Emerging Value: The Chick Chorioallantoic Membrane (CAM) Model in Oral Carcinogenesis Research

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Abstract
New questions and technological advances in cancer research have led to renewed relevance for the chicken Chorioallantoic Membrane (CAM) model. The CAM model is a powerful tool to study early events of tumor progression of head and neck cancer. Several advantages of the CAM model over other in vivo models include its clinical relevance, cost effectiveness and the wide accessibility of chicken eggs. These benefits make the CAM model of cancer progression an attractive in vivo option for global oral cancer researchers.

Keywords: Invasion; Tumor progression; In vivo models; Chicken embryo

The embryonic chicken is among the most well-characterized and useful in vivo biological systems [1]. The chorioallantoic membrane (CAM) assay is an emerging model of tumor progression using the embryonic chicken. The CAM is a very vascular structure that surrounds the chick embryo. It allows for exchange of dissolved oxygen and carbon dioxide, similar to the function of the placenta of a developing mammal. The CAM is comprised of an upper chorionic epithelium, intervening mesenchyme, and lower allantoic epithelium. The chorionic epithelium is structurally similar to human epithelium and has a collagen-IV-rich basement membrane. In the CAM assay, a small opening is made in the shell of a fertilized egg, allowing a tumor graft to be seeded directly on the chorionic epithelium. Grafted tumor cells invade through the basement membrane of the chorionic epithelium and into vascular structures in the underlying mesenchyme, thereby metastasizing to distant structures and organs including the liver and lungs of the developing chicken and the lower CAM [2].

The first tumor grafts on the CAM were successfully performed in 1913, establishing that the CAM readily accepts xenografts [2]. This discovery led to the development of multiple carcinogenesis assays using the CAM, which have become prominent methods to assess tumor angiogenesis, progression and metastasis [2,3]. Although the basic technique of grafting tumors to the CAM has changed very little, powerful technological advances allow scientists to use the CAM for assays of increasing complexity. These advances have led to renewed attention to this established model, particularly in the past decade (Figure 1). Since 2010, nearly one hundred cancer-related publications using the CAM model are listed on PubMed annually in peer-reviewed journals.

The CAM is used to study a wide range of cancers, including breast and prostate cancers. Our laboratory recently reported the use of the CAM model to simultaneously study multiple aspects of Head and Neck Squamous Cell Carcinoma (HNSCC) tumor progression, including tumor growth, invasion, metastasis and angiogenesis [2]. In this model, a small window is made through the shell of a fertilized egg early in development to expose the CAM. Tumor cells are seeded on the CAM of the developing chicken to provide an in vivo model of HNSCC progression. Within two to seven day of seeding, several parameters of HNSCC progression can be assessed. For example, invasion of the basement membrane is observable within two days of seeding whereas metastasis is investigated at days 5-7 after seeding of HNSCC cells on the CAM. This is similar to the sequence of clinical progression of HNSCC. For example, invasion of the basement membrane is required for transformation of precancerous lesions into HNSCC [4].

The CAM model has also been used to study epithelial dysplasia (pre-cancer) and peri-tumor lymphatic vessel density, as well as to test...
drugs for HNSCC. The study and related phenotypes are summarized in Table 1.

The CAM model of HNSCC has several benefits over murine in vivo models. The CAM closely replicates the tissue complexity of oral mucosa and therefore is an excellent model for HNSCC progression. Most murine models of HNSCC require subcutaneous or submucosal injection of cancer cells. Because the injected cells artificially bypass the basement membrane, injection-based murine models cannot replicate destruction of the basement membrane with subsequent invasion. This important phenotype is an essential step for transformation of a premalignant lesion into HNSCC [4]. However, in the CAM model of HNSCC, tumor cells must degrade the basement membrane of the chorionic epithelium to invade, closely replicating HNSCC development. Given that some recent work emphasizes the impact on invasion of structures and channels found in true extracellular matrix, it is attractive to study invasion in vivo [23,24].

Many technological advances are giving a fresh perspective on the value of CAM tumors. It is now possible to use stop-motion video-microscopy to continuously monitor a target’s activity over a period of days [25]. Unlike traditional murine models, the CAM model is able to exploit the promise of these new techniques. While both murine and CAM models provide in vivo results, tumors on the CAM are more readily observed and quantified. For similar reasons, the CAM model also offers easier study of angiogenesis.

Another advantage of the CAM model is the short duration (maximum 1 week) required to assess even late events in tumor progression, such as metastasis. This may take several weeks to months to assess in murine models [27]. This long duration increases the cost and time required for investigations. The rapid turnaround of results in the CAM model allows transient transfection studies, which cannot be performed in murine models. For example, the impact of an siRNA on downregulation of a protein lasts only a few days. This timeframe is insufficient for changes in tumor growth to be investigated in mice, which take several days to develop tumors.

Due to the lack of an immune system in the early chicken embryo, the CAM system readily accepts many types of xenografts. Very small numbers of cells are needed for xenograft experiments, and metastasis can be quantified very accurately through PCR-based methods [27]. The cost of mice limits the number of mammals that can be used for each experiment. The CAM model provides an opportunity to perform large in vivo experiments at a fraction of the cost of murine-based models of HNSCC. The cost effectiveness and easy accessibility of the CAM system enhance the appeal of this in vivo model, particularly when funding resources are limited.

The CAM model of tumor progression has some limitations. Perhaps the most significant limitation is that the mouse is currently accepted as the gold standard for in vivo biological studies. An initial investment of time and materials is necessary to set up the CAM protocol, which requires technical dexterity. This is also true of some murine HNSCC models such as the tongue and floor-of-mouth models [28].

The chick embryo becomes fully immunocompetent by day 18 [26]. The developing immune system of the embryonic chicken limits the duration of the study. Additionally, the lack of an immune system prevents the investigation of tumor-host immune system interactions. However, investigations of human HNSCC in mouse also require the use of immunodeficient mice. The larger sample sizes used for CAM experiments yield a large amount of data from multiple phenotypes requiring extensive time for analysis.

Overall, the scientific benefits of the CAM model make it an attractive option to all cancer biologists. The practical advantages of the CAM model over rodent models make it an accessible option to scientists in developing institutions. For these reasons, and because the CAM brings the study of these processes into a uniform setting where interactions can be examined, the popularity of the CAM model will continue to increase.

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References


### Table 1: The use of the CAM model in HNSCC research. The terms “chorioallantoic” and “head and neck cancer” were used to identify articles listed on Pubmed that use the CAM model to study HNSCC. The articles from the resulting search are organized according to the phenotype investigated using the CAM model system.

<table>
<thead>
<tr>
<th>HNSCC Cancer Phenotype Studied</th>
<th>Reference(s)</th>
</tr>
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<tbody>
<tr>
<td>Angiogenesis</td>
<td>[2,5-13]</td>
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<tr>
<td>Biomarker Expression</td>
<td>[2,6,7]</td>
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<tr>
<td>Drug Testing</td>
<td>[6-9,12-15]</td>
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<tr>
<td>Dysplasia Study</td>
<td>[16]</td>
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<tr>
<td>Invasion</td>
<td>[2,6,10,17-19]</td>
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<tr>
<td>Lymphatic Vessel Density</td>
<td>[20]</td>
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<tr>
<td>Metastasis</td>
<td>[13-15,17,21]</td>
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<tr>
<td>Tumor Growth</td>
<td>[2,6,7,10,22]</td>
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