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RESEARCH ARTICLE

Targeting inhibitors of apoptosis signaling proteins in leiomyomas: the potency of proapoptotic derived peptides to affect growth and apoptosis.

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ABSTRACT

Purpose: To depict the pattern of Inhibitors of Apoptosis signaling proteins controlling survival and/or apoptosis, and to investigate the impact of the following metabolic stimuli: ovarian steroids, estrogen and progesterone at challenge with Epidermal Growth Factor, and the potential Inhibitors of Apoptosis-derived peptides affecting growth and apoptosis of leiomyoma.

Methods: Paired cell cultures of leiomyoma and normal myometrium from premenopausal women undergoing hysterectomy were obtained. Western blot analysis, the Wilcoxon signed rank test, the Kolmogorov-Smirnov nonparametric test, the Two Tailed Analysis and the Student t test were applied. Statistical significance was established at $P \leq 0.05$

Results: The Western blot results clearly demonstrate that the level of proapoptotic protein ARTS and Caspase 9 proteins was significantly higher in the leiomyoma cells compared with the myometrium. XIAP and Caspase 3 proteins level was similar in both cell cultures. Notably, exposure to estradiol at Epithelial Growth Factor challenge of the leiomyoma cells significantly decreased the level of ARTS and Caspase 9 proteins. Exposure to estradiol or progesterone separately, as well as to progesterone at Epithelial Growth Factor challenge with no significant effect on the activity of Inhibitors of Apoptosis proteins of the leiomyoma cells ARTS derived peptides AIBM P1 and ARTS derived peptides AIBM P3 affected growth of leiomyoma and myometrium cells in a dose and time dependent manner with variability among the samples. The expression of Inhibitors of Apoptosis proteins of paired leiomyoma and myometrium cells treated with ARTS derived peptides P1 and ARTS derived peptides P3 was variable. At exposure to ARTS derived peptides P3 the level of ARTS and Caspase 9 proteins was slightly higher in the leiomyoma cells compared to the myometrium cells, but not statistically significant.

Conclusion: Our results provide a clearer insight into Inhibitors of apoptosis signaling proteins, response to ovarian steroids at challenge with Epithelial Growth Factor, and notably to ARTS derived peptides affecting growth and apoptosis of leiomyoma cells.

Introduction

Uterine leiomyomas are the most common smooth muscle tumors in women. Leiomyomas are a major health concern that can severely impaired quality of life. Leiomyomas can cause infertility, miscarriage, menorrhagia, pain and an increased hysterectomy rate.[1,2,3] Leiomyomas are smooth muscle benign monoclonal tumors, developed by transformation of myometrium somatic stem cells, viewed as a multistep process.[4] Many risk factors have been associated with the development of leiomyomas, including chromosome abnormalities (chromothripsis), steroid hormones, demographic, reproductive and lifestyle factors.[5,6,7]

However, the precise pathologic mechanism of leiomyomas is still unresolved.3Pathological alterations of signaling pathways have been recognized as a key feature in a variety of human diseases.[8] Deregulation of signaling networks are crucial for leiomyomas development[9]. Studies have supported a role of growth factors and their receptors tyrosine kinases (RTKs) in uterine leiomyoma growth, their regulation by ovarian hormones, and the "cross talk" between the estrogen receptor alpha and RTK signaling pathways.[9,10] The successful development of protein tyrosine kinase inhibitors (PTK) has indicated them as candidates for "signal transduction therapy".[8,11] Tyrphostins were the first signal transduction agents to be used in clinical practice. The current challenge is to identify agents that need to be combined with Protein Tyrosine Kinases (PTK) inhibitors to increase their efficacy.[11] Indeed, we identified a tyrosine kinase inhibitor and a selective Epidermal Growth Factor (EGF) inhibitor AG1478, as an effective suppressor of leiomyoma cell growth, unaffected by ovarian steroid hormones.[12] We demonstrated that downstream signaling components of the PI3K/AKT pathway, GSK3 and cyclin D2 were significantly elevated in leiomyomas.[13] As well as the significant interaction between PTEN-PDK and between pakt-pGSK3b,are involved in the survival and proliferation of leiomyomas.[13] Further, we showed that All-Trans-Retinoic Acid (ATRA) induced changes in the expression and activation of Retinol and PI3K/AKT pathway proteins in leiomyoma cultured cells: a significant increase of ADH1 (a principal enzyme of the Retinol pathway) and cyclin D2 (a promotor of G1/S progression), a relative decrease of pGSK3β (pro-apoptotic), and a relative increase of BAX (pro-apoptotic). Thus, demonstrating that ATRA treatment at PI3K pathway suppression significantly affected growth, signaling pattern and interactions among PI3K/BCL2/Retinol proteins involved in growth, survival and apoptosis of leiomyomas.[14]

ARTS/XIAP is a survival-apoptosis system that interacts with PI3K/AKT. Interaction between PI3K/AKT and XIAP (X-linked IAP) pathways has been shown in tumor cells, e.g. ovarian carcinoma, leukemia and melanoma.[15,16,17] Whereas, high levels of XIAP found in many types of cancers, often correlate with poor prognosis.[18,19,20] The binding of ARTS to XIAP involves sequences that are distinct from all other known IAP antagonists.[21]

The Inhibitor of apoptosis proteins (IAPs) bind to pro-apoptotic proteases, keeping them inactive and preventing cell death. IAP proteins are characterized by the presence of the conserved baculoviral IAP repeat (BIR) domain that is involved in protein-protein interactions. IAPs were initially thought to be mainly responsible for caspase inhibition, acting as negative regulators of apoptosis, but later works have shown that IAPs also control a plethora of other different cellular pathways.[20,21,22] As X-linked IAP (XIAP), and other IAP, levels are often deregulated in cancer cells and have been shown to correlate with patients prognosis.[22] Many small molecules have been designed to target the BIR domains, the vast majority being inspired by the N-terminal tetrapeptide of Second Mitochondria-derived Activator of Caspases/Direct IAP Binding with Low pI (Smac /Diablo), which is the natural XIAP antagonist. These compounds are therefore usually referred to as Smac mimetics (SMs). Despite the fact that SMs were intended to specifically target XIAP, it has been shown that they also interact with cellular IAP-1 (cIAP1) and cIAP2, promoting their proteasome-dependent degradation. SMs have been tested in combination with several cytotoxic compounds and are now considered promising immune modulators which can be exploited in cancer therapy, especially in combination with immune checkpoint inhibitors.[18,19,20,21,22] ARTS derived peptides-IBM (AIBM) are sufficient to bind XIAP and promote apoptosis. Moreover, AIBM-based peptides can bind to BIR3/XIAP and reduce XIAP levels inducing apoptosis in a mechanism similar to

function of full-length ARTS protein[21]. AIBM peptides penetrate cancer cells, induce caspase activation, and apoptosis, thus becoming a basis for developing ARTS-based cancer therapeutics [18,19,20,21,22].

These findings promoted us to explore and determine: 1) the signaling pathway alternations among Inhibitors of Apoptosis-IAP central target proteins of leiomyoma and healthy myometrium cultured cells 2) the impact of metabolic changes/stimuli: the ovarian steroid hormones 17ß-estradiol (E2) and progesterone involved in growth and maintenance of leiomyomas, at challenge with the EGF and 3) the potency of ARTS-derived peptides-AIBM to affect growth and apoptosis of leiomyoma cells following treatment.

Material and Methods

CELL CULTURE MEDIUM

The antibodies used to monitor the level of signaling proteins and compounds are summarized in Table 1.

Table 1: Antibodies and compound details

Antibody	Company	Cat No
ATRS	Sigma Aldrich Co IL (Merck)	300215902
XIAP	BD Transduction Laboratories	610717
Caspase 3	Cell Signaling	#9662
Caspase 9	Cell Signaling	#9508
ß actin	Sigma Aldrich Co IL	BM#6552
a actin	Santa Cruz Biotechnology	sc-32251
17ß-estradiol (E2)	Sigma Aldrich Co IL	E 4389
Progesterone	Sigma Aldrich Co IL	P 7556

Fetal bovine serum-USA origin (cat#:10210-106) (Gibco Invitrogen,USA). Fetal bovine serum–charcoal stripped (cat# 04-210-1B), DMEM (cat#: 01-055-1A), DMEM without phenol red (cat# 01-053-1A), Trypsin-EDTA solution (cat#:03-052-1A) and antibiotics-Pen-Strep (cat#: 03-031-1B) are from Biological Industries (Beit Haemek, Israel). ARTS derived synthetic peptides: AIBM Pep1–YGPSLRLLA and AIBM Pep3– QEHQGQGCH. The peptides were kindly provided by Prof. Sarit Larisch. (Laboratory of Cell Death and Cancer Research, Biology & Human Biology Departments, Faculty of Natural Sciences, University of Haifa, Haifa 3498838, Israel).

The synthetic peptides AIBM P1 and AIBM P3 were synthesized, labeled, purified, and analyzed as described in Reingewertz and colleagues.[21] Stock solutions were made prior to each experiment and kept at – 80ºC until use.

STUDY PARTICIPANTS

Caucasian pre-menopausal women, 35-50 years of age, undergoing hysterectomy for symptomatic leiomyomas. These patients were not receiving any hormonal treatments at the time of surgery. Leiomyomas size ranged between 5-15 cm. All tumor sections were taken in the region next to the periphery of the tumor (1.0-1.5 cm away from periphery). The operations were not scheduled to a specific phase of the menstrual cycle, but pregnancy was excluded in all cases. Samples of corresponding myometrium were also collected.

The study was approved by the Hadassah Institutional Research Board (No 365-7.9.07). Informed consent was obtained from each patient.

CELL CULTURES

Paired cell cultures of leiomyoma and adjacent normal myometrial tissue samples were established as previously described.[13] Primary cell cultures were initiated in DMEM culture medium with 20% fetal bovine serum and antibiotics (penicillin 100 u/ml and streptomycin 100 µg/ml). Thereafter the cell cultures were propagated in DMEM phenol red-free medium and 10% charcoal-stripped fetal bovine serum. The experiments were performed on secondary and/or tertiary cultures. Practiced protocols apply cell cultures between 1-3 passages.[23] α-actin was used to verify that the culture derived from smooth muscle cells (SMC), remains unchanged up to 5 passages in vitro.[24] The cells cultures were maintained at 37oC in a humidified incubator, containing 5% CO2. Logarithmically growing cells were used in the experiments.

CELL PROLIFERATION ASSAY

Cells were seeded at 1x104/well in 96/well microplates and grown for 2-3 days. Thereafter, the medium was replaced with a medium containing the ARTS-derived peptides 100nM, 1000nM AIBM P1, AIBM P3, and the vehicle-only control and grown as indicated. Cell proliferation was determined using the colorimetric methylene blue assay.[12]

EXPOSURE TO 17 B-ESTRADIOL (E2) OR PROGESTERONE AND AT CHALLENGE WITH EGF

Cells were seeded at $2x10^5$ cells /35 mm plates in DMEM phenol red-free medium and 10% charcoal-stripped fetal bovine serum for 2 days. Thereafter, the cultures were washed, fed with medium without serum, containing the steroid: 10 ng/ml estradiol or 100 ng/ml progesterone for 48 hrs. Cells were than stimulated with 30 ng/ml EGF for 10 min. The reaction was stopped by placing the cultures on ice and washed with ice-cold PBS. Immunoblot of the relevant proteins was performed on whole cell-lysates.[12]

EXPOSURE TO ARTS DERIVED PEPTIDES AIBM P1 (YGPSLRLLA) AND AIBM P3 (QEHQGQGCH)
Leiomyoma and myometrium cells were seeded at 1x104/well in 96/well microplates or at 0.5-$0.7x10^6$ in $25cm^2$ flasks and grown for 2-3 days.

EFFECT ON GROWTH
AIBM P1 and AIBM P3 at 100,1000 nM were diluted in medium serum from a stock solution of 1mM. Thereafter, the medium was replaced with medium containing the peptides AIBM P1, AIBM P3 and the vehicle-only control, and further grown for 3 days.

EFFECT ON IAP SIGNALING PROTEINS
AIBM P1 and AIBM P3 at 100 nM were diluted in medium serum from a stock solution of 1mM. Thereafter, the medium was replaced with medium containing the peptides AIBM P1, AIBM P3 and the vehicle-only control, further grown for 24 hrs. and Western blot (WB) analyzed.

BIOCHEMICAL ACTIVITIES
WB analysis for relevant proteins was carried as previously described and according to manufacturer recommendation.[12] Briefly, secondary or tertiary cells were seeded 0.7- 0.9 x 10^6 cells/$25cm^2$ flasks in phenol red-free DMEM and 10% charcoal-stripped fetal bovine serum for 2-3 days. At the end of the experiment, the cell cultures were washed twice with 20 ml cold phosphate buffered saline, 0.1ml lysis buffer was added to each culture and incubated on ice for 5 min. Cell lysates were boiled for 5 min. and frozen for further use. The developed bands were scanned and quantified by a densitometer (Image Master VDS-CL machine and the BIS 303 PC, Bio imaging systems, and TINA 2.0 software) and expressed in arbitrary units. The level of each protein is normalized to β-actin.

STATISTICS
The Wilcoxon signed rank test was used to compare the WB intensity value of leiomyoma and myometrium cell cultures with respect to ARTS, XIAP Cas3 and Cas9 proteins. Analysis of the WB densitometric results was based on data from matched leiomyoma and myometrium cell cultures. Statistical significance was established at $p \leq 0.05$.

The Two Tailed test was applied to analyzes the data on the effect of ovarian steroids estradiol and progesterone, at challenge with EGF of leiomyoma and myometrium cell cultures with respect to ARTS, XIAP Cas3 and Cas9 proteins. Statistical significance was established at $p \leq 0.05$. Data obtained of the effect of AIBM P1 and AIBM P3 peptides on growth of leiomyoma and myometrium cell cultures was analyzed using the Kolmogorov-Smirnov (KS) nonparametric test. This test compares the cumulative distribution function for a variable with a specified distribution, recommended, when the size of the sample is small (n=3). The results showed that the two groups were sampled from populations with different distribution. The test was applied since the cell samples were small (n=3). Small P value indicates that the two groups were sampled from populations with different distributions. The populations may differ in median, variability or the shape of the distribution. Student t test was applied for the data obtained from determination of the WB intensity of ARTS, XIAP, Cas3 and Cas9 of leiomyoma and myometrium cell cultures (n=4). Statistical significance was established at $p \leq 0.05$.

Results

PATTERN OF IAP SIGNALING PROTEINS OF LEIOMYOMA AND NORMAL MYOMETRIUM CELLS.
The expression and activation profile of IAP family proteins, involved in growth and apoptosis of leiomyoma and normal myometrium cells were WB analyzed. The results demonstrate significant differences in the level of ARTS and Cas9 proteins of the leiomyoma compared with normal myometrium cells, analyzed by the Wilcoxon signed rank test. (Figure 1)

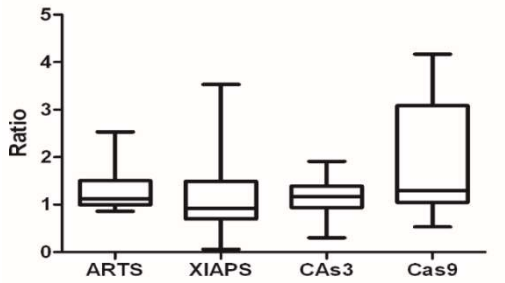

Fig 1: Expression profile of IAP signaling proteins associated with growth and apoptosis of leiomyoma and myometrium cells.

Expression of IAP signaling proteins ATRS (n=14), XIAP (n=15), Cas3 (n=12) and Cas9 (n=9) of the leiomyoma and myometrium cells. The proteins level of both cell populations was determined by WB, normalized to β-actin and expressed in arbitrary units (Wilcoxon signed rank test). Box plots indicate the upper and lower quartiles, with the horizontal line representing the median.

The level of ARTS (n=14), XIAP (n=15), Cas3 (n=12) and Cas9 (n=9) proteins between leiomyoma and myometrium cells is determined by densitometric measurement, normalized to ß-actin. The result clearly indicates that the ARTS and Cas9 proteins level was significantly higher in the leiomyoma compared to the myometrium cells ($p<0.020$ and $p<0.054$ respectively). The level of XIAP and Cas3 proteins of the leiomyoma cells was not significantly different compared to the myometrium cells.

EFFECTS OF PHYSIOLOGICAL AND METABOLIC CHANGES ON LEIOMYOMA CELLS SIGNALING
The effects of physiological and metabolic changes/stimuli on the leiomyoma IAP proteins ATRS, XIAP, Cas3 and Cas9 were examined and compared to the paired myometrium controls: Treatment with ovarian steroids estradiol and progesterone, as well as at challenge with EGF, a potent stimulator of leiomyoma growth that also mediates estrogen action.[26,38,39]

1) EFFECTS OF OVARIAN STEROIDS
Paired leiomyoma and myometrium cells were grown in medium for 2 days, washed and fed with medium without serum containing 10 ng/ml estrogen or 100 ng/ml progesterone for additional 2 days and stimulated with 30 ng/ml EGF for 10 min (n=3). Immunoblot of the relevant proteins was performed on whole cell-lysates.[12] The ovarian steroids concentrations are within the physiological tissue concentrations found in leiomyomas and myometrium.[12,25]

1a) Effect of estradiol at challenge with EGF
The pattern of ARTS proteins at treatment with estradiol was heterogenous. The results did not reach statistical significance. EGF challenge at estradiol treatment of the leiomyoma cells increased significantly the level of ARTS and Cas9 proteins ($p<0.04$ and $p<0.01$ respectively) (Two tailed analysis). (Figure 2a).

2B) EFFECT OF PROGESTERONE AT CHALLENGE WITH EGF
The signaling pattern of ARTS proteins at treatment with progesterone was heterogenous. However, there might be a possible indication that at challenge with EGF the level of ARTS and Cas9 proteins was slightly decreased in the leiomyoma cells (ARTS, XIAP), (Cas9, XIAP) (Figure 2b). Two tailed analysis indicated that the results did not reach statistical significance.

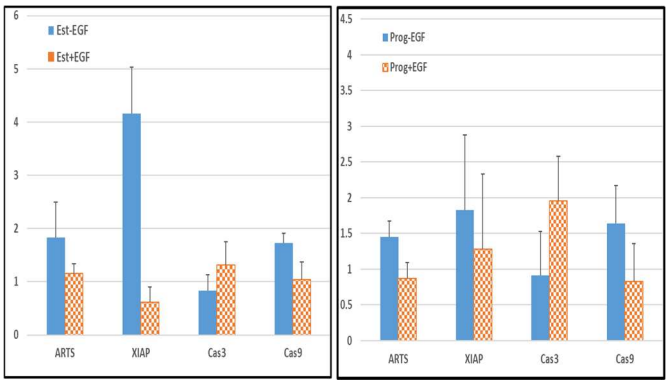

Fig 2: The effect of ovarian steroids at challenge with EGF on IAP proteins expression of leiomyoma and myometrium cells

Paired leiomyoma and myometrium cells grown with 10 ng/ml estrogen or 100 ng/ml progesterone and stimulated with 30 ng/ml EGF 2a) Estradiol at challenge with EGF affected significantly the level of ARTS and Cas9 proteins ($p<0.04$ and $p<0.01$ respectively) of the leiomyoma cells. 2b) Progesterone at challenge with EGF had no effect.

THE POTENCY OF AIBM P1 AND AIBM P3 PEPTIDES TO ARREST GROWTH OF LEIOMYOMA CELLS
The effect of AIBM P1 and AIBM P3 peptides on the growth of leiomyoma cells was examined. Paired leiomyoma and myometrium cells were

grown for 2-3 days, then exposed to AIBM P1, AIBM P3 peptides at 100 nM, 1000nM and the vehicle-only control (n=4). The growth pattern of both cell populations was determined on day 1 and on day 3. AIBM P1 and AIBM P3 affected the growth of leiomyoma and myometrium cells in a dose and time dependent manner with variability among the samples (Figure 3a-3c).

AIBM P1 and AIBM P3 at 1000nM decreased the growth of Sample 173 leiomyoma cells (≈57% and ≈57% respectively) at day 1 of treatment, not at day3, with no effect on the myometrium cell (Figure 3a). Whereas AIBM P1 at 100nM decreased the growth of the Sample 174 myometrium cells (≈61%) at day 1 of treatment, not at day3, with no effect on the leiomyoma cells (Figure 3b). Both AIBM P1and AIBM P3 had no effect on the growth of both cell population of Sample 175, for the duration of the experiment (Figure 3c). The KS test was applied to compare the cumulative distribution function for a variable with a specified distribution, recommended when the two groups were sampled from populations with different distribution. size of the sample is small (n=3). The results showed that the two groups were sampled from populations with different distribution.

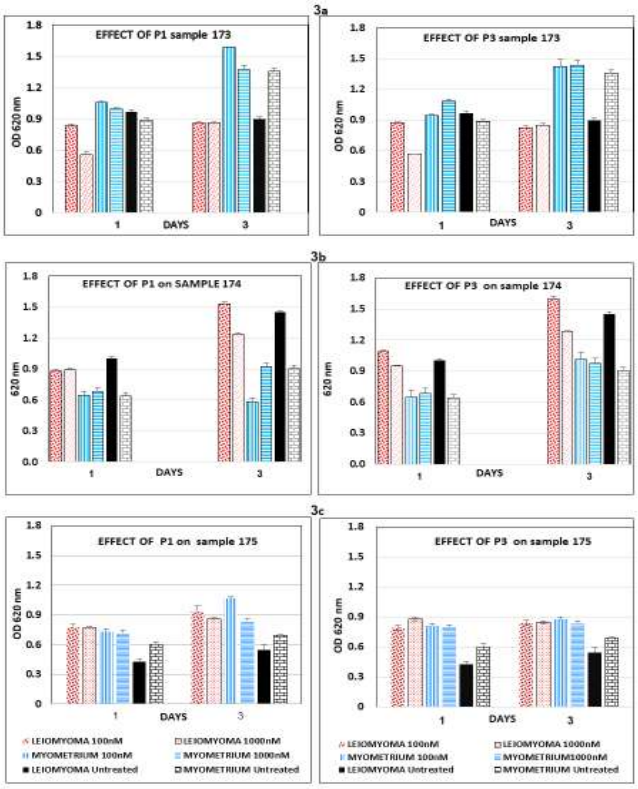

Fig 3: The effect of ARTS-derived peptides AIBM P1 and AIBM P3 on the growth of leiomyoma and myometrium cells.

Matched leiomyoma and myometrium cells were exposed to AIBM P1 and AIBM P3 at 100 nM, 1000 nM, the vehicle-only control. Cells proliferation and the ARTS-derived peptides effect on the leiomyoma compared to the myometrium cells were analyzed (The KS test).

EFFECTS OF AIBM P1 AND AIBM P3 PEPTIDES ON IAP SIGNALING PROTEINS OF LEIOMYOMA CELLS

The effect of AIBM P1 and AIBM P3 peptides on IAP signaling proteins of leiomyoma cells was examined. Paired leiomyoma and myometrium cells were grown for 2-3 days, exposed to to 100 nM AIBM P1, AIBM P3 peptides and the vehicle-only control for 24 hrs (n=4). The level of ARTS, XIAP, Cas3 and Cas9 proteins of the cells was Western blot determined. Although there was variability among the cultures, there might be a possible indication that AIBM P3 increased the level of ARTS and Cas9 of the leiomyoma cells slightly more compared to the myometrium (ARTS, X≈2.7, Cas9, X≈ 1.4 respectively) (Student t test) (Figure 4). To this point, as shown in Figure 1 ARTS and Cas9 proteins level is significantly higher in the leiomyoma cells compared to the myometrium ($p<0.020$ and $p<0.054$ respectively).

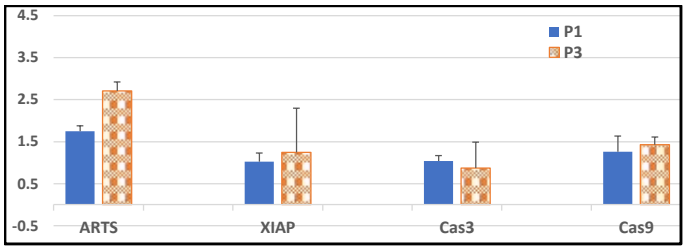

Fig 4: The effect of ARTS-derived peptides AIBM P1 and AIBM P3 on the growth of leiomyoma and myometrium cells.

Matched leiomyoma and myometrium cells were exposed to AIBM P1 and AIBM P3 at 100 nM, 1000 nM, the vehicle-only control. Cells proliferation and the ARTS-derived peptides effect on the leiomyoma compared to the myometrium cells were analyzed (The KS test).

Discussion

The present study demonstrates the significant alternations and impact of Inhibitors of Apoptosis-IAP signaling proteins, key regulators of growth and apoptosis on leiomyoma cell cultures, as well as the effect of metabolic

changes /stimuli: ovarian steroids and EGF on their expression and activation. ARTS and Caspase9 protein levels were significantly elevated in the leiomyoma cells compared with the myometrium cells. The level of XIAP and Casase3 proteins was similar in both cell cultures (WB analyzed). Noteworthy are the changes that ARTS-derived (AIBM) peptides induced on the proliferation of the leiomyoma cells.

The Inhibitor of apoptosis proteins (IAPs) bind to pro-apoptotic proteases, keeping them inactive and preventing cell death.[18] IAPs were initially thought to be mainly responsible for caspase inhibition, acting as negative regulators of apoptosis, but later works have shown that IAPs also control a plethora of other different cellular pathways.[18] ARTS proteins inhibit apoptosis by targeting XIAP and blocking caspases activation or activity. [19,20,21]

Caspases are proteolytic enzymes that belong to the cysteine protease family and play a crucial role in homeostasis and programmed cell death.[30] Caspases participate in both early and late stages of apoptosis.[30,31] Caspases involved in apoptosis have been subclassified by their mechanism of action as either initiator caspases (caspase-8 and caspase-9) or executioner caspases (caspase-3, caspase-6, and caspase-7). Caspases that participate in apoptosis are inhibited by proteins known as inhibitors of apoptosis (IAPs).[18] Cas9 is a key player in the intrinsic or mitochondrial pathway which is involved in various stimuli, including stress agents and radiation.[32] Cas9 is altered in pathological conditions, thus raising the possibility that it can act as a therapeutic target[33]

Several pharmacological agents have been suggested as effective alternative treatment for leiomyomas, through stimulation of Cas3 and Cas9, induction of apoptosis and growth arrest.[34,35,36]

Cell death regulation is vital for maintenance of homeostasis and proper development of multicellular organisms. IAP proteins implicated in cell death regulation, are frequently deregulated in cancer, contribute to disease initiation, tumor maintenance, and progression making them obvious targets for anticancer therapy.[18, 22] Support for the function of ARTS as a tumor suppressor protein came from studies in mice and in human patients.[20] Our present results demonstrated the impact of metabolic changes, the ovarian steroid hormones estradiol and progesterone, involved in the pathogenesis of leiomyomas, at challenge with the EGF on ARTS proteins level. Exposure to estradiol at EGF stimulation affected significantly ARTS and Cas9 proteins level of the leiomyoma cells. Exposure to estradiol or progesterone separately, as well as to progesterone at challenge with EGF had no significant effect on the activity of IAP proteins of the leiomyoma cells. Notably, in a previous study we showed that estradiol and progesterone treatment separately or in combination did not affect the proliferation of leiomyoma cell cultures.[12] The role of ovarian steroid hormones in the pathogenesis of leiomyomas is supported by epidemiological, clinical, and experimental evidence[5,25,26.]

Estradiol is involved in several factors, including progesterone, availability of progesterone receptors, growth factors, genetic and epigenetic factors.[27] The role of estrogen in leiomyomas is important for understanding their pathobiology and for the development of successful therapeutics. Estrogen-signaling pathways in leiomyomas include genomic (direct and indirect) and nongenomic including Ras-Raf-MEK (MAPK/Erk Kinase)-mitogen-activated protein kinase (MAPK) and phosphatidylinositide 3-kinase (PI3K)-phosphatidylinositol-3,4,5-trisphosphate (PIP3)-Akt (Protein kinase B)-mammalian target of rapamycin (mTOR) pathways; shortly Ras-Raf-MEK-MAPK and PI3K-PIP3-Akt-mTOR pathways.[27]

Progesterone plays a vital role in the structure, function and regulation of the female reproductive tract, including pregnancy. Progesterone mediates its function by interacting with the progesterone receptors, a member of a superfamily of almost 50 ligand-activated nuclear transcription factors.[27] In leiomyomas progesterone participates in development and proliferation processes.[29] Of notice, a recent review summarizing studies on progestogens and leiomyomas has concluded that the evidence points to lack of their efficacy.[37] An additional function of estrogen and progesterone is stimulation of the proliferation potential of leiomyomas through induction of EGR and EGF receptors (EGFR) expression.[38,39] Non-hormonal mediators such as growth factors, early life exposure and inflammation are also key factors in leiomyoma biology. Numerous agents

depend on those pathways and may find their place in the current and future therapy of leiomyomas.[40] Finding non-hormonal therapeutic targets will help to serve a group of patients who would prefer an alternative to surgical intervention.[40]

Importantly, we provided evidence that exposure to ARTS-derived (AIBM) peptides affected the proliferation of the leiomyoma cells. AIBM P1 and AIBM P3 peptides affected growth of the leiomyoma cells in a dose and time dependent manner, with variability among the samples.

Statistical analysis indicated that the two groups were sampled from populations with different distributions (The KS test). Further, AIBM P1 and AIBM P3 peptides induced changes in the level of IAP proteins of the leiomyoma cells with variability among the cultures. However, there might be a possible indication that AIBM P3 increased the level of ARTS and Cas9 of the leiomyoma cells slightly more compared to the myometrium cells (Student t test)

The pro-apoptotic ARTS protein acts as a dual antagonist of both XIAP and Bcl-2.[19,20] Binding of ARTS to XIAP is direct and leads to decreased XIAP protein levels and caspase activation, suggesting that it induces apoptosis by antagonizing IAPs.[19,20,21]ARTS is also important for killing cells by other pro-apoptotic factors, such as arabinoside etoposide and staurosporine. [20]

ARTS based short peptides derived from the C-terminus of ARTS (AIBM P2 and AIBM P3) were shown to bind XIAP and induce apoptosis in leukemia cell lines, K562 and CCRF-CEM.[22] Although all 3 peptides were efficiently taken up by the leukemia cells, AIBM P3 exhibited the highest apoptosis rate, AIBM P2 showed mild apoptotic effect, whereas AIBM P1 had no apoptotic effect. AIBM P3 was also most potent in promoting Caspase3 and Caspase9 activity, and in reducing XIAP levels, thus suggesting that these peptides exhibit a specific differential effect on these leukemia cells rather than nonspecific cytotoxicity.[22]

Recently a small molecule was described A4, that mimics the function of ARTS.[41] Like ARTS, A4 stimulated poly-ubiquitylation and the ubiquitin-proteasome system (UPS) mediated degradation of XIAP and Bcl-2, but not cIAP1, resulting in Caspase 9 and Caspase3 activation and apoptosis. The effect of A4 was selective as peripheral blood mononuclear cells and normal human breast epithelial cells were unaffected. Furthermore, cancer cell lines with high levels of XIAP were particularly sensitive to the killing effect of A4. A4 represents a novel class of dual-targeting compounds stimulating apoptosis by UPS-mediated degradation of important anti-apoptotic oncogenes.[41]

In view of the increasing understanding of leiomyomas management, of the interplay between genes, epigenetics, lifestyle and environment, leiomyomas management should be geared towards individualized preventive and treatment options, as well as to advanced design solutions Artificial Intelligence. Ideally, individualized therapies will offer primary prevention for women at high risk of leiomyomas and secondary prevention after initial treatment.[42,43]

Collectively, the present results highlight the impact of specific IAP signaling proteins, exposure to ovarian steroids at challenge with EGF, and notably to ARTS based peptides, derived from the C-terminus of ARTS (AIBM) affecting signaling and growth of leiomyoma cells. Interpretation of our results suggests that increasing knowledge of the role of signaling interplay in the pathogenesis of leiomyomas may present an opportunity to use specific signal transduction inhibitors for treating and preventing this tumor.

Conflicts of Interest:

None.

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