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Oestrogen receptor β over expression in males with non-small cell lung cancer is associated with better survival

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Summary
Background: Adenocarcinoma of the lung is more frequent in females than in males and the association with smoking is less pronounced than for the other histological subtypes of lung cancer. Oestrogen induction of cell proliferation has been found in breast adenocarcinomas, and since oestrogen receptors (ER) have been demonstrated in lung tumours, a similar role of oestrogens in the development of lung cancer has been suggested. We examined the expression of ERα, ERβ and progesterone in a well defined cohort of patients with NSCLC with more than 15 years of follow up, and related the results to gender and survival.
Methods: Paraffin embedded, histological material was collected from 104 patients (71 men and 33 women), operated in the period 1989—1992 for NSCLC (56 squamous cell carcinomas, 40 adenocarcinomas and 8 large cell carcinomas). ERα, ERβ and progesterone were immunohistochemically analysed. Staining frequency and intensity was scored semi-quantitatively. A tumour was defined as positive when more than 10% of the tumour cells were positive with at least a weak nuclear staining. Kaplan—Meier survival curves were generated to evaluate the significance of ERα, ERβ and progesterone expression for the prognosis.
Results: ERβ positivity was demonstrated in 69% (72 of 104) of the tumours. There was no statistically significant correlation between ERβ positivity and age, gender, stage, or histology. After adjusting for gender, age, stage at diagnosis and histology there was no difference in survival between subjects with ERβ positive and ERβ negative tumours. When stratifying by gender women with ERβ-negative tumours had a non-significant ($P = 0.26$) decrease in mortality compared with women with ERβ positive tumours. In contrast, men with ERβ positive tumours

Abbreviations: NSCLC, non-small cell lung cancer; ER, oestrogen receptor.

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had a significantly reduced mortality ($P = 0.035$) compared to men with ERβ negative tumours. Using multivariate regression analysis the interaction between gender and positive ERβ staining was the only significant prognostic factor. There was no correlation between the ERα immunohistochemical staining and any of the clinical variables, including survival. None of the 104 patients had tumours positive for progesterone.
Conclusion: The presence of ERβ in a tumour seems to be a positive prognostic factor for men with non-small cell lung cancer. The finding confirms another resent study and suggests that the relation between oestrogens and lung cancer be investigated further.

1. Introduction

During the last two decades the incidence of lung cancer in men has been levelling off, whereas the opposite trend has been observed in women. Lung cancer now kills more women than any other malignant disease [1]. This can be partly be explained by the increased incidence of female smokers [2], but beside that, differences in smoking habits and an increased susceptibility of females to tobacco smoke has been hypothesized [1,2]. Adenocarcinomas constitute a larger proportion of lung cancers in females than in males and the association between smoking and lung cancer is less pronounced for adenocarcinomas than for the other histological subtypes [3,4]. Tobacco is, beyond doubt, the main risk factor for lung cancer but approximately 20% of female lung cancer patients have never smoked [5]. Thus, other factors than smoking seem to be involved in the carcinogenesis of lung cancer and in this setting a role of oestrogen has been proposed. Oestrogen induction of cell proliferation is a well-known risk factor for the development of adenocarcinomas in other organs, e.g. breast, endometrium and ovary. Oestrogen receptors have been demonstrated in lung cancer and the role of oestrogens in the development and prognosis of lung cancer is the focus of an increasing number of studies [6,7]. The oestrogen receptor (ER) is a ligand-activated transcription factor that mediates the effect of 17β-estradiol both in males and females. Since 1996 two different ER have been identified, ERα and ERβ [8]. The tissue distribution and possible function of the two receptor types differ. Whereas ERα plays a key role in adenocarcinomas of the breast, the role for ERα in lung cancer is controversial and a more predominant role of ERβ has been suggested. Studies have demonstrated expression of ERβ but not ERα in human non-small cell lung cancer [9—11] while others have demonstrated that ERα mainly is localized to the cytoplasm of lung cancer cells [7,12] and to a lesser degree in the nucleoli, which is the case in, e.g. breast cancer. In vitro studies have demonstrated variable and often increased proliferation of lung cancer cell lines in response to 17β-estradiol and reduced growth in response to anti-oestrogens [7,9,13]. Recently it has been demonstrated, both in vitro and in vivo, that proliferation and growth of NSCLC cells can be reduced by means of anti-oestrogen [14]. The effect of such treatment has not been tested in clinical studies, but a number of observational studies have demonstrated an association between expression of ERβ and prognosis in patients with non-small cell lung cancer (NSCLC) [10—12]. In the present study we set out to examine the expression of ERα, ERβ and progesterone, and to correlate the results to sex and survival in a cohort of patients with NSCLC and more than 15 years follow up.

2. Materials and methods

2.1. Patients and tissue specimens

The present study is retrospective and based on histological material collected from a cohort of 120 patients operated for NSCLC at the Danish National Hospital, Rigshospitalet from 1989 to 1992. Suitable histological material was available from 104 chemo naive patients and the demographic features of these patients are summarized in Table 1. The median time since operation was 14.9 years (range, 13.7—16.9 years). Survival data were obtained from the Danish Death Certificate Registry. Collection of this patient material was initiated before the law of informed consent (law number 503) was implemented in Denmark on the 24th of June 1992.

Neither smoking history nor information on hormone replacement therapy was available. All tumours were classified according to Who's pathology classification [15] and the staging of the tumour was done according to the international staging system [16].

2.2. Immunohistochemical analysis

A representative tissue block of the resected lung cancer tissue, which contained tumour, was selected from each case for immunohistochemical studies. Formalin fixed, paraffin-embedded 4 μm thick tumour tissue sections were dried for 1 hur at 60 °C, de-paraffinized in Tissue Clear and 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 TEG pH 9 and micro-waving at high power for 15 min. Non-immune serum was used to block non-specific binding. Afterwards the sections were incubated with primary monoclonal antibodies to ER-alpha (ERα) (Oestrogen Receptor α Clone 1D5, Code 1545, Immunotech, USA) at a dilution of 1:100 for 60 min, ER-beta (ERβ) (Oestrogen Receptor β Clone PPG5/10, Code M7292, DakoCytomation, Denmark) at a dilution of 1:10 for 60 min, and progesterone (Clone 16, Code PgR-312, Novo Castra) at a dilution of 1:50 for 60 min. The antibody was visualised using the Dako EnVision + system and diaminobenzidine as a chromogen in an automated immunostainer