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Effect of *Argania spinosa* oil extract on proliferation and Notch1 and ERK1/2 signaling of T-cell acute lymphoblastic leukemia cell lines

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ABSTRACT

The Argan tree, called *Argania spinosa* (L.) Skeels, is a tropical plant, which belongs to the Sapotaceae family, it is exploited essentially for its fruits. The endosperm seed of the fruit constitutes a good potential source of edible oil for human consumption and is endowed with important medicinal properties such as antioxidant, antimalarial and anti-proliferative. The aim of the present work is to evaluate the anti-proliferative effect of the oil extracted from seeds of *A. spinosa* in T-cell acute lymphoblastic leukemia human (T-ALL) context. The activity was assessed through an *in vitro* test on three T-ALL cell lines: JURKAT, MOLT3 and DND41. The cytotoxicity effects of *A. spinosa* oil extract were checked by MTT assay and the change in the activity levels of two T-ALL proliferation-related proteins (Notch1 and ERK) was investigated by Western blot, the results demonstrate that treatment with *A. spinosa* oil extract at the dose of 100 µg/mL inhibited the growth of JURKAT, MOLT3 and DND41 cells, and reduced the expression levels and the activity of proliferation-related proteins such as ERK1/2 and Notch1 intracellular domain. *A. spinosa* oil extract could be a potential preventive and therapeutic approach recommended as anti-proliferative against leukemia.

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Argania spinosa oil extract; anti-proliferative effect; leukemia cell lines; Notch1; ERK1/2

Introduction

Cancer is the second leading cause of death in the world. It mainly relies in an uncontrolled growth and spread of cells that can affect almost any part of the body. T-cell acute lymphoblastic leukemia (T-ALL) is a highly aggressive hematologic malignancy characterized by the malignant clonal expansion of immature T-cell progenitors. T-cell transformation is a multi-step process in which different genetic alterations cooperate to alter the normal mechanisms that control cell growth, proliferation, survival and differentiation during thymocyte development. In this context, constitutive activation of Notch1 signaling is the most prominent oncogenic pathway in T-cell transformation (Vlierberghe & Ferrando, 2012).

Notch signaling plays a key role in the normal development of many tissues and cell types, through diverse effects on differentiation, survival and proliferation that are

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highly dependent on signal strength and cellular context. Because perturbation in the regulation of differentiation, survival and proliferation underlie malignant transformation, pathologic Noch signals potentially contribute to cancer development (Allenspach, Maillard, Aster, & Pear, 2002).

Furthermore, ERK1/2 is an important subfamily of mitogen-activated protein kinases that control a broad range of cellular activities and physiological processes. ERK1/2 can provide anti-apoptotic effects by down-regulating pro-apoptotic molecules via a decrease in their activity or a reduction of their protein expression by transcriptional repression. ERK1/2 can also promote cell survival by up-regulating anti-apoptotic molecules via enhancement of their activity or activation of their transcription (Lu & Xu, 2006).

Developing antitumor drugs from natural products is receiving increasing interest worldwide due to limitations and side effects of conventional therapy strategies for cancer (Cochrane, Nair, Melnick, Resek, & Ramachandran, 2008).

Argan tree or *Argania spinosa* (L). Skeels, is a rustic species, xero-thermophilic, which belongs to the tropical family of Sapotaceae, hence it is the only Northern representative in the Mediterranean region (Algeria and Morocco) where its endemism is marked at this region (Kechebar, Karoune, Belhamra, & Rahmoune, 2013).

The Argan tree is exploited essentially for its fruits. The endosperm seed of the fruit constitutes a good potential source of edible oil for human consumption and is endowed with important medicinal properties such as antioxidant, antimalarial and anti-proliferative (ElBabili et al., 2010).

The aim of the present investigation was to evaluate the anti-proliferative activity of the Argan oil on JURKAT, MOLT3 and DND41 human T-ALL cell lines and its effect on, Notch1 and ERK1/2 activity/expression levels.

Materials and methods

Reagents

The Argan oil was extracted from Algerian *A. spinosa* seeds by a traditional method. Cell growth determination Kit from Sigma Aldrich was used for MTT assay. Trypan blue from Sigma Aldrich was used for trypan blue exclusion assay and DMSO from Sigma Aldrich was used in both cell viability assays (trypan blue exclusion assay and MTT assay).

Cell culture

JURKAT, MOLT3, DND41 and pre T 2017 cell lines were cultured in complete RPMI supplemented with 10% fetal bovine serum, 2% antibiotic (penicillin/streptomycin) and 1% Glutamine. M31, HEK 293 and HaCat cells were maintained in Dulbecco's modified Eagle's medium (Gibco) containing 10% fetal bovine serum, 2% antibiotic (penicillin/streptomycin) and 1% Glutamine.

Trypan blue exclusion assay

To assess cell growth, 1×106 cells were incubated for 48 h with 100 µg/mL of Argan oil diluted in DMSO (dilution 1:10) or with DMSO alone. In detail, 1×10^6 cells

suspended in 1 mL was treated with 1.1 μL of Argan oil diluted 1:10 in DMSO and control cells were exposed to the same volume of DMSO. After incubation, trypan blue dye exclusion assay was performed by mixing 20 μL of cell suspension with 20 μL of 0.4% trypan blue dye (Sigma Aldrich) before injecting into a hemocytometer and cell counting. The numbers of cells that exclude the dye (viable cells) were counted.

MTT assay for cell proliferation

Cell proliferation was assessed with Cell Growth Determination Kit MTT Based (Sigma Aldrich) as previously described (Kumar et al., 2014). In brief, cells were seeded at 8×10^4 cells/mL and incubated with 100μg/mL Argan oil or vehicle alone for 72 h. After that, the culture medium was changed, and the MTT solution (stock solution: 5 mg/mL) was added in an amount equal to 10% of the culture volume and incubated for 4 h. The viable cell number was directly proportional to the production of formazan, which was solubilized with isopropanol and measured spectrophotometrically at OD560 with GloMax-Multi Microplate Multimode Reader (PROMEGA). The experiments were performed in triplicate.

The percentage of cell growth was calculated using the following formula (Patel, Gheewala, Suthar, & Shah, 2009):

$$\%\,\text{cell proliferation} = \frac{\text{At} - \text{Ab}}{\text{Ac} - \text{Ab}} \times 100,$$

where At is the absorbance value of test compound (Argan oil), Ab is the absorbance value of blank (Medium alone) and Ac is the absorbance value of control (DMSO).

Total protein extracts, immunoblotting and antibodies

Total protein extracts preparation was described elsewhere (Palermo et al., 2012). In brief, cell pellets were resuspended in lysis buffer (Tris 20 mM pH 7.5, NaCl 150 mM, NaF 1 mM, Triton X-100 1%, sodium-orthovanadate 1 mM, PMSF 1 mM, sodium butyrate 10 mM) plus protease inhibitors and incubated for 20 min on ice. After a centrifugation at 13,000 rpm for 20 min, supernatants were boiled for 5 min in Laemmli sample buffer 1× (Biorad). Concentration of protein was determined by Bradford assay (BioRad). Thirty microgram of protein was separated on 10% SDS-PAGE and transferred to nitrocellulose membranes (Perkin Elmer).

After the transfer, the blots were incubated for 2 h at room temperature with the following primary antibodies: mouse mAb against phospho-ERK (P-ERK) (Cell Signaling) (diluted 1:1000 in BSA 5%); rabbit mAb against ERK (ERK) (Cell Signaling) (diluted 1:1000 in BSA 5%); mouse mAb against Notch1 (N1) (Santa Cruz Biotecnology) (diluted 1:1000 in Milk 3%); and then incubated overnight at 4°C with goat HRP-conjugated anti-mouse IgG serum or a mouse HRP-conjugated anti-rabbit IgG serum as secondary antibodies. (Santa Cruz Biotecnology) (both diluted 1:3000 in Milk 3%); The membranes were visualized by chemiluminescence detection.

Statistical analysis

Results were expressed as the mean ± SD of *n* experiments as indicated in the figure legends. Statistical analysis was performed between *t*-test and ANOVA at a significance level of 0.05.

Results

In order to study the influences of Argan oil on T-ALL survival and proliferation, the proliferation and viability rate on JURKAT, MOLT3 and DND41 human T-ALL cell lines after 48 h of exposure to Argan oil was evaluated. As shown in Figure 1(a) and in Figure 2 Argan oil treatment strongly reduced the proliferation and the viability of all the three T-ALL cell lines. On the other hand, the same treatment did not affect neither the growth of murine preT 2017 and M31 immature T-cell lines nor of human HEK 293 and HaCat-keratinocytes (Figure 1(b)). Overall, these observations reveal a specificity of action of Argan oil in T-ALL context. The anti-proliferative activity of Argan oil on T-cell lymphoblastic leukemia cell lines was confirmed by testing the change in the expression/activity levels of the known pro-proliferative signaling Notch1 and ERK (Figure 3). As shown in Figure 3, Nocht and ERK signaling were differentially modulated by the Argan oil treatment in the three human T-ALL cell lines analyzed. The expression of the active intracellular domain of Notch1 (Notch1-IC) was strongly decreased by the Argan oil exposure both in MOLT3 and in DND41 while resulted increased in JURKAT T cells. On the other hand, the phosphorylation of ERK1/2 was impaired by Argan oil in JURKAT and MOLT3 while was not affected in DND41. Interestingly, ERK1/2 expression was not modulated by the treatment thus suggesting that the decreased phosphorylation in JURKAT and MOLT3 is not done by a deregulated expression, but by the inhibition of the post-translational mechanism.

Discussion

In order to investigate the possible therapeutic effects of Argan oil on leukemia, we evaluated the effect of Argan oil on the proliferation of JURKAT, MOLT3 and DND41 T-ALL cell lines, characterized by distinct immune-phenotype and molecular features.

According to the characterization by markers expression proposed by Burger and colleagues, JURKAT resemble a mature T-cell stage by expressing CD3, CD4 and TCR, while losing the CD1 antigen; MOLT3 have features of early cortical T-stage characterized by CD1 and low CD3 co-receptor expression; and DND41 represents the transition stage from the cortical to the mature T-stage by expressing CD3 and TCR, while still bearing the CD1 antigen (Burger et al., 1999). In addition, although the three cell lines analyzed display the constitutive activation of Notch1 signaling, MOLT3 and DND41 cells carried Notch1 gain of function mutations that generate an activated form of Notch, while JURKAT cells bear un-mutated Notch1. Interestingly, while MOLT3 and JURKAT are resistant to the treatment with the γ-Secretase Inhibitors (GSI), DND41 cells are GSI-sensitive (O'Neil et al., 2007).

Inhibition of proliferation has been a continuous effort in tumor treatment. Suppression of cell growth and induction of cell death are two major means to inhibit cancer

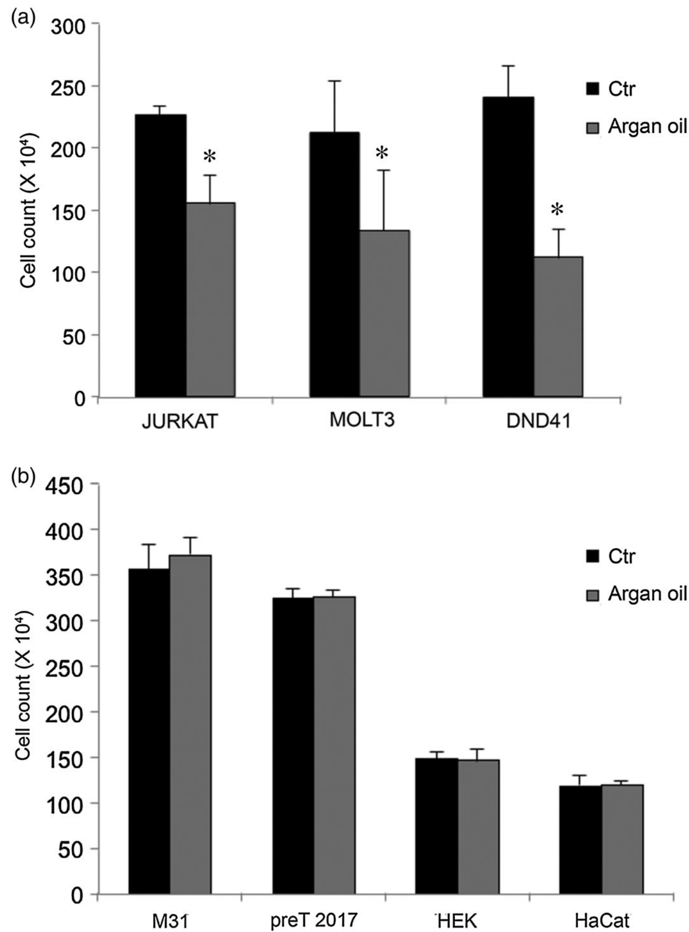

Figure 1. Effect of Argan oil on cell count by trypan blue exclusion assay. (a) Cell growth of JURKAT, MOLT3 and DND41 after 48 h of treatment with 100 µg/mL of Argan oil (Argan oil) compared to the DMSO vehicle exposed cells (Ctr) as shown by the cell counts by using trypan blue staining. Results shown in the figures are expressed as the means average deviations of three separate experiments and bars indicate SD $^*P < .05$. (b) The cell growth of M31, preT 2017, HEK and HaCat cell lines was not affected by 48 h of exposure with 100 µg/mL of Argan oil (Argan oil) when compared with the DMSO treated counterpart cells (Ctr) and as shown by the cell counts by using trypan blue staining. Results shown in the figures are expressed as the means average deviations of three separate experiments.

growth (Huang, Yang, Yang, Lee, & Pang, 2003). Natural antioxidants in vegetable food such as tea and olive oil are believed to reduce the risk of cancer (Norrish, Skeaff, Arribas, Sharpe, & Jackson, 1999; Paschka, Butler, & Young, 1998).

Our data indicated that Argan oil exerted an inhibitory effect on the proliferation of JURKAT, MOLT3 and DND41 cell lines, independent of their phenotypic or molecular differences.

Moreover, our results showed that Argan oil could influence in T-ALL the expression/activity of the oncogenic Notch1 and ERK pathways whose over activation is known to be a key event in the pathogenesis of several both solid and hematologic cancers.

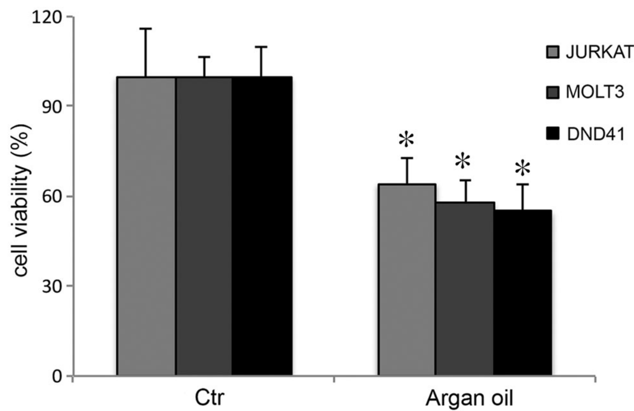

Figure 2. Effect of Argan oil cell viability in MTT assay. The Argan oil cytotoxicity was determined in JURKAT, MOLT3 and DND41 by MTT assay and calculated as percentage of inhibition of cell proliferation after 48 h of treatment with 100 µg/mL of Argan oil (Argan oil) compared to the DMSO vehicle exposed cells (Ctr). Results shown in the figures are expressed as the means average deviations of three separate experiments and bars indicate SD $^*P < .05$.

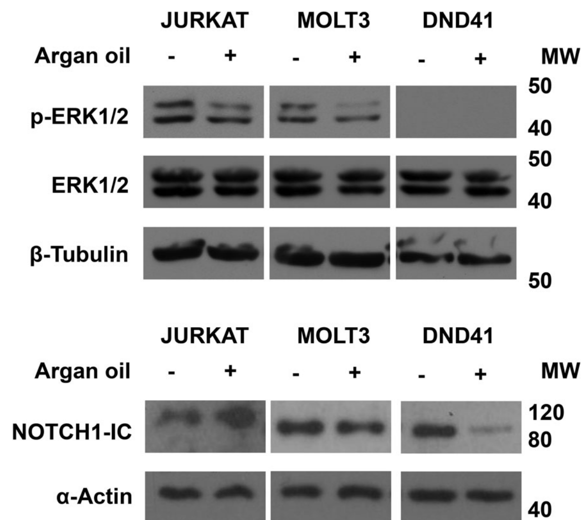

Figure 3. Total cell extracts from JURKAT, MOLT3 and DND41 treated with DMSO (−) or with Argan oil (+) were subjected to western blot by using antibodies against pospho-ERK1/2 (p-ERK1/2), ERK1/2 (ERK1/2) and Notch1 (Notch1-IC). The α-Actin and β-Tubulin expressions were used as loading controls.

We speculated that changes in ERK activity could be responsible for the growth inhibitory effects, given that many plant-derived components modulate ERK activities to elicit their antineoplastic actions (Hollosy & Keri, 2004). In addition, considering that activation and over-expression of Notch signaling were reported to have a high relevance in T-cell leukemogenesis (Ferrando, 2009; Screpanti, Bellavia, Campese, Frati, & Gulino, 2003), the decreased expression of Notch1 induced by Argan oil suggests further studies to evaluate its potential-anti-leukemic therapeutic action.

The results from this study showed a change in the levels of p-ERK and Notch1-IC expression suggesting that ERK and Notch signaling pathways could be intracellular targets for the biological activity by Argan oil in leukemia cells.

Algerian Argan oil represents a potential preventive and therapeutic plant-derived agent recommended as anti-proliferative against human leukemia. It should be noted that as a plant extract, Argan oil contains a variety of compounds that may act on different pathways of tumor cell growth and survival, such as Inhibition of ERK and Notch pathways.

Thus, the previous findings and the results presented here might indicate that the anti-proliferative activity of Argan oil could be due to the presence of a variety of antioxidant molecules such as sterols, polyphenols and tocopherols (Cadi, Mounaji, Amraoui, & Soukri, 2013), which may have beneficial effects against leukemia and may affect several oncogenic pathways including ERK and Notch pathways.

However, further investigations on a cellular or molecular level are necessary to describe the exact mechanisms that cause the anti-leukemic and anti-cancer effects of Argan oil extracts and their bioactive compounds responsible for these activities.

Conclusion

These findings showed the interest to elucidate the principle bioactive molecules of Algerian Argan oil and the exact mode of action and to evaluate their effectiveness in the treatment of leukemia in man. Taken together, our data are promising for the future use of Argan oil in patients developing leukemia.

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Disclosure statement

No potential conflict of interest was reported by the authors.

References

Allenspach, E. J., Maillard, I., Aster, J. C., & Pear, W. S. (2002). Notch signaling in cancer. *Cancer Biology and Therapy*, *1*, 466–476.

Burger, R., Hansen-Hagge, T. E., Drexler, H. G., & Gramatzki, M. (1999). Heterogeneity of T-acute lymphoblastic leukemia (T-ALL) cell lines: Suggestion for classification by immunophenotype and T-cell receptor studies. *Leukemia Research*, *23*, 19–27.

Cadi, R., Mounaji, K., Amraoui, F., & Soukri, A. (2013). Protective and antioxidant potential of the argan oil on induced oxidative stress in Tetrahymena pyriformis. *Journal of Medicinal Plants Research*, *7*, 1961–1968.

Cochrane, C. B., Nair, P. K., Melnick, S. J., Resek, A. P., & Ramachandran, C. (2008). Anticancer effects of Annonaglabra plant extracts in human leukemia cell lines. *Anticancer Research*, *28*, 965–971.

ElBabili, F., Bouajila, J., Fouraste, I., Valentin, A., Mauret, S., & Moulis, C. (2010). Chemical study, antimalarial and antioxidant activities, and cytotoxicity to human breast cancer cells (MCF7) of Argania spinosa. *Phytomedicine*, *17*, 157–160.

Ferrando, A. A. (2009). The role of NOTCH1 signaling in T-ALL Hematology. *American Society of Hematology Education Program*, *2009*, 353–361.

Hollosy, F., & Keri, G. (2004). Plant-derived protein tyrosine kinase inhibitors as anticancer agents: Current medicinal chemistry. *Current Medicinal Chemistry – Anticancer Agents*, *4*, 173–197.

Huang, S. T., Yang, R. C., Yang, L. J., Lee, P. N., & Pang, J. H. (2003). Phyllanthus urinaria triggers the apoptosis and Bcl-2 down-regulation in Lewis lung carcinoma cells. *Life Sciences*, *72*, 1705–1716.

Kechebar, M. S. A., Karoune, S., Belhamra, M., & Rahmoune, C. (2013). Characterization of the argan tree of southwestern Algeria. *International Journal of Current Microbiology and Applied Sciences*, *2*, 117–126.

Kumar, V., Palermo, R., Talora, C., Campese, A. F., Checquolo, S., Bellavia, D., … Tottone, L. (2014). Notch and NF-kB signaling pathways regulate miR-223/FBXW7 axis in T cell acute lymphoblastic leukemia. *Leukemia*, *28*, 2324–2335.

Lu, Z., & Xu, S. (2006). ERK1/2 MAP Kinases in cell survival and apoptosis. *IUBMB Life (International Union of Biochemistry and Molecular Biology: Life)*, *58*, 621–631.

Norrish, A. E., Skeaff, C. M., Arribas, G. L., Sharpe, S. J., & Jackson, R. T. (1999). Prostate cancer risk and consumption of fish oils: a dietary biomarker-based case-control study. *British Journal of Cancer*, *81*, 1238–1242.

O'Neil, J., Grim, J., Strack, P., Rao, S., Tibbitts, D., Winter, C., … Hardwick, J. (2007). FBW7 mutations in leukemic cells mediate NOTCH pathway activation and resistance to gamma-secretase inhibitors. *Journal of Experimental Medicine*, *204*, 1813–1824.

Palermo, R., Checquolo, S., Giovenco, A., Grazioli, P., Kumar, V., Campese, A. F., … Giorgi, A. (2012). Acetylation controls Notch3 stability and function in T-cell leukemia. *Oncogene*, *31*, 3807–3817.

Paschka, A. G., Butler, R., & Young, C. Y. (1998) Induction of apoptosis in prostate cancer cell lines by the green tea component, (-) epigallocatechin-3- gallate. *Cancer Letters*, *130*, 1–7.

Patel, S., Gheewala, N., Suthar, A., & Shah, A. (2009). In-vitro cytotoxicity activity of solanum nigrum extract against Hela cell line and Vero cell line. *International Journal of Pharmacy and Pharmaceutical Sciences*, *1*, 38–46.

Screpanti, I., Bellavia, D., Campese, A. F., Frati, L., & Gulino, A. (2003). Notch, a unifying target in T-cellacute lymphoblastic leukemia? *Trends in Molecular Medicine*, *9*, 30–35.

Vlierberghe, P. V., & Ferrando, A. (2012). The molecular basis of T cell acute lymphoblastic leukemia. *Journal of Clinical Investigations*, *122*, 3398–3406.