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HEALTH SCIENCES

Fatty acid synthase as a potential new therapeutic target for cervical cancer

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Abstract: Fatty acid synthase (FASN) is the rate-limiting enzyme for the de novo synthesis of fatty acids in the cytoplasm of tumour cells. Many tumour cells express high levels of FASN, and its expression is associated with a poorer prognosis. Cervical cancer is a major public health problem, representing the fourth most common cancer affecting women worldwide. To date, only a few in silico studies have correlated FASN expression with cervical cancer. This study aimed to investigate in vitro FASN expression in premalignant lesions and cervical cancer samples and the effects of a FASN inhibitor on cervical cancer cells. FASN expression was observed in all cervical cancer samples with increased expression at more advanced cervical cancer stages. The FASN inhibitor (orlistat) reduced the in vitro cell viability of cervical cancer cells (C-33A, ME-180, HeLa and SiHa) in a time-dependent manner and triggered apoptosis. FASN inhibitor also led to cell cycle arrest and autophagy. FASN may be a potential therapeutic target for cervical cancer, and medicinal chemists, pharmaceutical researchers and formulators should consider this finding in the development of new treatment approaches for this cancer type.

Key words: Fatty acid synthase, FASN, Cervical cancer, Orlistat.

INTRODUCTION

Cervical cancer represents a major public health problem, and it is the fourth most common cancer type affecting women worldwide (Bray et al. 2018). The main risk factor for the development of cervical cancer is persistent infection with high-risk human papillomavirus (HPV) (Walboomers et al. 1999). This virus infects cervical cells leading to cell transformation, which can progress to precursor lesions of cervical cancer (Ostor 1993). Despite this, the presence of HPV infection alone is not sufficient to trigger carcinogenesis, as additional alterations are also necessary (Hanahan & Weinberg 2011).

Fatty acid synthase (FASN) is the main enzyme responsible for the de novo synthesis of fatty acids through the condensation of malonyl-CoA and acetyl-CoA and the formation of palmitate, a 16-carbon fatty acid (Costello & Franklin 2005, Jayakumar et al. 1995). De novo synthesis plays an important role in energy homeostasis because it converts excessive ingested carbon into fatty acids, which are stored and used when needed to produce energy through oxidation. In addition, it contributes to the production of membrane lipids, which are essential for cell division and cell membrane formation. Because of the wide availability of dietary fatty acids, de novo synthesis and FASN activity are normally low in most tissues except the liver, adipose tissue and lactating mammary glands (Menendez & Lupu 2007, Weiss et al.

1986). On the other hand, studies have shown that many tumours have high levels of FASN, and its expression is associated with a poorer prognosis and resistance to chemotherapy (Kuhajda 2006, Wu et al. 2014, Haider et al. 2020).

Studies using FASN inhibitors have shown that inhibition leads to a decrease in cell proliferation, with subsequent death by apoptosis (Zhou et al. 2003). Furthermore, when FASN is inhibited, cell cycle arrest occurs in vitro and tumour growth is inhibited in xenograft models (Buckley et al. 2017). There are different inhibitors of FASN activity, including cerulenin, C75, orlistat (ORL), and TVB-264. Cerulenin and C75 are FASN inhibitors that have demonstrated antiproliferative activity in different types of cancer cells. However, the cerulenin presents chemical instability due to the presence of a highly reactive epoxy group in its chemical structure and consequently its toxic effects that limits its use in animal models (Lupu & Menendez 2006). C75 also shows severe side effects in animal models and non-specific binding to other proteins (Fhu & Ali 2020). On the other hand, TVB-2640 is an inhibitor that has recently entered in a clinical trial with promising results (Falchook et al. 2021). In our study, ORL (Figure 1) was chosen as a model of FASN inhibitor due to its commercial availability as capsules and therefore its easy access as well as its previous approval for the treatment of obesity. Moreover, ORL has been shown to be an irreversible inhibitor of FASN, with an important antiproliferative effect in different types of tumors (Czumaj et al. 2019, You et al. 2019, Schcolnik-Cabrera et al. 2018, Peng et al. 2018, Agostini et al. 2014, Seguin et al. 2012, Kridel et al. 2004).

Although overexpression of FASN is well established for some types of cancer, FASN expression has not yet been studied in cervical cancer. There are only a few in silico screening

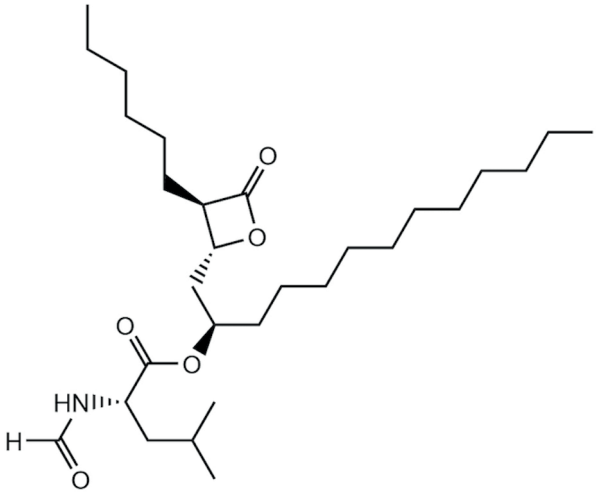

Figure 1. Chemical structure of orlistat (ChemDraw Professional®).

studies that have identified FASN as a marker for cervical cancer (Nisthul et al. 2018, Xia et al. 2018). Therefore, the aim of this study was to evaluate FASN expression in low- and high-grade squamous intraepithelial lesions and cervical cancer samples collected from patients. In addition, the consequences of in vitro FASN inhibition by ORL were evaluated using different cervical cancer cell lines.

MATERIALS AND METHODS

Samples

Samples were obtained from 36 patients who underwent gynaecological procedures at a reference hospital in the south of Brazil. The tissues were embedded in paraffin and cut at a thickness of 4 µm. Sections were mounted on microscope slides, fixed in 4% formalin (pH 7.0) and stained using haematoxylin and eosin to determine the pathological type and grade according to the Bethesda System for Reporting Cervical Cytology (Bethesda 2015). Samples were divided into four groups: nine samples of cervicitis (classified as control), nine samples of low-grade squamous intraepithelial lesions (LSIL), nine samples of high-grade squamous

intraepithelial lesions (HSIL), and nine cervical cancer samples. It is important to emphasise that it was not possible to obtain samples of normal cervical tissue for this study because patients without cytologic evidence of malignancy are not normally submitted to biopsy. This study was approved by the Research Ethics Committee of Hospital de Clínicas de Porto Alegre and Research Ethics Committee of Universidade Federal do Rio Grande do Sul (Approval protocol number: 2115382).

Immunohistochemistry

The expression of FASN was analyzed using the Starr Trek Universal HRP Detection System (Biocare Medical, Pacheco, USA). Briefly, sections were dewaxed with xylene, gradually hydrated with a graded alcohol series, then washed with phosphate-buffered saline (PBS). The sections were incubated with citrate buffer, 5% hydrogen peroxide and the background sniper reagent from the commercial kit. Afterwards, sections were incubated overnight with a primary anti-FASN antibody at 1:200 dilution (BD Transduction Laboratories, San Jose, USA). After washes, incubations were performed with a secondary biotinylated antibody, peroxidase enzyme and the betazoid diaminobenzidine chromogen revealing solution from the commercial kit. Tissue sections were counterstained with haematoxylin, then dehydrated and mounted. The intensity of staining was semiquantitatively graded as 0 (no staining), 1 (weak staining), 2 (moderate staining), or 3 (strong staining). Samples were divided into four groups based on the extent of immunostaining, assessed as the percentage of positive cells observed under a microscope: 0 (<10% positive cells), 1 (10–25% positive cells), 2 (26–75% positive cells), and 3 (>75% positive cells). The expression levels (final scores) were calculated by multiplying the score given by the percentage of positive cells by the grade of the intensity of staining. Sections with a final score of 1 or less were considered as (-), between 2 and 4 as (+), between 5 and 8 as (++), and 9 was considered as (+++).

Cell culture

In this study, four different cervical cancer cell lines (C-33A, ME-180, HeLa and SiHa) were used. They were obtained from American Type Culture Collection (ATCC® HTB-31™, ATCC® HTB-33™, ATCC® CCL-2™, ATCC® HTB-35™; Rockville, USA). The C-33A cell line is derived from cervical carcinoma and it does not contain copies of HPV integrated into its genome. The HeLa and SiHa cell lines contain HPV 18 and 16, respectively. The HeLa cell line is derived from a uterine adenocarcinoma and SiHa is from a cervical stage II squamous cell carcinoma. The ME-180 cell line is derived from a metastatic site in the peritoneum. It has copies of HPV with high homology to HPV 68, and it is derived from a cervical squamous cell carcinoma. Cervical carcinoma cell lines were maintained in cell culture flasks in low-glucose DMEM (Sigma-Aldrich, St. Louis, USA) supplemented with 10% foetal bovine serum (Gibco, Grand Island, USA), 1% penicillin/streptomycin and 0.1% amphotericin (Sigma-Aldrich, St. Louis, USA) at 37°C in a 5% CO_2 atmosphere. The ME-180 cell line was maintained under the same conditions but cultured with RPMI medium (Sigma-Aldrich, St. Louis, USA).

Preparation of orlistat solutions

For cell culture experiments, orlistat (ORL) was used as a FASN inhibitor and extracted from Xenical® (Roche, Basel, Switzerland) capsules according to Knowles et al. (2004). The content of each capsule was solubilised in 1 mL of ethanol, followed by centrifugation (12,000 *g* for 5 min) to separate insoluble products. The supernatant (250 mM of ORL) was stored at -80°C. The final concentration was determined

by high-performance liquid chromatography (Dolenc et al. 2010). Analyses were performed using a Shimadzu LC system (Shimadzu, Kyoto, Japan) equipped with a SPD-20AV ultraviolet (UV) detector. A C_{18} column was used as the stationary phase, and the mobile phase was water containing 0.1% (v/v) of phosphoric acid (85%) and acetonitrile (5:95, v/v), run at a flow rate of 1.5 $mL.min^{-1}$. Ultraviolet detection was carried out at 205 nm. The method was specific, linear (r = 0.999, n = 3) in the range of 10.00–30.00 $\mu g.mL^{-1}$, and precise (SD = 1.64% and 3.13% for intra- and interday precision, respectively).

Cell counting

To evaluate the effects of FASN inhibition on cell viability, the cell lines were treated with 100, 200, 300, 400 and 500 µM of ORL and incubated for 24, 48 and 72 h. These ORL concentrations were determined in preliminary tests by our research group and were based on previous studies on FASN inhibition with ORL (Carvalho et al. 2008). Cell lines (1×10^4 cells/well) were seeded on 24-well plates. Following incubation, the medium was removed, cells were washed with PBS, trypsinized with 0.25% trypsin/EDTA and counted using a FACSVerse flow cytometer (BD Biosciences, San Jose, USA). The results are expressed as the percentage of control.

Clonogenic cell survival assay

To evaluate the colony formation capacity of cells after FASN inhibition with ORL, the cell lines were seeded on 24-well plates (1×10^4 cells/well), treated with 300 µM of ORL, and incubated at 24, 48 and 72 h. The surviving adherent cells were washed with PBS, trypsinized, counted and replated in 6-well plates (500 cells/well). After 10 days of incubation in a complete culture medium, the colonies formed from each cell were fixed with methanol, stained with crystal violet and counted manually. Plating efficiency (PE) was evaluated, and the fraction of surviving cells was calculated.

$$\text{Plating efficiency (PE)} = (\text{number of colonies counted}/\text{number of cells plated}) \times 100$$

$$\text{Survival fraction} = (\text{PE of treated sample}/\text{PE of control}) \times 100$$

Annexin V and propidium iodide staining

The induction of apoptosis and necrosis was analyzed using an Annexin V-FITC Apoptosis Kit (QuatroG, Porto Alegre, BR) according to the manufacturer's instructions with some modifications. Briefly, the cell lines were plated in 24-well plates (1×10^4 cells/well) and treated with 300 or 400 µM of the FASN inhibitor (ORL). Afterwards, cells were harvested and incubated with 150 µL of annexin-binding buffer (10 mM 4-(2-hydroxyethyl)-1-pipera-zineethanesulfonic acid, 140 mM NaCl and 2.5 mM $CaCl_2$; pH 7.4), annexin V at 0.75 µL/sample and propidium iodide (PI) at 15 µL/sample for 15 min at room temperature in the dark, then cells were analyzed using a FACSVerse flow cytometer (BD Biosciences, San Jose, USA).

Cell cycle assay

The effects of FASN inhibition on cell cycle phase distribution were assessed using flow cytometry. Cells were plated in a 6-well plate (6×10^4 cells/well) then treated with 300 or 400 µM of ORL. After treatments, cells were harvested and fixed in cold ethanol 70% v/v in PBS for 2 h. Fixed cells were washed with PBS and marked with a solution containing 12 µg/L PI, Triton X-100 and RNAse for 30 min in the dark at room temperature. DNA content was analyzed using a FACSVerse flow cytometer (BD Biosciences, San Jose, USA).

Quantification of acidic vacuolar organelles by acridine orange staining

For the determination of acidic vacuolar organelles (a typical feature of autophagy), cells were plate in a 24-well plate (1 x 10^4 cells) and exposed to 200, 300, or 400 µM of ORL. After the incubation, cells were trypsinized and incubated with acridine orange (AO; 2.7 mM) for 15 min at room temperature, then the fluorescence emission was analyzed by flow cytometry using a FACSVerse flow cytometer (BD Biosciences, San Jose, USA).

Statistical analysis

Statistical analyses were performed with Prism 5 (GraphPad, La Jolla, CA). Data are expressed as the percentage of control and presented as the mean and standard deviation (SD) of at least three independent experiments. Statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Values were considered significant at $p < 0.05$.

RESULTS

FASN expression in cervical tissues

To evaluate FASN expression, immunohistochemistry was performed on cervicitis tissue (non-malignant control), LSIL, HSIL and cervical cancer samples collected from patients. The immunohistochemical staining showed that FASN was mainly localized in the cytoplasm of cervical cells, while the stroma exhibited no staining or weak focal staining (Figure 2). Weak staining was detected in cervicitis tissue samples. FASN expression in all LSIL, HSIL and carcinoma samples was more intense than in cervicitis tissue. LSIL showed moderate expression in 6/9 (66.7%) samples. High expression was detected in 4/9 (44%) HSIL and 4/9 (44%) carcinoma samples (Table I), with a similar expression profile in these two groups. No significant correlation was observed between the grade of the lesion and FASN expression, although there was a clear trend towards a progressive increase in expression as the stage of disease increased.

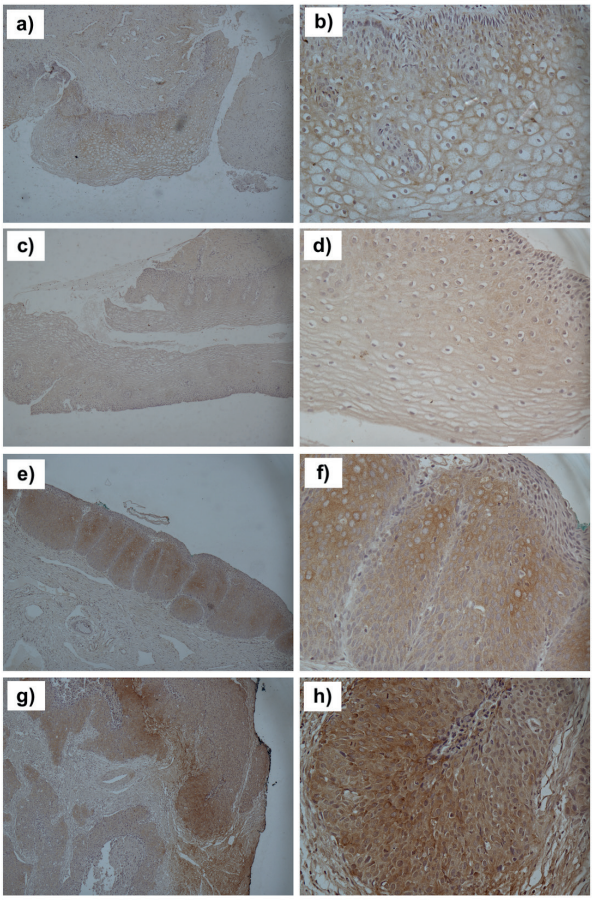

Figure 2. Representative immunohistochemistry images showing FASN expression in cervical tissue. FASN was detected in cervicitis (a, b), LSIL (c, d), HSIL (e, f) and carcinoma (g, h) samples. Images in panels a, c, e and g are at original magnification and 200X magnification; images in panels b, d, f and h are at original magnification and 400X magnification.

FASN inhibition affects the growth of cervical cancer cells

To evaluate whether the inhibition of FASN activity can modify the growth rate of cervical cancer cells, the HeLa, SiHa, ME-180 and C-33A cell lines were treated with increasing

Table I. FASN expression in cervical tissue.

Treatments (n)	Levels of staining			
	- (%)	+ (%)	++ (%)	+++ (%)
Control (9)	4 (44.4)	4 (44.4)	1 (11.1)	0 (0.0)
LSIL (9)	0 (0.0)	3 (33.3)	6 (66.7)	0 (0.0)
HSIL (9)	0 (0.0)	2 (22.2)	3 (33.3)	4 (44.4)
Carcinoma (9)	0 (0.0)	3 (33.3)	2 (22.2)	4 (44.4)

LSIL: Low-grade Squamous Intraepithelial Lesion; HSIL: High-grade Squamous Intraepithelial Lesion. The expression levels (final scores) were calculated by multiplying the score given by the percentage of positive cells by the grade of the intensity of staining. Sections with a final score of 1 or less were considered as (-), between 2 and 4 as (+), between 5 and 8 as (++), and 9 was considered as (+++).

concentrations of ORL. As shown in Figure 3, FASN inhibitor reduced the number of viable cells in all cell lines. HeLa and SiHa showed the highest reduction in the number of cells when compared to C-33A and ME-180 (Figure 3a-d). In addition, incubation with 300 μM of ORL for 24, 48 and 72 h reduced the number of viable cells in a time-dependent manner (Figure 3e). ORL had already significantly reduced the viability after 24 h of incubation. Furthermore, the surviving cells had reduced long-term viability, as evidenced by the reduced clonogenic survival of cells that survived 72 h of treatment, indicating a slow mechanism of cell death (Figure 3f). To better characterize the antiproliferative effects of FASN inhibition on cervical cancer cells, flow cytometry analysis was performed to evaluate the cell cycle. This analysis showed an increase in cells in the G0-G1 phase and a decline in cells in the S phase in all cell lines treated with 400 μM of ORL compared to untreated cells (Figure 4).

FASN inhibition induces apoptosis and autophagy

To investigate whether the decrease in viable cells by the FASN inhibition was related to apoptosis and necrosis mechanisms, flow cytometry analysis was performed for annexin V and PI (Figure 5). As shown in Figure 5, all cervical cancer cell lines showed an increase in the number of apoptotic cells for all treatments when compared to controls. The proportion of cells in apoptosis increased as the ORL concentration increased, with the exception of C-33A cells. In addition, the proportion of cells marked by PI was not significantly different, suggesting that the decrease in cell viability was not related to necrosis. Acridine orange was used to assess the acidic vacuolar organelles, used to indicate the extent of autophagy. As indicated in Figure 6, the percentage of AO-positive cells increased by the FASN inhibitor in all cervical cancer cells when compared to controls.

DISCUSSION

Tumour cells synthesise most of the fatty acids they need through de novo synthesis (Medes et al. 1953, Swinnen et al. 2003). These fatty acids are used as membrane lipids and to provide energy for the cells, and they can also stimulate signalling pathways through the post-translational modification of targets. FASN, the main enzyme for de novo fatty acid synthesis, produces palmitate through the condensation of malonyl-CoA and acetyl-CoA (Little & Kridel 2008). In most normal tissues, FASN is expressed

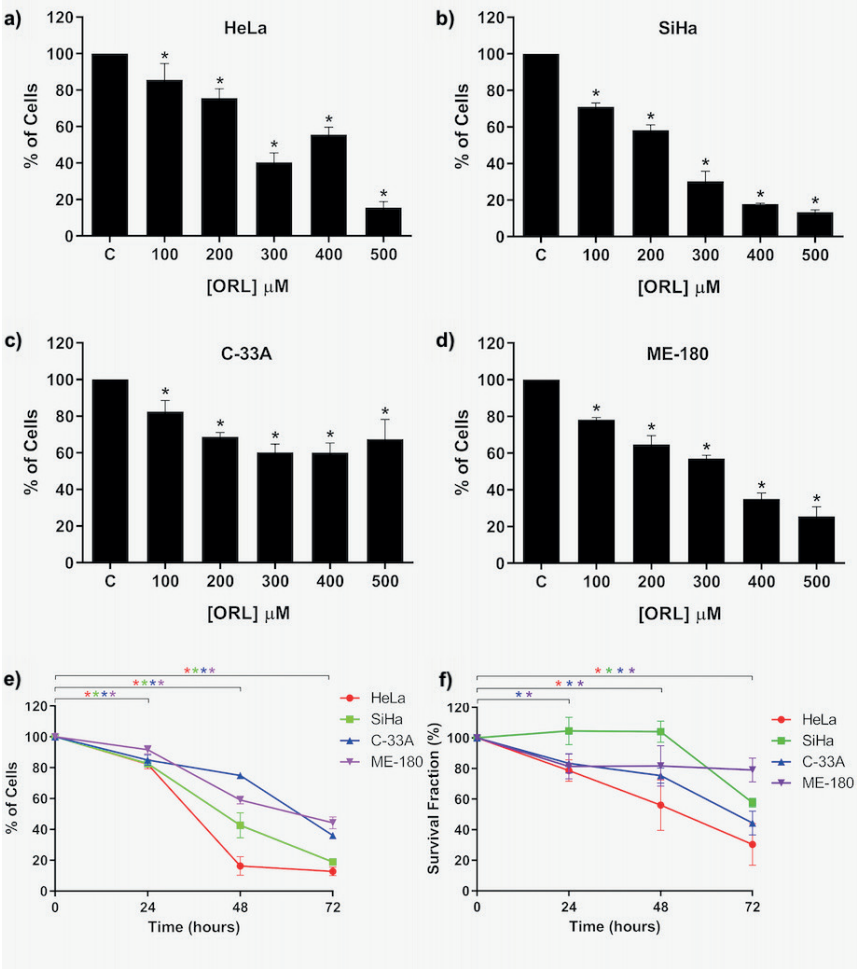

Figure 3. Fatty acid synthase (FASN) activity is necessary for the growth and survival of cervical cancer cell lines. FASN inhibitor (orlistat - ORL) reduced the number of viable cells in all cervical cancer cell lines HeLa (a), SiHa (b), C-33A (c) and ME-180 (d) compared to their respective controls. (e) FASN inhibitor (orlistat - ORL) reduced the number of viable cells in a time-dependent manner. (f) FASN inhibitor (orlistat - ORL) affected the colony formation capability of cell lines. Values refer to the average of three independent experiments ± standard deviation (SD). *p < 0.05 compared to control (one-way ANOVA followed by Tukey's test).

at low levels or not at all. On the other hand, most tumours show high FASN expression (Kuhajda 2006). Furthermore, studies that assessed the effect of FASN inhibition showed a decrease in proliferation, cell cycle arrest and apoptosis (Buckley et al. 2017).

In this study, cytoplasmic FASN expression was found in samples from all patient samples tested. Even the cervicitis samples, which were included as non-malignant controls, showed weak staining. These results demonstrate that FASN may also play an important role in inflammation. Indeed, some studies have observed that fatty acid synthesis is important for macrophage activation and for signalling the immune system (Qian et al. 2018, Carroll et al. 2018). In addition, FASN expression was progressively higher in LSIL, HSIL and carcinoma samples, which indicates a possible role of this enzyme in cervical carcinogenesis. Even though FASN expression did not show a statistical correlation with the grade of cervical lesions, our findings are consistent with many studies showing early FASN activation in the neoplastic processes of breast, gliomas, lung, colon and prostate cancers (Alo et al. 1999, Ogino et al. 2008, Grube et al. 2014, Piyathilake et al. 2000, Migita et al. 2009). Moreover, oesophageal squamous cell dysplasia, adenoma and metaplasia of the stomach also showed overexpression of FASN (Nemoto et al. 2001, Kusakabe et al. 2002). The overexpression of FASN in the early stages of cancer is relevant for carcinogenesis because, under hypoxia conditions, large amounts of

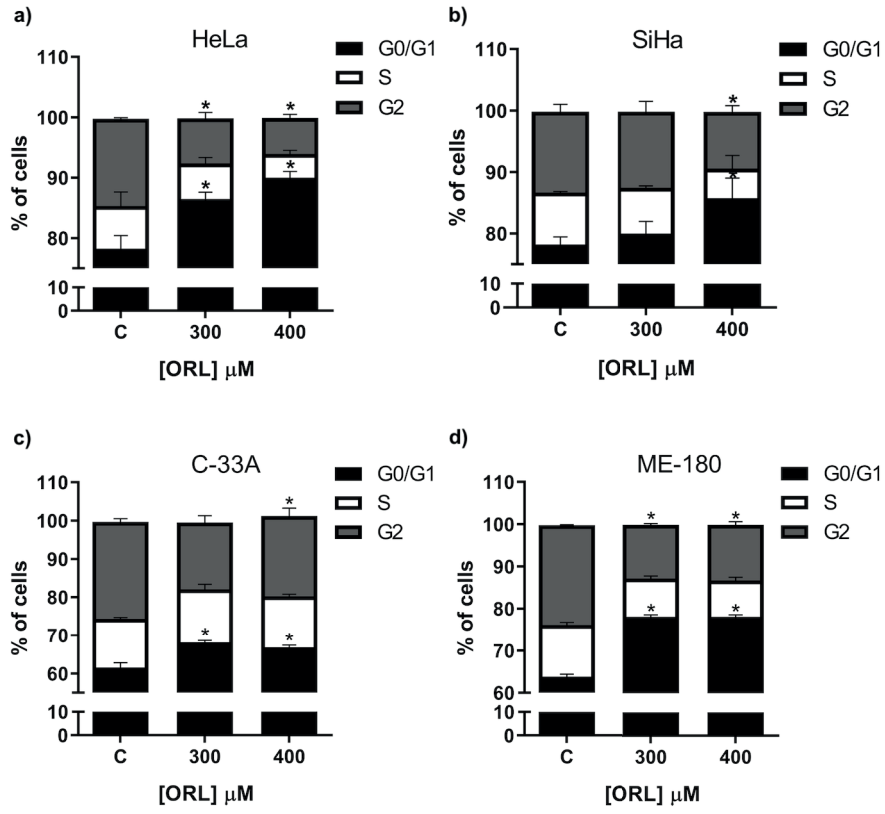

Figure 4. FASN inhibitor (orlistat - ORL) leads to cell cycle arrest. Cell cycle analysis by flow cytometry showed that the inhibition of FASN by ORL increased the G0-G1 population and reduced the number of cells in the S phase for the HeLa (a), SiHa (b), C-33A (c) and ME-180 (d) cell lines. Values refer to the average of three independent experiments ± standard deviation (SD). *p < 0.05 compared to control (one-way ANOVA followed by Tukey's test).

lactate are produced by aerobic glycolysis, damaging the respiratory chain. FASN, in turn, helps to achieve a redox balance by consuming excess NADPH, favouring oxidative respiration (Hosios & Vander Heiden 2018). Moreover, FASN expression has been correlated with the epithelial-mesenchymal transition (EMT). Li et al. (2014) showed that FASN mediates the EMT of breast cancer cells, and Hung et al. (2011) reported that FASN inhibition prevents the EMT process in breast cancer. This is important as EMT allows the cells to spread more quickly and aggressively through the loss of epithelial features and acquisition of mesenchymal characteristics. These advantages give cervical cancer a greater ability to proliferate and survive (Baron et al. 2004, Qureshi et al. 2015). Therefore, the FASN activation observed in premalignant lesions and cancers suggests that FASN may be decisive for early neoplastic transformation.

To better understand the role of FASN in cervical cancer carcinogenesis, ORL was used as a FASN inhibitor. Orlistat, a drug developed for the treatment of obesity, is an inhibitor of pancreatic lipases in the gastrointestinal tract. However, it has also been shown to be an irreversible inhibitor of FASN as it binds to the thioesterase domain, which is responsible for terminating palmitate synthesis (Kridel et al. 2004). Previous studies with ORL have demonstrated the anticancer activity of ORL by FASN inhibition in different types of cancer cells, including hepatoma, colorectal, non-small cell lung, ovarian, oral, prostate, pancreatic, and endometrial (You et al. 2019, Czumaj et al. 2019, Ali et al. 2018, Peng et al. 2018, Xiao et al. 2017, Wright et al. 2017, Sokolowska et al. 2017, Wysham et al. 2016).

In our study, the inhibition of FASN with ORL led to decreased cell viability, reduced

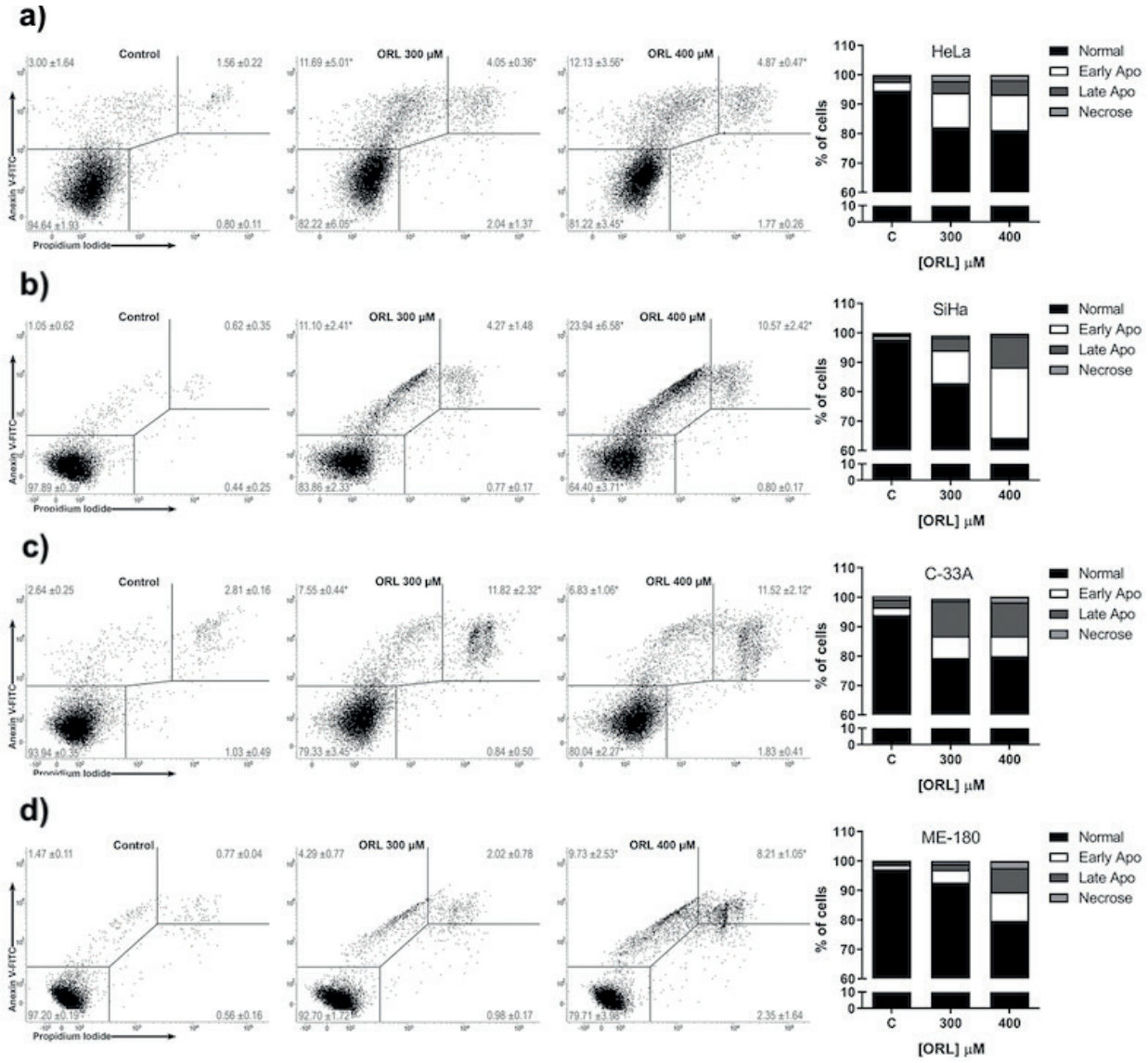

Figure 5. FASN inhibitor (orlistat - ORL) triggers apoptosis in cervical cancer cell lines. Annexin V and propidium iodide (PI) experiments revealed that ORL induces apoptotic cell death but not necrosis in HeLa (a), SiHa (b), C-33A (c) and ME-180 (d) cell lines. Values refer to the average percentage of cells in each gate for three independent experiments ± standard deviation (SD). *p < 0.05 compared to control (one-way ANOVA followed by Tukey's test).

colony formation, and triggered apoptosis and cell cycle arrest in all human cervical cancer cell lines tested (HeLa, SiHa, C-33A and ME-180). However, SiHa and HeLa cells, which are HPV 16-positive and HPV 18-positive, respectively, showed a greater reduction in cell proliferation when compared to the C-33A and ME-180 cell lines. It is important to emphasise that infection with HPV 16 or 18 is the factor most commonly associated with the development of human cervical cancer (Smith et al. 2007). However, to date, no studies have assessed the correlation between FASN expression and HPV infection. Despite this, different studies have shown that FASN expression is associated with several viral infections, including hepatitis C, HIV and respiratory syncytial virus (Yang et al. 2008, Aragones et al. 2010, Ohol et al. 2015). Therefore,

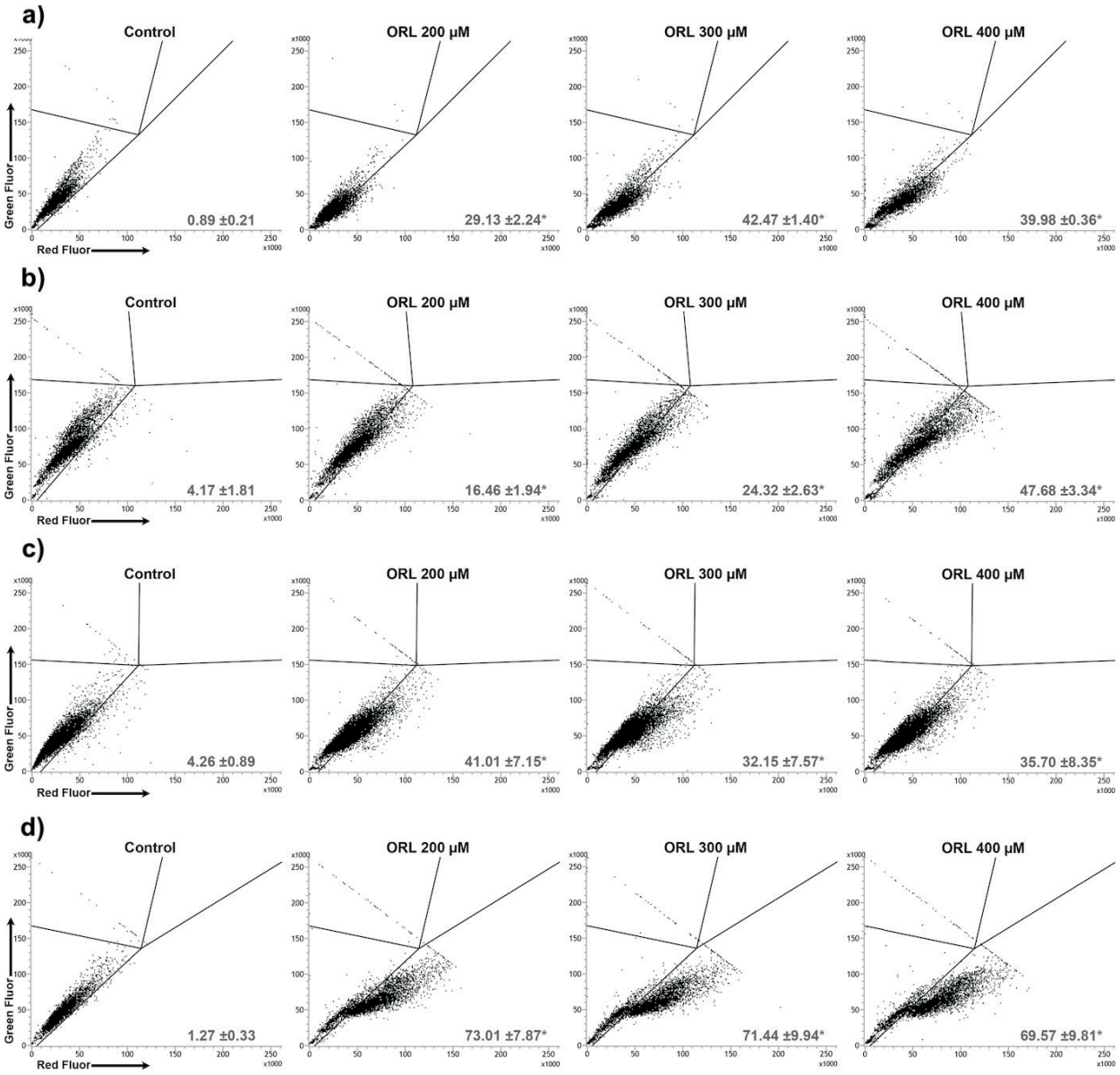

Figure 6. FASN inhibitor (orlistat - ORL) triggers autophagy in cervical cancer cell lines. Flow cytometry showed an increase in acridine orange (AO)-marked cells in the HeLa (a), SiHa (b), C-33A (c) and ME-180 (d) cell lines. Values refer to the average percentage of cells in each gate for three independent experiments ± standard deviation (SD). *p < 0.05 compared to control (one-way ANOVA followed by Tukey's test).

further studies of gene and protein expression by qPCR and western blot are needed to establish the relationship between FASN and HPV infection.

We also demonstrated that the decrease in cell viability by the FASN inhibitor occurred by apoptosis and not by necrosis, since the percentage of necrotic cells was low in all cell lines treated with ORL. These results are in agreement with other studies that report a decrease in viability and death by apoptosis for other cancer cell lines after treatment with ORL (Kridel et al. 2004, Carvalho et al. 2008, Grube et al. 2014). In our study, the FASN inhibitor was also able to induce autophagy in all cervical cancer cell lines tested. Although further studies are

required to confirm this finding, our data are in agreement with Grube et al. (2014), who showed that ORL induced autophagy in glioma cells. Also, Peng et al. (2018) showed that ORL can simultaneously induce apoptosis and autophagy in ovarian cancer cells. However, when cells were treated with an autophagy inhibitor, the number of apoptotic cells increased. Therefore, FASN inhibitors appear to have a cellular prosurvival role, and may be a potential adjuvant in cancer treatment (Peng et al. 2018).

Here, our approach was based on the use of ORL as a model for FASN inhibition. Despite the adverse effects that FASN inhibition can cause by inhibiting lipogenesis, many researches are interest in developing more specific and less toxic FASN inhibitors (Mullen & Yet 2015). However, to the best of our knowledge, there is only one FASN inhibitor under evaluation for human use in the treatment of advanced tumours. The TVB-2640 molecule is on phase II clinical studies and has demonstrated promising results on phase I clinical trial (Falchook et al. 2021). The objectives of the phase I clinical trial were to determine the TVB-2640 maximum dose and safety as monotherapy or in combination with other drugs. The TVB-2640 was given orally, daily and has demonstrated a favorable tolerability profile, with no significant gastrointestinal toxicities. The principal adverse events of TVB-2640 as monotherapy or in combination with taxanes were reversible skin and ocular effects. Other adverse events were alopecia, palmar plantar erythrodysesthesia syndrome, fatigue, decrease appetite, and dry skin. Therefore, the TVB-2640 demonstrated a manageable safety profile in phase I studies (Falchook et al. 2021).

On the other hand, ORL presents some drawbacks for its human use as antitumoral drug considering its very low aqueous solubility, low intestinal permeability and oral bioavailability (Lupu & Menendez 2006). Therefore, taking into account that FASN is a promising target for the treatment of cervical cancer, this study may pave the way to the design of new FASN inhibitors molecules with less adverse or toxic effects; or even for overcoming ORL drawbacks by the development of new formulation approaches, as its nanoencapsulation for improving its solubility and oral bioavailability (Pohlmann et al. 2013) or the development of local delivery systems.

In conclusion, our results showed increased FASN expression in early cervical cancer samples. Furthermore, inhibition of FASN by ORL decreased cell viability and triggered apoptosis, cell cycle arrest and autophagy in different cervical cancer cell lines. Taken together, these data suggest that FASN may be a potential therapeutic target for cervical cancer and should be considered in the development of new treatment approaches for this type of cancer.

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