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Potential Use of RNA Interference in Cancer Treatment: A Comparative Study of the Effect of STMN1 Inhibition on Behavior of Cancer Cells

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Abstract

Cancer as one of the most prevalent health issues accounts for 13 percent of all deaths worldwide. Based on a report by the American Cancer Society, breast, colorectal, and prostate cancers will be the three leading causes of death among Americans in 2018. The development of sequence-specific RNA molecules that may effectively knock out selected gene expression provides the chance of rational design for targeted cancer therapies. Stathmin 1/oncoprotein 18 may be a major regulator of microtubule dynamics. Stathmin1 is extremely overexpressed in a vast array of human malignancies, such as breast, colorectal, and prostate cancers. This study compares the effects of viable Stathmin1 knockdown, migration, and proliferation of pc3, mcf7 and sw480 cancer cell lines from breast, colorectal, and prostate cancer cells. Our result showed that response of the prostate cancer cell line to suppression of STMN1by siRNA is more considerable compared to that of breast and colorectal cancers' cell lines. Considering the importance of issue under study, further investigations are recommended to be done on STMN1 siRNA as a pharmacological intervention strategy in prostate cancer.

Keywords: siRNA, STMN1, PC3, MCF7, SW480, RNAi, oncoprotein 18

Introduction

Cancer as one of the most prevalent health issues accounts for 13 percent of all deaths worldwide. According to the Ministry of Health and Medical Education (MOHME) report, cancer is now the third major reason behind death in Iran after ischemic heart disease (IHD) and accidents (Amirkhah et al., 2017; Siegel et al., 2018).Based on a report by the American Cancer Society, breast, colorectal, and prostate cancers will be the three leading causes of death among Americans in 2018 (Siegel et al., 2018).

To successfully treat cancer, more novel therapeutic modalities should be developed in light of the worrying condition of cancer outbreak. The planned genomics-based strategies (genome-based therapeutics) have emphasized the gene downregulation as a leading cause of cancer development. This type of approach was expected to inhibit tumor expansion specifically and selectively with the least unexpected side effects for normal cells. Thanks to the identification of the target genes stimulating tumor growth and neoplastic transformation, the nucleotide sequence of cancer-relevant genes may contribute to the development of tailored anticancer agents lacking some toxic adverse effects of conventional cytotoxic drugs (Devi, 2006; Stella, 2013; Croce, 2008).Indeed, various studies have demonstrated that an effective oncogene knockdown (silencing) will enhance poor prognosis and tumor growth (Cao et al., 2015; Fatemian et al., 2014; Fatemian & Chowdhury, 2014)

In full view of increasing information on the human genome (i.e., the genome of Homo Sapiens), the development of sequence-specific RNA molecules that may effectively knock out selected gene expression provides the chance of rational design for targeted cancer therapies. Data collected shed light on the promising status of Antisense technology as a contemporary platform for controlled expression of oncogenes (Devi, 2006; Troegeler et al., 2014; Walther & Schlag, 2013).

Stathmin 1/oncoprotein 18 may be a major regulator of microtubule dynamics. Stathmin 1-based microtubule dynamics regulation is carried out by sequestration of free tubulin heterodimers or microtubule catastrophe-promoting. Stathmin/Op18 was ab initio known as a

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protein phosphorylated responding to a few extracellular signals (i.e., extracellular signal-regulated protein phosphorylation) (Cassimeris, 2002; Steinmetz, 2007; Chen et al/. 2013).

According to the reports, Stathmin1 is extremely overexpressed in a vast array of human malignancies, such as breast, colorectal, and prostate cancers (Ghosh et al., 2007; Bjorklund et al., 2010; Guo et al., 2016; Ogino et al., 2009; Kuang et al., 2005), and new studies demonstrate hopeful results for targeted cancer therapies by Stathmin1 expression knockdown (Hassan et al., 2015; Hemdan et al., 2014; Belletti & Baldassarre, 2011; Byrne et al., 2014).

This study compares the effects of viable Stathmin1 knockdown, migration, and proliferation of three cancer cell lines from breast, colorectal, and prostate cancer lineage cells. Therefore, future studies are expected to recognize the most liable cell line of these three cancer cell lines in an attempt to develop a modern Stathmin1 inhibition-based cancer treatment strategy.

Materials and Methods

Cell culture & cell line

All three cancer cell lines (i.e., PC3, SW480, and MCF7) were obtained from NCBI[1] (Pasteur Institute of Iran, Tehran, Iran). These cancer cell lines were kept in Gibco™ DMEM complemented with 10% Gibco™ Fetal Bovine Serum (FBS), 100U/ml penicillin, and 10mg/ml streptomycin at 37°C in a humidified atmosphere containing 5% carbon dioxide.

siRNA transfection and functional cell-based assay (CBA)

Stathmin1-siRNA transfection (sc-36128, Santa Cruz Biotechnology), Silencer™ Select Negative Control #1 siRNA (Invitrogen™, catalog number: Am4611), and Silencer™ Select GAPDH Positive Control siRNA (Invitrogen™, catalog number: 4390849) were carried out using Effectene Transfection Reagent (Qiagen catalog #301425) based on the manufacturer's instructions. In short, one day before transfection, the cells were seeded in 96-well plates at $2*10^4$ cells/well. On the day of transfection, the cells were 80% confluent (i.e., the confluency reached 80%), during which 50-200 nM siRNA concentration was utilized for transfecting all wells.

Six well tissue culture treated plates were utilized for RNA extraction. Initially, the cells ($30*10^4$ cells/well) were seeded in each well (i.e., 6-well plates) one day before transfection. Each well was transfected using 100 nM final siRNA concentration on the day of transfection. For transfection in Transwell migration assays, 100 and 150 nM concentrations of siRNAs were used.

RNA extraction and semi-quantitative real-time PCR

Total RNA extraction (or purification) was carried out via CinnaPure-RNA (Sinaclon, Cat. #PR891620) based on the manufacturer's instructions. For complementary DNA (cDNA) synthesis, the QuantiTect Reverse Transcription Kit (Pars Tous Biotechnology, Cat. #A101161) was exploited.

The Stathmin1 primer (VHPS-8955), GAPDH primer (forward primers: tcc, tgc, acc, acc, aac, tgc, ctt, ag; reverse primers: tcc, aca, gtc, ttc, tgg, gtg, gca, g), and Master Mix (Ampliqon, Cat. #A324406) were used for semi-quantitative real-time PCR (RT-PCR).

The normalization and calculation of the relative gene expression level were conducted via glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and $2^{-\Delta\Delta Ct}$ method, respectively. All of the qRT-PCR reactions were carried out in triplicates, and DNA templates ("water blanks") were contained as negative controls.

Cell proliferation assay

Cells were seeded in a 96-well cell culture plate overnight before transfecting with 50, 100, 150, and 200 nM final concentrations of siRNA on the following day. Cell proliferation assay was carried out by Cell Proliferation Kit I (MTT, 11 465 007 001, Roche) based on the manufacturer's instructions. Upon transfection, the cells were incubated for 48 hours in a CO2 incubator, and the plate optical density was calculated by Synergy™ 4 Multi-Detection Microplate Reader (BioTek, Winooski, Vermont) at 500 nm (690 nm as background). All of the qRT-PCR reactions were carried out in triplicates.

Cell migration assay

The Matrigel transmembrane invasion assay was carried out in a Transwell® plate (Corning, Incorporated, Corning, NY, USA) as previously delineated (Li et al.,2016). In short, complete 1% FBS-containing medium was added to the Transwell plate Matrigel-pretreated (BD Biosciences) upper chamber. Complete 10% FBS-containing medium was added to the lower chamber. The plates were

[1] National Cell Bank of Iran

incubated for 60 minutes at 37°C. The Stathmin 1-siRNA/scrambled-siRNAs-transfected cell suspension (i.e., with 1×10^5 cells/ml density) was added to the Transwell upper chamber (i.e., the ultimate volume of 200 µl).

Afterward, these Transwells were incubated for twenty-four hours at 37°C. Then, filter membrane-based cells were harvested, and the number of cells was determined using a microscope.

Statistical data analysis

The statistical data analysis was carried out by SPSS 16.0 (SPSS Incorporation., Chicago, Illinois, USA), and the data were expressed as the mean Â± standard deviation (SD). For the comparison of group means, the ANOVA was utilized.

Results

Stathmin 1 reduced viability knockdown (silencing) in all three cancer cell lines

According to Fig. 1, the specific siRNA-based STMN1 mRNA knockdown has led to small viability for cancer cell lines (i.e., PC3, SW480, and MCF7). The 100 nM final siRNA concentration had the largest effect on the cell viability. Based on the statistical data analysis, the 100 and 200 nM final siRNA concentrations were not significantly different for each cancer cell line; however, the viability of scrambled siRNA group and that of 100 nM final siRNA concentration cells were significantly different (P-value for PC3, SW480, and MCF7 cancer cell lines was 0.0004, 0.0002, and 0.003, respectively).

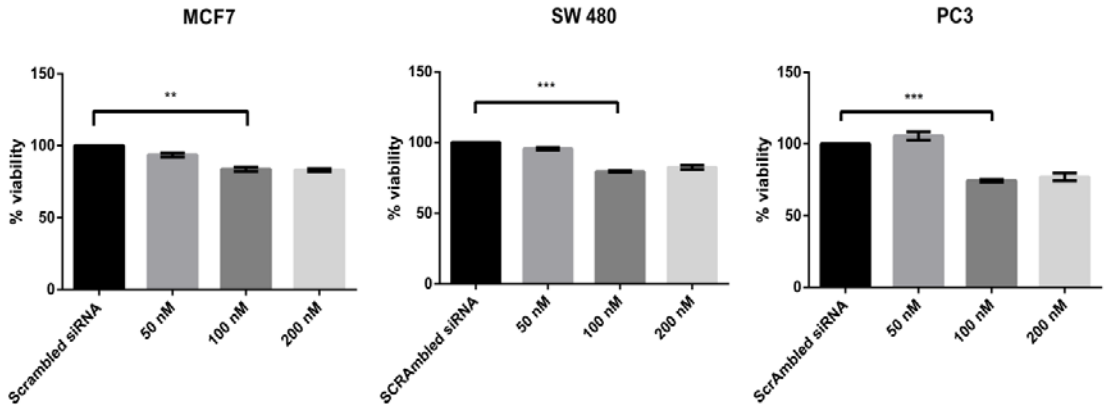

Fig. 1. The viability results from MTT assay (colorimetric assay for assessing cell metabolic activity) for PC3, SW480, and MCF7 cancer cell lines after knockdown of Stathmin 1-mRNA. The transfection of these cell lines was performed with diverse small interfering RNAs (siRNAs) concentrations, and the MTT-based evaluation of their viability was done one day after transfection. Scrambled siRNAs were utilized for cell line transfection as controls. The data are expressed as Mean ± SD (n=3±SD).

The successful reduction in cancer cell line migration is mediated by Stathmin1 Knockdown.

According to Fig. 2, the specific-siRNA-based Stathmin 1-mRNA knockdown has led to a reduction in cancer cell line migration (all three cancer cell lines under study) after twenty-four hours. The statistical data analysis shed light on the fact that cancer cell line ($p<0.0001$) and scrambled-siRNA vs. STMN1-siRNA (i.e., $p<0.0001$) were of great importance; however, scrambled-siRNA and STMN1-siRNA were significantly different in PC3 and SW480 ($p<0.0005$) in comparison to MCF7 ($p= 0.0082$).

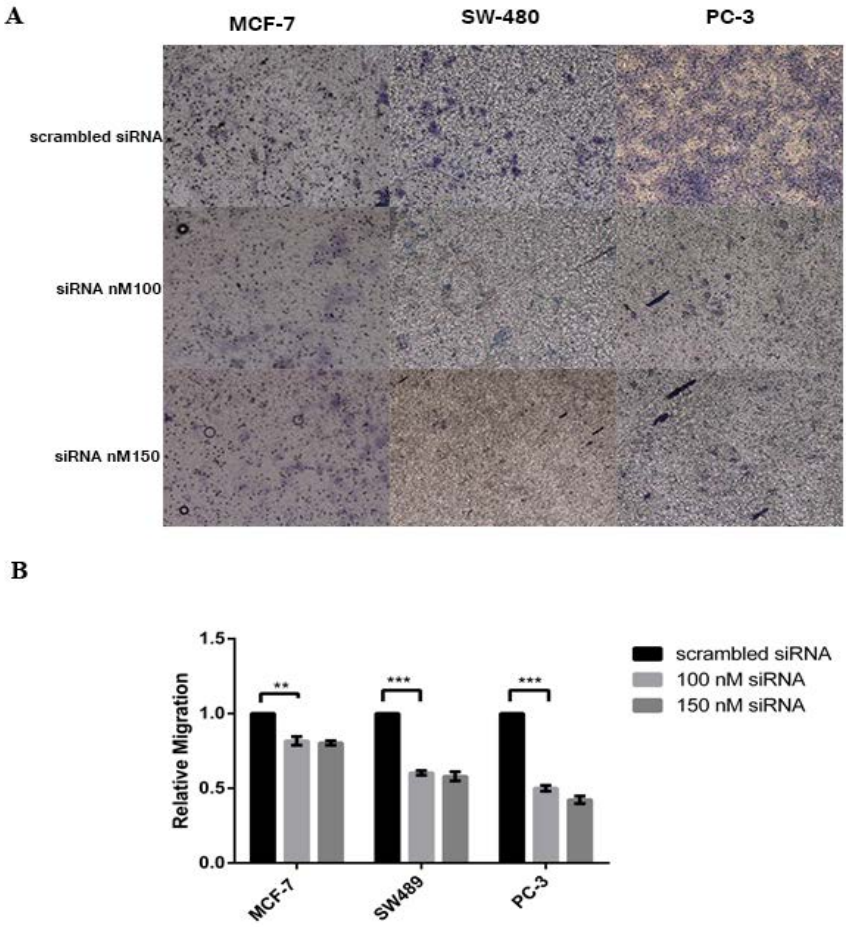

Fig. 2. The results of migration assay for the three cancer cell lines. The cells invaded by Transwell were detected on the downstream side of the filter by an inverted microscope, and then their amount was measured. A) The results of the Transwell® migration assay for each cancer cell line. B) The results were normalized to collect data from scrambled-siRNA-transfected cells. The values obtained are the Mean ± SD of at least three separate experiments.

The Stathmin 1-mRNA knockdown pattern was different in MCF7 in comparison to PC3 and SW480.

According to Fig. 3, siRNA succeeded to reduce the relative mRNA abundance for all three cancer cell lines. After two hours, the level of mRNA increased slowly to T0 in all three cancer cell lines. Nevertheless, based on the statistical data analysis, T0 and T2 were significantly different for PC3 and SW480 ($p < 0.0001$) in comparison to MCF7 ($p = 0.0004$). The statistical significance of T0 and T4 failed to change for PC3 and SW480 over time. However, according to Fig. 3, this statistical significance reduced for MCF7 ($p=0.0055$).

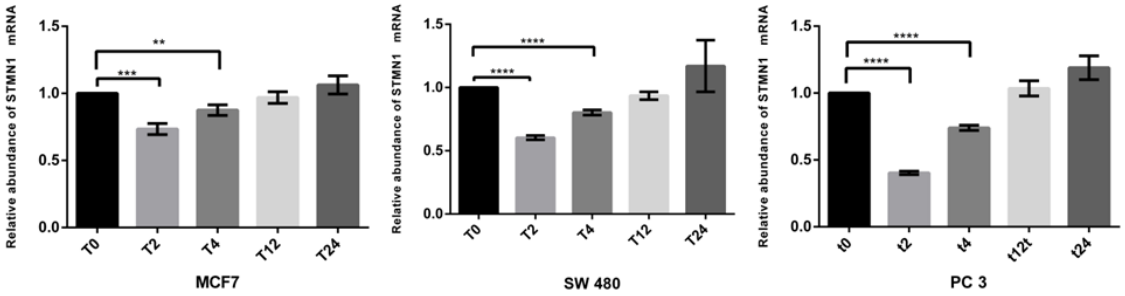

Fig. 3. Stathmin 1-RNA relative expression in PC3, SW480, and MCF7 (three cancer cell lines). The transfection of these cells was mediated by 100 nM concentration of siRNA. Extraction of total RNA was performed at appointed times. T0 RNA was chosen as the control group (n=3 ±SD).

Discussion

Inhibition of STMN1 mRNA in MCF-7, SW480, and PC-3 resulted in the reduction of viability and invasion in three cell lines. The highest sensitivity to the eliminated stmn1 was observed for PC-3. Our results suggest that stmn1 could be a suitable objective for RNAi therapy in prostate cancer relative to the colorectal and breast ones.

MTT results proved that STMN1 siRNA lessens the viability of MCF7, SW40, and PC3. We recognized the lowest viability in PC3 which was transfected with 100 nM of siRNA (74.48%).

The results shed light on the fact that in 50 nM concentration, there was an insignificant difference between the siRNA transfected and the control groups of the cell lines. As it was shown previously, STMN1 siRNA fails to augment the mortality of PC3 cells in 50 nM (Wegiel et al., 216). As previous studies revealed, the expression of stmn1 considerably augments during prostate morphogenesis period and cancer (Ghosh et al., 2007). Regarding the ability of 100 nM concentration of siRNA to optimally control the proliferation of PC3 cells, STMN1 inhibition can be considered as a therapeutic strategy for prostate cancer treatment. The usage of 200 nM siRNA failed to change the viability of the cell. This can be due to the saturation of the siRNA processing pathways in the cell (Kanasty et al., 2012). Relying on data obtained from MTT assay, pc3 cell line responsiveness to stmn1 siRNA-inhibition appears to be higher relative to its other counterparts.

STMN1 (Oncoprotein 18 and LAP18) leads to microtubule de-polymerization by tubulin sequestration and catastrophes stimulation (Cassimeris, 2002). It was mentioned that high STMN1 expression is related to cancer proliferation, resistance to taxanes (e.g., paclitaxel), and poorer prognosis in a wide range of human cancers (Nie et al., 214). Furthermore, STMN1 enacts a key role in numerous biologic processes associated with the control of cytoskeleton formation, cell cycle progression, mitotic division, cellular migration, and invasion, all of which significantly contribute to tumorigenesis. As demonstrated by some studies, STMN1 may act as a proliferation marker and integrate diverse signaling pathways such as tumor suppressor p27, p53, and phosphatidylinositol-3 kinase (PI3K/Akt) pathways (Myers, 2013)

The majority of studies have indicated that increased expression of stmn1, migration, and metastasis of malignant cells are considerably correlated. This phosphoprotein controls the cell's movement via regulating the microtubule dynamics in the cytoplasm. As it was proved earlier, inhibition of stmn1 decreases the lung cancer cells migration (Bao et al., 2017). To the best of our knowledge, no comparative study has been devoted so far to the impacts of STMN1 inhibition on prostate, breast, and colorectal cancers as the most prevalent cancers among both sexes. Considering the increasing interest of researchers in RNAi as a promising treatment approach, this study intends to identify the most sensitive cell line among breast cancer, colorectal, and prostate cancer cell lines as a promising direction to develop useful pharmacological interventions by STMN1 RNAi.

The findings of the invasion test exhibited that there is a significant difference between the cell lines apropos of sensitivity to siRNA ($p < 0.0001$) (Figure 2). Following medication with 150 nM of siRNA, the lowest and highest levels of invasion were observed for pc3 and mcf7 cells, respectively. According to the data analysis, 100 and 150 nM of siRNA failed to show any significant difference in three cell lines. In a study by Chakravarthi et al, miR-34a was used against stmn1, resulting in a 50% reduction in pc3 cells invasion. In line with the findings reported by them, this study also found that stmn1 knock-down lessens the movement of pc3 cells(Chakravarthi et al., 2018).

As shown in Figure 2, mcf7 cells have a lower response to stmn1-inhibition, as concluded from the invasion test results.

p53 mutation is the most frequent genetic irregularity observed in human cancer. Loss of p53 motion in cell lines is typically associated with several particular reasons including lack of growth inhibition or apoptosis following DNA injury and lack of induction of p53-regulated genes (Berglind et al., 2008).

As proved by studies, overexpression of p53 can decrease STMN1, whether directly or indirectly (Leroy et al., 2014). As MCF-7 expresses Wild-Type p53, the minimum effects of STMN1 inhibition are likely to be seen in MCF-7 (Figs. 1-3).

Due to the fact that SW 480 and PC-3 express mutated p53 (Aggarwal et al., 2018; Rochette et al., 2005), STMN1 inhibition considerably influences the invasion and viability of these cell lines (Figs. 1-3). It simply justifies the different reaction of mcf7 compared to its two other counterparts.

As it can be observed in Figure 3, siRNA managed to decrease cytoplasmic mRNA during 2-12 hr following transfection. Consequently, 2 hr following transfection, mRNA level showed the highest decrease in all cell lines. As it was revealed by the statistical analysis, the difference in SW480 and PC3 was relatively meaningful rather than MCF7 ($P<0.0001$ and $P = 0.0004$, respectively). For all cell lines, the cytoplasmic mRNA level began to go up after T2 and reached the mRNA levels of control group in T24. It is mostly due to lack of stably transfected cell lines for siRNA. Due to the unstable presence of siRNA and its destruction in the cell, transcription fails to be inhibited. Therefore, stably transfected cell lines for STMN1 siRNA seem to be a better approach to study the behavioral pattern of the cells.

In the end, according to findings of this study, response of the prostate cancer cell line to suppression of STMN1by siRNA is more considerable compared to that of breast and colorectal cancers' cell lines. Considering the importance of issue under study, further investigations are recommended to be done on STMN1 siRNA as a pharmacological intervention strategy in prostate cancer.

References

Aggarwal M, Saxena R, Asif N, Sinclair E, Tan J, Cruz I, et al. Reactivation of mutant p53 in human prostate cancer cells as a critical mechanism for inhibition of tumor growth by phenethyl isothiocyanate. AACR; 2018.

Amirkhah, R., Naderi-Meshkin, H., Mirahmadi, M., Allahyari, A., & Sharifi, H. R. (2017). Cancer statistics in Iran: Towards finding priority for prevention and treatment. *Cancer Press*, *3*(2), 27-38

Bao P, Yokobori T, Altan B, Iijima M, Azuma Y, Onozato R, et al. High STMN1 Expression is Associated with Cancer Progression and Chemo-Resistance in Lung Squamous Cell Carcinoma. Ann Surg Oncol. 2017;24(13):4017-24.

Belletti B, Baldassarre G. Stathmin: a protein with many tasks. New biomarker and potential target in cancer. Expert opinion on therapeutic targets. 2011;15(11):1249-66.

Berglind H, Pawitan Y, Kato S, Ishioka C, Soussi T. Analysis of p53 mutation status in human cancer cell lines: a paradigm for cell line cross-contamination. Cancer Biol Ther. 2008;7(5):699-708.

Bjorklund P, Cupisti K, Fryknas M, Isaksson A, Willenberg HS, Akerstrom G, et al. Stathmin as a marker for malignancy in pheochromocytomas. Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association. 2010;118(1):27-30.

Byrne FL, Yang L, Phillips PA, Hansford LM, Fletcher JI, Ormandy CJ, et al. RNAi-mediated stathmin suppression reduces lung metastasis in an orthotopic neuroblastoma mouse model. Oncogene. 2014;33(7):882-90.

Cao L, Fan L, Xu W, Li JY. Targeting MDM4 as a Novel Therapeutic Approach for Hematologic Malignancies. Current cancer drug targets. 2015;15(9):769-80.

Cassimeris L. The oncoprotein 18/stathmin family of microtubule destabilizers. Current opinion in cell biology. 2002;14(1):18-24.

Chakravarthi B, Chandrashekar DS, Agarwal S, Balasubramanya SAH, Pathi SS, Goswami MT, et al. miR-34a Regulates Expression of the Stathmin-1 Oncoprotein and Prostate Cancer Progression. Mol Cancer Res. 2018;16(7):1125-37.

Chen J, Abi-Daoud M, Wang A, Yang X, Zhang X, Feilotter HE, et al. Stathmin 1 is a potential novel oncogene in melanoma. Oncogene. 2013;32(10):1330-7.

Croce CM. Oncogenes and cancer. The New England journal of medicine. 2008;358(5):502-11.

Devi GR. siRNA-based approaches in cancer therapy. Cancer gene therapy. 2006;13(9):819-29.

Fatemian T, Chowdhury EH. Targeting oncogenes and tumor suppressors genes to mitigate chemoresistance. Current cancer drug targets. 2014;14(7):599-609.

Fatemian T, Othman I, Chowdhury EH. Strategies and validation for siRNA-based therapeutics for the reversal of multi-drug resistance in cancer. Drug discovery today. 2014;19(1):71-8.

Ghosh R, Gu G, Tillman E, Yuan J, Wang Y, Fazli L, et al. Increased expression and differential phosphorylation of stathmin may promote prostate cancer progression. The Prostate. 2007;67(10):1038-52.

Guo F, Luo Y, Mu YF, Qin SL, Qi Y, Qiu YE, et al. miR-193b directly targets STMN1 and inhibits the malignant phenotype in colorectal cancer. American journal of cancer research. 2016;6(11):2463-75.

Hassan MK, Watari H, Mitamura T, Mohamed Z, El-Khamisy SF, Ohba Y, et al. P18/Stathmin1 is regulated by miR-31 in ovarian cancer in response to taxane. Oncoscience. 2015;2(3):294-308.

Hemdan T, Linden M, Lind SB, Namuduri AV, Sjostedt E, de Stahl TD, et al. The prognostic value and therapeutic target role of stathmin-1 in urinary bladder cancer. British journal of cancer. 2014;111(6):1180-7.

Kanasty RL, Whitehead KA, Vegas AJ, Anderson DG. Action and reaction: the biological response to siRNA and its delivery vehicles. Mol Ther. 2012;20(3):513-24.

Kuang XY, Chen L, Zhang ZJ, Liu YR, Zheng YZ, Ling H, et al. Stathmin and phospho-stathmin protein signature is associated with survival outcomes of breast cancer patients. Oncotarget. 2015;6(26):22227-38.

Leroy B, Girard L, Hollestelle A, Minna JD, Gazdar AF, Soussi T. Analysis of TP53 mutation status in human cancer cell lines: a reassessment. Hum Mutat. 2014;35(6):756-65.

Li K, Zhou ZY, Ji PP, Luo HS. Knockdown of beta-catenin by siRNA influences proliferation, apoptosis and invasion of the colon cancer cell line SW480. Oncology letters. 2016;11(6):3896-900.

Myers AP. New strategies in endometrial cancer: targeting the PI3K/mTOR pathway--the devil is in the details. Clin Cancer Res. 2013;19(19):5264-74.

Nie W, Xu M-d, Gan L, Huang H, Xiu Q, Li B. Overexpression of stathmin 1 is a poor prognostic biomarker in non-small cell lung cancer. Laboratory Investigation. 2014;95:56.

Ogino S, Nosho K, Baba Y, Kure S, Shima K, Irahara N, et al. A cohort study of STMN1 expression in colorectal cancer: body mass index and prognosis. The American journal of gastroenterology. 2009;104(8):2047-56.

Rochette PJ, Bastien N, Lavoie J, Guerin SL, Drouin R. SW480, a p53 double-mutant cell line retains proficiency for some p53 functions. Journal of molecular biology. 2005;352(1):44-57.

Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA: a cancer journal for clinicians. 2018;68(1):7-30.

Steinmetz MO. Structure and thermodynamics of the tubulin-stathmin interaction. Journal of structural biology. 2007;158(2):137-47.

Stella Pelengaris MK. The Molecular Biology of Cancer :A Bridge From Bench to Bedside. 2013:633.

Troegeler A, Lastrucci C, Duval C, Tanne A, Cougoule C, Maridonneau-Parini I, et al. An efficient siRNA-mediated gene silencing in primary human monocytes, dendritic cells and macrophages. Immunology and cell biology. 2014;92(8):699-708.

Walther W, Schlag PM. Current status of gene therapy for cancer. Current opinion in oncology. 2013;25(6):659-64.

Wegiel B, Wang Y, Li M, Jernigan F, Sun L. Novel indolyl-chalcones target stathmin to induce cancer cell death. Cell Cycle. 2016;15(9):1288-94.