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Interferon-α receptors and activation pathways in lymphocytes and monocytes in the peripheral blood of patients with cervical intraepithelial neoplasia or invasive cancer

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Summary

Purpose of Investigation: There is substantial literature demonstrating the effects of type I interferons (IFNs) on viral infections. Here, the authors examined STAT1, IRF-7, and IFN receptors (IFNR1 and IFNR2) in patients with cervical intraepithelial neoplasia (CIN I, II, III) or invasive cervical cancer (ICC) and healthy women. *Materials and Methods:* Flow cytometry was used to monitor total T lymphocytes ($CD3^+$), T helper cells ($CD4^+$), cytotoxic T cells ($CD8^+$), and monocytes ($CD14^+$). *Results:* In patients, the numbers of CD3+, CD4+, and $CD14^+$ cells that were positive for IFN-α and the intracellular amounts of this cytokine increased in accordance with lesion progression. Patients showed increased numbers of cells expressing IFNR1 and IFNR2. Patients with neoplasia showed increased numbers of cells positive for STAT1 and IRF7 compared to controls. *Conclusion:* the antiviral response mediated by IFN-α is increased in the peripheral blood T cells of patients with different stages of cervical neoplasia.

Key words: Cervical cancer; IFN Receptors; Transcriptional factors.

Introduction

Cervical intraepithelial neoplasia (CIN) is a precursor lesion to cervical cancer and for the last 30 years, scientists have studied the association between cervical cancer and infection with human papillomavirus (HPV) [1]. Viral DNA from HPV has been found in about 90% of cervical cancer biopsies [2]. Certain HPV viral proteins interfere with the transmission of proteins and genes related to the immune response, such as E6 and E7, which block cytokine synthesis [3]. Presence of the viral capsid compromises antigen presentation, impeding the activation of Langerhans cells [4]. Blocking activation pathways is a means of evading the immune system, involving the loss of viral episomes and favoring the development of cells with HPV-integrated genomes [5].

Interferons (IFNs) are glycoproteins that were initially described with respect to their strong antiviral effects [6]. IFNs act on target cells by binding with specific receptors that consist of two subunits: IFNR1 and IFNR2 [7]. These receptors are members of the class II cytokine receptor family. Any change in IFNR1 and IFNR2 gene expression will modulate receptors that control the function of the cell, influencing differentiation, proliferation, and apoptosis [8]. Studying IFN-α signaling pathways to induce the expression of different genes led to the discovery of a family of proteins that directly bind cell surface receptors to cause nuclear events [9]. These proteins, called signal transducers and activators of transcription (STATs), are located in the cytoplasm. After the cell is stimulated with IFN-α, STATs are activated by tyrosine phosphorylation and, thereafter, recognize DNA regulatory sequences [10]. This phosphorylation event, which includes activation of the receptor associated with the Janus kinase (JAK) and tyrosine kinase (TYK) proteins, and the STAT1, STAT2, and STAT3 specifically promotes signal transduction and transcriptional activation. These three STAT proteins act as important regulators of the innate immune system in response to infections provoked by various virus RNA and DNA.

HPV suppresses the constitutive expression of STAT1, but not STAT2, IFN-9 regulating factor (IRF-9), or STAT3 [11]. STAT1 and STAT2 protein complexes are phosphorylated by IRF-9 and migrate to cell nucleus. There, this multimeric transcriptional factor (TF) binds with the IFN-stimulated response element in gene promoters responsive to IFN-α, promoting the synthesis of effector proteins [12-15]. A study by Müller *et al.* [16] concluded that cells deficient in JAK-1 and TYK are not responsive to type 1 IFNs (IFN-α/β).

Clinical and experimental data have shown that local and systemic cytokines can induce tumor regression. For patients with CIN II/III or other types of neoplasia, conservative treatment with IFN can preserve the woman's

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reproductive ability by avoiding the need for more invasive procedures, such as cold-knife conization. A study by Ramos *et al.* [17] demonstrated that IFN-α–treated patients with CIN III and with tumor regression expressed more type 1 T helper (Th1)-profile cytokines: IFN-γ, tumor necrose factor (TNF)-α, and IL-12 with a significant reduction in the high-risk HPV viral load. Patients whose therapy failed were smokers and had a higher expression of Th2-type (IL-4) or regulatory T cytokine: TGF-β2 and TGF-β3.

Immune response studies are important for understanding the systemic responsiveness to endogenous IFN-α in patients with CIN and invasive cervical cancer (ICC), as well as for developing new therapeutic protocols. Therefore, the objective of this study was to evaluate the production of IFN-α, its receptors (IFNR1 and IFNR2), and TFs associated with the activation pathway for IFN-α (IRF-7, STAT1) in total T lymphocytes ($CD3^+$), Th cells ($CD4^+$), cytotoxic T cells ($CD8^+$), and macrophages ($CD14^+$) from the peripheral blood of patients with CIN I–III and ICC.

Materials and Methods

Fifty-one female patients diagnosed with CIN (I–III) or ICC (confirmed by colposcopy-directed biopsy) were selected at the Research Institute for Oncology (IPON)/Discipline of Gynecology and Obstetrics at UFTM. Samples collected from patients with CIN I, II, III, and ICC totaled five, 16, 25, and five samples, respectively. The four patients diagnosed with ICC showed different Stages, namely: IIB, IIIB, IB1, and IB2. The control group included one sample from each of ten healthy women who had not been diagnosed with CIN or any autoimmune disease, and who were not immunosuppressed. All enrolled individuals were fully informed of the intentions of the research study. They signed a consent form to confirm their participation (case no. 759), in accordance with requirements of the research ethics committee.

Over the course of the study, peripheral blood samples were drawn from all participants. Cells were evaluated by flow cytometry, using protocols suggested by the manufacturer. Leukocytes were isolated from peripheral blood samples via centrifugation at 4°C with a standard cell lysing protocol, in accordance with the manufacturer's instructions. Cells were resuspended in PBS. The following antibodies for extracellular proteins were added: APC-labeled anti-CD14 (for macrophage staining), FITC-labeled anti-CD3 (for total T lymphocytes), Alexa-labeled anti-CD4 (for Th cells), and FITC-labeled anti-CD8 (for cytotoxic T cells). Cells were incubated at 4°C for 30 minutes, rinsed twice by centrifugation with PBS, fixed, and permeabilized with cytofix/cytoperm and perm/wash, according to the manufacturer's protocols. For intracellular protein staining, the cells were incubated with the following PE-labeled antibodies for 30 minutes at 4°C: anti-STAT1, anti-IRF7, anti–IFN-α, anti-IFNR1, and anti- IFNR2.

Finally, the cells were resuspended in PBS (500 μL) and analyzed with a cytometer. For an accurate determination of the cells corresponding to lymphocytes and no other cell type, the authors determined the region to be analyzed by constructing gates according to controls for relative size (forward scatter: FSC) and granularity/complexity (side scatter: SSC) in each experiment and for each patient.

An electronic database was developed for the statistical analysis. Variables were analyzed with the GraphPad Prism 4.0 program. Values were submitted to Student's *t*-test. Differences with $p \leq 0.05$ were considered to be statistically significant.

Table 1. — *Distribution in the expressions of IFN-α, IFNR1, IFNR2, IRF-7, and STAT1 in total T lymphocytes ($CD3^+$), helper T cells ($CD4^+$), cytotoxic T cells ($CD8^+$), and monocytes ($CD14^+$) obtained from the peripheral blood of patients and controls.*

Marker		Groups % gate				
		Control	CIN I	CIN II	CIN III	Invasive
IFN-alpha	CD3 * ¨	6.63	71.22	53.57	43.07	49.08
	CD4 Ψ °	3.94	34.22	18.61	15.16	25.00
	CD8 β	35.97	26.1	16.68	16.98	26.59
	CD14 *	0.04	7.91	7.05	8.12	20.34
IFNR1	CD3 *	7.201	53.99	53.91	47.29	50.67
	CD4 *	4.20	29.54	19.49	16.92	24.69
	CD8 γ δ	35.04	36.12	18.43	17.95	28.32
	CD14 Ψ	1.16Ψ	10.99	12.87	11.38	26.22
IFNR2	CD3 *	10.36	54.87	55.50	46.70	52.39
	CD4 β	2.80	28.51	20.28	15.80	22.78
	CD8 γ δ β	35.52	37.38	19.10	17.76	29.33
	CD14 *	1.07	10.08	8.25	11.20	24.99
STAT1	CD3 * ¨	9.59	72.60	51.59	44.94	52.70
	CD4 α	9.06	36.74	22.25	17.16	22.09
	CD8 β α °	38.20	27.71	17.65	16.80	29.74
	CD14 *	2.49	8.71	10.70	10.41	27.51
IRF7	CD3 * ¨	13.25	69.46	55.57	43.88	53.31
	CD4 # °	9.06	35.00	18.23	14.22	23.11
	CD8	37.52	26.21	17.92	17.57	29.04
	CD14 *	0.04	7.45	8.41	8.90	22.12

*Control × CIN I, II, III, ICC; ¨ CIN I × CIN III; # Control × CIN I; ψ Control × CIN I, II, III; ω CIN I × CIN II, III; β Control × CIN II, III; γ Control × CIN III; # Control × CIN I; ° CIN I× CIN III; α Control × CIN I, II.

Results

The authors evaluated the expressions of IFN-α, its receptors (IFNR1 and IFNR2), and TFs associated with its activation pathway (IRF-7 and STAT1) in cells related to the acquired immune response ($CD3^+$ total T lymphocytes, $CD4^+$ Th cells, and $CD8^+$ cytotoxic T cells) and cells related to the innate immune response ($CD14^+$ macrophages). Table 1 shows the results of the statistical analysis. In Figure 1, these data are represented as tendency curves for the proteins based on the lesion grade. The data are represented as % gate (number of cells positive for each marker) and as % fluorescence (quantity of the protein, independent of the quantity of positive cells).

In the patient groups (CIN I–III and ICC groups), the IRF-7, STAT1, and IFN-α markers were significantly increased in the total T lymphocytes and Th cells compared to the control group. These markers were significantly increased in the these cell populations in the CIN I group compared to the CIN III group, and in the CIN II group compared to the CIN III group (Table 1). Fluorescence intensities of IRF-7, STAT1, and IFN-α in the total T lym-

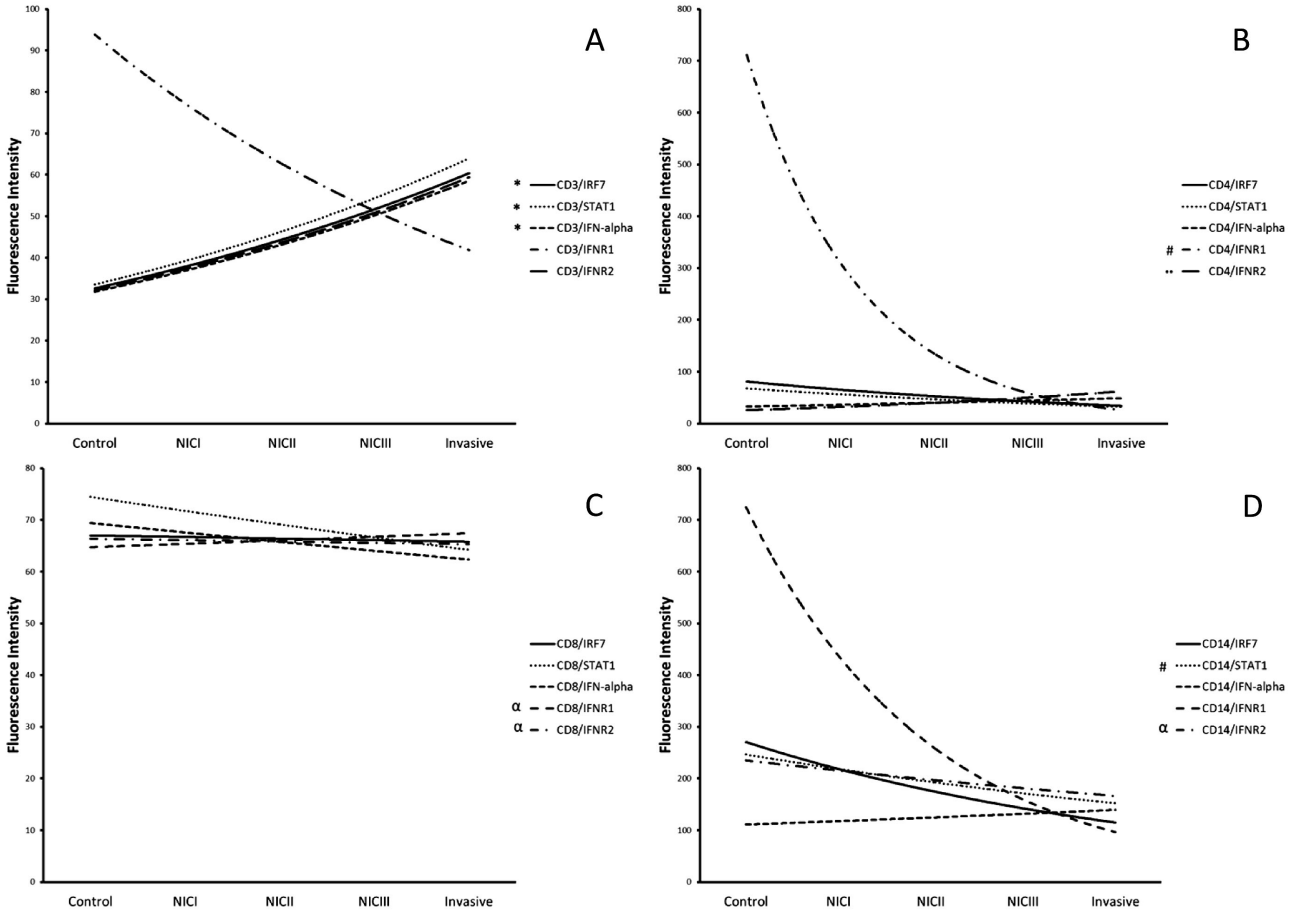

Figure 1. — Fluorescence intensity of IFN receptors and TFs in total T lymphocytes (A), Th cells (B), cytotoxic T lymphocytes (C), and monocytes (D). $p > 0.005$ for # Control *vs*. CIN I, II, III, ICC; * Control *vs*. CIN I, II, III, ¨ Control *vs*. CIN II, III; α Control *vs*. CIN I.

phocytes were significantly increased in the patient groups compared to the control group, except for IFN-α in the ICC group (Table 1 and Figure 1). Fluorescence intensities of STAT1 and IRF-7 showed decreasing tendencies in the $CD4^+$ lymphocytes (Figure 1). Analysis of the total lymphocytes showed similar behavior within the CIN I and II groups, with an increase in the markers. Similarly, the CIN III and ICC groups showed decreasing tendencies for STAT1, IRF-7, and IFN-α. In analyzing the fluorescence intensity of IFN-α, the present authors observed an increasing tendency towards cytokine production as the lesion evolved to a high grade (Figure 1A). This tendency was significant when comparing the CIN I–III groups to the control group.

The numbers of total T lymphocytes expressing IFNR1 and IFNR2 were significantly increased in the patient groups compared to the control group. The fluorescence intensities of IFNR2 and IFNR1 were significantly increased and decreased, respectively, in the total T lymphocytes in the CIN I–III groups compared to the control group. Significantly fewer cells in the CIN III group expressed IFNR2 compared to cells in the CIN I and II groups. Similarly, fewer cells expressed IFNR2 in the ICC group compared to the CIN II group. There was a tendency towards an increase in the number of total T lymphocytes positive for IFNR2 in the CIN II group compared to the CIN I group.

The percentage of Th cells expressing IFNR1 was significantly elevated in the patient groups compared to the control group. The authors observed an important increase in the fluorescence intensity of IFNR1 in the CIN III group compared to the CIN I group and a tendency towards an increase compared to the control group. Similarly, all of the patient groups showed an increase in the percentage of $CD4^+$ cells that expressed IFNR2 compared to the control group. There was no significant difference between the groups in terms of the fluorescence intensity of IFNR2 in the $CD4^+$ T lymphocytes (Figure 1B).

In the $CD8^+$ T cells, the percentage of cells expressing IFNR1 did not show statistically significant changes among the groups. However, the percentage of IFNR2-expressing cells was significantly reduced in the CIN II group compared to the CIN I group. Fluorescence intensities of IFNR1

and IFNR2 were significantly increased in $CD8^+$ T lymphocytes in the CIN II and III groups compared to the control group. No statistically significant differences in the quantity of $CD8^+$ T cells positive for IFN-α, IRF-7, or STAT1 were observed among the groups, except between the CIN II and III groups (Figure 1C).

All of the patient groups showed a greater number of macrophages that stained positively for IFNR1 and IFNR2 compared to the control group, but there were no significant differences in the fluorescence intensities of these receptors. Macrophages in the peripheral blood of the patient groups produced significantly more IFN-α, both in terms of % gate and % fluorescence, compared to the control group. This finding increased with increasing lesion grade. There was an increase in cells positive for STAT1 and IRF-7 during lesion progression in the patient groups compared to the control group, although the % fluorescence values of these molecules were reduced compared to the control group (Figure 1D).

Discussion

Type I IFN cytokines play important biological roles within the immune system. Their main function is to induce an antiviral state in infected and adjacent cells. They also act on innate immunity by activating natural killer cells, proinflammatory pathways, and cytokine production. They activate adaptive immunity by promoting the development of an antigen-specific response through the activation of T and B lymphocytes. The action of IFN-α begins when it binds to specific receptors, IFNR1 and IFNR2, in the target cells.

In general, patients with cervical neoplasia or cervical cancer showed increases in IFNR1 and IFNR2 in their peripheral blood cells. Although these patients, CIN and ICC had higher numbers of Th cells ($CD4^+$) and monocytes ($CD14^+$), there was a tendency towards a reduction in both receptors compared to the groups with neoplasia. These findings suggest that patients with tumors at a more advanced stage may have a greater number of these cells in circulation, as the immune system attempts to maximize its antitumor response through the action of IFN-α. In this process, the body may intensify the production of other immune response cytokines, which do not directly act to destroy neoplastic cells.

Whereas the number of total T lymphocytes in patients with neoplasia was increased compared to the control group, fewer of the cytotoxic T cells ($CD8^+$) expressed IFNR1 or IFNR2 (according to the gate %). The fluorescence intensities of these receptors were increased in the cytotoxic T cells, thus demonstrating an increase in IFNR1 and IFNR2 in these cells, in the CIN II and II groups, compared to the control. These results may demonstrate that the immune system is activated in an effort to increase the cytotoxic action of these lymphocytes.

Mutations in IFNR1 have been shown to harm the IFN response to the virus, to lessen its anti-proliferative ability, and to reduce the affinity of the receptor [18]. Very recent studies have shown that high-risk HPVs, such as HPV16, can interfere in the JAK-STAT signaling pathway, reducing gene regulation [19]. These findings suggest that direct contact between the cells and an oncogenic virus may cause mutations in important components, such as IFN receptors, thereby reducing their expression. It is possible that the generalized increase in the systemic expression of the receptors in the patient groups stemmed from the circulation of cells that were not in direct contact with the neoplasia and, therefore, did not undergo changes that would normally affect local immunity.

A study by Tirone *et al.* [20] showed that cervical samples from biopsies without cervical neoplasia had a higher expression of the IFN-α receptor than those with CIN. However, only the control group showed the simultaneous expression of both subunits of the IFN-α receptor. This finding associated with the present results might explain the inefficiency of local immune response, despite the existence of an efficient systemic immune response (as the present study demonstrated), possibly because of the presence of the virus.

In studying inflammatory infiltrate in the tumor microenvironment, Silva *et al.* [21] found a predominance of $CD3^+$ and $CD20^+$ lymphocytes in samples from patients with CIN III compared to samples from patients with ICC. They concluded that cell migration was proportional to lesion progression. Another study found the positive expression of $CD3^+$ T lymphocytes in patients with recurrence after conization for CIN III [22]. Fernandes *et al.* [23] investigated the number and function of circulating neutrophils in patients with cervical neoplasia, observing an increase in the number of cells in patients with microinvasion. Soluble mediators released by the tumor cells, such as nitric oxide, may have interfered with the ability of the neutrophils to migrate, thereby damaging the host immune response.

Various cytokines are produced after viral infection or activation of Toll-like receptors (TLRs). The most important modulators of the gene for IFN-α include two members of the IRF family, IRF-3 and IRF-7. Activation of these factors by TBK1/IKK promotes the expression of the IFN-α gene and maximizes the production of responsive genes, which are important for the development of an effective antiviral immune response [24]. The present authors observed a progressive increase in the quantity of cells positive for IRF-7 in total T lymphocytes, Th cells, and monocytes with increasing lesion progression, but not in cytotoxic T cells. However, when they analyzed the fluorescence intensity of IRF-7, they observed a significant increase in monocytes and cytotoxic T cells in the groups with neoplasia. This finding indicates that these cell populations may respond to IFN-α in an effort to eliminate the high-grade lesion. Furthermore, overexpression of aryl hydrocarbon receptor interacting protein (AIP), common in malignant cells,

interacts with IRF7, suppressing the production of IFN type I, avoiding antiviral response [25].

Various studies have shown an increase in the number of macrophages in patients with CIN and ICC, findings that corroborate with those of the present study [26, 27]. Transduction mediated by the active form of the adenovirus for IRF-7 in primary macrophages resulted in the production of type I IFN, overregulation of the target genes, including TRAIL, and an increase in macrophage tumoricidal activity [15]. In the present study, high-grade lesions showed an increase in IRF-7 in cytotoxic T cells, which was not accompanied by an increase in the number of cells positive for the IRF-7 marker. Moreover, although the number of total T lymphocytes that were positive for the IRF-7 marker significantly increased with lesion evolution, the present authors did not observe this increase in cytotoxic T cells. This finding may indicate a failure in antigen presentation by dendritic cells (DCs) or a tumor escape mechanism.

Colonna *et al.* [24] demonstrated that TLRs of myeloid DCs and macrophages induced a response to IFN-α/β, activating distinct pathways that allowed the immune system to adapt to different viral agents. Response elements to IFN-α, both in the systemic production of IFN-α in innate immunity and its local action based on the plasmocytoid DCs in adaptive immunity, were present in different pathways. This finding may indicate an effort to increase the possibility of responding to the antigen. Dupuis *et al.* [28] showed that viral replication was not inhibited by recombinant IFN-α in cells from two homozygotic newborns for STAT1 mutated alleles, indicating that the STAT1 pathway is indispensable to the responsiveness of IFN, and that an inherited STAT1 deficiency results in susceptibility to viral infection.

As the lesion progressed, there was a significant increase in total T lymphocytes and Th cells, but not cytotoxic T cells, with the STAT1 marker. In macrophages, the increase in STAT1-positive cells was accompanied by an increase in the quantity of STAT1, showing efficiency in the IFN-α activation pathway. The present authors conclude that cellular immunity, through the endogenous production of IFN-α, is concentrated in Th cells and macrophages, but not in cytotoxic T cells. The presence of STAT1 and IRF-7 is associated with a progressive increase in the number of IFN-α–producing cells, Th cells, and macrophages as the neoplastic lesion evolves.

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References

[1] Boulet G.B., Horvath C., Broeck D.V., Sahebali S., Bogers J.: "Human papillomavirus: E6 and E7 oncogenes." *Int. J. Biochem. Cell. Biol.*, 2007, *39*, 2006.

[2] Tindle R.W.: "Immune evasion in human papillomavirus-associated cervical cancer." *Nat. Rev. Cancer*, 2002, *2*, 59.

[3] Lee B.N., Follen M., Shen D.Y., Malpica A., Adler-Storthz K., Shearer W., *et al.*: "Depressed type 1 cytokine synthesis by superantigen-activated CD4+ T cells of women with human papillomavirus-related high-grade squamous intraepithelial lesions". *Clin. Diagn. Lab. Immunol.*, 2004, *11*, 2.

[4] Stanley M.: "Immune responses to human papillomavirus." *Vaccine*, 2006, *30*, 24.

[5] Rosty C., Sheffer M., Tsafrir D., Stransky N., Tsafrir I., Peter M., *et al.*: "Identification of a proliferation gene cluster associated with HPV E6/E7 expression level and viral DNA load in invasive cervical carcinoma." *Oncogene*, 2005, *24*, 7094.

[6] Friedman R.M.: "Interferon binding: the first step in establishment of antiviral activity." *Science.,* 1967, 156, 3783.

[7] Stark G.R., Kerr I.M., Williams B.R., Silverman R.H., Schreiber R.D.: "How cells respond to interferons." *Annu. Rev. Biochem.*, 1998, *67*, 227.

[8] Chawla-Sarkar M., Lindner D.J., Liu Y.F., Williams B. R., Sen G. C., Silverman R. H., *et al.*: "Apoptosis and interferons: Role of interferon-stimulated genes as mediators of apoptosis." *Apoptosis*, 2003, *8*, 237.

[9] Schindler C., Darnell J.E.: "Transcriptional responses to polypeptide ligands: the JAK-STAT pathway." *Annu. Rev. Biochem.*, 1995, *64*, 621.

[10] Levy D., Darnell J.E.: "Interferon-dependent transcriptional activation signal transduction without second messenger involvement." *New. Biol.*, 1990, *2*, 923.

[11] Chang Y.E., Laimins L.A.: "Microarray analysis identifies interferon-inducible genes and Stat-1 as major transcriptional targets of human papillomavirus type 31". *J. Virol.,* 2000, *74*, 4174.

[12] Darnell J.E., Kerr I.M., Stark G.R.: "JAK–STAT pathways and transcriptional activation in response to IFNs and other extracellular proteins." *Science*, 1994, *264*, 1415.

[13] Levy D.E.: "Interferon induction of gene expression through the Jak–Stat pathway." *Semin. Virology*, 1995, *6*, 181.

[14] Ihle J.N., Kerr I.M.: "Jaks and Stats in signaling by the cytokine receptor superfamily." *Trends Genetics*, 1995, *11*, 69.

[15] Solis M., Goubau D., Romieu-Mourez R., Genin P., Civas A., Hiscott J.: "Distinct functions of IRF-3 and IRF-7 in IFN-alpha gene regulation and control of anti-tumor activity in primary macrophages." *Biochem. Pharmacol.*, 2006, *72*, 1469.

[16] Muller M., Briscoe J., Laxton C., Guschin N., Ziemiecki A., Silvennoinen O., *et al.*: "The protein tyrosine kinase JAK1 complements defects in interferon-α/β and –γ signal transduction." *Nature*, 1993, 366, 129.

[17] Ramos M.C., Mardegan M.C., Peghini B.C., Adad S.J., Michelin M.A., Murta E.F.: "Expression of cytokines in cervical stroma in patients with high-grade cervical intraepithelial neoplasia after treatment with intralesional interferon alpha-2b." *Eur. J. Gynaec. Oncol.*, 2010, *31*, 522.

[18] Pan M., Kalie E., Scaglione B.J., Raveche E., Schreiber G., Langer J.A.: "Mutation of the IFNAR-1 receptor binding site of human IFN-α2 generates type I IFN competitive antagonists." *Biochemistry*, 2008, *47*, 12018.

[19] Kaczkowski B., Rossing M., Andersen D.K., Dreher A., Morevati M., Melissa A., *et al.*: "Integrative analyses reveal novel strategies in hpv11, 16 and 45 early infection." *Sci. Rep.*, 2012, *2*, 1.

[20] Tirone N.R., Peghini B.C., Barcelos A.C.M., Murta E.F., Michelin M.A.: "Local expression of interferon-alpha and interferon receptors in cervical intraepithelial neoplasia." *Cancer Immunol. Immunother.*, 2009, *58*, 2003.

[21] Silva C.S., Michelin M.A., Etchebehere R.M., Adad S.J., Murta E.F.: "Local lymphocytes and nitric oxide synthase in the uterine cervical

stroma of patients with grade III cervical intraepithelial neoplasia". *Clinics.*, 2010, *65*, 575.

[22] Maluf P.J., Michelin M.A., Etchebehere R.M., Adad S.J., Murta E.F.: "T lymphocytes (CD3) may participate in the recurrence of cervical intraepithelial neoplasia grade III." *Arch. Gynecol. Obstet.*, 2008, *278*, 525.

[23] Fernandes P.C.J., Garcia C.B., Micheli D.C., Cunha F.Q., Murta E.F., Tavares-Murta B.M.: "Circulating neutrophils may play a role in the host response in cervical cancer." *Int. J. Gynecol. Cancer*, 2007, *17*, 1068.

[24] Colonna M.: "TLR pathways and IFN-regulatory factors: To each its own." *Eur. J. Immunol.*, 2007, *37*, 306.

[25] Zhou,Q., Lavorgna,A.,Bowman, M., Hiscott, J., Harhaj, E.W.: "Aryl hydrocarbon receptor interacting protein targets IRF7 to suppress antiviral signaling and the induction of type I interferon." *J. Biol. Chem.*, 2015, *290*, 14729.

[26] Davidson B., Goldberg I., Kopolovic J.: "Inflammatory response in cervical intraepithelial neoplasia and squamous cell carcinoma of the uterine cervix." *Pathol. Res. Pract.*, 1997, *193*, 491.

[27] Tay S.K., Jenkins D., Maddox P., Hogg N., Singer A.: "Tissue macrophage response in human papillomavirus infection and cervical intraepithelial neoplasia." *Br. J. Obstet. Gynaecol.*, 1987, *94*, 1094.

[28] Dupuis S., Jouanguy E., Al-Hajjar S., Fieschi, C., Al-Mohsen, I.Z., Al-Jumaah, S. et al.: "Impaired response to interferon-α/β and lethal viral disease in human STAT1 deficiency." *Nat. Genet.*, 2003, *33*, 388.

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