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New tools for old drugs: Functional genetic screens to optimize current chemotherapy

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ABSTRACT

Despite substantial advances in the treatment of various cancers, many patients still receive anti-cancer therapies that hardly eradicate tumor cells but inflict considerable side effects. To provide the best treatment regimen for an individual patient, a major goal in molecular oncology is to identify predictive markers for a personalized therapeutic strategy. Regarding novel targeted anti-cancer therapies, there are usually good markers available. Unfortunately, however, targeted therapies alone often result in rather short remissions and little cytotoxic effect on the cancer cells. Therefore, classical chemotherapy with frequent long remissions, cures, and a clear effect on cancer cell eradication remains a corner stone in current anti-cancer therapy. Reliable biomarkers which predict the response of tumors to classical chemotherapy are rare, in contrast to the situation for targeted therapy. For the bulk of cytotoxic therapeutic agents, including DNA-damaging drugs, drugs targeting microtubules or antimetabolites, there are still no reliable biomarkers used in the clinic to predict tumor response. To make progress in this direction, meticulous studies of classical chemotherapeutic drug action and resistance mechanisms are required. For this purpose, novel functional screening technologies have emerged as successful technologies to study chemotherapeutic drug response in a variety of models. They allow a systematic analysis of genetic contributions to a drug-responsive or −sensitive phenotype and facilitate a better understanding of the mode of action of these drugs. These functional genomic approaches are not only useful for the development of novel targeted anti-cancer drugs but may also guide the use of classical chemotherapeutic drugs by deciphering novel mechanisms influencing a tumor's drug response. Moreover, due to the advances of 3D organoid cultures from patient tumors and *in vivo* screens in mice, these genetic screens can be applied using conditions that are more representative of the clinical setting. Patient-derived 3D organoid lines furthermore allow the characterization of the "essentialome", the specific set of genes required for survival of these cells, of an individual tumor, which could be monitored over the course of treatment and help understanding how drug resistance evolves in clinical tumors. Thus, we expect that these functional screens will enable the discovery of novel cancer-specific vulnerabilities, and through clinical validation, move the field of predictive biomarkers forward. This review focuses on novel advanced techniques to decipher the interplay between genetic alterations and drug response.

1. Introduction

Anti-cancer drug resistance is the major cause of death of cancer patients with disseminated tumors (Borst, 2012). In some patients intrinsic (or primary) drug resistance is already observed from the start (*i.e.* prior to chemotherapy) and tumors grow in the presence of chemotherapy (Holohan et al., 2013). Such intrinsic drug resistance can be a cancer-type specific or caused by individual cancer features (Gottesman, 2002). Frequently however, resistance arises in two steps. The tumor initially responds, but not all tumor cells are eradicated. From the residual disease the tumor regrows and eventually becomes resistant to all available chemotherapeutic drugs (Borst, 2012). We have recently reviewed various mechanisms that may cause minimal residual disease (Blatter and Rottenberg, 2015). Although residual disease may already contain selected drug-refractory tumor cells, it is also possible that the residual tumors are only transiently resistant due to cell cycle characteristics (Pajic et al., 2017). Then, drug resistance is acquired during the course of treatment (Housman et al., 2014). This secondary resistance is often due to (epi-)genetic alterations arising during the treatment that lead to, for instance, the activation of alternative signaling pathways, increased drug efflux, altered drug target availability, or rewiring of the DNA damage response (Holohan et al.,

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2013; Borst, 2012; Bouwman and Jonkers, 2012). To attenuate the development of drug resistance, combinational therapies of several drugs with different molecular mechanisms are frequently given to cancer patients (Al-Lazikani et al., 2012). Another approach is to re-sensitize resistant tumor cells by drugs targeting the resistance mechanism or the tumor microenvironment (De Henau et al., 2016; Callaghan et al., 2014). Unfortunately, we often lack knowledge about the mechanisms underlying resistance and therefore we usually lack a personalized strategy how to treat patients with (relapsing) tumors.

In the past decades, progress in the treatment of disseminated cancers has reduced cancer-related mortality (Kort et al., 2009). In addition to classical chemotherapy, also targeted anti-cancer drugs further improved cancer remission (Motzer et al., 2015; Zhou et al., 2011). Despite these advances, treatment failure due to drug resistance remains a substantial challenge in the clinical management of cancer. Treating a non-responsive tumor causes side effects without providing a benefit for the patient. Moreover, it incurs unnecessary costs and may even decrease the likelihood of success of subsequent treatments with other regimens (Siddiqui and Rajkumar, 2012).

To improve cancer therapy outcome, precision oncology is a promising strategy. Through the assessment of a tumor's specific genetic or proteomic changes, *i.e.* its biomarkers (Mehta et al., 2010), an individualized best treatment regimen can be chosen. Prognostic gene expression signatures are clinically well established, because prognosis of tumor recurrence directly depends on the altered expression of a number of genes involved in tumor progression and metastasis (Reyal et al., 2008; Wirapati et al., 2008; Cardoso et al., 2016). Conversely, a tumor's response to a particular treatment can fail due to the alteration of a single gene, such as the drug target or drug entry transporter (Borst and Wessels, 2010). Such alterations may not reliably be picked up by standard gene expression profiling. Thus, it is not surprising that only few predictive biomarkers are established, and even those remain imperfect in predicting therapy success. Currently, biomarkers are only available for targeted therapies, which block or stimulate specific pathways of tumor cells (Twomey et al., 2017) and usually yield good initial response with a modest effect on overall survival (Fojo and Parkinson, 2010). In contrast, classical cytotoxic chemotherapy interferes with all rapidly dividing cells, does not rely on oncogenic protein or pathway alterations, but often results in long-term remission and even cures some cancer types, and reduces cancer-related mortality. Unfortunately, not all patients benefit from the treatment and many eventually become resistant to all drugs available. Hence, there is a lack of clinically validated predictive biomarkers for classical chemotherapy.

Regarding targeted therapy, an early example of a predictive biomarker is HER2 expression status for trastuzumab treatment in metastatic breast cancer, an anti-cancer drug approved by the FDA in 1998. In combination with classical chemotherapy, trastuzumab efficiently decreases disease progression in HER2-amplified metastatic breast cancer (Cobleigh et al., 1999). In contrast, trastuzumab provides no benefit in breast cancer patients lacking HER2. Unfortunately, only about 30% of all HER2-positive breast cancer patients respond to trastuzumab-containing chemotherapy (De Palma and Hanahan, 2012). Thus, there are additional factors that influence therapy response, such as the intertumoral heterogeneity among a cancer (sub-)type, reflecting variations in molecular profiles of cancers in different patients. Additionally, the intratumoral heterogeneity complicates predictions of drug response (Ng et al., 2014). Molecular and genetic profiling of tumors has become cheaper and is often readily available. For mutations in specific genes, for instance *BRAF*, the effect on therapy response has been well characterized, so that sequencing of the corresponding genomic region will directly yield a predictive marker for therapy response. Unfortunately, the number of such well-defined biomarkers is limited, and to date only a small fraction of cancer patients directly benefit from established biomarkers. This is aggravated by the fact that not all patients bearing *BRAF* mutations do respond equally well to targeted BRAF inhibitors (Corcoran et al., 2015; Long et al., 2014; Prahallad et al., 2012). Thus, even such well-defined biomarkers are not sufficient, and additional characterization of the tumor is needed.

Several approaches have successfully identified novel molecular peculiarities which serve as predictive biomarkers. Hypothesis-driven approaches have, for instance, resulted in the establishment of *BRCA1/2* mutational status in predicting a positive response upon PARP inhibitor treatment in breast and ovarian cancer (Farmer et al., 2005; Tutt et al., 2010; Bryant et al., 2005). Analyses of large, population-based clinical trials have also identified subgroups of responsive patients (Uryniak et al., 2011), *e.g.* leukemia patients with the Philadelphia chromosome responded better to imatinib treatment (Druker et al., 2001). Predictive markers based on clinical data have also been suggested for classical chemotherapeutics, including high HER2 or low tau expression as markers for paclitaxel sensitivity (Pusztai, 2007). Besides *BRCA1/2* status, these markers have not entered the clinic, however, and still require additional validating clinical studies (Schork, 2015).

In recent years, advances in experimental genetic screening techniques have linked many genotypes to novel phenotypes in mammalian cells (Chen et al., 2015; Brockmann et al., 2017; Zhou et al., 2014b; Blomen et al., 2015; Wang et al., 2015b; Hart et al., 2015). Furthermore, genome-wide screens have broadened our understanding of molecular mechanisms responsible for therapy response (Ruiz et al., 2016; Berns et al., 2016; Planells-Cases et al., 2015; Wijdeven et al., 2015, for instance; and Table 1). Thus, these screens are valuable tools which can reveal novel mechanisms of resistance or hypersensitivity towards drugs, and facilitate a better understanding of drug response which might ultimately result in novel predictive biomarkers (Fig. 1). While most targeted anti-cancer therapeutics exploit gain-of-function alterations, *e.g.* in terms of oncogene addiction (Pagliarini et al., 2015), not all tumors bear targetable gain-of-function mutations. Inactivation of tumor suppressor genes is frequent, and cannot be directly targeted with a drug. However, as shown by the example of PARP inhibitor treatment in *BRCA1/2* mutated tumors, loss of a tumor suppressor can offer a treatment option with low side effects for healthy tissue. The study of synthetic lethality and context-dependent gene essentiality has been challenging in mammalian cells and was for long time limited to few model organisms. With the development of CRISPR/Cas9 genome editing and insertional mutagenesis in haploid human cells, it is now possible to efficiently study genetic interactions as well as the functional consequence of genetic mutations and possibly reveal new predictive biomarkers by linking drug-responsive phenotypes to genotypes.

Although some novel anti-cancer drugs have been successful and have yielded improvements for cancer patients, they remain imperfect (Fojo and Parkinson, 2010; Groenendijk and Bernards, 2014), and have also become a financial burden for the health system (Kantarjian et al., 2013; Aggarwal, 2010; Prasad and Mailankody, 2017; Fojo and Parkinson, 2010). During the course of treatment, most patients sooner or later also receive classical chemotherapy including platinum drugs, topoisomerase inhibitors, microtubule-targeting agents or antimetabolites as part of standard care (Gonen and Assaraf, 2012; Giovannetti et al., 2017). Their clinical use is based on empirical experience. However, these drugs are relatively cheap, effective and widely used. If clinical oncologists could be supported in their choice of classical chemotherapy based on molecular characteristics of a tumor, the therapeutic benefit of a standard treatment may increase and drugs to which the tumor is unlikely to respond would be avoided. To improve the proper selection of the treatment of choice and to expand our repertoire of drug response predictions, one needs to identify more molecular peculiarities of tumors which impact therapy response.

This review therefore elaborates on genome-wide screening techniques in mammalian cells with special emphasis on the response against classical cytotoxic drugs.