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Review Article

The chick embryo chorioallantoic membrane as a model for tumor biology

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ABSTRACT

Among the *in vivo* models, the chick embryo chorioallantoic membrane (CAM) has been used to implant several tumor types as well as malignant cell lines to study their growth rate, angiogenic potential and metastatic capability. This review article is focused on the major compelling literature data on the use of the CAM to investigate tumor growth and the metastatic process.

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Development and structure of the chick chorioallantoic membrane

The chicken is a well-known experimental model to study embryonic development. Chick embryos are readily accessible for visualization *in ovo* and experimental manipulation. Chick embryo development lasts 21 days before hatching [63].

Three extraembryonic membranes are formed during development: the yolk sac membrane, the amnion, and the chorioallantoic membrane (CAM). The CAM (Fig. 1) is formed on day 3–4 of incubation by the fusion of the chorion and the allantois and it consists of three layers, ectoderm (from the chorion), mesoderm, and endoderm (from the allantois) (Fig. 2) [63]. The CAM has a rich vascular system that develops within the mesodermal layer and is served by paired allantoic arteries and paired allantoic veins (Fig. 3). By 16 days of incubation, the CAM has become so large that it covers most yolk sac, and become closely pressed against the shell membranes, which enables it to act as a gas-exchange organ receiving oxygen and eliminating carbon dioxide through the pores in the shell [63]. The surface area of the CAM increases from approximately 6 cm^2 at day 6 to 65 cm^2 by day 14 [13].

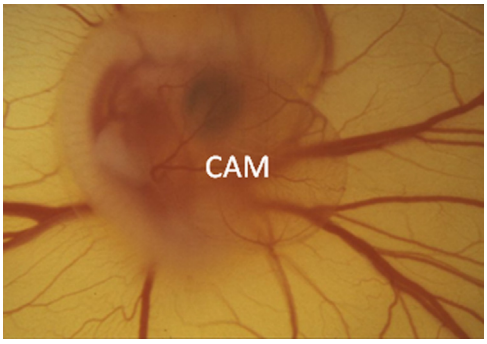

Fig. 1 – Macroscopic *in ovo* features of the chick chorioallantoic membrane (CAM) at day 5 of incubation. Original magnification, 30 ×.

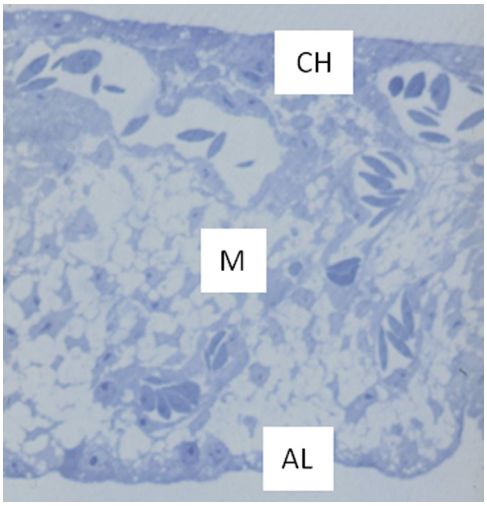

Fig. 2 – A semithin section of the CAM, showing the chorionic epithelium (CH), the intermediate vascularized mesenchyme (M), and the deep allantoic epithelium (AL). Original magnification, 160 ×.

Natural immunodeficiency of the developing chick

Being naturally immunodeficient, the chick embryo accepts transplantation from various tissues and species without immune response. Chickens are protected by a dual immune system composed of B and T cells, controlling the antibody and cell mediated immunity, respectively. The B cells are differentiated in the bursa of Fabricius, whereas T cells are differentiated in the thymus [63,12,18]. The chick immune system does not begin to develop to function until about 2 weeks into its development [22,78]. The presence of T cells can be first detected at day 11 and of B cells at day 12 [23], and by day 18 chicken embryos become immunocompetent [22,78].

By day 10 and, respectively by day 12, monocytes and macrophages are found in the yolk sac, spleen, bursa, gut, thymus, and in the liver [23]. The two major inflammatory cell types present in day 10–15 embryos are heterophils and monocytes. Heterophils are an avian analog of mammalian neutrophils and represent a main source of matrix metalloproteinase-9 (MMP-9) in the chick embryo, and could be identified by staining with a anti-chicken MMP-9 antibody [82]. On the other hand, monocytes/macrophages are the major source of MMP-13 in the chick embryo and could be identified by immunostaining with an anti-MMP-13 antibody [80].

Historical background

The CAM has long been used to study tumor growth because the chick's immunocompetent system is not fully developed and the conditions for rejection have not been yet established [34]. In 1911, Rous and Murphy demonstrated the growth of the Rous 45 chicken sarcoma transplanted onto the chick embryo CAM [64]. Murphy [44] in 1913 reported that mouse and rat tumors implanted onto the CAM could be maintained by continuous passage from egg to egg and described the effects of these transplantation on CAM and chick embryo. Murphy [44] in 1913 showed that rat tissues did not grow in adult chickens, while grew on the CAM until developmental day 18 [45,46]. Later, the CAM assay was improved through the removal of a square of the shell to expose the CAM surface [10]. The choice of the 9 day old embryo for tissue grafting and the selection of the junction point of two or more large blood vessels as a graft site was introduced by Willier in 1924 followed by the creation by Burnet in 1933 of an

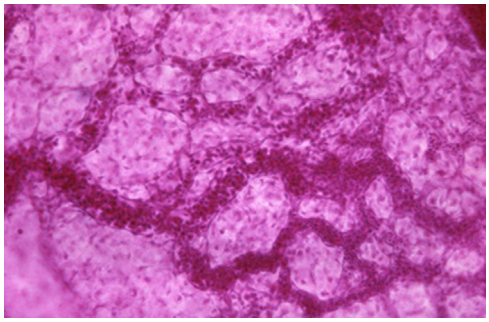

Fig. 3 – A macroscopic section of the chorioallantoic membrane (CAM) vascularized mesenchyme after *in toto* staining with paracarmine. Original magnification, 100 ×.