The IL-17 and Th17 cell immune response in cervical cancer : angels or demons : it depends on the context

Punt, B.S.

II

Role of IL-12p40 in cervical carcinoma

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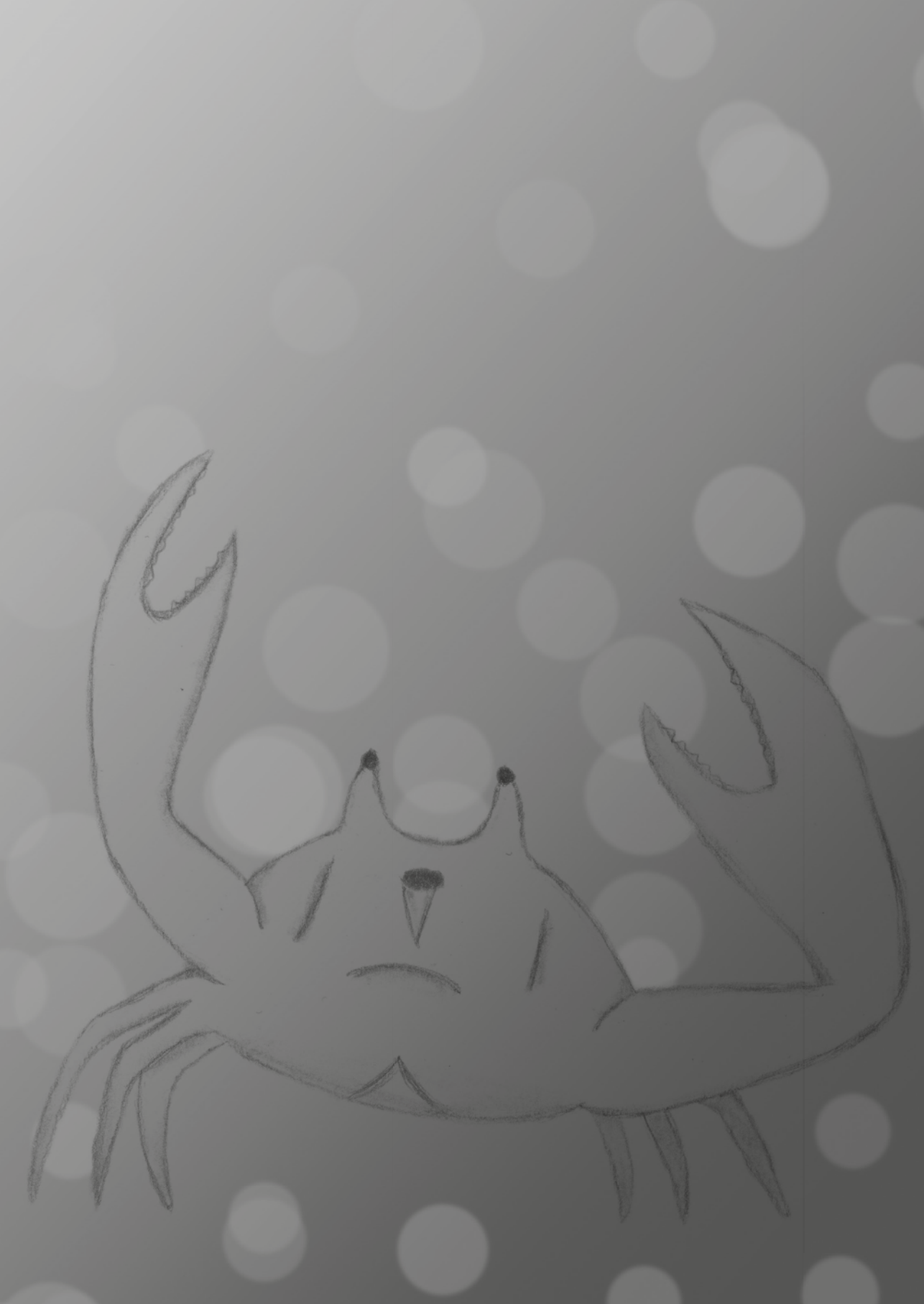

Abstract

Previously, we have shown that low *IL12p40* mRNA expression by cervical cancer cells is associated with poor survival of cervical cancer patients. As IL-12p40 is both a subcomponent of IL-12 and IL-23, the aim of this study was to elucidate the role of IL-12p40 in cervical cancer. We have measured the expression of *IL23p19*, *IL12p35* and *IL12p40* mRNA using mRNA *in situ* hybridization. As IL-23 is a component of the IL-17/IL-23 pathway, a pathway induced by IL-6 in humans, we have studied IL-1β and IL-6 expression. IL-1β and IL-6 were measured by immunohistochemistry. Only a high number of stromal IL-6^+ cells was correlated with poor disease-specific survival. The worst disease-specific survival was associated with a subgroup of patients that displayed a high number of IL-6^+ cells and low *IL12p40* expression ($p<0.001$). Both a high number of IL-6^+ cells and a high number of IL-6^+ cells plus low *IL12p40* expression were shown to be clinico-pathological parameters independent of lymph node metastasis, parametrial involvement and Sedlis score ($p=0.009$ and $p=0.007$, respectively). These results are in accordance with the hypothesis that the IL-17/IL-23 pathway has a tumor promoting role in cervical cancer.

Introduction

Cervical cancer is a leading cause of morbidity and mortality among women worldwide, especially in the developing countries.[1,2] Infection with oncogenic types of human papillomavirus (HPV) is an important factor in the development of cervical cancer.[3,4] The persistent HPV infection induces an inflammatory response. Inflammation is an important component in the majority of tumor types. The outcome of this inflammatory response surrounding the cancer cells is dependent on the composition of the inflammatory infiltrate and locally produced signaling molecules.[5] Although inflammatory cells within the neoplastic lesion are capable of generating an anti-tumor response this does not efficiently occur.[6,7]

Inflammatory cells are attracted to the tumor site by locally produced cytokines and chemokines.[8,9] Cervical cancer cells are known to produce an extensive range of cytokines and chemokines, such as CCL2, GM-CSF, TNFα and IL-12.[10-12] In addition to attracting inflammatory cells, these cytokines and chemokines influence the activation status and function of infiltrating antigen presenting cells and stromal cells, thus influencing the course of the disease.[9,13]

In a previous study, we have shown that high expression levels or undetectable levels of *IL12p40* mRNA in cervical carcinoma are associated with improved overall survival, as compared to low *IL12p40* levels that were associated with poor survival.[14] As IL-12 is known to stimulate effector cell populations such as cytotoxic T cells and natural killer cells,[15,16] our results suggest a dual role for IL-12p40.

The IL-12 cytokine family includes IL-12, IL-23, IL-27 and IL-35.[17] From this family IL-12 and IL-23 share the IL-12p40 subchain. IL-12 is composed of IL-12p40 and IL-12p35, whereas IL-23 is composed of IL-12p40 and IL-23p19. IL-23 plays, amongst others, an important a role in the IL-17/IL-23 pathway resulting in the maintenance and expansion of Th17 cells.[18] In addition to IL-23, IL-1β and IL-6 are thought to play an important role in the induction of Th17 cells in humans.[19] The effect of IL-23 on cancer progression or cancer eradication is still not clear.[20,21]

To further delineate the role of IL-12p40 in cervical carcinoma, we have quantified the mRNA expression levels of *IL23p19* and compared its expression level with *IL12p35* and *IL12p40* to investigate the relative importance of IL-12 and IL-23 in the tumor microenvironment. In addition, we have investigated the roles of IL-1β and IL-6 in the tumor microenvironment by determining the number of IL-1β^+ and IL-6$^+$ cells. Finally, we have assessed the correlations between *IL23p19, IL12p35, IL12p40,* IL-12 and IL-23 expression (low or high), a high number of IL-1β^+ and IL-6$^+$ cells and clinico-pathological parameters.

Materials and Methods

Patient material

Between 1985 and 1995, 254 untreated patients suffering from primary cervical carcinoma with stage IB and IIA underwent a radical hysterectomy type III with lymphadenectomy. From the tissue obtained, based on the availability of the material, 90 tissue samples were accessible for research. Tissues were routinely embedded in paraffin after 10% formalin fixation. The tissue samples of each patient were examined by a pathologist for the presence of tumor. Tumor percentage varied between 20% and 90%, median 60%. The characteristics of the patients are depicted in Table 1. Forty-seven patients received postoperative radiotherapy because of either tumor positive lymph nodes or the presence of positive risk factors described by the Sedlis criteria[22] (a combination of two of the following unfavorable prognostic parameters: depth of infiltration ≥ 15 mm (deep stromal invasion; middle or deep third), tumor size ≥ 40 mm and presence of vasoinvasion). Human tissue samples were used according to the guidelines of the Ethical Committee of the Leiden University Medical Center.

Table 1. Patient and tumor clinico-pathological characteristics

Clinico-pathological parameter	Category	N
Age	45 (mean)	90
	29 – 76 (range)	
FIGO stage[1]	IB	68
	IIA	21
Lymph node metastasis	No	66
	Yes	22
Tumor size[1]	< 40 mm	37
	≥ 40 mm	26
Depth of infiltration[1]	< 15 mm	58
	≥ 15 mm	26
Vascular space involvement[1]	No	39
	Yes	47
Sedlis criteria[2]	Positive	28
	Negative	52
Parametrial invasion[1]	No	74
	Yes	14
HPV status[3]	16, 18	66
	Other	15
Histology	Squamous	58
	Adenosquamous	18
	Adeno	7
	Other	6

[1]Data were not available for all patients. [2]Sedlis criteria (Sedlis et al, 1999): a combination of two of the following unfavorable prognostic parameters: depth of infiltration ≥ 15 mm (deep stromal invasion; middle or deep third), tumor size ≥ 40 mm and presence of vasoinvasion. [3]Only data for cervical carcinoma samples with a determined HPV type were included. HPV subtypes other than 16 and 18 included: HPV31 (n=2), HPV33 (n=6), HPV35 (n=1), HPV45 (n=3), HPV58 (n=1), HPV59 (n=2), HPV68 (n=1). Abbreviations: FIGO=Fédération Internationale de Gynécologie et d'Obstétrique

Preparation of IL12p35, IL12p40 and IL23p19 probes

RNA was isolated from frozen human spleen using TRIzol® (Invitrogen, Breda, The Netherlands) and first-strand cDNA was synthesized with oligoDT primers and Reverse Transcriptase AMV (both Roche Diagnostics GmbH, Mannheim, Germany), both according to manufacturer's instructions. Oligonucleotide primers for *IL23p19*, *IL12p35*

and *IL12p40* were chosen on the basis of known sequences (see Table 2) and cDNA encoding for the different cytokines was amplified. A pGEM®-3Zf(+) Vector (Promega, Madison, WI) was linearized with SmaI and the PCR products were cloned into the vector. After transferring the vector to E. coli strain Top 10 (Invitrogen Corp., San Diego, CA), the plasmids were isolated by using the QIAfilter Maxi KITS protocol (QIAGEN GmbH, Hilden, Germany). The sequence of the PCR product was confirmed by DNA sequencing. Plasmids were linearized with BamH1 and EcoR1 (both Boehringer, Mannheim, Germany) in case of *IL12p40,* BamH1 and SacI in case of *IL12p35* and SacII, SalI and SpeI (Boehringer, Mannheim, Germany) in case of *IL23p19* using One-Phor-All Buffer Plus (Amersham Biosciences, Roosendaal, The Netherlands). Both strands were translated in a digoxigenin (DIG) labeled RNA probe according to manufacturer's instructions (Roche Diagnostics GmbH, Mannheim, Germany). The concentration of the DIG-labeled sense and antisense RNA probes were determined on a 1% agarose gel stained with ethidium bromide (Sigma, St. Louis, MO). Probes were stored at -20°C until further use.

Table 2. RNA probes used and RNA *in situ* hybridization conditions

Target	Primer	Sequence (5'-3')	Product (bp)	Accession nr	Position	Hybr. temp. (ºC)
IL23p19	Fw	AGAGCCAGCCAGATTTGAGA	487	NM_016584.2	134-620	55
	Rev	GCAGATTCCAAGCCTCAGTC				
IL12p35	Fw	TGCTCCAGAAGGCCAGACAAAC	465	XM_003121	320-784	42
	Rev	CCCGAATTCTGAAAGCATGAAG				
IL12p40	Fw	GGACCAGAGCAGTGAGGTCTT	373	XM_004011	189-561	42
	Rev	CTCCTTGTTGTCCCCTCTGA				

Abbreviations: Fw=forward; Rev=reverse Hybr. temp.=hybridization temperature

RNA in situ hybridization

RNA *in situ* hybridization (RISH) was performed as previously described.[23,24] In short, 3 µm thick paraffin sections were pre-treated and hybridized with 100 ng/ml DIG labeled RNA probe diluted in hybridization mixture containing NaCl and saline-sodium citrate (SSC; Table 2). Hybridization was allowed for 16 hrs at either 55°C (*IL23p19*) or 42°C (*IL12p35* and *IL12p40*), in a humidified chamber. Slides were washed in 2x SSC for 30 min followed by 0.1x SSC with 20 mM β-mercaptoethanol for 45 min (Merck, Darmstadt, Germany), both at used hybridization temperature (see Table 2). Subsequently the slides were incubated with 2 U/ml ribonuclease (RNase) T1 (Roche Diagnostics GmbH, Mannheim, Germany) in 2x SSC, 1 mM EDTA at 37°C for 30 min.

RNA hybrids were detected using subsequently mouse anti-digoxigenin (1:2000, Sigma-Aldrich Chemie GmbH, Steinham, Germany), rabbit anti-mouse Ig (1:50, DAKO, Glostrup, Denmark) and mouse alkaline phosphatase anti-alkaline phosphatase (APAAP, DAKO, Glostrup, Denmark).[10]

Immunohistochemistry

Serial sections of formalin-fixed and paraffin-embedded tissue, 3 µm thick, were mounted on aminopropylethoxysilane-coated slides. Sections were deparaffinized, rehydrated and treated with 0.3% H_2O_2 in methanol for 20 min to block endogenous peroxidase activity.

Antigen retrieval was performed (0.01 M citrate, pH 6.0) and sections were rinsed in phosphate-buffered saline (PBS). Subsequently, sections were stained overnight using either a 1:100 dilution of an affinity-purified polyclonal goat anti-human IL-1β antibody (AF-201-NA; R&D Systems, Minneapolis, MN, USA) or a 1:300 dilution of anti-human polyclonal rabbit anti-IL-6 antibody (Abcam, Cambridge, UK). For anti-IL-1β, the slides were incubated with a goat HRP-polymer kit (Biocare Medical, Concord, CA, USA) according to the manufacturer's instructions. For anti-IL-6, the slides were incubated with a biotinylated swine anti-rabbit antibody (1:200; DAKO, Glostrup, Denmark) and subsequently with a biotinylated horseradish peroxidase-streptavidin complex (1:100, DAKO). Immune complexes were visualized with diaminobenzidine as previously described.[24]

Evaluation of RISH and immunohistochemistry

RISH was scored as previously described.[25] Intensity was scored as none (0), mild (1), moderate (2) or intense (3) at low magnification (100x). Furthermore, the percentage of positive tumor cells was determined and divided in six groups: 0% (0, absent), 1-5% (1, sporadic), 6-25% (2, local), 26-50% (3, occasional), 51-75% (4, majority) and 76-100% (5, large majority). The sum of both the percentage and the staining intensity of the positive cells resulted in an overall score (0 or 2 to 8). The scores were combined into three groups: category 0 (score 0, no expression), category 1 (scores 2, 3, 4 and 5, low expression) and category 2 (scores 6, 7 and 8, high expression). mRNA expression was scored by two independent researchers without knowing the identity and clinical outcome of patients. IL-1β^+ and IL-6$^+$ cells were quantified in the tumor by counting the number of stained cells per 6, randomly selected, high-power fields of view (HPF, 400x).

Statistical analysis

Data from immunohistochemistry as well as RISH are given as the mean $\pm$ the standard deviation. Statistical analysis was performed using SPSS 17.0 (SPSS Inc., Chicago, IL).

Data were processed using the Chi-square test. Kaplan-Meier survival curves were generated to assess differences in disease-free period (defined as the observation time in months from surgery to relapse of the disease (disease-free survival)) or cumulative disease-specific survival (defined as time in months from surgery to death due to cervical cancer). A Cox regression was used for multivariate survival analysis. P values below 0.05 were considered statistically significant.

Results

Patients

Of the group of 90 patients, 68 patients were diagnosed with FIGO stage IB and 21 with FIGO stage IIA and all underwent a radical hysterectomy combined with pelvic lymph adenectomy (Table 1). Fortyseven patients received postoperative radiotherapy because of either tumor positive lymph nodes or meeting the terms of the Sedlis criteria[22] (a combination of two of the following unfavorable prognostic parameters: depth of infiltration ≥ 15 mm, tumor size ≥ 40 mm and presence of vasoinvasion). Twentyfive patients suffered from recurrent disease. At the end of the study 70 patients were alive, 7 suffered from a recurrence and 18 patients had died of disease.

Expression of IL23p19, IL12p35 and IL12p40 in cervical cancer

As IL-12p40 is a subunit of both IL-12 and IL-23, we have determined the expression pattern of *IL23p19*. Both *IL23p19* and *IL12p40* were expressed by cervical cancer cells (Figure 1A, C). The expression of *IL12p40* was stronger than the expression of *IL23p19*. *IL23p19* was expressed in 63% of the samples (n=54), *IL12p40* was expressed in 54% of the samples (n=90) and *IL12p35* was expressed in 84% of the samples (n=90; Table 3). All samples that expressed either *IL23p19* or *IL12p40* also expressed *IL12p35*. In contrast, 13 out of 44 samples that expressed *IL12p40* did not express *IL23p19*.
A slightly positive correlation between *IL23p19* and *IL12p40* was found (n=54, r^2 = 0.117, p=0.011; data not shown). No statistically significant correlation between *IL23p19* and *IL12p35* was found (n=54, r^2 = 0.061, p=0.072; data not shown).

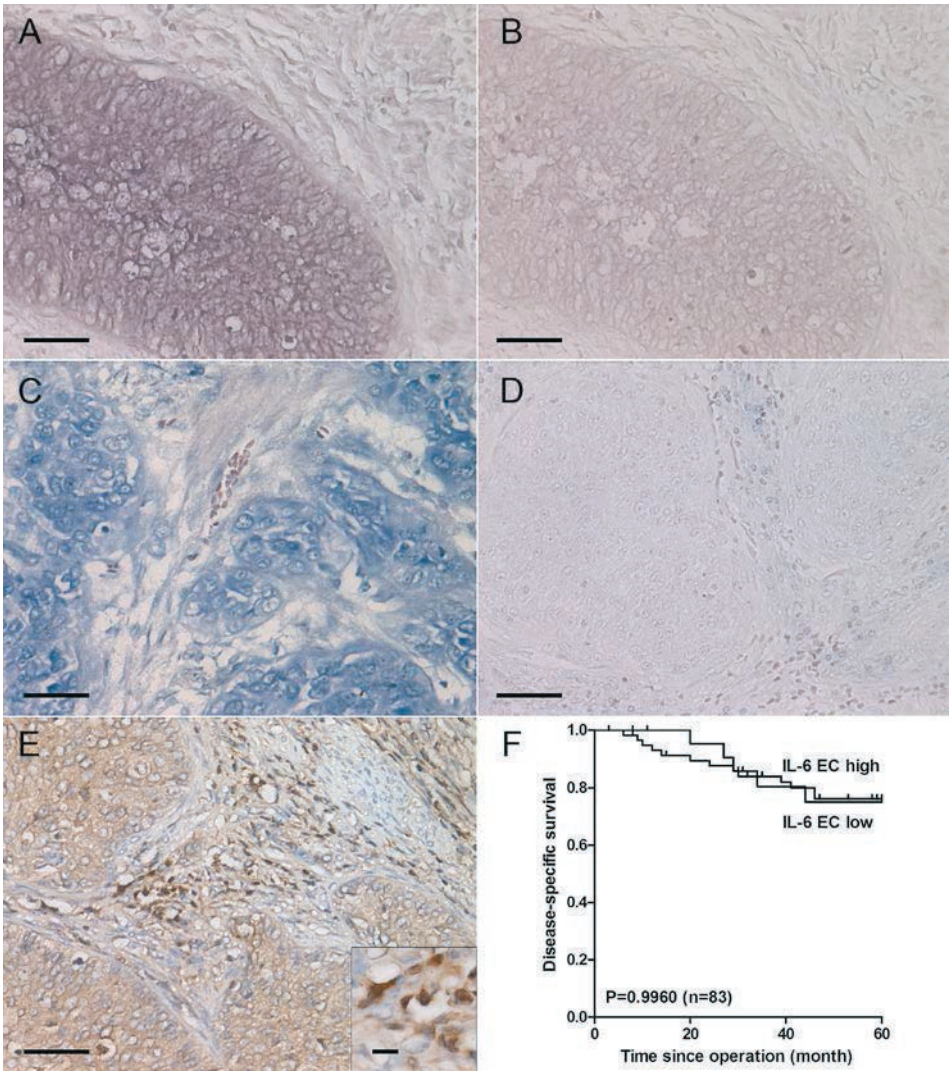

Figure 1. The expression of *IL23p19*, *IL12p40* and IL-6

Representative images of a cervical tumor expressing *IL23p19* (A) and *IL12p40* (C) determined using RISH (x250 magnification). Tumor cells stained positive (moderate) for *IL23p19* and positive (strong) for *IL12p40*. The negative (sense) controls of *IL23p19* and *IL12p40* RISH are shown in figures B and D, respectively. A representative image of cervical tumor expressing IL-6 determined by immunohistochemistry is shown in E. Both cells in the epithelial compartment (EC) as well as cells in the stroma expressed IL-6. Arrow indicates stromal IL-6^{+} cells, shown enlarged in the inset (x400 magnification). The association between cells in the epithelial compartment with low (IL-6 EC low) and high IL-6 (IL-6 EC high) expression and disease-specific survival is shown in F. Bars correspond to 50 mm in A–E and to 10 mm in the inset of E.

Table 3. Correlation between *IL23p19*, *IL12p35* and *IL12p40* expression in cervical cancer

Cytokine		***IL12p35***			
	Expression level	Absent	Low	High	Total n (%)
IL12p40	Absent	14	21	6	41 (46)
	Low	0	14	14	28 (31)
	High	0	3	18	21 (23)
Total	n (%)	14 (16)	38 (42)	38 (42)	90 (100)
p value					**<0.001**
		IL23p19			
		Absent	Low	High	Total n (%)
IL12p40	Absent	7	1	2	10 (19)
	Low	8	5	11	24 (44)
	High	5	4	11	20 (37)
Total	n (%)	20 (37)	10 (19)	24 (44)	54 (100)
p value					0.188
		IL23p19			
		Absent	Low	High	Total n (%)
IL12p35	Absent	1	0	0	1 (2)
	Low	8	5	8	21 (39)
	High	11	5	16	32 (59)
Total	n (%)	20 (37)	10 (19)	24 (44)	54 (100)
p value					0.619

The scores were combined into three groups: absent expression, low expression and high expression as described in the Materials and Methods. Statistically significant p values are shown in bold.

Association between IL-12 and IL-23 and disease-specific survival in cervical cancer

To investigate the relationship between the expression of *IL23p19, IL12p35* and *IL12p40* and disease-specific survival, Kaplan Meier plots were created. A log rank test was used to determine statistical differences in disease-specific survival. As the absence of *IL12p40* will result in neither IL-12 nor IL-23, we first confirmed that low expression of *IL12p40* was correlated with poor disease-specific survival (Figure 2A; n=48, log rank test 5.753, p=0.017) in this cohort. The expression of *IL12p35* (Figure 2B; n=74, log rank test 0.2019, p=0.653) and the expression of *IL23p19* (Figure 2C; n=33, log rank test 1.930, p=0.165) were both not significantly correlated with disease-specific

survival. The expression of *IL23p19, IL12p35* or *IL12p40* showed no significant difference in disease-free survival (data not shown).

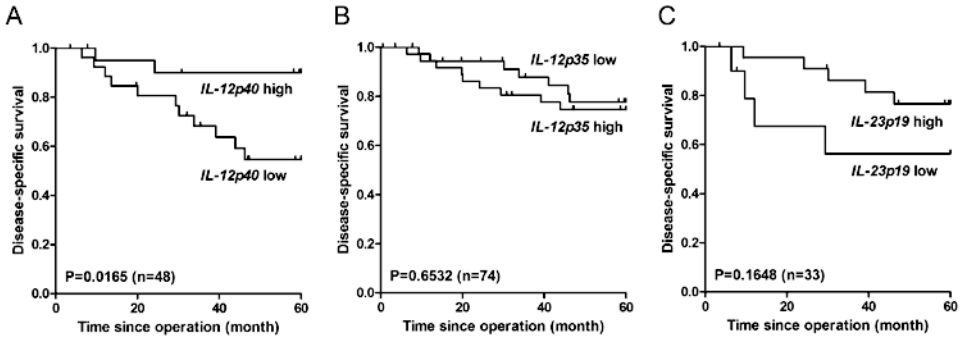

Figure 2. Correlation between IL-12 and IL-23 cytokine subunits and survival

The correlation between *IL12p40* (A), *IL12p35* (B) and *IL23p19* (C) expression and disease-specific survival in cervical cancer.

Presence of IL-6$^+$ cells and association with disease-specific survival in cervical cancer

The presence of *IL23p19* suggests that IL-23 may sustain a Th17 cell population in cervical cancer. As differentiation toward the IL-17/IL-23 pathway is thought to occur in the presence of IL-1β and IL-6 in humans,[19] we have determined the presence of IL-1β$^+$ and IL-6$^+$ cells using immunohistochemistry. IL-1β was predominantly expressed by cells in the stromal compartment. Occasionally, tumor cells also showed weak IL-1β expression. No statistically significant association between a low or high number of IL-1β-expressing cells and disease-specific survival was observed (Figure 3A). IL-6 was expressed by both cells in the epithelial (tumor cell) compartment as well as cells in the stromal compartment (Figure 1E). No significant association was observed between a low or high number of IL-6$^+$ cells in the epithelial compartment and disease-specific survival (Figure 1F). Subsequently, we quantified the number of IL-6$^+$ cells in the stroma. The presence of a high number of stromal IL-6$^+$ cells (median 17 IL-6$^+$ cells/HPF) was significantly correlated with disease-specific survival (Figure 3B; n=83, log rank test 12.57, $p<0.001$). No statistically significant difference was observed for disease-free survival (data not shown). We also determined whether the presence of both a high number of stromal IL-6$^+$ cells and low *IL12p40* expression was correlated with disease-specific survival. In this latter case an even stronger decrease in disease-specific survival was observed (Figure 3C; n=47, log rank test 20.38, $p<0.001$).

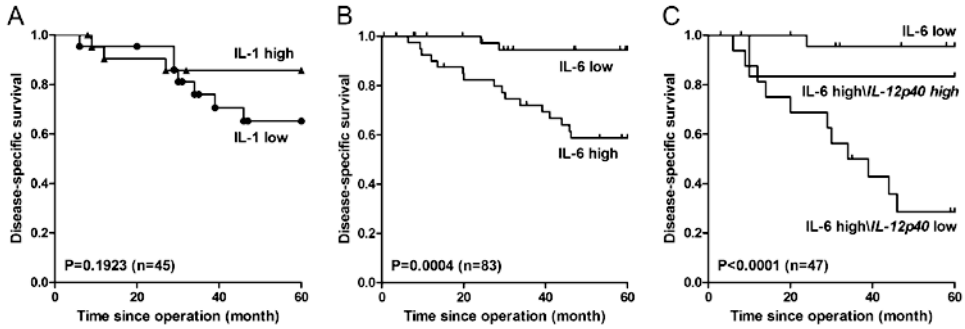

Figure 3. Correlations between IL-1β and IL-6 and survival

The correlation between a high number of IL-1β$^+$ cells (A), stromal IL-6$^+$ stromal cells (B) and *IL12p40* expression in combination with a high number of stromal IL-6$^+$ cells (C) and disease-specific survival.

Association between low IL12p40 expression, high number of stromal IL-6$^+$ cells and clinico-pathological parameters

To determine the relevance of our findings, we studied the correlations between our immunological findings and clinico-pathological parameters. First a univariate Cox analysis was performed, using the clinical parameters Sedlis criteria (two out of three of the following criteria positive: tumor size ≥ 40 mm, vasoinvasion and deep stromal invasion), lymph node metastasis and parametrial involvement and the immunological parameters low *IL12p40* expression, a high number of IL-6$^+$ cells and a high number of IL-6$^+$ cells plus low *IL12p40* expression. In the univariate Cox analyses, all the included parameters showed a significantly increased hazard ratio (HR; Table 4). Subsequently, a multivariate Cox analysis with the three clinico-pathological parameters and each of the statistically significant immunological parameters was performed. In this case two of the three immunological parameters were shown to be independent predictors of poor disease-specific survival: a high number of stromal IL-6$^+$ cells (HR 7.447, p=0.009) and a high number of stromal IL-6$^+$ cells and low *IL12p40* expression (HR 20.123, p=0.007).

Table 4. Cox regression of clinico-pathological variables and *IL12p40* and the number of IL-6-expressing cells in cervical cancer

Variable	Univariate hazard ratio (95% CI)	p value	Multivariate hazard ratio (95% CI)	p value	Multivariate hazard ratio (95% CI)	p value
Sedlis positive	4.354 (1.608-11.789)	**0.004**	3.848 (1.270-11.663)	**0.017**	0.706 (0.150-3.310)	0.659
Lymph node metastasis	3.266 (1.286-8.296)	**0.013**	1.333 (0.439-4.052)	0.612	2.887 (0.836-9.965)	0.094
Parametrial involvement	3.645 (1.411-9.411)	**0.008**	1.526 (0.542-4.444)	0.438	1.168 (0.283-4.822)	0.830
Low *IL12p40*	5.231 (1.156-23.661)	**0.032**				
Low number of IL-6$^+$ cells			Reference			
High number of IL-6$^+$ cells	8.975 (2.063-39.051)	**0.003**	7.447 (1.659-33.432)	**0.009**		
High number of IL-6$^+$ cells/low *IL12p40*	21.832 (2.805-169.915)	**0.003**			20.123 (2.248-180.147)	**0.007**

The hazard ratios and p values of compared expression levels are shown. Statistically significant p values are shown in bold.

Discussion

In a previous study, we found an association between low expression of *IL12p40* and poor disease-specific survival, whereas high expression of *IL12p40* or lack of expression of *IL12p40* were associated with a favorable disease-specific survival.[14] As IL-12p40 combines with both IL-12p35 and IL-23p19, to form IL-12 and IL-23, respectively, in the present study, we have further investigated the role of IL-12p40 in cervical cancer.

Both *IL23p19* and *IL12p35* were expressed in the majority of the samples. Of the 44 samples that expressed *IL12p40,* 13 samples did not express *IL23p19*. As *IL12p35* expression seems to be ubiquitous in cervical cancer,[14] the level of *IL12p40* or *IL23p19* expression most probably determines whether IL-12, IL-23 or both are expressed. In our study, we observed a trend between *IL23p19* and *IL12p35* expression, whereas in the study of Wolf *et al*. in ovarian cancer (n=112), a significant correlation between the expression of *IL23p19* and *IL12p35* was found.[26] The discrepancy between our results and the results of Wolf *et al*. may be due to the smaller size of our study group. Very few studies have investigated the association between local expression of IL-12 or IL-23

and prognosis. Using immmunohistochemistry, IL-12 has been associated with improved survival in patients with (advanced) gastric carcinoma.[27,28] In the study of Wolf *et al*., using RT PCR, both *IL12p35* and *IL23p19* were associated with a superior outcome.[26] In a multivariate analysis, *IL12p35* was found to be an independent factor for overall survival of ovarian carcinoma. As stated previously, we have observed a statistically significant correlation between low expression of *IL12p40* and poor disease-specific survival in cervical carcinoma.[14] In the present study and our previous study,[14] we did not find a significant correlation between either *IL23p19* or *IL12p35* expression and disease-specific survival. As *IL23p19* and *IL12p35* are both expressed, it is important to determine which cytokine, IL-23 or IL-12, has a dominant effect on the tumor microenvironment.

The molecular interaction between IL-23p19 and IL-12p40 has been studied by Beyer et al.[29] These authors reported that the interface region of IL-23p19 and IL-12p35 on IL-12p40 overlap. Due to different interresidue interactions of IL-12p35 and IL-23p19 with IL-12p40, these molecules interact with a different affinity with IL-12p40. Therefore, the availability of IL-12p40 in combination with the affinity for IL-12p35 and IL-23-p19 may result in skewing of the IL12/IL23 response. This is supported by experiments performed by Zwiers *et al*. These authors showed that in an experimental animal model, polymorphic variants of IL-12p40 can skew IL-12/IL-23 synthesis.[30] Thus, both differences in protein interactions between IL-23p19 and IL-12p35 on the one hand and IL-12p40 on the other hand and genetic polymorphisms in the protein chains, such as IL-12p40, contribute to the amounts of IL-12 and IL-23 formed. Our results support a previously suggested immunosuppressive role for IL-23.[20] This is further supported by a study in ovarian carcinoma were genetic differences in the IL-23 receptor have been reported to influence prognosis.[31]

In contrast, it has also been shown that overexpression of IL-23 reduces tumor growth and metastasis formation and that IL-23 is able to elicit a strong cytotoxic T-cell memory response,[21,32] underscoring our view that the level of expression of the different cytokines and chemokines plays an import role in the final outcome.

Our data suggest that in the presence of a limited amount of *IL12p40*, the biological effect of IL-23 dominate, whereas in the presence of a high amount of *IL12p40*, the biological effect of IL-12 prevails. As IL-12 polarizes the immune response toward an anti-viral response,[33] the favorable cumulative overall survival of patients with a high IL-12^{+} cell density,[27,28] high expression level of *IL12p35*[26] and *IL12p40*[14] can be explained by the capacity of this cytokine to increase the lytic activity and the production of interferon-γ of natural killer cells and cytotoxic T-lymphocytes.[33] Interestingly, we previously observed an association between high expression of *IL12p40* and high expression of *TGF-β* (p=0.024),[14] suggesting that the tumor cells are selected to counteract the effect of IL-12 or skew the response to the IL-17/IL-23 pathway.

In humans, in addition to TGF-β, IL-1β and IL-6 have been implicated to play a role in the IL-17/IL-23 pathway.[19,34] Previously, we have shown that it is likely that activated TGF-β is present in the tumor microenvironment, as PAI-1 expression, a target gene of TGF-β, is correlated with survival.[35] Furthermore, both the integrin avβ6 and active matrix metalloproteinase-2, known to activate TGF-β, are associated with poor disease-specific survival.[36,37] Even though the role of TGF-β in inducing Th17 in humans has been questioned, TGF-β may suppress Th1 and Th2 development thus favoring Th17 development.[38,39]

In our study, low or high numbers of IL-1β-positive cells were not correlated with disease-specific survival. The presence of a high number of stromal IL-6^+ cells was significantly associated with poor disease-specific survival. Previously, IL-6 has been implicated as an autocrine or paracrine growth factor for cervical cancer.[40,41] IL-6 has been shown to induce VEGF transcription via the STAT3 signaling pathway, thus promoting an angiogenic switch.[42] Indeed, blockade of the IL-6 receptor on cervical cancer cell lines was shown to interfere with cell survival signals and blocked expression of VEGF.[43]

As HPV vaccines will become available for the treatment of metastasized cervical carcinoma, the local cytokine/chemokine profile may be important to discriminate patients with a beneficial immune response from non-responding patients.

In conclusion, IL-12p40 plays at least a dual role in cervical carcinoma by associating with both IL-23p19 and IL-12p35. We have shown that low *IL12p40* expression was significantly correlated with poor disease-specific survival. Also, a high number of stromal IL-6^+ cells was shown to associate with poor disease-specific survival. The worst disease-specific survival was observed in a subgroup of patients that displayed a high number of stromal IL-6 expressing cells and low *IL12p40* expression. Furthermore, both a high number of stromal IL-6 expressing cells and a high number of stromal IL-6 plus low *IL12p40* expression were shown to be independent clinico-pathological parameters as compared to lymph node metastasis, parametrial involvement and Sedlis score. Our results support the hypothesis that IL-23 plays a tumor promoting role in cervical cancer.

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