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Materials-Driven Approaches to Understand Extrinsic Drug Resistance in Cancer

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Abstract

Metastatic cancer has a poor prognosis, because it is broadly disseminated and associated with both intrinsic and acquired drug resistance. Critical unmet needs in effectively killing drug resistant cancer cells include overcoming the drug desensitization characteristics of some metastatic cancers/lesions, and tailoring therapeutic regimens to both the tumor microenvironment and the genetic profiles of the resident cancer cells. Bioengineers and materials scientists are developing technologies to determine how metastatic sites exclude therapies, and how extracellular factors (including cells, proteins, metabolites, extracellular matrix, and abiotic factors) at metastatic sites significantly affect drug pharmacodynamics. Two looming challenges are determining which feature, or combination of features, from the tumor microenvironment drive drug resistance, and what the relative impact is of extracellular signals vs. intrinsic cell genetics in determining drug response. Sophisticated systems biology tools that can de-convolve a crowded network of signals and responses, as well as controllable microenvironments capable of providing discrete and tunable extracellular cues can help us begin to interrogate the high dimensional interactions governing drug resistance in patients.

Keywords: Extracellular matrix; carcinoma; systems biology; tissue engineering; microenvironment

Introduction

In drug development, potential chemotherapeutic molecules are screened in plastic multi-well plates, followed by animal models and clinical trials, a process that, on average, costs over one billion dollars and takes over a decade¹⁻³. This economic burden is not sustainable and is driven both by manufacturing in the scale-up of compounds, as well as inefficiencies and false positives during development. For example, pharmacological candidates often demonstrate efficacy in cells on tissue culture polystyrene surfaces but fail in pre-clinical or clinical trials. There is an opportunity here for materials scientists and bioengineers to insert model systems into this pipeline. Collectively, we could make a paradigm shift in the drug development field by accounting for a tumor cell's microenvironment as it responds to drugs in screening platforms. This could reduce the number of false positives that move through the pipeline, eliminating the otherwise wasted time in preclinical models on inefficient drug candidates. Accounting for tumor cell-microenvironment interactions requires the development of new biomaterial platforms, with the ability to capture human tissue properties in a controlled, reproducible, and economical fashion.

Drug resistance is a known, critical problem in drug development⁴. There is currently immense emphasis on the intracellular^{5,6} and genetic⁷ mechanisms of drug resistance (Fig. 1a). However, growing evidence is proving that the extracellular matrix (ECM), the collection of proteins and sugars that provides attachment, scaffolding, and receptor-mediated signaling to resident cells, plays a critical role in regulating tumor growth and metastasis⁸⁻¹². As one biophysical example, tumor ECM is notoriously stiff relative to healthy tissue¹³⁻¹⁶, which is associated with extensive changes in biochemistry^{17,18}, confinement¹⁹, and metastatic potential²⁰⁻²². This has motivated several groups to determine how the ECM drives drug resistance (Fig. 1b)^{23,24}.

Recent mathematical modeling of adaptive therapy has demonstrated the role of spatial competition in preventing resistant clones from growing out during therapy, and thus prolonging progression-free survival²⁵. Previous models have included the role of oxygen in the microenvironment²⁶, and reaction diffusion models of drug transport^{27,28}, stromal cells²⁹, tissue architecture, and now a few labs are incorporating other aspects of the tumor microenvironment^{30,31} and the ECM in drug response and tumor cell invasion³²⁻³⁴. The addition of ECM-driven resistance to this complex picture layers important information onto the elegant concepts of adaptive therapy. ECM cues that drive biological resistance are present in large regions of human tumors and could also work to increase spatial competition by altering the physical properties of the ECM or by creating privileged sectors of a tumor where genetically unmodified cells are competitively retained in a spatially heterogeneous manner. This spatial competition could also be further enhanced by the diversity of survival cues present in the metastatic niche (and potentially the synergy between diverse cues presented simultaneously). Investigating the evolutionary dynamics of resistance in mimetic microenvironments helps answer fundamental questions about clinical patterns and drug regimens. In this perspective, we discuss these topics, as well as point out needs in tool development: better experimental designs, experimental models, and computational approaches, to integrate and understand cell autonomous and microenvironmental driving factors in drug resistance.

The tumor microenvironment (TME)

A central goal in cancer research is the development of therapies that are specific and effective at eradicating cancer. A critical challenge is overcoming the diversity and heterogeneity found among cancer cells³⁵. Tumor cells are heterogeneous both because the malignant cells comprising a tumor are genetically (and epigenetically) distinct from each other, and because the tumor microenvironment (TME) drives additional phenotypic variation between these cells.

The TME is unique in drug resistance because it describes the non-tumor cell types (biological) and physiochemical features (abiotic) found within solid tumors. For instance, tumors are composed of many different types of cells, including normal epithelial cells, endothelial cells, fibroblasts, adipocytes, mesenchymal stem cells, and different types of immune cells. Additionally, solid tumors have other physiochemical features – enrichment for certain ECM proteins, altered elasticity/stiffness, increased interstitial pressure, hypoxia – that differ from normal organs. These unique features of the TME make important contributions to tumorigenesis, tumor growth, metastasis, and immune evasion. Unsurprisingly, the TME can also have strong effects on drug therapy, including altering drug pharmacokinetics³⁶, generating chemoresistance signals³⁷,³⁸, and promoting broad spectrum drug resistance by modulating apoptotic priming^{39, 40}. These phenotypes are more pronounced in metastatic microenvironments³⁹.

Therapeutic targeting of the TME

The TME is critical for a host of tumor phenotypes, and it has many distinct properties that are not commonly observed in normal tissues. These distinctions suggest a potential for targeting the TME, resulting in highly tumor-specific responses. Many therapies have been designed to target pro-tumorigenic aspects of the TME. Among the earliest concepts were therapies designed to target tumor vasculature. As tumors develop and grow, nutrient availability can become limiting in the absence of new vasculature. An “angiogenetic switch” is thought to facilitate rapid growth, coupled with the formation of new blood vessels innervating the tumor⁴¹. Inhibitors of VEGF signaling were developed to antagonize this process (Fig. 1c). Bevacizumab, a humanized anti-VEGF monoclonal antibody was approved in 2004 for the treatment of metastatic colorectal cancer⁴². Subsequently, this therapy was approved for several other cancers in combination with conventional chemotherapies⁴³.

Since those initial successes, therapies developed to target the TME have become as varied as the microenvironment itself. Therapies have been designed to target ECM proteins, proteins that degrade ECM, receptors that bind to ECM proteins, or downstream signaling pathways activated by ECM interactions⁴⁴. For example, tenascin-C is an ECM protein secreted by CAFs in the TME. It could be used as a tumor-specific target for targeted drug delivery⁴⁵. ECM-specific therapies are in early stages, and more studies will be needed to determine whether these will be effective^{46, 47}. Other features of the TME, such as hypoxia, are being targeted through the development of hypoxia activated prodrugs, or cellular responses to hypoxia, such as HIF1a or the unfolded protein response. As with ECM targeted therapies, these therapies have yet to achieve major success. In recent years, the most successful results have been achieved with the use of immune checkpoint inhibitors, such as those inhibiting PD1/PDL1 interaction, or CTLA4 activation⁴⁸ (Fig. 1c-d). These therapies are designed to disinhibit immune cells, allowing them to identify and destroy cancer cells in the TME. These therapies have huge potential and have been approved for use for many different types of cancer and as combinations. Although checkpoint inhibitors

result in remarkable responses in some patients, responses are extremely varied and understanding this issue remains an active area of research.

Targeting the TME: Needs

While some TME targeted therapies have been successful, responses vary considerably. Additionally, in some cases, the underlying mechanisms of action for TME targeted therapies remain unclear, which has led to unexpected, even paradoxical responses in some settings^{49, 50}. Clearly, much remains to be learned before TME targeting therapies achieve their full potential. The critical remaining need is to more deeply understand how the TME contributes to drug response. Of course, the TME is “complex”, but that complexity is not uniform or consistent. The TME varies across patients, across tumors within a single patient, and substantial variation even exists across a single tumor⁵¹. Furthermore, the TME is not static, but dynamically changing over time.

Studies of the TME have typically focused on the use of *in vivo* environments, e.g. tumors grown in mice. *In vivo* mouse models are preferred due to their perceived authenticity for modeling human disease. This is somewhat compromised by notable differences between mouse and human environments, as even the best PDX mouse systems fail to correctly mimic the environment and heterogeneity of human tumors in human environments⁵². Nonetheless, even for an *in vivo* model that accurately captures features seen in human tumors, due to the substantial variation that exists between individual people, any single model – no matter how accurate – will fail to model the diversity of environmental influences in patients. Thus, we propose that use of synthetic environments will offer complementary insights to those that can be generated using traditional *in vivo* mouse models. Rather than focus on a single authentic environment, synthetic systems can be *tunable*, allowing any/every environmental feature to be systematically varied.

This concept of “response” and “no-response” is a recurring concept in cancer therapy. Importantly, although drug sensitivity occurs in a graded manner, it is typically evaluated using a binary criterion that refers to the subset of patients who achieve an objective response by clinical criteria like RECISTv1.1⁵³. For instance, when using the RECIST criteria, a maximum of 5 lesions are used to quantitatively measure tumor volume before and after therapy. The reduction in tumor volume (below a single threshold percentage) across all lesions is used to create the binary metric of response versus non-response. These binary metrics obscure that the collection of lesions in a single patient that tumors at different metastatic sites, with unique TME inputs, can have quantitatively distinct levels of responses (or lack thereof).

Specifically, by analyzing a large number of radiographic images across multiple clinical trials across diverse cancers, it has been shown that changing the exact nature of the criteria (more or less target lesions) can have a large impact on how many patients are considered to have a “response”⁵⁴. In a recent metastatic urothelial carcinoma cohort treated with atezolizumab, different organ sites were shown to have

different response rates⁵⁵. Thus, only looking at binary outcomes fails to tell the full story about response at specific metastatic sites. A pressing need is a more comprehensive understanding of how individual lesions in individual organ

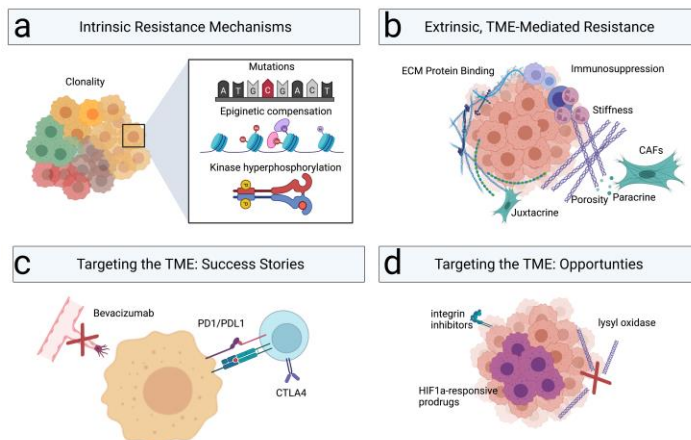


Figure 1: Intrinsic and Extrinsic Drug Resistance Mechanisms.

sites respond to specific drugs in patients.

Next generation materials for better drug screening

Synthetic environments catered to drug studies can allow for researchers to narrow the number of variables tested in pre-clinical models (hypothesis screening). Sampling tumors from a mouse to study an array of TME factors at high-resolution time points is unfeasible. Cell culture systems that are both controllable and more physiologically relevant than tissue culture plastic, with the ability to maintain compatibility with high-throughput drug screening are ideal for performing drug screens against numerous TME axes. With tunable, more realistic environments, one could perturb cell-ECM interactions systematically and screen across large swaths of conditions, drugs, and time points. Combined with systems-level analyses and computational modeling, these readouts could then be translated to *in vivo* studies in a predicted, heavily narrowed fashion to find new therapeutic targets. This is particularly important if we are to solve the challenge of understanding how drug resistance occurs at metastatic sites, with varied ECMs compared to the primary tumor.

The first-generation environments, collagen and Matrigel, are more realistic ECM environments for drug screening than plastic surfaces^{56, 57}. These allow cells to activate integrin-mediated signaling pathways typical of tumors (Fig. 1b). Despite their well-known role in cancer growth and metastasis⁵⁸⁻⁶², integrin antagonists have largely not worked to treat cancer⁶³, but have had some success in non-cancerous diseases⁶⁴⁻⁶⁶. To better understand this mystery, a few groups have pushed the frontier in making environments that can screen across many different integrin-binding ECM proteins simultaneously, while measuring drug responses⁶⁷. These drug screening environments have used surface chemistry (e.g. silanes, layer-by-layer deposition, and comb polymers and brushes) to tether either ECM proteins or peptides to present specific ECM proteins and combinations to cells. The LaBarge lab has used 2D hydrogels to simultaneously change the stiffness of polyacrylamide gels, growth factors, and integrin binding

ECM proteins on lapatinib response⁶⁸. The next critical step is to apply these drug screening approaches with materials that are both tunable like these 2D surfaces, while 3D like collagen and Matrigel, such as those based on hyaluronic acid, alginate, and poly(ethylene glycol) (PEG)⁶⁹⁻⁷³, and that model specific tissue environments to which cancers metastasize.

The development of these tunable materials environments has allowed our groups and others to reveal that cell response to chemotherapy and targeted drugs is sensitive to the stiffness of the surrounding ECM^{15, 16, 69, 74-82}. This is important information for drug development and prescription, as TME stiffness changes during tumor progression, TME stiffness varies across patients and tumors, and tumors metastasize to tissues of variable stiffness. One could infer then, that tailored drug regimens and dosages should account for TME stiffness in developing fully personalized medicine. The Weaver group has made progress in this area, proposing that lysyl oxidase (LOX) inhibitors could prevent collagen crosslinking in the TME, reducing its stiffness, delaying disease progression⁸³. Perhaps LOX inhibitors could be combined with standard of care to overcome stiffness-mediated drug resistance (Fig. 1d). Mechanotransduction activates pathways that increase cell growth, and our groups have found that co-administering chemotherapy and targeted drugs while inhibiting these stiffness-activated tyrosine kinases could overcome stiffness-mediated drug resistance^{69, 84}. The relative importance of the stiffness of the ECM, versus survival signals cells receive through integrin engagement or proteolysis of the ECM, is completely unknown. Parsing these individual factors is critical for designing potent drugs for patients with metastatic disease.

Maximizing Value from Materials approaches

There are numerous choices for materials environments that are excellent at representing features of the TME. The next most valuable step for the field is to translate these *in vitro* studies to useful pre-clinical and clinical studies by improving *in vitro* study design. To do this, we suggest increased collaborations between biomaterials synthesis experts with cancer biologists and systems biologists. This would result in experimental studies of drug responses with these *in vitro* platforms that will be 1) sufficiently powered, 2) paired with *in vivo* validation (using the most appropriate *in vivo* model), and/or 3) represented with statistically useful data analysis techniques.

By talking to systems biologists earlier on, ideally during the experimental design phase, we could best utilize and showcase the power of these TME-approximating materials systems. A simple, easy change groups doing drug studies with biomaterials systems can adopt is selecting the correct drug response metric. At present, it's challenging to compare drug response reports across different biomaterial studies. This is largely because researchers have chosen an improper drug response metrics and have not accounted for the vast differences in cell growth rates across biomaterials. There are key differences in reporting a drug's IC₅₀ (the inhibition concentration of a drug where the response is reduced by half), EC₅₀ (the effective concentration of a drug

that gives half-maximal response), E_{max} (the drug's maximum effect), GI_{50} (the concentration of a drug that reduces total cell growth by 50%), and the GR_{50} (the concentration of a drug that reduces cell growth rate by half)⁸⁵. The GR_{50} is an important contribution to the field of drug screening, because it accounts for the variable differences in growth rates between different cell lines and/or across different biomaterials. Labs must quantify differences in growth rates across their cells and materials before choosing one of the metrics above, or they risk presenting data that is difficult to translate to a preclinical model or to another lab's biomaterial system. Systems biologists have developed both experimental and computational tools to go even further, with ways to measure not only whether cells die, but how^{37, 86}, and how quickly⁸⁷⁻⁸⁹.

Connecting clinical and *in vitro* drug responses

Drug resistance is multifactorial. In the clinic, resistance mechanisms include mutations, copy number alterations, cell state transitions, microenvironmental factors and the physical exclusion of drugs (Fig. 1a-b)⁹⁰⁻⁹³. It is natural to assume that all of these mechanisms interact in complex and poorly understood ways in patients. However, the direct clinical studies tend to involve a detailed investigation of static biopsies of single modes of resistance at two time points (before and after therapy)⁹². The clinical reality in human patients is that sample sizes and sample timepoints are limited by practical issues associated with patient care and human subjects research. As a result, it is extraordinarily hard to directly observe the dynamic interactions of multiple diverse biological mechanisms of drug resistance within patients. We posit that this an important rationale for the role of tractable *in vitro* models of tumor biology.

3D cultures of patient derived tumor material that are grown as organoids have transformed cancer research⁹⁴. It is now clear that 3D organoids can recapitulate the structures and phenotypes of clinical cancer biology that were not previously possible in 2D culture. Some of these structures and properties can even alter drug responses *in vitro*. However, recapitulating specific *in vivo* characteristics in culture does not guarantee that the specific microenvironmental characteristic of interest is critical to understanding drug responses in real patients. Because of this, some recent studies have taken a more direct approach to understanding drug resistance in 3D patient-derived cultures. This direct design involves obtaining pre-therapy patient samples from individuals whose clinical responses to a given therapeutic are known. By assaying drug response *in vitro* in 3D patient derived organoids with clinical response information, one can directly assess the predictive power of *in vitro* systems for predicting clinical response in individual patients. The results from these direct comparison studies have been mixed. Some 3D cultures of primary tumor tissue predict the clinical therapeutic response to some drugs, while other drug responses cannot be predicted from *in vitro* systems⁹⁵.

Moving forward with better drug discovery approaches

Perhaps a possible answer to this challenge lies in recent observations of organoid cultures in pancreatic cancers⁹⁶. It was recently shown that these primary cultures of human

tumors retain the genetic heterogeneity of the primary tumor, but not the RNA-seq profile of the primary tumor. Is the RNA-seq profile different because of the differences in the microenvironment? This is a reasonable hypothesis that presents two paths forward for predicting drug resistance. One potential path forward is to try to engineer a microenvironment that completely recapitulates the biology of the primary tumor (and thus the RNA-seq profile). A second path forward is to try to only recapitulate the aspects of the microenvironment that are necessary to predict therapeutic response.

While both approaches seem challenging, the second approach appears more tractable from our perspective, and particularly suited to an engineering approach. What might an experimental approach look like here? Researchers could develop a culture bank of therapeutically responsive and non-responsive patient derived organoids. Then, by systematically engineering the microenvironment to retrospectively predict clinical responses *in vitro*, one could identify and tune the *in vitro* TME to recapitulate clinical response. If predictive power can be achieved, the engineered culture architecture could be tested in a new and completely independent clinical test set of drug responders and non-responders. Then eventually a prospective clinical trial could be designed to examine the predictive power in a newly enrolled cohort.

If clinical samples are not available, it should also be possible to leverage existing drug development knowledge to gain confidence in a novel microenvironmental model of drug response. This can be done by identifying positive and negative control compounds. For instance, an *in vitro* reconstruction of Renal Cell Carcinomas (RCC) to improve drug development should ideally recapitulate what is already known from randomized clinical trials in RCC. For instance, everolimus (targeting MTOR) has demonstrated efficacy in RCC in randomized clinical trials versus placebo controls⁹⁷. Subsequent clinical development has shown that single agent therapies like sunitinib⁹⁸ and cabozantinib⁹⁹ have greater clinical efficacy than everolimus alone⁹⁸. Finally, MK-2206, has been demonstrated to be inferior to everolimus in randomized clinical trials¹⁰⁰. Collectively, these clinical trials suggest that a clinically validated model (or collection of models) for RCC should be sensitive to everolimus at a dose that can be justified by the measurements of the pharmacokinetics of everolimus in its phase I trial. In addition, an ideally validated model would show qualitatively greater sensitivity to sunitinib and cabozantinib, but decreased sensitivity to MK-2206. All of these agents would ideally be dosed at a range of concentrations that are clinically plausible (given the known clinical exposures in phase I trials).

Outlook

The most important step for this community is to identify simple principles and paths forward that distill the intense complexity that arises at the intersection of innate and extrinsic drug resistance. Not only is this question complex *in vitro* and *in vivo* - we also don't know enough about how these compete in real patients. From a materials development perspective, the goal isn't as simple as "make

this look more like cancer” and then apply it to drug studies. A focus, instead, on partnering with cancer and systems biologists to better explain clinical findings is key. What may be needed here are models that can dynamically evolve with drug treatment and tumor growth, that include the immune component of the TME, and that are simultaneously high throughput, enable live tracking of individual cells and communities, and that can be paired with -omics techniques to understand drug resistance mutations and mechanisms.

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