Archives of Oral Biology (2006) **51**, 236–245

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Archives of Oral Biology

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Cloning of hamster osteopontin and expression distribution in normal tissues and experimentally induced oral squamous-cell carcinoma

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Accepted 22 June 2005

KEYWORDS
Osteopontin;
Hamster;
DMBA-induced carcinogenesis;
Oral squamous-cell carcinoma

Summary Osteopontin (OPN) is a non-collagenous extracellular matrix (ECM) protein expressed and secreted by several human cancers. This study investigated the expression pattern of OPN during development of oral squamous-cell carcinoma by using 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced squamous-cell carcinomas in buccal pouch of syrian golden hamsters. We first identified the hamster OPN cDNA sequence by screening of a hamster calvariae cDNA library with a rat OPN cDNA probe. The resulting 1449 bp of hamster OPN cDNA led to a deduced protein sequence of 305 amino acids containing several putative binding sites to integrins, CD44 receptors, calcium ions and hydroxyapatite, as well as multiple sites for phosphorylation, glycosylation and sulphation. Hamster OPN cDNA was then used as a probe to analyze the expression of OPN mRNA by Northern blot and in situ hybridization analyses of normal and malignant tissues. OPN mRNA was detected in several non-mineralized tissues as well as in mineralized tissues, but was not present in normal hamster buccal epithelium. DMBA-treated hamster buccal pouches expressed OPN mRNA as early as 4 weeks and displayed the highest level of expression at 15 weeks. The specimens treated with DMBA for 15 weeks exhibited histological features of squamous-cell carcinoma, presented microcrystalline deposits and showed OPN expression associated with malignant epithelium and tumor-associated macrophages. To summarize, our results suggest that buccal-pouch carcinogenesis of Syrian golden hamster may constitute an excellent experimental model to study the mechanisms by which OPN is associated with oral cancer pathogenesis, and to validate OPN-based therapeutic approaches to ameliorate oral cancer progression and metastasis.

Abbreviations: DMBA, 7,12-dimethylbenz[*a*]anthracene; ECM, extracellular matrix proteins; OPN, osteopontin

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doi:10.1016/j.archoralbio.2005.06.013

Introduction

OPN is an ECM protein that plays critical roles in the formation and remodeling of mineralized tissues,[1,2] inflammation,[2,3] cellular transformation and metastasis.[4–6]

OPN protein sequence is highly modified at the post-translational level by phosphorylation, glycosylation and sulphation.[1] It contains several structural domains that allow its interaction with cells through several integrin types and CD44 receptors.[1,3] Briefly, it has the arginine-glycine-aspartic acid (RGD) domain present in many ECM proteins that is critical for binding to $\alpha v\beta 3$, $\alpha v\beta 1$, $\alpha v\beta 5$ or $\alpha 5\beta 1$ integrins.[1,3] It also contains binding sites for $\alpha 9\beta 1$ and $\alpha 4\beta 1$ integrins[7,8] in close proximity to the RGD domain. Furthermore, it harbors one or two heparin-binding domains close the carboxyl-terminus of the protein involved in the binding to various isoforms of CD44.[1,3] In addition to binding to cells, OPN is thought to interact with hydroxyapatite and calcium ions through aspartate- (D-) rich sequences,[1] and to contain additional binding domains for calcium ions close to the carboxyl-terminus of the protein.[3] Binding to calcium and heparin, as well as cleavage of the protein through its two to three thrombin cleavage sites have also been suggested to regulate interactions with integrin-binding domains.[3]

Even more than 2 decades after its discovery as a marker of epithelial cells transformation,[9] there is considerable interest in the roles of OPN in tumorigenesis, both as a marker of malignancy and as a candidate for testing as a prognostic factor.[4–6] To study the association between cell transformation, metastasis and OPN expression in oral cancer, there is a need for an animal model where tumors exhibit similar markers than in human and where tumor progression can be analyzed reproducibly. The buccal pouch of the Syrian hamster is an excellent model to investigate oral cancer development, because squamous-cell carcinomas induced by the application of DMBA to the buccal pouch are morphologically and histologically similar to human tumors,[10] and express similar metabolic and molecular markers.[11–13] In this study, we have used this animal model to clone hamster OPN cDNA and to analyze OPN mRNA expression pattern in normal tissues and DMBA-induced oral squamous-cell carcinomas.

Materials and methods

Animals

Outbred 6-week-old male Syrian golden hamsters (*Mesocricetus auratus*) were purchased from the National Cancer Institute (Frederick, MD). All procedures using these animals were conducted in compliance with the state regulations, and approved by the Institutional Animal Care and Use program of the University of Texas Health Science Center (San Antonio, TX).

Generation of tumors in hamster buccal pouches

Animals were randomly divided into two groups. In the experimental group, the carcinogen DMBA (Sigma) dissolved in mineral oil (0.5%) was applied topically to the left buccal-pouch mucosa with a wool brush three times per week. By using pouches on only the left side, the unilateral formation of tumors allowed the experimental animals to eat and swallow normally. The control group was treated with Ringer's solution over the same time period as the experimental group. To obtain tissues at different stages of tumor development, animals were sacrificed at different times (2—15 weeks) after carcinogenesis had been initiated, by personnel blinded to the treatment assignment. During the study, animals were weekly monitored for gross abnormalities, including formation of tumors, change of surface color or texture alterations. Untreated pouches or Ringer's-treated control pouches did not exhibit apparent changes (data not shown). Thickened mucosa with a rough surface and whitish granular appearance was mostly observed in the 4- and 6-week DMBA-treated pouches. In the 15-week DMBA-treated pouches, solid tumors begun to appear in the pouch mucosa. Normal and tumor tissues were collected and immediately prepared for histological evaluation, in situ hybridization or immunohistochemistry, and the remainder immediately frozen in liquid nitrogen for subsequent RNA isolation.

Isolation of hamster OPN cDNA

An amplified UniZap™ XR Library of adult hamster calvaria was constructed and plated as previously described.[15] Initial screening of the library was performed with a rat OPN cDNA probe labeled with [α-^{32}P]dCTP (>3000 Ci/nmol; Dupont NEN) by using a T7 Quick Prime kit (Pharmacia) according to the Stratagene protocol. Filters were hybridized overnight at 65 °C and then washed in 0.1 × SSC and 0.1% SDS at 65 °C followed by autoradiography for detection of positive clones. Upon replating the positive clones, secondary screening allowed the identification of three positive plaques that were selected and grown to prepare cDNA for sequence analysis.