Research Article

Investigation of Interleukin-27 in the Sera of Nonmelanoma Skin Cancer Patients

Mehdi Ghahartars,1 Shiva Najafzadeh,2 Shabnam Abtahi,2 Mohammad Javad Fattahi,2 and Abbas Ghaderi2

1Department of Dermatology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Correspondence should be addressed to Abbas Ghaderi; ghaderia@sums.ac.ir

Received 28 August 2018; Revised 15 October 2018; Accepted 11 November 2018; Published 19 November 2018

Academic Editor: Gavin Robertson

Copyright © 2018 Mehdi Ghahartars et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IL-27 has been shown to have both tumor promoting and suppressing functions. IL-27, with its diverse influences on immune responses, has not been studied extensively in nonmelanoma skin cancers (NMSC), including Squamous and Basal Cell Carcinomas (SCC and BCC), and its probable use in NMSC treatment have yet to be unveiled. A cross-sectional analytical study was designed to investigate the serum levels of IL-27 in NMSC patients in comparison to normal individuals. Levels of IL-27 in the sera of 60 NMSC patients along with 28 healthy controls were measured by means of quantitative enzyme-linked immunosorbent assay (ELISA). In this study we observed that IL-27 serum levels were significantly higher in NMSC patients in comparison to healthy individuals (0.0134 versus 0.0008 ng/ml; P<0.001). Furthermore, when subcategorized based on pathological diagnosis, both BCC and SCC patients had higher levels of IL-27 in their sera compared to controls (P=0.002 and P=0.033; respectively). However, these levels were not different among SCC and BCC patients. According to our results, it seems that IL-27 is involved in antitumor immune responses in NMSCs. On the other hand, these observations might be indicative of this cytokine involvement in NMSC tumorigenesis and progression. Therefore, administration of this cytokine for therapeutic purposes in patients with such conditions should be exerted on the side of caution.

1. Introduction

Attempts for identifying mechanisms underlying carcinogenesis have resulted in discovery of many aspects of tumor immune responses, many of which are already being used in therapeutic protocols for different malignancies [1]. However, there are still aspects in need of being discovered and further clarified. Use of cytokines such as IL-2 and IFN-alpha for cancer immunotherapy is prominent milestones in the history of medical oncology [2], and attempts to identify other cytokines for this purpose have been continuing ever since. Several cytokines, such as IL-12, IL-15, IL-21, and granulocyte macrophage colony-stimulating factor (GM-CSF) have shown promising results for cancer therapy in both murine models and clinical trials [3].

Among cytokines being investigated for probable therapeutic applications, IL-27 has been shown to have both tumor promoting and suppressing functions, depending on the characteristics of the target neoplasm [4–6]. IL-27 is a member of the IL-6/IL-12 family and is considered to be a multifunctional cytokine with both pro- and anti-inflammatory properties [7]. This cytokine is a heterodimer composed of an IL-12 p40-related protein subunit, EBV-induced gene 3 (EBI3) and a unique IL-12p35-like protein, IL-27p28. This cytokine is mainly produced by activated antigen presenting cells (APCs) including dendritic cells (DCs) and macrophages [6, 7].

Several reports have demonstrated IL-27 exerting direct and indirect inhibitory effects on neoplastic cells. Studies have shown the tumor restricting effects of this cytokine on pediatric leukemias [8, 9], lymphomas [10], multiple myeloma (MM) [11], neuroblastoma [12], prostate cancer [13], non-small-cell lung cancer (NSCLC) [14], ovarian cancer (SKOV3 cell line) [15], colon cancer [16], esophageal cancer...
head and neck squamous cell carcinoma (SCC) [18], and melanoma [19]. These studies have suggested direct inhibition of cell growth/proliferation, migration, tumor angiogenesis, and IL-17 production, alongside with enhancement of NK cell responses, antibody-dependent cell-mediated cytoxicity (ADCC), generation of myeloid progenitor cells, promoting M1 macrophage differentiation, and most importantly activation and promotion of tumor specific cytotoxic T cell responses as means of antitumor mechanisms by IL-27 [5–7, 20].

In spite of well-documented antitumor activities for IL-27, tumor promoting effects have been reported for this cytokine, as well. In contrast to pediatric leukemias, a study has shown that IL-27 improves survival of adult Acute Myeloid Leukemia (AML) cells and decreases their responsiveness to chemotherapeutic agents [21]. Other studies have suggested that IL-27 exerts some of its tumor promoting effects through induction of immune regulatory phenotypes, such as increasing the expression of molecules like IL-18BP [22], PD-L1/2 [23, 24], IDO [23], CD39 [25], Tim3 [26], and IL-10 [26].

The significance of the immune system in nonmelanoma skin cancers (NMSC), including Squamous and Basal Cell Carcinomas (SCC and BCC), has been long recognized, mainly based on the increased incidence of these neoplasms in organ transplant patients receiving immune-suppressants and immunomodulation due to ultraviolet light [27]. IL-27, with its diverse influences on immune responses, has not been studied extensively in NMSCs and its roles in cancer initiation, progression, and its probable use in NMSC treatment have yet to be revealed. In an attempt to further clarify the roles of this cytokine in NMSC, we designed a cross-sectional study in order to compare serum levels of IL-27 in NMSC patients and healthy individuals.

2. Materials and Methods

A cross-sectional analytical study was designed to investigate the serum levels of IL-27 in NMSC patients in comparison to normal individuals. A total of 60 patients with histopathologic diagnosis of SCC or BCC, who consented to be involved in the study, were enrolled from a dermatology clinic affiliated with Shiraz University of Medical Sciences. Their demographics, past medical history, and family history were gathered from clinical documents. Patients with previous history of any neoplastic or autoimmune diseases, and those with metastatic NMSC were excluded from the study. The comparison group consisted of 28 age-sex matched individuals from the same geographic area with no history of malignant or autoimmune diseases and signs of infection at the time of sampling. The Medical Ethics Committee of Shiraz University of Medical Sciences approved that this study was in agreement with the Declaration of Helsinki principles [28].

5 ml of venous blood was collected from each participant. The blood samples were centrifuged and the obtained sera were stored at −80°C until analysis. Levels of IL-27 in the sera were measured by a quantitative enzyme-linked immunosorbent assay (ELISA) kit (Sigma-Aldrich; USA) according to the protocols described by the manufacturer.

3. Results

A total number of 60 NMSC patients and 28 healthy age-sex matched individuals, as controls, were involved in the study. The mean age of NMSC patients was 67.60±12.82 years, and male to female ratio was 3:1 (45:15). The most frequent diagnosis was SCC (n=40, 66.67%). Other clinicopathological features of the NMSC patients are presented in Table 1.

When comparing NMSC patients and controls, we observed that IL-27 serum levels were significantly higher in NMSC patients (Figure 1; 0.0134 ng/ml; P<0.001). In subgroup analysis according to pathologic diagnosis, serum levels of IL-27 were not different between SCC and BCC patients (P=0.100). However, we observed that SCC patients had higher levels of IL-27 in their serum in comparison to controls (Figure 1; 0.0134 versus 0.0008 ng/ml; P=0.002). The same was true when comparing IL-27 serum levels of BCC patients with controls (Figure 1; 0.0100 versus 0.0008 ng/ml; P=0.033). No other significant difference in IL-27 circulating levels was observed among different subgroups of patients (Table 1).

4. Discussion

Soon after its discovery by Pflanz in 2002 [29], IL-27 became a trendy subject in oncology-immunology, scientists started...
investigating its roles in carcinogenesis, use as a cancer biomarker, and designing novel immune therapies [5]. However the results of the conducted studies were controversial mostly depending on the type of neoplasm, its stage and many other known and unknown factors [4–6]. In this study we observed that IL-27 serum levels were significantly higher in NMSC patients in comparison to healthy individuals. Furthermore, when subcategorized based on pathological diagnosis, both BCC and SCC patients had higher levels of IL-27 in their sera compared to controls.

Studies investigating IL-27 roles in NMSC pathogenesis are scarce. In a study investigating IL-27 roles in skin tumorigenesis, Dibra et al. observed that increased levels of IL-27 enhance papilloma formation in the skin, help proliferation of mutated stem cells, sustain premalignant niche, increase angiogenesis, and augment vessel density, all of which lead to increased tumorigenesis [30]. However, in a survey on potential roles of IL-27 in head and neck SCC, Matsui et al. observed that this cytokine effects on murine NK cells resulted in longer survival, boosted cytotoxic activity, and probably ADCC of these cells, consequently leading to better antitumor responses [18].

Regarding Melanomas, Gonin et al. observed that IL-27 expression in melanomas was associated with tumor progression rather than regression [31]. They found that IL-27 might induce suppressive molecules such as PD-L1 and IL-10 and thus immunosuppressive responses and melanoma progression [31]. However, older studies on the melanomas have had converse results. These studies have shown that IL-27 exerts an antitumor effect on poorly immunogenic B16F10 melanoma by means of antiproliferative, antiangiogenic, Cytotoxic T lymphocyte (CTL), and NK cells activity [19, 32, 33].

NSCLCs are among the carcinomas that have been widely studied in this regard. In all studies, it has been proposed that IL-27 has tumor suppressing effects on NSCLCs [5]. The same seems to be true in cases of esophageal [17] and prostate [13] carcinomas and neuroblastomas [12, 34], and according to published studies IL-27 shows antitumor activity in these neoplasms. In case of hematologic malignancies, studies have shown the opposite roles for this cytokine. IL-27 seems to promote proliferation of human leukemic cell lines, suppresses sensitivity to chemotherapeutic agents [21], and induces the expression of immunosuppressive molecules like PDL-1/2 [24].

Regarding ovarian carcinomas, study results have been paradoxical. While Zhang et al. have observed that IL-27 expression by plasmid transfected SKOV3 cells leads to suppression of ovarian cancer cells’ proliferation and enhanced cytotoxicity [15], other studies have shown that IL-27 helps ovarian tumors’ progression by escalating production of IDO, PDL-1 [23], and CD39 [25] and thus induction of immunosuppressive environment in favor of ovarian cancer progression.

The observations of this study are indicative of IL-27 association with NMSC and the results could be both the cause and effect (following host immune responses) of NMSC presence.

5. Conclusion

Although many studies suggested IL-27 administration for cancer immunotherapy [6], its therapeutic use as an anticancer agent may not be effective and potentially even detrimental (in certain tumors where IL-27 has been associated with a protumor effect). To draw any definitive conclusion there is a need for studies with larger sample sizes, considering the amount of sun exposure, other skin cancer risk factors, and participants’ type of skin. Furthermore, correlating IL-27 levels to NMSC progression and prognosis requires longitudinal studies.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declared that they have no conflicts of interest.

Acknowledgments

This work was supported by a grant from Shiraz University of Medical Sciences (Grant no. 1396-01-01-16171).
References

[1] S. Farkona, E. P. Diamandis, and I. M. Blasutig, "Cancer immuno-
therapy: the beginning of the end of cancer?" *BMC Medicine*, vol. 14, no. 1, article 73, 2016.

[2] W. K. Decker, R. F. da Silva, M. H. Sanabria et al., "Cancer immuno-
therapy: historical perspective of a clinical revolution and emerging preclinical animal models," *Frontiers in Immunol-
ogy*, vol. 8, article 629, 2017.

[3] T. A. Waldmann, "Cytokines in Cancer Immunotherapy," *Cold Spring Harbor Perspectives in Biology*, vol. 10, no. 11, Article ID a028472, 2017.

[4] M.-S. Li, Z. Liu, J.-Q. Liu, X. Zhu, Z. Liu, and X.-F. Bai, "The Yin and Yang aspects of IL-27 in induction of cancer-specific T-cell responses and immunotherapy," *Immunotherapy*, vol. 7, no. 2, pp. 191–200, 2015.

[5] M. Fabb, G. Carbotti, and S. Ferrini, "Dual roles of IL-27 in cancer biology and immunotherapy," *Mediators of Inflammation*, vol. 2017, Article ID 3958069, 14 pages, 2017.

[6] G. Murugaiyan and B. Saha, "IL-27 in tumor immunity and immunotherapy," *Trends in Molecular Medicine*, vol. 19, no. 2, pp. 108–116, 2013.

[7] N. Orii, I. Mizoguchi, Y. Chiba et al., "Protective effects against tumors and infection by interleukin-27 through promotion of expansion and differentiation of hematopoietic stem cells into myeloid progenitors," *Oncoimmunology*, vol. 7, no. 5, Article ID e421892, 2018.

[8] A. Zorzoli, E. Di Carlo, and C. Cocco, "Interleukin-27 inhibits the growth of pediatric acute myeloid leukemia in NOD/SCID/ Il2rg-/- mice," *Clinical Cancer Research*, vol. 18, no. 6, pp. 1630–1640, 2012.

[9] S. Canale, C. Cocco, C. Frasson et al., "Interleukin-27 inhibits pediatric B-acute lymphoblastic leukemia cell spreading in a preclinical model," *Leukemia*, vol. 25, no. 12, pp. 1815–1824, 2011.

[10] C. Cocco, E. Di Carlo, S. Zufo et al., "Complementary IL-23 and IL-27 anti-tumor activities cause strong inhibition of human follicular and diffuse large B-cell lymphoma growth in vivo," *Leukemia*, vol. 26, no. 6, pp. 1365–1374, 2012.

[11] C. Cocco, N. Giuliani, E. Di Carlo et al., "Interleukin-27 acts as multifunctional antitumor agent in multiple myeloma," *Clinical Cancer Research*, vol. 16, no. 16, pp. 4188–4197, 2010.

[12] R. Salcedo, J. K. Stauffer, E. Lincoln et al., "IL-27 mediates complete regression of orthotopic primary and metastatic murine neuroblastoma tumors: role for CD8+ T cells," *The Journal of Immunology*, vol. 173, no. 12, pp. 7170–7182, 2004.

[13] E. Di Carlo, C. Sorrentino, A. Zorzoli et al., "The antitumor potential of Interleukin-27 in prostate cancer," *OncoTarget*, vol. 5, no. 21, pp. 10332–10341, 2014.

[14] I. Airoldi, M. G. Tupone, S. Esposito et al., "Interleukin-27 re-
educates intratumoral myeloid cells and down-regulates stem-
ess genes in non-small cell lung cancer," *OncoTarget*, vol. 6, no. 6, pp. 3694–3708, 2015.

[15] Z. Zhang, B. Zhou, K. Zhang, Y. Song, L. Zhang, and M. Xi, "IL-
27 suppresses SKOV3 cell proliferation by enhancing STAT3 and inhibiting the Akt signal pathway," *Molecular Immunology*, vol. 78, pp. 155–163, 2016.

[16] M. Hisada, S. Kamiya, K. Fujita et al., "Potent antitumor activity of interleukin-27," *Cancer Research*, vol. 64, no. 3, pp. 1152–1156, 2004.

[17] L. Liu, S. Wang, B. Shan et al., "IL-27-mediated activation of natural killer cells and inflammation produced antitumour effects for human oesophageal carcinoma cells," *Scandinavian Journal of Immunology*, vol. 68, no. 1, pp. 22–29, 2008.

[18] M. Matsu, T. Kishida, H. Nakano et al., "Interleukin-27 acti-
vates natural killer cells and suppresses NK-resistant head and neck squamous cell carcinoma through inducing antibody-
dependent cellular cytotoxicity," *Cancer Research*, vol. 69, no. 6, pp. 2523–2530, 2009.

[19] T. Yoshimoto, N. Morishima, I. Mizoguchi et al., "Antiprolifera-
tive activity of IL-27 on melanoma," *The Journal of Immunology*, vol. 180, no. 10, pp. 6527–6535, 2008.

[20] Y. Chiba, I. Mizoguchi, J. Furusawa et al., "Interleukin-27 exerts its antitumor effects by promoting differentiation of hemato-
poietic stem cells to MI macrophages," *Cancer Research*, vol. 78, no. 1, pp. 182–194, 2018.

[21] H. Jia, P. Dilger, C. Bird, and M. Wadhwa, "IL-27 promotes pro-
iferation of human leukemic cell lines through the MAPK/ERK signaling pathway and suppresses sensitivity to chemothera-
pedics drugs," *Journal of Interferon & Cytokine Research*, vol. 36, no. 5, pp. 302–316, 2016.

[22] G. Carbotti, G. Barisone, A. M. Orenge et al., "The IL-18 antag-
onist IL-18-binding protein is produced in the human ovarian cancer microenvironment," *Clinical Cancer Research*, vol. 19, no. 17, pp. 4611–4620, 2013.

[23] G. Carbotti, G. Barisone, I. Airoldi et al., "IL-27 induces the expression of IDO and PD-L1 in human cancer cells," *OncoTarget*, vol. 6, no. 41, pp. 43267–43280, 2015.

[24] H. Horlad, C. Ma, H. Yano et al., "An IL-27/Stat3 axis induces expression of programmed cell death 1 ligands (PD-L1/2) on infiltrating macrophages in lymphoma," *Cancer Science*, vol. 107, no. 11, pp. 1696–1704, 2016.

[25] S. M. d’Almeida, G. Kauffenstein, C. Roy et al., "The ecto-
ATPDase CD39 is involved in the acquisition of the immu-
noregulatory phenotype by M-CSF-macrophages and ovarian cancer tumor-associated macrophages: regulatory role of IL-27," *Oncoimmunology*, vol. 5, no. 7, Article ID e1178025, 2016.

[26] C. Zhu, K. Sakushi, S. Xiao et al., "An IL-27/Stat3 axis signalling axis drives Tim-3 and IL-10 expression and T-cell dysfunction," *Nature Communications*, vol. 6, article 6072, 2015.

[27] S. Rangwala and K. Y. Tsai, "Roles of the immune system in skin cancer," *British Journal of Dermatology*, vol. 165, no. 5, pp. 953–965, 2011.

[28] World Medical Association, "World Medical Association decl-
aration of Helsinki ethical principles for medical research involving human subjects," *Journal of the American Medical Association*, vol. 310, no. 20, pp. 2191–2194, 2013.

[29] S. Pflanz, J. C. Timans, J. Cheung et al., "IL-27, a heterodimeric cytokine composed of EB13 and p28 protein, induces proliferation of naive CD4+ T cells," *Immunity*, vol. 16, no. 6, pp. 779–790, 2002.

[30] D. Dibra, A. Mitra, M. Newman et al., "IL27 controls skin tumorigenesis via accumulation of ETAR-positive CD1b cells in the pre-malignant skin," *OncoTarget*, vol. 7, no. 47, pp. 77138–77151, 2016.

[31] J. Gonen, A. Carlotti, and C. Dietrich, "Expression of IL-27 by tumor cells in invasive cutaneous and metastatic melanomas," *PLoS ONE*, vol. 8, no. 10, Article ID e75694, 2013.

[32] S. Oniki, H. Nagai, T. Horikawa et al., "Interleukin-23 and inter-
leukin-27 exert quite different antitumor and vaccine effects on poorly immunogenic melanoma," *Cancer Research*, vol. 66, no. 12, pp. 6395–6404, 2006.
[33] Y. Chiba, I. Mizoguchi, K. Mitobe et al., "IL-27 enhances the expression of TRAIL and TLR3 in human melanomas and inhibits their tumor growth in cooperation with a TLR3 agonist Poly(I:C) partly in a TRAIL-dependent manner," *PLoS ONE*, vol. 8, no. 10, Article ID e76159, 2013.

[34] R. Salcedo, J. A. Hixon, J. K. Stauffer et al., "Immunologic and therapeutic synergy of IL-27 and IL-2: enhancement of T cell sensitization, tumor-specific CTL reactivity and complete regression of disseminated neuroblastoma metastases in the liver and bone marrow," *The Journal of Immunology*, vol. 182, no. 7, pp. 4328–4338, 2009.