C/CwB type adenocarcinomas cases revealed any microscopic metastasis (pleural involvement, vascular solid-type tumors. In 34 cases, Cells/F type adenocarcinomas revealed areas of tumor opacity on mediastinal window images of air-type and in each histological slide were selected for FISH analysis. A

Aneusomy by FISH analysis and histology as predictors of invasive lung cancer in bronchial biopsies from high risk subjects

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Background: The development of Lung Carcinoma (LC) is accompanied by changes in histological and chromosomal abnormalities in the airway mucosa. Neither histological grade nor markers of chromosomal abnormalities in preneoplastic epithelial lesions have been adequately validated as predictors of invasive LC.

Methods: Histological dysplasia score and chromosomal aneusomy measured by FISH analysis were compared as correlates of invasive LC in a case-control study of 44 individuals with LC (cases) and 90 individuals without LC (controls). We used bronchial biopsy samples from subjects found by LIFE or white light bronchoscopy to have had moderate dysplasia (MD), severe dysplasia (SD) or carcinoma in situ (CIS). Tissue samples were reviewed by the study pathologist, the grades of preneoplastic change were verified and the appropriate areas in each histological slide were selected for FISH analysis. A 4-color FISH probe was used for aneusomy detection targeting centromere 6, 5p15.2, 7p12 (EGFR) and 8q24 (CMYC).

No correlation was found between E2F1 and CUL1. In in vitro cellular studies need to examine these biomarkers prospectively, and to assess their interaction in predicting lung cancer risk.

Results: The population included 104 males and 30 females with a mean age of 64 years and a mean smoking history of 62 pack-years. There was no difference in mean age, sex distribution or pack-years of smoking between LC cases and controls, but cases had a higher frequency of current smokers (p=0.05). Thirty two had CIS as the highest histological grade of mucosal abnormality, 48 had SD and 54 MD. The strongest correlate with invasive LC was CIS by histological examination (OR=12.5, 95% CI 4.1 to 38.1). Chromosomal aneusomy was seen in 64% of the LC cases but in only 31% of the controls. (OR = 4.6, 95% CI 2.0 to 10.9). The proportion of subjects with chromosomal aneusomy increased from moderate dysplasia (22.2%) to severe dysplasia (41.7%) and CIS lesions (71.9%) and showed a similar trend for cases and controls. Presence of aneusomy slightly increased the risk for LC in MD (OR=1.91, 95% CI 0.26 to 13.8) but was a substantial impacting factor in subjects with SD (OR=7.06, 95% CI 0.82 to 60.1) and CIS (OR=5.93, 95% CI 0.5 to 69.7).

Conclusions: CIS on histological examination and abnormal FISH analysis are both associated with lung cancer cross-sectionally. Future studies need to examine these biomarkers prospectively, and to assess their interaction in predicting lung cancer risk.

SCF protein (Skp2, CUL1) regulate the E2F1 dependent transcriptional activity and cyclin E in human lung tumors

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Background: The E2F1 transcription factor is a cell cycle and oncogenic protein which plays ambiguous role in promoting proliferation or apoptosis depending on histological cell type of lung cancer (Salon et al. Cell Death and Differ. 2006). Accordingly, its activity is tightly controlled by transcriptional and post-transcriptional events in which the ubiquitin-proteasome pathway mediated by SCF complexes such as Skp2 and Cullin 1 (CUL1) proteins play a role in its degradation. We have previously identified differential pattern of E2F1 protein expression in human lung cancer with high expression in high grade neuroendocrine tumors, in contrast with low or no expression in non small cell carcinoma and in carcinoids (small cell lung carcinoma and large cell neuroendocrine carcinoma) (Eymin et al. Oncogene 2001). In order to investigate the role of proteasome degradation in E2F1 expression and activity we analyzed the components of SCF complex (Skp2 and CUL1) to understand their role on expression of E2F1 and its transcriptional targets cyclin E, important regulators of cell cycle at G1-S transition.

Methods: Using immunohistochemistry and immunoblotting we analyzed 128 lung tumors of all histological types for the relationship linking E2F1 and two components CUL1 and Skp2 of the ubiquitin-protein ligase SCFSkp2 <involved in E2F1 proteolysis and the consequence on cyclin E level expression.

Results: Skp2 protein was more often overexpressed in high grade neuroendocrine (HNGE) carcinoma (46/54; 86 %) than in NSCLC (16/50; 32 %) (p<0.0001), and undetectable in 25/25 carcinoids. Overexpression of E2F1 and Skp2 proteins were directly correlated in HNGE tumors where Skp2 overexpression was correlated with advanced stage (p<0.0001) and nodal metastasis (p<0.0001). There was a significant correlation between Skp2 and Ki67 across histological types (p=0.01). No correlation was found between E2F1 and CUL1. In in vitro cellular models we provided evidence that Skp2 is a novel transcriptional target of E2F1. Skp2 interacts with E2F1 and stimulates its transcriptional activity toward the cyclin E promoter. Consistently, we found a correlation between Skp2 and cyclin E (p<0.0001) and between E2F1 and cyclin E in neuroendocrine lung tumors (p<0.0001). In contrast, CUL1
correlated with downregulation and low cyclin E level (p=0.0003), and low Ki67 proliferative marker across histological types (p=0.005).

**Conclusions:** Our data from the first evidence of a direct and functional interconnection linking E2F1, the ratio of Skp2 / CUL1, and cyclin E oncoproteins in human tumors. Whereas Skp2 cooperate with E2F1 to activate its transcriptional function of E2F1 towards cyclin E, CUL1 was identified as a negative regulator of cyclin E suggesting that CUL1 could play a role in limiting the progression of neuroendocrine lung tumors. This indicates therapeutic target for inhibitors of proteasome in high grade neuroendocrine lung tumors.

**Results:** Furthermore, p53, EGFR, and K-ras mutation analysis were performed on the ratio of BAC component and micropapillary component in maximum cut surface of the tumor as histologic factors and compared their conveniences using same series of small-sized adenocarcinomas. Furthermore, we also examined the ratio of bronchioloalveolar carcinoma (BAC) component, micropapillary component in maximum cut surface of the tumor as histologic factors and genome abnormalities of p53, K-ras, and EGFR were also examined.

**Methods:** We conducted a retrospective review of 138 consecutive patients who underwent complete resection for small-sized adenocarcinoma and did not receive any chemotherapy and/or radiotherapy prior to surgery in National Cancer Center Hospital (Tokyo, Japan) from January 1993 to December 2000. We selected CEA, p53, MIB1, p27, EGFR, pEGFR, Cox2, Neuromatin, γH2AX, and TTF-1 and compared their conveniences using same series of small-sized adenocarcinomas. Furthermore, we also examined the ratio of bronchioloalveolar carcinoma (BAC) component, micropapillary component in maximum cut surface of the tumor as histologic factors and genome abnormalities of p53, K-ras, and EGFR were also examined.

**Results:** Among the 10 immunohistochemical factors, two histological factors, and three genomic factors, CEA, MIB1 index, and BAC component ratio were significantly associated with the prognosis of small-sized adenocarcinomas. Cases stained strongly with anti-CEA antibody showed significantly less favorable prognosis than negative cases (p=0.02). Cases that revealed more than 10% of MIB1 index showed less favorable prognosis than cases less than 10% of the index. And cases with more than 50% of BAC component showed more favorable prognosis than cases with less than 50% of BAC component (p=0.01). However, the other factors were not related with the prognosis.

**Conclusion:** Although numerous prognostic factors were reported about the lung adenocarcinoma, this study indicated that only three factors, namely, two immunohistochemical factors of CEA and MIB1 index and histological factor of the ratio of BAC component in the maximum cut surface of the tumor were significantly associated with the prognosis of small-sized adenocarcinoma of the lung. In order to construct a desirable and evidence-based selective diagnosis of the cases for reduction surgery, more extended study for searching convenient prognostic factors about small-sized adenocarcinoma should be performed.