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THE CLINICAL SIGNIFICANCE OF SOLUBLE E-CADHERIN IN NONSMALL CELL LUNG CANCER

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Aim: **Aberrant expression of the epithelial transmembrane adhesion molecule E-cadherin (E-cad) has been associated with many human malignancies. In the present study the clinical significance of serum levels of soluble E-cadherin (sE-cad) in newly diagnosed patients with non small cell lung cancer (NSCLC) was investigated.** ***Material and Methods:*** **An enzyme linked immunospecific assay (ELISA) to determine the circulating levels of sE-cad in 20 newly diagnosed patients with NSCLC as well as in 29 healthy volunteers (control group) was used.** ***Results:*** **NSCLC patients exerted increased circulating levels of sE-cad compared with individuals of the control group ($p < 0.001$). An association was also detected between serum sE-cad levels and the development of distant metastases. On the contrary, no statistically significant correlation could be established with histological type, gender and smoking habits. Patients with increased sE-cad levels at diagnosis had worser outcome, although multivariate analysis failed to demonstrate that sE-cad levels represent an independent prognostic factor of survival.** ***Conclusion:*** **Our data suggest that E-cad plays a role in the pathogenesis of NSCLC. sE-cad levels may be further studied as a potential prognostic biomarker.**

Key Words: **E-cadherin, transmembrane adhesion molecule, malignancy, non small cell lung cancer, prognostic biomarker.**

Lung cancer is the leading cause of cancer death in both women and men in the USA, Canada and China. In Europe and Australia it is the number one cause of death from cancer in men and the third cause — in women [1]. Cigarette smoke is by far the most significant factor in the causation of lung cancer. Approximately 85% of lung cancer cases occur in smokers or former smokers [2].

Epithelial tumors, which present the majority of lung tumors, are classified primarily into two subgroups: small cell lung cancer (SCLC) and nonsmall cell lung cancer (NSCLC). NSCLC is composed of squamous cell, adeno-, large cell and adeno-squamous carcinoma. A subtype of adenocarcinoma is the bronchioalveolar cell carcinoma. As the 5-year survival for lung cancer is currently 13% mainly due to metastasis the devastation caused by this single cancer type deserves special attention.

Cell adhesion is a key process because it is directly related to the differentiation, architecture and normal tissue development. It has been postulated that changes in cell-cell and cell-matrix interactions are responsible for the cancer cells transgression to normal tissue boundaries and their dispersion to distant sites [3–5]. The interactions among cells or among cells and substrate are mediated by molecules named adhesion molecules [6–8].

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Abbreviations used: CAM – cell-cell adhesion molecule; CEA –carcinoembryonic antigen; CIN – cervical intraepithelial neoplasia; E-cad – epithelial cadherin; N-cad – neural cadherin; NSCLC – non small cell lung cancer; P-cad – placental cadherin; SAM – substrate adhesion molecule; SCLC – small cell lung cancer; sE-cad – soluble epithelial cadherin; VE-cad – vascular endothelial cadherin.

Cadherins represent the most important superfamily of adhesion molecules. In their presence, even the absence of the rest of the adhesion molecules does not influence the whole adhesion process.

The epithelial transmembrane molecule E-cadherin (E-cad) is the prime mediator of epithelial cell-cell adhesion acting in a homotypic fashion. It participates in the development and architecture maintenance of epithelial tissues as well as in the signalling process. Cadherin superfamily consists of more than 40 members, which share common characteristics. Among cadherins E-cadherin is the most important molecule. E-cadherin (epithelial cadherin) is expressed in epithelial tissues being involved in formation and maintenance of the histoarchitecture. Other cadherins are N–cadherin (neural cadherin) which is found at the neural and muscular tissues, P-cadherin (placental cadherin) found in embryonic tissues and placenta, R-cadherin (retinal cadherin), VE-cadherin (vascular endothelial cadherin) etc [9, 10].

Loss of the function or/and the expression of any of the elements of the E-cadherin/catenins complex impair cell adhesiveness resulting to a loss of the normal tissue architecture [11]. Reduced/absent expression of E-cadherin has been found in a variety of human carcinomas including gastric, head and neck, bladder, prostate, colorectal and breast cancer [12–16]. Relatively recent immunohistochemical studies have clearly shown that downregulation of E-cadherin is also associated with lung cancer while prospective studies are also in process to investigate the role of adhesion molecules in various lung diseases [17]. Moreover, abnormal expression of E-cad has been detected in premalignant lesions such as Barrett's oesophagus [18], chronic active type B gastritis [19] and cervical intraepithelial neoplasia (CIN) [20].

In the present study an attempt to establish the role of the E-cad, in particular the one of its soluble fragment, in cases of NSCLC is made. SE-cad is a soluble 80 kDa fragment of E-cad that derives from shedding of this molecule.

Twenty patients (17 males, 3 females, mean age — 61 years) with recently diagnosed NSCLC were enrolled in the present study (the histological type — adenocarcinoma (n = 9), squamous cell carcinoma (n = 8), large cell carcinoma (n = 3)). 19 patients were smokers while 1 was a non-smoker (Table). A group of 29 healthy volunteers was used as control group.

Table. Clinicopathological data of patients with NSCLC and the sE-cadherin level in the blood serum

Histological type	Number of patients
Adenocarcinoma (AC)	9 (46%)
Squamous cell carcinoma (SCC)	8 (40%)
Large cell carcinoma (LCC)	3 (15%)

Number	Age	Gender	Histological type	Smoking	sE-cadherin (ng/ml)	Distant metastases
1	52	M	AC	+	4382	+
2	56	M	AC	+	3894	+
3	79	M	AC	+	4565	+
4	57	M	AC	+	2186	
5	62	M	AC	+	4877	+
6	54	M	AC	+	2386	
7	49	M	AC	+	2650	
8	66	F	AC	+	4667	+
9	51	F	AC	-	2376	
10	82	M	SCC	+	2586	
11	82	M	SCC	+	4580	+
12	75	M	SCC	+	2872	
13	74	F	SCC	+	2858	
14	48	M	SCC	+	4750	+
15	60	M	SCC	+	1942	
16	56	M	SCC	+	4662	+
17	48	M	SCC	+	2287	
18	57	M	LCC	+	3068	+
19	64	M	LCC	+	4777	+
20	72	M	LCC	+	2735	

Abbreviations: M – male, F – female;
Mean value of sE-cad: 3455 ng/ml;
Range: 1942–4877 ng/ml.

At the time of diagnosis blood samples were taken, centrifuged, routinely processed and kept at –70 °C until measurement. Measurement of sE-cad in serum of patients and healthy individuals was achieved with an ELISA method using commercial kit with specific monoclonal antibodies against E-cad (R&D systems, UK). Results were statistically analyzed using Student's *t*-test. Values of $p < 0.001$ were considered as statistically significant.

The major clinicopathological characteristics of the 20 patients are presented in the Table. Serum levels of E-cad were increased in cases of NCSLC compared to these in control group ($p < 0.001$). In particular, in the control group a mean value of sE-cad was 1015 ng/ml, whilst in the group of patients — 3455 ng/ml.

On the other hand, no statistically significant differ- ence could be established between the levels of sE cad and the histological type, sex and smoking habit (control group, only one non-smoker participated in the patients' group). On the contrary, increased serum levels of sE-cad correlated with the development of distant metastasis ($p < 0.001$); patients with generalized disease presented marked increase of sE-cad levels. Furthermore, the levels of sE-cad do not correlate with the histological type of tumor. Thus, E-cad can not be used as a marker for differential diagnosis of NSCLC subtypes. Finally, a statistically significant difference has not been found between men and women and/or smokers and non-smokers. Of course, the number of the patients studied herein is limited and we have to take under consideration the possibility of a not fully objective view due to the small number of patients. Patients with increased levels of sE-cad at the time of the diagnosis had worsier prognosis, although multivariate analysis failed to prove that E-cad levels are an independent prognostic indicator of survival.

According to a number of studies, the immunochemical analysis of E-cad complex in lung cancer patients showed also a downregulation of the E-cad expression. Particularly, in NSCLC cases low levels of E-cadherin expression in abnormal patterns — apart from its membranous expression seen normally — have been found in all histological types and especially in poorly and moderately differentiated carcinomas [21, 22]. In addition, E-cad expression has been shown to be reversely correlated with that of epidermal growth factor receptor that is overexpressed in the majority of lung cancers and correlates with poor prognosis [23]. At the present study, it has been demonstrated that the levels of circulating sE-cad are significantly increased in cases of NSCLC of all histological types when compared to healthy individuals. In particular, the degree of sE-cad increase correlated with the existence of distant metastases. Similar results with increased sE-cad levels have been reported in prostate cancer cases [24].

The question is what is the specific role that soluble E-cad plays in the whole process, what is its importance and how this can prove useful in cancer procedure. Probably, increased levels in serum reflect serious dysfunction of cellular E-cad since they are much higher that in healthy individuals. Changes in the expression levels of E-cad affect the adhesive ability of the tissues and in this way low expression results in weakening of the role of E-cad as an inhibitor of cancer invasion along with the capability of migration of cancer cells to distant organs.

In conclusion, further studies including a larger number of patients are needed in order to fully elucidate the role of sE-cad and to establish E-cadherin as a useful biomarker in diagnosis and prognosis of NSCLC. A very interesting view of this issue is being given by current studies in which the possibility of using sE-cad as a marker of response to chemotherapy is investigated.

REFERENCES

1. **Landis SH, Murray T, Bolden S, Wingo PA.** Cancer statistics 1998. CA Cancer J Clin 1998; **48**: 6–29.

2. American Thoracic Society Statement: cigarette smoking and health. Am J Respir Crit Care Med 1996; **153**: 861–5.

3. **Charalabopoulos K, Binolis J, Karkabounas S.** Adhesion molecules in carcinogenesis. Exp Oncol 2002; **24**: 249–57.

4. **Charalabopoulos K, Pignatelli M.** Adhesion molecules and cancer. Arch Hell Med 2001; **18**: 16–9.

5. **Charalabopoulos K, Papalimneou V, Charalabopoulos A.** Adhesion molecules in lung cancer. Exp Oncol 2003; **25**: 16–21.

6. **Gogali A, Charalabopoulos K, Constantopoulos S.** Integrins receptors in primary lung cancer Exp Oncol 2004; **26**: 106–10.

7. **Eideman GM, Crossin KL.** Cell adhesion molecules: implication for a molecular histology. Ann Rev Biochem 1991; **60**: 155–90.

8. **Takeichi M.** Cadherin cell adhesion receptors as a morphogenetic regulator. Science 1991; **251**: 1451–5.

9. **Takeichi M.** Cadherins in cancer: implications for invasion and metastasis. Curr Opin Cell Biol 1993; **5**: 806–911.

10. **Kemler R.** From cadherins to catenins; cytoplasmic protein interactions and regulation of cell adhesion. Trends Genet 1993; **9**: 317–21.

11. **Mayer B, Johnson JP, Leitl F, Jauch KW, Heiss MM, Schildberg FW, Birchmeier W, Funke I.** E-cadherin expression in primary and metastatic gastric cancer: down-regulation correlates with cellular dedifferentiation and glandular disintegration. Cancer Res 1993; **53**: 1690–5.

12. **Schipper JH, Frixen UH, Behrens J, Unger A, Jahnke K, Birchmeier W.** E-cadherin expression in squamous cell carcinomas of head and neck: inverse correlation with tumor dedifferentiation and lymph node metastasis. Cancer Res 1991; **51**: 6328–37.

13. **Charalabopoulos K, Tsambalas S, Syrigos K, Giannakopoulos X, Kalfakakou V, Kiortsis D, Alamanos J, Charalabopoulos A, Evangelou A.** Correlation of E-cadherin expression with clinicopathological data in patients suffering from transitional cell carcinoma of the bladder. Exp Oncol 2003; **25**: 180–5.

14. **Umbas R, Schalken JA, Aalders TW, Carter BS, Karthaus HF, Schaafsma HE, Debruyne FM, Isaacs WB.** Expression of the cellular adhesion molecule E-cadherin is reduced or absent in high-grade prostate cancer. Cancer Res 1992; **52**: 5104–9.

15. **Dorudi S, Sheffield JP, Poulsom R, Northover JM, Hart IR.** E-cadherin expression in colorectal cancer. An immunocytochemical and *in situ* hybridization study. Am J Pathol 1993; **142**: 981–6.

16. **Oka H, Shiozaki H, Kobayashi K, Inoue M, Tahara H, Kobayashi T, Takatsuka Y, Matsuyoshi N, Hirano S, Takeichi M.** Expression of E-cadherin cell adhesion molecules in human breast cancer tissues and its relationship to metastasis. Cancer Res 1993; **53**: 1696–701.

17. **Charalabopoulos K, Karachalios G.** Adhesion molecules and lung disease. Pneumon Med J 2000; **13**: 50–6.

18. **Syrigos KN, Karagiannakis AJ, Charalabopoulos K, Katirtzoglou N, Pignatelli M.** Abnormal expression of E-cadherin in dysplastic Barrett's oesophagus. Ann Gastroenterol 2000; **13**: 168.

19. **Charalabopoulos K, Papalimneou V, Euangelou A, Kalfakakou V, Kiortsis D, Kolettas E, Alamanos J, Charalabopoulos A, Agnantis NJ.** Is the E-cadherin downregulation observed in *Helicobacter pylori* infection associated with gastric cancer development? An additional element in the disease jigsaw. Exp Oncol 2003; **25**: 270–3.

20. **Vessey CJ, Wilding J, Folarin N, Hirano S, Takeichi M, Soutter P, Stamp GW, Pignatelli M.** Altered expression and function of E-cadherin in cervical intraepithelial neoplasia and invasive squamous cell carcinoma. J Pathol 1995; **176**: 151–9.

21. **Bohm M, Totzeck B, Birchmeier W, Wieland I.** Differences of E-cadherin expression levels and patterns in primary and metastatic human lung cancer. Clin Exp Metastasis 1994; **12**: 55–62.

22. **Toyoyama H, Nuruki K, Ogawa H, Yanagi M, Matsumoto H, Nishijima H, Shimotakahara T, Aikou T, Ozawa M.** The reduced expression of E-cadherin, α-catenin and γ-catenin but not β-catenin in human lung cancer. Oncol Rep 1999; **6**: 81–5.

23. **Al Moustafa AE, Yansouni C, Alaoui-Jamali M, O'Connor-McCourt M.** Upregulation of E-cadherin by an anti-epidermal growth factor receptor monoclonal antibody in lung cancer cell lines. Clin Cancer Res 1999; **5**: 681–6.

24. **Ahmed MI, Abd-Elmotelib F, Farag RM, Ziada NA, Khalifa A.** Evaluation of some tissue and serum biomarkers in prostatic carcinoma among Egyptian males. Clin Biochem 1999; **32**: 439–45.

КЛИНИЧЕСКОЕ ЗНАЧЕНИЕ ОПРЕДЕЛЕНИЯ СЕКРЕТИРУЕМОГО Е-КАДЕРИНА В СЫВОРОТКЕ КРОВИ БОЛЬНЫХ НЕМЕЛКОКЛЕТОЧНЫМ РАКОМ ЛЕГКОГО

Цель: **нарушения экспрессии трансмембранной молекулы адгезии эпителия Е-кадерина (E-cad) ассоциированы со злокачественными новообразованиями у человека. Цель исследования — оценить клиническое значение содержания секретируемого Е-кадерина (sE-cad) в сыворотке крови больных с диагнозом немелкоклеточного рака легкого (НМКРЛ).** ***Материалы и методы:*** **для определения уровня циркулирующего sE-cad в сыворотке крови 20 больных с НМКРЛ и 29 здоровых доноров применили метод ELISA.** ***Результаты:*** **у больных с НМКРЛ выявлено значительное повышение содержания циркулирующего sE-cad в сыворотке крови по сравнению с таковым в контрольной группе ($p < 0{,}001$). Установлена связь между уровнем sE-cad в сыворотке крови и появлением периферических метастазов. Не выявлено статистически достоверной корреляции между гистологическим типом опухоли, полом больного и курением. У пациентов с повышенным содержанием sE-cad наблюдалась тенеденция к худшему исходу заболевания, хотя результаты статистического анализа не подтвердили прогностического значения sE-cad.** ***Выводы:*** **полученные данные позволили предположить, что E-cad участвует в патогенезе НМКРЛ. Оценка содержания sE-cad в качестве прогностического биомаркера нуждается в дальнейшем исследовании.**

Ключевые слова: **Е-кадерин, молекула трансмембранной адгезии, малигнизация, немелкоклеточный рак легкого, прогностический биомаркер.**