

INFLUENCE OF SURGICAL RESECTION ON PLASMA ENDOGLIN (CD105) LEVEL IN NON-SMALL CELL LUNG CANCER PATIENTS

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Background and Aim: Endoglin is a proliferation-associated antigen on endothelial cells and essential for angiogenesis. Soluble endoglin (s-endoglin), formed by proteolytic cleavage of ectodomain of membrane receptor could be an indicator of tumor-activated endothelium. The aim of present study was to analyze changes of s-endoglin level in plasma of lung cancer patients following surgical resection and to estimate the correlation of s-endoglin with other soluble receptors, sTie2 and sVEGF R1. **Patients and Methods:** The study group consisted of 37 patients with stage I of non-small cell lung cancer. Plasma concentrations of s-endoglin, sTie2 and sVEGF R1 were evaluated by ELISA, three times: before surgical resection and on postoperative day 7 and 30. **Results:** The median of s-endoglin concentration decreased significantly on postoperative day 7 when compared with preoperative level and next increased on 30th day and it was comparable with that before surgery. s-Endoglin correlated with another soluble receptors, with sTie2 both before surgery ($r=0.44$) and on postoperative day 7 ($r=0.52$) and on 30th day ($r=0.58$), with sVEGF R1 — only on postoperative day 7 ($r=0.75$). **Conclusion:** The increased level of serum endoglin in lung cancer patients compared to controls and its changes after surgical treatment suggest potential application of soluble form of endoglin as potential tumor marker. **Key Words:** soluble endoglin, angiogenesis, lung cancer, surgery.

Endoglin (CD105) is a 180-kDa cell membrane glycoprotein which serves as a coreceptor for transforming growth factor (TGF)- β 1 or TGF- β 3, in the presence of the TGF- β type II receptor (T β RII) [1]. Endoglin is highly expressed by activated endothelial cells (ECs) [2]. Hypoxia and TGF- β are two main factors that cooperate to induce its expression [3]. Endoglin promotes angiogenesis mainly by activation of vascular ECs proliferation [4]. Endoglin overexpressed on endothelia of vessels in several human solid malignancies [5] and its overexpression is associated with lower patient survival rates, presence of nodes metastases and distant metastatic disease [6].

Tumor vascular endothelium shows up-regulation of various receptors, including the vascular endothelial growth factor receptor 2 (VEGF R2) and angiopoietin receptor, Tie2 [7]. Two other endothelial receptors for angiogenic factors, the VEGF receptor 1 (VEGF R1) and the orphan receptor Tie 1, are also up regulated by hypoxia [7]. So far, two different mechanisms are known which lead to the formation of soluble receptors [8]. Firstly, soluble receptors can be translated from differentially spliced pre-mRNA molecules lacking the transmembrane domain (e. g., sVEGF R1) [9] and the

second mechanism involves limited proteolysis in the extracellular domain of the membrane receptor leaving the ligand-binding domain intact (e. g., sTie2) [8]. The external domain can be cleaved or shed and released into the circulation [7].

Similarly to classical receptors, accessory receptor — endoglin exists in two forms: as a membrane-bound and a soluble (s-endoglin) in the circulation. Recent findings [10] suggest that the ectodomain of endoglin is released through proteolytic cleavage by membrane-type 1 matrix metalloproteinase (MT1-MMP) — matrix metalloproteinase-14 (MMP-14). Coexpression of endoglin with MMP-14 on the cell membrane leads to the cleavage of endoglin at the glycine-leucine bond at position 586, releasing the nearly complete endoglin extracellular domain [10]. Hawinkels' et al. [10] study also shows that MMP-14 is the most abundantly expressed MT-MMP in ECs and that knock-down of MMP-14 strongly reduces s-endoglin levels in the conditioned media of these cells cultures. Local up-regulation of endothelial MMP-14 expression may increase s-endoglin, decrease membrane-localized endoglin, and transform the endothelium to a quiescent state [10]. Similarly to endoglin, MMP-14 is highly expressed not only by ECs, but also by several other cell types, i. e. by cells of lung tumor [11]. In Atkinson's et al. [11] studies, among all MT-MMPs (MMPs 14-17, 24 and 25), MMP-14, -15 and -17 displayed higher expression in tumor relative to normal lung specimens. In addition MMP-14 mRNA expression strongly correlated to MMP-14 proteolytic activity in tumor models. Therefore, s-endoglin might be shed not only from endothelial

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Abbreviations used: ECs — endothelial cells; MMP-14 — matrix metalloproteinase-14; MT1-MMP — membrane-type 1 matrix metalloproteinase; s-endoglin — soluble endoglin; TGF- β 1 — transforming growth factor- β 1; T β RII — TGF- β type II receptor; Tie2 — angiopoietin receptor; VEGF R2 — vascular endothelial growth factor receptor 2.

cells, but also from tumor cells. To conclude, the level of s-endoglin could be an indicator of tumor-activated endothelium, but it depends on shedding proteases expression, mainly MMP-14.

The soluble receptor displays biological activity by acting as a specific endogenous antagonist complexing the corresponding ligand and thus preventing the ligand-mediated signal transduction [8]; soluble receptors are capable of scavenging circulating ligands, e. g. VEGF can be bound to sVEGF R1 [9], Ang1 and Ang2 — to sTie2 [8]. The role of s-endoglin is not yet clear, maybe it competes with TGF- β for T β RII binding [12]. But it is known that s-endoglin has antiangiogenic properties; it is capable of reducing spontaneous and VEGF-induced angiogenesis. Also, s-endoglin fused with the Fc fragment of human immunoglobulin G strongly reduces microvessel density in a mouse model of invasive ductal breast carcinoma [10]. Experiment of Le et al. [12] showed two different oligomeric forms of recombinant s-endoglin. The dimeric s-endoglin enhanced TGF- β signalling in U937 cells, in a dose-dependent fashion. However, tetrameric s-endoglin was not active in this system, thus, its biological relevance is not yet clear. This form of s-endoglin might be a resting inactive form that can undergo conformational changes into dimeric or other active forms under certain activating conditions. They concluded that the recombinant s-endoglin is capable of modulating TGF- β signal effectively, thus, can potentially be applied for therapeutic purposes.

The quantification of soluble forms of receptors might be interesting in terms of diagnostic and/or prognostic, but also therapeutic applications. The aim of present study was to analyze the changes of s-endoglin level in plasma of lung cancer patients and to estimate the correlation of s-endoglin with other soluble receptors, sTie2 and sVEGF R1.

MATERIALS AND METHODS

Study population. The study included patients with stage I non-small cell lung cancer, who underwent tumor resection without any preoperative therapy. These patients were treated in the University Hospital Department of Thoracic Surgery and Tumors, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń, during the years 2008–2010. Three blood samples were taken from each patient: one prior to surgery and others on postoperative days 7 and 30.

The study protocol was approved by the Ethical Committee of the Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń (Poland). All patients gave consent.

Blood sampling and processing. s-Endoglin, sTie2, sVEGF R1 concentrations were evaluated in plasma. Two millilitres of blood were taken from elbow vein. EDTA was used as an anticoagulant. Within 30 min after the collection, the blood samples were centrifuged at 2–8°C for 15 min at 1000 x g. The plasma was stored at -70°C.

s-Endoglin determination. s-Endoglin, sTie2, sVEGF R1 concentrations were assayed by commercially available sandwich enzyme-linked immunosorbent assay kits from R&D Systems (Quantikine Human Endoglin/CD105, sTie2, sVEGF R1 Immunoassay, R&D Systems Inc., Minneapolis, USA). Kit is designed to measure human endoglin/CD105, sTie2, sVEGF R1 in cell culture supernates, serum, and plasma.

Statistical analysis was done using Wilcoxon signed-rank test and Pearson's linear correlation. The results were considered statistically significant for $p < 0.05$.

RESULTS

In Table 1 the comparison of s-endoglin concentration before surgical treatment of lung cancer patients and on 7th day and 30th day after tumor resection is presented. The median of s-endoglin concentration decreased on 7th day when compared with preoperative level (3212.0 vs. 4112.0 pg/ml; $p < 0.0001$) and then it increased on 30th day to reach greater values than on 7th day (4447.0 vs. 3212.0 pg/ml; $p < 0.01$), but it was comparable with pretreatment level (4447.0 vs. 4112.0 pg/ml; $p = 0.478$).

Table 1. The plasma concentration of s-endoglin in lung cancer patients before and after surgical treatment (median, range)

	Time of determination			<i>p</i>
	Before surgery	On 7 th day after surgery	On 30 th day after surgery	
s-Endoglin [pg/ml]	4112.0	3212.0	4447.0	* <0.0001
lin [pg/ml]	2740.0–6576.0	2166.0–4077.0	3339.0–5665.0	** <0.001

Notes: * – Before vs. After 7; ** – After 7 vs. After 30

In this study the estimation of correlation between s-endoglin and other soluble receptors was accomplished (Table 2). The correlation between s-endoglin and sTie2, both before surgery ($r=0.44$) and on postoperative day 7 ($r=0.52$) and on 30th day ($r=0.58$) was high. However, s-endoglin was correlated with sVEGF R1 only on postoperative day 7, this correlation was very high ($r=0.75$).

Table 2. The correlation between soluble receptors levels before and after surgery ($p < 0.05$ for all correlations)

	sTie2			sVEGF R1		
	Before surgery	On 7 th day after surgery	On 30 th day after surgery	Before surgery	On 7 th day after surgery	On 30 th day after surgery
s-Endoglin	Before After 7 After 30	$r=0.44$	$r=0.52$	$r=-0.04$	$r=0.75$	$r=0.17$
			$r=0.58$			

DISCUSSION

Endoglin is primarily expressed in proliferating vascular endothelial cells, and its expression increases during tumor angiogenesis. Such properties have made endoglin a reliable marker of various solid tumors vasculature [13–21].

Soluble endoglin in serum is also elevated in various cancers, including breast, colorectal and liver cancers, and it correlates with the presence of metastatic disease [22–25].

Serum s-endoglin has also prognostic value. It is an indicator of prostate cancer metastasis to the pelvic lymph nodes and of biochemical recurrence after medical prostatectomy. In multivariate analysis,

only endoglin and Gleason score, but not PSA or clinical stage, were predictive of lymph node metastases [26–28]. Elevated pretreatment plasma s-endoglin level is predictive for decreased clinical benefit and a shorter overall survival in metastatic breast cancer patients treated with 2nd-line hormone therapy [29].

Besides that, the high level of s-endoglin decreased in patients receiving chemotherapy. Conventional chemotherapy regimens suppress endothelial cells in tumor vasculature and consequently inhibit the release of s-endoglin from endothelial cells [23].

In our study, after surgical resection of lung cancer the level of plasma s-endoglin decreased on 7th day when compared with preoperative level, and next on 30th day — increased and it was comparable with that before surgery intervention. This problem can be explained in the following way. The decrease of s-endoglin probably is a consequence of resection of tumor mass, highly vasculated and expressing both endoglin and shedding enzyme, MMP-14. However, increase of s-endoglin on 30th day in comparison with postoperative day 7 might be the result of stimulation of ECs and circulating progenitor endothelial cells (EPCs) by various tumor-derived angiogenic factors (e.g., VEGF, Ang2), which levels in circulation after surgical treatment are increased [30, unpublished own data].

Myśliwiec et al. [24] received similar results: after surgical treatment of colorectal cancer s-endoglin level on postoperative day 3 decreased when compared with preoperative level, then it increased on day 10 to reach greater values than on postoperative day 3, but lower than preoperative point. They explain these changes as follows: decrease of s-endoglin level after surgery might be at least in part due to the action of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-alpha). TNF-alpha has been reported to down-regulate endoglin at post-transcriptional level [31].

In our study there were interesting correlations between plasma s-endoglin levels with other soluble form of receptors. s-Endoglin correlated with sTie2 both before and after surgery (BEFORE: $r=0.44$, AFTER (day 7): $r=0.52$, AFTER (day 30): $r=0.58$) and with sVEGF R1 — only on postoperative day 7 ($r=0.75$). These two soluble receptors are formed in different manner: sTie2, similarly to s-endoglin is produced by proteolytic processing, however, sVEGF R1 derived predominantly from alternative splicing. This could explain above correlations of s-endoglin: with sTie2 — constant, and with sVEGF R1 — only in one investigated point.

The increased level of serum endoglin in various cancers compared to controls, prognostic value of this angiogenic factor and changes of its level after chemotherapy or surgical treatment suggest potential application of soluble form of endoglin as tumor marker in the future.

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