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REVIEW

The hamster model of sequential oral oncogenesis ☆

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Summary Oral squamous cell carcinoma (OSCC) is a common cancer characterised by low survival rate and poor prognosis. The multistep process of oral carcinogenesis is affected by multiple genetic events such as alterations of oncogenes and tumour suppressor genes. The use of appropriate experimental animal models that accurately represent the cellular and molecular changes which are associated with the initiation and progression of human oral cancer is of crucial importance. The Syrian golden hamster cheek pouch oral carcinogenesis model is the best known animal system that closely correlates events involved in the development of premalignant and malignant human oral cancers. Therefore, we established an experimental system of chemically induced oral carcinogenesis in hamsters, in order to study different stages of tumour formation: normal mucosa, hyperkeratosis, hyperplasia, dysplasia, early invasion, well differentiated OSCC and moderately differentiated OSCC. We investigated the expression of oncogenes EGFR, erbB2, erbB3, FGFR-2, FGFR-3, c-myc, N-ras, ets-1, H-ras, c-fos and c-jun, apoptosis markers Bax and Bcl-2, tumour suppressor genes p53 and p16, and cell proliferation marker Ki-67 in the sequential stages of hamster oral oncogenesis. Here, we describe the findings of the experimental model in regard to the involvement of signal transduction pathways in every stage of cancer development. Increased apoptosis and cell proliferation were observed in early stages of oral oncogenesis. Furthermore, the increased expression of transmembrane

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receptors (EGFR, erbB2, FGFR-2 and FGFR-3) as well as the increased expression of nuclear transcriptional factors in early stages of oral cancer indicates that these molecules may be used as early prognostic factors for the progression of OSCC. Since the expression of both H-ras and N-ras do not seem to affect signal transduction during oral oncogenesis, it can be assumed that a different signalling pathway, such as the PI3K and/or PLCγ pathway, may be implicated in the pathogenesis of OSCC.

Animal models of oral squamous cell carcinoma

Oral squamous cell carcinoma (OSCC) is a common cancer, characterised by poor prognosis and low survival rate.[1,2] Despite the large amount of research data in cell and molecular biology and the advances in oncology and surgery, the mortality and morbidity rates in OSCC patients remain unchanged.[1] The most important risk factors for OSCC remain tobacco and alcohol.[3] Viruses, most notably the human papillomavirus, have also been linked to oral carcinogenesis.[4] Genetic predisposition has also been suggested, due to the fact that the majority of the population exposed to these factors does not develop oral cancer, as well as the fact that sporadic cases of oral tumours occur in young adults and non-users of tobacco and alcohol.[3,5,6] However, the crux of the problem is the mechanisms by which environmental or genetic factors may change normal cells.

The multistep process of oral carcinogenesis is affected by multiple genetic events such as alterations of oncogenes and tumour suppressor genes.[7–9] Therefore, understanding the molecular mechanisms involved in the initiation and progression to malignancy of oral cancer will help to improve its prognosis and in the elaboration of new forms of treatment. The use of carcinogen-induced animal models to study the mechanisms of carcinogenesis is warranted, since chemical agents appear to be the dominant etiologic factor in various areas of the head and neck including oral cavity. Several animal models for development of OSCC have been generated, including hamster, rat and mouse models.[10]

One of the best characterised animal models for OSCC is the hamster cheek pouch system of oral carcinogenesis model that closely correlates with sequential common events involved in the development of premalignant and malignant human oral cancers.[11,12] These common events include mutations as well as changes in expression of oncogenes and tumour suppressor genes, such as p53, H-ras and p16,[13–16] expression of cell proliferation markers,[17,18] a response to immune-related cytokines, such as interleukins and tumour necrosis factors[19] and enhancement of growth in response to various factors, such as epidermal growth factor and transforming growth factor.[20] The major advantages of this model are similarity between hamster buccal pouch mucosa and keratinizing human oral mucosa, absence of spontaneous tumours, and susceptibility to systemic influences such as hormones, micronutrients, and others.[21,22]

The first successful production of tumours in this anatomic site was that of Salley who investigated the effects of the powerfully carcinogenic polycyclic hydrocarbon 7,12-dimethylbenzanthracene DMBA.[11,12] In mammalian cells, DMBA can be bioactivated via formation of a reactive diol-epoxide metabolite, which subsequently adducts to adenine and guanine residues in DNA.[21–23] The formation of these adducts has been suggested as the ultimate step in the carcinogenic mechanism of DMBA.[24–26] Another commonly used carcinogen is 4-nitroquinoline 1-oxide (4-NQO), a water-soluble quinoline derivative that can cause DNA adduct formation, resulting in adenosine substitution for guanosine.[27,28] 4-NQO also can undergo redox cycling to produce reactive oxygen species that result in mutations and DNA strand breaks.[28] 4-NQO has been shown to induce OSCC in rats[29] and mice[30–32] producing similar histological as well as molecular changes in sequential stages as seen in human oral carcinogenesis.[10,33]

It is generally agreed that experimental animal models representing accurately the cellular and molecular changes associated with the initiation and progression of human cancer are of crucial importance. Use of an animal model seems to be a virtually inevitable prelude to understanding development of carcinomas and assessing the efficiency of new therapeutic approaches.[33] Here, we describe a hamster model of oral carcinogenesis which was used in order to better understand the mechanisms involved in sequential stages towards malignancy.

An animal model of sequential oral oncogenesis

In order to study the molecular pathways involved in the sequential stages of OSCC development, we used a model of induced carcinogenesis in hamster. A brief description of the performed experimental procedures follows, since they have been described in detail elsewhere.[34,35]

Experimental carcinogenesis

Thirty seven Syrian golden hamsters (*Mesocricetus auratus*), five weeks old and 100 g weight were randomly divided into three experimental groups for carcinogen treatment (A, B and C) and one control group ($n = 7$). Three times per week the animals in groups A ($n = 10$), B ($n = 10$) and C ($n = 10$) were anesthetized with ether and their left buccal pouches were painted with 0.5% 9,10-dimethyl-1,2-benzanthracene (DMBA) (Sigma, St. Louis, MO) dissolved in paraffin oil, using a #4 camel's hair brush. The amount of carcinogen delivered to each animal was quite uniform using the ''wiped-brush'' method.[12] Treatment with this carcinogen was effected in group A animals for 10 weeks, and for animals of groups B and C for 14 weeks. The pouches of all animals were examined weekly in order to observe the growth of tumours on the mucosa. Animals were sacrificed by an overdose of ketamine hydrochloride (>30 mg/kg), given intraperitoneally, and the treated buccal pouches were removed at 10 weeks from the application of the carcinogen (group A), at 14