ERCC1 and Ki67 in Small Cell Lung Carcinoma and Other Neuroendocrine Tumors of the Lung

Distribution and Impact on Survival

Birgit Guldhammer Skov, MD, DrSci, Bente Holm, MD, PhD,† Anders Erreboe, MD,† Torsten Skov, MD, PhD,‡ and Anders Mellemgaard, MD, PhD†*

Background: Excision repair cross-complementation group 1 (ERCC1) is a key component of the platinum-DNA repair mechanism. Ki67 is associated with the clinical course of several malignancies. The associations of ERCC1 and Ki67, clinical features and survival in small cell lung carcinoma (SCLC), typical carcinoid (TC), atypical carcinoid (AC), and large cell neuroendocrine carcinoma (LCNEC) were determined.

Materials and Methods: We included a consecutive series of 186 patients with SCLC treated with platinum-based chemotherapy and surgically treated patients with TC ($n = 48$), AC ($n = 15$) and LCNEC ($n = 27$). ERCC1 and Ki 67 were measured by immunohistochemistry and scored using published criteria.

Results: The expression of ERCC1 was different among the different tumor types ($p < 0.001$). For patient with limited disease as well as extensive disease SCLC, no association of ERCC1 expression with survival was observed ($p = 0.59$). However, only 10% of SCLC tumors expressed ERCC1. For TC and AC, ERCC1 positive patients had better survival than ERCC1 negative patients. ERCC1 had no prognostic impact for LCNEC. A difference of the percentage of Ki67 LI was observed for the different tumor types ($p < 0.001$). The difference between TC and AC was significant ($p = 0.02$), as was the difference between low grade (TC+AC) and high grade NE (LCNEC + SCLC) ($p < 0.001$). For all included patients, a correlation between Ki67 and ERCC1 was observed (RSquare = 0.19, $p < 0.001$).

Conclusion: ERCC1 expression in SCLC treated with platinum-based chemotherapy has no impact on survival. High expression of ERCC1 in TC might represent a clue to the failure of platinum-based therapy in these patients. ERCC1 expression has prognostic impact in lung carcinoids. Ki 67 might be considered as a supplementary test to the histopatologic classification of NE tumors.

*Department of Pathology (Division Gentofte), Herlev University Hospital; †Department of Oncology Herlev Hospital, Herlev, Denmark; and ‡Broenshoej Kirkevej 49, Broenshoej, Denmark.

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Address for correspondence: Birgit Guldhammer Skov, MD, DrSci, Department of Pathology, Gentofte Hospital, DK-2900 Gentofte, Denmark. E-mail: bigu@heh.regionh.dk

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Small cell lung carcinoma (SCLC), large cell neuroendocrine carcinoma (LCNEC), typical carcinoid (TC), and atypical carcinoid (AC) comprise a group of lung tumors with neuroendocrine features (NE). Despite common classification, these NE are very different regarding natural course of the disease and treatment strategies. Management with platinum-based chemotherapy for SCLC results in initial response rates of 60 to 90%. This regimen is recommended for all stages of this disease and has been a standard first-line therapy for SCLC since the 1980s.[1,2] Early recurrence and subsequent resistance to therapy is the main cause of poor outcome in these patients and determination of who would benefit from therapy and who would not, has potential clinical implications.

In contrast, TC is resistant to platinum-based chemotherapy and the optimal treatment for patients with N0 and N1 status is complete surgical resection with preservation of as much normal lung as possible.[3,4] The optimal procedure for TC with N2 lymph node metastases is debated. For AC more extensive resections is commonly advocated[5] and metastases to N1 as well as N2 nodes have negative impact on survival.[5,6] Whether a subgroup of these patients might benefit from chemotherapy is largely unknown. Resection is preferred in early stage LCNEC,[7] but limited information exists regarding chemotherapy for more advanced stages.[8]

The mechanism of action of platinum drugs is the formation of platinum-DNA adducts, which cause inter- and intrastrand cross links, especially in highly proliferating cells. Unless these are repaired, the cell dies through apoptosis. Nucleotide excision repair (NER) is involved in the repair of platinum-induced DNA damage.[9,10] Excision repair cross-complementation group 1 (ERCC1) is one of several proteins involved in NER. It is the rate-limiting protein and has retrospectively been associated with resistance against platinum-based chemotherapy in different cancer types including non-small cell lung carcinoma (NSCLC),[11–15] although other studies could not confirm this observation.[16] Prospective studies in NSCLC are available with promising results.[17,18]

Only few studies have dealt with the impact of ERCC1 in SCLC.[19–21] A survival benefit was noted for patients with limited disease and low amount of ERCC1 in two of the studies.[19,20] However, the number of patients was rather small in both series ($n = 77$ and $n = 85$, respectively). Information on the distribution and the prognostic implication of ERCC1 in TC, AC, and LCNEC is lacking.

High proliferation rate is associated with tumor aggressiveness in many tumors, including NE.[22] This might be the result from genomic instability, which is the feature of tumors with low ERCC1 expression. Whether a correlation between ERCC1 expression and proliferation rate as measured by Ki67 exist in NE is unknown.

The purpose of this retrospective study was to determine the distribution of ERCC1 in tumor tissue from a large cohort of Danish patients with SCLC and in TC, AC, and LCNEC. We also aimed to evaluate the association between the level of ERCC1 and overall survival for SCLC treated with platinum-based chemotherapy, and the prognostic value of ERCC1 in patients with TC, AC, and LCNEC treated exclusively with surgery. Finally, we intended to determine the association between the proliferative status of NE as measured by Ki67, the ERCC1 status, and survival. To date, this is the largest group of NE patients investigated for these associations.

PATIENTS AND METHODS

Patients and Tissue Specimens

Consecutive, histologic specimens coded as SCLC (primary or metastatic) in the SNOMED system (M8041*) in the period January 1, 2000, to December 20, 2007, were retrieved from the Pathology System, Department of Pathology, Herlev University Hospital, Division Gentofte, Denmark.

A total of 238 specimens were retrieved from this single institution, which cover a population of about 600,000 inhabitants in the Copenhagen area. Most of patients from the region with pulmonary infiltrate were diagnosed at Gentofte Hospital. The diagnosis of SCLC in all specimens was confirmed by a single experienced pathologist (B.G.S.) before entering this study.

Sufficient material for immunohistochemical analyses for Ki67 and/or ERCC1 was available in 220 patients. For various reasons, 34 of these patients did not receive chemotherapy, leaving 186 patients with SCLC eligible for this study. The clinical information included age, gender, smoking history (smokers, former smokers, or never smokers [defined as <100 cigarettes over a lifetime] and number of pack-years), performance status (PS, according to Eastern Cooperative Oncology Group), serum lactate dehydrogenate (LDH), (measured as normal, elevated up to two times the upper limit, or elevated more than two times the upper limit), and stage of disease limited disease/extensive disease [LD/ED] for SCLC,[23] and tumor, node, metastasis stage for TC, AC, and LCNEC[24]).

All investigated tumor samples with SCLC were collected for routine histopathological examination before chemotherapy. The diagnostic material was endoscopic biopsies from the lung ($n = 160$) or metastatic tumor tissue from the liver ($n = 24$) or elsewhere ($n = 2$). No cytologic material was included.

All patients with SCLC were treated in a single department (Herlev University Hospital, Department of Oncology) and received at least one course of chemotherapy. The standard first-line treatment was a triplet consisting of carboplatin, etoposide, and vincristine. Patients diagnosed with LD were offered concomitant radiotherapy. At the end of treatment, patients with LD were offered prophylactic cranial irradiation. Second line treatment consists of either the same triplet as in the first-line treatment or topotecan. Second-line platinum-based chemotherapy was generally offered to patients with progression more than 90 days after ending first-line treatment. Topotecan was generally offered to patients with progression less than 90 days after ending first-line treatment. Palliative radiotherapy to chest and brain, bone or skin metastasis was administered in some patients. As this study was retrospective, information regarding response was not evenly reported and was not included in the analyses.

Forty-eight patients with TC, 15 with AC and 27 patients with LCNEC were also included, and the inclusion criteria for these patients are described elsewhere.[25] In short, information regarding age, gender, presence or absence of an endobronchial tumor component, stage, surgical treatment, adjuvant therapy, and survival was available. All included patients with TC, AC, or LCNEC were treated by surgery alone.

All material from TC, AC, and LCNEC was from the primary tumor in the lung. No cytologic material was included. The demographic properties of the patients are summarized in Table 1. The follow-up time for patients with SCLC was from the day the patient started chemotherapy until the date of death or January 2009 if alive. The follow-up time for patients with TC, AC, and LCNEC was from the day of diagnosis until the date of death or January 2009 if alive.

Survival data were obtained from the Danish Civil Registration System by linkage to a personal identification number. The study was approved by the Danish Ethics Committee on Research Involving Human Subjects (H-A-2008-120) and the Danish Data Protection Agency (J.nr.2008-41-2780).

Immunohistochemical Analysis

Formalin fixed, paraffin-embedded samples of tumor were selected for immunohistochemical studies. Tumor tissue sections 3 μm thick were dried for 1 hour at 60°C, deparaffinized in Tissue Clear, rehydrated in a graded ethanol series, and treated with 3% hydrogen peroxide to block endogenous peroxidase activity. Antigen retrieval was performed by immersing slides in citrate buffer at pH = 6 and microwaving at high power for 30 minutes. Nonimmune serum was used to block nonspecific binding. Afterward the sections were incubated with primary monoclonal antibodies to ERCC1 (mouse, clone 8F1, Neomarkers) at a dilution of 1:300 for 60 minutes and to Ki 67 (mouse, clone MIB 1, DAKO, Glostrup, Denmark) at a dilution of 1:1000. The antibodies were visualized using the Dako EnVision+ system and diaminobenzidine as a chromogen in an automated immunostainer (DakoCytomation, TechMate Horizon, Glostrup, Denmark). The sections were counterstained with hematoxylin. Positive controls included normal tonsil for ERCC1 and a lymph node for Ki 67. Negative controls had the antibody replaced by phosphate-buffered saline.