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# THE EFFECT OF RACEMIC GOSSYPOL AND AT-101 ON ANGIOGENIC PROFILE OF OVCAR-3 CELLS: A PRELIMINARY MOLECULAR FRAMEWORK FOR GOSSYPOL ENANTIOMERS

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***Aim*: To compare the effect of racemic gossypol with its (–)/(–) enantiomer (AT-101) on expression profiles of angiogenic molecules by mRNA levels in human ovarian cancer cell line OVCAR-3. *Methods:* Cell viability assay (2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide) was used to detect cytotoxicity of gossypol enantiomers. DNA fragmentation by an enzyme-linked immunosorbent (ELISA) assay was used to evaluate the rate of apoptosis. The mRNA expression levels of angiogenic molecules were investigated by Human Angiogenesis RT² Profiler™ PCR Array (SuperArray, Frederick, MD). *Results:* Both racemic form and AT-101 resulted in a significant cytotoxicity and induced apoptosis. This effect was observed in a dose- and time dependent manner. However, AT-101 was much more potent. In addition, the treatment of 10 μM of racemic gossypol alone and 3 μM of AT-101 alone resulted in significant down-regulation (⩾ 3 fold) in mRNA levels of some pivotal angiogenic molecules in OVCAR-3, but altered gene profiles were different by the treatment of each enantiomer. *Conclusion:* The efficacy of two gossypol enantiomers in OVCAR-3 cells showed distinction. AT-101 was much more potent than racemic gossypol, not only by means of cell death and apoptosis, but also by modulation of angiogenic molecules released from OVCAR-3 cells. Further studies with endothelial cells should be done to verify the anti-angiogenic effect of gossypol enantiomers in cancer treatment.**

***Key Words:* racemic gossypol, AT-101, angiogenesis, OVCAR-3, PCR array.**

Gossypol is a natural polyphenolic compound extracted from the cotton plant (*Gossypium species*) and the tropical tree, *Thespesia populnea.* In the late 1960's after several observations were done on gossypol's antifertility action in Chinese men, it attracted attention of many investigators trying to figure out the mechanisms lying beneath this property [1]. It was first demonstrated by Tuszynski and Cossu that gossypol has anti-proliferative effects against several cancer cell lines, the most sensitive of which are melanoma and colon carcinoma cells [2]. Although there are limited data about the molecular mechanisms induced and/or inhibited in gossypol exposed cancer cells, it was shown that gossypol induces apoptosis through inhibition of antiapoptotic Bcl-2 family members and loss of mitochondrial membrane potential and activation of caspase-3 [3, 4].There are also a number of clinical trials that gossypol show promising efficacy against some refractory human cancers [5].

Analysis of the enantiomeric ratios in different *Malvaceae* plants has shown an excess of the (+)/(–) enantiomer of gossypol (racemic gossypol) in most cottonseeds and in *Thespesia populnea*, with *Gossypium barbadense* being the main variety with an excess of the (–)/(–) enantiomer (AT-101) [6].

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*Abbreviations used*: ABTS – 2,29-azino-di-[3-ethylbenzthiazoline-sulfonate] diammonium salt; ELISA – enzyme-linked immunosorbent assay; FBS – fetal bovine serum; XTT – 2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide.

The antiproliferative effect of gossypol and its optical isomers on various human cell lines of reproductive and nonreproductive tissue origin was also studied and found that the (–)/(–) enantiomer of gossypol (AT-101) has more potent cytotoxic effects than the (+)/(–) enantiomer. Moreover, the cytotoxic effect of (–)/(–) gossypol (AT-101) was even observed in cells made resistant to adriamycin, vinblastin and cisplastin. Current investigations on the cisplatin-resistant head and neck squamous cell carcinoma (HNSCC) lines (UM-SCC-5PT and UM-SCC-10BPT) have also demonstrated the reversal of cisplatin resistance by AT-101 [6].

Although the mechanism for cytotoxic effect of gossypol on cancer cells has been widely studied, there is limited data for angiogenic properties of gossypol [7–9]. We have previously shown that gossypol potently inhibits some of angiogenic cytokines released from hormone- and drug refractory prostate cancer cell lines, at clinically achievable doses [10].

Angiogenesis is one of the major steps in the process of cancer progression. It has been demonstrated that tumor neovascularization strongly correlates with the risk of invasion and metastasis in nearly every type of human cancer [11]. Recent interest in the therapeutic potential of using angiogenesis as a target mechanism for anticancer therapy has led to the identification of various antiangiogenic agents that interfere at various stages of angiogenesis. In consideration of ovarian cancer, increased angiogenesis is also associated with rapid recurrence and decreased survival. Molecular-targeted therapeutics may potentially yield improvements in long-term disease control, and many

such agents are better tolerated than traditional cytotoxic drugs [12–14].

In this study, we aimed to compare the effect of gossypol enantiomers in OVCAR-3 cancer cells by means of cytotoxicity and apoptosis. OVCAR-3 is a highly metastatic, drug resistant human ovarian carcinoma cell line, and thus it is an ideal model to study the effects and mechanisms of various anticancer agents [15]. Moreover, to elucidate the underlying rationale of anticancer effects of gossypol enantiomers, we searched if any difference, by means of their effect on angiogenic molecules, exists between two enantiomeric forms of gossypol.

## MATERIALS AND METHODS

***Cell lines and reagents.*** Human OVCAR-3 ovarian cancer cells were obtained from ICLC (Genova, Italy). The cells were grown as monolayers in adherent cell lines and were routinely cultured in RPMI 1640 supplemented with 10% heat-inactivated FBS, 1% L-glutamine, 1% penicillin-streptomycin in 75 $cm^2$ polystyrene flasks (Corning Life Sciences, UK) and maintained at 37 °C in a humidified atmosphere with 5% $CO_2$. Growth and morphology were monitored and cells were passaged when they had reached 90% confluence. Cell culture supplies were obtained from Biological Industries (Kibbutz Beit Haemek, Israel). Racemic gossypol (98% > purity) was obtained from Sigma Chemical Co (USA). AT-101 was a generous gift from Ascenta Therapeutics (Nova Scotia, Canada). The stock solutions of racemic gossypol and AT-101 were prepared in DMSO (10 mM). The final dilutions were made immediately before use, and new stock solutions were made for each experiment. The DMSO concentration in the assay did not exceed 0.1% and was not cytotoxic to the tumor cells. All other chemicals, unless mentioned, were purchased from Sigma.

***XTT cell viability assay.*** After verifying cell viability using trypan blue dye exclusion test by Cellometer automatic cell counter (Nexcelom Inc.,USA.), cells were seeded at approximately $1x10^4$/ well in a final volume of 200 µl in 96-well flat-bottom microtiter plates with or without various concentrations of drugs. Plates were incubated at 37 °C in a 5% $CO_2$ incubator for the indicated time periods. At the end of incubation, 100 µl of XTT (Roche Applied Science, Mannheim, Germany) was added to each well, and plates were incubated at 37 °C for another 4 hours. Absorbance was measured at 450 nm against a reference wavelength at 650 nm using a microplate reader (Beckman Coulter, DTX 880 Multimode Reader). The mean of triplicate experiments for each dose was used to calculate the $IC_{50}$ values.

***Evaluation of apoptosis.*** Apoptosis was evaluated by an ELISA using Cell Death Detection ELISA Plus Kit (Roche Applied Science, Mannheim, Germany) according to the instruction manual. The relative amounts of mono- and oligonucleosomes generated from the apoptotic cells were quantified using monoclonal antibodies directed against DNA and histones by ELISA. Briefly, cytoplasmic fractions of the untreated control, racemic gossypol and AT-101 treated cells were transferred onto a streptavidin-coated plate and incubated for 2 hours at room temperature with a mixture of peroxidase conjugated anti-DNA and biotin labeled antihistone. The plate was washed thoroughly, incubated with ABTS, then absorbance was measured at 405 nm with a reference wavelength at 490 nm (Beckman Coulter, DTX 880 Multimode Reader).

***Cell treatment and RNA isolation.*** OVCAR-3 cells were treated with either 10 µM of racemic gossypol or 3 µM AT-101 (doses that were below $IC_{50}$ levels of both drugs) for 72 h, and then the total RNA from each sample was isolated by TridityG (Applichem) followed by chloroform according to the manufacturer's instructions. Samples were vigorously shaken for 15–20 s and were incubated for 15 min at room temperature to allow separation of aqueous layer with isopropanol, followed by a final wash in 75% ethanol. RNA pellets were air-dried and resuspended in RNase free water. RNA yield was determined spectrophotometrically by measuring the optical density at 260 nm and quality was determined by running samples on a 2% agarose gel and inspecting for distinct 18S, 28S and tRNA bands, indicating lack of degradation. Samples were frozen at –80 °C until use in cDNA synthesis.

***RT²Profiler™ human angiogenesis PCR array.*** 5 µg of total RNA was reverse transcribed in a final reaction mix of 20 µl using RT² First Strand Kit (SuperArray Bioscience) according to the manufacturer's instructions. cDNA was diluted by adding RNase free water. The PCR was carried out with a Light Cycler 480 instrument (Roche Applied Science, Mannheim, Germany). For one 96 well-plate of the PCR array, 2550 µl PCR master mix containing 2x SuperArray RT² qPCR Master Mix and 102 µl of diluted cDNA was prepared, and aliquat of 25 µl was added to each well. Universal cycling conditions (10 min at 95 °C, 15 s at 95 °C, 1 min 60 °C for 40 cycles) were used.

Five endogenous control genes beta-2-microglobulin (B2M), hypoxanthine phosphoribosyltransferase (HPRT1), ribosomal protein L13a (RPL13A), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and β-actin (ACTB) present on the PCR Array were used for normalization. Each replicate cycle threshold ($C_T$) was normalized to the average $C_T$ of 5 endogenous controls on a per plate basis. The comparative $C_T$ method was used to calculate the relative quantification of gene expression. The following formula was used to calculate the relative amount of the transcripts in the drugs treated samples and the control group, both of which were normalized to the endogenous controls: $\Delta\Delta C_T$ = $\Delta C_T$ (drugs treated) — $\Delta C_T$ (control) for RNA samples. $\Delta C_T$ is the $\log^2$ difference in $C_T$ between the target gene and endogenous controls by subtracting the average $C_T$ of controls from each replicate. The fold change for each treated sample was relative to the control sample = $2^{-\Delta\Delta CT.}$

***Sensitivity detection and identification of expressed genes.*** PCR Array quantification was based on the $C_T$ number. $C_T$ was defined as 35 for the $\Delta C_T$ calcula-

tion when the signal was under detectable limits. A list of differentially expressed genes was identified using a 2-tailed *t*-test. Changes in gene expression between drug treated cells and untreated controls were illustrated as a fold increase/decrease. The criteria were a *p* value less than 0.05 and a mean difference equal to or greater than 3-fold change in expression levels after treatment. The statistical calculation was based on the web-based program of RT²Profiler™ PCR Array Data Analysis. Alterations in mRNA levels which suited above criteria were considered to be up- or down regulated. The experiments were repeated three times.

***Statistical analysis.*** All experiments were conducted in triplicate and the results expressed as the mean ± SD, with differences assessed statistically with *p* values determined by Student's *t*-test.

## RESULTS

***Both racemic gossypol and AT-101 decreased the viability of human OVCAR-3 ovarian carcinoma cells in a time and dose dependent manner.*** To evaluate the effects of racemic gossypol and AT-101 on the viability of human ovarian cancer cells, OVCAR-3 cells were exposed to increasing concentrations of racemic gossypol (from 5- to 40 μM) and AT-101 (from 1- to 10 μM) for 24, 48 and 72 h, and XTT cell viability assay was performed. Both of the drugs decreased cell viability in a time- and dose dependent manner in OVCAR-3 cells (data not shown). As shown in figure 1, there were decreases in cell viability of 22-, 53-, and 65% in OVCAR-3 cells exposed to 5-, 15-, and 30 μM of racemic gossypol, respectively, when compared to untreated controls at 72 h. In addition, there were decreases in cell viability of 21-, 34-, and 78% in OVCAR-3 cells exposed to 2.5-, 5-, and 7.5 μM of AT-101, respectively, when compared to untreated controls at 72 h (Fig. 1). The highest cytotoxicity was observed at 72 h and $IC_{50}$ values of racemic gossypol and AT-101 in OVCAR-3 cells were calculated from cell viability plots and were found to be 16 and 4.2 μM, respectively.

As consistent with the literature, AT-101 was much more potent on reducing cell viability of ovarian cancer cells as compared to racemic gossypol.

***The evidence of apoptosis was more prominent in lower doses of AT-101 treated ovarian cancer cells as compared to racemic gossypol by DNA fragmentation analysis.*** As a marker of cell death, the levels of mono-oligo nucleosome fragments were measured using Cell Death Detection Plus Elisa Kit (Roche Applied Science, Mannheim, Germany). OVCAR-3 cells were treated with increasing concentrations of racemic gossypol or AT-101 for 72 h before analyzing DNA fragmentations. The results showed that when OVCAR-3 cells were exposed to 5, 15, and 30 μM of racemic gossypol, an increase in DNA fragmentation of 1.6, 2.9 and 7.2 times was observed (Fig. 2). However, when OVCAR-3 cells were exposed to 2.5, 5 and 7.5 μM AT-101, an increase in DNA fragmentation of 1.9, 2.6 and 4.9 times was observed (Fig. 2). These results clearly indicate that both of the drugs induce apoptosis in a dose- and time dependent manner (data not shown).

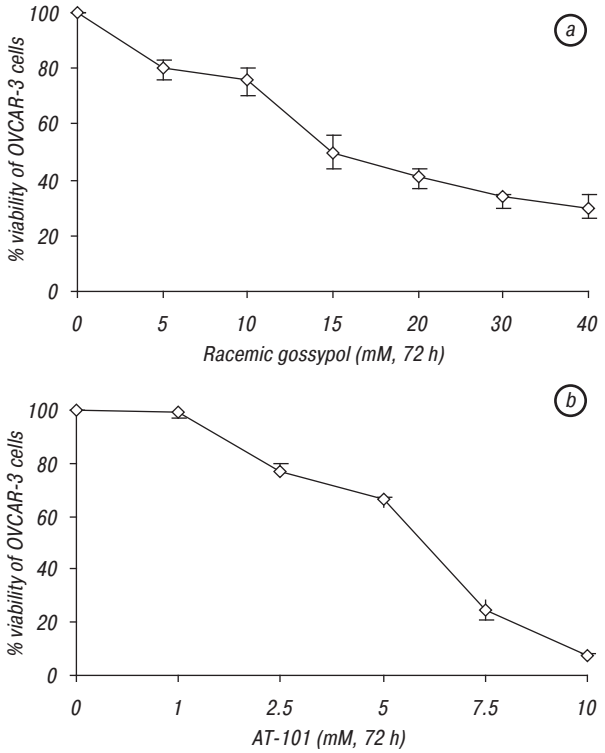


**Fig. 1.** Effect of single racemic gossypol and AT-101 on viability of OVCAR-3 cells at 72 h in culture. The data represents the mean of three different experiments ($p < 0.05$). The error bars represent the standard deviations, and where they are not seen, they are smaller than the thickness of the lines on the graphs.

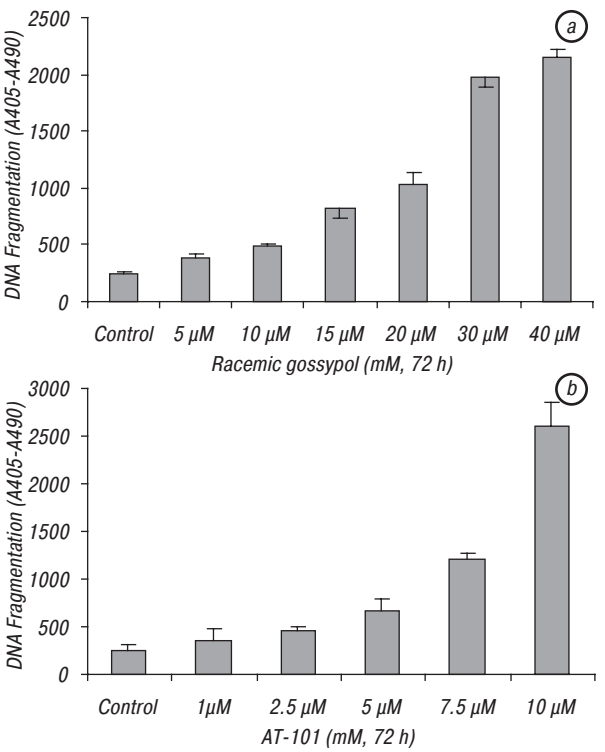


**Fig. 2.** Apoptotic effects of single racemic gossypol and AT-101 in OVCAR-3 cells through DNA fragmentation analyses. The data represents the mean of three different experiments ($p < 0.05$). The error bars represent the standard deviations, and where they are not seen, they are smaller than the thickness of the lines on the graphs.

***Comparison of angiogenic molecules of OVCAR-3 cells treated with either racemic gossypol or AT-101.*** To evaluate the effect of racemic gossypol and AT-101 on the angiogenic molecules in OVCAR-3 cells, we used RT²Profiler™ Human Angiogenesis PCR Array (SuperArray Bioscience). According to PCR array data, both of the drugs altered the expression profiles of some important angiogenic factors in OVCAR-3 cells as listed in Table 1. Changes in gene expression between drug treated cells and untreated controls were illustrated as a fold increase/decrease, and ⩾ 3 fold change was accepted as significant. While exposure of racemic gossypol (10 μM) caused down regulation of fibroblast growth factor 2 (FGF2), fibroblast growth factor receptor 3 (FGFR3), inhibitor of DNA binding 1, dominant negative helix-loop-helix protein (ID1), inhibitor of DNA binding 3, dominant negative helix-loop-helix protein (ID3), integrin alpha V (ITGAV), integrin beta 3 (ITGB3), midkine (MDK), matrix metalloproteinase 2 (MMP2), MMP9, plexin domain containing 1 (PLXDC1), transforming growth factor, beta 2 (TGFB2), TGFBR1, thrombospondin 1 (THBS1), THBS2 genes, AT-101 (3 μM) additionally caused down regulation of V-akt murine thymoma viral oncogene homolog 1 (AKT1), EPH receptor B4 (EPHB4), heparanase (HPSE), laminin, alpha 5 (LAMA5), neuropilin 1 (NRP1), tumor necrosis factor (TNF), tumor necrosis factor alpha-induced protein 2 (TNFAIP2), thymidine phosphorylase (TYMP), vascular endothelial growth factor A (VEGFA) and C genes apart from racemic gossypol. Furthermore, exposure of AT-101 caused to up-regulation of some genes that take role in inhibiting angiogenesis or inducing apoptosis (Table 1).

**Table 1.** Changes in mRNA levels of angiogenesis-related genes in OVCAR-3 cells exposed to racemic gossypol or AT-101. Changes ⩾ 3 fold was accepted as significant according to web-based program of RT²Profiler™ PCR Array Data Analysis.

| Racemic gossypol | | AT-101 | |
|---|---|---|---|
| Gene | Fold change | Gene | Fold change |
| FGF2 | –5.3 | AKT1 | –9.1 |
| FGFR3 | –3.9 | ANGPT2 | +7.2 |
| ID1 | –4.4 | BAI1 | +5.9 |
| ID3 | –3.6 | EPHB4 | –16.5 |
| ITGAV | –8.8 | FGF2 | –7.5 |
| ITGB3 | –4.7 | FGFR3 | –5.7 |
| MDK | –3.4 | HGF | –3.2 |
| MMP2 | –3.7 | HPSE | –5.5 |
| MMP9 | –4.1 | ID1 | –15.8 |
| PLXDC1 | –4.5 | ID3 | –18.0 |
| TGFB2 | –4.5 | ITGAV | –10.8 |
| TGFBR1 | –8.0 | ITGB3 | –4.9 |
| THBS1 | –6.0 | LAMA5 | –8.1 |
| THBS2 | –6.5 | JAG1 | +3.5 |
| VEGFC | –13.0 | LECT1 | +4.8 |
| | | MDK | –22.1 |
| | | MMP2 | –4.2 |
| | | MMP9 | –9.9 |
| | | NOTCH4 | +26.0 |
| | | NRP1 | –7.8 |
| | | PLXDC1 | –5.9 |
| | | TGFB2 | –4.9 |
| | | TGFBR1 | –10.9 |
| | | THBS1 | –14.7 |
| | | THBS2 | –8.1 |
| | | TIMP1 | +25.9 |
| | | TIMP2 | +16.8 |
| | | TNF | –15.2 |
| | | TNFAIP2 | –5.7 |
| | | TYMP | –15.3 |
| | | VEGFA | –11.3 |
| | | VEGFC | –30.7 |

## DISCUSSION

Gossypol is mainly found in two enantiomeric forms in nature: racemic form and (–)/(–) gossypol (AT-101). There is a body evidence that AT-101 was more potent than racemic form, although both enantiomeric forms of gossypol have cytotoxic and apoptotic effect for many types of cancer cells [16, 17]. Our study has demonstrated that the efficacy of two enantiomers was different from each other, and AT-101 was much more potent than gossypol, not only in cytotoxicity and apoptosis, but also by down-regulation of some crucial angiogenic molecules in OVCAR-3 cells.

Of angiogenic molecules that have been down-regulated by gossypol enantiomers were members of inhibitor of differentiation (ID), since upregulation of ID-1 is associated with advanced tumor stage and poor prognosis in ovarian cancer patients. Therefore ID-1 is one emerging targets for cancer treatment [18]. Furthermore, ID-3 as an important signaling molecule in vascularization may also serve as a therapeutic target in neovascularization [19]. Both racemic gossypol and AT-101 caused 4.4 and — 15.8 fold down-regulation on mRNA level of ID-1 respectively, whereas a –3.6 and 18 fold down regulation was observed in ID-3 (Table 2).

**Table 2.** Fold changes in mRNA levels of genes induced by both racemic gossypol and AT-101 treatment.

| Gene | Racemic gossypol | AT-101 |
|---|---|---|
| FGF2 | –5.3 | –7.5 |
| FGFR3 | –3.9 | –5.7 |
| ID1 | –4.4 | –15.8 |
| ID3 | –3.6 | –18.0 |
| ITGAV | –8.8 | –10.8 |
| ITGB3 | –4.7 | –4.9 |
| MDK | –3.4 | –22.1 |
| MMP2 | –3.7 | –4.2 |
| MMP9 | –4.1 | –9.9 |
| PLXDC1 | –4.5 | –5.9 |
| TGFB2 | –4.5 | –4.9 |
| TGFBR1 | –8.0 | –10.9 |
| THBS1 | –6.0 | –14.7 |
| THBS2 | –6.5 | –8.1 |
| VEGFC | –13.0 | –30.7 |

Heparin binding activity growth factors also show potent angiogenic properties, and their up-regulation is associated with a broad spectrum of mitogenic and angiogenic activities in some malignant tumors, including ovarian cancer [20]. Heparin binding activity growth factors (bFGF-2 and FGFR-3 and MDK) were down-regulated by both enantiomeric forms of gossypol; however the down regulation was much more evident in AT-101 exposure (Table 2). [21–23]. Moreover TGF-β2 and its receptors were also down regulated by both racemic gossypol and AT-101 [24].

VEGF has been correlated with poor disease-free survival and overall survival in patients with early or advanced stage ovarian cancer [25, 26]. Over expression of VEGF mRNA in the OVCAR-3 has been demonstrated previously. AT-101 caused a 30.7 fold decrease in VEGF C mRNA levels, while racemic gossypol resulted in only 13.0 fold decline. AT-101, alone, caused a –11.3 fold decrease in mRNA levels of VEGF A apart from racemic form.

Invasion and metastasis also require degradation of the basement membrane by members of the

MMP family proteins. MMP-2 and MMP-9 have been suggested to be critical in the metastatic process of ovarian cancer since they can degrade collagen IV [27]. The mRNA levels of these genes were down regulated by more than 3 fold by both racemic gossypol and AT-101.

EPHB2/B4 has been reported as a biomarker with negative prognostic value in ovarian carcinoma [28, 29]. AT-101 alone caused to down-regulate *EPBH4* gene (16.5 fold decrease) in OVCAR-3 cells. mRNA level of LAMA-5 was downregulated 8.1 fold. In many types of cancer, LAMA-5 has been observed to be highly expressed, implying that it is possible candidate for targeting angiogenesis in ovarian cancer [30].

Notch homolog 4 (NOTCH4), Angiopoietin 2 (ANGPT2), Brain-specific angiogenesis inhibitor 1(BAI1), Tissue inhibitor of metalloproteinase (TIMP1) and TIMP2 have been up-regulated significantly by 26.0, 7.2, 5.9, 10.9, and 14.7 fold respectively by AT-101 treatment. Up-regulation of these genes was shown to be associated with the inhibition of angiogenesis in cancer cells. For example activation of NOTCH4 modulates switching on angiogenesis [31]. ANGPT2 encodes a protein that disrupts the ability of vascular remodeling of angioprotein 1 and causes endothelial cell death [32]. BAI1 is an inhibitor of angiogenesis [33]. The activation of TIMPs results in inhibition of constructing new blood vessels [34].

As a conclusion, the efficacy of two gossypol enantiomers in OVCAR-3 cells showed distinction. AT-101 was much more potent than racemic gossypol, not only by means of cell death and apoptosis, but also by modulation of angiogenic molecules released from OVCAR-3 cells. These preliminary data highlights a possible role for future clinical trials with the potent form of gossypol enantiomer: AT-101. However, our study has some important limitations, since we have not yet obtained either *in vivo* results or studies with endothelial cells, which will certainly be very helpful in supporting our preliminary results. Further in-depth investigations should be done in xenograft models to confirm the efficacy and role of gossypol enantiomers in cancer treatment.

## REFERENCES

1. **Coutinho EM.** Gossypol: a contraceptive for men. Contraception 2002; **65**: 259–63.

2. **Tuszynski GP, Cossu G.** Differential cytotoxic effect of gossypol on human melanoma, colon carcinoma and other tissue culture cell lines. Cancer Res 1984; **44**: 768–71.

3. **Zhang M, Hongpeng L, Zhenkun T, *et al*.** Gossypol induces apoptosis in human PC-3 prostate cancer cells by modulating caspase-dependent and caspase-independent cell death pathways. Life Sci 2007; **80**: 767–74.

4. **Mohammmad RM, Wangs S, Banerjee S, *et al*.** Nonpeptidic small molecule inhibitor of Bcl-2 and Bcl-XL, (–) Gossypol, enhances biological effect of genistein against BxPC-3 human pancreatic cancer cell line. Pancreas 2005; **31**: 317–24.

5. **Liu G, Kelly WK, Wilding G, *et al*.** An open-label, multicenter, hase I/II study of single-agent AT-101 in men with castrate-resistant prostate cancer. Clin Cancer Res 2009; **15**: 3172–6.

6. **Dodou K.** Investigations on gossypol: past and present developments. Expert Opin Investig Drugs 2005; **14**: 1419–34.

7. **Zhang M, Liu H, Guo R, *et al*.** Molecular mechanism of gossypol-induced cell growth inhibition and cell death of HT-29 human colon carcinoma cells. Biochem Pharmacol 2003; **66**: 93–103.

8. **Mohammad RM, Wang S, Banerjee S, *et al*.** Nonpeptidic small-molecule inhibitor of Bcl-2 and Bcl-XL, (–)-Gossypol, enhances biological effect of genistein against BxPC-3 human pancreatic cancer cell line. Pancreas 2005; **31**: 317–24.

9. **Zhang M, Liu H, Tian Z, *et al*.** Gossypol induces apoptosis in human PC-3 prostate cancer cells by modulating caspase-dependent and caspase-independent cell death pathways. Life Sci 2007; **80**: 767–74.

10. **Karaca B, Kucukzeybek Y, Gorumlu G, *et al*.** Profiling of angiogenic cytokines produced by hormone- and drug-refractory prostate cancer cell lines, PC-3 and DU-145 before and after treatment with gossypol. Eur Cytokine Netw 2008; **19**: 176–84.

11. **Alonso Carrión L, González Sánchez FJ, *et al*.** The importance of angiogenesis in ovarian cancer. Rev Oncol 2004; **6**: 462–7.

12. **Cannistra SA, Matulonis UA, Penson RT, *et al*.** Phase II study of bevacizumab in patients with platinum resistant ovarian cancer or peritoneal serous cancer. J Clin Oncol 2007; **25**: 5180–6.

13. **Burger RA, Sill MW, Monk BJ, *et al*.** Phase II trial of bevacizumab in persistent or recurrent epithelial ovarian cancer or primary peritoneal cancer: a gynecologic oncology group study. J Clin Oncol 2007; **25**: 5165–71.

14. **Martin L, Schilder R.** Novel approaches in advancing the treatment of epithelial ovarian cancer: the role of angiogenesis inhibition. J Clin Oncol 2007; **25**: 2894–901.

15. **Hamilton TC, Young RC, McKoy WM, *et al*.** Characterization of a human ovarian carcinoma cell line (NIH:OVCAR-3) with androgen and estrogen receptors. Cancer Res 1983; **43**: 5379–89.

16. **Macpherson GR, Ng SSW, Lakhani NJ, *et al*.** Antiangiogenesis therapeutic strategies in prostate cancer. Cancer Metastasis Rev 2002; **21**: 93–106.

17. **Qiu J, Levın LR, Buck J, *et al*.** Different pathways of cell killing by gossypol enantiomers. Exp Biol Med Vol 2002; **227**: 398–401.

18. **Schindl M, Schoppmann SF, Ströbel T, *et al*.** Level of ID-1 protein expression correlates with poor differentiation, enhanced malignant potential, and more aggressive clinical behavior of epithelial ovarian tumors. Clin Cancer Res 2003; **9**: 779–85.

19. **Felty Q, Porther N.** Estrogen-induced redox sensitive Id3 signaling controls the growth of vascular cells. Atherosclerosis 2008; **198**: 12–21.

20. **Nissen LJ, Cao R, Hedlund EM, *et al*.** Angiogenic factors FGF2 and PDGF-BB synergistically promote murine tumor neovascularization and metastasis. J Clin Invest 2007; **117**: 2766–77.

21. **Mirkin BL, Clark S, Zheng X, *et al*.** Identification of midkine as a mediator for intercellular transfer of drug resistance. Oncogene 2005; **24**: 4965–74.

22. **Beenken A, Mohammadi M.** The FGF family: biology, pathophysiology and therapy. Nat Rev Drug Discov 2009; **8**: 235–53.

23. **Lozano RM, Redondo-Horcajo M, Jimenez MA, *et al*.** Solution structure and interaction with basic and acidic fibroblast growth factor of a 3-kDa human platelet factor-4 fragment with antiangiogenic activity. J Biol Chem 2001; **276**: 35723–34.

24. **Schlingensiepen KH, Fischer-Blass B, Schmaus S, *et al*.** Antisense therapeutics for tumor treatment: the TGF-beta2 inhibitor AP 12009 in clinical development against malignant tumors. Recent Results Cancer Res 2008; **177**: 137–50.

25. **Hartenbach EM, Olson TA, Goswitz JJ, *et al.*** Vascular endothelial growth factor (VEGF) expression and survival in human epithelial ovarian carcinomas. Cancer Lett 1997; **121**: 169–75.

26. **Barleon B, Sozzani S, Zhou D, *et al.*** Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor flt-1. Blood 1996; **87**: 3336–43.

27. **Brun JL, Cortez A, Commo F, *et al.*** Serous and mucinous ovarian tumors express different profiles of MMP-2, -7, -9, MT1-MMP, and TIMP-1 and -2. Int J Oncol 2008; **33**: 1239–46.

28. **Wu Q, Suo Z, Kristensen GB, *et al.*** The prognostic impact of EphB2/B4 expression on patients with advanced ovarian carcinoma. Gynecol Oncol 2006; **102**: 15–21.

29. **Spentzos D, Levine DA, Kolia S, *et al.*** Unique gene expression profile based on pathologic response in epithelial ovarian cancer. J Clin Oncol 2005; **23**: 7911–18.

30. **Hao J, Jackson L, Calaluce R, *et al.*** Investigation into the mechanism of the loss of laminin 5 (alpha3beta3-gamma2) expression in prostate cancer. Am J Pathol 2001; **158**: 1129–35.

31. **Leong KG, Hu X, Li L, *et al.*** Activated Notch4 inhibits angiogenesis: role of beta 1-integrin activation. Mol Cell Biol 2002; **22**: 2830–41.

32. **Maisonpierre PC, Suri C, Jones PF, *et al.*** Angiopoietin-2, a natural antagonist for Tie2 that disrupts *in vivo* angiogenesis. Science 1997; **277**: 55–60.

33. **Kang X, Xiao X, Harata M, *et al.*** Antiangiogenic activity of BAI1 *in vivo*: implications for gene therapy of human glioblastomas. Cancer Gene Ther 2006; **13**: 385–92.

34. **Reed MJ, Koike T, Sadoun E, *et al.*** Inhibition of TIMP1 enhances angiogenesis in vivo and cell migration *in vitro*. Microvasc Res 2003; **65**: 9–17.