A (4 studies), carotenoids (3 studies), vitamin E (3 studies), ZengShengPing (3 studies). More importantly, 15 agents including astaxanthin, beta-carotene, black raspberry, caffeic, canthaxanthin, COX-2 inhibitor, curcumin, EGFR inhibitor, ferulic acid, garlic, neem leaf extraction,

quercetin, vitamin C, xanthophylls, ZengShengPing were researched concurrently in both 4NQO- and DMBA-induced oral carcinogenesis models.

Discussion

OSCC is an important cause of malignancy death worldwide, the availability of preclinical models is likely to help elucidate the pathogenesis and facilitate interventions targeting this malignancy. Compare to *in vitro* study, experiments *in vivo* using animal models which are representative of whole organisms can reflect the tumor microenvironment and biological behaviors. Moreover, the use of animal models can avoid issues related to ethics, safety, and extended research cycles that arise in human experiments. An optimal animal model would exhibit spontaneously occurring oral cancer; however, spontaneous OSCC in genetically engineered mice models is extremely rare in laboratory animals. Tumor cell transplanted models generally use immunodeficient mice without accurate immune microenvironment. Chemical carcinogen-induced murine models can reproduce the multiple stages of OSCC development clinically and microscopically, enabling monitoring structural and architectural changes in the epithelium until invasion occurs.

Although 4NQO-induced rat/mouse tongue carcinoma and DMBA-induced hamster cheek pouch carcinoma are the classical animal models of oral carcinogenesis, the number
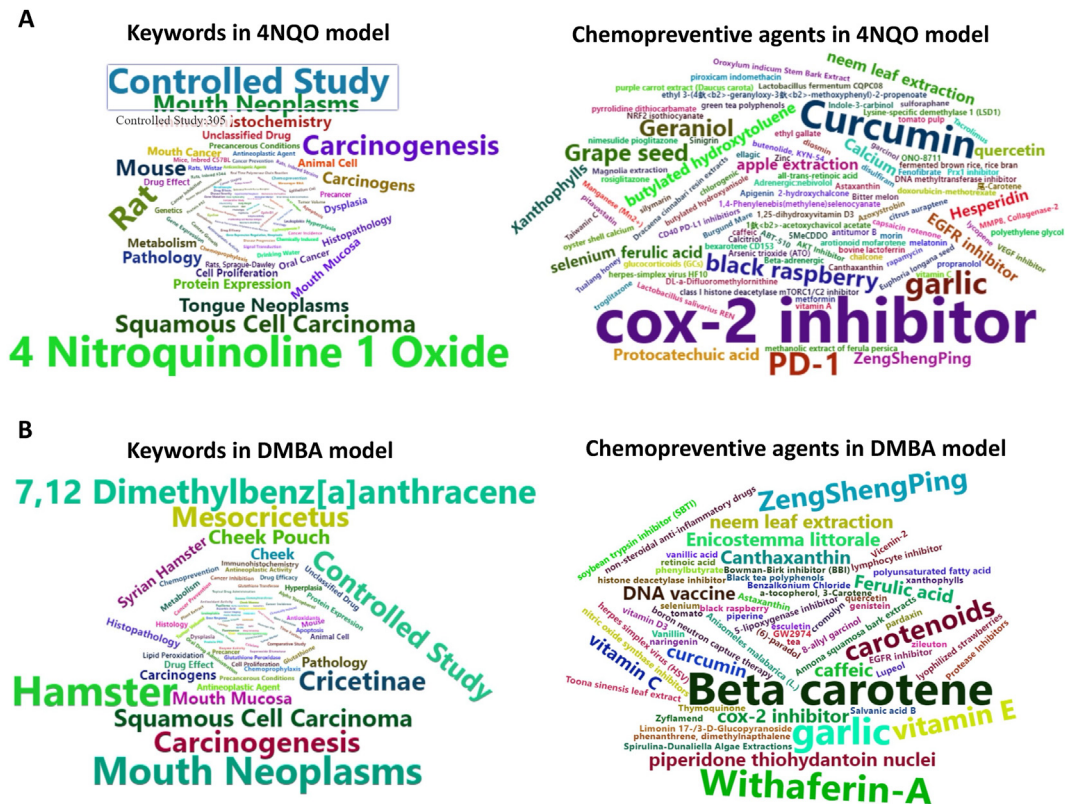
Figure 3 Cloud graphs of keywords and chemopreventive agents in the papers on (A) 4NQO- and (B) DMBA-induced experimental oral carcinogenesis.

and citations of papers on 4NQO are larger than those on DMBA (Fig. 1). DMBA-induced model is a questionable surrogate for the human oral cavity due to the hamster cheek pouch, which has inadequate lymphatic drainage and lacks cheek pouches in the human. Moreover, DMBA is not found in tobacco smoke or the environment but is an oxidatively activated carcinogen. Notably, 4NQO, a synthetic water-soluble carcinogen, is a mutagenic substance capable of generating changes in DNA that mimic tobacco-induced molecular events, resulting in genetic mutations and DNA strand breaks. 4NQO treatment serves as an alternative for tobacco exposure in animal experiments of OSCC; and 100% of 4NQO-treated murine exhibit precancerous lesions and tumors on the tongues. Moreover, mutational signatures of human OSCC and 4NQO-induced mouse OSCC overlap broadly. Compared with hamster and rat, the mouse has some advantages such as small size, a propensity to breed in captivity, a lifespan of 2—3 years, extensive physiological and molecular similarities to humans, and an entirely sequenced genome.[6]

The development of chemopreventive agents is a key topic in the community of oral cancer prevention, because there are no efficient pharmacological strategies to prevent oral cancer development.[12] It is important to make use of relevant animal models to develop prevention strategies for oral cancer development and even the entire field of cancerization. In this study, it is noteworthy that we provide a valuable profile for oral cancer chemopreventive agents (Fig. 3B). A total of 105 and 65 potential agents are identified in 4NQO- and DMBA-induced oral carcinogenesis model, respectively. Furthermore, 15 promising agents such as COX-2 inhibitor, curcumin, garlic are researched concurrently in both the two animal models. We hope that the potential profile of these agents will aid researchers and investigators in studying oral cancer chemoprevention.

Collectively, the scientometric characteristics of 4NQO- and DMBA-induced experimental oral carcinogenesis are firstly comprehensively provided in this study. The 4NQO-induced mouse model remains the relevant and reproducible model which mimics several morphological, histological, genomic and molecular features of human oral carcinogenesis, superior to DMBA-induced hamster model. It has clear benefits for studying oral cancer chemopreventive agents.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jds.2023.06.004.

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