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Previews

Cancer Cell Drug Response Transcriptomes in 3D

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The relevance of different in vitro culture models of cancer cells is a hot topic, but few systematic and definitive analyses in this area exist. In this issue of *Cell Chemical Biology*, Senkowski et al. (2016) address this issue by studying the transcriptomic profiles of drug-treated cancer cells cultured in two-dimensional and three-dimensional cultures. They describe biological findings with potential therapeutic implications and provide a unique data resource to mine.

The approach of using in vitro chemosensitivity profiling of cancer cells as a means to identify general and personalized cancer cell vulnerabilities has re-emerged as a popular research subject, both because it can complement and help us understand and implement personalized cancer cell genomics as well as because of recent significant advances in cell culture methodologies of primary and established cultures of cancer cells (Friedman et al., 2015). However, these approaches have also been challenged due to problems in data reproducibility, lack of standardized methods of following drug responses and questions about which cell culture models are relevant to use (Hatzis et al., 2014).

In this context, it follows simple logic that three-dimensional (3D) in vitro cultures in many ways can be expected to be more biologically relevant than cultures where cells are grown as monolayers on a stiff flat (2D) surface, such as in a conventional cell culture dish. The biological advantages of 3D culture can often be seen when cells that are cultured in proper 3D conditions form cellular morphological structures that are also observed in vivo. On the other hand, there are many strategies and schools of thought on biologically relevant culturing of cells in three-dimensional arrangements, ranging from spheroid non-adherent cultures, adherence-supportive extracellular matrices, growth on 3D scaffolds, to extremely advanced organ-on-a-chip models. A recent perspective by Horvath and coworkers thoroughly describes many of these key methodologies and how they can be used (Horvath et al., 2016).

Still, 2D culture assays are simpler, less costly, and typically more robust. Therefore, an important question is what the critical functional changes are between the culture conditions and the biological processes that may be relevant to study in 2D versus 3D, and, if it is the latter, what type of 3D culture is the most relevant. However, surprisingly few comprehensive comparative studies between monolayer and 3D culture conditions exist; instead, 3D culture superiority is often described with a near-religious conviction by its proponents as unquestionable and unchallengeable (although the type of 3D culture is not always specified). An additional challenge in understanding the role of cell culture conditions is that chemosensitivity testing, and especially high-throughput analyses of drug treatments, has most commonly been done with simple bioassays, such as those following overall cell viability, and the depth of the biological responses recorded have therefore been limited. In that sense, it is very clear that we need to use more advanced assays to follow drug responses and that these methods need to be standardized so that the results from one laboratory can be compared to the results from another.

With the vast amount of pre-existing information about transcriptomic profiles from in vitro and in vivo settings, studying transcriptomic responses in drug-treated cancer cells can be a powerful way of recognizing cellular responses and perhaps their relevance. This approach was first showcased in the seminal Connectivity Map study, published a decade ago by Lamb et al., 2006, in which the transcriptomic changes of three cancer cell lines when treated with over 1000 bioactive compounds were studied with microarray technology. Even though the drug treatments were done at a single dose, which were not always the most physiologically relevant, the data resource from the Connectivity Map study has been tremendously valuable for the research community and countless follow-up studies and analyses has been done by others based on the dataset. While the approach of using chip-based microarray transcriptome analysis was limiting for further broad scale analysis, new technologies that have since been developed have opened the door for high-throughput transcriptome profiling. The group behind the Connectivity Map developed a miniaturized Luminex bead-based methodology in which the abundance of a representative subset of mRNAs are used to infer the full transcriptome called L1000 (Duan et al., 2014). Multiplexed next-generation RNA sequencing is emerging as another feasible approach (Moyerbrailean et al., 2015).

In this issue of *Cell Chemical Biology*, Senkowski et al. (2016) provide the first study of its kind in which compound-induced transcriptomic changes in cells grown in different 2- or 3D conditions were explored. This work followed on a previous study by the authors (Senkowski et al., 2015) in which they performed one of the first comprehensive bioactive compound profiling campaigns comparing the cell viability effects on adenocarcinoma cells in monolayer growth versus a three-dimensional spheroid growth condition in which the cells were made quiescent by nutrient deprivation (Karlsson et al., 2012). Using this method, they discovered that although the quiescent cell spheroids generally had reduced sensitivity to cytotoxic chemotherapy, they also gained sensitivity to inhibitors of oxidative phosphorylation, suggesting that generally safe anti-helminthic drugs that target oxidative phosphorylation could be

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explored as anti-cancer agents. In the current study, they explore the L1000 methodology to profile the transcriptomic changes of cells grown either in 2D or in 3D spheroids that have been maintained in either nutrient-rich or nutrient-depleted media when treated with conventional cytotoxic chemotherapy and with oxidative phosphorylation inhibitors. First, Senkowski et al. (2016) show that the data generated is consistent with overlapping data from the LINCS project (http://www.lincsproject.org/) (Duan et al., 2014), highlighting that this approach is robust and reproducible between laboratories. Second, they discover that nutrient-deprived quiescent spheroidal culture induces mevalonate pathway gene expression, suggesting an increased dependence on cholesterol, and that oxidative phosphorylation inhibitors further enhance this expressional pattern. In agreement with these findings, HMG-CoA reductase inhibitors (statins) and other mevalonate metabolism inhibitors are found to synergize with the oxidative phosphorylation inhibitors, showing the power of identifying effective drug combinations through this deep biological profiling and arguing for further exploration of combinations of oxidative phosphorylation inhibitors and statins in cancer models. It is of interest that many of the compounds identified in the screens of quiescent spheroidal cultures have also been identified in screens against cancer stem cells, linking the quiescent spheroidal cultures to cholesterol metabolism and cancer cell stemness.

Perhaps most importantly, this study sets a new standard in biological profiling of drug sensitivity testing in 3D cell culture. Furthermore, the transcriptomic response data generated is expected to become a very valuable resource for further mining and generation of understanding of drug responses in different cell culture conditions.

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