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Bioengineering approaches to study multidrug resistance in tumor cells

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Abstract

The ability of cancer cells to become resistant to chemotherapeutic agents is a major challenge for the treatment of malignant tumors. Several strategies have emerged to attempt to inhibit chemoresistance, but the fact remains that resistance is a problem for every effective anticancer drug. The first part of this review will focus on the mechanisms of chemoresistance. It is important to understand the environmental cues, transport limitations and the cellular signaling pathways associated with chemoresistance before we can hope to effectively combat it. The second part of this review focuses on the work that needs to be done moving forward. Specifically, this section focuses on the necessity of translational research and interdisciplinary directives. It is critical that the expertise of oncologists, biologists, and engineers be brought together to attempt to tackle the problem. This discussion is from an engineering perspective, as the dialogue between engineers and other cancer researchers is the most challenging due to non-overlapping background knowledge. Chemoresistance is a complex and devastating process, meaning that we urgently need sophisticated methods to study the process of how cells become resistant.

Introduction

Cancer is the second leading cause of death in the United States, with 1 in 4 deaths being attributed to the disease.¹ Despite this sobering statistic, cancer treatment and survival rates have improved drastically over the past few decades. Less than 10% of all cancer related deaths are due to the effects of the primary tumor, indicating that initial treatments are usually highly effective.² The two main causes of death are distant metastases and tumor recurrence after remission, often associated with a resistance to chemotherapy. Both of these processes are highly complex, involving numerous cellular pathways which respond to a battery of endogenous and exogenous signals. Because of this complexity, it is incredibly difficult to study these processes *in vitro*. Acquired drug resistance in tumors was first observed almost 50 years ago, when it was noted that only 0.5% of patients achieved complete remission after treatment with a single therapeutic agent, and the majority of this sub-population eventually relapsed despite continuing treatment with the initially effective

therapy strategy.³ This failure of single agent therapies is attributed to a population of tumor cells which becomes insensitive to the single agent, rendering further treatment ineffective. Additionally, cancer cells may exhibit intrinsic resistance, which is characterized by a decreased sensitivity to a drug even without previous exposure. In the following decades, thousands of studies were undertaken with the goal of understanding and circumventing resistance in cancer cells. Although much has been learned, the ability to anticipate and prevent the development of chemoresistance is still not possible. Perhaps the most well-known recent case of chemoresistance foiling the success of a therapeutic is the case of imatinib mesylate (Gleevec®; Novartis). This drug was developed as a specific inhibitor of the Bcr-Abl fusion gene product which is the hallmark of Chronic Myeloid Leukemia (CML). It was initially considered a “magic bullet”⁴ for the treatment of CML, and produced a dramatic improvement in a large number of patients. This impressive early success led to rapid FDA approval and a huge amount of media attention. Unfortunately, this success was short lived, as many of the patients eventually relapsed. Not only did the leukemia return, but the re-emergent leukemic cells were resistant to Gleevec, precluding further Gleevec treatment for this subset of patients. Today Gleevec is still used to treat early stage CML, but it is no longer considered a miracle cure.⁵ This example illustrates the importance of considering chemoresistance during drug development and during patient care. Too often, the effects of acquired resistance are underestimated or ignored altogether during clinical trials. Sometimes these trials are not long enough to reflect the susceptibility of a drug to tumor resistance, leading to a drug being considered a cure when in reality it only postpones disease progression.

In this review we will discuss the biological and biochemical roots of drug resistance (Fig. 1), the strategies being developed to overcome them, and the future work that needs to be done. Specifically we will focus on the importance of translational research and the necessary collaborations between researchers in different fields that must be undertaken to further our understanding of chemoresistance. As this field moves forward, physicians, oncologists, biologists, biochemists and engineers will have to work together to understand this multifaceted behavior of malignant tumors. Towards this end, it is critical that an *in vitro* model of cancer be developed. This type of model can be applied to cheaper, high throughput assays unsuitable for animal models while still faithfully supporting normal cell behavior. Such a model will be incredibly complex and will require collaborations from many fields to become a reality. The benefits would absolutely justify this effort, as a biomimetic *in vitro* model will allow for the detailed study of molecular mechanisms of drug efficacy and the development of chemoresistance. This information is critical if we are to move forward. Treatment of cancer has improved greatly over the past decades, but if we want to move from cancer treatment to cancer cures, we will need to deepen our knowledge of complex tumor biology and apply this knowledge to the new therapeutics being developed.

Tumor microenvironment based resistance

Cancer stem cells

Although there have been many advances in chemotherapy over the years, advanced stage malignancies often remain stubbornly incurable. Treatment of these tumors is almost always associated with short term regression followed by relapse. Advanced tumors consist of a highly heterogeneous cell population, with only a small percentage of the cancer cells retaining proliferative activity when removed from their normal host.⁶ This sub-population of cells has recently been implicated in the persistence of tumors.⁷ These cells, called cancer stem cells (CSC), retain features of normal stem cells including self-renewal,⁸ pluripotency,⁹ and altered gene expression.¹⁰ Among the up-regulated genes found in CSCs are those that mediate exogenous drug export, DNA repair, anti-apoptosis and proliferation.¹¹ These are precisely the biochemical mechanisms implicated in chemoresistance,¹² suggesting that resistance to anti-tumor therapy may arise from the sub-population of CSCs.¹³ Further complicating the situation, the specific genetic and epigenetic state of individual CSCs is highly heterogeneous within the tumor CSC population.¹⁴ Heterogeneous cell populations are incredibly difficult to treat, as specificity and efficacy are not achievable across the entire population.^{15,16} Additionally, CSCs can give rise to poorly differentiated daughter cells which are highly susceptible to the epithelial-to-mesenchymal transition (EMT), a process which produces highly invasive and metastatic cells.¹⁷ The existence of CSCs also has many implications for the development of new anti-cancer agents. Currently, a major end-point goal of animal studies and clinical trials is the 50% reduction of tumor mass.¹⁸ CSCs, being more resistant to therapies, are most likely enriched in this 50% smaller tumor, meaning that aggressive relapse is highly probable despite the initial efficacy of treatment. This illustrates the necessity of developing new experimental metrics that will better predict long-term patient survival. CSCs could also be used for the molecular profiling of tumors. Recent evidence suggests that the behavior of tumors can be predicted by the early stage epigenetic state of the sub-population of CSCs.¹⁹ This application could also be extended to the analysis of metastasis. Many tumors spread to distant sites, but not all remote colonies turn malignant. The aggressiveness of a metastatic colony is associated with an enriched CSC population,²⁰ indicating that this could be used as a metric to assess whether a metastatic lesion is benign or malignant. Newly developed strategies for anti-CSC treatment are starting to be developed. The earliest strategy involved targeting CSC specific signaling pathways including EGFR, Wnt/ β -catenin and Hedgehog.²¹ Another promising strategy is the combination of a chemotherapeutic agent with a differentiation inducing agent. The goal is to induce differentiation of the CSC into a less aggressive, more sensitive phenotype to increase the activity of the anti-tumor agent.²² These strategies have yet to see any clinical success, but they are promising arenas of development.

Hypoxia and drug resistance

In normal tissue the design of the blood vessels is such that optimal blood flow occurs under virtually all circumstances meaning that most cells experience only transient exposure to low levels of oxygen. In tumors the newly formed blood vessels are much less effective at providing adequate oxygenation and hypoxia, a drastic reduction in oxygen levels, is a common finding.²³ These vessels are often lacking innervation, highly permeable, and are

prone to blind ends and excessive branching^{24,25} leading to hypoxia that can be long lasting (chronic) or transient (acute). Furthermore the oxygen delivery to tumor cells is very inconsistent and oxygenation levels within a tumor can be highly heterogeneous. Hypoxia can contribute to drug resistance in a number of ways. The most direct link is that the reduced blood flow and poor tissue perfusion result in decreased bioavailability of administered drugs. This leaves intracellular drug concentrations lower than anticipated. Additionally, many chemotherapeutic agents target DNA replication and cell cycle pathways and are thus more active against highly proliferative cells. Hypoxic cells tend to divide more slowly, and are therefore less sensitive to these types of therapeutics.²⁶ Radiation therapy suffers from similar problems, as a major method of radiotherapy-induced cell death is *via* the formation of reactive oxygen species (ROS). In hypoxia there is less oxygen to convert into ROS, so the cells are insensitive to this kind of treatment.²⁷ Additional mechanisms of hypoxia-mediated drug resistance are more indirect, but no less potent. Low oxygen levels stimulate genetic and epigenetic responses mediated by the activity of the hypoxia inducible factor (HIF)-1. HIF-1 is one of the most widely studied transcription factors, and has been implicated in the control of over 60 genes, most of which are involved in survival mechanisms including angiogenesis, proliferation, anti-apoptosis, and metabolism.²⁸ Many of these genes are also associated with a cancerous phenotype when overexpressed.²⁸ Tumor cells constitutively expressing HIF-1 are highly invasive, while HIF-1 silencing with small interfering RNA (siRNA) drastically reduces invasiveness.²⁹ Resistance to cytotoxic agents in hypoxia has been observed in a wide range of tumor types, and in most cases it is dependent upon the function of HIF-1. This resistance can be reversed by strategies that inhibit HIF-1.^{30–33} Therapies aimed at overcoming hypoxia-mediated resistance have taken two forms: prodrugs that are enzymatically converted to their active form preferentially under hypoxic conditions (otherwise known as bio-reductive agents) and small molecule inhibitors of HIF-1 activity. Bio-reductive agents rely upon the increased activity of the cytochrome P450 reductase system under hypoxic conditions, resulting in increased concentrations of the active metabolite within hypoxic tumors. By far the most successful example of this strategy is tirapazamine (TPZ). TPZ is activated across a wide range of oxygen levels, rather than being limited to only the most severely hypoxic regions of tumors.³⁴ The inhibition of HIF-1 has been explored in several reviews,^{35,36} but to date only one molecule has entered clinical trials.³⁷ This therapeutic strategy is still in its infancy and has a long way to go before it will appear in a clinical setting.

Cell adhesion mediated drug resistance

Cell adhesion mediated drug resistance (CAM-DR) is a multidrug resistant phenotype promoted by cell–cell and cell–ECM interactions.³⁸ CAM-DR that arises from cell–cell interactions has been coined multicellular resistance (MR),³⁹ and was first observed when experimental evidence showed that multicellular spheroids were more resistant to radiation than cells in a monolayer.⁴⁰ Subsequently it has been shown that these spheroids are also resistant to a range of mechanistically disparate cytotoxic drugs.^{41–44} This increased resistance is mediated through E-cadherin, a cell–cell adhesion molecule; multicellular spheroids treated with E-cadherin blocking antibodies become highly sensitive to cytotoxic agents, while E-cadherin blocking in 2D cell cultures has little effect on drug sensitivity.⁴⁵ This suggests an intriguing area for development of new therapeutics, but this strategy has

yet to be fully explored.⁴⁶ Cell–ECM interactions also have been shown to promote tumor cell survival and decreased sensitivity to cytotoxic agents. This is mediated through integrins and other surface proteins such as CD44.⁴⁷ Integrin expression has been implicated as an indicator of chemoresistance,⁴⁸ and CD44 is overexpressed in certain tumor cell lines.⁴⁹ Additionally, abolishing cell–ECM adherence leads to a specific form of cell death called anoikis, even without the activity of a cytotoxic drug.⁵⁰ Laminin, fibronectin and collagen have all been shown to have pro-survival abilities when interacting with cellular integrins.⁵¹ There has been minimal work done to date on exploiting this dependency for drug development, but perhaps this will be an avenue of exploration in the future.⁵²

Molecular mechanisms

ABC

The most extensively studied mechanism of multiple drug resistance (MDR) development is the ability of the tumor cell to reduce intracellular levels of the anti-cancer agent. This phenomenon is mainly due to the activity of the ATP-binding cassette (ABC) family of efflux-pumps. This family is further divided into subfamilies (ABCA–ABCG).⁵³ To date, 48 ABC transporters have been identified in humans, 15 of which have been implicated in chemoresistance.⁵⁴ The most extensively studied of the ABC family is ABCB2, also known as p-glycoprotein (PGP), which is encoded by the multiple drug resistance 1 (MDR1) gene.⁵⁵ This protein was first classified over 30 years ago due to its ability to confer drug resistance to Chinese hamster ovary cells.⁵⁶ PGP has a very broad specificity, and has been implicated in the development of cancer cell resistance to a number of structurally and mechanistically unrelated therapeutics.⁵⁷ Because PGP (and its family members) have such broad specificity, their activity has been shown to be a major contributor to drug resistance in numerous trials,^{58–60} and the expression of PGP is a predictive marker for how responsive a particular tumor will be to treatment.⁶¹ The importance of these efflux pumps in cytotoxic drug resistance has led to much interest in the development of anti-ABC drugs. These agents are designed to be used in combination with conventional cytotoxics to increase their potency and thus reduce their toxicity to patients.⁶² A large number of these drugs have been in clinical trials in recent years,^{63–66} although they have met with only minimal success. These drugs tend to be very specific in their action and thus the ABC levels in each individual patient's tumor needs to be established prior to therapy. Currently this level of individualized treatment is not standard, but could become more popular in the future.⁵⁹ An alternative strategy to circumvent multi-drug resistance is the development of novel cytotoxic agents that are not substrates for ABC transporters. This approach has been used in the recent development of novel taxane-like agents for the treatment of patients who have previously developed ABC-dependent resistance to other taxanes.^{67–69}

Anti-apoptotic resistance

The eventual goal of all cancer treatment is to kill all tumor cells. Many anti-cancer agents are potent inducers of apoptosis. In normal cells, apoptosis can be triggered by either the intrinsic or extrinsic pathway.⁷⁰ The extrinsic pathway is mediated by death receptors (DR) that respond to apoptotic signals in the extracellular environment. This leads to downstream assembly of the death-inducing signaling complex (DISC). One of the proteins in the DISC,

cysteine aspartyl-specific protease 8 (caspase 8), undergoes autocatalytic cleavage, causing it become active.⁷¹ This in turn activates downstream effector caspase proteins (3, 6, and 7) which lead to the characteristic changes of apoptosis. The intrinsic pathway is mediated by the release of apoptogenic factors, including cytochrome c, from the inter-membrane space of mitochondria. The release of these apoptogenic factors results in the formation of the apoptosome, leading to the auto-activation of the initiator caspase 9. Caspase 9 then activates effector caspases 3, 6, and 7 leading to cell death. The intrinsic pathway is controlled by interactions between anti- and pro-apoptotic Bcl-2 family members which share homology *via* their BH-3 domains.⁷² The multi-domain pro-apoptotic Bcl-2 family proteins Bax and Bak are essential for mitochondrial apoptosis and their activity is controlled by the BH-3-only pro-apoptotic Bcl-2 family proteins. Two models have been suggested for this activation of Bax and Bak. In the 'direct model', BH-3-only proteins directly activate Bax and Bak, whilst in the 'indirect model' BH-3-only proteins bind to anti-apoptotic family members (such as Bcl-2 and BclXL) and prevent them from binding to and inhibiting Bak and Bax.⁷³ Failure of apoptosis is a hallmark of cancer,⁷⁴ and numerous defects in the pathway described above have been reported in tumor cells. There has been considerable interest in developing strategies that target anti-apoptotic Bcl-2 family proteins, including Bcl-2 itself. A number of these, including antisense oligonucleotides and small molecule inhibitors, are in clinical trial.^{75,76}

DNA repair

The ability to detect and repair DNA damage is critical to the maintenance of cellular integrity. If normal cells cannot repair the damage, then they engage apoptosis to prevent the accumulation of cells with mutated DNA. Many chemotherapeutic agents cause cell death *via* DNA damage, but resistance is not always due to failure of the apoptotic pathway. Many tumor cells have increased capacity for DNA repair⁷⁷ as well as increased tolerance of damaged DNA without engaging apoptosis.⁷⁸ Increased tumor cell DNA repair can be mediated through the Fanconi anemia (FA) pathway.⁷⁹ Homology based DNA repair *via* this pathway involves the formation of a protein complex between FA proteins and the breast cancer proteins (BRCA).⁸⁰ The nonhomologous end-joining (NHEJ) DNA repair pathway, through the activity of DNA-dependent protein kinase (DNA-PK), is able to repair double strand breaks where sequence homology cannot be used to reform DNA.⁸¹ Increased DNA-PK activity has been linked to acquired resistance to DNA damage.⁸² The ability of tumor cells to continue to replicate DNA and proliferate even in the presence of DNA damage that would normally lead to cell cycle arrest or apoptosis seems also to be a major mechanism of developed chemoresistance.^{83,84} This cellular pathway has been a popular target for novel adjuvant therapies, mostly due to the myriad of potential target proteins involved in DNA repair. Despite this, only two classes of molecules have gone to clinical trials. The first group are inhibitors of O-6-methylguanine methyltransferase (MGMT) which is responsible for repairing alkylated guanine residues, the preferred DNA insult of many alkylating agents.⁸⁵ Despite this specificity, clinical trials were prematurely suspended due to observed bone marrow toxicity.⁸⁶ By far the more successful strategy has been the inhibition of poly(ADP-ribose) polymerase (PARP). In response to DNA insults, PARP synthesizes poly(ADP-ribose) (pADPr) which mediates the activity of a variety of DNA repair proteins.⁸⁷ Inhibitors of PARP are currently in phase II trials.^{88,89}

Drug detoxification

A further method for tumor cell resistance to cytotoxic drugs is through an increase in cellular detoxification. The main mediators of this process are glutathione (GSH)⁹⁰ and metallothioneins (MT).⁹¹ MTs are responsible for the detoxification of heavy metal containing therapeutics such as cisplatin⁹² and tumor cell MT expression levels have been shown to correlate with disease prognosis.⁹³ GSH conjugates to electrophilic centers in cytosolic compounds, catalyzed by the activity of Glutathione-S-Transferase (GST),⁹⁴ resulting in drug inactivation. Additionally, glutathione conjugation is associated with increased efflux of the drug, mediated by the ABC family of proteins.⁹⁵ Increased GST activity is seen in a range of tumor types and thus is a promising therapeutic target. Two approaches have been taken to date: either direct inhibition of GST as adjuvant therapy for other cytotoxic drugs or the administration of a pro-drug that is activated selectively in the tumor cell by glutathione conjugation. The former strategy has had only minimal success,⁹⁶ but the latter is much more promising. Two of the most successful pro-drugs to date have been canfosfamide⁹⁷ (TLK286) and PABA/NO,⁹⁸ which continue to undergo clinical testing.

Challenges and opportunities

As can be seen from this brief description of the varied mechanisms of acquired drug resistance one of the biggest challenges in cancer treatment is to develop the means to circumvent drug resistance. Such development requires a model system that is amenable to high throughput testing and is easily manipulable. The most important aim of any model system is that it can recapitulate the behavior of tumor cells *in vivo* in an arena that is easier to observe and study. Some of the earliest models, that are still employed to this day, rely on cultures of tumor cell lines grown in a monolayer. These systems are easy to maintain, easy to manipulate and give rapid and reproducible data. The vast majority of novel cancer therapeutics are screened for their efficacy against well-established cancer cell lines on high throughput, plate-based 2D systems. Despite their widespread use, these systems are suboptimal for a number of reasons. Firstly it is clear that cells grown in 2D monolayer cultures vary greatly from genetically identical cells grown in 3D cultures. Specifically, cells differ in their gene expression, morphology, proliferation, differentiation and metabolism.⁹⁹ Perhaps most importantly, cells grown in 2D monolayers are almost always less resistant to cytotoxic agents. This is a significant drawback for these systems, and drug efficacy in *in vitro* 2D systems is often not reflected by *in vivo* efficacy and resistance profiles.¹⁰⁰ Thus data on acquired resistance in 2D *in vitro* models may not be applicable to the *in vivo* situation.

From the above it seems clear that growing tumor cells in 3D is preferable when trying to model cancer accurately. Several 3D models of *in vitro* cancer growth have already been established. These models take one of two forms: tumor spheroids or gel embedded cultures. The former involves growing tumor cells in suspension and allowing them to self-organize into small clusters. These clusters can then be isolated until they grow to an appreciable size, at which point they can be used as surrogate tumors for experiments. Not all tumor cell types will form 3D cultures in this way, and in particular it is not applicable to cell types that

normally grow in suspension. The second method involves seeding cells into a solution which is then polymerized into a gel. The most commonly used substrates are collagen and Matrigel, but in theory a very wide range of substrates could be used. Cells in these 3D models behave more like native tumor cells and show increased drug resistance when compared to 2D culture systems. However one potential problem is that the increased drug resistance in 3D systems is attributed to cellular behavioral changes, when in reality it may also be affected by drug distribution. Cells that are part of the inner cell mass in these models are exposed to much lower drug concentrations than those experienced by the peripheral cells, and this may at least contribute to the relative drug resistance of these 3D cultures.

Another commonly used platform for cancer drug screening is the mouse xenograft model. This method involves injecting human cancer cells into a severe combined immunodeficient (SCID) mouse, usually sub-cutaneously. These cells will form a palpable tumor within a few weeks, and the effectiveness of anti-cancer drugs can be evaluated against these tumor bearing mice.¹⁰¹ There are a number of problems with this model, which combine to make this method unattractive for studying chemoresistance. Firstly, there are interspecies differences between mice and humans which can affect drug metabolism, drug delivery, drug toxicity and the natural history of the implanted tumor. In order for the mice to accommodate human tumor cell xenografts they must be profoundly immunodeficient. This lack of a functional immune system has two consequences: it makes testing immunomodulatory strategies impossible and it removes any role of the immune system in the efficacy of anti-cancer agents. The SCID mouse model may also underestimate the normal tissue toxicity of DNA-damaging strategies, as SCID mice have defective DNA repair pathways.¹⁰² The conventional metrics used to evaluate the performance of anti-cancer agents leave much to be desired. One of the classic end points used to evaluate drug efficacy is 50% reduction in solid tumor mass.¹⁰³ As discussed previously, reduction in tumor mass is relatively easy to accomplish, but the subsequent drug-resistance associated relapse is often ignored completely with these types of assays. Besides mass/volume measurements of the solid tumors, there are few quantitative measures that are taken from this model. This introduces issues for both reproducibility and comparisons between different agents. It also precludes investigations into the mechanism of a drug's action, which is often done in the 2D *in vitro* models. Perhaps the most important shortcoming of this model is its failure to recapitulate the complex and heterogeneous cellular and extracellular matrix components of a tumor. The injected cells are all identical, leading to a relatively homogenous tumor mass, unlike a real tumor where there is likely to be considerable heterogeneity between tumor cells. Additionally, these tumor cells are unable to interact with human stromal cells, which is an important characteristic of normal tumors. This problem is exacerbated by the fact that relatively few human cancer cell lines are usually used in xenograft models, thus failing to represent the vast range of genetic and epigenetic variations found in human cancers. These xenograft models are labor and animal intensive and not suited for high throughput assays, so concessions must be made on the number of cell lines that can be tested. Although prudent for cost and speed considerations, this often means that drugs with minimal efficacy in humans will show activity in mouse models.¹⁰⁴

Clinical trials are the last gauntlet that must be navigated by novel therapeutics, and they are clearly the most indicative of a drug's efficacy. Despite this, there are several problems with modern clinical trials for anti-neoplastic agents. The first problem is the ethical constraints imposed by working on humans. No trial would be able to justify having a true negative control group, as giving a group of cancer patients a placebo when they are expecting a front-line treatment would be unethical. Thus clinical trials usually test novel agents against patients who have already received conventional cytotoxic agents without success, or who have relapsed after conventional therapy. Clearly the cells in these patients' tumors are likely to be drug-resistant and less likely to respond than those in untreated patients. One way around this problem is to test new agents up-front against poor prognosis patients before giving them conventional treatment, referred to as a "window study". Although this gets around the problem of acquired drug resistance it still means that novel agents are usually tested against patients already known to have a poorer outcome and thus may still underplay any benefit of the new agent against a less selected population. Testing of combinations in clinical trials is also often problematic, not usually because of intrinsic issues about giving combinations of agents to patients (after all, virtually all successful chemotherapy regimens are combinations) but because the pre-clinical scheduling experiments needed to inform the optimum combination schedule of agents have frequently not been performed. Fortunately awareness of these issues is increasing and new trial designs are developing to allow better clinical testing of new agents.

The shortcomings of these techniques highlight the urgent need for a biomimetic, high throughput system that can accurately predict the success of a novel agent in treating human cancers. Such a system must be quantitative, which in combination with the development of standard efficacy metrics, will allow for rapid evaluation of performance. In addition these models need to be manipulable to allow the study of how cancer cells become resistant to therapies, and how we can combat these resistant cells. By being able to include all the major mechanisms of chemoresistance, we can begin to study how these signaling pathways interact, overlap and synergize to produce the drug-resistance observed in the clinic. The complexity of this model necessitates the collaboration between molecular biologists, systems biologists, clinicians, and engineers. Such an engineering approach to cancer modeling has only been conceived of in the past few years. With the continued input from both mechanical and tissue engineering, a comprehensive cancer model is a real, attainable goal (Fig. 2).

The role of engineers

Oncologists and clinicians are generally interested in end-point metrics that differentiate between efficacious and non-efficacious treatments. Although important, these types of studies often miss crucial information that could help inform future investigations. Engineers, on the other hand, are well versed in reductionist techniques aimed at decoupling the effects of several extrinsic factors. Although many mechanisms of chemoresistance have been identified and studied in depth, it is difficult to study one mechanism in isolation. Initial attempts at decoupling cellular pathways investigated the role of cell-matrix and cell-cell interactions on fibroblast proliferation. By using micropatterned substrates, cell localization could be tightly controlled, and cells could be kept in isolation or in small

clusters.¹⁰⁵ From this technique, researchers were able to elucidate the role of cell spreading *via* matrix attachment¹⁰⁶ and cell–cell interactions¹⁰⁷ on the proliferative activity of fibroblasts. By studying these processes individually and collectively, the downstream signaling pathways involved could be unambiguously identified. Other early studies were able to discern independent, pro-proliferative pathways active during endothelial remodeling. One pathway was activated in response to soluble growth factor signaling,¹⁰⁸ while the other was activated in response to cell morphology and cell–matrix interactions.¹⁰⁹

Recently, micropatterned poly(dimethylsiloxane) (PDMS) substrates have been synthesized that can tightly control cell adhesion and shape while maintaining 3D culture conditions.¹¹⁰ This substrate allows for independent control of dimensionality, protein coating, cluster size and cell shape. This PDMS membrane has been used to study the sensitivity of breast cancer cells to Taxol.¹¹¹ The study found that chemoresistance is independently related to specific cell–matrix adhesion proteins, cell–cell interactions and dimensionality. Although this study only investigated a single cancer line subjected to only a few different conditions, it is a step in the right direction. This type of study could easily be scaled up into a high-throughput analysis of susceptibility of many cancer lines under many different conditions to various anti-tumor agents. Because the mechanisms of acquired resistance can be studied independently, such an investigation would identify the major contributors to chemoresistance for each type of tumor. This could inform clinicians on what type of anti-resistance therapies would be most effective in their patients.

The interaction between tumors and host stromal cells can have a major impact on disease progression, regression, relapse and metastasis.^{112–114} Primitive cancer models use a homogenous tumor cell population, but recent studies have shown that multiple cell types are necessary to fully recapitulate the disease phenotype. Tissue engineers are very familiar with cell co-culture applications, having used them to grow bone,¹¹⁵ liver¹¹⁶ and bladder¹¹⁷ tissue *in vitro*. More recently, some groups have started co-culturing tumor and stromal cells in the same 3D model to try and elucidate the role of tumor–stromal interactions during disease progression. By comparing co-culture to single cell type models, the mechanisms of stromal cell mediated tumor survival can be identified. This type of analysis is already being done to identify how stromal cells effect tumor protein secretion for autocrine and paracrine signaling.^{118,119} From previous decoupling studies, engineers already know many of the signaling networks that are activated by various signaling molecules, and identifying which signaling molecules are present can help inform clinicians which pathways are being activated, and which pathways are the most attractive therapeutic targets. Newer co-culture models are using expression analysis as a predictive tool.¹²⁰ By simultaneously assaying the epigenetic state of different tumor sub-populations, researchers can identify which pathways are being activated. One group is using this type of study to investigate a breast cancer co-culture model.¹²¹ They analyzed co-cultures collected from 55 patients and identified how protein expression levels suggested future tumor behavior. They used this information as a form of personalized medicine, informing selection of an appropriate anti-tumor agent.

Another group has developed cell-specific *in vitro* bioluminescence imaging (CS-BLI) that allows for specific imaging of the tumor cells being cultured with stromal cells.¹²² This platform allows for the effect of stromal cells on drug resistance to be directly measured,

without a cell sorting protocol. The assay is based on tumor-cell specific luciferase expression, so by pairing the luciferase gene with different promoters, future studies could rapidly and quantitatively assay specific gene expression of transcription factor activity. Additionally, this platform was used as a drug screening assay, and was found to be a better predictor of efficacy than standard 2D models.¹²³ By using this assay and combining these types of studies, drug screening can be done with the knowledge of the specific epigenetic state of the tumor cells. This information could help identify which markers are the most predictive of patient response.

All of these studies emphasize how a detailed, unambiguous understanding of the underlying mechanisms of cell behavior is critical moving forward. In the most general sense, this is the area where engineers can contribute to the battle with cancer. We are by no means close to fully understanding tumor progression; indeed, new mechanisms of acquired resistance are still being discovered.^{124–126}

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Insight, innovation, integration

This paper, at its core, is about the necessity of translational initiatives for the progression of cancer drug research and development. The way that tumors react to chemotherapies is a complex process, with many pathways exerting simultaneous, overlapping and often subtle effects on cancer cell behavior and drug efficacy. Studying this phenomenon *in vitro* is advantageous for speed, reproducibility, cost and throughput considerations, but biologists and oncologists are lacking the necessary tools to design and execute such models. Chemoresistance, a widely observed clinical phenomenon, is highly dependent on dimensionality, cell–cell interactions and matrix mechanics, among many other factors, and an engineering perspective will be critical in recreating the native tumor microenvironment in an *in vitro* model.

Fig. 1. Multiple mechanisms (in red), under the control of multiple extracellular conditions, are responsible for the development of the resistant phenotype.

Fig. 2.

The expertise from oncologists and tissue engineers will need to be merged in order to create a successful *in vitro* cancer model that can recreate complex tumor processes such as chemoresistance.