ICAM-1 expression in patients with advanced non-small cell lung cancer treated with radiotherapy

Pinelopi Gkogkou[1], Evangelia Peponi[2], Dimitrios Ntaskagiannis[2], Asimo Demou[3], Elli Ioakeim[3], Briasoulis Evangelos[4], Periklis Tsekeris[2]

[1]Oncology Department, Norfolk and Norwich University Hospital, Conley Lane, NR4 7UY, Norwich, United Kingdom. [2]Department of Oncology, University Hospital of Ioannina, Stavros Niarchos Avenue 1, Ioannina, 45500, Greece. [3]Department of Pathology, "Hatzikosta" Community Hospital, Makrygianni Avenue, Ioannina, 45001, Greece. [4]Hematology Department and Interscience Molecular Laboratory, Cancer Biobank Center, University of Ioannina, University Campus, Ioannina, 45110, Greece.

Summary

Purpose: To investigate the possible clinical relevance of ICAM-1 molecule in patients with advanced non-small cell lung cancer (NSCLC) treated with radiotherapy.

Methods: The expression of ICAM-1 was examined immunohistochemically on tissue specimens of 62 patients with pathologically confirmed NCSLC. The median age at diagnosis was 62 years (range 49-84) with a male predominance (87.8%). All patients had stage III disease at presentation. The median follow up was 15.5 months (range 7-44). Obtained expression data were weighted against clinical and pathological parameters.

Results: Thirty-seven patients (60%) had no ICAM-1 staining, 16 patients (26%) had weak staining, while 6 patients (10%) expressed moderate staining and only 3 patients (5%) showed strong ICAM-1 staining. Moderate and high expressions were mostly observed in adenocarcinomas and undifferentiated carcinomas (n=8), that are considered more

aggressive than squamous cell carcinoma (n=1). The median overall survival (OS) was 15 months (range 11-20). There seemed to exist an inverse association between ICAM-1 expression and OS, since there was a trend in median survival in favor of no ICAM-1 expression (p=0.083). Moreover, in patients with no ICAM-1 expression, there was observed a statistically significant difference in OS, favoring the squamous cell subtype (p=0.006). Nevertheless, ICAM-1 expression did not confer any statistical significance regarding smoking status (p=0.128), metastatic potential (p=0.574) as well as with the site of metastasis (p=0.964).

Conclusion: Our findings may serve as a helping resource for further investigations of ICAM-1 as a molecular marker that could characterize treatment response and survival of tumor subpopulations.

Key words: ICAM, immunohistochemistry, non-small cell lung cancer, radiotherapy

Introduction

Non-small cell lung carcinoma (NSCLC) is one of the most aggressive types of cancer. During the last decade most patients with advanced disease receive chemoradiotherapy combined regimens which have improved the control of resistant tumors [1-3]. Nowadays, personalized treatments are being offered on the basis of molecular and pathologic tumor characteristics, therefore, iden-

tification of proteins and molecular mechanisms adjusting the pathway of relapse and metastases are important for effective long-term management of the disease [4].

Intercellular adhesion molecule-1 (ICAM-1) is a transmembrane glycoprotein belonging to the immunoglobulin superfamily of adhesion molecules [5-8]. ICAM-1 is traditionally known to play

a crucial role in a variety of benign diseases and malignant tumors, as an adhesion molecule and a stimulator of inflammatory cells during antigen presentation [9]. During inflammatory and immune response, ICAM-1 is activated and expressed on endothelial cells binding via lymphocyte function-associated antigen-1 (LFA-1) and macrophage antigen-1 (Mac-1), facilitating the invasion of damaged tissue by immune cells [7-10]. Furthermore, ICAM-1 can be induced by interleukin-1 (IL-1), and tumor necrosis factor (TNF) and is expressed in the vascular endothelium, macrophages and lymphocytes [5,10].

ICAM-1 has also been implicated in elucidating tumor prognosis and progression in various types of cancer [5,6,11]. Furthermore, ICAM-1 is expressed on the surface of many cancer cell types [5,12] and is also present in a soluble form circulating in the plasma of cancer patients at elevated levels [6,11]. It has also been proposed that ICAM-1 may be involved in the process of cancer metastases, promoting the spread of metastatic cancer cells to secondary sites [12-14].

There are studies having evaluated the novel role of ICAM-1 in cancer patients receiving radiotherapy, showing differences in expression of the molecule for those that showed treatment response [15,16]. This remark could be explained by the increased expression of ICAM-1 on tumor cells, correlating with T-lymphocytes and interacting with them as an adhesion molecule. This co-stimulation causes binding and killing of the tumor cells and could possible predict a relationship between this molecule and radiotherapy effect [17].

In the current study, we investigated whether ICAM could play a prognostic-predictive role in a cohort of patients with advanced NSCLC treated with chemoradiation. We also examined the relationship of ICAM with other clinicopathologic factors.

Methods

Study design and patient population

Sixty-two patients with advanced stage NSCLC were retrospectively evaluated and all of them had signed the informed consent form. Data were collected regarding host (age, sex, smoking history), tumor (TNM classification, histological grade and type) and treatment regimen (chemotherapy and radiotherapy). All the patients received radiotherapy. Sixty six (66%) of the patients received platinum-based chemotherapy. Ethical approval was obtained from University Hospital of Ioannina, Greece. Formalin-fixed paraffin-embedded tissue specimens were available. Histological typing was based on WHO classification. Immunostaining with the ICAM-1 monoclonal antibody was performed on formalin-fixed,
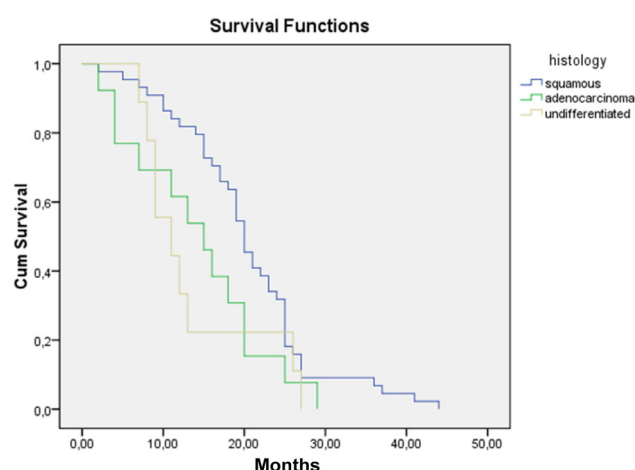
Figure 1. Univariate analysis of histological subtype (squamous vs adenocarcinoma vs undifferentiated) with overall survival (p=0.006).

paraffin-embedded tissue sections using the EnVision System (DAKO Corp, Netherlands). All tumor specimens were obtained before therapy. Tumor specimens were reviewed by two pathologists blinded to any clinical information. For accuracy reasons, one pathologist scored all the samples and the second pathologist confirmed observed scores by spot checking. Individuals cores were evaluated and scored for intensity of ICAM-1 immunoreactivity on a 0-3 rating scale. A score of zero was given to tissues with no-ICAM-1 staining, a score of one delineated weak ICAM-1 staining, a score of two expressed moderate ICAM-1 staining and a score of three was given to tissues with strong ICAM-1 staining (Figure 1).

Statistics

The immunohistochemical expression of ICAM-1 was investigated as a predictive factor for the radiotherapy response. It was also associated with pathological and clinical variables such as patient, tumor and treatment characteristics. The study endpoints included OS, progression-free survival (PFS), time to locoregional recurrence (LC) and time to distant metastasis (DM). OS was defined as time to death from any cause. PFS was defined as time to events including death or disease progression at local, regional or distant sites. The date of progression was defined as the date of radiological or histological confirmation if available, whatever occurred earlier. LC was defined as time to documented recurrence in the lung or regional mediastinum lymphadenopathy. DM was defined as time to documented recurrence in distant sites outside the locoregional sites of disease. Actuarial values of the endpoints were evaluated by the Kaplan-Meier method, and compared with the log-rank test for equality of survival functions. Cox regression analysis was used for univariate analysis and multivariate analysis and parameters were compared with log-rank tests. Only variables that had prognostic significance in the univariate analysis were included in the multivariate model. P values of <0.05 were considered statistically significant; all p values were two-tailed. All time-related

outcomes were calculated from the day of diagnosis. Statistical calculations were performed using SPSS software version 22.0 for Windows (IBM,Chicago, IL, USA).

Results

Patient, disease and treatment characteristics

The median age at diagnosis was 62 years (range 49-84). All patients had stage III disease at presentation. Patient and disease characteristics are listed in Table 1, while treatment characteristics are outlined in Table 2. The median overall survival was 15 months (range 11-20). The 2-year OS was 75.8% (Figure 2).
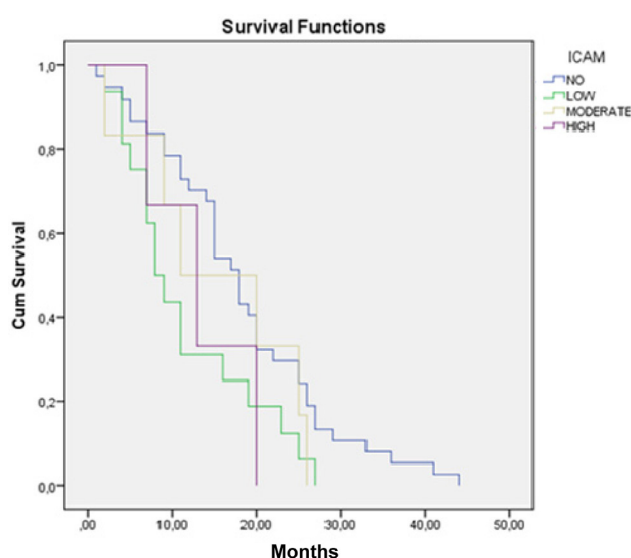
Figure 2. Univariate analysis of ICAM-1 expression (no vs moderate vs strong) with overall survival (p=0.083).
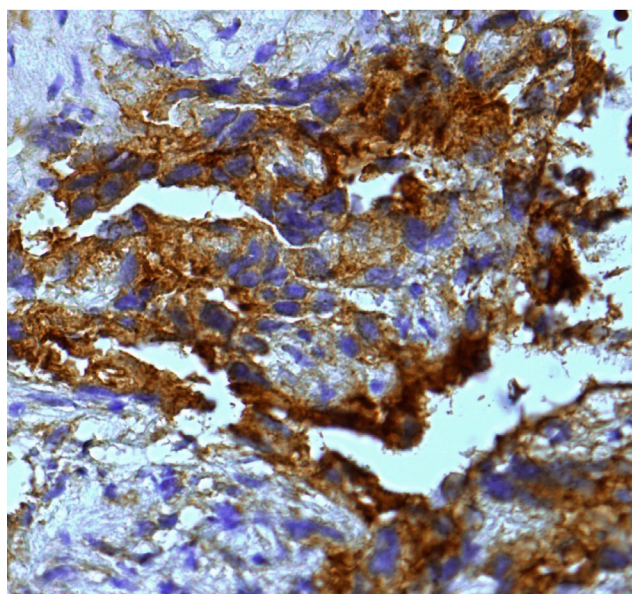
Figure 3. Strong ICAM-1 staining in non-small cell lung cancer cells.

ICAM-1 immunohistochemical analysis- Prognostic role

Thirty seven patients (60%) had no ICAM staining, 16 patients (26%) had weak staining, while 6 patients (10%) expressed moderate staining and only 3 patients (5%) showed strong ICAM staining. Moderate and high expression was observed mostly in adenocarcinomas and undifferentiated carcinomas (n=8), that are considered more aggressive histological types than squamous cell carcinomas (n=1).

Univariate analysis was performed to examine the impact of ICAM-1 expression. There seemed to exist an inverse association between ICAM-1 expression and OS, since there was a trend in median survival in favor of no ICAM-1 expression (p=0.083). Moreover, in patients with no ICAM-1 expression, a statistically significant difference in OS was observed, favoring the squamous cell subtype (p=0.006) (Figure 3). Nevertheless, ICAM-1 expression did not confer any statistical significance regarding smoking status (p=0.128), metastatic potential (p=0.574) as well as with the site of metastasis (p=0.964). In the multivariate analysis neither ICAM-1 expression (p=0.122) nor histology subtype (p=0.471) were significantly associated with OS.

Discussion

Although many studies have been conducted about the treatment efficacy of radiotherapy with the use of modern techniques in combination with the new chemotherapeutic regimens, still the prognosis of advanced stage NSCLC remains dismal. A lot of studies have been trying to identify the molecular profile of NSCLC and correlate biological markers to the response to radiotherapy. This retrospective study was performed in order to evaluate if the ICAM-1 adhesion molecule could act as a predictive factor for OS, PFS, LC and free DM survival in patients with advanced stage NSCLC treated by radiotherapy ± chemotherapy. We also examined the correlation between clinicopathological parameters and ICAM-1 expression.

Compared to other studies [16,18] our cohort showed no correlation between sex and ICAM-1 expression. There was also no correlation between the smoking effect and the absence of ICAM-1 expression. In other publications, although smoking is considered to be a robust factor correlated to lung cancer, no study could be found showing any correlation with ICAM-1 expression [19]. Notably, in the study of Lensamr et al [20], it was observed that in smokers, the ICAM-1 expression was decreased almost to zero in most of the specimens.

It was reported that the percentage of macrophages expressing ICAM-1 was significantly lower in smokers in comparison to non-smokers. On the other hand, in another observational study, ICAM had an increased expression in smokers, with a possible explanation of inflammation effect that is caused by smoking [21].

In this study, there was a statistically significant correlation between lack of ICAM-1 expression and histological subtype. While most of the squamous cell carcinomas did not express ICAM-1, high ICAM-1 expression was found in adenocarcinomas and undifferentiated subtypes. This has been also examined in two studies that reported no difference between histologic subtypes and the expression of ICAM-1 [16,19]; however, in a study by Schardt et al [22], an interaction between ICAM-1 expression and large cell subtype has been noted.

In the present study no correlation was found between ICAM-1 expression and the metastatic potential of the tumor, as well as the sites of metastasis. Other authors have reported an increased metastatic potential in positive expression of ICAM-1 [16,23,24]. Also, in a study of Sprenger et al [19], patients with higher levels of ICAM-1 expression were likely to sustain liver and brain metastatic potentiality.

Regarding our results, there seems to exist an inverse association between ICAM-1 expression and OS. Other studies have implied that ICAM-1 expression may have a predictive role and is associated with poor prognosis [16,19,23]. Patients with high levels of ICAM-1 expression were likely to have a more advanced stage of disease and metastatic sites. In a study by Kotteas et al [16], high expression of ICAM-1 in patients with small cell lung cancer would predict this molecule marker for

response to treatment in patients with extensive disease.

Our study noted that patients without ICAM-1 staining had better survival. This might mean that these patients would possibly benefit from RT treatment. Perhaps this would indicate a trend for using ICAM-1 as a predictive marker for response to radiotherapy in NSCLC. On the other hand, thoracic irradiation due to inflammation was reported to significantly increase the expression of ICAM-1, which was indicative of being associated with lung injury from radiotherapy [25-27].

The most important limitation of the present study is its retrospective design. It is therefore limited by the bias inherent in this type of analysis. Nevertheless, despite the small number of patients analyzed, patients were treated relatively consistently and data were collected with meticulous follow-up.

In conclusion, according to our findings, ICAM-1 expression might be a prognostic factor for OS and a predictive factor for radiotherapy response in advanced stage NSCLC patients. Additionally, ICAM-1 lack of expression was a significant prognostic factor concerning histological subtype favoring the squamous cell subtype.

Ethical standards

The institutional ethics review committee at the University Hospital of Ioannina, Greece, approved this study. This work complied with principles of the Helsinki Declaration.

Conflict of interests

The authors declare no conflict of interests.

References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J , Thun MJ . Cancer statistics, 2009. CA Cancer J Clin 2009;59:225-49.

2. Pfister DG, Johnson DH, Azzoli CG et al. American Society of Clinical Oncology treatment of unresectable non-small-cell lung cancer guideline: update 2003. J Clin Oncol 2004;15:333-53.

3. Schaake-Koning C, van den Bogaert W, Dalesio O et al. Effects of concomitant cisplatin and radiotherapy in inoperable non-small cell lung cancer. N Engl J Med 1992;326:524-30.

4. Kulesza P1, Ramchandran K, Patel JD. Emerging concepts in the pathology and molecular biology of advanced non-small cell lung cancer. Am J Clin Pathol 2011;136:228-38.

5. Dymicka-Piekarska V, Kemona H. Does colorectal cancer clinical advancement affect adhesion molecules (sP-selectin, sE-selectin and ICAM-1) concentration? Thromb Res 2009;124:80-3.

6. Bella J, Kolatkar PR, Marlor CW, Greve JM, Rossmann MG. The structure of the two amino-terminal domains of human ICAM-1 suggests how it functions as a rhinovirus receptor and as an LFA-1 integrin ligand. Proc Natl Acad Sci USA 1998;95:4140-45.

7. Staunton DE, Marlin SD, Stratowa C, Dustin ML, Springer TA. Primary structure of ICAM-1 demonstrate interaction between members of the immunoglobulin and integrin supergene families. Cell 1988;52:925-33.

8. Springer TA. Adhesion receptors of the immune sys-

tem. Nature 1990;346:425-34.

9. Hubbard AK, Rothlein R. Intercellular adhesion molecule-1 (ICAM-1) expression and cell signaling cascades. Free Radic Biol Med 2000;28:1379-86.

10. Rothlein RD. A human intercellular adhesion molecule (ICAM-1) distinct from LFA-1. J Immunol 1986;137:1270-74.

11. Dowlati A, Gray R, Sandler AB, Schiller JH, Johnson DH. Cell adhesion molecules, vascular endothelial growth factor, and basic fibroblast growth factor in patients with non-small cell lung cancer treated with chemotherapy with or without bevacizumab–an Eastern Cooperative Oncology Group Study. Clin Cancer Res 2008;14:1407-12.

12. Lin Y-C, Shun C-T, Wu M-S, Chen C-C. A novel anticancer effect of Thalidomide: Inhibition of intercellular adhesion molecule-1 mediated cell invasion and metastasis through suppression of nuclear factor-kB. Clin Cancer Res 2006;12:7165-73.

13. Brooks KJ, Coleman EJ, Vitetta ES. The antitumor activity of an anti-CD54 antibody in SCID mice xenographed with human breast, prostate, non-small cell lung, and pancreatic tumor cell lines. Int J Cancer 2008;123:2438-45.

14. Liang S, Slattery MJ, Wagner D, Simon SI, Dong C. Hydrodynamic shear rate regulates melanoma-leukocyte aggregation, melanoma adhesion to the endothelium, and subsequent extravasation. Ann Biomed Eng 2008;36:661-71.

15. Grothey A1, Heistermann P, Philippou S, Voigtmann R. Serum levels of soluble intercellular adhesion molecule-1 (ICAM-1, CD54) in patients with non-small-cell lung cancer: correlation with histological expression of ICAM-1 and tumour stage. Br J Cancer 1998;77:801-7.

16. Kotteas EA, Gkiozos I, Tsagkouli S et al. Soluble ICAM-1 levels in small-cell lung cancer: prognostic value for survival and predictive significance for response during chemotherapy. Med Oncol 2013;30:662.

17. Slavin-Chiorini DC, Catalfamo M, Kudo-Saito C, Hodge JW, Schlom J, Sabzevari H. Amplification of the lytic potential of effector/memory CD8+ cells by vector-based enhancement of ICAM-1 (CD54) in target cells: implications for intratumoral vaccine therapy. Cancer Gene Ther 2004;11:665-80.

18. Kawano T, Yanoma S, Nakamura Y et al. Evaluation of soluble adhesion molecules CD44 (CD44st, CD44v5, CD44v6),ICAM-1, and VCAM-1 as tumor markers in head and neck cancer. Am J Otolaryngol 2005;26:308-13.

19. Sprenger A, Schardt C, Rotsch M et al. Soluble intercellular adhesion molecule-1 in patients with lung cancer and benign lung disease. J Cancer Res Clin Oncol 1997;123:632-38.

20. Lensmar C, Elmberger G, Skold M, Eklund A. Smoking alters the phenotype of macrophages in induced sputum. Respir Med 1998;92:415-20.

21. Campos JL, Calero C, Arellano-Orden E et al. Increased levels of soluble ICAM-1 in chronic obstructive pulmonary disease and resistant smokers are related to active smoking. Biomark Med 2012;6:805-11.

22. Schardt C, Heymanns J, Schardt C, Rotsch M, Havemann K. Differential expression of the intercellular adhesion molecule-1 (ICAM-1) in lung cancer cell lines of various histological types. Eur J Cancer 1993;29A:2250-55.

23. Haghi AR, Vahedi A, Shekarchi AA, Kamran A. Correlation of serum intercellular adhesion molecule 1 and vascular endothelial growth factor with tumor grading and staging in breast cancer patients. J Cancer Res Ther 2017;13:257-61.

24. DeVita F, Infusino S, Auriemma A, Orditura M, Catalano G. Circulating levels of soluble intercellular adhesion molecule-1 in non small cell lung cancer patients. Oncol Rep 1998;5:393-6.

25. Kesanakurti D, Chetty C, Rajasekhar Maddirela D, Gujrati M, Rao JS. Essential role of cooperative NF-κB and Stat3 recruitment to ICAM-1 intronic consensus elements in the regulation of radiation-induced invasion and migration in glioma. Oncogene 2013;32:5144-55.

26. Raoufi-Rad N, McRobb LS, Lee VS et al. In vivo imaging of endothelial cell adhesion molecule expression after radiosurgery in an animal model of arteriovenous malformation. PLoS One 2017;12:e0185393.

27. Guo L, Ding G, Xu W et al. Prognostic biological factors of radiation pneumonitis after stereotactic body radiation therapy combined with pulmonary perfusion imaging. Exp Ther Med 2019;17:244-50.