Detection of Chromosomal Instability by Fluorescence in Situ Hybridization in Surgical Specimen of Non-Small Cell Cancer

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Purpose: The aim of this study was to evaluate prognostic importance of chromosomal instability (CIN) in non-small cell lung cancers (NSCLC). We examined the relationship between CIN detected by fluorescence in situ hybridization and survival in patients with NSCLC and subgroup.

Experimental Design: 132 surgical specimens of NSCLC were studied. The patients included 109 men and 23 women, with an median age of 59 years. Tumors included 64 adenocarcinomas (AC), 68 squamous cell carcinomas (SCC). The pathologic stage was IA in 22, IB in 53, IIA in 5, IIB in 28, IIIA in 18, and IIIB in 6 cases. Multi-target DNA FISH assay (LAVision, Vysis) was used to determine which tumors carried CIN. Survival were compared according to the following factors: gender, age, histology, T factor, N factor, CIN and smoking status.

Results: Fifty tumors (37.9%) showing numerical heterogeneity in all four examined chromosomes were judged to be carrying CIN. The percentage of CIN was significantly higher in Adenocarcinoma group than squamous cell carcinoma group. (54.7% vs 22.1%, p = 0.001)

The rate of lymph node involvement was lower in CIN positive group. (26% vs 43.9%, p = 0.039) The CIN positive group had lower smoking history. (26.0 ± 24.0 vs 35.1 ± 24.2, p = 0.036) In multivariate analysis, there was no significant differences two groups. Kaplan-Meier survival curves according to CIN status shows no significant differences. Log-rank test revealed only gender factor predicted a poor survival.

Conclusions: Our study demonstrates that CIN is more frequent in AC than SCC. But CIN is not an independent prognostic factor in the group of patients with NSCLC and in the subgroups: both with AC and SCC.

CD63 as a biomarker for predicting the clinical outcomes in non-small cell lung cancers

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Background: The prognosis of lung cancer is still poor, since there are few early detection tools available yet. So, it is important to identify more efficient and clinically applicable biomarkers associated with the prognosis in as earlier stages as possible.

Method: In this study, we firstly observed the expression profile of CD63 in 33 cases of non-small cell lung cancers (NSCLC) using real-time quantitative RT-PCR. To explore the potential of this molecule as a prognostic biomarker for lung cancer subtypes, we constructed tissue microarrays with 90 NSCLCs. Then immunohistochemistry analysis was performed with anti-CD63 antibody. Significance of the association between CD63 expression status and clinicopathological parameters was tested by Chi-square test and two-sided Fisher’s exact test using SPSS version 12.0 and STATA version 7.0 software.

Result: Majority of NSCLCs (75.8%) showed lower CD63 RNA level (less than half) than normal tissue. Notably, all SqCs showed low-expression except one case, while AdCs showed diverse range of expression. CD63 protein expression level was largely compatible with RNA level. Totally, 63.3% of NSCLC were CD63 protein expression negative. All SqCs were negative, while majority (70.2%) of adenocarcinomas (AdCs) were positive. CD63 protein negativity was significantly associated with SqCs type, larger tumor size, and advanced stage. In AdCs, CD63 negativity was associated with poor survival (p=0.008). This association was also significant in earlier stage (I and II) AdCs (p=0.041), but not in advanced stage AdCs. After being adjusted for age and sex by Cox regression and stratified by stages, CD63 negativity still showed borderline association with poor survival as an independent predictor (p=0.076, HR=2.3).

Conclusion: Taken together, these results suggest that CD63 can be a biomarker for predicting the prognosis in earlier stage of lung ADCs. Our findings can be a clue to investigate the role of CD63 in tumorigenesis of ADCs of lung and other cancers.
tive Real-time PCR to analyse EGFR gene copy number using the ABI 7000 sequence detection system. The same specimens are analysed to identify expression of EGFR using standard immunohistochemistry techniques.

**Results:** 100 samples have been analysed to date. EGFR mutations occurred in ~6% of these. K-ras mutations occurred in ~8%. EGFR overexpression occurred in ~40%. Data including gene copy number from the complete cohort of patients will be linked to survival and other clinical parameters.

**Conclusion:** The level of mutations in the EGFR are low. This may be explained by the fact that our study population was entirely Caucasian, predominantly smokers or ex smokers and the tumour sample population was not enriched for adenocarcinoma. The K-ras mutation rate is consistent with a predominantly smoking population. The detection of these mutations in the future may become important determinant in the selection of therapy as there is some evidence to suggest that K-ras mutated tumours are not only resistant to the TKI’s but also their growth may be accelerated with TKI treatment. The relevance of determining EGFR gene copy number using RT-PCR technology is as yet unclear but a planned survival analysis may determine its prognostic significance.

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**BSTB: Prognostic Factors Posters, Tue, Sept 4**

Elevated expression of Carcinoembryonic Antigen-related Cell Adhesion Molecule 1 (CEACAM-1) is associated with increased angiogenic potential in non-small cell lung cancer

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**Purpose:** Recent studies have challenged the previously postulated concept of a tumor-suppressive effect of Carcinoembryonic Antigen-related Cell Adhesion Molecule 1 (CEACAM-1). A possible angiogenic influence of CEACAM-1 in non small-cell lung cancer (NSCLC) has not been investigated so far. Therefore, we examined microvesel density (MVD) and CEACAM-1 expression in primary NSCLC and analyzed their possible correlations under consideration of their prognostic effects.

**Patients and methods:** Specimens from 82 consecutive patients with completely resected NSCLC were stained immunohistochemically using the monoclonal anti-CEACAM-1 antibody 4D1/C2 and the monoclonal anti-CD31 antibody JC70A. The prognostic relevance of CEACAM-1 expression and MVD was evaluated by univariate Kaplan-Meier and multivariate Cox regression analysis. The median follow-up period was 75 months (range 10 to 156 months).

**Results:** A high MVD (i.e.≥31 microvessels/x400 microscopic field) was observed more frequently in tumors with high CEACAM-1 expression (i.e.≥66% stained tumor cells) than in tumors with low CEACAM-1 expression (61.8 versus 33.3%, respectively; p=0.01). In univariate survival analyses, high CEACAM-1 expression and high MVD were associated with development of distant metastasis (p=0.011 and 0.022 respectively) and decreased cancer-related survival (p=0.046 and p=0.006 respectively). Multivariate Cox regression analysis demonstrated that the prognostic impact of CEACAM-1 depended on the prognostic influence of MVD, while MVD itself represented an independent prognosticator for unfavorable cancer-related survival (p=0.021; relative risk, 2.1; 95% confidence interval, 1.1-4.0).

**Conclusion:** Here we show for the first time that high CEACAM-1 expression is associated with increased angiogenic activity in NSCLC, and that the prognostic influence of CEACAM-1 might be derived from this association.

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**PLOD2 and TTF1 expression as prognostic variables in selection for adjuvant treatment in early stage lung adenocarcinoma**

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**Background:** To examine recent gene and protein expression profiling reports and a meta analysis of Thyroid Transcription Factor 1 protein expression for reproducibility as prognostic factors for survival, using two online Lung Cancer Micro array data sources as having prognostic potential in early Adenocarcinoma of the Lung. To assess whether a poor prognostic grouping can be divided into treatment prognostic subgroups by genes selected for 5 Fluorouracil sensitivity or resistance.

**Methods:** Two online micro array data sets were used to assess reproducibility of reported profiles of prognostic signatures. The gene probe for TTF1 was examined for survival association in the two data sets. Genes found to be significant for survival in the first data set were tested in a separate test group. Gene probes found to be significant in both groupings were assessed, as a categorized variables, for predicting survival.

**Results:** The high expression of a probe for the gene procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 (PLOD2) and the low expression of a probe for TTF1 gene were significant and increased the hazard for a poor survival. The significance was seen in both micro array data sets. The hazard for PLOD2, 95% CI 1.14 to 2.13 and for TTF1 0.51 to 0.98. PLOD2 was a better discriminator for survival to 18, 24 or 30 months. PLOD2 gene expression but not TTF1 remained an important variable if age, sex, grade, size or bronchoalveolar features were examined as covariables.

That genes possibly important in 5FU sensitivity can be used to cluster patients with a poor prog nostic high PLOD2 expression or low TTF1 expression.

**Conclusion:** That TTF1 has achieved further proof as a prognostic factor in early Adenocarcinomas of the lung. That PLOD2 a gene associated with a fibroblastic response to serum might be important in early cancer progression in Adenocarcinoma of the lung and deserves further investigation. That patient categorization using prognostic genes for 5FU sensitivity is possible and should be considered in a prospective trial.

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