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Personalized in vitro cancer models to predict therapeutic response: Challenges and a framework for improvement

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ABSTRACT

Personalized cancer therapy focuses on characterizing the relevant phenotypes of the patient, as well as the patient's tumor, to predict the most effective cancer therapy. Historically, these methods have not proven predictive in regards to predicting therapeutic response. Emerging culture platforms are designed to better recapitulate the in vivo environment, thus, there is renewed interest in integrating patient samples into in vitro cancer models to assess therapeutic response. Successful examples of translating in vitro response to clinical relevance are limited due to issues with patient sample acquisition, variability and culture. We will review traditional and emerging in vitro models for personalized medicine, focusing on the technologies, microenvironmental components, and readouts utilized. We will then offer our perspective on how to apply a framework derived from toxicology and ecology towards designing improved personalized in vitro models of cancer. The framework serves as a tool for identifying optimal readouts and culture conditions, thus maximizing the information gained from each patient sample.

Contents

Abbreviations: AOP, adverse outcome pathway; ATP, adenosine triphosphate; CAF, carcinoma associated fibroblast; CSRA, chemotherapy sensitivity and resistance assay; CYP, cytochrome P450; ECM, extracellular matrix; ER, estrogen receptor; ERE, estrogen response element; MTT, 3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide; 2D, two dimensional; 3D, three dimensional; OECD, Occupational Economic Cooperation Development; TOP, therapeutic outcome pathway.

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1. Introduction: need for personalized medicine, current approaches and in vitro models

Classically, patients have relied on systemic treatments and invasive procedures such as chemotherapy, radiation therapy, and surgery to combat cancer. Choosing the right cancer treatment is difficult because limited tools, money and time are available to guide this decision. The therapeutic index for most cancer treatments is extremely narrow, requiring a balance between drug efficacy and toxicity tolerance. In a given population, drug efficacy and tolerance can differ greatly between individuals and even across an individual tumor (Leonard, Williams,

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Tulpule, Levine, & Oliveros, 2009; Gerlinger et al., 2012). The mechanisms underlying cancer development and progression vary drastically from patient to patient and a cure for one patient can be ineffective or harmful to another. Researchers have shifted from trying to identify silver bullet "cures" for a given cancer to attempting to find solutions to combat patient specific cancer subtypes or in other words personalized medicine (Ginsburg & McCarthy, 2001; Veer & Bernards, 2008).

Personalized medicine encompasses both tumor and non-tumor systemic patient phenotypes that contribute to the effectiveness of a treatment/therapeutic (Chin, Andersen, & Futreal, 2011; Bartlett et al., 2014). Human populations can exhibit profound systemic differences in drug disposition, which can also contribute to therapeutic response. Identifying polymorphisms in genes that encode for drug metabolizing enzymes can help determine if a patient will experience adverse effects or even no effects in response to a drug (Tomalik-Scharte, Lazar, Fuhr, & Kirchheiner, 2007; Johansson & Ingelman-Sundberg, 2011). For instance, individuals that carry the uridine diphosphate glucuronosyltransferase (UGT) 1A1*28 gene variant produce less of the UGT1A1 enzyme and are at high risk for irinotecan associated morbidity and mortality (Iyer et al., 2002; Bosch, Meijerman, Beijnen, & Schellens, 2006).

Assessment of tumor cells themselves provides a lens into susceptibilities of a tumor to targeted therapies. One method to evaluate tumor cells is through profiling the tumor's DNA, RNA, or protein, to identify molecular biomarkers that are predictive of patient response. A second method is to integrate tumor cells into chemosensitivity and resistance assays (CSRAs), a term used to describe an in vitro functional assay that measures response to a drug ex vivo, which will be discussed in detail later. Investigating the molecular profile of cancer cells to identify biomarkers can help predict drug resistance, and has identified gene expression profiles that correlate with cancer recurrence after specific drug treatments (Chang et al., 2003; Estes, Lovato, Khawaja, Winter, & Larson, 2007; Marchionni et al., 2007). Small molecule kinase inhibitors such as gefitinib or erlotinib are the suggested drug treatment for lung cancer patients whose tumors have mutations in epidermal growth factor receptor. However, if those patients also have mutations in KRAS they are at high risk for gefitinib and erlotinib resistance (Pao et al., 2005). Gene expression combined with protein secretion profiles of cancer cells also identifies biomarkers for targeted therapies (Sawyers, 2004; Harris et al., 2007; Dias-Santagata et al., 2010). One study of patients with advanced cancer including over 15 different tumor types found that patients who underwent targeted therapy had a significant increase in overall response rate, time to treatment failure, and survival duration (Tsimberidou et al., 2012). While molecular profiling of the tumor is a powerful approach, the focus of this review is to discuss the technological advancement of CSRAs and methods to improve their clinical use.

Beyond tumor cells themselves, some cancer therapeutics do not target the tumor alone but also the associated microenvironment. In breast, colorectal and prostate carcinomas, stromal components actively participate in cancer development and are continuously modified as the disease progresses. It has been demonstrated that tumor-associated stroma undergoes extensive changes in gene expression and that a stromal transcript signature correlates with histological tumor grade (Tuxhorn et al., 2002; Chang et al., 2003; Calon et al., 2015). Accordingly, targeting stromal changes is gaining acceptance as an alternative option for treating cancer. For example, vascular endothelial growth factor (VEGF) inhibitors such as bevacizumab combat cancer by targeting tumor angiogenesis (Ferrara, Hillan, Gerber, & Novotny, 2004). These examples of tumor and tumor microenvironment contributions to response underscore the complexity of individual tumors and highlight the need for an expanded view when considering therapeutic response of individuals to cancer therapies.

While patient profiling can improve patient outcome, cancer mortality remains high and it is clear that we need additional approaches (Hidalgo et al., 2011). In response, there is once again increasing interest in integrating patient samples into in vitro models to predict in vivo response. These models, most commonly referred to as chemosensitivity and resistance assays (CSRAs), have the potential to provide a rapid, high throughput and inexpensive approach to predicting therapy for individuals, but have thus far faced challenges that have hindered their success (Bartlett et al., 2014). Poor in vitro culture conditions (Samson, Seidenfeld, Ziegler, & Aronson, 2004; Bartlett et al., 2014), the limited information offered by traditional in vitro readouts (Burstein et al., 2011; Wilmes et al., 2013), and tumor tissue heterogeneity (Samson et al., 2004; Burstein et al., 2011; Gerlinger et al., 2012; Bartlett et al., 2014) have been invoked to explain discordance between in vivo and in vitro therapeutic response.

In 2004 and 2011 the American Society of Clinical Oncology reviewed CSRAs in both instances, they recommended not to use these models to identify appropriate therapeutic agents outside of clinical trials but stressed their potential importance (Schrag et al., 2004; Burstein et al., 2011). Advances in molecular biology, toxicology, biomedical/tissue engineering and other fields offer advances that may help move CSRAs into clinical use.

In an effort to improve CSRAs, we propose a guiding framework coined a therapeutic outcome pathway (TOP), to select culture conditions, cellular readouts and key components to include in a CSRA. The TOP concept is based on the adverse outcome pathway (AOP) framework used in toxicology to map the molecular, cellular and tissue level targets of a toxin or in this case a therapeutic. We will begin our discussion by addressing challenges with in vitro personalized models and the emerging solutions. This will familiarize the reader with the advantages of personalized in vitro models and the obstacles that hamper their success. We will then introduce the TOP framework and describe how it might be used to overcome these obstacles and maximize the information retrieved from a patient sample. The TOP framework will facilitate the development of personalized in vitro models because it will highlight what should be incorporated into models and what readouts will be most predictive of response. To illustrate the usefulness of the framework, we will provide an example of estrogen receptor (ER) positive breast cancer and tamoxifen treatment.

2. The nature of patient derived samples: heterogeneity, sample acquisition, and challenges with primary cell culture

Intertumor heterogeneity (heterogeneity between tumors of different patients) has driven the need for personalized medicine (Burrell, McGranahan, Bartek, & Swanton, 2013). Even within the same cancer subtype, each patient's tumor can have distinctly different gene expression profiles, tumor microenvironments and behaviors (Ogino, Fuchs, & Giovannucci, 2012). Early attempts to predict in vivo response made use of immortalized cell lines in vitro. However, immortalized cell lines are highly selected subpopulations and do not adequately reflect the heterogeneous function and behavior of tumors (Dairkee et al., 2004; Cree, Glaysher, & Harvey, 2010). Consequently, there is increased interest in using primary cells in personalized in vitro models for evaluating drug response (Liotta & Petricoin, 2000; Gerlinger et al., 2012; Longo, 2012).

While ideal, patient derived primary cells present their own set of challenges in areas such as sample acquisition, variability and culture. First, they are difficult to obtain in the standard diagnostic workflow. Traditionally, standard of care dictates that tissue removed during biopsy be immediately sent for pathological examination where it is assessed for morphologic and molecular markers that are indicative of disease stage and cancer subtype (Ivshina et al., 2006). Pathological review is well established and is a reliable method to gain valuable information about a patient's cancer, but it offers limited predicted power (Hirsch et al., 2008; Gravendeel et al., 2009; Sequist et al., 2011). Integrating patient samples into in vitro models requires that some part of the sample, which would ordinarily go to pathology, be relinquished for research purposes. With early detection and small