

Transforming Growth Factor β 1 (TGF β) mRNA Expression and Macrophage Migration Inhibitory Factor (MIF) in Endometrial Cancer Versus Normal Endometrial Tissue

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Received: October 03, 2020; **Published:** February 25, 2021

Abstract

Objectives: Tumoral microenvironment inflammatory cells play a main role in cancer progression. The peritumoral Tumor Associated Macrophages (TAMs) infiltration depends on the kind of chemokine, cytokines and growth factors secreted by tumor cells or stroma in response to the tumor. At initial stages TAMs could promote anti-tumoral response but in advanced stages could promote neovascularization and metastatisation. Among the microenvironment chemo-attractants MIF has been shown correlated with increased TAMs recruitment in many human cancers modulating immune responses and promoting angiogenesis. Transforming Growth Factor β 1 (TGF β 1) is a cytokine that may exert tumor suppression during the first stages of tumorigenesis, while at the advanced stages tumor progression, invasion and metastasis. In this study we compared TGF β with MIF mRNA gene expression in endometrial cancer (EC) tissue versus normal endometrium (NE).

Materials and Methods: Fresh specimens from 15 patients with EC and corresponding NE were stored at -80° . One mcg of mRNA was reverse-transcribed in cDNA. A Real-Time PCR determined relative cDNA levels of targeted gene mRNA.

Results: We observed a down-regulation of TGF β 1 mRNA in 81% of samples ($P < .01$) and over-expression of MIF mRNA expression in 100% of samples ($p < .01$). The down regulation of TGF β 1 mRNA was statistically significantly inversely related to MIF mRNA ($P < .01$).

Conclusion: MIF mRNA over-expression significantly directly related to TGF β down-regulation could be an expression of inhibition of invasive potential in endometrial cancer cells modulated by macrophages. Detailed analysis needed to identify exactly in which cell subpopulation of tumoral microenvironment this cytokines are over/down - expressed.

Keywords: Endometrial Cancer; Peritumoral Microenvironment; mRNA; Macrophage Inhibitory Factor; Macrophages; Transforming Growth Factor β 1

Abbreviations

MIF: Macrophage Inhibitory Factor; TNF β 1: Transforming Growth Factor β 1; EC: Endometrial Cancer; NE: Normal Endometrium Cancer Cell; NSLCC: Non Small Cell Lung Cancer; TAMs: Tumor Associated Macrophages

Introduction

Increasing scientific evidence confirms that inflammatory response in the tumor microenvironment plays an important role in the development and behavior of solid tumors. In general, the prognosis of endometrial carcinoma depends on histotype, lymph node metastases, stage, histological degree and invasion of the lymphovascular spaces but in the near future the analysis of the peritumoral microenvironment could provide us with other important prognostics factors with important implications in the therapeutic field [1,2]. Chronic inflammation and immune diseases can predispose the individual to various types of neoplasms. On the other side, tumor itself, induced by an oncogenetic event, develops a peri-tumor microenvironment formed by inflammatory cells (granulocytes, macrophages, etc.) and soluble mediators such as chemokines, cytokines, prostaglandins, tissue remodeling factors and growth factors that together interact with tumor cells and vessels, being able to favor or counteract development and metastatic spread [3-5].

Among all soluble mediators of tumor microenvironment, Transforming Growth factor β 1 (TGF β 1) is a cytokine that may exert a role in tumor progression [6]. Fluorene-9-bisphenol (BHPF), a new derivative of bisphenol A, has been found significantly inhibit the endometrial cancer cells by blocking transforming growth factor- β (TGF- β) signaling pathway [7]. The Tumor Associated Macrophages (TAMS) infiltration is a prominent component of the mononuclear leukocyte population in solid tumors which are recruited and modulated by many growth factors and chemokines/cytokines productions by tumor cells or stroma in response to the tumor itself [8]. At an initial stage this signal can promote antitumor response but in an advanced stage the chemokine secreted by the tumor can stimulate metastases and neoangiogenesis [5,9]. Among the tumor microenvironment chemo-attractants, Macrophage Migration Inhibitory factor (MIF) has been found to enhance tumor cells metastases by modulating immune responses and by promotion of angiogenesis in melanoma, lymphoma, pancreatic cancer, colon carcinoma, glioblastoma and NSLCC (non-small lung cancer carcinoma) [10-17]. Vice versa, in breast cancer versus normal tissue, up-regulated MIF was reported statistically significantly directly related with progesterone and estrogen receptors expression (markers of a favorable prognosis) and indirectly related with tumor size [18].

In this study we decided to compare in human endometrial cancer versus normal endometrium counterparts TGF β 1 mRNA with MIF mRNA expression.

Materials and Methods

Immediately after surgery, fresh samples of endometrial cancer (EC) and their normal endometrial counterpart (NE) were obtained from patients submitted to primary surgery for endometrial cancer at RCCS Humanitas Clinical Institute in Milan (Italy). Parts of the samples were used for the histologic diagnosis and other parts were immediately treated with RNA later (Ambion) for 24 - 36h at 4°C and subsequently dried and stored at 80°. The study was approved by the Ethical Committee of Humanitas Research Institute and informed, written consent was obtained for all patients. All the clinical and surgical data were recorded on a data base. The total RNA was isolated both from endometrial cancer and normal endometrial specimen using TRI Reagent (Ambion). RNA was quantified by Nanodrop spectrophotometer ND-1000 and its quality was examined by 1.5% agarose gel electrophoresis. According to the manufacturer's instructions, 1 mg of total RNA was reverse-transcribed using the High-Capacity cDNA Archive kit (Applied Biosystems), treated with DNase I, quantified and reverse transcribed into cDNA using random primers. A real-time quantitative polymerase chain reaction, using SyberGreen I (Applied Biosystem) as detection dye, was used to determine the relative cDNA levels of genes in each samples. The amplification protocol was used as following: 2 minutes at 50°C to activate uracil-DNA glycosylase, 10 minutes at 94.5°C (activation), 40 cycles of denaturation at 97°C for 30s and annealing and extension at 59.7°C for 1 minute. The relative amount of each target gene mRNA to the housekeeping gene (18S) was calculated as 2(DCt), where DCt@Ct gene ACt housekeeping gene. The fold-change of each target gene mRNA to the corresponding normal tissue was calculated as 2(DDCt), where DDCt@DCt target gene in tumor tissue - DCt target gene in normal tissue. The threshold cycle Ct was automatically given by the SDS2.2 software package (Applied Biosystems).

We analyzed MIF and TGF β 1 gene expression.

Statistical analysis

Statistical significance was determined by T- test and considered significant at a P value of < 0.05.

Results and Discussion

We collected tissue samples from endometrial cancer (EC) and from normal corresponding endometrium (NE) in 15 patients with endometrial cancer FIGO stage I-IIIc. All patients were submitted to primary laparoscopic total hysterectomy and bilateral salpingectomy with pelvic lymphadenectomy. Four patients dropped out from the study: two because the endometrial sample was damaged during the storage making it impossible to process, and two because no residual tumor was found in the samples, despite an initial histologic diagnosis by endometrial biopsy. Table 1 and 2 describe the clinical characteristics. Three patients (27%) underwent adjuvant chemotherapy and pelvic radiotherapy and one patient (9%) underwent adjuvant pelvic radiotherapy (Table 2). At a median 3 years follow-up, the median disease free survival was 25 months (range 18 - 36). Only one patient with clear cell adenocarcinoma FIGO stage IIIA and no residual disease after surgery relapsed at 18 months (Table 2).

No. of patients	11
Median Age	63 (range 53 - 81)
Median BMI (Kg/m ²)	28 (range 25 - 31)
FIGO stage I	8 (73%)
IA	7 (64%)
IB	1 (9%)
FIGO stage III	3 (27%)
III A	2 (18%)
III C	1 (9%)
Histotype	
Endometrioid	7 (64%)
Clear Cell	2 (18%)
Villoglandular	1 (9%)
Endometrioid with squamous differentiation	1 (9%)

Table 1: Evaluable patients' clinical characteristics.

BMI: Body Mass Index.

Pt.	Age	FIGO stage	LVS	N	G	Histotype	ADJ	DFS months
1	66	IA	-	-	G3	AE	FU*	30
2	65	IA	-	-	G3	ACC	PAC + RT	32
3	75	IA	-	-	G1	AV	FU	36
4	63	IA	-	-	G2	AE	FU	35
5	58	IA	-	-	G2	AE	FU	23
6	68	IA	-	-	G2	AE	FU	24
7	61	IA	-	-	G2	AE	FU	36
8	81	IB	-	-	G2	AE	FU	22
9	53	IIIA	-	-	G2	AS	PAC + RT	25
10	81	IIIA	+	-	G2	ACC	CT +RT	18°
11	63	IIIC	+	+	G2	AE	RT	23

Table 2: Clinic characteristics of 11 evaluable patients.

LVS: Lymphovascular Space; N: Lymph Nodes; G: Histological Grade; ADJ: Adjuvant Therapy; DFS: Disease Free Survival in Months; PAC: Cisplatin, Paclitaxel; CT: Carbo Taxol; RT: Radio Therapy; AE: Endometrioid Adenocarcinoma; ACC: Clear Cell Adenocarcinoma; AV: Villoglandular Adenocarcinoma; AS: Squamous Adenocarcinoma; *Patient refused RT; ° Abdomino-pelvic relapse after 18 months.

We observed a significant up-regulation of MIF mRNA in the endometrial cancer tissues versus normal endometrium counterpart ($P < 0.01$) in all samples (Figure 1). TGF $\beta 1$ mRNA was significantly down-regulated in 81% of samples (Figure 2, $P < .01$). TGF $\beta 1$ and MIF mRNA expression were significantly inversely related in 81% of samples ($p < .001$).

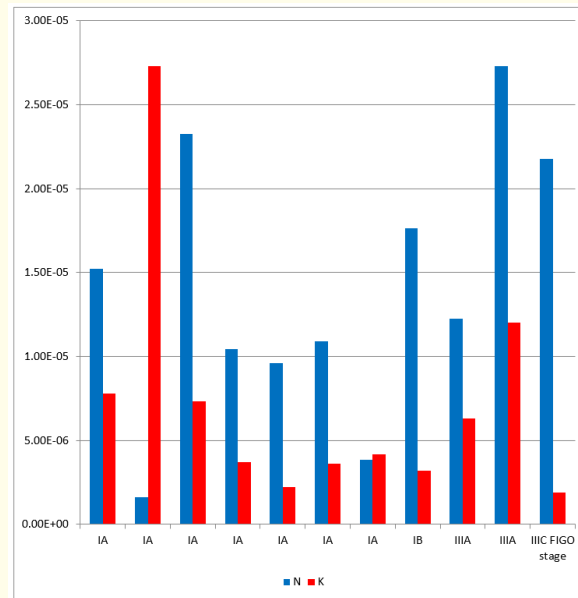


Figure 1: Transforming growth factor beta (TGF β) mRNA gene expression in normal endometrium (N) versus endometrial cancer samples counterpart (K).

In 81% endometrial cancer versus (K) normal endometrium (N) counterparts TGF β mRNA gene expression was down regulated ($P < .01$).

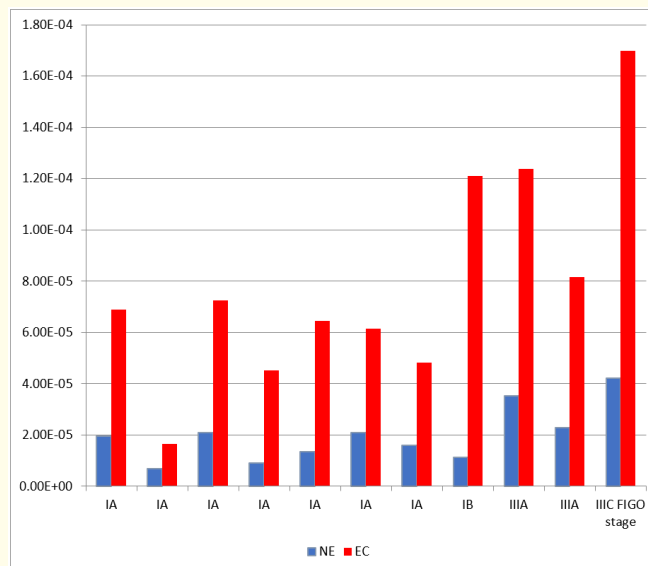


Figure 2: Macrophage inhibitory factor (MIF) mRNA gene expression in normal endometrium (N) versus endometrial cancer samples counterpart (K).

In 100% endometrial cancer versus normal endometrium counterparts MIF mRNA gene expression was up-regulated ($P = < .000$).

This report is, at our knowledge, the first study investigating in endometrial human cancer samples, compared with their normal endometrium counterparts, the mRNA gene expression of TGF β 1 and MIF. We chose the mRNA gene expression evaluation because many mechanisms can interfere during the transcription process from cellular DNA to the final product. Therefore, we decided to evaluate the mRNA expression level of the two genes examined, as mRNA expression is the primary index of gene activity. The significant over-expression of MIF mRNA in endometrial cancer in comparison with normal endometrium counterpart, confirms the data reported by other authors on other tumor types [19-22]. We already reported that over-expression of MIF mRNA was associated with low histologic grade, less aggressive type, no lymphovascular invasion and improved disease free survival rate [23]. These data seemed to suggest that, in patients with endometrial cancer, the up-regulation of MIF might be related to the inhibition of metastatic spread. TGF mRNA expression, statistically significantly in directed related with MIF mRNA expression in 81% samples, seems to confirm (being TGF β 1 associated with tumor progression) that MIF over-expression can have a role in the inhibition of TGF β 1. Understanding the tumour microenvironment behaviors and how it affects tumour cells could represent an attractive therapeutic target. The results displayed in this preliminary study open new sceneries for further researches, identifying in which subpopulation of tumoral microenvironment this proteins mediator are over-expressed.

Conclusion

MIF mRNA over-expression significantly directly related to TGF β 1 down-regulation could be an expression of inhibition of invasive potential in endometrial cancer cells modulated by macrophages. Detailed analysis needed to identify exactly in which cell subpopulation of tumor microenvironment this cytokines are over/down - expressed.

Acknowledgements

Our thanks to Paola Allavena MD PhD and Marco Erreni PhD.

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Volume 10 Issue 3 March 2021

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