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Neoadjuvant Therapy Affects Tumor Growth Markers in Early Stage Non-Small-Cell Lung Cancer

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Abstract

Introduction: While adjuvant therapy of early-stage non-small-cell lung cancer (NSCLC) is widely accepted, literature data concerning neoadjuvant treatment provide contradictory results with both improved and unaffected survival rates. Also, data concerning potential effects of neo-adjuvant therapy on cellular level are scarce.

Objective: The aim of present study was to analyze the effect of chemotherapy followed by surgical resection on several key biological markers of tumor growth (TGF-β, VEGF), apoptosis (sAPO-1/Fas/CD95) and invasiveness (TIMP-1) assessed in the sera of NSCLC early-stage patients (IB-IIIA).

Material and methods: Measurements were performed by ELISA method in blood serum from 24 NSCLC patients (I-IIIA) collected prior therapy, one day before surgery and 3 days after.

Results: TGF-β serum concentrations were significantly lower after both chemotherapy ($P<0.05$) and surgery ($P<0.01$) in comparison to the baseline. VEGF levels decreased following NEO therapy with subsequent significant up-regulation after surgery ($P<0.001$). Interestingly, post-surgery serum VEGF strongly correlated with TGF-β concentration ($r = 0.52$, $P = 0.014$). No significant differences were observed for serum sAPO-1/CD95/FAS as well as TIMP-1 concentrations at any of three evaluated time-points.

Conclusion: Neoadjuvant treatment of early-stage NSCLC affects mostly mechanisms responsible for tumor growth and vascularization. Its effect on cancer cells apoptotic activity needs further evaluation.

Key words: lung cancer, neoadjuvant therapy, TGF-β, VEGF, sAPO-1, TIMP-1

Introduction

Lung cancer is recognized as the leading cause of cancer-related deaths with more than 1 200 000 cases per year worldwide. High mortality rates with less than 10% of patients surviving their malignancy are attributed both to delayed diagnosis (70% in stage IIIB and IV) and consequently less than satisfactory treatment effectiveness. Thus, new strategies in lung cancer therapy have been introduced in recent years, promising better outcomes in certain patient groups. While adjuvant, post-surgery, therapy of early- stage non-small-cell lung cancer (NSCLC) is now widely accepted, literature data concerning neoadjuvant, pre-surgery, treatment (NEO) provide contradictory results with both improved or unaffected survival rates [1]. A multicenter study of the French Thoracic Cooperative Group with 355 NSCLC patients demonstrated longer 2-year overall survival rate in patients on neo-adjuvant treatment (59% vs. 52%) with particular benefit in early-stage disease (N0-N1) [2]. On the other hand, American Intergroup (S9900) with 354 patients showed no significant benefit of that strategy [3]. Importantly enough, data concerning potential effects of neo-adjuvant therapy on the cellular or molecular levels are scarce. Therefore, the aim of present study was to analyze the effect of chemotherapy followed by surgical resection on several key biological markers of tumor growth (TGF-β, VEGF), apoptosis (sAPO-1/Fas/CD95) and invasiveness (TIMP-1) assessed in the sera of NSCLC early-stage patients (IB-IIIA).

Material and Methods

Study Design

The study was approved by a local Ethics Committee and informed consent was obtained from all participating subjects.

The study group consisted of 24 patients (22-male; 2-female), mean age 62 ±21 years, with diagnosed early stage NSCLC (IA-1, IB-1, IIA-1, IIB-9, IIIA-12). Neoadjuvant treatment (cisplatin and vinarelbin) was administered prior to radical surgery in all patients. Blood samples were collected prior therapy, one day before surgery and 3 days after. Serum was separated by centrifugation, aliquoted and frozen at -70°C. Tumor and normal tissue samples obtained during surgery were immediately processed: sonicated on ice and centrifuged. Supernatants were aliquoted and frozen at -70°C.

Cytokine Assessment

Cytokines measurements in serum and tissues were performed by quantitative enzyme immunoassay tech-

nique (ELISA) using commercial kits (R&D Systems, USA) according to manufacturer recommendations. Optical density was measured at 450 nm using spectrophotometric reader Elx800 (Biotek Instruments, USA). Cytokines serum concentration was expressed as pg/ml or ng/ml. Tissue concentrations were recalculated and expressed as pg per mg of tissue.

Statistical Evaluation

Data were presented as means ±SD. The Kruskal-Wallis analysis of variance by ranks was used to test statistical significance of differences between groups while the Spearman test was used to analyze correlations.

Results

Serum

Selected biological markers in serum were evaluated at three distinct time points: before any treatment, after NEO therapy one day prior to surgery and at the 3rd day following radical treatment. TGF-β serum concentrations were significantly lower after both chemotherapy and surgery in comparison to the baseline before any treatment as shown in Table 1. No significant correlation was observed for TGF-β serum levels before and after NEO (r=0.18, P=0.112) as well as before and after surgery (r=0.21, P=0.39). VEGF levels demonstrated non-significant decrease following NEO therapy, with considerable increase after surgery. Correlation analysis revealed tendency towards significance for VEGF in serum before and after NEO (r= 0.600; P=0.08). Interestingly, strong correlation between post-surgery VEGF and TGF-β serum concentrations was find out (r=0.52, P=0.014). No significant differences were observed for serum sAPO-1/ CD95/FAS and TIMP-1 concentrations at any of three evaluated time-points. However, relationship between TIMP-1 levels before and after NEO (r=0.71; P =0.015), TIMP-1 before and after surgery (r=0.67, P =0.002) and sAPO-1/CD95/FAS (r=0.42, P=0.054) were observed. Interestingly, strong correlation between post-NEO sAPO-1/CD95/FAS levels and circulating total free DNA measured by direct fluorescent PicoGreen staining was revealed (r=-0.87; P= 0.002; detailed data not shown).

Tissue

TGF-β and VEGF tissue levels were evaluated in samples obtained during surgery, i.e., following the NEO treatment. As expected, differences were demonstrated between tumor and normal lung tissue for both cytokines, respectively for TGF-β 5.22 ± 4.74 pg/mg vs. 2.69 ± 1.07 pg/mg (P = 0.023), for VEGF: 6.43 ± 6.60 pg/mg vs. 3.88 ± 4.34 pg/mg (P = 0.059). However, there was no relationship between both TGF-β and VEGF serum and tumor concentrations, respectively r = 0.17 and r = 0.07. Moreover, TGF-β. levels in tumor tissue correlated strongly with circulating total free DNA (r = -0.93; P = 0.003; detailed data not shown).

Discussion

It has been postulated that discrepancies in preoperative therapy clinical effectiveness assessment might be due to the patients characteristics (early versus advanced disease), as well as the drug profile [1, 3]. Besides, it is well known that not only progression stage but also individual cancer cells phenotype affects treatment outcomes. Therefore, better understanding of the basic mechanisms responsible for therapy success or failure might allow more targeted and effective approach. Markers evaluated in present study represent key tumor biological activities responsible for its growth and invasiveness.

Enhanced TGF-β. expression and serum concentration are often observed in NSCLC. Its importance is considered fundamental for tumor cells survival and loss of response to physiological growth control. Moreover, its connection to drug resistance has been demonstrated [4]. Data concerning chemotherapy effects, including cisplatine or vinarelbine treatment, are contradictory, with none or direct suppressive effect [5, 6]. Present study have shown significant inhibition of TGF-β production following induction therapy with further, though non-significant, drop after surgery. Similar changes following NEO has been observed for VEGF, well known proangiogenic factor, key regulator of tumor-induced angiogenesis. Accordingly, predictive role of serum VEGF drop in reaction to therapy has been proven by Ludovini et al [7]. Down-regulation of both TGF-β and VEGF production after NEO therapy seems to reflect mechanism of

Table 1. Effects of neoadjuvant therapy and subsequent surgery on the serum concentration of evaluated tumor markers.

	Before neoadjuvant therapy	**After neoadjuvant therapy**	**After surgery**
TGF-β (ng/ml)	20888 ± 6059	15769 ±6428 P=0.044	14503 ±5219 P=0.007
VEGF (pg/ml)	414 ±191	326 ±236	636 ±409 P=0.001
sAPO-1 (pg/ml)	4.2 ±1.4	3.7 ±1.5	4.1 ±2.4
TIMP (pg/ml)	543 ±87	509 ±131	563 ±124

Data are means ±SD. Kruskal-Wallis analysis of variance was applied to test for statistical significance.

NEO beneficiary effect allowing better tumor growth control. The rapid increase in serum VEGF observed at the last time-point – 3 days after resection was most definitely due to the surgery trauma and wound-healing mechanisms. Therefore, it did not reflect the tumor-related VEGF-pool.

Surprisingly enough, no effect of NEO therapy on the sAPO-1/CD95/FAS were shown. Chemotherapeutic drugs, cisplatine but also anti-mitotic drug as vinarelbine, cause DNA damage and kill cancer cells mainly by apoptosis induction. Therefore, one could expect increased levels of sAPO-1/CD95/FAS – very much involved in regulation of apoptosis, even mentioned as a key controller of the drug-induced apoptosis. Unexpectedly, above mechanism has been confirmed by a strong correlation between serum sAPO-1 and free circulating DNA levels, as mentioned above. Lack of direct effect of NEO on the serum sAPO-1 levels was in line with results of Koomagi et al [8] who proved that APO-1 presented significant prognostic but not predictive role in NSCLC treatment.

No effect of induction therapy on serum TIMP-1 has been demonstrated as well. Meanwhile, Li et al [9] confirmed that cisplatine acted by suppressing tumor metastasis via down-regulating the expression of TIPM-1 in serum. However, our study group consisted of patients with early stage metastasis-free disease. It might be possible that above mechanism operates exclusively when tissue/serum TIMP-1 levels increase due to the disease progression. Also, strong correlations reported for the TIPM-1 serum levels at all time-points suggest that treatment did not exert any effect on this protein production.

Cytokines levels in normal and tumor tissue differed significantly as expected. Observed correlation between tumor TGF-β and serum free DNA concentrations goes along with data suggesting that TGF-β.-dependent apoptosis is at least partially responsible for the chemotherapy effects [10].

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