The profiling, identification, quantification and analysis of differentially expressed genes (DEGs) in response to drug treatment in lung cancer

Rahaba Marima, Rodney Hull, Zodwa Dlamini, Clement Penny

Abstract

The profiling and identification of genes that are differentially expressed is frequently used to underpin the underlying molecular mechanisms of biological conditions and provides a molecular foothold on biological questions of interest. However, this can be a daunting task since there is a cross talk and overlap of some of the components of the signalling pathways. The deregulation of the cell cycle signalling pathway is a hallmark of cancer, including lung cancer. Proper regulation of the cell cycle results in cellular homeostasis between cell proliferation and cell death. The comprehension of the cell cycle regulation in drug metabolism studies is of significance. This study aimed at elucidating the regulation of cell cycle genes' in response to LPV/r in lung cells. Thus, this study describes methodology for revealing molecular mechanisms employed by LPV/r to induce stress on genomic DNA. This approach is based on the interrogation of a panel of 84 genes related to the cell cycle pathway, and how the differentially expressed genes' expression pattern corroborates loss in nuclear integrity (phenotypic observation). MAD2L2, AURKB and CASP3 gene expressions were further confirmed by RT-qPCR. Furthermore, the use of in-silico bioinformatics tools integrates the molecular profiles and phenotypic changes. This approach revealed the activation of the DNA damage response (DDR) pathway in response to LPV/r treatment. The proposed methodology will aid in the comprehension of drug metabolism at genotypic and phenotypic levels.

- Gene profiling often reveals the underlying molecular mechanisms.
- RT² PCR gene arrays have integrated patented quality controls and allow reliable gene expression analysis.
- In-silico bioinformatics analysis help reveal pathways affected, that often correspond to phenotypic changes/features.

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* Corresponding author.
E-mail address: rahaba.marima@up.ac.za (R. Marima).

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Specifications table

| Subject Area               | Biochemistry, Genetics and Molecular Biology |
|----------------------------|---------------------------------------------|
| More specific subject area | Molecular Oncology                          |
| Protocol name              | PCR gene arrays                             |
| Reagents/tools             | RT² Profiler Human PCR Arrays (Qiagen PAHS-020Z)-Cell Cycle |
| Experimental design        | Human lung cells were cultured, split and synchrosined prior to LPV/r drug treatment for nuclear integrity interrogation and gene expression profiling and analysis. |
| Trial registration         | N/A                                         |
| Ethics                     | N/A.                                        |
| Value of the Protocol      | • Gene profiling often reveals the underlying molecular mechanisms
|                            | • RT² PCR gene arrays have integrated patented quality controls and allow reliable gene expression analysis
|                            | • In-silico bioinformatics analysis help reveal pathways affected, that often corresponds to phenotypic changes/features |

Introduction

Lung cancer is one of the leading cause of deaths in both men and women, globally and in South Africa [1,2]. The increase in lung cancer incidence rates is particularly observed in the HIV population. As a non-AIDS defining cancer (NADC), lung cancer is rising as a colliding epidemic with HIV. While antiretroviral (ARV) treatment improves the health related quality of life (HRQoL) for HIV positive patients on ARV treatment, the rise in lung cancer cases in the ARV treatment (ART) era remains to be fully elucidated [3]. While the cell cycle is a tightly regulated process, anti-cancer drugs targeting key role players in cell cycle regulation, cell cycle checkpoints and DNA damage response continue to emerge [4–6]. Lopinavir/ritonavir (LPV/r) is a WHO approved dual protease inhibitor used against HIV infection [7]. Previous reports revealed the cytotoxic properties of this protease inhibitor against cancer cells. However, the effects of LPV/r treatment in non-cancerous healthy cells remains elusive . Nelfinavir is also one of the PIs showing cytotoxic effects [8,9]. Although LPV/r is a well established PI has been approved for HIV/AIDS treatment as per [7] guidelines, this study reveals how novel anti-cancer drugs could be mechanistically evaluated in vitro for cytotoxicity. Such novel work can include the eco-friendly preparation of nanomaterials and investigation of their properties in pharmaceutical and medical applications or cancer therapeutics as well as energy and environmental remediation [10–17]. This study aimed at determining the changes in the expression of cell cycle related genes in response to LPV/r in normal healthy and adenocarcinoma lung cells. This study employed a human cell cycle 84-gene array panel, accompanied by pathway analysis and nuclear interrogation approaches.

Method details

Cell culture

The lung cell lines MRC-5 (normal lung fibroblast (ATCC CCL171) and A549 (lung adenocarcinoma (ATCC CCL185) were purchased from the American Type Culture Collection (ATCC). These cell lines are adaptable to a single growth medium and they have been previously demonstrated to show reproducible profiles for growth and drug sensitivity. MRC-5 and A549 cell lines were treated...
with a range of concentrations, including the plasma concentration level, of LPV/r at 24h and 48h time intervals, for screening purposes. Cells were grown in Dulbecco’s Modified Eagle’s Medium (DMEM):F12, 10% serum and 1% pen/strep and left to grow to ~70% confluency in a humid chamber at 37°C and a 5% CO$_2$ level for 48h to 72h. This was followed by trypsinization, to detach cells from the flask, centrifuging to the cells and remove the supernatant, washing with PBS and re-suspending the cells in 5ml of fresh DMEM media. (For gene expression studies, cells’ scraping can sometimes yield improved nucleic acid amounts, as indicated under RNA extraction). The cells were split in a 1:2 ratio and sub-cultured. Cell culture medium containing 10% FBS and 1% pen/strep was replaced every 2-3 days until cells reached 70-80% confluency. Cells were then serum-starved for 24h to synchronise the cell cycle. The following day, the cells were pharmacologically treated with LPV/r at 32 and 80 μM, for 24h to 48h. Vehicle control cells were exposed to growth medium and vehicle only (methanol 0.1% v/v).

**DNA staining using 4, 6-diamidino-2-phenylindole (DAPI)**

A variety of fluorescent dyes are available to label DNA and allow for easy visualization of the nucleus in fluorescent dyes are available to label DNA and allow for easy visualization of the nucleus in interphase cells and chromosomes in mitotic cells. These stains include Hoechst, 4,6-diamidino-2-phenylindole (DAPI), ethidium bromide, propidium iodide, and acridine orange. Compared to the Hoechst DNA staining, DAPI has greater photostability [18]. It is assumed that DAPI associates with the minor groove of double-stranded DNA, with a preference for the adenine-thymine clusters. For DAPI to enter the cell and intercalate into DNA, cells must be first permeabilized with a detergent before or after fixation. Fluorescence increases approximately 20-fold when DAPI is bound to double-stranded DNA. Here, cells (MRC-5 and A549) were treated for 48h with LPV/r at 32 and 80 μM. The two concentrations were selected as a plasma relevant and an experimental dose. DAPI was used to assess the effects of the ARVs on the cellular DNA using the following protocol:

One (1) × 10$^4$ cells were seeded on the coverslips and allowed to grow overnight in 6-well plates. Cells were then rinsed with PBS and serum-starved for 24h. This was then followed by the treatment of cells with 32 μM and 80 μM of LPV/r. A 1 × PBS would be required for the preparation of DAPI solution. The DAPI (Millipore-Sigma) solution was then diluted 1:5000 in PBS for staining in the later steps. Next, the cell culture medium was removed from the cells grown on the coverslips, the cells were then washed three time using PBS. The cells were fixed for 10min in 3.7% formaldehyde in PBS. The fixative was then removed and the cells were rinsed three times, 5min each, in PBS. The cells were then permeabilized in 0.2% Triton X-100 in PBS for 5min. The triton-X was then aspirated, and the cells were rinsed three times, 5min each, in PBS. Cells were then incubated for 10min at room temperature with 100 μl of the diluted DAPI labelling solution The. DAPI was then removed and the cells were rinsed three times in PBS. The coverslips were then mounted with aqueous FluoroMount mounting medium (Sigma) and allowed to dry. The cells were then viewed on a Zeiss LSM 780 confocal microscope.

**Human Cell-Cycle Gene (PCR) Arrays**

A gene array panel was used to analyze the cell-cycle response pre- and post- LPV/r treatments of each cell-line. This method provides for a highly reliable and sensitive gene expression profiling of focused panels of genes in signal transduction, biological processes, or disease research pathways using real-time PCR methodology. In this study, RT$^2$ Profiler Human Cell-Cycle PCR Arrays (PAHS-020Z) were used (Qiagen). This pathway-focused array consists of 84 genes related to the cell-cycle, in a 96-well plate format as illustrated in Table 1. Aside from the pathway-focused genes, the panel contains 5 housekeeping (reference) genes and additionally a panel of proprietary controls that monitor genomic DNA contamination (GDC), first strand synthesis (RTC) and real-time PCR efficiency (PPC). Use of such a panel requires the isolation of high quality cellular RNA and sufficient cDNA synthesis to provide for the PCR amplification and analysis of gene expression. These steps are described below.
Table 1
The Human cell cycle PCR gene array layout.

|   | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 |
|---|----|----|----|----|----|----|----|----|----|----|----|----|
| A | ABL1| ANAPC2| ATM | ATR | AURKA | AURKB | BCCIP | BCL2 | BIRC5 | BRCA1 | BRCA2 | CASP3 |
|   | NM_005157 | NM_013366 | NM_000051 | NM_001184 | NM_003600 | NM_004217 | NM_016567 | NM_00633 | NM_01168 | NM_007294 | NM_000059 | NM_004346 |
| B | CCNA2 | CCNB1 | CCNB2 | CCNC | CCND1 | CCND2 | CCND3 | CCNE1 | CCNF | CCNG1 | CCNG2 | CCNH |
|   | NM_001237 | NM_031966 | NM_004701 | NM_005190 | NM_003056 | NM_001759 | NM_001760 | NM_001238 | NM_001761 | NM_004060 | NM_004354 | NM_001239 |
| C | CCNT1 | CDC16 | CDC20 | CDC25A | CDC25C | CDC34 | CDC6 | CDK1 | CDK2 | CDK4 | CDK5R1 | CDK5RAP1 |
|   | NM_001240 | NM_003903 | NM_001255 | NM_001789 | NM_004359 | NM_001254 | NM_001786 | NM_001798 | NM_000075 | NM_003885 | NM_016408 | NM_016408 |
| D | CCND1 | CDK7 | CDK8 | CDKN1A | CDKN1B | CDKN2A | CDKN2B | CDKN3 | CHEK1 | CHEK2 | CKS1B | CKS2 |
|   | NM_001259 | NM_001799 | NM_001260 | NM_00389 | NM_004064 | NM_000077 | NM_004936 | NM_005192 | NM_001274 | NM_007194 | NM_001826 | NM_001827 |
| E | CUL1 | CUL2 | CUL3 | E2F1 | E2F4 | GADD45A | GTSE1 | H5U1 | KNTC1 | KRPNA2 | MAD2L1 | MAD2L2 |
|   | NM_003592 | NM_003591 | NM_003590 | NM_005225 | NM_001950 | NM_001924 | NM_016426 | NM_004507 | NM_014708 | NM_002266 | NM_002358 | NM_006341 |
| F | MCM2 | MCM3 | MCM4 | MCM5 | MDM2 | MKI67 | MNAT1 | MRE11A | NBN | RAD1 | RAD17 | RAD51 |
|   | NM_004526 | NM_002388 | NM_005914 | NM_006739 | NM_002392 | NM_002417 | NM_002431 | NM_005590 | NM_002485 | NM_002853 | NM_002873 | NM_002875 |
| G | RAD9A | RB1 | RBBP8 | RBL1 | RBL2 | SERTAD1 | SKP2 | STN1 | TDP1 | TDP2 | TP53 | WEE1 |
|   | NM_004584 | NM_000321 | NM_002894 | NM_002895 | NM_005611 | NM_013376 | NM_005983 | NM_005563 | NM_007111 | NM_006286 | NM_000546 | NM_003390 |
| H | ACTB | B2M | GAPDH | HPRT1 | RPLPO | HGDG | RTTC | RTTC | RTTC | RTTC | RTTC | RTTC |
|   | NM_001101 | NM_004048 | NM_002046 | NM_000194 | NM_000102 | NM_000105 | NM_001004 | NM_001004 | NM_001004 | NM_001004 | NM_001004 | NM_001004 |
**Table 2**
gDNA elimination mixture.

| Reagent                      | Volume (μl) |
|------------------------------|-------------|
| Total RNA                    | 1 μg        |
| GE (5x gDNA Elimination Buffer) | 2          |
| RNase-free water to a final volume | 10         |

* The volume in μl depends on concentration of the RNA in ng/μl. The gDNA mixture was incubated at 42°C for 5 min and immediately placed on ice for 1 min.

**Table 3**
Reverse transcription (RT) cocktail.

| Reagent                      | Volume (μl) |
|------------------------------|-------------|
| - Quantscript RT Buffer      | 4           |
| - Quantscript Reverse Transcriptase | 1         |
| - RT Primer Mix              | 2           |
| RNase-free water             | 3           |
| Final volume                 | 10          |

***RNA Extraction***

RNA was extracted from cells treated for 48 h at drug plasma levels 32 μM LPV/r and from control (vehicle-methanol) cells, using the RNeasy Mini Kit (Qiagen) following the manufacturer’s instructions. Briefly, cells were washed with PBS twice, and then lysed directly by adding 350 μl of Buffer RLT, to T25 cell culture flasks (for A549 cells) and adding 600 μl of Buffer RLT to T75 cell culture flasks (for MRC-5 cells). Cell scrapers (Lonza) were used to detach the cells, as trypsinisation has been reported to interfere with quality of RNA [19]. The lysate was then transferred into sterile 2ml micro-centrifuge tubes. The tubes were then briefly vortexed before equal volumes (350 μl or 600 μl) of 70% ethanol was added to the lysate. Up to 700 μl of the sample was transferred into an RNeasy Mini spin column placed in a 2ml collection tube. These were centrifuged for 15 s at 8000 g. The flow-through was then discarded and 700 μl of Buffer RW1 was added to the RNeasy spin column and centrifuged for 15 s at 8000 g. Once again the flow through was discarded and 500 μl of Buffer RPE was added to the RNeasy spin column, which was centrifuged for 15 s at 8000 g. The flow through was discarded again, and 500 μl of Buffer RPE was added and the tubes were centrifuged for 2 min at 8000 g. The flow-through was discarded and the RNeasy spin-column was centrifuged at 8000 g for 1 min. The RNeasy spin-column was inserted into a new 1.5ml collection tube and 30 μl of RNase-free water was added directly to the spin column membrane. This was incubated at room temperature for 1 min to allow the RNase free water to cover the spin column membrane. Total RNA was eluted by centrifuging at 8000 x g for 1 min. The quality and quantity of RNA was measured using a Nanodrop (Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies)). For highly pure RNA, the 260/280 absorption ratio is ~2, and the 260/230 ratio is 2.0-2.2.

***cDNA synthesis***

cDNA was synthesized using the RT² First Strand cDNA synthesis kit (Qiagen) following the manufacturer’s instructions. Briefly, two mixtures were prepared separately, firstly the Genomic DNA (gDNA) elimination mixture (Table 2) for each RNA sample and secondly, the reverse transcription (RT) cocktail (Table 3).

The RT cocktail was briefly centrifuged and added to the gDNA mixture to provide a final volume of 20 μl. This mixture was then incubated at 42°C for 15 min, 95°C for 5 min and then at 4°C for 5 min. cDNA was diluted to 111 μl by adding nuclease-free water, according to the manufacturer’s instructions. The cDNA was used immediately for the PCR Arrays or stored at -20°C for future use.
PCR Array plates

The synthesized cDNA was mixed with the Qiagen RT² SYBR Green/ROX qPCR master mix, to which nuclease-free water was also added. Thereafter, 25 μl was loaded per well into the 96-well plates which contained pre-dispensed primer sets of the RT² Profiler PCR Array, as indicated in Table 4.

The PCR Array plates were centrifuged briefly (Boeco) before they were loaded on the ABI 7500 RT PCR instrument (Applied Biosystems). The following cycling conditions were used: 10min at 95°C, followed by 40 cycles of 15s at 95°C and 1min at 60°C. Data (CT values) was exported as Excel files to (Qiagen) GeneGlobe Database for analysis.

Data Analysis using GeneGlobe

The online GeneGlobe (Qiagen) analysis tool was used to analyse the PCR array data of all treated and untreated samples. A cycle threshold (Ct) of 35 was set as the cut-off value for gene expression. Thus genes with Ct values greater than 35 cycles were considered non-detectable. Five house-keeping genes, including (ACTB-Beta Actin (ACTB); Beta-2-microglobulin (BM2); Glyceraldehyde-3-phosphate dehydrogenase (GAPDH); HPRT1-Hypoxanthine phosphoribosyltransferase (HPRT1) and RPLPO-Ribosomal protein, large, P0 (RPLPO)) were used to obtain the change in cycle threshold (ΔCT) value for each gene of interest, where ΔCT is calculated as: CT value of gene of interest less the CT value of reference gene. The difference between the ΔCT of the treatment group and that of the vehicle control group is represented by ΔΔCT values, where the fold-change is calculated by $2^{(-\Delta\Delta CT)}$. [20]. This value represents the level of the expression of each gene in the drug-treated (EFV or LPV/r) sample versus that in the vehicle control sample. By convention, the fold difference of ±2 up-or-down regulation was used as a basis of target selection. The PCR array results were confirmed by RT-qPCR.

Validation of Cell-cycle Array Data

Real-Time quantitative Polymerase Chain Reaction (RT-qPCR) has been proven to be a more sensitive method for gene expression studies, compared to the conventional method [21]. To validate the PCR Array data, Real-Time quantitative PCR (RT-qPCR) was performed at least three independent times in triplicate. Three representative genes of the array were rationally selected according to their level of expression (above or below two-fold expression).

Primer Design

The Primer 3 tool (http://primer3.wi.mit.edu/) was used to design primers for the selected gene targets, including the house-keeping gene (HKG) GAPDH, based on NCBI accession numbers provided by Qiagen (for all three selected targets), following PCR Array analysis. The target genes selected included MAD2L2 (accession number NM_006341), CASP3 (accession number NM_004346), AURKB (accession number NM_004217) and (HKG) GAPDH (accession number NM_002046). The amplicons’ sizes were as follows; 157bp for MAD2L2, 149bp for CASP3, 150bp for AURKB and 87bp for GAPDH. Primer sequences of the selected gene targets and GAPDH as a reference gene (one of the internal controls’ panel in the PCR Array) are shown below, Table 5.

Total RNA from LPV/r treated and control cells, treated for 24h and 48h, was extracted using the RNeasy mini kit (Qiagen), as described above. The extracted RNA (treated and control) samples were used for reverse transcription in RT-qPCR experiments. Briefly, RNA was reverse transcribed to
cDNA using the Maxima First Strand cDNA synthesis kit for RT-qPCR with dsDNase (Thermo Fischer Scientific), following the manufacturer’s instructions as shown in Tables 6 and 7.

The contents were gently mixed and centrifuged. This was followed by incubation of samples at 37°C for 2 min, chilling samples on ice, another brief centrifuge and then the samples were placed on ice. Components of Table 2.5 were added to those listed in table 3.5. The two cocktails were gently mixed and briefly centrifuged. The reactions were incubated at 25°C for 10 min and 50°C for 15 min. The reactions were terminated by heating at 85°C for 5 min. This was followed by 4°C hold for 5 min. The cDNA was used immediately Table 8 or was stored at -20°C for future use.

The reactions each consisting of a total volume of 10 μl were then loaded into a 96-well PCR plate, which was centrifuged at 1000 rpm for 20 s, (Boeco) before loading onto the ABI 7500 RT-qPCR Instrument (ABI,) The following thermal profile was used: denaturation at 95°C for 10 min; followed by 40 cycles of 95°C for 15 s, then 60°C for 1 min (data collection point); and finally the dissociation stage, which is pre-set on the ABI 7500 system at 95°C for 15 s, Reps1, 60°C for 1 min, 95°C for 15 s.

RT-qPCR data analysis

Following the RT-qPCR experimental run on the ABI 7500 light cycler, the CT values were exported as an Excel file to create a table of CT values. This table was then uploaded on to the data analysis web portal at http://www.qiagen.com/geneglobe. Samples were assigned to controls and test groups. CT values were then normalized based on a manual selection of the GAPDH reference gene. The

### Table 5
Primer sequences of target genes.

| Target | Forward | Reverse |
|--------|---------|---------|
| MAD2L2 | 5'-CGA GTT CCT GGA GTA GCA TGT GCA TC-3' | 5'-GTA GCA CAG CTT GTC GAT TTA A-3' |
| Caspase 3 (CASP3) | 5'-GCT CAT ACC TGT GGG TGT GTA-3' | 5'-ATG AGA ATG GGG GAA GAG GCA -3' |
| AURKB | 5'-AGC AGC GAA CAG GCA CG-3' | 5'-GCC GAA GTC AGC AAC CA-3' |
| GAPDH | 5'-TGCACCACCAAATGCTTGAGC-3' | 5'-GCATGACTGTGTCATGAG-3' |

### Table 6
Digestion of gDNA.

| Reagent       | Volume (µl) |
|---------------|-------------|
| 10 × dsDNase Buffer | 1          |
| dsDNase       | 1           |
| Template RNA  | 1 µg*       |
| Nuclease-free water | Up to 10   |
| Total volume  | 10          |

* Added volume of RNA template varies, to amount to 1 µg.

### Table 7
Reverse Transcription Components.

| Reagent       | Volume (µl) |
|---------------|-------------|
| 5X reaction mix | 4          |
| Maxima enzyme mix | 2         |
| Nuclease-free water | 4         |
| Total volume  | 10          |
Table 8
RT-qPCR master mix cocktail.

| Reagent                          | Volume (μl) |
|----------------------------------|-------------|
| Fwd Primer                       | 0.4         |
| Rv Primer                        | 0.4         |
| 2X SYBR Green master mix         | 5.0         |
| cDNA                             | 0.8²        |
| Nuclease-free water              | 3.4         |
| Total volume                     | 10.0        |

* 10 μM of each primer (for all different gene targets) was used.

² No template reactions were also included, for negative control(s).

data analysis web portal calculates fold change/regulation using the delta delta CT method [20,22] in which delta CT is calculated between genes of interest, (GOI) and HKG, followed by delta-delta CT calculations (delta CT (test/experiment)-delta CT (control/calibrator)). Fold Change is then calculated using $2^{\Delta\Delta CT}$ formula, shown below.

$$\Delta CT = CT_{\text{Test,target}} - CT_{\text{HKG}}$$

$$\Delta CT = CT_{\text{Calibrator,target}} - CT_{\text{Calibrator,HKG}}$$

$$2^{\Delta\Delta CT} = 2^{((\Delta CT_{\text{Test}})-\Delta CT_{\text{Calibrator}})}$$

To understand the pattern of differentially expressed genes, the Ingenuity Pathway Analysis (IPA) bioinformatics tool was used.

**Bio-informatics for Pathway analysis**

*In-silico* analyses using various bioinformatics tools provide an important means for analyzing genes, their products and the (disease) pathways they are involved in [23]. The gene-lists represented by the GenBank accession numbers of selected gene targets (from the gene array data) were up-loaded on the IPA database.

Qiagens’s Ingenuity Pathway Analysis (IPA) has been widely used to model, analyse and understand the complex biological and chemical systems by the scientific community. In this study, IPA was used to help build a more complete regulatory picture and gain a better understanding of the biology underlying the studied gene expression profiles in response to LPV/r drug treatment. To achieve this, the canonical pathway function and the core analysis function of target genes were used. The GenBank accession numbers of the differentially expressed genes were uploaded on IPA. The representation of canonical pathways was achieved by the use of the z score. The z core was primarily used to indicate the degree of expression levels, with positive z score denoting upregulation, negative z core representing down-regulation, while zero (0) z core illustrate unchanged gene expression. Next, the core analysis function of IPA helps build a biological picture of the observed differential expression patterns.

**Results and discussion**

DDR is one of the cell’s mechanisms to maintain genome integrity following genotoxic insults. Furthermore, gene profiling of DEGs often reveals the underlying molecular mechanisms. The cytotoxic effects of protease inhibitors such as LPV/r have been previously reported [24]. Furthermore, various HAART components’ anti-proliferative effects have been previously reported [25]. However, to the best of our knowledge, our study is the first to reveal the upregulated p53 DDR pathway in response to LPV/r treatment in lung adenocarcinoma cells. This was accompanied by loss in nuclear architecture. Examination of genes involved in regulation of the cell cycle revealed how LPV/r treatment induces
the expression of genes that negatively regulate the cell cycle such as the inhibitors of the cyclin dependent kinases (CDKs), while repressing cell cycle positive regulators. The Aurora transcript variants, Aurora Kinase A (AURKA) and AURKB, reported to be overexpressed in various cancers, were also observed to be upregulated in A549 cancer cells and downregulated by LPV/r treatment [26]. In addition, MAD2L2 was downregulated while CASP3 was upregulated by LPV/r treatment in cancer cells. Profiling of 84 genes related to the cell cycle in response to LPV/r treatment has been insightful, compared to single-gene targeted studies. The IPA bioinformatics tool has also played a significant role in building a biological picture of the observed differential gene expression patterns. Additional mechanistic studies to reveal LPV/r anti-cancer properties will help shed more light on this drug’s potential use as an anti-cancer therapeutic.

In clinical settings, patients are exposed to ARV treatment for prolonged periods. Although this study potentially demonstrates the genotoxic effects of LPV/r on normal MRC-5 lung cells, and anti-proliferative effects on A549 adenocarcinoma cells, comparing these in vitro findings with in vivo results would be beneficial. Future research could include patient sample studies, protein expression level studies of target proteins and microRNA targets regulating the identified pathways.

Conclusions

The research approach employed in this study revealed the genotoxic effect of LPV/r on lung cancer and normal cells. The mechanisms used by this dual protease inhibitor in healthy and cancerous cells are similar, although most of the gene targets in normal cells remain repressed. Further studies are warranted to reveal the severity of LPV/r genotoxicity not only in normal lung cells, but on other types of healthy cells, as this could limit the potential use of LPV/r as an anti-cancer agent. Nonetheless, emerging research reveals the repurposing of antiretroviral drugs as anti-cancer drugs. Despite the cutting-edge diagnostic methods and therapeutics, cancer still remains a leading cause of death globally. There is therefore an urgent need for improved cancer therapeutic options. Due to time and financial constraints in anti-cancer novel drug development, drug repurposing of the already FDA approved drugs holds promising potential to cancer management, as nelfinavir is a promising PI for cancer drug repurposing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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