LncRNAs GIHCG and SPINT1-AS1 Are Crucial Factors for Pan-Cancer Cells Sensitivity to Lapatinib

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Lapatinib is a small molecule inhibitor of EGFR (HER1) and ERBB2 (HER2) receptors, which is used for treatment of advanced or metastatic breast cancer. To find the drug resistance mechanisms of treatment for EGFR/ERBB2 positive tumors, we analyzed the possible effects of lncRNAs. In this study, using CCLE (Cancer Cell Line Encyclopedia) database, we explored the relationship between the lncRNAs and Lapatinib sensitivity/resistance, and then validated those findings through in vitro experiments. We found that the expression of EGFR/ERBB2 and activation of ERBB pathway was significantly related to Lapatinib sensitivity. GO (Gene Oncology) analysis of top 10 pathways showed that the sensitivity of Lapatinib was positively correlated with cell keratin, epithelial differentiation, and cell-cell junction, while negatively correlated with signatures of extracellular matrix. Forty-four differentially expressed lncRNAs were found between the Lapatinib sensitive and resistant groups (fold-change > 1.5, \( P < 0.01 \)). Gene set variation analysis (GSVA) was performed based on 44 lncRNAs and genes in the top 10 pathways. Five lncRNAs were identified as hub molecules. Co-expression network was constructed by more than five lncRNAs and 199 genes in the top 10 pathways, and three lncRNAs (GIHCG, SPINT1-AS1, and MAGI2-AS3) and 47 genes were identified as close-related molecules. The three lncRNAs in epithelium-derived cancers were differentially expressed between sensitive and resistant groups, but no significance was found in non-epithelium-derived cancer cells. Correlation analysis showed that SPINT1-AS1 (\( R = −0.715, P < 0.001 \)) and GIHCG (\( R = 0.557, P = 0.013 \)) were correlated with the IC50 of epithelium-derived cancer cells. In further experiments, GIHCG knockdown enhanced cancer cell susceptibility to Lapatinib, while high level of SPINT1-AS1 was a sensitive biomarker of NCI-N87 and MCF7 cancer cells to Lapatinib. In conclusions, IncRNAs GIHCG and SPINT1-AS1 were involved in regulating Lapatinib sensitivity. Up-regulation of GIHCG was a drug-resistant biomarker, while up-regulation of SPINT1-AS1 was a sensitive indicator.

Keywords: pan-cancer, computational analysis, LncRNAs, lapatinib, targeted therapy
INTRODUCTION

Lapatinib is a small molecular drug that has been shown to be a dual tyrosine kinase inhibitor, which is involved in the EGFR/HER1 and ERBB2/HER2 pathways and suppresses the autophosphorylation of these receptors. Clinically, it has been used in combination therapy with capecitabine in patients with advanced or metastatic breast cancer that overexpressed ERBB2/HER2 in the cases of previous treatment with anthracyclines, taxanes, or trastuzumab (Herceptin) (Geyer et al., 2006). In addition, a satisfactory response rate has also been found with Lapatinib treatment for ERBB2-positive progressive gastric cancer (Cetin et al., 2014; Satoh et al., 2014). However, in patients with head and neck squamous cell carcinoma, Lapatinib combined with radiotherapy did not show therapeutic effects (Harrington et al., 2015). Similarly, in ERBB2/EGFR positive metastatic bladder cancer patients who underwent first-line chemotherapy didn't get benefit from Lapatinib maintenance treatment (Powles et al., 2017). Therefore, uncovering the drug-resistant mechanism of Lapatinib targeted therapy and find new sensitive biomarkers.

Long non-coding RNAs (lncRNAs) are a large class of transcribed RNA molecules that are longer than 200 nucleotides but do not encode proteins. In addition to the regulation of diverse cellular processes, such as epigenetics, cell cycle, and cell differentiation, they have been found to play important roles in carcinogenesis, tumor development, and treatment resistance (Heery et al., 2017; Peng et al., 2017; Hahne and Valeri, 2018; Wang et al., 2018; Wu et al., 2018). For instance, Ma et al. found that lncRNAs CASC9 and EWSAT1 were two crucial molecules associated to EGFR-TKIs resistant in non-small cell lung cancer (Ma et al., 2017).

The Cancer Cell Line Encyclopedia (CCLE) database (https://portals.broadinstitute.org/ccle) is an open access resource with the most completely integrated datasets of cancer cells genomes and drug effectiveness. It includes the experimental datasets of drug treatment of 24 kinds of chemical compounds in almost 1,000 cancer cell lines of various human cancers (Barretina et al., 2012). Kim et al. used CCLE database in their recent publication. They found that high levels of FGFR and integrin β3 are resistant to crizotinib treatment, suggesting that FGFR, and integrin β3 could be predictive markers for Met-targeted therapy (Kim et al., 2015). To date, there is a limited number of studies (Jiang et al., 2014; Niknafs et al., 2016; Bester et al., 2018; Li D. et al., 2018; Sun et al., 2018) to explore lncRNAs by CCLE database. In this study, we analyzed the lncRNAs of whole-genome datasets of CCLE after treatment with Lapatinib on pan-cancer cell lines, and proposed crucial lncRNAs GIHCG and SPINT1-AS1 involved in regulating Lapatinib sensitivity.

MATERIALS AND METHODS

Data Extraction From CCLE

There are 5,344 IncRNA probes and 49,331 non-IncRNA probes in the whole-genome gene expression profile chip used in CCLE (Barretina et al., 2012). There are 1,037 cell lines of various cancer types in the database. Among those, 504 cell lines had been treated with Lapatinib and got IC50 (half maximal inhibitory concentration) data and 501 cell lines were examined by microarrays. Since the study focused on solid tumors, we deleted cell lines of hematopoietic and lymphoid cell lines. Finally, 420 solid tumor cell lines were enrolled in the study (Table 1).

### Cancer Cell Lines and Cell Culture

Nineteen cancer cell lines were used for validating experiments in vitro. Four of those were gastric cancer cell lines (NCI-N87, SGC-7901, AGS, and MKN-45), three were melanoma cell lines (MuM-2C, MV3, and A-375), three were hepatocarcinoma cell lines (LM3, 97L, and Huh7), three were thyroid cancer cell lines (KHM-5M, CAL-62, and C643), two were breast cancer cell lines (MCF7 and SK-BR-3), two were pancreatic cancer cell lines (TCC-PAN2 and BxPC3), and two were colorectal cancer lines (DLD-1 and NCI-H-747). Cell lines NCI-N87, MuM-2C, LM3, MV3, Huh7, SGC-7901, CAL-62, AGS, MCF7, C643, 97L, SK-BR-3, KHM-5M, A-375, TCC-PAN2, MKN-45, and BxPC3 were purchased from The Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). Cell lines DLD-1 and NCIH-747 were purchased from The Global Bioresource Center ATCC (Maryland, USA). The cell lines were cultured in RPMI-1640 supplemented with 10% fetal bovine serum in a humidified incubator at 37°C with 95% air and 5% CO2.

### Transient Transfection of siRNAs

SPINT1-AS1 and GIHCG siRNAs were transfected into cancer cells by Lipofectamine 2000 (Invitrogen, Carlsbad, California).

| TABLE 1 | The distribution of 420 cancer cell lines of solid tumors. |
|---|---|
| Cancer types | Count |
| Autonomic ganglia | 10 |
| Biliary tract | 1 |
| Bone | 11 |
| Breast | 29 |
| Central nervous system | 29 |
| Endometrium | 20 |
| Kidney | 9 |
| Large intestine | 23 |
| Liver | 19 |
| Lung | 91 |
| Esophagus | 15 |
| Ovary | 28 |
| Pancreas | 28 |
| Pleura | 7 |
| Prostate | 3 |
| Salivary gland | 1 |
| Skin | 40 |
| Soft tissue | 12 |
| Stomach | 18 |
| Thyroid | 5 |
| Upper aerodigestive tract | 7 |
| Urinary tract | 14 |
| CCLE cell line names | Cell type         | IC50 (µM)* |
|----------------------|-------------------|------------|
| SNU1                 | Stomach           | 8          |
| KMRC2                | Kidney            | 8          |
| HEY8                 | Ovary             | 8          |
| NCIH1915             | Lung              | 8          |
| SH10TC               | Stomach           | 8          |
| JMSU1                | Urinary tract     | 8          |
| UACC62               | Skin              | 8          |
| SKLU1                | Lung              | 8          |
| ES2                  | Ovary             | 8          |
| SNU398               | Liver             | 8          |
| MSTO211H             | Pleura            | 8          |
| HMC18                | Breast            | 8          |
| HS229T               | Lung              | 8          |
| HS895T               | Skin              | 8          |
| NCIH1092             | Lung              | 8          |
| 8505C                | Thyroid           | 8          |
| RKO                  | Large intestine   | 8          |
| SW1573               | Lung              | 8          |
| NCIH2172             | Lung              | 8          |
| IGR37                | Skin              | 8          |
| T24                  | Urinary tract     | 8          |
| NCIH1581             | Lung              | 8          |
| HLF                  | Liver             | 8          |
| MG63                 | Bone              | 8          |
| HS840T               | Upper aerodigestive tract | 8      |
| DMS114               | Lung              | 8          |
| HS936T               | Skin              | 8          |
| FU37                 | Stomach           | 8          |
| NCIH2052             | Pleura            | 8          |
| 3050C                | Thyroid           | 8          |
| RERFLCAI             | Lung              | 8          |
| SW579                | Thyroid           | 8          |
| TOV112D              | Ovary             | 8          |
| HS729                | Soft tissue       | 8          |
| KMRC1                | Kidney            | 8          |
| SJSA1                | Bone              | 8          |
| HUH1                 | Liver             | 8          |
| 1321N1               | Central nervous system | 8      |
| TC71                 | Bone              | 8          |
| KELLY                | Autonomic ganglia | 8          |
| NCIH520              | Lung              | 8          |
| IGR39                | Skin              | 8          |
| EN                   | Endometrium       | 8          |
| U118MG               | Central nervous system | 8     |
| 639V                 | Urinary tract     | 8          |
| HGC27                | Stomach           | 8          |
| UMUC3                | Urinary tract     | 8          |
| 42MGBC               | Central nervous system | 8      |
| SKNBE2               | Autonomic ganglia | 8          |

(Continued)

| CCLE cell line names | Cell type         | IC50 (µM)* |
|----------------------|-------------------|------------|
| CALU1                | Lung              | 8          |
| NCIH2111             | Lung              | 8          |
| HEC59                | Endometrium       | 8          |
| BFTC909              | Kidney            | 8          |
| RPMI17951            | Skin              | 8          |
| IPC298               | Skin              | 8          |
| NCIH1651             | Lung              | 8          |
| MDA-MB-436           | Breast            | 8          |
| SKN72                | Autonomic ganglia | 8          |
| DKMG                 | Central nervous system | 8    |
| IALM                 | Lung              | 8          |
| NCIH1702             | Lung              | 8          |
| JHH6                 | Liver             | 8          |
| PSN1                 | Pancreas          | 8          |
| HOS                  | Bone              | 8          |
| CAL78                | Bone              | 8          |
| U87MG                | Central nervous system | 8     |
| G11                  | Central nervous system | 8   |
| NCIH1155             | Lung              | 8          |
| SBC5                 | Lung              | 8          |
| IMR32                | Autonomic ganglia | 8          |
| NCIH4140             | Lung              | 8          |
| WM2664               | Skin              | 8          |
| MEWO                 | Skin              | 8          |
| BT549                | Breast            | 8          |
| SKMEL30              | Skin              | 8          |
| NCIH1703             | Lung              | 8          |
| HEP3B217             | Liver             | 8          |
| TT2609C02            | Thyroid           | 8          |
| HEPG2                | Liver             | 8          |
| SKNAS                | Autonomic ganglia | 8          |
| NCIH1944             | Lung              | 8          |
| SW1271               | Lung              | 8          |
| COLO679              | Skin              | 8          |
| DAOY                 | Central nervous system | 8    |
| SHP77                | Lung              | 8          |
| NCIH1299             | Lung              | 8          |
| VMRCRC2              | Kidney            | 8          |
| LOXIMVI              | Skin              | 8          |
| NCIH1339             | Lung              | 8          |
| HS746T               | Stomach           | 8          |
| SKHEP1               | Liver             | 8          |
| NCIH1694             | Lung              | 8          |
| COV504               | Ovary             | 8          |
| NCIH1793             | Lung              | 8          |
| SNU423               | Liver             | 8          |
| JHUEM2               | Endometrium       | 8          |
| CALU6                | Lung              | 8          |
| JB2                  | Urinary tract     | 8          |
| CCLE cell line names | Cell type          | IC50 (µM)* |
|----------------------|--------------------|------------|
| UACC257              | Skin               | 8          |
| G402                 | Soft tissue        | 8          |
| MESSA                | Soft tissue        | 8          |
| HT1080               | Soft tissue        | 8          |
| MPP89                | Pleura             | 8          |
| OVTOKO               | Ovary              | 8          |
| SUIT2                | Pancreas           | 8          |
| SIMA                 | Autonomic ganglia  | 8          |
| H4                   | Central nervous system | 8     |
| WM1799               | Skin               | 8          |
| A673                 | Bone               | 8          |
| NCIH1975             | Lung               | 8          |
| MDAMB157             | Breast             | 8          |
| SKMEL5               | Skin               | 8          |
| SKE31                | Bone               | 8          |
| NCIH2452             | Pleura             | 8          |
| NCIH647              | Lung               | 8          |
| SAOS2                | Bone               | 8          |
| NCIH2023             | Lung               | 8          |
| NCIH226              | Lung               | 8          |
| SF295                | Central nervous system | 8     |
| SW620                | Large intestine    | 8          |
| NCIH661              | Lung               | 8          |
| HS939T               | Skin               | 8          |
| HS758T               | Breast             | 8          |
| HCC44                | Lung               | 8          |
| EFO21                | Ovary              | 8          |
| KPNS19S              | Autonomic ganglia  | 8          |
| SF126                | Central nervous system | 8     |
| HS739T               | Breast             | 8          |
| NCIH19693            | Lung               | 8          |
| TOV21G               | Ovary              | 8          |
| KALS1                | Central nervous system | 8     |
| A375                 | Skin               | 8          |
| CHP212               | Autonomic ganglia  | 8          |
| SW1990               | Pancreas           | 8          |
| LOUNH91              | Lung               | 8          |
| OV90                 | Ovary              | 8          |
| SKMEL2               | Skin               | 8          |
| NCIH23               | Lung               | 8          |
| YKG1                 | Central nervous system | 8     |
| WM88                 | Skin               | 8          |
| ACHN                 | Kidney             | 8          |
| SKNRF                | Autonomic ganglia  | 8          |
| DU145                | Prostate           | 8          |
| GAMG                 | Central nervous system | 8     |
| MDAMB435S            | Skin               | 8          |
| NCIH2087             | Lung               | 8          |
| NCIH1563             | Lung               | 8          |
| HEC06                | Endometrium        | 8          |

(Continued)
| CCLE cell line names | Cell type           | IC50 (µM)* |
|---------------------|---------------------|------------|
| SW480               | Large intestine     | 8          |
| NCIH522             | Lung                | 8          |
| NCIH650             | Lung                | 8          |
| OC314               | Ovary               | 8          |
| COV318              | Ovary               | 8          |
| HS852T              | Skin                | 8          |
| NCIH727             | Lung                | 8          |
| EFO27               | Ovary               | 8          |
| SJRH30              | Soft tissue         | 8          |
| KNS81               | Central nervous system | 8     |
| SNU449              | Liver               | 8          |
| A2058               | Skin                | 8          |
| HS294T              | Skin                | 8          |
| SNU182              | Liver               | 8          |
| COLO205             | Large intestine     | 8          |
| HUCCT1              | Biliary tract       | 8          |
| WIKAWAHERAKLIO02ER | Endometrium         | 8          |
| LS411N              | Large intestine     | 8          |
| PATU8902            | Pancreas            | 8          |
| PC3                 | Prostate            | 8          |
| SKMEL24             | Skin                | 8          |
| C3A                 | Liver               | 8          |
| AN3CA               | Endometrium         | 8          |
| SN3M                | Endometrium         | 8          |
| TE1                 | Esophagus           | 8          |
| NCIH1573            | Lung                | 8          |
| HCT116              | Large intestine     | 8          |
| NCIH1568            | Lung                | 8          |
| HPAc                | Pancreas            | 8          |
| HEC151              | Endometrium         | 8          |
| OVMANA              | Ovary               | 8          |
| HCC56               | Large intestine     | 8          |
| HEC1A               | Endometrium         | 8          |
| CAK2                | Kidney              | 8          |
| CAPAN2              | Pancreas            | 8          |
| NCIH1373            | Lung                | 8          |
| NCIH1048            | Lung                | 8          |
| CAS1                | Central nervous system | 8     |
| HCC1569             | Breast              | 8          |
| SNU475              | Liver               | 8          |
| LS123               | Large intestine     | 8          |
| NCIH1341            | Lung                | 8          |
| PANC0403            | Pancreas            | 8          |
| MOG0066             | Central nervous system | 8     |
| IM95                | Stomach             | 8          |
| ONCODG1             | Ovary               | 8          |
| NCIH747             | Large intestine     | 8          |
| WM115               | Skin                | 8          |
| D853105MG3          | Central nervous system | 8     |
| EFE184              | Endometrium         | 8          |

(Continued)
| CCLE cell line names | Cell type          | IC50 (µM)* |
|---------------------|--------------------|------------|
| KYSE150             | Esophagus          | 8          |
| UACC812             | Breast             | 8          |
| ONS76               | Central nervous system | 8      |
| KNS62               | Lung               | 8          |
| PANC1005            | Pancreas           | 7.987659   |
| ISTMES2             | Pleura             | 7.889111   |
| NOCIH1355           | Lung               | 7.860067   |
| KYSE30              | Esophagus          | 7.858886   |
| 22RV1               | Prostate           | 7.847305   |
| MIAAPCA2            | Pancreas           | 7.469959   |
| JHOS4               | Ovary              | 7.408363   |
| A2780               | Soft tissue        | 7.399833   |
| HCC70               | Breast             | 7.36332    |
| NOCIH2286           | Lung               | 7.359588   |
| MALME3M             | Skin               | 7.325411   |
| GCY                 | Stomach            | 7.255416   |
| PK1                 | Pancreas           | 7.236271   |
| 786O                | Kidney             | 7.170835   |
| T3M10               | Lung               | 7.170651   |
| A2780               | Ovary              | 7.146877   |
| SKLMS1              | Soft tissue        | 7.136584   |
| HT1376              | Urinary tract      | 7.080406   |
| HUPT4               | Pancreas           | 7.0557     |
| PANCO3273           | Pancreas           | 6.904092   |
| SW1088              | Central nervous system | 6.737086 |
| SNU16               | Stomach            | 6.697771   |
| PLCPRF5             | Liver              | 6.669433   |
| HARA                | Lung               | 6.656741   |
| MELHO               | Skin               | 6.552444   |
| RT112               | Urinary tract      | 6.525924   |
| K029AX              | Skin               | 6.444433   |
| EBC1                | Lung               | 6.372372   |
| MCAS                | Ovary              | 6.3241     |
| COLO320             | Large intestine    | 6.295312   |
| PK9                 | Pancreas           | 6.190494   |
| HT29                | Large intestine    | 5.884947   |
| TE9                 | Esophagus          | 5.855279   |
| WM963B              | Skin               | 5.68912    |
| KClIM0H1            | Pancreas           | 5.619114   |
| TYKNJ               | Ovary              | 5.343411   |
| 8MGGA               | Central nervous system | 5.226626 |
| PANCO2003           | Pancreas           | 5.197284   |
| NOCIH1650           | Lung               | 5.152449   |
| NIHOCAR3            | Ovary              | 5.117735   |
| OVCAR8              | Ovary              | 5.095931   |
| JHH7                | Liver              | 4.92477    |
| HMCB                | Skin               | 4.767848   |
| MKN74               | Stomach            | 4.689733   |
| HCT15               | Large intestine    | 4.666833   |
| WM793               | Skin               | 4.641666   |

(Continued)
**TABLE 2** Continued

| CCLE cell line names | Cell type                      | IC50 (µM)* |
|----------------------|-------------------------------|------------|
| FAU                  | Upper aerodigestive tract     | 0.823073   |
| SKCO1               | Large intestine               | 0.71562    |
| KYSE140             | Esophagus                     | 0.68893    |
| CAL27               | Upper aerodigestive tract     | 0.688771   |
| CHL1                | Skin                          | 0.675993   |
| TE11                | Esophagus                     | 0.63775    |
| JHH5                | Liver                         | 0.569108   |
| CALU3               | Lung                          | 0.494588   |
| MDAMB157              | Breast                        | 0.488741   |
| NCIH1668            | Lung                          | 0.386496   |
| NCIH1648            | Lung                          | 0.373409   |
| HCC287              | Lung                          | 0.372134   |
| NCIH2255            | Lung                          | 0.333763   |
| NCIH2170            | Lung                          | 0.300981   |
| TE617T             | Soft tissue                   | 0.242928   |
| CCK81               | Large intestine               | 0.240195   |
| SKBR3              | Breast                        | 0.196392   |
| AU665              | Breast                        | 0.18321    |
| NUGC4              | Stomach                       | 0.171543   |
| ZR7530             | Breast                        | 0.166593   |
| BT474              | Breast                        | 0.116183   |
| NCIN87             | Stomach                       | 0.066107   |

*Extracted from CCLE database (https://portals.broadinstitute.org/ccle).

IC50 (µM) is half maximal inhibitory concentration (IC50), which is defined as a drug concentration producing absolute 50% inhibition of growth in cell proliferation assay. By definition, this metric relies on the assumption, that at a high concentration of the drug, 100% effect is achieved as all cells die in a proliferation assay.

RESULTS

**Lapatinib IC50 From Pan-Cancer Cell Lines Analysis**

The CCLE data of Lapatinib IC50 of the selected 420 cell lines was shown in Table 2. The upper limit of IC50 was originally determined as 8 µM for those cancer cell lines in the database. There were 302 cancer cell lines with IC50 higher than 8 µM, which were insensitive to Lapatinib drug. There were 118 cancer cell lines with IC50 lower than 8 µM, which were relatively sensitive to Lapatinib drug. Taking 8 µM of IC50 as a threshold, we categorized 420 cancer cell lines into two groups, high_IC50 (n = 302) and low_IC50 (n = 118). Since EGFR and ERBB2 are the targets of the Lapatinib drug, the expression levels of EGFR, and ERBB2 in high_IC50 and low_IC50 groups were analyzed. The expression levels of EGFR and ERBB2 were significantly higher in low-IC50 group than in high_IC50 (Figure 1A, P = 0.006 and P < 0.001, respectively). The distribution tendency of 22 types of solid cancer cell lines in high-IC50 (up to 8 µM) and low_IC50 (lower than 8 µM) groups is presented in Figure 1B. GSEA analysis showed that ERBB pathway-related genes were enriched in low_IC50 group (Figure 1C, ERBB signaling pathway NES = −1.81, P < 0.002, p. adjust = 0.064; regulation of ERBB signaling pathway NES = −1.69, P < 0.002, p. adjust = 0.064).

Pathway Analysis Involved in Lapatinib Sensitivity

To illustrate the mechanism of Lapatinib resistance, we selected genes with fold-change >1.5 times to perform GO analysis (Table S2). In the top 10 involved pathways, Lapatinib sensitivity was positively associated with cell keratin, epithelial differentiation,
FIGURE 1 | The correlation of mRNA expression levels of EGFR and ERBB2 and Lapatinib IC50. (A) The bar charts of mRNA expression levels of EGFR (left) and ERBB2 (right) of cancer cell lines between the high IC50 and low IC50 groups of Lapatinib drug. The expression levels of EGFR and ERBB2 are significantly higher in the low IC50 group than that in the high IC50 group (p < 0.01). (B) The distribution tendency of 22 types of solid cancer cell lines in high IC50 (up to 8 µM) and low IC50 (lower than 8 µM). The red lines represent mean value of Lapatinib IC50. (C) The enrichment analysis of ERBB signaling pathway reveals that ERBB signaling pathway is significantly enriched in Lapatinib low IC50 group. "Y" axis indicates the enrichment score (ES) value, and "X" axis indicates genes according to differential expression value between high IC50 and low IC50 groups. The blue and red dot curves represent ES value. The bottom barcodes represent the leading gene set that strongly contributed to ES value. The positive ES value represents positive correlation to Lapatinib IC50, and minus ES value represents negative correlation to Lapatinib IC50.
and cell-cell junction, while negatively related to signatures of extracellular matrix (Figure 2, $P < 0.001$, $P$ adjust < 0.001).

**Analysis of LncRNAs Involved in Lapatinib Sensitivity**

We further screened the differentially expressed lncRNAs, and 44 lncRNAs were identified between the high IC50 group and low IC50 group (Figure 3A and Table 3, fold-change > 1.5, $P < 0.01$). Then, we selected genes in the top 10 pathways and 44 differential lncRNAs for the construction of the co-expression network. The enrichment scores of the top 10 pathway genes in every cancer cell lines were calculated and determined by GSVA analysis. Five lncRNAs were highlighted as the hub factors in the top 10 regulating pathways (Figure 3B). The association of the 5 lncRNAs with 199 genes in the top 10 pathways was further analyzed, and a molecular network of co-expression was established, which included top 50 key molecules closely associated to Lapatinib sensitivity. Three crucial lncRNAs, GHIHG, SPINT1-AS1, and MAGI2-AS3, still remained in the co-expression network (Figure 3C).

**Differential Expressing Analysis of Three LncRNAs Between Epithelial and Non-epithelial Cancer Groups**

We divided the 420 cancer cell lines into epithelium derived group ($n = 278$) and non-epithelium derived group ($n = 142$; including nervous system, bone, cartilage, and pleura). The differential expression levels of the three lncRNAs between the
two groups are presented in Figure 4A. In the epithelium-derived group, the differential expression levels of the three IncRNAs between Lapatinib high IC50 and low IC50 groups were significantly different (Figure 4B, P < 0.05). In the non-epithelium groups, there was no significant difference of the three IncRNAs between Lapatinib high IC50 and low IC50 groups. Higher expressing level of SPINT1-AS1 was found in epithelium-derived cancer cells, and higher expressing levels of MAGI2-AS3 and GIHCG were observed in the non-epithelium group.

Differentially expressed genes (1.5-fold change) between the Lapatinib high IC50 and low IC50 groups in epithelial group (Table S3) were utilized to perform GO analysis. Enhanced signatures of cell keratin, epithelial differentiation, and cell-cell junction were observed in Lapatinib low IC50 group, and decreased signature of extracellular matrix were observed in Lapatinib high IC50 group (Figure 5, P < 0.001, P adjust < 0.001).

Correlation of LncRNAs SPINT1-AS1, GIHCG, or MAGI2-AS3 and Lapatinib Sensitivity in Epithelial Group

Correlation analysis revealed that Lapatinib IC50 of the non-epithelial group was higher than that of the epithelial group (Figure 6A). Of the three critical IncRNAs, SPINT1-AS1, and GIHCG were the IncRNAs most correlated to Lapatinib sensitivity (Figure 6B). SPINT1-AS1 and GIHCG were selected as key factors of affecting Lapatinib sensitivity of epithelial cancers. The up-regulation of SPINT1-AS1 was found in low IC50 group and increased GIHCG was found in high IC50 group (Figure 6C).

Validating Study of GIHCG and SPINT1-AS1 on Regulating Lapatinib Sensitivity in vitro

In validating experiments, we examined expression levels of GIHCG and SPINT1-AS1 in seven types of cancer cell lines (thyroid cancer, pancreatic cancer, liver cancer, melanoma, gastric cancer, breast cancer, and colorectal cancer) and Lapatinib IC50 of the same cancer cell lines. Correlation analysis showed that higher expression levels of SPINT1-AS1 were significantly associated with lower Lapatinib IC50 (Figure 7A, R = −0.715, P < 0.001), while higher expression levels of GIHCG were significantly related to higher Lapatinib IC50 (Figure 7A, R = 0.557, P = 0.013).

The sensitive cancer cell lines of NCI-N87 (gastric cancer) and MCF7 (breast cancer), as well as the resistant cancer cell lines of NCIH-747 (colon cancer) and BxPC3 (pancreatic cancer)
**TABLE 3** | Differentially expressed lncRNAs between Lapatinib high_IC50 and low_IC50 groups of 420 cancer cell lines (fold-change > 1.5, \( P < 0.01 \)).

| Probes          | Title                                                                 | Symbol                          | Ensemble transcript id version | Log FC   | \( P \)-value | Adj. \( P \)-value |
|-----------------|-----------------------------------------------------------------------|---------------------------------|--------------------------------|----------|---------------|------------------|
| 225381_at       | mir-100-let-7a-2 cluster host gene (non-protein coding)               | MIR100HG                        | ENSG00000255248.7              | 1.399024 | 4.98E-08      | 1.48E-05         |
| 226546_at       | uncharacterized LOC100506844                                          | G1HCG                           | ENSG00000257698.1              | 1.19665  | 1.52E-15      | 8.13E-12         |
| 228564_at       | Long intergenic non-protein coding RNA 1116                           | LINCO1116                       | ENSG00000163634.9              | 1.12280  | 4.24E-06      | 0.000493         |
| 227554_at       | MAGI2 antisense RNA 3                                                  | MAGI2-AS3                       | ENSG00000234456.7              | 1.096172 | 2.73E-07      | 5.84E-05         |
| 1566482_at      | NA                                                                     | RP11-305O6.3                    | ENSG00000250280.2              | 0.961776 | 3.96E-08      | 1.24E-05         |
| 213156_at       | Zinc finger and BTB domain containing 20                              | ZBTB20                          | ENSG00000259976.3              | 0.942404 | 6.68E-06      | 0.000649         |
| 213158_at       | Zinc finger and BTB domain containing 20                              | ZBTB20                          | ENSG00000259976.3              | 0.908785 | 1.6E-05       | 0.001179         |
| 224741_s_at     | ZNF667 antisense RNA 1 (head to head)                                 | ZNF667-AS1                      | ENSG00000166770.10             | 0.873077 | 0.000703      | 0.019471         |
| 229480_at       | MAGI2 antisense RNA 3                                                  | MAGI2-AS3                       | ENSG00000234456.7              | 0.870971 | 4.07E-07      | 8.05E-05         |
| 229493_at       | HOXD cluster antisense RNA 2                                           | HOXD-AS2                        | ENSG00000237380.6              | 0.795366 | 2.89E-07      | 5.94E-05         |
| 227082_at       | Zinc finger and BTB domain containing 20                              | ZBTB20                          | ENSG00000259976.3              | 0.780225 | 5.64E-05      | 0.003174         |
| 226587_at       | Prader Willi/Angelman region RNA 8                                     | PWAR6                           | ENSG00000257151.1              | 0.777959 | 0.0002        | 0.008638         |
| 242358_at       | RASSF8 antisense RNA 1                                                 | RASSF8-AS1                      | ENSG00000246695.7              | 0.770905 | 9.02E-08      | 2.29E-05         |
| 236075_s_at     | Uncharacterized LOC101928000                                          | LOC101928000                    | ENSG00000234327.7              | 0.76675  | 6.6E-06       | 0.000649         |
| 221974_at       | Imprinted in Prader-Willi syndrome (non-protein coding) /// uncharacterized LOC101930404 /// Prader Willi/Angelman region RNA, SNRPN neighbor /// small nucleolar RNA, C/D box 107 /// small nucleolar RNA, C/D box 115-13 /// small nucleolar RNA, C/D box 115-26 /// small nucleolar RNA, C/D box 116-22 /// small nucleolar RNA, C/D box 116-28 /// small nucleolar RNA, C/D box 116-4 /// small nuclear ribonucleoprotein polypeptide N | IPW /// LOC101930404 /// PWARSN /// SNORD115-13 /// SNORD115-26 /// SNORD115-7 /// SNORD116-22 /// SNORD116-28 /// SNORD116-4 /// SNRPN | ENSG00000224078.13 | 0.719911 | 0.000535      | 0.016616         |
| 227099_s_at     | Chromosome 11 open reading frame 96                                    | C11orf96                        | ENSG00000254409.2              | 0.688826 | 0.001963      | 0.037596         |
| 217520_x_at     | Uncharacterized LOC101929232 /// PDCD6IP pseudogene 2                  | PDCD6IP2                        | ENSG00000274253.4              | 0.671638 | 1.03E-05      | 0.000862         |
| 226591_at       | Prader Willi/Angelman region RNA 6                                     | PWAR6                           | ENSG00000257151.1              | 0.665136 | 0.000597      | 0.018108         |
| 233562_at       | Long intergenic non-protein coding RNA 839                            | LINCO0839                       | ENSG00000185904.11             | 0.644287 | 0.000226      | 0.009658         |
| 228370_at       | Imprinted in Prader-Willi syndrome (non-protein coding) /// uncharacterized LOC101930404 /// Prader Willi/Angelman region RNA, SNRPN neighbor /// small nucleolar RNA, C/D box 107 /// small nucleolar RNA, C/D box 115-13 /// small nucleolar RNA, C/D box 115-26 /// small nucleolar RNA, C/D box 115-7 /// small nucleolar RNA, C/D box 116-22 /// small nucleolar RNA, C/D box 116-28 /// small nucleolar RNA, C/D box 116-4 | IPW /// LOC101930404 /// PWARSN /// SNORD115-13 /// SNORD115-26 /// SNORD115-7 /// SNORD116-22 /// SNORD116-28 /// SNORD116-4 | ENSG00000224078.13 | 0.63548  | 0.004004      | 0.056605         |
| 230272_at       | Long intergenic non-protein coding RNA 461 /// microRNA 9-2            | LINCO0461 /// MIR9-2            | ENSG00000245526.10             | 0.633241 | 0.000333      | 0.011874         |
TABLE 3 | Continued

| Probes   | Title                                                                 | Symbol          | Ensemble transcript id version | Log FC  | P-value  | Adj. P-value |
|----------|-----------------------------------------------------------------------|-----------------|--------------------------------|---------|----------|-------------|
| 227121_at | Zinc finger and BTB domain containing 20                              | ZBTB20          | ENSG00000259976.3              | 0.622039| 6.47E-05 | 0.003438    |
| 228438_at | Uncharacterized LOC100132891                                         | LOC100132891    | ENSG00000235351.9              | 0.610992| 0.00111  | 0.026335    |
| 213447_at | Imprinted in Prader-Willi syndrome (non-protein coding)              | IPW || LOC101930404 || PWARN || SNORD107 || SNORD115-13 || SNORD115-26 || SNORD115-7 || SNORD116-22 || SNORD116-28 || SNORD116-4 || SNRPN  | ENSG00000224078.13 | 0.603999 | 0.000792 | 0.021388 |
| 224646_x_at | H19, imprinted maternally expressed transcript (non-protein coding) || microRNA 675 | ENSG00000260265.1             | −0.58771| 0.000615| 0.089285   |
| 235921_at | Uncharacterized LOC100506119                                         | RP11-44F21.5    | ENSG00000130600.18            | −0.66521| 0.008633| 0.089285   |
| 232202_at | Family with sequence similarity 83, member B                           | RP11-747H7.3    | ENSG00000260711.2             | −0.68534| 2.63E-09| 1.08E-06   |
| 227985_at | Uncharacterized LOC100506098                                         | LOC10273721     | ENSG000002233834.6             | −1.04243| 7.5E-08 | 2.0E-05     |
| 232202_at | Family with sequence similarity 83, member B                           | FAM83B          | ENSG00000261111.6             | −1.07231| 2.29E-10| 1.22E-07   |
| 227985_at | Uncharacterized LOC100506098                                         | LOC100506098    | ENSG00000260711.2             | −0.92003| 9.63E-11| 6.43E-08   |
| 213447_at | Imprinted in Prader-Willi syndrome (non-protein coding)              | LOC100506098    | ENSG000002333834.6             | −1.04243| 7.5E-08 | 2.0E-05     |
| 227985_at | Uncharacterized LOC100506098                                         | LOC100506098    | ENSG00000260711.2             | −0.92003| 9.63E-11| 6.43E-08   |
| 229223_at | Uncharacterized LOC100506098                                         | LOC100506098    | ENSG00000260711.2             | −0.92003| 9.63E-11| 6.43E-08   |

log FC, log2 of fold-change. Positive value indicates increased expression in high IC50 group, and negative value indicates decreased expression in high IC50 group. NA, Not available.

were selected for a subsequent validating study. After knocking-down expression levels of GIHCG and SPINT1-AS1 by small interfering RNAs, Lapatinib IC50, and inhibitory rate of cancer cells were detected. Among three small interference sequences of GIHCG and SPINT1-AS1 mRNAs, siRNA sequence 3 of GIHCG (Si3, Figure 7B), and siRNA sequence 1 of SPINT1-AS1 (Si1, Figure 7C) were identified as effective siRNAs for further experiments.

Knocking-down of GIHCG could significantly enhance the sensitivity to Lapatinib in MCF7 and BxPC3 cancer cell lines (Figure 7D), while down-regulation of SPINT1-AS1 could promote resistance to Lapatinib in NCI-N87 and MCF7 cancer cell lines (Figure 7E). To clarify whether there is a mutual regulatory relationship between GIHCG and SPINT1-AS1, we detected the expression level of SPINT1-AS1 after GIHCG knockdown and vice versa. As shown in Figures 7F,G...
suppression of GIHCG in Lapatinib resistant cancer cell lines NCIH-747 and BxPC3 could induce up-regulation of SPINT1-AS1 \((P < 0.05)\), while knockdown of SPINT1-AS1 did not change the expression level of GIHCG \((P > 0.05)\).

**DISCUSSION**

LncRNA is an important regulatory molecule in drug resistance during chemotherapy or gene targeted therapy (Li et al., 2016; Dong et al., 2018; Wu et al., 2018; Zhou et al., 2018). In this study, we analyzed Lapatinib sensitivity to EGFR and ERBB2 targeted therapy pan-cancer cell line wide. We noticed that Lapatinib sensitivity was not only positively correlated to the activation of EGFR and ERBB2 signaling pathways, but also positively associated to cell keratin, epithelial differentiation, and cell-cell junction. The Lapatinib sensitivity of cancer cell lines was negatively associated to extracellular matrix signature. By screening differentially expressed lncRNAs and establishing co-expression network between Lapatinib high IC50 and low IC50 groups, three key lncRNAs, SPINT1-AS1, GIHCG, and MAGI2-AS3, were found. Of those, GIHCG and SPINT1-AS1 were only differentially expressed in epithelial derived cancers. SPINT1-AS1 was negatively related to Lapatinib IC50, whereas GIHCG was positively associated to Lapatinib IC50. By siRNAs treatment, downregulation of SPINTA-AS1 could promote Lapatinib resistance, while downregulation of GIHCG promoted Lapatinib sensitivity. The combination of bioinformatical approach and experimental study confirmed that lncRNAs were involved in regulating sensitivity to Lapatinib targeted therapy.

PI3K/Akt, Ras/Raf/MEK/ERK1/2, and PLCγ pathways are downstream pathways of EGFR and ERBB2 and play important roles in cell proliferation and survival of multiple cancers.
FIGURE 5 | Pathway analysis of Lapatinib sensitivity related genes. The genes in the top 10 pathways with fold-change more than 1.5 are used between Lapatinib high, IC50 and low, IC50 groups. The middle brown dot of each network indicates the name of a gene set, and the small dots surrounding it indicate the genes of the gene set. The red dots represent the up-regulated genes in the high, IC50 group, and the green dots represent the up-regulated genes in the low, IC50 group. The darker red or green spot are the larger fold-change of differential genes. The black spots with different sizes and numbers on the right side indicate the gene numbers in the gene clusters.

(Roskoski, 2014). The expression levels of EGFR and ERBB2 are positively correlated to Lapatinib sensitivity (Rusnak et al., 2007; Xiang et al., 2018). Trastuzumab (Herceptin) is a molecular targeted drug of ERBB2-positive metastatic/advanced breast cancer and gastric cancer (Bang et al., 2010; Loibl and Gianni, 2017). Lapatinib is a small molecule chemical, which proved effective for ERBB2-positive advanced or metastatic breast cancer when combined with capecitabine after previous treatment with anthracyclines, paclitaxel, or trastuzumab (Geyer et al., 2006). In gastric cancer, treatment with Lapatinib plus capecitabine and oxaliplatin also revealed anti-cancer effects on HER2-amplified gastroesophageal adenocarcinoma, especially in Asian and younger patients (Hecht et al., 2016). LncRNAs emerged as one of the new resistance mechanisms to chemotherapy or molecule targeted therapy. By bioinformatics analysis, Lapatinib sensitive cancer cells exhibited enrichment of genes related to cell keratin, epithelial differentiation, and cell-cell junction. The ERBB family plays an important role in regulating cell differentiation (Pellat et al., 2017). We noticed that Lapatinib sensitivity is positively correlated to ERBB pathway activation. It means that cancer cells sensitive to Lapatinib drug often showed enrichment of cell differentiation-related genes, while Lapatinib-resistant cancer cells are often accompanied by enrichment of extracellular matrix pathway (D’Amato et al., 2015; Khan et al., 2016; Lin et al., 2017; Watson et al., 2018). Furthermore, increases of extracellular matrix could further induce epithelial-mesenchymal transition of cancer cells (Tzanakakis et al., 2018).
Although the role of lncRNAs in cancer progression and Lapatinib resistance have been reported in other studies (Russell et al., 2015; Li et al., 2016; Liang et al., 2018; Ma et al., 2018), this is the first study that proved that lncRNAs GIHCG and SPINT1-AS1 are involved in regulating therapeutic sensitivity to Lapatinib. Based on pan-cancer cell lines analysis, Lapatinib IC50 is significantly different between non-epithelial cancer cell lines, and epithelial cancer cell lines. As the inhibitor of miR-200b/200a/429, LncRNA GIHCG was shown effectively promoting the progression of liver cancer through inducing methylation of miR-200b/200a/429 promoter (Sui et al., 2016). GIHCG is also involved in promoting cancer proliferation and migration in tongue and renal cancers (D’Aniello et al., 2018; Ma et al., 2018). However, there is no study on whether or not GIHCG could regulate Lapatinib drug sensitivity in cancers. LncRNA SPINT1-AS1 is a Kunitz type 1 antisense RNA1, belonging to serine peptidase inhibitor. An increased expression of SPINT1-AS1 has been observed in colorectal cancer (Li C. et al., 2018). It is also the first time that lncRNA SPINT1-AS1 has been found regulating Lapatinib drug sensitivity on multiple cancer cells. In validating experiments, the knockdown of SPINT1-AS1 did not result in the up-regulation of GIHCG. We speculated that GIHCG may regulate SPINT1-AS1 expression through regulating promoter methylation or by manner of competitive endogenous RNA (ceRNA) (Zhang G. et al., 2018; Zhang L. et al., 2018). However, the mutual regulatory mechanisms of lncRNA GIHCG and SPINT1-AS1 remain to be studied in the future.

CONCLUSION

In conclusion, the current study proposed a group of lncRNAs related to Lapatinib sensitivity based on pan-cancer cell lines analysis. By subsequent experimental study, lncRNAs GIHCG and SPINT1-AS1 were firstly identified as crucial lncRNAs in regulating Lapatinib resistance or sensitivity in epithelial-derived cancer cell lines. SPINT1-AS1 is a Lapatinib sensitivity predictor, while GIHCG is a predictive molecule for Lapatinib resistance.

ETHICS STATEMENT

The protocols used in this study were approved by Rui Jin Hospital Ethics Review Boards. Written
informed consents were obtained from all human material donors in accordance with the Declaration of Helsinki. Animals were used according to the protocols approved by Rui Jin Hospital Animal Care and Use Committee.

**AUTHOR CONTRIBUTIONS**

KL and YY conceived and designed the experiments. ZX, ShS, ZZ, JG, and QL performed the experiments. ZX, ZZ, SaS, WS, YY, and KL analyzed the data. ZX, ShS, ZZ, SaS, WS, YY, and KL...
contribute reagents, materials, and analysis tools. ZX, YY, and KL wrote the paper.

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