REVIEW

Long-noncoding RNAs (lncRNAs) in drug metabolism and disposition, implications in cancer chemo-resistance

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Abstract Drug metabolism is an orchestrated process in which drugs are metabolized and disposed through a series of specialized enzymes and transporters. Alterations in the expression and/or activity of these enzymes and transporters can affect the bioavailability (pharmacokinetics, or PK) and therapeutic efficacy (pharmacodynamics, or PD) of drugs. Recent studies have suggested that the long non-coding RNAs (lncRNAs) are highly relevant to drug metabolism and drug resistance, including chemo-resistance in cancers, through the regulation of drug metabolism and disposition related genes. This review summarizes the regulation of enzymes, transporters, or regulatory proteins involved in drug metabolism by lncRNAs, with a particular emphasis on drug metabolism and chemo-resistance in cancer patients. The perspective strategies to integrate multi-dimensional pharmacogenomics data for future in-depth analysis of drug metabolism related lncRNAs are also proposed. Understanding the role of lncRNAs in drug metabolism will not only facilitate the identification of novel regulatory mechanisms, but also enable the discovery of lncRNA-based biomarkers and drug targets to personalize and improve the therapeutic outcome of patients, including cancer patients.

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1. Introduction

Chemotherapy is one of the common treatment options for cancer patients. It is conceivable that the efficacy of a chemotherapy regimen relies on the maintenance of a drug at the effective dose and duration. Upon their administration, the chemotherapeutic agents need to go through the intracellular drug metabolism and disposition within the cancer cells and the liver, or other metabolic organs and tissues. Since most of the chemotherapeutic agents have a steep toxicity curve and a narrow therapeutic window, the intracellular drug retention is critical to ensure the therapeutic efficacy of these anti-neoplastic drugs. Decreased intracellular drug retention, usually caused by the dysregulation of enzymes and transporters responsible for the metabolism and disposition (uptake and efflux) of the anti-tumor agents, is one of the primary factors that limit effective cancer therapy.

Drug metabolizing enzymes (DMEs) and transporters are crucial in the metabolism, elimination and detoxification of xenobiotics, including the clinical drugs. There are three major phases of drug metabolism and disposition: the phase I and phase II drug metabolism and the phase III drug disposition. Different tissues and organs in the human body are well-equipped with a variety of DMEs and transporters. The phase I enzymes are mostly cytochrome P450 (CYPs) that either activate or inactivate the drugs. The enzymes that fall into the category of phase II enzymes include uridine diphosphate glucuronosyltransferases (UGTs), sulfotransferases (SULTs), and glutathione S-transferases (GSTs). The key role of the phase II enzymes is to detoxify xenobiotics or mediate their bioactivation hence potentially toxic metabolites through a process called conjugation. In the phase III drug disposition, the parent drugs or their metabolites are eliminated and excreted by transporters.

Recent studies have demonstrated that long noncoding RNAs (lncRNAs) are highly relevant to drug metabolism pathways and multidrug resistance in various types of cancers. LncRNAs represent a major type of noncoding RNAs. They are RNA transcripts larger than 200 nucleotides, but do not have protein-coding potentials. LncRNAs exert their biological functions as regulatory RNAs, serving as signals, guides, decoys, or scaffolds to regulate the expression of a wide range of target genes. Accumulating evidences suggest that dysregulation of lncRNAs is strongly associated with the development of chemo-resistance of various cancers. Interestingly, it has been shown that the dysregulation of chemo-resistance-related lncRNAs can be effectively detected in body fluids of cancer patients, suggesting that lncRNAs can be used as diagnostic and prognostic biomarkers in patients. It is conceivable that functional studies of lncRNAs that are involved in drug metabolism and disposition will help to better understand and/or manage chemo-resistance in the clinic.

2. Regulation of drug metabolism and disposition by lncRNAs

2.1. Three phases of drug metabolism

The phase I metabolism reaction may involve either of the following sequential, yet competitive chemical processes: oxidation, reduction, and hydrolysis. The human bodies are constantly exposed to a variety of chemicals, including agrochemicals, environmental pollutants and pharmaceutical products. These pharmacologically active substances are metabolized in the body, usually through the phase I metabolism catalyzed by the cytochrome P450 enzymes. In addition to the pharmacologically active substances (drugs), the pharmacologically inactive substances (prodrugs), after administration, are metabolized into active drugs by phase I enzymes, especially by the P450 enzymes. The P450 enzymes alter the pharmacologic activities of many drugs and prodrugs and they also play an important role in their eliminations.

The phase II metabolic reactions can be considered chiefly as the detoxifying steps with some exceptions. The enzymes that fall under this category are broadly classified as transferases, including UDP-glucuronosyltransferases, sulfotransferases, N-acetyltransferases, glutathione S-transferases and methyltransferases. Besides playing a crucial role in the inactivation of pharmacologically active compounds, phase II reactions are involved in the biotransformation of endogenous compounds as well as xenobiotics to chemical forms that are more readily eliminated from the body due to increased water solubility upon conjugations. A compromised phase II metabolism may lead to increased toxicity of clinical drugs.

The phase III reactions mainly involve drug transporters that participate in the absorption, distribution, and elimination of drugs. Drug transporters include uptake and efflux transporters. Examples of the efflux transporters include the ATP-binding cassette (ABC) family such as P-glycoprotein (P-gp) and multidrug resistance-associated proteins (MRPs)24. The drug transporters are widely distributed in tissues and cells, determining the absorption and intracellular concentrations of drugs and hence directly affecting their pharmacokinetics. Increased expression and/or activity of efflux drug transporters represent a major mechanism for the development of cancer chemo-resistance. At present, the major members of the ABC transporters linked to multidrug resistance (MDR) in cancer cells include P-gp (ABCB1/MDR1), MRP1 (ABCC1), MRP2 (ABCC2), MRP4 (ABCC4) and BCRP (ABCG2). These transporters can efflux numerous structurally diverse, mainly hydrophobic compounds from cells, but each transporter has its preferred substrates. These transporters are the most widely studied in the context of drug response and toxicity. In some cancers, the dysregulation of these transporters is closely associated with poor overall prognosis and poor response to drug therapies.

2.2. Regulation of phase I enzymes by lncRNAs

Quite a few studies have reported that lncRNAs are involved in the regulation of phase I drug metabolism by affecting the transcription of CYP genes. In one study, a transcriptional regulatory network containing nuclear receptors and lncRNAs that controls both the basal and drug inducible expression of CYPs was identified in the HepaRG cells. HNF1α-AS1 and HNF4α-AS1, genes encoding two of the lncRNAs involved in this regulatory network, are located closely to the gene loci of hepatocyte nuclear factors 1α and 4α (HNF1α and HNF4α), two transcription factors essential for the regulation of CYP enzyme genes. A knockdown of HNF1α-AS1 decreased the mRNA expression of CYPs, the nuclear receptors and HNF4α-AS1, but knockdown of HNF4α-AS1 exhibited opposite regulatory effects on CYP gene expression. A subsequent study confirmed that knockdown or overexpression
of HNF1α-AS1 can significantly alter the expression of pregnane X receptor (PXR), a master regulator of DMEs including the CYPs, as well as the basal and rifampicin inducible mRNA expression of multiple CYPs, including CYP2B6, 2C8, 2C9, 2D6, 3C1 and 3A4. Notably, the regulation of CYP3 by HNF1α-AS1 was independent of its coding gene HNF1α. HNF1α is a well-established nuclear receptor that regulates the transcription of CYPs and can induce the expression of HNF1α-AS1. However, HNF1α-AS1 does not affect the expression of HNF1α. Further overexpression and knockdown experiments suggested that HNF1α can regulate the expression of aryl hydrocarbon receptor (AhR), another xenobiotic receptor that can regulate the expression of selected CYPs; whereas the effect of HNF1α-AS1 was more specific on PXR. The induction of CYP1A2, 2C8 and 2C19 by HNF1α-AS1 knockdown was also different from HNF1α knockdown. These results suggested that HNF1α-AS1 is involved in the regulation of P450s and their regulatory nuclear receptors in the human liver cells through a mechanism that is different from that of HNF1α.

Studies also identified other mechanisms concerning the regulatory role of lncRNAs on phase I metabolism. lnc-HC has been reported to negatively regulate cholesterol metabolism in hepatocytes through its direct interaction with hnrRNP2B1. Mechanistically, the lnc-HC-hnrRNA2B1 complex binds to the mRNA of the mouse Cyp7a1 or Abca1 gene and decreases the protein translation of these two genes. Since CYP7A1 or ABCA1 are involved in the conversion of cholesterol to bile acids and cholesterol efflux, respectively, the down-regulation of CYP7A1 and ABCA1 resulted in reduced cellular cholesterol excretion. In contrast, knockdown of lnc-HC restored the cholesterol homeostasis in mice. Interestingly, the expression of lnc-HC itself can be regulated by high cholesterol exposure through the transcriptional factor CCAAT/enhancer-binding protein beta. In another example, LncLSTR, a liver-enriched lncRNA in mouse termed liver-specific triglyceride regulator, was found to down-regulate the expression of ApoC2 through its direct interaction with hnRNPA2B1. Mechanistically, the lnc-HC-LncSTR and TDP-43 (a RNA and DNA binding protein) can form complexes that directly enhance the transcription of Cyp8b1, another key enzyme to convert cholesterol to bile acids, which engenders a bile pool and affects the ApoC2 expression through the bile acid receptor FXR.

In addition to the CYP enzymes, lncRNAs have also been reported to regulate the expression of other phase I enzymes. Among examples, lncRNA H19 can regulate the methylation of long interspersed nuclear elements-1 (LINE-1) through interacting with 5-adenosylhomocysteine hydrolase (SAHH) in response to benzo[a]pyrene exposure. H19 was also associated with the expression of aldehyde dehydrogenase 1 (ALDH1) in colorectal cancer stem cells and H19 is highly expressed in ALDH1-positive breast cancer patients. Maternally expressed gene 3 (MEG3) is a lncRNA that has been reported to regulate the expression of alcohol dehydrogenases (ADHs). While MEG3 is generally down-regulated in hepatocellular carcinoma, overexpression of MEG3 can increase alcohol dehydrogenase 4 (ADH4) expression through competitive sponging of miR-664, which will further inhibit the proliferation of tumor cells, suggesting its role as a tumor suppressor.

2.3. Regulation of phase II enzymes by lncRNAs

Less is known about the regulation of phase II enzymes by lncRNAs. However, there have been reports suggesting that lncRNAs may contribute to the regulation of phase II enzymes. CHST15, a chondroitin sulfotransferase, is among the targets of HOX transcript antisense intergenic RNA (HOTAIR) and is required for HOTAIR-mediated invasiveness in breast cancer cell lines. Being closely correlated with the expression of CHST15 in primary and metastatic tumor lesions, overexpression of HOTAIR is necessary and sufficient for the transcription and downstream function of CHST15. The expression of HNF1α-AS1 and HNF4α-AS1 is also associated with the expression of sulfotransferases (SULTs) and UDP-glucuronosyltransferases (UGTs). In a study aiming to determine the impact of lncRNAs on acetaminophen (APAP)-induced hepatotoxicity, knockdown of these two lncRNAs significantly altered APAP-induced hepatotoxicity pathways, including APAP detoxification by UGTs and sulfation by SULTs, and detoxification through glutathione conjugation by glutathione-S-transferases (GSTs). In the bacteria strain Plutella xylostella (L.), 64 lncRNAs were found to be differentially expressed between chlorantraniliprole-resistant and -sensitive strains. These lncRNAs exhibited a significant co-expression pattern with UGTs and P450 enzymes, suggesting their potential role in regulating UGTs and drug resistance. Another lncRNA that is found to be associated with the expression of phase II enzymes is lncRNA NONMMUG032898.1, which is an intronic lncRNA transcribed from the intron region of its neighboring protein coding gene Ugt2b1. NONMMUG032898.1 and Ugt2b1 were found to be up-regulated in the liver of male mice exposed to BDE-47, an environmental chemical that belongs to the polybrominated diphenyl ethers (PBDEs). These lncRNAs may contribute to the regulation of phase II enzymes.

2.4. Regulation of drug transporters by lncRNAs

Many lncRNAs were identified as potential regulators for the drug transporters, including in the context of cancer chemotherapy. Among examples, lncRNA MALAT1 can regulate the expression of the efflux transporters MRP1 and MDR1 via the activation of the transcriptional factor STAT3. The expression of MALAT1 is elevated in cisplatin-resistant A549 lung cancer cells. MALAT1 overexpression promotes the expression of MRP1 and MDR1, which in turn decreases cisplatin sensitivity both in vitro and in vivo. Niclosamide, a specific STAT3 inhibitor, can abolish the upregulation of MRP1 and MDR1 by MALAT1, suggesting that the regulation is STAT3-dependent. ANRIL is another lncRNA that is implicated in the regulation of MDR1 and MRP1. ANRIL is highly expressed in cisplatin-resistant cells and 5-FU-resistant gastric cancer tissues and cells. Knockdown of ANRIL decreased the cell proliferation and the expression of MDR1 and MRP1. A strong association between the expression of ANRIL and MDR1/ MRP1 was also observed in gastric cancer tissues from patients. Further experiments demonstrated that knockdown of ANRIL reversed the cisplatin- and 5-FU-resistance in gastric cancer cells. MRUL is a lncRNA that up-regulates ABCB1 and induces multidrug resistance. The expression of MRUL is elevated in multidrug-resistant gastric cancer cell lines. Knockdown of MRUL in these cell lines led to increased apoptosis and reduced doxorubicin efflux. Moreover, MRUL depletion reduced ABCB1 mRNA expression in a dose- and time-dependent manner.
suggested that ABCB1 is likely the regulatory target of MRUL to induce multidrug-resistance in gastric cancer. The lncRNA Linc-VLDLR is believed to be associated with the expression of ABCG2 and contributes to cell stress response. The expression of this lncRNA was significantly upregulated in the extracellular vesicles (EVs) derived from malignant human hepatocellular cancer tissues. Exposure of the hepatocytes to anti-neoplastic drugs, such as sorafenib, camptothecin and doxorubicin, increased the expression of Linc-VLDLR both inside the cells and in the EVs. Linc-VLDLR knockdown decreased the cell viability and reduced the expression of ABCG2, suggesting that Linc-VLDLR may be involved in cellular response to the anti-tumor drugs as an EV-enriched lncRNA.

Additionally, there were several studies showing that lncRNAs can regulate the expression of drug transporters through microRNA sponging. For instance, lncRNA XIST was shown to positively regulate SGK1, which is a positive regulator of transporters, through direct sponging the SGK1-targeting microRNA miR-124, leading to doxorubicin resistance in colorectal cancer tissues and cell lines. LncRNA KCNQ1OT1 has been reported to regulate oxaliplatin resistance through the miR-7-5p/ABCC1 pathway. LINC00518 was found to be over-expressed in breast cancer tissues and chemo-resistant breast cancer cell lines. LINC00518 can act as a molecular sponge of the MRPI (ABCC1)-targeting miR-199a, leading to increased MRPI expression and chemoresistance. In contrast, knockdown of LINC00518 enhanced the chemosensitivity to adriamycin, vincristine and paclitaxel. The lncRNA bladder cancer associated transcript-1 (BLACAT1) has also been shown to accelerate oxaliplatin-resistance of gastric cancer through promoting ABCB1 protein expression by sponging miR-361.

Besides the ATP binding cassette family of transporters, lncRNAs are also reported to regulate the function of solute carrier family of transporters. For example, the trace element copper (Cu) is essential for life in numerous biological processes, the major copper importer in humans is the high-affinity copper transporter 1 (CTR1) and 2. In addition to copper, CTR1 has the ability to transport platinum-containing drugs. Recent studies reported that lncRNA nuclear enriched abundant transcript 1 (NEAT1) can regulate the CTR1 and could induce cisplatin sensitivity in non-small cell lung cancer (NSCLC) cells. Upregulation of NEAT1 could competitively bind to hsa-mir-98-5p, which enhanced the green tea polyphenol (EGCG)-induced CTR1 expression and increased the drug accumulation in NSCLC cells. Further study from the same group showed that the expression of CTR1 was decreased, but the expression of NEAT1 was increased in the

| Studies | Perturbation | Phenotype | Data Type | Species | Accession ID |
|---------|--------------|-----------|-----------|---------|--------------|
| Targeting palbociclib-resistant estrogen receptor-positive breast cancer cells via oncolytic virotherapy | Palbociclib | Drug resistance | RNA-seq | Human | GSE130437 |
| Transcriptional changes in the breast cancer cell line MCF7 rendered resistant to the cationic drug siramesine | Siramesine | Drug resistance | RNA-seq | Human | GSE130363 |
| Inhibition of the aryl hydrocarbon receptor/polyamine biosynthesis axis suppresses multiple myeloma and prostate cancer progression | AHR inhibitor | Drug resistance | RNA-seq | Human | GSE117160 |
| Venetoclax with azacitidine disrupts energy metabolism and targets leukemia stem cells in patients with acute myeloid leukemia | Venetoclax/azacitidine | Metabolism disruption | RNA-seq | Human | GSE116567 |
| Patient adipose stem cell-derived adipocytes reveal genetic variation that predicts antidiabetic drug response | Not available | Drug response | RNA-seq/DNA-seq | Human | GSE115421 |
| Transcriptome sequencing (RNA-Seq) of non-tumor kidney tissues from 36 patients undergoing nephrectomy for exploring the metabolic mechanism of sorafenib and identifying the major transcriptional regulation factors in sorafenib metabolism in kidney | Not available | Drug metabolism | RNA-seq | Human | GSE93069 |
| Quantitative profiling of the UGT transcriptome in human drug metabolizing tissues | Not available | Not available | RNA-seq | Human | GSE82292 |
| The PGC-1α/ERRα axis represses one-carbon metabolism and promotes sensitivity to anti-folate therapy in breast cancer | AMPK activation | Drug sensitivity | ChIP-seq | Human | GSE75877 |
| Genome-wide analysis of human constitutive androstanoreceptor (CAR) transcriptome in wild-type and CAR-knockout HepaRG cells | CAR knockout | Not available | RNA-seq | Human | GSE71446 |
| 8p Loss of heterozygosity triggers metastasis and drug resistance | 8p LOH | Metastasis and drug resistance | RNA-seq | Human | GSE68042 |
| Impact of CAR agonist ligand TCPOBOP on transcription factor binding in adult male mouse liver | CAR agonist | Not available | ChIP-seq | Mouse | GSE121915 |
| Dissecting the effect of genetic variation on the hepatic expression of drug disposition genes across the collaborative cross mouse strains | Not available | Not available | RNA-seq | Mouse | GSE77715 |
enriched lung cancer stem cells. Knockdown or overexpression NEAT1 decreased or increased the cancer stem cells (CSC) function in NSCLC/CSCs, which could modulate the chemoresistance of NSCLC53.

3. Combining multi-omics data and machine learning to identify lncRNAs involved in chemoresistance through drug metabolism and disposition

By far, most of the lncRNA studies in drug metabolism have been using “bottom-up” strategies, which generate hypotheses based on documented lncRNA functions and then investigate the role of a particular lncRNA in a specific drug metabolism process. However, few studies were able to identify novel lncRNAs that have a direct relationship with drug metabolism. Since the majority of lncRNAs are poly-A tailed54, their expression information is buried within most of the RNA-seq data, which usually measures the abundance of poly-A tailed RNA molecules55. Many of the existing RNA-seq analyses have focused on the protein-coding mRNAs. In this regard, repurposing the RNA-seq, DNA-seq and pharmacogenomic data, which have already been generated and deposited into public databases, can accelerate the discovery of novel lncRNAs that are master regulators of drug metabolism. We have summarized a list of publicly accessible datasets, including DNA-seq, RNA-seq and ChIP-seq data, that can be used to repurpose the lncRNA profiles for their potential effect on drug metabolism and drug resistance (Table 1). Recently, we integrated the pharmacogenomics data with lncRNA expression data in 5605 tumor samples and 505 cancer cell lines from 27 cancer types4. We constructed lncRNA-based drug response prediction models and identified novel lncRNAs that are master regulators of cancer drug responses. Our analysis identified 27,341 lncRNA–drug interactions for 265 chemotherapy drugs. Moreover, the computational analyses have identified a group of “multi-drug resistant” lncRNAs, whose up-regulation is associated with resistance to more than 100 chemotherapy compounds, suggesting they may play important roles in drug metabolism and disposition. Indeed, our further pathway analysis indicated that the up-regulation of these “multi-drug resistant” lncRNAs is associated with the dys-regulation of xenobiotic nuclear receptor target genes, including drug-metabolizing enzymes and transporters56.

The increasingly adopted Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-associated Protein 9 (CRISPR-Cas9) system can also provide tremendous data resources to study the regulation of drug metabolism and disposition by lncRNAs. CRISPR system has been successfully used in high throughput screening to discover lncRNAs that are associated with cancer drug resistance56. A study in melanoma cells developed a genome-scale CRISPR-Cas9 gain-of-function activation system that targets more than 10,000 lncRNA transcriptional start sites. This study has identified 16 lncRNA loci whose induction can facilitate resistance to BRAF inhibitors by melanoma cells57. Another study developed a CRISPR activation of lncRNA (CaLR) strategy to target 14,701 lncRNA genes in acute myeloid leukemia cell lines, in which 2874 lncRNAs were identified for their high therapeutic relevance to Ara-C drug resistance58. Although the CRISPR screening for lncRNAs is still at its infant stage, these studies have reinforced the notion that lncRNAs can mediate cancer drug response. It is our believe that through integrating lncRNA profiling with drug metabolism pathway analysis will help to identify lncRNAs that affect drug resistance through their effect on drug metabolism and disposition.

Besides efforts that focused on genomic and epigenetic regulation of drug metabolism through lncRNAs, emerging metabolomic datasets may provide new direction for clarifying the landscape of drug metabolism in diseases. A recent study has profiled 225 metabolites related to cell growth, immune response, and xenobiotic metabolism in 928 cancer cell lines across 20 cancer types in the Cancer Cell Line Encyclopedia (CCLE)59. With the paired genomic, transcriptomic, epigenetic and pharmacologic profiles available in the CCLE database, these datasets will enable unbiased association analysis to identify dependencies among cancer drug resistance, cell metabolism, and the lncRNA alterations.

In addition to lncRNAs that can directly regulate DMEs and transporters, some studies proposed that lncRNAs can also mediate the regulation of drug metabolism and disposition by xenobiotic receptors, such as PXR and CAR. For instance, a RNA-seq analysis on adult male C57BL/6 mouse livers treated with PXR and CAR agonists revealed that, among 3843 hepatic lncRNAs, 193 of them were differentially regulated by PXR or CAR60. Genomic annotation found that most of these PXR- or CAR-responsive lncRNAs are produced from the introns and 3′-UTRs of protein coding genes, and only a small fraction belong to intergenic lncRNAs. Further integration analysis with published PXR ChIP-seq data identified 774 lncRNAs with direct PXR-DNA binding sites, and 26.8% of them had significant changes in the binding after agonist exposure. Notably, most of these lncRNAs had positive enrichment of H3K4me2 and PXR near their gene loci, indicating a potential interaction between H3K4me2 and PXR to maintain the constitutive expression of liver-enriched lncRNAs. Knowing xenobiotic receptors are master regulators of drug metabolism and disposition, these results suggested a potentially important role of lncRNAs in mediating the PXR or CAR effect on xenobiotic metabolism. Table 2 summarizes some of the publicly available transcriptome data that may help to facilitate the discovery of those lncRNAs that can directly participate in the drug metabolism.

| Studies   | Species | Sample size | Molecules   | Treatment |
|-----------|---------|-------------|-------------|-----------|
| GSE68365  | Mouse   | 30 (5/group)| CAR/PXR    | Knockout  |
| GSE104734 | Mouse   | 9 (3/group) | CAR/PXR    | Activation|
| GSE76148  | Human   | 24 (6/group)| CAR/PXR/PPARz| Activation|
| GSE71446  | Human   | 12 (4/group)| CAR        | Knockout  |
| GSE95685  | Mouse   | 22 (3–4/group)| CAR      | Activation|

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4. Conclusions and perspectives

Drug metabolism and disposition represent a highly complex system that relies on an orchestrated regulation of DMEs and transporters at the cellular or organism levels. The regulations are relevant in both general drug metabolism, as well as drug metabolism and disposition in cancer chemotherapies. Since knowing the precise regulation of this process could help improve drug efficacy and reduce drug toxicity, great efforts have been made to improve the understanding of the mechanism of regulation. Previous studies have largely focused on the regulation of drug metabolism by regulatory proteins such as xenobiotic receptors, while recent and ongoing studies have suggested a tremendous potential of lncRNAs in regulating the expression and/or activity of DMEs and transporters. Mechanistically and as summarized in Fig. 1, lncRNAs may have exerted their regulatory functions through: 1) their yet to be defined functional cross-talk with the xenobiotic receptors; 2) a post-transcriptional mechanism such as sponging the DME- and transporter-targeting miRNAs; and 3) functioning as xenobiotic receptor responsive genes. The studies on lncRNAs related to drug metabolism are still in their infancy; further understanding the role of lncRNAs in drug metabolism and disposition will help to identify novel regulatory mechanisms, enable the discovery of lncRNA-based biomarkers and drug targets, and personalize and improve the therapeutic outcome in patients, including the cancer patients.

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Figure 1  Proposed model of regulation of drug metabolism and disposition through lncRNAs. lncRNAs may exert their regulatory functions through: 1) their yet to be defined functional cross-talk with the xenobiotic receptors; 2) a post-transcriptional mechanism such as sponging the DME- and transporter-targeting miRNAs; and 3) functioning as xenobiotic receptor responsive genes. XRE, xenobiotic response element.

4. Conclusions and perspectives

Drug metabolism and disposition represent a highly complex system that relies on an orchestrated regulation of DMEs and transporters at the cellular or organism levels. The regulations are relevant in both general drug metabolism, as well as drug metabolism and disposition in cancer chemotherapies. Since knowing the precise regulation of this process could help improve drug efficacy and reduce drug toxicity, great efforts have been made to improve the understanding of the mechanism of regulation. Previous studies have largely focused on the regulation of drug metabolism by regulatory proteins such as xenobiotic receptors, while recent and ongoing studies have suggested a tremendous potential of lncRNAs in regulating the expression and/or activity of DMEs and transporters. Mechanistically and as summarized in Fig. 1, lncRNAs may have exerted their regulatory functions through: 1) their yet to be defined functional cross-talk with the xenobiotic receptors, or through a post-transcriptional mechanism such as sponging the DME- and transporter-targeting miRNAs. Last but not least, lncRNAs may have participated in mediating the effect of xenobiotic receptors on drug metabolism and disposition as xenobiotic receptor responsive genes. The studies on lncRNAs related to drug metabolism are still in their infancy; further understanding the role of lncRNAs in drug metabolism and disposition will help to identify novel regulatory mechanisms, enable the discovery of lncRNA-based biomarkers and drug targets, and personalize and improve the therapeutic outcome in patients, including the cancer patients.

Author contributions

Yue Wang and Zihui Fang participated in the initial drafting of the manuscript. All authors participated in the revision and finalization of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

Appendix A. Supporting information

Supporting data to this article can be found online at https://doi.org/10.1016/j.apsb.2019.09.011.

References

1. Leonessa F, Clarke R. ATP binding cassette transporters and drug resistance in breast cancer. Endocr Relat Cancer 2003;10:43–73.
2. Xu C, Li CY, Kong AN. Induction of phase I, II and III drug metabolism/transport by xenobiotics. Arch Pharm Res 2005;28:249–68.
3. Crettol S, Petrovic N, Murray M. Pharmacogenetics of phase I and phase II drug metabolism. Curr Pharmaceut Des 2010;16:204–19.
4. Kim J, Piao HL, Kim BJ, Yao F, Han Z, Wang Y, et al. Long non-coding RNA MALAT1 suppresses breast cancer metastasis. Nat Genet 2018;50:1705–15.
5. Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, et al. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature 2009;458:223–7.
6. Mathieu EL, Belhocine M, Dao LT, Puthier D, Spicuglia S. Functions of lncRNA in development and diseases. Med Sci (Paris) 2014;30:790–6.
7. Qi W, Song X, Li L. Long non-coding RNA-guided regulation in organisms. Sci China Life Sci 2013;56:891–6.
8. Ulitsky I, Bartel DP. lincRNAs: genomics, evolution, and mechanisms. Cell 2013;154:26–46.
9. Bolha L, Ravnik-Glavac M, Glavac D. Long noncoding RNAs as biomarkers in cancer. Dis Markers 2017;2017:7243968.
10. Prensner JR, Iyer MK, Balbin OA, Dhanasekaran SM, Cao Q, Brenner JC, et al. Transcriptome sequencing across a prostate cancer
cohort identifies PCAT-1, an unannotated lncRNA implicated in disease progression. *Nat Biotechnol* 2011; 29:742–9.

Kotake Y, Nakagawa T, Kitagawa K, Suzuki S, Liu N, Kitagawa M, et al. Long non-coding RNA ANRIL is required for the PRC2 recruitment to and silencing of p15(INK4B) tumor suppressor gene. *Oncogene* 2011; 30:1956–62.

Liu J, Wan L, Lu K, Sun M, Pan X, Zhang P, et al. The long noncoding RNA MEG3 contributes to cisplatin resistance of human lung adenocarcinoma. *PLoS One* 2015; 10:e0114586.

Xia Y, He Z, Liu B, Wang F, Chen Y. Downregulation of Meg3 enhances cisplatin resistance of lung cancer cells through activation of the WNT/beta-catenin signaling pathway. *Mol Med Rep* 2015; 12:4530–7.

Zhang J, Liu J, Xu X, Li L. Curcumin suppresses cisplatin resistance development partly via modulating extracellular vesicle-mediated transfer of MEG3 and miR-214 in ovarian cancer. *Cancer Chemother Pharmacol* 2017; 79:479–87.

Akers JC, Gonda D, Kim R, Carter BS, Chen CC. Biogenesis of extracellular vesicles (EVs): exosomes, microvesicles, retrovirus-like vesicles, and apoptotic bodies. *J Neuro Oncol* 2013; 113:1–11.

Silva A, Bullock M, Calin G. The clinical relevance of long non-coding RNAs in cancer. *Cancers* (Basel) 2015; 7:2169–82.

Furge LL, Guengerich FP. Cytochrome P450 enzymes in drug metabolism and chemical toxicology: an introduction. *Biochem Mol Biol Educ* 2006; 34:66–74.

Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacol Ther* 2013; 138:103–41.

Hu L, Zhuo W, He YJ, Zhou HH, Fan L. Pharmacogenetics of P450 oxidoreductase: implications in drug metabolism and therapy. *Pharmacogenomics Genom* 2012; 22:812–9.

Ortiz de Montellano PR. Cytochrome P450-activated prodrugs. *Future Med Chem* 2013; 5:213–28.

Jornada DH, dos Santos Fernandes GF, Chiba DE, de Melo TR, dos Santos JL, Chung MC. The prodrug approach: a successful tool for improving drug solubility. *Molecules* 2015; 21:42.

McCarver DG, Hines RN. The ontogeny of human drug-metabolizing enzymes: phase II conjugation enzymes and regulatory mechanisms. *J Pharmacol Exp Ther* 2002; 300:361–6.

Kensler TW, Egner PA, WB, Zhu YR, Zhang BC, Lu PX, et al. Chemoprevention of hepatocellular carcinoma in aflatoxin endemic areas. *Gastroenterology* 2004; 127:S310–8.

DeGorter MK, Xia CQ, Yang JJ, Kim RB. Drug transporters in drug efficiency and toxicology. *Ann Rev Pharmacol Toxicol* 2012; 52:240–73.

Shi Z, Tiwari AK, Shukla S, Robey RW, Singh S, Kim IW, et al. Sildenafil reverses ABCB1- and ABCG2-mediated chemotherapeutic drug resistance. *Cancer Res* 2011; 71:3029–41.

Bellon Caneiro JM, Forcada Jimenez M, Gomez Oliveros L. The ileocecal sphincter in the functional dynamics of the digestive tract: collective review. *Rev Esp Enferm Apar Dig* 1987; 72:745–8.

Chen L, Bao Y, Piekos SC, Zhu K, Zhang L, Zhang XB. A transcriptional regulatory network containing nuclear receptors and long noncoding RNAs controls basal and drug-induced expression of cytochrome P450s in HepaRG cells. *Mol Pharmacol* 2018; 94:749–59.

Wang Y, Yan L, Liu J, Chen S, Liu G, Nie Y, et al. The HNF1alpha-regulated IncRNA HNF1alpha-AS1 is involved in the regulation of cytochrome P450 expression in human liver tissues and Huh7 cells. *J Pharmacol Exp Ther* 2019; 368:353–62.

Lan X, Yan J, Ren J, Zhong B, Li J, Li Y, et al. A novel long non-coding RNA Inc-HC binds hnRNP2B1 to regulate expressions of Cyp7a1 and Abca1 in hepatic cholesterol metabolism. *Hepatology* 2016; 64:58–72.

Li P, Ruan X, Yang L, Kiesewetter K, Zhao Y, Luo H, et al. A liver-enriched long non-coding RNA, IncLSTR, regulates systemic lipid metabolism in mice. *Cell Metabol* 2015; 21:455–67.

Fu Y, Wang W, Li X, Liu Y, Niu Y, Zhang B, et al. LncRNA H19 interacts with S-adenosylhomocysteine hydrolase to regulate LINE-1 methylation in human lung-derived cells exposed to benzo[a]pyrene. *Chemosphere* 2018; 207:84–90.

Ren J, Ding L, Zhang D, Shi G, Xu Q, Shen S, et al. Carcinoma-associated fibroblasts promote the stemness and chemoresistance of colorectal cancer by transferring exosomal IncRNA H19. *Theranostics* 2018; 8:3932–48.

Shima H, Kida K, Adachi S, Yamada A, Sugae S, Nariu K, et al. LncRNA H19 is associated with poor prognosis in breast cancer patients and promotes cancer stemness. *Breast Canc Res Treat* 2018; 170:507–16.

He JH, Han ZP, Liu JM, Zhu JB, Zou MX, Lv YB, et al. Over-expression of long non-coding RNA MEG3 inhibits proliferation of hepatocellular carcinoma Huh7 cells via negative modulation of miRNA-664. *J Cell Biochem* 2017; 118:3713–21.

Liu LC, Wang YL, Lin PL, Zhang X, Cheng WC, Liu SH, et al. Long noncoding RNA HOTAIR promotes invasion of breast cancer cells through chondroitin sulfotransferase CHST15. *Int J Cancer* 2019; 145:2478–87.

Chen L, Manauutoe JE, Zhong X-b. Impact on acetylamino-phen-induced hepatotoxicity by long non-coding RNAs HNF1a-AS1 and HNF4a-AS1 in HepRG cells. *FASEB J* 2019; 33:506.6.

Zhu B, Xu M, Shi H, Gao X, Liang P. Genome-wide identification of lncRNAs associated with chlorantraniliprole resistance in diamond-back moth *Platella xylostella* (L.). *BMJ Genomics* 2017; 18:380.

Li CY, Cui JY. Regulation of protein-coding gene and long noncoding RNA pairs in liver of conventional and germ-free mice following oral PBDE exposure. *PLoS One* 2018; 13:e0201387.

Fang Z, Chen W, Yuan Z, Liu X, Jiang H. LncRNA-MALAT1 contributes to the cisplatin-resistance of lung cancer by upregulating MRP1 and MDR1 via STAT3 activation. *Biomed Pharmacother* 2018; 101:536–42.

Tirosh I, Izar B, Prakash SM, Wadsworth 2nd MH, Trombetta JJ, et al. Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. *Science* 2016; 352:189–96.

Wang Y, Zhang D, Wu K, Zhao Q, Nie Y, Fan D. Long noncoding RNA MRUL promotes ABCB1 expression in multidrug-resistant gastric cancer cell sublines. *Mol Cell Biol* 2014; 34:3182–93.

Takahashi K, Yan IK, Wood J, Haga H, Patel T. Involvement of extracellular vesicle long noncoding RNA (linc-VLDLR) in tumor cell responses to chemotherapy. *Mol Cancer Res* 2014; 12:1377–87.

Zhu J, Zhang R, Yang D, Li J, Yan X, Jin K, et al. Knockdown of long non-coding RNA XIST inhibited doxorubicin resistance in colorectal cancer by upregulation of miR-124 and downregulation of SGG1. *Cell Physiol Biochem* 2018; 51:113–28.

Hu H, Yang L, Li L, Zeng C. Long non-coding RNA KCNQ1OT1 modulates oxaliplatin resistance in hepatocellular carcinoma through miR-7-5p/ABCC1 axis. *Biochem Biophys Res Commun* 2018; 503:2400–6.

Chang L, Hu Z, Zhou Z, Zhang H. linc00518 contributes to multidrug resistance through regulating the MiR-199a/MLR1 axis in breast cancer. *Cell Physiol Biochem* 2018; 48:16–28.

Wu X, Zheng Y, Han B, Dong X. Long noncoding RNA BLACAT1 modulates ABCB1 to promote oxaliplatin resistance of gastric cancer via sponging miR-361. *Biomed Pharmacother* 2018; 99:832–8.

Eisses JF, Kaplan JH. Molecular characterization of hCTR1, the copper transporter 1. The human copper uptake protein. *J Biol Chem* 2002; 277:29162–71.

Lee J, Fena MM, Nose Y, Thiele DJ. Biochemical characterization of the human copper transporter Ctr1. *J Biol Chem* 2002; 277:4380–7.

Ishida S, Lee J, Thiele DJ, Hershkovitz I. Uptake of the anticancer drug cisplatin mediated by the copper transporter Ctr1 in yeast and mammals. *Proc Natl Acad Sci U S A* 2002; 99:14298–302.

Jandial DD, Farshchi-Heydari S, Larson CA, Elliott GI, Wrasidlo WJ, Howell SB. Enhanced delivery of cisplatin to intraperitoneal ovarian carcinomas mediated by the effects of bortezomib on the human copper transporter 1. *Clin Cancer Res* 2009; 15:553–60.

Kim ES, Tang X, Peterson DR, Kilari D, Chow CW, Fujimoto J, et al. Copper transporter CTR1 expression and tissue platinum concentration in non-small cell lung cancer. *Lung Cancer* 2014; 85:88–93.
52. Jiang P, Wu X, Wang X, Huang W, Feng Q. NEAT1 upregulates EGCG-induced CTR1 to enhance cisplatin sensitivity in lung cancer cells. *Oncotarget* 2016;7:43337–51.

53. Jiang P, Chen A, Wu X, Zhou M, Ul Haq I, Mariyam Z, et al. NEAT1 acts as an inducer of cancer stem cell-like phenotypes in NSCLC by inhibiting EGCG-upregulated CTR1. *J Cell Physiol* 2018;233:4852–63.

54. Batista PJ, Chang HY. Long noncoding RNAs: cellular address codes in development and disease. *Cell* 2013;152:1298–307.

55. Iyer MK, Niknafs YS, Malik R, Singhal U, Sahu A, Hosono Y, et al. The landscape of long noncoding RNAs in the human transcriptome. *Nat Genet* 2015;47:199–208.

56. Esposito R, Bosch N, Lanzos A, Polidori T, Pulido-Quetglas C, Johnson R. Hacking the cancer genome: profiling therapeutically actionable long non-coding RNAs using CRISPR-Cas9 screening. *Cancer Cell* 2019;35:545–57.

57. Joung J, Engreitz JM, Konermann S, Abudayyeh OO, Verdine VK, Aguet F, et al. Genome-scale activation screen identifies a lncRNA locus regulating a gene neighbourhood. *Nature* 2017;548:343–6.

58. Bester AC, Lee JD, Chavez A, Lee YR, Nachmani D, Vora S, et al. An integrated genome-wide CRISPRa approach to functionalize lncRNAs in drug resistance. *Cell* 2018;173:649–664 e20.

59. Li H, Ning S, Ghandi M, Kryukov GV, Gopal S, Deik A, et al. The landscape of cancer cell line metabolism. *Nat Med* 2019;25:850–60.

60. Dempsey JL, Cui JY. Regulation of hepatic long noncoding RNAs by pregnane X receptor and constitutive androstane receptor agonists in mouse liver. *Drug Metab Dispos* 2019;47:329–39.