Modulation of non-coding RNAs by resveratrol in ovarian cancer cells: In silico analysis and literature review of the anti-cancer pathways involved

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Abstract
Background and aim: Non-coding RNAs control cell functioning through affecting gene expression and translation and their dysregulation is associated with altered cell homeostasis and diseases, including cancer. Nutraceuticals with anti-cancer therapeutic potential have been shown to modulate non-coding RNAs expression that could impact on the expression of genes involved in the malignant phenotype.

Experimental procedure: Here, we report on the microarray profiling of microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) and on the associated biochemical pathways and functional processes potentially modulated in OVCAR-3 ovarian cancer cells exposed for 24 h to Resveratrol (RV), a nutraceutical that has been shown to inhibit carcinogenesis and cancer progression in a variety of human and animal models, both in vitro and in vivo. Diana tools and Gene Ontology (GO) pathway analyses along with Pubmed literature search were employed to identify the cellular processes possibly affected by the dysregulated miRNAs and lncRNAs.

Results and conclusion: The present data consistently support the contention that RV could exert anti-neoplastic activity via non-coding RNAs epigenetic modulation of the pathways governing cell homeostasis, cell proliferation, cell death and cell motility.

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1. Introduction

Ovarian cancer remains among the deadliest gynecological cancer in women worldwide. Based on a recent statistic, it is predicted that in 2019 in US there will be more than 22,000 new cases of ovary cancer, with about 14,000 deaths that represent 5% of all deaths for cancer.1 Ovarian cancer is frequently diagnosed in the late stage because it develops asymptotically in the early stage and manifests its presence after it has spread in the peritoneum and distant organs.2 In most cases, surgery and chemotherapy elicit an initial good response, which however is followed by relapse of chemoresistant clones that inevitably lead to death the patient.3,4 The tumor microenvironment, with its unique composition in stromal- and immune cell-derived cytokines and of blood and lymphatic vessels that determine the availability of nutrients, growth factors and oxygen, plays a pivotal role in ovarian cancer cell metabolism and progression.5–12 There is an urgent need for understanding the molecular history of ovarian carcinogenesis in order to identify novel pharmacologic targets. Numerous oncogenes and tumor suppressor driver genes are found mutated in chemoresistant ovarian cancers.13 In addition to these mutations, also the altered epigenetic regulation of oncogenes and tumor suppressor genes contributes to ovarian carcinogenesis.14,15 Epigenetic regulation of carcinogenic driver genes
includes abnormally hypermethylation of the tumor suppressor gene promoter, abnormal post-translational modifications of the histones and the production of non-coding RNAs, either microRNAs (miRNA, of approximately 20 nucleotides) and long non-coding RNAs (lncRNA, of 200–250 or more nucleotides). Studies have implicated epigenetic dysregulation in ovarian carcinogenesis.16–21

However, our understanding of the involvement of non-coding RNAs in ovarian cancer cell biology remains limited. More importantly, we still need to understand how we can correct these epimutations pharmacologically.

In recent decades there has been a renewed interest for the possible exploitation of natural products in the prevention and cure of cancer. Indeed, a variety of therapeutic phytochemicals found in food stuff (known as nutraceuticals) have shown anti-cancer activity, either in vitro and in animal studies, and thus have great potentials for repositioning as complementary drugs for improving the efficacy of chemo- and immune-therapeutics as well as for attenuating the adverse side effects of conventional therapies.22–25

The anti-cancer effects of such nutraceuticals include induction of cell death, block of cell proliferation, modifications of cancer cell metabolism and of tumor microenvironment.26–28

Resveratrol [3,4’5-trihydroxy-trans-stilbene (RV)], a nutraceutical found in black and red berries, grape and nuts, is one such epigenetic modulator.29–31

In this work, we analyzed the profiling of miRNAs and lncRNAs in ovarian cancer OVCAR-3 cells exposed for 24 h to RV. The cellular processes associated with RV-modulated non-coding RNAs were identified by in silico analyses with appropriate software. Based on literature data, our findings support the view that RV elicits its antineoplastic activity also via non-coding RNAs epigenetic modulation of the pathways that govern cell homeostasis (particularly protein synthesis, organelle turnover and autophagy), cell metabolism (e.g., glucose uptake and Warburg effect), cell proliferation, cell death and cell motility.

2. Materials and methods

2.1. Cell culture, reagents and treatments

NIH-OVCAR-3 (simply referred as to OVCAR-3) ovarian cancer cells were maintained in standard conditions (37 °C, 95 v/v% air: 5 v/v% CO2) in RPMI 1640 medium (cod. R8758; Sigma–Aldrich, St. Louis, MO) containing 10% heated-inactivated FBS (cod. ECS0180L; Euroclone, Milano, Italy), supplemented with 1% Glutamine (cod. GLS175; Sigma–Aldrich) and 1% Penicillin/Streptomycin (cod. P0781; Sigma–Aldrich). The cells adherent on plastic dishes and at approx. 80% confluence were treated in complete medium for 24 h with 100 μM Resveratrol (RV, cod. R5010; Sigma–Aldrich; stock dissolved in DMSO). At the end, the cell monolayer was washed and processed for RNA extraction.

2.2. One color microarray genome-wide gene expression analysis

Total RNA was isolated from the cells using Absolutely RNA mRNA kit (Agilent Technologies, Palo Alto, CA). mRNA was amplified and labeled by Amino Allyl MessageAmp II aRNA Kit (Ambion, Austin, TX) using NHS ester Cy3 dye (Amersham Biosciences, Arlington Heights, IL). Total RNA quality and labeling was checked by means of RNA 6000 Nanochip assays and run on the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Total RNA amplified and labeled mRNA concentrations were calculated using the NanoDrop ND–1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE). Equal amounts (0.2 mg) of labeled specimens were fragmented and hybridized to Human Whole Genome Oligo Microarrays 860 K v2 (Agilent Technologies), representing 27958 Entrez Gene RNAs and 7419 lincRNAs. Each step was performed using the In Situ Hybridization Kit-Plus (Agilent Technologies) and following the 60-mer oligo microarray processing protocol. Slides were then washed with SSPE and scanned using an Agilent Scanner version C (G2505C, Agilent Technologies). Images were analyzed using the Feature Extraction software v10.7. Raw data elaboration was carried out with Bioconductor (www.bioconductor.org),32 using R statistical language. Background correction was performed with the normexp method and quantile was used for between-array normalization. The Linear Models for Microarray Analysis (LIMMA) package was then used to identify differentially expressed genes between the different experimental conditions. The empirical Bayes method was used to compute a moderated t-statistics.33 Transcripts with a log base two-fold change (logFC) greater than +0.20 or lower than −0.20 were considered as differentially expressed.

2.3. One color microarray microRNA expression analysis

One hundred nanograms of total RNA from cells at different experimental conditions were treated following the miRNA microarray protocol (Agilent Technologies, Placerville, CA). Briefly, RNA was dephosphorylated and denatured, followed by a ligation and labeling step. Samples were hybridized to Human miRNA Microarray 8 × 60K glass arrays from the Sanger miRBase database release 16 (2006 human miRNAs represented, Agilent Technologies). After hybridization, slides were washed following the Agilent procedure and scanned with the dual-laser Agilent Scanner version C (G2505C, Agilent Technologies). Images were analyzed using the Feature Extraction software v10.7. Raw data elaboration was carried out with Bioconductor (www.bioconductor.org),32 using R statistical language. The LIMMA package was then used to identify differentially expressed miRNAs between the different experimental conditions. The empirical Bayes method was used to compute a moderated t-statistics.33 miRNAs with a log base two-fold change (logFC) greater than +0.58 or lower than −0.58 were considered as differentially expressed.

2.4. Bioinformatic analyses for target processes prediction

TCGA (www.cbioportal.org/) was interrogated for the oncprint of lncRNAs. This tool allows to obtain the genomic profile of the genes of interest in a cohort of patients by selecting the specific type of cancer. The oncprint represents the percentage of genetic alterations and permits the comparison of the status of several genes in the same patient.

DIANA TOOLS (diana.imis.athena-innovation.gr/) was used to retrieve predicted microRNA targets and Gene Ontology (GO) processes in which it was predicted its involvement.

For these analyses, DIANA-mirPath v3.0 has been applied to obtain miRNA and pathway-related information. mirPath utilizes predicted miRNA targets (in CDS or 3’UTR regions) provided by the DIANA algorithms (TarBase v.7.0, microT-CDS v.5.0 and TargetScan) or even experimentally validated miRNA interactions.

The reverse search tool has been used in order to identify all miRNAs targeting a specific GO pathway. The module takes as input a GO biological process name or an identification code. Based on the algorithm and the specie of interest, a list of the miRNAs targeting the selected pathways and the relative target genes is generated. 
3. Results

3.1. MicroRNAs modulated by resveratrol and pathways potentially affected

In a first analysis, where the statistical analysis for differential expression of miRNAs between control and RV-treated cells was based on a log2 fold-change >0.2 (for up-regulated) or < -0.2 (for down-regulated) with adjusted p-value <0.01, a total of forty-four up-regulated miRNAs and fifty-four down-regulated miRNAs were identified (heat-map in Fig. S1). In a more stringent statistical analysis, where the criterion for differentially expressed miRNAs in control and RV-treated cells was a log2 fold-change >0.585 (for up-regulated) and <-0.585 (for down-regulated) with adjusted p < 0.01, six miRNAs and one miRNA were found up- and down-regulated, respectively (heat-map in Fig. 1). We used the DIANA software to get a first insight on the predicted pathways in which these miRNAs could be involved. The unsupervised hierarchical clustering analysis of the cellular processes affected by these miRNAs is shown in Fig. 2. The trend of miRNAs up-regulated by RV appears to cluster together, and consistently indicates ‘organelle’ process as the major process implicated in their regulation. Other processes significantly associated with the modulation of these miRNAs include protein metabolism and function, catabolic processes, phosphatidylinositol signaling and gene expression regulation (Fig. 2). The unequivocal in silico identification of the miRNA

Fig. 1. Heat-map of microRNAs affected by Resveratrol (RV) treatment. Heat-map showing the OVCAR-3 expression profiles of microRNAs differently modulated upon RV treatment (third and fourth column) compared to control condition (first and second column). Green and red bars represent down-regulation and up-regulation, respectively.

Fig. 2. miRNAs versus pathways heat-map. Darker and lighter colors show lower and higher significance values. The dendrogram exhibits hierarchical clustering for miRNAs and pathways, based on similar pathway targeting patterns.
Table 1
Opposite impact on OVCA-3 miRNome by Resveratrol (RV) treatment and pathways versus target genes. Resveratrol (RV) positively modulates seven miRNAs and negatively affects one miRNA. The table is created as a reverse search, starting with a biological process of interest as input (first column) to catch miRNAs targeting the selected pathway (here we just filtered miRNAs modulated by Resveratrol, showing in the second column). Along each pathway and their relative miRNAs, we identify the corrispective miRNAs target genes (fourth column).

| Pathway | miRNA | logFC | Target |
|---------|-------|-------|--------|
| EMT Cell migration | hsa-miR-1207-5p | 1.345089789 | HBEGF, SH3BP1, DGKZ, ODXL |
| | hsa-miR-3665 | 1.330782842 | NOTCH1, CPG6 |
| | hsa-miR-1225-5p | 0.962634046 | LIFT |
| | hsa-miR-3663-3p | 0.951629515 | TGFβ2 |
| | hsa-miR-1915-3p | 0.870670136 | GSK3B |
| | hsa-miR-4271 | 0.696628837 | TGFβR3, FGFR2 |
| | hsa-miR-494 | -0.668439833 | PTEN |
| Glucose metabolic process | hsa-miR-1207-5p | 1.345089789 | GAPDH, PFKL, HK1, PGAM1, SLC25A1 |
| | hsa-miR-4281 | 1.192915761 | GAPDH, PGCB |
| | hsa-miR-4271 | 0.696628837 | PPFRB2, SORD |
| Autophagy | hsa-miR-1207-5p | 1.345089789 | RPLP1, GOSR2, ACG12, PDDN1, IGF1 |
| | hsa-miR-3665 | 1.330782842 | RPL26, RPL5 |
| | hsa-miR-4281 | 1.192915761 | TBCD |
| | hsa-miR-3663-3p | 0.951629515 | ZKSCAN3, TMEM208, LAMTOR1, MGA75, RPS11 |
| | hsa-miR-1915-3p | 0.870670136 | RPL18A, EIF4G1, S6ENP5 |
| | hsa-miR-4271 | 0.696628837 | IGF1, SYVN1 |
| | hsa-miR-494 | -0.668439833 | EP5G, USP13, ATG4A, ATG4C, RNF152 |
| Cell death Apoptotic process | hsa-miR-1207-5p | 1.345089789 | BIRC6, PIM1, NUAK2, ARE1, DHCR24, EGLN3 |
| | hsa-miR-3663-3p | 0.951629515 | APC |
| | hsa-miR-1915-3p | 0.870670136 | FAMM2, BCL2 |
| | hsa-miR-4271 | 0.696628837 | S100A14, BCL2L1, FGF1, MAPK1 |
| | hsa-miR-494 | -0.668439833 | ROCK1, KLF11, CLPTM1L, SMNDC1, PAWR, RNF152, BBIC3, PTEN |
| Stem cell maintenance Stem cell proliferation | hsa-miR-1207-5p | 1.345089789 | WNT7B |
| | hsa-miR-4271 | 0.696628837 | WNT7B, TRIM71 |
| Drug metabolic process Drug export | hsa-miR-1207-5p | 1.345089789 | CYFSE1 |
| | hsa-miR-3663-3p | 0.951629515 | FMOD1 |
| | hsa-miR-4271 | 0.696628837 | EPXQ2 |

Table 2
miRNAs role in cancer. The table shows miRNAs modulated by Resveratrol (Table 2A for up-regulated and Table 2B for down-regulated, respectively); their epigenetic mechanism in cancer is indicated along the bibliographic references. For full references refer to Supplementary file 1 Reference List.

A. Up-regulated miRNAs.

| miRNA | Mechanism | Gene Target | Cancer | Reference |
|-------|-----------|-------------|--------|-----------|
| miR-1207-5p | Inhibits tumor growth, invasion and metastasis | hTERT, CSFI | Gastric | [64] |
| | Inhibits EMT induced by TGF-β and EGF, by indirectly down-regulating PI3K/AKT pathway, STAT3 and some important inflammatory mediators | | Lung | [65] |
| | Suppresses invasion and metastasis by targeting genes related to cell migration. | CD151 | Nasopharyngeal | [66] |
| | Prevents tumor growth and invasion through the inhibition of AKT/mTOR signaling pathway. | FASN | Hectocellular | [67] |
| | Increases sensitivity to gemcitabine and reduces cancer growth. | SRC | Pancreatic | [68] |
| miR-1225-5p | Lower expressed in stage III and IV compared to I and II; suppresses cell migration and invasion. | IRS1, SIRT3 | Glioblastoma | [69] |
| | Prevents tumor cell proliferation and metastasis by inhibiting the activation of Wnt/β-catenin pathway. | | Thyroid | [70] |
| | Acts as tumor suppressor by preventing tumor growth, metastasis and invasion through down-regulation of β-catenin. | IRS1 | Gastric | [71] |
| miR-1915-3p | Reduces cell migration and proliferation. | SETD1A, BCL-2 | Breast | [72] |
| | Inhibits tumor progression and promotes apoptosis. | | Gastric | [73] |

B. Down-regulated miRNAs.

| miRNA | Mechanism | Gene Target | Cancer | Reference |
|-------|-----------|-------------|--------|-----------|
| miR-494 | Inhibits apoptosis and promotes cell growth and invasiveness through PTEN/AKT signaling. | PTEN, GSK3B | Colon | [74] |
| | Stimulates tumor progression and proliferation by activating Wnt/β-catenin pathway. | | Colon | [75] |
| | Promotes tumorigenesis through the inhibition of apoptosis induced by cisplatin. | CASP2 | Lung | [76] |
| | Associated to poor prognosis and bad clinical outcome, promotes carcinogenesis, tumor growth and proliferation by up-regulating PI3K/AKT pathway. | | Cervical | [77] |
| | Contributes to cell cycle progression, cell viability, invasion and migration via activation of PI3K/AKT pathway. | PTEN | Hepatocellular | [78] |
targets and pathways is challenging, because the miRNAs interacting networks have not yet been fully mapped. To get more insights on the relevance of the miRNAs modulated by RV we have pursued a practical direct approach by checking whether these miRNAs were indeed involved in the regulation of malignant features. We chose to focus on the processes that mainly influence the progression and recurrence of ovarian cancers. The following processes were considered: cell metabolism (essentially of glucose), macromolecular cell homeostasis (essentially organelle and protein turnover mediated by autophagy), drug resistance, cell death, stemness, cell migration and Epithelial-to-Mesenchymal Transition (EMT). The miRNAs of interest and their targets involved in these processes were selected and used to build Table 1. All the miRNAs modulated by RV appear involved in the malignant features that characterize cancer. To further substantiate the potential involvement in cancer biology of the miRNAs modulated by RV we made a literature search using as key words the ‘miRNA name’ of interest and ‘cancer’. The data are reported in Table 2. Surprisingly, only a few of these miRNAs have been explored for their involvement in cancer. The most relevant publications were referring only to three of the miRNAs up-regulated (namely, miR-1207-5p, miR-1225-5p, and miR-1915-3p) and to the only one down-regulated (miR-494) by RV.

3.2. Long non-coding RNAs modulated by resveratrol and pathways potentially involved

Microarray analysis of lncRNAs differentially expressed in control and RV-treated OVCAR-3 cells selected for differences in the expression of logFC > 0.2 or < -0.2 for up- and down-regulation, respectively, revealed changes in a total of fifteen lncRNAs, of which five were up-regulated and ten were down-regulated (heatmap in Fig. 3 and Table 3). The literature search revealed that three of the lncRNAs up-regulated by RV were involved in processes inhibiting cancer progression through facilitating apoptosis, blocking cell proliferation and cell migration, and by inducing autophagy (Table 4A) and, vice versa, ten of the lncRNAs down-regulated by RV were acting in oncogenic pathways favoring the progression of several types of cancers (Table 4B). To further understand the clinical relevance of these lncRNAs in ovarian cancer pathogenesis and progression, we interrogated the TCGA database for the presence of altered expression in human samples. The oncoprint relative to the fifteen lncRNAs of interest in one hundred-eighty-two patients is shown in Fig. 4. It appears evident that PVT1 presents alterations in 45% of the cases, UCA1 is altered in 14% of the cases, and HULC is altered in 11% of the cases, XIST is altered in just one case, while HNF1-AS1 and ARHGAP27P1 (also known as LOC146880) show no alterations at all, and all the others present alterations comprised between is 1% and 7%. To be noted, while NBR2 tends to be expressed at very low level all other alterations essentially consist in gene amplification. Approximately 6% of the cases presents both UCA1 and PVT1 or both PVT1 and HULC genes amplification.

3.3. Cancer-related processes regulated by resveratrol-modulated non-coding RNAs

Based on the data above, we have summarized in a visual form the pathways and biological processes in which the non-coding RNAs modulated by RV in ovarian cancer cells are involved and through which they can impinge on the cancer features. The cartoons in Figs. 5 and 6, respectively, illustrate how RV may effectively contrast the malignant behaviour of cancer cells through the up- or down-regulation of miRNAs (Fig. 5) or of lncRNAs (Fig. 6).

4. Discussion

It is now well demonstrated that cancer genesis and progression result not only from gene mutations but also from epimutation in genes that control cell behaviour and cell-to-cell communication. Epimutations consist in the regulation of gene expression through mechanisms that involve the accessibility of the gene, its transcription as well as the stability and translation of the messenger RNA. Non-coding RNAs, which include miRNAs and lncRNAs among others, are part of the third epigenetic mechanism.34 Non-coding
Table 4

LncRNAs role in cancer. The table shows lncRNAs modulated by Resveratrol (Table 4A for up-regulated and Table 4B for down-regulated lnc-RNAs respectively); their epigenetic mechanism in cancer is indicated along the bibliographic references. For references refer to Supplementary List.

### A. Up-regulated lncRNAs.

| LncRNA | Mechanism | Cancer | Reference |
|--------|-----------|--------|-----------|
| HOTAIR | Induces ATG7 up-regulation promoting autophagy as a protective mechanism of radioresistance. | Pancreatic | [103] |
|        | Acts under stress conditions interacting with AMPK promoting its activation. | Hepatocellular | [103] |
| NBR2   | Acts as tumor suppressor preventing proliferation, invasion and migration through NOTCH1 regulation. | Osteosarcoma | [105] |

### B. Down-regulated lncRNAs.

| LncRNA | Mechanism | Cancer | Reference |
|--------|-----------|--------|-----------|
| GAS5   | Decreases miR-106a-5p expression levels to control cell proliferation, invasion and migration by inactivating the Akt/mTOR pathway. | Gastric | [93] |
|        | Enables apoptosis and prevents cell proliferation through a negative regulation of miR-182-5p expression in order to inhibit FOX03a degradation. | Colorectal | [94] |
|        | Inhibits cell growth and migration by sponging miR-21 and increasing SPRY2 transcription. | Ovarian | [95] |
|        | Inhibits proliferation and invasion directly binding miR-196a-5p with a negative interaction to prevent downstream FOXO1/PI3K/AKT pathway activation. | Breast | [96] |
|        | Prevents tumor cell proliferation and invasion through PI3K/AKT/mTOR pathway down-regulation. | Esophageal | [97] |
|        | Suppresses tumor progression and cell proliferation by reducing the expression and the secretion of IL-10 and VEGF-A through NF-κB and Erk1/2 pathway regulation. | Colorectal | [98] |
|        | Enhances chemosensitivity and promotes G0/G1 cell cycle arrest and apoptosis by modulating PARP1 expression through a direct interaction with EZF4 to its promoter. | Ovarian | [99] |
|        | Decrease tumor growth and proliferation via regulating the Akt/mTOR pathway by sponging miR-103. | Prostate | [100] |
|        | Inhibits tumor growth and increases radiosensitivity down-regulating miR-135b expression levels. | Lung | [101] |
| lncRNA | Mechanism | Cancer | Reference |
|-------|-----------|--------|-----------|
| LINC00092 | Stimulates CAI-mediated cancer progression and enhances glycolysis by directly interacting with PFKFB2. | Ovarian | [140] |
| | Promotes glucose metabolism and the Warburg effect by activating mTOR/STAT3 signaling which prevents mTOR-143 mediated HK2 degradation. | Bladder | [141] |
| | Induces aggressive radio-resistance phenotype, cell cycle progression and cell growth by promoting PI3K/AKT signaling. | Prostate | [142] |
| | Stimulates tumor growth and metastasis through an epigenetic control mediated by EZH2 leading to E-cadherin and p21 expression repression. | Gastric | [143] |
| | Expression induced by TGF-β pathway to promote EMT and stemness in a Slug positively dependent manner, downstream factor of TGF-β signaling. | Gliona | [144] |
| | Transcription factor C/EBPβ promotes its expression in order to maintain tumor progression and development. | Bladder | [145] |
| | Promotes carcinoma development preventing PD1 expression repressed by miR-25-2a/δ, -193a and -214. | Gastric | [146] |
| | Increases tumor progression with Wnt/β-catenin activation pathway by up-regulating p-GSK3-β protein levels. | Breast | [147] |
| | Stimulates cell proliferation and invasion by up-regulating GSK3-β and β-catenin. | Thyroid | [148] |
| | Hampers apoptosis and cell cycle arrest by sponging miR-143 to exert a positive regulation on MAPK1. | Lung | [149] |
| | Directly interacts with miR-203 by preventing BE2 degradation in order to induce migration, invasion and metastasis. | Gastric | [150] |
| | Down-regulates miR-182 to promotes cell viability, invasion and proliferation up-regulating TIMP2. | Osteosarcoma | [151] |
| | Stimulates cancer proliferation and cell cycle progression by repressing p21 through methylation promoter repressing miR-405. | Renal | [152] |
| | Negatively modulates miR-122 promoting cell proliferation, invasion and migration. | Gliona | [153] |
| | Acts as oncogene to promote cell growth and metastasis by sponging miR-204 and activating CXCR4. | Prostate | [154] |
| | Increases chemoresistance and induces apoptosis up-regulating SF1 through sponging miR-184. | Oral | [155] |
| | Enhances CREB1 expression to promote proliferation and invasion by directly interacting with miR-99a-3p. | Gastric | [156] |
| | Induces cell migration and invasion acting on Wnt/β-catenin pathway by preventing miR-122 expression. | Oral | [157] |
| | Facilitates cell proliferation and cell cycle phases transition by directly binding with EZH2 to increase cyclin D1 expression. | Gastric | [158] |
| | Leads to cell proliferation, invasion and migration activating Wnt/β-catenin signaling pathway. | Laryngeal | [159] |
| | Promotes cell viability and enhances cisplatin resistance by increasing Wnt6 expression to stimulates Wnt pathway. | Bladder | [160] |
| | Increases cancer glycolysis through preventing PFKFB2 degradation mediated by miR-182. | Gliona | [161] |
| | Positively regulates proliferation, invasion and migration and suppresses apoptosis by repressing miR-96 and up-regulating FOXO3. | Pancreatic | [162] |
| UCA1 | Supports chemoresistance by enhancing glycolysis acting as a molecular sponge on miR-125a preventing HK2 repression. | Leukemia | [163] |
| | Promotes cell viability, migratory and invasiveness properties and inhibits apoptosis by negatively affecting miR-182 to prevent TIMP2 degradation. | Gastric | [164] |
| | Represses miR-28-5p to increase cell proliferation and invasion mediated by HOXB3 activity. | Colon | [165] |
| | Regulates cell proliferation and inhibits apoptosis through a positive regulation of autophagy pathway. | Colorectal | [166] |
| | Enhances cell cycle progression and cell viability by directly interacting with EZH2 and facilitating p21 promoter methylation. | Breast | [167] |
| | Increases TGFβ1-induced EMT through JAG1 and Notch signaling by negatively affecting miR-124. | Tongue | [168] |
| | Facilitates apoptosis inhibition, cell viability and EMT process throughmiR-15a repression and Hippo JNK pathway promotion. | Thyroid | [169] |
| | Increases cisplatin resistance and inhibits apoptosis by targeting miR-143 and positively modulating FOSL2. | Ovarian | [170] |
| | Activates ERK signaling pathway and promotes FGFRI-mediated cell growth and metastasis through miR-216b repression. | Hepatocellular | [171] |
| | Positively regulates tumorogenesis by modulating PI3K/AKT/mTOR signaling mediators. | Ovarian | [172] |
| | Correlates with poor clinical outcomes and facilitates cancer growth and development AKT/GSK3β/CDCN1 signaling pathway. | Ovarian carcinoma | [173] |
| | Induces cell growth by silencing PTEN/AKT signaling pathway. | Osteosarcoma | [174] |
| | Targets oncosuppressor miR-129 preventing SOX4 repression to promotes proliferation, invasion and apoptosis inhibition. | Renal | [175] |
| | Positively modulates JGBPs expression by sponging miR-204 to enhances proliferation and invasion. | Thyroid | [176] |
| | Acts as a competing endogenous RNA targeting miR-145 to increase cancer cell invasion and migration. | Nasopharyngeal | [177] |
| | Increases drug resistance by positively modulating autophagy through ATG7 expression repressing miR-582-5p. | Bladder | [178] |
| |Regulates proliferation and metastasis by directly binding miR-144 and preventing PBX3 degradation. | Lung | [179] |
| | Positively impacts on drug resistance by repressing miR-129 and promoting ABCB1 expression. | Ovarian | [180] |
| | Enhances HMGBl expression by repressing anti-tumor miR-193a to sustain cancer cell proliferation and migration. | Lung | [181] |
| | Stimulates colony formation, proliferation, EMT and radiosensitivity. | Colorectal | [182] |
| | Increases cell viability by promoting AKT and mTOR activating phosphorylation to maintain tamoxifen resistance. | Breast | [183] |

**Continued on next page**
### B. Down-regulated lncRNAs.

| lncRNA | Mechanism | Cancer | Reference |
|--------|-----------|--------|-----------|
| MEG3   | Increases cell growth, invasion and metastasis by down-regulating miR-127 to reduce ZEB1 down-regulation. | Osteosarcoma | [237] |
|       | Enables chemo-resistance through SIRT1-mediated autophagy acting as miR-194 sponge to prevent SIRT1 miRNA-mediated inhibition. | Colorectal | [238] |
|       | Promotes migratory capability and metastasis by interacting with miR-200a to positively modulate ZEB1/2-induced EMT. | Lung | [239] |
|       | Promotes tumor growth and migration and inhibits apoptosis by silencing miR-143 so that its direct target RUNX2 can activate PI3K/akt pathway. | Retinoblastoma | [240] |
|       | Reduces chemosensitivity inhibiting apoptosis and promoting cell survival by activating AKT pathway. | Breast | [241] |
|       | Induced by oxidative stress acts sponging miR-let-7a/7b to promotes inflammatory role of IL-6 in tumor microenvironment. | Cholangiocarcinoma | [242] |
|       | Positively regulates STAT3 expression and its downstream targets to enhance cell proliferation, migration and invasion. | Esophageal | [243] |
|       | Enhances tamoxifen chemoresistance through autophagy by reducing methylation in Beclin-1 promoter. | Breast | [244] |
|       | Promotes FOXM1-mediated cancer invasion and cell cycle by negatively regulating miR-342-3p. | Gastric | [245] |
|       | Increases EMT process by preventing miR-194-5p-induced FOXM1 degradation. | Colorectal | [246] |
|       | Regulates cancer development through β-catenin/ck4-3 expression and EMT markers up-regulation. | Tongue | [247] |
|       | Promotes tumor growth and migration and reduces miR-138-5p expression to increase SIRT1 activity. | Cervical | [249] |
|       | Facilitates cell viability and EMT process through STAT3 up-regulation by sponging miR-29b-3p. | Lung | [250] |
|       | Activates as a molecular sponge to miR-152 increasing cell proliferation and invasion through DNMT1 over-expression. | Breast | [251] |
|       | Plays an oncogenic role promoting cell migration, invasion and EMT process by negatively regulating miR-484 expression, preventing ROCK2 repression. | Lung | [252] |
|       | Stimulates cancer cell proliferation, migration and invasion induced by STAT3 by antagonizing miR-93-5p. | Breast | [253] |
|       | Down-regulates E-cadherin expression through methylation mechanism and promotes EMT-related factors. | Lung | [254] |
|       | Increases cancer development directly interacting with miR-17 to prevent STAT3 repression. | Lung | [255] |
|       | Positively affects proliferation, invasion and migration by stimulating NF-κB and PI3K/AKT pathways. | Melanoma | [256] |
| PVT1   | Increases glycolysis in cancer metabolism by preventing miR-497-mediated HK2 degradation, acting as miRNA sponge. | Osteosarcoma | [212] |
|       | Up-regulates SOX2 promoting cancer cell invasion and proliferation. | Ovarian | [213] |
|       | Act as a negative regulator of miR-133a, promoting cell proliferation, cell cycle progression and tumor growth. | Ovarian | [214] |
|       | Stimulates cancer cell proliferation and inhibits apoptosis up-regulating BCL-2 expression proteins by sponging miR-497. | Lung | [215] |
|       | Positively regulates apoptosis and autophagy by targeting miR-216b to prevent Beclin-1 degradation in order to reduce cisplatin sensitivity. | Lung | [216] |
|       | Down-regulates p21 to promote EMT, cell proliferation and migration. | Pancreatic | [217] |
|       | Represses LATS2 transcription by recruiting EZH2 on its promoter and increases cancer cell proliferation. | Lung | [218] |
|       | Induces cell propagation and prevents apoptosis stabilizing EZH2 and MDM2 proteins and repressing p53 expression. | Hepatocellular | [219] |
|       | Stimulates glucose metabolism by sponging miR-143 and enhancing HK2 expression levels. | Gallbladder | [220] |
|       | Promotes radioresistance by directly interaction with miR-195 acting as a molecular sponge. | Lung | [221] |
|       | Inhibits miR-200b expression by recruiting EZH2 on its promoter and promotes cancer proliferation, cell cycle progression and migration. | Cervical | [222] |
|       | Correlates with poor clinical outcome and increases proliferation and invasion by up-regulating EZH2. | Glioma | [223] |
|       | Promotes tumorigenesis, EMT and metastasis by increasing TWIST1 expression levels through miR-186 sponging. | Prostate | [224] |
|       | Induces proliferation and metastatic capability via GREM1 by acting as molecular sponge on miR-128-3p. | Glioma | [225] |
|       | Negatively regulates miR-424 to increases cancer cells proliferation, migration and invasion abilities. | Cervical | [226] |
|       | Acts as a competitive endogenous DNA by modulating miR-497 expression to increase cell viability and invasion. | Lung | [227] |
|       | Promotes tumorigenesis and cancer progression by directly interacting with miR-128 to prevent its binding to VEGF. | Bladder | [228] |
|       | Positively affects cancer colony formation, migration and invasion by repressing miR-31 and enhancing CDK1 expression. | Bladder | [229] |
|       | Increases drug-resistance by inhibiting apoptosis through BCL-2 activation. | Gastric | [230] |
|       | Stimulates tumor growth, invasion and metastasis acting as a competitive endogenous DNA on miR-26b. | Colon | [231] |
|       | Sustains cancer progression and development via p38 phosphorylation. | Prostate | [232] |
|       | Inhibits apoptosis and cancerc cell proliferation by positively modulating M CL-1 stability. | Breast | [233] |
|       | Acts as oncogene in cancer progression promoting cancer cell development by enhancing chemoresistance, through HIF-1α stabilization and acetyltransferase induction. | Nasopharyngeal | [234] |
|       | Promotes cancer cell growth and EMT process by increasing migratory markers expression and activating TGF-β/Smad pathway. | Pancreatic | [235] |
|       | Stimulates cancer progression by angioinvasion through STAT3 binding and VEGF activation. | Gastric | [236] |
| H19   | Correlates with migration and invasion and positively modulates EMT through PI3K/AKT pathway activation. | Breast | [196] |
|       | Increases cyclin D1 expression by sponging miR-34a to support cell viability, invasion and migration. | Osteosarcoma | [197] |
|       | Stimulates proliferation, invasion, migration and angiogenesis by increasing FGF2 protein levels secretion from tumor-associated macrophages. | Thyroid | [198] |
|       | Induces EGFR-mediated cell growth and motility by negatively regulating miR-195. | Hepatocellular | [199] |
|       | Promotes tumor progression and development through preventing STAT3-degradation mediated by miR-125b. | Oral | [200] |
|       | Positively regulates cancer progression and development by modulating CD99 through miR-206 repression. | Osteosarcoma | [201] |
|       | Enhances Wnt/β-catenin signaling activation to sustain EMT process. | Tongue | [202] |
|       | Promotes invasion and metastasis preventing SLAIN2 degradation by directly binding to miR-106b-5p. | Colorectal | [203] |
|       | Play an oncogenic role in tumorigenesis acting as a metastatic enhancing RNA for miR-429. | Renal | [204] |
|       | Promotes cancer initiation and progression through EZH2 and β-catenin up-regulation. | Esophageal | [205] |
|       | Associated with metastasis and low overall survival, increases proliferation and invasion by sponging miR-206 to prevent ANXA2 and KRAS suppression. | Gallbladder | [206] |
|       | Stimulates invasion and migration increasing EMT process through β-catenin and NF-κB signaling pathway activation. | Oral | [207] |
|       | Correlates with migration and invasion and positively modulates EMT through PI3K/AKT pathway activation. | Cholangiocarcinoma | [208] |
|       | Accelerates EMT and cancer progression by negatively affecting miR-124. | Lung | [209] |
|       | Promotes tumor development under hypoxia condition by repressing the onco-suppressor miR-200a. | Hepatocellular | [210] |
|       | Correlates to bad clinical outcome, facilitates tumor development and cell cycle progression by directly interacting with miR-129-5p. | Breast | [211] |
RNAs arise from gene expression that is influenced by environmental stimuli. Cell-to-cell interactions as well as availability of nutrients, oxygen, cytokines and drugs in the tumour microenvironment have a great epigenetic impact on cancer cell behaviour through modulation of non-coding RNAs.35

Herbal- and dietary products-derived phytochemicals with therapeutic potential, also known as nutraceuticals, are attracting the interest of cancer biologists because of their ability to impact on the epigenome of cancer cells, thus opening to novel effective and less toxic therapeutic strategies in the prevention and cure of cancer.27,36

Ovarian cancer is among the leading causes of death in the field of gynecological cancers in most developed countries. Epigenetics clearly plays a role in ovarian cancer pathogenesis and progression.16,17,19–21

RV is a nutraceutical polyphenol with anti-cancer potential.22,23,24 RV has been shown effective in causing cancer cell death and cancer cell senescence38–46 and to inhibit cancer cell invasion and metastasis30,47–49 in several in vitro and in vivo models, including ovarian cancer.30,42,49 It has been shown that autophagy contributes to RV-induced apoptosis in ovarian cancer cells.50

Further, RV was shown to antagonize EMT and invasion of ovarian cancer cells by activating the SIRT1 pathway.51

Table 4 (continued)

| LncRNA          | Mechanism                                                                 | Cancer                  | Reference |
|-----------------|---------------------------------------------------------------------------|-------------------------|-----------|
| **B. Down-regulated lncRNAs.** |                                                                           |                          |           |
| **HULC**        | Promotes carcinogenesis inducing PTEN degradation via autophagy-mediated system by inhibiting miR-15a maturation and stimulating LC3 expression. | Ovarian                  | [270]     |
| **HOTAIRM1**    | Increases proliferation and invasion stimulating HOXA1 oncogene by keeping away histone and DNA methyltransferase from its promoter. | Promotes cell viability and metastasis modulating DLGAP1 by sponging miR-148a from its promoter. | Glioblastoma | [278] |
| **HNF1A-AS1**   | Oncogene inhibiting apoptosis and promoting autophagy by sponging mir-30b-5p preventing BCL-2 and ATM5 degradation. | Promotes cell proliferation, migration and apoptosis by sponging miR-30b-5p. | Bladder    | [281] |
|                 | EGR-1-induced transcription increases cell proliferation, migration and apoptosis by promoting cell cycle progression. | STAT3-mediated expression stimulates EMT by positively regulating NOTCH signaling pathway. | Prostate   | [282] |
|                 | STAT3-mediated expression stimulates EMT by positively regulating NOTCH signaling pathway. | STAT3-mediated expression stimulates EMT by positively regulating NOTCH signaling pathway. | Prostate   | [282] |
|                 | STAT3-mediated expression stimulates EMT by positively regulating NOTCH signaling pathway. | STAT3-mediated expression stimulates EMT by positively regulating NOTCH signaling pathway. | Prostate   | [282] |
|                 | STAT3-mediated expression stimulates EMT by positively regulating NOTCH signaling pathway. | STAT3-mediated expression stimulates EMT by positively regulating NOTCH signaling pathway. | Prostate   | [282] |
|                 | Promotes cancer development increasing CDK6 expression through miR-149-5p repression. | Promotes cancer development increasing CDK6 expression through miR-149-5p repression. | Lung       | [285] |
| **RTKN**        | Induces cell migration and invasion by positively affecting Wnt/β-catenin pathway. | Positively correlated with H19, enhances proliferation, migration, invasion and cell cycle progression. | Prostate    | [287] |
|                 | RV increases cell cycle progression by directly interacting with EZH2 and repressing NKO1 and p21 protein levels. | RV increases cell cycle progression by directly interacting with EZH2 and repressing NKO1 and p21 protein levels. | Prostate    | [288] |
| **HMGA2**       | RV increases cell cycle progression by directly interacting with EZH2 and repressing NKO1 and p21 protein levels. | RV increases cell cycle progression by directly interacting with EZH2 and repressing NKO1 and p21 protein levels. | Prostate    | [289] |

Phytochemicals have been shown to elicit anti-cancer activities via epigenetics.32–35 In this work we focus on the modulatory activity of non-coding RNAs in ovarian cancer cells exposed to RV. The OVCAR-3 cell line, isolated from the ascites of a malignant and multi-drug resistant ovarian adenocarcinoma, was chosen as representative ovarian cancer cells.

We found that a 24 h incubation of OVCAR-3 cells with 100 nM RV, a dose clinically relevant,56 results in the modulation of several miRNAs and lncRNAs that potentially target molecular pathways involved in the malignant phenotype. Using a log base 2-fold change (logFC) greater than 0.58 or lower than –0.58 (corresponding to 1.5-fold expression) as a threshold for differentially
expressed non-coding RNAs, it was found that RV up-regulated seven miRNAs and five lncRNAs and down-regulated two miRNAs and ten lncRNAs. The miRNAs modulated by RV have been found dysregulated and involved in the malignant aspects of ovarian cancer.17 Interestingly, the three miRNAs mostly up-regulated by RV, i.e. miR-1207-5p, miR-3665 and miR-4281, consistently regulate autophagy and glucose metabolism (Table 1), two pathways that are dysregulated in ovarian cancers.10,11,16,57,58 It is worth noting that two miRNAs up-regulated by RV, namely miR-1207-5p and miR-1225-5p were shown to limit EMT and cancer metastasis in gastric, lung and nasopharyngeal cancers (the former) and to inhibit progression and metastasization of glioblastomas (the latter). For miR-494, contradictory results were found. In some cases, this miRNA acts as an oncomiRNA by targeting the onco-suppressor PTEN (Table 2), while in other cases it seems to act as a tumor-suppressive miRNA since its over expression can inhibit apoptosis and promote cell proliferation of cancer cells. Such apparent contradiction may have at least two possible explanations, both very likely. First, the phenotypic outcome arising from the modulation of a given miRNA is cell-context in that it depends on the genetic background and metabolic status of the cell. Thus, miR-494 may be oncogenic or tumor suppressive in cancer cell lines of different origin and in different environmental conditions. Another possible explanation is that miR-494-3p and miR-494-5p have specificity for different targets thus eliciting different functional effects, and in some studies the 5p or 3p strand was not specified.
Among the fifteen lncRNAs modulated by RV, five were found up-regulated in ovarian cancer TCGA database because of gene amplification (PVT1, UCA1, HULC and GASS) or of mRNA hyper-expression (MEG3). Interestingly, PVT1, UCA1, HULC and MEG3 have been shown to act as oncogenic lncRNAs in a variety of cancers by promoting cell proliferation, cell migration, metastasis, glycolysis, multi-drug resistance (Table 4), and RV down-regulates their expression in ovarian cancer cells. On the other hand, GASS acts as a tumor-suppressive non-coding RNA in a variety of cancer types (Table 4), and RV up-regulated its expression in ovarian cancer (Table 3). To be noted, NBR2 that is found expressed at low level in ten patients (out of 182) in the TCGA database (Fig. 4) and that acts as a tumor suppressor (Table 4) was up-regulated in OVCAR-3 cells exposed to RV. The treatment also down-regulated the expression of XIST, LINC00092, H19, MALAT1 that were shown to act as oncogenic lncRNAs.

Taken together, RV was found to modulate in OVCAR-3 cells non-coding RNAs that consistently opposed oncogenic pathways (summarized in Figs. 5 and 6). One limitation of the present study is that these effects have not been validated in the cells. To this end, experiments are in progress. Further studies shall identify the relevant target molecules of these non-coding RNAs.

Nonetheless, the data here reported substantiate the view that RV has the potential to counteract the progression of ovarian cancer and add to the known anti-cancer mechanisms of this nutraceutical, thus supporting its potential harnessing as an adjuvant therapeutic. In this regard, RV appears well tolerated in both animals and humans and no marked toxicity has been reported in the ongoing clinical trials (recorded on clinicaltrial.gov) testing its anti-cancer effectiveness. RV is an hormetic drug that promotes opposite effects depending on the concentration used. Thus, in the clinical practice the choice of the appropriate dose for obtaining the desired effect depends on some characteristics and habits of the patient in terms of microbiota, hormones, gender, etc. One caveat for the clinical exploitation of RV is its poor bioavailability in the systemic circulation, since it is efficiently absorbed after oral administration and rapidly and extensively metabolized. To overcome such limitations and improve its anti-cancer benefits and pharmacokinetic profile, novel analogs of RV and nano-platforms for its targeted delivery are under development.

Section

Special Issue "Nutraceuticals and Diet in Human Health and Disease”.

Declaration of competing interest

No conflict of interest, financial or otherwise, to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcme.2020.02.006.

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