Could miR-34a Inhibition be Used as a Tool to Overcome Drug Resistance in MCF-7 Cells Treated with Synthesized Steroidal Heterocycles?

Shaymaa M M Yahya¹, Mervat M Abd-Elhalim¹, Abdou O Abdelhamid², Emad F Eskander¹, Ghada H Elsayed¹*

Abstract

Background: Progesterone derivatives have explored an improved effect on human cancer cells through combination of the explored heterocycles with progesterone moiety. miRNAs have an important role in moderating cancer cell survival, proliferation and drug resistance. The current study tested the hypothesis “whether miR-34a inhibitor has a negative impact on apoptosis and angiogenesis in MCF-7 cells treated with newly synthesized progesterone derivatives”. Methods: MCF-7 cells were treated with progesterone derivatives individually and in combination with miR-34a inhibitor. miR-34a expression levels were measured in MCF-7 cells treated with progesterone derivatives using QRT-PCR. MCF-7 cells treated with progesterone derivatives individually showed increased miR-34a expression levels. miR-34a deficient cells were treated with the newly synthesized progesterone derivatives, after that, apoptotic and angiogenic gene expression levels were determined using QRT-PCR. The studied genes were as follows: apoptotic (Bcl-2, survivin, CCND1, CDC2, P53 and P21) and angiogenic (VEGF, Hif-1α, MMP-2, Ang-1, Ang-2, and FGF-1).

Results: The results showed that miR-34a deficient MCF-7 cells treated with the newly progesterone derivatives still have promising effects on apoptotic and angiogenic genes. Besides, results revealed that miRNA-34a deficient MCF-7 cells exhibited improved effect of tested compounds in some apoptotic and angiogenic genes such as CDC-2, MMP-2.

Conclusion: These results revealed that miR-34a inhibitor did not have remarkable negative effect on apoptosis and angiogenesis. On contrary, it showed an improved effect on some genes. And consequently, miR-34a inhibitor could be used safely as a tool to tackle drug resistance in breast cancer cells.

Keywords: Breast cancer- progesterone derivatives- miR-34a inhibitor- drug resistance- apoptotic and angiogenic genes

Introduction

Breast cancer was found to account for 22% of newly discovered cancer cases each year in women. Besides, it is the most leading cause of mortality and the second most commonly diagnosed cancer (DeSantis et al., 2014). Drug resistance represents the major cause for unsuccessful chemotherapeutic strategies (Pasquier et al., 2011). Etoposide, doxorubicin, paclitaxel, topotecan, and 5-fluorouracil are commonly used chemotherapeutic agents for breast cancer; however, multidrug resistance can develop against these agents. It was reported that MicroRNAs (miRNAs) are key players in drug resistance (Yu et al., 2015). miRNAs have pivotal roles in moderating cancer cell survival, proliferation and drug resistance development, however, the exact mechanisms of chemotherapy response control by miRNAs needs further investigations and their therapeutic benefits have not been fully evaluated (Yahya et al., 2014).

Recently, microRNA-34a (miR-34a) has attracted extensive research interests due to its involvement in myriad of oncogenic pathways in different cancers (Ito et al., 2017; Wen et al., 2017; Shi et al., 2014; Adams et al., 2016; Li et al., 2014a; Li et al., 2014b). Besides, it is a candidate for diagnostic as well as a prognostic biomarker (Imani et al., 2017; Raptiet al., 2017; Chen et al., 2017). In a recent work (Yahya et al., 2017), our group introduced newly synthesized pyridine, thiazole, thiazolopyridine, pyrazole, and pyrazolopyridine progesterone derivatives. These compounds were prepared and tested for their anti-proliferative effects where they proved a noticeable anti-proliferative action on breast cancer cells. This study found that compounds 2, 3, 4, 6, 7, 8 and 9 affected positively on apoptosis by the suppression of Bcl-2, however, survivin and CCND1 expression levels were down regulated by compounds 3, 4,
Moreover, Compound 4 enhanced the apoptosis process by the enhancement of P53 gene expression levels. Concerning the angiogenic process, these compounds affected angiogenesis by the suppression of VEGF, Ang-2, MMP-9 and FGF-1; and the enhancement of HIF-1α and Ang1.

Interestingly, in a previous work of our research group (Yahya et al., 2018), it was reported that miR-34a expression levels were up-regulated on treating MCF-7 cells with modified steroid derivatives as a simultaneous response to drug resistance. Besides, it was previously reported that overexpression of miR-34a inhibited the proliferation, migration, and invasion of breast cancer cells (Avtanski et al., 2016). It also modulates drug sensitivity of breast cancer by affecting some of anti-apoptotic genes such as BCL-2 and CCND1. However, Kastl et al., (2012) reported that miR-34a over expression is associated with docetaxel resistance and enhance breast cancer stemness and drug resistance (Kim et al., 2016), on contrary, by having NOTCH 1 and PRKD1 as its targets, miR-34a affect chemo-resistance of breast cancer cells to adriamycin (Li et al., 2012). The former findings together motivated us to test the hypothesis whether knocking down miR-34a will have a negative impact on apoptotic and angiogenic pathways in cells treated with the newly synthesized progesterone derivatives.

Materials and Methods

Cell propagation, maintenance and treatment

Breast cancer MCF-7 cells were purchased from ATCC (American Type Culture Collection) and maintained in the proper conditions. The cells were cultured in Dulbecco’s modified Eagle’s Medium (DMEM) (Lonza, Belgium) supplemented by 10 % fetal bovine serum (FBS), 4 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin sulfate at 37˚C in a humidified incubator with 5 % CO2. The cells harvested after trypsinization (0.025 % trypsin and 0.02 % EDTA) and washed twice with Dulbecco’s phosphate-buffered saline (DPBS) (Yahya et al., 2016; Hamed et al., 2018). When the cells density reached approximately 80%, cells were split for further culture. The experiments were made up when the cells were in the logarithmic growth phase. Before transfection, 1.5x10^6 cells were seeded per well of 24-well plate in 1.5 ml of DMEM culture medium containing 10% FBS serum and antibiotics. The second day, cells were transfected with 100nM miR-34a-5p inhibitor (Qiagen, USA) in a serum free media using 1μl of the Hiperfect transfection reagent. MiScript Inhibitor negative control which has no homology to any known mammalian gene was used as negative control. The third day, the media were changed with fresh complete media and the cells in the 1st plate were incubated at the proper conditions for another 48 hrs with the tested compounds (Yahya et al., 2016; Hamed et al., 2018). While, the cells in the 2nd plate were subjected to miRNA extraction to verify successful transfection. These compounds were synthesized and subject to cytotoxicity analysis in a previous work (Yahya et al., 2017). The cells were incubated with various concentrations of the test compounds (6.25, 12.5, 25 and 50 μM) for 48 h at a cell density of 104 cells/well of 96 well plate. Using the relation between used concentrations and neutral red intensity value, IC50 of tested compounds was calculated. Four parameter equation logistic curve was used (log concentration vs. % cell growth as compared to control cells). The structures of these compounds are illustrated in Figure 1 (Yahya et al., 2017) and their IC50 values are listed in Table 1.

Quantitative miRNA determination

miR-34a expression levels were determined using the Qiagen miscript system (Qiagen, USA) according to manufacturer instructions. miRNeasy kit was used for Purification of RNA containing miRNA, after that cDNA generation from RNA containing miRNA was performed using miscript RT-kit. Real-Time PCR was used for detection of mature miRNA using miRNA-specific primers to hsa-miR-34a. The cycling conditions were as follow: denaturation for 15 s at 94˚C, annealing for 30 s at 55˚C and extension for 30 s at 70˚C. Fluorescence data were collected using MiniOpticon Bio-Rad Real Time Thermal Cycler.

RNA isolation, clean up and Quantitative real time RT-PCR

RNA was isolated using Qiazol buffer (Qiagen, USA) according to manufacturer instructions. RNA was subsequently cleaned up using RNAeasy Mini Kit (Qiagen, USA). β-actin, BCL-2, survivin, CCND1, CDC2, P53 and P21, VEGF, HIF-1α, MMP-2, Ang-1, Ang-2, and FGF-1 genes copy numbers were quantified using QuantiFastSybergreen RT-PCR. The copy numbers were normalized to 100,000 copies of the house keeping beta-actin gene. Primer sequences are listed in Table 2. The RT and subsequent PCR cycling conditions were as follow, 50°C for 10 min, 95°C for 5 min, 95°C for 15s, then 60°C for 30 s, the number of cycles were 40 cycles. MiniOpticonTM Bio-Rad Real Time Thermal Cycler was used for gene expression quantitation.

Statistical analysis

The data were analyzed using Microsoft Excel. All the data are expressed as Mean ± standard error mean. Analysis of the data was done using student t-test to detect the significant difference between the studied compounds. A level of P < 0.05 was defined as statistically significant. All data were reproducible.

Results

In the current work, all tested progesterone derivatives significantly elevated the expression levels of miR-34a (Figure 2). MiRNA-34a expression level was reduced to 7.42% as compared to negative control treatment. The effect of miR-34a inhibition on progesterone derivatives treated MCF-7 cells is summarized in Figure 3. In a previous work of our research group all tested progesterone derivatives (compounds 2, 3, 4, 6, 7, 8, and 9) revealed noticeable down-regulation of BCL-2 gene expression level as compared to control cells (Yahya et al., 2017). However, MCF-7 cells treated with tamoxifen showed only a non-significant reduction on BCL-2 expression.
level. On the other hand, treating MCF-7 cells with miR-34a inhibitor synergistically with tamoxifen and progesterone derivatives, reduced BCL-2 expression level in case of tamoxifen, compounds 2, 3, 6, and 7. However, this reduction was not significant in case of compound 8 and 9 treatments (Table 3). Yahya et al., (2017) showed that treating MCF-7 cells with compounds 3, 4, 6, 7 and 9 dramatically reduced survivin expression level. However, upon knocking down miR-34a only compounds 2 and 3 significantly reduced survivin expression levels. Besides, tamoxifen, compounds 6, 7, and 9 resulted in a non-significant reduction in survivin levels (Table 3). Similarly, treating MCF-7 with miR-34a inhibitor synergistically with compounds 7 and tamoxifen resulted in reduction in CCND1 expression level while compounds 2, 4, 6, 7, 9 produced a non significant reduction in this gene expression levels (Table 3). Previously (Yahya et al., 2017), treating MCF-7 cells with compounds 3, 4, 8 individually resulted in significant reduction in CCND1 gene expression levels, meanwhile compounds 6, 7, and 9 resulted in non-significant reductions in the expression levels of CCND1 gene. Interestingly, CDC2 gene expression was dramatically decreased in miR-34a suppressed MCF-7 cells treated with compounds 3, 7, 8, 9, in addition to a non significant reduction in cells treated with tamoxifen, compounds 2, 4, and 6 (Table 3). P53 as an important tumor suppressor gene was only up regulated

Table 1. The in vitro Cytotoxic Activity of the Newly Synthesized Compounds on MCF-7 Cancer Cell Line

| Compd.No | IC\textsubscript{50} (\textmu M) |
|----------|----------------------|
| 4.12     | 2                    |
| 4.71     | 3                    |
| 5.87     | 4                    |
| 5.28     | 6                    |
| 3.82     | 7                    |
| 5.28     | 8                    |
| 5.87     | 9                    |
| 3.53     | Tamoxifen            |

IC\textsubscript{50} Concentration required to inhibit cell viability by 50%

Figure 1. Chemical Structure of the Newly Synthesized Heterosteroids (Yahya et al. 2017).

in MCF-7 cells treated with compound 4 individually; however, upon synergistically inhibiting miR-34a, P53 was significantly up regulated in MCF-7 cells treated with tamoxifen, compounds 7, and 8 (Table 4). The current findings suggest that miR-34a inhibition did not affect the promising action of the novel compounds in rising P53 expression levels. P21 gene expression level was not affected in MCF-7 cells subjected to novel compounds

Table 2. Primers Used for Apoptotic and Angiogenic Pathway Analyses

| Gene       | Primer forward (5’-3’)            | Primer reverse (5’-3’)            |
|------------|----------------------------------|----------------------------------|
| β-actin    | CTTTCTGGGCGATGGAGCTCT            | GGAAGAATGATCTTGTATCTTC           |
| BCL-2      | CTTGGTGGAACACATCAGCC            | AATCAACAGAGGCCG CATGC           |
| Survivin   | AGGACGCGCCCTTCTTGGAGG           | CTTTTATGTTCCCTATGGGGTC          |
| CDC2       | CAAATATAGTCAGTCTTCAGATTG        | CCTGTAAGATTTGGTGTTAAAATAC        |
| CCND1      | GAGGAAGAGGAAGAGAGAGG             | GAGATGGAAGGGGAGAAAGAG           |
| P53        | AGA GTC TAT AGG CCC ACC CC       | GCT CGA CGC TAG GAT CTG AC       |
| P21        | AAG ACC ATG TGG ACC TGT         | GGT AGA AAT CTG TCA TGC TG      |
| VEGF       | TACCTCCACCATGCAAGTG             | ATGATTCTGGCCTCTCCTCTTC           |
| HIF-1a     | GCAAGGACCTGAAAAAGG              | GGCCTTCAGACCTTGA                |
| MMP-2      | ATGCCCTCAAAACTTCAGCTCT-         | CAGGGTTTCCATCAGCATT             |
| Ang-1      | AAAAGGTCACTGGGACACG             | TACTCACTGGGCACTTCA              |
| Ang-2      | TCAAGCAGAAAAATCCATATTG          | GCCTCAGCAGTTCCAGTGT             |
| FGF-1      | AAGCCCGTCGTTGTCCTAGG           | GATGGCAGTGGGAGTTGGGAC           |

Figure 1. Chemical Structure of the Newly Synthesized Heterosteroids (Yahya et al. 2017).
individually. However, it was down regulated in MCF-7 treated with tamoxifen and miR-34a inhibitor (Table 4). In the current study, MCF-7 treated with compounds 2, 3, 6, 7, 8, 9, alongside with miR-34a inhibitor significantly resulted in down regulation in VEGF expression levels, and only compound 4 and tamoxifen did not show this effect (Table 4). MCF-7 cells treated with tamoxifen, compounds 2 and 9 showed significant elevation in HIF-1 alpha expression levels (Yahya et al., 2017), in contrary, when miR-34a was inhibited, cells treated with compounds 3, 6, 7, 8, 9 showed significant down regulation in this gene expression levels (Table 4). In the current study, MCF-7 cells treated with the tested compounds alone did not produce significant reduction in MMP-2 expression levels, however, when it was treated with miR34a inhibitor alongside with tamoxifen, compounds 2, 3, 6, 9, MMP-2 expression levels was dramatically reduced (Table 5). This effect could be attributed to the observed reduction in HIF-1alpha in miR-34a knocked cells.

MCF-7 cells treated with miR-34a inhibitor and compounds 3, 6, 8 and 9 resulted in significant reduction in Ang-1 expression levels which is consistent with its physiological role as a promoter of angiogenesis, however its levels were significantly over expressed when treated with tamoxifen, compounds 2, 7, and 9 individually (Table 5) (Yahya et al., 2017). Similarly, all tested compounds showed significant reduction in Ang-2 expression levels except compounds 6 and 9 (showed a non-significant reduction), which is relatively comparable to Ang-2 levels when treating cells with the tested compounds individually (all tested compounds showed significant reduction in Ang-2 expression levels (Table 5).

FGF-1 which was found to mediate tumor initiation, progression and metastasis, did not show significant changes in its expression levels when treating MCF-7 cells with miR-34a inhibitor with the tested compounds (Table 5). This opposes what happened when this cells was treated with compounds 2, 3, and 8 individually, as FGF-1 expression levels were significantly reduced (Yahya et al., 2017). This could suggest possible role of miR-34a in modulating FGF-1 levels which needs further

---

**Table 3. Effect of miRNA-34a Inhibition Synergistically with Tested Compounds on BCL-2, survivin, CCND1, and CDC2 Gene Expression Levels.** NC, MiScript Inhibitor negative control treated cells; Tx, Tamoxifen treated cells. Data are represented as mean ±SE; Data were reproducible, *P<0.05.

| Compounds | Normalized copy numbers of BCL-2 gene | Normalized copy numbers of survivin gene | Normalized copy numbers of CCND1 gene | Normalized copy numbers of CDC2 gene |
|-----------|--------------------------------------|-----------------------------------------|---------------------------------------|--------------------------------------|
| NC        | 16323.3±1328.9                       | 12566.7±1315.19                        | 4362574.5±531367.73                  | 764621.9±111428.39                   |
| Tamoxifen | 3670.4±956.1*                        | 12146.7±653.72                         | 92745.0±7873.84*                     | 455488.6±45366.17                    |
| 2         | 6163.4±624.1*                        | 4346.4±573.41*                         | 3528464.9±605266.78                  | 657286.3±69796.17                    |
| 3         | 4883.3±69.3*                         | 310.3±18.69*                           | 4405250.9±704416.75                  | 380827.8±65095.22*                   |
| 4         | 41337.6±5169.3*                      | 15200.7±5940.53                        | 3872463.5±457621.15                  | 560245.7±75715.85                    |
| 6         | 5108.9±134.5*                        | 12518.6±1397.87                        | 2762058.4±247331.67                  | 369646.7±22845.35                    |
| 7         | 5170.9±498.5*                        | 11463.4±870.863                       | 2140033.8±45209.63*                  | 186787.3±8259.18*                    |
| 8         | 11992.7±1415.9                       | 13340.2±387.49                         | 3684999.0±2205126.08                 | 105688.1±5640.14*                    |
| 9         | 11562.1±651.9                        | 10830.6±365.13                         | 4135436.5±1894751.06                 | 241498.9±35490.38*                   |

---

**Figure 2. Effect of Tested Compounds on miRNA-34a Expression Levels.** C, Control cells; Tx, Tamoxifen treated cells; Data are represented as mean ±SE, Data were reproducible, *P<0.05.
many miRNAs were found to be a key player in the progression of breast cancer chemoresistance by regulation of apoptosis, estrogen resistance, drug transporters modulation, epithelial mesenchymal transition (EMT), and cancer stem cells (Yalcin and Gunduz, 2016). Apoptosis is a very important mechanism which is responsible for eliminating abnormal cells in normal tissues as well as cancerous tissues (Kaplan et al., 2020). The positive role of miR-34a in antagonizing malignancy consequences, like the down regulation of cancer cell differentiation, proliferation, invasion and migration is well established (Saito et al., 2015; Imani et al., 2017; Wang et al., 2017). The definite role played by miR-34a in breast cancer has many controversies. Indeed miR-34a may affect positively

Table 4. Effect of miRNA-34a Inhibition Synergistically with Tested Compounds on P53, P21, VEGF, and Hif-1α Gene Expression Levels. NC, MiScript Inhibitor negative control treated cells; Tx, Tamoxifen treated cells; Data are represented as mean ±SE, Data were reproducible, *P<0.05.

| Compounds | Normalized copy numbers of P53 gene | Normalized copy numbers of P21 gene | Normalized copy numbers of VEGF gene | Normalized copy numbers of Hif-1α gene |
|-----------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|
| NC        | 87.8±9.0                            | 676652.4±46463.56                    | 1032935.2±515018.4                   | 271226.8±42606.55                   |
| Tamoxifen | 894.3±129.5*                        | 926948.7±67943.13*                   | 10926537.0±473735.0                  | 13281.9±474.05*                     |
| 2         | 63.5±8.3                            | 385834.4±47514.07*                   | 5833701.1±875074.4                   | 173003.7±22458.14                   |
| 3         | 25.1±1.6*                           | 117823.5±15648.62*                   | 3388792.0±449572.7*                  | 47305.7±917.46*                     |
| 4         | 65.3±6.1                            | 331034.8±9472.42*                    | 31763403.8±2217195.4*                | 283276.9±24351.39                   |
| 6         | 58.4±12.3                           | 248011.9±29900.24*                   | 4377939.5±446935.4                   | 84645.5±6637.07*                    |
| 7         | 183.3±1.7*                          | 160652.9±4757.99*                    | 1821693.0±299328.1*                  | 17937.7±1274.72*                    |
| 8         | 259.0±25.3*                         | 185973.0±42125.24*                   | 6861655.5±663298.0*                  | 110716.7±10815.01*                  |
| 9         | 241.8±50.8                          | 274048.4±13694.67*                   | 7543390.1±984681.7                   | 42780.5±3173.73*                    |

Figure 3. The Effect of miR-34a Inhibition on Progesterone Derivatives Treated MCF-7 Cells
Table 5. Effect of miRNA-34a Inhibition Synergistically with Tested Compounds on MMP-2, Ang-1, Ang-2, and FGF-1 Gene Expression Levels. NC, MiScript Inhibitor negative control treated cells; Tx, Tamoxifen treated cells; Data are represented as mean ±SE, Data were reproducible, *P<0.05.

| Compounds | Normalized copy numbers of P MMP-2 gene | Normalized copy numbers of Ang-1 gene | Normalized copy numbers of Ang-2 gene | Normalized copy numbers of FGF-1 gene |
|-----------|----------------------------------------|--------------------------------------|---------------------------------------|---------------------------------------|
| NC        | 72.6±4.44                              | 496.4±22.55                          | 4956.5±276.73                         | 4167.8±767.01                        |
| Tamoxifen | 11.4±1.83*                             | 1009.9±275.98                        | 4574.1±153.74                         | 3592.6±329.86                        |
| 2         | 12.2±1.59*                             | 399.9±62.41                          | 3023.7±359.85*                        | 3004.3±277.34                        |
| 3         | 3.7±0.21*                              | 95.2±4.71*                           | 1306.0±114.46*                        | 1511.7±61.89                        |
| 4         | 67.5±20.19*                            | 454.4±34.13                          | 2206.1±137.71*                        | 4096.2±465.23                        |
| 6         | 1.0±0.17*                              | 224.5±19.25*                         | 4182.5±150.18                         | 1966.3±111.67                        |
| 7         | 64.6±5.21                              | 465.9±57.72                          | 1421.2±84.55*                         | 4613.5±52.51                         |
| 8         | 79.7±9.26                              | 160.3±22.45*                         | 1237.9±159.17*                        | 6583.1±1264.45                       |
| 9         | 110.7±5.41*                            | 167.4±28.23*                         | 3728.3±990.12                         | 2843.8±188.01                        |

in the modulation of drug sensitivity in breast cancer by suppression of NOTCH1, BCL-2, and CCND1 (Li et al. 2017). Kastl et al., (2012) reported that miR-34a over expression is associated negatively with docetaxel resistance and drug sensitivity could be restored upon miR-34a modulation.

In the current work, all tested progesterone derivatives significantly up-regulated the expression levels of miR-34a which is consistent with our previous findings (Yahya et al., 2018) where the treatment of MCF-7 cells with steroid derivatives significantly up regulated miR-34a expression levels. This was attributed to the evoked drug resistance against tested compounds.

It is well established that miR-34a targets BCL-2 and various cyclins (Li et al., 2013). However knocking down miR-34a did not affect on BCL-2 reduction by the tested compounds. This means that the knocking down of miR-34a did not affect negatively on the promising effect of the most of novel derivatives on BCL-2 gene expression levels. Yahya et al., (2017) showed that treating MCF-7 cells with compounds 3, 4, 6, 7 and 9 dramatically reduced survivin expression level. However, upon knocking down miR-34a only compounds 2 and 3 significantly reduced survivin expression levels. Besides, tamoxifen, compounds 6, 7, and 9 resulted in a non-significant reduction in survivin levels. miR-34a was found to target survivin. This effect could be indirect regulation through the downregulation of survivin upstream activators or transcriptional factors, which are targets of miR-34a; or Direct regulation (Huang et al., 2015). Moreover, over expression of miR-34a significantly down regulated survivin in HNSCC cell line, UM-SCC-74A (Kumar et al., 2012), in non-small cell lung cancer (NSCLC) cells (Ji et al., 2012), laryngeal squamous cell carcinoma cell lines (Cao et al., 2013), a gastric cancer cell line (Chen et al., 2010), murine melanoma cells (Shen et al. 2012). Interestingly, CDC-2 gene expression was significantly down regulated in MCF-7 cells treated with miR-34a inhibitor synergistically with compounds 3, 7, 8, 9, in addition to a non significant reduction in cells treated with tamoxifen, compounds 2, 4, and 6 This reduction was not achieved in MCF-7 cells treated with these compounds individually. Chen et al., (2013) found that CDK1 (encoded by CDC2 gene) inhibition-induced cell death in neuroblastoma cells was dependent on the suppressed MYCN levels and miR-34a-mediated. Where the down regulation of MYCN resulted from CDK1 inhibition which consequently decreased the transcriptional activation of MYCN on the survivin promoter. CDK1 inhibition resulted in elevation of miR-34a, meanwhile miR-34a suppression up-regulated the expression of MYCN and enhanced cell survival of neuroblastoma cells treated with CDK1 antagonist. These findings may interpret the promising effect achieved in CDC2 reduction upon miR-34a inhibition. P53 as an important tumor suppressor was only up regulated in MCF-7 cells treated with compound 4 individually; however, upon synergistically inhibiting miR-34a, P53 was significantly up regulated in MCF7cells treated with tamoxifen, compounds 7, and 8. The miR-34a gene is located at lp36.23. MiR-34a was firstly introduced as a target of P53 which acted as an important tumor suppressor (He et al., 2007; Misso et al., 2014). Ye et al., (2016) stated that the anti-tumor effect of miR-34a was originally dependent on the modulation of SIRT1 and p53/p21 protein and not apoptosis-related proteins. In our previous study (Yahya et al., 2017); it was found that all tested progesterone derivatives produced significant down regulation in vascular endothelial growth factor (VEGF) expression levels. VEGF was identified as a target gene for miR-34a (Yu et al., 2014). In the current study, MCF-7 treated with compounds 2, 3, 6, 7, 8, 9, alongside with miR-34a inhibitor significantly resulted in down regulation in VEGF expression levels, and only compound 4 and tamoxifen did not show this effect. MCF-7 cells treated with tamoxifen, compounds 2 and 9 showed significant elevation in HIF-1 alpha expression levels, in contrary, when miR-34a was inhibited, cells treated with compounds 3, 6, 7, 8, 9 showed significant down regulation in this gene expression levels. This could be explained by the findings of Lin et al., (2017) who found that miR-34a up regulation significantly up regulates HIF-1alpha and down regulates SIRT1 and consequently promoted apoptosis. Previous observations found that hypoxia and HIF-1 enhances the expression levels and/or activities of MMP-2 and MMP-9 (Krishnamachary et al., 2006; Munoz-Najar et al., 2006). In the current
study, MCF-7 cells treated with the tested compounds alone did not produce significant reduction in MMP-2 expression levels, however, when it was treated with miR34a inhibitor alongside with tamoxifen, compounds 2, 3, 6, 9, MMP-2 expression levels was dramatically reduced. This effect could be attributed to the observed reduction in HIF-1alpha in miR-34a knocked cells. FGFR-1 which was found to mediate tumor initiation, progression and metastasis; did not show significant changes in its expression levels when treating MCF-7 cells with miR-34a inhibitor with the tested compounds. This opposes what happened when this cells was treated with compounds 2, 3, and 8 individually, as FGFR-1 expression levels were significantly reduced. This could suggest possible role of miR-34a in modulating FGFR-1 levels which needs further investigations.

MCF-7 cells treated with miR-34a inhibitor and compounds 3, 6, 8 and 9 produced significant reduction in Ang-1 expression levels which is consistent with its physiological role as a promoter of angiogenesis, however its levels were significantly over expressed when treated with tamoxifen, compounds 2, 7, and 9 individually. Similarly, all tested compounds showed significant reduction in Ang-2 expression levels except compounds 6 and 9 (showed a non-significant reduction), which is relatively comparable to Ang-2 levels when treating cells with the tested compounds individually (all tested compounds showed significant reduction in Ang-2 expression levels. Unfortunately, little is known about the role of miR-34a in modulating Angiopoietins which needs further studies to be elucidated.

In conclusion, the current study revealed that miRNA-34a inhibitor could be used as a tool to tackle drug resistance in breast cancer. The observed results in this study points out that miR-34a inhibition did not have a noticeable negative impact on apoptosis and angiogenesis. On contrary, miRNA-34a deficient MCF-7 cells showed improved effect of the tested progesterone derivatives in some apoptotic and angiogenic genes such as CDC-2, MMP-2.

Abbreviations

miR-34a: microRNA-34a; DMEM: Dulbecco’s modified Eagle’s Medium; FBS: fetal bovine serum; DPBS: Dulbecco’s phosphate-buffered saline; EMT: epithelial mesenchymal transition; NSCLC: non-small cell lung cancer; VEGF: vascular endothelial growth factor.

Author Contribution Statement

YS, suggested the point of research, designed the experiment, wrote the manuscript, and participated in the statistical analysis of the data. EG, participated in the design of the experimental work, participated in writing the manuscript, and participated in the statistical analysis of the data. AM, participated in the design of the experimental work and writing the manuscript. AA, participated in the design of the experimental work and writing the manuscript. EE, revised the manuscript. The authors read and approved the final manuscript.

Acknowledgements

Not applicable.

Funding statement

This work was funded by research support grant (project ID: 5328) from Science and technology development fund (STDF), Cairo, Egypt.

The Science and Technology Development Fund (STDF) has stimulated the Egyptian scientific society by funding distinguished research papers and establishing scientific partnerships with scientists from many advanced countries in order to keep track of quickly advancing technology, and be open to different societies, as well as, new economic unions, compete on the international arena, link scientific research to technological development and cooperate with civil society institutions to activate their role in the integrated scientific research system. STDF build and strengthen S&T through capacity building of community and infrastructure in the valued strategic areas of Egypt’s long-term competitiveness and development.

Ethical committee

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest

The authors declare that are no conflicts of interest.

References

Adams BD, Parsons C, Slack FJ (2016). The tumor-suppressive and potential therapeutic functions of mir-34a in epithelial carcinomas. Expert OpinTher Targets, 20, 737–53.

Avtanski DB, Nagalingam A, Tomaszewski JE, et al (2016). Indolo-pyrido-isouquinolin based alkaldoid inhibits growth, invasion and migration of breast cancer cells via activation of P53-miR34a axis. Mol Oncol, 10, 1118–32.

Cao W, Fan R, Wang L, et al (2013). Expression and regulatory function of miRNA-34a in targeting sur-vivin in gastric cancer cells. Tumour Biol, 34, 963-71.

Chen AH, Qin YE, Tang WF, et al (2017). Mir-34a and mir-206 act as novel prognostic and therapy biomarkers in cervical cancer. Cancer Cell Int, 17, 63.

Chen Y, Tsai YH, Tseng SH (2013.) Inhibition of cyclin-dependent kinaseA 1-induced cell death in neuroblastoma cells through the microRNA-34a-MYCN-survivin pathway. Surgery, 153, 4-16.

Chen Y, Zhu X, Zhang X, Liu B, Huang L (2010). Nanoparticles modified with tumor-targeting scFv deliver siRNA and miRNA for cancer therapy. Mol Ther, 18, 1650-6.

DeSantis C, Ma J, Bryan L, Jemal A (2014). Breast cancer statistics, 2013. CA Cancer J Clin, 64, 52–62.

Hamed AR, Emara M, Soltan MM, et al (2018). Investigating the role of miRNA-98 and miRNA-214 in chemoresistance of HepG2/Dox cells: studying their effects on predicted ABC transporters targets. Med Chem Res, 27, 531-7.

He L, He X, Lim LP, et al (2007). A microRNA component of the P53 tumour suppressor network. Nature, 447, 1130–4.

Huang J, Lu, Y, Wang J, Liu B (2015). MicroRNA regulation and therapeutic targeting of survivin in cancer. Am J Cancer Res, 5, 20-31.

Imani S, Zhang X, Hosseinifar H, Fu S, Fu J (2017). The diagnostic role of microRNA-34a in breast cancer: A
Shaymaa M M Yahya et al

systematic review and meta-analysis. Oncotarget, 8, 23177–87.

Imani S, Wei C, Cheng J, et al (2017). MicroRNA-34a targets epithelial to mesenchymal transition-inducing transcription factors (EMT-TFs) and inhibits breast cancer cell migration and invasion. Oncotarget, 8, 21362–79.

Ito Y, Inoue A, Seers T, et al (2017). Identification of targets of tumor suppressor microRNA-34a using a reporter library system. Proc Natl Acad Sci USA, 114, 3927–32.

Ji X, Wang Z, Geamanu A, et al (2012). Delta-tocotrienol suppresses Notch-1 pathway by upregulating miR-34a in nonsmall cell lung cancer cells. Int J Cancer, 131, 2668-77.

Kaplan A, Kutlu MH (2020). Investigation of silver nitrate on cytotoxicity and apoptosis in MCF7 human breast carcinoma cells. Asian Pac J Cancer Biol, 5, 49-56.

Katli L, Brown I, Schofield AC (2012). miRNA-34a is associated with docetaxel resistance in human breast cancer cells. Breast Cancer Res Treat, 131, 445–54.

Kim Y, Park EY, Chang E, et al (2016). A novel miR-34a target, protein kinase D1, stimulates cancer stemness and drug resistance through GSK3/β-catenin signaling in breast cancer. Oncotarget, 7, 14791–802.

Krishnamachary B, Zagzag D, Nagasawa H, et al (2006). Hypoxia-inducible factor-1-dependent repression of E-cadherin in von Hippel-Lindau tumor suppressor-null renal cell carcinoma mediated by TCF3, ZFHX1A, and ZFHX1B. Cancer Res, 66, 2725-31.

Kumar B, Yadav A, Lang J, Teknoe TN, Kumar P (2012). Dysregulation of microRNA-34a expression in head and neck squamous cell carcinoma promotes tumor growth and tumor angiogenesis. PLoS ONE, 7, e37601.

Li Z, Weng X, Xiong Q, et al (2017). miR-34a expression in human breast cancer is associated with drug resistance. Oncotarget, 8, 106270-82.

Li L (2014a). Regulatory mechanisms and clinical perspectives of miR-34a in cancer. J Cancer Res Ther, 10, 805–10.

Li XJ, Ren ZJ, Tang JH (2014b). MicroRNA-34a: A potential therapeutic target in human cancer. Cell Death Dis, 5, e1327.

Li L, Yuan L, Luo J, et al (2013). MiR-34a inhibits proliferation and migration of breast cancer through down-regulation of Bcl-2 and SIRT1. Clin Exp Med, 13, 109–17.

Li XJ, Ji MH, Zhong SL, et al (2016). MicroRNA-34a modulates chemosensitivity of breast cancer cells to adriamycin by targeting NOTCH1. Arch Med Res, 43, 514–21.

Lin Y, Shen J, Li D, et al (2017). MiR-34a contributes to diabetes-related cochlear hair cell apoptosis via SIRT1/HIF-1α signaling. Gen Comp Endocrinol, 246, 62–70.

Miss G, Di Martino MT, De Rosa G, et al (2014). MiR-34: a new weapon against cancer?. Mol Ther Nucleic Acids, 3, e194.

Munoz-Najar UM, Neurath KM, Vumbaca F, Claffey KP (2006). Hypoxia stimulates breast carcinoma cell invasion through MT1-MMP and MMP-2 activation. Oncogene, 25, 2379-92.

Pasquier J, Magal P, Boulange-Lecomte C, Webb G, Le Foll F (2011). Consequences of cell-to-cell P-glycoprotein transfer on acquired multidrug resistance in breast cancer: a cell population Dynamics model. Biol Direct, 6, 5.

Rapti SM, Kontos CK, Christodoulou S, Papadopoulos IN, Scorilas A (2017). MiR-34a overexpression predicts poor prognostic outcome in colorectal adenocarcinoma, independently of clinicopathological factors with established prognostic value. Clin Biochem, 50, 918-24.

Saito Y, Nakaoka T, Saito H (2015). MicroRNA-34a as a therapeutic agent against human cancer. J Clin Med, 4, 1951-9.

Shen Z, Zhan G, Ye D, et al (2012). MicroRNA-34a affects the ocurrence of laryngeal squamous cell carcinoma by targeting the antiapoptotic gene surviving. Med Oncol, 29, 2473-80.

Shi Y, Liu C, Liu X, Tang DG, Wang J (2014). The microRNA-miR-34a inhibits non-small cell lung cancer (NSCLC) growth and the CD44H stem-like NSCLC cells. PLoS One, 9, e90022.

Wang Y, Zhang X, Chao Z, et al (2017). MiR-34a modulates ErbB2 in breast cancer. Cell Biol Int, 41, 93-101.

Wen D, Peng Y, Lin F, Singh RK, Mahato RI (2017). Micellar delivery of mir-34a modulator rubone and paclitaxel in resistant prostate cancer. Cancer Res, 77, 3244–54.

Yahya SMM, Elmegeed GA, Mohamed MS, et al (2018). The effect of newly synthesized heterosteroids on miR34a, 98, and 214 expression levels in MCF-7 breast cancer cells. Int J Clin Biochem, 33, 32-38.

Yahya SMM, Abdelhamid AO, Abd-EIhalim MM, Elsayed GH, Eskander EF (2017). The effect of newly synthesized progesterone derivatives on apoptotic and angiogenic pathway in MCF-7 breast cancer cells. Steroids, 126, 15-23.

Yahya SMM, Hamed AR, Emara M, et al (2016). Differential effects of c-myc and ABCB1 silencing on reversing drug resistance in HepG2/Dox cells. Tumor Biol, 37, 5925–32.

Yahya SMM, Elsayed GH (2014). A summary for molecular regulations of miRNAs in breast cancer. Clin Biochem, 48, 388-96.

Yalcin S, Gunduz U (2016). Nanoparticle based delivery of miRNAs to overcome drug resistance in breast cancer. J Nanomed. Nanotechnol, 7, 6.

Ye Z, Fang J, Dai S, et al (2016). MicroRNA-34a induces a senescence-like change via the down-regulation of SIRT1 and up-regulation of p53 protein in human esophageal squamous cancer cells with awild-type p53 gene background. Cancer Lett, 370, 216-21.

Yu DD, Lv MM, Chen WX, et al (2015). Role of miR-155 in drug resistance of breast cancer. Tumor Biol, 36, 1395-401.

Yu G, Yao W, Xiao W, et al (2014). MicroRNA-34a functions as an anti-metastatic microRNA and suppresses angiogenesis in bladder cancer by directly targeting CD44. J Exp Clin Cancer Res, 33, 779.

This work is licensed under a Creative Commons Attribution-Non Commercial 4.0 International License.