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A Microphysiological System Model of Therapy for Liver Micrometastases

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Abstract

Metastasis accounts for almost 90% of cancer-associated mortality. The effectiveness of cancer therapeutics is limited by the protective microenvironment of the metastatic niche and consequently these disseminated tumors remain incurable. Metastatic disease progression continues to be poorly understood due to the lack of appropriate model systems. To address this gap in understanding, we propose an all-human microphysiological system that facilitates the investigation of cancer behavior in the liver metastatic niche. This existing LiverChip is a 3D-system modeling the hepatic niche; it incorporates a full complement of human parenchymal and non-parenchymal cells and effectively recapitulates micrometastases. Moreover, this system allows for real-time monitoring of micrometastasis and assessment of human-specific signaling. It is being utilized to further our understanding of the efficacy of chemotherapeutics by examining the activity of established and novel agents on micrometastases under conditions replicating diurnal variations in hormones, nutrients and mild inflammatory states using programmable microdispensers. These inputs affect the cues that govern tumor cell responses. Three critical signaling groups are targeted: the glucose/insulin responses, the stress hormone cortisol and the gut microbiome in relation to inflammatory cues. Currently, the system sustains functioning hepatocytes for a minimum of 15 days; confirmed by monitoring hepatic function (urea, α-1-

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Author contributions

AMC and AW conceived and wrote the article. CLY wrote a subsection on systems biology. All authors reviewed and edited the article. SEW, DPT, VCP, CLY, RP-B, TN and DSB generated data included in the figures. JTB, DAL, RV, LG and AW provided oversight to experimental and engineering in their laboratories.

Competing Interests

All authors declare that they have no competing interests except the following authors: A. Wells: Patent on liverchip now being commercialized by Zyoxel Ltd. L. Griffith: Patent on liverchip now being commercialized by Zyoxel Ltd.; consulting fees paid by Zyoxel Ltd in 2012 but no current relationship.

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antitrypsin, fibrinogen, and cytochrome P450) and injury (AST and ALT). Breast cancer cell lines effectively integrate into the hepatic niche without detectable disruption to tissue and preliminary evidence suggests growth attenuation amongst a subpopulation of breast cancer cells. xMAP technology combined with systems biology modeling are also employed to evaluate cellular crosstalk and illustrate communication networks in the early microenvironment of micrometastases. This model is anticipated to identify new therapeutic strategies for metastasis by elucidating the paracrine effects between the hepatic and metastatic cells, while concurrently evaluating agent efficacy for metastasis, metabolism and tolerability.

Keywords

Micrometastasis; chemotherapeutics; mammary carcinoma; liver

INTRODUCTION

Metastasis is the leading cause of cancer-associated mortality. The development of metastases involves a series of sequential biological processes that allow the spread of cancer cells from a primary site to secondary organs. Cells escape from the primary tumor by intravasating into the circulation followed by extravasation into the parenchyma of a distant organ (1). Those cells that successfully disseminate may either outgrow immediately or lay dormant, as small or pre-malignant micrometastases, for years to decades before becoming clinically evident (2, 3). This is especially daunting in the case of breast cancer where even though the primary tumor is often successfully treated, up to 30% of women with early stage breast cancer will eventually relapse with metastatic disease (4). Due to the widespread distribution of metastatic tumors and the protective effects of the metastatic microenvironment, the effectiveness of cancer therapeutics is limited and consequently recurrent cancers remain largely incurable.

One of the major hurdles impeding the development of cancer therapeutics to target micrometastases is the limitations of current model systems. Animal models are not suitable for this type of study as they generally only provide endpoint analyses in addition to issues of relevance for the human condition (5, 6). Typically, immunocompromised murine models are used (7–9), yet studies have demonstrated that immune systems are crucial to the micrometastatic microenvironment (10, 11). Those animal studies that do use syngeneic models are not fully representative of the human situation due to interspecies differences in cytokines and metabolism (6). While in vitro culture investigations can avoid the cross species issues, the current 2D culture systems lack important aspects which impact tumor behavior, such as 3D architecture to provide tissue depth for tumor intercalation; functional aspects, including fluid flow and control of oxygen content, and do not allow for extended culture. There is also a distinct absence of models capable of recreating micrometastasis while concurrently providing for the evaluation of agent efficacy, toxicity and metabolism. For these reasons, a number of investigators have utilized organotypic cultures in bioreactors as investigative tools to overcome such issues (12–17).

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THE LIVER AS THE METASTATIC TARGET

The liver represents an ideal organ system to study both micrometastasis and the efficacy of cancer therapeutics. Firstly, it is a major site of metastasis for a wide range of carcinomas (e.g. breast, lung, colon, prostate, brain, melanomas). Depending on the primary tumor type, 30–70% of patients dying from cancer have hepatic metastases (18). Secondly, the liver is the major site for drug metabolism (both activation and detoxification), a significant factor in determining efficacy and limiting toxicities of cancer therapies (19). Further, there is evidence that metastatic disease alters liver function, potentially increasing toxicity, as well as changing efficacy of the agent upon the tumor (20).

The liver architecture is suited for its diverse functions such as metabolism, detoxification, and regulation of multifaceted defense mechanisms. It is composed of a complex assembly of highly specialized parenchymal and non-parenchymal cell types (Figure 1). The parenchymal hepatocytes are responsible for most metabolic functions along with the production of the majority of the circulating plasma proteins, transporters, protease inhibitors, blood coagulation factors and immune modulators. The non-parenchymal cellular portion represents 20 – 40% of the liver and is primarily comprised of liver sinusoidal endothelial, Kupffer and stellate cells. The non-parenchymal cells are essential components, releasing substances under both normal and pathological conditions that regulate many hepatocyte functions (21).

As mentioned above, the liver is a major site for drug metabolism. The metabolic processes generally involve converting drugs into more hydrophilic compounds in order for them to be excreted in the bodily fluids (e.g. urine or bile). The cytochrome P450 family of enzymes is primarily responsible for the metabolism of several essential chemotherapy agents. There are more than 50 cytochrome P450 enzymes with approximately 90% of drugs metabolized through CYP3A4/5, CYP2C9, CYP2C19, CYP2D6 and CYP1A2 (22). In particular, the CYP3A subfamily has been identified to play a predominant role in metabolizing chemotherapeutics (23, 24).

The liver is also one of the primary sites of systemic regulation of circadian rhythms via modulations of hormones, nutrients and inflammatory cytokines. Cell proliferation and metabolic rhythms are regulated by circadian cycles, and often show asynchronies between normal and malignant tissues (25). It is currently unknown how the molecular signals that change on a diurnal basis at both the systemic and local level influence the complex micrometastatic microenvironment. However, these circadian rhythms are known to alter the metabolic capabilities of the liver at the transcription, protein, and enzymatic levels (26). It is reasonable to expect that such rhythms would alter micrometastases and/or how chemotherapeutic agents are handled.

LIVERCHIP MICROPHYSIOLOGICAL MODEL

A major challenge in the field of hepatic tissue engineering is the development of ex vivo and in vitro hepatic tissues that both exhibit a stable phenotype and maintain liver parenchymal function. Further, investigating metastatic seeding and survival of cells requires examination over a period of days-to-weeks. Numerous 2D and 3D in vitro liver-

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based systems have been developed and are extensively reviewed (27). Extended evaluation, required for metastasis research, is not recreated in conventional 2D cultures which generally only remain viable and functional for <7 days (28); the addition of a second layer of collagen for a 'sandwich' can extend hepatocyte functioning for longer in select settings (28a, 29). Additionally, dedifferentiation of hepatocytes in these standard 2D culture is well established which subsequently causes a reduction in liver functions (e.g. downregulation of enzymes and reduced production of plasma proteins such as albumin) (30–34). Numerous developments in recent years, notably microfluidics and cell positioning techniques, have overcome some of the aforementioned disadvantages of conventional 2D cultures, primarily by controlling metabolite accumulation and non-steady-state conditions allowing for extended culturing (35–37).

For our investigations and as a possible approach going forward, we utilize an all-human LiverChip microphysiological 3D system (Zyoxel, Ltd; Oxford, UK) that faithfully models both the hepatic niche and micrometastatic tumor cells (34). The liver bioreactor employed by our laboratory has already provided insight into the phenotypic plasticity of both breast and prostate carcinoma cells (38–41). Notably, the bioreactor milieu provides for a greater chemoresistance that cannot be extrapolated from 2D studies (42). We continue to develop and improve this system as detailed below. A more detailed overview of the technical aspects of the LiverChip system and the bioengineering behind the model is described in Wheeler et al., (43).

Functional hepatic niche

We aimed to replicate the fundamental physiologic functions and conditions of the hepatic niche, including multi-cellular composition, metabolism, protein production, and circadian cycles (Figure 2 and 3A and B). The LiverChip bioreactor successfully recreates and maintains all-human hepatic tissues with structural integrity and functional complexity for weeks in culture. It also provides adequate samples for multiple downstream assays while avoiding materials that adsorb steroid hormones and drugs. The system incorporates fresh human hepatocytes and a full complement of non-parenchymal cells, at physiologically relevant ratios. Cultures are maintained for a minimum of 15 days in defined, serum-free medium that supports cell differentiation in culture and can be modified to reflect circadian changes (i.e., insulin, glucagon, glucose and cortisol). The human hepatocytes and non-parenchymal cells are sourced mainly from therapeutic partial hepatectomies for metastatic colorectal carcinoma or other benign diseases such as focal nodular hyperplasia. Many of the tissue isolations are consequently from patients who previously had chemotherapy for colorectal liver metastases. However, prior work has demonstrated that the viability and function of hepatocytes previously exposed to such agents remains unaltered (44).

A thorough characterization of isolated hepatocytes is essential to ensure that functionality is sustained during culture. The investigation of morphology in combination with protein secretion, predominantly albumin, is frequently used as verification of hepatocyte functionality. However, these parameters alone do not necessarily correlate with the existence of other hepatocyte-specific functions (45, 46). Subsequently, a high level of importance and attention has been placed upon ensuring the health and functionality of the

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hepatic tissue in the LiverChip, and as such a comprehensive set of biomarkers has been developed (Figure 2). A key measurement of functionality for our system is active cytochrome P450 metabolism. Previously, we have developed and validated an assay method to simultaneously evaluate the activity of 5 different cytochrome P450 enzymes with the rate of metabolism extrapolated as picomoles of substrate metabolized or metabolite formed per minute per million cells (47). Functionality is further assessed through determining levels of glucose consumption/production, urea catabolism as well as the secretion of positive acute phase proteins, such as alpha-1-antitrypsin (A1AT) and fibrinogen. Maintained health is verified via the production of aspartate transaminase (AST) and alanine aminotransferase (ALT), intracellular proteins released from hepatocytes during times of injury and are monitored clinically. Importantly, all these measurements can be monitored in real-time over the course of an experiment. A variety of imaging modalities are also employed for additional confirmation of hepatocyte health and functionality, including confocal or multiphoton assessment of post-immunolabeled reactors for viability (e.g. calcein AM/ethidium bromide, albumin), maintained presence of non-parenchymal components (e.g. CD45, Lyve-1, CD68, desmin), cell-cell interactions (e.g. E-cadherin, F-actin, α-smooth muscled actin) etc. Each reactor scaffold can be uniquely processed to collect a wide variety of visual information. Some initial data pertaining to these assays is presented in Figure 3B (I, ii and iii).

The LiverChip system is continually being assessed for ways to bring it closer to recapitulating an in vivo liver. Although the system allows for the generation of sufficient effluent samples for the plethora of downstream assays described above, this comes with the limitation of requiring a considerable number of cells per well. Further, while functionality is successfully achieved, we have not yet recapitulated liver structure within the present scaffolding matrices. The scaffolds are of a rigid nature which is associated with non-specific inflammatory responses in tissues (48). Investigations are currently underway which are perusing new functionalized matrices and scaffolding that more accurately reflect the rheology of the liver as well as aid in recreating liver architecture in vitro.

Recapitulating micrometastasis

In order to develop rational approaches to target growing cancer cells and promote clinically undetectable micrometastases towards a dormant state, integrated in vitro systems are needed that can support the initial micrometastatic nodules and follow such nodules for extended periods in culture.

The LiverChip, and other simular moderate-throughput organotypic systems offer the ability to evaluate the role of the the diversity of the human population in the micrometastatic tumor microenvironment in an all-human system in which the liver environment comes from individual patients. We have previously characterized other bioreactor systems to examine the metastatic behaviors of breast and prostate cancer cells (38–41). These microphysiological systems not only effectively recapitulate metastasis but also concurrently allows for real-time monitoring of metastasis and assessment of human-specific signaling. Moreover, the system is evaluable over weeks, having been tested through 30 days, with computer programmable inputs or delivery of modifiers (e.g. hormones, proteins,

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metabolites, inhibitors) that allow one to define specific required signals that either promote or hinder specific metastatic properties (43). The main breast cancer cell lines utilized are the well-characterized metastatic MDA-MB-231 and non-aggressive MCF-7 breast carcinoma cell lines. In order to track and monitor tumor burden within the hepatic niche, the cells lines have been modified to express fluorescent labels.

Upon seeding individual carcinoma cells (the number per well determined by cell concentration and number introduced into each unit) into engineered hepatic tissue, individual cells survive, form nodules, and can grow into mm-size tumor masses under the constant perfusion flow. A tissue is generated that comprises a hybrid of unaffected host tissue and tumor boundary, which is representative of the histological distinctions observed in human tumors (40, 42). Importantly, the breast cancer cell lines, MDA-MB-231 and MCF-7, successfully integrate into and alongside the hepatic niche without detectable disruption to tissue. Markers of health (AST, ALT) and function (cytochrome P450 activity, glucose consumption, urea catabolism, acute phase protein production) remain at levels comparable to that of unburdened hepatic tissue (unpublished data). Furthermore, preliminary evidence suggests growth attenuation amongst a subpopulation of the highly proliferative and invasive MDA-MB-231 cell line when these cells intercalate as individual cells within the tissue parenchyma (unpublished data).

Previous work also demonstrates that in the context of the 3D liver microbioreactor, breast carcinoma cells undergo a phenotypic shift to a more epithelioid phenotype with intimate connections to the hepatic cells (40, 42). This was noted not just with human cell lines, but also with primary explants from breast, prostate and melanoma (42). This phenotype is often noted in small metastatic nodules of patients, wherein the metastases appear more differentiated than the primary tumor (39, 49–52). Furthermore, the reversion to a more epithelioid phenotype in the MDA-MB-231 was associated with enhanced chemoresistance. This was observed though reduced cell death by chemotherapies in MDA-MB-231 cells over-expressing E-cadherin compared to E-cadherin negative MDA-MB-231 and MDA-MB-231-shEcad cells; this protection from death was abrogated by an E-cadherin antibody. Conversely, driving the epithelial MCF-7 cells towards a proliferative mesenchymal phenotype increased their sensitivity (42). The greater chemoresistance conferred by re-expression of E-cadherin mimics the resistance noted in patients (39). The extent of resistance (as determined by LD_{50}) is orders of magnitude greater for tumors grown in the 3D liver system (unpublished data) compared to those grown in 2D cultures either with hepatocytes or with forced E-cadherin expression alone (42). This difference in chemotherapeutic efficacy between 3D and 2D models is well established within the literature (53–55). Many critical signals, regulators and the micrometastatic architecture are not present when cells are cultured under standard 2D conditions (56). The cellular architecture and growth conditions recreated by 3D cultures more accurately mimics the in vivo behavior of metastatic cancer cells and is key for accurate prediction of drug efficacy during the discovery process.

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EVALUATION AND DEVELOPMENT OF CANCER THERAPIES

The most challenging step in the development of therapies for metastasis relates to treating the small and pre-malignant micrometastases. Therapies are required which are either directly toxic towards the proliferating metastatic cells or lock the clinically irrelevant micrometastases in their dormant state. The different biology of the metastatic site requires an understanding as to how the efficacy and toxicity of chemotherapy is linked in the metastatic niche. This niche is affected directly by the molecular signals and cellular behaviors of the cancer cells themselves as well as the surrounding microenvironment, which is also altered based upon circadian fluctuations of metabolites and inflammatory mediators.

A fundamental element important to the investigating the efficacy of chemotherapies is the simple fact that the levels of hormones, nutrients and immune regulators are not static in nature, they fluctuate based the circadian cycles. Going forward, we therefore ask if circadian fluctuations that modulate metabolism and hormones also influence the efficacy and toxicity/detoxification of chemotherapy agents against metastatic tumors. Such questions can be analyzed via the LiverChip as it intimately links the efficacy and hepatic metabolism of therapeutic agents while concurrently controlling diurnal variations as well as mild inflammatory states.

Influence of physiological parameters on metastases and efficacy of chemotherapies

The LiverChip model is being utilized to further our understanding of the efficacy of cancer therapeutics towards these micrometastases by examining the activity of established and novel agents under conditions replicating circadian variations in key components of the portal circulation (nutrients, insulin), the systemic circulation (cortisol) and mild inflammatory states using programmable microdispensors (43). Three critical physiologic parameters are assessed: the glucose/insulin responses, the stress hormone cortisol and the gut microbiome in relation to inflammatory cues. We aim to capture unique insights into the responses of the tumor and parenchymal cells to the complexity of key circadian/diurnal cycles of hormones and other signals.

Impact of physiological diurnal fluctuations in the microenvironment— Investigations into the mechanisms by which the circadian fluctuations affect tumor cell proliferation as well as the parenchymal functions of the liver may lead to innovative therapeutic targets or regimes. Chronotherapy, the administration of drugs at a certain time of day, has already shown promise in treating cancer with evidence to suggest that there may be a window of time when a cytotoxic drug could kill the malignant cells more effectively than the normal host cells (26). Importantly, the peak time for the chronomodulated delivery of chemotherapy has been shown to determine the tolerability of patients with metastatic disease (57). There is also accumulating epidemiological data indicating that disruption of circadian rhythms promotes tumorigenesis and progression (25). Recent work has indicated that, contrary to existing dogma, fasting may be more efficacious in chemotherapy treatments by protecting healthy cells at high doses of chemotherapy (58). However, whether chronobiological dosing will be more effective in treating micrometastases is

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unknown, due to our inability to probe responses of these small tumors in the clinical setting. A large gap exists in linking the complex metastatic microenvironment to molecular signals that change on a diurnal basis at both the systemic and local level. We have developed and demonstrated a modification to the LiverChip system that integrates onto the platform multiplexed microdispensers capable of programmable delivery of compounds with high precision, thereby enabling accurate, flexible and programmable diurnal control (Figure 3C and D). This modified LiverChip model is capable of filling this gap as both phenotypes and mechanisms can be investigated.

Diurnal control of nutrients and hormones is anticipated to have a significant impact upon both tumor growth characteristics and hepatocyte metabolism. It is likely to give rise to both macroscopic and molecular behaviors that are different from those in standard culture. Under diurnal conditions, it is anticipated that a more attenuated growth phenotype in the tumor cells will be observed. Circadian rhythms also have an important impact on drug effectiveness and toxicity as drug metabolism by the liver is regulated by circadian influences upon the activity of xenobiotic-metabolizing enzymes (e.g. cytochrome P450) (59). Subsequently, the timing of treatment chemotherapeutic agents will be of great importance due to the influence of circadian rhythms over efficacy (26). Therefore, diurnal control should bring us closer to more effectively recapitulating physiologically relevant conditions reflective of the in vivo micrometastatic nodule and subsequently direct a more accurate assessment of the efficacy of chemotherapeutic agents.

Presently, the vast majority of standard culture systems conventionally employ medium that contains supra-physiological levels of cortisol, insulin and glucose. We anticipate that these supra-physiological conditions of standard cultures stimulate uncontrolled metastases resulting in easier eradication by chemotherapeutic agents that target proliferation. This then causes overestimation of therapeutic efficacy (59a). Therefore, the levels utilized to investigate diurnal fluctuations in hormones and nutrients will mimic those in the human body.

Impact of mild inflammatory states in the microenvironment—The greatest clinical conundrum for all cancers, but particularly for breast carcinoma, is the ability for disseminated cells to lie dormant for years before emerging as a relentlessly aggressive disease. While the mechanisms currently remain unknown, evidence is emerging that inflammatory cytokines and matrix components may play at least a part in driving small and pre-malignant micrometastasis into a proliferative state (60–62). These signals can emerge from a variety of insults or even physiological situations.

The liver receives blood from the portal circulation that has bacterial inflammatory initiators from the gut microbiome, in addition to inflammatory cytokines and chemokines from the spleen and Peyer’s patches (21). We look to establish the influence of mild inflammatory states on tumor phenotype and chemoresistance. In the assessment of such studies, the common portal blood contaminant lipopolysaccharide will serve as the exogenous stimulant. Although the liver clearly handles such challenges routinely, higher loads may overwhelm the adaptive response and lead to overt inflammation, with activation of the non-parenchymal cells. The subsequent production of inflammatory cytokines and matrix

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components by non-parenchymal cells may then drive the cancer cells into a proliferative state. Of the non-parenchymal cells, stellate cells have been shown to promote the growth and invasion of colon cancer metastases in the liver of mice through the secretion of SDF-1 (63). Additionally, Kupffer cells secrete an arsenal of cytokines, growth factors and matrix-degrading enzymes that are known to support extravasation, motility and invasion (64). Endothelial cells are known to be important for tumor outgrowth as demonstrated by the continuing clinical trials involving angiogenesis inhibitors as targeted therapies (65), and have also been associated with promoting immunotolerance by rendering T cells non-responsive to tumor antigen-specific stimulation (66). Importantly, from a broader perspective, immune and stromal cells are well established to play pivotal roles in regulating pre-metastatic niches (67)

A second initiator of an inflammatory or stressed milieu is tissue stiffness (29, 68). It has been established that a collagen-rich fibrotic microenvironment drives epithelial cells towards a mesenchymal phenotype in which the cells can proliferate and migrate inappropriately. Chronic inflammation leads to such a situation in the liver in particular, wherein there is a feed-forward mechanism by which fibrosis leads to increased pressure in the liver sinusoid, which in turn leads to further fibrosis.

This mild inflammatory milieu would also affect the liver functioning, and likely cancer chemoresponsiveness through altered agent metabolism. However, given the plethora of cell types and signals involved, it is not possible to predict the sensitivity of cancer cells to chemotherapy during chronic exposure to mild inflammatory states. In theory, the greater proliferative fraction should increase chemoresponsiveness, while the inflammatory milieu should promote resistance, making this model crucial in order to elucidate effective avenues for metastatic therapies.

Communication network in the early microenvironment of the hepatic niche

The metastatic microenvironment is composed of a complex milieu of external cues arising from the tumor, stromal, and parenchymal cells. To reconstitute intercellular communication in the early microenvironment of micrometastases, the all-human cellular composition of the LiverChip facilitates the investigation of human-specific cellular crosstalk between tumor and hepatic tissues. Yet, to model and ultimately predict responses of multiple cell types requires the integration of experimental and computational approaches. In recent years, a systems biology framework has advanced molecular, cell, and tissue biology towards a more predictive capability.

“Data-driven” models have emerged as standard tools for systems-level research of signaling networks. With specific applications towards understanding disease pathophysiology, data-driven models are employed to elucidate relationships, interactions, and influences among multivariate components. These perspectives offer more robust insights compared to reductionist studies focused on individual entities. Such models enable the integration of data obtained from different metrics assessed at diverse physiological scales. Within our experimental system, integration of complex cytokine, acute phase protein, phenotypic responses (e.g., death or survival) and metabolic data arising from the growth of small micrometastatic nodules is attainable (Figure 4).

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Conceptually, data are assessed by multivariate analyses such as hierarchical clustering, principal component analysis (PCA), and partial least squares (PLS, e.g. PLSR and PLSDA) by extracting groups of molecular activities that are statistically associated with an established cell behavior or phenotype. As a result, insights gleaned from multivariate analyses generate new hypotheses, which are then tested by experiments.

Although a systems biology framework continues to revolutionize our understanding of disease pathophysiology and accelerate drug discovery efforts (8, 69, 70), we anticipate that data-driven models will identify markers in early metastatic disease, as well as during evaluations of drug efficacy and toxicity.

CLINICAL IMPLICATIONS

Whilst progress has been made in our understanding and treatment of metastatic disease, it currently remains incurable. An advantage of this physiologically representative, all-human liver bioreactor is the concurrent assessment of new as well as existing agents against micrometastases. Moreover, the base organotypic system was originally developed for and is now in use as a consistent and robust drug testing model in which human metabolism of agents can be recreated (71). Generally, chemotherapeutic drug testing against cancer cells in ex vivo or in vitro settings fail to simultaneously capture agent metabolism as well as the contribution of the microenvironment on carcinoma cell responsiveness (e.g. circadian variations, tumor-parenchymal interactions, immunological perturbations and fluid flow). This LiverChip is capable of recapitulating both critical aspects for human efficacy prediction. Additionally, primary breast carcinoma cells as previously described (40, 42) can be incorporated into the system to bring us closer to the patient experience. Animals models of Patient-Derived Xenografts (PDx) have shown that human tumor grafts transplanted into mice can be predictive of patient response to therapy (72). Therefore, once established, this system may also be used to assess responses of human patient tumor samples to chemotherapies. This would alleviate the need for animal involvement and also serve as an excellent screen for ascertaining the most effective personalized treatment regime for individuals afflicted with cancer.

SUMMARY

We focus on occult micrometastatic behaviors as these represent substantial challenges in preclinical development due to the complexities of identifying these nodules and monitoring their response to therapies in the clinical setting. The LiverChip has the unique inherent advantage of intimately linking the evaluation of therapeutic efficacy for metastasis with metabolism and tolerability. Not only is it a major organ for xenobiotic detoxification, but it is also a main site for metastasis. Cancer cells produce many factors and metabolites that alter the surrounding tissue, it is likely that metabolism of agents are altered by hepatocyte crowding and loss, as well as the signals from tumors (73). These reciprocal paracrine effects likely alter both efficacy and toxicity/detoxification. The essential point of linkage would be even more labile in the face of cyclical changes in hormones, nutrients and inflammatory modulators. Subsequently, investigations of these essential parameters are anticipated to yield markers of micrometastatic behavior that will enable better clinical

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monitoring, and guide the design of clinical studies. Additionally, this metastatic model is readily transferable to other organs and cancer type. This system has the potential to be used to evaluate the mechanisms leading to and promoting the escape and outgrowth of dormant micrometastatses. Additionally, this metastatic model could be readily transferable to other organs and cancer types after optimizing microenvironmental conditions.

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References

1. Christiansen JJ, Rajasekaran AK. Reassessing epithelial to mesenchymal transition as a prerequisite for carcinoma invasion and metastasis. Cancer Res. 2006; 66:8319–8326. [PubMed: 16951136]
2. Taylor DP, Wells JZ, Savol A, Chennubhotla C, Wells A. Modeling boundary conditions for balanced proliferation in metastatic latency. Clin Cancer Res. 2013; 19:1063–1070. [PubMed: 23329811]
3. Weinberg RA. The many faces of tumor dormancy. APMIS. 2008; 116:548–551. [PubMed: 18834401]
4. O'Shaughnessy J. Extending survival with chemotherapy in metastatic breast cancer. Oncologist. 2005; 10(Suppl 3):20–29. [PubMed: 16368868]
5. Hackam DG, Redelmeier DA. Translation of research evidence from animals to humans. JAMA. 2006; 296:1731–1732. [PubMed: 17032985]
6. Mestas J, Hughes CC. Of mice and not men: differences between mouse and human immunology. J Immunol. 2004; 172:2731–2738. [PubMed: 14978070]
7. Fantozzi A, Christofori G. Mouse models of breast cancer metastasis. Breast Cancer Res. 2006; 8:212. [PubMed: 16887003]
8. Khanna C, Hunter K. Modeling metastasis in vivo. Carcinogenesis. 2005; 26:513–523. [PubMed: 15358632]
9. Teicher BA. Tumor models for efficacy determination. Mol Cancer Ther. 2006; 5:2435–2443. [PubMed: 17041086]
10. Junttila MR, de Sauvage FJ. Influence of tumour micro-environment heterogeneity on therapeutic response. Nature. 2013; 501:346–354. [PubMed: 24048067]
11. Slaney CY, Rautela J, Parker BS. The emerging role of immunosurveillance in dictating metastatic spread in breast cancer. Cancer Res. 2013; 73:5852–5857. [PubMed: 24062312]
12. Domenech M, Bjerregaard R, Bushman W, Beebe DJ. Hedgehog signaling in myofibroblasts directly promotes prostate tumor cell growth. Integr Biol (Camb). 2012; 4:142–152. [PubMed: 22234342]
13. Gomez EW, Chen QK, Gjorevski N, Nelson CM. Tissue geometry patterns epithelial-mesenchymal transition via intercellular mechanotransduction. J Cell Biochem. 2010; 110:44–51. [PubMed: 20336666]
14. Nelson CM, Vanduijn MM, Inman JL, Fletcher DA, Bissell MJ. Tissue geometry determines sites of mammary branching morphogenesis in organotypic cultures. Science. 2006; 314:298–300. [PubMed: 17038622]
15. Ridky TW, Chow JM, Wong DJ, Khavari PA. Invasive three-dimensional organotypic neoplasia from multiple normal human epithelia. Nat Med. 2010; 16:1450–1455. [PubMed: 21102459]
16. Shaw KR, Wrobel CN, Brugge JS. Use of three-dimensional basement membrane cultures to model oncogene-induced changes in mammary epithelial morphogenesis. J Mammary Gland Biol Neoplasia. 2004; 9:297–310. [PubMed: 15838601]
17. Stroock AD, Fischbach C. Microfluidic culture models of tumor angiogenesis. Tissue Eng Part A. 2010; 16:2143–2146. [PubMed: 20214470]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

18. Pickren, J.; Tsukada, Y.; Lane, W. Liver metastasis. In: Weiss, L.; Gilbert, L., editors. Liver metastasis. Boston, MA: G.K. Hall Medical Publishers; 1982.

19. Grever, MR.; Grieshaber, CK. Toxicology by Organ System. 5. Bast, RC., Jr; Kufe, DW.; Pollock, RE., editors. Hamilton, ON: BC Decker; 2000.

20. Guengerich FP, Turvy CG. Comparison of levels of several human microsomal cytochrome P-450 enzymes and epoxide hydrolase in normal and disease states using immunochemical analysis of surgical liver samples. J Pharmacol Exp Ther. 1991; 256:1189–1194. [PubMed: 2005581]

21. Crispe IN. The liver as a lymphoid organ. Annu Rev Immunol. 2009; 27:147–163. [PubMed: 19302037]

22. Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. J Pharmacol Exp Ther. 1994; 270:414–423. [PubMed: 8035341]

23. Slaughter RL, Edwards DJ. Recent advances: the cytochrome P450 enzymes. Ann Pharmacother. 1995; 29:619–624. [PubMed: 7663035]

24. Wilkinson GR. Drug metabolism and variability among patients in drug response. N Engl J Med. 2005; 352:2211–2221. [PubMed: 15917386]

25. Fu L, Lee CC. The circadian clock: pacemaker and tumour suppressor. Nat Rev Cancer. 2003; 3:350–361. [PubMed: 12724733]

26. Levi F, Schibler U. Circadian rhythms: mechanisms and therapeutic implications. Annu Rev Pharmacol Toxicol. 2007; 47:593–628. [PubMed: 17209800]

27. Godoy P, Hewitt NJ, Albrecht U, Andersen ME, Ansari N, Bhattacharya S, Bode JG, Bolleyn J, Borner C, Bottger J, Braeuning A, Budinsky RA, Burkhardt B, Cameron NR, Camussi G, Cho CS, Choi YJ, Craig Rowlands J, Dahmen U, Damm G, Dirsch O, Donato MT, Dong J, Dooley S, Drasdo D, Eakins R, Ferreira KS, Fonsato V, Fraczek J, Gebhardt R, Gibson A, Glanemann M, Goldring CE, Gomez-Lechon MJ, Groothuis GM, Gustavsson L, Guyot C, Hallifax D, Hammad S, Hayward A, Haussinger D, Hellerbrand C, Hewitt P, Hoehme S, Holzhutter HG, Houston JB, Hrach J, Ito K, Jaeschke H, Keitel V, Kelm JM, Kevin Park B, Kordes C, Kullak-Ublick GA, LeCluyse EL, Lu P, Luebke-Wheeler J, Lutz A, Maltman DJ, Matz-Soja M, McMullen P, Merfort I, Messner S, Meyer C, Mwinyi J, Naisbitt DJ, Nussler AK, Olinga P, Pampaloni F, Pi J, Pluta L, Przyborski SA, Ramachandran A, Rogiers V, Rowe C, Schelcher C, Schmich K, Schwarz M, Singh B, Stelzer EH, Stieger B, Stober R, Sugiyama Y, Tetta C, Thasler WE, Vanhaecke T, Vinken M, Weiss TS, Widera A, Woods CG, Xu JJ, Yarborough KM, Hengstler JG. Recent advances in 2D and 3D in vitro systems using primary hepatocytes, alternative hepatocyte sources and non-parenchymal liver cells and their use in investigating mechanisms of hepatotoxicity, cell signaling and ADME. Arch Toxicol. 2013; 87:1315–1530. [PubMed: 23974980]

28. Debnath J, Brugge JS. Modelling glandular epithelial cancers in three-dimensional cultures. Nat Rev Cancer. 2005; 5:675–688. [PubMed: 16148884]

28a. Dunn JC, Tompkins RG, Yarmush ML. Long-term in vitro function of adult hepatocytes in a collagen sandwich configuration. Biotechnol Prog. 1991; 7:237–245. [PubMed: 1367596]

29. Khetani SR, Bhatia SN. Microscale culture of human liver cells for drug development. Nat Biotechnol. 2008; 26:120–126. [PubMed: 18026090]

30. Bissell DM, Arenson DM, Maher JJ, Roll FJ. Support of cultured hepatocytes by a laminin-rich gel. Evidence for a functionally significant subendothelial matrix in normal rat liver. J Clin Invest. 1987; 79:801–812. [PubMed: 3546380]

31. Clayton DF, Harrelson AL, Darnell JE Jr. Dependence of liver-specific transcription on tissue organization. Mol Cell Biol. 1985; 5:2623–2632. [PubMed: 3841792]

32. Godoy P, Hengstler JG, Ilkavets I, Meyer C, Bachmann A, Muller A, Tuschl G, Mueller SO, Dooley S. Extracellular matrix modulates sensitivity of hepatocytes to fibroblastoid dedifferentiation and transforming growth factor beta-induced apoptosis. Hepatology. 2009; 49:2031–2043. [PubMed: 19274752]

33. Koide N, Shinji T, Tanabe T, Asano K, Kawaguchi M, Sakaguchi K, Koide Y, Mori M, Tsuji T. Continued high albumin production by multicellular spheroids of adult rat hepatocytes formed in

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

the presence of liver-derived proteoglycans. Biochem Biophys Res Commun. 1989; 161:385–391. [PubMed: 2730666]

34. Tong JZ, De Lagausie P, Furlan V, Cresteil T, Bernard O, Alvarez F. Long-term culture of adult rat hepatocyte spheroids. Exp Cell Res. 1992; 200:326–332. [PubMed: 1572400]
35. Takeshita K, Bowen WC, Michalopoulos GK. Three-dimensional culture of hepatocytes in a continuously flowing medium. In Vitro Cell Dev Biol Anim. 1998; 34:482–485. [PubMed: 9661052]
36. Allen JW, Khetani SR, Bhatia SN. In vitro zonation and toxicity in a hepatocyte bioreactor. Toxicol Sci. 2005; 84:110–119. [PubMed: 15590888]
37. Novik E, Maguire TJ, Chao P, Cheng KC, Yarmush ML. A microfluidic hepatic coculture platform for cell-based drug metabolism studies. Biochem Pharmacol. 2010; 79:1036–1044. [PubMed: 19925779]
38. Chao Y, Wu Q, Acquafondata M, Dhir R, Wells A. Partial mesenchymal to epithelial reverting transition in breast and prostate cancer metastases. Cancer Microenviron. 2012; 5:19–28. [PubMed: 21892699]
39. Chao YL, Shepard CR, Wells A. Breast carcinoma cells re-express E-cadherin during mesenchymal to epithelial reverting transition. Mol Cancer. 2010; 9:179. [PubMed: 20609236]
40. Yates C, Shepard CR, Papworth G, Dash A, Beer Stolz D, Tannenbaum S, Griffith L, Wells A. Novel three-dimensional organotypic liver bioreactor to directly visualize early events in metastatic progression. Adv Cancer Res. 2007; 97:225–246. [PubMed: 17419948]
41. Yates CC, Shepard CR, Stolz DB, Wells A. Co-culturing human prostate carcinoma cells with hepatocytes leads to increased expression of E-cadherin. Br J Cancer. 2007; 96:1246–1252. [PubMed: 17406365]
42. Chao Y, Wu Q, Shepard C, Wells A. Hepatocyte induced re-expression of E-cadherin in breast and prostate cancer cells increases chemoresistance. Clin Exp Metastasis. 2012; 29:39–50. [PubMed: 21964676]
43. Wheeler S, Borenstein J, Clark AM, Ebrahimkhani M, Fox IJ, Griffith L, Inman W, Lauffenburger DA, Nguyen T, Pillai VC, Prantil-Baun R, Stolz DB, Taylor DP, Ulrich T, Venkataramanan R, Wells A, Young C. All-Human Microphysical Model of Metastasis Therapy. Stem Cell Res Ther. 2013 in press.
44. Hewes JC, Riddy D, Morris RW, Woodrooffe AJ, Davidson BR, Fuller B. A prospective study of isolated human hepatocyte function following liver resection for colorectal liver metastases: the effects of prior exposure to chemotherapy. J Hepatol. 2006; 45:263–270. [PubMed: 16635536]
45. Hengstler JG, Brulport M, Schormann W, Bauer A, Hermes M, Nussler AK, Fandrich F, Ruhnke M, Ungefroren H, Griffin L, Bockamp E, Oesch F, von Mach MA. Generation of human hepatocytes by stem cell technology: definition of the hepatocyte. Expert Opin Drug Metab Toxicol. 2005; 1:61–74. [PubMed: 16922653]
46. Knobeloch D, Ehnert S, Schyschka L, Buchler P, Schoenberg M, Kleeff J, Thasler WE, Nussler NC, Godoy P, Hengstler J, Nussler AK. Human hepatocytes: isolation, culture, and quality procedures. Methods Mol Biol. 2012; 806:99–120. [PubMed: 22057448]
47. Pillai VC, Strom SC, Caritis SN, Venkataramanan R. A sensitive and specific CYP cocktail assay for the simultaneous assessment of human cytochrome P450 activities in primary cultures of human hepatocytes using LC-MS/MS. J Pharm Biomed Anal. 2013; 74:126–132. [PubMed: 23245243]
48. Wells RG. The role of matrix stiffness in regulating cell behavior. Hepatology. 2008; 47:1394–1400. [PubMed: 18307210]
49. Chaffer CL, Brennan JP, Slavin JL, Blick T, Thompson EW, Williams ED. Mesenchymal-to-epithelial transition facilitates bladder cancer metastasis: role of fibroblast growth factor receptor-2. Cancer Res. 2006; 66:11271–11278. [PubMed: 17145872]
50. Kowalski PJ, Rubin MA, Kleer CG. E-cadherin expression in primary carcinomas of the breast and its distant metastases. Breast Cancer Res. 2003; 5:R217–222. [PubMed: 14580257]
51. Tarin D, Thompson EW, Newgreen DF. The fallacy of epithelial mesenchymal transition in neoplasia. Cancer Res. 2005; 65:5996–6000. discussion 6000-5991. [PubMed: 16024596]

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52. Wells A, Yates C, Shepard CR. E-cadherin as an indicator of mesenchymal to epithelial reverting transitions during the metastatic seeding of disseminated carcinomas. Clin Exp Metastasis. 2008; 25:621–628. [PubMed: 18600305]

53. Horning JL, Sahoo SK, Vijayaraghavalu S, Dimitrijevic S, Vasir JK, Jain TK, Panda AK, Labhasetwar V. 3-D tumor model for in vitro evaluation of anticancer drugs. Mol Pharm. 2008; 5:849–862. [PubMed: 18680382]

54. Mitra M, Mohanty C, Harilal A, Maheswari UK, Sahoo SK, Krishnakumar S. A novel in vitro three-dimensional retinoblastoma model for evaluating chemotherapeutic drugs. Mol Vis. 2012; 18:1361–1378. [PubMed: 22690114]

55. Sung JH, Shuler ML. A micro cell culture analog (microCCA) with 3-D hydrogel culture of multiple cell lines to assess metabolism-dependent cytotoxicity of anti-cancer drugs. Lab Chip. 2009; 9:1385–1394. [PubMed: 19417905]

56. Lee GY, Kenny PA, Lee EH, Bissell MJ. Three-dimensional culture models of normal and malignant breast epithelial cells. Nat Methods. 2007; 4:359–365. [PubMed: 17396127]

57. Levi F, Focan C, Karaboue A, de la Valette V, Focan-Henrard D, Baron B, Kreutz F, Giacchetti S. Implications of circadian clocks for the rhythmic delivery of cancer therapeutics. Adv Drug Deliv Rev. 2007; 59:1015–1035. [PubMed: 17692427]

58. Lee C, Raffaghello L, Brandhorst S, Safdie FM, Bianchi G, Martin-Montalvo A, Pistoia V, Wei M, Hwang S, Merlino A, Emionite L, de Cabo R, Longo VD. Fasting cycles retard growth of tumors and sensitize a range of cancer cell types to chemotherapy. Sci Transl Med. 2012; 4:124ra127.

59. Ohdo S. Circadian rhythms in the CNS and peripheral clock disorders: chronopharmacological findings on antitumor drugs. J Pharmacol Sci. 2007; 103:155–158. [PubMed: 17299245]

59a. Bernabe DG, Tamae AC, Biasoli ER, Oliveira SH. Stress hormones increase cell proliferation and regulates interleukin-6 secretion in human oral squamous cell carcinomas. Brain Behv Immun. 2011; 25:574–583.

60. Aguirre-Ghiso JA. Models, mechanisms and clinical evidence for cancer dormancy. Nat Rev Cancer. 2007; 7:834–846. [PubMed: 17957189]

61. Barkan D, El Touny LH, Michalowski AM, Smith JA, Chu I, Davis AS, Webster JD, Hoover S, Simpson RM, Gauldie J, Green JE. Metastatic growth from dormant cells induced by a col-I-enriched fibrotic environment. Cancer Res. 2010; 70:5706–5716. [PubMed: 20570886]

62. Wendt MK, Taylor MA, Schiemann BJ, Schiemann WP. Down-regulation of epithelial cadherin is required to initiate metastatic outgrowth of breast cancer. Mol Biol Cell. 2011; 22:2423–2435. [PubMed: 21613543]

63. Matsusue R, Kubo H, Hisamori S, Okoshi K, Takagi H, Hida K, Nakano K, Itami A, Kawada K, Nagayama S, Sakai Y. Hepatic stellate cells promote liver metastasis of colon cancer cells by the action of SDF-1/CXCR4 axis. Ann Surg Oncol. 2009; 16:2645–2653. [PubMed: 19588204]

64. Paschos KA, Majeed AW, Bird NC. Role of Kupffer cells in the outgrowth of colorectal cancer liver metastases. Hepatol Res. 2010; 40:83–94. [PubMed: 19788686]

65. Gao D, Nolan DJ, Mellick AS, Bambino K, McDonnell K, Mittal V. Endothelial progenitor cells control the angiogenic switch in mouse lung metastasis. Science. 2008; 319:195–198. [PubMed: 18187653]

66. Hochst B, Schildberg FA, Bottcher J, Metzger C, Huss S, Turler A, Overhaus M, Knoblich A, Schneider B, Pantelis D, Kurts C, Kalff JC, Knolle P, Diehl L. Liver sinusoidal endothelial cells contribute to CD8 T cell tolerance toward circulating carcinoembryonic antigen in mice. Hepatology. 2012; 56:1924–1933. [PubMed: 22610745]

67. Sceneay J, Smyth MJ, Moller A. The pre-metastatic niche: finding common ground. Cancer Metastasis Rev. 2013; 32:449–464. [PubMed: 23636348]

68. Erler JT, Weaver VM. Three-dimensional context regulation of metastasis. Clin Exp Metastasis. 2009; 26:35–49. [PubMed: 18814043]

69. Ideker T, Winslow LR, Lauffenburger AD. Bioengineering and systems biology. Ann Biomed Eng. 2006; 34:257–264. [PubMed: 16474915]

70. Joughin BA, Naegle KM, Huang PH, Yaffe MB, Lauffenburger DA, White FM. An integrated comparative phosphoproteomic and bioinformatic approach reveals a novel class of MPM-2

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motifs upregulated in EGFRvIII-expressing glioblastoma cells. Mol Biosyst. 2009; 5:59–67. [PubMed: 19081932]

71. Dash A, Inman W, Hoffmaster K, Sevidal S, Kelly J, Obach RS, Griffith LG, Tannenbaum SR. Liver tissue engineering in the evaluation of drug safety. Expert Opin Drug Metab Toxicol. 2009; 5:1159–1174. [PubMed: 19637986]
72. Siolas D, Hannon GJ. Patient-derived tumor xenografts: transforming clinical samples into mouse models. Cancer Res. 2013; 73:5315–5319. [PubMed: 23733750]
73. Hirschhaeuser F, Sattler UG, Mueller-Klieser W. Lactate: a metabolic key player in cancer. Cancer Res. 2011; 71:6921–6925. [PubMed: 22084445]
74. Miller MA, Barkal L, Jeng K, Herrlich A, Moss M, Griffith LG, Lauffenburger DA. Proteolytic Activity Matrix Analysis (PrAMA) for simultaneous determination of multiple protease activities. Integr Biol (Camb). 2011; 3:422–438. [PubMed: 21180771]

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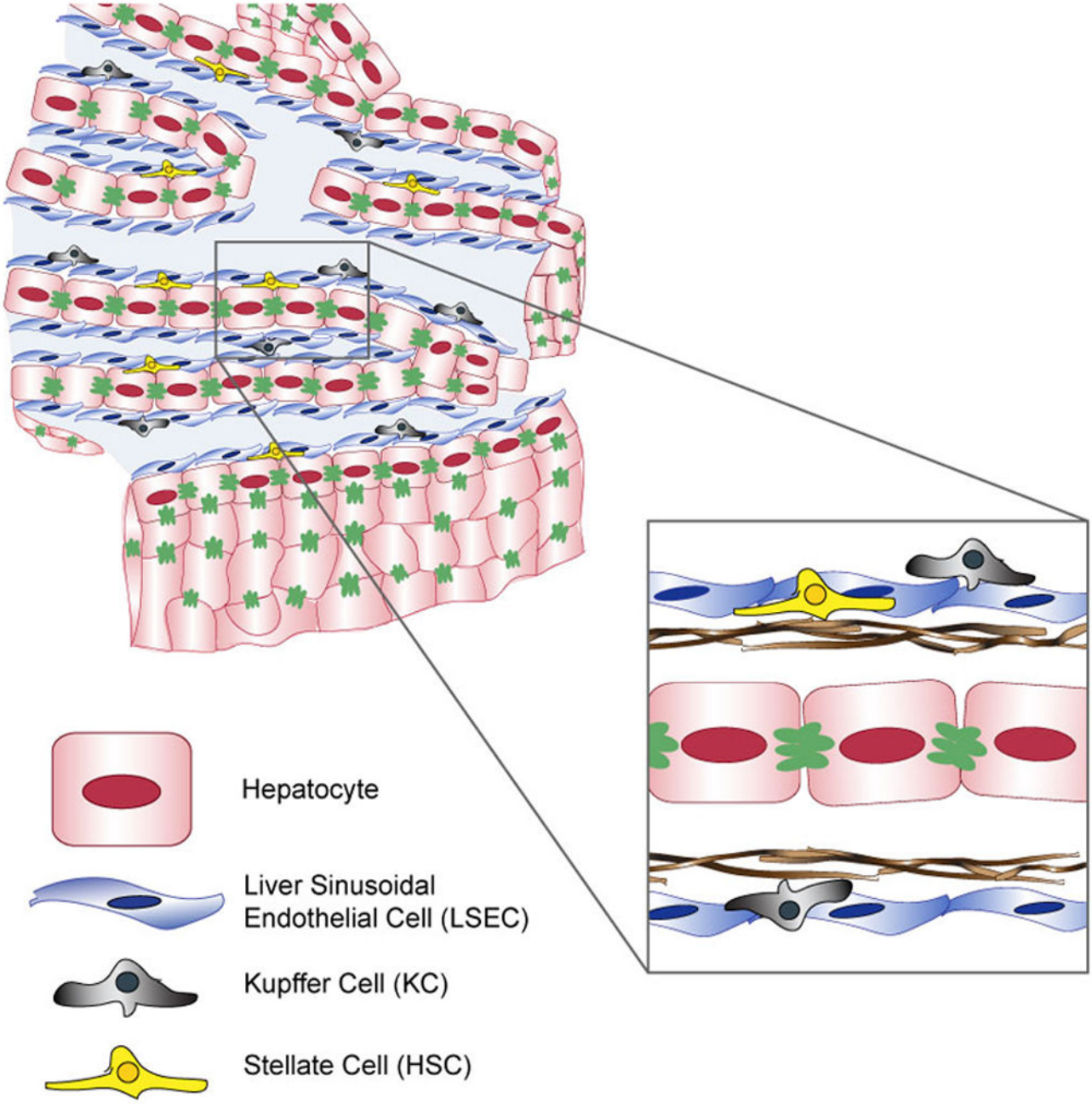

Figure 1. Cellular composition of the liver

Highly specialized parenchymal and non-parenchymal cell types populate the liver. The hepatocytes form the parenchymal portion, while the non-parenchymal portion comprises multiple cellular types, predominantly liver sinusoidal endothelial, Kupffer and stellate cells.

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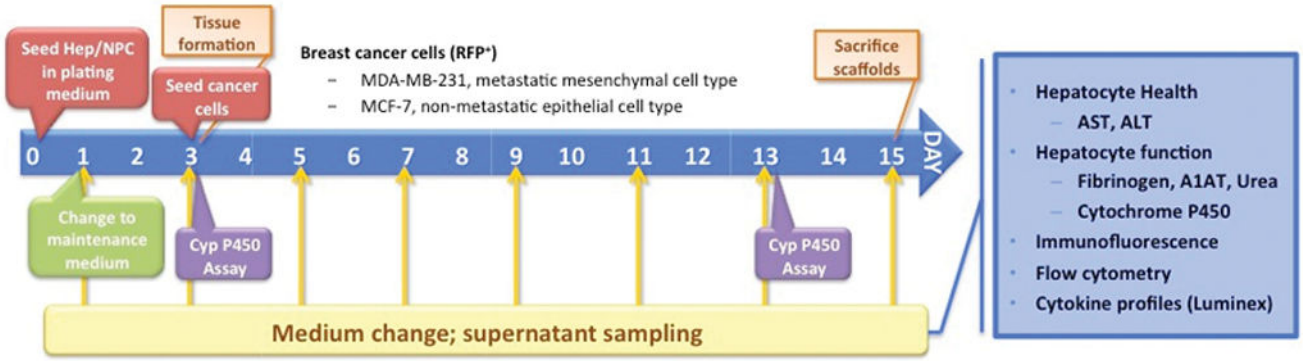

Figure 2. LiverChip experimental schematic to probe for function and tumor outgrowth

Day 0, the LiverChip system (Zyoxel, Ltd.) is seeded with hepatocytes and non-parenchymal cells. Day 1, the medium is changed to serum free maintenance medium. Day 3, the hepatic tissue has formed and is seeded with RFP^+ breast cancer cells (MCF-7 or MDA-MB-231). Cytochrome P450 activity is assessed on day 3 and 13. Medium was changed every 2 days and effluent samples taken routinely. To monitor the influence of circadian rhythms, sampling frequency is increased to at least 7 times per day. The hepatic niche is maintained for a minimum of 15 days. Assays to assess hepatocyte health and function, cytokine profiles as well as tissue morphology and cellular phenotype are performed on the tissue and effluent samples.

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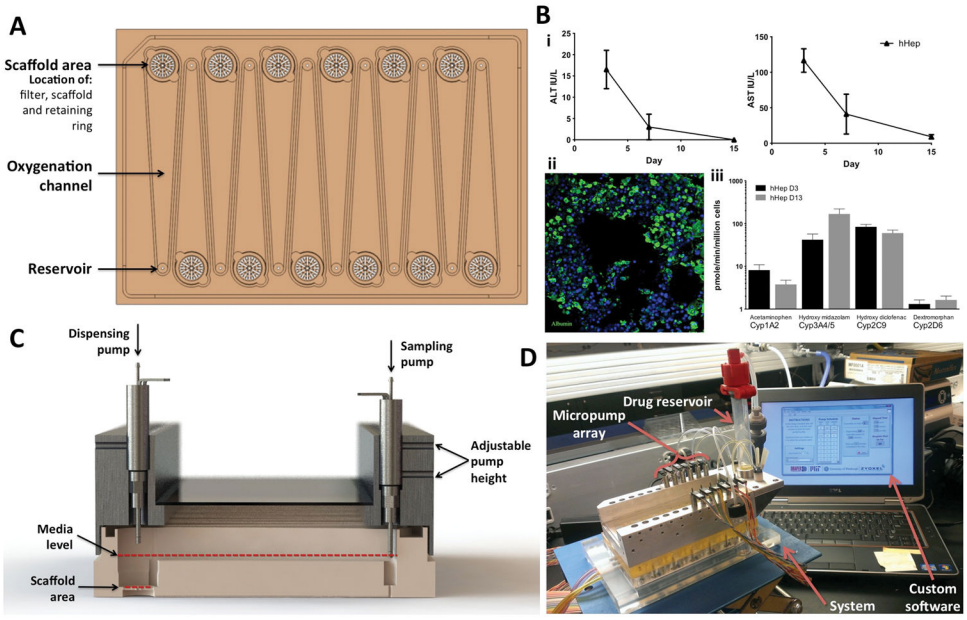

Figure 3. The LiverChip microphysiological system

A) Aerial view of the LiverChip plate. Cells are seeded into the scaffold area, fluid flows up through this region across the oxygenation channel and is then recirculated via the reservoir. B) Functional and healthy hepatic tissue is formed and maintained for 15 days in the LiverChip. i – Hepatocyte injury markers, AST and ALT, continue to decrease as tissue formation matured in the LiverChip system (n = 2 donors). Following isolation and seeding into the system, the levels are high but as the cells establish tissue they drop to near undetectable levels. ii, iii – Hepatocytes still produce albumin (day 15) and maintain cytochrome P450 activity (day 3 and 13) over the culture period (n= 2 donors). C) An array of microdispensers can be mounted over the LiverChip system providing for precise delivery of drug, hormone and nutrient boluses as small as 100 nL. D) When used in conjunction with custom-made software and electronic controllers, programmable diurnal control of drug concentrations within the bioreactor can be achieved.

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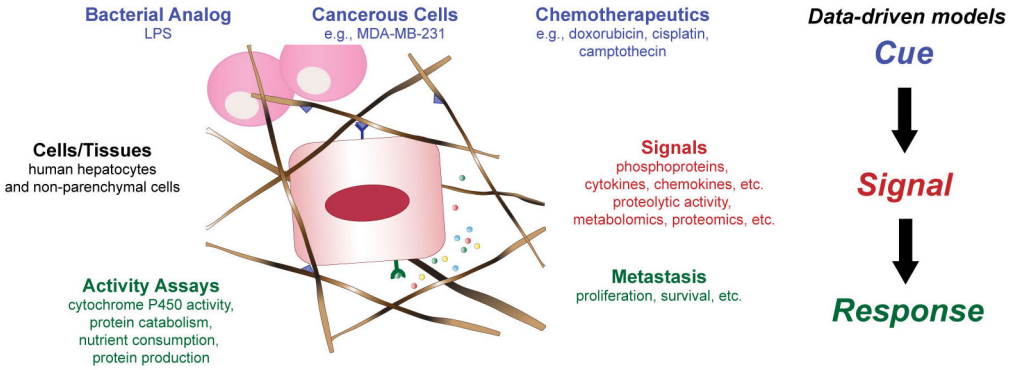

Figure 4. General overview of the systematic approach for analyzing communication networks in the early micrometastatic microenvironment

The cues, signals and responses are color coded. Adapted from (74).