Topic Introduction

Culturing Mouse Tumor Cells

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Genetically engineered mouse models (GEMMs) are attractive for the study of cancer; however, they can be time-consuming and expensive to produce and maintain. Thus, in certain contexts, the use of in vitro culture systems of tumor cells may provide an efficient and effective means to test hypotheses before assessment in or to complement discoveries in GEMMs. This introduction will briefly review the issues pertaining to in vitro analyses of primary cancer cells and highlight several “best practice” protocols that can be used when working with diverse types of carcinomas.

INTRODUCTION

Over the past decade, genetically engineered mouse models (GEMMs) of numerous neoplasms have proven to be a faithful and enlightening way to study cancer biology. Tumors that arise within GEMMs engender a microenvironment that resembles closely the human condition, unlike any other model systems, either in vivo or in vitro. Given the significant accumulating evidence underscoring the importance of the tumor microenvironment in cancer cell behavior, GEMMs are supplanting most in vitro approaches. However, this technological advancement comes with a significant trade-off: Mouse colonies require extensive investment in time and money, especially in preclinical evaluation of novel therapeutics. Thus, selected in vitro assays are practical and necessary tools to understand tumor biology that can be translated to in vivo studies or complement them, such as the study of treatment efficacy of newly developed drugs, discovery of novel drug targets, and dissection of cancer cell–stromal interactions.

QUESTIONS TO ASK BEFORE ESTABLISHING A CULTURE

Several questions need to be addressed before selecting the proper in vitro assays for cancer cells. First, which tumor cells need to be studied? Tumors comprise many types of cells, including the tumor cells themselves, fibroblasts, endothelial cells, and immune cells, such as macrophages and T cells. For many years, cell lines have been created by dissociating tumors and placing the resulting suspension in culture. Although it has been assumed that this method yields relatively pure populations of cancer cell lines after a few passages in culture, recent data indicate that certain noncancer cells adapt to these conditions and persist in primary cultures, and some have been shown to fuse with cancer cells in vitro

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TABLE 1. Selected applications of 3D culture models

Application	Cancer types	References
Branching morphogenesis	Lung, esophagus, prostate, pancreas, breast	Tyson et al. 2007; Kalabis et al. 2008, 2012; Wescott et al. 2009; Franzdottir et al. 2010; Andersen et al. 2011; Lo et al. 2012
Invasiveness/EMT	Breast, esophagus, skin, prostate, glioblastoma/glioma, lung	Kalabis et al. 2008, 2012; Sarkar and Yong 2009; Harma et al. 2010; Natsuizaka et al. 2010; Aref et al. 2012; Gurski et al. 2012; Hirata et al. 2012; Xiong et al. 2012
Stromal–epithelial interactions	Esophagus, breast, ovarian, lung, colon	Okawa et al. 2007; Kalabis et al. 2008, 2012; Vaira et al. 2010; Cai et al. 2012; Moin et al. 2012; Sameni et al. 2012
Effect of extracellular matrix	Breast, lung, prostate, ovarian	Rosines et al. 2007; Sodek et al. 2008; Dhimolea et al. 2010; Robertson et al. 2010; Gurski et al. 2012
Effect of pharmacologic treatment	Prostate, lung, breast, endometrial, lung, colon	Harma et al. 2010; Vaira et al. 2010; Aref et al. 2012; Chitcholtan et al. 2012; Debeb et al. 2012; Fallica et al. 2012

EMT, epithelial-to-mesenchymal transition.

(Ferrand et al. 2011; Powell et al. 2011). Thus, strategies to purify primary cancer cells before culture may lead to more faithful analysis. One method for isolating pure populations of cancer cells from tumors is fluorescence-activated cell sorting (FACS) of lineage-labeled tumors (see Protocol: **Creation of Primary Cell Lines from Lineage-Labeled Mouse Models of Cancer**) (Rhim 2014).

Next, how “physiological” does your assay need to be? The answer to this question lies in the type of question to be studied. For example, if one plans to analyze downstream effectors of a particular pathway within a cancer cell or if particular chemotherapeutics and/or targeted agents are cytotoxic to a particular type of cancer cell, then traditional 2D culture is an acceptable choice, given its ease of use. However, if one wishes to study the effect of certain genetic manipulations on cell polarity or branching morphogenesis or the effect of biologic therapies (e.g., inhibitory antibodies) that are dependent on polarized receptor expression, then different tools are required. Over the past decade, several groups, including our own, have developed “organotypic” model systems, which place cells into a 3D matrix that mimics the in vivo environment with high fidelity. Multiple variables can be manipulated in these systems, including the addition of stromal cells and immune cells, allowing the tumor microenvironment to be modeled ex vivo. Three of these model systems are described in accompanying protocols: Protocol: **Organotypic Culture of Untransformed and Tumorigenic Primary Mammary Epithelial Cells**, Protocol: **Culturing Adult Stem Cells from Mouse Small Intestinal Crypts**, and Protocol: **Three-Dimensional Organotypic Culture of Stratified Epithelia** (Hamilton et al. 2014; Jechlinger 2014; Rhim and Rustgi 2014). The drawbacks to these 3D systems are the complexity and cost required. However, it is generally agreed that, compared with traditional 2D cultures, organotypic cultures provide data that resemble more closely what is observed in vivo. In preclinical drug treatment trials, 3D cultures more effectively modeled in vivo effects of drugs compared to 2D cultures (see Table 1).

The final question is the following: Does the in vitro assay truly reflect the in vivo property it purports to measure? In vitro assays are correlative models of the in vivo situation, and one should be cautious about overstating conclusions derived from these studies. Table 1 highlights examples in which 3D cultures have been used to address specific questions that such models are able to answer.

There are a number of protocols that address the aforementioned issues. Some of these have been cited above, and although they are based on using specific types of tumors, the protocols can be generalized for use with different types of cancers. For example, Protocol: **Culturing Primary Mouse Pancreatic Ductal Cells** (Reichert et al. 2014) was designed for studying pancreatic ductal adenocarcinoma, but can be applied toward other carcinomas that resemble ductal structures.

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