RESEARCH ARTICLE

Serum Kynurenic Acid: Possible Association with Invasiveness of Non-small Cell Lung Cancer

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Abstract

The lung adenocarcinoma is considered more aggressive than other types of non-small cell lung cancer. As metabolites of tryptophan degradation along the kynurenine pathway, including kynurenic acid, have been shown to induce immunosuppression and facilitate escape of tumor cells from immune surveillance, a hypothesis was set up that differences in biological behavior between types of lung cancer may be associated with altered activity of the kynurenine metabolic pathway. The aim of the study was to determine kynurenic acid levels in the serum of patients with bronchial adenocarcinoma for comparison with other types of non-small cell lung cancer. A total of 227 patients with non-small cell lung cancer were enrolled in the study, including 71 with adenocarcinoma and 96 with squamous cell carcinoma. Serum kynurenic acid concentration was determined with use of high performance liquid chromatography and fluorometry. The level of kynurenic acid in the serum of patients with adenocarcinoma was significantly higher than in those with squamous cell lung cancer (107.1 ± 62.8 pmol/ml; 95% CI: 92.4 to 132.3 pmol/ml versus 82.1 ± 47.6 pmol/ml; 95% CI: 78.5 to 91.2 pmol/ml, respectively; p = 0.027). Differences between other histological types of lung cancer were insignificant. We conclude that increased activity of kynurenine metabolic pathway manifested by elevated serum kynurenic acid level may be one of the factors associated with clinically distinct biological behavior of adenocarcinoma, in particular high invasiveness and rapid progression.

Keywords: Lung cancer - kynurenine - antiproliferative - kynurenic acid - invasiveness

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Introduction

Lung adenocarcinoma is widely recognized as biologically more aggressive than other types of non-small cell lung cancer (NSCLC). Clinical observation indicates that adenocarcinoma is characterized by high loco-regional invasiveness and rapid progression. Adenocarcinoma histology has been identified as an independent risk factor for recurrence in stage IIN0 and stage IIN1 of NSCLC (Maeda et al., 2011). Moreover, recent studies proved that adenocarcinoma histology is associated with a higher risk of distant recurrence, when compared to squamous cell carcinoma (HR=1.74; CI: 1.24-2.44) (Tomaszek et al, 2011). Differences in overall survival after lobectomy for stage I and II disease in patients with adenocarcinoma vs. other histologic types of lung cancer have also been reported (Cook et al., 2010). The causes of this biological aggressiveness remain unknown.

Overall invasiveness of malignant tumors is dependent on the properties of the tumor at the molecular level (Watanabe et al., 2011), as well as on the proper response of the immune system (Dai et al., 2010). Preliminary scientific evidence suggests that a fundamental role in control over specific immune responses against solid tumors, including lung cancer, may be attributed to metabolites of tryptophan degradation via kynurenine pathway (Munn and Mellor, 2007; Suzuki et al., 2010). It is the main pathway for tryptophan metabolism catalyzed by indoleamine 2,3-dioxygenase (IDO) and kynurenine aminotransferases (KATs), resulting in synthesis of kynurenic acid (KYNA) (Figure 1) (Fulop et al., 2007).

KYNA has previously been proven to play an important role in neurotransmission in the central nervous system as an antagonist of N-methyl-D-aspartate (NMDA) and α7 nicotinic acetylcholine receptors (Foster, 1986). Recent results of molecular studies showed that kynurenic acid is a ligand for G protein-coupled receptor 35 (GPR35) and for aryl hydrocarbon receptor (AHR), which are abundantly expressed both in malignant cells and in immune tissues (Wang et al., 2006; DiNatale et al., 2010). Moreover, very recent experimental research disclosed

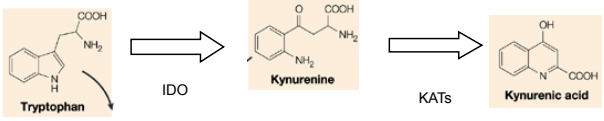

Figure 1. Kynurenine Metabolic Pathway-Synthesis of Kynurenic Acid; IDO = indoleamine-2,3-dioxygenase; KATs = kynurenine aminotransferases

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antiproliferative effect of kynurenic acid at micro- and milimolar concentrations against cancer cells in vitro (Walczak et al., 2011).

Based on the above considerations we set up a hypothesis that differences in biological behavior between various histologic types of lung cancer may be associated with altered activity of kynurenine metabolic pathway. The present study aimed to determine kynurenic acid levels in the serum of patients with bronchial adenocarcinoma and compared it to patients with other types of NSCLC, to shed more light on the background of biological characteristics of this devastating disease.

Materials and Methods

A total of 227 patients with non-small cell lung cancer undergoing surgical treatment or surgical invasive diagnostic procedures in the Department of Thoracic Surgery, Medical University of Lublin between July 2008 and December 2010 were enrolled in the study. Eligibility criteria included histopathologically confirmed NSCLC, whereas patients with small cell lung carcinoma, concomitant malignancies other than NSCLC, autoimmune diseases, viral hepatitis or renal failure were excluded. The study has been approved by the Ethics Committee at our institution, and informed consent has been obtained from all participants prior to the enrollment into the study. Detailed clinical and demographic characteristics of the study group are presented in Table 1.

Venous blood for measurements was collected from peripheral vein in the operating room immediately before the operation, after at least 12 hours of fasting. Blood samples were centrifuged, and the separated serum samples were immediately deep frozen and stored at -80°C until further analyses. After thawing, samples were acidified with trichloroacetic acid, and precipitated proteins were removed by centrifugation. The supernatants were analyzed for KYNA content by application to cation exchange Dowex 50W columns. Eluted KYNA was subjected to high performance liquid chromatography (HPLC) using Hewlett Packard 1050 HPLC system with C18 reverse phase column, and quantified fluorometrically (Hewlett Packard 1046A fluorescence detector).

Pathological staging (pTNM) according to the seventh edition of the lung cancer stage classification system was used for determination of staging (Detterback et al., 2009). Histopathological diagnosis of NSCLC was performed on the resection specimens according to World Health Organization (WHO) criteria (Travis et al., 2004). Clinical and laboratory data were prospectively collected and stored in a computer database. Statistical analysis was performed using computer software. Results are presented as mean values ± standard deviation (SD), median, minimum and maximum values, unless stated otherwise. As Wilk-Shapiro test showed that distribution of values in compared groups were different from normal distribution, non-parametric tests were applied. U Mann-Whitney test was used for comparisons between two groups. Kruskall-Wallis ANOVA rank test with Dunn's post hoc test were used for comparisons between multiple groups. Pearson's χ^2 test was used for assessment of differences in staging distribution, age, gender, or lesion location pattern between groups. Probability p value less than 0.05 was considered statistically significant.

Table 1. Detailed Clinical and Demographic Characteristics of the Study Group

Sex	Male		Female
	164 (72%)		63 (28%)
Age (years)	Mean ± SD	Min	Max
	64.1 ± 8.1	44	76
Type of NSCLC	Number of patients	% of the study group	
Adenocarcinoma	71		31.3
Squamous cell	96		42.3
Large cell	24		10.6
Mixed + undifferentiated	16		7
Total	227		100

Results

KYNA level in the serum of patients with adenocarcinoma was significantly higher than in squamous cell cancer (110.41 ± 61.4 pmol/ml versus 84.60 ± 44.42 pmol/ml; H = 10.4; p = 0.039). Differences between adenocarcinoma and large cell, adenocarcinoma and mixed, squamous and large cell, squamous and mixed, or large cell and mixed types were insignificant (p > 0.05). Mean and median serum KYNA levels in adenocarcinoma were the highest of all histologic types of NSCLC. Detailed results of serum KYNA level measurements in patients with various types of NSCLC are presented in Table 2.

Comparison of serum KYNA levels between types

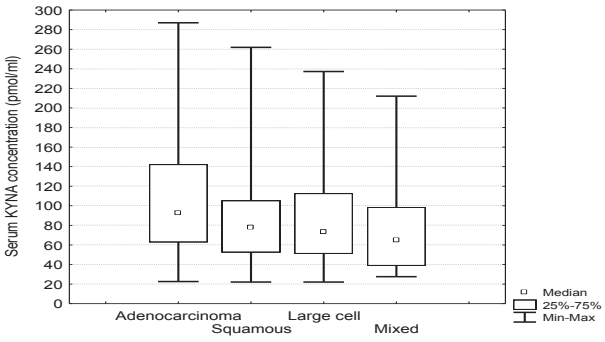

Figure 2. Comparison of Serum KYNA Levels Between Histologic Types of NSCLC; Kruskall-Wallis ANOVA rank test: H = 11.2; p = 0.027; Post-hoc Dunn's test: adenocarcinoma > squamous cell carcinoma (p <0.05)

Table 2. The Level of KYNA in the Serum of Patients with Various Types of NSCLC

	Adenocarcinoma	Squamous cell	Large cell	Mixed + undiffer.
Number of patients	71	96	24	16
Mean (pmol/ml) ± SD	107.1 ± 62.8	82.1 ± 47.6	86.2 ± 51.3	69.5 ± 46.2
95% CI (pmol/ml)	92.4 – 132.3	78.5 – 91.2	64. 6 –101.6	54.4 – 87.1

of NSCLC is presented graphically in Figure 2. To avoid possible bias from uneven distribution of staging in compared groups the stage of the disease in patients with adenocarcinoma was compared to those with squamous cell carcinoma. Pearson's χ^2 test showed that the differences between the groups were due to a chance and were insignificant ($\chi^2 = 3.94$; df = 6; $p = 0.57$). No significant differences regarding age, gender and location of the lesion (left vs. right lung) were detected either ($p = 0.34$; $p = 0.18$ and $p = 0.46$, respectively).

Discussion

The present study revealed that KYNA level in the serum of patients with adenocarcinoma was significantly higher than in patients with squamous cell lung cancer. It has been previously shown that bronchial adenocarcinoma is characterized by aggressive development and poor prognosis compared to other histologic types of NSCLC (Cooke et al., 2010; Maeda et al., 2011; Tomaszek et al., 2011). This behavior may be attributed to the properties of the tumor itself, as well as to impaired anti-tumor immune response (Dai et al., 2010; Watanabe et al., 2011). Tryptofan degradation along the kynurenine pathway has been shown to be a major factor contributing to escape of tumor cells from immune surveillance (Munn et al., 1999). Metabolites of tryptophan catabolism via kynurenine pathway suppress antitumor responses and possibly promote tumor immune escape by inducing an immunoregulatory or an anergic T cell phenotype at a systemic level (Mellor et al., 2002; Schroecksnadel et al., 2005). These findings suggest that increased activation of kynurenine metabolic pathway reflected in elevated levels of KYNA may be one of possible factors contributing to clinically recognized biological behavior of adenocarcinoma, including high invasiveness and rapid progression.

To date, in available literature, there have been no reports on the concentration of KYNA in the serum of patients with various histologic types of lung cancer. Only single studies investigated KYNA in other malignancies. Increased plasma and bone marrow KYNA concentrations have been detected in monoclonal gammopathy of undetermined significance and multiple myeloma patients (Zdzisinska et al., 2010). The authors found that multiple myeloma patients with more aggressive IgG hypergammaglobulinemia had higher blood KYNA lelves than patients with IgA class of monoclonal protein.

A few studies focused on the level of kynurenine metabolic pathway activation in malignant diseases. Increased tryptophan catabolism was observed in adult T-cell leukemia, gynecological tumors and colorectal cancers (Huang et al., 2002; Schroecksnadel et al., 2005; Hoshi et al., 2009). Karanikas and colleagues assessed IDO gene expression in tumor tissues of 28 patients with NSCLC using quantitative real-time polymerase chain reaction (Karanikas et al., 2009). They found that patients with adenocarcinoma showed higher levels of IDO mRNA expression than patients with squamous cell carcinoma. Activity of IDO is considered a rate–limiting step of the kynurenine pathway, and increased mRNA expression in adenocarcinoma may ultimately result in higher tissue and serum KYNA levels, which reliably reflect overall activity of the kynurenine pathway. These findings, together with our results, support the hypothesis that biological behavior of adenocarcinoma, in particular high invasiveness and rapid progression, may be associated with excessive activity of kynurenine pathway.

In contrast, Suzuki and colleagues found no significant histologic-type-related differences in 123 patients with lung cancer. The authors measured serum kynurenine and tryptophan concentrations and estimated IDO activity by calculating the kynurenine to tryptophan ratio (Suzuki et al., 2010). However, the assessment was indirect and based on kynurenine and tryptophan concentrations which are relatively unstable substances. We believe that measurement of KYNA as a biomarker of kynurenine pathway activity is much more reliable due to its chemical and thermal stability, as well as because of its direct interactions with GPR35 and AHR abundantly expressed in malignant tumor cells and in the immune system cells.

Nevertheless, the question whether elevated KYNA levels in adenocarcinoma are the cause or the result of aggressive properties of this tumor remains to be answered. The above considerations approached toward the former. However, the latter is also hypothetically possible, because the accumulation of kynurenine metabolites in blood and tissues may result from excessive immune stimulation by a more aggressive disease, as was demonstrated in infective, autoimmune and malignant diseases (Witkiewicz et al., 2008; Lob et al., 2009). Another theory speculated that KYNA is an effector constituent of an immune feedback control loop. Overstimulation of the immune system leads to increased KYNA production, which, in turn, downregulates the immune system in a mechanism similar to a feedback loop providing control over the entire system (Zdzisinska et al., 2010).

In conclusion, our study, for the first time, revealed significantly higher serum levels of KYNA in patients with adenocarcinoma compared to squamous cell lung cancer. The difference appears to be due to excessive stimulation of kynurenine metabolic pathway for tryptophan degradation in patients with adenocarcinoma. Our findings suggest that increased activity of this metabolic pathway may be one of factors associated with clinically distinct biological behavior of adenocarcinoma, in particular high invasiveness and rapid progression, as metabolites of tryptophan degradation along kynurenine pathway have been shown to induce immunosuppression and facilitate escape of tumor cells from immune surveillance. Nevertheless, given previously demonstrated antiproliferative properties of KYNA against cancer cells in vitro, the role of KYNA as a defensive response of the immune system against adenocarcinoma should also be considered. Further research in this subject is required. It might open new perspectives for clinical application of KYNA or its synthetic analogues as anti-tumor agents.

Acknowledgements

The author(s) declare that they have no competing interests.

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