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Novel Holistic Approaches for Overcoming Therapy Resistance in Pancreatic and Colon Cancers

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Abstract
Gastrointestinal (GI) cancers, such as of the colon and pancreas, are highly resistant to both standard and targeted therapeutics. Therapy-resistant and heterogeneous GI cancers harbor highly complex signaling networks (the resistome) that resist apoptotic programming. Commonly used gemcitabine or platinum-based regimens fail to induce meaningful (i.e. disease-reversing) perturbations in the resistome, resulting in high rates of treatment failure. The GI cancer resistance networks are, in part, due to interactions between parallel signaling and aberrantly expressed microRNAs (miRNAs) that collectively promote the development and survival of drug-resistant cancer stem cells with epithelial-to-mesenchymal transition (EMT) characteristics. The lack of understanding of the resistance networks associated with this subpopulation of cells as well as reductionist, single protein-/pathway-targeted approaches have made 'effective drug design' a difficult task. We propose that the successful design of novel therapeutic regimens to target drug-resistant GI tumors is only possible if network-based drug avenues and agents, in particular 'natural agents' with no known toxicity, are correctly identified. Natural agents (dietary agents or their synthetic derivatives) can individually alter miRNA profiles, suppress EMT pathways and eliminate cancer stem-like cells that derive from pancreatic cancer and colon cancer, by partially targeting multiple yet meaningful networks within the GI cancer resistome. However, the efficacy of these agents as combinations (e.g. consumed in the diet) against this resistome has never been studied. This short review article provides an overview of the different challenges involved in the understanding of the GI resistome, and how novel computational biology can help in the design of effective therapies to overcome resistance.

Introduction

Gastrointestinal (GI) cancers, like pancreatic cancer (PC) and colon cancer (CC), account for approximately 30% of the total cancer patient population in the USA [1, 2]. Gemcitabine, the standard drug for PC, has not improved the dismal survival rate (it increases median sur-

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vival by only a few weeks) [3], and 5-fluorouracil with oxaliplatin for CC has shown limited clinical utility [4], especially due to a high rate of tumor recurrence. Alternative platinum-based regimens incorporating oxaliplatin have demonstrated only marginal benefits for PC and CC patients [5]. Emerging evidence suggests that the poor response to the current treatment modalities for GI cancer is linked to aberrations in multiple signaling pathways together with the presence of a small subpopulation of drug-resistant cancer stem cells/cancer stem-like cells (CSCs/CSLCs) that have the propensity to promote tumor recurrence, invasion and metastasis [6]. Although genotoxic chemotherapies target the majority of tumor cells, the CSCs/CSLCs in the tumor mass are nonresponsive, resulting in tumor recurrence [7]. While the successful isolation and characterization of CSCs/CSLCs has been achieved, the molecular networks supporting their resistant behavior are poorly understood. It has therefore been suggested that the identification of the resistant signatures (termed resistomes) associated with CSCs/CSLCs using next-generation computational technologies could have a significant impact on the successful design of any effective therapies against drug-resistant GI cancers [8].

GI Cancers Harbor Inherently Robust Networks

In general, aggressive and therapy-resistant cancers sustain complex signaling networks that are inherently robust and resistant to changes such as those caused by single pathway-targeted agents [9–17]. Numerous multimodel molecular analyses have shown that the GI resistome is multifactorial, harboring an intricate cross-talk between parallel signaling with cues coming from both genetic and epigenetic [including microRNAs (miRNAs)] sources [18, 19]. These multiple cues within the tumor microenvironment affect CSCs/CSLCs, which exhibit molecular signatures distinct from those of bulk tumor cells [20]. The resultant heterogeneity demarcates GI tumors into 'niche within niche' subcompartments that are self-sustaining. This self-sufficiency of CSC/CSLC niches within the tumor is one of the primary reasons for their capacity for self-renewal and their propensity to form secondary tumors at distant sites, giving rise to recurring tumors and distant metastasis [21]. Therefore, study approaches that examine the molecular compartmentalization of network niches within tumors hold great significance for the overall understanding of the GI cancer resistome.

Status quo of GI Cancer Drug Discovery

The process of drug discovery has essentially remained the same for the last 2 decades [22]. The process starts with the discovery of a therapeutic biomarker from appropriate disease datasets. This is followed by the identification of a selective agent that targets the biomarker using the high-throughput screening of chemical libraries. Once a lead hit compound is identified, it is modified to enhance its potency, pharmacokinetics and bioavailability parameters as well as to reduce any associated toxicity. These lead agent(s) are then evaluated for their potency (anticancer activity) using different laboratory assays in appropriate cellular and animal models. If their efficacy and potency are established in a fair number of in vitro and ex vivo (usually animal) models, the lead compounds (now termed preclinical drugs) undergo a very rigorous toxicity and pharmacokinetic profiling evaluation in higher animals such as rats, monkeys, rabbits and dogs. If the preclinical agent passes these rigorous toxicity checkpoints, it is successful in being approved as an investigational new drug. An approved investigational new drug paves the way for phase I dose-escalation studies in humans. These early-phase clinical studies verify the tolerability and activity of new drugs, and lead to phase II and III clinical development prior to final market approval. Over the years, the cost of the above-listed steps has been steadily increasing. Furthermore, each of these steps in the drug discovery procedure acts as a screen that filters agents from >1,000 candidate compounds down to 2–3 clinically approved drugs. The burgeoning cost of bringing 1 drug from the laboratory bench to the clinic for administration to patients is roughly USD 1 billion [23]. While it is recognized that a huge amount of money is spent on promotion, this cost also includes the different preclinical and clinical phases of drug development. The entire discovery process is usually projected to take approximately 2–10 years. Despite this rigorous and highly expensive process, in many instances (>90%), the drugs fail to have a meaningful impact on patients, resulting in attrition [24]. Aside from a few 'magic bullet' drugs, most of the single pathway-targeted therapies have not lived up to expectations.

It is unfortunate to note that, despite considerable investment in targeted drug discovery over the last 2 decades, multitargeted chemotherapeutic agents remain the most effective treatment modalities against cancer, with some very small exceptions. Targeted drugs fail to induce a meaningful impact on robust cancer networks, suggesting that newer alternative strategies are needed [25].

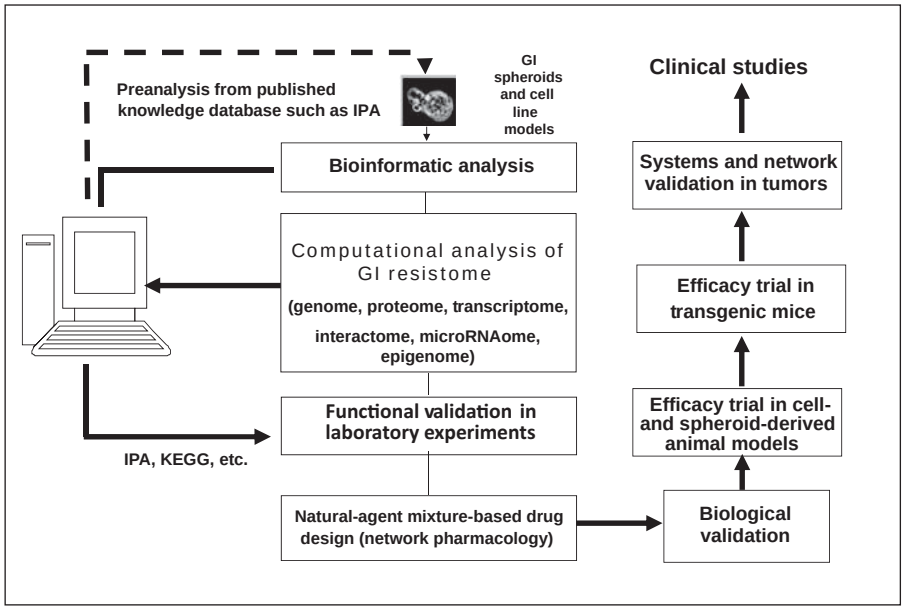

Fig. 1. The role of the GI resistome in reducing the efficacy of chemotherapy in PC and CC can be assessed through a holistic computational analysis, which considers the entire set of interacting pathways (genetic and epigenetic). It is hypothesized that once the underlying interacting pathways supporting the resistome are identified, employing a combination of natural agents (i.e. a network pharmacology-type strategy) will effectively target and reverse the resistance hubs (that support the resistome), and lead to the elimination of the resistant fraction of tumor cells (particularly GI CSCs/CSLCs and associated miRNAs). IPA = Ingenuity Pathway Analysis; KEGG = Kyoto Encyclopedia of Genes and Genomes.

Here, we discuss some of the novel approaches that are being evaluated in order to perturb the GI resistome in a meaningful way, with the hope of better treatment outcome.

Why Are Network Pharmacology-Based Approaches Needed against the GI Resistome?

Network pharmacology is defined as the approach involving pathway/network analysis to determine the set of genes/proteins/miRNAs that are the most crucial for any disease system, which will then facilitate the identification of molecules capable of targeting the identified set of targets [26]. This serves as an ideal approach to tackle the complexity associated with the GI resistome, which requires analyses at the network level that are holistic and pathway-centric. Reductionist therapies designed against one pathway or a set of pathways that are restricted to the bulk of the tumor network, or CSCs/CSLCs per se, may not have the desired impact. Recently, we showed that network modeling can be used to develop new combination therapies for an efficacious outcome in PC [27]. However, much needs to be learned about GI cancers, by utilizing these multiple approaches, which, it is anticipated, will shed light on the underlying mechanisms of therapy resistance (fig. 1; table 1). Such studies would likely help in cataloging promiscuous agents (such as natural products) that could induce desired polypharmacological perturbations in the resistome to overcome resistance, and could thus constitute new therapeutic strategies for GI cancers.

Network Pharmacology in Nature

For a long time, natural products (particularly those found in our diets, dietary derivatives and their chemical analogs) have been evaluated for their polypharmacological health benefits. It is interesting to note that >50% of antibiotics and cancer drugs come from natural products or are generated from lead compounds originally identified in nature [28]. These drugs have been identified primarily due to the extensive epidemiological evidence demonstrating that diet or dietary behavior can influence the vulnerability to acquire disease or to overcome it. There is convincing published knowledge from our own and other laboratories showing that natural products can impact most of the hallmarks of cancer [29]. Nevertheless, a major hurdle that keeps natural products at the margin of mainstream clinical application is the absence of a concrete/defined mechanism of action. Furthermore, most of the single pathway/reductionist laboratory investigations on natural agents have been performed at physiologically inappropriate high doses that cannot be applied in the clinic due to the associated issue of poor bioavailability. This poses two important questions. (1) Are the pleiotropic mechanisms of action of natural products

Table 1. List of computational and network studies that have been used to understand and overcome cancer drug resistance

Study title	Computational methodology used	Reference
Proteomic analysis of gemcitabine-induced drug resistance in PC cells	2D-DIGE and MALDI-TOF mass spectrometry were performed to compare the proteomic alterations of a panel of differential gemcitabine-resistant PANC-1 cells with gemcitabine-sensitive pancreatic cells	49
Deciphering molecular determinants of chemotherapy in GI malignancy using systems biology approaches	Review on integrating high-throughput techniques and computational modeling to explore biological systems at different levels, from gene expressions to networks; systems biology approaches have been successfully applied in various fields of cancer research	50
Personalized-medicine approaches for CC driven by genomics and systems biology: OncoTrack	Attempt to comprehensively map the CC molecular landscape in tandem with crucial, clinical, functional annotation for systems biology analysis, thereby providing predictive power for CC management	51
Gene signatures of drug resistance predict patient survival in colorectal cancer	Resistant and sensitive CC patient stratification based on gene signatures	52
Pathway-gene identification for PC survival via doubly regularized Cox regression	Application of a doubly regularized Cox regression model to identify both the genes and the signaling pathways related to PC survival	53
Computational modeling of PC reveals the kinetics of metastasis, suggesting optimum treatment strategies	Application of the mathematical framework of metastasis in comprehensive data on 228 PC patients	54
Predictive modeling of the in vivo response to gemcitabine in PC	Application of a mathematical model of tumor growth based on a dimensionless formulation describing tumor biology	55
Chemoprevention, chemotherapy and chemoresistance in colorectal cancer	Transcriptomic signature of chemoresistance in CC	56
Strategies for overcoming chemotherapy resistance in enterohepatic tumors	Comprehensive review of the various genomic strategies to overcome chemoresistance in cancer	57

good enough to impact diseases as complex as cancer and, thus, worthy of being included in mainstream therapeutic strategies? (2) Are the appropriate preclinical models and proper technologies being applied to investigate these agents in a more comprehensive manner?

As proposed recently, answering these questions requires a major paradigm shift in our analysis tools, from reductionism (single proteins/pathways) to holism (protein-protein interaction and studies on multiple interacting pathways) [30]. Most interestingly, it has been proposed that the limitations of low bioavailability can be overcome by enhancing the polypharmacology targets of recognized natural products by combining these with other natural agents [31, 32]. Despite initial efforts, this field urgently requires an interdisciplinary analysis which studies natural-product-induced changes in the genome, epigenome, proteome, kinome, miRNAome and transcriptome of complex and therapy-resistant disease models such as GI cancer. Cutting-edge genomic, epigenomic, proteomic, systems-biology, network-modeling and molecular-biology methods are needed to understand the transcriptional and translational regulatory mechanism(s) of action. The development of superior formulations and combinations of lead natural products or their derivatives for clinical application, either to inhibit or to treat aggressive GI cancers, is also needed. These can be accomplished through comprehensive cross-disciplinary interactions between different research fields including molecular and computations biology. Such an exercise would merge molecular biology to next-generation systems and network-level interrogations of the large-scale omic data obtained from natural-agent-exposed normal and cancer cell lines, their corresponding xenograft animal tumor models and neutraceutical clinical trial specimens.

Identifying Promiscuous Agents from Nature's Bounty Using Network Pharmacology for Therapy-Resistant GI Cancers

Evaluation of the published literature in the last few years has confirmed the growing interest in the use of network pharmacology strategies, specifically in the area of natural product research. A PubMed search using the key words 'natural product' and 'network pharmacology' showed >1,500 hits. These published studies demonstrated that the multitargeted/pleiotropic activity of different natural products and their chemically synthesized analogs is somewhat similar to that of chemotherapeutic drugs (i.e. impacting multiple meaningful nodes within cancer-associated networks, and leading to the reprogramming of the tumor prosurvival pathways towards prodeath signaling pathways).

One cannot doubt that the interaction of natural products with some of the major hallmarks of cancers is weak. Such interactions may or may not be sufficient to significantly alter the associated network and induce any purposeful phenotypic end point. Furthermore, the poor bioavailability associated with these agents has also been linked to their low benefits as anticancer agents. However, to get around these problems, it has been proposed that the network-targeting capabilities of many such natural products can be enhanced by using them in combination, i.e. in ways in which they occur naturally in dietary items. Supporting this idea, 1 study [33] showed that traditional Chinese herbal formulations (containing many different bioactive constituents) can induce activity against disease networks which is superior to that of single agents. These combinations can also help in resensitizing resistant tumors to standard chemotherapeutics.

Drug Repurposing Principles of Network Pharmacology for GI Cancer

Drug repurposing is one of the major areas where network pharmacology has impacted in a positive way [34]. With the failure rates of the drugs coming through the pipeline being high, there has been a shift in the drug discovery strategy, whereby researchers are now looking for new applications for existing drugs or are reevaluating existing applications of previously shelved drugs for different indications. This mirrors the sudden surge in the use of 'old' drugs for disease indications which are totally unconnected. Nelfinavir is a prominent example initially used as an anti-HIV agent, but then revealed to possess a weak kinase inhibitory activity, which led to its evaluation for the treatment of a number of tumor indications [35]. Similarly, the antidiabetic agent metformin is now being intensively evaluated for its growth-inhibitory potential in various tumor models [36, 37]. Our laboratory previously demonstrated that metformin could suppress PC proliferation and also inhibit the survival of highly resistant fractions of CSCs/CSLCs screened from PC cells [38]. Based on preliminary work from our laboratory and others [39, 40], metformin has been entered into multiple clinical trials (e.g. NCT01579812). A search of the key words on the clinicaltrials.gov website for 'metformin' and 'cancer' showed >150 trials. A similar search using the key words 'nelfinavir' and 'cancer' returned 32 clinical studies. These are not the only examples, and a number of different agents are available that have been investigated for therapeutic benefits against diseases other than the intended targets. Despite the acceptance of the pleiotropic, multitargeted activity of synthetic drugs by the drug industry, the same principles are not being applied to investigate the benefits of the multitude of activities exhibited by various plant-derived anticancer agents present in nature. It is anticipated that the repurposing and network pharmacology principles, if applied to natural dietary or related agents, will reveal the hidden potential of some of the agents that have not yet entered clinical evaluation.

Overcoming GI Stem-Cell Resistance Biology Using Promiscuous Natural Agents

As mentioned above, the presence of highly resistant CSCs/CSLCs has been attributed to the therapy resistance observed in GI cancers [41, 42]. Standard or targeted therapeutics can eliminate the bulk of the tumor cells; however, the CSCs/CSLCs are not responsive to any form of treatment, and they thus give rise to secondary tumors and metastasis. Therefore, targeting these resistant CSCs/CSLCs should be a high priority for any therapeutic regimen to be successful against aggressive and resistant cancers. As the GI CSC/CSLC resistome is sustained by a highly complex and intertwined network of multiple prosurvival signaling, drugs with very narrow mechanisms of action are bound to fail. In order to have a meaningful impact (i.e. perturbations in the GI CSC/CSLC resistome), pleiotropic agents are needed. Working in this direction, our laboratory was among the first to show the activity of natural products, in particular, the curcumin derivative difluorocurcumin, in PC [43, 44] and CC stem cells [45].

As predicted, the curcumin derivative difluorocurcumin induces the regression of GI CSCs/CSLCs, resulting in the inhibition of multitude cancer hallmarks and therefore, in essence, induces network pharmacology effects.

Targeting GI miRNA Networks Using Natural Products

miRNAs are a class of noncoding RNAs that mediate the posttranscriptional regulation of protein-coding genes through binding at the 3′ untranslated region of target mRNAs, and they cause translational inhibition, mRNA destabilization and degradation [46]. One miRNA can downregulate hundreds of target mRNAs. While there is context dependency for specificity, such regulation can cause a very weak gene expression in diverse cellular functions, such as cellular development, differentiation, proliferation, apoptosis and metabolism. There is ample evidence to demonstrate that miRNA deregulation results in the emergence and sustenance of GI CSCs/CSLCs and their associated resistome [47]. It has been observed that the differential expression of miRNA genes in bulk tumor cells to that of CSCs could, in part, be explained by the localization of these genes in CSC/CSLC-specific genomic regions, the microenvironment and epigenetic mechanisms, and may also cause deregulations of the miRNA processing machinery. miRNA expression appears to serve as a hub of the regulatory networks underlying complex diseases. Along these lines, we previously showed that isolated CD44(+)/CD133(+)/EpCAM(+) cells (triple-marker-positive cells) from the human PC cell lines, MiaPaCa-2 and L3.6pl, display aggressive characteristics such as increased cell growth, clonogenicity, cell migration and a capacity for self-renewal; this is consistent with overexpression of CSC-CSLC signatures/markers [48]. We also found deregulated expression of >400 miRNAs, including let-7, miR-30, miR-125b and miR-335, in CSCs/CSLCs. As a proof-of-concept, knockdown of miR-125b resulted in the inhibition of tumor cell aggressiveness of CSCs/CSLCs (triple-marker-positive cells), consistent with the downregulation of CD44, EpCAM, EZH2 and Snail. These results clearly suggest the importance of miRNAs in the regulation of CSC-CSLC characteristics, and their potential to serve as novel targets for therapy. These and other findings suggest that systems- and network pharmacology-based approaches would be useful for the preclinical evaluation of novel miRNA-targeted agents in order to design personalized therapies to overcome the GI CSC/CSLC resistome.

Conclusion

The field of GI cancer-drug discovery has remained somewhat stagnant for over 50 years. This is confirmed by the fact that there are very few effective drugs that make any meaningful impact on GI cancer. It also points to the need for drastic changes in the way that drugs are being discovered for cancers in general. On the one hand, a number of FDA approvals have been granted to plant-derived cytotoxic combinations, such as the Taxol derivative, nab-paclitaxel. Taxol or related compounds work by inhibiting multiple cancer hallmarks, some of which are known and others yet to be identified. Despite their incremental benefits, these compounds have gained clinical acceptance. One thing that can be learned from these FDA approvals is that, in order to have a meaningful impact on cancers that are supported by complex signaling networks, one needs multitargeted agents. This is especially true for GI cancers that are seeded by very complex interaction networks not impacted by very targeted-type approaches. The minimal benefits observed for drugs like nab-paclitaxel proves that agents that hit multiple tumor-associated networks may have a better chance than single protein- or pathway-inhibiting agents. The drug discovery arena has been chasing targeted therapy with the focus on increasingly high binding affinity and improving specificity towards the binding pocket in the biomarker. However, as revealed by such high attrition rates in the discovery of precision medicine, these approaches need a complete revamp. Sadly, barring very few success stories, most of these single pathway-targeted drugs have been shelved as they did not show the efficacy expected or else were too toxic for clinical use, resulting in the recent spike in the drug attrition rates. The cost of bringing one drug to the market is estimated at around USD 1 billion. This staggering amount covers both preclinical studies as well as advanced, multiphase clinical testing. In spite of the highly rigorous and expensive preclinical research that supports their potential, these drugs rarely pass the ultimate test at in the clinic. This has been a routine observation during the last 10 years or so, whereby thousands of candidate molecules in the laboratory have been reduced to just a couple for clinical use. Such a high failure rate indicates that something is amiss in the drug discovery field, and that a complete overhaul is required in the approach to designing new molecules. The reasons for the high failure rate center around certain recurring themes, including: (1) the disease arises from the aberration of one protein or pathway and the assumption is that a drug interacts only with the said target of interest, (2) drug dis-

covery is restricted to the Lipinski's rule of 5, in spite of the knowledge that the promiscuous behavior of chemotherapeutic agents or weakly pleiotropic natural agents can induce a meaningful impact on cancer-associated networks. Network pharmacology is one such advancement that holds promise for revolutionizing our approach towards next-generation cancer drug development. Deeper evaluation of network dynamics has the potential to predict novel anticancer drug targets. The incorporation of personalized information, such as mutation signatures or metabolomic profiles, into the molecular networks, is expected to enhance the disease stage-specific drug targeting in times to come. The current increase in network methodologies may lead to the discovery of the truly novel targets of the cellular community, which are the hidden masterminds that drive GI cancer initiation, progression and therapy resistance. It is recognized that such holistic approaches are still restricted to cell-cell interaction or the microenvironment, and we do not have the tools to look into higher-order tissue-tissue or organ-organ interactions. Nevertheless, the proponents of this technology are producing more compelling evidence that will allow the promotion of the technologies that are emerging at the forefront of conventional drug discovery. These strategies may help revive some hastily discontinued drugs and could also cut the cost of bringing new drugs through the developmental pipeline. It is predicted that, within a decade, newer computational models will have evolved, that will allow for quicker and cheaper study of heterogeneous GI tumors. This, in turn, will create an attractive environment for molecular biologists and pharmaceutical researchers alike, who will actively use these tools for the drug discovery process. In conclusion, computational methodologies, particularly network pharmacology, certainly have the potential to revamp the way drug discovery is being performed. If used correctly, it is predicted that they will result in the development of clinical beneficial drug combinations for therapy-resistant GI cancers.

Disclosure Statement

The authors have no disclosures.

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