The *in ovo* chick chorioallantoic membrane (CAM) assay as an efficient xenograft model of hepatocellular carcinoma

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Resulting Publication or Report:

The *In Ovo* Chick Chorioallantoic Membrane (CAM) Assay as an Efficient Xenograft Model of Hepatocellular Carcinoma Li M*, Pathak R*, Lopez-Rivera E, Friedman S.L., Aguirre-Ghiso J, Sikora A.G. (2015) JoVE (Journal of Visualized Experiments), e52411-e52411. *Equal Contribution.

Plain Language Summary of Work:

Hepatocellular carcinoma (HCC) is the 3rd leading cause of cancer mortality in the world. Currently only 30% of HCC patients are eligible for potentially curative surgical treatments, and systemic chemotherapy is not efficacious. Therefore, there is a pressing unmet clinical need for novel HCC therapies, and development of model systems suitable for efficient testing of new therapeutic approaches. The chick chorioallantoic membrane (CAM) assay provides a reproducible, cost-effective, and fast medium-throughput method of testing potential anti-tumor drugs *in vivo*.

The CAM assay has been used extensively to study angiogenesis. It has also been successfully developed into a tumor xenograft model of cancers, including glioblastoma, pancreatic cancer, and melanoma. Both *in ovo* and *ex ovo* techniques have been utilized in the literature, with details varying from protocol to protocol. One major challenge to the CAM xenograft model is the relatively high incidence of embryonic death after manipulation of the egg, with published chick embryo mortality rates ranging from 25-50 percent. In this project, I developed an *in ovo* xenograft model of HCC that reliably produce growth of three-dimensional, vascularized tumors that histologically resemble undifferentiated HCC.

Significance:

The novelty of this method is that it can be used to prepare and maintain a perpetual personalized tumor bank, which can be used for testing an individual's response to particular anti-cancer drug(s). Compared to the existing standard mouse AVATAR mouse models where tumor takes 3-6 weeks to grow, CAM is cheaper and faster (2-5 days). A very important advantage of the CAM model is that it closely replicates cancer development. Successful establishment of tumor xenografts onto the CAM model will also allow researchers to propagate tumors in large numbers, eliminating the need to obtain frequent biopsy samples from patients. An added advantage will be the ability to

screen the individual for potential resistance towards existing chemotherapy agent. This will enable researchers and clinicians to have perpetual access to tumor samples, develop a fast and cost effective biological assay to study the effect of anti-cancer drugs, and facilitate choice of the most effective personalized treatment regimen for patients.

Technical Summary of Work:

The chick chorioallantoic membrane (CAM) begins to develop by day 7 after fertilization and matures by day 12. The CAM is highly vascularized and is involved in gas exchange as well as bone formation. Furthermore, the CAM contains extracellular matrix proteins such as fibronectin, laminin, collagen, integrin alpha(v)beta3, and MMP-2, making it an attractive model to study tumor invasion and metastasis. Scientists have long taken advantage of the physiology of the CAM by using it as a model of angiogenesis. More recently, the CAM assay has modified to work as an *in vivo* xenograft model system for various cancers that bridges the gap between basic *in vitro* work and more complex animal cancer models. The CAM assay allows for the study of tumor growth, anti-tumor therapies, and pro-tumor molecular pathways in a biologically relevant system that is both cost- and time-effective. In the current project, I successfully described the development of CAM xenograft model of hepatocellular carcinoma (HCC) with embryonic survival rates of up to 93% and reliable tumor take leading to growth of three-dimensional, vascularized tumors**.**

My Specific Contribution:

1. Co-development of the basic assay to successfully graft cancer cells onto the egg.
2. Optimizing the scaffold matrix to promote, and support the growth of cancer cells.
3. Optimizing the ratio of cancer cell to scaffold matrix.
4. Performing all the experimental procedures described in the publication, and preparing the manuscript.
5. Coordinating scripting and filming of the procedure as described in the JoVE video.